Cell. Mol. Life Sci. 65 (2008) 2493 – 2506
1420-682X/08/162493-14
DOI 10.1007/s00018-008-8030-5

Cellular and Molecular Life Sciences

Review

Cardiolipin, the heart of mitochondrial metabolism

R. H. Houtkooper and F. M. Vaz*

Laboratory of Genetic Metabolic Diseases, University of Amsterdam, Academic Medical Center, Departments of Clinical Chemistry and Pediatrics, P.O. Box 22700, 1100 DE Amsterdam (The Netherlands),
Fax: +31 20 6962596, e-mail: f.m.vaz@amc.nl

Received 16 January 2008; received after revision 26 February 2008; accepted 26 March 2008
Online First 21 April 2008

Abstract. Cardiolipin is a unique phospholipid, which is almost exclusively localized in the mitochondrial inner membrane where it is synthesized from phosphatidylglycerol and cytidinediphosphate-diacylglycerol. After primary synthesis, the mature acyl chain composition of cardiolipin is achieved by at least two remodeling mechanisms. In the mitochondrial membrane cardiolipin plays an important role in energy metabolism, mainly by providing stability for the individual enzymes and enzyme complexes involved in energy production. Moreover, cardiolipin is involved in different stages of the mitochondrial apoptotic process and in mitochondrial membrane dynamics. Cardiolipin alterations have been described in various pathological conditions. Patients suffering from Barth syndrome have an altered cardiolipin homeostasis caused by a primary deficiency in cardiolipin remodeling. Alterations in cardiolipin content or composition have also been reported in more frequent diseases such as diabetes and heart failure. In this review we provide an overview of cardiolipin metabolism, function and its role in different pathological states.

Keywords. Cardiolipin, phospholipid, acyl chain composition, energy production, mitochondrial apoptosis, Barth syndrome.

Introduction

The lipid bilayer surrounding cells and organelles is mainly comprised of phospholipids, resulting in the spatial separation of the different subcellular compartments. Typical phospholipids consist of a glycerol backbone, a polar head group and hydrophobic acyl chains, resulting in their amphipatic character [for example phosphatidylglycerol (PG) in Fig. 1]. These phospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), PG, and phosphatidic acid (PA). The mitochondrial phospholipid cardiolipin (CL) contains three glycerol backbones and four acyl chains (Fig. 1), resulting in a very specific ultrastructure and role in mitochondrial function. CL was first isolated from bovine heart by Mary Pangborn in 1942 as 'a substance essential for the reactivity of beef heart antigens in the serologic test for syphilis' [1]. In 1964, LeCocq and Ballou established the structure of CL by comparison with synthetic analogs [2]. The diphosphatidylglycerol structure combined with the four acyl chains gives CL its dimeric nature, which is unique among phospholipids and results in a highly specific conical structure. This cone shape favors a hexagonal H_{II} phase of the membrane which is implicated in membrane fusion [3]. Subcellular fractionation studies revealed that CL is primarily localized to mitochondria [4], where it has been shown

* Corresponding author.

to be highly important for optimal activity of several mitochondrial enzymes. In mitochondria, CL is important for the formation of contact sites between the inner and outer mitochondrial membrane by its ability to adopt the hexagonal phase [5]. More recently, CL has been implicated in the formation of enzyme complexes of the respiratory chain and in the mitochondrial apoptotic pathway. Insight with respect to the importance of both CL content and CL acyl chain composition for mitochondrial metabolism has increased, as illustrated by the involvement of CL in various pathological conditions. In this review, we discuss the current state of knowledge on the importance of CL, mainly focusing on the metabolism of CL and its functional role in critical mitochondrial processes.

CL subcellular distribution

The distribution of phospholipids among cellular and organellar membranes is highly variable. CL is predominantly found in the mitochondrial inner membrane and in contact sites between the inner and outer mitochondrial membrane of various organisms [5–7]. Small amounts are found in the outer mitochondrial membrane of *Neurospora crassa* [8] and recently it has been reported that CL is also found in the peroxisomal membrane of the yeast *Pichia pastoris* [9]. A mitochondrial phospholipid scramblase, termed PLS3, has been described which is involved in the translocation of CL from the mitochondrial inner membrane to the mitochondrial outer membrane, a process which is important during the initial phase of mitochondrial apoptosis [10]. Changes in PLS3 activity by overexpression of either normal PLS3 or an inactive PLS3 have been reported to result in increased CL synthesis rates to compensate for the altered distribution within mitochondria [11]. Clustered localization of CL in contact sites is reported to be mediated by several mitochondrial proteins, including the octameric form of mitochondrial creatine kinase [12]. This protein is also reported to be involved in lipid transfer between the mitochondrial bilayers [13], but whether or not CL is also transferred via the creatine kinase-mediated membrane bridge is not known.

CL acyl chain composition

The acyl chain composition of CL is very diverse, both between organisms and between tissues and cell types within one organism. CL of *Saccharomyces cerevisiae* is dominated by mono-unsaturated acyl chains (18 : 1, 16 : 1) [14], whereas human CL mainly contains di-unsaturated acyl chains (18 : 2) [14], and CL from other organisms, such as various bivalves and sea urchin sperm, contains longer poly-unsaturated acyl chains (22 : 6, 20 : 5) [15, 16]. It has been suggested that this difference in composition is an adaptation to the environmental conditions (e.g. temperature, salt concentration) specific to the habitat of the species [15]. Beside the differences between organisms, there are also prominent variations in CL composition between various tissues and cell types within the same organism. For example, mouse CL predominantly contains linoleic acid side chains, but the relative abundance of this fatty acid in CL ranges from ~60% in skeletal muscle to more than 80% in heart and liver [17]. Other fatty acids in CL include oleic acid (18 : 1) and docosahexaenoic acid (22 : 6), while palmitic acid (16 : 0) is virtually absent in CL. This also holds true for CL from various rat and human tissues [16]. Variation in CL acyl composition appears to be age dependent. A study in rat heart showed that during aging, there is a large shift in fatty acid composition in CL and PE toward longer and more unsaturated acyl chains, mainly docosahexaenoic acid [18].

Recent studies have shown that although the predominant fatty acid species in CL of organisms may differ, there is a high degree of symmetry in the acyl chain distribution of CL in different organisms and in tissues within the same organism [16]. This symmetric distribution of acyl chains is thought to promote the structured organization of the mitochondrial membrane and to be of importance for the function of CL, as was recently reviewed [19].

CL synthesis, remodeling and degradation

CL synthesis

The first step in phospholipid synthesis is the acylation of glycerol-3-phosphate by a glycerol-3-phosphate acyltransferase [20] (Fig. 1). The resulting acylglycerol-3-phosphate can subsequently be acylated by an acylglycerol-3-phosphate acyltransferase resulting in PA. PA is converted to cytidinediphosphate-diacylglycerol (CDP-DAG) by the enzyme CDP-DAG synthase, which is localized in both mitochondria and microsomes [20]. From this branchpoint in the phospholipid biosynthetic pathway, CDP-DAG can be incorporated into all different phospholipids. In contrast to most phospholipid biosynthetic pathways, which are localized at the endoplasmic reticulum (ER), CL biosynthesis from PA takes place in mitochondria. The first, rate-limiting step in the dedicated CL biosynthetic pathway is the conversion of CDP-DAG to phosphatidylglycerol phosphate

