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Cell-free transfer of phospholipids between the endoplasmic reticulum and the Golgi apparatus of leek seedlings

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The transfer of lipids between the endoplasmic reticulum and the Golgi apparatus was investigated in vitro using a cell-free system from leek seedlings. Lipids of the donor membranes (endoplasmic reticulum) were radiolabeled either by incubating leek seedlings with [1-^{14}C]acetate or [^{3}H]acetate. Acceptor membranes (Golgi apparatus) were unlabeled and immobilized on nitrocellulose strips. The assay measured the lipid transfer resulting from both an ATP-independent process and an ATP- and cytosol-dependent process. A significant ATP- and cytosol-dependent lipid transfer was observed only in the case of the endoplasmic reticulum as donor and the Golgi apparatus as acceptor. Lipids transferred in an ATP-dependent manner were chiefly phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. The stimulation of lipid transfer by ATP as compared to the ATP-independent process was + 79% (PC), + 123% (PS) and + 69% (PE). On the other hand, PI was not transferred in an ATP-dependent manner (the stimulation by ATP was only 20%). This supports the theory that a sorting of phospholipids takes place in the donor membrane. Moreover, a formation of lysoPC was observed only in the presence of ATP (+330%). The ATP-dependent lipid transfer was inhibited by *N*-ethylmaleimide, indicating the involvement of cytosolic (but no phospholipid transfer proteins) or membrane proteins in the transfer process. The ATP-dependent transfer of lipids was also diminished at 12°C showing the sensitivity to low temperatures of the transfer of lipids between the endoplasmic reticulum and the Golgi apparatus.

Introduction

Leek seedlings are a useful model to investigate in vivo the transfer of intracellular membrane lipids to the plasma membrane. The intracellular transport of lipids and particularly of very long chain fatty acid (VLCFA)-containing lipids to the plasma membrane of leek cells and the vesicular nature of this transport were demonstrated [1–3]. Moreover, the transfer of VLCFA-containing lipids has been proven to follow the endoplasmic reticulum (ER) to Golgi apparatus to plasma membrane pathway [1–3]. In order to investigate the molecular mechanisms of the vesicular transfer of lipids at the level of the endoplasmic reticulum to Golgi apparatus, an in vitro approach that reconstitutes this vesicular transfer is required.

Investigations of membrane trafficking have been aided greatly by development of cell-free systems [4,5]. Rothman and colleagues [4] reconstituted transport of the protein G of the vesicular stomatitis virus in homogenates of chinese hamster ovary cells. Reconstitution of the transport of this protein between the endoplasmic reticulum and the Golgi apparatus was also achieved by using perforated cells [6]. The reconstitution of transport processes from the ER to the Golgi apparatus has also been achieved in *Saccharomyces cerevisiae* [5]. These cell-free systems have indicated requirements for various cytosolic or membranous factors involved in the vesicular transport of proteins between the ER and the Golgi apparatus or between the different regions of the Golgi apparatus [4,5].

Until recently, these cell-free systems were primarily used to study transfer of specific proteins based on a processing step rather than transport of lipids and membrane constituents in general. Only the transfer of glycosphingolipids within the Golgi apparatus was characterized in vitro in Chinese hamster ovary cells [7]. Morré et al. have developed another cell-free system from rat liver that reconstitutes the cell-free transfer of proteins between the ER and the Golgi apparatus [8,9] but also that of lipids [10–12]. This approach

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uses the donor membranes (ER) in solution and the acceptor (Golgi apparatus) fixed on nitrocellulose strips.

Using the latter cell-free system approach with the ER and the Golgi apparatus enriched fractions from leek seedlings, we have reconstituted an ATP-dependent transfer of lipids between the endoplasmic reticulum and the Golgi apparatus. This transfer was specific for the ER as donor and the Golgi apparatus as acceptor, as already found in spinach leaves [12]. In addition, we demonstrate that this ATP-dependent transfer of lipids was cytosol-dependent, sensitive to *N*-ethylmaleimide (NEM) and partially blocked at 12°C. The latter point is in agreement with in vivo studies where a partial block at 12°C between the ER and the Golgi apparatus was demonstrated (Moreau, P. et al., unpublished results). The sensitivity to low temperatures, the cytosol requirement and the sensitivity to NEM all favor involvement of a vesicular intermediate in the ATP-dependent transfer of phospholipids reconstituted in vitro.

