

How lipids modulate mitochondrial protein import

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Abstract Mitochondria have to import the vast majority of their proteins, which are synthesized as precursors on cytosolic ribosomes. The translocase of the outer membrane (TOM complex) forms the general entry gate for the precursor proteins, which are subsequently sorted by protein machineries into the mitochondrial subcompartments: the outer and inner membrane, the intermembrane space and the mitochondrial matrix. The transport across and into the inner membrane is driven by the membrane potential, which is generated by the respiratory chain. Recent studies revealed that the lipid composition of mitochondrial membranes is important for the biogenesis of mitochondrial proteins. Cardiolipin and phosphatidylethanolamine exhibit unexpectedly specific functions for the activity of distinct protein translocases. Both phospholipids are required for full activity of respiratory chain complexes and thus to maintain the membrane potential for protein import. In addition, cardiolipin is required to maintain structural integrity of mitochondrial protein translocases. Finally, the low sterol content in the mitochondrial outer membrane may contribute to the targeting of some outer membrane proteins with a single α -helical membrane anchor. Altogether, mitochondrial lipids modulate protein import on various levels involving precursor targeting, membrane potential generation, stability and activity of protein translocases.

Keywords Mitochondria · Protein import · Cardiolipin · Phosphatidylethanolamine

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Introduction

Mitochondria fulfill crucial functions in the eukaryotic cell like ATP production via oxidative phosphorylation, synthesis of lipids, amino acids, heme and iron-sulfur clusters and act as a signaling platform for apoptosis. Mitochondrial functions rely on the presence of about 1000 (yeast) or 1500 (human) different proteins (Reinders et al. 2006; Pagliarini et al. 2008). The vast majority of these proteins is nucleus-encoded, translated on cytosolic ribosomes as precursors and imported into the organelle in a posttranslational manner. Specific targeting signals direct the precursors to the mitochondrial surface. During their journey through the cytosol the precursor proteins are kept in an unfolded state by cytosolic chaperones (Neupert and Herrmann 2007; Chacinska et al. 2009). The translocase of the outer membrane (TOM complex) forms the general entry gate for most of the mitochondrial precursor proteins. The TOM complex consists of the pore-forming Tom40, the receptor proteins Tom20, Tom22 and Tom70 and three small Tom proteins, which are required for stability and assembly of the TOM machinery. After passage through the TOM channel specific protein machineries sort the precursors into the mitochondrial subcompartments: outer and inner membrane, intermembrane space and matrix (Fig. 1) (Koehler 2004; Dolezal et al. 2006; Neupert and Herrmann 2007; Chacinska et al. 2009; Endo and Yamano 2009). The import pathways and mechanisms were studied in baker's yeast *Saccharomyces cerevisiae*, but are largely conserved in human mitochondria. Recent studies indicate that the mitochondrial protein machineries are integrated into a sophisticated protein network (Becker et al. 2012). The protein translocases not only directly cooperate with each other to channel precursor proteins, but are also linked to distinct protein complexes like the respiratory chain, the mitochondrial contact site and cristae organizing system (MICOS) and the endoplasmic reticulum-mitochondria encounter structure (ERMES) (van der Laan et al. 2006; Meisinger et al. 2007; von der Malsburg et al. 2011; Harner et al. 2011a). In addition, protein import via the TOM complex is regulated by phosphorylation and thereby closely connected

