Reconstitution of Phosphatidylserine Transport from Chemically Defined Donor Membranes to Phosphatidylserine Decarboxylase 2 Implicates Specific Lipid Domains in the Process*

Received for publication, October 22, 2003, and in revised form, December 3, 2003
Published, JBC Papers in Press, December 4, 2003, DOI 10.1074/jbc.M311570200

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Phosphatidylserine (PtdSer) is transported from its site of synthesis in the endoplasmic reticulum to the locus of PtdSer decarboxylase 2 (Psd2p) in the Golgi/vacuole and decarboxylated to form phosphatidylethanolamine. Recent biochemical and genetic evidence has implicated the C2 domain of Psd2p and a membrane-bound form of the phosphatidylinositol binding/transfer protein, PstB2p, as essential for this transport process. We devised a reconstituted system in which chemically defined donor membranes function to transfer PtdSer to the biological acceptor membranes containing Psd2p. The transfer of PtdSer is poor when the donor membranes have a high degree of curvature but markedly enhanced when the membranes are relatively planar (≥400-nm diameter). PtdSer transfer is also dependent upon both the bulk and the surface concentrations of the lipid, with pure PtdSer vesicles acting as the most efficient donors at all concentrations. The lipid transfer from donor membranes containing either 100% PtdSer or 50% PtdSer at a fixed concentration (e.g. 250 μM Ptd-Ser) differs by a factor of 20. Surface dilution of PtdSer by choline, ethanolamine, glycerol, and inositol phospholipids markedly inhibits PtdSer transfer, whereas phosphatidic acid (PtdOH) stimulates the transfer. Most importantly, the transfer of PtdSer from liposomes to Psd2p fails to occur in acceptor membranes from strains lacking PstB2p or the C2 domain of Psd2p. These data support a model for PtdSer transfer from planar domains highly enriched in PtdSer or in PtdSer plus PtdOH.

The mechanisms responsible for transporting newly synthesized glycerophospholipids among the different subcellular compartments within eukaryotic cells remain largely undefined at the genetic and biochemical level (1). In many instances, the data support a process that is relatively rapid and independent of a requirement for ATP or other nucleotides and largely independent of factors essential for vesicle movement that are an integral part of protein traffic between membrane compartments (2–9). Morphological and biochemical experiments have suggested that one possible mechanism may consist of the formation of zones of apposition between donor and acceptor membranes that facilitate lipid transport between membranes (3, 10–15). We have developed a series of genetic screens and biochemical assays to study the transport of PtdSer to the loci of PtdSer decarboxylases in an effort to define some of the molecules involved in glycerophospholipid transport processes (16). These screens and assays follow the synthesis of PtdSer in donor membranes and its subsequent decarboxylation to form phosphatidylethanolamine (PtdEtn) in acceptor membranes. The formation of this PtdEtn provides a distinct biochemical marker for the transfer reaction. In yeast, there are two PtdSer decarboxylases, Ptd1p and Ptd2p, that are located in the mitochondria and the Golgi/vacuole, respectively (17–20). Combined genetic and biochemical experiments have thus far implicated one protein in transport of PtdSer between a subfraction of the endoplasmic reticulum (known as the mitochondria-associated membrane) and mitochondria (21). This latter protein is a subunit of ubiquitin ligase, Met30p (22, 23), and its action affects the ability of mitochondria-associated membrane to act as a PtdSer donor and mitochondria to act as a PtdSer acceptor (21). Thus far, three proteins have been implicated in the transfer of PtdSer to the locus of Ptd2p. One of these proteins is the phosphatidylinositol-4-kinase, Stt4p (24, 25). A second protein is a phosphatidylinositol (PtdIns) transfer/binding protein, PstB2p (7). The third protein motif is the C2 domain of the Psd2p (26). Our previous data provide some support for a model in which docking reactions occur between donor/acceptor pairs of membranes that enable lipid transport to occur between them (16). These docking reactions may require protein modification (e.g. protein ubiquitination), lipid modification (e.g. phosphorylation of PtdIns), and specific lipid binding reactions, by the actions of PstB2p and the C2 domain of Ptd2p and/or other proteins. One current hypothesis is that macromolecular assemblies form on and between both the donor and acceptor membranes to create zones of adhesion, within which specific proteins function to transport phospholipids (16).

