Abstract: Mitochondria import about 1000 precursor proteins from the cytosol. The translocase of the outer membrane (TOM complex) forms the major entry site for precursor proteins. Subsequently, membrane-bound protein translocases sort the precursor proteins into the outer and inner membrane, the intermembrane space, and the matrix. The phospholipid composition of mitochondrial membranes is critical for protein import. Structural and biochemical data revealed that phospholipids affect the stability and activity of mitochondrial protein translocases. Integration of proteins into the target membrane involves rearrangement of phospholipids and distortion of the lipid bilayer. Phospholipids are present in the interface between subunits of protein translocases and affect the dynamic coupling of partner proteins. Phospholipids are required for full activity of the respiratory chain to generate membrane potential, which in turn drives protein import across and into the inner membrane. Finally, outer membrane protein translocases are closely linked to organelar contact sites that mediate lipid trafficking. Altogether, intensive crosstalk between mitochondrial protein import and lipid biogenesis controls mitochondrial biogenesis.

Keywords: mitochondria; phospholipids; protein import; TOM complex; SAM complex

1. Introduction

Mitochondria emerged from an endosymbiotic event two billion years ago. A prokaryotic cell was incorporated by an eukaryotic ancestor cell and eventually developed to today’s mitochondrion. Over the course of evolution, the genetic information of the endosymbiont was either lost or transferred to the host genome. The endosymbiotic roots of mitochondria are important for their shape, propagation and functions within the cell. Mitochondria contain two membranes, the outer and inner membranes, as well as the intermembrane space and matrix. The inner membrane forms large invaginations, which are termed cristae and harbor respiratory chain complexes. Mitochondria are essential organelles that perform several biochemical functions for the cell, such as ATP production via oxidative phosphorylation, synthesis of co-factors such as iron-sulfur clusters or coenzyme Q, steps of urea cycle and heme biosynthesis, and biosynthesis of lipids and amino acids. The mitochondrial surface constitutes a signaling platform for programmed cell death, apoptosis and inflammation in innate immunity [1,2]. Dysfunctions of mitochondria have been linked to several diseases, including neurodegenerative disorders [3–5].

Mitochondrial functions and biogenesis depend on a large set of proteins. They contain about 900 proteins in baker’s yeast Saccharomyces cerevisiae and about 1100 proteins in humans [5–9]. Due to their endosymbiotic origin, mitochondria harbor their own genomes. However, this circular DNA encodes just for a small number of proteins: 8 proteins in yeast and 13 proteins in human mitochondria. About 99% of mitochondrial proteins are produced as precursors on cytosolic ribosomes. The precursor proteins contain cleavable or non-cleavable targeting signals that mediate their targeting to the mitochondrial surface [10,11]. The translocase of the outer membrane (TOM complex) imports the vast majority of mitochondrial proteins. Subsequently, membrane-bound multisubunit protein translocases...
sort the incoming precursor proteins into the outer and inner membrane, intermembrane space and matrix [12–17].

Mitochondrial functions also depend on the import and biosynthesis of phospholipids. The phospholipid composition of mitochondrial membranes is critical for several different processes, including mitochondrial dynamics, apoptosis, oxidative phosphorylation and membrane architecture for a more detailed overview [18–22]. Mitochondrial membranes contain the characteristic phospholipid cardiolipin (CL). The capability of mitochondria to produce CL is a relic of their endosymbiotic origin. Defects of biosynthesis of CL have been associated with cardiovascular diseases [23–25]. We propose that there is a close link between mitochondrial protein import and lipid biosynthesis. Protein translocases are linked to organelle contact sites that mediate lipid trafficking between mitochondria and other cellular compartments. Furthermore, the phospholipid composition of mitochondrial membranes also affects protein import into the mitochondria. Phospholipids are important for the integrity and functions of protein translocases, as well as the maintenance of the mitochondrial membrane potential that drives protein transport across and into the inner membrane. Structural characterizations of protein translocases have revealed how phospholipids affect protein transport. We focus on recent structural and biochemical data to point out the role of lipids in protein import and highlight the crosstalk between protein and lipid biogenesis.

2. Mitochondrial Phospholipids

Mitochondrial membranes in yeast contain about 40–45% phosphatidylcholine (PC), 25–30% phosphatidylethanolamine (PE), 10–15% of phosphatidylinositol (PI), 10–15% CL, 3–5% phosphatidylserine (PS) and up to 2% phosphatidic acid (PA) [26,27]. Sphingolipids and sterols are present only in low amounts [27]. The lipid composition of the outer and inner membranes differs strongly. Whereas the outer membrane has a low protein content, the inner membrane is the most protein-enriched membrane in the cell [26,27]. Furthermore, CL is strongly enriched in the inner membrane, whereas it is a low-abundant lipid in the outer membrane [28]. The charge of the headgroup and the fatty acid chain composition are important features of phospholipids that can affect the functions of protein machineries (Figure 1). PI, PS, PA and CL have a negatively charged headgroup, whereas the most abundant phospholipids, PE and PC, possess a neutrally charged headgroup. Mitochondria are rich in so-called non-bilayer phospholipids, such as PE and CL. The headgroup of these lipids has a smaller diameter than the fatty acid tail. Depending on pH and cation concentrations, isolated non-bilayer phospholipids form non-lipid bilayer structures [26,29,30]. In contrast, the headgroup and the fatty acid domain of bilayer-forming phospholipids like PC, PI and PS have a similar diameter. Non-bilayer phospholipids affect membrane curvature and locally destabilize the lipid bilayer, which are both important features for the function of membrane-bound protein machineries [26,29]. The presence of non-bilayer lipids is critical for mitochondrial functions. Supporting this view, the parallel loss of CL synthase and mitochondrial PE synthesis is lethal [31].

Mitochondria synthesize the non-bilayer phospholipids CL and PE. CL is produced at the mitochondrial inner membrane in a multistep pathway. The precursor PA is transported from the endoplasmic reticulum (ER) to the mitochondrial inner membrane, where the CDP-diacylglycerol (DAG) synthase Tam41 catalyzes the reaction of PA with cytidine phosphate to CDP-DAG [32]. Pgs1 combines glycerol-phosphate with CDP-DAG to produce phosphatidylglycerolphosphate (PGP). Subsequent removal of phosphate leads to the production of phosphatidylglycerol (PG). The CL synthase Crd1 adds a second CDP-DAG to PG to form CL. The produced CL undergoes remodeling of its acyl chain composition by the sequential activity of the CL-specific deacylase Cld1 and the monolysos-acyltransferase Taz1, termed Taffazin, in humans [20,23–27,33]. Mutations of human Taffazin have been found in patients suffering from Barth syndrome, which is characterized by dilated cardiomyopathy, neutropenia, growth retardation and 3-methyl-glutaconic aciduria [20,23–27,33].
Mitochondrial PS decarboxylase 1 (Psd1) decarboxylates PS to produce the majority of cellular PE in yeast. Additional pathways for the production of PE exist [27,34]. First, ethanolamine is activated in a two-step reaction to CDP-ethanolamine, which reacts with DAG to form PE. In human cells, the Kennedy pathway typically represents the dominant pathway to generate PE. Second, the vacuolar Psd2 decarboxylates PS to form PE in the vacuolar membrane. Third, the acyltransferases Tge3 and Ale1 generate minor amounts of PE [27,34]. The biogenesis of Psd1 involves autocatalytic cleavage into the membrane anchor and the catalytic subunit. This autocatalytic cleavage is critical for activating the enzyme. Both subunits remain stably bound to each other to allow PE biosynthesis on the intermembrane space side of the inner membrane [35,36]. Other phospholipids such as PC, PS, PA and PI are produced in the ER membrane and have to be imported into mitochondria. Organelle contact sites, such as the ER mitochondria encounter structure (ERMES) or vacuole mitochondria patch (vCLAMP), play an important role in the exchange of phospholipids between mitochondria and other cellular compartments [37–40].

3. Overview of Mitochondrial Protein Import Pathways

Mitochondrial precursor proteins are produced on cytosolic ribosomes. According to our current knowledge, protein import into mitochondria occurs predominantly in a post-translational manner [10,11]. Cytosolic factors guide precursor proteins to the mitochondrial surface. Proteins are imported in a largely unfolded stage to allow the passage of the translocation channel. The TOM complex is the general entry gate for most precursor proteins. The β-barrel protein Tom40 forms the protein-conducting channel [41–45]. All other TOM subunits contain a single α-helical membrane anchor. Structural analyses of yeast and human TOM complexes revealed a highly similar molecular organization, including two Tom40 molecules linked via two Tom22 subunits. Each Tom40 is associated with

![Figure 1. Mitochondrial phospholipids. Phospholipids contain a hydrophilic head with a glycerol backbone as the central structural element and distinct headgroups, each linked by a phosphate group. The headgroup is characteristic for each class of phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), and cardiolipin (CL). It varies in its net charge and is linked via a phosphate group to glycerol. Acyl chains are also attached to the glycerol backbone via ester bonds and form the hydrophobic tail of the phospholipids. The acyl chains can vary in length and saturation. Figure 1 is adapted from [19].](image-url)
three small Tom proteins, Tom5, Tom6 and Tom7 [46–50]. This TOM core complex associates with the receptors Tom20 and Tom70, which recognize different types of incoming precursor proteins [51–55]. The cytosolic domain of Tom22 forms the docking site for both receptor proteins in the TOM complex [56].

