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The functions of cardiolipin in cellular metabolism–potential modifiers of the Barth syndrome phenotype

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ABSTRACT

The phospholipid cardiolipin (CL) plays a role in many cellular functions and signaling pathways both inside and outside of mitochondria. This review focuses on the role of CL in energy metabolism. Many reactions of electron transport and oxidative phosphorylation, the transport of metabolites required for these processes, and the stabilization of electron transport chain supercomplexes require CL. Recent studies indicate that CL is required for the synthesis of iron–sulfur (Fe–S) co-factors, which are essential for numerous metabolic pathways. Activation of carnitine shuttle enzymes that are required for fatty acid metabolism is CL dependent. The presence of substantial amounts of CL in the peroxisomal membrane suggests that CL may be required for peroxisomal functions. Understanding the role of CL in energy metabolism may identify physiological modifiers that exacerbate the loss of CL and underlie the variation in symptoms observed in Barth syndrome, a genetic disorder of CL metabolism.

1. Introduction

Cardiolipin (CL) (1,3-diphosphatidyl-sn-glycerol) is the signature phospholipid of the mitochondrial membrane. First isolated from beef heart (Pangborn, 1942), it is ubiquitous in eukaryotes and also present in prokaryotes. Studies in yeast utilizing well-characterized deletion mutants of CL synthesis (Fig. 1) indicate that CL regulates many cellular functions and signaling pathways, both inside and outside of the mitochondria. The ubiquitous association of CL with energy transducing membranes is consistent with the role of this lipid in bioenergetics (reviewed by Joshi et al., 2009). In fact, CL synthesis and mitochondrial bioenergetics are inter-dependent, as CL synthesis is both required for and stimulated by oxidative phosphorylation (Gohil et al., 2004). Within the mitochondria, the effects of CL deficiency extend beyond bioenergetics to decreased mitochondrial protein import and perturbation of mitochondrial fusion (Jiang et al., 2000; Gebert et al., 2009; Joshi et al., 2012). The deleterious effects of CL deficiency outside the mitochondria include perturbation of the PKC-Slt2 cell integrity and high osmolarity glycerol (HOG) signaling pathways and decreased vacuolar function (Zhong et al., 2005; Zhong et al., 2007; Chen et al., 2008b; Zhou et al., 2009). Perturbation of CL synthesis has long been associated with aging (Paradies et al., 2010), and loss of CL was found to decrease longevity in yeast cells (Zhou et al., 2009). The significance of CL in human health is apparent from clinical findings that perturbation of CL metabolism leads to the life-threatening disorder known as Barth syndrome (BTHS).

In addition to the cellular functions listed above, recent studies indicate that CL is intricately involved in cellular metabolism (Fig. 2). These studies are the focus of the current review. CL interacts with components of the electron transport chain and is required for stabilization of electron transport chain supercomplexes and for optimal respiratory control (Bazan et al., 2013; Pfeiffer et al., 2003; Zhang et al., 2002; Zhang et al., 2005). Perturbation of iron–sulfur (Fe–S) biogenesis has been reported in CL deficient yeast cells, suggesting that iron homeostasis as well as enzymatic activities requiring Fe–S cofactors are dependent on CL biosynthesis (Patil et al., 2013). CL is also required for activities of carrier proteins that transport metabolites for energy metabolism (Kadenbach et al., 1982; Fiermonte et al., 1998; Sedlak

Abbreviations: CL, cardiolipin; Fe–S, iron–sulfur; PKC-Slt2, Protein kinase C-Slt2 mitogen activated protein kinase; HOG, high osmolarity glycerol; BTHS, Barth syndrome; RC R, respiratory control ratio; ROS, reactive oxygen species; ALCAT1, acyl-CoA: lysocardiolipin acyltransferase-1; ISC, iron-sulfur cluster; CIA, cytosolic Fe–S protein assembly; TIM, translocase of the inner membrane; CDP-DG, CDP-diacylglycerol; AAC, ADP/ATP translocase; PiC, phosphate carrier; FATP, fatty acid transport protein; MDV, mitochondrial derived vesicles; DMCA, dilated cardiomyopathy with ataxia; PGP, phosphatidylglycerolphosphate; PGPS, phosphatidylglycerolphosphate synthase; PG, phosphatidylglycerol; MLCL, monolysocardiolipin; TOM, translocase of the outer membrane; SAM, sorting and assembly machinery; CAT, carnitine acyltransferase; CRC, carnitine/acylcarnitine translocase.