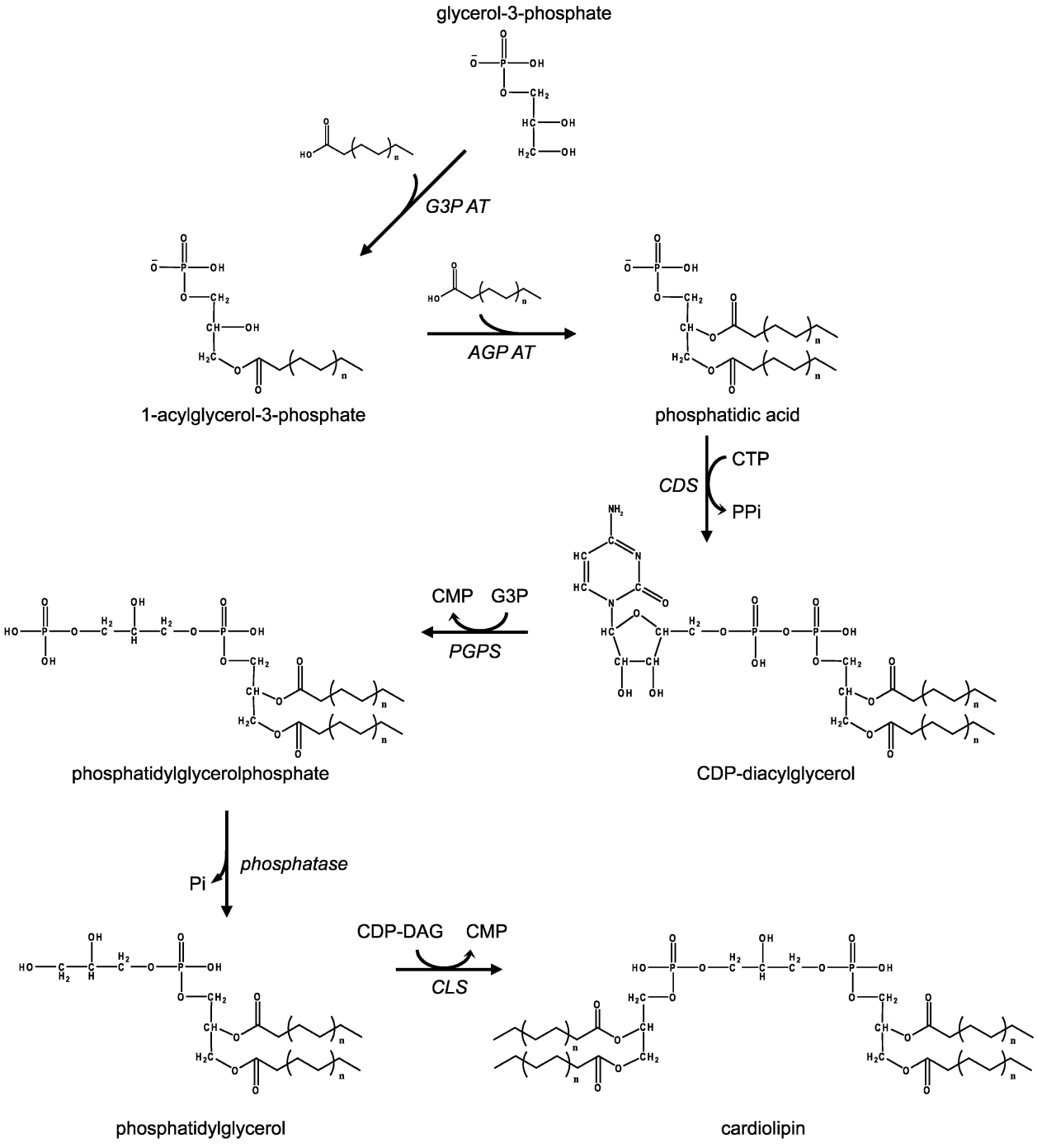

Figure 1. Cardiolipin synthesis in eukaryotes. The common precursor of CL biosynthesis is glycerol-3-phosphate (G3P) which is derived from several metabolic reactions. G3P is then acylated by a G3P acyltransferase (AT), yielding 1-acylglycerol-3-phosphate (AGP), which is subsequently acylated to phosphatidic acid (PA) by an AGP AT. In the next step, cytidinediphosphate-diacylglycerol (CDP-DAG) is formed by condensation of CTP and PA catalyzed by the enzyme CDP-DAG synthase (CDS). CDP-DAG serves as a branchpoint in phospholipid biosynthesis. For the CL pathway, CDP-DAG is converted to phosphatidylglycerolphosphate (PGP) by the enzyme PGP synthase (PGPS), and PGP is dephosphorylated by a phosphatase yielding phosphatidylglycerol (PG). In the final step of CL biosynthesis, condensation of one molecule of PG and one molecule of CDP-DAG by the enzyme CL synthase (CLS) results in the formation of CL.

(PGP) by the enzyme PGP synthase [21], followed by dephosphorylation by a yet unknown phosphatase to form PG. Finally, CL is synthesized by the condensation of PG and a molecule of CDP-DAG at the mitochondrial inner membrane, a reaction catalyzed by the enzyme cardiolipin synthase (CLS) [22]. The effectiveness of this reaction is indicated by the presence of only small amounts of PG in the mito-

chondrial membrane [6]. CLS is an evolutionarily highly conserved enzyme. All eukaryotic CLSs have been shown to have an alkaline pH optimum and require divalent cations for activity [22]. Studies on rat liver CLS [23], *Arabidopsis thaliana* CLS [24], and human CLS [25] have shown that the enzyme has no strong preference for precursors with a particular acyl chain composition. The substrate preference of CLS can therefore not explain the specific *in vivo* CL composition, indicating that remodeling of acyl chains is needed after CL has been synthesized.

CL remodeling; monolyso-CL acylation

To achieve the specific set of acyl chains required for each organism or tissue, additional remodeling steps are applied, since CLS has no strong substrate preference. This can be achieved in a coenzyme A (CoA)-dependent deacylation-reacylation cycle, also known as Lands cycle [26] or via CoA-independent transacylation between different phospholipids [27]. The Lands cycle consists of a phospholipase-mediated deacylation of *de novo* synthesized phospholipids, followed by a reacylation reaction, which is CoA dependent. Most phospholipid-remodeling enzymes described to date play a role in PC metabolism, acylating lyso-PC to PC [28]. Although different enzyme activities have been described to reacylate monolyso-CL *in vitro*, the exact remodeling pathway of CL remains unknown.

Schlame and Rüstow [29] described a CoA-dependent enzyme activity from rat liver mitochondria that reacylates monolyso-CL using linoleoyl-CoA. They were the first to propose a deacylation/reacylation reaction, i.e. a Lands cycle, as a potential mechanism for CL remodeling. Ma et al. [30] reported a CoA-dependent monolyso-CL acyltransferase activity which was present in mitochondria of various rat tissues. This enzyme activity was optimally active at alkaline pH and had a preference for linoleoyl-CoA and oleoyl-CoA, implying an important role in achieving the final structure of CL [30]. The same group purified a 74-kDa CoA-dependent monolyso-CL acyltransferase from pig liver mitochondria [31]. In contrast to the monolyso-CL acyltransferase from rat [30], this protein had a pH optimum of 7.0, but the substrate preference was similar using linoleoyl-CoA and oleoyl-CoA [31]. Unfortunately, the authors did not report the identification of the purified monolyso-CL acyltransferase at the molecular level. Although there are minor differences between the optimal assay conditions for the reported monolyso-CL acyltransferase activities, it is likely that these represent the same enzyme.

Xu et al. [27] reported a novel CL-remodeling activity, namely CoA-independent transacylation of monolyso-CL using PC as acyl donor, which was present in rat liver and human lymphoblasts. In contrast to the CoA-dependent monolyso-CL acyltransferase activity, the transacylase activity almost exclusively used linoleoyl-containing PC, not oleoyl- or arachidonoyl-containing PC. Interestingly, this activity was reduced by ~50% in Barth syndrome lymphoblasts, suggesting this could represent the enzymatic activity that is deficient in Barth syndrome, i.e. tafazzin (see below) [27].

An ER-associated monolyso-CL acyltransferase from mouse (ALCAT1) has been demonstrated to acylate monolyso-CL *in vitro* [32]. The physiological relevance, however, is doubtful, since CL is localized in mitochondria and not in the ER. In addition, the ALCAT1 sequence has been shown to be identical to the acylglycerolphosphate acyltransferase-8 (AGPAT8) sequence, suggesting that this enzyme is involved in the acylation of lyso-PA in the ER [33]. Although ALCAT1 might not be directly involved in CL acyl chain remodeling, remodeled PA could, possibly derived from mitochondria-associated membranes, serve as a precursor of CL synthesis [34, 35] and thereby have its effect on CL acyl chain composition. The contribution of the different PA pools (mitochondrial or ER) to CL synthesis, and the transport mechanism of extra-mitochondrial PA to the site of CL synthesis (i.e. the mitochondrial inner membrane) remain to be elucidated.

CL remodeling; tafazzin

The only enzyme/gene which has been proven to be involved in the remodeling of CL acyl chains *in vivo* is tafazzin [36]. Based on sequence homology, tafazzin was designated a putative acyltransferase [37]. Since Barth syndrome patients, who lack proper tafazzin function due to mutations in the corresponding gene, display an altered CL composition in various tissues/cell types [38, 39], tafazzin involvement in CL remodeling was assumed. Tissues as well as cells like fibroblasts and lymphocytes from Barth syndrome patients are deficient in CL but contain high levels of monolyso-CL, and the degree of unsaturation of the acyl side chains of CL is lower than in healthy individuals (as reflected by a lower linoleic acid content) [38]. In 2005, Testet and colleagues reported that YPR140w, the yeast ortholog of human tafazzin, displays lyso-PC acyltransferase activity [40]. The significance of their findings remains to be clarified, however, especially since no abnormalities in PC have been observed in tafazzin-deficient yeast cells. A more recent report indicates that tafazzin from *Drosophila melanogaster* functions as a 1-palmitoyl-2-linoleoyl-PC:monolyso-CL linoleoyltransferase, i.e. a transacylase transferring linoleic acid from PC to monolyso-CL yielding CL and lyso-PC [41]. This enzyme activity

corresponds to the previously reported enzymatic activity that was diminished in Barth syndrome cells [27].

Even though tafazzin is highly conserved in evolution, from the unicellular eukaryote *S. cerevisiae* to primates [42], the dominant fatty acid in CL is different between these organisms. Interestingly, *Drosophila* tafazzin is highly specific for PC containing linoleic acid [41], just as the transacylase activity in rat and human tissues/cells [27]. It would therefore be interesting to investigate if tafazzin orthologs in organisms where linoleic acid is not the predominant CL acyl chain evolved to have a different acyl chain substrate specificity, or that additional mechanisms such as the interplay/balance between the different remodeling systems play a role in these processes.

Although various enzymes/activities have been described for the remodeling of CL, it is still unclear how differences in CL composition arise in different cell types. This could result from environmental conditions such as the availability of fatty acids or the differential expression of the various remodeling enzymes in different tissues. For example, in the developing zebrafish embryo, spatial and temporal expression of tafazzin is strictly regulated [43]. During early development, tafazzin is expressed ubiquitously; however, in later embryonic stages, tafazzin expression is more restricted to the head and heart region, whereas upon hatching, tafazzin is mainly expressed in the heart [43]. Although no data were presented for adult animals, the study nicely underlines the importance of tissue-specific, age-dependent expression of tafazzin, which might also hold true for other remodeling enzymes.

Another interesting issue is that the linoleic acid specificity that is described for most remodeling enzymes does not fit with the age-related enrichment of arachidonic acid and docosahexaenoic acid in the rat heart [18] and the 16-fold increase in docosahexaenoic acid-containing CL in the diabetic mouse heart [44]. Since the plasma concentration of unesterified linoleic acid is much higher than the concentrations of unesterified arachidonic acid and docosahexaenoic acid, there must be a selective, active mechanism for the incorporation of these latter fatty acids in CL in the aging rat heart [18]. Remodeling enzymes (or activities) specific for these fatty acids have thus far not been described. With the age-related changes in CL composition in mind, it would be interesting to analyze their age-dependent expression, obviously after identification of the different remodeling systems.

