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Effect of low temperatures on the transfer of phospholipids with various acyl-chain lengths to the plasma membrane of leek cells

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Abstract

The intracellular transport of lipids with very-long-chain acyl moieties (C_{20}–C_{24} fatty acid-containing lipids) to the plasma membrane of leek cells follows the vesicular ER-Golgi apparatus-plasma membrane pathway. Here we report on the effect of low temperatures on the vesicular transport of lipids and especially C_{20}–C_{24} fatty acid-containing lipids to the plasma membrane of leek cells. These lipids, normally transported through the ER-Golgi apparatus-plasma membrane pathway at 24°C, accumulated in the ER and the Golgi apparatus at 12°C with a related deficit in the plasma membrane. As lipids with long chain acyl moieties (C_{16} and C_{18} fatty acid-containing lipids) were still transferred to the plasma membrane at 12°C, the data demonstrate a specific effect of low temperatures on the vesicular transfer of C_{20}–C_{24} fatty acid-containing lipids to the plasma membrane of leek cells. Therefore, evidence is provided for distinct pathways and/or mechanisms transferring lipids to the plasma membrane of leek cells which differ in their sensitivity to low temperatures.

Key words: Phospholipid transfer; Very-long-chain fatty acid; Plasma membrane; Lipid accumulation

1. Introduction

Low temperatures affect drastically the vesicular transport of proteins along the ER-Golgi apparatus-plasma membrane pathway in homeotherms [1–4]. Temperatures between 10°C and 16°C block the vesicular transport at the ER-to-*cis*-Golgi apparatus step and temperatures around 20°C stop the transport of proteins beyond the medial and *trans* cisternae of the Golgi apparatus [1–4].

Transport in poikilotherms seems also to be reduced by low temperatures. The secretion of amylases in barley aleurone layers [5] and rice scutellum [6] is diminished below 20°C although the basis for the temperature effect has not been investigated. However, no data are available concerning the effect of low temperatures on the vesicular transport of lipids from the ER to the plasma membrane of higher plants.

Leek seedlings offer a convenient system in which to investigate membrane trafficking in poikilotherms because of several advantages: (i) The transfer of lipids to the plasma membrane has been demonstrated in vivo [7–9]; (ii) Kinetic evidence for the intermembrane vesicular transfer of lipids containing very-long-chain fatty acids to the plasma membrane [7,9] are available; (iii) VLCFA are synthesized from stearoyl-CoA in the ER and from icosanoyl-CoA in the Golgi apparatus, whereas the plasma membrane, which accumulates VLCFA-containing lipids, is devoid of any elongating activity [7]; (iv) The transport of neosynthesized lipids to the plasma membrane is blocked by monensin, a carboxylic ionophore known to block protein transport at the level of the Golgi apparatus [10]. The lipids which are blocked accumulate in the Golgi apparatus [9,11]. In addition, the deficit of lipids in the plasma membrane is due chiefly to a decrease of VLCFA-containing lipids whereas C_{18} fatty acid-containing lipids are not affected by monensin treatment [9].

Abbreviations: BCA, bicinchoninic acid; LRV, lipid-rich vesicles; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; VLCFA, very-long-chain fatty acids (having more than 20 carbon atoms); VSV, vesicular stomatitis virus.

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In this report we describe the synthesis and transfer of VLCFA-containing phospholipids and C_{16} and C_{18} fatty acid-containing phospholipids to the plasma membrane of leek seedlings in response to temperature. Our findings suggest the existence of distinct pathways or mechanisms for the transfer of phospholipids with various fatty acyl-chain lengths to the plasma membrane. One is the conventional ER-Golgi apparatus-plasma membrane pathway which is highly sensitive to temperature and mediates specifically the transfer of VLCFA-containing phospholipids. In contrast, the transfer of the C_{16} and C_{18} fatty acid-containing phospholipids to the plasma membrane is not sensitive to low temperatures.

2. Materials and methods

2.1. Plant material

Leek seeds stored overnight at 4°C were surface sterilized with sodium hypochlorite in the presence of Triton X-100 for 2 min and washed three times with distilled water. They were then grown for seven days in the dark at 24°C as described [7–9].

