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Transport of phosphatidylinositol to rat hepatocyte plasma membrane catalyzed by phosphatidylinositol transfer protein

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Plasma membrane sheets were isolated from fresh rat liver and characterized by electron microscopy and marker enzyme activities. Plasma membrane sheets were used as the acceptor membrane in the measure of transport of phosphatidyl[^{3}H]inositol from small unilamellar phospholipid vesicles or rough endoplasmic reticulum donor membranes. Catalysis of this transport was achieved with phosphatidylinositol transfer protein purified from rat or bovine brain. Assays were designed to separate donor and acceptor membranes by density gradient centrifugation. Rates of transfer were directly proportional to incubation time and the amounts of transfer protein and plasma membrane sheet added. These results are discussed in terms of cellular phosphatidylinositol metabolism, membrane phospholipid composition, and vesicle trafficking in rat hepatocytes.

Introduction

In eukaryotic cells the principal site of *de novo* phospholipid synthesis is the endoplasmic reticulum; most phospholipid species, however, are found in membranes throughout the cell [1]. One mechanism believed to be responsible for intracellular lipid transport involves protein-mediated transport of single lipid molecules between membranes through the cytoplasm [2,3]. Proteins which catalyze *in vitro* the transfer of phosphatidylinositol within donor-acceptor membrane systems have been characterized in bovine, human, and rat tissues [4–6], as well as in yeast [7]. Thus far, without exception, phosphatidylinositol transfer proteins are cytosolic proteins that exhibit remarkable similarities in molecular mass (32–37 kDa), isoelectric point (pH 4.6–5.6), phospholipid specificity (phosphatidylinositol and phosphatidylcholine), and catalytic activity [8]. Despite these functional and gross structural similarities, there is a surprising absence of primary sequence homology between rat and yeast phosphatidylinositol transfer proteins [9,10]. Protein-mediated intermembrane phosphatidylinositol transport has been demonstrated using unilamellar and multilamellar vesicles and microsomes as donor membrane and unilamellar and multilamellar vesicles, mitochondria, myelin and human platelet plasma membranes as acceptor membrane [8].

Within the eukaryotic cell a major route of phosphatidylinositol transport could occur between endoplasmic reticulum and plasma membrane. In rat hepatocytes endoplasmic reticulum-derived phosphatidylinositol acquires additional phosphate groups at the plasma membrane and participates in agonist-coupled signal transduction pathways [11,12]. This and other metabolic and regulatory processes contribute to the dramatic gradient of phosphatidylinositol in these two membranes: approx. 11 mol% in rough endoplasmic reticulum to 6 mol% in plasma membrane [13–17]. To test the hypothesis that phosphatidylinositol transfer protein could provide catalysis along this route, we took advantage of a straightforward isolation of intact sheets of rat hepatocyte plasma membrane described by Hubbard and colleagues [18]. We assessed the ability of phosphatidylinositol transfer protein to transfer phosphatidylinositol from small unilamellar vesicles or rat hepatocyte endoplasmic reticulum to plasma membrane sheets.

Materials and Methods

Isolation of plasma membrane sheets

Male Sprague-Dawley rats (*Rattus norvegicus*), weighing 110–150 g, were obtained from Sasco (Omaha,

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NE). Following overnight deprivation of food, the animals were killed by CO_2 asphyxiation. Livers were excised intact and perfused with ice-cold 0.9% (w/v) NaCl through branches of the portal vein. Subsequent steps were performed on ice or at 4°C. Plasma membranes were prepared as described [18]. Each liver, weighing 5–8 g, was minced and processed in 4 volumes of 0.25 M sucrose, 5 mM Tris-HCl, 1 mM $MgCl_2$ (pH 7.4), hereafter referred to as 0.25 M STM, in a Dounce homogenizer equipped with a loose-fitting pestle. The homogenate was filtered through cheesecloth. The filtrate was centrifuged at $280 \times g$ for 5 min to sediment unbroken cells, the pellet was washed, and the supernatants were combined and centrifuged at $1500 \times g$ for 10 min. This step separates larger structures, such as nuclei and membrane sheets, from vesicular and soluble elements. The resulting pellets were pooled and resuspended in one part 0.25 M STM, followed by the addition of two parts 2.0 M sucrose, 5 mM Tris-HCl, 1 mM $MgCl_2$ (pH 7.4), hereafter referred to as 2.0 M STM. The density of the suspension was adjusted to 1.18 g cm^{-3} ($n_D^{25} = 1.4010$) by adding either 0.25 M STM or 2.0 M STM. The crude membrane fraction was apportioned into centrifuge tubes (30–35 ml aliquots), overlaid with 2–3 ml 0.25 M sucrose, and centrifuged at $82000 \times g$ (r_{av}, 11.8 cm) for 1 h without brake. Plasma membrane sheets floated to the interface. The opalescent pellicle was collected, resuspended in enough 0.25 M sucrose to obtain a density less than 1.05 g cm^{-3} ($n_D^{25} = 1.3500$), and centrifuged at $1500 \times g$ for 10 min. The washed plasma membrane sheets were resuspended in 0.25 M STM and, if not used immediately, stored at −70°C. Aliquots were analyzed for protein content and phospholipid composition.