CL degradation

Little is known about the catabolism of CL. Acyl chain hydrolysis is especially important with respect to the deacylation-reacylation cycle of CL as described in the previous section to obtain the preferred acyl chain composition. A phospholipase A_2 activity from rat liver mitochondria has been shown to hydrolyze CL, although this was not its preferred substrate [45]. Human cytosolic 85 kDa PLA_2 ($cPLA_2$) has been shown to hydrolyze monolyso- and dilyso-CL but not CL [46]; however, the physiological relevance for CL metabolism is unclear since the enzyme has a cytosolic localization. Recently, a new $cPLA_2$ splice variant, the 100-kDa $cPLA_2\beta3$, was identified which is localized in mitochondria [47]. Whether or not this PLA_2 variant is capable of hydrolyzing CL was not reported. Another interesting enzyme with respect to CL catabolism could be $PLA_2\gamma$. This calcium-independent enzyme is present in the myocardium, has a dual localization in mitochondria and peroxisomes and is capable of PC and PE hydrolysis which is accompanied by reorganization of mitochondrial cristae [48]. It would be interesting to analyze whether this enzyme is capable of hydrolyzing CL.

A recently described human phospholipase D, termed MitoPLD, has been shown to hydrolyze CL [49]. By generating the fusogenic lipid PA, MitoPLD is involved in the mitochondrial fusion mechanism. A more detailed description of its function will be discussed below. The role of MitoPLD in CL homeostasis, however, remains to be elucidated.

CL function

It has long been known that phospholipids are important for maintaining the structural integrity of biological membranes, which in turn serve as a protection mechanism against harmful influences from the exterior. These membranes also compartmentalize the cell, which is essential to create different environmental conditions such as pH and other ion gradients.

CL as stimulatory co-factor of mitochondrial enzymes

Beside the structural function of CL, several mitochondrial enzymes have been shown to require CL for optimal activity. Cholesterol side chain cleavage by the enzyme cytochrome $P\text{-}450_{scc}$ (or CYP11A1) has been shown to be optimal after CL binding although binding, of the CL precursor PG also resulted in a near optimal activity [50]. The mitochondrial phosphate carrier [51] and the mitochondrial creatine kinase [52, 53] require CL for their activity; however, in contrast to cytochrome $P\text{-}450_{scc}$, other phospholipids cannot

substitute for CL [52]. Similarly, reconstitution of the carnitine acylcarnitine translocase [54] and the pyruvate carrier [55] in proteoliposomes has been shown to be most efficient in the presence of CL and this could not be achieved by addition of other phospholipids. The mitochondrial glycerol-3-phosphate dehydrogenase also has been reported to bind to CL which influences the catalytic properties of this enzyme [56]. The tricarboxylate carrier has been demonstrated to be stimulated in the presence of CL [57]. The same stimulatory effect, although less pronounced, is observed in the presence of other acidic phospholipids, i.e. PS and PI [57]. The enzyme that synthesizes CL, CLS, has been shown to require CL for full activity, which is only achieved in combination with PE [58]. Interestingly, the activity of the ADP/ATP carrier has been shown to be optimal only in the presence of tetralinoleoyl-CL [59]. Other CL species, such as tetraoleoyl-CL and monolyso-CL, but also other phospholipids were not effective in stimulating the ADP/ATP carrier activity [59]. In addition to its stimulatory association with discrete mitochondrial enzymes, CL is intimately involved in different cellular processes including energy metabolism and apoptosis. Below we describe recent insights into CL function, focusing on CL involvement in various metabolic pathways.

CL and energy metabolism

CL is associated with different complexes of the respiratory chain, which are involved in the transduction of electrons and the production of ATP in the mitochondrial inner membrane (Fig. 2). Restoration of the enzymatic function of phospholipid-depleted complex I and III has been shown to require CL [60]. More recently, complex II from *Escherichia coli* has been shown to be associated with two acyl chains of one CL molecule [61]. Transfer of electrons between complex III and IV is dependent on cytochrome *c*, which has been shown to interact loosely with CL [62, 63]. Complex IV has been shown to require two associated CL molecules in order to maintain its full enzymatic function. This dependence cannot be substituted by monolyso-CL or dilyso-CL [64]. Complex V of the oxidative phosphorylation machinery also displays high binding affinity for CL, binding four molecules of CL per molecule of complex V [65].

Deletion of the yeast *crd1* gene, which encodes CLS, results in a complete depletion of CL. This *crd1*Δ yeast model has been widely used to assess CL dependency of mitochondrial function. Complete absence of CL in *crd1*Δ results in a decreased complex IV and complex V activity, and a partially defective protein import into mitochondria [66]. These deficiencies are partially compensated by high levels of the CL precursor PG, which is present in *crd1*Δ. The deficient ADP/ATP carrier activity and decreased mitochondrial membrane potential also observed in *crd1*Δ, however, are not restored by the elevated levels of PG [66]. CL is not only associated with individual mitochondrial respiratory chain enzymes but also with respiratory chain supercomplexes. Supercomplex formation, clustering of respiratory chain complexes in closely related units, has been suggested as opposed to a free diffusion model [67]. These supercomplexes have a functional role in the efficient channeling of electrons between the individual complexes. Moreover, the unique structure of CL is suggested to function as a proton trap within the mitochondrial membranes, thereby strictly localizing the proton pool and minimizing the changes in pH in the mitochondrial intermembrane space [68]. In yeast, a dimer of complex III is assembled with a monomer or dimer of complex IV. In mammalian mitochondria, the supercomplex mainly consists of complex I, complex III and multiple complex IV molecules [69]. Although the formation of the supercomplexes is independent of CL, its stability is impaired in *crd1*Δ mutant yeast [70]. Recent studies have shown that the supercomplexes are also destabilized in tafazzin-deficient yeast and human, i.e. Barth syndrome, cells [71, 72], which have decreased CL levels, increased monolyso-CL levels and a lower degree of unsaturation, as will be discussed below.

CL and apoptosis

Besides its function in the transfer of electrons in the respiratory chain, cytochrome *c* also has important functions in the apoptosome-mediated apoptosis pathway (Fig. 3). Cytochrome *c* is thought to play a role in apoptosis via the release of apoptotic factors from the mitochondria, the activation of caspases in the cytosol and the oxidation of lipids in the plasma membrane for clearance of apoptotic cells [73]. The mitochondrial enhancement loop of type II apoptosis is partly mediated by CL [for reviews specifically about the role of CL in apoptosis see 74, 75]. During the apoptotic process in type II cells (i.e. mitochondrial apoptosis), the pro-apoptotic Bcl-2 family protein Bid is cleaved by caspase 8, producing truncated Bid (or tBid), which is considered the active form of Bid [76]. tBid is subsequently recruited to the mitochondria, a process mediated by CL localized at contact sites between the mitochondrial inner and outer membrane [77]. CL translocation from the inner membrane to the contact sites is mediated by phospholipid scramblase 3 and has been shown to be an important step for the efficient propagation of the apoptotic signal [10]. Other studies showed that monolyso-CL might be involved in the recruitment

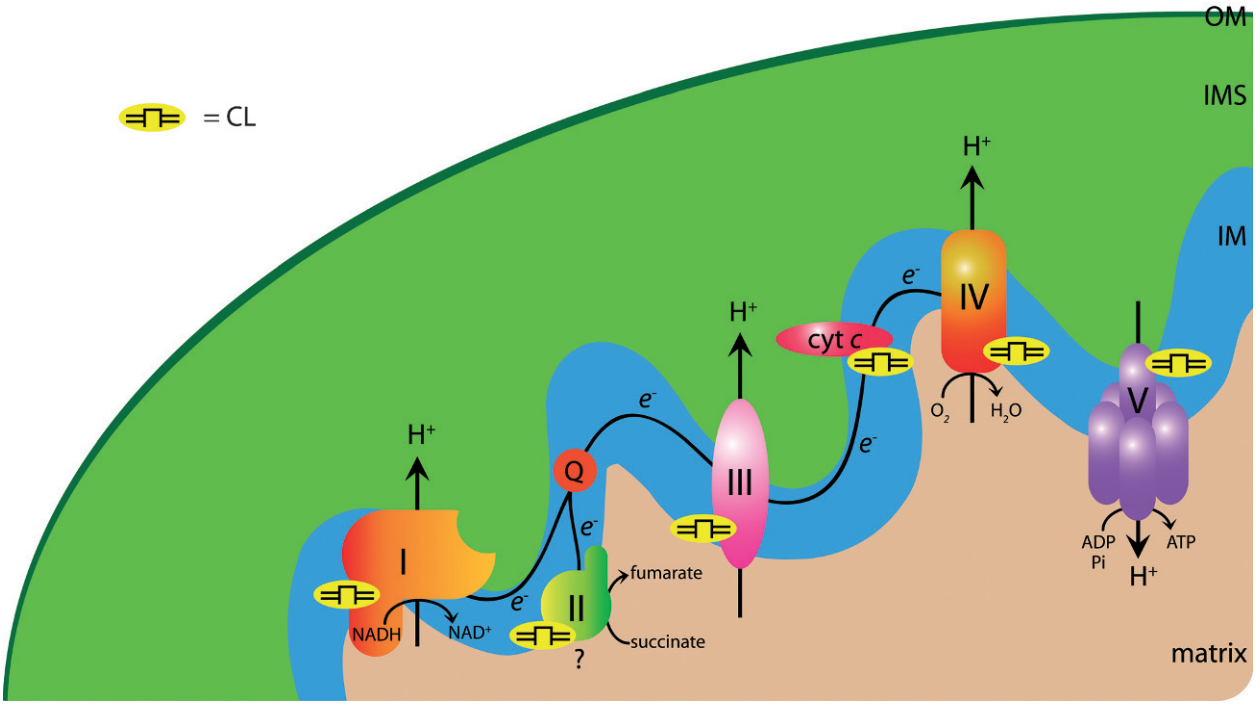

Figure 2. Association of CL with the oxidative phosphorylation system. Oxidative phosphorylation (OXPHOS) and ATP synthesis are localized at the mitochondrial inner membrane of eukaryotes. Electrons are delivered to either complex I or II, transferred via the lipid-soluble carrier ubiquinone (Q), complex III and cytochrome *c* (cyt *c*) to complex IV where these electrons are transferred to molecular oxygen thereby producing H_2O. Due to the electron flow/transfer through the respiratory chain, complexes I, III and IV pump protons from the mitochondrial matrix into the intermembrane space generating both a pH gradient and a concomitant membrane potential. These two gradients are used by complex V (ATP synthase) to synthesize ATP from ADP and organic phosphate. All individual enzyme complexes have been reported to bind/associate with CL. Furthermore, CL is essential for the maintenance of OXPHOS supercomplexes, which consist of multiple OXPHOS complexes (e.g. in human: complex I, III and IV) resulting in efficient clustering of this metabolic pathway. Finally, the electron carrier cyt *c* is associated with CL, which is important for the association of this protein with the mitochondrial inner membrane.