Previous studies have established conditions for the transport of nascent PtdSer from its site of synthesis to the locus of Ptd2p in intact cells, permeabilized cells, and isolated membranes (7, 8, 24, 26). In all cases, the transport of PtdSer required the presence of the PstB2p and the C2 domain of Ptd2p on the acceptor membrane. In an effort to further deconstruct and reconstitute the transport reaction, we have now addressed the problem of defining the basic molecular require-

* This work was supported by National Institutes of Health Grant 2R37 GM 32453. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: PtdSer, phosphatidylserine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; NBD-Ptd[1-14C]Ser, 1-acyl-2-[6-(7-nitro-2,1,3-benzoxadiazol-4y)aminocaproyl-P-hosphatidy|1-14C]serine; PtdCho, phosphatidylcholine; PtdGro, phosphatidylglycerol; PtdIns-4,5-P2, PtdIns 4,5-bisphosphate; Ptd2p, PtdSer decarboxylase 2.
ment for producing competent donor membranes. These studies focused upon defining 1) the minimal physical requirements, 2) the lipid compositional requirements, 3) the ionic requirements, and 4) the fidelity of defined donors in the PtdSer transport reaction. Our results demonstrate that planar PtdSer-rich domains function optimally in the transport reaction with the same genetic and molecular constraints observed for the process in vivo.

EXPERIMENTAL PROCEDURES

Chemicals—Simple salts, buffers, and amino acids were from Fisher and Sigma. Yeast media components were from Difco. Zymolyase-100T was purchased from U.S. Biological. The 1-14C-cisternine was obtained from American Radiolabeled Chemicals. Phospholipids were obtained from Avanti Polar Lipids. The radiolabeled substrates for Ptd2p, 1-acetyl-2-[6-(7-nitro-2-1,3-benzoxadiazol-4-yl)aminocaproyl]-Phosphatidyl-1-14C-cisternine (NBD-Ptd[1-14C]-Ser) and Ptd[1-14C]-Ser were synthesized as described previously (17, 20).

Yeast Strains and Media—The PTD1-deficient strain, RY52 (MATα ura3 his3 trp1 met14 leu2 lys2 suicide plasmid::URA3) was derived from W303. The plasmid cloned the cisternine (1-14C)-cisternine (NBD-Ptd[1-14C]-Ser) and Ptd[1-14C]-Ser were synthesized as described previously (17, 20).

Preparation of Membranes—Yeast in 1-liter cultures were grown to mid-exponential growth phase, their medium was collected, adjusted to pH 8.0; 0.5 mM EDTA; 0.3 M sucrose; 1 mM 2-mercaptoethanol; 10 mM KCl at 30 °C for 1 h. The lipid suspension was subjected to vortex mixing for 5 min at room temperature to create multilamellar liposomes. Unilamellar liposomes were prepared either by sonication or membrane extrusion. For sonication, the multilamellar liposomes were placed in glass tubes and probe-sonicated on ice for 30 s followed by cooling for 30 s. The sonication–cooling cycle was performed three times. The sonicated lipid suspension was briefly centrifuged at 1000 × g for 3 min to remove titanium particles shed from the probe. For membrane extrusion, we used an Avestin Laposof apparatus. The multilamellar liposome suspensions were converted to unilamellar vesicles of defined size, using 25 passes through polycarbonate filters of either 50-, 100-, 200-, 400-, or 1000-nm diameter, as specified in individual experiments.

