thematic review

New perspectives on the regulation of intermembrane glycerophospholipid traffic

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Abstract **In eukaryotes, phosphatidylserine (PtdSer) can serve as a precursor of phosphatidylethanolamine (PtdEtn) and phosphatidylcholine (PtdCho), which are the major cellular phospholipids. PtdSer synthesis originates in the endoplasmic reticulum (ER) and its subdomain named the mitochondria-associated membrane (MAM). PtdSer is transported to the mitochondria in mammalian cells and yeast, and decarboxylated by PtdSer decarboxylase 1 (Psd1p) to form PtdEtn. A second decarboxylase, Psd2p, is also found in yeast in the Golgi-vacuole. PtdEtn produced by Psd1p and Psd2p can be transported to the ER, where it is methylated to form PtdCho. Organelle-specific metabolism of the aminoglycerophospholipids is a powerful tool for experimentally following lipid traffic that is now enabling identification of new proteins involved in the regulation of this process. Genetic and biochemical experiments demonstrate that transport of PtdSer between the MAM and mitochondria is regulated by protein ubiquitination, which affects events at both membranes. Similar analyses of PtdSer transport to the locus of Psd2p now indicate that a membrane-bound phosphatidylinositol transfer protein and the C2 domain of Psd2p are both required on the acceptor membrane for efficient transport of PtdSer. Collectively, these recent findings indicate that novel multiprotein assemblies on both donor and acceptor membranes participate in interorganelle phospholipid transport.**—Voelker, D. R. **New perspectives on the regulation of intermembrane glycerophospholipid traffic.** ***J. Lipid Res.*** **2003.** 44: **441–449.**

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Intracellular phospholipid transport in eukaryotes is one of the most fundamental aspects of organelle biogenesis, yet it remains poorly understood with respect to the genes that are involved in the process and the mechanism of action of the gene products. Most phospholipid synthesis originates in the endoplasmic reticulum (ER) and these lipids must be disseminated throughout the cell for the assembly of new organelles (1, 2). Five of the most prominent phospholipids synthesized in the ER (in decreasing order of abundance) are phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), and phosphatidic acid (PtdOH). Although the pool size of PtdOH is usually only 1–2% of the ER membrane lipid, the flux through this pool is extremely high, since it primarily functions as a precursor for all the other phospholipids and triacylglycerol. The role of the ER in phospholipid synthesis is dominant but not exclusive. Significant rates of PtdCho and PtdIns synthesis can also occur in the Golgi (3–5). In addition, the mitochondria can synthesize their own pool of PtdOH that is believed to function as an important major precursor for mitochondrial phosphatidylglycerol (PtdGro) and cardiolipin (Ptd_2Gro) (6). Both PtdGro and Ptd_2Gro are retained within the mitochondria of most eukaryotic cells.

Following synthesis, the phospholipids must be transported to membranes that lack the synthetic machinery to generate their own full repertoire of lipids. In **Fig. 1**, selective aspects of interorganelle glycerophospholipid traffic, which are the major focus of this review, are shown. Mitochondria must import PtdCho, PtdIns, and PtdSer. The PtdSer imported into mitochondria is rapidly metabolized to PtdEtn by PtdSer decarboxylase 1 (Psd1p) (7, 8). The PtdEtn produced within the mitochondria is essential for the function of the organelle (9, 10). PtdEtn produced in the ER by the Kennedy pathway (i.e., the sequential conversion of ethanolamine → phosphoethanolamine → CDP-ethanolamine → PtdEtn) (11) is only poorly transported-imported into mitochondria, and cannot substitute for the essential function of the pool produced within the mitochondria (9, 10, 12). Quite remarkably, the PtdEtn produced by Psd1p within the mitochondria is

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Abbreviations: ER, endoplasmic reticulum; MAM, mitochondria-associated membrane; PAM, plasma membrane-associated membrane; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; Ptd_2Gro, cardiolipin; PtdIns, phosphatidylinositol; PtdOH, phosphatidic acid; PtdSer, phosphatidylserine.

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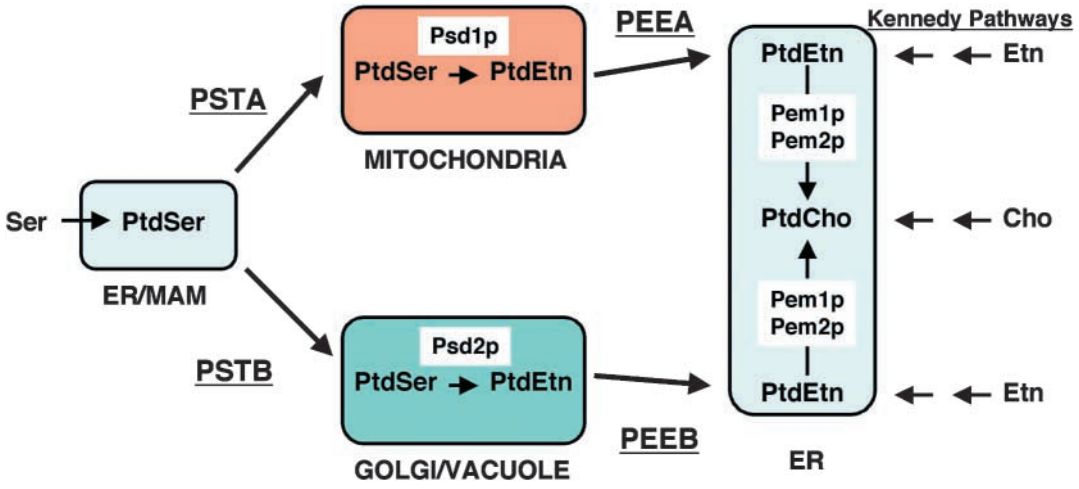

