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Monensin blocks the transfer of very long chain fatty acid containing lipids to the plasma membrane of leek seedlings. Evidence for lipid sorting based on fatty acyl chain length

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Delivery of newly synthesized fatty acids and lipids to the plasma membrane in leek seedlings via the endoplasmic reticulum (ER)-Golgi apparatus pathway is primarily by bulk transport (without sorting). However, pulse-chase experiments revealed kinetics of transport of lipids with VLCFA (very long chain fatty acids having more than 18 carbon atoms) in favor of a preferential transfer of these molecules to the plasma membrane. Use of monensin showed the accumulation of lipids in the Golgi apparatus and a related decrease of the amount of lipids transported to the plasma membrane. Lipid and fatty acid analyses revealed that transport of VLCFA-containing phospholipids was most strongly inhibited by the monensin block. These results taken together with an inability of the plasma membrane to synthesize VLCFA support a role for the Golgi apparatus in VLCFA delivery to the plasma membrane and leads to the hypothesis of a sorting function as well, based on fatty acyl chain length.

Introduction

Morré and Mollenhauer proposed the endomembrane flow concept [1] to link vectorially and functionally the endoplasmic reticulum (ER), the Golgi apparatus and the plasma membrane. While the trafficking of proteins and glycoproteins has been studied widely [2–4], little is known about the intracellular traffic of lipids (for review, see Ref. 5), particularly for plant cells.

Lipids of the plasma membrane of higher plants are primarily phospholipids (essentially PC, PE and to a lesser extent, PS and PI) and neutral lipids including phytosterols. The latter are the most characteristic lipids of the plasma membrane [6,7]. In leek cells, saturated very long chain fatty acids (VLCFA, having more than 18 carbon atoms) also are characteristic components of the plasma membrane [8]. These molecules are synthesized in the ER [8] and to a lesser extent in the Golgi apparatus [9]. Moreover, the plasma membrane is unable to synthesize these molecules [9,10]. For these reasons, leek cells are a particularly interesting system to investigate lipid transfer to the plasma membrane [8–11].

Previously, intracellular transfer of acyl-lipids and VLCFA, in 7-day-old etiolated leek seedlings, was determined to start in the ER and to reach the plasma membrane after a lag of about 30 min [9–11]. Monensin, a carboxylic ionophore known to block transport of glycoproteins at the Golgi apparatus led to the accumulation of fatty acids in the Golgi apparatus [12].

In this paper, we report that lipid traffic to the plasma membrane via the ER-GA-plasma membrane pathway can be dependent upon fatty acyl chain length in a manner that even more clearly establishes a Golgi apparatus to plasma membrane relationship.

A preliminary account on this work was presented at the 9th International Symposium on Plant Lipids, held at Wye College, Kent, July 1990.

Materials and Methods

Plant material, substrates and reagents

Leek seeds stored overnight at 4°C were surface sterilized with sodium hypochlorite in the presence of Triton X-100 for 2 min and then washed three times

Abbreviations: ER, endoplasmic reticulum; GA, Golgi apparatus; LRV, lipid-rich vesicles; PM, plasma membrane; VLCFA, very long chain fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PEG, poly(ethylene glycol); TLC, thin-layer chromatography.

