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Molecular basis for low temperature compartment formation by transitional endoplasmic reticulum of rat liver

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The molecular basis for temperature compartment formation was investigated using a cell-free system from rat liver. The donor was from liver slices prelabeled with [^{3}H]acetate. Unlabeled Golgi apparatus membranes were immobilized on nitrocellulose as the acceptor. When transfer was determined as a function of temperature, a transition in transfer activity was observed at low temperatures (≤ 20°C) similar to that seen in vivo. The decrease in transfer efficiency correlated with a decrease in phosphatidylethanolamine and phosphatidylserine content of the transition vesicles formed. By adding lipid mixtures enriched in these lipids to the vesicles, their ability to fuse with the *cis* Golgi apparatus was reconstituted. These findings provide evidence for a role for lipids in low temperature compartment formation.

Membrane traffic; Low temperature compartment; Golgi apparatus; Phospholipid

1. INTRODUCTION

The bulk flow of lipids and proteins between the endoplasmic reticulum and the Golgi apparatus is mediated by 50–70 nm transition vesicles [1–3] that form from transitional elements of the endoplasmic reticulum [2] and fuse with membranes of the *cis* face of the Golgi apparatus. The formation and fusion of endoplasmic reticulum-derived transition vesicles have been reconstituted in cell-free systems from rat liver [4–6], cultured cells [7] and yeast [8]. Semi-intact cells permeable to macromolecules have also been employed [9,10].

Tartakoff [11] demonstrated that secretory proteins of the exocrine pancreas accumulated in a pre-Golgi apparatus compartment at 10°C, while at 22°C or above, these proteins progressed through the Golgi apparatus and into condensing vacuoles. Similar findings were obtained with hepatic cells [12], rat pancreatic cells [13], in rat liver slices and cultured hamster kidney cells [14].

In this report, we provide evidence for a low temperature pre-Golgi apparatus compartment the formation of which accompanies reduced cell-free transport in a cell-free system. A role for lipid sorting in the temperature regulation of trafficking of membranes between the endoplasmic reticulum and the Golgi apparatus is indicated.

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Abbreviations: HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

2. MATERIALS AND METHODS

2.1. *Materials*

Rats were 180 g males from Harlan Industries Inc. (Indianapolis, IN), provided with rat chow and drinking water ad libitum. Livers were removed and used for preparation of Golgi apparatus and transitional endoplasmic reticulum. Nitrocellulose was from S & S Scientific (Keene, NY). All chemicals were from Sigma Chemical Co. (St. Louis, MO).

2.2. *Preparation of Golgi apparatus (acceptor fraction)*

Golgi apparatus were isolated unlabeled from liver portions by sedimentation on 1.2 M sucrose [15]. The acceptor Golgi apparatus were then adsorbed on 1 cm^2 nitrocellulose strips. To do so, the Golgi apparatus were resuspended at a final concentration of 1–2 mg of protein per ml in 33 mM HEPES (pH 7.0) containing 2.5 mM magnesium acetate and 33 mM KCl (HEPES/Mg(OAc)$_2$/KCl). The nitrocellulose strips (10 strips per ml) were added to the Golgi apparatus suspension and incubated at 37°C for 1 h with continuous shaking. To block unoccupied sites, the strips then were transferred to HEPES/Mg(OAc)$_2$/KCl containing 5% bovine serum albumin and incubated for an additional 1 h at 4°C with shaking. The strips, each loaded with about 100 μg of Golgi apparatus protein, were then rinsed through four changes of HEPES/Mg(OAc)$_2$/KCl, blotted, and added to the reconstituted system, as described below.

