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Cardiolipin remodeling: a regulatory hub for modulating cardiolipin metabolism and function

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Abstract

Cardiolipin (CL), the signature phospholipid of mitochondria, is involved in a plethora of cellular processes and is crucial for mitochondrial function and architecture. The *de novo* synthesis of CL in the mitochondria is followed by a unique remodeling process, in which CL undergoes cycles of deacylation and reacylation. Specific fatty acyl composition is acquired during this process, and remodeled CL contains predominantly unsaturated fatty acids. The importance of CL remodeling is underscored by the life-threatening genetic disorder Barth syndrome (BTHS), caused by mutations in tafazzin, which reacylates monolysocardiolipin (MLCL) generated from the deacylation of CL. Just as CL-deficient yeast mutants have been instrumental in elucidating functions of this lipid, the recently characterized CL-phospholipase mutant *cld1*Δ and the tafazzin mutant *taz1*Δ are powerful tools to understand the functions of CL remodeling. In this review, we discuss recent advances in understanding the role of CL in mitochondria with specific focus on the enigmatic functions of CL remodeling.

Keywords

Cardiolipin; Cardiolipin remodeling; Mitochondria; Bioenergetics; Tafazzin; Phospholipase; Barth syndrome

Introduction

In eukaryotes, cardiolipin (CL) is predominantly localized and exclusively synthesized *de novo* in the mitochondria (Hostetler et al. 1972; Joshi et al. 2009). As the signature phospholipid of mitochondria, CL is involved in numerous mitochondrial functions, including bioenergetics, apoptosis, mitochondrial dynamics, and mitochondrial structure (Joshi et al. 2009; Ren et al. 2014; Houtkooper & Vaz 2008; Schug & Gottlieb 2009; Schlame & Ren 2009). The diverse functions of CL in the mitochondria highlight the unique nature of this anionic phospholipid. CL contains four fatty acyl chains and two phosphatidyl moieties bridged by a glycerol (Pangborn 1947; Lecocq & Ballou 1964). The hydrophobicity of four acyl groups and negative charges of two phosphate groups confer a wide variety of interactions with mitochondrial proteins (Schlame & Ren 2009; Schlame et al. 2000; Claypool 2009; Klingenberg 2009; Gohil & Greenberg 2009). In addition, the

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dimeric structure confers a conical shape that favors a hexagonal H_{II} phase in membranes (Cullis et al. 1986). Regulation of CL *de novo* synthesis, acyl remodeling, degradation, oxidation, and trafficking controls CL content, acyl composition, and membrane distribution. Recent reviews have described cellular functions (Joshi et al. 2009; Ren et al. 2014; Houtkooper & Vaz 2008) and physicochemical properties of CL (Schlame & Ren 2009; Lewis & McElhaney 2009). In this review, we focus on the mechanisms and functions of CL remodeling.

CL biosynthesis

Most membrane lipids in the mitochondria are synthesized in the endoplasmic reticulum. In contrast, CL biosynthesis occurs exclusively in the mitochondrial inner membrane. The *de novo* synthesis of CL (Fig. 1) is a highly conserved pathway from yeast to mammals (Tian et al. 2012) and is well-characterized in the yeast *Saccharomyces cerevisiae*. The first reaction of CL biosynthesis is the conversion of mitochondrial phosphatidic acid (PA) to CDP-diacylglycerol (CDP-DAG) by the mitochondrial CDP-DAG synthase Tam41 (Tamura et al. 2013; Kutik et al. 2008). Pgs1 catalyzes the committed step of CL synthesis by transferring the phosphatidyl group of CDP-DAG to glycerol-3-phosphate, generating phosphatidylglycerolphosphate (PGP) (Chang et al. 1998a). The subsequent dephosphorylation of PGP to phosphatidylglycerol (PG) is catalyzed by the PGP phosphatase Gep4 in yeast (Osman et al. 2010; Kelly & Greenberg 1990) and PTPMT1 in mammals (Zhang et al. 2011; Xiao et al. 2011). CL synthase (Crd1) catalyzes the final reaction of the *de novo* synthesis of CL by adding a phosphatidyl group from CDP-DAG to PG (Tamai & Greenberg 1990; Jiang et al. 1997; Tuller et al. 1998; Chang et al. 1998b). Thus, CL contains two phosphatidyl groups that are linked by a glycerol molecule. The final reaction of CL synthesis in the prokaryote *Escherichia coli* is different from that in eukaryotes. In *E. coli*, PG incorporates the other phosphatidyl group from phosphatidylethanolamine (PE) (Tan et al. 2012) or another PG (Hirschberg & Kennedy 1972) to generate CL. The distinct mechanisms of action of CL synthase suggest that CL synthesis has evolutionarily differentiated paths (Tian et al. 2012).

