Review

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Lipid homeostasis in mitochondria

<https://doi.org/10.1515/hsz-2020-0121>

Received January 30, 2020; accepted March 10, 2020

Abstract: Mitochondria are surrounded by the two membranes, the outer and inner membranes, whose lipid compositions are optimized for proper functions and structural organizations of mitochondria. Although a part of mitochondrial lipids including their characteristic lipids, phosphatidylethanolamine and cardiolipin, are synthesized within mitochondria, their precursor lipids and other lipids are transported from other organelles, mainly the ER. Mitochondrially synthesized lipids are re-distributed within mitochondria and to other organelles, as well. Recent studies pointed to the important roles of inter-organelle contact sites in lipid trafficking between different organelle membranes. Identification of Ups/PRELI proteins as lipid transfer proteins shuttling between the mitochondrial outer and inner membranes established a part of the molecular and structural basis of the still elusive intra-mitochondrial lipid trafficking.

Keywords: cardiolipin; cristae; ERMES; inter-organelle contact; mitochondria; phosphatidylethanolamine; phospholipids; Ups/PRELI.

Introduction

Mitochondria are central in cellular energy production, metabolism and signaling. Mitochondria have two membranes, the outer membrane (OM) and inner membrane (IM), and two aqueous compartments, the intermembrane space (IMS) between the OM and IM and the innermost

matrix, and consist of ~1000 different proteins and >400 different lipids, including fatty acids, glycerophospholipids, glycerolipids, sphingolipids and prenols (Bird et al., 2013). Since mitochondria cannot be made *de novo*, synthesis and transport of proteins and lipids are a prerequisite for mitochondrial biogenesis. Transport of mitochondrial proteins, most of which are synthesized outside mitochondria, has been extensively studied mainly by the Jeff Schatz group and the Walter Neupert group in the beginning and later by many other groups worldwide, yet studies on lipid transport have lagged behind those on protein transport. The systems and mechanisms of lipid transport have thus been overlooked for a long time.

Reflecting the endosymbiotic origin of mitochondria, the IM of mitochondria show similarity in its lipid composition to those of bacterial cell membranes. The inner cell membrane of bacteria like *Escherichia coli* contains a high content of phosphatidylethanolamine (PE; ~75% of membrane lipids) and anionic lipids phosphatidylglycerol (PG; ~10–20%) and cardiolipin (CL; 5–10%), but lacks phosphatidylcholine (PC) and sterols. Mammalian cytoplasmic membranes contain mainly PC (40%), PE (25%), phosphatidylinositol (PI; 10%), phosphatidylserine (PS; 10%), phosphatidic acid (PA; 1%) as well as sphingolipids (20%) and sterols. On the other hand, mitochondrial IMs have high contents of PE (~35%), CL (~20%), PC (~40%) and PI (~5%) in mammals and PE (~25%), CL (~15%), PC (~40%) and PI (~15%) in yeast (Horvath and Daum, 2013). CL is required for optimal activities of various IM proteins including the translocator complexes and respiratory-chain complexes, and a small fraction of CL in the OM (~5–10%; Gebert et al., 2009) was suggested to be important for regulation of mitochondria-specific autophagy or mitophagy (Chu et al., 2013). As CL and PE are non-bilayer forming phospholipids, they contribute to destabilization as well as high membrane curvatures of the IM, which would be favorable for efficient fusion and fission of mitochondrial membranes (Basu Ball et al., 2018).

Mitochondrial lipid homeostasis or an optimum lipid composition of the OM and IM relies on the balance between synthesis and trafficking of mitochondrial lipids. Mitochondria can synthesize CL and PE, phospholipids essential for mitochondrial functions, and some minor lipids, including CDP-diacylglycerol (DAG), phosphatidylglycerol

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phosphate (PGP), and PG, while other phospholipids are synthesized mainly in the endoplasmic reticulum (ER). Therefore, vast amounts of phospholipids other than PE and CL, including precursor lipids for the CL and PE synthesis, are imported from other organelles, primarily the ER, but partly from the vacuole as well. Although we are only in the beginning of understanding the cellular mechanism behind the mitochondrial lipid homeostasis, we summarize here the recent findings on phospholipid synthesis in mitochondria and lipid transport through mitochondria, and discuss their prospects.

Phospholipid synthesis in mitochondria

While most of the enzymes involved in cellular phospholipid synthesis reside in the secretory-pathway organelles, including the ER, mitochondria can synthesize phospholipids, PE and CL, with the aid of groups of lipid synthetic enzymes located in the mitochondrial IM (Figure 1). CL and PE are essential phospholipids for mitochondrial respiratory functions (Baker et al., 2016; Calzada et al., 2019). CL is a reverse cone-shaped phospholipid with the structure of dimeric phospholipid, which is indispensable for the functional integrity of mitochondria (Joshi et al., 2009; Ren et al., 2014). In particular, CL plays an important role in stabilizing the protein complexes localized

in the IM such as respiratory-chain complexes and the translocators like the TIM23 complex (Pfeiffer et al., 2003; Malhotra et al., 2017). PE and CL have common properties as non-bilayer forming phospholipids, and lowering the levels of both PE and CL leads to serious growth defects of cells (Joshi et al., 2012; Basu Ball et al., 2018).

PE is generated from PS by PSD (Psd1 in yeast) in the IM, a PS decarboxylase conserved from bacteria to humans (Figure 1). Psd1 is synthesized as an inactive precursor form with the N-terminal presequence in the cytosol. Psd1 is imported into mitochondria with the aid of the translocators in the OM and IM, the TOM complex and the TIM23 complex, respectively. Psd1 is anchored to the IM by its transmembrane (TM) segment by the stop-transfer mechanism at the TIM23 complex upon processing of the presequence by the matrix-localized processing peptidases MPP and Oct1 (Horvath et al., 2012). Then the conserved LGST sequence near the C-terminus of Psd1 is autocatalytically cleaved to form the C-terminal α -subunit and N-terminal β -subunit, which interact noncovalently with each other after the cleavage. Formation of the pyruvate residue at the N-terminus of the α -subunit upon the cleavage renders the heterodimeric Psd1 complex enzymatically active (Li and Dowhan, 1990; Horvath et al., 2012).

The precursor lipid PS for generating PE is synthesized by PS synthetase (PSS, Cho1 in yeast) in the ER membrane (Bae-Lee and Carman, 1984). PS is imported into mitochondria, and then decarboxylated to PE by Psd1

Figure 1: Current model for phospholipid synthesis and transport in the ER and mitochondria in yeast.

Arrows with solid and dotted lines indicate phospholipid conversion and transport, respectively. Red arrows indicate direct involvement in lipid transfer or phospholipid synthesis. Psd1 localized in the ER membrane is drawn by dotted line because the ER-localization is carbon-source dependent (Friedman et al., 2018). The decreased PE level may activate the alternative CL synthetic pathway by, for example, facilitating the transport of PA (and other lipids) from the OM to IM through the action of Mdm31, Mdm32, Fmp30 and/or the Mdm31-Por1 interaction (Miyata et al., 2017, 2018).

(Choi et al., 2000a). This PE further moves back from mitochondria to the ER, and is converted to PC by methylation by Cho2 and Opi3 methyltransferases localized in the ER (Kodaki and Yamashita, 1987). On the other hand, it was reported that Psd1 is also localized in the ER membrane in a metabolic-state-dependent manner, and that the ER-resident Psd1 plays a critical role in cellular phospholipid homeostasis (Friedman et al., 2018) (Figure 1).

Mitochondrial Psd1 is responsible for the majority of PE synthesis in wild-type yeast cells, whereas a part of PE is also synthesized outside mitochondria through the Kennedy pathway and PS decarboxylation by Psd2 at the Golgi and vacuole membranes and/or endosomes (Trotter and Voelker, 1995; Bürgermeister et al., 2004; Gulshan et al., 2010). Extra-mitochondrially synthesized PE tends to stay outside mitochondria (Bürgermeister et al., 2004). However, a recent study revealed that PE synthesized via the Kennedy pathway in the ER can be imported into mitochondria and partly restores the defective respiratory functions due to loss of Psd1 and that Vps39 plays a critical role in the transport of PE to mitochondria probably via vacuole-mitochondria contact sites (Iadarola et al., 2020).

