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REVIEW

Biological Diversity and Remodeling of Cardiolipin in Oxidative Stress and Age-Related Pathologies

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Abstract—Age-related dysfunctions are accompanied by impairments in the mitochondrial morphology, activity of signaling pathway, and protein interactions. Cardiolipin is one of the most important phospholipids that maintains the curvature of the cristae and facilitates assembly and interaction of complexes and supercomplexes of the mitochondrial respiratory chain. The fatty acid composition of cardiolipin influences the biophysical properties of the membrane and, therefore, is crucial for the mitochondrial bioenergetics. The presence of unsaturated fatty acids in cardiolipin is the reason of its susceptibility to oxidative damage. Damaged cardiolipin undergoes remodeling by phospholipases, acyltransferases, and transacylases, creating a highly specific fatty acyl profile for each tissue. In this review, we discuss the variability of cardiolipin fatty acid composition in various species and different tissues of the same species, both in the norm and at various pathologies (e.g., age-related diseases, oxidative and traumatic stresses, knockouts/knockdowns of enzymes of the cardiolipin synthesis pathway). Progressive pathologies, including age-related ones, are accompanied by cardiolipin depletion and decrease in the efficiency of its remodeling, as well as the activation of an alternative way of pathological remodeling, which causes replacement of cardiolipin fatty acids with polyunsaturated ones (e.g., arachidonic or docosahexaenoic acids). Drugs or special diet can contribute to the partial restoration of the cardiolipin acyl profile to the one rich in fatty acids characteristic of an intact organ or tissue, thereby correcting the consequences of pathological or insufficient cardiolipin remodeling. In this regard, an urgent task of biomedicine is to study the mechanism of action of mitochondria-targeted antioxidants effective in the treatment of age-related pathologies and capable of accumulating not only *in vitro*, but also *in vivo* in the cardiolipin-enriched membrane fragments.

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Mitochondria are not only the main source, but also the target of reactive oxygen species (ROS). ROS-induced oxidative damage to these cellular structures underlies many degenerative diseases and age-related pathologies [1, 2]. Since ROS production increases with aging, their higher content hinders normal functioning of cell macromolecules and corresponding signaling pathways, being a leading factor in the aging of cells, tissues, and entire body [3-5].

An increased sensitivity of cells to ROS manifested, for example, as an age-related activation of lipid peroxidation, leads to an increase in the rigidity of cell membranes, which might be associated with changes in their lipid composition [6, 7]. Thus, a gradual decrease in the linoleic acid (18:2) content was observed in the rodent liver microsomal and mitochondrial membrane fractions with aging, which correlated with an increase in the con-

Abbreviations: BTHS, Barth syndrome; CL, cardiolipin; DHA, docosahexaenoic acid; FA, fatty acid; MLCAT, monolysocardiolipin acyltransferase; MLCL, monolysocardiolipin; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; *TAZ*, tafazzin gene; TLCL, tetralinoleoylcardiolipin; TPP, triphenylalkylphosphonium.

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tent of long-chain polyunsaturated fatty acids (PUFAs) (22:4 and 22:5), a subclass of lipids that are more unsaturated and more susceptible to oxidation than linoleic acid (C18:2) [8]. In many cases, linoleic acid-containing phospholipids are predominantly oxidized even in the presence of phospholipids containing fatty acids (FAs) more sensitive to the oxidation (e.g., C20:4, C22:5, and C22:6) [9-11].

In this review, we analyze recent achievements in studying the structure and functional features of cardiolipin (CL), the mechanisms of acyl remodeling, and the distribution of CL in mitochondrial membranes. Particular attention is paid to the mechanism of CL remodeling, as well as the effects of drugs on the restoration of damaged CL structure, leading to the mitigation of the severity of the corresponding pathological conditions.

ROLE AND MAJOR FUNCTIONS OF CARDIOLIPIN

Cardiolipin [1,3-bis(sn-3′-phosphatidyl)-sn-glycerol, CL] has been found in eukaryotes and bacteria [12, 13], but not in archaea which contain only its analogues [14].

Unlike other phospholipids, in eukaryotic cells, CL was found almost exclusively in the inner mitochondrial membrane. CL plays an important role in maintaining the optimal structure and function of the mitochondria and is essential for biogenesis of cristae [15, 16] and fusion/division of mitochondria [17]. It interacts with many proteins of the inner mitochondrial membrane, thereby promoting formation of respiratory supercomplexes and optimizing mitochondrial bioenergetics [18-20]. Besides, CL as a component of proteolipids is indirectly involved in the import of mitochondrial proteins [21], biogenesis of Fe-S clusters, and tricarboxylic acid cycle [22]. Although indirectly, CL participates in the regulation of protein translation on the mitoribosomes by promoting the anchoring of membrane proteins in the inner mitochondrial membrane [23, 24].

In fact, CL can be considered as a functional "glue" that binds components of the mitochondrial respiratory chain into an integrated system providing efficient transfer of electrons and protons. Due to its characteristic cone-shaped structure, CL localizes to the regions of negative curvature of the crista membrane and promotes assembly and interaction of complexes and supercomplexes of the mitochondrial respiratory chain which supports the transmembrane proton gradient [25, 26].

When mitochondrial membrane is damaged, CL can translocate to the outer mitochondrial membrane in a process mediated by nucleoside diphosphokinase (NDPK-D) and/or phospholipid scramblase 3 [27]. Externalized CL can then serve as a signal for initiating mitochondria-mediated apoptosis and mitochondria-specific autophagy (mitophagy) [28]. In many pathologies, CL oxidation triggers cell death [29]. The synergistic effect of Ca^{2+} ions and oxidized CL in the mitochondrial pore induction and cytochrome *c* release can be important for the regulation of the initial phase of apoptosis, as well as results in significant consequences in pathologies characterized by the accumulation of oxidized CL in mitochondria, for example, ischemic tissue damage, stroke, chronic inflammation, aging, and age-related degenerative diseases [30].