2.3. *Isolation of the transition element fraction*

Transition elements were isolated from freshly prepared rat liver slices radiolabeled with [^{3}H]acetate. Slices were cut by hand with a razor blade and incubated for 30 min at 37°C in a total volume of 1 ml with 1 mCi of [^{3}H]acetate (Amersham Corp.). Then, 5–10 ml of phosphate-buffered saline buffer, pH 6.2, was added, and the liver slices were incubated for an additional hour at 37°C. The slices were collected on a Miracloth (Chicopee Mills, NY) filter and rinsed to remove unincorporated radioactivity. The washed slices were homogenized in 2 vols. of 37.5 mM Tris-maleate, pH 6.5, 0.5 M sucrose, 5

mM $MgCl_2$ and 1% dextran (average mol. wt. 225 kDa) for 45 s with a Polytron 20 ST operated at 6,000 rpm. The homogenates were centrifuged for 15 min at 6,000 × *g* and the pellets used as a source of Golgi apparatus as described [15]. The supernatant was diluted 1:5 with the homogenization medium and, following a second centrifugation at 10,000 × *g* to remove mitochondria, was layered onto a discontinuous sucrose gradient consisting of 2.0, 1.5 and 1.3 M sucrose layers. After centrifugation at 85,000 × *g* for 90 min, membranes collecting at the 1.3 M sucrose/sample interface were removed using a Pasteur pipette, pelleted by centrifugation for 20 min at 70,000 × *g* and resuspended as the donor fraction.

2.4. *Reconstituted membrane transfer*

Incubations were in 8-ml glass shell vials. The reconstituted cell-free system (1 ml vol.) contained 250 μl of resuspended radiolabeled transition element membrane (3 mg/ml, ca. 100,000 dpm), 250 μl of cytosol, 250 μl of an ATP-regenerating system and 250 μl of HEPES/$Mg(OAc)_2$/KCl, pH 7.0. The cytosol fraction was the > 10 kDa M_r fraction prepared by filtration of a microsome-free supernatant (90,000 × *g* for 60 min) of rat liver through a Centricon YM filter (Amicon). The strips were arranged vertically in the vial in the form of a triangle around the circumference. All solutions were maintained at 4°C until initiation of the reaction by addition of ATP and transfer to 37°C. At the end of the incubation, the strips were rinsed through four changes of HEPES/$Mg(OAc)_2$/KCl, blotted on Whatman No. 1 filter paper, dried and radioactivity determined. All values were corrected for non-specific absorption of radioactivity at $t = 0$ (ca. 0–25 cpm/min/strip). These control strips, which were loaded with acceptor, were handled in exactly the same way as the experimental strips: they were immersed in the donor mixture for several seconds but were not incubated.

2.5. *Isolation of transition vesicles*

For isolation of transition vesicles, the donor fractions were incubated for 1 h in the absence of acceptor strips at 37°C to induce the formation of transition vesicles. Transition vesicle-enriched fractions were obtained by preparative free-flow electrophoresis [6] using a VAP-22 continuous free-flow electrophoresis unit (Bender and Hobein, Munich, Germany). Absorbance was measured at 280 nm and appropriate peak fractions [6] were pooled and concentrated by centrifugation at 85,000 × *g* for 30 min. Proteins were estimated by the BCA [16] procedure.

2.6. *Lipid reconstitution*

For lipid reconstitution experiments, phospholipids at a final total concentration of 50 μM were added to resuspended transition vesicles in HEPES/$Mg(OAc)_2$/KCl and sonicated for 30 s using a Branson Model B-22-4 bath sonifier. The phospholipids used were PC (dioleoylphosphatidylcholine, 98% pure, PE (dioleoylphosphatidylethanolamine, 99% pure or dipalmitoylphosphatidylethanolamine, 99% pure), PS (phosphatidylserine, mixture from bovine brain, 98% pure), PI (phosphatidylinositol, mixture from soybean, 98% pure) and PA (dioleoylphosphatidic acid, 98% pure). All were from Sigma (St. Louis, MO).

2.7. *Analysis of lipids*

Lipids from the various membranes were extracted by $CHCl_3$/CH_3OH (1/1) at 60–70°C for 20 min. The lipid extracts were washed and then analyzed by thin layer chromatography according to Heape et al. [17].

2.8. *Electron microscopy*

Transition vesicle fractions were prepared for electron microscopy by fixation in 2.5% glutaraldehyde in 0.1 M sodium phosphate (pH 7.2), followed by post-fixation in osmium tetroxide in the same buffer. Dehydration was through an acetone series with embedment in Epon. Thin sections were observed and photographed using a Philips EM 200 electron microscope. Diameters of all vesicles were measured. Only those vesicles with diameters in the size range 50–70 nm were scored as transition vesicles, and composition was expressed as a percentage of total vesicles. We have shown previously that the only vesicles formed in response to ATP + cytosol were in the 50–70 nm size range [18].