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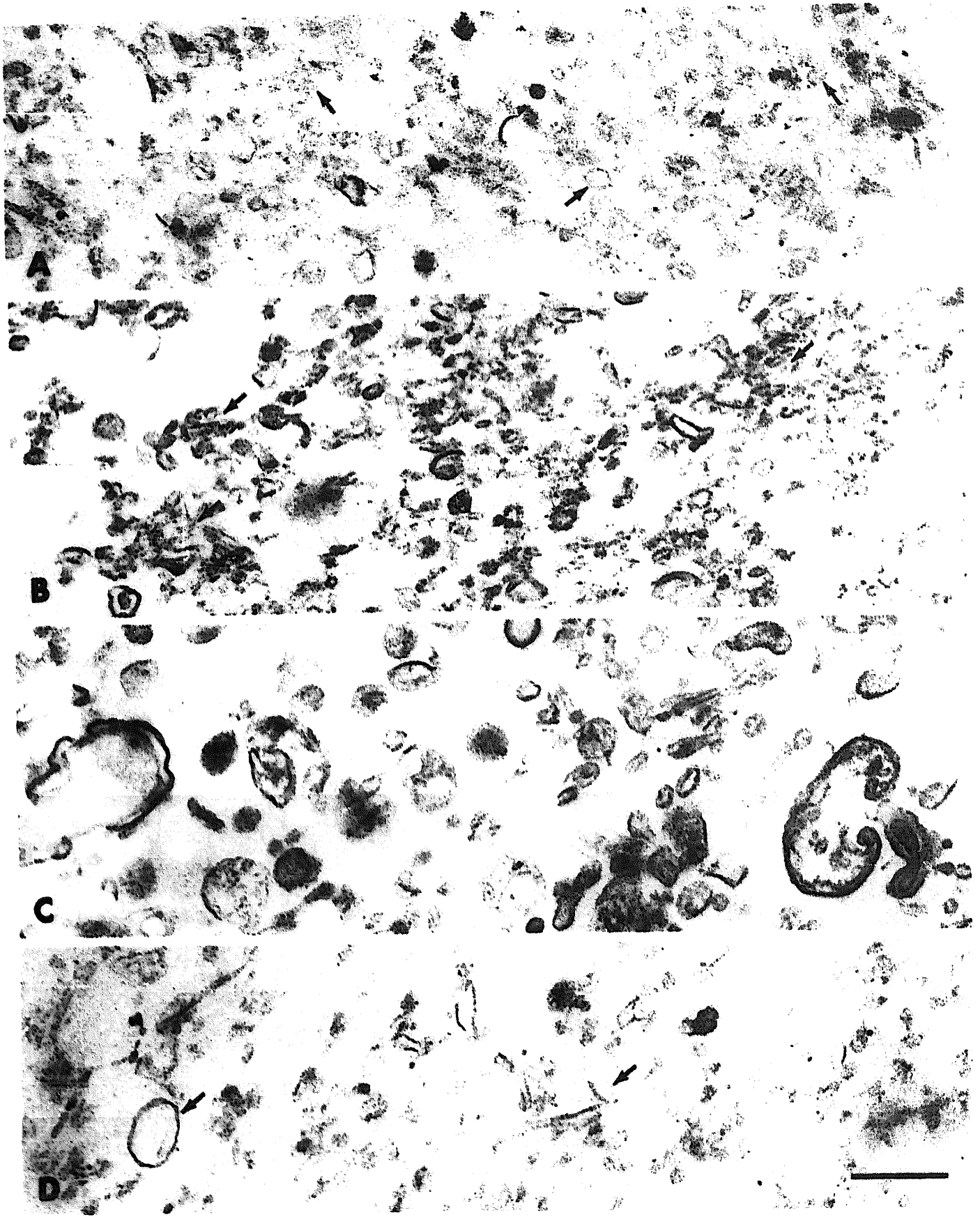
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with distilled water. They were then grown for seven days in the dark at 25°C as described [9–12].

Chemicals were from Sigma (St-Louis, MO, U.S.A.). [1-^{14}C]Acetate was from the Commissariat à l'énergie atomique (Saclay, France).

In vivo incorporation of [1-^{14}C]acetate in the presence of monensin

Batches of 200–800 sliced seedlings received 75–550 μCi [1-^{14}C]-acetate (55 mCi/mmol) and were incubated in vivo for 30 min, with or without 1 μM monensin [9–12]. This incubation followed, when indicated, a 30-min labeled acetate-free preincubation with or without 1 μM monensin. The final volume of each incubation medium was 5 ml for 200 seedlings. Monensin dilutions were with a 5 mM stock solution of monensin in 95% ethanol. All incubations were with a constant final ethanol concentration of 0.5%

Subcellular fractionation on linear sucrose gradients

Leek seedlings were homogenized in a grinding buffer consisting of 0.5 M sorbitol and 10 mM KH_2PO_4 (pH 7.8). The homogenate was centrifuged for 20 min at 12000 × *g* and the 12000 × *g* supernatant was centrifuged at 150000 × *g* for 1 h. The resulting microsomal pellet was resuspended in the grinding buffer and loaded onto a 11 ml linear sucrose gradient (1.07–1.20 g/cm^3) and centrifuged for 22 h at 130000 × *g*. The gradient was then collected in 45 fractions of 250 μl each. The various membrane fractions obtained, ER (endoplasmic reticulum), GA (Golgi apparatus), LRV (lipid rich vesicles) and PM (plasma membrane) were characterized by marker enzymes [9,10,15] and morphology. Proteins were estimated according to Bradford [13], using serum albumin as standard.

Isolation of the plasma membrane by phase partition

The microsomal pellet was mixed with 0.5 M sorbitol containing 10 mM KH_2PO_4 and 40 mM NaCl (pH 7.8) to obtain a final PEG 4000 and dextran T500 concentrations of 6.0% (w/w). The mixture (final volume: 28 ml) was centrifuged 15 min at 1000 × *g* and the PEG-enriched upper phase (12 ml) was recovered without disturbing the interface. Membranes were then recovered after centrifugation at 150000 × *g* for 60 min and resuspended in the grinding buffer. According to the marker enzymes the plasma membrane fraction obtained by phase partition is more purified than that obtained from sucrose gradients. For a question of restraint of time, in some experiments the plasma membrane obtained on sucrose gradients was used instead of that prepared by phase partition. In the case of the comparison of microsomes and phase-partitioned plasma membrane (for example Tables III, VI and Fig. 3), it is noteworthy that the microsomes (crude 150000 × *g* pellet) includes the plasma membrane.

