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Low Temperature-Induced Alterations in the Chloroplast and Microsomal Membranes of *Dunaliella salina*[1]

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ABSTRACT

The metabolic regulation of membrane lipid composition has been examined using the cell wall-less, unicellular green alga *Dunaliella salina* (UTEX 1644) as a model system. Low temperature stress was employed to initiate and study the regulatory response.

When cultures growing logarithmically at 30°C were chilled to 12°C, cell division ceased for approximately 100 hours, and then the cells resumed logarithmic growth at a slower rate. The phospholipid, glycolipid and protein content, on a per cell basis, was, in each case, approximately 20% higher in cells grown at 12°C. The volume of the 12°C-acclimated cells was 2.8 times that of 30°C-grown cells. The quantity of chloroplast membrane, as determined by morphometric analysis, was 20% greater, whereas the content of microsomal membrane material was more elevated, being approximately 2.8 times that of 30°C-grown cells.

Lipid compositional analyses were carried out on purified chloroplasts and microsomes isolated from *Dunaliella* grown at 30 and 12°C and also from cells 12 and 60 hours following a shift from 30 to 12°C. In both chloroplast and microsomal phospholipids fatty acid unsaturation increased during acclimation to low temperature. Generally, microsomal phospholipids responded more quickly and to a greater extent than did chloroplast phospholipids. Despite these alterations, little change in the relative proportions of phospholipid classes was observed in either cell fraction.

In sharp contrast to the pattern of phospholipid change, chloroplast glycolipids responded to low temperature by significantly increasing the proportion of one specific class, digalactosyl diglycerides, relative to monogalactosyl diglycerides, while showing minimal change in fatty acid distribution within any given glycolipid class.

The ease and rapidity with which *Dunaliella* cells can be manipulated with respect to environmental stress and isolation of intact cell organelles makes it particularly well suited for research on intermembrane lipid dynamics within the plant cell.

In recent years, much progress has been made in understanding the lipid metabolism, physical properties, and physiological role of biological membranes (25). Experimental evidence, derived mainly from microorganisms and higher animals, strongly suggests that the physiological functioning of a membrane is dependent upon its physical state (fluidity) and that the physical state is itself a function of the membrane lipid composition (24).

Progress toward understanding the role of lipids in determining properties of plant membranes has been more difficult for several reasons. The cellular heterogeneity of plant tissues and the presence of a rigid wall around most cells hinder cell fractionation and isolation of homogeneous membrane preparations for examination of their specific chemical and physical properties. To further complicate matters, plant cells contain two major lipid biosynthetic compartments, the chloroplasts and the endoplasmic reticulum (17, 23, 26). The two function interdependently by as yet poorly defined mechanisms to regulate membrane lipid composition (21, 23, 26).

We have undertaken a comprehensive study of membrane metabolism and physical properties in the eukaryotic plant cell, using the unicellular green alga *Dunaliella salina* as a model system. This alga has many unique properties which make it a useful experimental system. For example, it grows rapidly under axenic conditions to yield a population of very homogeneous cells. Being naturally wall-less, *Dunaliella* is easily disrupted, facilitating cell fractionation. The lipid composition of *Dunaliella* is typical of green algae and similar to that of higher plants. It is able to tolerate a wide range of temperatures and salinities (6), making it useful for investigating the role of the membrane in response to environmental stresses.

A certain amount of information concerning the lipids of *Dunaliella* is already available (13). In this communication we report additional data of the type needed for a detailed analysis of intracellular membrane lipid dynamics. We have employed a shift in growth temperature as a means of altering membrane properties and lipid metabolism. Although a thorough understanding of the effects of low temperature on plant cells is lacking, it is clear that the cell membranes and their constituent lipids change in response to chilling (14). Studying the rate and the extent of lipid change in chloroplasts and microsomes during low temperature acclimation affords a sensitive tool for identifying the contributions of the two lipid synthesizing organelles to the plant cell's membrane economy.