Another important phospholipid synthesized exclusively in mitochondria is CL. CL is synthesized from PA through multiple modification steps (Figure 1). PA is first converted to CDP-DAG as a high-energy intermediate phospholipid. In the beginning, a conserved CDP-DAG synthase Cds1 was thought to catalyze the PA-to-CDP-DAG conversion in both the ER membrane and mitochondrial IM in yeast (Kuchler et al., 1986; Kelley and Carman, 1987). However, Cds1 was found to be exclusively localized in the ER, indicating the existence of another CDP-DAG synthase in mitochondria. A candidate of the mitochondrial CDP-DAG synthase was Tam41, which was initially identified as a protein facilitating the assembly of the IM translocator, the TIM23 complex, and thus the mitochondrial protein import (Gallas et al., 2006; Tamura et al., 2006). Tam41 is a peripheral membrane protein present on the matrix side of the IM, and its loss leads to the CL depletion (Kutik et al., 2008). We therefore purified Tam41 from yeast cells and demonstrated that Tam41 directly catalyzed the CDP-DAG synthesis from PA, which indicates that Tam41 is the mitochondria-specific CDP-DAG synthase (Tamura et al., 2013; Jiao et al., 2019).

Then, PGP synthase (Pgs1 in yeast), a peripheral membrane protein on the matrix side of the IM, conjugates CDP-DAG and glycerol 3-phosphate to form PGP (Janitor et al., 1995; Chang et al., 1998a). PGP is then dephosphorylated by PGP phosphatase (Gep4 in yeast), another peripheral membrane protein on the matrix side of the IM,

to be converted to PG (Osman et al., 2010). Finally, CL synthase (CLS, Crd1 in yeast), an integral membrane protein with multiple TM segments in the IM, combines PG and CDP-DAG to generate an acidic phospholipid with four acyl chains, CL (Chang et al., 1998b; Tuller et al., 1998). Newly synthesized CL undergoes a further remodeling process that generates more symmetric CL molecules with unsaturated acyl chains by removal of one acyl chain by a deacylase (Cld1 in yeast) (Beranek et al., 2009; Baile et al., 2014) and subsequent transacylation of the resultant mono-lyso CL (MLCL) by the transacylase Taz1 (yeast) or tafazzin TAZ (mammal) (Gu et al., 2004). Taz1 is present in both the OM and IM, exposing the catalytic domain to the IMS (Claypool et al., 2006). Mutations in the *TAZ* genes cause Human Barth syndrome (BTHS), an X-linked genetic disorder, primarily affecting males (Barth et al., 2004). As cells with defective Taz1 can be rescued by deletion of the *CLD1* gene in yeast, accumulation of MLCL or the decreased CL/MLCL ratio appears to cause BTHS (Ye et al., 2014). Recently, mutations in *PISD* encoding the mitochondrial PE synthase were also reported to lead to a human disease, which is similar to “mitochondrial chaperonopathies”, causing skeletal dysplasia, cataracts and white matter changes (Zhao et al., 2019). This report highlights the important roles of non-bilayer phospholipids in the maintenance of normal mitochondrial functions.

A small fraction of CL was reported to be present in the OM. CL in the OM may be important for the functions of, for example, the translocators TOM and SAM/TOB complexes in the OM (Gebert et al., 2009) and as a cue to eliminate mitochondria by mitophagy (Chu et al., 2013). CL in the OM may result from direct transport of CL from the IM or transport of MLCL from the IM followed by transacylation by OM-localizing Taz1. CL in the OM can be converted to PA by phospholipase D named mitoPLD, which regulates mitochondrial fusion-division dynamics (Choi et al., 2000b; Huang et al., 2011; Zhang et al., 2016; Adachi et al., 2016, 2018).

Phospholipid transport between mitochondria and other organelles

As mitochondria are not linked to the endomembrane system for the secretory pathway, mitochondrial lipids cannot be supplied by vesicular transport from the secretory-pathway organelles like the ER. Soluble lipid transfer proteins could facilitate transport from the ER to mitochondria as in the case of mammalian VAT1 for PS transport (Junker and Rapoport, 2015; Watanabe et al., 2020).

However, accumulated evidence points to the roles of inter-organellar contact sites in phospholipid transport between mitochondria and other organelles. Inter-organellar contact sites are distinct regions where different organelle membranes are closely apposed to each other through specific protein-protein interactions (Scorrano et al., 2019) (Figure 2). The mitochondrial OM and the ER membrane of yeast *Saccharomyces cerevisiae* are physically connected by a protein complex called the ER-mitochondria encounter structure (ERMES) complex, generating mitochondria-ER contact sites (Kornmann et al., 2009). The ERMES complex consists of four core subunits Mmm1, Mdm10, Mdm12 and Mdm34, and accessory proteins including Tom7, Gem1 and Lam6 (Meisinger et al., 2006; Yamano et al., 2010; Kornmann et al., 2011; Stroud et al., 2011; Elbaz-Alon et al., 2015; Gatta et al., 2015; Murley et al., 2015). Mmm1 is an ER membrane protein with a single TM segment at the N-terminus and a large C-terminal domain exposed to the cytosol (Kornmann et al., 2009). On the other hand, Mdm10 and Mdm34 are membrane proteins in the mitochondrial OM; Mdm10 is a β -barrel membrane protein, whereas Mdm34 has no obvious TM regions (Sogo and Yaffe, 1994; Youngman et al., 2004; Flinner et al., 2013). Mdm12 is a soluble protein but binds to Mmm1 and likely Mdm34 (Boldogh et al., 2003; Stroud et al., 2011). The interactions among these factors result in formation of the mitochondria-ER contact sites. The contact sites formed by ERMES can be visualized as discrete foci in cells when Mmm1, Mdm12, Mdm34 or Lam6 is expressed as fusion proteins with a fluorescent protein (Hobbs et al., 2001; Boldogh et al., 2003; Kornmann et al., 2009; Elbaz-Alon et al., 2015; Gatta et al., 2015; Murley et al., 2015). The number of the ERMES foci is four to seven or less per cell, suggesting that each ERMES complex tends to form large

aggregates or clusters at the contact-site regions (Kornmann et al., 2011; Kakimoto et al., 2018).

In addition to the role of the organelle tethering point, ERMES was suggested to provide a route for lipid transport between the ER and mitochondria. However, in the beginning, phospholipid transfer by ERMES was controversial because *in vivo* and *in vitro* analyses showed that loss of an ERMES subunit led to only minor defects in phospholipid transport from the ER to mitochondria as measured by the PS to PE conversion (Nguyen et al., 2012; Voss et al., 2012). This could be partly due to the presence of similar contacts between the ER and mitochondria and between mitochondria and the vacuole. An *in vitro* assay system for inter-organellar lipid transport was recently developed, by using isolated ER membranes and mitochondria (Kojima et al., 2016; Tamura et al., 2019a). This *in vitro* assay system is free from secondary effects owing to the presence of other organelle membranes from, for example, endosomes or the vacuole, which could also be the sources of phospholipid supply *in vivo*. By using this *in vitro* assay system, it was revealed that ERMES plays a major role in PS transport from the ER to mitochondria, but a less important role in PE transport from mitochondria to the ER (Kojima et al., 2016).

Amino-acid sequence comparison showed that Mmm1, Mdm12 and Mdm34 contain a lipid binding domain called the SMP domain (Alva and Lupas, 2016; Wong and Levine, 2017). Indeed, X-ray structures of the SMP domains of Mmm1 and Mdm12 showed that these proteins have a groove or deep cavity which can accommodate a phospholipid molecule(s) (Jeong et al., 2016, 2017; AhYoung et al., 2017; Kawano et al., 2018). Furthermore, the recombinant Mmm1-Mdm12 complex had a phospholipid transport activity between liposomes *in vitro*, although Mmm1

Figure 2: Inter-organellar contact sites in yeast.

Schematic diagrams of the ER-mitochondria (ERMES), the ER/nucleus-vacuole (NVJ), and mitochondria-vacuole (vCLAMP) contacts.

and Mdm12 alone showed only weak lipid transfer activities (Kawano et al., 2018). Mutations inside or at the outlet of the hydrophobic pockets of the SMP domains of recombinant Mmm1 or Mdm12 impaired the lipid transfer activities of the Mdm12-Mmm1 complex and furthermore caused defective PS transport from the ER to mitochondrial membranes via ERMES *in vitro* (Kawano et al., 2018). The structures of Mdm12 with bound phospholipid show that Mdm12 accommodates the acyl-chain tails of phospholipid in the hydrophobic pocket while the head group of the bound phospholipid is exposed to the aqueous phase from the outlet of the pocket. The structure of the Mmm1-phospholipid complex also indicates the presence of a hydrophobic pocket or groove like Mdm12, to which phospholipid binds in a tail-in manner (Jeong et al., 2017). As the high-resolution structure of Mdm34 is not available, the mode of phospholipid binding to the SMP domain of Mdm34 is unclear. Accommodation of mainly the acyl chains of phospholipid in the hydrophobic pocket of Mdm12 indicates that phospholipid recognition by Mdm12 is not highly selective. This finding is consistent with the idea that the ERMES complex may be a relatively unselective phospholipid transport factor for bulk phospholipids rather than specific phospholipids while some preference (less efficient for PE) for the phospholipid transport and binding by ERMES was suggested from *in vitro* lipid transport and binding assays as described above (Kojima et al., 2016; Jeong et al., 2017).