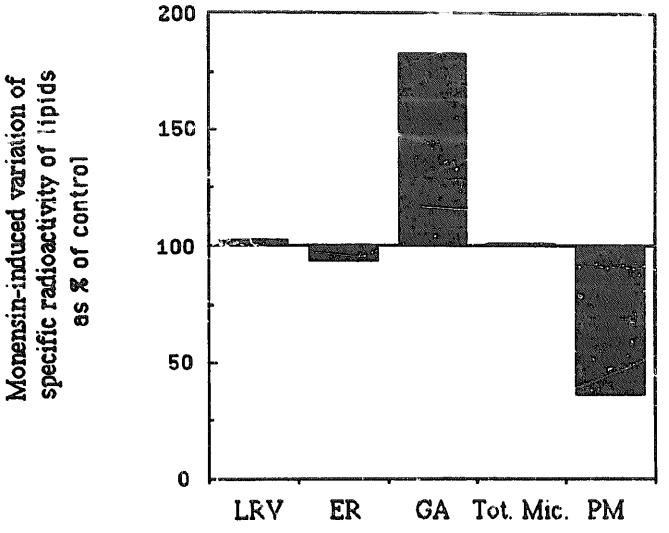

Fig. 2. Effect of monensin on the intracellular distribution of neosynthesized lipids. Seedlings were preincubated 30 min with 1 μM monensin or 0.5% ethanol for control. Then, 550 μCi of [1-^{14}C]acetate were added for 30 min. LRV, lipid-rich vesicles; ER, endoplasmic reticulum; GA, Golgi apparatus; Tot. Mic., total microsomes; PM, plasma membrane. Radioactivities of lipids of the various membrane fractions were calculated as dpm/mg of proteins. Results obtained in the presence of monensin were expressed as percent of the radioactivities found in the membrane fractions for the control incubation.

Lipid extraction and quantification

Lipids of membrane fractions were extracted by chloroform/methanol (2:1, v/v) as described [9–11]. The radioactivity incorporated into lipids was determined by liquid scintillation methods. Lipids were resolved by thin-layer chromatography (TLC) using two solvent systems in series: methyl acetate/*n*-propanol/

Fig. 1. Electron micrographs of leek fractions. (A) Endoplasmic reticulum-enriched fraction from the sucrose gradient. The fraction consists of small vesicles, many of which contain attached ribosomes (arrows), with thin (6 nm) membranes. A few vesicles with thick (9–10 nm) membranes, possibly plasma membrane or tonoplast, are the only recognizable contaminant. (B) Golgi apparatus-enriched fraction from the sucrose gradient. The major composition of the fraction are systems of tubular membranes and vesicles of a thickness overlapping or intermediate between that of the endoplasmic reticulum and plasma membrane. Some of the assemblies of tubules and vesicles are still organized into aggregate structures probably representing what were once Golgi apparatus stacks (arrows). (C) Plasma membrane-enriched fraction prepared by aqueous two phase partition. The fractions consisted nearly exclusively of vesicles with thick (9–10 nm) membranes representative of the plasma membrane. (D) The 'light' membrane fraction (LRV) from the sucrose gradient containing membranes of undetermined origins. The major membranes present were vesicles and sheets of membranes with a thickness and staining characteristics greater than those of endoplasmic reticulum but less than for plasma membrane (arrows) and very small vesicles with thin membranes. Scale bar = 0.5 μm.