Fig. 1. Aminoglycerophospholipid transport in eukaryotes. The major steps in interorganelle transport of phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn) are shown as a composite of reactions occurring in both yeast and mammalian cells. PtdSer is synthesized in the endoplasmic reticulum (ER) and mitochondria-associated membrane (MAM) by isozymes of PtdSer synthase. The nascent PtdSer is transported to the mitochondria or the Golgi-vacuole via pathways designated PSTA and PSTB, respectively. Upon arrival at the inner membrane of the mitochondria or the Golgi-vacuole, the PtdSer is decarboxylated by isozymes of PtdSer decarboxylase (Psd1p or Psd2p). The PtdEtn formed can be retained or exported to other organelles. Export of PtdEtn from the mitochondria or the Golgi-vacuole is predicted to occur via pathways designated PEEA and PEEB, respectively. Exported PtdEtn that arrives at the ER can be methylated to form phosphatidylcholine (PtdCho) by the methyltransferase enzymes (Pem1p and Pem2p). The sequential conversion of PtdSer to PtdEtn and PtdCho can be used as an indicator of interorganelle lipid transfer in intact and permeabilized cells as well as isolated organelles. Thus far, only the mitochondrial Psd1p isozyme has been described in mammalian cells. Furthermore, in mammals, the methyltransferase reactions are essentially restricted to liver tissue. Eukaryotes can also synthesize PtdEtn in the ER, and PtdCho in the ER, and Golgi using Etn or Cho precursors, and the Kennedy pathways. The PtdEtn and PtdCho generated by the Kennedy pathways can at least partially substitute for defects in the PSTA and PSTB pathways. Genetic strategies for isolating mutants along the PSTA-PEEA pathway utilize mutagenesis of strains lacking an intact PSTB-PEEB pathway followed by rescue with Etn or Cho. Likewise, strategies for isolating mutants along the PSTB-PEEB pathway use mutagenesis of strains lacking an intact PSTA-PEEA pathway, followed by rescue with Etn or Cho.

not static and restricted to this organelle, but can be exported to all other membranes of eukaryotes to fulfill their requirement for PtdEtn (13). In eukaryotes such as yeast that can efficiently methylate PtdEtn to PtdCho in the ER (2), the flux of PtdSer into the mitochondria, and PtdEtn out of the mitochondria, can be used to generate all of the PtdEtn and PtdCho required for all cell membranes. These findings clearly indicate that mitochondria have the capacity to produce large amounts of phospholipid and play a very dynamic role in the biogenesis of other cell membranes.

The Golgi apparatus has a limited capacity to synthesize some phospholipids (3–5), but is generally thought to require the transport of PtdEtn, PtdIns, and PtdSer in most eukaryotes. It remains unclear how much of the requirement for PtdCho and PtdIns is fulfilled autonomously by the organelle and how much must be transported from the ER. In both lower and higher eukaryotes, the availability of choline enables the Kennedy pathway (choline → phosphocholine → CDP-choline → PtdCho) to be used for PtdCho synthesis in the ER and Golgi. In yeast, PtdSer transported to the Golgi can also be a substrate for PtdSer decarboxylase 2 (Psd2p) (12). The Psd2p is also found to colocalize with the vacuole compartment in yeast. The flux of PtdSer through the Golgi-vacuole is also quite high. The resultant PtdEtn can fulfill most cellular needs for this lipid, but is incompetent to supply the pool required by mitochondria under respiratory conditions (9, 10). When required, the PtdEtn produced in the Golgi-vacuole can also be transported to the ER for methylation to PtdCho, and fulfill all the cellular needs for these two lipids under nonrespiratory conditions (glucose medium) (12).

The plasma membrane, lysosomes, and endosomes appear essentially incapable of the synthesis of PtdCho, PtdEtn, PtdIns, and PtdSer (14). Compositional analyses of these membranes reveal that they all contain the aforementioned lipids (15, 16). Thus, the biogenesis of these membranes and the maintenance of their structural and functional integrity require lipid transport.