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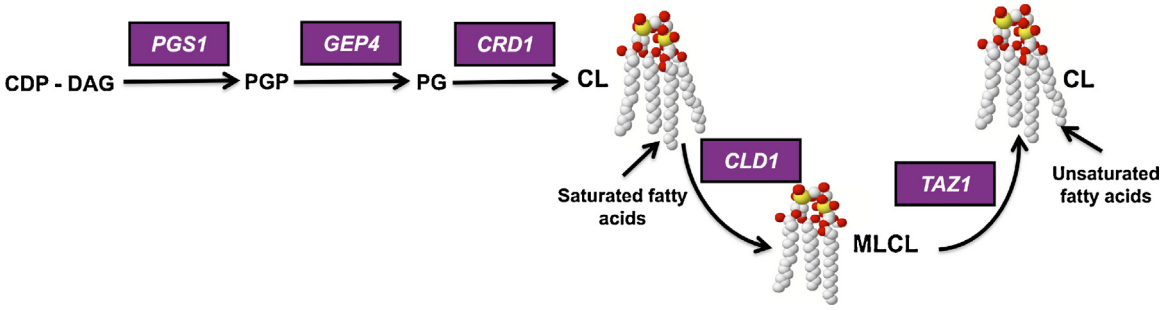

Fig. 1. Synthesis and remodeling of cardiolipin (CL) in yeast. CDP-DAG is converted to phosphatidylglycerolphosphate (PGP) by phosphatidylglycerolphosphate synthase (PGPS), encoded by *PGS1* (Chang et al., 1998; Dzugasova et al., 1998). PGP phosphatase (Gep4) catalyzes the conversion of PGP to phosphatidylglycerol (PG) (Osman et al., 2010). PG is converted to cardiolipin (CL) by CL synthase (Crd1) (Jiang et al., 1997; Chang et al., 1998; Tuller et al., 1998). CL is remodeled in a two-step process in which the CL specific deacylase encoded by *CLD1* removes a fatty acyl group, forming monolysocardiolipin (MLCL) (Beranek et al., 2009), and tafazzin (Taz1) reacylates MLCL to form a generally more unsaturated CL (Xu et al., 2003). In mammalian cells, CL is deacylated by more than one enzyme (Kiebish et al., 2013). Tafazzin is the enzyme that is mutated in Barth syndrome.

and Robinson, 1999; Lange et al., 2001; Hoffmann et al., 1994; Jiang et al., 2000; Bisaccia and Palmieri, 1984), as well as for enzymes in the carnitine shuttle (Pande et al., 1986; Noel and Pande, 1986). In addition, CL might also be required for cellular metabolism outside mitochondria. CL is present in the membrane of peroxisomes (Zinser et al., 1991) and may affect β-oxidation and other metabolic activities of this organelle. The role of CL in mitochondrial protein import is discussed as a potential mechanism underlying the metabolic defects associated with CL deficiency. We speculate that defects in these functions may be physiological modifiers that account for the wide disparity of clinical phenotypes observed in BTHS, and conclude with a discussion of important unanswered questions that are exciting directions for future research.

