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Transport of phospholipids between subcellular membranes of wild-type yeast cells and of the phosphatidylinositol transfer protein-deficient strain *Saccharomyces cerevisiae sec 14*

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The transfer of glycerophospholipids between microsomes and mitochondria, and from internal membranes to the plasma membrane of *Saccharomyces cerevisiae* was characterized. Cellular energy production was found to be essential for intracellular translocation of phospholipids, but neither a membrane potential nor an intact cytoskeleton are required for this process. Using the temperature-sensitive mutant strain *Saccharomyces cerevisiae sec 14*, which is defective in the phosphatidylinositol transfer protein, it could be demonstrated that this protein is not involved in the transport of phosphatidylinositol and phosphatidylcholine from internal membranes to the plasma membrane. Our results also confirm earlier findings that phosphatidylinositol and phosphatidylcholine can be delivered to the plasma membrane in a process independent of the flux of vesicles competent for protein secretion.

Introduction

In yeast, as in higher eukaryotes, the endoplasmic reticulum and the inner mitochondrial membrane are major sites of membrane phospholipid synthesis [1,2]. Other membranes, e.g., the plasma membrane, are devoid of phospholipid synthesizing enzymes. Because none of the subcellular compartments can produce all its constituent phospholipids, efficient intermembrane lipid traffic is essential for cellular function. Mechanisms of intracellular phospholipid transfer are only poorly understood, mainly because it is extremely difficult to decide which of the mechanisms proposed [1,3,4] is operating in an intact cell in a specific case. Spontaneous or protein-catalyzed transport of monomeric phospholipids through the aqueous phase, vesicle flux or controlled organelle fusion might govern certain steps of intracellular lipid transport, e.g., the transport of phospholipids between the endoplasmic reticulum and mitochondria, or from internal membranes to the plasma membrane.

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The fact that phosphatidylserine synthesized in the endoplasmic reticulum [2] is decarboxylated to phosphatidylethanolamine after transport to the inner mitochondrial membrane can be exploited to study this transfer process in living cells. Applying this technique to intact and permeabilized cultured cells, Voelker [5–7] found that the rate of phosphatidylserine transfer to mitochondria was greatly reduced by azide and fluoride and was stimulated by ATP. Membrane collision and/or vesicle flux were discussed as possible mechanisms of this transport process. Previously, De Silva and Siu [8] had suggested that transport of phosphatidylethanolamine and phosphatidylcholine to the plasma membrane of *Dictyostelium* was mediated by specific vesicles. The authors' assumption was based on the finding that disruption of the microtubule network by colchicine interrupted phospholipid transport and led to the accumulation of phospholipid rich vesicles. On the other hand, transport of phosphatidylcholine [9] and phosphatidylethanolamine [10] to the plasma membrane was found to be independent of metabolic energy in mammalian cells, suggesting a mechanism different from vesicle flow.

Making use of the metabolic conversion mentioned above we designed assays to investigate in vivo the intracellular transport of phosphatidylserine and phos-

phatidylethanolamine in the yeast, *Saccharomyces cerevisiae*. Experiments were extended to studies of the translocation of phospholipids from internal membranes to the plasma membrane. The availability of yeast secretory mutants allowed us to apply genetic methods to the problem of intracellular lipid traffic. In a previous study, we had found that interruption of the flow of secretory vesicles in temperature-sensitive secretory mutants had no effect on the transport of phosphatidylinositol and phosphatidylcholine to the plasma membrane [11]. More recently, Aitken et al. [12] succeeded in the construction of a yeast mutant defective in the gene encoding the phosphatidylinositol transfer protein (*PIT 1*). This mutant is unable to grow thus pointing to an essential function of the gene product. Bankaitis and co-workers [13,14] then demonstrated that the *PIT 1* gene product was identical to the *SEC 14* protein which had previously been identified as being absolutely required for protein secretion at the level of the late Golgi complex. However, the role played by the phosphatidylinositol transfer protein is still far from clear. Here, we report that the phosphatidylinositol transfer protein which is not functioning in a temperature-sensitive *pit1/sec14* yeast mutant at the non-permissive temperature is not required for the transport of phosphatidylinositol and phosphatidylcholine to the plasma membrane of yeast cells.