of tBid to the mitochondrial membrane [78], which is in line with the hydrolysis of CL in apoptotic cells [79]. Translocation of tBid to the mitochondria leads to the oligomerization of the Bcl-2 family proteins Bax or Bak [80], which form pores in the mitochondrial membrane properties, enabling release of (pro-apoptotic) large molecules from the intermembrane space. In *in vitro* liposome systems, this process is completely dependent on the presence of CL [81], further stressing the important role of CL in type II apoptosis. The molecules released from mitochondria during apoptosis include AIF, Smac/Diablo, endonuclease G, Omi/HtrA2 and cytochrome *c* [82]. Since cytochrome *c* is associated with CL in the intermembrane space, dissociation of this cytochrome *c*-CL complex is required in combination with the membrane alteration by the Bcl-2 family proteins to achieve release of the downstream pro-apoptotic factors into the cytosol [63]. The mechanism by which cytochrome *c* is released from CL has recently been elucidated [83]. Normally, cytochrome *c* functions as an electron carrier in the mitochondrial respiratory chain, but can undergo a conformational change which will alter its function to a CL-specific peroxidase activity. This CL oxidation occurs prior to cytochrome *c* release during the early phase of apoptosis and is believed to be essential for the release of the downstream apoptotic factors. The oxidation of CL highly depends on the degree of unsaturation of the CL acyl chains, since a higher degree of unsaturation results in a higher susceptibility to cytochrome *c*-induced CL oxidation [83]. The importance of CL in apoptosis is stressed by the different effects on sensitivity to apoptotic stimuli of CLS knockdown cell lines as will be discussed below.

Altogether, CL serves as a central switch in the mitochondrial apoptotic program, controlling the initiation of this process on different levels. This makes CL a potentially interesting target for therapeutical intervention in diseases in which cell death is dysregulated, such as cancer.

Mitochondrial membrane fusion/fission and apoptosis; a role for CL?

Membrane fusion/fission is crucial for mitochondrial morphology and function and is strictly regulated by a

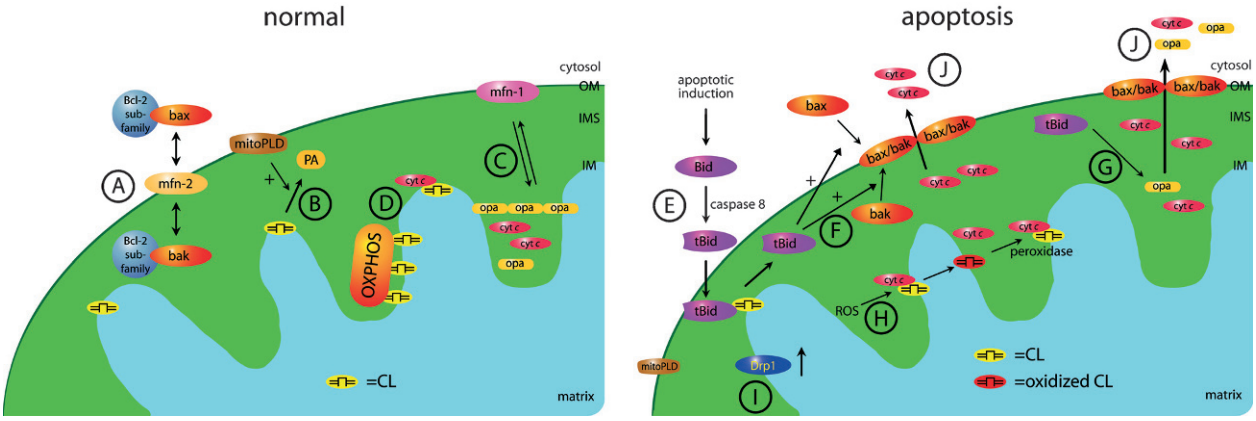

Figure 3. The role of CL in apoptosis and mitochondrial membrane dynamics. The mitochondrial pathway of apoptosis is important in type II cells in which the release of pro-apoptotic molecules from the mitochondria serves as an amplification loop for apoptosis. CL plays a role in several steps of mitochondrial apoptosis but also in mitochondrial membrane dynamics, i.e. fusion and fission. A shift in this delicate balance toward enhanced fission (or reduced fusion) is also implicated in mitochondrial apoptosis. In normal mitochondria, the pro-apoptotic Bcl-2 family members Bak and Bax are inactive and interact with mitofusin-2 (Mfn-2) (A). Furthermore, MitoPLD converts CL to PA, which can function as a fusogenic lipid, promoting mitochondrial fusion (B). Mitofusin-1 (Mfn-1) interacts with the inner membrane-localized OPA1, which controls the tight structure of the mitochondrial cristae, keeping most cytochrome *c* (cyt *c*) in the cristae (C). On the mitochondrial inner membrane, CL is needed for the correct function of oxidative phosphorylation (OXPHOS), for example by its association with the cyt *c* involved in electron transport (D). Induction of apoptosis results in the cleavage of Bid to truncated Bid (tBid) by caspase-8 (E). tBid translocates to CL in the mitochondria and induces the translocation of Bak and Bax to the mitochondrial outer membrane (F), a process dependent on the presence of CL. tBid formation also results in the disruption of OPA1-mediated tight structure of the cristae (G). Disruption of the cristae structure results in the release of cyt *c* into the intermembrane space, facilitating its release from the mitochondria. Reactive oxygen species (ROS) that are formed during apoptotic progression disrupt the association of cyt *c* with CL by oxidation of CL (H). This cyt *c* can act as a CL-specific peroxidase which can oxidize the CL acyl chains, enhancing its own effect. Mitochondrial fission is promoted by the activation of the fission protein Drp1 (I). Altogether, these activities result in the release of cyt *c* and other pro-apoptotic proteins from the mitochondria through Bak/Bax pores in the mitochondrial outer membrane (J), resulting in apoptosome formation and progression of the apoptotic signal downstream of the mitochondria. OPA1 release from the mitochondria, together with reduced activity of the mitofusins and MitoPLD and enhanced mitochondrial fission results in a balance toward mitochondrial fragmentation, an important hallmark of mitochondrial apoptosis.

growing family of proteins that drive this process [84]. Fusion and fission are important for normal cellular homeostasis but are also required during the apoptotic program which involves carefully orchestrated fragmentation of mitochondria. Although MitoPLD is the only known protein that directly links CL to mitochondrial fusion/fission, it is likely that CL is more generally involved in this process which is why we discuss this topic briefly below.

Under normal conditions, MitoPLD resides in the mitochondrial outer membrane, facing the cytosol. Although the exact mechanism is unclear, it is suggested that during fusion, two mitochondria are brought together and tethered by mitofusin proteins [see ref. 85 for a review], followed by MitoPLD-mediated generation of PA at the site of fusion by hydrolysis of CL [49]. Subsequently, PA and/or one of its derivatives acts as a fusogenic lipid enabling the fusion of the mitochondria, a process closely resembling the phospholipase D-mediated membrane fusion during exocytosis. Downregulation of MitoPLD, which results in reduced fusogenic activity, potentially plays a role in the apoptotic process [49]. The reduction in mitochondrial fusion results in fragmented mitochondria, which is a hallmark of mitochondrial apoptosis.

Although the direct involvement of CL has not been established, fusion of mitochondria is dependent on several protein systems, also during apoptosis, which may at least partly involve CL. Beside the Bax/Bak-dependent release of cytochrome *c* from the intermembrane space and the consequent effects on apoptosome formation and the caspase cascade, a less studied and understood Bax/Bak-independent pathway can enhance apoptosis [86]. Binding of tBid to the mitochondria, which occurs in a CL-dependent manner, results in transient opening of the permeability transition pore but also in mitochondrial cristae remodeling [86]. Cristae remodeling is mediated by the mitochondrial inner membrane protein OPA1, which controls the tight structure of the cristae junctions [87]. Disruption of the membrane integrity, for example by deletion of OPA1, facilitates the release of cytochrome *c* from the cristae which can subsequently be released from the mitochondria, promoting apoptosis [88].

Disruption of the balance between fusion and fission appears to be an important mechanism during the apoptotic program, both by increasing fission and inhibiting fusion leading to the characteristic mitochondrial fragmentation observed in apoptosis [84].

For example, release of OPA1 from the mitochondria during apoptosis leads to a decrease in mitofusin-1-mediated mitochondrial fusion [89, 90]. In addition, the CL-dependent recruitment [81] and conformational change of Bax and Bak during apoptosis [91] is suggested to alter their normal interaction with mitofusin-2 leading to inactivation of mitofusin-2. This inactivation has been suggested to contribute to a decrease in mitochondrial fusion, thereby supporting continued apoptosis [92]. Beside inhibition of fusion, there is an increase in the activity of Drp1, which mediates mitochondrial fission [93, 94].

