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Glycerolipid synthesis and lipid trafficking in plant mitochondria

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Abstract

Lipid trafficking between mitochondria and other organelles is required for mitochondrial membrane biogenesis and signaling. This lipid exchange occurs by poorly understood nonvesicular mechanisms. In yeast and mammalian cells, this lipid exchange is thought to take place at contact sites between mitochondria and the ER or vacuolar membranes. Some proteins involved in the tethering between membranes or in the transfer of lipids in mitochondria have been identified. However, in plants, little is known about the synthesis of mitochondrial membranes. Mitochondrial membrane biogenesis is particularly important and noteworthy in plants as the lipid composition of mitochondrial membranes is dramatically changed during phosphate starvation and other stresses. This review focuses on the principal pathways involved in the synthesis of the most abundant mitochondrial glycerolipids in plants and the lipid trafficking that is required for plant mitochondria membrane biogenesis.

Keywords

lipid trafficking; membrane biogenesis; membrane contact sites; mitochondria; phosphate starvation; plants

Introduction

Mitochondria are organelles present in nearly all eukaryotic cells and play important roles in multiple fundamental processes including energy production, metabolic syntheses, and programmed cell death. Mitochondria are delineated by two membranes, the outer (OM) and the inner (IM) membrane, which are composed of phospholipids and proteins. The biogenesis of mitochondria requires the import not only of proteins from the cytoplasm but also of lipids from extramitochondrial membranes as mitochondria cannot synthesize all the lipids necessary for membrane biogenesis. While mechanisms of protein import into mitochondria are now well described [1], much less is known about processes involved in

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Author Contributions

All the authors wrote the paper.

phospholipid trafficking to and within mitochondria [2,3]. In yeast, the enzymes involved in mitochondrial phospholipid synthesis are well described and the biogenesis of mitochondrial membranes requires extensive lipid exchanges with the endoplasmic reticulum (ER) and the vacuole [2,4–6]. In addition, phospholipids have to be distributed between the two mitochondria membranes implying a transfer of lipids not only between mitochondria and other organelles but also between the OM and the IM. In plants, few data are available concerning the synthesis of mitochondria membranes. Interactions between mitochondria and ER are suspected to play a role in this process but this has never been demonstrated. In addition, in the absence of phosphate (Pi), plastids transfer galactoglycerolipids to mitochondria indicating that plastids are also involved in mitochondria membrane biogenesis at least during Pi starvation [7]. Recently, a mitochondrial protein involved in lipid trafficking has been identified in *A. thaliana*, opening new perspectives in the understanding of this process in plants [8]. In this minireview, we describe the principal synthesis pathways of the main mitochondria glycerolipids and their intracellular localization in plants. Then, we discuss the possible fluxes of lipids between mitochondria and other organelles and the putative proteins that could be involved in such processes.

Mitochondrial glycerolipid synthesis in plants

Overview of mitochondrial glycerolipid synthesis and composition in plants

Glycerolipids are composed of a glycerol backbone esterified with two fatty acids (FAs) in position *sn-1* and *sn-2*. In plants, *de novo* FAs synthesis occurs mainly in plastids [9]. Thereafter, these FAs are incorporated into plastid glycerolipids to build photosynthetic membranes and are also exported outside the plastids where they are incorporated into extraplastidial glycerolipids in the ER [9]. Thus in plants, there are two main sites for glycerolipids synthesis: the plastids and the ER. Different families of glycerolipids are defined according to the polar head group present at the *sn-3* position of the glycerol backbone. Here, we focus on the phospholipid and galactoglycerolipid families.

Phospholipids are glycerolipids containing a Pi moiety in their polar head. They are mainly synthesized in the ER and are the major constituents of extraplastidial membranes including mitochondria in normal growth conditions (Table 1). The two most abundant **classes** of phospholipids in plant mitochondria are phosphatidylcholine (PC) and phosphatidylethanolamine (PE), like in yeast and mammals (Table 1) [2]. Mitochondria membranes also contain phosphatidylinositol (PI), phosphatidylglycerol (PG), and cardiolipin (CL), a mitochondrial-specific lipid that is necessary for a number of critical processes. Phosphatidylserine (PS) is a minor constituent of mitochondrial membranes (up to 3% in yeast) and is not detected in plant mitochondria (Table 1) [2,10]. However, PS is an important phospholipid in mitochondria because it is a precursor of PE (see below).

Galactoglycerolipids are nonphosphorous glycerolipids containing galactose residues in their polar head and are the major constituents of plastid membranes [11]. They are synthesized in plastids and are found mainly in this organelle in normal growth conditions [11]. However, during Pi starvation, a situation that is common in plants, a specific lipid remodeling occurs leading to drastic changes in the glycerolipid composition of plant cells [7,12]. Indeed, phospholipids such as PC and PE are degraded to release Pi necessary for

other cellular processes and are recycled to form the galactoglycerolipid digalactosyldiacylglycerol (DGDG). DGDG synthesized in plastids is then transferred to mitochondria and other extraplastidial membranes such as the plasma membrane (PM) and the tonoplast [7,13]. Thus, during Pi starvation, DGDG become one of the major glycerolipids of mitochondria, composing up to 18% of the membrane lipids (Table 1). The exact role of DGDG in mitochondria during Pi starvation (structural and/or functional) is still elusive. Low amounts (< 3%) of galactoglycerolipids are also detected in mitochondria purified from plants grown in normal (i.e., not Pi starved) conditions (Table 1) [7,8]. Whether these galactoglycerolipids are actual components of mitochondrial membranes or derive from remnants of plastids that copurify with mitochondria is still under debate.

Phospholipid synthesis in plants

In this part of the review, we briefly describe the main pathways involved in phospholipid synthesis in plants and we highlight the intracellular localization of the enzymes involved. More detailed information about plant lipid synthesis can be found in other reviews [2,9].