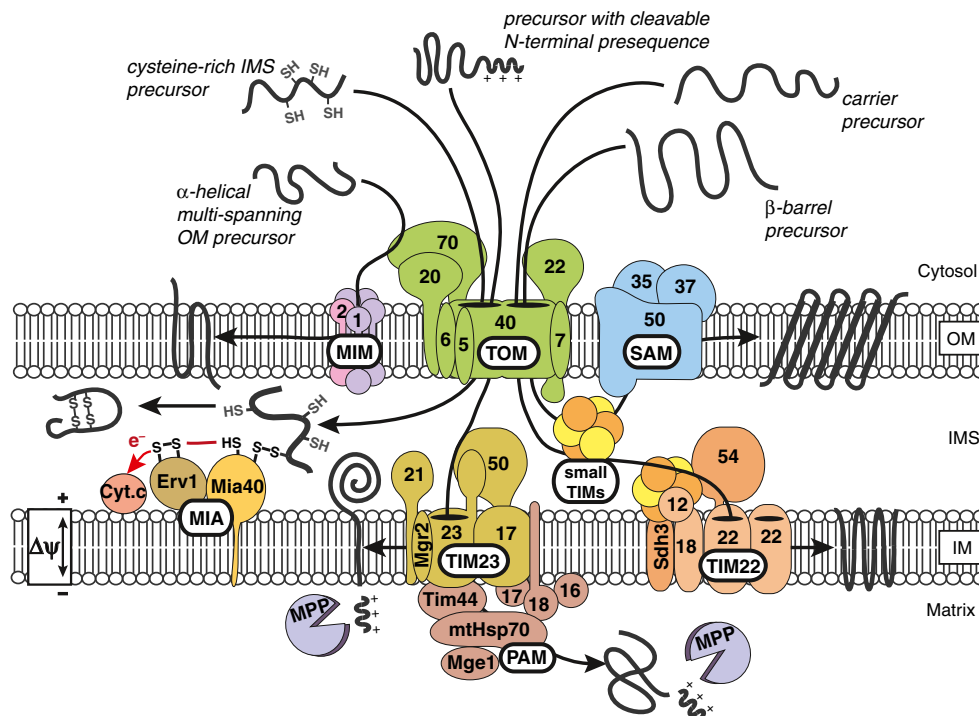


Fig. 1 Five pathways sort mitochondrial precursor proteins into the mitochondrial subcompartments. Precursors of multi-spanning outer membrane proteins are recognized by Tom70 and transferred to the MIM machinery, which mediates their integration and assembly into the membrane. The TOM complex forms the general entry gate for most of the other mitochondrial precursors. After passage through the Tom40 channel four sorting pathways branch off. β -barrel precursors are directly transferred to the SAM complex, which mediates their integration into the outer membrane (OM). During the transport small TIM chaperones of the intermembrane space (IMS) shield hydrophobic segments of the β -barrel precursors. Precursors with a cleavable presequence are transferred to the TIM23 complex, which mediates

either lateral release into the inner membrane (IM) or transport into the mitochondrial matrix. Precursor translocation via the TIM23 complex into the matrix additionally requires the ATP-consuming action of the PAM module. The presequence is removed upon import by the mitochondrial processing peptidase (MPP). The small TIM chaperones guide precursors of multi-spanning carrier proteins to the TIM22 complex, which facilitates their insertion into the inner membrane. The membrane potential across the inner membrane ($\Delta\psi$) drives TIM23 and TIM22 dependent import. Mia40 sorts cysteine-rich precursors into the intermembrane space and mediates their oxidative folding. Erv1 shuttles the electrons from Mia40 to cytochrome *c* of the respiratory chain

to the metabolic state of the cell (Schmidt et al. 2011; Gerbeth et al. 2013). These observations indicate that various processes can modulate mitochondrial protein import on different levels. Studies of the last years demonstrated that protein biogenesis is not only influenced by protein interactions and modifications, but also by the lipid composition of mitochondrial membranes. The role of mitochondrial lipids for protein biogenesis was underestimated for long time. We are just beginning to understand how the lipid composition affects the membrane-bound protein translocases. Recent studies revealed unexpectedly specific roles of certain lipids for the activity and stability of mitochondrial protein complexes.

Mitochondrial protein import

Protein sorting into the mitochondrial outer membrane

Proteins are integrated into the outer mitochondrial membrane by α -helical or β -barrel domains. These two types of

transmembrane proteins follow different import pathways. Precursors of β -barrel proteins are translocated through the TOM channel and transferred with the help of the small TIM chaperones to the sorting and assembly machinery (SAM complex; Fig. 1) (Hoppins and Nargang 2004; Wiedemann et al. 2004). TOM and SAM form a supercomplex to promote efficient biogenesis of β -barrel precursors (Qiu et al. 2013). The SAM complex mediates β -barrel folding and insertion into the outer membrane (Paschen et al. 2003; Ishikawa et al. 2004; Qiu et al. 2013). It consists of the essential β -barrel protein Sam50 (also known as Tob55), which forms the core of the protein translocase. Sam50 associates with the two peripheral subunits Sam35 (Tob38) and Sam37 (Mas37) on the cytosolic surface (Wiedemann et al. 2003; Paschen et al. 2003; Gentle et al. 2004; Klein et al. 2012). While Sam35 has been implicated in promoting precursor recognition, Sam37 was reported to mediate the release of the β -barrel precursor into the outer membrane (Chan and Lithgow 2008; Kutik et al. 2008a).