After passage of the TOM complex, specific protein translocases sort the precursor proteins into the outer and inner membranes, the intermembrane space, and the matrix (Figure 2) [12–15]. First, the presequence translocase (TIM23 complex) transports precursor proteins containing a cleavable presequence into the matrix and inner membrane. Second, the carrier translocase (TIM22 complex) integrates proteins with several transmembrane segments, such as carrier proteins, into the inner membrane. Small TIM chaperones guide these hydrophobic precursor proteins toward the TIM22 translocase. The activity of the respiratory chain generates a membrane potential across the inner membrane, which drives protein transport via the presequence and carrier translocase [57,58]. Third, Mia40 (mitochondrial intermembrane space import and assembly machinery) oxidatively folds cysteine-rich proteins, which trap them in the intermembrane space. Fourth, β-barrel precursors are transported from the TOM complex to the sorting and assembly machinery (SAM complex, also termed the TOB complex for topogenesis of β-barrel proteins), which inserts them into the outer membrane. Finally, the mitochondrial import (MIM) machinery inserts proteins with α-helical membrane anchor into the outer membrane [12–15]. The MIM complex has so far only been identified in fungi. A functional paralog was described in Trypanosoma brucei [59], but the human equivalent awaits identification. OXA1 (oxidase assembly) integrates mitochondrially encoded proteins into the inner membrane [12–15]. OXA1 also promotes the insertion of some nuclear encoded precursor proteins into the inner membrane [60–62].

**Figure 2.** Protein import pathways into mitochondria. Mitochondria have to import the majority of their proteins, which are synthesized as precursors on cytosolic ribosomes. The translocase of the outer membrane (TOM) forms the general entry gate for mitochondrial precursor proteins. After passing the
TOM channel, β-barrel precursors are guided by small TIM chaperones of the intermembrane space to the sorting and assembly machinery complex (SAM), which mediates their folding and insertion into the outer membrane. The presequence translocase of the inner membrane (TIM23) transports precursors with a cleavable presequence into and across the inner membrane. Translocation into the matrix depends on the ATP-depending activity of the presequence translocase-associated motor (PAM). After import, mitochondrial processing peptidases (MPP) remove the cleavable presequence. In the carrier pathway, small TIM chaperones transfer the hydrophobic carrier precursor from the TOM complex to the carrier translocase (TIM22), which inserts them into the inner membrane. The membrane potential (Δψ) drives protein import into and across the inner membrane. The mitochondrial intermembrane space import and assembly machinery (MIA) directs cysteine-rich precursors into the IMS and mediates their oxidative folding. The mitochondrial import machinery (MIM complex) inserts precursors of single and multi-spanning proteins into the OM. Finally, the oxidase assembly machinery (OXA) inserts mitochondrially encoded proteins into the mitochondrial IM.

4. Role of Lipids in Protein Import into Mitochondria

Protein import into mitochondria is modulated by changes in the phospholipid composition of the mitochondrial membranes (Figure 3). The phospholipid composition is important for the stability and operation of protein translocases and has an impact on dynamic protein–protein interactions. Phospholipids affect the activity of respiratory chain complexes, which in turn leads to reduced membrane potential and impaired protein import. The combination of structural and biochemical studies provides first insights into how phospholipids affect mitochondrial protein transport on a molecular level.

4.1. Role of Lipids in the TOM Complex

The TOM complex forms the entry gate for the majority of mitochondrial precursor proteins and is therefore essential for life. The central β-barrel protein Tom40 is associated with Tom22 and the small Tom proteins, which all contain a single α-helical membrane anchor [46–50]. Two Tom22 subunits link two Tom40 subunits and are critical for the integrity of the translocase. Remarkably, the structural analyses revealed the presence of a phospholipid between Tom40 and Tom22, which could be PE in yeast or PC in humans [47–50]. A structure of the human TOM complex revealed the presence of further phospholipids, such as PE and PC, which were detected between Tom40 and Tom6, Tom7 and Tom22 [50]. These data indicate that phospholipids could play an important role in the integrity and function of the TOM complex. Indeed, the binding of a model precursor protein to the TOM complex is impaired in PE-deficient yeast mitochondria [63,64]. Supporting this view, the transport of β-barrel proteins across the outer membrane is decreased in PE-deficient mitochondria, whereas TOM-independent protein import remains unaffected [63]. The binding of precursor proteins to the TOM complex is similarly affected in the absence of CL in yeast mitochondria [28]. Thus, non-bilayer-forming phospholipids are important for the function of the TOM complex. In contrast, depletion of the bilayer-forming PC does not interfere with precursor binding to the translocase [64]. However, the molecular mechanisms by which non-bilayer phospholipids affect the binding of precursor proteins to the TOM complex remains unknown. CL also stabilizes the association of Tom20 with the TOM complex [28]. Structural data of the TOM complex containing Tom20 and Tom70 are missing. Therefore, it remains enigmatic how CL affects the docking of Tom20 to the TOM complex.

4.2. Effects of Lipids on the Import of β-Barrel Proteins

Yeast mitochondria contain five β-barrel proteins that are essential for the transport of proteins (Tom40, Sam50, Mdm10) and metabolites and ions (Porin isoforms: Por1 and Por2) across and into the outer membrane and are part of organelle contact sites (Mdm10). Precursors of β-barrel proteins are first transported across the outer membrane via the TOM complex and subsequently integrated into the outer membrane by the SAM complex [13–15,65]. TOM and SAM complexes form a supercomplex to facilitate the
transfer of precursor proteins [66,67]. The small TIM chaperones stabilize the precursor at the TOM-SAM complex [66–70]. Structural and biochemical analyses of the past few years have provided important insights into the operation of the SAM complex on a molecular level [71–73]. The SAM complex contains two peripheral subunits, Sam37 and Sam35, and the β-barrel protein Sam50. Sam50 belongs to the Omp85 protein family, which is present in the outer membrane of bacteria, mitochondria and plastids from plants [74–76]. It contains one polypeptide translocation-associated domain (POTRA) that faces the intermembrane space and a 16-stranded transmembrane β-barrel. The β-barrel of Sam50 forms a lateral gate between the first and the last β-strands. Hairpins of two β-strands of the incoming precursor protein are inserted sequentially into the lateral gate, leading to the stepwise formation of the β-barrel [77]. The short β-strands of the lateral gate induce thinning and distortion of the lipid bilayer to facilitate insertion of the growing β-barrel [71,72]. The maturation and release of the newly formed β-barrel proteins involves a β-barrel switching mechanism [72]. In the idle state, the SAM complex contains two Sam50 monomers. The second Sam50 functions as a placeholder to facilitate the insertion and folding of the β-barrel precursor protein. The insertion of a β-barrel precursor into the lateral gate of the Sam50 displaces the second Sam50 to allow the new β-barrel to form [72]. Finally, the efficient release of the Tom40 precursor from the SAM complex depends on the binding of Mdm10 to the SAM complex [72,78,79].

Altogether, the formation of β-barrel proteins at the SAM complex involves massive molecular rearrangements at the protein translocases, such as coupling of partner proteins and formation of β-barrel that occurs within the lipid bilayer. Therefore, it is not surprising that protein sorting via the SAM complex strongly depends on the native phospholipid composition of the outer membrane. Import of β-barrel proteins is impaired in yeast mitochondria deficient of either CL, PE or PC [28,63,64]. Inspection of different biogenesis steps of Tom40 revealed that CL and PE are particularly important for the initial binding of the precursor to the SAM complex [28,63]. Interestingly, two phospholipids were found in the interface between the two Sam50 molecules and between Sam50 and Mdm10 [72]. However, their identity remains unknown. We speculate that these phospholipids may affect the molecular dynamics of the SAM complex. Future studies are required to define the biophysical properties of phospholipids or the membrane environment that are important for protein transport via SAM machinery.

The SAM complex also constitutes a platform for the assembly of the Tom40 precursors with other TOM subunits. Biochemical and structural data revealed that Tom5 and Tom6 assemble with Tom40 at the SAM complex [73,80–82]. Furthermore, the SAM-Mdm10 complex promotes the insertion and assembly of the Tom22 precursor [78,80,83]. The assembly of Tom22 with Tom40/small Tom modules is a critical step in the formation of a mature TOM complex [56]. The assembly of these single-spanning TOM subunits is defective in PC-deficient yeast mitochondria [64], whereas depletion of neither CL nor PE impairs their assembly steps [28,63]. We propose that the important role of phospholipids in the assembly of the TOM complex could be explained by the presence of lipids in the interface between the β-barrel of Tom40 and further TOM subunits [47,48,50]. Altogether, the activity of the dynamic SAM complex in protein biogenesis strongly depends on the proper lipid composition of the outer membrane.

4.3. Lipids and the Import of α-Helical Outer Membrane Proteins

The outer membrane contains several proteins with single or multiple α-helical transmembrane segments. Different import mechanisms involving TOM, SAM subunits or protein-independent insertion have been reported [13–15,65]. The MIM complex is the major protein translocase for single and multi-spanning proteins in yeast mitochondria [84–92]. It is a highly dynamic protein machinery that cooperates with different partner proteins, such as the SAM and TOM subunits [84–92]. Therefore, we speculate that the lipid environment may be important for MIM-dependent protein import. Indeed, negatively charged phospholipids CL and PA promote the import of multi-spanning proteins [93,94]. The
non-bilayer forming properties of these lipids seem not to be critical since depletion of the most abundant non-bilayer phospholipid PE does not affect import of these proteins [63]. Finally, high sterol content impairs the integration of the C-terminally anchored Fis1 into the outer membrane [95,96]. The molecular mechanisms of the MIM-dependent protein insertion into the outer membrane remain to be determined. Therefore, it is unclear how lipids affect this import pathway at the molecular level.

![Figure 3. Role of phospholipids in mitochondrial protein import.](image)

Figure 3. Role of phospholipids in mitochondrial protein import. Alterations in the composition of phospholipids of the mitochondrial membranes affect protein import pathways and protein translocases at different levels. The phospholipids CL, PE and PC are important for the stability and function of outer and inner membrane protein translocases. The depletion of CL or PE affects the respiratory chain, which causes a reduction of the membrane potential and impaired protein translocation across and into the inner membrane. Figure 3 is adapted from [19].