While extensive analysis of ERMES in *S. cerevisiae* advanced our understanding of the ER-mitochondria contacts, higher eukaryotes including humans lack obvious ERMES protein homologs. However, as proteins responsible for cellular phospholipid synthesis are well conserved from yeast to humans, there must be proteins that transport lipid molecules between the ER and mitochondria. In 2017, PDZD8 containing an SMP domain was reported as a functional ortholog of yeast Mmm1 (Hirabayashi et al., 2017). Although lipid transfer functions of PDZD8 was not experimentally tested, the loss of PDZD8 was reported to lead to a significant decrease in the mitochondria-ER contacts, resulting in the abnormal Ca^{2+} transport between the ER and mitochondria. However, phylogenetic analyses indicated that PDZD8 is a paralog of Mmm1 rather than an ortholog (Wiedeman et al., 2018). Besides, a recent study reported that PDZD8 is localized at contact sites between the ER and late endosomes/lysosomes in a Rab7-dependent manner (Guillén-Samander et al., 2019). Therefore, it is still controversial whether PDZD8 primarily functions as a molecular tether between the ER and mitochondria.

What is the mechanism of lipid transport between the ER and mitochondrial membranes by ERMES? Generally,

in order for the lipid to be transported by SMP-domain containing protein(s) between the membranes, the lipid should first partially pop out from the membrane and captured by the SMP domain (desorption step). Then, the SMP-domain containing protein(s) carrying the lipid moves or diffuse to the acceptor membrane (diffusion step), and the lipid is released from the SMP domain for insertion into the membrane (absorption step) (Figure 3A). The rate-limiting step is likely the desorption step (Dittman and Menton, 2017), the energy barrier of which may be surmounted by the SMP domain by disrupting lipid-membrane interactions and providing a hydrophobic lipid-binding pocket. In the case of ERMES, the diffusion step corresponds to the transfer of the lipid through a chain of the SMP-domain containing EREMS subunits, Mmm1-Mdm12-Mdm34. For this step, two possible mechanisms can be considered (Figure 3B) (Kawano et al., 2018). In the lipid carrier model, Mmm1, Mdm12 and Mdm34 change their orientations and positions relative to the other components to bring the outlet of the lipid-binding pocket of one component to the outlet of the next component for transfer of lipid molecules. The continuous conduit model assumes the presence of a continuous hydrophobic route running from Mmm1 to Mdm34 via Mdm12 for processive movement of lipid molecules between Mmm1 and Mdm34. The recently reported X-ray structure of the complex of the Mmm1 and Mdm12 SMP domains from *Zygosaccharomyces rouxii* and *S. cerevisiae*, respectively, showed the presence of a continuous tunnel, but the revealed tunnel is not wide enough to allow lipid transfer between Mmm1 and Mdm12. A variation of this model assumes a continuous cleft instead of a tunnel (Figure 3B). As the conformations of the bound lipids and the sizes of the outlets of the lipid-binding cavities vary for different reported structures of Mmm1 and Mdm12, partly due to crystal packing effects (Jeong et al., 2017; Kawano et al., 2018), the outlet of the SMP domain may be flexible enough to transiently get connected to the outlet of the neighboring SMP domain, forming a continuous hydrophobic cleft. In that case, a phospholipid molecule may slide through the continuous cleft to move from one SMP domain to the next, exposing the hydrophilic head group to the aqueous phase. As the ERMES complex form a large aggregate or cluster, phospholipid molecules could move laterally between the SMP domains as well as in the bilayer-to-bilayer direction. Then it would be an attractive idea that phospholipid molecules are pooled in the SMP domains in the ERMES cluster between the ER and mitochondria. Dynamic nature of the conformations of and interactions among the SMP domain-containing proteins of ERMES should be thus important for lipid transfer between the ER and mitochondria.

Figure 3: Transfer of phospholipid between distinct organelle membranes by SMP-domain-containing protein (s).

(A) A phospholipid (PL) molecule in the bilayer membrane (1) transiently and partially pops out from the membrane to the aqueous phase (2). The partially popping out PL molecule is energetically unstable and is primarily reinserted into the membrane. However, the popping out PL molecule has a chance of being trapped by the hydrophobic cavity of the SMP domain if the SMP domain is close to the membrane. The SMP domain may further help to break the PL-membrane interactions by direct binding to the membrane, as well. Then the SMP domain diffuses to the nearby acceptor membrane (3, 4) and releases the bound phospholipid molecule, which will be, via the partially inserted state (5), fully inserted into the membrane (6). The Gibbs free energy of each state is at the bottom of the panel. (B) Only a single SMP domain-containing protein is shown in (A) for simplicity, but multiple SMP domain-containing proteins can be involved in the inter-membrane PL transfer process, like ERMES (Mmm1, Mdm12 and Mdm34). In such a case, the trapped PL molecule can be exchanged between the multiple SMP domain-containing proteins by the lipid carrier model (left) or the continuous conduit model (central and right). The continuous conduit model assumes a continuous tunnel or continuous cleft for lipid transfer (see text).

The lack of one of the ERMES components leads to multiple abnormalities *in vivo*; (1) the characteristic clusters of ERMES are no longer formed; (2) the phospholipid transport is compromised; (3) mitochondrial morphology becomes aberrant; (4) the cell growth is significantly slowed. Interestingly however, these abnormalities, due to dysfunctions of ERMES, are rescued by the specific mutation (e.g. D716H) introduced to *VPS13* (Lang et al., 2015). This finding suggests that *Vps13* can somehow function redundantly with ERMES (Figure 2).

Interestingly, *Vps13* facilitates the formation of the contact between mitochondria and the vacuole by interacting with a mitochondrial OM protein *Mcp1* (John Peter et al., 2017). Besides, the N-terminal domain of *Vps13* was shown to have a lipid-transport ability (Kumar et al., 2018) and the homologous domain in *ATG2*, an autophagy factor, also has a similar lipid transfer activity (Osawa et al., 2019; Valverde et al., 2019). These findings suggest that phospholipid transport from the vacuole to mitochondria through *Vps13* is sufficient to maintain

the cellular phospholipid homeostasis if the phospholipid trafficking between the ER and mitochondrion is reduced by the absence of ERMES.

Several different proteins in addition to Vps13 and Mcp1 were reported to constitute mitochondrial-vacuolar contacts (Figure 2). Vps39, which also functions as a component of the homotypic fusion and protein sorting complex, interacts with Tom40 in the mitochondrial OM and with Ypt7 on the vacuolar membrane, thereby facilitating formation of the mitochondria-vacuolar contact called vCLAMP (Elbaz-Alon et al., 2014; Hönscher et al., 2014; González Montoro et al., 2018). The growth defect of ERMES-deficient cells is partly rescued by the overexpression of Vps39, suggesting that the lipid transport from the vacuole to mitochondria is promoted by the formation of vCLAMP (Hönscher et al., 2014).

It was shown that mitochondria contact with peroxisomes in addition to the ER and vacuole. By expressing split-fluorescent protein fragments on different organelle membranes, organelle contact sites can be visualized by fluorescence from the assembled fluorescent proteins (Kakimoto et al., 2018; Shai et al., 2018). Assessment of organelle contact sites using such a split-fluorescent protein, in combination with yeast genetic screening, led to the identification of Fzo1 and Pex34 as mitochondria-peroxisome tethering factors (Shai et al., 2018). At present, it is not yet clear whether these factors are involved in the lipid transport, like the cases of ERMES, Vps13 and vCLAMP.

Lam6, an accessory protein of ERMES, is a conserved protein containing GRAM and VASt domains and is thought to mediate sterol transport at organellar contact sites (Elbaz-Alon et al., 2015; Gatta et al., 2015; Murley et al., 2015). The GRAM and VASt domains are structurally similar to the PH domain with a lipid binding property, and to the StART domain having a hydrophobic cavity often seen in lipid transport proteins, respectively (Horenkamp et al., 2018). As Lam6 is localized not only at the mitochondria-ER contact site but also at the mitochondria-vacuole and ER-vacuole contact sites, it may mediate sterol transport between these different pairs of organelle membranes (Elbaz-Alon et al., 2015; Gatta et al., 2015; Murley et al., 2015).

Phospholipid transport within mitochondria

As PE and CL synthases function in the mitochondrial IM, facing the IMS and matrix, respectively, their precursor lipids must reach the IMS side of the OM or IM for the PE

synthesis, and the matrix side of the IM for the CL synthesis (for reviews, Dimmer and Rapaport, 2017; Tatsuta and Langer, 2017; Tamura et al., 2019b). However, molecular mechanisms of lipid transport within mitochondria had been unclear until assignment of the elusive intramitochondrial PA transport function to the mitochondrial IMS protein Ups1 (PRELID1 in mammals) (Figure 1).