MATERIALS AND METHODS

Culture Conditions. Axenic cultures of *Dunaliella salina* (UTEX 1644) were grown in synthetic medium composed of the following: NaCl, 1.7 M; $MgSO_4$, 10 mM; KCl, 10 mM; $CaCl_2$, 2.5 mM; NaH_2PO_4:Na_2HPO_4 70:30 (M/M), 2 mM; $NaNO_3$, 2 mM; H_3BO_3, 100 μM; Na_2EDTA, 40 μM; $FeCl_3 \cdot 6H_2O$, 12 μM; $MnCl_2 \cdot 4H_2O$, 8 μM, $ZnCl_2$, 0.8 μM; $Na_2MoO_4 \cdot 2H_2O$, 0.6 μM; $CuSO_4 \cdot 5H_2O$, 0.2 μM; and $CoCl_2 \cdot 6H_2O$, 0.17 μM. Cultures were grown under continuous light (50 μE m^{-2} s^{-1}) in 1-L Erlenmeyer flasks containing 500 ml medium bubbled with 0.5% CO_2-enriched air at either 30 or 12°C. In temperature shift experiments the culture temperature was decreased over 2.5 h. Cell population density was measured using a Coulter Counter model ZB.

Cell Fractionation. Cells in middle to late logarithmic growth phase (1×10^6 cells ml^{-1}) were harvested by centrifugation at 365*g* for 7 min and resuspended in cold 400 mM mannitol/ 25 mM Tris-HCl (pH 8.2), 2 mM EDTA, 1 mM $MgCl_2$. As observed by phase microscopy, the cells became more spherical, but the flagella

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remained active. The concentrated cell suspension (20–50 × 10^6 cells ml^{-1}) was placed in a cold Parr cell disruption bomb and equilibrated for 10 min at 120 lb in^{-2} N_2 gas. The suspension was released from the bomb at a flow rate of 200 ml min^{-1}. This treatment resulted in 90 to 95% cell breakage as estimated by microscopic observation.

The suspension was then centrifuged at 2,000*g* for 3 min. The resulting pellet (designated the chloroplast fraction) was highly enriched in chloroplasts (Fig. 3A). Centrifugation of the 2,000*g* supernatant at 20,000*g* for 15 min yielded a pellet (intermediate fraction) containing mitochondria, Golgi structures, flagella, and other membrane components (not shown). The 20,000*g* supernatant was centrifuged at 98,000*g* for 90 min to yield a homogeneous microsomal pellet (Fig. 3B).

Lipid Extraction and Chemical Analysis. Lipids were extracted from whole cells and cell fractions using the procedure of Bligh and Dyer (5). Lipids were separated into classes (NL, GL, PL)[2] by silicic acid chromatography (22). TLC of polar lipids was routinely performed on silica gel H in the solvent system $CHCl_3$:acetic acid:methanol:H_2O (70:25:5:2.2, v/v/v/v). Individual lipids were identified by their TLC mobility, by specific spray reagents, and by comparison with authentic standards. Chl content was determined spectrophotometrically (2). Total GL content was quantified according to Dubois (9). After separation by TLC, individual GL were quantified in the presence of silica gel (20). Lipid phosphorus was determined using the procedure of Bartlett (3) following digestion with $HClO_4$ (15). Individual PL separated by TLC were quantified using the method of Rouser *et al.* (22).

The DGTH content was estimated from the data of lipid composition. Towards this end, the fatty acids 16:1 of PG and 14:2 of PE were used as internal standards for the chloroplast and microsomal fractions, respectively. For example, knowing the relative proportion of 16:1 in PG (Fig. 4) and the PG content of the chloroplast fraction (Table III), the expected 16:1 content in total PL could be calculated. This value was compared to the actual content of 16:1 obtained in a column chromatographic fraction containing total PL + DGTH (Table IV). The relative difference was an approximation of DGTH content in chloroplasts. A similar analysis was performed to obtain an estimation of DGTH content in microsomes.

GLC of fatty acid methyl esters (18) was performed as described previously (19). Identification of fatty acids was achieved by GC-MS analysis and by comparison with standards before and after catalytic hydrogenation (1).

Protein content was determined using Folin-Ciocalteu reagent (12).

Electron Microscopy and Morphometric Techniques. Whole cells were fixed in growth medium containing 2% glutaraldehyde. Each cell fraction was fixed as a pellet in buffered mannitol (above) containing 2% glutaraldehyde. Samples were washed, postfixed in OsO_4, dehydrated in an ethanol-acetone series and embedded in low-viscosity embedding medium. Thin sections were poststained with uranyl acetate and lead citrate.