Phosphatidylethanolamine is synthesized by two major pathways: the PS decarboxylation pathway, probably minor in plants, and the CDP-ethanolamine (CDP-Etn) pathway, essential in plants (Fig. 1A). The PS decarboxylation pathway involves the direct decarboxylation of PS by PS decarboxylase (PSD) (Fig. 1A). *A. thaliana* has three distinct PSDs. Their subcellular localizations were determined by GFP fusion: PSD1 is in the IM of mitochondria, PSD2 is in the tonoplast, and PSD3 is in the ER [14]. PSD2 and PSD3 represent nearly two-third of the PSD activity in leaves. Single, double, or triple *psd* mutant plants do not have major defects in growth or development [14,15]. Only a slight accumulation of PS and a small decrease of PE contents are observed in the mitochondria of the triple *psd1psd2psd3* mutant, indicated either that the PS decarboxylation pathway is a minor contributor to total PE synthesis in plants or that plants compensate for the loss of all three PSDs [14]. The first step of PE biosynthesis by the CDP-Etn pathway is the phosphorylation of ethanolamine (Etn) by the Etn kinase (EK) (for a review see [16]) (Fig. 1A). Phospho-ethanolamine (P-Etn) is used to form CDP-Etn by phospho-ethanolamine-cytidyl transferase (PECT) and finally, CDP-Etn is used, together with diacylglycerol (DAG), by the aminoalcoholphosphotransferase (AAPT) to form PE. *A. thaliana* is able to synthesize Etn by the decarboxylation of free L-serine catalyzed by a serine decarboxylase (SDC), a soluble enzyme localized in the cytosol when fused to GFP [17,18]. In plants, EKs are soluble enzymes with specificity for Etn phosphorylation [19]. PECT activity has been detected in both mitochondrial and ER fractions in castor bean endosperm, with 80% of the activity associated to the surface of mitochondria [19]. In *A. thaliana*, only one PECT gene has been identified (PECT1). PECT1 encodes a protease-sensitive protein enriched in mitochondria fractions. The PECT1-GFP fusion protein forms a ring-like pattern characteristic of mitochondria OM proteins when expressed in *Arabidopsis* protoplasts [20,21]. These findings suggest that PECT1 is a membrane protein localized on the cytoplasmic leaflet of the mitochondria OM in *A. thaliana* [20,21]. Homozygous *pect1* T-DNA KO plants are embryo-lethals, demonstrating the importance of this OM-localized PECT1 enzyme in *A. thaliana* PE synthesis [20]. In wheat, GFP fused to AAPT localizes in the ER and the Golgi complex [22]. Most of the studied plant AAPTs present a dual specificity

for both CDPEtn and CDP-Choline (CDP-Cho) with sometimes a preference for one of these two substrates [22,23]. Two AAPT genes with a dual specificity are encoded in the *A. thaliana* genome [23]. Plants with mutations in either *aapt1* or *aapt2* genes do not present any growth phenotype, whereas the double mutant *aapt1aapt2* is embryo-lethal showing these two enzymes have redundant functions and that the CDP-Etn and CDP-Cho pathways are essential for plant development [24].

Two main pathways lead to PC synthesis: the methylation pathway and the CDP-Cho pathway [2]. The CDP-Cho pathway involves a similar line of reactions as the CDP-Etn pathway described above (Fig. 1A) (for a review see [16]). Choline is phosphorylated by choline kinase (CK) to form phospho-choline (P-Cho) that is then activated into CDP-Cho by the phospho-choline cytidyltransferase (CCT) and finally transferred onto a DAG backbone by AAPTs. CKs seem to be soluble enzymes encoded by several isoforms in soybean and in *A. thaliana* [25]. Plant CCTs were found to be associated with the surface of the ER and two isoforms exist in *A. thaliana* [26,27]. The final step of the CDP-Cho pathway is catalyzed in plants by the dual-specificity AAPT enzymes localized in the ER (see previous section). In the methylation pathway for synthesizing PC in yeast and metazoans, the PE polar head is methylated three times by the PE-methyltransferase (PEMT) using S-adenosyl-methionine (SAM) as a methyl donor group [2]. However, plants are not able to directly methylate PE and use an alternative pathway in which free P-Etn is methylated (Fig. 1A). After a first methylation, the phospho-monomethyl-ethanolamine (P-MMEtn) formed can be subsequently methylated to form phospho-dimethyl-ethanolamine (P-DMEtn) and phospho-choline (P-Cho) which can be used by AAPTs to synthesize PC. These methylations are catalyzed by phospho-ethanolamine methyltransferase (PEAMT). On the other hand, each of these methylated phospho-intermediates can be converted to a CDP-activated form, charged on DAG and fully methylated at the level of the phosphatidyl molecule by a phospholipid methyltransferase (PLMT) (Fig. 1A). The relative contribution of each pathway to total PC content differs in various plant species [28]. In *A. thaliana*, two PEAMT genes have been identified. One (PEAMT1) is able to catalyze the three consecutive methylations of P-Etn, whereas the second (PMEAMT) can use only P-MMEtn and PDMEtn as substrates [29,30]. A PLMT enzyme has also been identified but it seems to play only a minor role in *A. thaliana* PC synthesis [31]. PEAMTs are soluble enzymes that are predicted to be cytoplasmic, whereas PLMT harbors transmembrane domains and appears to be localized in the ER membrane [30].

Phosphatidylglycerol is only a minor constituent of mitochondria membranes but is an important precursor for cardiolipin synthesis. PG is formed in two steps and starts with the synthesis of phosphatidylglycerolphosphate (PGP) from CDP-DAG and glycerol-3-phosphate catalyzed by PGP synthase (PGPS) (Fig. 1A). Then PGP is dephosphorylated to form PG by a PGPS phosphatase. Two PGPS enzymes have been found in *A. thaliana*. Using GFP fusion, it has been shown that PGPS1 is dually targeted to both mitochondria and chloroplasts, whereas PGPS2 is localized only in the ER [32,33]. *Pgps1* mutant plants present a drastic chloroplast phenotype with reduced PG content and impairment of thylakoid biogenesis and photosynthesis. However, no defects in mitochondria structure or lipid content were observed, suggesting that mitochondria, unlike plastids, can import PG synthesized by PGPS2 in the ER [32,34]. Recently, a PGP phosphatase, named CrPGPP1,

has been characterized in the microalgae *Chlamydomonas reinhardtii* [35]. CrPGPP1 complements the growth and lipid defects of the yeast PGPP mutant *gpp4* [35]. The homolog of CrPGPP1 in *A. thaliana* could be At3g58830 [35]. The At3g58830 protein contains a putative PGPP domain (PF09419) and is predicted to be targeted to mitochondria and/or chloroplasts.