Enzyme Assays—The transport-coupled decarboxylation assay was performed in a volume of 40 μl containing 25 mM Tris-Cl, pH 7.0, 10 mM 2-mercaptoethanol, 150 mM KCl, and substrates ranging from 50 to 500 μM total lipid. Membranes containing Ptd2p and 60 μg of total protein were added to the incubations containing the lipidosome substrates. The specific radioactivity of the Ptd[1-14C]-Ser substrate added to the reaction was 0.9 μCi/μmol of total lipid. The reactions were performed in gas-tight tubes with an insert well for trapping CO2 on 2 M KOH-impregnated filter paper. Reactions were performed at 30 °C for 20 min and terminated by the addition of 50 μl of 0.25 M H2SO4 introduced with a syringe and yeast cell through a septum. The evolved 14CO2 was collected at 30 °C for 1 h. The filter paper was recovered from each reaction tube and radioactivity quantified by liquid scintillation spectrometry. Ptd2p activity that is independent of transport processes was measured using NBD-Ptd[1-14C]-Ser as described previously (8, 26).

RESULTS

Donor Membranes with a Low Degree of Curvature Facilitate PtdSer Transport to Ptd2p—Since its discovery, the Ptd2p enzyme activity has always been difficult to measure using its native substrate (19, 20). Measurement of catalytic activity with Ptd[1-14C]-Ser in the presence of detergents routinely gave very low activity, presumably due to detergent-mediated inactivation of the enzyme. The use of a membrane-partitioning substrate, NBD-Ptd[1-14C]-Ser, provided a means to measure Ptd2p activity in the absence of detergents (19, 20), but this substrate was not useful for reconstituting transport, because it spontaneously inserts into all membranes (27). Sonicated preparations of Ptd[1-14C]-Ser liposomes routinely proved to be very poor or inactive substrate donors for Ptd2p, although biological membranes harboring Ptd[1-14C]-Ser functioned as reasonably good substrates (7, 8, 26). In addition, liposomes prepared from biological membranes that functioned as substrates were also poor substrate donors for the enzyme. We initially sought to understand the discrepancies between biological membranes and liposomes as substrate donors. In preliminary studies, we discovered that large, multilamellar Ptd[1-14C]-Ser liposomes could function as reliable substrates for the enzyme, whereas the activity from small unilamellar Ptd[1-14C]-Ser liposomes was nearly undetectable. From these initial observations, we refined the biochemical assay using Ptd[1-14C]-Ser liposomes of defined size, prepared by the polycarbonate filter extrusion method. Following the refinements of the assay, we returned to the initial comparisons between small unilamellar and large multilamellar vesicles assayed under optimal conditions. These results are presented in Fig. 1 and demonstrate that at a fixed concentration of 0.2 mM PtdSer, there is a 3-fold difference in the activity of the enzyme measured with the multilamellar liposomes, compared with the unilamellar liposomes. This magnitude of difference is likely to be an underestimate, because the multilamellar vesicles have only their outermost lamellar layer of PtdSer available for transfer to the enzyme, whereas the small unilamellar vesicles contain only one phospholipid bilayer. Thus, with the smaller vesicles, there is far more PtdSer available to interact with the acceptor membranes. A direct comparison of unilamellar lipo-
Liposomes

FIG. 1. Large multilamellar liposomes are preferred substrates for transport-dependent PtdSer decarboxylation. Small unilamellar liposomes were prepared by sonication of lipid suspensions. Large unilamellar liposomes were prepared by vortexing lipid films. The liposomes containing 0.1 mM Ptd[1-14C]Ser were incubated with acceptor membranes for 20 min at 30 °C. The 14CO2 generated by the reaction was trapped on filter paper and quantified by liquid scintillation spectrometry. Values are means ± S.E. for six experiments.

When compared with membranes with lower PtdSer content. Fig. 3C is another replot of the data that expresses the transport-dependent decarboxylation of PtdSer as a function of the surface concentration of PtdCho. This analysis of the data demonstrates the differences in rates with respect to the surface concentration of PtdCho and PtdSer at different bulk concentrations of total lipid. The striking decline in transport activity occurs in an exponential manner with respect to the reduction in surface concentration of the PtdSer and corresponding increase in PtdCho. The exponential decline in activity occurs systematically and in parallel for all of the concentrations of total lipid examined in the reaction. These data indicate that the surface concentration of PtdSer is a critical parameter in regulating the transport of this lipid to the locus of Psd2p. In Fig. 3D, the data from Fig. 3B are shown as a double reciprocal plot. This latter treatment of the data demonstrates that the apparent Vmax for the transport-coupled reaction is dramatically affected by reduction in the surface concentration of PtdSer, but the apparent Km of the process is not significantly altered (see inset in Fig. 3D). These kinetic parameters are consistent with the Psd2p being substrate-limited as a consequence of reduced PtdSer transport to the enzyme. Collectively, the information in Fig. 3 supports a mechanism in which PtdSer domains in the donor membrane are recognized by proteins present in the acceptor membrane that transport the substrate to Psd2p.