For morphometric analysis, cell profiles were photographed at × 4,200 and printed at a magnification of × 6,300. The micrographs were used to determine the percent relative volume (V_v) and the surface density (S_v) of the cellular components using a coherent multipurpose test system as described by Weibel (27). Briefly, V_v was obtained directly from the ratio of points falling on a particular component to points falling on protoplasm. S_v was obtained by determining the number of intersections of membrane components with lines of the test system and relating that to the total number of points (or, more precisely, to the total length of line) falling in the protoplasm. Sufficient points required to generate a standard error of ten percent or less for components comprising more than five percent relative volume were counted. Standard errors were obtained by randomly grouping data from individual cell profiles. Other more complicated statistical procedures were employed on some data with similar results.

Cell volumes were determined using a calibrated ocular micrometer by measuring the dimensions of live cells in culture medium containing Ficoll to retard mobility.

RESULTS

Effects of Temperature on Growth Rates and Cell Properties. Under the conditions of illumination, CO_2-enrichment and NaCl concentration specified above, *Dunaliella* grew with a generation time of approximately 20 h at 30°C and 80 h at 12°C. At both temperatures, cultures displayed logarithmic growth unil reaching a density of approximately 1.6 × 10^6 cells ml^{-1}, at which point the

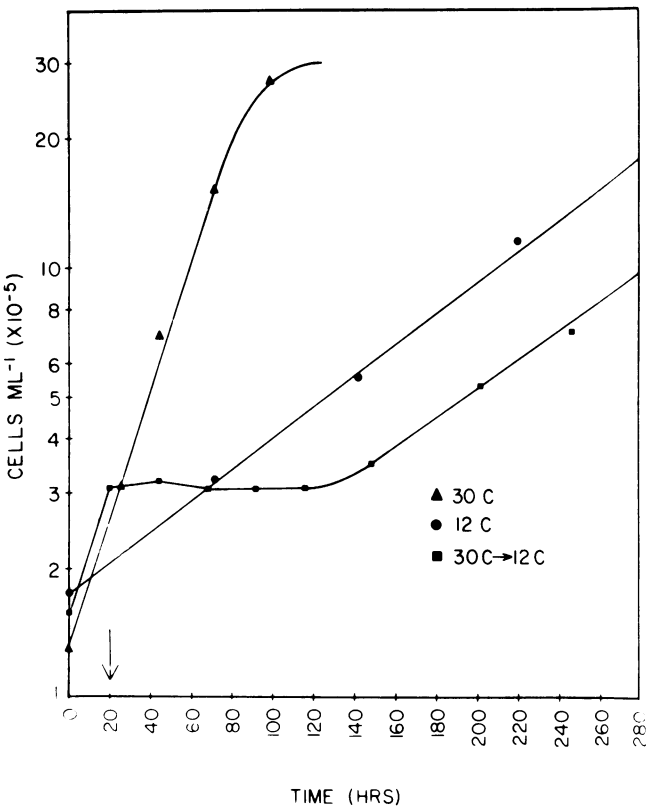

FIG. 1. Effect of temperature on growth rate. The curves marked by triangles and circles represent growth in cultures maintained isothermally at 30 and 12°C, respectively. The curve marked by squares represents actively growing cells shifted from 30 to 12°C. Arrow indicates time of shift.

Table I. *Lipid and Protein Content of Dunaliella Cells Grown at 30 and 12°C*

Values represent mean ± SD of three separate experiments.

Parameter	30°C	12°C
Protein (mg/10^9 cells)	112. ± 4	127. ± 2
PL (μmol/10^9 cells)	7.74 ± 0.3	9.68 ± 0.3
GL (μmol/10^9 cells)	23.9 ± 1.1	28.9 ± 2.8
Chl (μmol/10^9 cells)	7.10 ± 0.1	5.48 ± 0.7
Chl *a*/*b*	2.77 ± 0.2	4.09 ± 0.3

[2] Abbreviations: NL, neutral lipid(s); GL, glycolipid(s); L, phospholipid(s); DGTH, diacylglyceryltrimethylhomoserine; PG, phosphatidylglycerol; PE, phosphatidylethanolamie; CL, cardiolipin DGDG, digalactosyl diglyceride; PC, phosphatidylcholine; Mgdg, monagalactosy diglyceride; PI, phosphatidylinositol; SL, sulfolipid(s).