Given the specific mitochondrial localization of CL, its importance for mitochondrial morphology [95] and the fact that an increasing number of proteins associate with CL to focus different processes to the mitochondrial membrane, this lipid is likely to function in other aspects of mitochondrial dynamics that remain to be explored.

CL and disease

Emerging insights have linked deficiencies in CL to disease. In this part of the review we focus on Barth syndrome, the only genetic disorder known to date which is primarily caused by a defect in CL metabolism. We will not discuss the role of anti-CL antibodies in primary or secondary antiphospholipid syndrome. For a review of this topic the reader is referred to Levine et al. [96].

Barth syndrome

Barth syndrome (MIM 302060) is an X-linked recessive disorder, clinically characterized by cardioskeletal myopathy, neutropenia and abnormal growth [97, 98]. Barth syndrome is caused by mutations in the tafazzin gene, which in humans is believed to encode six different tafazzin proteins as a result of alternative splicing [36]. Only two of these variants, full-length tafazzin and tafazzin lacking exon 5, are thought to be relevant *in vivo*, as shown by complementation analysis in tafazzin-deficient yeast [99]. Only human tafazzin lacking exon 5, however, is fully capable of restoring the tafazzin-deficient yeast phenotype [99]. Based on protein sequence homology, this variant is highly conserved throughout evolution [99]. These results are confirmed by phylogenetic sequence analysis [42], which shows that a splice acceptor site for exon 5 is absent in New and Old World monkeys, resulting in the existence of tafazzin lacking exon 5 only. In contrast, in all primates analyzed, this splice acceptor site has evolved and tafazzin containing exon 5 (at least at the mRNA level) is also observed in these species. In human mRNA, other splice variants have also been found in which exon 6 and 7 are spliced out in various combinations [42, 99]. The importance of these splice variants is at the least debatable, since these exons are highly conserved between mammals and not alternatively spliced in closely related monkey species. The presence of these different splice variants has complicated Barth syndrome research because it is still not known whether the different splice variants produce functional proteins and, if so, whether these proteins have different or similar functions.

At the biochemical level, Barth syndrome is characterized by decreased levels of CL [39]. Moreover, highly increased levels of monolyso-CL have been shown in tafazzin-deficient yeast and in patient tissues and cells [38, 99, 100]. In addition, a shift is observed in the degree of unsaturation of CL acyl chains. The remaining CL species in Barth syndrome cells are more saturated than those in control cells. The same holds true for the monolyso-CLs, which contain more saturated acyl chains in Barth syndrome cells when compared to the very small amount of monolyso-CL present in control cells [38, 99]. The aberrations in CL are used as a diagnostic parameter for the identification of individuals suffering from Barth syndrome. Recently, a new method was developed which uses the ratio of monolyso-CL to CL as a diagnostic marker in bloodspots which can be used to quickly screen for Barth syndrome [101]. In our laboratory, this is confirmed by (monolyso-)CL analysis in lymphocytes or fibroblasts followed by sequencing of the tafazzin gene.

As discussed above, tafazzin of *D. melanogaster* has been shown to be involved in the remodeling of CL acyl chains by transferring linoleic acid from PC to monolyso-CL [41]. Although there is no experimental evidence, the fact that CL levels are lower and monolyso-CLs accumulate in Barth syndrome suggests that human tafazzin(s) has (have) a similar function.

The pathogenesis of Barth syndrome is still not well understood. It has been demonstrated that the activity of selected respiratory chain complexes is reduced in muscle biopsies of Barth syndrome patients [102–104]. Recent studies have confirmed these findings and have shown that the reduced respiratory chain activity is caused by reduced stability of the oxidative phosphorylation supercomplexes [71, 72]. This reduction in energy production is likely to be the primary cause of the (cardio)myopathy.

The cause of the neutropenia in Barth syndrome and the role of CL therein is as yet unclear. Although neutrophils of Barth syndrome patients bind annexin-V, and in that respect appear to be in apoptosis, these cells do not show enhanced apoptosis when compared to normal neutrophils [105]. This therefore does not

explain the observed neutropenia observed in Barth syndrome patients.

Barth syndrome and apoptosis

The lower degree of unsaturation of the acyl chains could play a role in the apoptotic pathway of Barth syndrome cells. One would expect that in Barth syndrome cells apoptosis is inhibited by the reduced susceptibility of CL to oxidative damage leading to reduced cytochrome *c* release. On the other hand, Barth syndrome cells have elevated levels of monolyso-CL, which might enhance tBid recruitment to the mitochondria and promote apoptosis [78]. Two studies, in Barth syndrome lymphoblasts [38] and neutrophils [105], showed that there is no evidence of increased apoptosis, despite the high monolyso-CL levels. It is important to realize that the monolyso-CL used for the binding experiments with tBid in the paper by Esposti et al. [78] is trilinoleoyl-monolyso-CL, whereas monolyso-CL species in Barth syndrome cells are (just as the CL species) much less unsaturated. This will likely influence the initiation of the mitochondrial pathway of apoptosis in Barth syndrome.

In contrast to the finding that Barth syndrome cells have reduced apoptosis, a recent study showed that a decrease in CL levels by the knockdown of CLS results in a higher susceptibility to apoptotic stimuli [106]. Although it might be expected that apoptosis is inhibited due to reduced binding of tBid to the mitochondria, this is not observed, possibly because the residual amount of CL is sufficient for this process. The increase in cell death, which in the initial phase is apoptotic in nature and in later stages predominantly necrotic, could be caused by a number of factors. These include impaired energy homeostasis, a lowered membrane potential and decreased association of cytochrome *c* to CL in the mitochondrial inner membrane. The latter will result in facilitated release of cytochrome *c* from the mitochondria thereby enhancing apoptosis [106].

CL involvement in other disorders

Besides Barth syndrome, no other pathological conditions have been described which have a primary deficiency in CL metabolism due to mutations in a single gene. Several pathological conditions, however, have been linked to CL abnormalities, including Tangier disease, diabetes and heart disease. For an extensive review on the latter two topics, see Chicco and Sparagna [107].

CL alterations have been found in Tangier disease (MIM 205400). Tangier disease is characterized by very low plasma levels of high-density lipoprotein cholesterol, accumulation of cholesteryl esters in tissues and an increased risk for developing cardiovascular disease [108]. Genetic analysis showed that mutations in the plasma membrane ATP-binding cassette transporter A1 (ABCA1) cause Tangier disease [109–111]. This transporter is believed to mediate reverse cholesterol and phospholipid transport, that is from cells to the plasma apolipoprotein A-1 [112]. Phospholipid analysis of cultured Tangier disease fibroblasts showed a three- to fivefold increase in the levels of CL, monolyso-CL and dilyso-CL [113]. The authors suggested that increased CL levels result in enhanced cholesterol oxidation and thereby the formation of oxysterols which increase cholesterol efflux. This process could function as an escape mechanism to remove excess cholesterol from the cell [113]. More recent studies concerning Tangier disease have not focused on the increased levels of CL and its breakdown products, leaving the implications on mitochondrial metabolism undiscussed.

Diabetic cardiomyopathy is thought to be caused by a combination of altered substrate utilization and mitochondrial dysfunction. Alterations in CL content have been hypothesized to precede these processes. Although results concerning CL abnormalities in diabetic tissues are at least contradictory [107], two recent reports indicate that in the hearts of streptozotocin-treated (i.e. type I diabetic) mice, CL levels are decreased and there is altered CL acyl chain remodeling resulting in increased incorporation of the highly unsaturated docosahexaenoic acid [44, 114]. Whether (and if so, how) these specific CL alterations influence the development or progression of diabetic cardiomyopathy is unknown.

Recently, decreased CL levels have been suggested to play a role in the mitochondrial dysfunction observed in nonalcoholic fatty liver disease [115] and in heart failure [116]. As in diabetes, the role of CL in aging and ischemia/ reperfusion is still controversial. Although some studies indicate a loss of CL content or a change in acyl chain composition, other studies did not observe any changes in CL [107]. The different observations are likely to be caused by different methodologies with regard to animal strain, (sub)cellular fractionation and quantification of CL content and acyl chain composition.

CL quantification/determination

Since the first isolation of CL in the 1940s, several methods have been developed for the measurement of CL and the identification of the acyl side chains. Early methods to detect phospholipids included thin-layer chromatography [117, 118] and are still widely used for the quantitative measurement of total CL. A more

recently developed method which was thought to work via specific binding of the dye 10-*N*-nonyl acridine orange (NAO) to CL, and which unfortunately is still sometimes used to detect/measure CL, has been shown to be nonspecific. The dye still accumulated in mitochondria of the yeast *crd1*Δ (i.e. CLS) mutant that completely lacks CL [119]. This implies that NAO is unsuitable to analyze CL levels or to detect the lipid in cells using NAO fluorescence.

Beside the quantitative analysis of CL per se, CL acyl chain composition has become more of a focus. Newly developed analytical methods to measure acyl chain composition, such as HPLC-mass spectrometry (MS), are suitable for these studies and are now more commonly used [120–122]. Although qualitative analysis of the acyl chain composition of phospholipids can be performed by the HPLC-MS methods, these methods are less suitable for quantitative analysis because the response of the mass spectrometer is different for the various phospholipid subspecies making this method primarily useful for detecting relative changes within phospholipid classes.

Conclusion

Since its discovery in 1942, CL has been extensively studied, initially for its involvement in the serologic test for syphilis. Later, CL was implicated in several mitochondrial processes, and emerging insights in recent years have shown that CL functions as a key mediator of mitochondrial metabolism. The finding of Barth syndrome as a primary metabolic deficiency of CL not only provided researchers with an interesting model to study CL metabolism and its dysregulation, but more importantly with an extra incentive to investigate disease pathology, with the ultimate goal to improve treatment for patients suffering from this serious disorder. The role of CL in energy transduction and apoptosome-mediated apoptosis is currently the subject of many research groups, as is the importance of CL content and composition in various common diseases, such as diabetes and heart failure. Based on increasing knowledge on the importance of CL, and more specifically its acyl chain composition, for different mitochondrial processes, we advocate detailed CL analysis for studies on the mitochondrial membrane but more importantly also for patients suffering from mitochondrial disease with unknown origin, since CL abnormalities might be their underlying cause.