2. CL and mitochondrial bioenergetics

CL is enriched in the membranes of bacteria, mitochondria, and hydrogenosomes, which play a role in ATP synthesis through the generation of a transmembrane electrochemical gradient (Daum, 1985; Dowhan, 1997; de Andrade Rosa et al., 2006). The association of CL with energy transducing membranes is consistent with the crucial role of this lipid in cellular bioenergetics (reviewed by Schlame et al., 2000; Hoch, 1992). The physical interaction between CL and mitochondrial respiratory chain complexes and other components of membranes also helps in the formation of a lipid scaffold, which functions to stabilize, tether, and increase the enzyme activity of interacting proteins (Beyer and Klingenberg, 1985; Beyer and Nuscher, 1996; Sedlak and Robinson, 1999). In this light, it is not surprising that perturbation of CL synthesis affects the structure and function of mitochondrial respiratory chain complexes and transporters.

2.1. CL and respiration

Analyses of CL function in yeast have been facilitated by the availability of yeast mutants of each step in CL synthesis (Fig. 1). In particular, the CL synthase null mutant *crd1*Δ, which lacks CL (Jiang et al., 1997; Tuller et al., 1998; Chang et al., 1998), has been the focus of numerous studies. Although *crd1*Δ cells can grow in non-fermentable carbon sources, indicating that CL is not essential for respiration, the ADP/O and respiratory control ratios (RCR) of *crd1*Δ mitochondria are reduced in these conditions (Koshkin and Greenberg, 2002). CL is required for optimal RCR and ADP/O ratios and for maintenance of the mitochondrial membrane potential (Jiang et al., 2000; Claypool et al., 2008), especially during unfavorable conditions such as increased temperature and osmolarity (Koshkin and Greenberg, 2002; Koshkin and Greenberg, 2000). The role of CL in respiration has been recently reviewed (Joshi et al., 2009; Patil and Greenberg, 2013).

2.2. CL is required for stabilization of supercomplexes

The electron transport chain complexes are organized into supramolecular structures referred to as supercomplexes (Schagger and Pfeiffer, 2000). *S. cerevisiae* lacks complex I (NADH complex) but contains NADH dehydrogenase composed of a single subunit (Ndi1). Yeast supercomplexes are formed by the association of two units of complex III with units of complex IV. Supercomplexes in mammalian cells are formed by the association of complex I with two units of complex III and multiple units of complex IV (Schagger, 2002). The proposed role of supercomplexes is that of efficient substrate channeling between the individual complexes. The *crd1*Δ mutant exhibits destabilization of the supercomplexes (Pfeiffer et al., 2003; Zhang et al., 2002; Zhang et al., 2005). Bazan and co-workers reported the in vitro reconstitution of supercomplexes and showed that supercomplex III_2IV_2 reconstitution is dependent on CL (Bazan et al., 2013). The loss of CL decreases activity of ADP/ATP carrier protein activity and its association with the supercomplexes (Claypool et al., 2008; Jiang et al., 2000). Destabilization of supercomplexes was also reported in tafazzin-deficient human fibroblasts (McKenzie et al., 2006) and, more recently, in tafazzin-deficient induced pluripotent stem cells (Dudek et al., 2013).

2.3. Loss of CL leads to increased generation of reactive oxygen species (ROS)

Destabilization of supercomplexes is expected to result in increased electron leakage and ROS production (Maranzana et al., 2013). Not surprisingly, the absence of CL in yeast cells leads to increased protein carbonylation, a hallmark of increased ROS (Chen et al., 2008a). The primary sites of ROS generation are complexes I and III (Turrens et al., 1985; Barja, 1999; Kushnareva et al., 2002; Grivennikova and Vinogradov, 2006). The CL acyl chains, which are in close proximity to these ROS generating sites, are susceptible to peroxidation (Li et al., 2010, 2012; Liu et al., 2012). The superoxides generated by respiratory complex III were shown to cause peroxidation of CL and to reduce the activity of cytochrome c oxidase (Paradies et al., 1998, 2000, 2001). The exogenous supplementation of CL, but not peroxidized CL or other phospholipids, rescued both reduced activity of cytochrome c oxidase and increased generation of ROS in reperfused heart (Paradies et al., 2001; Petrosillo et al., 2007). As CL is extensively remodeled by the transacylase tafazzin (Malhotra et al., 2009), we speculate that this may be a mechanism whereby damaged