PtdSer Transport to Psd2p Is Not Generally Enhanced by Anionic Phospholipids—The preceding data demonstrate that donor membranes highly enriched in PtdSer, efficiently transfer this lipid to Psd2p. We next sought to determine whether this was a general property of anionic lipids or if it was specific only for PtdSer. We utilized liposomes containing 70 mol % PtdSer and 30 mol % of other phospholipids and compared the transport activity with that of 100 mol % PtdSer liposomes. The results of these studies are shown in Fig. 4. As described above, optimal transport-dependent decarboxylation occurred with 100 mol % PtdSer liposomes, and this activity was markedly diminished (~75%) with liposomes containing 30 mol % PtdCho. Substitution of PtdEtn, phosphatidylinositol (PtdIns), phosphatidylinositol-4-phosphate, and PtdIns-4,5-P2 for PtdCho failed to restore the transport activity to the level of 100 mol % PtdSer. However, there were some modest, reproducible increases in the transport, relative to PtdCho addition, when the...
added lipid was PtdGro or PtdIns 4-phosphate. When the data are expressed as a percentage of the activity obtained with pure PtdSer vesicles, the added lipids and their corresponding activities are as follows: PtdSer, 100%; PtdCho, 25%; PtdOH, 104%; PtdEtn, 40%; PtdGro, 48%; PtdIns, 26%, PtdIns-4-P, 54%; PtdIns-4,5-P2, 26%. These data clearly demonstrate that the transport of PtdSer is not generally stimulated by the presence of other anionic phospholipids, or non-choline-containing lipids within the donor membrane. However, when the liposomes contained 70% PtdSer and 30% PtdOH, PtdSer transport to Psd2p was fully restored to the levels found for 100% PtdSer. Thus, the PtdSer transport reaction does not display an absolute requirement for 100 mol % PtdSer in the donor membrane to reach maximum efficiency, but only PtdOH appears to function as a useful substitute for PtdSer.

**The Transport System Distinguishes between Homotypic and Heterotypic Donor Membranes**

The inhibition of PtdSer transport by lipids other than PtdOH could simply reflect the sensitivity of the reconstituted system to the addition of extraneous lipids or it could be a direct effect of surface dilution of PtdSer. In order to examine the nature of the inhibition, we compared the effects of mixtures of homotypic liposomes and heterotypic liposomes upon the coupled transport/decarboxylation reaction. For homotypic conditions, we measured the effects of adding different populations of liposomes, each composed of 100 mol % of the lipids studied, upon PtdSer transport. For heterotypic conditions, we created liposomes harboring 70 mol % PtdSer and 30 mol % of either PtdCho or PtdOH. The results of these experiments are shown in Fig. 5. In each case, the final PtdSer concentration was 140 μM, and the concentration of the other lipids was 60 μM. The heterotypic samples...
Mn$^{2+}$ in the presence of Mn$^{2+}$ data from Fig. 4. The lipids and the relative transport activities of Psd2p and the C2 domain of PstB2p were compared with similar analyses performed with the C2 domain of Psd2p. Unilamellar liposomes of 400-nm diameter containing 100 mol % of PtdSer, PtdCho or PtdOH were used as homotypic liposomes. In all conditions, the final concentration of PtdSer was 140 μM. When PtdCho and PtdOH were added under either heterotypic or homotypic conditions, their final concentration was 60 μM. Values are means ± S.E. for three experiments.