2.2. Labeling and isolation of plasma membranes

Batches of 300 leek seedlings each were incubated in vivo for 120 min with 0.3 mCi [1-^{14}C]acetate (55 mCi/mmol) at 4°C, 12°C, 16°C or 24°C, respectively. After incubation the seedlings were homogenized by mashing with mortar and pestle in a grinding buffer consisting of 0.5 M sorbitol and 10 mM KH_2PO_4 (pH 7.8). Each homogenate was centrifuged for 5 min at $1000 \times g$. The resulting supernatant was centrifuged for 20 min at $12000 \times g$ and the $12000 \times g$ supernatant was centrifuged at $150000 \times g$ for 60 min. The resulting membrane pellet was resuspended in 1 ml grinding buffer and 900 μl were used to isolate the plasma membrane by phase partition. Briefly, 0.9 ml of each membrane pellet was mixed with a solution of PEG 4000 (6.3%, w/w) and Dextran T-500 (6.3%, w/w) in 0.5 M sorbitol containing 10 mM KH_2PO_4 and 40 mM NaCl (pH 7.8) to obtain final PEG/Dextran concentrations of 6% (w/w). The mixture (final volume of 28 ml) was centrifuged 15 min at $500 \times g$ and the PEG-plasma membrane enriched upper phase (12 ml) was recovered without disturbing the interface. Plasma membranes were then recovered after centrifugation at $150000 \times g$ for 60 min and resuspended in the grinding buffer. The purity of plasma membranes assessed by marker enzymes [9] indicated that 80% of the glucan synthetase II and 90% of the vanadate-sensitive K^+-stimulated Mg^{2+}-ATPase activities of the membrane pellets were recovered in the plasma membranes whereas only 5–10% of ER and Golgi membrane markers (CDP-choline-diglyceride phosphotransferase, inosine diphosphatase) activities were present. Inhibition of ATPase activities by nitrate indicated that contamination by tonoplast and mitochondria could not exceed 10% [8]. The enrichment of glucan synthetase II [8], the high degree of inhibition of Mg^{2+}-ATPase by vanadate [8], the absence of any elongase activity [7,8], and the enrichment of sterols and VLCFA [8] are indicative that the plasma membrane fraction is highly enriched.

2.3. Isolation of endoplasmic reticulum (ER), lipid-rich vesicles (LRV) and Golgi apparatus (GA) fractions

Two batches of 300 leek seedlings received 0.3 mCi of [1-^{14}C]acetate (55 mCi/mmol) each and were incubated in vivo at 12°C or 24°C, respectively. The seedlings were then homogenized in a grinding buffer consisting of 0.5 M sorbitol and 10 mM KH_2PO_4 (pH 7.8). Each homogenate was centrifuged as described for the isolation of membrane pellets. The resulting membrane pellets were resuspended in 2.2 ml of grinding buffer and 1 ml of each were loaded onto 11 ml linear sucrose gradients (1.07–1.20 g/cm^3) and centrifuged for 22 h at $130000 \times g$. The gradients were then collected in 45–50 fractions of 250 μl each. This fractionation resolved lipid-rich vesicles (1.08–1.09 g/cm^3), endoplasmic reticulum (1.11–1.12 g/cm^3) and Golgi apparatus (1.13–1.14 g/cm^3) fractions. Purity of ER and Golgi apparatus membranes were as already published [8,9] and the lipid-rich vesicle fraction, involved in the transfer of lipids to the plasma membrane [7,9], was constituted by vesicles and sheets of membranes with a thickness and staining characteristics greater than the ER and less than for the plasma membrane and very small vesicles with thin membranes [9].

2.4. Lipid extraction and analyses

Lipids of the different membranes fractions were extracted by chloroform/methanol (2:1, v/v) as described earlier [12] and labeling was determined by liquid scintillation methods. The fatty acid methyl esters were prepared according to Lepage and Roy [13], all lipid classes containing fatty acids lead to the formation of fatty acid methyl esters with this method. Analyses by radio-GLC was with a Intersmat IGC 120 DFL chromatograph fitted with a 10% CP-SIL 58 columm (1/4″ × 2 m) on WHP (100–200 mesh size). The temperature was programmed at 8°C/min between 190°C and 290°C. The effluent gases were continuously monitored for radioactivity in a Packard 894 unit. Identification of fatty acids was achieved by comparison of retention times with those of standards.

2.5. Determination of proteins

Proteins of the various membrane fractions were measured by the BCA procedure [14] using BSA as a standard.

3. Results

3.1. Lipid synthesis at low temperature

Leek seedlings were incubated in vivo with labeled acetate for 120 min either at 24°C or at 12°C. Based on several experiments, the total de novo fatty acid biosynthesis was lowered by 30–40% at 12°C as compared to 24°C. The radioactivities of the fatty acids having from 16 to 24 carbon atoms were determined. Fatty acids having 16, 20, 22 and 24 carbon atoms were saturated, the C_{18} fatty acids were essentially unsaturated (less than 1% of 18:0, over 85% of 18:2, the complement being equally distributed between 18:1 and 18:3). The decrease of lipid synthesis was rather equally accounted for by decreases in saturated C_{16}, unsaturated C_{18} and saturated very long chain fatty acid synthesis (Table 1), thus indicating that VLCFA synthesis was not specifically affected at low temperature as compared to C_{16} and C_{18} fatty acid syntheses. However, the synthesis of unsaturated C_{18} fatty acids (12°C/24°C ratio of 0.76) was significantly less affected by low temperature than that of C_{16} (12°C/24°C ratio of 0.61) and very-long-chain fatty acids (12°C/24°C ratio of 0.68). This could explain the low decrease of radioactivity observed in PC (which is known to have a rather high content of unsaturated C_{18} fatty acids) as compared to the other phospholipids PA, PS, PI and PE and other polar lipids which migrated similarly as animal cerebrosides (Table 2).

We have also determined that the labeling of the neutral lipids was decreased by the same order of magnitude than that of the phospholipids. Two important points must be noticed, the labeling of sterols was considerably lowered at 12°C (12°C/24°C ratio of 0.40), and the amount of labeled free fatty acids was always low and represented less than 5% of the total lipid label.