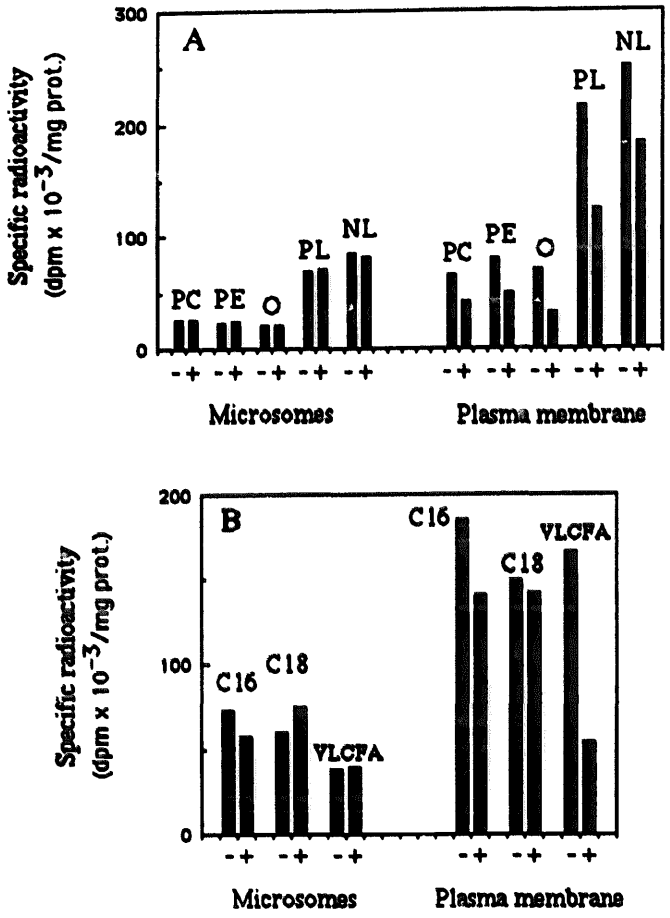

Fig. 3. Effect of monensin on lipid (A) and fatty acid (B) compositions of microsomes and plasma membrane. Treatment ± monensin and [1-^{14}C]acetate incorporation were as indicated in Table III. Results shown are from conditions described for experiment 5 (Table III), similar results have been obtained for each condition of Table III. PC, phosphatidylcholine; PE, phosphatidyl-ethanolamine; O, other phospholipids; PL, total phospholipids; NL, neutral lipids; C_{16}, palmitic acid; C_{18}, saturated and unsaturated fatty acids having 18 carbon atoms; VLCFA, very long chain fatty acids. −, control incubation − monensin; +, + monensin 1 μM.

chloroform/methanol/0.25% aqueous KCl (25:25:28:10:7:, v/v) followed by hexane/diethyl ether/acetic acid (90:15:2, v/v). This technique allows the resolution of most of the phospholipids and neutral lipids. Acyl-CoAs remained at the origin. After autoradiography of the plates, labeling of the different lipids was estimated by densitometry calibrated from various amount (5–1000 dpm) of a labelled standard analysed in parallel [14]. Lipid bands were visualized by copper acetate/phosphoric acid charring. The bands corresponding to free fatty acids (FFA) and fatty alcohols (FA) were very close and have been combined in Table VI. However, the densitometry analyses revealed that FA appeared more radioactive than FFA. No detectable labeling was associated with the major lysophospholipids.

Radio-GLC of the fatty acid-methyl esters

The fatty acid methyl esters were prepared from the lipid extracts according to Lepage and Roy [16]. The radio-gas liquid chromatography of the fatty acid-methyl esters was performed using a Packard 429 chromatograph fitted with a 10% CP-SIL 58 column (1/8" × 2 m) on WHP (100–200 mesh size) as described [11]. The effluent gases were-continuously monitored for radioactivity in a Packard 894 unit.

Electron microscopy

Subcellular membrane fractions were fixed by immersion in 2.5% glutaraldehyde in 0.1 M sodium phosphate (pH 7.2 at 4°C), followed by postfixation in 1% osmium tetroxide in the same buffer. Dehydration was through an acetone series, with embedment in Epon. Thin sections were examined and photographed with a Phillips EM/200 electron microscope.

Results

Lipid transfer analysed by pulse-chase experiments

The membranes prepared by subfractionation on sucrose gradients (ER, Golgi apparatus and lipid-rich vesicles) and the plasma membrane prepared by phase partition are identified by marker enzyme activities (Table I) and by electron microscopy (Fig. 1). The lipid-rich vesicle fraction corresponds to the light fraction (1.08–1.09 g/cm^3) already shown to transfer lipids

TABLE I

Distribution of marker enzymes and elongase activities between the various subcellular fractions

Data presented are combined from Moreau et al. [10] and from Bertho [15]. Enzyme assays were performed as already described [10]. C_{18}-CoA elongase (elongase using C_{18}-CoA as substrate) and C_{20}-CoA elongase (elongase using C_{20}-CoA as substrate) are defined as the enzymes responsible for the synthesis of very long chain fatty acids [17]. ER, endoplasmic reticulum; LRV, lipid-rich vesicles; GA, Golgi apparatus; PM, plasma membrane.

	Percentage of total activity of microsomes			
Membrane fractions:	ER	LRV	GA	PM
CDP choline-diglyceride phosphotransferase	59	3	17	3
C_{18}-CoA elongase	64	3	25	0
C_{20}-CoA elongase	23	4	63	0
Inosine diphosphatase	18	14	58	7
Glucane synthetase II	3	5	5	84
Vanadate-sensitive K^+-stimulated, Mg^{2+}-ATPase	1	1	3	93

TABLE II

Specific radioactivities of total lipids of the various membrane fractions as a function of chase time following a 120 min labeling period

The various membrane fractions were prepared by centrifugation of the microsomal pellet on linear density sucrose gradients. Lipid radioactivities were determined as explained in material and methods. Data are means ± S.D. of three experiments. Abbreviations as in Table I.