CARDIOLIPIN: STRUCTURE, COMPOSITION, AND ASYMMETRY

Cardiolipin is a unique component of the inner mitochondrial membrane, accounting for up to 20% of the total phospholipid content [13, 31]. It is the third most common mitochondrial glycerophospholipid after phosphatidylcholine and phosphatidylethanolamine [32-34].

Unlike other glycerophospholipids, the two glycerol terminal hydroxyl groups in CL are replaced by two phosphatide fragments, resulting in the formation of anionic phospholipid with four esterified fatty acyl chains. The hydrophobicity of the acyl groups and the negative charges of the two phosphate groups ensure CL interactions with a wide range of mitochondrial proteins. Because of the four acyl chains connected to the negatively charged polar fragment, CL has the "conical" shape with the polar fragment at the top and flexible acyl chains at the base of the "cone" [35]. This structure allows CL to form local microdomains in the membrane, which are necessary for the formation of the curved mitochondrial cristae [36].

The length, degree of unsaturation, and extent of oxidation of the CL side chains also affect CL shape, stability, and nature of its interactions with proteins [37, 38]. As mentioned above, the collapse of membrane asymmetry during CL transfer to the outer membrane is a promitophageal mechanism in which externalized CL acts as a signal for organelle degradation [39].

ACYL COMPOSITION OF CARDIOLIPIN CHAINS

CL acyl chains vary greatly in tissues in organisms from different taxa [40] and can even depend on the diet [41].

Prokaryotic CL lacks polyunsaturated FAs (PUFAs) and contains instead saturated or monounsaturated FAs with relatively short chains (usually, 16 carbon atoms long). In eukaryotes, CL contains longer unsaturated chains (18-22 carbon atoms) [13]. Monounsaturated FAs

(18:1, 16:1), including *tetra*-oleyl-CL, prevail in CL of *Saccharomyces cerevisiae* yeast [35]. In tissues of higher eukaryotes, CL contains mono- or di-unsaturated chains 16-22 carbon atoms in length [35], which makes it more sensitive to oxidative stress. In rodents and humans, CL mainly contains linoleic (18:2), oleic (18:1) and docosahexaenoic (22:6; DHA) acids, while palmitic acid (16:0) is rare [35]. In zebrafish (*Danio rerio*) at all ages (3-24 months), CL contains mostly DHA (22:6) (~45%), stearic acid (18:0) (~16%), and oleic (18:1) acid [35]. Mollusks stand apart in the animal kingdom: eicosapentaenoic acid and DHA make up to 73 mol.% in the tissues and organs of the Manila clam *Ruditapes philippinarum*; the content of DHA (including that in the content of *tetra*-DHA-CL) reaches 80 mol.% in the scallop *Pecten maximus*, Pacific oyster *Crassostrea gigas*, and blue mussel *Mytilus edulis*. It is assumed that such a high PUFA content in mollusks is an adaptation to the environmental conditions specific to their living environment (temperature and salt concentration) [42, 43].

FATTY ACID COMPOSITION OF CARDIOLIPIN IN MAMMALIAN TISSUES AND ORGANS

The total CL content in tissues varies in proportion to the needs for oxidative metabolism, which is also reflected in a higher content of mitochondria. In tissues with periodic oxidative activity, for example, in skeletal muscle, the CL content is very high (10-20% of the total amount of phospholipids) [44]. In the heart, the CL content is ~12-15%, while in other organs it is much lower: ~6-7% in the kidneys, ~5-6% in the liver, and ~2-3% in the testes [31, 45]. Moreover, since the energy requirement per mass unit in the brain is almost half of that in the heart muscle or kidneys, the brain has the smallest proportion of CL relative to the total phospholipid content (~1-2%). The relative content of CL in different tissues is similar in humans, rat, and guinea pig [31, 45].

As noted above, eukaryotes are characterized by the asymmetry in the CL distribution among organs, tissues, cells, and even between the inner and outer mitochondrial membranes. Also, there is an asymmetry in the substituents (FAs) in CL itself (CL molecule asymmetry) [13]. In most tissues and cells, CL contains a variety of FAs in, with one or two FAs prevailing [10, 26, 35, 46, 47].

In almost all tissues, except the brain and the testes, the most common FA in the CL is linoleic acid (18:2n-6) $[CH_3(CH_2)_3\text{-}(CH_2CH{=}CH)_2(CH_2)_7COOH]$, which is an essential ω-6-unsaturated FA that can be converted to oleic acid upon reduction and arachidonic acid upon oxidation. Its relative content in the heart and liver is over 80% [24, 32, 46, 48-50], ~60% in skeletal muscles [24, 32, 46, 48-50], ~61% in kidneys, and ~49% in spleen [46].

The next most common FAs in CL are oleic acid (18:1n-9) $[CH_3(CH2)_7CH{=}CH(CH_2)_7COOH$, an essential ω-9-monounsaturated FA] [32, 46, 48, 49, 51] and saturated stearic acid (18:0) $[CH_3(CH_2)_{16}COOH]$ [32, 48]. Their content is ~44, ~40 and ~6% in the lungs, skeletal muscles, and liver, respectively) [46, 51]. In bovine heart, CL is enriched with ω-3-unsaturated α-linolenic acid (18:3n-3) $[CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH]$ [49].