CL remodeling enzymes

In yeast and metazoans, a few species of unsaturated acyl groups predominate in CL (Houtkooper et al. 2009a; Schlame et al. 2002), which is highly symmetric (Schlame et al. 2005). Because the enzymes for *de novo* CL synthesis do not exhibit acyl specificity (Ren et al. 2014; Houtkooper & Vaz 2008), remodeling in which acyl chains are removed and replaced with other acyl chains plays a key role in generating symmetric CL with characteristic fatty acid residues following *de novo* synthesis. The remodeling of phospholipids can occur by two mechanisms. In the two-step deacylation-reacylation Lands cycle (Fig. 2a), a phospholipid is deacylated by phospholipase to lysophospholipid. Subsequently, acyl groups from acyl-CoA or neighboring phospholipids are added to the lysophospholipid by acyltransferases or transacylases (Lands 1960). In an alternative mechanism of acyl remodeling, acyl chains are exchanged between adjacent phospholipids in a single transacylase-catalyzed step (Xu et al. 2003; Yamashita et al. 1997) (Fig. 2b). CL remodeling may occur by both mechanisms (Fig. 2c). In the two-step mechanism, CL is

remodeled via the deacylation-reacylation cycle, in which CL is deacylated by phospholipases, and MLCL is reacylated to CL by either transacylase or acyltransferase activity. One-step CL remodeling is the reaction that remodels CL via only transacylation, in which deacylation of phospholipids is not necessary. The importance of CL remodeling is underscored by the life-threatening genetic disorder Barth syndrome (BTHS), which results from mutations in tafazzin, the transacylase that remodels CL (Bione et al. 1996; Barth et al. 1983).

Tafazzin

Tafazzin was identified in 1996 as the gene that is defective in BTHS (Bione et al. 1996; Barth et al. 1983). Homology to acyltransferase sequence motifs suggested that the enzyme was an acyltransferase (Neuwald 1997). Tafazzin is now well-characterized as a phospholipid-lysophospholipid transacylase (Xu et al. 2003) and can catalyze CL remodeling by both mechanisms (Schlame et al. 2002; Xu et al. 2003; Vreken et al. 2000). As a transacylase, tafazzin can catalyze CL acyl remodeling in a single step, in which acyl groups are exchanged between phospholipid and lysophospholipid. In the one-step transacylation of CL remodeling, CL phospholipases are not required for generating MLCL, as a transacylation reaction between a lyso-phospholipid and a CL can generate a phospholipid and MLCL. The subsequent transacylation between MLCL and an adjacent phospholipid generates a remodeled CL and a monolyso-phospholipid. Tafazzin can also participate in a deacylation-reacylation cycle to remodel CL (Fig. 2c). The involvement of tafazzin in this type of remodeling is underscored by the fact that tafazzin deficiency, which abrogates the reacylation step, leads to decreased total CL and increased MLCL, the intermediate generated from deacylation of CL (Vreken et al. 2000; Gu et al. 2004; Schlame et al. 2003; Valianpour et al. 2002; Xu et al. 2006; Acehan et al. 2011; Houtkooper et al. 2009b). The decreased CL/MLCL ratio illustrates that remodeling is initiated by deacylation, which consumes total CL and accumulates MLCL in the absence of tafazzin-mediated reacylation. Coincident with a decreased CL/MLCL ratio, unsaturated CL is also decreased in the absence of tafazzin. These changes in CL profiles are observed in all studied eukaryotes with tafazzin mutations, including yeast (Gu et al. 2004), fruit fly (Xu et al. 2006), mouse (Acehan et al. 2011; Soustek et al. 2011), and human (Vreken et al. 2000; Schlame et al. 2003; Valianpour et al. 2002; Houtkooper et al. 2009b).

Tafazzin-mediated CL remodeling shifts acyl composition towards unsaturation. Interestingly, initial findings showed that the purified recombinant tafazzin enzyme lacks acyl specificity and can react with nearly all phospholipids and lysophospholipids (Xu et al. 2009; Testet et al. 2005; Malhotra et al. 2009a). How, then, does tafazzin remodel CL with specific acyl groups in vivo? Recent studies indicate that the substrate specificity of tafazzin is affected by the membrane lipid phase (Schlame et al. 2012; Gawrisch 2012). In vitro, tafazzin-catalyzed CL transacylation occurs only in nonbilayer lipid micelles and is probably limited to mitochondrial microdomains (Schlame et al. 2012). This underlying mechanism suggests that CL remodeling may be spatially activated at specific mitochondrial membrane domains where tafazzin localizes to shape mitochondrial architecture.