Preparation of endoplasmic reticulum

After perfusing a rat liver with 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4), and 1 mM EDTA, a 20% homogenate was prepared in the same buffer with a motor-driven Potter-Elvehjem apparatus and subjected to centrifugations of $1500 \times g$ for 10 min, $11000 \times g$ for 30 min, and $150000 \times g$ for 1 h. The microsomal pellet was resuspended in the same buffer. Approximately 70 mg of microsomes were incubated with 50 μCi [2-^{3}H]inositol (555 GBq $mmol^{-1}$, Amersham, Arlington Heights, IL) to label the phospholipid fraction [19]. The crude microsomal fraction was recovered by centrifugation at $150000 \times g$ for 1 h; it was then resuspended in 0.25 M sucrose and applied to the top of discontinuous gradients consisting of the following sucrose solutions: 1.25 g cm (2.0 ml), 1.18 g cm^{-3} (3.0 ml), and 1.06 g cm^{-3} (4.5 ml). Samples were centrifuged at $150000 \times g$ (r_{av}, 12.3 cm) for 4 h. The rough endoplasmic reticulum fraction at the 1.18–1.25 g cm^{-3} interface was collected, washed once with homogenization buffer, and stored in 1-ml aliquots at −70°C. Lipid analysis of the endoplasmic reticulum indicated greater than 95% of the ^{3}H-radioactivity was associated with phosphatidylinositol, yielding a radiospecific activity of 7180 dpm $nmol^{-1}$. Neither alkaline phosphodiesterase I nor 5'-nucleotidase activity could be detected in the endoplasmic reticulum preparation (data not shown).

Lipid analysis

Aliquots of membrane fractions were extracted with chloroform/methanol (1:2, v/v) containing 0.05% (w/v) butylated hydroxytoluene [20]. After addition of equal volumes of chloroform and 2.4 M HCl and centrifugation, the aqueous upper phase was extracted twice more with chloroform. The combined organic phases were washed twice with methanol/1 M HCl (1:1, v/v), taken to dryness under N_2, redissolved in chloroform/methanol (2:1, v/v), and stored at −20°C. Phospholipids were resolved by thin-layer chromatography on 0.25-mm silica gel G plates developed in isopropanol/ propionic acid/chloroform/ water (3:2:2:1, v/v) as described by Higgins and Fieldsend [16]. Phospholipid phosphorus was determined according to Rouser et al. [21]. Unpaired data were compared using Student's *t*-test.

Enzyme assays

All assays were performed on aliquots of plasma membrane sheets or rough endoplasmic reticulum stored at −70 °C for up to 2 weeks. Alkaline phosphodiesterase I (EC 3.1.4.1) activity was determined by measuring the released *p*-nitrophenol from thymidine 5'-monophosphate *p*-nitrophenyl ester [22]. 5'-Nucleotidase (EC 3.1.3.5) activity was assayed by measuring the release of inorganic phosphate from 5'-AMP [23]. Glucose-6-phosphatase (EC 3.1.3.9) activity was also assayed by measuring the release of inorganic phosphate [24]. NADH-cytochrome-*c* reductase (EC 1.6.2.1) activity was determined by measuring the rate of cytochrome *c* reduction [25].

Electron microscopy

Equal volumes of the resuspended plasma membrane sheets (50–100 μg protein) and 2% glutaraldehyde in phosphate-buffered saline were fixed for 30 min on ice, and the suspension was centrifuged at $15600 \times g$ for 5 min. The resulting pellet was post-fixed in equal volumes of 2% osmium tetroxide and 0.9% (w/v) NaCl containing 10 mM potassium phosphate (pH 7.4) for 15–30 min. After rinsing with 40% and 70% ethanol, the pellet was stained en bloc with 3 parts saturated uranyl acetate and 7 parts 100% ethanol overnight at 4 °C. The pellet was dehydrated through a graded series of ethanol and embedded in LR White resin. Thin sections were stained with aqueous uranyl

acetate and lead citrate and examined in a Philips model 300 electron microscope at 60 kV.