Cardiolipin is an unusual membrane lipid because it contains four acyl-chains and is synthesized by the condensation of CDP-DAG and PG molecules (Fig. 1A). This reaction is catalyzed by the CL synthase (CLS) localized in the IM of mitochondria [36–38]. CLS is encoded by one gene (CLS1) in *Arabidopsis* [39]. Similar to yeast *cls1* cells, *A. thaliana cls1* mutant plants show defects in growth, mitochondria ultrastructure, and assembly of respiratory chain complexes [38,40]. They also present defects in mitochondria fission and are more sensitive to stresses inducing programmed cell death. In *Arabidopsis*, CL can be enriched in specific domains of mitochondria called CL-enriched membrane domains (CMDs) [38,41]. These domains have been observed in bacteria membranes but not in yeast and mammals [41]. In addition, similar to mammals, plant CL acyl-chains are longer and highly unsaturated compared to those found in yeast and bacteria CL [42]. All these results suggest that CL in plants has similar functions to CL in yeast and mammals but could also have particular roles illustrated by the presence of CMDs and the acyl-chain composition of CL.

Phosphatidylinositol is synthesized by PI synthase (PIS) from CDP-DAG and *myo-inositol* in plants (Fig. 1A). There are two of these enzymes in *A. thaliana*, AtPIS1 and AtPIS2, and GFP fusions indicate that they localize in the ER and perhaps also the Golgi complex [43].

Phosphatidylserine can be synthesized by two different pathways catalyzed by two different PS synthases (PSSs) associated with the endomembrane system in plants (Fig. 1A) [44,45]. The base-exchange (BE) pathway corresponds to the substitution of the head group of PE or PC with serine. The CDP-DAG (CD) pathway leads to the formation of PS by the condensation of CDP-DAG with serine. Both pathways can occur in plants depending on the species but only the BE-pathway is present in *A. thaliana* [44,45].

Galactoglycerolipids synthesis

The two galactoglycerolipids present in plastid membranes [i.e., monogalactosyldiacylglycerol (MGDG) and DGDG] are synthesized by the MGDG synthase (MGD) and the DGDG synthase (DGD) enzymes present in the plastid envelope (Fig. 1B) (for a review see [11]). Three MGD isoforms are found in *A. thaliana* and they catalyze the formation of MGDG by the transfer of a galactose molecule from UDP-galactose to a DAG moiety [46]. MGD1 is localized in the inner envelope of plastids and is responsible for the bulk synthesis of MGDG. MGD2 and MGD3 are present in the outer envelope at the surface of the plastid and are mainly involved in the synthesis of galactoglycerolipids during Pi starvation and in roots and pollen tube [46,47]. Two genes encode DGD enzymes in *A. thaliana*. DGD1 and DGD2 are localized in the outer envelope at the surface of plastids and are able to transfer a galactose residue from UDP-galactose to MGDG to form DGDG (Fig. 1B) [48]. In leaf, DGD1 is involved in the bulk synthesis of

DGDG, whereas DGD2 seems to act mainly during Pi starvation in association with MGD2/3 [48].

Mitochondria glycerolipid trafficking in plants

What is known in nonplant organisms

As described above, mitochondria membrane biogenesis requires lipid exchanges between mitochondria and other organelles, such as the ER and vacuoles in yeast or the plastids in plants. There is also lipid exchange between the outer and the inner membranes of mitochondria. Mitochondria are not connected to the vesicular pathway. Therefore, lipid transport is thought to occur at contact sites between membranes. Organelle contact sites have important functions in addition to lipid trafficking. Indeed, in yeast and mammals, ER–mitochondria contact sites also play a role in mitochondria division, calcium exchange, and mitochondria inheritance (for a review see [49]). Most of the proteins identified at contact sites between mitochondria and ER or vacuoles in yeast are involved in the process of membrane tethering and the proteins and mechanisms involved in the transfer of lipids *per se* are still elusive (Table 2) (for a review see [2,3]). In mammals, most of the complexes identified between ER and mitochondria seem to be involved in Ca²⁺ exchanges (Table 2).

Traffic between mitochondria and other organelles

The most studied tethering complex in yeast is the ER–mitochondria encounter structure (ERMES) complex, composed of four core subunits (Mdm10, Mdm12, Mdm34, and Mmm1), which tethers ER and mitochondria membranes (Table 2) [4,50]. Deletion of ERMES subunits leads to an impairment in ER–mitochondria phospholipid trafficking [4,51]. However, the role of this complex in phospholipid translocation, in addition to its role in membrane tethering, and the nature of the transported lipids are still under debate. Recently, structural analyses suggest that the ERMES complex is able to bind glycerolipids and may have a preference for PC [52]. It has been suggested that ERMES may form a hydrophobic tunnel between ER and mitochondria, supporting a role of the ERMES complex in phospholipid translocation [52].

The second complex identified in yeast as a ER–mitochondria tether is the ER membrane complex (EMC)–Tom5 complex (Table 2) [53]. This complex is composed of five ER membrane subunits that are able to interact with the small OM protein Tom5, which is part of the translocase of outer membrane (TOM) complex involved in protein import across the OM [53]. Disruption of the EMC–Tom5 interaction interferes with the transfer of PS from ER to mitochondria suggesting this complex facilitates the mitochondria import of PS [53]. The EMC–Tom5 complex is involved in ER–mitochondria tethering but the role of this complex in the direct translocation of lipids between membranes is still unknown [53].

Recently, interactions between mitochondria and vacuoles, called vacuole and mitochondria patches (vCLAMPs), were identified and it was suggested that these interactions could be involved in the transfer of lipids to mitochondria (Table 2) [5,6,54]. The vCLAMPs are suspected to have redundant functions with the ERMES complex in lipid trafficking because deletion of a subunit involved in vCLAMPs formation increases the number of ERMES-

mediated ER–mitochondria contact sites and inversely, deletion of ERMES subunits leads to an increase of mitochondria–vacuole contact sites [6]. Furthermore, the number of each contact site is regulated in function of the physiological cell demand with an increase of ERMES complex-mediated ER–mitochondria contact sites when cells are grown in the presence of glycerol concomitant with a decrease of vCLAMP-mediated mitochondria–vacuoles junctions [5]. These results show a coregulation of the contact sites between mitochondria and other organelles in response to environmental changes [5,6]. Recently, the uncharacterized protein Lam6 was shown to be present at several organelle contact sites [55,56]. Indeed, Lam6 is present at the ER–mitochondria junctions where the protein interacts with the ERMES complex and the OM proteins Tom70/71 [55,56]. Lam6 has also been found at the mitochondria–vacuoles contact sites and at the nucleovacuolar junctions [55,56]. Lam6 is not required for the formation of these different contact sites but regulates the extent of association between these organelles opening new perspectives in the understanding of the regulation of contact sites between organelles [55,56].