Membrane fractions	Total lipids (dpm × 10^{-3}/mg protein) as a function of chase time (min)				
	0	5	15	30	60
ER	750 ± 120	600 ± 120	450 ± 100	300 ± 80	280 ± 80
LRV	300 ± 50	370 ± 60	430 ± 70	270 ± 30	350 ± 70
GA	250 ± 40	320 ± 50	380 ± 60	420 ± 70	320 ± 50
PM	130 ± 20	160 ± 30	190 ± 40	240 ± 50	490 ± 80

[9–11,15]. Glucane synthetase II and the vanadate-sensitive K^+-stimulated Mg^{2+}-ATPase activities indicate a high enrichment of the plasma membrane fraction with plasma membrane vesicles. The ER (59% of CDP-choline phosphotransferase activity) and Golgi apparatus fractions (58% of inosine diphosphatase activity) present 90% of elongase activities and, more important, the plasma membrane is totally devoid of elongase activity. These membrane fractions were prepared after pulse-chase experiments to study the sequence of transfer of lipids and VLCFA from their site(s) of synthesis to the plasma membrane.

Table II shows the radioactivity of total lipids as a function of chase time following a 120 min labeling period. The ER fraction was the most radioactive fraction after the labeling period. Following a 15 min chase, a high increase of the radioactivity associated with lipid-rich vesicles is observed. Then after a 30 min chase, the Golgi apparatus fraction showed the highest specific radioactivity. Finally, after a 60 min chase, the plasma membrane fraction was the most radioactive fraction. As shown for total lipids, we found also a sequential transfer of the VLCFA from the ER to the plasma membrane as already shown in Ref. 9. These results are in agreement with an ER-GA-PM pathway as a main route for a bulk transport of lipids and VLCFA.

Effects of monensin

Monensin effects on the intracellular distribution of neosynthesized lipids between the Golgi apparatus, the plasma membrane and different intracellular membranes are shown in Fig. 2. A failure of neosynthesized lipids to be delivered to the plasma membrane was observed and was correlated with an accumulation of these lipids in the Golgi apparatus. Similarly, after a 30 min preincubation of the seedlings with or without 1 μM monensin, [1-^{14}C]acetate was added for 30 min and crude microsomes and the highly purified plasma membrane fraction were compared (Table III). Upon monensin addition, the amount of neosynthesized lipids in the plasma membrane decreased by 41.5 ± 11.7% ($n = 5$) as compared to the control incubation (Table III). At the same time, the level of neosynthesized lipids in the total microsomes was unchanged by the addition of monensin (−2.7 ± 7.6%, $n = 5$). Table IV shows the effect of increasing concentration of monensin (without pre-incubation) on the distribution of labeled lipids between the various membrane fractions. Increasing the external concentration of monensin led to a higher accumulation of radioactive lipids in the Golgi apparatus and a higher decrease of these lipids in the plasma membrane. Together with the results shown in Fig. 2 and Table III (obtained after pre-incubation with monensin), these results demonstrate a

TABLE III

Monensin-induced nondelivery of neosynthesized lipids to the plasma membrane

Seedlings were preincubated 30 min with 1 μM monensin or 0.5% ethanol for controls. For experiment 1 and 2, 200 seedlings were further incubated 30 min with 75 μCi of [1-^{14}C]acetate. For experiment 3 and 4, 400 seedlings were further incubated 30 min with 170 μCi of [1-^{14}C]acetate. For experiment 5, 800 seedlings were further incubated 30 min with 550 μCi of [1-^{14}C]acetate. Values are expressed as dpm × 10^{-3}/mg of protein.

Expt. No.	Control		Monensin		Monensin effect on crude microsomes (%)	Monensin effect on plasma membrane (%)
	crude microsomes	plasma membrane	crude microsomes	plasma membrane		
1	19	56	22	38	+15.8	−32.1
2	19	56	19	39	0	−30.3
3	45	101	43	46	−4.4	−54.4
4	47	84	35	36	−25.5	−57.1
5	170	502	171	335	+0.6	−33.5

GA-PM relationship during the transport of lipids to the plasma membrane. Finally, Table V shows the effect of adding monensin during the chase following a 120 min labeling period on the transport of lipids to the plasma membrane. Monensin at 1 μM inhibited the transport of labeled lipids to the plasma membrane during the chase by 75% (Table V).

Qualitative analyses of lipids destinated to the plasma membrane

The monensin-induced decrease of lipid delivery to the plasma membrane was analysed qualitatively. The lipids were analysed using TLC and autoradiography and the fatty acyl moieties of the lipids were analyzed using radio-GLC after preparation of the fatty acid methyl esters from the whole lipid extract.