Materials and Methods

Plant material. Leek seeds stored overnight at 4°C were sterilized with sodium hypochlorite in the presence of Triton X-100 for 5 min and then washed five times with distilled water. They were then grown for 7 days in the dark at 24°C on a growth medium already described [1–3,13].

Labeling and isolation of donor ER membranes. Ten batches of 20 seedlings were incubated each either with 10 μCi of [1-^{14}C]acetate (54 Ci/mol) or with 10 μCi of [^{3}H]acetate (5–6 Ci/mmol) for 120 min at 24°C. Leek seedlings were homogenized in a grinding buffer consisting of 50 mM Hepes, 10 mM KCl, 1 mM EDTA, 10 mM ascorbate, 0.1% BSA, 0.4 M sucrose, 5% PVP 40, 2 mM SHAM, 1 mM PMSF, 2.5 mM potassium pyrosulfite (pH 8.2). The homogenate was centrifuged at 1000 $\times g$ for 5 min. The supernatant was centrifuged for 10 min at 12000 $\times g$ and the resulting supernatant was loaded onto a discontinuous sucrose gradient consisting of 2 ml of 37% (w/v) sucrose and 4 ml of 21.5% (w/v) sucrose. After centrifugation at 80000 $\times g$ for 60 min, the donor ER membranes at the homogenate/21.5% sucrose interface were collected, diluted with buffer consisting of 30 mM Hepes, 2.5 mM magnesium acetate, 30 mM KCl (pH 6.8) and centrifuged at 100000 $\times g$ for 45 min. The pellet was resuspended in the appropriate volume of Hepes 30 mM, 2.5 mM magnesium acetate, 30 mM KCl, 10 mM β-mercaptoethanol (pH 6.8).

Isolation and immobilization of acceptor membranes on nitrocellulose strips. 300–400 unlabeled leek seedlings were homogenized as described above. Subfractionation procedures were identical with those for the donor ER membranes. The acceptor Golgi membranes were collected at the 21.5%/37% interface of the sucrose gradient, diluted with buffer consisting of 30 mM Hepes, 2.5 mM magnesium acetate, 30 mM KCl (pH 6.8) and centrifuged at 100000 $\times g$ for 45 min. The pellet was resuspended in Hepes 30 mM, 2.5 mM magnesium acetate, 30 mM KCl, 10 mM β-mercaptoethanol (pH 6.8) for immobilization of Golgi membranes on nitrocellulose strips (1 ml of acceptor membranes for 50 strips). Strips (1 cm $\times$ 1 cm) were cut from nitrocellulose sheets (Schleicher and Schuell BA-S 85), added to the resuspended acceptor Golgi membranes and incubated at 4°C for 15 min with continuous shaking. Two strips were removed, rinsed through three changes of cold Hepes buffer and kept for protein determination. The remaining strips were incubated with 5% BSA for 30 min to block sites on the nitrocellulose unoccupied by membranes. The strips, each loaded with 60 μg of Golgi membrane protein, were rinsed through three changes of cold Hepes buffer to remove excess of BSA and were ready to be used in the cell-free transfer assay.

Preparation of the cytosolic fraction. The homogenate of one hundred leek seedlings was first centrifuged as described above. The 12000 $\times g$ supernatant was then centrifuged at 150000 $\times g$ for 15 min in an ultracentrifuge Hitachi Himac CS100. The supernatant was strained through centrisart filters (SM 13249) and centrifuged for 30 min at 2000 $\times g$. Cytosolic proteins were then recovered and concentrated in the Hepes buffer at 16 mg/ml and stored at -20°C. The step using centrisart filters allowed to discard proteins having a molecular mass below 20 kDa and consequently was likely to eliminate phospholipid transfer proteins from the cytosolic fraction used for the lipid transfer assays. Various amounts up to 320 μg of cytosolic proteins were analysed by SDS-PAGE [14] and no proteins below 25 kDa were detected. The effect of the cytosolic fraction alone (in the absence of ATP) on the lipid transfer was checked and no stimulation was observed, suggesting the absence of any phospholipid transfer protein activity.