Other outer membrane proteins contain a single (single-spanning) or several (multi-spanning) transmembrane α -

helices. Precursors of multi-spanning outer membrane proteins are recognized by the receptor protein Tom70 of the TOM complex, but are not translocated through the TOM channel (Otera et al. 2007; Becker et al. 2011; Papic et al. 2011; Wenz et al. 2014). Instead, the mitochondrial import machinery (MIM complex; Fig. 1) mediates membrane insertion and assembly of these precursors (Becker et al. 2011; Papic et al. 2011; Dimmer et al. 2012). The MIM complex consists of several copies of Mim1 and the recently identified Mim2 (Becker et al. 2008; Hulett et al. 2008; Popov-Čeleketić et al. 2008; Dimmer et al. 2012). In contrast, no common import pathway for the precursors of single-spanning α -helical outer membrane proteins was found. A role of TOM, SAM and MIM complexes in the import of different precursors was reported (Ott et al. 2007; Stojanovski et al. 2007; Becker et al. 2008; Hulett et al. 2008; Popov-Čeleketić et al. 2008; Thornton et al. 2010; Harner et al. 2011b). Particularly interesting is the biogenesis of the outer membrane protein of 45 kDa (Om45), which is embedded by an N-terminal α -helix and exposes a large soluble domain into the intermembrane space (Lauffer et al. 2012; Song et al. 2014; Wenz et al. 2014). The biogenesis of the Om45 precursor involves three protein translocases: TOM and TIM23 complexes drive the translocation of the soluble domain, while the MIM machinery assembles the precursor into the outer membrane (Song et al. 2014; Wenz et al. 2014). Finally, the insertion of a few α -helical outer membrane proteins was reported to occur in a protein-independent manner (Meineke et al. 2008; Kemper et al. 2008).

Protein sorting into inner mitochondrial compartments

The majority of mitochondrial precursors contain an N-terminal cleavable presequence, which is removed upon import by the mitochondrial processing peptidase (MPP). The receptor proteins Tom20 and Tom22 recognize this type of precursors at the mitochondrial surface (Yamano et al. 2008). After passage through the TOM channel the precursors are directly transferred to the presequence translocase (TIM23 complex; Fig. 1) (Chacinska et al. 2003). The highly dynamic TIM23 complex mediates the sorting of the precursors either into the mitochondrial matrix or into the inner membrane. The intermembrane space domains of Tim23, Tim50 and Tim21 are involved in the interaction with the TOM complex and in precursor recognition (Chacinska et al. 2005; Tamura et al. 2009a; Mokranjac et al. 2009; Marom et al. 2011; Shiota et al. 2011; Schulz et al. 2011; Lytovchenko et al. 2013). Tim23 forms a voltage-sensitive channel, which can be closed by Tim50 to prevent proton leakage (Meinecke et al. 2006; Alder et al. 2008). Tim17 plays a role in motor recruitment and the pore architecture of the TIM23 channel (Chacinska et al. 2005; Martinez-Caballero et al. 2007). The membrane potential drives transport of the positively charged

presequence through the TIM23 channel. This step involves activation of the TIM23 channel through membrane-potential mediated conformational changes (Malhotra et al. 2013). Tim21 associates with respiratory chain complexes and thereby couples protein import directly to generation of the membrane potential (van der Laan et al. 2006). The recently identified subunit Mgr2 stabilizes the binding of Tim21 to the TIM23 complex (Gebert et al. 2012). Inner membrane precursors additionally contain an internal hydrophobic sorting sequence, which stops translocation and induces lateral insertion of the preprotein into the membrane. Presequence-containing precursors that have to be fully transported into the mitochondrial matrix require an additional driving force, which is provided by the presequence translocase-associated motor (PAM; Fig. 1) (Neupert and Herrmann 2007; Chacinska et al. 2009). The core component of this import motor is the mitochondrial Hsp70 (mtHsp70) that binds to the TIM23 complex via Tim44. The chaperone facilitates precursor translocation into the matrix by multiple cycles of ATP-dependent precursor binding and release. Thereby, mtHsp70 is alternating between the ADP-bound state with high affinity and the ATP-bound state with low affinity for incoming precursor proteins. This mtHsp70 cycle is controlled by the interaction with several co-factors within the PAM module. The J-domain of Pam18 (Tim14) enhances the ATPase activity of mtHsp70, whereas the nucleotide exchange factor Mge1 stimulates the ADP/ATP exchange of the chaperone. Pam18 forms a stable heterodimer with Pam16 (Tim16), a J-like protein that exhibits an inhibitory role on Pam18 function (Li et al. 2004; Mokranjac et al. 2006). The non-essential Pam17 promotes the association of the Pam16/Pam18 module with the presequence translocase (van der Laan et al. 2005).