3.2. The amount of neo-synthesized VLCFA in the plasma membrane is highly decreased at low temperatures

Leek seedlings were incubated in vivo with labeled acetate for 120 min at 4°C, 12°C, 16°C and 24°C. The corresponding membrane pellets (which contain membranes originating from the ER, the Golgi apparatus, the plasma membrane and all the organelles) and plasma membrane fractions were then prepared and analysed for their fatty acid labeling as mentionned in the experimental section. The level of labeling of C_{16}, C_{18} and very-long-chain acyl moieties of the plasma membrane lipids were compared relative to the lipids of the total membrane pellets as a function of temperature (4°C, 12°C, 16°C and 24°C). Relatively little change was observed for the radioactivity of C_{16} and C_{18} fatty acids (Fig. 1). However, a relatively large decrease in the ratio of labeling of the plasma membrane relative to that of the membrane pellets was found for the VLCFA when the temperatures were lowered below 12°C (Fig. 1). Since the synthesis of VLCFA was not dramatically affected as compared to that of C_{16} and C_{18} fatty acyl moieties (Table 1), it appeared that the delivery of VLCFA-containing lipids to the plasma membrane was slowed at low temperatures. The slope calculated between 12°C and 24°C for VLCFA was almost three times greater than that between 4°C and 12°C for VLCFA, which demonstrates a critical sensitivity of the accumulation of labeled VLCFA in the plasma membrane between 12°C and 24°C.

3.3. Accumulation of neo-synthesized lipids in intracellular membranes at low temperature

To determine the site of intracellular lipid accumulation, leek seedlings were incubated with [1-

Table 1
Effect of low temperature (12°C) on the synthesis of the total C_{16}, C_{18} and very-long-chain fatty acyl moieties

	Labeling of the fatty acyl moieties (dpm × 10^{-3}/mg prot.)		12°C/24°C ratios	Distribution of radioactivity between the acyl moieties (%)	
	12°C	24°C		12°C	24°C
C_{16}	132 ± 10.6	216 ± 17.3	0.61 ± 0.05	35.1 ± 2.4	39.8 ± 3.3
C_{18}	210 ± 38	276 ± 49	0.76 ± 0.14	55.8 ± 10.2	50.9 ± 9.4
VLCFA	34 ± 3.1	50 ± 4.5	0.68 ± 0.06	9.0 ± 0.8	9.2 ± 0.9
Total	376 ± 25	542 ± 32	0.69 ± 0.09		

Etiolated leek seedlings were incubated in vivo with 0.3 mCi of [1-^{14}C]acetate for 120 min either at 24°C or at 12°C. Total lipids were extracted as described in Materials and methods and fatty acid methyl esters were prepared and analysed by radio-GLC as described in the Section 2. Identification of the chain length was according to standards. The radioactivities of the fatty acids having from 16 to 24 carbon atoms were determined. Fatty acids having 16, 20, 22 and 24 carbon atoms were saturated. The C_{18} fatty acids were essentially unsaturated (less than 1% of 18:0 and over 85% of 18:2). These results are mean values ± S.D. of three experiments.

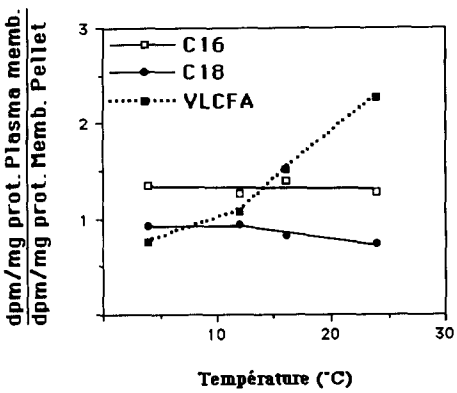

Fig. 1. Effect of temperature on the ratio of radioactivity for plasma membrane to that of the total membrane pellets for C_{16} and C_{18} fatty acids and the very-long-chain fatty acids (VLCFA). Etiolated leek seedlings were incubated in vivo with 0.3 mCi of [1-^{14}C]acetate for 120 min at 4 different temperatures (4°C, 12°C, 16°C and 24°C). The total membrane pellets were prepared and plasma membranes isolated. Lipids from the total membrane pellets and the plasma membrane fractions were then extracted, the fatty acid methyl esters prepared from each fraction were analysed by radio-gas liquid chromatography. Results are mean values ± S.D. of three experiments.