Materials and Methods

Yeast strains and culture conditions

The wild-type yeast strain *Saccharomyces cerevisiae* D273-10 B was grown aerobically at 30°C on YPGal medium containing 2% galactose, 2% peptone and 1% yeast extract. Before labelling with [^{3}H]serine, [^{3}H]inositol, [^{3}H]choline or [^{3}H]methionine, respectively, cells grown on YPGal overnight were transferred to serine-, inositol-, choline- or methionine-free medium [15], containing 2% galactose and incubated for 2 h prior to labelling.

The temperature-sensitive mutant *Saccharomyces cerevisiae sec 14* and other secretory mutants used as a control were grown aerobically at 24°C overnight either on YPD (2% glucose) or YPGal (2% galactose). For the induction of the secretory block, the temperature was shifted to 37°C for 2 h. In control experiments, the corresponding wild-type strain *Saccharomyces cerevisiae* X-2180 was incubated under identical conditions.

Labelling of wild-type yeast cells with lipid precursors

Cells grown overnight in YPGal were preincubated for 2 h in serine-free medium (see above) at a concentration of 20 mg wet weight per ml. Then, 5 μCi [^{3}H]serine (20 Ci/mmol) were added to 15 ml of this suspension and incubation was continued for 20 min at 30°C under vigorous shaking. The incorporation of serine was stopped by the addition of 5 ml ice-cold 20% TCA. Cellular material was harvested by centrifugation (2 min, 3000 rpm) on a table top centrifuge and washed once with ice-cold 5% TCA. Then, 1 g glass beads (0.3–0.4 mm diameter) and 250 μl 10 mM Tris-HCl (pH 7.4) were added and cells were disintegrated by vortexing three times for 1 min with intermittent cooling on ice. 10 ml chloroform/methanol (2:1, v/v) was added and lipids were extracted according to Folch et al. [16]. Glass beads and cell debris were removed by centrifugation. The resulting organic phase was washed twice with 2 M KCl/methanol (4:1, v/v) and once with methanol/water/chloroform (47:48:3, per vol.). Aliquots of the resulting lipid extract were used for further analysis.

Pulse-chase experiments using [^{3}H]serine as a phospholipid precursor were carried out following a different incubation protocol. Cells grown overnight on YPGal medium were preincubated for 15 min in a serine-free medium (2.5 g cell wet weight in 75 ml). 50 μCi [^{3}H]serine at a specific activity of 25 Ci/mol were added and cells were labelled for 60 min at 30°C. Then, the radioactive medium was removed by centrifugation, cells were washed once with sterile distilled water and further incubated in 75 ml fresh YPGal medium. At this point, azide and fluoride were added at a final concentration of 5 mM each where indicated. At time points indicated 15 ml of the culture were removed and added to 5 ml 20% TCA. Cells were disintegrated and lipids were extracted as described above.

Labelling of secretory mutants with lipid precursors

Labelling experiments with temperature-sensitive secretory mutants and the corresponding wild-type strain, *Saccharomyces cerevisiae* X-2180, were modified insofar as part of the culture was shifted to the non-permissive temperature of 37°C during preincubation in inositol-, choline- or methionine-free medium, respectively, whereas the other part was left at the permissive temperature (24°C). When transport of phospholipids to the plasma membrane or to mitochondria was tested, cells (13 g wet weight) were preincubated in 2 l inositol-, choline- or methionine-free medium, respectively, for 2 h at 24°C or 37°C. Then, cells were pulse-labelled with 120 μCi [^{3}H]inositol, [^{3}H]choline or [^{3}H]methionine (60–80 Ci/mmol each) at the respective temperatures for 15 min. Then, unlabelled inositol, choline or methionine, respectively, were added to a final concentration of 10 mM. During the subsequent chase, samples (600 ml of the culture) were taken at time points indicated and chilled on ice. Azide and fluoride were added to a final concentration of 5 mM in order to stop the cellular metabolism and plasma membrane [17] and mitochondria [18] were isolated by published procedures.