Precursors of multi-spanning inner membrane proteins lacking a cleavable presequence are escorted by cytosolic chaperones to the mitochondrial surface, where they are recognized by the Tom70 receptor. After passage through the TOM channel the small TIM chaperones Tim9/Tim10 guide the precursors through the aqueous intermembrane space to the carrier translocase (TIM22 complex; Fig. 1). Tim9 and Tim10 form a hexameric complex, which assembles with Tim12 at the carrier translocase (Lionaki et al. 2008; Gebert et al. 2008). The soluble domain of Tim54 of the TIM22 complex might provide a docking site for the small TIM chaperones (Kerscher et al. 1997). Tim22 is the central component of the carrier translocase and forms a voltage-activated twin-pore in the inner membrane (Rehling et al. 2003; Peixoto et al. 2007). The Tim22 channel facilitates membrane insertion of the precursor proteins in a membrane potential-dependent manner. The subunits Tim18 and Sdh3 play a role in the stability and assembly of the carrier translocase (Wagner et al. 2008; Gebert et al. 2011; Okamoto et al. 2014). Interestingly, Sdh3 is also present in complex II (succinate dehydrogenase) of the respiratory chain, providing an evolutionary link

between inner membrane protein sorting and respiration (Gebert et al. 2011).

Precursor proteins with a cysteine-rich motif are transferred after passage of the TOM channel to Mia40, the central component of the mitochondrial intermembrane space import and assembly machinery (MIA; Fig. 1). Mia40 acts as an oxidoreductase to drive oxidative folding by the introduction of disulfide-bridges within its substrates (Banci et al. 2009; Kawano et al. 2009). Mia40 itself is re-oxidized by the sulfhydryl oxidase Erv1 (essential for respiration and viability), which shuttles the electrons towards cytochrome *c* of the respiratory chain (Mesecke et al. 2005; Bihlmaier et al. 2007; Tienson et al. 2009; Böttinger et al. 2012a).

Biosynthesis and function of mitochondrial lipids

The major phospholipids of mitochondrial membranes are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and the dimeric phospholipid cardiolipin (CL). Sterols like ergosterol in yeast and sphingolipids are only present in minor amounts (van Meer et al. 2008; Horvath and Daum 2013). The signature phospholipid of mitochondria CL is unequally distributed between both mitochondrial membranes. Whereas the mitochondrial inner membrane shows especially high levels of CL, the outer membrane only contains about one quarter of the total mitochondrial CL pool (Gebert et al. 2009). Formation of mitochondrial membranes depends on the import of PC, PS and PI, which are generated in the endoplasmic reticulum. In yeast, the ERMES complex forms a molecular tether between the mitochondrial outer membrane and the endoplasmic reticulum, which may facilitate exchange of lipids between both organelles (Kornmann et al. 2009). Mitochondria are capable of synthesizing CL and PE autonomously (Fig. 2). For PE synthesis, PS is decarboxylated by the phosphatidylserine decarboxylase 1 (Psd1) of the mitochondrial inner membrane (Voelker 1997; Osman et al. 2011; Horvath et al. 2012). Although other biosynthesis pathways exist, the mitochondrially generated PE represents the major fraction of the total PE pool in the yeast cell (Bürgermeister et al. 2004). The biosynthesis

of CL comprises a multistep reaction that has been studied in detail (Gohil and Greenberg 2009; Osman et al. 2011; Claypool and Koehler 2012; Horvath and Daum 2013). The CL-precursor phosphatidic acid (PA) is imported from the ER into the mitochondrial outer membrane and further transported via Ups1 (unprocessed 1) across the intermembrane space to the inner mitochondrial membrane (Connerth et al. 2012). Subsequently, Tam41 (translocator assembly and maintenance) converts PA to cytidine diphosphate-diacylglycerol (CDP-DAG) (Tamura et al. 2013), which is used for the generation of phosphatidylglycerolphosphate (PGP) by Pgs1 (PGP synthase). Gep4 dephosphorylates PGP to phosphatidylglycerol (PG), and the cardiolipin synthase (Crd1) catalyzes the formation of CL from PG and CDP-DAG. Finally, the premature CL undergoes remodeling of its acyl-chains involving two enzymes, the CL-specific deacylase Cld1 and the transacylase taffazin (Taz1) (Fig. 2) (Osman et al. 2011; Horvath and Daum 2013).