Ups1 was originally identified as a protein involved in the processing of the mitochondrial IM protein Mgm1 in mitochondria, which functions for the IM fusion (Sesaki et al., 2006). After import into mitochondria, Mgm1 generates two distinct forms, a long form (l-Mgm1) and a short form (s-Mgm1), depending on the pulling force exerted by the motor components of the IM translocator, the TIM23 complex (Herlan et al., 2004). On the basis of the relative amount of l-Mgm1 to s-Mgm1, the motor function of the TIM23 complex was found to be compromised when Ups1 was depleted. Subsequent studies revealed that loss of Ups1 resulted in the decrease in the CL level, which impaired the TIM23 complex function (Osman et al., 2009a; Tamura et al., 2009).

Yeast has two more Ups1 homologues, Ups2 (PRELID3b in mammals) and Ups3, and these Ups proteins (PRELID proteins in mammals) all form functional complexes with a soluble cysteine-rich IMS protein Mdm35 (TRIAP1 in mammals) (Potting et al., 2010; Tamura et al., 2010). Like Ups1 affecting the CL level, loss of Ups2 but not Ups3 led to the decreased level of PE in mitochondria (Osman et al., 2009a; Tamura et al., 2009, 2010, 2012; Potting et al., 2010). Therefore, Ups1 and Ups2 were suggested to be involved in the maintenance of the normal levels of CL and PE, respectively, but it was still unclear whether these proteins were directly involved in their synthesis or involved in the transport of PA and PS, which were precursor phospholipids of CL and PE. Besides more puzzlingly, the double deletion strain of Ups1 and Ups2 (*ups1Δups2Δ*) and the *mdm35Δ* strain, in which neither Ups1 nor Ups2 could function, maintained nearly normal levels of CL, but with decreased PE levels. Ups1 and Ups2 thus appeared to regulate the mitochondrial lipid composition in a complex manner.

In 2012, the Langer group revealed that Ups1 is a lipid transfer protein that shuttles PA between the OM and IM in mitochondria (Connerth et al., 2012). In particular, they showed that the purified Ups1-Mdm35 complex was bound to acidic lipid-containing liposomes and transferred PA between them *in vitro*. Then, X-ray structures of yeast and human Ups1-Mdm35 complexes were reported from several groups (Miliara et al., 2015; Watanabe et al., 2015; Yu et al., 2015). The determined Ups1-Mdm35 structures revealed that Ups1 and Mdm35 form a single compact domain, the

Ups1 part of which is similar to the START (StAR-related lipid-transfer) domains in several lipid transfer proteins, despite the lack of the sequence homology (Yoder et al., 2001; Tilley et al., 2004; Kudo et al., 2008). Mdm35 dissociates from Ups1 to expose the hydrophobic region upon membrane binding, and in turn, Mdm35-free Ups1 bound to the membrane can leave for the aqueous solution upon re-association with soluble Mdm35 (Connerth et al., 2012; Watanabe et al., 2015). The Ups1-Mdm35 complex has an amphiphilic pocket that can accommodate a phospholipid molecule in a head-in manner. Positively charged residues are located at the bottom of the pocket, which is essential for recognition of the negatively charged head group of PA. Besides, the hydrophobic pocket has an Ω -loop structure at its outlet, which functions as a lid to regulate lipid binding to the pocket and to hide the hydrophobic acyl chains of bound PA from the aqueous solvent. Preferential binding of the Ups1-Mdm35 complex to liposomes rich in acidic phospholipids *in vitro* suggests the operation of negative feedback regulation of the Ups1-mediated PA transfer function. When the level of CL is sufficiently high, Ups1-Mdm35 likely stays preferentially at the IM, thereby rendering its PA transfer function inactivated. On the other hand, decrease in the CL level in the IM promotes the leaving of Ups1-Mdm35 from the IM, allowing its shuttling between the OM and IM for PA transfer from the OM to the IM. Interestingly, a fraction of Mdm35-free Ups1 in the IM is constantly degraded by the AAA protease in the IM, Yme1, which may contribute to keeping the Ups1 level optimum for the CL-sensitive PA transfer (Potting et al., 2010).

A similar lipid transfer function was revealed for the Ups2-Mdm35 complex (Figure 1). *In vitro* phospholipid transport experiments showed that recombinant Ups2-Mdm35 was capable of transferring PS between liposomes (Aaltonen et al., 2016; Miyata et al., 2016). As PS, the precursor lipid for PE synthesis, is exclusively made in the ER by the PS synthase (Cho1 in yeast), PS is transported from the ER to mitochondria, and then from the OM to the IM by Ups2-Mdm35 for the PE synthesis by IMS-facing IM protein, Psd1. Interestingly, this Ups2-mediated PE synthesis is significantly activated only upon shifting from fermentable to nonfermentable growth conditions of yeast (Miyata et al., 2016). Under fermentable conditions, Psd1-mediated PE synthesis can take place independently of Ups2. This PE synthesis in the absence of the Ups2-mediated PS transfer is suggested to take place by Psd1 acting *in trans* on PS in the OM, likely at OM-IM contact sites (Aaltonen et al., 2016) (Figure 1). Such contact sites are formed by the mitochondrial contact site and cristae-organizing system (MICOS) complex in mitochondria (Rabl et al.,

2009; Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011). The majority of PE synthesized in the Psd1-dependent and Ups2-independent manner was found to be transported to the ER for further conversion to PC while PE synthesized in the IM may stay in the IM for its coordinated functions with CL.

How do Ups1 and Ups2 discriminate PA and PS? The recently revealed X-ray structures of PRELID1-TRIAP1 and PRELID3b-TRIAP1 (human Ups1-Mdm35 and Ups2-Mdm35) show similar structural organizations of the complexes (Miliara et al., 2019). Miliara et al. (2019) applied a reverse genetic screen to identify determinants of lipid specificity between Ups1 and Ups2, on the basis of the fact that the synthetic lethality caused by the loss of Ups2 and an IM scaffold protein prohibitin (Phb1 or Phb2; Osman et al., 2009a,b) can be rescued by the expression of Ups2, but only to a limited extent by the expression of Ups1. The expression of *UPS1* variants with random polymerase chain reaction (PCR) mutagenesis in *ups2 Δ phb1 Δ* cells thus allowed to reveal “hot spots” for changing the substrate specificity of Ups1 and Ups2. Interestingly several mutations that allow Ups2 variants to substitute for Ups1 are mapped in the Ω loop, a lid for the lipid binding pocket, and the Ups2 variant carrying Ω loop from Ups1 is able to transfer PA, highlighting an important role for the Ω loop in the substrate selection.

The observation that the decreased CL level caused by the Ups1 dysfunction can be recovered by the simultaneous Ups2 dysfunction is puzzling at a glance. However, as the decrease in the CL level in the absence of Ups1 is restored when the PE level is decreased by the loss of Psd1 or PS synthetase Cho1, as well, the decreased PE level rather than the Ups2 dysfunction is critical for the restoration of the decreased CL level by the loss of Ups1 (Miyata et al., 2017). Therefore, an alternative PA transport pathway or CL synthesis is upregulated independently of Ups1 when the PE level is lowered (Figure 1). Based on this consideration, Miyata et al. (2017) found that Mdm31, Mdm32 and Fmp30 in the mitochondrial IM are involved in the CL synthesis under the decreased PE conditions. In particular, Mdm31 in the IM directly interacts with porin in the OM, a channel for small metabolites and ions, suggesting the presence of an alternative PA transport route at the OM-IM contact sites (Miyata et al., 2018). Consistently, we showed that overexpression of Mdm31 partly rescued the defects in growth and CL accumulation observed in cells lacking Ups1 or one of the ERMES subunits (Mmm1, Mdm10, Mdm12 and Mdm34), suggesting the role of Mdm31 in PA transport (Tamura et al., 2012). Further, it is interesting that amino-acid sequence analysis showed that Mdm31 contains the Chorein-N domain, which

appears to transfer lipids between membranes (Levine, 2019). Therefore, it is attractive to assume that Mdm31 can directly transfer PA from the OM to the IM independently of Ups1-Mdm35 when the PE level is lowered.