The cell-free transfer assay. The transfer medium in a total volume of 1 ml consisted of 250 μl of labeled donor ER membranes (700 μg protein), 50 μl of cytosol fraction (800 μg protein), 100 μl of ATP-ATP regenerating system (5 mM ATP, 3 mM UTP, 2 mM phosphocreatine and 10 U/ml creatine-phosphokinase) and 600 μl (for incubations in the presence of ATP) or 700 μl (for incubations in the absence of ATP) of Hepes buffer (pH 7.0). Incubations were in 4 ml glass vials at 24, 12 or 4°C (ice-bath temperature) with 4 strips per vial. Transfer assays were started by introducing the acceptor membranes immobilized on the strips into the incubation media. Following incubations for 5, 10, 15, 20, 30 or 60 min, the strips were

removed from the incubation media, rinsed through three changes of cold Hepes buffer and transferred to scintillation vials. Radioactivity transferred to the strips was determined by liquid scintillation counting in a Packard 2000 CA. For each experiment, transfer in the presence or in the absence of ATP at 4°C (on ice) was also measured and found to be the same and these background values were substracted from the other experimental values.

Protein determination. Proteins of donor ER membranes, acceptor Golgi membranes immobilized on nitrocellulose strips and of the cytosolic fraction were determined according to the BCA procedure [15] using BSA as standard.

Lipid extraction and analyses. Lipids transferred to the nitrocellulose strips were extracted by chloroform/methanol (2/1) for 2 min at room temperature, washed with distilled water three times and were then separated on HPTLC plates (Merck 60F254) eluted by methyl acetate/n-propanol/ chloroform/ methanol/ 0.25% aqueous KCl (25:25:28:10:7, v/v) according to Heape et al. [16].

Lipids from the donor ER membranes were extracted by chloroform/methanol (1:1, v/v) for 30 min at room temperature and then washed and analysed as indicated above.

Autoradiography was performed using Hyperfilms MP (Amersham).

Results

ATP-dependent and ATP-independent lipid transfers from endoplasmic reticulum to Golgi apparatus

Lipids were transferred in vitro from endoplasmic reticulum to Golgi apparatus immobilized on nitrocellulose strips. The cell-free transfer of lipids from endoplasmic reticulum to Golgi apparatus was time and ATP dependent (Fig. 1). The requirement for ATP, as previously found with rat liver [10,11] and spinach leaves [12], distinguished two types of lipid transfer which were also different kinetically. The ATP-dependent transfer increased with time up to 30 min and reached a plateau at 60 min. The other component of transfer was ATP-independent and linear with time (5–60 min). The most important information from the results presented in Fig. 1 was that the ATP-dependent transfer was saturable whereas the ATP-independent transfer was not.

Fig. 2 shows the ATP dependency for incubations of 10 min, 20 min and 30 min. First, we did not observe any stimulation by 40 μM ATP for a 10 min incubation but this could be due to a matter of sensitivity. Longer times were required to detect an effect of ATP at this concentration. For higher ATP concentrations (80–200 μM), a stimulation was observed at 10 min and increased quite linearly with time. With ATP concentrations of 500 μM and 1 mM, the efficiency of the stimulation by ATP was also evident and not significantly different from that obtained for ATP concentrations ranging from 80 to 200 μM. These results indi-

