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Mitochondrial cardiolipin/phospholipid trafficking: The role of membrane contact site complexes and lipid transfer proteins

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ABSTRACT

Historically, cellular trafficking of lipids has received much less attention than protein trafficking, mostly because its biological importance was underestimated, involved sorting and translocation mechanisms were not known, and analytical tools were limiting. This has changed during the last decade, and we discuss here some progress made in respect to mitochondria and the trafficking of phospholipids, in particular cardiolipin. Different membrane contact site or junction complexes and putative lipid transfer proteins for intra- and intermembrane lipid translocation have been described, involving mitochondrial inner and outer membrane, and the adjacent membranes of the endoplasmic reticulum. An image emerges how cardiolipin precursors, remodeling intermediates, mature cardiolipin and its oxidation products could migrate between membranes, and how this trafficking is involved in cardiolipin biosynthesis and cell signaling events. Particular emphasis in this review is given to mitochondrial nucleoside diphosphate kinase D and mitochondrial creatine kinases, which emerge to have roles in both, membrane junction formation and lipid transfer.

1. Phospholipid/cardiolipin trafficking in mitochondria

Lipids are important for mitochondrial morphology and function, including bioenergetics, biosynthetic activities, and cell signaling (reviewed in Claypool and Koehler, 2011; Nunnari and Suomalainen, 2012; Schug et al., 2012; and this issue of CPL). However, while synthesis and trafficking of mitochondrial proteins has been described in much detail during the last two decades, much less is known in this respect about mitochondrial lipids. This is certainly due to the methodological difficulties of isolating and analyzing lipids, or labeling and manipulating their levels *in vivo*. Nevertheless, there has been recent progress in understanding some aspects of synthesis and trafficking of mitochondrial lipids, related in particular to three aspects:

(i) Some phospholipids are synthesized within mitochondria. While the major part of cellular lipid biosynthesis takes place in the ER, mitochondria also participate. For the mitochondrial synthesis of the non-bilayer phospholipids cardiolipin (CL; Fig. 1) and phosphatidylethanolamine (PE), all involved enzymes and mostly also their localizations are known now (reviewed in Claypool and Koehler, 2011; Horvath and Daum, 2013; Osman et al., 2011). Their precursors, phosphatidic acid (PA) and phosphatidylserine (PS), respectively, have to be imported from the ER, while the products CL and PE can again be exported to the mitochondrial surface and even the ER.
(ii) Mitochondrial lipids are heterogeneously distributed. Such heterogeneity exists between the mitochondrial inner (MIM) and outer membranes (MOM), but also between leaflets of a single membrane, thus creating strong asymmetries. This concerns in particular CL, which is highly enriched in MIM, in contrast to MOM and other cellular membranes that are virtually devoid of this phospholipid. Even more, the inner MIM leaflet, where CL biosynthesis proceeds, is thought to have a higher CL content as compared to the outer MIM leaflet. Breakdown

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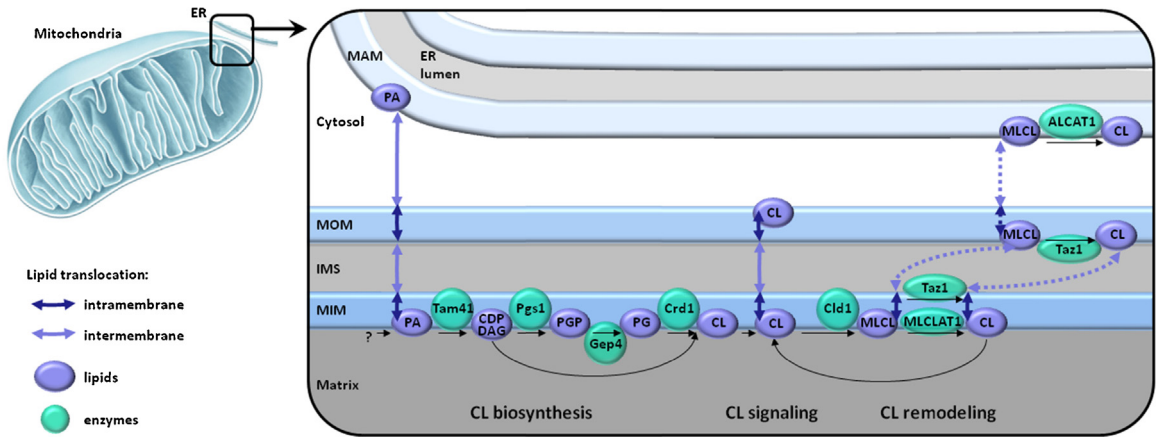