POTENTIAL TRANSPORT ROUTES FOR GLYCEROPHOSPHOLIPIDS

For many years, phospholipid transfer-exchange proteins were proposed as likely candidates for executing lipid transport reactions required for new membrane assembly (17). However, convincing evidence that these proteins are primary effectors for de novo membrane assembly has not been forthcoming. In addition, critical genetic tests of the function of these proteins have failed to establish an essential role as soluble carriers of phospholipid for the purpose of membrane biogenesis (18–20). One of the most extensively studied phospholipid transfer proteins is the PtdCho-PtdIns transfer protein in yeast named Sec14p (21). Current evidence suggests that Sec14p

acts principally as a regulator of PtdCho synthesis, especially in the Golgi apparatus (22).

In many independent studies of transport of newly synthesized phospholipids between organelles, classical inhibitors of vesicle traffic have proven ineffective at arresting the process. Monensin (a disruptor of protein traffic through the Golgi) failed to alter PtdCho transport from the ER to the plasma membrane (23). In these same studies, the cytoskeletal poisons nocodazole and colchicine, as well as ATP depletion, were also without measurable effect. Similar findings were also reported for PtdEtn transport between the ER and the plasma membrane (24). Additional lines of investigation examining PtdEtn export from the mitochondria to the plasma membrane found this process was insensitive to brefeldin A intoxication (25), which disrupts Golgi structure and function. Genetic experiments using yeast temperature-sensitive *sec14* mutants also provide results that indicate PtdIns traffic between the ER and the plasma membrane is not altered under nonpermissive conditions (37°C) that block protein transport through the Golgi (26). Experiments examining PtdSer transport between the ER and the mitochondria in permeabilized cells also demonstrate the process is insensitive to agents that can disrupt vesicle traffic, such as GTPγS (27). Collectively, these findings indicate that the transport of phospholipids between organelles can follow some specialized and poorly defined routes that are different from those followed by membrane proteins. This observation is surprising because phospholipids must necessarily accompany proteins in vesicle budding and fusion events. However, the majority of existing evidence indicates that when membrane protein traffic is arrested by either genetic manipulation or the use of metabolic poisons, traffic of newly synthesized glycerophospholipids, in most cases, proceeds unabated. These findings indicate that novel processes that are independent of membrane protein traffic must regulate the interorganelle movement of many phospholipids.

One potential route for phospholipid transport that could account for the above observations is lipid movement via specialized zones of apposition that occur between different subcellular membranes. Several morphological studies performed decades ago identified specialized regions of close association of mitochondria with the ER (28, 29). More recent studies have also described these associations both morphologically and biochemically (30–34). In addition, studies with permeabilized cells provided evidence that PtdSer movement between the ER and mitochondria requires a physical interaction between the organelles that is not easily disrupted (27). Subfractionation of mitochondrial preparations has also revealed the presence of a specialized subpopulation of the ER that is tightly associated with the mitochondrial outer membrane in mammalian cells and yeast (31, 33). The specialized region of the ER is now referred to as the mitochondria-associated membrane, and given the acronym MAM. Strikingly, the MAM fraction is enriched in PtdSer synthase relative to the bulk ER (31, 33). This enrichment in PtdSer synthase might be expected if one function of the MAM is to provide a selected pool of PtdSer to the mitochondria. Additional morphological studies in mammalian cells have also described similar close associations among other organelles including those between Golgi stacks and the ER in three-dimensional reconstructions (35). In yeast, a new subpopulation of the ER has recently been described that is closely associated with the plasma membrane (36). This population of the ER has been given the name plasma membrane-associated membrane and is referred to by the acronym PAM. The PAM also shows enrichment in PtdSer synthase relative to the bulk ER pool, and has been proposed as a transfer sight for PtdSer to the cell surface. Currently, we know very little about these zones of contact between different organelle membranes. A number of obvious critical questions about these zones of apposition between organelles need to be addressed including: *1*) How are they formed? *2*) How are they stabilized? *3*) What are their molecular constituents? *4*) How are these membrane contacts regulated? *5*) If they are sites of lipid transport, are all lipids transferred, or only a select population? *6*) Are the lipid transfers all ATP independent? *7*) Can the association between the membranes be disrupted by metabolic poisons? *8*) Can genetic screens be developed to isolate mutants defective in their formation?

A GENETIC APPROACH TO THE GLYCEROPHOSPHOLIPID TRANSPORT PROBLEM

One approach to examining interorganelle lipid transport is to exploit the powerful genetic tools available in the yeast *Saccharomyces cervisiae*. Work in the author's laboratory has mainly focused on using this approach. An outline of the salient features of the cytogeography of aminoglycerophospholipid synthesis, transport, and metabolism is shown in Fig. 1. In yeast, PtdSer synthesized in the ER is transported to the mitochondria, the Golgi-vacuole, or other organelles. When PtdSer arrives at the mitochondria, it is imported to the inner membrane and decarboxylated by Psd1p to form PtdEtn (37). Likewise, when PtdSer arrives at the Golgi-vacuole, it is decarboxylated to form PtdEtn at the locus of Psd2p (37). The PtdEtn produced in either the mitochondria or the Golgi-vacuole is exported from these organelles and returned to the ER for methylation by Pem1p and Pem2p to form PtdCho (2). Mammalian cells also possess the pathways for PtdSer transport into the mitochondria and PtdEtn export out of the mitochondria. However, in mammalian cells the methylation of PtdEtn to PtdCho is primarily restricted to liver tissue. Both yeast and mammalian cells can also synthesize PtdEtn and PtdCho in the ER by the Kennedy pathways that originate with cytosolic Etn and Cho (37). Null alleles have been constructed for the yeast PtdSer decarboxylases and the strains are denoted *psd1Δ* and *psd2Δ*, respectively (38, 39).