3. RESULTS

3.1. *Response of ATP-dependent transfer to temperature*

At temperatures of 20°C or above, transfer from [^{3}H]acetate-labeled donor to unlabeled Golgi apparatus acceptor immobilized on nitrocellulose exhibited an ATP + cytosol-dependent component (Fig. 1). The magnitude of the ATP + cytosol-dependent transfer was proportional to time of incubation for at least 60 min (Fig. 1, inset). However, at incubation temperatures below 20°C, little or no net ATP-dependent transfer of [^{3}H]acetate-labeled membrane constituents occurred. The transition began at about 18°C and was proportional to temperature at temperatures above 18°C. Re-

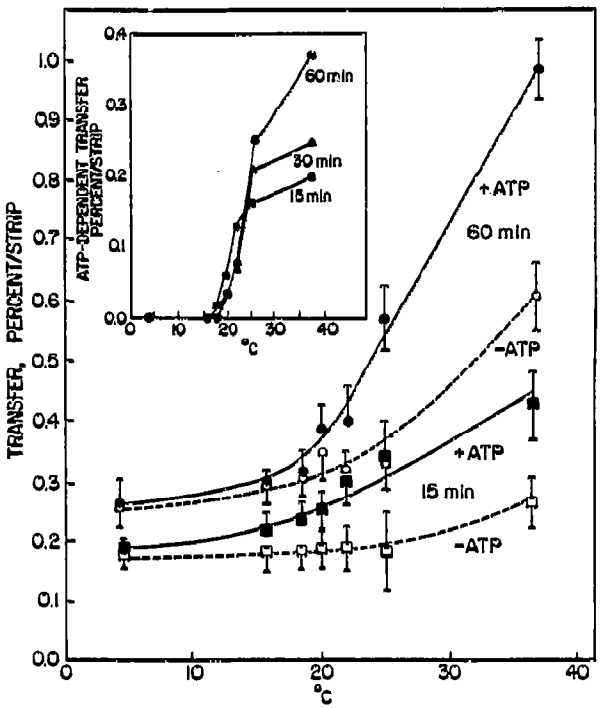

Fig. 1. Cell-free transfer of lipids after 15 (squares) and 60 (circles) min between transitional endoplasmic reticulum and Golgi apparatus in the presence (solid symbols and lines) and absence (open symbols, dashed lines) of ATP. The incubation medium (total volume 1 ml) contained 250 μl of donor membranes (2 mg/ml) prepared from rat liver slices incubated for 60 min with [^{3}H]acetate as described [13]. Golgi apparatus membranes (~ 160 μg of proteins) were absorbed to nitrocellulose strips as the acceptor. No ATP, or ATP at a final concentration of 80 μM, and liver cytosol (250 μl of a > 10 kDa fraction, 0.5 mg protein) were added. The inset shows the ATP-dependent transfer for each temperature (transfer in the absence of ATP was subtracted from transfer with ATP) after 15, 30 and 60 min of incubation. Results are means of 4–10 independent experiments. ATP-dependent cell-free transfer of lipids between labeled transitional endoplasmic reticulum membranes and Golgi apparatus occurred only at temperatures above 20°C.