Fig. 1. Lipid trafficking linked to cardiolipin biosynthesis, signaling and remodeling. Biosynthesis of cardiolipin (CL), the roles of CL in signaling, and the CL remodeling process involve transfer of CL or intermediates between different membranes (Claypool and Koehler, 2011; Osman et al., 2011; Schlame, 2008). *Biosynthesis*: CL synthesis proceeds in the inner leaflet of the mitochondrial inner membrane (MIM). The CL-precursor phosphatidic acid (PA) is mainly synthesized in the ER and has to be imported from mitochondria-associated ER membranes (MAM) *via* the mitochondrial outer membrane (MOM) to this MIM inner leaflet by multiple inter- and intramembrane translocation steps. In mammals, some PA may be synthesized in mitochondria. The cytidinediphosphate-diacylglycerol (CDP-DAG) synthase Tam41 (translocator assembly and maintenance), the phosphatidylglycerolphosphate (PGP) synthase Pgs1 and the phosphatidylglycerophosphatase Gep4 (genetic interactors of prohibitins) then generate the intermediates CDP-DAG, PGP and phosphatidyl glycerol (PG), respectively, that will not accumulate under normal conditions. CL is produced in a final step from PG and CDP-DAG by the cardiolipin synthase Crd1. *Signaling*: CL and oxidized derivatives of CL traffic to the mitochondrial surface under conditions that induce mitophagy or apoptosis, respectively. This process, which includes one inter- and two intramembrane translocation steps, seems to participate in pro-mitophagic or -apoptotic signaling (Chu et al., 2013; Kagan et al., 2009; Schlattner et al., 2013). *Remodeling*: The nascent CL carries mostly saturated acyl chains and is symmetric (carries the same acyl chaines on its two glycerol groups). Acyl chains are exchanged in a process called CL remodeling, which is initiated by the cardiolipin-specific deacylase Cld1 that removes an acyl chain from CL, generating monolyso-CL (MLCL). This intermediate can be used in different remodeling pathways by transacylation reactions, the by far most important being catalyzed in MIM by taffazin (Taz1; probably in the outer leaflet), and less so by MLCL acyltransferase 1 (MLCLAT1; probably in the inner leaflet). However, MLCL seems to be able to traffic also to MOM and MAM, where Taz1 and acyl-CoA lysocardiolipin acyltransferase ALCAT1, respectively, also catalyze acyl transfer to MLCL. Such ALCAT1 remodeling of CL seems to occur under pathological conditions, generating "bad" CL containing C22:6 acyl chains. Except for MLCLAT1 and ALCAT1, the specific yeast enzymes are indicated.

of these asymmetries, as observed during apoptosis, emerges as a potential signaling event (Chu et al., 2013).

(iii) Mitochondrial lipids can be secondarily modified. Again, CL is a good example, since modifications concern its acyl chain composition (by a process called CL remodeling; Fig. 1 (Schlame and Ren, 2009; Yang et al., 2012)) as well as oxidative modifications of unsaturated acyl chains (Korytowski et al., 2011; Samhan-Arias et al., 2012). These modifications may not only provide specific functions for the lipid bilayer and protein interactions or be a simple product of oxidative stress, respectively. In particular oxidative modifications may again provide specific signals (Kagan et al., 2005).

Common to these properties is the need of phospholipids to travel between specific cellular membranes and also between leaflets within these membranes. This issue has much less been studied in mitochondria, in contrast to other cellular compartments. Only recently it emerged that formation of contact sites between adjacent membranes, MIM, MOM and mitochondria-associated membranes (MAM) of the ER are a prerequisite and possibly even sufficient for intermembrane lipid transfer (reviewed in Helle et al., 2013; Kornmann, 2013; Michel and Kornmann, 2012). In addition, first proteins in the mitochondrial intermembrane space (IMS) have been identified that could play a role as specialized lipid transfer proteins (reviewed in Tatsuta et al., 2014). We will review these issues in the following, with an emphasis on the trafficking of CL within the CL life cycle, comprising biosynthesis, remodeling, and signaling functions (Fig. 1).

2. Intermembrane lipid transfer

Lipid transfer involving mitochondrial membranes does not seem to occur *via* vesicle trafficking, although budding of vesicles at the mitochondrial surface has been observed (reviewed in Soubannier et al., 2012) and the inverse fusion of cytosolic vesicles with MOM cannot be excluded. For effective phospholipid transfer, rather other pathways have to be considered (Fig. 2A and B). Simple diffusion between membranes is much too slow, but different factors could accelerate such non-protein assisted transfer.

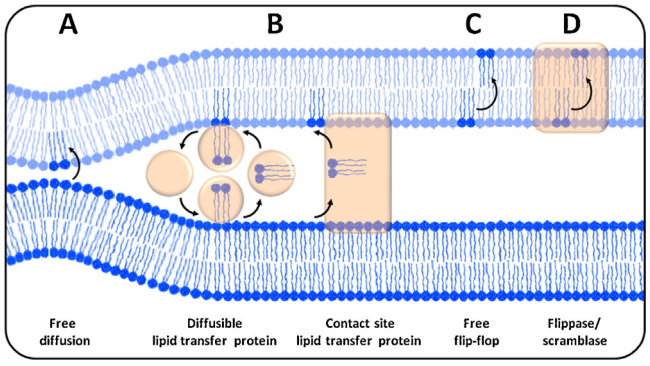

Fig. 2. Potential lipid/cardiolipin transfer mechanisms in mitochondria. (A) Transmembrane transfer of monomeric lipid/CL *via* free diffusion is very slow (half-times in the order of days). This may be accelerated when membranes come very close, when aqueous phase solubility is increased (as *e.g.* when acyl chains are removed like in mono lyso-CL), or membranes fuse and/or create inverted micelles with hexagonic phases allowing lipid/CL to diffuse between MIM and MOM leaflets (Lev, 2010). (B) Lipid transfer proteins (center, orange) can greatly facilitate this process by both lipid/CL extracting/inserting and carrier activities (Lev, 2010). It is conceivable that small proteins with hydrophobic cavity, as known in other cellular compartments, shuttle lipids, *e.g.* between MIM and MOM. They could be freely diffusible or anchored *via* a flexible domain in the MIM as proposed for the PRELI family (Potting et al., 2013). Alternatively, proteins that are known to crosslink MIM-MOM or MOM-MAM could provide transfer routes as has been proposed for NDPK-D/Nm23-H4 (Schlattner et al., 2013). (C) Intra-membrane lipid transfer between the two leaflets is also rather slow when occurring spontaneously. (D) This could be again accelerated by specialized enzymes like scramblase (mixing between leaflets, ATP-independent) or flippases (directed transfer, mostly ATP-dependent). Alternatively, lipid flip-flop could possibly occur as a side-process with different transmembrane proteins.