The basic genetic strategies employed to isolate potential yeast mutants in lipid traffic fall into two categories. In the first category, *psd2Δ* strains are used. These strains

must rely on either the mitochondrial Psd1p pathway for the synthesis of PtdEtn and PtdCho or the Kennedy pathways present in the ER. The *psd2Δ* cells are mutagenized and Etn requiring cells (auxotrophs) are selected. The logic of this approach is to create mutations in genes encoding proteins that participate in or regulate PtdSer transport to the mitochondria or PtdEtn export out of the mitochondria. The Kennedy pathway present in the ER allows the PtdEtn and PtdCho needs of the cell to be partially or fully satisfied in the mutants when they are supplemented with Etn (37). The second category of mutants is produced from *psd1Δ* strains. These strains must rely on either the Golgi-vacuole Psd2p pathway for the production of PtdEtn and PtdCho, or the Kennedy pathways present in the ER. These strains are mutagenized to create defects in PtdSer transport to the Golgi-vacuole, or PtdEtn export from these organelles. The cells harboring these transport defects are also identified as Etn auxotrophs. The Etn supplementation under these conditions allows the PtdEtn and PtdCho requirements of the cells to be either fully or partially satisfied.

In the original development of the genetic approach to isolate lipid transport mutants in yeast, several simplifying hypotheses were made. One hypothesis assumes that specific gene products regulate lipid traffic to and from the mitochondria and to and from the Golgi-vacuole. At the outset, the pathways for PtdSer transport to the mitochondria and the Golgi-vacuole were named PSTA and PSTB, respectively. In a similar manner, the pathways for PtdEtn export from the mitochondria and the Golgi-vacuole were named PEEA and PEEB, respectively. By convention in yeast nomenclature, genes appear as upper-case italic (*PSTA*), and mutations in lower-case italic (*pstA*), and proteins are designated in upper and lower case standard text with a terminal letter p (PstAp). Thus far we have identified mutations and genes affecting the PSTA and PSTB pathways. A second hypothesis generated in the genetic approach was that PtdEtn and PtdCho produced by the Kennedy pathways would be sufficient to rescue defects in phospholipid transport along the PSTA and PSTB pathways. The results of genetic screening indicate that the assumptions of this second hypothesis are only partially true. In general the mutants we isolated only have partial defects in lipid transport, but usually strong growth phenotypes. We interpret this to mean that the functioning Kennedy pathways are adequate to rescue mutants that are relatively "leaky" (i.e., allow some reduced level of transport to occur) but are insufficient to compensate for mutants completely arrested in lipid transport. In addition, several genes implicated in lipid transport also appear to be involved in other cellular functions. Work with some of these mutants is described below and now provides new insights into the mechanisms regulating the interorganelle transport of PtdSer. A summary of mutant strains, complementing genes, and gene product functions is given in **Table 1**.

UBIQUITINATION REGULATES PHOSPHATIDYLSERINE TRANSPORT TO MITOCHONDRIA

Examination of PtdSer transport between the ER-MAM and the mitochondria has proven to be a versatile system for uncovering some of the details involved in glycerolipid traffic. A schematic summary of the findings is shown in **Fig. 2**. Clear evidence derived from both yeast and mammalian systems indicates that PtdSer synthesized in the MAM is taken up by the mitochondria for conversion to PtdEtn (32, 33, 40). In mammalian systems, there is an ATP requirement for PtdSer transport to the mitochondria that has been demonstrated in both intact and permeabilized cells (27, 41, 42). Subcellular fractionation demonstrates that in mammalian cells, the ATP is required for PtdSer egress from the MAM compartment (32). Experiments with nucleotide analogs indicate that ATP must be hydrolyzed for PtdSer transport to occur, but it remains unclear precisely how the ATP is used (42). In contrast to the findings in mammalian cells, studies with permeabilized yeast have not demonstrated an ATP requirement for PtdSer exit from the MAM (34). We currently do not understand the basis for differences in nucleotide requirements between yeast and mammalian cells. Aside from the ATP requirement for PtdSer transport to mitochondria observed in mammalian cells, there is no other requirement for soluble factors. However, Kuge et al. (43) purified a low-molecular-weight protein from brain extracts that is capable of enhancing the transport reaction several-fold. The protein, named S100B, binds Ca^{2+} and contains an EF hand motif. Currently the site and mechanism of action of S100B are unknown. However, the action of the protein provides clear evidence that the lipid transport process can be specifically modulated by protein factors.

PtdSer transport-import into the mitochondria can be reconstituted in vitro with purified donor membranes and acceptor mitochondria (8, 44). The process shows incomplete susceptibility to proteolysis of mitochondria (8, 45).