Phosphatidylinositol transfer assays

Phosphatidylinositol transfer activity was measured by determining the rate of phosphatidyl[^{3}H]inositol transfer from donor to acceptor membranes. Phosphatidylinositol transfer protein (31.9 kDa) was purified to near-homogeneity from postmicrosomal supernatants of bovine or rat brain homogenates [4,6]. Transfer protein (33.5 μg ml^{-1}) was stored at $-70\,^{\circ}$C in 50 mM Hepes-Na, 50 mM NaCl, 1 mM EDTA (pH 7.4) to

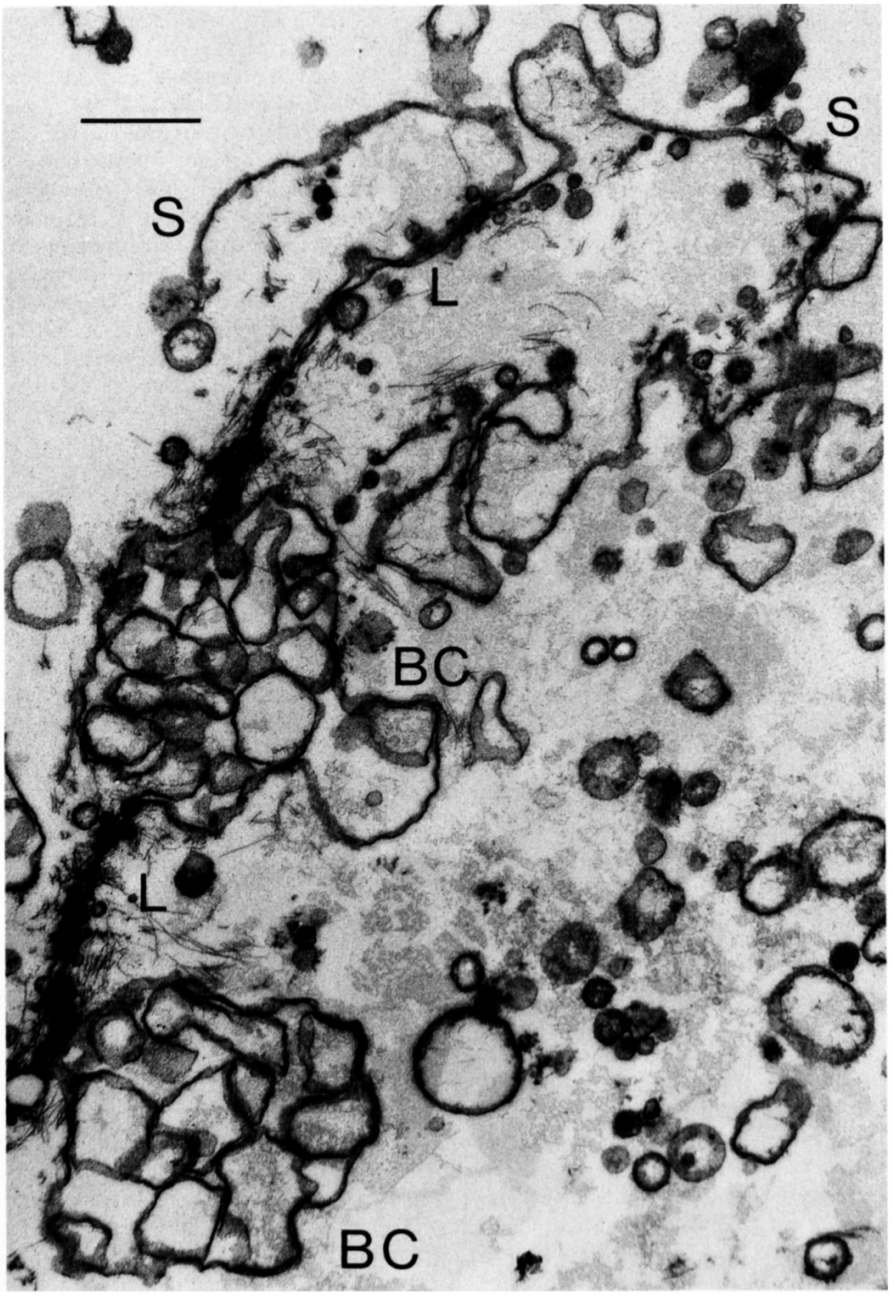

Fig. 1. Electron micrograph of rat hepatocyte plasma membrane sheet preparation. Three morphologically distinct domains can be identified: bile canalicular membrane (BC), lateral membrane (L), and sinusoidal membrane (S). Bar length, 0.5 μm.

TABLE I

Biochemical characterization of plasma membrane sheets

Marker enzymes for specific rat liver cell membranes (PM, plasma membrane; ER, endoplasmic reticulum) were assayed as described in Materials and Methods. Values are reported as the mean (μmol product h^{-1} (mg protein)$^{-1}$) ± S.D.; the number of determinations is in parentheses.