Cld1, the CL-specific phospholipase

Although tafazzin alone can mediate CL remodeling in the presence of lyso-phospholipids, leading to acyl specificity via the transacylation reaction (Ren et al. 2014; Schlame et al. 2012), the dramatic decrease in the CL/MLCL ratio in the tafazzin mutant underscores the importance of CL remodeling via the deacylation-reacylation cycle. In the yeast *S. cerevisiae*, deletion of the cardiolipin-specific phospholipase Cld1, identified in 2009 (Beranek et al. 2009), reduces acyl unsaturation specifically in CL and also prevents MLCL accumulation in tafazzin mutants (Beranek et al. 2009; Baile et al. 2014). These findings indicate that Cld1 is the only CL-specific phospholipase in yeast that initiates CL remodeling by deacylation. Blocking Cld1-mediated deacylation of CL in tafazzin mutants prevents the decreased CL/MLCL ratio, while unremodeled CL remains primarily saturated. Therefore, the *cld1*Δ mutant is a powerful tool with which to distinguish between decreased CL/MLCL and decreased unsaturated (remodeled) CL as the cause of cellular defects in tafazzin-deficient cells. Deletion of *CLD1* rescues respiratory growth defects (Ye et al. 2014; Baile et al. 2014) and decreased chronological lifespan (Ye et al. 2014) in *taz1*Δ, indicating that the decreased CL/MLCL ratio, but not decreased unsaturated CL, leads to the defects in tafazzin-deficient cells. These findings have implications for BTHS, because if a decreased CL/MLCL ratio is the cause of the pathology, attenuation of CL-specific phospholipase activity may be a potential strategy to treat BTHS patients.

Interestingly, *CLD1* is the most highly regulated gene in the CL biosynthetic and remodeling pathways (Ye et al. 2014). Its transcription is repressed in logarithmically growing cells and upregulated about 30-fold in the stationary phase (Ye et al. 2014). This regulation is coordinated with cellular respiratory activity, as *CLD1* transcriptional expression is not increased in the stationary phase in cells lacking mitochondrial DNA (Ye et al. 2014). In addition, respiratory conditions such as growth in non-fermentable carbon sources or glucose depletion also induce *CLD1* expression (Ye et al. 2014; Baile et al. 2013). Controlling *CLD1* expression in response to physiological conditions is important, as increased expression of *CLD1* impairs mitochondrial integrity (Ye et al. 2014).

Cld1 has a higher affinity for saturated than unsaturated acyl groups; thus, Cld1-catalyzed CL remodeling contributes to a more unsaturated CL acyl composition (Beranek et al. 2009; Baile et al. 2014). Interestingly, the *cld1*Δ mutant contains more unsaturated CL in the stationary phase than the logarithmic phase (Ye et al. 2014), suggesting that additional mechanisms alter CL acyl composition in the absence of Cld1-mediated CL remodeling. A likely possibility is that CL is remodeled by tafazzin alone via the one-step transacylation, which is independent of Cld1-involved deacylation-reacylation cycles. Another possibility is that CL precursors PA or PG may be remodeled in the absence of Cld1, leading to changes in CL acyl profiles. Further studies are needed to distinguish among these possibilities.

Mammalian phospholipases that deacylate CL

CL-specific phospholipases in mammals have not been identified, although several mammalian phospholipases are reported to have CL-hydrolyzing activities. These CL phospholipases, including iPLA$_2$β, iPLA$_2$γ, cPLA$_2$, and sPLA$_2$ (Buckland et al. 1998; Hsu et al. 2013; Dennis et al. 2011), belong to a large phospholipase A$_2$ superfamily. Among

these enzymes, $sPLA_2$ (the secreted phospholipase A_2) displays the highest CL deacylase activity in vitro (Hsu et al. 2013).

$iPLA_2\beta$ (Group VIA PLA_2; PNPLA9) and $iPLA_2\gamma$ (Group VIB; PNPLA8) are calcium-independent phospholipase A_2s. They are both found in membrane fractions, including mitochondria (Dennis et al. 2011). $iPLA_2\beta$ is required for integrity of the mitochondrial membrane and influences the release of cytochrome c under oxidative stress (Gadd et al. 2006; Seleznev et al. 2006). Genetic suppression of this enzyme partially restores the CL/MLCL ratio in *Drosophila* tafazzin mutants (Malhotra et al. 2009b). Interestingly, male sterility caused by tafazzin deficiency in *Drosophila* is also rescued by inactivation of $iPLA_2\beta$ (Malhotra et al. 2009b).

$iPLA_2\gamma$ plays critical roles in mitochondrial lipid metabolism and mitochondrial function. Genetic ablation of $iPLA_2\gamma$ in mice leads to tissue-specific phenotypes. In the heart, it decreases CL content and increases CL containing arachidonic (20:4) and docosahexenoic acids (22:6) (Mancuso et al. 2007), accompanied by decreased mitochondrial bioenergetics and defective myocardial function (Mancuso et al. 2007). However, deletion of iPLA2γ in tafazzin knockdown mice increases immature CL species containing 16:0, 16:1, and 18:1 acyl chains and decreases MLCL species containing 18:2–18:2–18:2 and 18:2–18:2–18:1. Although deletion of iPLA2γ in tafazzin knockdown mice partially rescues the abnormal CL profile caused by tafazzin deficiency, it does not prevent a decrease in the predominant CL species (tetra-18:2) or increase in MLCL, suggesting that iPLA2γ is not the only phospholipase that participates in CL remodeling (Kiebish et al. 2013). This finding also suggests that iPLA2γ-catalyzed deacylation of CL is partially responsible for the decreased CL/MLCL ratio in tafazzin mutants. Interestingly, the decreased CL/MLCL ratio in tafazzin-deficient BTHS lymphoblast cells is almost restored to normal after treatment with bromoenol lactone (Malhotra et al. 2009b), an $iPLA_2$ chemical inhibitor that can inhibit both $iPLA_2\beta$ and $iPLA_2\gamma$. Therefore, it is probably safe to extrapolate that both $iPLA_2\beta$ and $iPLA_2\gamma$ participate in initiating CL remodeling by deacylation of CL and contribute to the decreased CL/MLCL ratio in tafazzin-deficient cells. Interestingly, in the hippocampus, deletion of $iPLA_2\gamma$ increases CL content and increases CL with shorter saturated acyl groups (Mancuso et al. 2009), coinciding with enlarged and degenerating mitochondria and cognitive dysfunction (Mancuso et al. 2009). What remains unclear are the specificities of these enzymes, how they are regulated, and their physiological functions. Furthermore, the CL phospholipase activities of the other PLA_2s have not been studied.

