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Review

Biosynthesis, remodeling and turnover of mitochondrial cardiolipin

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ABSTRACT

Among mitochondrial lipids, cardiolipin occupies a unique place. It is the only phospholipid that is specific to mitochondria and although it is merely a minor component, accounting for 10-20% of the total phospholipid content, cardiolipin plays an important role in the molecular organization, and thus the function of the cristae. This review covers the formation of cardiolipin, a phospholipid dimer containing two phosphatidyl residues, and its assembly into mitochondrial membranes. While a large body of literature exists on this topic, the review focuses on papers that appeared in the past three years. This article is part of a Special Issue entitled: Lipids of Mitochondria edited by Guenther Daum

1. Introduction

Cardiolipin (CL, diphosphatidylglycerol) is a dimeric phospholipid that is specific to mitochondria and can thus be used to identify and quantify mitochondria in subcellular fractions. CL interacts with various proteins, which plays an important role in the lateral organization of mitochondrial membranes. The presence of CL in mitochondria and its involvement in mitochondrial function, are conserved in all eukaryotes, among them yeast, animals, protozoans, and plants. Even in plants, where energy metabolism does not primarily rely on mitochondria but on chloroplasts, deletion of cardiolipin synthase has detrimental consequences, a phenomenon probably related to increased oxidative stress [1].

The life of a CL molecule can be divided into 3 phases. The first is its biosynthesis in the inner mitochondrial membrane; the second is the remodeling of its fatty acids and its assembly into the inner membrane; and the third is its degradation and oxidative modification and its potential translocation out of the inner membrane. While the first step is relatively well established, the other two are still under scrutiny as their mechanisms are not fully understood and their functional implications are not very clear. A lot of effort has been spent on elucidating the mechanism and the function of the acyl remodeling of CL because deficient CL remodeling causes Barth syndrome. Recently, CL remodeling has also been discovered in bacteria [2]. Since several review articles have been published on this subject, the present paper has the character of an update, focusing mostly on the literature between 2013 and 2016.

2. Biosynthesis of CL

The biosynthetic pathway of CL has been established in the 1970s [3]; it takes place on the matrix face of the inner mitochondrial membrane [4]. The first step of this pathway is catalyzed by Tam41 [5], which was initially described as an activity required for the assembly and the maintenance of protein translocases [6,7]. Eventually, Tam41 was found to be essential for CL biosynthesis [8] and to have CDP-diacylglycerol synthase activity [5]. The second step is catalyzed by Pgs1 [9], an enzyme that forms phosphatidylglycerophosphate by transferring a phosphatidyl group from CDP-diacylglycerol to the sn-1 hydroxyl group of glycerol-3-phosphate. The conversion of an anhydride bond into an ester bond produces a large drop in free energy, which makes this reaction a suitable target for biological rate control. For instance, Pgs1 can be phosphorylated in yeast, which reduces its activity [10]. The third step on the CL pathway is catalyzed by Gep4 in yeast [11] or PTPMT1 in mammalian cells [12]. These enzymes remove the terminal phosphate group from phosphatidylglycerophosphate to form phosphatidylglycerol. The fourth and final step of CL biosynthesis is catalyzed by CL synthase (Crd1), which uses phosphatidylglycerol and CDP-diacylglycerol, to form CL. CL synthase was discovered in yeast [9,13,14] and later identified in humans [15–17] and plants [18,19]. The CL pathway is illustrated in Fig. 1.

The CL pathway is different in prokaryotes, where phosphatidyl transfer occurs from one phosphatidylglycerol molecule to another (for a review, see ref. [20]) or, as recently discovered, from phosphatidylethanolamine to phosphatidylglycerol [21]. A bacterial-type CL synthase is also present in some unicellular eukaryotes [22]. Not surprisingly, the CL pathway is regulated by PGC-1, the transcription factor that controls the biogenesis of mitochondria [23]. Another regulatory activity may be associated with ATP citrate lyase, a cytosolic

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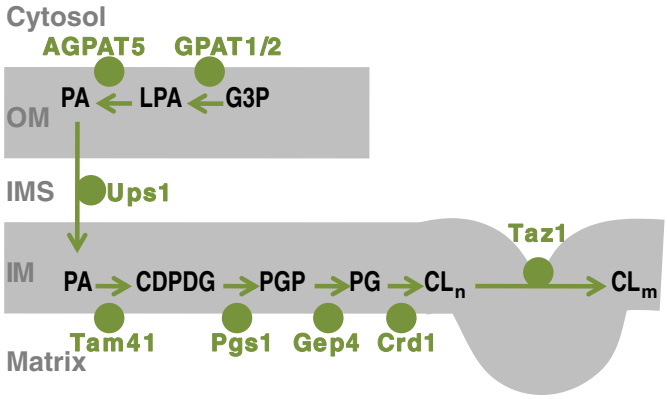

Fig. 1. Cardiolipin biosynthesis in mitochondria. Glycero-3-phosphate (G3P) is acylated to lysophosphatidic acid (LPA) and then to phosphatidic acid (PA). PA formation occurs on the outer face of the outer mitochondrial membrane (OM) and in the endoplasmic reticulum. PA is transferred from the outer to the inner mitochondrial membrane (IM) via the intermembrane space (IMS) and is converted to CL on the matrix face of the IM via the intermediates CDP-diacylglycerol (CDPDG), phosphatidylglycerophosphate (PGP), and phosphatidylglycerol (PG). The final step of the pathway is the remodeling of nascent cardiolipin (CL_n) to mature cardiolipin (CL_m). Remodeling occurs in the outer leaflet of the inner membrane and requires disturbance of the bilayer packing order. Mammalian genes are shown for the enzymes of PA synthesis and yeast genes are shown for all other enzymes.

enzyme that is placed at the junction between carbohydrate and lipid metabolism. It was proposed that the activation of this enzyme stimulates the formation of CL [24].