Marker enzyme	Specific activity	Relative enrichment (compared to homogenate)
Alkaline phosphodiesterase I (PM)	70.6 ± 20.7 (3)	32.0 ± 9.5
5'-Nucleotidase (PM)	63.6 (1)	28.0
Glucose-6-phosphatase (ER)	3.0 (1)	1.1
NADH–cytochrome-*c* reductase (ER)	6.8 ± 2.8 (3)	0.7 ± 0.3

which 5 mg ml^{-1} bovine albumin had been added. In most experiments the donor membranes were small unilamellar vesicles containing, in the ratio 90:10:0.2 (mol%), phosphatidylcholine/phosphatidyl[^{3}H]inositol/cholesteryl [1-^{14}C]oleate (2.1 GBq $mmol^{-1}$, New England Nuclear, Boston, MA). The acceptor membranes were isolated plasma membrane sheets. Transfer assays contained 110 nmol phospholipid vesicles, 50–500 μg protein of plasma membrane sheets, 1.6–3.3 μg phosphatidylinositol transfer protein, 0.25 M sucrose, 5 mM Tris-HCl, 1 mM EDTA (pH 7.4) in a total volume of 0.3–0.7 μl. After incubation for 30 or 60 min at 37 °C, the mixture was placed on a two-step sucrose gradient (1.06 g cm^{-3} and 1.18 g cm^{-3}) and centrifuged at 190000 × *g* (r_{av}, 8.5 cm) for 80 min to separate the donor and acceptor membranes. After centrifugation, 0.3-ml aliquots were taken from the top of the gradient and analyzed by liquid scintillation spectrometry and lipid extraction. Assays were performed in duplicate; control incubations were carried out in the absence of phosphatidylinositol transfer protein. Activity is calculated as pmol phosphatidylinositol transferred per 30 min or 1 h. In other experiments the donor membrane was rough endoplasmic reticulum (138 μg protein) containing phosphatidyl[^{3}H]inositol and the acceptor membrane was plasma membrane sheet (200 μg protein). The assay buffer was 50 mM Hepes-Na, 50 mM NaCl, 1 mM EDTA (pH 7.4); phosphatidylinositol transfer protein (3.3 or 6.6 μg) was added where indicated. The total volume was 0.6 ml, incubation was for 1 h at 37 °C. The reaction mixture was then layered on top of a 12-ml continuous sucrose gradient, 1.06–1.31 g cm^{-3}, and centrifuged at 150000 × *g* (r_{av}, 12.3 cm) for 3.5–4 h. Aliquots of 0.5 ml were collected and analyzed for radioactivity or enzyme activities. In some assays utilizing biological membranes, recovery was monitored by marker enzyme activity, alkaline phosphodiesterase I for plasma membrane and NADH–cytochrome *c* reductase for endoplasmic reticulum. Transfer experiments were performed in duplicate. Protein-catalyzed phospholipid transfer between small unilamellar vesicles has been described in detail [26].

Results

Plasma membrane isolation and characterization

Plasma membrane sheets were isolated and examined morphologically by transmission electron mi-

TABLE II

Phospholipid composition of rat liver plasma membrane sheets and rough endoplasmic reticulum

Membrane fractions, before and after one-hour incubations for phospholipid transfer, were subjected to lipid extraction, as outlined in Materials and Methods. Aliquots were analyzed for total lipid phosphorus and, following thin-layer chromatography, quantitation of individual phospholipid classes. Acidic species include phosphatidic acid, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate, all unresolved. Values, reported as the percentage of total lipid phosphorus, represent the mean ± S.D. of 3–6 determinations.

Phospholipid class	Plasma membrane sheets		Rough endoplasmic reticulum
	not incubated or incubated in presence of small unilamellar vesicles	incubated in presence of phosphatidylinositol transfer protein and small unilamellar vesicles	
Lysophosphatidylcholine	5.2 ± 2.1	2.7 ± 1.4	2.9 ± 0.8
Sphingomyelin	17.6 ± 3.0	16.6 ± 1.1	1.9 ± 0.6
Phosphatidylcholine	38.7 ± 3.0	48.4 ± 1.4	56.3 ± 3.7
Phosphatidylinositol	4.3 ± 1.2	2.5 ± 1.7	11.0 ± 1.8
Phosphatidylserine	4.2 ± 1.7	2.9 ± 0.2	5.4 ± 3.8
Phosphatidylethanolamine	18.9 ± 0.7	18.6 ± 1.4	18.6 ± 1.0
Acidic species	6.2 ± 1.4	5.8 ± 1.2	2.2 ± 0.6
Lipid phosphorus/protein (nmol/mg)	335 ± 48	not determined	583 ± 15