Mammalian CL acyltransferases: ALCAT1 and MLCLAT1

In addition to tafazzin, two other acyltransferases can reacylate MLCL in mammalian cells, including acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) and MLCL acyltransferase (MLCLAT1). ALCAT1 is an acyl-CoA-dependent acyltransferase that localizes to the mitochondria-associated membrane, which is a sub-compartment of the endoplasmic reticulum (Cao et al. 2004; Li et al. 2010). It preferentially transfers acyl groups from linoleoyl-CoA or oleoyl-CoA to polyglycerophospholipids that are metabolic intermediates for the synthesis of CL, including MLCL, dilysocardiolipin (DLCL), phosphatidylglycerol (PG), and bis (monoacylglycero) phosphate (Cao et al. 2004; Cao et al. 2009). Therefore,

ALCAT1-involved remodeling can convert saturated CL into more unsaturated CL. ALCAT1 expression is increased in response to oxidative stress (Li et al. 2010). Interestingly, overexpression of ALCAT1 causes oxidative stress (Liu et al. 2012), mitochondrial fragmentation, and instability of mitochondrial DNA (Li et al. 2012). Mitochondrial DNA depletion was also observed in yeast cells overexpressing *CLD1* (Ye et al. 2014). It is possible that both ALCAT1 and *CLD1*-regulated CL remodeling share a common function of modulating CL composition for mitochondrial activity.

MLCLAT1-catalyzed acylation of MLCL was first detected in rat heart mitochondria (Ma et al. 1999) and subsequently purified from pig liver mitochondria (Taylor & Hatch 2003). Purified recombinant MLCLAT1 has a higher affinity for linoleoyl-CoA than oleoyl-CoA or palmitoyl-CoA for the acylation of MLCL to CL. Interestingly, increased expression of MLCLAT1 in BTHS lymphoblast cells increases both incorporation of linoleate into CL and total CL levels (Taylor & Hatch 2009). Although ALCAT1 and MLCLAT1 can reacylate MLCL to CL, the accumulation of MLCL in the absence of tafazzin suggests that ALCAT1 and MLCLAT1-mediated acylation of MLCL is not functionally redundant with tafazzin.

Localization of CL remodeling enzymes

CL *de novo* synthesis occurs on the matrix side of the mitochondrial inner membrane where all the biosynthetic enzymes are located (Tamura et al. 2013; Osman et al. 2010; Tamai & Greenberg 1990; Dzugasova et al. 1998). The post-synthetic remodeling of CL is dependent on the localization of specific remodeling enzymes. Yeast tafazzin is an integral membrane protein of the mitochondria (Claypool et al. 2006), where it binds to the outer face of the inner membrane and the inner face of the outer membrane (Claypool et al. 2006). Submitochondrial fractionation analysis indicates that yeast tafazzin is associated with the mitochondrial inner and outer membrane as well as the contact sites of the mitochondrial inner and outer membrane (Claypool et al. 2006). Therefore, tafazzin can preferentially remodel CL facing the intermembrane space or in the contact sites. In contrast, Cld1 is located in the inner membrane facing the matrix side (Baile et al. 2013). How MLCL generated by Cld1 deacylation on the matrix side of the mitochondrial inner membrane is reacylated by tafazzin in the mitochondrial inner and outer membranes facing the intermembrane space is not understood. In mammals, CL remodeling enzymes tafazzin, $iPLA_2\beta$, $iPLA_2\gamma$, and MLCLAT1 are present in the mitochondria, and ALCAT1 is in the mitochondria-associated membrane (Li et al. 2010). The sub-mitochondrial localization of these enzymes has not been characterized. Understanding the precise localization of mammalian CL remodeling enzymes will help to clarify the mechanism of CL remodeling.