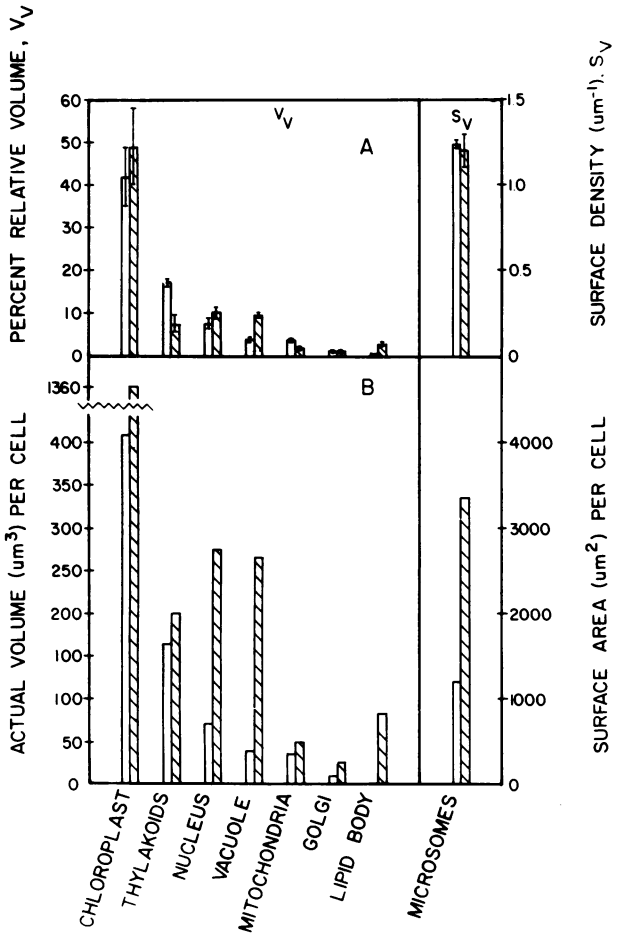

FIG. 2. Morphometric analysis of cells grown at 30°C (open bars) and 12°C (hatched bars). A, relative percent volume (V_v) of protoplasm occupied by cell organelles and surface density (S_v) of microsomal components. B, actual volumes and surface area. Values were obtained by multiplying V_v and S_v by cell volumes given in text.

growth rate began to decrease, so that the cultures reached a stationary phase at a density of 2.5 to 3 × 10^6 cells ml^{-1}. When cultures maintained at 30°C were shifted to 12°C, cell division ceased for approximately 96 h (Fig. 1). Following this lag, cell division resumed, with a generation time of 80 h.

A comparison of lipid and protein content of cells grown at 30 and 12°C is given in Table I. These values were obtained from cultures growing logarithmically at 30°C or from cultures which had resumed logarithmic growth following a shift in growth temperature, *i.e.* 120 h to 180 h after the shift to 12°C. The protein, PL, and GL content, on a per cell basis, were all higher by 15 to 25% in the 12°C-grown cells. Chl content was 30% lower in 12°C cultures. The ratio of Chl *a* to Chl *b* was 50% higher in cells grown at 12°C.

Such changes in the chemical content of the cells with temperature would suggest possible alterations in cell structure. To determine if structural changes were occurring, cell volumes were measured and morphometric analyses were performed.

Cell dimensions were obtained using an ocular micrometer. Cells grown at 30°C were generally pear-shaped or ellipsoidal, while those grown at 12°C were usually spherical. Therefore, in generating values for cell volume, the equations for relating diameters to volumes of a prolate spheroid and sphere, respectively, were employed. The cell volume of 30°C-grown cells was found to be 976 ± 89 μm^3, while that of the 12°C-grown cells was 2,775 ± 208 μm^3, representing a 2.8-fold increase.

A quantitative ultrastructural comparison of cells grown at the two temperatures was performed using morphometric techniques. This permitted the relative percent volume of protoplasm (V_v) occupied by certain structures and, in some cases, the surface area per unit volume of protoplasm (S_v) of particular cell components and microsomal constituents to be determined (Fig. 2A). The relative volumes occupied by the chloroplast, nucleus and Golgi apparatus did not change with temperature. The relative volumes of chloroplast thylakoid material and mitochondria decreased in 12°C-grown cells whereas the vacuolar and lipid body volumes increased. The surface density of microsomal components remained proportionally the same at both temperatures.