When secretory mutants and the corresponding wild-type strain were used to study the secretion of the water-soluble deacylation products of phosphatidylinositol and phosphatidylcholine into the medium, cells (100 ml culture volume) were grown overnight in the presence of 50 μCi [^{3}H]inositol or [^{3}H]choline (60–80 Ci/mmol each), respectively, in an otherwise inositol- or choline-free medium. Then, cells were harvested, washed with sterile distilled water and transferred to a fresh YPD medium (100 ml). At time-points indicated, samples of 1 ml culture were taken, cells and culture medium were separated by centrifugation on a table top centrifuge and analyzed for their radioactive components (see below).

Analytical procedures

Phospholipids were separated from each other by 1-D thin-layer chromatography on silica gel 60 plates (Merck) using chloroform/methanol/25% NH_3 (65:35:5, per vol.) as a developing solvent. Lipid phosphorus was quantitated by the method of Broekhuyse [19]. Radioactively labelled phospholipids were scraped off the plates and measured by liquid scintillation counting using Ready Protein (Beckman), containing 5% water, as a scintillation cocktail.

Glycerophosphoinositol was separated from inositol and inositol phosphate by column chromatography using AG1-X2 as described by Paltauf et al. [20]. Glycerophosphocholine was separated from choline and choline phosphate by thin-layer chromatography using chloroform/methanol/25% NH_3 (6:10:5; per vol.) as a solvent.

Protein was quantitated by the method of Lowry et al. [21] using bovine serum albumin as a standard. SDS-PAGE was carried out as described by Laemmli [22] and used routinely for the characterization of subcellular fractions. Monospecific antisera against plasma membrane ATPase (a gift from R. Serrano, Heidelberg) and mitochondrial porin were employed as markers for measuring cross-contamination of organelle preparations.

Results and Discussion

Transport of phosphatidylserine and phosphatidylethanolamine between the endoplasmic reticulum and mitochondria

Shortly after labelling yeast cells with [^{3}H]serine, radioactivity can be detected not only in phosphatidylserine, but also in phosphatidylethanolamine and phosphatidylcholine (Table I). The relatively high percentage of radioactivity in the two latter phospholipids points to an efficient translocation cascade. Newly synthesized phosphatidylserine made in the endoplasmic reticulum is transported to mitochondria, where it is converted to phosphatidylethanolamine by the inner mitochondrial membrane enzyme phosphatidylserine decarboxylase; phosphatidylethanolamine produced in the inner mitochondrial membrane is translocated to the endoplasmic reticulum, where conversion to phosphatidylcholine occurs by stepwise methylation catalyzed by phosphatidylethanolamine *N*-methyltransferase and phospholipid *N*-methyltransferase. Both enzymes have been localized exclusively to the endoplasmic reticulum [2].

TABLE I

Transport and metabolic conversion of glycerophospholipids in vivo

Wild-type yeast cells were labelled for 15 min with [^{3}H]serine in the presence or absence of inhibitors, total lipids were extracted and analyzed as described in Materials and Methods. Mean values are from three experiments. CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

	cpm/aliquot * in total lipids	PS (%)	PE (%)	PC (%)
Control	9630	45 ± 2	15 ± 2	38 ± 4
+ Nocodazole (0.1 mM)	7080	40 ± 3	17 ± 1	41 ± 2
+ CCCP (0.1 mM)	13960	35 ± 2	11 ± 2	51 ± 2

* Data from one representative experiment are shown. Aliquots contain identical cell numbers.