The mitochondrial IM has two distinct types of crista structures, lamellar cristae and tubular cristae. While lamellar crista is generated by the fusion of two distinct IMs by Mgm1 after fusion of the OM (Harner et al., 2016), tubular crista could be formed by the substantial increase in the amount of the IM through the supply of proteins and phospholipids to the IM. Indeed, this latter idea was found to be the case, that is, loss of Mdm35, which inactivates the Ups1 and Ups2-mediate PA and PS transport, led to a decrease in crista structures, suggesting the potential role of intramitochondrial phospholipid transport in the crista formation (Kojima et al., 2019). Besides, the triple-deletion *mgm1Δdnm1Δmdm35Δ* cells (deletion of *DNM1* in addition to the *MGM1* deletion is required to suppress the loss of mtDNA, which causes possible secondary effects) show significantly altered IM structures or even empty mitochondria without internal IM structures, which leads to the decreased CL level and severe growth defects. Thus, intra-mitochondrial phospholipid transport is coupled to the development of crista IM structures as well as the optimum CL content in the IM. On the other hand, it is to be noted that CL in the IM does not in turn facilitate formation of cristae structures through the induced high membrane curvature of the IM because *crd1Δ* cells do not affect cristae structures (Baile et al., 2014; Basu Ball et al., 2018).

Conclusions and perspectives

Our understanding of synthesis and transport of mitochondrial phospholipids has advanced in recent years. Proteins facilitating the phospholipid transfer between organelle membranes across the aqueous phase have been identified; such proteins include Ups proteins for the phospholipid transport between the mitochondrial OM and IM, and ERMES between the ER membrane and mitochondrial OM. In particular, identification of inter-organelle contacts between various pairs of organelle membranes, which often contain proteins with lipid-binding domains, points to the increasing importance of those contact sites in the transfer of lipids between different organelles.

Nevertheless, it is still unclear how lipid compositions of different membranes are constantly monitored and optimized in response to metabolic conditions by inter-membrane lipid trafficking. Besides, little is known

for flippases and scramblases within mitochondria, which would facilitate lipid translocation from one leaflet to the other leaflet of the membrane; such transmembrane movement of lipids achieves asymmetric distribution of lipids across the membrane bilayer, which is essential for lipid trafficking, cell signaling, vesicular trafficking, organelle morphology dynamics, etc. Another elusive question is related to likely non-uniform lateral distribution of phospholipids in the mitochondrial membranes. In particular, non-bilayer forming phospholipids PE and CL in the IM would segregate into distinct domains, which may contribute to membrane fusion and fission as well as membrane protein biogenesis in the highly protein-rich IM. Such lipid segregation may be connected to the membrane scaffolds mediated by prohibitins, the assembly of which form ring structures in the membranes (Osman et al., 2009b), yet molecular mechanisms behind the lateral lipid organization within the membrane is still poorly understood.

Trafficking of proteins and lipids appear to operate under distinct principles. Protein trafficking is highly specific, precise and efficient, reflecting the allocation of unique pathways to individual proteins. In contrast, pathways for lipid trafficking are often redundant and equipped with multiple back-up pathways, thereby rendering the lipid trafficking versatile and flexible as well as robust, which would be essential for the cellular lipid homeostasis. In the coming several years, we anticipate witnessing the discovery of more new factors and pathways for mitochondrial lipid trafficking, which will lead to answers to the many fundamental questions described here and establishing the still vague principle of cellular lipid trafficking.

Acknowledgments: This work was supported by JSPS KAKENHI to T.E. (Funder Id: <http://dx.doi.org/10.13039/501100001691>, 15H05705 and 2222703) and Y.T. (17H06414 and 19H03174), a JST CREST grant to T.E. (Funder Id: <http://dx.doi.org/10.13039/501100003382>, JPMJCR12M1), and an AMED-Prime grant to Y.T. (Funder Id: <http://dx.doi.org/10.13039/100009619>, JP19gm5910026).

References

- Aaltonen, M.J., Friedman, J.R., Osman, C., Salin, B., di Rago, J.P., Nunnari, J., Langer, T., and Tatsuta, T. (2016). MICOS and phospholipid transfer by Ups2-Mdm35 organize membrane lipid synthesis in mitochondria. *J. Cell Biol.* 213, 525–534.
- Adachi, Y., Itoh, K., Yamada, T., Cerveny, K.L., Suzuki, T.L., Macdonald, P., Frohman, M.A., Ramachandran, R., Iijima,

- M., and Sesaki, H. (2016). Coincident phosphatidic acid interaction restrains Drp1 in mitochondrial division. *Mol. Cell* 63, 1034–1043.
- Adachi, Y., Iijima, M., and Sesaki, H. (2018). An unstructured loop that is critical for interactions of the stalk domain of Drp1 with saturated phosphatidic acid. *Small GTPases* 9, 472–479.
- AhYoung, A.P., Lu, B., Cascio, D., and Egea, P.F. (2017). Crystal structure of Mdm12 and combinatorial reconstitution of Mdm12/Mmm1 ERMES complexes for structural studies. *Biochem. Biophys. Res. Commun.* 488, 129–135.
- Alva, V. and Lupas, A.N. (2016). The TULIP superfamily of eukaryotic lipid-binding proteins as a mediator of lipid sensing and transport. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1861, 913–923.
- Bae-Lee, M.S. and Carman, G.M. (1984). Phosphatidylserine synthesis in *Saccharomyces cerevisiae*. Purification and characterization of membrane-associated phosphatidylserine synthase. *J. Biol. Chem.* 259, 10857–10862.
- Baile, M.G., Sathappa, M., Lu, Y.W., Pryce, E., Whited, K., McCaffery, J.M., Han, X., Alder, N.N., and Claypool, S.M. (2014). Unremodeled and remodeled cardiolipin are functionally indistinguishable in yeast. *J. Biol. Chem.* 289, 1768–1778.
- Baker, C.D., Basu Ball, W., Pryce, E.N., and Gohil, V.M. (2016). Specific requirements of nonbilayer phospholipids in mitochondrial respiratory chain function and formation. *Mol. Biol. Cell* 27, 2161–2171.
- Barth, P.G., Valianpour, F., Bowen, V.M., Lam, J., Duran, M., Vaz, F.M., and Wanders, R.J.A. (2004). X-linked cardioskeletal myopathy and neutropenia (Barth syndrome), an update. *Am. J. Med. Genet.* 126A, 349–354.
- Basu Ball, W., Neff, J.K., and Gohil, V.M. (2018). The role of nonbilayer phospholipids in mitochondrial structure and function. *FEBS Lett.* 592, 1273–1290.
- Beranek, A., Rechberger, G., Knauer, H., Wolinski, H., Kohlwein, S.D., and Leber, R. (2009). Identification of a cardiolipin-specific phospholipase encoded by the gene *CLD1 (YGR110W)* in yeast. *J. Biol. Chem.* 284, 11572–11578.
- Bird, S.S., Marur, V.R., Stavrovskaya, I.G., and Kristal, B.S. (2013). Qualitative characterization of the rat liver mitochondrial lipidome using LC-MS profiling and high energy collisional dissociation (HCD) all ion fragmentation. *Metabolomics* 8, 67–83.
- Boldogh, I.R., Nowakowski, D.W., Yang, H.-C., Chung, H., Karmon, S., Royes, P., and Pon, L.A. (2003). A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. *Mol. Biol. Cell* 14, 4618–4627.
- Bürgermeister, M., Birner-Grünberger, R., Nebauer, R., and Daum, G. (2004). Contribution of different pathways to the supply of phosphatidylethanolamine and phosphatidylcholine to mitochondrial membranes of the yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 1686, 161–168.
- Calzada, E., Avery, E., Sam, P.N., Modak, A., Wang, C., McCaffery, J.M., Han, X., Alder, N.N., and Claypool, S.M. (2019). Phosphatidylethanolamine made in the inner mitochondrial membrane is essential for yeast cytochrome bc1 complex function. *Nat. Commun.* 10, 1432.
- Chang, S.-C., Heacock, P.N., Clancey, C.J., and Dowhan, W. (1998a). The *PEL1* gene (Renamed *PGS1*) encodes the phosphatidylglycerophosphate synthase of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 273, 9829–9836.
- Chang, S., Heacock, P.N., Mileykovskaya, E., Voelker, D.R., and Dowhan, W. (1998b). Isolation and characterization of the gene (*CLS1*) encoding cardiolipin synthase in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 273, 14933–14941.
- Choi, J.-Y., Riekhof, W.R., Wu, W.-I., and Voelker, D.R. (2000a). Macromolecular assemblies regulate nonvesicular phosphatidylserine traffic in yeast. *Biochem. Soc. Trans.* 34, 404–408.
- Choi, S.-Y., Huang, P., Jenkins, G.M., Chan, D.C., Schiller, J., and Frohman, M.A. (2000b). A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. *Nat. Cell Biol.* 8, 1255–1262.
- Chu, C.T., Ji, J., Dagda, R.K., Jiang, J.F., Tyurina, Y.Y., Kapralov, A.A., Tyurin, V.A., Yanamala, N., Shrivastava, I.H., Mohammadyani, D., et al. (2013). Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat. Cell Biol.* 15, 1197–1205.
- Claypool, S.M., McCaffery, J.M., and Koehler, C.M. (2006). Mitochondrial mislocalization and altered assembly of a cluster of Barth syndrome mutant tafazzins. *J. Cell Biol.* 174, 379–390.
- Connerth, M., Tatsuta, T., Haag, M., Klecker, T., Westermann, B., and Langer, T. (2012). Intramitochondrial transport of phosphatidic acid in yeast by a lipid transfer protein. *Science* 338, 815–818.
- Dimmer, K.S. and Rapaport, D. (2017). Mitochondrial contact sites as platforms for phospholipid exchange. *Biochim. Biophys. Acta – Mol. Cell Biol. Lipids* 1862, 69–80.
- Dittman, J.S. and Menton, A.K. (2017). Speed limits for nonvesicular intracellular sterol transport. *Trends Biochem. Sci.* 42, 90–97.
- Elbaz-Alon, Y., Rosenfeld-Gur, E., Shinder, V., Futerman, A.H., Geiger, T., and Schuldiner, M. (2014). A dynamic interface between vacuoles and mitochondria in yeast. *Dev. Cell* 30, 95–102.
- Elbaz-Alon, Y., Eisenberg-Bord, M., Shinder, V., Stiller, S.B., Shimon, E., Wiedemann, N., Geiger, T., and Schuldiner, M. (2015). Lam6 regulates the extent of contacts between organelles. *Cell Rep.* 12, 7–14.
- Flinner, N., Ellenrieder, L., Stiller, S.B., Becker, T., Schleiff, E., and Mirus, O. (2013). Mdm10 is an ancient eukaryotic porin co-occurring with the ERMES complex. *Biochim. Biophys. Acta* 1833, 3314–3325.
- Friedman, J.R., Kannan, M., Toulmay, A., Jan, C.H., Weissman, J.S., Prinz, W.A., and Nunnari, J. (2018). Lipid homeostasis is maintained by dual targeting of the mitochondrial PE biosynthesis enzyme to the ER. *Dev. Cell* 44, 261–270.e6.
- Gallas, M.R., Dienhart, M.K., Stuart, R.A., and Long, R.M. (2006). Characterization of Mmp37p, a *Saccharomyces cerevisiae* mitochondrial matrix protein with a role in mitochondrial protein import. *Mol. Biol. Cell* 17, 4051–4062.
- Gatta, A.T., Wong, L.H., Sere, Y.Y., Calderón-Noreña, D.M., Cockcroft, S., Menon, A.K., and Levine, T.P. (2015). A new family of StART domain proteins at membrane contact sites has a role in ER-PM sterol transport. *Elife* 4, 1–21.
- Gebert, N., Joshi, A.S., Kutik, S., Becker, T., McKenzie, M., Guan, X.L., Mooga, V.P., Stroud, D.A., Kulkarni, G., Wenk, M.R., et al. (2009). Mitochondrial cardiolipin involved in outer-membrane protein biogenesis: implications for Barth Syndrome. *Curr. Biol.* 19, 2133–2139.
- González Montoro, A., Auffarth, K., Hönscher, C., Bohnert, M., Becker, T., Warscheid, B., Reggiori, F., van der Laan, M., Fröhlich, F., and Ungermann, C. (2018). Vps39 interacts with Tom40 to establish one of two functionally distinct vacuole-mitochondria contact sites. *Dev. Cell* 45, 621–636.e7.