4.4. The Role of Lipids in Inner Membrane Protein Transport Processes

The inner mitochondrial membrane harbors two major protein translocases, the TIM23 translocase, which sorts presequence-containing proteins, and the TIM22 complex, which integrates carrier proteins into the inner membrane. The membrane potential is critical for driving protein import via both protein import pathways [57,58]. Respiratory chain complexes transport protons across the inner membrane into the intermembrane space to build up the membrane potential. Several studies have revealed that phospholipids are important for the stability and function of respiratory chain complexes. Different phospholipids have been found in the structures of respiratory chain complexes [97,98]. In yeast, complex III
(cytochrome bc1 complex) and complex IV (cytochrome c oxidase) associate in respiratory chain supercomplexes [99–101], whereas mammalian complex I (NADH dehydrogenase), complex III and complex IV form high molecular weight supercomplexes [100]. CL stabilizes the association of respiratory chain complexes III and IV into supercomplexes in yeast mitochondria [102–106]. In contrast, depletion of PE does not interfere with structural integrity, but is required for the full activity of respiratory chain complexes [107–110]. Yeast mutant strains that are defective in PE biosynthesis frequently display reduced membrane potential, which in turn affects protein transport into and across the inner membrane [108]. Depending on the growth conditions, depletion or loss of CL also affects respiratory activity, membrane potential, and consequently protein import [111–115]. In contrast, the activity and stability of respiratory chain complexes are not impaired in PC-deficient cells [110,116]. In our view, phospholipids differentially affect the function and stability of respiratory chain complexes.

Phospholipids are important for the structural integrity of the TIM23 complex and its dynamic coupling of partner proteins. The presequence translocase consists of seven subunits. The essential components Tim23 and Tim17 form a pore that releases proteins into the membrane and promotes protein translocation into the matrix [117–120]. Mgr2 closely associates with Tim17 and Tim23 and monitors the lateral gate to control the release of membrane proteins into the inner membrane [121]. Tim23 exposes a soluble domain into the intermembrane space that coordinates with Tim50 and Tim21 recognition of incoming precursor proteins and their transfer to the translocation pore [54,122–125]. Lateral release into the inner membrane depends on the membrane potential as a driving force and is supported by the presence of CL in the membrane [126]. Protein transport into the matrix also involves the dynamic association and ATP-consuming activity of the presequence translocase-associated motor (PAM). Here, Tim44 forms a docking site for mitochondrial Hsp70 that powers via ATP-hydrolysis protein translocation into the matrix. Co-chaperones of the PAM module control the reaction cycle of mitochondrial Hsp70 [127–129]. Altogether, the presequence translocase undergoes dynamic molecular switches to promote protein transport into the matrix or inner membrane.

CL stabilizes the TIM23 complex in different ways [115,130–133]. First, CL supports the interaction of the PAM module with the TIM23 complex [113,115]. Second, the association of Tim44 with membranes is promoted when CL is present [130,131]. Finally, the association of Tim50, Tim21 and Tim17 with Tim23 is destabilized in CL-deficient mutant mitochondria [115,133]. Similarly, the amount and integrity of the presequence translocase are reduced in PC- and PE-deficient mitochondria [108,116]. Remarkably, membrane potential and respiratory activity are only mildly decreased upon the loss of PC, but protein import via the presequence pathway is reduced, indicating a direct role of PC in protein transport via the TIM23 complex [116]. Further structural and detailed biochemical studies should reveal how PC and PE affect TIM23 stability and function.

The carrier translocase integrates multi-spanning proteins into the inner membrane. The central subunit Tim22 inserts proteins into the inner membrane [57]. Other subunits of the TIM22 complex mediate the assembly of the translocase and docking of small TIM chaperones. Defects in the membrane potential are a major cause for reduced import of carrier proteins in PE- and CL-deficient mitochondria [108,111]. Although the membrane potential is intact, the import of carrier proteins is reduced in PC-deficient mitochondria [116], pointing to a more direct role of PC in protein translocation. How phospholipids affect TIM22-mediated protein insertion remains unknown. Recently reported cryo-electron microscopic structures did not reveal phospholipids in the yeast and human TIM22 complex [134,135]. The TIM22 complex remains largely intact in lipid mutant mitochondria [108,114,116], but the assembly of the imported carrier proteins like ADP/ATP carrier depends on the presence of CL [111,114,115,136,137]. Remarkably, acylglycerolkinase (AGK) is a subunit of the human TIM22 complex. AGK is involved in lipid metabolism and phosphorylates monoacylglycerol and diacylglycerol to lysophosphatidic acid and phosphatidic acid, respectively [135,138,139]. Mutations of AGK have been linked to patients suffering from
Sengers syndrome [140]. Why the enzyme AGK is a component of the TIM22 complex remains to be clarified. Inactivation of kinase activity does not affect its function in protein import, indicating that the structural properties of AGK are important for its function in protein transport [138,139]. The dual role of AGK might coordinate protein translocation and lipid metabolism in mitochondria.

5. Connection of Protein Transport and Organelle Contact Sites

Most phospholipids are produced in the endoplasmic reticulum, while mitochondria synthesize CL and parts of PE [19,26,27,39,40]. Consequently, mitochondrial biogenesis depends on the import of phospholipids from the ER. Mitochondria do not receive lipids via vesicular trafficking, since they are not part of the endomembrane system. Instead, molecular contact sites allow the exchange of lipids with different cellular compartments. In yeast, the ERMES complex mediates lipid trafficking between ER and mitochondria, whereas the vacuole and mitochondria patch (vCLAMP) facilitates the exchange of lipids with the vacuolar membrane [141–147].

Remarkably, there are several connections between protein import and organelle contact sites, pointing to a close link between protein and lipid transport [148]. The TOM complex is linked to organelle contact sites (Figure 4). In a split-Venus approach, Tom5 was found as an interaction partner of the ER membrane complex (EMC) and a function of EMC in lipid transport to mitochondria was reported [149]. The molecular mechanism by which lipids are transported via EMC and Tom5 remains to be demonstrated. Interestingly, EMC inserts proteins into the ER membrane [150,151]. How the activities of the EMC complex are coordinated remains unknown. The Tom70 receptor interacts with the sterol transport protein Lam6/Ltc1 of the ER membrane [152]. Lam6/Ltc1 is present at different organellar contact sites between ER, mitochondria and the vacuole and coordinates the formation of ERMES and vCLAMP [153]. Finally, Tom40 is the mitochondrial binding partner of Vps39 in the vCLAMP structure [154]. Whether or not the Tom40 β-barrel is involved in lipid trafficking remains enigmatic.

![Image](image_url)

**Figure 4.** Connections of protein transport and organelle contact sites. Organelle contact sites allow lipid trafficking between mitochondria and other cellular organelles such as the endoplasmic reticulum (ER). The ER–mitochondria encounter structure (ERMES) forms a molecular bridge between ER and...
mitochondria. It consists of the ER-anchored protein Mmm1, which is linked via Mdm12 and Mdm34 to Mdm10. Gem1 and Tom7 are associated with ERMES. Mdm10 also binds to the SAM complex to promote protein biogenesis. Binding of Tom7 detaches Mdm10 from the SAM complex and promotes its association with ERMES. The ER membrane protein complex (EMC) and ER-localized Lam6 interact with the Tom5 and Tom70 of the TOM complex, respectively. The vacuole and mitochondria patch (vCLAMP) link mitochondria to the vacuolar membrane. vCLAMP consists of the vacuolar membrane (VM) protein Ypt7 and the vacuolar protein sorting 39 (Vps39), which binds to Tom40 to mediate the exchange of lipids between the vacuole and mitochondria.

Mdm10 has dual localization. It is associated with the SAM complex [72,78,79,83,155] and forms the mitochondrial anchor of the ERMES complex [78,156]. Tom7 controls the distribution of Mdm10 between both protein complexes. Binding of unassembled Tom7 dissociates the SAM-Mdm10 complex and promotes the association of Mdm10 with ERMES [78,79,157]. The ERMES complex consists of the synaptotagmin-like mitochondrial lipid-binding protein (SMP) domains containing Mmm1, Mdm12 and Mdm34 and the mitochondrial Mdm10. Such SMP domains have been implicated in mediating lipid transport. Structural data reveal that the ER-resident Mmm1 and Mdm34 form a hydrophobic tunnel that allows lipid transport [145,146]. Interestingly, the inner wall of the Mdm10 β-barrel contains hydrophobic patches [158]. It remains to be demonstrated whether the β-barrel of Mdm10 transports lipids into the outer membrane.

Altogether, outer membrane protein translocases are closely linked to organelle contact sites. The functional implications of these connections remain elusive. We speculate that these molecular interactions are important for coordinating protein and lipid transport during mitochondrial biogenesis and functions.

6. Conclusions

Protein import is closely linked to the phospholipid composition of mitochondrial membranes. Remarkably, the individual depletion of the different phospholipid classes has distinct effects on the different protein import routes. Whereas CL and PE are important in maintaining respiratory chain function and membrane potential-dependent import pathways, depletion of PC did not affect the activity of respiratory chain complexes [108,111,116]. CL and PE, but not PC, promote precursor binding to the TOM complex [28,63,64]. We propose that the non-bilayer features of CL and PE are important for the function of the TOM complex. The function of dynamic protein translocases, such as the SAM complex and the TIM23 translocase, is strongly dependent on the native lipid composition [28,63,64,113,115,126]. Structural data reveal that phospholipids are present in the interface of translocase subunits and dynamically associated partner proteins [47,48,50,72]. Studies addressing the impact of the negatively charged PI and PS on protein transport are currently missing and will be important to define which features of lipids are important for their function in protein import. A recent study reported that the knockdown of PS synthase in Drosophila melanogaster leads to reduced mitochondrial PS and CL and impaired localization of mitochondria-targeted EYFP [159]. However, whether PS affects mitochondrial protein import on molecular levels remains to be determined. Protein translocases dynamically interact with each other and with several partner proteins to fine-tune protein transport to cellular needs [13–15]. The impact of lipids on the organization of such molecular interactions remains to be determined. Lipids are also important for cellular signaling, which can regulate protein import into mitochondria. In human cells, a lipid signaling cascade is initiated upon starvation and hypoxia, which results in the downregulation of the mitochondrial PE content [160]. The decreased PE content in turn stimulates the inner membrane AAA protease YME1L, which degrades inner membrane-bound protein translocases, lipid transfer proteins and metabolic enzymes [160]. Altogether, we propose that protein and lipid biogenesis are closely connected to each other to control mitochondrial biogenesis and function.
References

Author Contributions: J.J.H. and T.B.: conceptualization and designing the figures. T.B. writing the manuscript. All authors contribute to the editing and corrections of the text and figures. All authors have read and agreed to the published version of the manuscript.