TABLE 1. Summary of mutations/genes involved in interorganelle transport of glycerophospholipids

Pathway	Mutation	Gene	Protein Function	Role in Transport
PSTA	*pstA1*	*MET30*	SCF-Ubiquitin ligase	Regulates MAM-to-mitochondria transport of PtdSer
	CHO-R41	Unknown	Unknown	Regulates outer-to-inner membrane transport of PtdSer
PSTB	*pstB1*	*STT4*	PtdIns-4-kinase	Regulates PtdSer transport to Psd2p
	pstB2	*PSTB2/PDR17/SFH4*	PtdIns transfer-binding protein	Required on acceptor membrane for PtdSer transport to Psd2p
	psd2-C2Δ	*PSD2*	PtdSer decarboxylase	C2 domain is essential for PtdSer transport in vivo

MAM, mitochondria-associated membrane; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine.

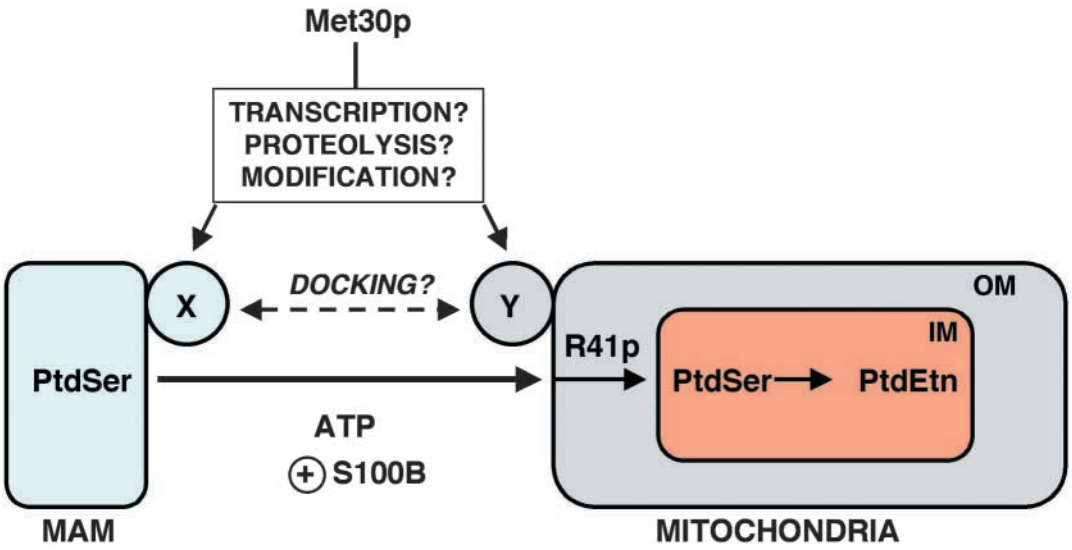

Fig. 2. Regulation of PtdSer transport from the MAM to the inner mitochondrial membrane. PtdSer synthesized in the MAM is transported to the outer mitochondrial membrane (OM) and subsequently imported to the inner membrane (IM), where it is decarboxylated to form PtdEtn. The egress of PtdSer from the MAM requires ATP in mammalian cells, but not yeast. Although transport does not require any soluble proteins, the rate of transport can be increased by a Ca^{2+} binding protein, S100B. The transport reaction between the MAM and mitochondria in yeast is regulated by Met30p, an SCF-ubiquitin ligase. The action of Met30p affects targets on both the MAM and the mitochondria, as indicated by the X and Y components appearing on the membranes. It is not yet known if the action of Met30p is direct or indirect, and the white box highlights levels at which ubiquitin ligases are known to function in a way that could ultimately affect either the expression, the proteolysis, or the direct modification of elements X and Y. Reconstitution studies with isolated MAM and mitochondria indicate that the X and Y constituents are likely to participate in docking complexes between the organelles. The import of PtdSer into the inner membrane is ATP independent in both yeast and mammalian cells. A mammalian cell line, CHO-R41, that is resistant to a cytolytic toxin is defective in the import reaction, and the site of action of the putative gene product (R41p) is indicated in the figure.

The donor membranes can be either microsomes, MAM preparations, or liposomes. It is also possible to use lipid analogs such as 1-acyl,2-NBD-aminocaproyl-PtdSer to load the outer membrane of mitochondria and monitor import of the lipid to the inner membrane by following its decarboxylation to PtdEtn (46).

Genetic analysis of PtdSer transport to the mitochondria is now providing exciting new information about the process. Emoto et al. (47) isolated a mutant line of CHO-K1 cells, named R41, that expresses low levels of PtdEtn in its plasma membrane, and is resistant to a cytolytic toxin that binds this lipid. The R41 cell line exhibits a defect in transport of PtdSer between the outer and inner mitochondrial membrane as measured by outer membrane loading with 1-acyl,2-NBD-aminocaproyl-PtdSer, followed by import and decarboxylation reactions. The lesion in PtdSer import does not affect protein import into the organelles, indicating that some of the machinery for the two processes is distinct.