- Gu, Z., Valianpour, F., Chen, S., Vaz, F.M., Hakkaart, G.A., Wanders, R.J.A., and Greenberg, M.L. (2004). Aberrant cardiolipin metabolism in the yeast taz1 mutant: a model for Barth syndrome. *Mol. Microbiol.* 51, 149–158.
- Guillén-Samander, A., Bian, X., and de Camilli, P. (2019). PDZD8 mediates a Rab7-dependent interaction of the ER with late endosomes and lysosomes. *Proc. Natl. Acad. Sci. U.S.A.* 116, 22619–22623.
- Gulshan, K., Shahi, P., and Moye-Rowley, W.S. (2010). Compartment-specific synthesis of phosphatidylethanolamine is required for normal heavy metal resistance. *Mol. Biol. Cell* 21, 443–455.
- Harner, M., Körner, C., Walther, D., Mokranjac, D., Kaesmacher, J., Welsch, U., Griffith, J., Mann, M., Reggiori, F., and Neupert, W. (2011). The mitochondrial contact site complex, a determinant of mitochondrial architecture. *EMBO J.* 30, 4356–4370.
- Harner, M.E., Unger, A.-K., Geerts, W.J., Mari, M., Izawa, T., Stenger, M., Geimer, S., Reggiori, F., Westermann, B., and Neupert, W. (2016). An evidence based hypothesis on the existence of two pathways of mitochondrial crista formation. *eLife* 5, e18853.
- Herlan, M., Bornhövd, C., Hell, K., Neupert, W., and Reichert, A.S. (2004). Alternative topogenesis of Mgm1 and mitochondrial morphology depend on ATP and a functional import motor. *J. Cell Biol.* 165, 167–173.
- Hirabayashi, Y., Kwon, S.-K., Paek, H., Pernice, W.M., Paul, M.A., Lee, J., Erfani, P., Raczkowski, A., Petrey, D.S., Pon, L.A., et al. (2017). ER-mitochondria tethering by PDZD8 regulates Ca^{2+} dynamics in mammalian neurons. *Science* 358, 623–630.
- Hobbs, A.E.A., Srinivasan, M., McCaffery, J.M., and Jensen, R.E. (2001). Mmm1p, a mitochondrial outer membrane protein, is connected to mitochondrial DNA (Mtdna) nucleoids and required for Mtdna stability. *J. Cell Biol.* 152, 401–410.
- Hönscher, C., Mari, M., Auffarth, K., Bohnert, M., Griffith, J., Geerts, W., van der Laan, M., Cabrera, M., Reggiori, F., and Ungermann, C. (2014). Cellular metabolism regulates contact sites between vacuoles and mitochondria. *Dev. Cell* 30, 86–94.
- Hoppins, S., Collins, S.R., Cassidy-Stone, A., Hummel, E., DeVay, R.M., Lackner, L.L., Westermann, B., Schuldiner, M., Weissman, J.S., and Nunnari, J. (2011). A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane organization in mitochondria. *J. Cell Biol.* 195, 323–340.
- Horenkamp, F.A., Valverde, D.P., Nunnari, J., and Reinisch, K.M. (2018). Molecular basis for sterol transport by StART-like lipid transfer domains. *EMBO J.* 37, e98002.
- Horvath, S.E. and Daum, G. (2013). Lipids of mitochondria. *Prog. Lipid. Res.* 52, 590–614.
- Horvath, S.E., Böttinger, L., Vögtle, F.-N., Wiedemann, N., Meisinger, C., Becker, T., and Daum, G. (2012). Processing and topology of the yeast mitochondrial phosphatidylserine decarboxylase 1. *J. Biol. Chem.* 287, 36744–36755.
- Huang, H., Gao, Q., Peng, X., Choi, S.-Y., Sarma, K., Ren, H., Morris, A.J., and Frohman, M.A. (2011). piRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. *Dev. Cell* 20, 376–387.
- Iadarola, D.M., Basu Ball, W., Trivedi, P.P., Fu, G., Nan, B., and Gohil, V.M. (2020). Vps39 is required for ethanolamine-stimulated elevation in mitochondrial phosphatidylethanolamine. *Biochim. Biophys. Acta* 1865, 158655.
- Janitor, M., Jarosch, E., Schweyen, R.J., and Subík, J. (1995). Molecular characterization of the *PEL1* gene encoding a putative phosphatidylserine synthase. *Yeast* 11, 1223–1231.
- Jeong, H., Park, J., and Lee, C. (2016). Crystal structure of Mdm12 reveals the architecture and dynamic organization of the ERMES complex. *EMBO Rep.* 17, 1857–1871.
- Jeong, H., Park, J., Jun, Y., and Lee, C. (2017). Crystal structures of Mmm1 and Mdm12–Mmm1 reveal mechanistic insight into phospholipid trafficking at ER-mitochondria contact sites. *Proc. Natl. Acad. Sci. U.S.A.* 114, E9502–E9511.
- Jiao, H., Yin, Y., and Liu, Z. (2019). Structures of the mitochondrial CDP-DAG synthase Tam41 suggest a potential lipid substrate pathway from membrane to the active site. *Structure* 27, 1258–1269.e4.
- John Peter, A.T., Herrmann, B., Antunes, D., Rapoport, D., Dimmer, K.S., and Kornmann, B. (2017). Vps13-Mcp1 interact at vacuole–mitochondria interfaces and bypass ER-mitochondria contact sites. *J. Cell Biol.* 216, 3219–3229.
- Joshi, A.S., Zhou, J., Gohil, V.M., Chen, S., and Greenberg, M.L. (2009). Cellular functions of cardiolipin in yeast. *Biochim. Biophys. Acta Mol. Cell Res.* 1793, 212–218.
- Joshi, A.S., Thompson, M.N., Fei, N., Hüttemann, M., and Greenberg, M.L. (2012). Cardiolipin and mitochondrial phosphatidylethanolamine have overlapping functions in mitochondrial fusion in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 287, 17589–17597.
- Junker, M. and Rapoport, T.A. (2015). Involvement of VAT-1 in Phosphatidylserine transfer from the endoplasmic reticulum to mitochondria. *Traffic* 16, 1306–1317.
- Kakimoto, Y., Tashiro, S., Kojima, R., Morozumi, Y., Endo, T., and Tamura, Y. (2018). Visualizing multiple inter-organelle contact sites using the organelle-targeted split-GFP system. *Sci. Rep.* 8, 6175.
- Kawano, S., Tamura, Y., Kojima, R., Bala, S., Asai, E., Michel, A.H., Kornmann, B., Riezman, I., Riezman, H., Sakae, Y., et al. (2018). Structure-function insights into direct lipid transfer between membranes by Mmm1-Mdm12 of ERMES. *J. Cell Biol.* 217, 959–974.
- Kelley, M.J. and Carman, G.M. (1987). Purification and characterization of CDP-diacylglycerol synthase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 262, 14563–14570.
- Kodaki, T. and Yamashita, S. (1987). Yeast phosphatidylethanolamine methylation pathway. Cloning and characterization of two distinct methyltransferase genes. *J. Biol. Chem.* 262, 15428–15435.
- Kojima, R., Endo, T., and Tamura, Y. (2016). A phospholipid transfer function of ER-mitochondria encounter structure revealed *in vitro*. *Sci. Rep.* 6, 30777.
- Kojima, R., Kakimoto, Y., Furuta, S., Itoh, K., Sesaki, H., Endo, T., and Tamura, Y. (2019). Maintenance of cardiolipin and crista structure requires cooperative functions of mitochondrial dynamics and phospholipid transport. *Cell Rep.* 26, 518–528.e6.
- Kornmann, B., Currie, E., Collins, S.R., Schuldiner, M., Nunnari, J., Weissman, J.S., and Walter, P. (2009). An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* 325, 477–481.
- Kornmann, B., Osman, C., and Walter, P. (2011). The conserved GTPase Gem1 regulates endoplasmic reticulum-mitochondria connections. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14151–14156.
- Kuchler, K., Daum, G., and Paltauf, F. (1986). Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in *Saccharomyces cerevisiae*. *J. Bacteriol.* 165, 901–910.
- Kudo, N., Kumagai, K., Tomishige, N., Yamaji, T., Wakatsuki, S., Nishijima, M., Hanada, K., and Kato, R. (2008). Structural basis for specific lipid recognition by CERT responsible for