Funding: The work was funded by grants from the Deutsche Forschungsgemeinschaft to T.B. (BE 4679/2-2 project ID 269424439; SFB1218 project ID 269925409).

Conflicts of Interest: The authors declare no conflict of interest.

1. Spinelli, J.B.; Haigis, M.C. The multifaceted contributions of mitochondria to cellular metabolism. Nat. Cell Biol. 2018, 20, 745–754. [CrossRef] [PubMed]
2. Moehlman, A.T.; Youle, R.J. Mitochondrial quality control and restraining innate immunity. Annu. Rev. Cell Dev. Biol. 2020, 36, 265–289. [CrossRef] [PubMed]
3. Nunnari, J.; Suomalainen, A. Mitochondria: In sickness and in health. Cell 2012, 148, 1145–1159. [CrossRef] [PubMed]
4. Picard, M.; Wallace, D.C.; Burelle, Y. The rise of mitochondria in medicine. Mitochondrion 2016, 30, 105–116. [CrossRef] [PubMed]
5. Morgenstern, M.; Peikert, C.D.; Lübbert, P.; Alka, O.; Steiert, C.; Naumenko, N.; Schendzielorz, A.; Melchionda, L.; et al. Quantitative high-confidence human mitochondrial proteome and its dynamics in cellular context. Cell Metab. 2021, 33, 2464–2483. [CrossRef]
6. Sickmann, A.; Reinders, J.; Wagner, Y.; Joppich, C.; Zahedi, R.; Meyer, H.E.; Schönfisch, B.; Perschil, I.; Chacinska, A.; Guiard, B.; et al. The proteome of Saccharomyces cerevisiae mitochondria. Proc. Natl. Acad. Sci. USA 2003, 100, 13207–13212. [CrossRef]
7. Pagliarini, D.J.; Calvo, S.E.; Chang, B.; Sheth, S.A.; Vafai, S.B.; Oring, S.E.; Walford, G.A.; Sugiana, C.; Boneh, A.; Chen, W.K.; et al. A mitochondrial protein compendium elucidates complex I disease biology. Cell 2008, 134, 112–123. [CrossRef]
8. Morgenstern, M.; Stiller, S.B.; Lübbert, P.; Peikert, C.D.; Dannenmaier, S.; Drepper, F.; Weill, U.; Höß, P.; Feuerstein, R.; Gebert, M.; et al. Definition of a high-confidence mitochondrial proteome at quantitative scale. Cell Rep. 2017, 29, 2836–2852. [CrossRef]
9. Rath, S.; Sharma, R.; Gupta, R.; Ast, T.; Chan, C.; Durham, T.J.; Goodman, R.P.; Grabarek, Z.; Haas, M.E.; Hung, W.H.W.; et al. MitoCarta3.0: An updated mitochondrial proteome now with sub-organelle localization and pathway annotations. Nucleic Acids Res. 2021, 49, 1541–1547. [CrossRef]
10. Becker, T.; Song, J.; Pfanner, N. Versatility of preprotein transfer from the cytosol to mitochondria. Trends Cell Biol. 2019, 29, 534–548. [CrossRef]
11. Bykov, Y.S.; Rapaport, D.; Herrmann, J.M.; Schuldiner, M. Cytosolic events in the biogenesis of mitochondrial proteins. Trends Biochem. Sci. 2020, 45, 650–667. [CrossRef] [PubMed]
12. Endo, T.; Yamano, K.; Kawano, S. Structural insight into the mitochondrial protein import system. Biochim. Biophys. Acta. 2011, 1808, 955–970. [CrossRef]
13. Wiedemann, N.; Pfanner, N. Mitochondrial machineries for protein import and assembly. Annu. Rev. Biochem. 2017, 86, 685–714. [CrossRef]
14. Eaglesfield, R.; Tokatlidis, K. Targeting and insertion of membrane proteins in mitochondria. Front. Cell Dev. Biol. 2021, 9, 803205. [CrossRef]
15. Hansen, K.G.; Herrmann, J.M. Transport of proteins into mitochondria. Protein J. 2019, 38, 330–342. [CrossRef] [PubMed]
16. Endo, T.; Sakaue, H. Multifaceted roles of porin in mitochondrial protein and lipid transport. Biochem. Soc. Trans. 2019, 47, 1269–1277. [CrossRef] [PubMed]
17. Grevel, A.; Becker, T. Porins as helpers in mitochondrial protein translocation. Biol. Chem. 2020, 401, 699–708. [CrossRef]
18. Ren, M.; Phoon, C.K.; Schlame, M. Metabolism and function of mitochondrial cardiolipin. Prog. Lipid Res. 2014, 55, 1–16. [CrossRef]
19. Mårtensson, C.U.; Doan, K.N.; Becker, T. Effects of lipids on mitochondrial functions. Biochim. Biophys. Acta. 2017, 1862, 102–113. [CrossRef]
20. Falabella, M.; Vernon, H.J.; Hanna, M.G.; Claypool, S.M.; Piteathly, R.D. Cardiolipin, mitochondria, and neurological disease. Trends Endocrinol. Metab. 2021, 32, 224–237. [CrossRef]
21. Gohil, V.M.; Greenberg, M.L. Mitochondrial membrane biogenesis: Phospholipids and proteins go hand in hand. J. Cell Biol. 2009, 184, 469–472. [CrossRef] [PubMed]
22. Rampelt, H.; Zerbes, R.M.; van der Laan, M.; Pfanner, N. Role of the mitochondrial contact site and cristae organizing system in membrane architecture and dynamics. Biochim. Biophys. Acta. 2017, 1864, 737–746. [CrossRef] [PubMed]
23. Shen, Z.; Ye, C.; McCoy, K.; Greenberg, M.L. The role of cardiolipin in cardiovascular health. BioMed Res. Int. 2015, 2015, 891707. [CrossRef] [PubMed]
24. Claypool, S.M.; Koeberl, C.M. The complexity of cardiolipin in health and disease. Trends Biochem. Sci. 2012, 37, 32–41. [CrossRef]
25. Paradies, G.; Paradies, V.; Ruggiero, F.M.; Petrosillo, G. Role of cardiolipin in mitochondrial function and dynamics in health and disease: Molecular and pharmacological aspects. Cells 2019, 8, 728. [CrossRef]
26. Osman, C.; Voelker, D.R.; Langer, T. Making heads or tails of phospholipids in mitochondria. J. Cell Biol. 2011, 192, 7–16. [CrossRef]
27. Horvath, S.E.; Daum, G. Lipids of mitochondria. Prog. Lipid Res. 2013, 52, 590–614. [CrossRef]
28. Gebert, N.; Joshi, A.S.; Kutik, S.; Becker, T.; McKenzie, M.; Guan, X.L.; Mooga, V.P.; Stroud, D.; Kulkarni, G.; Wenk, M.R.; et al. Mitochondrial cardiolipin involved in outer-membrane protein biogenesis: Implications for Barth syndrome. *Curr. Biol.* **2009**, *19*, 2133–2139. [CrossRef]

29. Killian, J.A.; de Kruijff, B. Nonbilayer lipids affect peripheral and integral membrane proteins via changes in the lateral pressure profile. *Biochim. Biophys. Acta* **2004**, *1666*, 275–288.

30. Ball, W.B.; Neff, J.K.; Gohil, V.M. The role of nonbilayer phospholipids in mitochondrial structure and function. *FEBS Lett.* **2018**, *592*, 1273–1280. [CrossRef]

31. Gohil, V.M.; Thompson, M.N.; Greenberg, M.L. Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine and cardiolipin biosynthetic pathways in Saccharomyces cerevisiae. *J. Biol. Chem.* **2005**, *280*, 35410–35416. [CrossRef] [PubMed]

32. Tamura, Y.; Harada, Y.; Nishikawa, S.I.; Yamano, K.; Kamiya, M.; Shiota, T.; Kuroda, T.; Kuge, O.; Sasaki, H.; Imai, K.; et al. Tom41 is a CDP-diacylglycerol synthase required for cardiolipin biosynthesis in mitochondria. *Cell Metab.* **2013**, *17*, 709–718. [CrossRef] [PubMed]

33. Dudek, J.; Hartmann, M.; Rehling, P. The role of mitochondrial cardiolipin in heart function and its implication in cardiac disease. *Biochim. Biophys. Acta* **2019**, *1865*, 810–821. [CrossRef]

34. Calzada, E.; Onguka, O.; Claypool, S.M. Phosphatidylethanolamine metabolism in health and disease. *Int. Rev. Cell Mol. Biol.* **2016**, *321*, 29–88.