As already observed in previous experiments [12], sterols were not labeled after 30 min and radioactivities measured in the membrane fractions were accounted for by fatty acid-containing lipids. In the absence of monensin (Table VI), the label incorporation in the lipids of either microsomes or plasma membrane was distributed equally between polar and neutral lipids. In the plasma membrane, the radioactivity of PC was reduced while that of PS and to a lesser extent PE was increased. The proportion of labeled VLCFA increased in the plasma membrane (33% of the total labeled fatty acids) as compared to the microsomes (22%). Triacylglycerols especially were reduced in the plasma membrane. Since the microsomes (microsomal 150000 $\times g$ pellet) contains the plasma membrane, the enrichment of VLCFA in the plasma membrane is even higher.

TABLE IV

Effect of different monensin concentrations on the distribution of labeled lipids among membrane fractions

Monensin incorporations were realised without any pre-incubation. For each monensin concentration, ethanol was adjusted to the same 0.5% final concentration. 200 seedlings were used and incubated 30 min with 550 μCi of [1-^{14}C]acetate. ER, LRV and GA fractions were prepared by centrifugation on sucrose density gradient and PM was prepared by phase partition of the same microsomal pellet. Radioactivities of lipids were calculated as dpm/mg of proteins. Results for monensin are expressed as % increase or decrease of the total radioactivity of lipids relative to controls. At 0.5 and 1 μM monensin lipid synthesis is not inhibited, and at 2 μM, lipid synthesis is lowered only by 10–15% [12]. Abbreviations as in Table I.

Membrane fractions	Evolution (%) of radioactivity of lipids as a function of monensin concn. (μM)		
	0.5 ($n=1$)	1	2 ($n=2$)
ER	+3	−2±3 ($n=3$)	−12
LRV	−16	−17±9 ($n=3$)	−26
GA	+23	+66±10 ($n=3$)	+87
PM	−22	−41±7 ($n=5$)	−73
Microsomes	+13	−2±6 ($n=5$)	+15

TABLE V

Effect of monensin, added during the chase, on the transport of lipids to the plasma membrane following a 120 min labeling period

Seedlings were incubated 120 min with [1-^{14}C]acetate as usually [9–11] and then 60 min with unlabeled acetate (acetate buffer 0.2 M, pH 5.5) and with or without 1 μM monensin. Radioactivities of total lipids were determined as explained in Material and Methods. Results shown are mean±S.D. of three experiments. PM, plasma membrane, purified by phase partition; Microsomes, 150000 $\times g$ microsomal pellet.

Membrane fraction	Lipids (dpm $\times 10^{-3}$/mg of protein)		
	120 min labeling period	60 min chase, monensin (μM)	
		0	1
Microsomes	880±110	840± 80	830± 60
PM	560±120	760±140	610±110

Upon monensin addition, no variation of lipid labeling was observed in the crude microsomal fraction (Fig. 3). As expected, a monensin-induced deficit of labeled phospholipids and neutral lipids was found for the plasma membrane (Fig. 3). This deficit was greater for phospholipids (−42.3%) than for neutral lipids (−26.7%) and was −35%, −37% and −54% for PC, PE and other phospholipids (PS, PI, PG) respectively.

The radio-GLC analyses of the lipids of crude microsomes revealed a small decrease of labeled C_{16} fatty acids (−21%) that correlated with a small increase of labeled C_{18} fatty acids (+25%). However, no variation of the labeling of VLCFA was observed.

The radio-GLC analyses of the lipids of the plasma membrane showed a decrease of the labeling of VLCFA compared to C_{16} and C_{18} fatty acids (Fig. 3). The deficit of labeled VLCFA was −67% and only −24% and −5% for labeled C_{16} and C_{18} fatty acids, respectively.

The higher decrease of labeled phospholipids than neutral lipids in the plasma membrane after monensin treatment, suggests that the deficit of labeled VLCFA is rather correlated to the deficit of labeled phopholipids. Consequently, it is likely that the monensin block concerns preferentially VLCFA-containing phospholipids.

Discussion

We previously designed a methodology to quantify how much monensin enters the cells. We found that only a very small amount of external monensin applied

TABLE VI

Incorporation of [1-^{14}C]acetate in the lipids of crude microsomes and plasma membrane

After incubation of seedlings with [1-^{14}C]acetate for 30 min, subcellular fractionations and lipid extraction were carried out as described in Material and Methods. Lipid and fatty acid radioactivities were determined by autoradiography of TLC plates and radio-GLG of total fatty acid methyl esters. Results are expressed as % of total neosynthesized lipids and fatty acids; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DG, diglycerides; FFA + FA, free fatty acids + fatty alcohols; TG, triglycerides; N.I., not identified. C_{16}–C_{24}, fatty acid chains having 16 to 24 carbon atoms. C_{16}, C_{20}, C_{22} and C_{24} are saturated fatty acids. C_{18} represents the sum of saturated and unsaturated fatty acids. VLCFA, very long chain fatty acids (C_{20}–C_{24}).