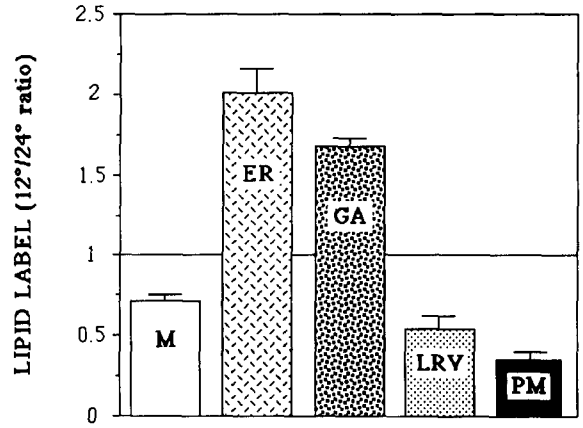

Fig. 2. Effect of temperature upon the lipid labeling of the various membrane fractions of leek seedlings. Etiolated leek seedlings were incubated in vivo with 0.3 mCi of [1-^{14}C]acetate for 120 min at 12°C or 24°C. Various membrane fractions (M, total membrane pellets; LRV, lipid-rich vesicles; ER, endoplasmic reticulum; GA, Golgi apparatus and PM, plasma membrane) were then isolated and the radioactivities of their lipids were determined and expressed as dpm × 10^{-3}/mg proteins. The ratios 12°C/24°C were calculated for each membrane fraction and are shown in the figure. The radioactivities of the lipids of the membrane fractions (dpm × 10^{-3}/mg proteins) at 12°C and 24°C, respectively were: M (270 ± 62 and 380 ± 73), LRV (32 ± 8 and 59 ± 6), ER (126 ± 19 and 63 ± 13), GA (125 ± 10 and 92 ± 11), PM (216 ± 49 and 519 ± 127). These results are means of three (LRV, ER and GA fractions) or five (M and PM fractions) experiments ± S.D.

^{14}C]acetate at 12°C and 24°C for 120 min, the different membrane fractions were isolated and the distribution of radioactivity within lipids was determined. Lowering the temperature to 12°C resulted in a 25–30% decrease of radiolabeled acetate incorporated into the total lipids of the total membrane pellets (Fig. 2). For the plasma membrane, a 60% decrease of radioactivity was observed in total lipids, more than twice the decrease of lipid synthesis.

The analysis of the radioactivity of the lipids of the ER and Golgi apparatus fractions revealed an accumulation of radioactive lipids in these fractions at 12°C as compared to 24°C. A two fold increase in the total radioactivity of lipids was observed for the ER and a 1.4 fold increase for the Golgi apparatus at 12°C compared to 24°C. In a typical experiment, the total lipid label was 23000 dpm at 24°C compared to 53000 dpm at 12°C in the ER and 60000 dpm at 24°C compared to 84000 dpm at 12°C in the Golgi apparatus. Meanwhile, the lipid label in the plasma membrane and the lipid-rich vesicles was 78000 dpm at 24°C and 38000 dpm at 12°C and 19000 dpm at 24°C and 10000 dpm at 12°C, respectively. The total accumulation of labeled lipids in the ER Golgi apparatus reached 54000 dpm at 12°C and the difference in labeled lipids in the plasma

Table 2
Effect of low temperature (12°C) on the synthesis of the various phospholipids

	Labeling of the phospholipids (dpm × 10^{-3}/mg prot.)		12°C/24°C ratios	Distribution of radioactivity between the phospholipids (%)	
	12°C	24°C		12°C	24°C
PC	81.8 ± 8.2	112.0 ± 11.0	0.73 ± 0.11	51.1 ± 4.8	44.8 ± 2.7
PS	8.5 ± 1.1	14.8 ± 1.9	0.58 ± 0.06	5.3 ± 0.5	5.9 ± 1.3
PI	11.9 ± 1.8	20.3 ± 3.0	0.59 ± 0.07	7.4 ± 0.7	8.1 ± 2.0
PA	6.6 ± 1.5	11.2 ± 2.5	0.59 ± 0.10	4.1 ± 0.7	4.5 ± 1.6
PE	42.3 ± 4.1	75.5 ± 7.6	0.56 ± 0.07	26.4 ± 1.7	30.2 ± 3.4
UPL *	8.9 ± 1.2	16.2 ± 2.3	0.55 ± 0.07	5.5 ± 0.7	6.5 ± 1.1
Total	160.0 ± 5.6	250.0 ± 7.8	0.64 ± 0.10		

Etiolated leek seedlings were incubated in vivo with 0.3 mCi of [1-^{14}C]acetate for 120 min either at 24°C or at 12°C. Total lipids were extracted as described in Materials and methods and phospholipids were separated by thin layer chromatography using methyl-acetate/n propanol/chloroform/methanol/0.25% aqueous KCl (25/25/28/10/7) according to Bertho et al. [9]. Phospholipids were scraped off from the HPTLC plates and their radioactivities were determined by scintillation counting. These results are mean values ± S.D. of five experiments.
* Unidentified polar lipids.

membrane plus lipid-rich vesicles at 12°C compared to 24°C was 49000 dpm. Although lipid synthesis was decreased at 12°C, the accumulation of labeled lipids in both the ER and the Golgi apparatus at this temperature and their concomittant decrease in the plasma membrane, suggest that the transport of some labeled lipids from the ER and the Golgi apparatus to other cellular compartments and especially the plasma membrane was reduced.