croscopy (Fig. 1). Three major functional domains are readily identified: bile canalicular membrane with numerous cross sections of microvilli; lateral membrane with tight junctions and desmosomes; and sinusoidal membrane with coated pits. Some small microsomes, studded with ribosomes, were present in the plasma membrane sheet preparation, along with a few mitochondria. The activity of plasma membrane marker enzymes was assessed to characterize further our plasma membrane sheet isolation. 5'-Nucleotidase and alkaline phosphodiesterase I averaged a 30-fold enrichment over the initial homogenate (Table I). Membrane derived from the endoplasmic reticulum appeared to be the major organelle contaminant. Based upon the activities of two enzyme markers, glucose-6-phosphatase and NADH–cytochrome-*c* reductase (Table I) and their relative enrichments, we estimated that the plasma membrane sheets contained approximately 15–19% contaminating endoplasmic reticulum, a value which is comparable to that originally reported [18]. The yield of plasma membrane sheets was 0.18–0.68 mg (g tissue)$^{-1}$ for seven preparations. The phospholipid content and composition of the plasma membrane sheets (Table II) were similar to values reported for rat hepatocyte plasma membranes prepared from larger animals and by different techniques [13,14]. Attempts to reduce further endoplasmic reticulum contamination by techniques similar to those used in membrane separation following phospholipid transfer assays (i.e., pelleting rather than flotation) resulted in significantly lower yields of intact plasma membrane sheets.

Phosphatidylinositol transfer from vesicles to plasma membrane sheets

A donor-acceptor assay system was designed to analyze the transfer of phosphatidyl[^{3}H]inositol from small unilamellar phospholipid vesicles to isolated plasma membrane sheets. Vesicles contained 10 mol% phosphatidylinositol, a proportion similar to that found in rough endoplasmic reticulum (Table II). A non-transferable lipid, cholesteryl [^{14}C]oleate, was added to the vesicles to evaluate donor vesicle recovery. The entire density gradient used for the separation of donor and acceptor membranes was analyzed by liquid scintillation spectrometry. The results of a typical assay are presented in Fig. 2. The donor vesicles, and transfer protein when present, were located in the low-density fractions, while the plasma membrane sheets were found at the interface and in the high-density fractions. The contaminating endoplasmic reticulum in the plasma membrane sheet preparations formed a small pellet under these centrifugation conditions and was not further analyzed. To quantitate transfer activity, we analyzed the ratio of ^{3}H to ^{14}C radioactivity. The overwhelming majority of radioactivity was present in fractions 1–3 of the gradient. The donor membranes,

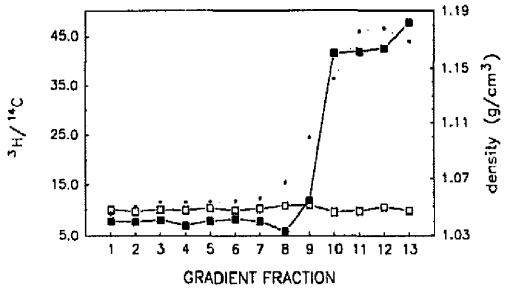

Fig. 2. Separation of phospholipid vesicles and plasma membrane sheets following protein-mediated transfer of phosphatidylinositol. Small unilamellar vesicles (110 nmol containing 9.0 nmol phosphatidyl[^{3}H]inositol and < 0.2 nmol cholesteryl [^{14}C]oleate) were mixed with plasma membrane sheets (2.9 nmol phosphatidylinositol) and, where indicated, phosphatidylinositol transfer protein (3.3 μg) in a total volume of 0.35 ml. After 1 h at 37 °C, the mixture was placed on a discontinuous sucrose gradient. The dotted line depicts the density of the gradient fractions (0.5 ml). The ratio of phosphatidylinositol to cholesteryl oleate (dpm ^{3}H/dpm ^{14}C) is determined for gradient-separated membrane mixtures incubated in the absence (□) or presence (■) of transfer protein.

as prepared, exhibited a ^{3}H/^{14}C ratio of 10. In the absence of phosphatidylinositol transfer protein, this ratio remained unchanged throughout the gradient. In the presence of phosphatidylinositol transfer protein, the plasma membrane sheet fractions showed an increased ^{3}H/^{14}C ratio, indicating transfer of phosphatidyl[^{3}H]inositol to the plasma membrane sheets. Likewise, in the presence of transfer protein, the small unilamellar vesicles yielded a decreased ^{3}H/^{14}C ratio, indicating a selective loss of phosphatidyl[^{3}H]inositol. As depicted in Fig. 3, the amount of phosphatidyl[^{3}H]inositol transferred to plasma membrane sheets increased in direct proportion with the addition of plasma membrane sheets (acceptor membrane). From data not shown we also observed linear relationships between phospholipid transfer and incubation