The presence of the cytoskeleton inhibitor nocodazole and the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) during the incubation of cells with [^{3}H]serine did not inhibit total lipid synthesis and interconversion (Table I), indicating that neither an intact cytoskeleton nor a membrane potential are required for phospholipid synthesis and translocation. When the energy-dependence of intermembrane translocation of phosphatidylserine and phosphatidylethanolamine was studied, cells were labelled with [^{3}H]serine in a 15 min pulse. After depleting cells of energy by addition of azide and fluoride, pre-formed phosphatidylserine was no longer converted to phosphatidylethanolamine in the chase (Fig. 1), although the action of phosphatidylserine decarboxylase is energy-independent and the enzyme is insensitive to azide and fluoride in vitro [23]. This result indicates that translocation of phosphatidylserine from the specific subfraction of the endoplasmic reticulum where it is produced [2] to the inner mitochondrial membrane requires energy. This result is in good agreement with data presented by Voelker [6,7], who proposed an ATP requirement for the translocation of phosphatidylserine to mitochondria.

Similarly, conversion of phosphatidylethanolamine to phosphatidylcholine came to an halt, when yeast cells were poisoned with azide and fluoride (Fig. 1). Since methylation of phosphatidylethanolamine requires cellular energy, we cannot decide from results shown in Fig. 1, if translocation of phosphatidylethanolamine from mitochondria to microsomes or the

enzymatic conversion of phosphatidylethanolamine to phosphatidylcholine is the limiting energy-dependent step blocked in this experiment. In a previous study [11], we were able to demonstrate by subfractionation of yeast cells that phosphatidylethanolamine remained in mitochondria and was no longer translocated to microsomes when cells were deenergized. This result gave raise to the view that not only methylation of phosphatidylethanolamine, but also its intracellular transport is energy-dependent.

Transport of phospholipids to the plasma membrane

After incubating yeast cells for 5 min with [^{3}H]serine radioactively labelled phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine can be recovered from isolated plasma membrane preparations (Table II). The total amount of radioactivity in all three phospholipids increases in the plasma membrane during a subsequent 15 min chase. Interestingly, most of the radioactivity was associated with phosphatidylserine. This result reflects the unusually high phosphatidylserine content of the yeast plasma membrane [2], although the percentage of radioactivity (> 70%) exceeds the phosphatidylserine content (approx. 30% of total glycerophospholipids). Contamination of plasma-membrane preparations with other subcellular fractions was negligably low (less than 5%) and could not account for the high radioactivity in phosphatidylserine. As shown in Table II, the distribution of radioactivity among phospholipids was quite different in the plasma membrane and mitochondria. In comparison to total cellular membranes with their ratio of label

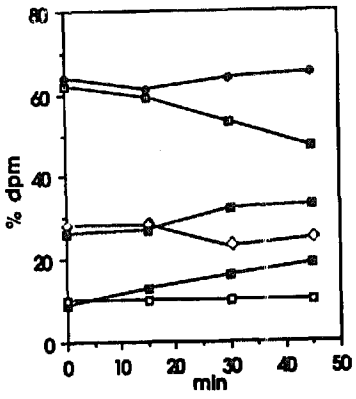

Fig. 1. Energy-dependence of the transport of aminoglycerophospholipids in vivo. Wild-type yeast cells, *Saccharomyces cerevisiae* D273-10B, were grown overnight on a complex medium, transferred to a serine-free medium and pulse-labelled for 60 min with [^{3}H]serine as outlined in Materials and Methods. During the subsequent chase in complex medium, no further incorporation of label into glycerophospholipids occurred. Azide and fluoride were added at the zero time-point to a final concentration of 5 mM each. At time points indicated, cells were harvested and lipids were extracted and analyzed. ⊡, phosphatidylserine; ◆, phosphatidylserine (N_3^- + F^- present); ■, phosphatidylethanolamine; ◇, phosphatidylethanolamine (N_3^- + F^- present); ■, phosphatidylcholine; □, phosphatidylcholine (N_3^- + F^- present).

TABLE II

Transport of phospholipids to the plasma membrane and to mitochondria in vivo

Wild-type yeast cells were pulse-labeled with [^{3}H]serine for 5 min. Before and after a chase period of 15 min organelles were isolated, and their phospholipids were analyzed as described in Materials and Methods. The enrichment of plasma membrane over the homogenate was 70–80-fold as judged by immunotitration with antiserum against plasma membrane ATPase and 4–5-fold for mitochondria using antiserum against porin. The contamination of plasma membrane with other organelles was marginal; mitochondria were contamined with microsomes (25–30%).