35. Horvath, S.E.; Böttinger, L.; Vögtle, F.N.; Wiedemann, N.; Meisinger, C.; Becker, T.; Daum, G. Processing and topology of the yeast mitochondrial phosphatidylserine decarboxylase 1. *J. Biol. Chem.* **2012**, *287*, 36744–36755. [CrossRef]

36. Sam, P.N.; Calzada, E.; Acoba, M.G.; Zhao, T.; Watanabe, Y.; Nejatfard, A.; Trinidad, J.C.; Shutt, T.E.; Neal, S.E.; Claypool, S.M. Impaired phosphatidylethanolamine metabolism activates a reversible stress response that detects and resolves mutant mitochondrial precursors. *iScience* **2021**, *24*, 102196. [CrossRef]

37. Eisenberg-Bord, M.; Shai, N.; Schuldiner, M.; Bohnert, M. A tether is a tether is a tether: Tethering at membrane contact sites. *Dev. Cell* **2016**, *39*, 395–409. [CrossRef] [PubMed]

38. Becker, L.; Bannwarth, M.; Meisinger, C.; Hill, K.; Model, K.; Ryan, M.T.; Dietmeier, K.; Martin, F.; Wagner, R.; Pfanner, N. Tom40 forms the hydrophilic channel of the protein-conducting TOM channel in the outer membrane of mitochondria. *J. Biol. Chem.* **2001**, *153*, 1151–1160. [CrossRef] [PubMed]

39. Ball, W.B.; Neff, J.K.; Gohil, V.M. The role of nonbilayer phospholipids in mitochondrial structure and function. *FEBS Lett.* **2018**, *592*, 1273–1280. [CrossRef]

40. Tamura, Y.; Kawano, S.; Endo, T. Lipid homeostasis in mitochondria. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 321–330. [CrossRef] [PubMed]

41. Hill, K.; Model, K.; Ryan, M.T.; Dietmeier, K.; Martin, F.; Wagner, R.; Pfanner, N. Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins. *Nature* **1998**, *395*, 516–521. [CrossRef] [PubMed]

42. Ahtiing, U.; Thieffry, M.; Engelhardt, H.; Hegerl, R.; Neupert, W.; Nussberger, S. Tom40, the pore-forming component of the protein-conducting TOM channel in the outer membrane of mitochondria. *J. Cell Biol.* **2001**, *153*, 1151–1160. [CrossRef] [PubMed]

43. Becker, L.; Bannwarth, M.; Meisinger, C.; Hill, K.; Model, K.; Krimmer, T.; Casadio, R.; Truscott, K.N.; Schulz, G.E.; Pfanner, N.; et al. Preprotein translocase of the outer mitochondrial membrane: Reconstituted Tom40 forms a characteristic TOM pore. *J. Mol. Biol.* **2005**, *353*, 1011–1020. [CrossRef]

44. Suzuki, H.; Kadowaki, T.; Maeda, M.; Sasaki, H.; Nabekura, J.; Sakaguchi, M.; Mihara, K. Membrane-embedded C-terminal segment of rat mitochondrial TOM40 constitutes protein-conducting pore with enriched β-striucture. *J. Biol. Chem.* **2004**, *279*, 50619–50629. [CrossRef]

45. Shiota, T.; Imai, K.; Qiu, J.; Hewitt, V.L.; Tan, K.; Shen, H.H.; Sakiyama, N.; Fukasawa, Y.; Hayat, S.; Kamiya, M.; et al. Molecular architecture of the active mitochondrial protein gate. *Science* **2015**, *349*, 1544–1548. [CrossRef]

46. Bausewein, T.; Mills, D.J.; Langer, J.D.; Nitschke, B.; Nussberger, S.; Kühlbrandt, W. Cryo-EM structure of the TOM core complex from Neurospora crassa. *Cell* **2017**, *170*, 693–700. [CrossRef]

47. Araiso, Y.; Tsutsumi, A.; Qiu, J.; Imai, K.; Shiota, T.; Song, J.; Lindau, C.; Wenz, L.; Sakaue, H.; Yunoki, K.; et al. Structure of the mitochondrial import gate reveals distinct preprotein paths. *Nature* **2019**, *575*, 395–401. [CrossRef]

48. Tucker, K.; Park, E. Cryo-EM structure of the mitochondrial protein-import channel TOM complex at near-atomic resolution. *Nat. Struct. Mol. Biol.* **2019**, *26*, 1158–1166. [CrossRef]

49. Wang, W.; Chen, X.; Zhang, L.; Yi, J.; Ma, Q.; Yin, J.; Zhuo, W.; Gu, J.; Yang, M. Atomic structure of human TOM core complex. *Cell Discov.* **2020**, *6*, 67. [CrossRef]

50. Guan, Z.; Yan, L.; Wang, Q.; Qi, L.; Hong, S.; Gong, Z.; Yan, C.; Yin, P. Structural insights into assembly of human mitochondrial translocase TOM complex. *Cell Discov.* **2021**, *7*, 22. [CrossRef]

51. Brix, J.; Dietmeier, K.; Pfanner, N. Differential recognition of preproteins by the purified cytosolic domains of the mitochondrial import receptors Tom20, Tom22, and Tom70. *J. Biol. Chem.* **1997**, *272*, 20730–20735. [CrossRef] [PubMed]

52. Yamamoto, H.; Fukui, K.; Takahashi, H.; Kitamura, S.; Shiota, T.; Terao, K.; Uchida, M.; Esaki, M.; Nishikawa, S.; Yoshihisa, T.; et al. Roles of Tom70 in import of presequence-containing mitochondrial proteins. *J. Biol. Chem.* **2009**, *284*, 31635–31646. [CrossRef] [PubMed]

53. Yamano, K.; Yatsukawa, Y.; Esaki, M.; Hobbs, A.E.A.; Jensen, R.E.; Endo, T. Tom20 and Tom22 share the common signal recognition pathway in mitochondrial protein import. *J. Biol. Chem.* **2008**, *283*, 3799–3807. [CrossRef] [PubMed]
54. Gomkale, R.; Linden, A.; Neumann, P.; Schendzielorz, A.B.; Stoldt, S.; Dybkov, O.; Kilisch, M.; Schulz, C.; Cruz-Zaragoza, L.D.; Schwappach, B.; et al. Mapping protein interactions in the active TOM-TIM23 supercomplex. *Nat. Commun.* **2021**, *12*, 5715. [CrossRef]

55. Backes, S.; Bykov, Y.S.; Flohr, T.; Räsche, M.; Zhou, J.; Lenhard, S.; Krämer, L.; Mühlhaus, L.; Bibi, C.; Jann, C.; et al. The chaperone-binding activity of the mitochondrial surface receptor Tom70 protects the cytosol against mitoprotein-induced stress. *Cell Rep.* **2021**, *35*, 108936. [CrossRef]

56. van Wilpe, S.; Ryan, M.T.; Hill, K.; Maarse, A.C.; Meisinger, C.; Brijs, J.; Dekker, P.J.T.; Moczo, M.; Wagner, R.; Meier, M.; et al. Tom22 is a multifunctional organizer of the mitochondrial preprotein translocase. *Nature* **1999**, *401*, 485–489. [CrossRef]

57. Rehling, P.; Model, K.; Brandner, K.; Kovermann, P.; Sickmann, A.; Meyer, H.E.; Kühlbrandt, W.; Wagner, R.; Truscott, K.N.; Pfanner, N. Protein insertion into the mitochondrial inner membrane by a twin-pore translocase. *Science* **2003**, *299*, 1747–1751. [CrossRef]

58. Malhotra, K.; Sathappa, M.; Landin, J.S.; Johnson, A.E.; Alder, N.N. Structural changes in the mitochondrial Tim23 channel are coupled to the proton-motive force. *Nat. Struct. Mol. Biol.* **2003**, *20*, 965–972. [CrossRef]

59. Vitali, D.G.; Käser, S.; Kolb, A.; Dimmer, K.S.; Schneider, A.; Rapaport, D. Independent evolution of functionally exchangeable mitochondrial outer membrane import complexes. *elife* **2018**, *7*, e34488. [CrossRef]

60. Meier, S.; Neupert, W.; Herrmann, J.M. Proline residues of transmembrane domains determine the sorting of inner membrane proteins in mitochondria. *J. Cell Biol.* **2005**, *170*, 881–888. [CrossRef]

61. Bohnert, M.; Rehling, P.; Guiard, B.; Herrmann, J.M.; Pfanner, N.; van der Laan, M. Cooperation of stop-transfer and conservative sorting mechanisms in mitochondrial protein translocation. *Curr. Biol.* **2010**, *20*, 1227–1232. [CrossRef] [PubMed]

62. Stiller, S.B.; Höpker, J.; Oeljeklaus, S.; Schütze, C.; Schrempp, S.G.; Vent-Schmidt, J.; Horvath, S.E.; Frazier, A.E.; Gebert, N.; van der Laan, M.; et al. Mitochondrial OXA translocase plays a major role in biogenesis of inner-membrane proteins. *Cell Metab.* **2016**, *23*, 901–908. [CrossRef] [PubMed]

63. Becker, T.; Horvath, S.E.; Böttiger, L.; Gebert, N.; Daum, G.; Pfanner, N. Role of phosphatidylethanolamine in the biogenesis of mitochondrial outer membrane proteins. *J. Biol. Chem.* **2013**, *288*, 16451–16459. [CrossRef] [PubMed]

64. Schuler, M.H.; Di Bartolomeo, F.; Böttinger, L.; Horvath, S.E.; Wenz, L.S.; Daum, G.; Becker, T. Phosphatidylcholine affects the role coupled to the proton-motive force. *Science* **2013**, *344*, 901–908. [CrossRef] [PubMed]

65. Dukanovic, J.; Rapaport, D. Multiple pathways in the integration of proteins into the mitochondrial outer membrane. *Biochim. Biophys. Acta* **2011**, *1808*, 971–980. [CrossRef] [PubMed]

66. Qiu, J.; Wenz, L.S.; Zerbes, R.M.; Oeljeklaus, S.; Bohnert, M.; Stroud, D.A.; Wirth, C.; Ellenrieder, L.; Thornton, N.; Kutik, S.; et al. Coupling of mitochondrial import and export translocases by receptor-mediated supercomplex formation. *Cell* **2013**, *154*, 596–608. [CrossRef] [PubMed]

67. Wenz, L.S.; Ellenrieder, L.; Qiu, J.; Bohnert, M.; Zufall, N.; van der Laan, M.; Pfanner, N.; Wiedemann, N.; Becker, T. Sam37 is crucial for formation of the mitochondrial TOM–SAM supercomplex, thereby promoting β-barrel biogenesis. *J. Cell Biol.* **2015**, *209*, 26523–26532. [CrossRef] [PubMed]

68. Wiedemann, N.; Truscott, K.N.; Pfannschmidt, S.; Guiard, B.; Meisinger, C.; Pfanner, N. Role of phosphatidylethanolamine in the biogenesis of mitochondrial β-barrel proteins. *J. Cell Biol.* **2014**, *164*, 18188–18194. [CrossRef]