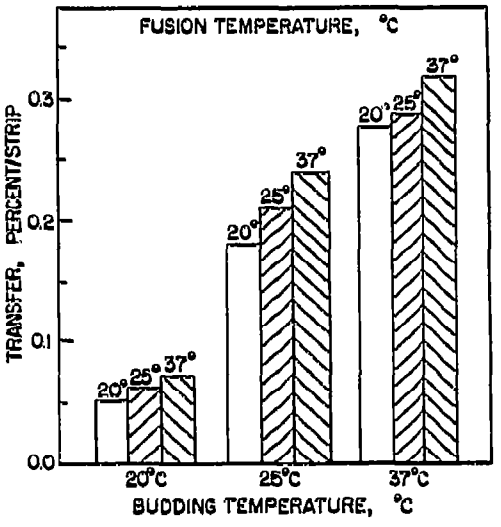

Fig. 2. The low temperature block is on the fusion step with Golgi apparatus rather than on the budding of transition vesicles from transitional endoplasmic reticulum (TER). Radiolabeled TER membranes were first incubated for 15 min in the presence of ATP (80 μM) and cytosol at the different temperatures (20, 25 and 37°C). The TER membranes were removed by centrifugation (Eppendorf 5412 centrifuge, 5 min at 10,000 rpm). Supernatants corresponding to each temperature were incubated at 20, 25 or 37°C with Golgi apparatus acceptor membranes on nitrocellulose strips for 30 min. Radioactivity associated with the strips was then determined. Results were means of two experiments with consistent results. As determined from electron microscopy and estimates from protein content, the number of transition vesicles present in the supernatant at the end of the first incubation was hardly affected by temperature (Table I), such that the temperature block was on attachment rather than formation. In the absence of ATP, the transfers in percentage strip were 0.24 ± 0.07 at 37°C, 17 ± 0.03 at 25°C and equivalent to plus ATP, i.e. 0.5 ± 0.1 at 20°C in this experiment.

sults are expressed as percentage transfer, which is the ratio of radioactivity transferred per cm^2 of nitrocellulose to the total amount of donor radioactivity present in the vial for each transfer assay × 100.

3.2. *Basis for the low temperature block*

To determine if budding, transfer, or both, were affected by the low temperatures, radiolabeled transitional endoplasmic reticulum membranes were incubated at 20, 25 and 37°C in the presence of ATP and cytosol for 15 min to promote vesicle formation. These experiments were with [^{3}H]acetate-labeled donor membranes. After 30 s on ice, the bulk of the transitional endoplasmic reticulum was removed by centrifugation. The resultant three supernatants, containing the transition vesicles and corresponding to each of the three temperatures, were incubated at 20, 25 or 37°C with Golgi apparatus acceptor membranes. After 30 min, the radioactivity of the acceptor strips was determined in order to measure transfer (Fig. 2). With budding at 20°C or lower, little or no transfer occurred in the subsequent incubation, even at 25°C or 37°C (Fig. 2). In

Table I

Quantitation of cell-free transition vesicle formation at 20, 25 and 37°C in the experiments of Fig. 2

Temperature (°C)	Total protein (μg)	Transition vesicles (%)	Transition vesicle protein (μg)
20	224 ± 10	14 ± 2	31
25	232 ± 24	15 ± 5	35
37	262 ± 20	14 ± 4	37

Results are averages of three determinations ± S.D.

contrast, with budding at 25°C or 37°C, subsequent transfer showed only a modest response to temperature and no evidence for a sharp transition between 20°C and 25°C. Thus, the lack of transfer at 20°C was due either to reduced production of vesicles, a decreased ability of the vesicles produced to fuse with the Golgi apparatus, or both.

A relatively normal rate of vesicle production at temperatures of 20°C or below was indicated from measurements of the quantities of transition vesicles produced at 20°C and 25°C. Both from measurements of total protein and from electron microscopy, the quantity of transition vesicles produced at 20°C was on average 89% of the quantity produced at 25°C (Table I). Thus the reduced efficiency of transfer observed with the cell-free system at 20°C could not be adequately explained by a reduction in the production of vesicles at 20°C, raising the possibility that the transition vesicles produced were fusion incompetent.

To determine if the transition vesicles formed at 20°C in the cell-free system might be fusion incompetent, transition vesicles were produced from radiolabeled transitional endoplasmic reticulum either at 20 or 37°C in the presence of ATP and cytosol and then purified by preparative free-flow electrophoresis and used as the donor in the cell-free system. Equivalent amounts of vesicles were incubated at 20 or 37°C with Golgi acceptor membranes fixed on nitrocellulose strips. With transition vesicles formed at 20°C, the percentage transfer under these conditions was reduced to 0.35 of that with transition vesicles formed at 37°C.