Traffic between the OM and IM of mitochondria

Several proteins have been demonstrated to be involved in lipid trafficking and/or in OM–IM tethering in yeast and mammals (Table 2) (for a review see [2,3]). The lipid transfer proteins UPS1/UPS2 are soluble proteins of the IMS that are involved in the transfer of PA and other phospholipids between mitochondria membranes [57,58]. They also somehow play a role in the regulation of mitochondria CL and PE levels [57,58]. The transport of PA by Ups1 requires its association with a chaperone, Mdm35, which maintain Ups1 in a lipid transport competent conformation [59].

The mitochondrial nucleotide diphosphate kinase (NDPK-D) is a hexameric enzyme playing a role in both OM–IM tethering and CL transfer [60]. NDPKD is involved in the transfer of c-phosphate between nucleoside triphosphates and diphosphates and is able to bind CL present in both the OM and IM allowing the tethering of these membranes. The binding of NDPK-D to membranes promotes the transfer of CL from the IM to the OM concomitant with a decrease of the phosphotransferase activity of the enzyme [60]. It has been proposed that the transfer of CL to the OM mediated by NDPK-D could be involved in the activation of apoptosis [60]. A similar mode of action is also observed for the octameric form of the mitochondrial creatine kinase (mtCK) in mammals [60].

The tethering of the OM and the IM of mitochondria can also be mediated by different complexes (Table 2). In mitochondria, two complexes localized respectively in the OM and the IM, the TOM and the translocase of inner membrane (TIM) complexes, are involved in the import of proteins into mitochondria [1]. These complexes are also able to mediate the tethering of mitochondrial membranes *via* the interaction of the Tom22 and Tim21 subunits (Table 2) [61]. This interaction is required for the import of mitochondrial proteins [61]. The mitochondria contact site and cristae organizing system (MICOS) is a complex localized in the IM and involved in several processes such as membrane contact sites, cristae morphology, or protein import [62]. One of the core subunits of this complex, Mic60, is able to interact with several proteins of the OM like Tom40, Sam50, VDAC, Metaxin, or Ugo1, and mediates by this way contact sites between the IM and the OM [63–67]. In addition, the

MICOS complex shows genetic interactions in yeast with components of the ERMES complex and consequently, a role of the MICOS complex in lipid trafficking from ER to the IM of mitochondria has been proposed [66]. This hypothesis is supported by a recent study in plants showing the involvement of Mic60 in mitochondria lipid trafficking (see below) [8].

Glycerolipids trafficking in plant mitochondria

Putative pathways involved in plant mitochondria glycerolipids trafficking

As described above, plant mitochondria are able to synthesize only a small fraction of their membrane phospholipids and obtain most of them from extramitochondrial membranes (Fig. 2).

The contact sites involved in lipid trafficking to plant mitochondria are still poorly characterized. ER–mitochondria contact sites have been observed in plants [68–70]. Two recent papers suggest a role of these contacts in the regulation of mitochondria division and morphology [69,70] but their role in lipid exchanges, although suspected, has never been demonstrated. According to the localization of the enzymes involved in glycerolipid synthesis, plant mitochondria should import PC, PI, PS, and most of the PE from the ER (Fig. 2, green arrows). Plant mitochondria are able to synthesize PG but can also import this lipid from ER when the mitochondrial PGPS1 enzyme is nonfunctional [32]. Plant mitochondria can also establish contact with plastids. These contact sites are thought to be involved in the transfer of DGDG from plastids to mitochondria during Pi starvation suggesting that plastids can also be a source of lipids for mitochondria, at least in this stress condition (Fig. 2) [7]. The number of mitochondria–plastids junctions increases during Pi starvation showing that the extent of contact sites between mitochondria and plastids is regulated by environmental changes in a similar way to what has been described for mitochondria contact sites in yeast [5,7]. Yeast mitochondria seem to also exchange lipids with the vacuole [5,6,54]. An analysis of the intracellular localization of the phospholipase D PLDf2 in *A. thaliana* suggests that the vacuolar membrane (also called tonoplast) could play a role in the lipid remodeling occurring during Pi starvation [71]. PLDξ2, which removes the polar head of glycerolipids to form phosphatidic acid, is evenly distributed in the tonoplast membrane in Pi sufficient condition but is enriched to some domains of the tonoplast when Pi availability is limited [71]. Interestingly, these PLDξ2-enriched domains are positioned close to mitochondria and plastids suggesting that tonoplast–mitochondria and tonoplast–plastid contact sites might exist in plants and might be involved in the remodeling of lipids during Pi starvation [71].

It should be noted that the extent of lipid exchanges between ER and mitochondria in plants is probably different from that in yeast. As described above, the CDP-Etn pathway mainly mediates the synthesis of PE in plants, whereas in yeast, the mitochondrial PSD1 plays a key role in the synthesis of PE in mitochondria [72]. Consequently, yeast mitochondria have to massively import PS from the site of synthesis, the ER [2]. Thus, the flux of PS coming from the ER is probably less important in plant mitochondria compared to yeast. Nevertheless, one of the key enzymes of the CDP-Etn pathway in plants, PECT1, is localized at the surface of mitochondria, whereas the enzymes catalyzing the following and final step of this