Tissue-specific profiles of CL. The correspondence of FA composition and structure of CL to the metabolic load in mammalian tissues eliminates interspecific differences, resulting, for example, in the similarity in the FA composition of CL in liver of rodents and cows [46]. In other types of tissues, e.g., rodent testes, the CL composition is dominated by saturated palmitic (16:0) $[CH_3(CH_2)_{14}COOH]$ (~55%) and stearic acid [46]. Finally, rat testes contain a large amount of *tetra*-palmitate-CL [52]. In contrast, heart CL has a characteristic FA profile dominated by the linoleic acid (18:2n-6). Approximately 80% CL molecules in the heart are esterified with this acid at all four positions with the formation of *tetra*-linoleoylcardiolipin (TLCL) [32]. This molecular feature is also highly conserved in mammals: a similar FA composition is observed in CL from the bovine, rodent, and human hearts [32-34, 48, 49, 53-56].

TLCL deficiency causes mitochondrial dysfunction, which can lead to autophagy, apoptosis, and development of pathologies [28]. CL, in which one of the four residues is esterified with oleic acid instead of linoleic acid (18:2–18:2)-(18:2–18:1), is the second most common CL species in the bovine, rodent, and human hearts [32, 48, 49]. It is assumed that oleic and linoleic acids can provide "molecular symmetry" in the mitochondria, which is disturbed upon pathological CL remodeling. However, the content of TLCL and even CL with 18-carbon FAs varies widely in different tissues [26].

Mammalian brain demonstrates an unprecedented (in comparison to other tissues) diversification of mitochondria-specific FAs in CL: hundreds of CL species with PUFAs were identified in the brain tissue [57-60]. It is interesting that not only the brain has a completely different CL acyl profile compared to the heart, but the differences between the CL acyl profiles are pronounced between different species. Oleic acid is the most common FA in CL in the bovine [61] and human [40] brains, where it constitutes ~54 and 32% of the total FA content, respectively. In rats, the most common FA is stearic acid, which accounts for almost half of the fatty acyl groups in CL [46]. There are also interspecific differences in the second most represented FA in the brain CL. In humans, it is stearate, while in rodents, it is oleate [40]. Linoleate typically accounts for <10% of total FAs in CL in the brain [40]. Brain CL is believed to contain less unsaturated FAs than cardiac CL, probably to ameliorate the accumulation of lipid peroxidation products during aging [62]. At the same time, mammalian central nervous system is rich in

two long-chain essential PUFAs – ω-3-unsaturated DHA [$CH_3(CH_2)(CH{=}CH\text{-}CH_2)_6(CH_2)COOH$] and arachidonic acid [$CH_3(CH_2)_4(CH{=}CH\text{-}CH_2)_4(CH_2)_2COOH$] [40, 62].

Currently, biologically active substances based on the most common FAs are actively developed that can be used in oxidative stress models as either damaging agents, e.g., oxidized TLCL ($TLCL_{ox}$) [59], or protective drugs, e.g., oleic acid modified by replacement with imidazole group and conjugated to triphenylphosphonium (see "Cardiolipin-Targeted Therapy" below).

CARDIOLIPIN SYNTHESIS AND REMODELING

CL is synthesized from two phosphatidylglycerol molecules in bacteria and from phosphatidylglycerol and diacylglycerol cytidine diphosphate (CDP-DAG) in eukaryotes [64]. In eukaryotes, CL is located predominantly and *de novo* synthesized exclusively in mitochondria [23], which allows to use this phospholipid as a reliable molecular marker of mitochondria in the cellular physiology studies.

In eukaryotes (unlike bacteria), a significant portion of CL undergoes remodeling [13]. The newly synthesized CL is deacylated by a CL-specific phospholipase or calcium-independent phospholipase A2 to form monolysocardiolipin (MLCL) [24]. Then, MLCL is reacylated by CoA-independent tafazzin [65] or acyl-CoA:lysocardiolipin acyltransferase 1 [66] and converted into mature CL. Through this process, a high degree of symmetry of CL acyl chains is created. In addition to acyl CoA:lysocardiolipin acyltransferase, mitochondrial monolysocardiolipin acyltransferase catalyzes MLCL acylation in a reaction using linoleoyl-CoA as a substrate [13, 35]. Single-stage remodeling involves only CL transacylation (deacylation is not required), since the transacylation reaction between lysophospholipid and CL can generate phospholipid and MLCL. Subsequent transacylation between MLCL and adjacent phospholipid generates remodeled CL and monolysophospholipid [13, 35].

Significance of CL remodeling. Since enzymes of CL *de novo* synthesis do not exhibit acyl specificity [24, 67], remodeling, in which some acyl chains are replaced by others, plays a key role in the generation of symmetric CL. During this process, a specific composition of FAs (mostly unsaturated) is created [7]. In some organs and tissues (e.g., heart, muscles, and liver), CL maturation eliminates the diversity of substituents, limiting them predominantly to tetralinoleyl (TLCL) (~80-85%) [13]. It is assumed that TLCL is a structurally homogeneous and molecularly symmetric form of CL, which is necessary to ensure high energy consumption during heart beat [13, 68].

Thus, remodeling may be a key regulatory mechanism for maintaining consistent CL composition. Firstly, CL remodeling can alter the extent of unsaturation of the CL content, as well as to generate free FAs and MLCL, which is necessary for specific CL functions. Secondly, remodeling can occur in individual mitochondrial domains containing the involved enzymes, which would limit specific CL functions to the corresponding compartments. Thirdly, the physicochemical properties of CL can change during remodeling, which might affect its interaction with proteins and functioning of the respiratory chain as a whole.

TAFAZZIN

Tafazzin, one of the key CL-remodeling proteins, has been found in the mitochondrial intermembrane space in all the studied eukaryotes [26, 69, 70]. Tafazzin is associated with multi-protein complexes of 10^5-10^6 Da, which is important for its functional activity. It was also found at the sites of contact between the inner and outer mitochondrial membranes [36].