The function of CL remodeling

CL is important for mitochondrial architecture and organization, as it is required for cristae biogenesis (Xu et al. 2005; Acehan et al. 2009) and mitochondrial fusion and fission (Joshi et al. 2012; Xu et al. 2010). As a result of CL-protein interactions, CL promotes respiratory supercomplex formation and optimizes mitochondrial bioenergetics (Pfeiffer et al. 2003; Zhang et al. 2002; Claypool et al. 2008a; Mileykovskaya & Dowhan 2014; Paradies et al. 2014). In addition, CL functions in mitochondrial protein import (Jiang et al. 2000; Gebert et

al. 2009), iron-sulfur biogenesis (Patil et al. 2013), and the TCA cycle (Patil et al. 2013; Raja & Greenberg 2014) and acts as a signal for apoptosis (Schug & Gottlieb 2009) and mitophagy (Chu et al. 2013). The diverse mitochondrial functions of CL highlight its important role as a structural component of the organelle as well as a signaling lipid. A major unanswered question is how CL is regulated during all these processes. CL remodeling may be a pivotal regulatory hub for CL functions. First, CL remodeling can occur in discrete mitochondrial domains where CL remodeling enzymes are localized. Specific CL functions may thus be controlled by regulation of the remodeling enzymes, which may be spatially restricted. Second, CL remodeling can alter CL unsaturation and CL content and generate MLCL and free fatty acids, any or all of which may be important for specific CL functions. Third, the physicochemical properties of CL are subject to change during remodeling, which is likely to affect the affinity of CL-protein interactions. These and possibly other mechanisms that regulate CL remodeling may affect CL-dependent functions. Currently, little is known about the function of CL remodeling, primarily because defective CL remodeling is coupled with deficiency in both CL acyl composition and CL/MLCL production. Thus, it is difficult to distinguish cellular defects caused by decreased CL content and CL remodeling. While the *cld1*Δ mutant may facilitate these studies in yeast, addressing the question awaits the identification of CL-specific phospholipase in mammalian cells.

Unremodeled and remodeled CL

As discussed above, deletion of the CL-specific yeast phospholipase *CLD1* blocks CL remodeling and leads to decreased unsaturated acyl species in CL (Beranek et al. 2009; Baile et al. 2014; Ye et al. 2014). Nevertheless, respiratory growth (Baile et al. 2014; Ye et al. 2014), fermentative growth (Ye et al. 2014), and lifespan (Ye et al. 2014) are similar in *cld1*Δ and wild type cells, suggesting that unremodeled CL in *cld1*Δ and remodeled CL in wild type cells can similarly support these functions. Mitochondrial morphology and cristae size, respiratory supercomplex stability, mitochondrial respiration, and the mitochondrial membrane potential are also similar in *cld1*Δ and wild type cells (Baile et al. 2014). These findings indicate that CL remodeling is not essential in yeast, particularly with respect to bioenergetics. Although unsaturated CL is greatly diminished when CL is not remodeled (Beranek et al. 2009; Baile et al. 2014; Ye et al. 2014), we cannot conclude that Cld1-mediated alteration of acyl composition has no effect on cellular functions, as other genes and pathways may compensate for the loss of Cld1.

CL remodeling and bioenergetics

The role of CL in bioenergetics is well-studied, including mitochondrial respiration, electron transport, and oxidative phosphorylation. CL interacts with respiratory complexes (Lange et al. 2001; Palsdottir et al. 2003; Eble et al. 1990; Shinzawa-Itoh et al. 2007) and ADP/ATP carrier (Claypool et al. 2008a; Beyer & Klingenberg 1985), and the CL-protein interactions stabilize respiratory supercomplexes (Pfeiffer et al. 2003; Zhang et al. 2002; Claypool et al. 2008a). It is thus not surprising that mitochondrial respiration and energy production are correlated with CL biosynthesis (Claypool et al. 2008a; Jiang et al. 2000; Gohil et al. 2004), and that CL deficiency resulting from abnormal CL remodeling disrupts energy dynamics. For example, tafazzin mutations lead to decreased CL content and defective bioenergetics in

yeast (Ma et al. 2004; Claypool et al. 2008b) and mammals (Xu et al. 2005; McKenzie et al. 2006). As discussed above, this defect in yeast *taz1*Δ is due to the decrease in CL/MLCL, not to the decrease in unsaturated CL (Baile et al. 2014; Ye et al. 2014).

The interplay between CL remodeling and bioenergetics is further underscored by the regulation of the yeast remodeling enzyme Cld1. *CLD1* expression increases under respiratory conditions (Ye et al. 2014; Baile et al. 2013). It is also controlled by the Hap2/3/4/5 transcription factor complex that mediates activation of respiratory gene expression (Ye et al. 2014). Furthermore, dissipating the mitochondrial membrane potential in *taz1*Δ decreases the CL/MLCL ratio, suggesting that the activity of Cld1-catalyzed CL deacylation is associated with energy coupling (Baile et al. 2013). The respiratory control of Cld1 activity emphasizes the importance of Cld1-mediated remodeling in energy dynamics. Interestingly, *CLD1* overexpression increases ATP content, and the energy supply is shifted from respiration to glycolysis (Ye et al. 2014). However, how alterations in CL properties resulting from Cld1-mediated remodeling influence energy dynamics remains unclear.