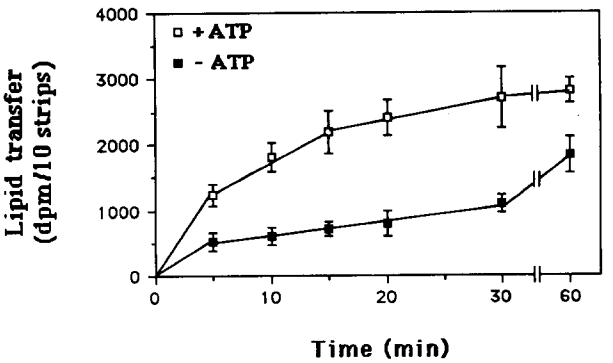

Fig. 1. ATP-independent and ATP-dependent cell-free lipid transfer between the endoplasmic reticulum and the Golgi apparatus of leek cells. The reticulum donor fraction was isolated from leek seedlings incubated 120 min with 200 μCi of [1-^{14}C]acetate at 24°C and the Golgi acceptor fraction was isolated from unlabeled seedlings and immobilized on nitrocellulose strips (each strip was loaded with 60 μg of Golgi membrane proteins) as explained in the experimental section. The transfer medium consisted of 250 μl of labeled donor ER membranes (700 μg of proteins), 50 μl of cytosol fraction (800 μg of proteins), 100 μl or not of ATP (500 μM final) and UTP (300 μM final) plus an ATP regenerating system made of 2 mM phosphocreatine and creatine-phosphokinase 10 U/ml, and Hepes buffer up to 1 ml (pH 7.0). Incubations were at 24°C and 4°C and 10 strips were used for each condition. Radiolabeled lipids transferred to the strips were quantitated by scintillation counting and counts obtained at 4°C were substracted from those obtained at 24°C. Values are means of five experiments ± S.D.

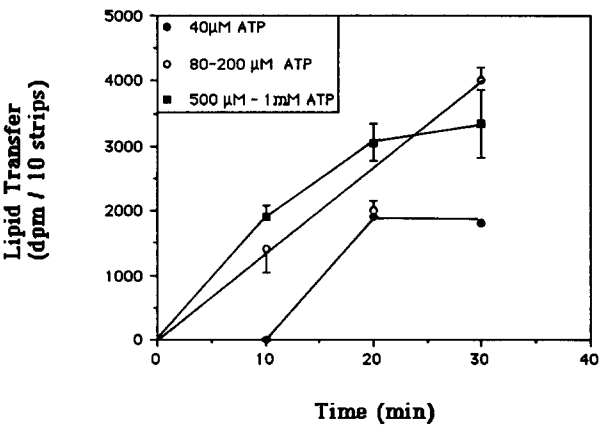

Fig. 2. ATP- and time-dependence of cell-free lipid transfer between the endoplasmic reticulum and the Golgi apparatus of leek cells. The experimental conditions were as in Fig. 1. ATP was added to the complete regenerating system to give different final concentrations of 40 μM, 80 μM, 200 μM, 500 μM and 1 mM. Incubations were for 10 min, 20 min and 30 min. Results represent the actual ATP-dependent lipid transfer calculated by the difference between transfer obtained in the presence of ATP and that obtained in its absence. Values are means of two experiments plus or minus mean average deviations.

cated that there was a threshold in the concentration of ATP under which no or a lower stimulation by ATP was observed. Moreover, whatever the ATP concentration from 80 μM to 1 mM, the same plateau was reached at 30 or 60 min, the stimulation could not be increased by an increase of the ATP concentration.

To avoid any limiting conditions due to the ATP concentration, the following experiments were with 500 μM ATP.

Characteristics of the ATP-dependent and ATP-independent lipid transfer

The experiments described above were carried out in the presence of cytosolic proteins, prepared as described in Materials and Methods, in order to avoid any cytosolic protein having a molecular mass below 20 kDa. This was required to avoid the presence of phospholipid transfer proteins in the cytosolic fraction. We have checked the effect of the cytosolic fraction on the efficiency of both the ATP-dependent and the ATP-independent transfer of lipids between the endoplasmic reticulum and the Golgi apparatus isolated from leek seedlings. Eliminating the cytosolic fraction from the incubation medium decreased the ATP-dependent transfer by 70%, whereas no effect was detected on the ATP-independent transfer. These results indicate a potential requirement of cytosolic proteins for the ATP-dependent transfer of lipids; these proteins do not correspond to classical phospholipid transfer proteins.

In a second step we have determined the donor and acceptor specificity of the cell-free transfer of lipids. We used either endoplasmic reticulum or Golgi apparatus as donor and both these membranes or BSA as acceptor in the cell-free assay and measured for each combination the ATP-dependent and ATP-independent components of the lipid transfer. The stimulation by ATP was observed only with endoplasmic reticulum as donor and the Golgi apparatus as acceptor. In contrast, the ATP-independent transfer was not acceptor specific.