By multiplying the relative volumes by the cell volumes, the absolute volumes of the cell components were determined (Fig. 2B). Although the actual volume of chloroplasts increased dramatically with low temperature, the chloroplast membrane material (thylakoids) increased by only 20%. The difference in chloroplast volume was occupied by storage material, *i.e.* starch (data not shown). This small increase in chloroplast membrane material was in accord with the approximately 20% increase in GL content (Table I) of 12°C-grown cells. The quantity of microsomal material increased from approximately 1,200 μm^2/cell at 30°C to approximately 3,360 μm^2/cell at 12°C. These changes in the relative amounts of chloroplast membrane and microsomal membrane with temperature are reflected in the chemical analysis of isolated fractions (see below).

Cell Fractionation. As a first step in determining the role of the two lipid synthesizing compartments, the chloroplast and the microsomes, during acclimation to low temperature, we attempted to isolate the two components for lipid characterization.

Because *Dunaliella* lacks a cell wall, cell disruption was greatly facilitated. Exposing cells in 400 mM mannitol to a relatively mild drop in pressure resulted in almost complete cell breakage and a high yield (up to 70%) of intact chloroplasts.

Centrifugation of the cell homogenate at 2,000*g* yielded a pellet, designated the chloroplast fraction, which contained primarily intact chloroplasts, with a few broken chloroplasts and traces of other cellular contamination as determined by electron microscopy (Fig. 3A). This chloroplast fraction was utilized without further purification. Centrifugation of the supernatant at 20,000*g* resulting in a pellet (not shown) containing mitochondria, Golgi bodies, flagella and unidentified vesicles. In the case of 12°C-grown cells, this intermediate fraction also contained chloroplast fragments. The 20,000*g* supernatant was centrifuged at a higher speed to obtain a microsomal fraction composed of a homogeneous population of small vesicles (Fig. 3B). Mitochondrial or flagellar contamination was absent.

The relative proportions of various lipid classes in these three cellular fractions isolated from 30°C-grown cells, 12°C-grown cells, and from cells harvested 12 and 60 h following a shift in growth temperature from 30 to 12°C are shown in Table II. The percentage of total cellular Chl recovered in the chloroplast fraction decreased steadily following the temperature shift and was only 84% in 12°C-grown cells. The concomitant appearance of Chl in the intermediate fraction correlated with the increased fragility of the chloroplasts at low temperature, as observed by microscopy. A similar pattern was observed in the distribution of GL.

The quantitative distribution of PL in the fractions changed dramatically with growth temperature. The 3-fold increase in microsomal PL content in 12°C-grown cells as compared to that in 30°C-grown cells is in agreement with morphometric data (Fig. 2B), which demonstrates an enrichment in microsomal membrane material relative to the amount of chloroplast membrane material.

The molar ratios of Chl to GL to PL in the isolated fractions showed relatively few differences among 30°C-grown, 12°C-grown, and shifted cells. The low Chl content of chloroplasts