CL remodeling and mitochondrial architecture

Mitochondrial morphology is disrupted in CL remodeling-deficient mutants (Xu et al. 2005). BTHS lymphoblast cells that contain tafazzin mutations display clusters of fragmented mitochondria and dysmorphic cristae (Acehan et al. 2007). However, how tafazzin-catalyzed remodeling shapes mitochondrial architecture is unclear. While tafazzin-catalyzed transacylation is activated by specific physical properties of the membrane (Schlame et al. 2012), tafazzin-mediated remodeling is proposed to reshuffle acyl groups between CL and adjacent membrane lipids and create tightly packed membrane curvature (Schlame et al. 2012). In yeast, tafazzin is localized in the outer face of the inner membrane and the inner face of the outer membrane (Claypool et al. 2006). Therefore, tafazzin-mediated remodeling may participate in the biogenesis of curvature for both the mitochondrial inner and outer membranes. In addition, tafazzin is also localized to the contact sites (Claypool et al. 2006) where the mitochondrial inner and outer membranes are closely tethered together. The mitochondrial contact sites are a newly recognized feature of mitochondrial architecture and are organized by the large protein complex, MICOS. Although CL is enriched in the contact sites (de Kroon et al. 1997; Daum 1985; Zinser et al. 1991; Hovius et al. 1990), it is unclear if CL remodeling is important for their formation. Interestingly, when the integrity of the MICOS complex is disrupted, CL remodeling is also deficient (Harner et al. 2014). Specifically, *AIM24* encodes a yeast inner membrane protein that is required for maintenance of MICOS subunit levels (Harner et al. 2014). Genetic manipulation of MICOS subunits in *aim24*Δ depletes mitochondrial cristae and decreases tafazzin protein, resulting in CL profiles similar to those of tafazzin-deficient mutants (Harner et al. 2014). This finding suggests that tafazzin may function at the contact sites. Although the mechanisms linking remodeling to mitochondrial architecture have not been investigated, tafazzin-catalyzed remodeling is probably important for many features of mitochondrial structure, including the mitochondrial inner and outer membrane, the contact sites, and the cristae.

CL remodeling, CL oxidation, and apoptosis

CL is susceptible to oxidative damage because it is predominantly localized in the mitochondrial inner membrane, where reactive oxygen species (ROS) are generated during respiration (Paradies et al. 2002; Tyurina et al. 2006). Fatty acyl groups of CL containing two or more double bonds are susceptible to oxygenation or CL peroxidation. Tetralinoleoyl-CL (18:2) is the most abundant CL in the heart and most other tissues, whereas brain CL is more complex and contains polyunsaturated fatty acids such as arachidonic (20:4) and docosahexaenoic acids (22:6) (Houtkooper et al. 2009a; Samhan-Arias et al. 2012). These CL species are subject to passive or selective oxidation. Passive oxidation of CL is a non-selective process in which CL is peroxidized, leading to decreased activity of complexes I, III, and IVas well as decreased supercomplex formation (Paradies et al. 2002; Paradies et al. 2001; Paradies et al. 2000; Genova et al. 2008). Selective CL peroxidation by ROS occurs during apoptosis, and CL is the only mitochondrial phospholipid that undergoes peroxidation during this process (Kagan et al. 2005). The release of cytochrome c is an apoptogenic step. CL is bound to cytochrome c by insertion of one acyl chain into a hydrophobic pocket of the protein (Bayir et al. 2006; Ott et al. 2007; Kalanxhi & Wallace 2007). Binding is fortified by electrostatic interactions between the phosphate groups of CL and lysine residues of cytochrome c (Sinibaldi et al. 2008). The tertiary structure of cytochrome c is altered by binding to CL, and the CL-bound cytochrome c complex manifests peroxidase activity (Kagan et al. 2005; Kagan et al. 2009), which is responsible for CL peroxidization by the superoxide dismutation product H_2O_2 (Kagan et al. 2005). Oxidation of CL decreases the CL-cytochrome c interaction, resulting in a decrease in membrane-bound cytochrome c (Iverson et al. 2004; Ostrander et al. 2001; Nomura et al. 2000; Ott et al. 2002). Thus, CL peroxidation is critical for the release of cytochrome c during apoptosis (Belikova et al. 2007; Paradies et al. 2009). Interestingly, double bonds in CL acyl chains increase the CL-cytochrome c binding affinity (Belikova et al. 2006). Thus, cytochrome c preferentially binds to polyunsaturated CL, which is highly susceptible to oxidation by ROS (Belikova et al. 2006). CL remodeling can both modulate the affinity of cytochrome c for CL and replace oxidized fatty acids with non-oxidized acyl groups, restoring the interaction. Therefore, CL remodeling may be important for controlling cytochrome c release during apoptosis.