After having shown the effect of cytosolic proteins and ATP on lipid transfer, we have studied the effect of *N*-ethylmaleimide (NEM) on the efficiency of both ATP-dependent and ATP-independent lipid transfer. The ATP-dependent lipid transfer from the endoplasmic reticulum to the Golgi apparatus was found to be inhibited by *N*-ethylmaleimide (Fig. 3). NEM concentrations of 5 and 10 mM were required to inhibit the ATP-dependent lipid transfer whereas NEM 1 mM was ineffective (Fig. 3). NEM has already been shown to block the cell-free vesicular transfer of proteins and lipids in various systems [4,7,10]. That the ATP-dependent transfer of lipids (Fig. 3) was inhibited by NEM strongly suggested that cytosolic, or membranous proteins are involved in this transfer. ATP-independent

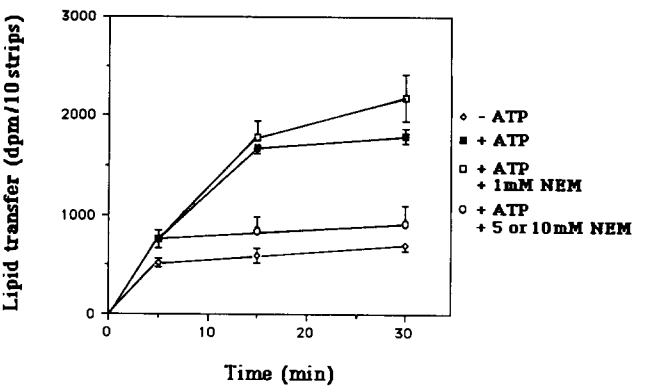

Fig. 3. *N*-Ethylmaleimide inhibition of cell-free lipid transfer between endoplasmic reticulum and Golgi apparatus of leek cells. The experimental conditions were as for Fig. 1, *N*-ethylmaleimide treatment was as follows. Donor membranes resuspended in Hepes buffer (without βmercaptoethanol), the cytosol fraction and the acceptor membranes immobilized on nitrocellulose strips were incubated for 10, 20 or 30 min in the presence or absence of ATP and an ATP-regenerating system and NEM (1 mM, 5 mM or 10 mM). No inhibition of the ATP-independent lipid transfer by NEM was observed. All other procedures were as already described. Values are means of three experiments ± S.D.

lipid transfer was not inhibited by NEM even at 10 mM.

The sensitivity of the two components of lipid transfer to temperature was investigated. At 12°C, the ATP-dependent transfer represented only 25% of that observed at 24°C (Fig. 4). In contrast, lowering the temperature had only a limited effect on the ATP-independent transfer of lipids. We had previously observed that at 12°C there was a total block of the vesicular transfer of VLCFA-lipids from the Golgi apparatus to the plasma membrane of leek cells in vivo, and a partial block of the endoplasmic reticulum to Golgi apparatus transfer step (Moreau, P. et al., unpublished results). The same could be true in vitro

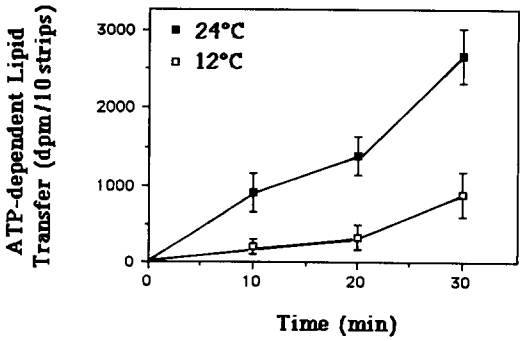

Fig. 4. Effect of temperature on the ATP-dependent lipid transfer between endoplasmic reticulum and Golgi apparatus of leek cells. Same experimental conditions as in Fig. 1 except that incubations were at 24°C or at 12°C. The ATP-dependent transfer was calculated as the difference between transfer obtained in the presence of ATP and that obtained in its absence. Results are mean values of four experiments ± S.D.

since we have also found a partial block of the endoplasmic reticulum to Golgi apparatus transfer (Fig. 4).

The donor and acceptor specificity, the cytosol requirement and the sensitivities to NEM and low temperature of the ATP-dependent lipid transfer strongly suggest that the nature of this transfer was vesicular. Moreover, we have found that the transfer stimulated by ATP was saturable after 30 min of incubation. A plausible explanation would be that the endoplasmic reticulum was unable to form transfer vesicles after a 30 min incubation. This hypothesis was investigated by the following experiment. Three successive sets of 4–10 strips (with immobilized Golgi acceptor membranes) were added for 20 min to the same reaction medium containing labeled endoplasmic reticulum as donor membranes. The ATP-dependent transfer of lipids, present for the two first sets of Golgi membranes acceptor strips was no longer observed with the third set of strips.