69. Hoppins, S.C.; Nargang, F.E. The Tim8-Tim13 complex of Neurospora crassa functions in the assembly of proteins into both mitochondrial membranes. *J. Mol. Biol.* **2004**, *340*, 12396–12405. [CrossRef] [PubMed]

70. Weinhäupl, K.; Lindau, C.; Hessel, A.; Wang, Y.; Schütze, C.; Jores, T.; Melchionda, L.; Schönfisch, B.; Kalbacher, H.; Bersch, B.; et al. Structural basis of membrane protein chaperoning through the mitochondrial intermembrane space. *Cell* **2018**, *175*, 1365–1379. [CrossRef] [PubMed]

71. Diederichs, K.A.; Ni, X.; Rollauer, S.E.; Botos, I.; Tan, X.; King, M.S.; Kunji, E.R.S.; Jiang, J.; Buchanan, S.K. Structural insight into mitochondrial β-barrel outer membrane protein biogenesis. *Nat. Commun.* **2020**, *11*, 3290. [CrossRef] [PubMed]

72. Takeda, H.; Tsutsui, A.; Nishizawa, T.; Lindau, C.; Busto, J.V.; Wenz, L.S.; Ellenrieder, L.; Imai, K.; Straub, S.P.; Mossmann, W.; et al. Mitochondrial sorting and assembly machinery operates by β-barrel switching. *Nature* **2021**, *590*, 163–169. [CrossRef] [PubMed]

73. Wang, Q.; Guan, Z.; Qi, L.; Zhuang, J.; Wang, C.; Hong, S.; Yan, L.; Wu, Y.; Cao, X.; Cao, J.; et al. Structural insight into the SAM-mediated assembly of the mitochondrial TOM core complex. *Science* **2021**, *373*, 1377–1381. [CrossRef] [PubMed]

74. Gentle, I.; Gabriel, K.; Beech, P.; Waller, R.; Lithgow, T. The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria. *J. Cell Biol.* **2004**, *164*, 19–24. [CrossRef] [PubMed]

75. Zeth, K. Structure and evolution of mitochondrial outer membrane proteins of β-barrel topology. *Biochim. Biophys. Acta* **2010**, *1797*, 1292–1299. [CrossRef]

76. Diederichs, K.A.; Buchanan, S.K.; Botos, I. Building better barrels—β-barrel biogenesis and insertion in bacteria and mitochondria. *J. Mol. Biol.* **2021**, *433*, 166894. [CrossRef]

77. Höhr, A.I.; Lindau, C.; Wirth, C.; Qiu, J.; Stroud, D.A.; Kutik, S.; Guiard, B.; Hunte, C.; Becker, T.; Pfanner, N.; et al. Membrane protein insertion through a mitochondrial β-barrel gate. *Science* **2018**, *359*, eaah6834. [CrossRef] [PubMed]
78. Ellenrieder, L.; Opaliński, Ł.; Becker, L.; Krüger, V.; Mirus, O.; Straub, S.P.; Ebell, K.; Flinner, N.; Stiller, S.B.; Güirad, B.; et al. Separating mitochondrial protein assembly and endoplasmic reticulum tethering by selective coupling of Mdm10. Nat. Commun. 2016, 7, 13021. [CrossRef]

79. Yamano, K.; Tanaka-Yamano, S.; Endo, T. Tom7 regulates Mdm10-mediated assembly of the mitochondrial import channel protein Tom40. J. Biol. Chem. 2010, 285, 41222–41231. [CrossRef]

80. Thornton, N.; Stroud, D.A.; Milenkovic, D.; Güirad, B.; Pfanner, N.; Becker, T. Two modular forms of the mitochondrial sorting and assembly machinery are involved in biogenesis of α-helical outer membrane proteins. J. Mol. Biol. 2010, 396, 540–549. [CrossRef]

81. Becker, T.; Güirad, B.; Thornton, N.; Zufall, N.; Stroud, D.A.; Wiedemann, N.; Pfanner, N. Assembly of the mitochondrial protein import channel: Role of Tom5 in two-stage interaction of Tom40 with the SAM complex. Mol. Biol. Cell. 2010, 21, 3106–3113. [CrossRef] [PubMed]

82. Dukanovic, J.; Dimmer, K.S.; Bonnefoy, N.; Krümpke, K.; Rapaport, D. Genetic and functional interactions between the mitochondrial outer membrane proteins Tom6 and Sam37. Mol. Cell. Biol. 2009, 29, 5975–5988. [CrossRef] [PubMed]

83. Meisinger, C.; Rissler, M.; Chacinska, A.; Szklarz, L.K.S.; Milenkovic, D.; Kozjak, V.; Schönfisch, B.; Lohaus, C.; Meyer, H.E.; Yaffe, M.P.; et al. The mitochondrial morphology protein Mdm10 functions in assembly of the preprotein translocase of the outer membrane. Dev. Cell. 2004, 7, 61–71. [CrossRef] [PubMed]

84. Becker, T.; Pfannschmidt, S.; Güirad, B.; Stojanovski, D.; Milenkovic, D.; Kütik, S.; Pfanner, N.; Meisinger, C.; Wiedemann, N. Biogenesis of the mitochondrial TOM complex: Mim1 promotes insertion and assembly of signal-anchoranchored receptors. J. Biol. Chem. 2008, 283, 120–127. [CrossRef]

85. Hulett, J.M.; Lueker, F.; Chan, N.C.; Perry, A.J.; Wolynec, P.; Likić, V.A.; Gooley, P.R.; Lithgow, T. The transmembrane segment of Tom20 is recognized by Mim1 for docking to the mitochondrial TOM complex. J. Mol. Biol. 2008, 387, 694–704. [CrossRef] [PubMed]

86. Hulett, J.M.; Lueker, F.; Chan, N.C.; Perry, A.J.; Wolynec, P.; Likić, V.A.; Gooley, P.R.; Lithgow, T. The transmembrane segment of Tom20 is recognized by Mim1 for docking to the mitochondrial TOM complex. J. Mol. Biol. 2008, 387, 694–704. [CrossRef] [PubMed]

87. Becker, T.; Wenz, L.S.; Krüger, V.; Lehmann, W.; Muller, J.M.; Goroncy, L.; Zufall, N.; Lithgow, T.; Güirad, B.; Chacinska, A.; et al. The mitochondrial import protein Mim1 promotes biogenesis of multispanning outer membrane proteins. J. Biol. Chem. 2011, 194, 387–395. [CrossRef]

88. Papic, D.; Krumpe, K.; Dukanovic, J.; Dimmer, K.S.; Rapaport, D. Multispan mitochondrial outer membrane protein Ugo1 follows a unique Mim1-dependent import pathway. J. Cell Biol. 2011, 194, 397–405. [CrossRef]

89. Dimmer, K.S.; Papic, D.; Schumann, B.; Sperl, D.; Krumpe, K.; Walther, D.M.; Rapaport, D.A. Crucial role for Mim2 in the biogenesis of mitochondrial outer membrane proteins. J. Cell Sci. 2012, 125, 3464–3473. [CrossRef]

90. Doan, K.N.; Grevel, A.; Märtensson, C.U.; Ellenrieder, L.; Thornton, N.; Wenz, L.S.; Opaliński, Ł.; Güirad, B.; Pfanner, N.; Becker, T. The mitochondrial import complex Mim functions as main translocase for α-helical outer membrane proteins. Cell Rep. 2020, 31, 107567. [CrossRef]

91. Vitali, D.G.; Drwesh, L.; Cichocki, B.A.; Kolb, A.; Rapaport, D. The biogenesis of mitochondrial outer membrane proteins show variable dependence on import factors. iScience 2020, 23, 100779. [CrossRef] [PubMed]

92. Zhou, J.; Jung, M.; Dimmer, K.S.; Rapaport, D. The multi-factor modulated biogenesis of the mitochondrial multi-span protein Om14. J. Cell Biol. 2022, 221, e202112030. [CrossRef] [PubMed]

93. Sauerwald, J.; Jores, T.; Eisenberg-Bord, M.; Chauritzman, S.G.; Schuldiner, M.; Rapaport, D. Genome-wide screens in Saccharomyces cerevisiae highlight a role for cardiolipin in biogenesis of mitochondrial outer membrane multispan proteins. Mol. Cell. Biol. 2015, 35, 3200–3211. [CrossRef] [PubMed]

94. Vögtle, F.N.; Keller, M.; Taskin, A.A.; Horvath, S.E.; Guan, X.L.; Prinz, C.; Opalińska, M.; Zorzin, C.; van der Laan, M.; Wenk, M.R.; et al. The fusogenic lipid phosphatidic acid promotes the biogenesis of mitochondrial outer membrane protein Ugo1. J. Cell Biol. 2020, 210, 951–960. [CrossRef] [PubMed]

95. Kemper, C.; Habib, S.J.; Engl, G.; Heckmeyer, P.; Dimmer, K.S.; Rapaport, D. Integration of tail-anchored proteins into the mitochondrial outer membrane does not require any known import components. J. Cell Sci. 2008, 121, 1990–1998. [CrossRef] [PubMed]

96. Krumpe, K.; Frumkin, I.; Herzig, Y.; Rimon, N.; Ozbalci, C.; Brügger, B.; Rapaport, D.; Schuldiner, M. Ergosterol content specifies targeting of tail-anchored proteins to mitochondrial outer membranes. Mol. Biol. Cell. 2012, 23, 3927–3935. [CrossRef] [PubMed]

97. Lang, C.; Nett, J.H.; Trumpower, B.L.; Hunte, C. Specific roles of protein-phospholipid interactions in the yeast cytochrome bc1 complex structure. EMBO J. 2001, 20, 6591–6600. [CrossRef] [PubMed]