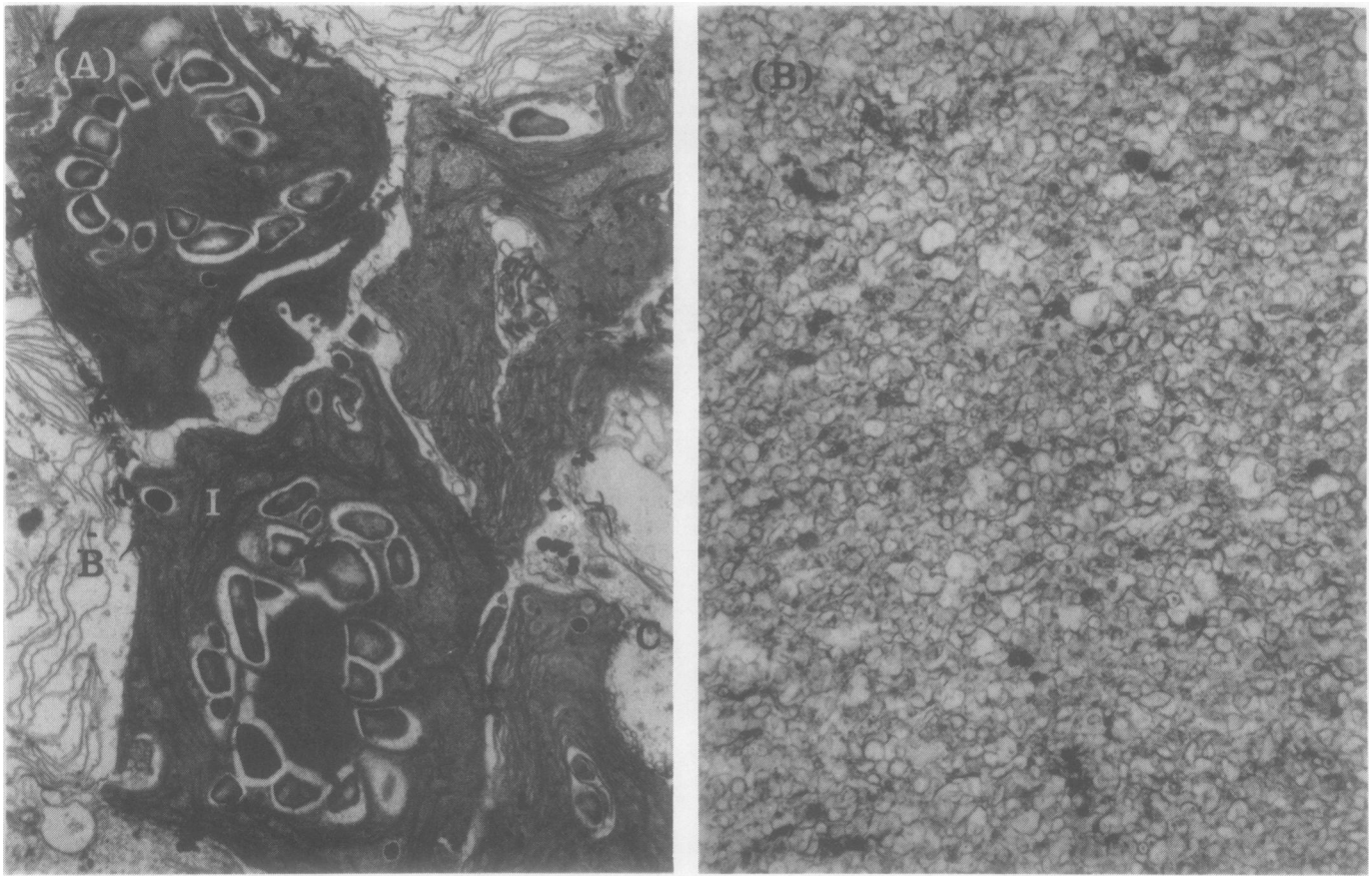

FIG. 3. Electron micrographs of the chloroplast fraction (A) and microsomal fraction (B) obtained as described in "Materials and Methods." A, chloroplast fraction contains intact chloroplasts (I), broken chloroplasts (B), and some cellular contamnation (C). × 7,500. B, microsomal fraction contains homogeneous small vesicles free of contaminating mitochondria and flagella. × 7,500.

Table II. *Lipid Analysis of Cell Fractions from Cells Grown at 30°C, 12°C, or Following a Shift in Growth Temperature*

Values represent mean of two or three separate experiments.

Fraction Analyzed	Chl[a]	GL	PL	Chl:GL:PL[b]
		%		*ratio*
30°C-grown cells				
Chloroplast	100	94.0	71.0	1.6:5.5:1
Intermediate	ND[c]	4.2	17.0	—:0.6:1
Microsomes	ND	1.7	8.8	—:0.5:1
30°C→12°C (12 h)				
Chloroplast	99.6	92.8	54.4	1.6:6.4:1
Intermediate	0.4	6.0	33.0	0.01:0.7:1
Microsomes	ND	1.3	9.7	—:0.5:1
30°C→12°C (60 h)				
Chloroplast	94.2	80.9	46.8	1.5:6.1:1
Intermediate	5.3	15.5	33.9	0.1:1.6:1
Microsomes	0.4	3.6	19.4	0.02:0.7:1
12°C-grown cells				
Chloroplast	84.0	73.6	47.8	0.8:4.1:1
Intermediate	12.9	20.7	28.0	0.2:2.0:1
Microsomes	3.2	5.8	24.2	0.06:0.6:1

[a] Values are given for Chl, GL, and PL as % of total amount recovered in each case.