CL remodeling and disease

BTHS is the most direct example of a human disorder resulting from perturbation of CL remodeling. BTHS is a severe X-linked genetic disorder caused by mutations in tafazzin, resulting in cardiomyopathy, skeletal myopathy, growth retardation, and neutropenia (Barth et al. 1999; Christodoulou et al. 1994; Clarke et al. 2013). Cardiac problems prevalent in BTHS patients include dilated, hypertrophic, and noncompaction cardiomyopathy and heart failure. Abnormal CL profiles resulting from tafazzin deficiency (Vreken et al. 2000; Schlame et al. 2003; Valianpour et al. 2002; Houtkooper et al. 2009b) affect energy metabolism and heart function, as CL is required for optimal activity of oxidative phosphorylation. As discussed above, defects in yeast tafazzin mutants are rescued by blocking CL deacylation. If these findings are conserved in mammalian cells, the identification and characterization of human CL phospholipases may help to develop

therapeutic strategies for BTHS patients. BTHS is also characterized by a wide disparity of clinical presentations, ranging from severe incapacitating disease to nearly asymptomatic, even in patients carrying identical tafazzin mutations (Joshi et al. 2009; Ren et al. 2014; Clarke et al. 2013). The lack of genotype-phenotype correlation suggests that genetic modifiers may play a role in BTHS pathology. While the molecular basis underlying the pathology of BTHS is not understood, potential modifiers that inhibit CL deacylation may prevent the decreased CL/MLCL. Identifying such modifiers may shed light on other avenues for BTHS treatment.

The clinical presentations in BTHS are similar to those of an autosomal recessively inherited human disorder, dilated cardiomyopathy with ataxia (DCMA), which is caused by mutations in DNAJC19 (Davey et al. 2006; Ojala et al. 2012). DNAJC19 encodes a mitochondrial inner membrane chaperone protein that is thought to function in mitochondrial protein import (Davey et al. 2006). Interestingly, a recent study suggested that the DNAJC19 protein may function in CL remodeling by regulating tafazzin activity via the association with prohibitin, a ring-like scaffold protein located in the inner membrane of the mitochondria (Richter-Dennerlein et al. 2014). Defective CL remodeling has also been implicated in both Type I and II diabetes (Han et al. 2005; Watkins et al. 2002; He & Han 2014). At the very early stage of pathological development in the diabetic mouse model, cardiac CL undergoes abnormal remodeling, resulting in a decrease in total CL content, depletion of the major cardiac CL, tetralinoleoyl-CL (18:2), and an increase in CL species containing longer and polyunsaturated fatty acids (Han et al. 2007). Interestingly, these abnormal CL presentations are ameliorated by treatment with the antidiabetic drug rosiglitazone, which increases both total CL and tetralinoleoyl-CL but decreases polyunsaturated CL in diabetic mice (Watkins et al. 2002; Pan et al. 2006). In summary, CL remodeling plays a role in the pathology of human disease. Therefore, elucidating the functions of CL remodeling in metabolic and physiological process may provide novel strategies for treating human disorders.

Coda

CL was first isolated from beef heart in 1947 (Pangborn 1947). After more than six decades, a plethora of cellular and mitochondrial functions have been linked to CL through genetic, biochemical, and cellular studies. CL is now recognized as a crucial phospholipid for optimal cellular physiology and human health. Important as CL homeostasis is, CL remodeling may be a critical regulatory hub for controlling CL content, acyl composition, and distribution and, thus, for its versatile functions. The functions of CL remodeling are just beginning to be explored. While it is involved in bioenergetics, mitochondrial structure, and apoptosis, additional functions and detailed mechanisms of CL remodeling have yet to be elucidated.

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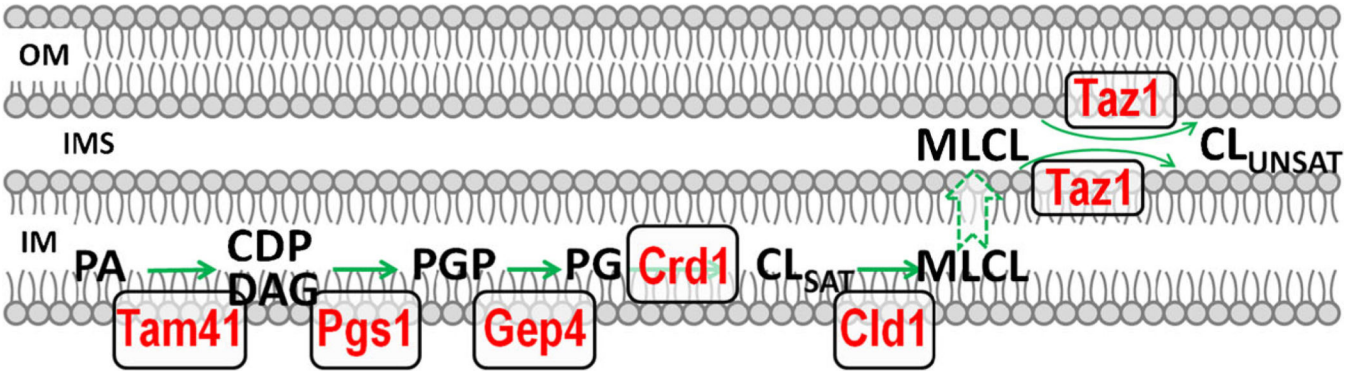

Fig. 1.