Acknowledgements. The authors thank R.J. Wanders, E. Gottlieb and F. Gonzalvez for helpful discussions and comments on the manuscript. F.M.V. is supported by a grant of the Prinses Beatrix Fonds, grant number WAR-05-0126 and a grant of the Barth Syndrome Foundation.

1 Pangborn M. C. (1942) Isolation and purification of a serologically active phospholipid from beef heart. J. Biol. Chem. 143, 247–256.
2 Lecocq J. and Ballou C. E. (1964) On the structure of cardiolipin. Biochemistry 3, 976–980.
3 Cullis P. R., Hope M. J. and Tilcock C. P. (1986) Lipid polymorphism and the roles of lipids in membranes. Chem. Phys. Lipids 40, 127–144.
4 Zambrano F., Fleischer S. and Fleischer B. (1975) Lipid composition of the Golgi apparatus of rat kidney and liver in comparison with other subcellular organelles. Biochim. Biophys. Acta, 380, 357–369.
5 Ardail D., Privat J. P., Egret-Charlier M., Levrat C., Lerme F. and Louisot P. (1990) Mitochondrial contact sites: lipid composition and dynamics. J. Biol. Chem. 265, 18797–18802.
6 Daum G. (1985) Lipids of mitochondria. Biochim. Biophys. Acta 822, 1–42.
7 Zinser E., Sperka-Gottlieb C. D., Fasch E. V., Kohlwein S. D., Paltauf F. and Daum G. (1991) Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote *Saccharomyces cerevisiae*. J. Bacteriol. 173, 2026–2034.
8 de Kroon A. I., Dolis D., Mayer A., Lill R. and de K. B. (1997) Phospholipid composition of highly purified mitochondrial outer membranes of rat liver and *Neurospora crassa*: is cardiolipin present in the mitochondrial outer membrane? Biochim. Biophys. Acta 1325, 108–116.
9 Wriessnegger T., Gubitz G., Leitner E., Ingolic E., Cregg J., de la Cruz B. J. and Daum G. (2007) Lipid composition of peroxisomes from the yeast *Pichia pastoris* grown on different carbon sources. Biochim. Biophys. Acta 1771, 455–461.
10 Liu J., Dai Q., Chen J., Durrant D., Freeman A., Liu T., Grossman D. and Lee R. M. (2003) Phospholipid scramblase 3 controls mitochondrial structure, function, and apoptotic response. Mol. Cancer Res. 1, 892–902.
11 Van Q., Liu J., Lu B., Feingold K. R., Shi Y., Lee R. M. and Hatch G. M. (2007) Phospholipid scramblase-3 regulates cardiolipin de novo biosynthesis and its resynthesis in growing HeLa cells. Biochem. J. 401, 103–109.
12 Epand R. F., Tokarska-Schlattner M., Schlattner U., Wallimann T. and Epand R. M. (2007) Cardiolipin clusters and membrane domain formation induced by mitochondrial proteins. J. Mol. Biol. 365, 968–980.
13 Epand R. F., Schlattner U., Wallimann T., Lacombe M. L. and Epand R. M. (2007) Novel lipid transfer property of two mitochondrial proteins that bridge the inner and outer membranes. Biophys. J. 92, 126–137.
14 Hoch F. L. (1992) Cardiolipins and biomembrane function. Biochim. Biophys. Acta 1113, 71–133.
15 Kraffe E., Soudant P., Marty Y., Kervarec N. and Jehan P. (2002) Evidence of a tetradocosahexaenoic cardiolipin in some marine bivalves. Lipids 37, 507–514.
16 Schlame M., Ren M., Xu Y., Greenberg M. L. and Haller I. (2005) Molecular symmetry in mitochondrial cardiolipins. Chem. Phys. Lipids 138, 38–49.
17 Han X., Yang K., Yang J., Cheng H. and Gross R. W. (2006) Shotgun lipidomics of cardiolipin molecular species in lipid extracts of biological samples. J. Lipid Res. 47, 864–879.
18 Lee H. J., Mayette J., Rapoport S. I. and Bazinet R. P. (2006) Selective remodeling of cardiolipin fatty acids in the aged rat heart. Lipids Health Dis. 5, 2.
19 Schlame M. (in press) Cardiolipin synthesis for the assembly of bacterial and mitochondrial membranes. J. Lipid Res.
20 Voelker DR (2004) Lipid synthesis and transport in mitochondrial biogenesis. In: Mitochondrial Function and Biogenesis, pp. 267–291, Koehler, C. M. and Bauer, M. F. (eds), Springer, Berlin.

21 Chang S. C., Heacock P. N., Clancey C. J. and Dowhan W. (1998) The PEL1 gene (renamed PGS1) encodes the phosphatidylglycero-phosphate synthase of *Saccharomyces cerevisiae*. J. Biol. Chem. 273, 9829–9836.
22 Schlame M. and Hostetler K. Y. (1997) Cardiolipin synthase from mammalian mitochondria. Biochim. Biophys. Acta 1348, 207–213.
23 Hostetler K. Y., Galesloot J. M., Boer P. and Van Den B. H. (1975) Further studies on the formation of cardiolipin and phosphatidylglycerol in rat liver mitochondria: effect of divalent cations and the fatty acid composition of CDP-diglyceride. Biochim. Biophys. Acta 380, 382–389.
24 Nowicki M., Muller F. and Frentzen M. (2005) Cardiolipin synthase of *Arabidopsis thaliana*. FEBS Lett. 579, 2161–2165.
25 Houtkooper R. H., Akbari H., van L. H., Kulik W., Wanders R. J., Frentzen M. and Vaz F. M. (2006) Identification and characterization of human cardiolipin synthase. FEBS Lett. 580, 3059–3064.
26 LANDS W. E. (1960) Metabolism of glycerolipids. 2. The enzymatic acylation of lysolecithin. J. Biol. Chem. 235, 2233–2237.
27 Xu Y., Kelley R. I., Blanck T. J. and Schlame M. (2003) Remodeling of cardiolipin by phospholipid transacylation. J. Biol. Chem. 278, 51380–51385.
28 de Kroon A. I. (2007) Metabolism of phosphatidylcholine and its implications for lipid acyl chain composition in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1771, 343–352.
29 Schlame M. and Rustow B. (1990) Lysocardiolipin formation and reacylation in isolated rat liver mitochondria. Biochem. J. 272, 589–595.
30 Ma B. J., Taylor W. A., Dolinsky V. W. and Hatch G. M. (1999) Acylation of monolysocardiolipin in rat heart. J. Lipid Res. 40, 1837–1845.
31 Taylor W. A. and Hatch G. M. (2003) Purification and characterization of monolysocardiolipin acyltransferase from pig liver mitochondria. J. Biol. Chem. 278, 12716–12721.
32 Cao J., Liu Y., Lockwood J., Burn P. and Shi Y. (2004) A novel cardiolipin-remodeling pathway revealed by a gene encoding an endoplasmic reticulum-associated acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) in mouse. J. Biol. Chem. 279, 31727–31734.
33 Agarwal A. K., Barnes R. I. and Garg A. (2006) Functional characterization of human 1-acylglycerol-3-phosphate acyltransferase isoform 8: cloning, tissue distribution, gene structure, and enzymatic activity. Arch. Biochem. Biophys. 449, 64–76.
34 Chakraborty T. R., Vancura A., Balija V. S. and Haldar D. (1999) Phosphatidic acid synthesis in mitochondria: topography of formation and transmembrane migration. J. Biol. Chem. 274, 29786–29790.
35 Voelker D. R. (2003) New perspectives on the regulation of intermembrane glycerophospholipid traffic. J. Lipid Res. 44, 441–449.
36 Bione S., D'Adamo P., Maestrini E., Gedeon A. K., Bolhuis P. A. and Toniolo D. (1996) A novel X-linked gene, G4.5. is responsible for Barth syndrome. Nat. Genet. 12, 385–389.
37 Neuwald A. F. (1997) Barth syndrome may be due to an acyltransferase deficiency. Curr. Biol. 7, R465–R466.
38 Valianpour F., Mitsakos V., Schlemmer D., Towbin J. A., Taylor J. M., Ekert P. G., Thorburn D. R., Munnich A., Wanders R. J., Barth P. G. and Vaz F. M. (2005) Monolysocardiolipins accumulate in Barth syndrome but do not lead to enhanced apoptosis. J. Lipid Res. 46, 1182–1195.
39 Vreken P., Valianpour F., Nijtmans L. G., Grivell L. A., Plecko B., Wanders R. J. and Barth P. G. (2000) Defective remodeling of cardiolipin and phosphatidylglycerol in Barth syndrome. Biochem. Biophys. Res. Commun. 279, 378–382.
40 Testet E., Laroche-Traineau J., Noubhani A., Coulon D., Bunoust O., Camougrand N., Manon S., Lessire R. and Bessoule J. J. (2005) Ypr140wp, 'the yeast tafazzin', displays a mitochondrial lysophosphatidylcholine (lyso-PC) acyltransferase activity related to triacylglycerol and mitochondrial lipid synthesis. Biochem. J. 387, 617–626.
41 Xu Y., Malhotra A., Ren M. and Schlame M. (2006) The enzymatic function of tafazzin. J. Biol. Chem. 281, 39217–39224.
42 Gonzalez I. L. (2005) Barth syndrome: TAZ gene mutations, mRNAs, and evolution. Am. J. Med. Genet. A 134, 409–414.
43 Khuchua Z., Yue Z., Batts L. and Strauss A. W. (2006) A zebrafish model of human Barth syndrome reveals the essential role of tafazzin in cardiac development and function. Circ. Res. 99, 201–208.
44 Han X., Yang J., Yang K., Zhao Z., Abendschein D. R. and Gross R. W. (2007) Alterations in myocardial cardiolipin content and composition occur at the very earliest stages of diabetes: a shotgun lipidomics study. Biochemistry 46, 6417–6428.
45 de Winter J. M., Lenting H. B., Neys F. W. and Van Den B. H. (1987) Hydrolysis of membrane-associated phosphoglycerides by mitochondrial phospholipase A2. Biochim. Biophys. Acta 917, 169–177.
46 Buckland A. G., Kinkaid A. R. and Wilton D. C. (1998) Cardiolipin hydrolysis by human phospholipases A2. The multiple enzymatic activities of human cytosolic phospholipase A2. Biochim. Biophys. Acta 1390, 65–72.
47 Ghosh M., Loper R., Gelb M. H. and Leslie C. C. (2006) Identification of the expressed form of human cytosolic phospholipase A2beta (cPLA2beta): cPLA2beta3 is a novel variant localized to mitochondria and early endosomes. J. Biol. Chem. 281, 16615–16624.
48 Mancuso D. J., Han X., Jenkins C. M., Lehman J. J., Sambandam N., Sims H. F., Yang J., Yan W., Yang K., Green K., Abendschein D. R., Saffitz J. E. and Gross R. W. (2007) Dramatic accumulation of triglycerides and precipitation of cardiac hemodynamic dysfunction during brief caloric restriction in transgenic myocardium expressing human calcium-independent phospholipase A2gamma. J. Biol. Chem. 282, 9216–9227.
49 Choi S. Y., Huang P., Jenkins G. M., Chan D. C., Schiller J. and Frohman M. A. (2006) A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. Nat. Cell Biol. 8, 1255–1262.
50 Pember S. O., Powell G. L. and Lambeth J. D. (1983) Cytochrome P-450scc-phospholipid interactions. evidence for a cardiolipin binding site and thermodynamics of enzyme interactions with cardiolipin, cholesterol, and adrenodoxin. J. Biol. Chem. 258, 3198–3206.
51 Bisaccia F. and Palmieri F. (1984) Specific elution from hydroxylapatite of the mitochondrial phosphate carrier by cardiolipin. Biochim. Biophys. Acta 766, 386–394.
52 Muller M., Moser R., Cheneval D. and Carafoli E. (1985) Cardiolipin is the membrane receptor for mitochondrial creatine phosphokinase. J. Biol. Chem. 260, 3839–3843.
53 Schlattner U. and Wallimann T. (2000) A quantitative approach to membrane binding of human ubiquitous mitochondrial creatine kinase using surface plasmon resonance. J. Bioenerg. Biomembr. 32, 123–131.
54 Noel H. and Pande S. V. (1986) An essential requirement of cardiolipin for mitochondrial carnitine acylcarnitine translocase activity: lipid requirement of carnitine acylcarnitine translocase. Eur. J. Biochem. 155, 99–102.
55 Nalecz K. A., Bolli R., Wojtczak L. and Azzi A. (1986) The monocarboxylate carrier from bovine heart mitochondria: partial purification and its substrate-transporting properties in a reconstituted system. Biochim. Biophys. Acta 851, 29–37.
56 Beleznai Z. and Jancsik V. (1989) Role of cardiolipin in the functioning of mitochondrial L-glycerol-3-phosphate dehydrogenase. Biochem. Biophys. Res. Commun. 159, 132–139.
57 Bisaccia F., De P. A., Prezioso G. and Palmieri F. (1990) Kinetic characterization of the reconstituted tricarboxylate carrier from rat liver mitochondria. Biochim. Biophys. Acta 1019, 250–256.