Tafazzin is a phospholipid-lysophospholipid transacylase capable of catalyzing CL remodeling; the deficiency of this enzyme abolishes the reacylation step and leads to a decrease in the level of total CL and increase in the level of MLCL (an intermediate form of lipid formed during CL diacylation) [65, 71-74]. Importantly, tafazzin-mediated remodeling shifts the CL acyl composition towards unsaturation.

Clinically, the importance of CL remodeling was demonstrated in the Barth syndrome (BTHS), a life-threatening phenotype that results from mutations in the tafazzin gene (*TAZ*) [69, 70]. This X-chromosome-linked recessive disease is characterized by a triad of clinical symptoms: cardiomyopathy, skeletal myopathy, and neutropenia combined with lactic acidosis and increased urinary content of 3-methylglutaconic acid [75]. The main diagnostic feature of BTHS, along with the mutation in the *TAZ* gene, is a change in the CL/MLCL ratio in the blood. A reduced CL/MLCL ratio indicates that remodeling is initiated by deacylation upon MLCL accumulation in the absence of tafazzin-mediated reacylation. These changes in the CL profile are observed in all studied eukaryotes with tafazzin mutations, including yeast [65], *Drosophila* fruit fly [53], mouse [73], and humans [71, 72].

Characteristic changes in the content and composition of CL in *TAZ* mutants indicate that reactions mediated by acyl-CoA:lysocardiolipin acyltransferase 1 and monolysocardiolipin acyltransferase are not able to fully compensate for the loss of tafazzin.

***TAZ* gene sequence.** Since the acyl modification of CL by tafazzin is one of the main regulators of its functional activity, it can be assumed that the structural and functional characteristics of tafazzin affect the functional state of mitochondria and species-specific lifespan.

Comparison of nucleotide sequences of the *TAZ* gene and several of its mRNA transcripts revealed the existence of two alternative sites for the transcription initiation and several splicing variants corresponding to several tafazzin isoforms [70, 74]. In addition, it was found that in some species (including hominids), tafazzin has exon 5 [74]. In many isoforms of mammalian tafazzin, new conserved regions have been found closer to the *C*-terminus that resulted from a shift in the reading frame relative to the full-length transcript of the *TAZ* gene via skipping the exon 9 or preserving the intron between the exons 10 and 11 in the gene transcript [74]. These changes are very specifically distributed among mammalian orders, and their presence correlates with the species-specific lifespan and body weight, as well as with the intensity of mitochondrial metabolism. Probably, tafazzin isoforms are needed to achieve the optimal balance between the increase in the biochemical activity of mitochondria required under certain environmental conditions and maintenance of longevity, since the functional purpose of these isoforms is partly related to the changes in the primary and secondary structures of their *C*-terminal sequences.

Two tafazzin fragments are critical for its function: a hydrophobic 30-a.a. motif in the *N*-terminal region that acts a membrane anchor, and the hydrophilic domain in the middle part of the polypeptide chain that is presumably involved in the interaction with other proteins. As a result of alternative splicing of the primary *TAZ* transcript, four different mRNAs are formed: full-length (FL) mRNA and mRNAs lacking exon 5 (Δ5), exon 7 (Δ7), or both exons 5 and 7 (Δ5Δ7), respectively [76]. The FL and Δ5 isoforms of tafazzin possess the transacylase activity but differ in their topology (immersion in the membrane) [77]. The shortest tafazzin isoforms do not contain the hydrophobic region and are apparently cytoplasmic proteins, while the slightly longer forms resulting from the alternative splicing at exons 5-7 differ in the length of the hydrophilic domain. The most common tafazzin isoform is the enzyme lacking exon 5 (Δ5).

Mutations in the *TAZ* gene decrease the formation of TLCL in favor of CL molecules with different acyl composition, which affects the structure and functional activity of the mitochondria. The study of the ratio between various forms of tafazzin in the blood cells of BTHS patients and healthy individuals showed that, in addition to two functionally active isoforms (FL and Δ5), there are many mRNA variants encoding inactive forms of the protein [77].

The enzymatic activity of tafazzin in people with *TAZ* mutations (BTHS patients) is reduced or absent. If a particular *TAZ* mutation leads to the formation of tafazzin protein with residual enzymatic activity, it can be expected that changes in the content and composition of CL will be less pronounced resulting in a milder disease phenotype [7], while mutations leading to the loss of transacylase activity will cause significant changes in the structure of the inner mitochondrial membrane and functional activity of proteins located in it. Finally, for genocopies with the same *TAZ* sequence, the differences in the severity of the disease may be determined by one or more phenotypic modifiers, from environmental to biochemical [78]. The presence of the evolutionarily conserved motif HxxxxD (where x is any amino acid) characteristic of glycerolipid acyltransferases in the *TAZ* sequence indicates the likely role of tafazzin in the remodeling of newly synthesized CL. Indeed, in patients with BTHS, the efficiency of linoleic acid (C18:2) incorporation into CL was significantly reduced, unlike other FAs [7]. In BTHS, defective remodeling of CL correlates with changes in the ultrastructure of cristae [16, 79, 80]. Huang et al. showed that the electron transfer chain (ETC) supercomplexes are unstable in CL-depleted heart mitochondria from the *TAZ*-knockdown mice [81]. Similarly, Kiebish et al. reported that a decrease in the activity of tafazzin in the myocardium leads to changes in the mitochondrial lipidome, for example, to an increase in the content of PUFA oxidized derivatives. The eventual effect of this impairment is a decrease in the ability of respiratory chain to oxidize NADH and $FADH_2$ [82].