3.3. *Lipid composition of fusion-incompetent and -competent transition vesicles*

A biochemical basis for the inability of transition vesicles formed at 20°C to function in cell-free transfer was sought from the phospholipid composition of the transition vesicles formed at the low temperature compared to those formed at higher temperatures. The phospholipid composition (as a % of the total phospholipids) at the three temperatures (16–20°C, 25°C and 37°C) showed no significant differences from the starting transitional endoplasmic reticulum (Table II). Transition vesicles formed at 37°C were characterized by a high content of PE and were enriched in PS. The content

Table II

Phospholipid distribution as a percentage of the total in transitional endoplasmic reticulum (TER) membranes and of transition vesicles formed from TER at three different temperatures

Phospholipids	Fraction of total phospholipids (%)				
	Transition vesicles			Endoplasmic reticulum	
	37°C	25°C	16–20°C	Transitional	Rough
PC	71.4 ± 2.924[a]	75.3 ± 4.224[a]	74.6 ± 2.1[a]	62.5 ± 4.6[b]	61.5 ± 3.0[b]
PS	7.2 ± 1.6[c]	4.0	3.5 ± 2.9[d]	3.3 ± 0.9[d]	3.8 ± 0.6[d]
PI	5.4 ± 0.1[c]	3.8	10.7 ± 2.0[e]	11.6 ± 0.4[e]	7.7 ± 2.4[e]
PE	16.0 ± 3.0[f]	16.9 ± 1.4[f]	11.2 ± 1.6[e]	22.5 ± 3.9[f]	26.9 ± 2.5[f]

TER membranes were prepared, transition vesicles were induced to form [5] and the vesicles were concentrated by preparative free-flow electrophoresis [6]. Lipid analyses were according to Heape et al. [17]. Data are means ± S.D. of 3 different determinations and compared to conventional rough endoplasmic reticulum. The lipid composition of the TER was unaffected by temperature, and average values combining the three temperatures are reported. Values not followed by the same letter are significantly different ($P < 0.06$).

of PI was reduced correspondingly compared to transitional endoplasmic reticulum.

With transition vesicles produced at 16–20°C, a different phospholipid composition was observed compared to that of vesicles produced at 37°C and 25°C. Specifically, there was a decrease in the contents of PE and PS and a corresponding increase in the content of PI (Table II).

3.4. *Lipid reconstitution*

Reconstitution experiments were employed to determine if the reduced PE and PS contents of the transition vesicles formed at low temperature were related to the inability of those vesicles to combine with *cis* Golgi apparatus membranes. The fusion efficiency of transition vesicles produced at 20°C was restored by altering the phospholipid content through the addition of exogenous PE and PS. Various mixtures of phospholipids in the cell-free system (solubilized by sonication in the same buffer used in the transfer experiments) were compared (Table III). Restoration of transfer was obtained from PC/PE and PC/PE/PS mixtures, and to a lesser extent with a PC/PS mixture (Table III). Adding PC/PI or PC/PA, inhibited rather than activated, ATP-dependent transfer. For PC/PE and PC/PE/PS mixtures added to fusion-incompetent transition vesicles formed at 20°C, transfer was restored to 60–80% of that obtained at 25°C, a temperature where the low temperature block was no longer operative (Fig. 1) and to a level equal to or greater than that predicted for 20°C, from the Arrhenius equation, at which there was no low temperature-specific block (Table II, Fig. 3).

In the reconstitution experiments of Table III and Fig. 3, the source of PE was dioleoylphosphatidylethanolamine. This phospholipid exhibits a lamellar (L)-to-hexagonal (H_{II}) phase transition temperature of about 10°C with water in excess [19]. On the other hand, when dipalmitoylphosphatidylethanolamine, which has much higher L_β–L_α and L_α–H_{II} phase transition temperatures, the PE was no longer able to restore transfer competency to vesicles formed at 20°C (data not shown).

4. DISCUSSION

The results reported here, for transfer of lipids as a function of temperature, duplicate in the cell-free system the reduced ability of the vesicles produced at low temperatures to fuse with the Golgi apparatus [14]. ATP-dependent transfer occurred only at temperatures above 18°C. The strict temperature dependency of the transfer process also points to the specificity of the cell-free transfer of membrane from transitional endoplasmic reticulum to the *cis* Golgi apparatus. The temperature dependency of transfer among successive Golgi apparatus compartments in a cell-free system reconstituted from VSV-infected Chinese hamster ovary cells showed no such marked transition [20]. Therefore, the cell-free system described here afforded an opportunity to investigate the molecular basis for the low temperature block.