Yeast *pstA1* mutants defective in PtdSer transport along the mitochondrial pathway have also recently been identified (48). These mutants are defective in transporting PtdSer between the MAM compartment and the mitochondria, but remain able to transport 1-acyl,2-NBD-aminocaproyl-PtdSer between the outer and inner membrane, normally. Consistent with a lesion in lipid transport between the MAM and mitochondria, the *pstA1* mutants produce mitochondria of abnormally high density with a significantly reduced phospholipid-protein ratio. The initial *pstA1* mutant was isolated as an Etn auxotroph in a strain with a *psd2Δ* mutation. The Etn auxotrophy provided a strong selection for the gene complementing the mutation. The complementing gene is *MET30,* which encodes a subunit of a multicomponent ubiquitin ligase. This type of ubiquitin ligase consists of the proteins Skp1p (suppressor of kinetochore protein 1), Cdc53p (cell division cycle 53, a homolog of the mammalian cullins), Cdc34p (cell division cycle 34, an E2 ubiquitin conjugating enzyme), and Hrt1p (high copy reduction of Ty3 transposition), and any of several F-box-containing proteins that directly interact with Skp1p (49). Members of this family of ubiquitin ligase are given the acronym SCF-ubiquitin ligases to denote Skp1, cullin proteins, and F-box proteins as common features. The *MET30* gene encodes the Met30p product that contains an F-box domain for direct interactions with Skp1. In addition, Met30p contains five WD 40 repeats (50) that are modules for protein-protein interaction, which participate in direct binding reactions with the substrates for ubiquitination. In vivo measurement of ubiquitination by the *pstA1* mutant demonstrates that this strain is defective in the ubiquitination of Met4p, one of the known substrates for SCF-Met30p. It is currently not known how many additional protein substrates Met30p can recognize. Complementation of the *pstA1* mutant with *MET30* not only alleviates the Etn auxotrophy of the strain but also restores PtdSer transport to the mitochondria (48). Reconstitution studies have also enabled identification of the membranes affected by ubiquitination in

the *pstA1* mutants. In these studies, MAM and mitochondria were prepared from both parental and *pstA1* mutant strains, and different combinations of the organelle preparations were used to examine PtdSer transport. MAM derived from the mutant was unable to function as a PtdSer donor to wild-type mitochondria. In addition, mitochondria derived from the mutant were unable to act as an acceptor for PtdSer produced in wild-type MAM. These findings demonstrate that the terminal site of action of the *pstA1* mutation resides in both the MAM and the mitochondria. Thus, the data reveal that Met30p regulates PtdSer transport on both the donor and the acceptor membranes.

The involvement of protein ubiquitination in regulating PtdSer transport was completely unanticipated. However, there is now well-established evidence that ubiquitination plays a direct role in regulating multiple membrane trafficking events (51). Thus far, protein ubiquitination has been directly implicated in endocytosis (52), protein sorting at the Golgi apparatus (53), and viral budding events at the plasma membrane (54). In addition, ubiquitination is now also recognized to regulate gene transcription both positively and negatively (55–57). We currently do not know at what level Met30p regulates PtdSer transport between the MAM and mitochondria or whether the effects are direct or indirect. One provocative hypothesis that we are currently testing is whether protein targets present on both the MAM and mitochondria are directly ubiquitinated. Such protein targets could theoretically serve as the basis for forming docking sites between the two membranes and assembling zones of apposition.

REQUIREMENTS FOR PTDSER TRANSPORT TO THE LOCUS OF PSD2P

The genetic approach to identifying components involved in PtdSer transport to Psd2p along the PSTB pathway is also providing new information about the molecular components required for this process. A schematic summary of the information is given in **Fig. 3**. The first mutant described in the pathway, *pstB1*, is an Etn auxotroph defective in PtdEtn synthesis from PtdSer (58). The gene that complements the growth defect is *STT4*, which encodes a PtdIns-4-kinase (59, 60). The *pstB1* strain is defective in PtdIns-4-kinase activity, and this catalytic activity as well as PtdEtn synthesis is restored in the mutant following transformation with a plasmid harboring the wild-type *STT4* gene. These findings suggest that PtdIns4P or PtdIns4,5P_2 can play a role in regulating PtdSer transport to Psd2p, although the mechanism and sight of action of the polyphosphoinositides remains to be elucidated. Recently, the Stt4p has been localized to the plasma membrane and directly implicated in the production of PtdIns4P and PtdIns4,5P_2 in this organelle (60). However, there is still some uncertainty about whether the entire PtdIns4P pool produced by Stt4p remains restricted to the plasma membrane, or if a fraction of the pool can be selectively distributed to internal cell membranes. These current ambiguities raise the possibility that PtdSer generated in the ER may need to transiently pass through the plasma membrane prior to reaching Psd2p. Alternatively, the PtdIns4P generated in the plasma membrane may need to be transported to the ER (perhaps at the PAM) to facilitate PtdSer movement to Psd2p.