FIG. 5. The inhibitory effect of PtdCho and the stimulatory effect of PtdOH upon transport-dependent PtdSer decarboxylation require the presence of these lipids in the same membrane as PtdSer. Unilamellar liposomes of 400-nm diameter containing 70 mol % PtdSer and 30 mol % of either PtdCho or PtdOH, as indicated, were used as heterotypic liposomes. Unilamellar liposomes of 400-nm diameter containing 100 mol % of PtdSer, PtdCho or PtdOH were used as homotypic liposomes. In all conditions, the final concentration of PtdSer was 140 μM. When PtdCho and PtdOH were added under either heterotypic or homotypic conditions, their final concentration was 60 μM. Values are means ± S.E. for three experiments.

FIG. 6. Mn$^{2+}$ enhances the rate of transport-dependent PtdSer decarboxylation. Unilamellar liposomes of 400-nm diameter containing 100 mol % PtdSer at 200 μM concentration were used as substrate for the reaction. Values are means ± S.E. for three experiments.

FIG. 7. Mn$^{2+}$ cannot reverse the inhibition of transport-dependent PtdSer decarboxylation. Unilamellar liposomes of 400-nm diameter containing either 100 mol % PtdSer or 70 mol % PtdSer and 30 mol % of the indicated lipids were used as substrates for the reaction. The final concentration of PtdSer in all cases was 200 μM. The final concentration of Mn$^{2+}$ was 0.25 mM. Values are means ± S.E. for three experiments.

Liposome Compositions

FIG. 7. Mn$^{2+}$ cannot reverse the inhibition of transport-dependent PtdSer decarboxylation. Unilamellar liposomes of 400-nm diameter containing either 100 mol % PtdSer or 70 mol % PtdSer and 30 mol % of the indicated lipids were used as substrates for the reaction. The final concentration of PtdSer in all cases was 200 μM. The final concentration of Mn$^{2+}$ was 0.25 mM. Values are means ± S.E. for three experiments.

47%; PtdOH, 97%; PtdEtn 48%, PtdGro, 45%; PtdIns, 47%; PtdIns-4-P, 60%; and PtdIns-4,5-P$_2$. The Mn$^{2+}$ is not absolutely required for events at the acceptor membrane but can increase the apparent efficiency of PtdSer transport. The action of Mn$^{2+}$ is insufficient to overcome the inhibitory effects of the surface dilution of PtdSer.

PtdSer Transport from Defined Liposomes to PstB2p Is Constrained by the Same Genetic and Molecular Factors Observed in Vivo—In vivo studies identify PstB2p and the C2 domain of PstB2p as essential elements for the transfer of nascent PtdSer to the locus of PstB2p (7, 8, 26). These requirements are recapitulated in permeabilized cell systems used for examining PtdSer transport (8, 26). If the reconstituted transport system from defined donor membranes described in this report is a valid model for interorganelle PtdSer transport, it should also conform to the requirements seen in vivo. In order to test the validity of the transport reactions described above, we prepared acceptor membranes from strains harboring a C2 domain deletion (psdB2-C2Δ) in PstB2p and from strains with a null allele (pstB2Δ) for PstB2p. The units of PstB2p activity present in these membrane samples were determined independently of lipid transport using NBD-Ptd[1,4,5]C-Ser. Subsequently, we determined the transport-dependent decarboxylation of Ptd[1-
The transport-dependent decarboxylation of PtdSer from defined donor membranes requires the presence of the C2 domain of Psd2p and PstB2p on the acceptor membranes. Unilamellar liposomes of 400-nm diameter containing 100 mol % PtdSer at 200 μm were used as the substrate. In A, the acceptor membranes were generated from yeast strains harboring psd1Δ psd2Δ alleles and expressing PFD2 (wild type) or PSD2/C2Δ from a YEp352 plasmid as indicated. In B, acceptor membranes were generated from strains harboring a psd1Δ allele and a chromosomal copy of PSD2 in conjunction with either a chromosomal copy of PSTB2 (wild type) or the corresponding null allele pstB2Δ. Values are means ± S.E. for three experiments.

**DISCUSSION**

In this paper, we describe and characterize a defined donor membrane system that is competent to transport PtdSer to the locus of Psd2p for decarboxylation to form PtdEtn. The establishment of this donor membrane system is an important first step toward elucidating the minimum required components involved in interorganelle PtdSer transport and identifying their mechanisms of action. This system now provides important new insights into structural elements of both the donor and acceptor membranes that are important for membrane recognition and PtdSer transport.