pathway, AAPT1/2, are in the ER membrane (Fig. 2) [21]. This suggests an intimate cooperation between ER and mitochondria for the regulation of cellular PE synthesis. In addition, most of the models about the biogenesis of mitochondria postulate that the lipids synthesized in extramitochondria membranes have to be translocated to the mitochondria membrane (Fig. 2, green arrows). However, another interesting hypothesis is that enzymes present in one membrane can act on a second membrane if the two membranes are close enough (Fig. 2, red arrows). This mechanism, also referred as *in trans* activity, has been suggested by a few studies in yeast and in *Arabidopsis* [73–75]. In *Arabidopsis*, Mehrshahi and colleagues developed a transorganellar complementation strategy to show that mutant plants for different plastid-localized enzymes involved in tocopherol and carotenoid synthesis are complemented by the expression of the corresponding enzymes retargeted in the ER [75]. These results show that the enzymes present in the ER can access the hydrophobic precursors of tocopherol and carotenoid present in plastid envelope supporting a model of action *in trans* of these enzymes in an artificial system [75]. In yeast, the PEMT enzyme Opi3 localized in the ER directly methylates PE present in the PM to form PC at contact sites between both organelles [74]. In a same way, the PI-4-phosphate (PI4P) present in PM can be directly hydrolyzed to form PI by the PI4P phosphatase Sac1 localized in the ER allowing the regulation of PI4P level into the PM [76]. The mechanism of action *in trans* requires the tethering of two membranes but not the transfer of lipids from one membrane to the other. Thus, the plants ER-localized AAPTs may act *in trans* on mitochondria OM DAG to directly synthesized PC and PE on the surface of mitochondria (Fig. 2, red arrows). Also during Pi starvation, the induced MGD2/3 and DGD2 enzymes present at the surface of plastids might synthesize DGDG directly on mitochondria OM from DAG substrates (Fig. 2, red arrows). If these ER enzymes do operate *in trans* at ER-mitochondria contact sites in plants, direct transfer of lipids between mitochondria and other organelles might only be required for some classes of glycerolipids such as the precursor molecules DAG or CDP-DAG. Similarly, lipid remodeling during Pi starvation might not necessarily involve massive transfer of lipids but rather the removal of mitochondrial phospholipids polar head by phospholipases and formation of DGDG *in trans* by MGDs and DGDs present in plastids.

Proteins involved in plant mitochondria lipid trafficking

Table 2 shows the few proteins that have been found to be involved in mitochondria lipid trafficking and/or membrane tethering in plants and also a list of the putative homologs of the yeast and mammalian proteins that can be found in *Arabidopsis*.

Only one protein in plants, called mitochondria–ER-localized LEA-related LysM domain protein 1 (MELL1) has been identified at contact sites between ER and mitochondria (Table 2) [70,77]. MELL1 over-expression increases the extent of mitochondria–ER contact sites and modulates the shape and number of mitochondria [70]. It was proposed that this protein could regulate the process of mitochondria fusion or fission in moss by directly or indirectly affecting the proteins present at mitochondria–ER contact sites [70]. Thus, isolating proteins that interact with MELL1 or are regulated by it may be a good way to identify other proteins present at ER–mitochondria junctions in plants.

Of all the protein complexes thought to tether mitochondria to other organelles in yeast and mammals, only the yeast EMC–Tom5 complex and proteins of the vCLAMP may be present in plants (Table 2). Indeed, many putative tethering complexes are poorly conserved between species (Table 2). For example, the first tethering complex identified in yeast (i.e., ERMES [4]), is not found in mammalian cells. This poor conservation probably reflects the fact that: (a) the nature and extent of lipids that traffic to mitochondria vary from one species to another and (b) contact sites between mitochondria and other organelles such as ER are not only involved in lipid trafficking but also in other processes such as calcium signaling or apoptosis, which can be restricted to particular species [78].

As shown in Table 2, *Arabidopsis* homologs can be found for most of the proteins involved in OM–IM lipid trafficking in yeast and mammals, suggesting that intramitochondrial lipid trafficking pathways are better conserved than transport pathways between mitochondria and other organelles. Indeed, homologs can be found for UPS1/2, NDPK-D, Tom22, and Tim21 suggesting that the interaction between the TOM and TIM complexes is also conserved in plants (Table 2). Interestingly, one of the IM subunits of the TIM complex, Tim17.2, presents a C-terminal extension of 85 amino acids in *A. thaliana* compared to yeast and mammalian homologs [79]. This extension is inserted into the OM and consequently, Tim17.2 is able to bridge the two mitochondrial membranes together [79]. This Ct domain is essential for the import of Tim17.2 into mitochondria but its exact role is still poorly understood [79]. The core components of the MICOS complex, Mic60, is also present in *Arabidopsis* mitochondria IM and is able to interact with the OM protein Tom40 showing that the tethering capacity of Mic60 is also conserved in plants (Table 2) [8]. Furthermore, AtMic60 is involved in the export of PE from mitochondria and in the import of DGDG from plastids during Pi starvation, attributing a new function for this protein in mitochondria lipid trafficking [8]. AtMic60 is able to destabilize liposomes *in vitro* and it was proposed that AtMic60 could be involved in mitochondria lipid trafficking by regulating the tethering of the IM with the OM and by destabilizing membranes, which is likely to promote lipid desorption from membranes [8]. Interestingly, AtMic60 was found to be part of a large lipid-binding complex called the mitochondrial transmembrane lipoprotein (MTL) complex, which also contains the TOM complex [8]. The quantity of AtMic60 and Tom40 increases in the MTL complex during Pi starvation similar to the content of DGDG suggesting that the MTL complex, *via* AtMic60 and OM partners such as Tom40, could mediate the traffic of DGDG in mitochondria during Pi starvation [8]. In addition, some proteins, of unknown functions, that localize to the outer envelope of plastids have been characterized as components of the MTL complex [8]. These proteins are putative candidates to mediate membrane tethering and/or lipid trafficking between mitochondria and plastids. Furthermore, if, as in yeast, the plant TOM complex is able to interact with proteins present in the ER, such as the EMC complex [53], the MTL complex, *via* AtMic60 and/or other partners, might also be involved in the mitochondria–ER tethering and/or in the transfer of lipids between these membranes. This work opens new perspectives to understand the mechanisms involved in plant mitochondria membrane biogenesis and further investigations are needed to define the partners of AtMic60 in lipid trafficking and to understand the role of the MTL complex in such process.

Importance of mitochondria lipid trafficking in plants

The importance of lipid trafficking to plant mitochondria, particularly during stress condition, is still poorly understood because few of the key proteins have been identified. We know that the morphology and the respiration of plant mitochondria are not altered during Pi starvation [7]. This demonstrates that the drastic modification of the mitochondrial membranes lipid composition does not alter the structure and the function of this organelle during this stress. So far, aside from AtMic60, no protein involved in mitochondria lipid transport has been characterized in plants. However, in the absence of AtMic60, plants have a partial defect in lipid trafficking and have no visible phenotype in the presence or absence of Pi (M. Michaud and J. Jouhet, unpublished data) [8]. Further studies are necessary to explore the effect of the absence of AtMic60 at the subcellular level. The other putative candidates identified in the MTL complex, such as Tom40, are multifunctional proteins that are essential in plants and cannot be investigated by conventional genetic approaches. Thus, it is a challenge to characterize new mutant plants that are more strongly affected in plant mitochondria lipid trafficking without being lethal.