A second mutant defective in PtdSer transport to Psd2p is *pstB2* (61). The *pstB2* mutant shows a pronounced defect in conversion of nascent PtdSer to PtdEtn in both intact and permeabilized cells. The gene complementing the mutant is *PSTB2*, and has also been named *PDR17* (pleiotropic drug resistance 17) (62) and *SFH4* (Sec14p homolog) (63), based on other studies. The encoded protein, PstB2p, is homologous to the major PtdIns-PtdCho transfer protein of yeast, Sec14p (21). The PstB2p can act as a lipid transfer-exchange protein in vitro with PtdIns, but not PtdCho. Most importantly, the PstB2p does not transfer PtdSer between membranes in vitro, indicating that it is unlikely to act as a soluble carrier of the lipid in vivo (61). The PstB2p is amphitropic, with one population of the protein found in the soluble fraction and the other associated with membranes. The bound fraction of PstB2p is tightly associated with membranes and is resistant to removal with 1 M KCl, suggesting that the protein interacts with a high affinity ligand(s). The nature of the putative ligands is not clear, but could be either lipid or protein or a combination of the two. The in vitro PtdIns transfer activity of PstB2p clearly demonstrates that the protein can bind PtdIns, and this lipid may contribute to the membrane binding.

Reconstitution studies with membrane fractions derived from permeabilized cells demonstrate that it is possible to spatially and temporally segregate PtdSer synthesis from transport in vitro (64). Donor membranes (derived from the ER) containing PtdSer synthase and largely depleted of Psd2p are initially used to synthesize radiolabeled PtdSer. Subsequently, acceptor membranes devoid of PtdSer synthase but enriched in Psd2p (derived from the Golgi-vacuole) are mixed with the prelabeled donors and are used to measure transport-dependent decarboxylation of PtdSer. To test the site of action of PstB2p, donor and acceptor membranes were prepared from both wild-type and *pstB2* mutant cells. The PtdSer pools of the donor membranes were radiolabeled and independently combined with acceptor membranes from either the wild-type or *pstB2* mutant strains, and PtdEtn formation was used to measure transport of the labeled PtdSer. Donor membranes derived from either wild-type or mutant cells were able to transfer PtdSer to wild-type acceptor membranes. In contrast, acceptor membranes from *pstB2* mutant cells were unable to decarboxylate PtdSer from either wild-type or mutant donors. These data demonstrate that PstB2p must be present in the acceptor membranes for PtdSer transport to occur from the donor.

The requirement for PstB2p on the acceptor membrane prompted additional studies examining the role of other protein components on the acceptor membrane. One logical protein to examine in more detail was Psd2p. The Psd2p contains a C2 domain that could potentially in-

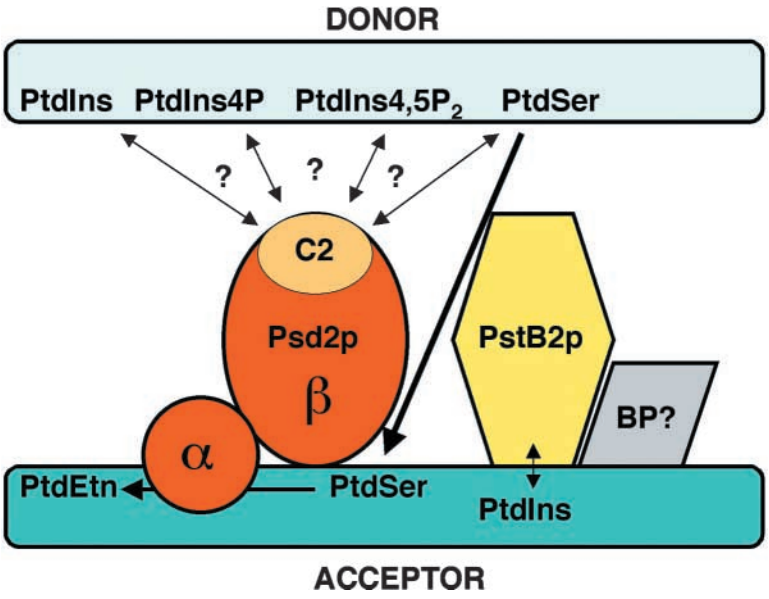

Fig. 3. Regulation of PtdSer transport to the locus of Psd2p. PtdSer produced in the donor ER must be transported to the acceptor membrane for formation of PtdEtn. For efficient PtdSer transfer to occur, the acceptor membrane requires the presence of both PstB2p and Psd2p. The PstB2p is an amphitropic phosphatidylinositol (PtdIns) binding protein, as indicated by the double arrow on the acceptor membrane. The high affinity association of PstB2p with microsomal membranes in vitro suggests that a protein ligand (BP) may also facilitate the membrane binding. The Psd2p requires an intact C2 domain for PtdSer transfer. The C2 domain of Psd2p is predicted to bind PtdSer and other anionic lipids that may include PtdIns, PtdIns4P, and PtdIns4,5P_2 as indicated by the double arrows between the C2 domain and the donor membrane. The question marks in the figure indicate interactions that are suspected but have yet to be directly demonstrated.