Tafazzin specificity. Unlike *in vivo*, purified recombinant tafazzin exhibits no specificity for any particular acyl group and displays transacylase activity not only toward CL, but also toward other phospholipids, such as phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, and their lyso-(L)-derivatives, because it is able to transfer acyl groups 7-19 carbon atoms in length containing up to three double bonds [77]. Therefore, tafazzin should be considered not only as an enzyme that converts one pair of phospholipids to another, but as an enzyme that creates a certain equilibrium matrix of phospholipid/lysophospholipid molecules. Theoretically, such broad specificity of tafazzin should lead to similar acyl composition of all phospholipids in the corresponding membrane compartments [26].

Recent studies showed that *in vivo* substrate specificity of tafazzin may depend on the phase state of membrane lipids [83]. It was initially assumed that CL remodeling can be spatially activated in certain domains of mitochondrial membranes, where tafazzin is located, to form the mitochondrial architecture [7, 75]. However, it was found that tafazzin is very specific *in vivo*, first of all, toward the mitochondrial membrane CL. Apparently, such specificity of the *in vivo* transacylation reactions is determined not so much by the properties of tafazzin itself, as by the features of the mitochondrial membrane organization and availability of acyl groups. It is assumed that the main function of tafazzin is optimization of phospholipid packing in the membranes by maintaining the possibility of conformational transitions of mitochondrial membrane lipids [26]. As suggested by Kagan et al.,

CL and its numerous metabolites form the basis of mitochondrial communication, i.e., act as yet unknown "mitochondrial language" that is especially important for coordinating complex brain function. Diversified four chains in CL form a quaternary number system analogous to the DNA genetic code formed by four nucleotides. If there are >20 residues of FAs available for the integration into CL, the total theoretical number of possible isomers will be $>20^4$. In addition to this, oxidation of FA chains in CL can occur together with the enzymatic cleavage of oxidized FA chains to produce lyso-CL, which will additionally increase the diversity and strength of the transmitted signals [4, 26]. Thus, it is assumed that CL and its oxidation and hydrolysis products constitute a rich "language of communication" used by the mitochondria of eukaryotic cells for the regulation of cellular physiology and metabolism, as well as for cell-cell interactions. Kagan et al. [4, 5] described two main ways for the mitochondrial CLs to perform signaling functions: 1) via asymmetric distribution across the membranes and translocation leading to surface externalization of CL and 2) via oxidation with the formation of special products recognized by the cell executive mechanism.

CARDIOLIPIN METABOLISM DISORDERS. CHANGES IN CARDIOLIPIN IN AGING AND PATHOLOGIES

The three well-known CL-related pathological are the loss of CL content, peroxidation, and impaired acyl chain remodeling [38, 55, 84, 85]. A decrease in the CL content can be caused by its degradation due to the increased activity of phospholipases or decrease in its *de novo* synthesis as a result of decrease in the activity of corresponding enzymes [84, 86], as well as CL oxidation by external ROS [87] or cytochrome *c* in the content of a complex with peroxidase activity [88]. Disorders of CL metabolism, including age-related ones, lead to changes in the structure and function of mitochondria, including the loss of cristae, decrease in the ability of mitochondria to divide and fuse, and reduced mitophagy and apoptosis, leading to pathological consequences [3, 48, 53, 55, 62, 72, 73, 89-93].

CL oxidation and loss of symmetry. Due to the presence of unsaturated acyls and proximity to the redox centers of the respiratory chain complexes I and III and other known sites of ROS generation in the mitochondria, CL is potentially capable of oxidation upon active functioning of the ETC.

Moreover, in the brain, CL oxidation mediates generation of neuronal death signals [59, 62]. By changing FAs to less unsaturated ones, it is possible to reduce the likelihood of CL peroxidation in the brain and to promote the survival of neurons (cells that do not proliferate and are functionally indispensable). A high degree of CL unsaturation in the cardiac activity may be necessary to achieve a high rate of heart oxidative metabolism [91].

The loss of symmetry as a result of CL oxidation or hydrolysis also occurs in cancer, mitophagy, and apoptosis [4, 5]. It was found that dysregulation of CL synthesis and remodeling leads to the loss of symmetry in rat brain tumors [60]. The mitochondrial morphology is disturbed in mutant individuals with CL remodeling deficiency [15]. Thus, clusters of fragmented mitochondria and dysmorphic cristae were observed in the lymphoblasts of BTHS patients [79].

Age-related CL decrease in heart. Paradies et al. found a 40% decrease in the CL content, along with a 35% decrease in the level of cytochrome oxidase activity, in cardiac mitochondria in 26-month-old Fisher 344 rats compared to the 5-month-old animals [94]. Pepe et al. reported a 1.4-fold decrease in the molar percentage of CL in 24-month-old Wistar rats compared to the 6-month-old rats [92]. McMillin et al. found a decrease (~23%) in the CL content in the heart mitochondria: from 39 ± 2 nmol/mg mitochondrial protein in 6-month-old Fischer 344 rats to 30 ± 2 nmol/mg mitochondrial protein in 30-month-old rats [95]. However, Moghaddas et al., who studied the FA composition and percentage content of CL separately in the subsarcolemmal and interfibrillar heart mitochondria in 6- and 24-month-old rats, revealed no differences in either CL percentage content (~13% in young and old animals) or FA composition of CL, despite the decrease in the level of oxidative phosphorylation in the interfibrillar mitochondria of old rats [96]. According to the authors, this could be due to the fact that the mortality of Fischer 344 rats increases only between 28 and 30 months of age (median survival is 29 months) [97], while the study used rates aged 24 months as "old" animals [96].