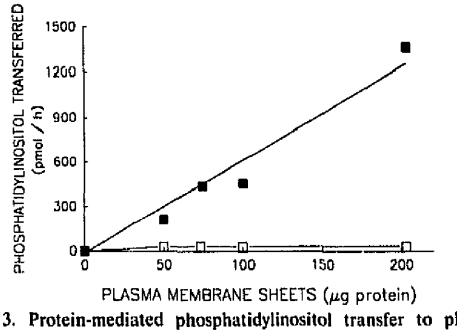

Fig. 3. Protein-mediated phosphatidylinositol transfer to plasma membrane sheets. Experimental conditions are similar to those described in Fig. 2. The amount of phosphatidyl[^{3}H]inositol transferred to plasma membrane sheets is determined as a function of increased amount of plasma membrane sheets in the assay system (open symbols, without transfer protein; filled symbols, with transfer protein). Data points represent the means of duplicate assays.

TABLE III

Phosphatidylinositol transfer protein activity with various assay systems

Phosphatidylinositol transfer activity was measured as detailed in Materials and Methods. Transfer activities for 30-min incubations were corrected for controls containing no phosphatidylinositol transfer protein. Small unilamellar vesicle acceptor vesicles contained phosphatidylcholine (87 mol%), phosphatidylinositol (5 mol%), and lactosylceramide (8 mol%). Initial membrane phosphatidylinositol content was determined chemically before incubations; transferred phosphatidylinositol was based on quantitation of ^{3}H-radioactivity.

Donor membrane	Initial phosphatidylinositol in membrane (nmol)	Acceptor membrane	Initial phosphatidylinositol in membrane (nmol)	Transfer protein (pmol)	Transfer activity (pmol/30 min)
Small unilamellar vesicles	2.36	Small unilamellar vesicles	3.00	2.1	320
				6.5	400
				10.8	677
	10.6	Plasma membrane sheets	2.60	108	1047
				216	1268
	176		38	2160	11710

time and phosphatidylinositol transfer protein; in addition, we noted no significant differences between plasma membrane sheets used immediately after isolation and those stored at −70 °C.

Plasma membrane sheets were analyzed for phospholipid composition before and after incubation with phosphatidylinositol transfer protein and small unilamellar vesicles (Table II). The most striking change occurred in the content of phosphatidylcholine, which underwent a statistically significant ($P < 0.05$) increase of 25% in the presence of phosphatidylinositol transfer protein. The observed alterations in the membrane content of lysophosphatidylcholine, phosphatidylinositol, and phosphatidylserine were not significant. One mechanism of accounting for these membrane composition changes would be an exchange of phosphatidylinositol and a net transfer of phosphatidylcholine from vesicles to plasma membrane sheets catalyzed by phosphatidylinositol transfer protein.

The catalytic activity of phosphatidylinositol transfer protein was compared in two assay systems (Table III). When small unilamellar vesicles were employed as both donor and acceptor membranes and the initial pools of phosphatidylinositol were nearly equivalent, specific activities between 70 and 160 mol phosphatidylinositol transferred/mol phosphatidylinositol transfer protein were calculated for the 30-min incubation period. When plasma membrane sheets served as acceptor membrane, the amount was adjusted to present a 5-fold smaller pool of phosphatidylinositol in order to favor a net transfer. Under these conditions specific transfer activities between 5 and 10 mol/mol were determined for the 30-min incubations. Clearly, catalysis in the vesicle-plasma membrane assay system is less efficient than in the vesicle-vesicle assay system. Moreover, as the data in Table II indicate, a net transfer of phosphatidylinositol was unlikely.

Phosphatidylinositol transfer from endoplasmic reticulum to plasma membrane sheets

Somewhat more challenging to undertake were protein-mediated transfers of phosphatidylinositol from endoplasmic reticulum to plasma membrane. A major difficulty lay with the separation of these two mem-