The formation at 16°C of a compartment between the endoplasmic reticulum and the Golgi apparatus has been reported for cultured cells undergoing viral replication [21–24]. Reduced temperature has been shown to also affect post-transitional processing and secretion in tissues. Tartakoff [11] demonstrated that secretory proteins of the exocrine pancreas accumulated in pre-Golgi apparatus vesicles at 10°C, while at temperatures of 22°C or greater the proteins progressed through the Golgi apparatus and into condensing vacuoles. With rat hepatocytes, secretory proteins were blocked in a pre-Golgi apparatus compartment at 18°C but not at 20°C [12]. This was similar to rat pancreas cells where at 16°C most of the labeled secretory proteins remained in the endoplasmic reticulum, while at 20°C the medial Golgi apparatus region was reached [13]. Both in vitro and in vivo, the vesicle migration was blocked completely at temperatures of 16°C and lower [21,24].

Table III

Restoration of transfer capability of vesicles formed at 20°C by different lipid mixtures

Lipid addition	Temperature (°C)	ATP	dpm	% transfer	ATP-dependent transfer
No addition	20	−	420 ± 24	0.50 ± 0.03	
		+	441 ± 33	0.52 ± 0.04	0.02
PC/PE 50 μM (1/4)		−	411 ± 8	0.49 ± 0.01	
		+	546 ± 34	0.65 ± 0.04	0.16
PC/PS 50 μM (1/4)		−	444 ± 37	0.53 ± 0.05	
		+	516 ± 34	0.61 ± 0.04	0.08
PC/PS/PE 50 μM (1/2/2)		−	456 ± 25	0.54 ± 0.03	
		+	594 ± 29	0.71 ± 0.03	0.17
PC/PI 50 μM (1/4)		+	387 ± 29	0.45 ± 0.03	
PC/PA 50 μM (1/4)		+	390 ± 27	0.46 ± 0.03	
No addition	25	−	450 ± 21	0.54 ± 0.03	
		+	633 ± 37	0.75 ± 0.05	0.21
	37	−	489 ± 20	0.58 ± 0.02	
		+	726 ± 38	0.86 ± 0.05	0.28

Lipids were prepared as vesicles sonicated in the same buffer used to resuspend the membranes. Incubations were for 30 min. Results are averages ± S.D. from 3 independent experiments.

With slices of rat liver, incubation at 18°C and below, resulted in the accumulation of transition vesicles [14]. Except for a slightly larger diameter, the transition vesicles that accumulated at low temperature in the liver slices appeared normal [14]. The transitional endoplasmic reticulum of the Golgi apparatus region apparently was able to form vesicles at 16–18°C, but at least some of the vesicles did not fuse, either with the *cis* Golgi apparatus or with each other, to form an intermediate compartment. A similar low temperature block was observed at 16°C for the green alga, *Micrasterias americana* [25]. Similarly, in a cell-free system to reconstitute endoplasmic reticulum to Golgi apparatus transfer, ATP-dependent transfer was not observed at temperatures of 16°C and below [23].

Whereas known recognition and attachment signals of membrane trafficking are proteins or glycoproteins, membrane fusions have been suggested to be controlled, at least in part, by phospholipids [26,27]. In liposomes and artificial membrane models, non-lamellar phase-forming lipids promoted fusion [26,27]. PE and PS were among the most efficient phospholipids to trigger the lamellar–non-lamellar (H_{II}) phase transition. Proteoliposomes containing cytochrome *c* oxidase, cardiolipin and varying proportions of PC and PE fused only when PE contents exceeded 10% [28]. Since lipid membranes lacking proteins responded similarly, the implication was that fusion required only sufficient proportions of PE and/or PS [26,27]. Indeed, ATP-dependent fusion of liposomes with Golgi apparatus has been reported for permeabilized cells [29].