Age-related changes in the FA composition of CL. The content of CL, in particular, TLCL $(18:2)_4$, in muscles decreases with age, together with the replacement of linoleic acid (18:2) in CL by other FAs. This is accompanied by a decrease in the ETC function and activation of ROS generation. It can be assumed that the depletion of TLCL $(18:2)_4$ during aging can cause mitochondrial dysfunction, which, in turn, can lead to sarcopenia [98]. Thus, FAs in the CL of young rats [e.g., linoleic acid (18:2n-6)] are replaced with more unsaturated FAs, such as arachidonic acid (20:4n-6) and DHA (22:6n-3) in old animals [48].

Lee et al. found age-related changes in the CL FAs (mostly represented by linoleic acid) in the heart of 4-, 12-, and 24-month-old rats, who received the same FA-balanced diet throughout their lives [48]. The content of linoleic acid decreased ~1.5 times in 24-month-old rats compared to 4-month-old animals (3965 ± 617 and 5525 ± 656 nmol/g, respectively), while the contents of arachidonic acid and DHA, in contrast, increased in old rats (79 ± 9 vs. 178 ± 27 for arachidonic acid and 104 ± 16 vs. 307 ± 68

for DHA in 4- and 24-month-old rats, respectively). No similar changes were observed for ethanolamine glycerophospholipids or unesterified FAs, which indicates the specificity of these effects with respect to CL [48]. In another work, age-related changes in the composition of PUFAs in the liver, kidneys, and heart were evaluated in rats of different age (3, 12, and 24 months) either fed *ad libitum* every other day or given every day only 60% amount of food consumed by the age-matched control animals. The content of saturated FAs did not significantly change with age; mono- and bis-unsaturated FAs decreased in the liver and heart, and the ratio of the former to the latter increased in the liver, kidneys, and heart. The content of PUFAs in the liver and heart increased. The content of the most common PUFAs (n-6 family) decreased in all three organs, while the content of PUFAs of the n-3 family increased in the liver and kidneys. Moreover, the content of 20:4n-6 and 22:6n-3 either remained the same or increased with age [93]. Food restriction largely leveled out most of these changes and prevented development of sarcopenia, which is an age-related loss of muscle mass, strength, and physical exercises [93].

Pathology. CL undergoes significant changes in pathological conditions, such as cancer, diabetes, heart disease, neurodegenerative disorders, Parkinson's disease, and BTHS, as well as aging [20, 38, 55, 60, 71, 84, 86, 99]. Post-mortem analysis of brain tissues from patients with Alzheimer's disease revealed an increased palmitate (16:0) content in CL isolated from the frontal cortex and reduced DHA (22:6n-3) content in CL isolated from the temporal cortex compared to the brain of healthy people [40]. A decrease in the total CL content in brain mitochondria was found in Parkinson's disease [28], Alzheimer's disease, and age-related dementia [100]. The concentration of CL decreased by 50% after ischemia and subsequent reperfusion injury to the heart [101]. It is also known that brain oxidation of CL generates neuronal death signals during trauma [102].

CARDIOLIPIN-TARGETED THERAPY. ACTION OF ANTIOXIDANTS

Diet. Nutrient deficiency and exogenous nutritional supplements dynamically change the content and composition of CL [103]. Restriction of proliferation and transition of tumor cells to the stationary phase caused by vincristine and mitomycin C were accompanied by CL accumulation [104]. Recovery of cell growth after serum deprivation stimulated the synthesis of CL with longer acyl chains [103].

Inclusion of hydrogenated coconut oil in rat diet increased the content of oleic and palmitoleic acids in CL *in vivo*, which was associated with a decrease in the oxidative and phosphorylating ability of liver mitochondria using glutamate and malate as substrates [41]. Conversely, in rats fed with safflower oil, the content of oleic and arachidonic acids in CL in the liver increased, which was associated with a greater ability for oxidative phosphorylation. Apparently, these effects are explained by the disturbance in the mitochondrial ultrastructure caused by changes in the FA profile of CL [41].

Antioxidants. In general, antioxidants help preserve both the structure and the content of mature CL. Thus, in the brain, the antioxidant melatonin helps maintain the structural integrity of CL and prevents an increase in the level of CL peroxidation with age [105].

The CL content in heart mitochondria decreases (by about 40%) in old rats, with the main decrease occurring during the second year of life [91]. Acetyl-L-carnitine reverses the age-related decrease in mitochondrial metabolism by restoring the CL content to the level observed in young control animals, and changes in the CL content correlate with changes in the rate of pyruvate transport and oxidation [91, 99].

Synthetic antioxidants. It was previously shown in several laboratories that CL oxidation in response to oxidative stress can be prevented by adding mitochondria-targeted antioxidants, both peptides (SS-02 and SS-31) [106, 107] and conjugates of penetrating cations with quinols, for example, XJB-5-131, a conjugate of 4-amino-TEMPO and chemically modified gramicidin S, which effectively delivers nitroxide to mitochondria [59, 106], 5-MitoQ [10-(2,3-dimethoxy-5-methyl-1,4-benzoquinonyl-6)decyltriphenylphosphonium] [108], and SkQ1 [10-(6′-plastoquinonyl)decyltriphenylphosphonium] [109-112].