teract with lipids or proteins in the donor membrane and contribute to the formation of a docking complex (39, 65–67). The C2 domain is located on the large β-subunit of the enzyme, whereas the active site is located on the small α-subunit (68). To test the role of the C2 domain in PtdSer transport, a deletion mutant, *psd2-C2Δ*, was constructed and characterized (69). The mutant protein, Psd2-C2Δp, was expressed from a high-copy plasmid in a genetic background devoid of PtdSer decarboxylases (*psd1Δ psd2Δ*). Measurement of Psd2-C2Δp enzyme activity in cell extracts revealed that the level of catalysis was 10-fold greater than that normally produced by the chromosomal copy of the wild-type enzyme. In these experiments, the catalytic activity of both mutant and wild-type Psd2p was measured using a 1-acyl,2-NBD-aminocaproyl-PtdSer substrate that freely partitions into all membranes present in the extract. Subcellular fractionation using density gradients further revealed that the localization of the wild-type enzyme and Psd2-C2Δp was identical. Despite the high catalytic activity and normal subcellular localization, the *pstB2-C2Δ* mutant was inviable under conditions that required a functional PtdSer decarboxylase. Examination of the lipid metabolism of the *psd2-C2Δ* mutant demonstrates that the cells are defective in the conversion of PtdSer to PtdEtn. These findings reveal that the C2 domain of Psd2p has an essential in vivo function that is independent of catalysis.

One likely requirement for the C2 domain is the direct participation in a docking reaction with the donor membrane and/or transport of nascent PtdSer to the membrane in which the catalytic subunit of enzyme resides. To examine this essential role, permeabilized cells were prepared, and the transport of nascent PtdSer from the ER to the locus of Psd2p was measured. In permeabilized cells, nascent PtdSer was inaccessible to the Psd2-C2Δp, but freely accessible to the wild-type enzyme. Furthermore, in permeabilized cells, 1-acyl,2-NBD-aminocaproyl-PtdSer, which partitioned into all membranes, was readily decarboxylated by Psd2-C2Δp. Collectively, these findings provide strong support for a model in which the C2 domain of Psd2p participates in the transfer of the nascent PtdSer from the donor to the acceptor membrane, but not catalysis.

The salient features of our current PtdSer transport model involving Psd2p and PstB2p appear in Fig. 3. The acceptor membrane harbors Psd2p and PstB2p, and both proteins are required for PtdSer transport. PtdIns is a known ligand for PstB2p and may contribute to the binding on the acceptor membrane (61). It is also possible that PtdIns present on the donor membrane may contribute to a docking reaction between the donor and acceptor. Since the binding of PstB2p to the acceptor membrane is resistant to elution with 1 M KCl, proteins on the acceptor membrane are also suspected to play a role in this association. The C2 domain of Psd2p is required for PtdSer transport. Known ligands for C2 domains include PtdSer, PtdIns, and PtdIns4,5P_2 (67, 70, 71), but there is not yet any direct evidence for their interaction with the C2 domain of Psd2p. The genetic data identifying a role for Stt4p in the PSTB pathway would be consistent with either PtdIns4P or PtdIns4,5P_2 participating in a docking process between the donor and acceptor membranes.

CONCLUDING COMMENTS

Examination of the site-specific metabolism of the aminoglycerophospholipids is now providing new information about some of the molecules involved in regulating interorganelle traffic of these lipids following their synthesis. The current data are most consistent with a mechanism involving zones of apposition between donor and acceptor membranes. We currently do not know if these zones are stable or relatively short lived. Evidence is mounting to support the idea that protein complexes assemble on either the donor or the acceptor membranes, or both, to create docking and lipid transport machinery.

For MAM and mitochondria in the PSTA pathway, protein ubiquitination functions as a regulatory event that controls the interaction of the membranes. The regulatory role could be direct and involve ubiquitination of proteins on both the MAM and mitochondria and lead to the formation of docking complexes between the organelles. Alternatively, ubiquitination could regulate the transcription of molecules that directly participate in the formation of docking complexes between the membranes. Yet another possibility is that ubiquitination regulates the proteolysis of docking and transport complexes on the membranes. In the PSTB pathway, the PtdIns binding protein PstB2p and the lipid-binding C2 domain of Psd2p have been directly implicated in the transport reaction at the acceptor membrane. The full lipid-binding spectrum of these proteins is not yet known. However, work with the *pstB1* mutant indicates that PtdIns4P or a derivative is also likely to play a role in regulating PtdSer transport. These findings in the PSTB pathway support a model in which a multiprotein complex assembles on the acceptor membrane and docks with a donor membrane to facilitate lipid transport. Docking between the donor and acceptor membranes could occur with either proteins or lipid ligands, or both. Collectively, the recent findings from genetic and biochemical studies of aminoglycerophospholipid transport point to a new way of thinking about phospholipid transport between organelles. In its most simplified version, acceptor membranes contain protein assemblies capable of effecting phospholipid transport on their surface. These protein assemblies are proposed to be activated upon recognition and direct interaction with the donor membrane.

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