Both the PE and the PS contents of the transition vesicles formed at 16–20°C were reduced and the content of PI was increased compared to transition vesicles formed at 37°C. Thus the altered composition of the phospholipids might be sufficient to prevent productive interaction with the *cis* Golgi apparatus of the transition vesicles formed at reduced temperatures.

The transition vesicles labeled with radioactive phospholipids, chiefly PC coming from endoplasmic reticulum, have been reported to fuse with the *cis* Golgi apparatus in the cell-free system used here [30]. Evidence for fusions came from studies where radiolabeled PC transported by the vesicles became accessible to phospholipase A activity found within the *cis* Golgi apparatus cisternae. Phospholipase processing occurred

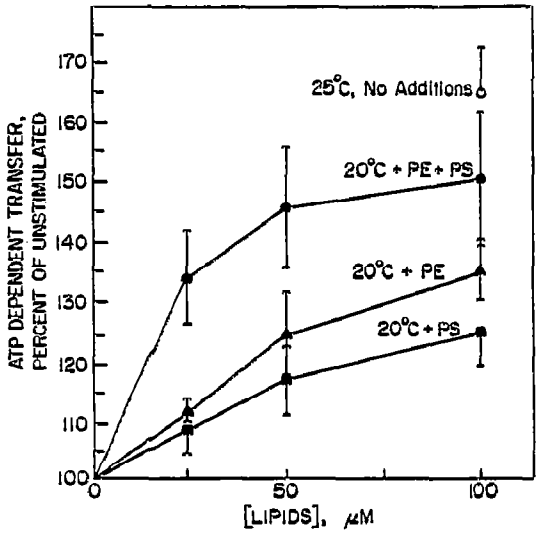

Fig. 3. Lipid mixtures restore the cell-free transfer capacity of vesicles formed from transitional endoplasmic reticulum at 20°C. Experimental conditions were those described for Table III. Lipids were added in the ratios 1/4 for PC/PS and PC/PE, and 1/2/2 for PC/PE/PS. Comparisons were based on the amount of transfer obtained at 20°C in the presence of ATP as equal to 100.

only with transfers carried out above the transition temperature for vesicle fusion at 20°C and in the presence of ATP [30]. Phospholipase processing was specific for fractions derived from the *cis* Golgi apparatus and was not given by Golgi apparatus subfractions enriched in *trans* markers [30].

That the transition vesicles formed were not simply fragments of the parent transitional endoplasmic reticulum has been proved by more detailed lipid analyses. Both triglycerides and ceramides were present in the transitional endoplasmic reticulum but both triglycerides [30] and ceramides (P. Moreau, C. Cassagne, T.W. Keenan and D.J. Morré, unpublished results) were reduced or absent from transition vesicle-enriched fractions. If the transfer experiments were carried out with conventional rough endoplasmic reticulum, no vesicles were formed and no ATP-dependent transfer was observed [5].

The totality of the findings support the concept of a role for PE and/or PS as a facilitator of attachment and/or fusion of transition vesicles from endoplasmic reticulum with *cis* Golgi apparatus membranes, possibly by lowering the required activation energy. The relative amounts of PE and PS may become rate limiting to fusion as the temperature is lowered, and thereby serve to determine the temperature where the low temperature block is exerted in a given cell type or cell-free system. A decrease of PC and PI (lipids stabilizing bilayers) and an increase of PE and PA have been reported, as well, to precede myoblast fusion [32].

There are few reports of a functional consequence of phospholipid sorting in endomembrane trafficking. Previously, van Meer and colleagues [33] suggested a role for glycosphingolipids in protein sorting at the *trans* Golgi apparatus. The putative temperature dependence of PE and PS transfer to membranes of transition vesicles, and reduced transfer at 20°C and below, may explain the basis for low temperature compartment formation, and would support the concept of a critical role for these lipids in membrane fusion. Equally important, the findings document that transition vesicles formed in a cell-free system from transitional endoplasmic reticulum, duplicate, in a remarkable way, the properties of their counterparts produced in vivo as deduced from electron microscopy.