	Specific activity (cpm/mg protein)					
	Plasma membrane			Mitochondria		
	PS	PE	PC	PS	PE	PC
0 min	70	30	6	51	85	31
15 min, control	220	50	12	115	204	78
15 min, + N_3 + F (5 mM)	92	34	7	58	103	31

of 51:30:19 in phosphatidylserine:phosphatidylethanolamine:phosphatidylcholine, the plasma membrane showed an enrichment of radiolabelled phosphatidylserine (ratio 68:25:7), whereas in mitochondria the majority of radioactivity appeared in phosphatidylethanolamine (ratio 33:47:19). This result lends further support to the idea that phosphatidylserine is transported to the plasma membrane more efficiently than phosphatidylethanolamine or phosphatidylcholine. The possible involvement of phosphatidylserine in the flux of secretory vesicles to the cell periphery is under investigation. Depleting cells of energy completely blocks translocation of phospholipids to the plasma membrane (Table II), supporting the view that also in this case translocation of phospholipids is an energy-linked process.

Involvement of the phosphatidylinositol transfer protein in translocation of phosphatidylinositol and phosphatidylcholine to the plasma membrane

Ever since the discovery of cytosolic phospholipid transfer proteins some two decades ago, the question has been asked as to the physiological role of these proteins. In particular, the question has to be answered whether or not such proteins contribute to the supply of phospholipids to membranes during membrane biogenesis and maintenance of membrane phospholipid patterns. The availability of the temperature-sensitive *sec 14* mutant strain which is defective in the phosphatidylinositol transfer-protein offered the possibility to test whether or not this protein contributes to the translocation of phosphatidylinositol and phosphatidylcholine between subcellular membranes, e.g., from internal membranes to the plasma membrane. Under non-permissive conditions (37°C), the phosphatidyl-

choline transfer activity in the cytosol of the *sec 14* mutant is decreased to 4% of the control at the permissive temperature (Daum, G., unpublished results). The total lack of phosphatidylinositol transfer activity in the *sec 14* mutant at the non-permissive temperature has been described before [14].

Two types of experiments were performed to address the problems mentioned above. In one set of experiments, cells were pulse-labelled with [^{3}H]choline or [^{3}H]inositol, respectively, and the appearance of radiolabelled phosphatidylcholine or phosphatidylinositol in the plasma membrane was measured after a chase period. Data shown in Fig. 2 clearly demonstrate that the specific radioactivity of phosphatidylcholine in the plasma membrane increased under conditions (cultivation of cells at 37°C) where the phosphatidylinositol transfer-protein was inactive. Similar results were obtained after prelabelling cells with [^{3}H]inositol (Fig. 3). The increase of radioactivity was not due to a contamination of the plasma membrane with Golgi membranes, which accumulate under non-permissive conditions in the *sec 14* mutant. An antiserum raised against the KEX 2 proteinase, a Golgi-specific protein [26], reacted equally with plasma membranes of *sec 14* cells grown at 24 or 37°C (data not shown). Wild-type cells used as a control behaved at both 24°C or 37°C similar to the *sec 14* mutant strain cultivated under permissive conditions (see Figs. 2 and 3), indicating