	Crude microsomes	Plasma membrane	Plasma membrane/ microsome ratio
Polar lipids	50.7 ± 6.8	50.4 ± 1.8	0.99 ± 0.06
Acyl-CoAs	2.8 ± 1.8	1.7 ± 0.8	0.79 ± 0.20
PC	15.0 ± 4.2	13.0 ± 1.8	0.87 ± 0.15
PS	2.6 ± 0.9	4.2 ± 1.7	1.62 ± 0.17
PI	4.7 ± 1.5	4.6 ± 1.2	0.98 ± 0.06
PA	0.7 ± 0.5	0.7 ± 0.6	1.00 ± 0.02
PG	3.5 ± 1.9	4.0 ± 1.0	1.14 ± 0.21
PE	12.6 ± 4.4	16.2 ± 0.5	1.28 ± 0.09
DGDG	2.9 ± 0.7	2.0 ± 0.9	0.69 ± 0.11
MGDG	2.0 ± 0.5	0.9 ± 0.7	0.45 ± 0.07
Neutral lipids	49.3 ± 6.8	49.6 ± 1.8	1.01 ± 0.07
DG	15.1 ± 4.2	16.3 ± 3.7	1.08 ± 0.16
FFA + FA	20.6 ± 3.2	20.5 ± 3.2	0.99 ± 0.1
TG	2.3 ± 1.0	0.7 ± 0.6	0.32 ± 0.19
N.I.	15.2 ± 3.1	15.2 ± 2.5	1.00 ± 0.18
C_{16}	42.7 ± 1.6	37.2 ± 1.4	0.87 ± 0.04
C_{18}	35.2 ± 4.8	29.7 ± 1.9	0.85 ± 0.07
VLCFA	22.1 ± 4.1	33.1 ± 0.9	1.51 ± 0.26
C_{20}	6.6 ± 0.9	9.8 ± 1.7	1.53 ± 0.26
C_{22}	9.5 ± 1.9	15.4 ± 0.8	1.58 ± 0.39
C_{24}	5.7 ± 1.8	7.7 ± 1.4	1.41 ± 0.58

was recovered in endomembranes [12]. The involvement of the Golgi apparatus in the intracellular transport of lipids was suggested by incubating leek seedlings in the presence of monensin [12]. However, no information concerning the final destinations of these lipids was obtained. In order to determine whether the lipids transiting via the Golgi apparatus are 'en route' to the plasma membrane, we have studied the effect of monensin on the intracellular distribution of neosynthesized lipids after in vivo incubations. It was determined that monensin, at the concentrations used, had no effect on the quality of subcellular fractionations. In the experiments reported previously [12], monensin was added at the same time as the substrate [1-^{14}C]acetate. We determined that monensin was effective after a lag of about 15 min. Consequently, neosynthesized lipids could transit through the Golgi apparatus and reach post Golgi transport vesicles and the plasma membrane before monensin action. To prevent this, the labeled substrate was added in the incubation medium after a 30 min preincubation with or without monensin.

That lipids and particularly VLCFA-containing lipids are transported along an ER-GA-plasma membrane pathway in leek cells is emphasized by the following points. (i) VLCFA are synthesized in the ER (chiefly C_{20}) and in the Golgi apparatus (chiefly C_{22} and C_{24}) (Ref. 9, Table 1). Thus, the VLCFA can be channeled through the ER-GA-plasma membrane pathway because of the localization of the elongases. (ii) Kinetically, a sequence of transfer is observed from the site(s) of lipid and VLCFA synthesis to the plasma membrane (Ref. 9, Table 2). (iii) Monensin leads to an accumulation of lipids in the Golgi apparatus (Table IV and Fig. 2) and a decrease in the plasma membrane (Table III and Fig. 2). Moreover, we observed a slower increase in the transfer of label from the Golgi apparatus to the plasma membrane following pulse-chase experiments in the presence of monensin (Table V). (iv) Total VLCFA synthesis in the microsomes is not changed upon monensin addition, whereas the transport of VLCFA-containing lipids to the plasma membrane is blocked by monensin (Fig. 3).