CL and PE are both so-called non-bilayer forming phospholipids. They are characterized by a relatively small diameter of their head group and a comparably large diameter of their hydrophobic fatty acid tails. Depending on pH and cation concentration, non-bilayer forming phospholipids have the tendency to form local hexagonal phase structures, which can induce an increase of membrane tension and thus substantially influence the properties and architecture of the lipid bilayer (van den Brink-van der Laan et al. 2004; Osman et al. 2011). Consequently, non-bilayer forming phospholipids have been implicated in a multitude of crucial processes such as membrane fusion, transbilayer transport of lipids as well as folding and transport of proteins (van den Brink-van der Laan et al. 2004; Dowhan and Bogdanov 2009). It has been shown that simultaneous deletion of mitochondrial CL and PE synthases is synthetically lethal for the cell, implicating overlapping functions of the two phospholipids (Gohil et al. 2005). Indeed, yeast mitochondria defective in respiration are more frequently developed when PE and CL levels are reduced (Birmer et al. 2003; Zhong et al. 2004) indicating that CL and PE are important for mitochondrial functions. Simultaneous depletion of both CL and PE synthases affects mitochondrial morphology, which is likely connected to defects of the mitochondrial fusion machinery (DeVay et al. 2009; Joshi

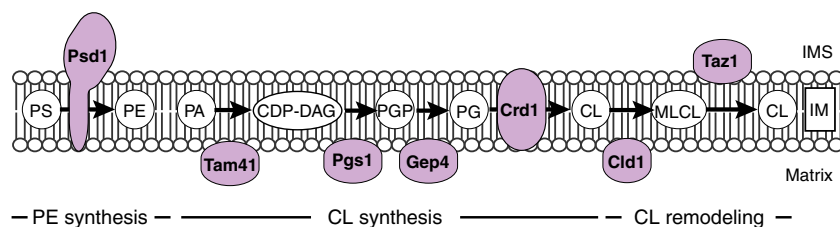


Fig. 2 Mitochondrial phospholipid biogenesis in yeast. Mitochondria synthesize cardiolipin (CL) and phosphatidylethanolamine (PE) in the inner membrane (IM). Psd1 decarboxylates phosphatidylserine (PS) to

produce PE. The synthesis of CL involves several steps and is followed by alterations of the acyl-chain composition in a process termed CL remodeling. For details see text. *IMS* intermembrane space

et al. 2012). Furthermore, a genetic interaction of both CL and PE synthases with prohibitins has been detected (Bimer et al. 2003; Osman et al. 2009). Prohibitins have been implicated in regulating inner mitochondrial membrane organization by functioning as protein and lipid scaffolds. A recent study reported a role of prohibitins in the remodeling of CL (Richter-Dennerlein et al. 2014). Finally, CL is required for apoptosis, mitophagy and iron-sulfur cluster biogenesis (Gonzalvez et al. 2008; Chu et al. 2013; Patil et al. 2013). Altogether, phospholipids are important for the maintenance of various mitochondrial functions and thus for cell survival.

Role of lipids in protein import

The sorting of precursor proteins into mitochondria involves the action of several membrane-embedded protein transport machineries (Fig. 1). Numerous studies helped to elucidate the composition and function of these translocases, yet the impact of the lipid composition of the mitochondrial membranes on protein sorting was for long time only poorly understood. Recent studies revealed a remarkable importance of certain lipids for specific protein import routes, in which the best-studied lipids are CL and PE.