58 Schlame M. and Hostetler K. Y. (1991) Solubilization, purification, and characterization of cardiolipin synthase from rat liver mitochondria. Demonstration of its phospholipid requirement. J. Biol. Chem. 266, 22398–22403.
59 Hoffmann B., Stockl A., Schlame M., Beyer K. and Klingenberg M. (1994) The reconstituted ADP/ATP carrier activity has an absolute requirement for cardiolipin as shown in cysteine mutants. J. Biol. Chem. 269, 1940–1944.
60 Fry M. and Green D. E. (1981) Cardiolipin requirement for electron transfer in complex I and III of the mitochondrial respiratory chain. J. Biol. Chem. 256, 1874–1880.
61 Yankovskaya V., Horsefield R., Tornroth S., Luna-Chavez C., Miyoshi H., Leger C., Byrne B., Cecchini G. and Iwata S. (2003) Architecture of succinate dehydrogenase and reactive oxygen species generation. Science 299, 700–704.
62 Nicholls P. (1974) Cytochrome c binding to enzymes and membranes. Biochim. Biophys. Acta 346, 261–310.
63 Ott M., Robertson J. D., Gogvadze V., Zhivotovsky B. and Orrenius S. (2002) Cytochrome c release from mitochondria proceeds by a two-step process. Proc. Natl. Acad. Sci. USA 99, 1259–1263.
64 Robinson N. C., Zborowski J. and Talbert L. H. (1990) Cardiolipin-depleted bovine heart cytochrome c oxidase: binding stoichiometry and affinity for cardiolipin derivatives. Biochemistry 29, 8962–8969.
65 Eble K. S., Coleman W. B., Hantgan R. R. and Cunningham C. C. (1990) Tightly associated cardiolipin in the bovine heart mitochondrial ATP synthase as analyzed by 31P nuclear magnetic resonance spectroscopy. J. Biol. Chem. 265, 19434–19440.
66 Jiang F., Ryan M. T., Schlame M., Zhao M., Gu Z., Klingenberg M., Pfanner N. and Greenberg M. L. (2000) Absence of cardiolipin in the crd1 null mutant results in decreased mitochondrial membrane potential and reduced mitochondrial function. J. Biol. Chem. 275, 22387–22394.
67 Boumans H., Grivell L. A. and Berden J. A. (1998) The respiratory chain in yeast behaves as a single functional unit. J. Biol. Chem. 273, 4872–4877.
68 Haines T. H. and Dencher N. A. (2002) Cardiolipin: a proton trap for oxidative phosphorylation. FEBS Lett. 528, 35–39.
69 Schagger H. and Pfeiffer K. (2000) Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. EMBO J. 19, 1777–1783.
70 Pfeiffer K., Gohil V., Stuart R. A., Hunte C., Brandt U., Greenberg M. L. and Schagger H. (2003) Cardiolipin stabilizes respiratory chain supercomplexes. J. Biol. Chem. 278, 52873–52880.
71 Brandner K., Mick D. U., Frazier A. E., Taylor R. D., Meisinger C. and Rehling P. (2005) Taz1, an outer mitochondrial membrane protein, affects stability and assembly of inner membrane protein complexes: implications for Barth syndrome. Mol. Biol. Cell 16, 5202–5214.
72 McKenzie M., Lazarou M., Thorburn D. R. and Ryan M. T. (2006) Mitochondrial respiratory chain supercomplexes are destabilized in Barth syndrome patients. J. Mol. Biol. 361, 462–469.
73 Bayir H., Fadeel B., Palladino M. J., Witasp E., Kurnikov I. V., Tyurina Y. Y., Tyurin V. A., Amoscato A. A., Jiang J., Kochanek P. M., DeKosky S. T., Greenberger J. S., Shvedova A. A. and Kagan V. E. (2006) Apoptotic interactions of cytochrome c: redox flirting with anionic phospholipids within and outside of mitochondria. Biochim. Biophys. Acta 1757, 648–659.
74 Gonzalvez F. and Gottlieb E. (2007) Cardiolipin: setting the beat of apoptosis. Apoptosis. 12, 877–885.
75 Kagan V. E., Tyurina Y. Y., Bayir H., Chu C. T., Kapralov A. A., Vlasova I. I., Belikova N. A., Tyurin V. A., Amoscato A., Epperly M., Greenberger J., Dekosky S., Shvedova A. A. and Jiang J. (2006) The 'pro-apoptotic genies' get out of mitochondria: oxidative lipidomics and redox activity of cytochrome c/cardiolipin complexes. Chem. Biol. Interact. 163, 15–28.
76 Gross A., Yin X. M., Wang K., Wei M. C., Jockel J., Milliman C., Erdjument-Bromage H., Tempst P. and Korsmeyer S. J. (1999) Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death. J. Biol. Chem. 274, 1156–1163.
77 Lutter M., Fang M., Luo X., Nishijima M., Xie X. and Wang X. (2000) Cardiolipin provides specificity for targeting of tBid to mitochondria. Nat. Cell Biol. 2, 754–761.
78 Esposti M. D., Cristea I. M., Gaskell S. J., Nakao Y. and Dive C. (2003) Proapoptotic Bid binds to monolysocardiolipin, a new molecular connection between mitochondrial membranes and cell death. Cell Death. Differ. 10, 1300–1309.
79 Matsko C. M., Hunter O. C., Rabinowich H., Lotze M. T. and Amoscato A. A. (2001) Mitochondrial lipid alterations during Fas- and radiation-induced apoptosis. Biochem. Biophys. Res. Commun. 287, 1112–1120.
80 Wei M. C., Zong W. X., Cheng E. H., Lindsten T., Panoutsakopoulou V., Ross A. J., Roth K. A., MacGregor G. R., Thompson C. B. and Korsmeyer S. J. (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292, 727–730.
81 Kuwana T., Mackey M. R., Perkins G., Ellisman M. H., Latterich M., Schneiter R., Green D. R. and Newmeyer D. D. (2002) Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 111, 331–342.
82 van Loo G., Saelens X., van Gurp M., MacFarlane M., Martin S. J. and Vandenabeele P. (2002) The role of mitochondrial factors in apoptosis: a Russian roulette with more than one bullet. Cell Death. Differ. 9, 1031–1042.
83 Kagan V. E., Tyurin V. A., Jiang J., Tyurina Y. Y., Ritov V. B., Amoscato A. A., Osipov A. N., Belikova N. A., Kapralov A. A., Kini V., Vlasova I. I., Zhao Q., Zou M., Di P., Svistunenko D. A., Kurnikov I. V. and Borisenko G. G. (2005) Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. Nat. Chem. Biol. 1, 223–232.
84 Detmer S. A. and Chan D. C. (2007) Functions and dysfunctions of mitochondrial dynamics. Nat. Rev. Mol. Cell Biol. 8, 870–879.
85 Santel A. (2006) Get the balance right: mitofusins roles in health and disease. Biochim. Biophys. Acta 1763, 490–499.
86 Scorrano L., Ashiya M., Buttle K., Weiler S., Oakes S. A., Mannella C. A. and Korsmeyer S. J. (2002) A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. Dev. Cell 2, 55–67.
87 Griparic L., van der Wel N. N., Orozco I. J., Peters P. J. and van der Bliek A. M. (2004) Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. J. Biol. Chem. 279, 18792–18798.
88 Frezza C., Cipolat S., Martins de B. O., Micaroni M., Beznoussenko G. V., Rudka T., Bartoli D., Polishuck R. S., Danial N. N., De S. B. and Scorrano L. (2006) OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. Cell 126, 177–189.
89 Arnoult D., Grodet A., Lee Y. J., Estaquier J. and Blackstone C. (2005) Release of OPA1 during apoptosis participates in the rapid and complete release of cytochrome c and subsequent mitochondrial fragmentation. J. Biol. Chem. 280, 35742–35750.
90 Cipolat S., Martins de B. O., Dal Z. B. and Scorrano L. (2004) OPA1 requires mitofusin 1 to promote mitochondrial fusion. Proc. Natl. Acad. Sci. USA 101, 15927–15932.
91 Desagher S., Osen-Sand A., Nichols A., Eskes R., Montessuit S., Lauper S., Maundrell K., Antonsson B. and Martinou J. C. (1999) Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. J. Cell Biol. 144, 891–901.
92 Karbowski M., Norris K. L., Cleland M. M., Jeong S. Y. and Youle R. J. (2006) Role of Bax and Bak in mitochondrial morphogenesis. Nature 443, 658–662.