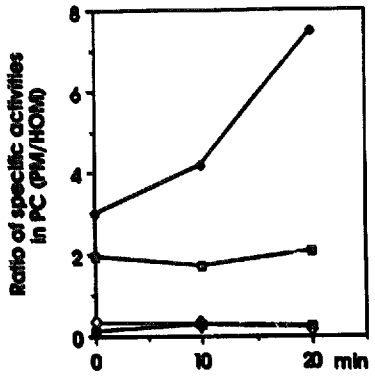

Fig. 2. Transport of phosphatidylcholine from internal membranes to the plasma membrane. *Saccharomyces cerevisiae sec 14* and the corresponding wild-type strain, *Saccharomyces cerevisiae* X-2180, were grown aerobically overnight at 24°C on YPD medium. Then, cells were washed and transferred to a choline-free medium [15]. Half of the culture was further incubated at 24°C, the other half was shifted to 37°C (non-permissive temperature) for 2 h. Then, pulse-labelling with [^{3}H]choline and the subsequent chase were carried out at the respective temperatures. Procedures for the isolation of the plasma membrane and lipid analysis are described in Materials and Methods. Specific activities (dpm phosphatidylcholine/mg protein) were calculated for the plasma membrane and for the homogenate. The ratio of these two specific activities (PM/HOM) at different time points was taken as a measure for the rate of transport of phosphatidylcholine to the plasma membrane. The homogenate contains approx. 60–70% membrane protein. ⊡, *sec 14*, 24°C; ◆, *sec 14*, 37°C; ■, wild-type, 24°C; ◇, wild-type, 37°C.

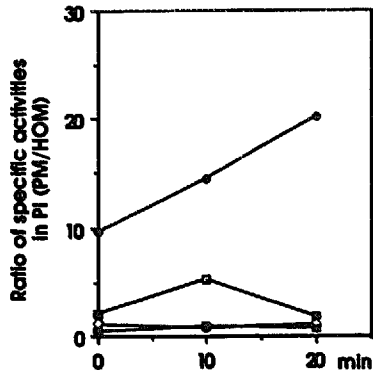

Fig. 3. Transport of phosphatidylinositol from internal membranes to the plasma membrane. Labelling of *Saccharomyces cerevisiae sec 14* with [^{3}H]inositol was carried out following the protocol outlined for Fig. 2, except that preincubation and labelling were carried out in an inositol-free medium. ⊡, *sec 14*, 24°C; ◆, *sec 14*, 37°C; ■, wild-type, 24°C; ◇, wild-type, 37°C.

that the increase of radioactivity in phosphatidylcholine and phosphatidylinositol in the plasma membrane of *sec 14* cells at 37°C is specific for the *sec 14* mutation. These results, however, provide convincing evidence that a functional phosphatidylinositol transfer protein is not required for the transfer of phosphatidylcholine or phosphatidylinositol to the plasma membrane.

In a second set of experiments, the fact was exploited that yeast possesses a phospholipase B which is associated with the plasma membrane and the periplasma [27,28]. This enzyme hydrolyzes phosphatidylinositol and phosphatidylcholine to fatty acids and the respective glycerophosphodiesters, glycerophosphoinositol or glycerophosphocholine, which are released into the growth medium. Since the total phospholipid content of the plasma membrane is constant, phosphatidylinositol and phosphatidylcholine serving as substrates for phospholipase B must be transported to the plasma membrane from internal membranes. Over a period of 6 h about 20–30% of radiolabel associated with cellular phospholipids is released into the medium. Since plasma membrane phospholipids comprise only about 7% of total cellular phospholipids, the release of radioactive glycerophosphoinositol or glycerophosphocholine into the culture medium can be taken to measure the transport of phosphatidylinositol and phosphatidylcholine to the plasma membrane. Data summarized in Figs. 4 and 5 demonstrate that the release of radioactive glycerophosphoinositol or glycerophosphocholine from *sec 14* mutant cells is increased rather than decreased at the non-permissive temperature of 37°C. In the case of glycerophosphoinositol (Fig. 4), the increase was identified as a temperature effect, which was also observed with wild-type cells at 37°C. In the case of glycerophosphocholine (Fig. 5), the increase was typical of the mutant strain. These data confirm