[b] M ratio in each cell fraction.

[c] Not detected.

isolated from 12°C-grown cells reflects the 50% decrease in the Chl to PL ratio in whole cells (Table I). The apparent contamination of microsomal PL by chloroplast PL was never more than 5 to 7%, as estimated from the amount of Chl and GL appearing in the microsomes. The apparent contamination of the chloroplast fraction, as determined by the presence of CL or by the appearance of other organelles in electron micrographs, was low, certainly less than 10 to 15%.

Table III. *Lipid Composition of Whole Cells, Chloroplasts (CHLP), and Microsomes (MIC)*

Values represent the mean of two to four analyses from one to three separate experiments.

Lipid	30°C			12°C		
	Cell	CHLP	MIC	Cell	CHLP	MIC
			mol % PL			
PI	11	8	13	12	12	15
PC	25	31	32	20	24	28
PG	37	46	15	34	47	17
PE	15	15	41	25	17	40
CL	3	tr	0	3	tr	0
			mol % GL			
SL	12	15		18	14	
DGDG	21	19		27	28	
MGDG	67	66		55	58	

Alterations in Lipid Composition during Acclimation. The polar lipid composition of whole cells, isolated chloroplasts, and microsomes is shown in Table III. Analysis of PL from whole cells grown at 30 and 12°C indicated a decrease in PC and PG, and a substantial increase in PE with low temperature. However, these

Table IV. *Fatty Acid Composition of Total PL (plus DGTH) from Chloroplasts and Microsomes*

Growth temperature regimes are described in the text. Values represent mean ± SD of eight to ten analyses from three separate experiments.

Fatty Acid	30°C	30°C→12°C (12 h)	30°C→12°C (60 h)	12°C
		Chloroplasts		
14:2	2.6 ± 0.4	2.4 ± 0.3	1.5 ± 0.4	1.5 ± 0.3
16:0	24.8 ± 1.2	22.4 ± 1.5	22.4 ± 0.5	25.9 ± 1.9
16:1	17.2 ± 1.4	18.4 ± 3.3	16.6 ± 2.2	12.1 ± 1.1
18:1	8.8 ± 0.8	11.1 ± 0.4[a]	11.6 ± 0.9	13.0 ± 0.7
18:2	26.0 ± 1.2	25.5 ± 0.7	23.9 ± 0.3	19.6 ± 1.1
18:3 (γ)	2.9 ± 0.2	3.0 ± 0.1	5.4 ± 0.8	7.0 ± 0.3
18:3 (α)	16.8 ± 1.2	15.4 ± 0.6	17.9 ± 0.8	20.0 ± 1.1
		Microsomes		
14:2	8.5 ± 0.4	6.0 ± 0.2	4.4 ± 0.7	3.7 ± 0.6
16:0	43.5 ± 0.8	37.7 ± 1.7	37.2 ± 1.8	36.6 ± 3.1
18:1	11.5 ± 0.5	16.8 ± 0.9	15.3 ± 0.7	14.6 ± 0.6
18:2	21.7 ± 0.6	21.8 ± 1.9	22.2 ± 0.4	18.8 ± 0.4
18:3 (γ)	3.4 ± 0.2	4.3 ± 0.7	7.9 ± 0.5	9.9 ± 0.7
18:3 (α)	7.8 ± 0.3	7.7 ± 0.5	9.4 ± 0.2	12.7 ± 1.1

[a] Underlined values differ significantly ($t_\alpha = 0.05$ one-tailed) from 30°C value.

changes were not reflected in the analytical data for isolated microsomes and chloroplasts. This apparent discrepancy can be explained by the increased quantity of microsomal membranes (which are enriched in PE) in the 12°C-grown cells.

The polar lipid DGTH, present in the PL fractions of chloroplasts and microsomes, could not be quantified by the routine phosphorus analysis employed since it lacks phosphorus (for structure see ref. 19). Employing the procedure described in "Material and Methods" using specific fatty acids as internal standards, the chloroplast DGTH content was estimated to be approximately 25% of the chloroplast PL content. The microsomal DGTH content was determined to be between 15 and 20% of the microsomal PL content.