98. Shinzawa-Itoh, K.; Aoyama, H.; Muramoto, K.; Terada, H.; Kurauchi, T.; Tadehara, Y.; Yamasaki, A.; Sugimura, T.; Kurono, S.; Tsujimoto, K.; et al. Structures and physiological roles of 13 integral lipids of bovine heart cytochrome c oxidase. EMBO J. 2007, 26, 1713–1725. [CrossRef]

99. Cruciat, C.M.; Brunner, S.; Baumann, F.; Neupert, W.; Stuart, R.A. The cytochrome bc1 and cytochrome c oxidase complexes associate to form a single supramolecular complex in yeast mitochondria. J. Biol. Chem. 2000, 275, 18093–18098. [CrossRef]

100. Schägger, H.; Pfeiffer, K. Supramolecular complexes in the respiratory chains of yeast and mammalian mitochondria. EMBO J. 2000, 19, 1777–1783. [CrossRef]
101. Böttger, L.; Guiard, B.; Oeljeklaus, S.; Kulawiak, B.; Zufall, N.; Wiedemann, N.; Warscheid, B.; van der Laan, M.; Becker, T. A complex of Cox4 and mitochondrial Hsp70 plays an important role in the assembly of the cytochrome c oxidase. *Mol. Biol. Cell.* 2013, 24, 2609–2619. [CrossRef] [PubMed]

102. Zhang, M.; Mileykovskaya, E.; Dowhan, W. Gluing the respiratory chain together. Cardiolipin is required for supercomplex formation in the inner mitochondrial membrane. *J. Biol. Chem.* 2002, 277, 43535–43556. [CrossRef] [PubMed]

103. Pfeiffer, K.; Gohil, V.; Stuart, R.A.; Hunte, C.; Brandt, U.; Greenberg, M.L.; Schägger, H. Cardiolipin stabilizes respiratory chain supercomplexes. *J. Biol. Chem.* 2003, 278, 52873–52880. [CrossRef] [PubMed]

104. Zhang, M.; Mileykovskaya, E.; Dowhan, W. Cardiolipin is essential for organization of complexes III and IV into a supercomplex in intact yeast mitochondria. *J. Biol. Chem.* 2005, 280, 29403–29408. [CrossRef] [PubMed]

105. McKenzie, M.; Lazarou, M.; Thorburn, D.R.; Ryan, M.T. Mitochondrial respiratory chain supercomplexes are destabilized in Barth Syndrome patients. *J. Mol. Biol.* 2006, 361, 462–469. [CrossRef] [PubMed]

106. Wenz, T.; Hielscher, R.; Hellwig, P.; Schägger, H.; Richers, S.; Hunte, C. Role of phospholipids in respiratory cytochrome bc1 complex catalysis and supercomplex formation. *Biochem. Biophys. Acta* 2009, 1787, 609–616. [CrossRef] [PubMed]

107. Calzada, E.; Avery, E.; Pingdewinde, N.S.; Modak, A.; Wang, C.; McCaffrey, J.M.; Han, X.; Alder, N.N. Phosphatidylethanolamine made in the inner mitochondrial membrane is essential for yeast cytochrome bc1 complex formation. *Nat. Commun.* 2019, 10, 1432. [CrossRef]

108. Böttger, L.; Horvath, S.E.; Kleinschroth, T.; Hunte, C.; Daum, G.; Pfanner, N.; Becker, T. Phosphatidylethanolamine and cardiolipin differentially affect the stability of mitochondrial respiratory chain supercomplexes. *J. Mol. Biol.* 2012, 423, 677–686. [CrossRef]

109. Tasseva, G.; Bai, H.D.; Davidescu, M.; Haromy, A.; Michelakis, E.; Vance, J.E. Phosphatidylethanolamine deficiency in mammalian mitochondria impairs oxidative phosphorylation and alters mitochondrial morphology. *J. Biol. Chem.* 2013, 288, 4158–4173. [CrossRef]

110. Baker, C.D.; Ball, W.B.; Pryce, E.N.; Gohil, V.M. Specific requirements of nonbilayer phospholipids in mitochondrial respiratory chain function and formation. *Mol. Biol. Cell.* 2006, 27, 2161–2171. [CrossRef]

111. Jiang, F.; Ryan, M.T.; Schlame, M.; Zhao, M.; Gu, Z.; Klingenberg, M.; Pfanner, N.; Greenberg, M.L. Absence of cardiolipin in the crd1 null mutant results in decreased mitochondrial membrane potential and reduced mitochondrial function. *J. Biol. Chem.* 2000, 275, 22387–22394. [CrossRef] [PubMed]

112. Gallas, M.R.; Dienhart, M.K.; Stuart, R.A.; Long, R.M. Characterization of Mmp37p, a Saccharomyces cerevisiae mitochondrial matrix protein with a role in mitochondrial protein import. *Mol Cell. Biol.* 2006, 17, 4051–4062. [CrossRef] [PubMed]

113. Tamura, Y.; Harada, Y.; Yamano, K.; Watanabe, K.; Ishikawa, D.; Ohshima, C.; Nishikawa, S.-I.; Yamamoto, H.; Endo, T. Identification of Tam41 maintaining integrity of the TIM23 protein translocator complex in mitochondria. *J. Cell Biol.* 2006, 174, 631–637. [CrossRef] [PubMed]

114. Kutik, S.; Rissler, M.; Guan, X.L.; Guiard, B.; Shui, G.; Gebert, N.; Heacock, P.N.; Rehling, P.; Dowhan, W.; Wenk, M.R.; et al. The translocator maintenance protein Tam41 is required for mitochondrial cardiolipin biosynthesis. *J. Cell Biol.* 2008, 183, 1213–1221. [CrossRef]

115. Tamura, Y.; Endo, T.; Iijima, M.; Sesaki, H. Upslp and Ups2p antagonistically regulate cardiolipin metabolism in mitochondria. *J. Cell Biol.* 2009, 185, 1029–1045. [CrossRef]

116. Schuler, M.H.; Di Bartolomeo, F.; Mårtensson, C.U.; Daum, G.; Becker, T. Phosphatidylcholine affects inner membrane protein translocases of mitochondria. *J. Biol. Chem.* 2016, 291, 18718–18729. [CrossRef]

117. Truscott, K.N.; Kovermann, P.; Geissler, A.; Meier, M.; Driessen, A.J.; Pfanner, N.; Wagner, R. A presenec-and voltage-sensitive channel of the mitochondrial preprotein translocase formed by Tim23. *Nat. Struct. Biol.* 2001, 8, 1074–1082. [CrossRef]

118. Martinez-Caballero, S.; Grigoriev, S.M.; Herrmann, J.M.; Campo, M.L.; Schuler, M.H.; Herrmann, J.M. A disulfide bond in the TIM23 complex is crucial for voltage gating and mitochondrial protein import. *J. Biol. Chem.* 2006, 281, 29403–29408. [CrossRef]

119. Meinecke, M.; Wagner, R.; Kovermann, P.; Guiard, B.; Mick, D.U.; Hutu, D.P.; Voos, W.; Truscott, K.; Chacinska, A.; Pfanner, N.; et al. Tim50 maintains the permeability barrier of the mitochondrial inner membrane. *Science* 2006, 312, 1523–1526. [CrossRef]

120. Ieva, R.; Schrempp, S.G.; Opaliński, Ł.; Wollweber, F.; Hoë, P.; Heiβwolf, A.K.; Gebert, M.; Zhang, Y.; Guiard, B.; Rospert, S.; et al. Mgr2 functions as lateral gatekeeper for preprotein sorting in the mitochondrial inner membrane. *Mol. Cell.* 2013, 56, 641–652. [CrossRef] [PubMed]

121. Martínez-Caballero, S.; Frazier, A.E.; Dudek, J.; Meisinger, C.; Geissler, A.; Sickmann, A.; Meyer, H.E.; Truscott, K.N.; Guiard, B.; et al. Mitochondrial presequence translocation: Switching between TOM tethering and motor recruitment involves Tim21 and Tim17. *Cell* 2005, 120, 817–829. [CrossRef] [PubMed]

122. Chacinska, A.; Lind, M.; Mileykovskaya, E.; Dowhan, W. Protein import into mitochondria. *Biochem. Soc. Trans.* 2005, 33, 1019–1023. [CrossRef] [PubMed]

123. Waegemann, K.; Popov-Čeleketa, D.; Neupert, W.; Azem, A.; Mokranjac, D. Cooperation of TOM and TIM23 complexes during translocation of proteins into mitochondria. *J. Mol. Biol.* 2015, 427, 1075–1084. [CrossRef]
125. Lytvovenko, O.; Melin, J.; Schulz, C.; Kilisch, M.; Hutu, D.P.; Rehling, P. Signal recognition initiation reorganization of the presequence translocase during protein import. *EMBO J.* 2013, 32, 886–898. [CrossRef]

126. van der Laan, M.; Meinecke, M.; Dudek, J.; Hutu, D.P.; Lind, M.; Perschil, I.; Guiard, B.; Wagner, R.; Pfanner, N.; Rehling, P. Motor-free mitochondrial presequence translocase drives membrane integration of preproteins. *Nat. Cell Biol.* 2007, 9, 1152–1159. [CrossRef]

127. Schulz, C.; Schendzielorz, A.; Rehling, P. Unlocking the presequence import pathway. *Trends Cell Biol.* 2015, 25, 265–275. [CrossRef]

128. Moulin, C.; Caumont-Sarcos, A.; Ieva, R. Mitochondrial presequence import: Multiple regulatory knobs fine-tune mitochondrial biogenesis and homeostasis. *Biochim. Biophys. Acta* 2019, 1866, 930–944. [CrossRef]

129. Mokranjac, D. How to get to the other side of the mitochondrial inner membrane—the protein import motor. *Biol. Chem.* 2020, 401, 723–736. [CrossRef]

130. Weiss, C.; Oppliger, W.; Vergères, G.; Demel, R.; Jenö, P.; Horst, M.; de Kruijff, B.; Schatz, G.; Azem, A. Domain structure and lipid interaction of recombinant yeast Tim44. *Proc. Natl. Acad. Sci. USA* 1996, 8890–8894. [CrossRef]