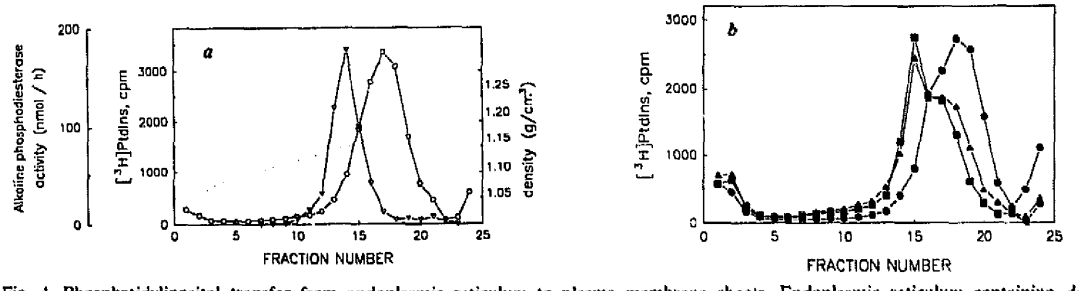

Fig. 4. Phosphatidylinositol transfer from endoplasmic reticulum to plasma membrane sheets. Endoplasmic reticulum containing *de novo* synthesized phosphatidyl[^{3}H]inositol (2.9 nmol) and plasma membrane sheets (8.9 nmol phosphatidylinositol) were incubated with increasing phosphatidylinositol transfer protein; mixtures were subsequently separated on a linear sucrose gradient. (a) Analysis of gradient fractions (0.5 ml) for plasma membrane alkaline phosphodiesterase I activity (▽), endoplasmic reticulum phosphatidyl[^{3}H]inositol radioactivity (○), and density (· · · · · ·) in the absence of transfer protein. (b) Analysis of gradient radioactivity from membrane mixtures containing no transfer protein (●), 3.3 μg transfer protein (▲), or 6.6 μg transfer protein (■).

branes following incubation. We achieved partial separation with a shallow linear sucrose gradient, illustrated in Fig. 4a; plasma membrane sheets were centrifuged to a density of 1.14–1.16 g cm^{-3}, indicated by the initial profile of ^{3}H-radioactivity, and rough endoplasmic reticulum were centrifuged to a density of 1.17–1.19 g cm^{-3}, depicted by the activity of phosphodiesterase I. In the absence of transfer protein, essentially all ^{3}H-radioactivity remained associated with endoplasmic reticulum, shown in Fig. 4b. However, with increasing amounts of transfer protein in the assay system, there was a pronounced increase in radioactivity in the lower density fractions which coincide with the plasma membrane sheets. Concomitantly, there was a decrease in radioactivity associated with rough endoplasmic reticulum. Such shifts in radioactivity profiles could most probably be attributed to an accelerated transport through the aqueous medium catalyzed by phosphatidylinositol transfer protein. Although nearly impossible to quantitate, we estimate that 30–40% of endoplasmic reticulum phosphatidyl[^{3}H]inositol had been transported to the plasma membrane sheet preparation. While the relatively small amount of contaminating endoplasmic reticulum in the plasma membrane sheet preparation could, in theory, account for the measured phosphatidylinositol transfer described in other experiments (Figs. 2 and 3, Table III), this experiment demonstrated that plasma membrane sheets can function as an acceptor membrane in the presence of endoplasmic reticulum donor membranes. Furthermore, plasma membrane and endoplasmic reticulum have been separated on a continuous, rather than discontinuous density gradient.

Discussion

From a wide spectrum of *in vitro* investigations the phenomenon of protein-mediated intermembrane phospholipid movement has been elaborated. By forming a stoichiometric complex with a phospholipid molecule for diffusion through the aqueous phase, phosphatidylinositol transfer protein catalyzes fluxes of phosphatidylinositol and phosphatidylcholine between membrane surfaces, both biological and artificial [8]. Within the eukarytotic cell the catalytic activity of phosphatidylinositol transfer protein has been proposed as one means by which membranes, other than the endoplasmic reticulum where phosphatidylinositol is synthesized, acquire this phospholipid [3]. To satisfy the transfer protein's mechanistic preference for lipid exchange, rather than unidirectional net transfer [26], the more abundant and widely distributed phosphatidylcholine molecule is likely transported, in the reverse direction, to the endoplasmic reticulum. Van Paridon and co-workers [27] have suggested that the dual substrate specificity and relative affinities toward phosphatidylinositol and phosphatidylcholine are consistent with a role of phosphatidylinositol transfer protein in maintaining phospholipid levels in intracellular membranes. That such catalytic events may occur *in vivo* is supported by the recent seminal observations by Cleves et al. [28,29]: mutation of phosphatidylinositol transfer protein in yeast disrupts the exocytic transport of proteins from a late Golgi compartment and leads to cell death; remarkably, mutations to enzymes in the CDP-choline pathway of phosphatidylcholine synthesis permit a bypass of the phosphatidylinositol transfer protein requirement for cell viability; similarly and even more remarkably, mutation of the *SAC*1 gene product, most likely an actin- and anionic phospholipid-binding protein, also supresses the lethality of the phosphatidylinositol transfer protein mutation.