It has been shown that the lipid-rich vesicles (LRV fraction) are involved in the transfer of lipids (and chiefly VLCFA-containing lipids) to the plasma membrane of leek seedlings [7–9]. Kinetically, pulse-chase experiments and monensin treatment have indicated that lipid-rich vesicles exhibit characteristics intermediate between the ER and the Golgi apparatus and also between the latter and the plasma membrane [7,9,15], suggesting that the LRV fraction could contain lipid-rich vesicles of both origins. Consequently, the evolution of the lipid labeling of the LRV fraction is more difficult to interpret.

3.4. Accumulation of VLCFA in the ER and Golgi membranes at low temperature

The decrease of the transfer of VLCFA-containing lipids to the plasma membrane at 12°C (Fig. 1) and the increase of lipid labeling both in the ER and the Golgi apparatus at this temperature (Fig. 2) imply that the fatty acyl moieties of the VLCFA-containing lipids should accumulate at the ER and Golgi apparatus at 12°C. An accumulation of the total labeled fatty acids and especially the labeled VLCFA (C_{20}, C_{22} and particularly C_{24} fatty acids) in the intracellular membranes (ER and Golgi apparatus) was effectively observed (Fig. 3). At the same time, there was a deficit of labeled VLCFA in the plasma membrane as compared to the labeled C_{16} and particularly C_{18} fatty acids (Fig. 3).

The response of the C_{20}-containing lipids was of interest because of the differential localization of the elongases (C_{18}-CoA elongase in the ER and C_{20}-CoA elongase in the Golgi apparatus) [7]. The results obtained for the C_{20}-containing lipids indicate at least the existence of a partial block of the transfer of lipids from the ER to the Golgi apparatus. Moreover, this partial block could explain why a higher increase of labeled C_{22} and C_{24} fatty acids was observed in the ER than in the Golgi apparatus (Fig. 3). Effectively, C_{20} fatty acids, retained in the ER, could be elongated by the C_{18}-CoA elongase of the ER to form C_{22} and C_{24} fatty acids to some extent [16].

The decrease of labeled VLCFA moieties in the lipid-rich vesicles (Fig. 3) provided additional evidence for a role of these vesicles in the transfer of VLCFA-containing lipids to the plasma membrane.

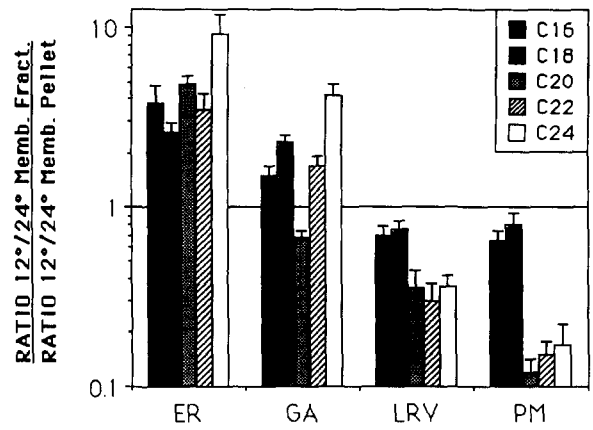

Fig. 3. Effect of temperature upon fatty acid labeling of various membrane fractions. Etiolated leek seedlings were incubated in vivo as described in Fig. 2. The membrane fractions were then prepared according to the procedures detailed in Materials and methods. Lipid extraction and fatty acid analyses were as described in the experimental section. The radioactivities of the fatty acids having from 16 to 24 carbon atoms were determined. Fatty acid having 16, 20, 22 and 24 carbon atoms were saturated. The C_{18} fatty acids were essentially unsaturated (less than 1% of 18:0 and more than 85% of 18:2). For each fatty acid of each membrane fraction the ratios of radioactivity were calculated for 12°C compared to 24°C. For membranes other than the membrane pellets these ratios were then divided by the ratio obtained for the membrane pellets since the latter (value of 0.75, Fig. 2) was similar to that of total synthesis (value of 0.69, Table 1). Consequently, this calculation corrects for the effect of temperature on synthesis and shows directly the accumulation or reduction of labeled fatty acids in each of the membrane fractions compared to the total membrane pellets. Data presented are mean values of two experiments ± average mean deviation.

The 12°C temperature block for VLCFA transport to the plasma membrane was further confirmed by pulse-chase experiments where the pulse (120 min) was at 24°C and the chases were realised at 4°C, 12°C or 24°C for 120 min. The results are shown in Table 3. During the chase, lipids containing C_{16} and C_{18} fatty acyl moieties were transferred to the plasma membrane at 12°C and 24°C but less efficiently at 4°C whereas the transfer of lipids containing VLCFA moieties to the plasma membrane was blocked at 12°C and below. We did not observe any increase of VLCFA labeling in the plasma membrane during the chase at 12°C (Table 3).

3.5. To which lipids are VLCFA associated?

We have already observed that VLCFA are predominantly found in the major phospholipids PC and PE [17] and also in the neutral lipids such as glycerides and wax esters. It has also been determined that only low amounts of VLCFA are present in the free fatty acids which moreover represent less than 5% of the total labeled lipids.