Outer membrane protein sorting

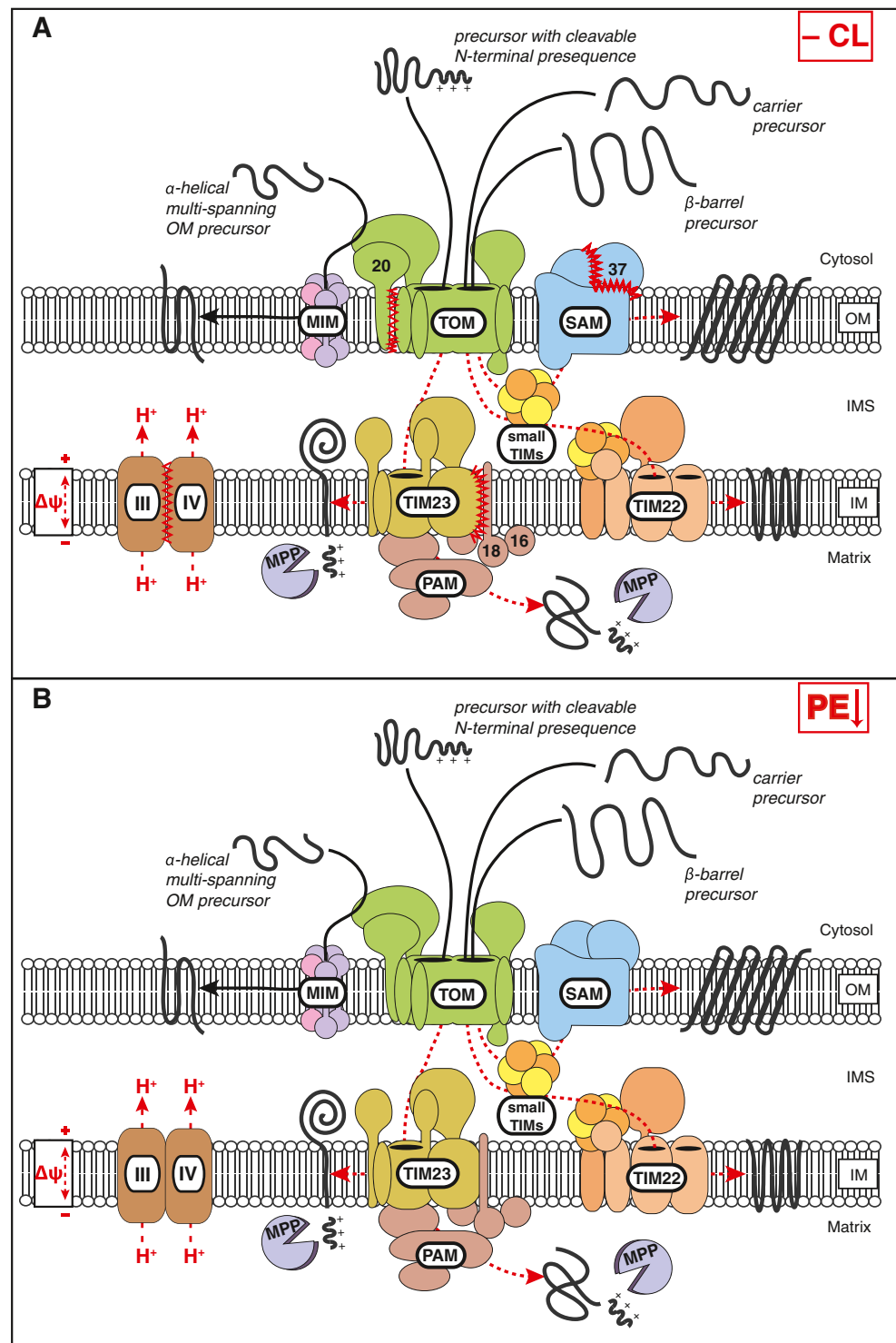
The biogenesis of β -barrel precursors is sensitive towards alterations of the phospholipid composition of the mitochondrial outer membrane. Particularly, CL and PE are important for efficient import of β -barrel precursors. In CL- and PE-deficient mitochondria early biogenesis steps are impaired (Gebert et al. 2009; Becker et al. 2013). Experimental dissection of the import pathway in PE-depleted mitochondria revealed that already translocation of the β -barrel precursors across the outer membrane is reduced (Becker et al. 2013). The observed import defect for β -barrel precursors in the phospholipid mutants can be partially explained by a decreased activity of the TOM complex. Supporting this view the binding of an arrested precursor to the TOM complex is impaired in both CL- and PE-deficient mitochondria (Gebert et al. 2009; Becker et al. 2013). Moreover, a reconstituted TOM complex requires the lipid composition of the outer mitochondrial membrane for full activity in liposomes (Stan et al. 2000). The molecular role of CL and PE for the function of the TOM complex differs. In the absence of CL the association of the Tom20 receptor with the core of the TOM complex is weakened (Fig. 3) (Gebert et al. 2009). In contrast, depletion of PE does not affect the stability of the TOM complex (Becker et al. 2013). The impaired TOM function contributes to the reduced precursor binding to the SAM complex in mitochondria with decreased PE and CL content (Gebert et al. 2009; Becker et al. 2013). Since CL stabilizes the