The hepatocyte is a polarized epithelial cell whose plasma membrane consists of multiple functionally and morphologically distinct domains [30,31]. The sinusoidal domain is specialized for hormone reception and exchange of metabolites with the blood and is characterized by irregular microvilli and coated pits. The lateral domain, which is in direct contact with the adjacent hepatocyte, is specialized for cell attachments and communication and is characterized by tight junctions, desmosomes, and gap junctions. The bile canalicular domain, which is separated from the lateral domain by tight junctions, is specialized for bile secretion and is characterized by numerous microvilli. Each of these three domains is readily identifiable in our plasma membrane sheet preparations (Fig. 1). The observations presented in this communication are significant because they describe phosphatidylinositol transport to a plasma membrane preparation of defined morphology. Although the relative (30-fold) enrichment of this preparation is quite reasonable with respect to plasma membrane, there is, nonetheless, largely unavoidable contaminating endoplasmic reticulum (Table I). To rule out transfer of phosphatidylinositol from small unilamellar vesicles to contaminating endoplasmic reticulum is impossible (Figs. 2 and 3, Table III). In the series of experiments in which the donor membrane is purified rough endoplasmic reticulum, we demonstrate a very significant protein-mediated transfer of phosphatidylinositol to gradient-separated plasma membranes (Fig. 4). Thus, we have succeeded in recreating a highly probable in vivo path of intracellular phosphatidylinositol transport, namely endoplasmic reticulum to plasma membrane.

We selected rat liver for membrane isolations since this tissue was well-characterized and had served as a membrane source in many early phosphatidylinositol transport studies [8]. The open plasma membrane sheet preparation is particularly advantageous as an acceptor membrane in protein-mediated phospholipid transport: this extended, open membrane [18] presents to phos-

phatidylinositol transfer protein its cytoplasmic surface, i.e., the same surface which the protein would encounter in the cell. On the other hand, we cannot exclude the possibility that transfer occurred (exclusively) at the exoplasmic surface of these open membrane sheets. The topological localization of phosphatidylinositol to the cytoplasmic surface of rat liver plasma membranes [15,17] and our observation of a slight loss of that phospholipid during incubations in the presence of transfer protein (Table II) together suggest some association between phosphatidylinositol transfer protein and the cytoplasmic surface of the plasma membrane. An earlier investigation of plasma membrane acceptor membranes utilized vesiculated human platelet membranes [5], a membrane preparation which most likely exposed only the exoplasmic surface to phosphatidylinositol transfer protein.

Our experiments indicate that hepatocyte plasma membrane sheets can accept phosphatidylinositol from small unilamellar vesicle donor membranes or from hepatocyte rough endoplasmic reticulum. It is clear from the essentially linear relationships between the amount of phosphatidylinositol transferred and the amount of transfer protein (Fig. 3), incubation time, and the quantity of plasma membrane added that initial rates of transfer could be calculated. Despite the complex design of these experiments and, especially, the necessity to separate donor and acceptor membranes by density gradient centrifugation, we compared such transfer rates with those determined by more conventional assay systems [32]. The markedly diminished catalytic efficiency of phosphatidylinositol transfer protein toward plasma membrane sheets (Table III) is not unlike that observed whenever biological membranes have been employed in the measurement of intermembrane phospholipid transfer activity [32] and may reflect both membrane compositional as well as membrane shape variables. Also noteworthy is the apparent unidirectional net transfer of phosphatidylcholine from small unilamellar vesicles to plasma membranes (Table II). Similar unidirectional fluxes of phosphatidylcholine, catalyzed by phosphatidylinositol transfer protein, have been described in monolayer-monolayer and vesicle-vesicle assay systems [19,26].

For both bovine and yeast phosphatidylinositol transfer proteins, the affinity to bind (and presumably transfer) phosphatidylinositol was at least an order of magnitude greater than that for phosphatidylcholine [27,33]. The ability of phosphatidylinositol transfer protein to modulate the amounts of both phosphatidylinositol and phosphatidylcholine in membranes is intrinsic to its *in vitro* catalytic properties [8] and is consistent with its proposed participation in vesicular lipid-protein trafficking in yeast [10]. Extrapolation of such a function to other eukaryotic cells as well as to other cellular membranes, including plasma membrane, must now be given serious consideration. The development of a simple phospholipid transfer assay utilizing relevant biological membranes holds great promise in defining more precisely the role of phosphatidylinositol transfer protein in intracellular membrane structure and function. For example, we are currently investigating the fractionation of rat hepatocyte plasma membrane into structural/functional domains in an attempt to identify the primary destination of phosphatidylinositol molecules imported by phosphatidylinositol transfer protein and to describe the relationships among these domains during phosphatidylinositol metabolism.

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