93 Jagasia R., Grote P., Westermann B. and Conradt B. (2005) DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. Nature 433, 754–760.
94 Youle R. J. and Karbowski M. (2005) Mitochondrial fission in apoptosis. Nat. Rev. Mol. Cell Biol. 6, 657–663.
95 Acehan D., Xu Y., Stokes D. L. and Schlame M. (2007) Comparison of lymphoblast mitochondria from normal subjects and patients with Barth syndrome using electron microscopic tomography. Lab. Invest 87, 40–48.
96 Levine J. S., Branch D. W. and Rauch J. (2002) The antiphospholipid syndrome. N. Engl. J. Med. 346, 752–763.
97 Hauff K. D. and Hatch G. M. (2006) Cardiolipin metabolism and Barth syndrome. Prog. Lipid Res. 45, 91–101.
98 Schlame M. and Ren M. (2006) Barth syndrome, a human disorder of cardiolipin metabolism. FEBS Lett. 580, 5450–5455.
99 Vaz F. M., Houtkooper R. H., Valianpour F., Barth P. G. and Wanders R. J. (2003) Only one splice variant of the human TAZ gene encodes a functional protein with a role in cardiolipin metabolism. J. Biol. Chem. 278, 43089–43094.
100 van Werkhoven M. A., Thorburn D. R., Gedeon A. K. and Pitt J. J. (2006) Monolysocardiolipin in cultured fibroblasts is a sensitive and specific marker for Barth Syndrome. J. Lipid Res. 47, 2346–2351.
101 Kulik W., van Lenthe H., Stet F. S., Houtkooper R. H., Kemp H., Stone J. E., Steward C. G., Wanders R. J. and Vaz F. M. (2007) Bloodspot assay using HPLC tandem mass spectrometry for detection of Barth syndrome. Clin. Chem. 54, 371–378.
102 Ades L. C., Gedeon A. K., Wilson M. J., Latham M., Partington M. W., Mulley J. C., Nelson J., Lui K. and Sillence D. O. (1993) Barth syndrome: clinical features and confirmation of gene localisation to distal Xq28. Am. J. Med. Genet. 45, 327–334.
103 Barth P. G., Scholte H. R., Berden J. A., Van der Klei-Van Moorsel J. M., Luyt-Houwen I. E., Van 't Veer-Korthof ET, Van der Harten J. J. and Sobotka-Plojhar M. A. (1983) An X-linked mitochondrial disease affecting cardiac muscle, skeletal muscle and neutrophil leucocytes. J. Neurol. Sci. 62, 327–355.
104 Christodoulou J., McInnes R. R., Jay V., Wilson G., Becker L. E., Lehotay D. C., Platt B. A., Bridge P. J., Robinson B. H. and Clarke J. T. (1994) Barth syndrome: clinical observations and genetic linkage studies. Am. J. Med. Genet. 50, 255–264.
105 Kuijpers T. W., Maianski N. A., Tool A. T., Becker K., Plecko B., Valianpour F., Wanders R. J., Pereira R., Van H. J., Verhoeven A. J., Roos D., Baas F. and Barth P. G. (2004) Neutrophils in Barth syndrome (BTHS) avidly bind annexin-V in the absence of apoptosis. Blood 103, 3915–3923.
106 Choi S. Y., Gonzalvez F., Jenkins G. M., Slomianny C., Chretien D., Arnoult D., Petit P. X. and Frohman M. A. (2007) Cardiolipin deficiency releases cytochrome c from the inner mitochondrial membrane and accelerates stimuli-elicited apoptosis. Cell Death. Differ. 14, 597–606.
107 Chicco A. J. and Sparagna G. C. (2006) Role of cardiolipin alterations in mitochondrial dysfunction and disease. Am. J. Physiol Cell Physiol. 292, C33–C44.
108 Oram J. F. (2000) Tangier disease and ABCA1. Biochim. Biophys. Acta 1529, 321–330.
109 Bodzioch M., Orso E., Klucken J., Langmann T., Bottcher A., Diederich W., Drobnik W., Barlage S., Buchler C., Porsch-Ozcurumez M., Kaminski W. E., Hahmann H. W., Oette K., Rothe G., Aslanidis C., Lackner K. J. and Schmitz G. (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat. Genet. 22, 347–351.
110 Brooks-Wilson A., Marcil M., Clee S. M., Zhang L. H., Roomp K., van D. M., Yu L., Brewer C., Collins J. A., Molhuizen H. O., Loubser O., Ouelette B. F., Fichter K., shbourne-Excoffon K. J., Sensen C. W., Scherer S., Mott S., Denis M., Martindale D., Frohlich J., Morgan K., Koop B., Pimstone S., Kastelein J. J., Genest J., Jr. and Hayden M. R. (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat. Genet. 22, 336–345.
111 Rust S., Rosier M., Funke H., Real J., Amoura Z., Piette J. C., Deleuze J. F., Brewer H. B., Duverger N., Denefle P. and Assmann G. (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat. Genet. 22, 352–355.
112 Vaughan A. M. and Oram J. F. (2006) ABCA1 and ABCG1 or ABCG4 act sequentially to remove cellular cholesterol and generate cholesterol-rich HDL. J. Lipid Res. 47, 2433–2443.
113 Fobker M., Voss R., Reinecke H., Crone C., Assmann G. and Walter M. (2001) Accumulation of cardiolipin and lysocardiolipin in fibroblasts from Tangier disease subjects. FEBS Lett. 500, 157–162.
114 Han X., Yang J., Cheng H., Yang K., Abendschein D. R. and Gross R. W. (2005) Shotgun lipidomics identifies cardiolipin depletion in diabetic myocardium linking altered substrate utilization with mitochondrial dysfunction. Biochemistry 44, 16684–16694.
115 Petrosillo G., Portincasa P., Grattagliano I., Casanova G., Matera M., Ruggiero F. M., Ferri D. and Paradies G. (2007) Mitochondrial dysfunction in rat with nonalcoholic fatty liver: involvement of complex I, reactive oxygen species and cardiolipin. Biochim. Biophys. Acta 1767, 1260–1267.
116 Sparagna G. C., Chicco A. J., Murphy R. C., Bristow M. R., Johnson C. A., Rees M. L., Maxey M. L., McCune S. A. and Moore R. L. (2007) Loss of cardiac tetralinoleoyl cardiolipin in human and experimental heart failure. J. Lipid Res. 48, 1559–1570.
117 Poorthuis B. J., Yazaki P. J. and Hostetler K. Y. (1976) An improved two dimensional thin-layer chromatography system for the separation of phosphatidylglycerol and its derivatives. J. Lipid Res. 17, 433–437.
118 Weerheim A. M., Kolb A. M., Sturk A. and Nieuwland R. (2002) Phospholipid composition of cell-derived microparticles determined by one-dimensional high-performance thin-layer chromatography. Anal. Biochem. 302, 191–198.
119 Gohil V. M., Gvozdenovic-Jeremic J., Schlame M. and Greenberg M. L. (2005) Binding of 10-N-nonyl acridine orange to cardiolipin-deficient yeast cells: implications for assay of cardiolipin. Anal. Biochem. 343, 350–352.
120 Pulfer M. and Murphy R. C. (2003) Electrospray mass spectrometry of phospholipids. Mass Spectrom. Rev. 22, 332–364.
121 Han X. and Gross R. W. (2005) Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. Mass Spectrom. Rev. 24, 367–412.
122 Hsu F. F., Turk J., Rhoades E. R., Russell D. G., Shi Y. and Groisman E. A. (2005) Structural characterization of cardiolipin by tandem quadrupole and multiple-stage quadrupole ion-trap mass spectrometry with electrospray ionization. J. Am. Soc. Mass Spectrom. 16, 491–504.