3. Remodeling of CL fatty acids

By definition, remodeling is a structural modification that follows the biosynthesis of CL. In the wake of this process, CL acquires a new set of fatty acids, which is organism and tissue specific. In some tissues, such as mammalian brain, the CL pattern is very diverse [25,26] but in others, especially in tissues with high energy turnover, CL contains surprisingly few molecular species, and those species are typically dominated by only one or two unsaturated acyl groups [27].

Remodeling is catalyzed by tafazzin, an enzyme that transfers fatty acids from phospholipids to lysophospholipids [28] (Fig. 1). However, tafazzin does so non-specifically, which does not provide an explanation for the fatty acid pattern observed in CL; tafazzin is simply a vehicle that allows fatty acids to exchange between phospholipid molecules. In order for tafazzin to produce a specific molecular pattern rich in tetralinoleoyl-CL (the dominant CL molecule of human heart), the free energy of tetralinoleoyl-CL must be lower than the free energy of other molecular species. Such energy differences are dependent on the packing properties of the entire lipid assembly. For instance, we have shown in vitro that negative curvature creates conditions that favor the formation of tetralinoleoyl-CL because the shape of this lipid naturally fits the geometry of negatively curved monolayers [29].

Recently, Abe et al. proposed that purified yeast tafazzin catalyzes acyl-specific transacylations in bilayers [30]. However, the claim that the reaction took place in rigid bilayers is contradicted by the fact that the medium contained up to 0.08% (1.28 mM) Triton X-100, which amounts to a 2-fold excess of the detergent over the phospholipids (0.59 mM). Furthermore, the data analysis (Guggenheim plot to determine pseudo-first order rate constants) did not carefully distinguish the properties of the enzyme (rate of catalysis) from the properties of the chemical equilibrium (concentration of substrate and products after completion of the reaction). This is more difficult for tafazzin than for many other enzymes because of the small difference in free energy between substrates and products. As the reaction approaches its equilibrium, the rates of forward and reverse reactions become similar and eventually identical, which causes an underestimation of the actual forward conversion between two time points. Essentially all acyl specificities observed by Abe et al. [30] can be explained by differences in the equilibrium composition, which basically confirms that the transacylation specificities are driven by the physical properties of the lipids rather than the enzyme. Finally, transgenic experiments have not supported the idea of intrinsic acyl specificity of tafazzin, because human tafazzin replicates the Drosophila-type CL pattern in flies and the yeast-type CL pattern in yeast [31,32], although these patterns are different from human CL and from each other.

Thus, when considering the tafazzin reaction in vivo, the question becomes what in the environment of the enzyme gives acyl specificity to the otherwise promiscuous acyl exchange? An unequivocal answer to this question will require further studies, but at this point it seems likely that the process that is driving CL remodeling is the assembly of the large protein complexes and supercomplexes of the inner mitochondrial membrane (see below).

A better understanding of CL remodeling will ultimately hinge on knowing the precise localization and the physical interactions of tafazzin. It has been shown that tafazzin is a non-integral membrane protein that resides on the outer face of the inner and perhaps also on the inner face of the outer mitochondrial membrane [33,34]. There it is associated with several protein complexes that range in size from roughly 100 to over 600 kDa [33–36]. Among its interaction partners are the ATP synthase and the ADP-ATP carrier but they hardly account for the entire spectrum [35]. In H9c2 cell, we found the half-life of tafazzin to be shorter than the half-life of other mitochondrial proteins implying that tafazzin is either a temporary resident of protein assemblies or the assemblies themselves are short-lived [36]. However, the half-life of tafazzin varies between cell types and is very sensitive to certain mutations [34]. Given these ambiguities, a simple narrative of the tafazzin function has remained elusive up to now.

4. Assembly of CL in protein complexes

CL strongly interacts with many different proteins. They constitute a long list (reviewed in ref. [37]) to which recently the uncoupling protein [38], multi-spanning proteins of the outer mitochondrial membrane [39], and the dynamin-related protein 1 [40,41] were added. Structural features of CL-binding proteins have been defined [42], but high affinity for CL is present in too many proteins to make a convincing argument for true specificity. Rather, the physical basis of this tight association seems to be rooted in the unique shape of CL and its charge state, which together produce a tendency to cluster and a tendency to form strong non-covalent bonds. Crucial herein is the large acyl moiety, which enforces hydrophobic interactions, and the small, motion-restricted head group, which leaves unshielded the two negatively charged phosphates and therefore increases their radius of interaction [43]. Studies in isolated mitochondria have shown that most if not all CL is associated with proteins [44,45]. In other words, mitochondrial membranes contain virtually no free CL in the bulk lipid phase.

Preliminary evidence has emerged for the involvement of protein complex assembly in the remodeling of CL. This applies specifically to three groups of proteins, namely (i) prohobitins, (ii) the MICOS complex, and (iii) the complexes of oxidative phosphorylation (OXPHOS).

(i) Prohibitins form large complexes in the inner mitochondrial membrane. They interact with other proteins and play a role in phospholipid homeostasis although their function has not been clearly defined [46]. Knockdown of prohibitin 2 in HEK 293 cells led to alterations in the molecular composition of CL [47]. Proteins that interact with prohibitins, such as DNAJC19 and MDM33, also affect CL [47,48].

(ii) MICOS stands for Mitochondrial Contact site and Cristae Organizing System. It comprises a large heterogeneous complex that localizes to cristae junctions and connects the inner with the outer mitochondrial membrane [49]. The MICOS complex plays a central role in both the

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