The importance of lipid remodeling during Pi starvation can also be evaluated with mutant plants affected in galactoglycerolipid synthesis. In plants that lack MGD3 (*mgd3*) or MDG2 and MDG3 (*mgd2mgd3*), the remodeling of lipids is highly impaired in the absence of Pi, particularly in roots [47]. In this situation, *mgd3* and *mgd2mgd3* plants have a reduced fresh weight and root growth and an alteration of photosynthesis compared to wild-type plants [47]. However, mitochondrial respiration is not altered in *mgd3* and *mgd2mgd3* mutant roots grown in the absence of Pi [47]. These results indicate that the inability of plants to increase their cellular level of galactoglycerolipids when Pi availability is low affects plant development and the function of plastids but only modestly reduces mitochondria respiration capacity.

Outstanding questions and future prospects

Communication between mitochondria and other organelles is essential for plant cell viability. This dialog is mediated *via* contact sites between the mitochondria and the ER and plastids and perhaps other organelles as well. Whether plant mitochondria are also able to interact with other organelles, such as vacuoles or PM, to obtain lipids for membrane biogenesis is still an open question. The recent identification of MELL1 at contact sites between ER and mitochondria in moss and of AtMic60 and its role in mitochondria lipid trafficking in *Arabidopsis* have allowed us to start to dissect mitochondria–organelle contact sites and lipid trafficking in plant mitochondria. However, a lot of more work will be necessary to increase our knowledge of mitochondria contact sites, their role in lipid trafficking and membrane biogenesis, as well as their conservation and regulation in plants. A current challenge in the study of plant mitochondria is the lack of marker proteins for contact sites like the ERMES complex or vCLAMP in yeast. These proteins will be useful tools to study the dynamic of contact sites *in vivo* and also to find additional proteins involved in organelle junctions and/or lipid trafficking. However, in plants, we can take advantages of the lipid remodeling that occurs during different stress conditions to study the regulation of organelle contact sites and to find new partners involved in contact sites

establishment or lipid trafficking, for example by looking at proteins, like PLD ξ 2, that are up-regulated or that change their localization during stress. Finally, working with mutants affected in lipid trafficking or that are unable to remodel their lipid composition during stress may help us to better understand the importance and impact of mitochondria lipid remodeling during environmental stress in plants.

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Abbreviations

AAPT	aminoalcoholphosphotransferase
BE-PSS	base exchange phosphatidylserine synthase
CCT	phospho-choline cytidyltransferase
CDPSS	CDP-DAG phosphatidylserine synthase
Cho	choline
CK	choline kinase
CL	cardiolipin
CLS	cardiolipin synthase
CMD	cardiolipin-enriched membrane domain
DAG	diacylglycerol
DGD	digalactosyldiacylglycerol synthase
DGDG	digalactosyldiacylglycerol
EK	ethanolamine kinase
EMC	ER membrane complex
ER	endoplasmic reticulum
ERMES	ER–mitochondria encounter structure
Etn	ethanolamine
FA	fatty acid
GFP	green fluorescent protein
IM	inner membrane
IMS	intermembrane space

MGDG	monogalactosyldiacylglycerol
MGD	monogalactosyldiacylglycerol synthase
MICOS	mitochondria contact site and cristae organizing system
MTL	mitochondrial transmembrane lipoprotein complex
OM	outer membrane
P-Cho	phospho-choline
PC	phosphatidylcholine
P-DMEtn	phospho-dimethyl-ethanolamine
PdtDMEtn	phosphatidyl-dimethyl-ethanolamine
PdtMMEtn	phosphatidyl-monomethylethanolamine
PEAMT	phospho-ethanolamine methyltransferase
PECT	phospho-ethanolamine-cytidyltransferase
P-Etn	phosphoethanolamine
PG	phosphatidylglycerol
PGP	phosphatidylglycerolphosphate
PGPP	phosphatidylglycerolphosphate phosphatase
PGPS	phosphatidylglycerolphosphate synthase
Pi	phosphate
PI	phosphatidylinositol
PIS	phosphatidylinositol synthase
PLMT	phospholipid methyltransferase
PMEAMT	phospho-monomethyl-ethanolamine methyltransferase
P-MMEtn	phospho-monomethyl-ethanolamine
PM	plasma membrane
PSD	PS decarboxylase
PS	phosphatidylserine
SAM	S-adenosyl-methionine
SDC	serine decarboxylase
Ser	serine

TIM	translocase of the inner membrane of mitochondria
TOM	translocase of the outer membrane of mitochondria
UDP-Gal	UDP-galactose
vCLAMP	vacuole and mitochondria patch