- nonvesicular trafficking of ceramide. *Proc. Natl. Acad. Sci. U.S.A.* 105, 488–493.
- Kumar, N., Leonzino, M., Hancock-Cerutti, W., Horenkamp, F.A., Li, P., Lees, J.A., Wheeler, H., Reinisch, K.M., and De Camilli, P. (2018). VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites. *J. Cell Biol.* 217, 3625–3639.
- 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. (2008). The translocator maintenance protein Tam41 is required for mitochondrial cardiolipin biosynthesis. *J. Cell Biol.* 183, 1213–1221.
- Lang, A.B., Peter, A.T.J., Walter, P., and Kornmann, B. (2015). ER–mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. *J. Cell Biol.* 210, 883–890.
- Levine, T.P. (2019). Remote homology searches identify bacterial homologues of eukaryotic lipid transfer proteins, including Chorein-N domains in TamB and AsmA and Mdm31p. *BMC Mol. Cell Biol.* 20, 43.
- Li, Q.X. and Dowhan, W. (1990). Studies on the mechanism of formation of the pyruvate prosthetic group of phosphatidylserine decarboxylase from *Escherichia coli*. *J. Biol. Chem.* 265, 4111–4115.
- Malhotra, K., Modak, A., Nangia, S., Daman, T.H., Gunsell, U., Robinson, V.L., Mokranjac, D., May, E.R., and Alder, N.N. (2017). Cardiolipin mediates membrane and channel interactions of the mitochondrial TIM23 protein import complex receptor Tim50. *Sci. Adv.* 3, e1700532.
- Meisinger, C., Wiedemann, N., Rissler, M., Strub, A., Milenkovic, D., Schönfisch, B., Müller, H., Kozjak, V., and Pfanner, N. (2006). Mitochondrial protein sorting: differentiation of beta-barrel assembly by Tom7-mediated segregation of Mdm10. *J. Biol. Chem.* 281, 22819–22826.
- Miliara, X., Garnett, J.A., Tatsuta, T., Abid Ali, F., Baldie, H., Pérez-Dorado, I., Simpson, P., Yague, E., Langer, T., and Matthews, S. (2015). Structural insight into the TRIAP1/PRELI-like domain family of mitochondrial phospholipid transfer complexes. *EMBO Rep.* 16, 824–835.
- Miliara, X., Tatsuta, T., Berry, J.-L., Rouse, S.L., Solak, K., Chorev, D.S., Wu, D., Robinson, C.V., Matthews, S., and Langer, T. (2019). Structural determinants of lipid specificity within Ups/ PRELI lipid transfer proteins. *Nat. Commun.* 10, 1130.
- Miyata, N., Watanabe, Y., Tamura, Y., Endo, T., and Kuge, O. (2016). Phosphatidylserine transport by Ups2–Mdm35 in respiration-active mitochondria. *J. Cell Biol.* 214, 77–88.
- Miyata, N., Goda, N., Matsuo, K., Hoketsu, T., and Kuge, O. (2017). Cooperative function of Fmp30, Mdm31, and Mdm32 in Ups1-independent cardiolipin accumulation in the yeast *Saccharomyces cerevisiae*. *Sci. Rep.* 7, 16447.
- Miyata, N., Fujii, S., and Kuge, O. (2018). Porin proteins have critical functions in mitochondrial phospholipid metabolism in yeast. *J. Biol. Chem.* 293, 17593–17605.
- Murley, A., Sarsam, R.D., Toulmay, A., Yamada, J., Prinz, W.A., and Nunnari, J. (2015). Ltc1 is an ER-localized sterol transporter and a component of ER-mitochondria and ER-vacuole contacts. *J. Cell Biol.* 209, 539–548.
- Nguyen, T.T., Lewandowska, A., Choi, J.-Y., Markgraf, D.F., Junker, M., Bilgin, M., Ejsing, C.S., Voelker, D.R., Rapoport, T.A., and Shaw, J.M. (2012). Gem1 and ERMES do not directly affect phosphatidylserine transport from ER to mitochondria or mitochondrial inheritance. *Traffic* 13, 880–890.
- Osawa, T., Kotani, T., Kawaoka, T., Hirata, E., Suzuki, K., Nakatogawa, H., Ohsumi, Y., and Noda, N.N. (2019). Atg2 mediates direct lipid transfer between membranes for autophagosome formation. *Nat. Struct. Mol. Biol.* 26, 281–288.
- Osman, C., Haag, M., Potting, C., Rodenfels, J., Dip, P. V., Wieland, F.T., Brügger, B., Westermann, B., and Langer, T. (2009a). The genetic interactome of prohibitins: coordinated control of cardiolipin and phosphatidylethanolamine by conserved regulators in mitochondria. *J. Cell Biol.* 184, 583–596.
- Osman, C., merkwirth, C., and Langer, T. (2009b). Prohibitins and the functional compartmentalization of mitochondrial membranes. *J. Cell Sci.* 122, 3823–3830.
- Osman, C., Haag, M., Wieland, F.T., Brügger, B., and Langer, T. (2010). A mitochondrial phosphatase required for cardiolipin biosynthesis: the PGP phosphatase Gcp4. *EMBO J.* 29, 1976–1987.
- Pfeiffer, K., Gohil, V., Stuart, R.A., Hunte, C., Brandt, U., Greenberg, M.L., and Schägger, H. (2003). Cardiolipin stabilizes respiratory chain supercomplexes. *J. Biol. Chem.* 278, 52873–52880.
- Potting, C., Wilmes, C., Engmann, T., Osman, C., and Langer, T. (2010). Regulation of mitochondrial phospholipids by Ups1/ PRELI-like proteins depends on proteolysis and Mdm35. *EMBO J.* 29, 2888–2898.
- Rabl, R., Soubannier, V., Scholz, R., Vogel, F., Mendl, N., Vasiljev-Neumeyer, A., Körner, C., Jagasia, R., Keil, T., Baumeister, W., et al. (2009). Formation of cristae and crista junctions in mitochondria depends on antagonism between Fcj1 and Su e/g. *J. Cell Biol.* 185, 1047–1063.
- Ren, M., Phoon, C.K.L., and Schlame, M. (2014). Metabolism and function of mitochondrial cardiolipin. *Prog. Lipid Res.* 55, 1–16.
- Scorrano, L., De Matteis, M.A., Emr, S., Giordano, F., Hajnóczky, G., Kornmann, B., Lackner, L.L., Levine, T.P., Pellegrini, L., Reinisch, K., et al. (2019). Coming together to define membrane contact sites. *Nat. Commun.* 10, 1287.
- Sesaki, H., Dunn, C.D., Iijima, M., Shepard, K.A., Yaffe, M.P., Machamer, C.E., and Jensen, R.E. (2006). Ups1p, a conserved inter-membrane space protein, regulates mitochondrial shape and alternative topogenesis of Mgm1p. *J. Cell Biol.* 173, 651–658.
- Shai, N., Yifrach, E., van Roermund, C.W.T., Cohen, N., Bibi, C., Ijlst, L., Cavellini, L., Meurisse, J., Schuster, R., Zada, L., et al. (2018). Systematic mapping of contact sites reveals tethers and a function for the peroxisome-mitochondria contact. *Nat. Commun.* 9, 1761.
- Sogo, L.F. and Yaffe, M.P. (1994). Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J. Cell Biol.* 126, 1361–1373.
- Stroud, D.A., Oeljeklaus, S., Wiese, S., Bohnert, M., Lewandowski, U., Sickmann, A., Guiard, B., Van Der Laan, M., Warscheid, B., and Wiedemann, N. (2011). Composition and topology of the endoplasmic reticulum-mitochondria encounter structure. *J. Mol. Biol.* 413, 743–750.
- Tamura, Y., Harada, Y., Yamano, K., Watanabe, K., Ishikawa, D., Ohshima, C., Nishikawa, S., Yamamoto, H., and Endo, T. (2006). Identification of Tam41 maintaining integrity of the TIM23 protein translocator complex in mitochondria. *J. Cell Biol.* 174, 631–637.
- Tamura, Y., Endo, T., Iijima, M., and Sesaki, H. (2009). Ups1p and Ups2p antagonistically regulate cardiolipin metabolism in mitochondria. *J. Cell Biol.* 185, 1029–1045.
- Tamura, Y., Iijima, M., and Sesaki, H. (2010). Mdm35p imports Ups proteins into the mitochondrial intermembrane space by functional complex formation. *EMBO J.* 29, 2875–2887.
- Tamura, Y., Onguka, O., Hobbs, A.E.A., Jensen, R.E., Iijima, M., Claypool, S.M., and Sesaki, H. (2012). Role for two conserved intermembrane space proteins, Ups1p and Up2p, in intra-