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REFERENCES

[1] Zeigel, R.F. and Dalton, A.J. (1962) J. Cell Biol. 15, 45–54.
[2] Morré, D.J., Mollenhauer, H.H. and Bracker, C.E. (1971) in: Results and Problems in Cell Differentiation: Origin and Continuity of Cell Organelles, vol. 2 (Reinert, J. and Ursprung, H. eds.) pp. 82–126, Springer-Verlag, Berlin.
[3] Morré, D.J., Kartenbeck, J. and Franke, W.W. (1979) Biochim. Biophys. Acta 559, 71–152.
[4] Morré, D.J., Paulik, M. and Nowack, D. (1986) Protoplasma 132, 110–113.
[5] Nowack, D.D., Morré, D.M., Paulik, M., Keenan, T.W. and Morré, D.J. (1987) Proc. Natl. Acad. Sci. USA 84, 6098–6102.
[6] Paulik, M., Nowack, D.D. and Morré, D.J. (1988) J. Biol. Chem. 263, 17738–17748.
[7] Balch, W.E., Wagner, K.R. and Keller, D.S. (1987) J. Cell Biol. 104, 749–760.
[8] Haselbeck, A. and Shekman, R. (1986) Proc. Natl. Acad. Sci. USA 83, 2017–2021.
[9] Beckjers, C.J., Keller, D.S. and Balch, W.E. (1987) Cell 50, 523–534.
[10] Simons, K. and Virta, H. (1987) EMBO J. 6, 2241–2247.
[11] Tartakoff, A.M. (1986) EMBO J. 5, 1477–1482.
[12] Fries, E. and Lindstrom, E. (1986) Biochem. J. 237, 33–39.
[13] Saraste, J., Palade, G.E. and Farquhar, M.G. (1986) Proc. Natl. Acad. Sci. USA 83, 6425–6429.
[14] Morré, D.J., Minnifield, N. and Paulik, M. (1989) Bio. Cell (Paris) 67, 51–60.
[15] Morré, D.J., Cheetham, R.D., Nyquist, S.E. and Ovtracht, L. (1972) Prep. Biochem. 2, 61–69.
[16] Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallaik, A.K., Gartner, F.H., Porvenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, F.J. and Klenk, O.C. (1985) Anal. Biochem. 100, 76–85.
[17] Heape, M.A., Juguelin, H., Boiron, F. and Cassagne, C. (1985) J. Chromatogr. 332, 391–395.
[18] Nowack, D.D., Paulik, M., Morré, D.J. and Morré, D.M. (1990) Biochim. Biophys. Acta 1051, 250–258.
[19] Tilock, C.P.S. and Cullis, P.R. (1982) Biochim. Biophys. Acta 684, 212–218.
[20] Balch, W.E. and Rothman, J.E. (1985) Arch. Biochim. Biophys. 240, 413–425.
[21] Saraste, J. and Kuismanen, E. (1984) Cell 38, 535–549.
[22] Holmes, K.V., Doller, E.W. and Struman, L.S. (1981) Virology 115, 334–344.
[23] Tooze, J., Tooze, S.A. and Warren, G. (1984) Eur. J. Cell Biol. 33, 291–293.
[24] Tooze, S., Tooze, J. and Warren, G. (1988) J. Cell Biol. 106, 1475–1487.
[25] Noguchi, T. and Morré, D.J. (1991) Protoplasma 162, 128–139.
[26] Seddon, J.M. (1990) Biochim. Biophys. Acta 1031, 1–69.
[27] Chernomordik, L.V., Melikyan, G.B. and Chizmadzhev, Y.A. (1987) Biochim. Biophys. Acta 906, 309–352.
[28] Gad, A.E., Broza, R. and Cullis, P.R. (1982) Biochim. Biophys. Acta 556, 181–195.
[29] Kobayashi, T. and Pagano, R.E. (1988) Cell 55, 797–805.
[30] Moreau, P. and Morré, D.J. (1991) J. Biol. Chem. 266, 4329–4333.
[31] Moreau, P., Rodriguez, M., Cassagne, C., Morré, D.M. and Morré, D.J. (1991) J. Biol. Chem. 266, 4322–4328.
[32] Santini, M.T., Indovina, P.L., Cantafora, A. and Blotta, I. (1990) Biochim. Biophys. Acta 1023, 298–304.
[33] van Meer, G. (1989) Annu. Rev. Cell Biol. 5, 247–275.