These results confirmed that only a limited amount of membrane lipids could be transferred via an ATP-dependent mechanism, suggesting that a limited amount of transfer vesicles could be produced by the endoplasmic reticulum donor membranes during the time of the experiments.

Analysis of the lipids transferred in the cell-free system

The composition of the lipids transferred from the endoplasmic reticulum donor membranes to the Golgi apparatus acceptor membranes (immobilized on nitrocellulose strips) was compared in the presence or absence of ATP plus ATP regenerating system as described above. The labeled lipids transferred to the strips were extracted by chloroform/methanol (2:1) for 2 min and then separated on HPTLC plates as described in the experimental section. The quantitative analyses of the lipids transferred to the Golgi apparatus acceptor membranes (shown in Table I) revealed that ATP stimulated particularly the transfer of the phospholipids PC (+79%), PE (+67%) and PS (+123%). On the other hand, the transfer of PI was not significantly stimulated by ATP (only 20%), indicating a differential transfer of phospholipids which occurred between the ER and the Golgi apparatus in vitro.

Moreover, the appearance of a great amount of lysoPC was observed in the presence of ATP (Table I). The 4-times increase of lysoPC in the presence of ATP needs further comments. We have calculated that lysoPC represented around 2.5% of the total labeled lipids of the ER donor membranes. For this calculation, we have taken into account the radioactivity associated with the origin of migration on HPTLC plates (see Fig. 5) and the amount of labeled neutral lipids which are not included in the Table I (no significant ATP-dependent transfer was observed for these lipids).

TABLE I

Effect of ATP on the transfer of various phospholipids from the ER to the Golgi apparatus

Experimental conditions were the same as those described in Fig. 1. Donor and acceptor membranes were incubated at 24°C for 30 min. Lipid analyses were performed as described in Materials and Methods. The first band observed (see Fig. 5) corresponding to the origin of migration is constituted of undetermined material (acyl-CoAs contribute to the radioactivity of this spot). The data presented correspond to the total dpm transferred to the Golgi per 10 strips in the absence and presence of ATP. The values corresponding to the ER donor membranes are also indicated. These data are from four independent experiments ± SD. LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.

Phospholipids	Golgi acceptor (dpm transferred per 10 strips)		ER donor (total dpm per assay)
	−ATP	+ATP	
LPC	90 ± 40	390 ± 80	2010 ± 220
PC	620 ± 120	1110 ± 180	24200 ± 5000
PS	130 ± 20	290 ± 50	2500 ± 450
PI	100 ± 20	120 ± 20	3100 ± 370
PE	490 ± 90	820 ± 130	11300 ± 1500

Moreover, the percentage of lysoPC was similar for the isolated ER membranes before or after any incubation and for the ER membranes incubated in the absence or the presence of ATP.

In the absence of ATP, the amount of lysoPC found on the Golgi acceptor membranes accounted for 4.3% of the total labeled lipids transferred to the strips. The transfer of lysoPC in the absence of ATP could cer-

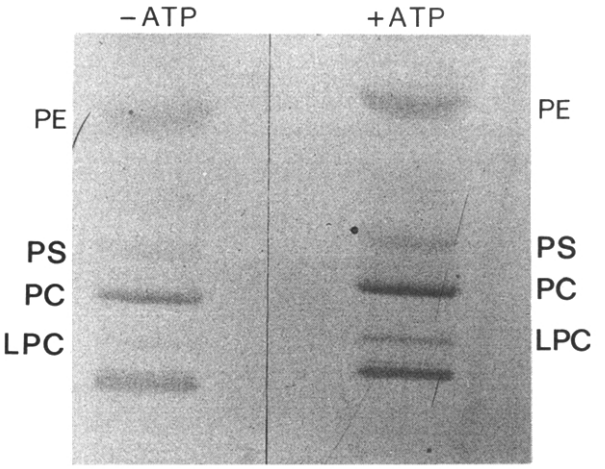

Fig. 5. HPTLC analysis of the lipids transferred to the Golgi acceptor membranes. Experimental conditions as described in Fig. 1. Donor and acceptor membranes were incubated at 24°C for 30 min. Lipid analyses were performed as described in Materials and Methods. The first band corresponds to the origin of migration and its nature has not been determined. LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine. The autoradiography shown is representative of two other analyses that gave similar results.