association of the peripheral subunit Sam37 to Sam50/Sam35 the lipid composition is important for the integrity of this protein translocase (Fig. 3) (Gebert et al. 2009). However, the function of the SAM complex is not generally compromised in CL- or PE-deficient mitochondria, since the assembly of the α -helically anchored Tom22 is not affected (Gebert et al. 2009; Becker et al. 2013). The assembly of the Tom22 precursor with other TOM subunits involves all three SAM components (Stojanovski et al. 2007). The biogenesis of β -barrel precursors is also affected in mitochondria lacking the transacylase Taz1 involved in CL remodeling (Gebert et al. 2009). Impaired function of yeast Taz1 or of its human homolog taffazin causes an altered acyl composition of CL (Ye et al., 2014). Mutations in the human homolog taffazin lead to Barth syndrome, a severe disease that is characterized by cardiac and skeletal myopathies as well as neutropenia (Barth et al. 1983; Claypool et al. 2006). Interestingly, the assembly of β -barrel precursors is impaired in mitochondria isolated from Barth syndrome patients (Gebert et al. 2009). Thus, the phospholipid composition of mitochondrial membranes affects import of β -barrel precursors. Since biogenesis of the β -barrel protein Tom40 is essential for cell survival, the impaired β -barrel biogenesis likely contributes to the synthetically lethal phenotype of the double deletion of the mitochondrial CL and PE synthases (Gohil et al. 2005).

Interestingly, there is a close link between outer membrane protein biogenesis and the ERMES complex, which is important for the maintenance of normal CL and PE levels (Osman et al. 2009; Kornmann et al. 2009). Mitochondria of ERMES mutants are defective in the biogenesis of β -barrel proteins (Meisinger et al. 2007; Wideman et al. 2010). Moreover, one of the core components of the ERMES complex, Mdm10 (mitochondrial distribution and morphology), associates with the SAM complex to mediate the assembly of the TOM complex (Meisinger et al. 2007; Thornton et al. 2010). Thus, Mdm10 provides a molecular link between ERMES and outer membrane protein biogenesis.

In contrast to β -barrel proteins, the biogenesis of outer membrane proteins with α -helical membrane anchors is largely unaffected in mitochondria with defective PL or CL synthesis (Gebert et al. 2009; Becker et al. 2013). However, recent reports indicate an important role of the low ergosterol content in yeast mitochondrial membranes for the targeting of some outer membrane proteins with a C-terminal α -helical membrane anchor (tail-anchored) (Kemper et al. 2008; Krumpe et al. 2012). First, in yeast mutants where the ergosterol content between mitochondria and ER membranes is comparable mitochondrial tail-anchored proteins mislocalize to the ER (Krumpe et al. 2012). Second, an increasing ergosterol content inhibits the insertion of a mitochondrial tail-anchored precursor into liposomes (Kemper et al. 2008). These studies imply a role of the lipid content of the mitochondrial outer membrane in protein targeting.

Fig. 3 Cardiolipin (CL) and phosphatidylethanolamine (PE) modulate different steps of mitochondrial protein biogenesis. In the absence of CL (–CL) or upon reduction of PE levels (PE↓), the binding of precursor proteins to the TOM complex is affected. Consequently, the TOM-dependent biogenesis of β -barrel proteins is impaired. In contrast, TOM-independent import of multi-spanning outer membrane (OM) proteins is not disturbed by the altered lipid composition. The activity of the respiratory chain complexes III and IV is decreased, which causes a reduced membrane potential ($\Delta\psi$) and impaired protein import across or into the inner membrane (IM). Deletion of CL affects the association of Tom20, Sam37 and Pam16/Pam18 with TOM, SAM and TIM23 complexes. In addition, the stability of the respiratory chain supercomplexes is decreased. Transport defects are indicated by *dashed red lines*, whereas disturbances of protein complex stability is indicated by *jagged red lines*



Inner membrane protein sorting

Protein transport into and across the mitochondrial inner membrane by TIM23 and TIM22 complexes essentially requires the presence of a membrane potential. The membrane potential is generated by the activity of the respiratory chain

complexes, which form supercomplexes in the mitochondrial inner membrane (Schägger and Pfeiffer 2000; Cruciat et al. 2000). Structural investigations on cytochrome *c* reductase (complex III) and cytochrome *c* oxidase (complex IV) revealed that, among other phospholipids, CL and PE specifically bind to both complexes (Lange et al. 2001; Shinzawa-