CL *de novo* synthesis and remodeling in *S. cerevisiae*. The first reaction of CL *de novo* synthesis is the conversion of phosphatidic acid (PA) to CDP-diacylglycerol (CDP-DAG) by the mitochondrial CDP-DAG synthase Tam41. The committed step of CL synthesis is catalyzed by Pgs1, which converts CDP-DAG to phosphatidylglycerolphosphate (PGP). PGP is subsequently dephosphorylated to phosphatidylglycerol (PG) by the *GEP4*-encoded PGP phosphatase. CL synthase, encoded by *CRD1*, condenses PG and CDP-DAG to form CL. CL synthesized *de novo* has primarily saturated acyl chains (CL_{SAT}). CL_{SAT} is deacylated by the CL-specific phospholipase Cld1 to monolysocardiolipin (MLCL), which is reacylated by tafazzin (the *TAZ1* gene product) to CL containing more unsaturated acyl chains (CL_{UNSAT}). All the CL biosynthetic enzymes are localized in the mitochondrial inner membrane (IM), whereas tafazzin is localized in the outer face of the (IM) and the inner face of the outer membrane (OM). IMS: intermembrane space

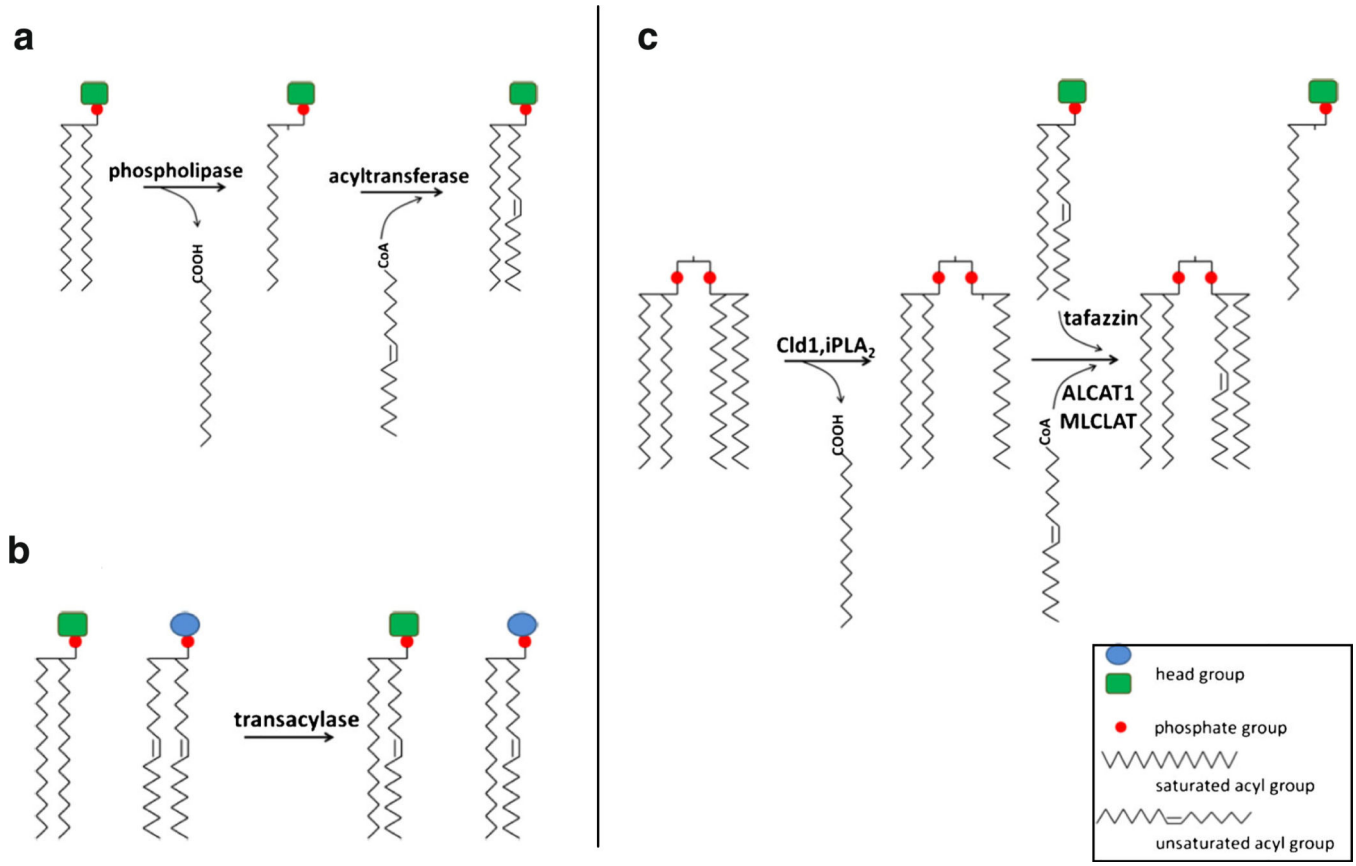

Fig. 2.

phospholipid remodeling. The remodeling of phospholipids can occur by two mechanisms, (**a**) a two-step deacylation-reacylation Lands cycle, and (**b**) a single-step transacylation. CL remodeling (**c**) can occur by both mechanisms. Tafazzin-catalyzed transacylation can remodel CL in a single step or reacylate MLCL to CL in the two-step mechanism