The first set of experiments in this study was designed to address discrepancies among permeabilized cells, isolated organelles, and liposomes in the transport-dependent decarboxylation of PtdSer. Successful donor/acceptor membrane systems have previously been described for nascent PtdSer transport to the locus of Psd2p using biological membranes (7, 8). However, liposomes derived from the donor membranes consistently failed to function as donors in the transport reaction. These prior results along with the lack of success using small unilamellar liposomes of PtdSer, prepared by sonication, suggested that specific protein components were required in the donor membranes to effect PtdSer transport. However, further manipulation of the liposomes revealed that both the curvature and the composition of the donor membrane were important factors in producing competent donor membranes. The data in Figs. 1 and 2 demonstrate that the optimum donors are PtdSer liposomes with diameters of ≥400 nm. There is a significant reduction in PtdSer transport when the diameters of the liposomes are reduced. The dependence of the transport upon donor membrane diameter was unanticipated. However, this preference for a more planar surface on the donor membrane may reflect the donor/acceptor membrane alignments found in other membranes that occur at zones of apposition seen between the mitochondria and ER (3, 10, 14, 28), between the plasma membrane and ER (3), or between the Golgi and the ER (30). In yeast, these zones of apposition appear to be up to 40 nm in length between ER and mitochondria and 40–100 nm in length between ER and the plasma membrane. For spherical donor membranes with a 50-nm diameter, a zone of apposition of 40 nm would require alignment with 25% of the cross-sectional circumference that would be described by an arc of 90°. In contrast, for spherical donor membranes of 400-nm diameter, a zone of apposition of 40 nm would require alignment with 3% of the cross-sectional circumference described by an arc of just 10°. These physical aspects of donor and acceptor membranes may be important features that affect the recognition, binding affinity, and transfer of lipids and are plausible explanations of the vesicle curvature-dependent differences in transport that we observe in our reconstituted system.

A second unanticipated finding described in this report is the relatively stringent dependence of the transport system upon the surface concentration of PtdSer. Our studies using homotypic and heterotypic liposomes described in Fig. 5 provide strong evidence that surface concentration and dilution of PtdSer are critical properties affecting lipid transport and that the process is not subject to nonspecific inhibition by the addition of extraneous lipids to the in vitro reaction. As shown in Fig. 3C, the decline of PtdSer transport occurs exponentially with the surface dilution of the lipid. The data implicate high local concentrations of PtdSer as important features of the lipid transport process. These high concentrations could be essential requirements for acceptor membrane recognition of donors and for docking of the membranes prior to transport of the lipid. Alternatively, high surface concentrations of PtdSer may be required to drive the action of inefficient transporters that are
assembled on the acceptor membrane. It is noteworthy that studies of PtdSer metabolism to PtdEtN in mammalian cells describe the preferential utilization of newly synthesized PtdSer for transport between the endoplasmic reticulum and mitochondria (31, 32). It is possible that newly synthesized pools of this lipid are highly concentrated in specialized subdomains that are uniquely competent for transport. Although surface dilution of PtdSer by many lipids inhibits transport, PtdOH can replace up to 30% of the PtdSer without loss of transport efficiency. It is not yet clear if PtdOH can simply substitute for PtdSer or if it plays a more important role in promoting interactions between donor and acceptor membranes. PtdOH is centrally positioned in the regulation of membrane trafficking events insofar as its production is regulated by ARF1 through stimulation of phospholipase D (33, 34), and its increased production stimulates the production of PtdIns-4,5-P2 (35), which in turn can regulate secretory and endocytic vesicle traffic (36). Both PtdOH and PtdIns-4,5-P2 also play an important role in maintaining the Golgi apparatus and preventing its fragmentation (37). Our current results with PtdOH suggest that it may also play an important role in regulating PtdSer traffic.