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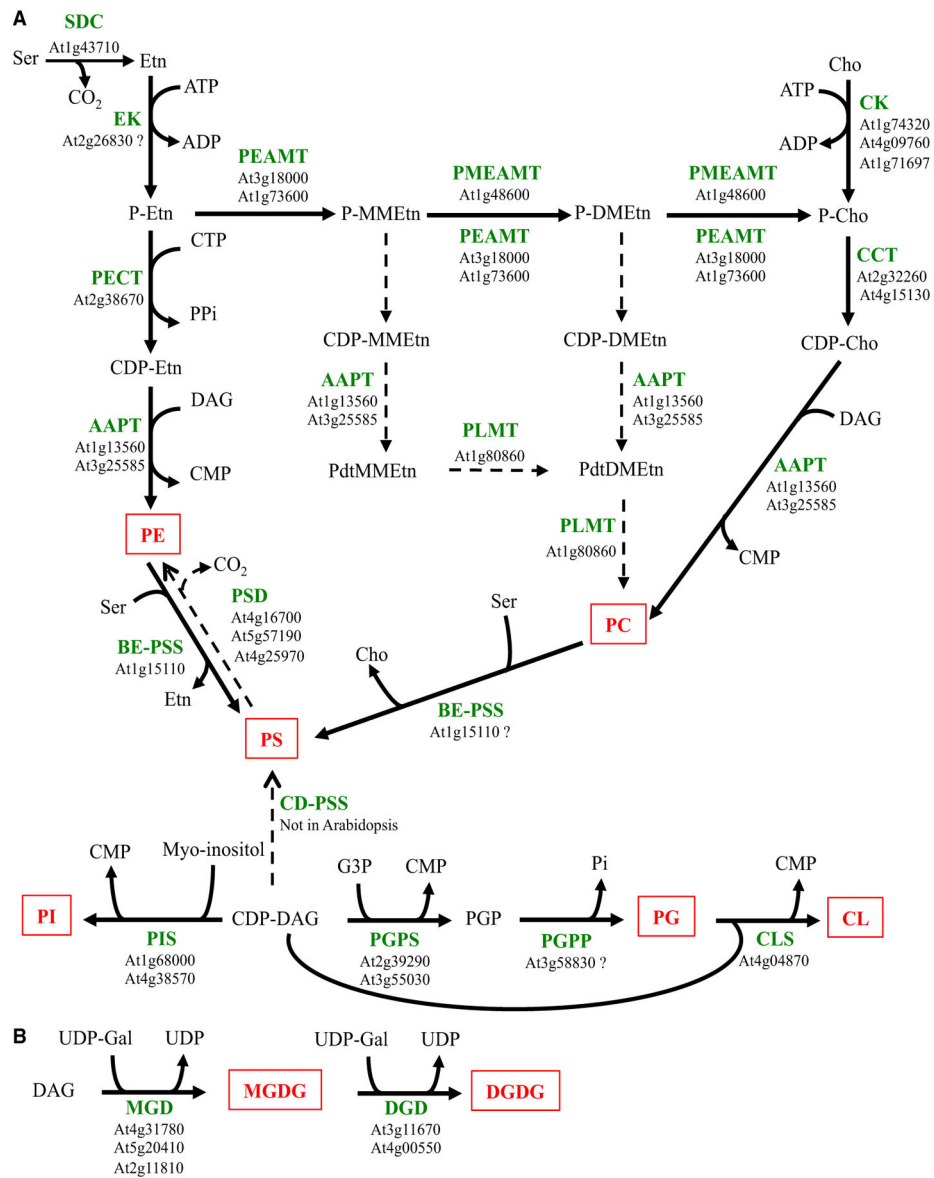


Fig. 1. Pathways involved in the synthesis of the main glycerolipids present in Arabidopsis mitochondrial membranes. (A) Pathways involved in phospholipid synthesis. Full arrows indicate the main reactions occurring in Arabidopsis, whereas dashed arrows represent minor pathways. (B) Pathway involved in galactoglycerolipids synthesis in Arabidopsis. PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; CL, cardiolipin; DAG, diacylglycerol; PGP, phosphatidylglycerolphosphate; Ser, Serine; Etn, ethanolamine; P-Etn, phospho-Etn; P-MMEtn, phospho-monomethyl-Etn; P-DMEtn, phospho-dimethyl-Etn; Cho, Choline; P-Cho, phospho-choline; CDP, cytidyldiphosphate; CMP, cytidylmonophosphate; PdtMMEtn, phosphatidyl-monomethyl-Etn; PdtDMEtn, phosphatidyl-dimethyl-Etn; G3P, glycerol-3-phosphate; SDC, Ser decarboxylase; EK, Etn kinase; PECT, phospho-Etn-cytidyltransferase; AAPT, aminoalcoholphosphotransferase; BE-PSS, base exchange PS synthase; CD-PSS,

CDP-DAG PS synthase; PSD, PS decarboxylase; PEAMT, P-Etn methyltransferase; PMEAMT, PMMEtn methyltransferase; PLMT, phospholipid methyltransferase; CK, Cho kinase; CCT, P-Cho cytidyltransferase; PIS, PI synthase; PGPS, PGP synthase; PGPP, PGP phosphatase; CLS, CL synthase; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; UDP, uridyldiphosphate; UDP-Gal, UDP-galactose; MGD, MGDG synthase; DGD, DGDG synthase.

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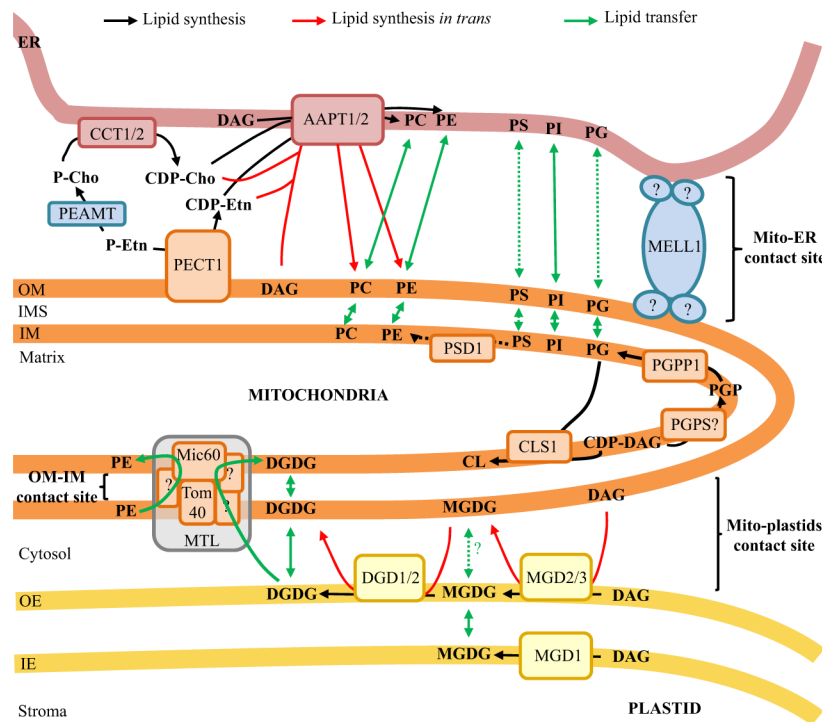


Fig. 2. Routes of lipid trafficking between mitochondria and ER or plastid membranes and between mitochondrial outer (OM) and inner (IM) membranes in Arabidopsis. Lipid trafficking to and into mitochondria is thought to occur at contact sites between membranes. Two mechanisms have been proposed for the role of contact sites in the biogenesis of mitochondrial membranes: transfer of lipids from one membrane to another (green arrows) and enzymes present in a membrane and acting on another membrane in close proximity (red arrows). Dashed arrows indicated pathways that are probably minor. The proteins and complexes recently identified at membrane contact sites and/or involved in plant mitochondria lipid trafficking are also represented. IMS, intermembrane space; OE, outer envelope; IE, inner envelope; MTL, mitochondria transmembrane lipoprotein complex. The other legends are the same as in Fig. 1.