tainly be accounted for by the rapid exchange of this lysophospholipid between membranes. In the presence of ATP, we observed that the amount of lysoPC reached 10.6% of the total labeled lipids recovered from the Golgi acceptor membranes. The ATP stimulation of phospholipid transfer (for PC, PE and PS) had an average of + 90% whereas the ATP-stimulated increase of lysoPC reached +330%. This high increase strongly suggested that, in addition to the transfer of lysoPC observed in the absence of ATP, there was an ATP-dependent formation and/or transfer of lysoPC. Since there was no ATP-dependent formation of lysoPC in the ER membranes, we conclude that the ATP-dependent formation of labeled lysoPC from labeled PC could have occurred during the transfer and/or at the level of the Golgi acceptor membranes.

Discussion

It is generally admitted that protein transport from the endoplasmic reticulum to the Golgi apparatus proceeds through the budding of transition vesicles from the endoplasmic reticulum and their subsequent targeting to and fusion with the Golgi apparatus [5,17,18]. Various cell-free systems have reconstituted those steps in vitro [5,6,8–12]. In this paper, using a cell-free transfer assay directly adapted from those already developed from rat liver [8–11] and spinach leaves [12], we have studied the cell-free transfer of phospholipids from the endoplasmic reticulum to the Golgi apparatus isolated from leek cells. We have evidenced, as previously observed for rat liver [10,11] and spinach leaves [12], an ATP-independent and an ATP-dependent transfer of lipids. As for the cell-free assay described for spinach leaves [12], we have shown the donor and acceptor specificity of the ATP-dependent transfer of lipids between the endoplasmic reticulum and the Golgi apparatus isolated from leek cells. In addition, we have determined that this transfer is stimulated by a cytosolic fraction free of phospholipid transfer protein, is inhibited by NEM and is reduced significantly at low temperature (12°C). These characteristics, together with the saturability of the ATP-dependent transfer of lipids strongly favor a vesicular nature of the ATP-dependent process that was reconstituted in the cell-free assay. The effect of cytosol and the inhibition by NEM of the ATP-dependent transfer of lipids suggest that cytosolic and/or membrane proteins could be involved in the ATP-dependent process. The decrease of the ATP-dependent transfer of lipids at 12°C is in agreement with previous in vivo studies which showed a partial block of the transfer of lipids between the endoplasmic reticulum and the Golgi apparatus at 12°C (Moreau, P. et al, unpublished results). Additional arguments in support of the vesicular nature of the ATP-dependent process were obtained by using a membrane fraction partially enriched in putative endoplasmic reticulum-derived transfer vesicles as donor in our cell-free assay. This membrane fraction was prepared from endoplasmic reticulum membranes incubated with 500 μM ATP as described for rat liver [19]. We have found that, using this fraction as donor increased significantly the efficiency of transfer as compared with the endoplasmic reticulum as donor. This preliminary result is strongly in agreement with the vesicular nature of the ATP-dependent transfer process.

The identity of the proposed vesicular intermediate between the ER and the Golgi apparatus is now under active investigation by a morphological approach to prove the vesicular nature of the ATP-dependent transfer observed. The isolation of the putative transfer vesicles is under progress in our laboratory and the in vitro fusion of these vesicles with the Golgi apparatus will be investigated.

We have calculated that around 5% ± 1 of the labeled lipids of 1 mg of endoplasmic reticulum membranes were transferred in an ATP-dependent manner per mg of Golgi acceptor membranes. The fact that the ATP-dependent process is saturable in vitro is in agreement with a limited size of the pool of membrane lipids that are transferable by the ATP-dependent mechanism. A consequence of the saturability of the ATP-dependent process in vitro is that only a limited amount of transfer vesicles can be produced in vitro in our conditions. Hence, it is not surprising that the in vitro situation differs markedly from the in vivo situation where transition vesicles flow continuously from the endoplasmic reticulum.

The ATP-independent transfer component (of undetermined origin) was found to be kinetically different from the ATP-dependent transfer component since it was linear and continued even after a 60 min incubation period. Consequently, we assume that this pool of transferable lipids is not limiting as for the ATP-dependent transfer and originates from the total endoplasmic reticulum and not from a small-sized domain.