We have analysed the fatty acid content of the major phospholipids PC and PE at 12°C and 24°C, the

Table 3
Effect of the temperature of the chase on the transfer of the various fatty acids to the plasma membrane of leek seedlings after a 120 min pulse

Fatty acids (chain length)	Labeling of the fatty acyl chains (dpm × 10^{-3}/mg prot.)		
	16	18	20–24
Pulse (120 min)	243	65	33
Chase at 4°C (120 min)	260	75	33
Chase at 12°C (120 min)	320	106	34
Chase at 24°C (120 min)	377	134	55

Etiolated leek seedlings were incubated in vivo with 0.3 mCi of [1-^{14}C]acetate for 120 min at 24°C. Then, they were chased for 120 min in vivo by unlabeled acetate (0.2 M acetate buffer, pH 5.5) at various temperatures as described [9]. The membrane pellets were prepared and the plasma membranes corresponding to each experimental condition were then isolated as described in Section 2. In these experiments, we have ensured that the level of labeling of the total membrane pellets was unchanged during the various chases and, therefore, that no further de novo synthesis occured during the chases [9,17]. Lipids of the plasma membrane fractions were extracted and the label associated with C_{16}, C_{18} and C_{20}–24 fatty acids was determined for each experimental condition by radio-GLC analysis. Fatty acids having 16, 20, 22 and 24 carbon atoms were saturated. The C_{18} fatty acids were essentially unsaturated (less than 1% of 18:0 and over 85% of 18:2). These data are mean values of two experiments with reproducible results.

results are presented in Table 4. The 12°C/24°C ratios indicate that very few labeled C_{20}–C_{24} fatty acids were found in both PC and PE of the plasma membrane whereas these fatty acids were still synthesized and inserted into PC and PE of the total membrane pellets.

These results demonstrate that most of PC and PE as carriers of VLCFA did not reach the plasma membrane at 12°C as it normally occurs at 24°C.

Moreover, another interesting point is the slight increase of unsaturated C_{18} fatty acids in PC of the total membrane pellets and the fact that unsaturated C_{18}-containing PC was still efficiently transferred to the plasma membrane at low temperature.

Table 4
12°C/24°C ratios of fatty acid labeling (dpm/mg prot.) of the major phospholipids PC and PE in the total membrane pellets and plasma membranes

Membranes	Phospho-lipids	12°C/24°C ratios of fatty acid labeling (fatty acid chain length (carbon atoms))		
		16	18	20–24
Total membrane pellets	PC	0.35	1.12	0.41
	PE	0.52	0.68	0.67
Plasma membrane	PC	0.45	0.98	< 0.1
	PE	0.43	0.78	< 0.1

Etiolated leek seedlings were incubated in vivo with 0.3 mCi of [1-^{14}C]acetate for 120 min either at 24°C or at 12°C. Phospholipid isolation was as explained in the legend of Table 1. Fatty acid methyl esters of PC and PE were obtained by the method of Lepage and Roy [13] and were analysed as described in Materials and methods. The radioactivities of the fatty acids having from 16 to 24 carbon atoms were determined. Fatty acids having 16, 20, 22 and 24 carbon atoms were saturated. The C_{18} fatty acids were essentially unsaturated (less than 1% of 18:0 and over 85% of 18:2). For each fatty acid of each membrane fraction the ratios of radioactivity were calculated for 12°C compared to 24°C. These data are mean values of two experiments with reproducible results.

4. Discussion

In this paper, we have studied the effect of low temperatures on the transfer to the plasma membrane of leek seedlings of fatty acyl chain-containing lipids as a function of their fatty acyl chain length and unsaturation. At low temperature, the vesicular transport of VLCFA-containing lipids to the plasma membrane of leek cells was seriously decreased whereas that of C_{16} and C_{18} fatty acid-containing lipids still occurred. It could be argued that low temperature will just block the spontaneous diffusion of VLCFA-containing lipids and not that of C_{16} and C_{18} fatty acid-containing lipids. However, it has already been demonstrated that VLCFA-containing lipids are transferred to the plasma membrane through the ER to Golgi apparatus to plasma membrane pathway [7–9].

We have previously shown that the transport of VLCFA-containing lipids to the plasma membrane of leek cells was blocked by monensin treatment [9,11] whereas that of C_{16} and C_{18} fatty acid-containing lipids was not. Interestingly, we have observed at 12°C a similar pattern to that obtained after monensin treatment. We have also shown that the decrease of the transfer of VLCFA-containing lipids to the PM at 12°C was accompanied by an accumulation of these molecules at the level of the endoplasmic reticulum and the Golgi apparatus. Moreover, we have determined that the VLCFA were particularly esterified to PC and PE. Consequently, we conclude that lowering the temperature to 12°C decreased the transport of VLCFA-containing lipids and especially that of VLCFA-PC and VLCFA-PE (Table 4) through the vesicular ER-Golgi apparatus-plasma membrane pathway.

We have also found that C_{16} and C_{18} fatty acid-containing lipids were still transferred to the plasma membrane at 12°C and that unsaturated C_{18}-PC was preferentially transferred to the plasma membrane at this temperature (Table 4). It has been shown that lowering the temperature lead to an increase of unsaturated C_{18}-fatty acids (Ref. [18] and references therein). Interestingly, it has been suggested that changes in the level of desaturase activity induced by low temperature would act primarily on newly formed (labeled) fatty acids rather than on existing lipids [19]. Moreover, lower temperatures also lead to an increase of PC among the phospholipid species [20].