TPP-n-ISA [3-(12-imidazol-1-yl)octadecanoyl-propyl-triphenylphosphonium bromide], a compound capable of supporting CL in a structural state that impedes peroxidation, has a protective effect upon brain damage and irradiation [113]. SS-31 (Bendavia), which is the most studied of the Szeto-Schiller tetrapeptides (SS) that localize mainly to the inner mitochondrial membrane regardless of the mitochondrial potential gradient, reduces ROS generation and prevents the damage caused by ischemia/reperfusion in different models of heart attack. Thus, it reduces the size of the heart attack *in vivo* in sheep by 15% ($p = 0.02$) and in guinea pigs in the *ex vivo* model by 38-42% ($p < 0.05$) [114, 115]. It has also been shown that SS-31 acts on CL, affecting its interaction with cytochrome *c* and leading to the optimization of electron transfer, inhibition of ROS formation, and suppression of cytochrome *c* peroxidase activity. In some cases, SS-31 helps preserve the density of mitochondrial cristae, presumably by maintaining the TLCL pool [107, 116]. Currently, the protective effect of SS-31 is being studied in several phase II trials in various models of oxidative stress, including patients with mitochondrial diseases (e.g., BTHS) [107, 116].

The triphenylalkylphosphonium ion (TPP^+) conjugated to plastoquinone (SkQ1) or coenzyme Q (MitoQ)

ensures voltage-dependent delivery of quinone analogues to the inner mitochondrial membrane. Combination therapy with MitoQ and losartan (angiotensin receptor blocker) improved cardiovascular function in the rat model of hypertension. The systolic and pulse pressures were ~23% lower in rats treated with MitoQ (500 μM) and losartan (2.5 mg/kg per day) (167.1 ± 2.9 and 50.2 ± 2.05 mm Hg, respectively) than in control rats with hypertension [206.6 ± 9 mm Hg ($p < 0.001$) and 63.7 ± 2.7 mm Hg ($p = 0.001$), respectively] [117].

SkQ1 is a mitochondria-targeted antioxidant that, as we showed earlier, increases the lifespan of male BALB/c mice and dwarf hamsters [110] in the animal models of ischemia-induced cardiac dysfunction. It has a positive effect by reducing post-ischemic complications caused by the oxidative damage of mitochondria [109, 118].

As shown by V. P. Skulachev et al., the advantage of mitochondria-targeted antioxidants, such as SkQ1, is that, unlike other antioxidants, they directly prevent CL oxidation and have a positive effect in nanomolar concentrations, which indicates high specificity of these compounds [2, 119].

Effect of antioxidants on CL oxidation. Brain injury in rats was found to result in the oxidation of about half of CL molecules and appearance of more than 150 new oxidized CL molecular species [59], as well as a 16-fold increase in the content of oxidized forms of CL compared to the control. This was prevented by addition of the mitochondria-targeted antioxidant XJB-5-131 (50 mg/kg) [59]. RNA_i-mediated modification of the levels of CL synthase increased the mechanical resistance of primary rat cortex neurons to mechanical stretching (*in vitro* model of traumatic neuronal damage). XJB-5-131 in a concentration of 1-25 μM inhibited in a dose-dependent manner the death of primary neurons caused by $TLCL_{ox}$ [59]. TPP-IOA [9-(Z)-(3-(12-imidazol-1-yl)-octadeca-9-enoyloxy)propyl-triphenylphosphonium bromide; oleic acid modified by replacement of imidazole fragment and conjugated with TPP in the presence of 3-hydroxypropyl linker] interacts directly with cytochrome *c* in the mitochondria, preventing both cytochrome *c* release and peroxidase activity of cytochrome *c*, and inhibits CL oxidation and apoptosis in SH-SY5Y cells [63].

Using homozygous knockout mice with impaired error correction in mitochondrial DNA and mutant mtDNA phenotype (mutator mice; model of progeria) [120] that express a version of gamma A polymerase (PolgA), we showed that SkQ1 neutralizes the effect of mutations and slows down the appearance of signs of aging in the mtDNA of mutator mice. When studying the effect of SkQ1 on the phospholipid composition of mitochondria from different tissues, neither the mutation nor the treatment with SkQ1 had a noticeable effect on the content of most classes of phospholipids, except CL, whose content was relatively lower in the mutant mice compared to the wild-type animals. SkQ1 restored the CL level in the mutant mice to the levels observed in the wild-type mice. In addition, we analyzed the acyl composition of all mitochondrial membrane phospholipids and revealed that the content of polyunsaturated (n-6) FAs in mtDNA mutator mice was markedly reduced to 2/3 of the levels in the wild-type mice, both in the skeletal muscle and liver mitochondria. At the same time, the decrease in the content of PUFAs was compensated by the increase in the content of saturated FAs. SkQ1 treatment completely prevented this remodeling, returning the unsaturated/saturated FA ratio to the level observed in the wild-type mice. Since CL usually contains four linoleic (18:2n-6) chains per molecule (see above), a decrease in the amount of CL (by ~5 mol.%) is likely to correspond mostly to the loss of ~10 mol.% of (n-6) PUFA, the preservation of which is due to the SkQ1-induced increase in the CL level in mutant mice to the level typical of wild-type mice. Thus, we found that when mutant mice fed with the SkQ1-containing diet not only exhibited a lesser decrease in the content of mature CL in various tissues than the control (without SkQ1), but also the FA composition of lipids in these animals normalized and approached that observed in the wild-type mice. The normalization of mitochondrial morphology, respiration, and parameters of oxidative phosphorylation in mutant mice observed in this case supports the hypothesis on the leading role of CL in maintaining the structural and functional state of mitochondria. The protection of mitochondrial CL from oxidative damage, therefore, might be the reason for preserving mitochondrial ultrastructure necessary to maintain the bioenergetic function of mitochondria at the required level [121].

Mulkidjanian et al. showed that, unlike artificial amphiphilic antioxidants (MitoQ and SkQ), natural hydrophobic antioxidants, such as ubiquinol and α-tocopherol, cannot protect CL molecules located within respiratory supercomplexes [122] and inaccessible to ubiquinol or α-tocopherol and water-soluble polar antioxidants, such as glutathione [29].