Glycerolipid composition of mitochondria extracted from different types of *A. thaliana* cells grown in the presence or absence of phosphate (Pi). Cell suspensions are green photosynthetic cells, whereas callus are nonphotosynthetic cells. The quantity of each glycerolipid is indicated in mol%. PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin; PI, phosphatidylinositol; PG, phosphatidylglycerol; PA, phosphatidic acid; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; SQDG: sulfoquinovosyldiacylglycerol.

Table 1.

	PC	PE	CL	PI	PG	PA	DGDG	MGDG	SQDG	Ref.
Cell suspension 6 days 1 mM Pi	49	33	11	5	2	-	-	-	-	[10]
Cell suspension 3 days 1 mM Pi	39.4	41.2	10.2	3.3	3	-	1.5	1.4	0	[7]
Cell suspension 3 days 0 mM Pi	32.6	29	14.7	2.3	0.3	-	18.2	2.3	0.6	[7]
Callus 8 days 1 mM Pi	39.1	30.6	6.4	8.7	2.9	6.3	2.4	2	-	[8]
Callus 8 days 0 mM Pi	31.9	22.1	7	6.2	3.4	7.1	15.9	3.2	-	[8]

Table 2.

Proteins identified at contact sites between mitochondria and other organelles or at contact sites between the outer (OM) and the inner (IM) membranes of mitochondria. For each protein, their name, the complex they belong, the organism, and their cellular localization are indicated. The membranes tethered by the complex are also indicated. Putative homologs in Arabidopsis were found by a blast search in TAIR database using the yeast, human, or moss protein sequences as a query and default parameters. *A. thaliana* proteins with a E-value < 0.01 are presented in this table. ERMES, ER-mitochondria encounter structure; TOM, translocase of the outer membrane; TIM, translocase of the inner membrane; EMC, ER membrane complex; vCLAMP, vacuole and mitochondria patch; MICOS, mitochondria contact sites and cristae organizing system; IMS, intermembrane space; C, cytosol; V, vacuole.

Protein	Complex	Organism	Loc.	Ref.	Membrane contact site	Role(s)	Putative homologs in Arabidopsis				
Mdm10	ERMES	Yeast	OM	[4]	M-ER	Membrane tethering, lipid transfer, mitochondria division and inheritance, mitophagy	No				
Mdm12			OM				No				
Mdm34			OM				No				
Mmm1			ER				No				
Gem1p			OM				[50]	Miro1 (At5g27540), Miro2 (At3g63150) Miro3 (At3g05310)			
Lam6	TOM	Yeast	ER	[55]	M-ER	Membrane tethering, lipid transfer	VAD1 (At1g02120), At3g59660, At1g03370				
Tom5			OM	[53]			At5g08040				
EMC1			EMC	ER				At5g11560			
EMC2				ER				No			
EMC3				ER				At4g12590			
EMC5				ER				No			
EMC6				ER				No			
Vps39			vCLAMP	Yeast			V	[5,6]	M-V	Membrane tethering, lipid transfer	EMB2754 (At4g36630)
Ypt7							V	[5]			Yes (more than 10)
Vps13							V	[54]			At4g17140, At1g48090, At5g24740
Lam6	ER	[55]			VAD1 (At1g02120), At3g59660, At1g03370						
Lam6		Yeast	ER	[56]	M-ER	Membrane tethering	VAD1 (At1g02120), At3g59660, At1g03370				
Tom70			OM				No				
Tom71			OM				No				
Mmr1				Yeast			OM/ER	[80]	M-ER	Membrane tethering, mitochondria inheritance	No
VDAC1				Mammals			OM	[81]	M-ER	Membrane tethering, calcium exchange	VDAC1 (At3g01380), VDAC2 (At5g67500), VDAC3 (At5g15090), VDAC4 (At5g57490)
Grp75			C				mtHSP70-1 (At4g37910), mtHSP70-2 (At5g09590)				
IP ₃ R			ER				No				
PTPIP51			Mammals	OM			[82]	M-ER	Membrane tethering, calcium exchange	No	
VAPB				ER				VAP27-1 (At3g60600), PVA12 (At2g45140), At5g47180, At4g00170, At2g23830, VAP27-2 (At1g08820)			

Protein	Complex	Organism	Loc.	Ref.	Membrane contact site	Role(s)	Putative homologs in Arabidopsis
Fis1		Mammals	OM	[83]	M-ER	Membrane tethering, calcium exchange	FIS1A (At3g57090), FIS1B (At5g12390)
BAP31			ER				At5g42570, At1g11905
MELL1		Moss	OM/ER	[70,77]	M-ER	Membrane tethering, mitochondria morphology	At5g44310, At4g21020, At1g72100
Ups1		Yeast/mammals	IMS	[59]		PA transfer between OM-IM	At5g13070
Mdm35			IMS				At4g33100
Ups2		Yeast	IMS	[58]		PL transfer between OM-IM	At5g13070
NDPK-D		Mammals	IMS	[60]	OM-IM	CL transfer between OM-IM	NDPK3 (At4g11010)
mtCK		Mammals	IMS	[84]	OM-IM	CL transfer between OM-IM	No
Tom22	TOM	Yeast	OM	[61]	OM-IM	Protein import, membrane tethering	Tom9.1 (At1g04070), Tom9.2 (At5g43970)
Tim21	TIM		IM				Tim21 (At4g00026)
Mic60	MICOS	Yeast/mammals	IM		OM-IM	OM-IM tethering, protein import, mitochondria morphology and inheritance, lipid transfer	AtMic60 (At4g39690)
Tom40	TOM	Yeast/plants	OM	[8,64,65]			At3g20000
SAM50		Yeast/mammals	OM				SAM50.1 (At3g11070), SAM50.2 (At5g05520)
VDAC		Yeast	OM	[66]			VDAC1 (At3g01380), VDAC2 (At5g67500), VDAC3 (At5g15090), VDAC4 (At5g57490)
Ugo1		Yeast	OM	[67]			No
Metaxin		Mammals	OM	[63]			Metaxin (At2g19080)
Tim17.2	TIM	Plants	IM/OM	[79]	OM-IM	Protein import, membrane tethering	
AtMic60	MICOS	Plants	IM	[8]	OM-IM	Membrane tethering, lipid transfer, mitochondria morphology	