From the results shown in Fig. 3 and Tables 3 and 4, we can conclude that lowering the temperature to 12°C had two principal consequences on the transport of fatty acid-containing lipids to the plasma membrane of leek seedlings:

(i) VLCFA-containing lipids and particularly VLCFA-PC and VLCFA-PE were not transferred to the plasma membrane at 12°C;

(ii) Unsaturated-C_{18}-PC was preferentially transferred to the plasma membrane at this temperature.

An important consequence of these data is the possible existence of distinct lipid transfer mechanisms or pathways that could be distinguished by their sensitivity to temperature and the nature of the lipids transferred.

VLCFA-containing lipids follow the ER-Golgi apparatus-plasma membrane pathway above 12°C and C_{16} and C_{18} fatty acid-containing lipids are transported to the plasma membrane by mechanism(s) not sensitive to low temperatures. The nature of the mechanism(s) transferring C_{16} and C_{18} fatty acid-containing lipids is unknown but spontaneous diffusion, lipid-transfer proteins or other potential vesicular pathway(s) can be considered.

Monomer diffusion can almost be totally ruled-out on the basis of kinetic data. In contrast, phospholipid-transfer proteins, assumed to be cytosolic and/or associated with membranes [21], could be responsible for the transfer of C_{16}- and C_{18}-containing lipids to the plasma membrane. However, it has recently been determined that lipid-transfer proteins may have exclusively an extracellular location in some plant cells [22–24], in disagreement with an intracellular role in lipid transport.

According to the kinetics of transfer of total lipids between intracellular membranes and the plasma membrane, the transfer of C_{16} and C_{18} fatty acid-containing lipids was as slow as that of VLCFA-containing lipids [8,9] and not as fast as it could have been expected if lipid transfer proteins were effectively involved.

Recent findings in the yeast SEC 14 mutant [25] showed that the transport of phosphatidylcholine and phosphatidylinositol to the plama membrane could not be accounted for by a vesicular transfer involving secretory vesicles or the PI/PC transfer protein. The involvement of membrane collision-contact in the translocation of phospholipids was considered [25]. Such a mechanism can eventually be considered in our case.

On another hand, one can also suggest that the existence of other vesicular pathway(s) transferring C_{16} and C_{18} fatty acid-containing lipids is not unlikely. The absence of monensin effect in various systems has led to propose the existence of exocytic pathways which bypass the Golgi apparatus [10]. In leek cells, the results obtained following both monensin [9] and low temperature (this paper) treatments can support such an hypothesis.

Multiple pathways to transfer different molecules to the same target membrane have been suggested previously. In chinese hamster ovary cells, the transfers of the VSV G protein and cholesterol to the plasma membrane were shown to follow different pathways [26]. The VSV G protein was transported through the ER to Golgi apparatus to plasma membrane pathway as expected. The latter was blocked by brefeldin A whereas cholesterol was transferred to the plasma membrane via a vesicular route insensitive to brefeldin A. The brefeldin-insensitive pathway was proposed to be a direct ER to plasma membrane route [26].

The postulate that plant Golgi apparatus could have at least two exit sites was developed recently [27]. It has been shown in cultured carrot cells and clover root tip cortical cells that the synthesis of polygalacturonic acid-rhamnogalacturonan-I was confined to the *cis* and medial cisternae of the Golgi apparatus and that of xyloglucan was confined to the *trans* cisternae of the Golgi apparatus and the *trans* Golgi apparatus network [27]. It has been proposed that different secretory vesicles emerging from different stacks of the Golgi apparatus could be targeted to specific domains of the plasma membrane [27]. In sycamore maple cells, however, the *trans* Golgi apparatus network was proposed as the major site of exit of secretory vesicles carrying complex polysaccharides [28].

How explain that distinct mechanisms and/or pathways are involved in the transfer of VLCFA-containing lipids (vesicular transport through the Golgi apparatus) and C_{16} and C_{18} fatty acid-containing lipids (unknown transfer mechanism but, certainly not, that used by VLCFA-containing lipids) to the same target membrane? This question may imply that specific membrane domains exist. Although no experimental data are yet available on our system, membrane domains enriched either with VLCFA-containing lipids or C_{16} and/or C_{18} fatty acid-containing lipids can be expected in the Golgi apparatus and the ER. Hence, the possibility emerges that differences in temperature sensitivity for transfer among different classes of lipids with different acyl chains could be related to the formation of specific domains in the intracellular membranes. We have already proposed that the lipids to be transferred to the leek seedling plasma membrane are sorted at the level of the Golgi apparatus and that an important criterion of this sorting is the chain length of the fatty acyl moieties of these lipids [9]. In the literature, it has been strongly suggested that glycolipid 'clustering' would be an essential event in the formation of membrane domains to sort both lipids and proteins at the level of the *trans* side of the Golgi apparatus of epithelial cells [29,30], and it has also

been proposed that glycosyl phosphatidylinositol-anchored proteins partition into apical glycosphingolipid domains [31]. Recently, glycosphingolipid rafts were proposed to be at the origin of lateral segregation of lipids and proteins within the membrane and that the formation of such microdomains with a specific protein and lipid composition will control the sorting and delivery of the right molecules to the right target membrane [32].