- mitochondrial phospholipid trafficking. *J. Biol. Chem.* **287**, 15205–15218.
- Tamura, Y., Harada, Y., Nishikawa, S., Yamano, K., Kamiya, M., Shioota, T., Kuroda, T., Kuge, O., Sesaki, H., Imai, K., et al. (2013). Tam41 is a CDP-diacylglycerol synthase required for cardiolipin biosynthesis in mitochondria. *Cell Metab.* **17**, 709–718.
- Tamura, Y., Kojima, R., and Endo, T. (2019a). Advanced *in vitro* assay system to measure phosphatidylserine and phosphatidylethanolamine transport at ER/mitochondria interface. *Methods Mol. Biol.* **1949**, 57–67.
- Tamura, Y., Kawano, S., and Endo, T. (2019b). Organelle contact zones as sites for lipid transfer. *J. Biochem.* **165**, 115–123.
- Tatsuta, T. and Langer, T. (2017). Intramitochondrial phospholipid trafficking. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1862**, 81–89.
- Tilley, S.J., Skippen, A., Murray-Rust, J., Swigart, P.M., Stewart, A., Morgan, C.P., Cockcroft, S., and McDonald, N.Q. (2004). Structure-function analysis of human [corrected] phosphatidylinositol transfer protein alpha bound to phosphatidylinositol. *Structure* **12**, 317–326.
- Trotter, P.J. and Voelker, D.R. (1995). Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (*PSD2*) in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **270**, 6062–6070.
- Tuller, G., Hrasnik, C., Achleitner, G., Schieffaler, U., Klein, F., and Daum, G. (1998). YDL142c encodes cardiolipin synthase (Cslp) and is non-essential for aerobic growth of *Saccharomyces cerevisiae*. *FEBS Lett.* **421**, 15–18.
- Valverde, D.P., Yu, S., Boggavarapu, V., Kumar, N., Lees, J.A., Walz, T., Reinisch, K.M., and Melia, T.J. (2019). ATG2 transports lipids to promote autophagosome biogenesis. *J. Cell Biol.* **218**, 1787–1798.
- Voss, C., Lahiri, S., Young, B.P., Loewen, C.J., and Prinz, W.A. (2012). ER-shaping proteins facilitate lipid exchange between the ER and mitochondria in *S. cerevisiae*. *J. Cell Sci.* **125**, 4791–4799.
- von der Malsburg, K., Müller, J.M., Bohnert, M., Oeljeklaus, S., Kwiatkowska, P., Becker, T., Loniewska-Lwowska, A., Wiese, S., Rao, S., Milenkovic, D., et al. (2011). Dual role of Mitofilin in mitochondrial membrane organization and protein biogenesis. *Dev. Cell* **21**, 694–707.
- Watanabe, Y., Tamura, Y., Kawano, S., and Endo, T. (2015). Structural and mechanistic insights into phospholipid transfer by Ups1-Mdm35 in mitochondria. *Nat. Commun.* **6**, 7922.
- Watanabe, Y., Tamura, Y., Kakuta, C., Watanabe, S., and Endo, T. (2020). Structural basis for inter-organelle phospholipid transport mediated by VAT-1. *J. Biol. Chem.* **295**, 3257–3268.
- Wideman, J.G., Balacco, D.L., Fieblinger, T., and Richards, T.A. (2018). PDZD8 is not the 'functional ortholog' of Mmm1, it is a paralog. *F1000Res.* **7**, 1088.
- Wong, L.H. and Levine, T.P. (2017). Tubular lipid binding proteins (TULIPs) growing everywhere. *Biochim. Biophys. Acta – Mol. Cell Res.* **1864**, 1439–1449.
- Yamano, K., Tanaka-Yamano, S., and Endo, T. (2010). Tom7 regulates Mdm10-mediated assembly of the mitochondrial import channel protein TOM40. *J. Biol. Chem.* **285**, 41222–41231.
- Ye, C., Lou, W., Li, Y., Chatzisprou, I.A., Hüttemann, M., Lee, I., Houtkooper, R.H., Vaz, F.M., Chen, S., and Greenberg, M.L. (2014). Deletion of the cardiolipin-specific phospholipase Cld1 rescues growth and life span defects in the tafazzin mutant: implications for Barth syndrome. *J. Biol. Chem.* **289**, 3114–3125.
- Yoder, M.D., Thomas, L.M., Tremblay, J.M., Oliver, R.L., Yarbrough, L.R., and Helmkamp, G.M. (2001). Structure of a multifunctional protein. Mammalian phosphatidylinositol transfer protein complexed with phosphatidylcholine. *J. Biol. Chem.* **276**, 9246–9252.
- Youngman, M.J., Hobbs, A.E.A., Burgess, S.M., Srinivasan, M., and Jensen, R.E. (2004). Mmm2p, a mitochondrial outer membrane protein required for yeast mitochondrial shape and maintenance of mtDNA nucleoids. *J. Cell Biol.* **164**, 677–688.
- Yu, F., He, F., Yao, H., Wang, C., Wang, J., Li, J., Qi, X., Xue, H., Ding, J., and Zhang, P. (2015). Structural basis of intramitochondrial phosphatidic acid transport mediated by Ups1-Mdm35 complex. *EMBO Rep.* **16**, 813–823.
- Zhang, Y., Liu, X., Bai, J., Tian, X., Zhao, X., Liu, W., Duan, X., Shang, W., Fan, H.Y., and Tong, C. (2016). Mitoguardin regulates mitochondrial fusion through MitoPLD and is required for neuronal homeostasis. *Mol. Cell* **61**, 111–124.
- Zhao, T., Goedhart, C.M., Sam, P.N., Sabouny, R., Lingrell, S., Cornish, A.J., Lamont, R.E., Bernier, F.P., Sinasac, D., Parboosingh, J.S., et al. (2019). *PISD* is a mitochondrial disease gene causing skeletal dysplasia, cataracts, and white matter changes. *Life Science Alliance* **2**, e201900353.