How the lipids which follow the ER-GA-PM pathway are sorted from other lipids and from other pathways is of critical importance. In epithelial cells, it has been proposed that lipid interactions could facilitate and direct the trafficking of sphingolipids and choles-

terol through the Golgi apparatus to the plasma membrane [5,18]. In this model, lipid–protein interactions also were considered. Recently, Wattenberg [19] using a cell-free system with mutants from Chinese hamster ovary cells, showed that glycolipid and glycoprotein transport through the Golgi complex were similar biochemically and kinetically. The conclusions were that glycolipid and glycoprotein sorting mechanisms could be similar and/or simultaneously operational.

Concerning phospholipids, data came out to propose that phospholipids also are sorted to different compartments and that multiple pathways are available [5,20]. For example, PS is preferentially sorted to the plasma membrane in different cell types [5] and also transported to the mitochondria [21]. Its transport to the plasma membrane is proposed to follow the ER-GA-PM pathway [5] and its translocation to the mitochondria from the ER was shown to be ATP-dependent [21]. In contrast, the transport of PE in Chinese hamster V 79 lung cell fibroblasts was shown to be too fast to be mediated by vesicular carriers [22].

Recently, we have used a cell-free system from rat liver to study the trafficking of lipids between the ER and the Golgi apparatus [23,24]. We have shown that lipid transport between these two organelles was similar to that of proteins for the ATP-dependent (vesicular) process [23]. From different cell types and approaches [5,18–24], it is likely that polar heads of phospholipids, lipid–lipid interactions and lipid-protein interactions are potentially involved in lipid sorting and targeting. The results presented in this paper suggest that lipid sorting could also be operational at another level, i.e. the fatty acyl moieties of the lipids.

We have found that monensin blocked predominantly VLCFA-containing lipids. The results suggest a role for chain length of the fatty acyl moieties in the export of these molecules through the Golgi apparatus to the plasma membrane. In many, if not all cells, the thickness of the plasma membrane is higher than any other intracellular membrane [1,2]. VLCFA-containing lipids targeted to the plasma membrane may be a contributing factor to this increase in membrane thickness. We propose, as well, that the fatty acyl chains of lipids could function as a sorting signal to help ensure their delivery to the plasma membrane. This exciting hypothesis needs further investigations which are under way in our laboratory.

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References

1 Morré, D.J. and Mollenhauer, H.H. (1974) in Dynamic Aspect of Plant Ultrastructure (Robards, A.W., ed.), pp. 84–137, Mc Graw-Hill, London.
2 Morré, D.J., Kartenbeck, J. and Franke, W.W. (1979) Biochim. Biophys. Acta 559, 71–152.
3 Farquhar, M.G. (1985) Annu. Rev. Cell Biol. 1, 447–488.
4 Griffiths, G. and Simons, K. (1986) Science 234, 438–443.
5 Van Meer, G. (1989) Annu. Rev. Cell Biol. 5, 247–275.
6 Benveniste, P. (1986) Annu. Rev. Plant Physiol. 37, 275–308.
7 Benveniste, P. and Hartmann, M.A. (1987) Methods Enzymol. 148, 632–650.
8 Cassagne, C. and Lessire, R. (1978) Arch. Biochem. Biophys. 191, 146–152.
9 Moreau, P., Bertho, P., Juguelin, H. and Lessire, R. (1988) Plant Physiol. Biochem. 26, 173–178.
10 Moreau, P. Juguelin, H., Lessire, R. and Cassagne, C. (1988) Phytochemistry 27, 1631–1638.
11 Moreau, P., Juguelin, H., Lessire, R. and Cassagne, C. (1986) Phytochemistry 25, 387–391.
12 Bertho, P., Moreau, P., Juguelin, H., Gautier, M. and Cassagne, C. (1989) Biochim. Biophys. Acta 973, 91–96.
13 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
14 Juguelin, H. and Cassagne, C. (1984) Anal. Biochem. 142, 329–335.
15 Bertho, P. (1991) Ph.D. Thesis, Université de Bordeaux 2, Bordeaux.
16 Lepage, G. and Roy, C.C. (1986) J. Lipid Res. 27, 114–120.
17 Lessire, R., Juguelin, H., Moreau, P. and Cassagne, C. (1985) Arch. Biochem. Biophys. 239, 260–269.
18 Simons, K. and Van Meer, G. (1988) Biochemistry 27, 6197–6202.
19 Wattenberg, B.W. (1990) J. Cell Biol. 111, 421–428.
20 Sleight, R.G. (1987) Annu. Rev. Physiol. 49, 193–208.
21 Voelker, D.R. (1990) J. Biol. Chem. 265, 14340–14346.
22 Sleight, R.G. and Pagano, R.E. (1983) J. Biol. Chem. 258, 9050–9058.
23 Moreau, P., Rodriguez, M., Cassagne, C., Morré, D.M. and Morré, D.J. (1991) J. Biol. Chem. 266, 4322–4328.
24 Moreau, P. and Morré, D.J. (1991) J. Biol. Chem. 266, 4329–4333.