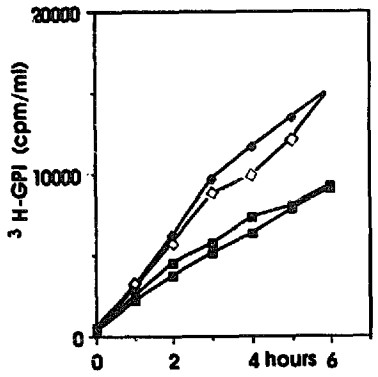

Fig. 4. Secretion of glycerophosphoinositol into the growth medium. Cells were labelled overnight at 24°C with [^{3}H]inositol as described in Materials and Methods. More than 95% of the label was associated with cellular lipids. After three careful washes, cells were transferred into a fresh unlabelled medium and incubated for further 6 h at the permissive (24°C) or restrictive (37°C) temperature. At time points indicated, aliquots of the culture were harvested and cells were sedimented by centrifugation. Radiolabelled glycerophosphoinositol (GPI) in the medium was separated from inositol and inositol phosphate by ion-exchange chromatography [20]. ⊡, *sec 14*, 24°C; ◆, *sec 14*, 37°C; ■, wild-type, 24°C; ◆, wild-type, 37°C.

the assumption that the phosphatidylinositol transfer protein is not required for the transportation of phosphatidylcholine or phosphatidylinositol to the plasma membrane. The increased release of [^{3}H]glycerophosphocholine from *sec 14* cells under non-permissive conditions was puzzling for several reasons. Firstly, it was more pronounced when cells were prelabelled with [^{3}H]choline than with [^{3}H]methionine (data not shown). In the first case, phosphatidylcholine synthesized via CDP-choline is labelled, whereas with [^{3}H]methionine phosphatidylcholine synthesized de novo by methylation of phosphatidylethanolamine becomes labelled. Obviously, the two pools of phosphatidylcholine are not equivalent with respect to production of phosphatidylcholine for phospholipase-B-catalyzed degradation. Secondly, this result deserves consideration in view of the finding of Cleves et al. [29] that several extragenic suppressor mutations of the *sec 14* defect were identified as mutations affecting steps of the CDP-choline pathway of phosphatidylcholine synthesis, namely choline kinase and choline phosphotransferase. For these reasons, we were tempted to speculate that preferential degradation of phosphatidylcholine coming through this route of synthesis might have something to do with the lack of phosphatidylcholine transfer activity in the *sec 14* mutant at the elevated temperature. It was disappointing to learn from experiments with other temperature-sensitive secretory mutants (*sec 53*, *sec 61*, *sec 18* and *sec 1*) that blocks at all stages of the secretory pathway gave essentially the same result (data not shown). The increased secretion of glycerophosphocholine at the non-permissive temperature seems to be a general effect associated with an impaired secretory pathway of proteins rather than a specific effect of phosphatidylinositol transfer protein deficiency.

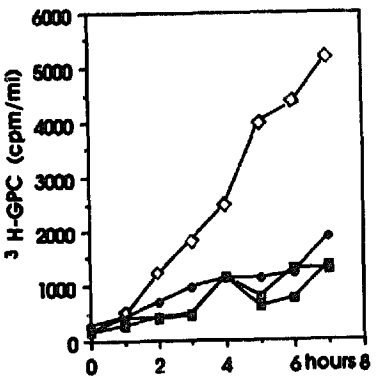

Fig. 5. Secretion of glycerophosphocholine into the growth medium. The incubation protocol of cells with [^{3}H]choline was the same as described for Fig. 4. Glycerophosphocholine was separated from choline and choline phosphate by thin-layer chromatography as described in Materials and Methods. ⊡, *sec 14*, 24°C; ◆, *sec 14*, 37°C; ■, wild-type, 24°C; ◆, wild-type, 37°C.

Nevertheless, data obtained with secretory mutants described above corroborated our previous finding based on experiments with *sec 18* and *sec 7* mutants [11], that secretory vesicles involved in protein transport do not contribute to the translocation of phosphatidylinositol and phosphatidylcholine from internal membranes to the plasma membrane. The fact that the phosphatidylinositol transfer protein is also not responsible for this process leaves us with the question which mechanism then applies to this transport pathway. The existence of phospholipid transport vesicles has been proposed [30], but lacks experimental support. Membrane contact and controlled fusion is still an alternative, but difficult to prove in vivo. Preferential utilization of newly synthesized phospholipids for interorganelle movement [23,31] point to specific topographical requirements which could be reconciled with the involvement of membrane collision-contact in phospholipid transport processes.

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