Apparently, CL molecules inside the supercomplexes may be accessible to small, mobile, amphiphilic artificial antioxidants that have a specific affinity for the membrane/water interface and, therefore, are able to suppress peroxidation reactions mediated by cytochrome *c* on the membrane surface [122]. Mulkidjanian et al. indicated a pronounced heterogeneity of the inner mitochondrial membrane in terms of sensitivity to oxidative stress [29]. On one hand, this membrane apparently contains CL-rich respiratory supercomplexes (CL islands) [19, 123]. On the other hand, these CL islands are separated by the phospholipid bilayer sections lacking CL. The CL molecules in the CL islands are sensitive to ROS, while the lipids between the CL islands are protected from oxidation by ubiquinol molecules. This explains why mainly

CL molecules are oxidized under oxidative [2] or traumatic stress conditions [29, 59].

Cardiolipins are ubiquitous membrane phospholipids in both prokaryotes and eukaryotes. Most FAs in eukaryotic CL are unbranched FAs with the number of carbon atoms from 18 to 22. In some cases, the diversity of FAs in CL is sharply reduced under normal conditions. For example, almost all FA molecules in CL in the mammalian heart and muscles are linoleic acid. In marine mollusks, which experience fluctuations in salinity, temperature, and pressure when inhabiting different depths, FAs in CL are mainly represented by arachidonic and docosahexaenoic acids. Diet also affects the FA composition of CL [124]. There is a hypothesis based on archaeological evidence that it was the use of "ready-to-eat" PUFA-rich food resources in coastal marine and lake areas that became a prerequisite for the emergence of large modern brain unique in its level of complexity, which made the origin of *H. sapiens* possible [125]. Indeed, unlike muscles and heart, mammalian brain is significantly enriched with long PUFAs necessary to maintain normal functioning of an adult brain. Humans would have hardly developed a large, complex, metabolically expensive brain in an environment that had not provided enough PUFAs in the diet. Conversion of plant PUFAs with 18 carbon atoms into arachidonic and docosahexaenoic acids is energetically disadvantageous due to the combination of rapid PUFA oxidation in the course energy production and slow enzymatic transformation of short FAs into longer ones [59, 125].

Mutations of the *TAZ* gene correlate with characteristic changes in the CL content and composition. Therefore, other CL synthesis pathways cannot fully compensate for the loss of tafazzin.

In addition, despite the universal nature of the described molecular mechanism, phenotypic disorders in BTHS affect only certain tissues. For example, morphological abnormalities in the mitochondria of embryonic stem cells are observed only after differentiation of these cells into cardiomyocytes [16]. Apparently, very active mitochondria with a high density of cristae are most sensitive to the defects in tafazzin. Be that as it may, defects in the structural organization of mitochondria are not an inevitable consequence of the tafazzin absence; rather only a proportion of defective mitochondria increases. Perhaps this explains the variability of phenotypic disorders in BTHS. Since heart has one of the highest metabolic rates among all organs [31], increased energy requirements can cause additional stress on the heart tissue, increasing the likelihood of damage/failure even in the absence of congenital pathologies [16].

While tafazzin-catalyzed transacylation is activated by certain physical properties of the membrane, tafazzin-mediated remodeling exchanges acyl groups between CL and adjacent membrane lipids to create a certain curvature of the membrane. This is confirmed by the morphological changes in the inner mitochondrial membrane in BTHS [79] and the restoration of the mitochondrial ultrastructure upon CL protection from the ROS-induced damage by mitochondria-targeted antioxidant [119, 121].

CL also undergoes significant changes in a variety of pathologies, such as cancer, diabetes, heart disorders, Parkinson's disease, and BTHS [13, 14, 24, 64-66], as well as aging [3, 48, 91-93, 121]. Modification and depletion of CL in various pathologies can be explained by impairments in the CL remodeling or activation of alternative pathological remodeling pathways that cause replacement of CL FA with more unsaturated ones, such as arachidonic acid or DHA [47]. The presence of four acyl groups in a CL molecule creates the possibility of a huge variety of stereochemically different CL molecules. This diversity can be further enhanced by the oxidation of individual FAs, resulting in the loss of symmetry even by the molecules initially containing identical acyl groups. It is quite possible that myriads of various asymmetric CL molecules are used as a special signaling language that ensures the "dialogue" between mitochondria and other intracellular or extracellular compartments [4, 5]. Recently, we have come to understanding that, in addition to their role as cell energy plants, mitochondria are important regulatory components involved in many cellular and extracellular functions – from metabolism coordination and cell death to immune response. Different CL variants are considered important signaling molecules. It seems intuitively obvious that the huge diversification of CL in eukaryotes in comparison to prokaryotes is a language of communication between mitochondria and other cell components. The biochemical principles of this language, including the meaning of its "words", are still waiting to be decoded. Most tissues have several basic CL variants. An unprecedentedly large variety (hundreds) of CL variations were found in the brain ([4, 5] and references therein). It was suggested that this diversity of CL is a mitochondrial language, especially important for ensuring coordination of complex brain functions.

Mulkidjanian et al. indirectly confirmed this hypothesis, noting that, if CL were only the object of oxidation rather than the origin of a signaling pathway, even random oxidation of one CL molecule in the cell by active ROS would ultimately lead to the elimination of the entire cell. They suggested that CL oxidation simultaneously serves as a signal that triggers a chain of anti-apoptotic reactions that unfold faster than the CL-mediated apoptotic cascade [29].

Changing the diet can only partially restore the FA profile of CL to its "normal" state, rich in FAs characteristic of a particular organ, and improve its function by counteracting consequences of pathological or insufficient CL remodeling. The most important area of anti-

aging medicine, therefore, is the development of mitochondria-targeted antioxidants that can reach CL not only *in vitro*, but also *in vivo*.

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