Itoh et al. 2007). In addition, the activities of complex III and in particular of complex IV depend on the presence of CL and PE (Pfeiffer et al. 2003; Zhong et al. 2004; Wenz et al. 2009; Böttinger et al. 2012b; Tasseva et al. 2013). Yet, both phospholipids might affect the respiratory activity via different mechanisms. CL stabilizes the association of complex III and complex IV within the respiratory chain supercomplexes (Fig. 3) (Zhang et al. 2002; Zhang et al. 2005; Wenz et al. 2009; Bazán et al. 2013). The negatively charged head group of CL was shown to facilitate the interaction of complex III and complex IV (Wenz et al. 2009). In contrast, the role of PE in the stability of respiratory chain complexes is less clear. In mammalian mitochondria, the depletion of PE causes decreased levels of respiratory supercomplexes (Tasseva et al. 2013), whereas in PE-deficient yeast mitochondria higher oligomeric assemblies comprising complexes III and IV can be detected (Böttinger et al. 2012b). Also mitochondria from a *taz1* deletion yeast mutant and from Barth syndrome patients show defective assembly and reduced stability of respiratory chain supercomplexes (Brandner et al. 2005; McKenzie et al. 2006). Altogether, the phospholipid composition of the mitochondrial inner membrane is crucial for the activity of the respiratory chain.

The impaired activity of the respiratory chain complexes causes a reduced membrane potential in CL- or PE-deficient mitochondria. Consequently, the membrane potential-dependent import pathways via the TIM23 and TIM22 complexes are impaired (Fig. 3) (Jiang et al. 2000; Tamura et al. 2006; Gallas et al. 2006; Kutik et al. 2008b; Tamura et al. 2009b). Additionally, the presence of CL is important for the stability of the TIM23 complex. In particular, the association of the Pam16/Pam18 module to the TIM23 complex is affected in CL-deficient mitochondria (Fig. 3) (Tamura et al. 2006; Gallas et al. 2006; Kutik et al. 2008b; Tamura et al. 2009b). Oligomerization of the model precursor ADP/ATP carrier (AAC) and its interaction with respiratory chain complexes depend on the presence of CL (Claypool et al. 2008; Kutik et al. 2008a; Tamura et al. 2009b). In contrast, the oligomerization of the AAC precursor and the integrity of the TIM23 complex remain largely unaffected upon reduction of the PE level (Böttinger et al. 2012b). Altogether, both non-bilayer forming phospholipids CL and PE are crucial for the membrane potential-dependent protein import into and across the inner membrane. In addition, deletion of CL affects stability and assembly of inner membrane protein complexes.

Conclusions

In the last years several studies indicated that the lipid bilayer of biological membranes is much more than a hydrophobic border between cellular compartments. Individual lipids modulate the

function of a versatile set of mitochondrial proteins and thereby affect central mitochondrial processes like the import of precursor proteins. Unexpectedly, recent studies revealed that both CL and PE exert specific roles for the functionality of several protein translocases. The TOM complex depends on both phospholipids for optimal function. Furthermore, membrane potential-dependent import across and into the inner membrane is affected in CL- and PE-depleted mitochondria, since both phospholipids are required for full respiratory activity. However, the molecular mechanisms by which CL and PE promote the functionality of distinct protein complexes appear to be different. While CL seems to confer stability to multimeric membrane complexes, depletion of PE does not affect the formation of protein complexes. Thus, the non-bilayer forming character of CL and PE cannot be the only important property modulating protein import machineries. Therefore, it will be important to analyze whether bilayer forming phospholipids such as PC, PS or PI play a role for activity and stability of protein transport machineries and for maintenance of the membrane potential. In addition, structural information on the membrane-embedded protein translocases will likely provide important insights how phospholipids are bound to protein complexes to modulate their activities. Moreover, studies on cellular ergosterol content revealed that the lipid composition affects protein targeting to the mitochondrial outer membrane (Krumpe et al. 2012). Future studies will have to elucidate how the specific lipid composition of membranes is recognized by the incoming precursor to warrant proper targeting. Although we are just at the beginning to understand the role of lipids in mitochondrial protein biogenesis, the first studies already revealed fascinating insights into the specific modulation of protein biogenesis steps by lipids.

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