131. Marom, M.; Safonov, R.; Amram, S.; Avneon, Y.; Nachliel, E.; Gutman, M.; Zohary, K.; Azem, A.; Tsfadia, Y. Interaction of the Tim44 C-terminal domain with negatively charged phospholipids. *Biochemistry* 2009, 48, 11815–11819. [CrossRef] [PubMed]

132. Bajaj, R.; Jaremko, Ł.; Jaremko, M.; Becker, S.; Zweckstetter, M. Molecular basis of the dynamic structure of the TIM23 complex in the mitochondrial intermembrane space. *Structure* 2014, 22, 1501–1511. [CrossRef] [PubMed]

133. Malhotra, K.; Modak, A.; Nangia, S.; Daman, T.H.; Gunsel, U.; Robinson, V.L.; Mokranjac, D.; May, E.R.; Alder, N.N. Cardiolipin mediates membrane and channel interactions of the mitochondrial TIM23 protein import complex receptor Tim50. *Sci. Adv.* 2017, 3, e1700532. [CrossRef]

134. Zhang, Y.; Ou, X.; Wang, X.; Sun, D.; Zhou, X.; Wu, X.; Li, Q.; Li, L. Structure of the mitochondrial TIM22 complex from yeast. *Cell Res.* 2021, 31, 366–368. [CrossRef] [PubMed]

135. Qi, L.; Wang, Q.; Guan, Z.; Wu, Y.; Shen, C.; Hong, S.; Jianbo, C.; Zhang, X.; Yan, C.; Yin, P. Cryo-EM structure of the human mitochondrial translocase TIM22 complex. *Cell Res.* 2021, 31, 369–372. [CrossRef] [PubMed]

136. Claypool, S.M.; Oktay, Y.; Boontheung, P.; Loo, J.A.; Koehler, C.M. Cardiolipin defines the interactome of the major ADP/ATP carrier protein of the mitochondrial inner membrane. *J. Cell Biol.* 2008, 182, 937–950. [CrossRef]

137. Senoo, N.; Kandasamy, S.; Ogubona, O.B.; Baile, M.G.; Lu, Y.; Claypool, S.M. Cardiolipin, conformation, and respiratory complex-dependent oligomerization of the major mitochondrial ADP/ATP carrier in yeast. *Sci. Adv.* 2020, 6, eabb0780. [CrossRef]

138. Kang, Y.; Stroud, D.A.; Baker, M.J.; De Souza, D.P.; Frazier, A.E.; Liem, M.; Tull, D.; Mathivanan, S.; McConvilje, M.J.; Thorburn, D.R.; et al. Sengers syndrome-associated mitochondrial acylglycerol kinase is a subunit of the human TIM22 protein import complex. *Mol. Cell.* 2017, 67, 457–470. [CrossRef]

139. Vukotic, M.; Nolte, H.; König, T.; Saita, S.; Ananjew, M.; Krüger, M.; Tatsuta, T.; Langer, T. Acylglycerol kinase mutated in Sengers syndrome is a subunit of the TIM22 protein translocase in mitochondria. *Mol. Cell.* 2017, 67, 471–483. [CrossRef]

140. Mayr, J.A.; Haack, T.B.; Graf, E.; Zimmermann, F.A.; Wieland, T.; Haberberger, B.; Superti-Furga, A.; Kirschner, J.; Steinmann, B.; Baumgartner, M.P.; et al. Lack of the mitochondrial protein acylglycerol kinase causes Sengers syndrome. *Am. J. Hum. Genet.* 2012, 90, 314–320. [CrossRef]

141. Tatsuta, T.; Langer, T. Intramitochondrial phospholipid trafficking. *Biochim. Biophys. Acta* 2017, 1862, 81–89. [CrossRef] [PubMed]

142. Kornmann, B.; Currie, E.; Collins, S.R.; Schuldiner, M.; Nunnari, J.; Weissman, J.S.; Walter, P. An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* 2009, 325, 477–481. [CrossRef] [PubMed]

143. Elbaz-Alon, Y.; Rosenfeld-Gur, E.; Shinder, V.; Futerman, A.H.; Geiger, T.; Schuldiner, M. A dynamic interface between vacuoles and mitochondria in yeast. *Dev. Cell.* 2014, 30, 95–102. [CrossRef] [PubMed]

144. Hönscher, C.; Mari, M.; Auffarth, K.; Bohnert, M.; Griffith, J.; Geerts, W.; van der Laan, M.; Cabrera, M.; Reggiori, F.; Ungermann, C. Cellular metabolism regulates contact sites between vacuoles and mitochondria. *Dev. Cell.* 2014, 30, 86–94. [CrossRef] [PubMed]

145. AhYoung, A.P.; Jiang, J.; Zhang, J.; Dang, X.K.; Loo, J.A.; Zhou, Z.H.; Egea, P.F. Conserved SMP domains of the ERMES complex bind phospholipids and mediate tether assembly. *Proc. Natl. Acad. Sci. USA* 2015, 112, E3179–E3188. [CrossRef]

146. Jeong, H.; Park, J.; Jun, Y.; Lee, C. Crystal structures of Mmm1 and Mdm12–Mmm1 reveal mechanistic insight into phospholipid trafficking at ER-mitochondria contact sites. *Proc. Natl. Acad. Sci. USA* 2017, 114, E9502–E9511. [CrossRef]

147. Renne, M.F.; Bao, X.; Hokken, M.W.; Bierhuizen, A.S.; Hermansson, M.; Sprenger, R.R.; Ewing, T.A.; Ma, X.; Cox, R.C.; Brouwers, J.F.; et al. Molecular species selectivity of lipid transport creates a mitochondrial sink for di-unsaturated phospholipids. *EMBO J.* 2022, 41, e106837. [CrossRef]

148. Ellenrieder, L.; Rampelt, H.; Becker, T. Connection of protein transport and organelle contact sites in mitochondria. *J. Mol. Biol.* 2017, 429, 2148–2160. [CrossRef]

149. Lahiri, S.; Chao, J.T.; Tavassoli, S.; Wong, A.K.; Choudhary, V.; Young, B.P.; Loewen, C.J.R.; Prinz, W.A. A conserved endoplasmic reticulum membrane protein complex (EMC) facilitates phospholipid transfer from the ER to mitochondria. *PLoS Biol.* 2014, 12, e1001969. [CrossRef]

150. Guna, A.; Volkmar, N.; Christianson, J.C.; Hegde, R.S. The ER membrane protein complex is a transmembrane domain insertase. *Science* 2018, 359, 470–473. [CrossRef]
151. Pleiner, T.; Tomaleri, G.P.; Januszyk, K.; Inglis, A.J.; Hazu, M.; Voorhees, R.M. Structural basis for membrane insertion by the human ER membrane protein complex. *Science* 2020, 369, 433–436. [CrossRef] [PubMed]

152. Murley, A.; Sarsam, R.D.; Toulmay, A.; Yamada, J.; Prinz, W.A.; Nunnari, J. Ltc1 is an ER-localized sterol transporter and a component of ER–mitochondria and ER–vacuole contacts. *J. Cell Biol.* 2015, 209, 539–548. [CrossRef] [PubMed]

153. Elbaz-Alon, Y.; Eisenberg-Bord, M.; Shinder, V.; Stiller, S.B.; Shimon, E.; Wiedemann, N.; Geiger, T.; Schuldiner, M. Lam6 regulates the extent of contacts between organelles. *Cell Rep.* 2015, 12, 7–14. [CrossRef] [PubMed]

154. Montoro, A.G.; Auffarth, K.; Hönscher, C.; Bohnert, M.; Becker, T.; Warscheid, B.; Reggiori, F.; van der Laan, M.; Fröhlich, F.; Ungermann, C. Vps39 interacts with Tom40 to establish one of two functionally distinct vacuole-mitochondria contact sites. *Dev. Cell.* 2018, 45, 621–636. [CrossRef] [PubMed]

155. Klein, A.; Israel, L.; Lackey, S.W.K.; Nargang, F.E.; Imhof, A.; Baumeister, W.; Neupert, W.; Thomas, D.R. Characterization of the insertase for β-barrel proteins of the outer mitochondrial membrane. *J. Cell Biol.* 2012, 199, 599–611. [CrossRef]

156. Meisinger, C.; Pfannschmidt, S.; Rissler, M.; Milenkovic, D.; Becker, T.; Stojanovski, D.; Youngman, M.J.; Jensen, R.E.; Chacinska, A.; Guiard, B.; et al. The morphology proteins Mdm12/Mmm1 function in the major β-barrel assembly pathway of mitochondria. *EMBO J.* 2007, 26, 2229–2239. [CrossRef]

157. Meisinger, C.; Wiedemann, N.; Rissler, M.; Strub, A.; Milenkovic, D.; Schönfisch, B.; Müller, H.; Kozjak, V.; Pfanner, N. Mitochondrial protein sorting: Differentiation of β-barrel assembly by Tom7-mediated segregation of Mdm10. *J. Biol. Chem.* 2006, 281, 22819–22826. [CrossRef]

158. Flinner, N.; Ellenrieder, L.; Stiller, S.B.; Becker, T.; Schleiff, E.; Mirus, O. Mdm10 is an ancient eukaryotic porin co-occurring with the ERMES complex. *Biochim. Biophys. Acta* 2013, 1833, 3314–3325. [CrossRef]

159. Yang, X.; Liang, J.; Ding, L.; Li, X.; Lam, S.M.; Shui, G.; Ding, M.; Huang, X. Phosphatidylserine synthase regulates cellular homeostasis through distinct metabolic mechanisms. *PLoS Genet.* 2019, 15, e1008548. [CrossRef]

160. MacVicar, T.; Ohba, Y.; Nolte, H.; Mayer, F.C.; Tatsuta, T.; Sprenger, H.G.; Lindner, B.; Zhao, Y.; Li, J.; Bruns, C.; et al. Lipid signalling drives proteolytic rewiring of mitochondria by YME1L. *Nature* 2019, 575, 361–365. [CrossRef]