The formation of specific membrane microdomains is certainly as crucial for lipid sorting as for protein sorting. Thus the existence of membrane domains enriched in C_{20}–C_{24} fatty acid-containing lipids and C_{16} and C_{18} fatty acid-containing lipids will have to be investigated. In addition, it will be important to determine whether the effect of low temperature on the transport of C_{20}–C_{24}-containing lipids to the plasma membrane is related to the phase transition temperature of these membrane lipid domains.

References

[1] Matlin, K.S. and Simons, K. (1983) Cell 34, 233–243.
[2] Saraste, J. and Kuismanen, E. (1984) Cell 38, 535–549.
[3] Saraste, J., Palade, G.E. and Farquhar, M.G. (1986) Proc. Natl. Acad. Sci. USA 83, 6425–6429.
[4] Tartakoff, A.M. (1986) EMBO J. 5, 1477–1482.
[5] Fadeel, A., Moll, B.A. and Jones, R.L. (1980) Plant Physiol. 66, 466–470.
[6] Mitsui, T. and Akazawa, T. (1986) Plant Physiol. 82, 880–884.
[7] Moreau, P., Bertho, P., Juguelin, H. and Lessire, R. (1988) Plant Physiol. Biochem. 26, 173–178.
[8] Moreau, P., Juguelin, H., Lessire, R. and Cassagne, C. (1988) Phytochem. 27, 1631–1638.
[9] Bertho, P., Moreau, P., Morré, D.J. and Cassagne, C. (1991) Biochim. Biophys. Acta 1070, 127–134.
[10] Mollenhauer, H.H., Morré, D.J. and Rowe, L.D. (1990) Biochim. Biophys. Acta 1031, 225–246.
[11] Bertho, P., Moreau, P., Juguelin, H., Gautier, M. and Cassagne, C. (1989) Biochim. Biophys. Acta 978, 91–96.
[12] Heape, M.A., Juguelin, H., Boiron, F. and Cassagne, C. (1985) J. Chromatogr. 322, 391–395.
[13] Lepage, G. and Roy, C.C. (1986) J. Lipid Res. 27, 114–120.
[14] 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.
[15] Moreau, P., Sturbois, B., Maneta-Peyret, L., Morré, D.J. and Cassagne, C. (1993) in Molecular Mechanisms of Membrane Traffic (Morré, D.J., Howell, K.E. and Bergeron, J.J.M., eds.), NATO ASI series H, Vol. 74, pp. 187–195, Springer-Verlag.
[16] Lessire, R., Juguelin, H., Moreau, P. and Cassagne, C. (1985) Phytochem. 24, 1187–1192.
[17] Moreau, P. (1986) Ph. D Thesis, University of Bordeaux II, Bordeaux, France.
[18] Mazliak, P. (1979) in Low Temperature Stress in Crop Plants (Lyons, J.M., Graham, D. and Raison, J.K., eds.), pp. 391–404, Academic Press, New York.
[19] Williams, J.P., Mobashsher, U.K. and Wong, D. (1992) Biochim. Biophys. Acta 1128, 275–279.
[20] Willemot, C. (1979) in Low Temperature Stress in Crop Plants (Lyons, J.M., Graham, D. and Raison, J.K., eds.), pp. 411–430, Academic Press, New York.
[21] Arondel, V. and Kader, J.C. (1990) Experientia 46, 579–585.
[22] Bernhard, W.R., Thoma, S., Botella, J., Somerville, C.R. (1991) Plant Physiol. 95, 164–170.
[23] Sterk, P., Booij, H., Schellekens, G.A., Van Kammen, A., De Vries, S.C. (1991) Plant Cell 3, 907–921.
[24] Thoma, S., Kaneko, Y., Somerville, C. (1993) Plant J. 3, 427–436.
[25] Gnamusch, E., Kalaus, C., Hrastnick, C., Paltauf F. and Daum, G. (1992) Biochim. Biophys. Acta 1111, 120–126.
[26] Urbani, L. and Simoni, R.D. (1990) J. Biol Chem. 265, 1919–1923.
[27] Moore, P.J., Swords, K.M.M., Lynch, M.A. and Staehelin, A. (1991) J. Cell Biol. 112, 589–602.
[28] Zhang, G.F. and Staehelin, A. (1992) Plant Physiol. 99, 1070–1083.
[29] Van Meer, G., Stelzer, E.H.K., Wijnaendts-van-Resandt, R.W. and Simons, K. (1987) J. Cell Biol. 105, 1623–1635.
[30] Simons, K. and Van Meer, G. (1988) Biochemistry 27, 6197–6202.
[31] Brown, D. and Rose, J. (1992) Cell 68, 533–544.
[32] Fiedler, K., Kobayashi, T., Kurzchalia, T.V. and Simons, K. (1993) Biochemistry 32, 6365–6373.