A critical test of the significance of any in vitro reconstitution system is its fidelity to the in vivo situation and permeabilized cell systems. In previous work, we described mutant strains of yeast that are defective in transporting nascent PtdSer to the locus of Ptd2p (7, 8, 24, 26). These studies identify the PtdIns transfer/binding protein PstB2p as essential for PtdSer transport (7). Additional studies also identify the C2 domain of Ptd2p as essential for PtdSer transport in vitro (26). In vitro studies with permeabilized cells corroborate the in vivo findings about PstB2p and the C2 domain of Ptd2p. Furthermore, studies with isolated organelles reveal the same properties for PstB2p found in both permeabilized cells and living cells (8). The studies described above have led to a model for PtdSer transport that requires the presence of PstB2p and the C2 domain of Ptd2p on the acceptor membrane, as essential elements required for PtdSer transport to the Ptd2p enzyme for catalysis (16). In the current study, we have endeavored to replace the biological donor membrane with defined liposomes. Our data demonstrate that synthetic donor membranes with relatively planar characteristics and a high surface concentration of PtdSer can productively interact with biological acceptor membranes in the transfer of lipid. The fidelity of this artificial donor system to the characteristics of previously described in vivo, permeabilized cell, and isolated organelle systems was demonstrated in several experiments. Both the permeabilized cell system and the isolated organelle system require Mn2+ for optimal PtdSer transport. In addition, all of the PtdSer transport studies involving Ptd2p described previously require the presence of the C2 domain of Ptd2p and PstB2p on the acceptor membrane (8, 26). In Fig. 8, we demonstrate that the transport of lipid from the liposomes to Ptd2p requires the presence of both the C2 domain of Ptd2p and the presence of PstB2p. These latter results are extremely important for multiple reasons. First, the findings emphatically demonstrate that there is no artificial fusion occurring between the donor and acceptor membranes. We know this to be true, because we can quantify the transport-independent activity of Ptd2p with the NBD-Ptd[1-14C]serine substrate. Since the NBD-modified substrate spontaneously inserts into the acceptor membrane without a requirement for the transport machinery, it provides us with a measure of activity to be expected if spontaneous fusion occurs between donor and acceptor membranes. Second, these results make it extremely unlikely that PstB2p is acting upon substrates while they are resident in the donor membrane. This conclusion is deduced from the findings that neither the C2 domain nor PstB2p are required for catalysis by Ptd2p, and the activity of the enzyme is normal when the NBD substrate is inserted into the acceptor membrane. Despite this normal activity, the C2 domain and PstB2p are essential for catalysis of substrates that must be transported from other membranes to Ptd2p either in vivo or in vitro (8, 26).

We have put forward the hypothesis that PstB2p and Ptd2p act in concert on the acceptor membrane to form part of a recognition and transport module that interacts with the donor (16). We anticipate that additional proteins and lipids on both the donor and donor membrane can also contribute to this complex. The previously demonstrated requirement for the PtdIns-4-kinase, Stt4p (24), suggests that polyphosphoinositides are required for some step in the overall transport process. In addition, this report also suggests a role for PtdOH in the process. We hypothesize that the net result is to form a macromolecular assembly that transiently bridges the two membranes and executes lipid transport. Morphological and biochemical studies support such a model for lipid transport between the ER and mitochondria (3, 10–12, 14, 15) as well as the ER and plasma membrane (13). The findings in this report implicate specialized PtdSer-rich domains in the donor membrane as required elements for productive lipid transfer to the acceptor. These specialized domains might be a consequence of the action of other effectors such as lipid-binding proteins present in the donor membranes. The formation of PtdSer-rich domains may also be regulated by the polyphosphoinositides or PtdOH present in either the donor or the acceptor membrane.

In summary, we describe a new system for examining the interorganelle transport of PtdSer between defined synthetic donor membranes and biological acceptor membranes. The findings implicate PtdSer domains within the donor as critical elements required for transport of the lipid. PtdOH may also act in concert with PtdSer to facilitate docking and recognition between membranes. The in vitro studies also demonstrate that planar membranes function optimally in lipid transport, and these may further facilitate the formation of zones of apposition between donor and acceptor membranes. This system recapitulates the in vivo lipid transport process and is constrained by the same genetic and molecular elements required in living cells.

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