The HPTLC analyses of the lipids transferred to the Golgi apparatus acceptor membranes indicated ATP-dependent transfer of phospholipids PC, PE and PS. These phospholipids were found as major constituents of the endoplasmic reticulum-derived transition vesicles isolated from rat liver [19,20] and also were preferentially transferred in the cell-free assays described for rat liver [10,11] and spinach leaves [12]. Interestingly, PI transfer was low and not significantly stimulated by ATP as compared to PC, PE and PS. This result is one of the first evidences [10,12] of a differential transfer of phospholipids between the ER and the Golgi apparatus in vitro. More investigations will be required to determine whether our observations are related to sorting events of phospholipid molecules.

A characteristic of the transfer from the ER to the Golgi apparatus reconstituted in rat liver was the ATP-dependent formation of lysoPC from PC by an A-type phospholipase of the *cis* Golgi apparatus [11]. We have also observed the ATP-dependent formation of lysoPC in our experiments (Table I). However, there was probably both an ATP-dependent formation and an ATP-dependent transfer of lysoPC. Consequently, the origin(s) of lysoPC formation in the plant cell-free system studied here is not as clear as that determined in the rat liver cell-free system [11].

However, the fact that some labeled lysoPC is formed from part of the labeled PC transferred to the acceptor membranes, in both rat liver and leek seedlings, introduces the possibility that this conversion could play a role in the transfer process. This exciting hypothesis will have to be investigated carefully in both systems.

References

1 Moreau, P., Bertho, P., Juguelin, H. and Lessire, R. (1988) Plant Physiol. Biochem. 26, 173–178.
2 Bertho, P., Moreau, P., Juguelin, H., Gautier, M. and Cassagne, C. (1989) Biochim. Biophys. Acta 978, 91–96.
3 Bertho, P., Moreau, P., Morré, D.J. and Cassagne, C. (1991) Biochim. Biophys. Acta 1070, 127–134.
4 Rothman, J.E. and Orci, L. (1992) Nature 355, 409–415.
5 Pryer, N.K., Werestehube, L.J. and Scheckman, R. (1992) Annu. Rev. Biochem. 61, 471–516.
6 Balch, W.E., Wagner, K.R. and Keller, D.S. (1987) J. Cell. Biol. 104, 749–760.
7 Wattenberg, B.W. (1990) J. Cell. Biol. 111, 420–428.
8 Nowack, D.D., Morré, D.M., Paulik, M., Keenan, T.W. and Morré, D.J. (1987) Proc. Natl. Acad. Sci. USA 84, 6098–6102.
9 Paulik, M., Nowack, D.D. and Morré, D.J. (1988) J. Biol. Chem. 263, 17738–17748.
10 Moreau, P., Rodriguez, M., Cassagne, C., Morré, D.M. and Morré, D.J. (1991) J. Biol. Chem. 266, 4332–4328.
11 Moreau, P. and Morré, D.J. (1991) J. Biol. Chem. 266, 4329–4333.
12 Morré, D.J., Penel, C., Morré, D.M., Sandelius, A.S., Moreau, P. and Andersson, B. (1991) Protoplasma 160, 49–64.
13 Moreau, P., Juguelin, H., Lessire, R. and Cassagne, C. (1988) Phytochemistry 27, 1631–1638.
14 Laemmli, U.K. (1970) Nature 227, 680–685.
15 Smith, P.K., Krohn,R.I., Hermanson, G.T., Mallio, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klonk, D.J. (1985) Anal. Biochem. 150, 76–85.
16 Heape, M.A., Juguelin, H., Boiron, F. and Cassagne, C. (1985) J. Chromatogr. 322, 391–395.
17 Morré, D.J., Kartenbeck, J. and Francke, W.W. (1979) Biochim. Biophys. Acta 559, 71–152.
18 Pelham, H.R.B. (1991) Annu. Rev. Cell. Biol. 5, 1–23.
19 Moreau, P., Cassagne, C., Keenan, T.W. and Morré, D.J. (1993) Biochim. Biophys. Acta 1146, 9–16.
20 Moreau, P., Juguelin, H., Cassagne, C. and Morré, D.J. (1992) FEBS Lett. 310, 223–228.