The relative proportions of cellular GL responded to temperature change in a more dynamic fashion than did those of PL. In extracts of whole cells and of isolated chloroplasts, the main site of GL localization, the relative content of DGDG increased at low temperature while that of MGDG decreased (Table III). Thus, the ratio of MGDG to DGDG decreased from 3.4 in 30°C chloroplasts to 2.1 in 12°C chloroplasts. The alteration in the MGDG to DGDG ratio occurred mainly between 12 and 60 h following a shift to 12°C (data not shown). The sulfolipid content showed no significant changes.

The degree of phospholipid fatty acid unsaturation increased in both cellular compartments at low temperature (Table IV). The major changes in the fatty acid composition of chloroplast PL occurred in the C_{18} fatty acids, where increases in 18:1 and 18:3 were partially offset by a decrease in 18:2. The majority of changes occurred later than 60 h following a temperature shift.

The C_{14} and C_{16} fatty acids as well as the C_{18} fatty acids of the microsomal PL changed with temperature. In fact, 14:2 and 16:0 decreased within 12 h following a temperature shift. The trienoic C_{18} fatty acid content increased significantly between 12 and 60 h following a shift in growth temperature. By the time cells had resumed logarithmic growth at low temperature, the percentages of all major fatty acids of microsomal PL were significantly different from the values found in 30°C preparations. In almost every case, the microsomal compartment responded more rapidly and to a greater degree than did the chloroplast compartment. Interestingly, the first C_{18} chain fatty acid to change in both fractions was 18:1, which increased.

The fatty acid compositions of the major individual PL of chloroplast and microsomal fractions of 30 and 12°C-grown cells are shown in Figure 4. Patterns of changes in fatty acids of chloroplast DGTH and microsomal DGTH were qualitatively similar but quantitatively different. Decreases in 16:0 and 18:2, and increases in 18:3 (both isomers) were more pronounced in microsomal DGTH. PC, the other major lipid common to both fractions, showed little change in chloroplasts, whereas in microsomes, 16:0 and 18:2 decreased and 18:1 increased. In the case of chloroplast PG, 16:1 and 18:2 decreased and 16:0 and 18:3 increased with low temperature. Microsomal PE displayed a large decrease in 14:2 and 16:0, whereas 18:1, 18:2, and 18:3 (γ and α) increased.

An analysis of the fatty acid composition of the individual GL revealed marked differences from one GL class to another (Table V). However, the alterations in fatty acid composition in each class during acclimation were small. SL, DGDG, and MGDG all sustained slight decreases in 18:2 and similar increases in 18:3 with low temperature. The fatty acids 16:3 (isomer) and 16:4 of DGDG increased. Such changes were reflected in the fatty acid composition of total GL (data not shown). The fatty acids 16:2 and 16:3 (isomer) of MGDG, consistently present in small amounts in 30°C chloroplasts, were not detectable in 12°C chloroplast MGDG.

DISCUSSION

The lipid composition of *Dunaliella*, as established in these studies, is generally similar to that of other green algae and higher plants. The typical mixture of PL and GL is present (Table III), and these are distributed within the functionally different membrane systems in the usually observed proportions. Some distinctive constituents are present, the most prominent being DGTH, a lipid which has also been reported to occur in several other species of algae (7, 10, 19) and in a pathogenic fungus (29). The fatty acid components of *Dunaliella* lipids are typical of eukaryotic plant cells. Under the axenic culture conditions employed here C_{14}, C_{16}, and C_{18} fatty acids predominate, and these include varying degrees of unsaturation (Table IV, V, Fig. 4). The close resemblance of *Dunaliella's* lipids to those of more complex plants coupled with the manipulative advantages of using this cell in the laboratory have encouraged us to view it as a highly desirable model system for studying plant membrane metabolism.

Plant cells are considerably more complex than their animal counterparts in the sense that they depend heavily upon two cellular compartments, the chloroplast and ER, for the production of membrane lipid constituents. PL are for the most part formed in the ER (16, 21, 24) while GL are believed to arise in the chloroplast (8). The blending of products from these two centers yields the mixed lipid populations characteristic of each membrane type within the cell. Despite considerable study (17), the respective roles of the two synthetic compartments in regulating cellular lipid composition are still largely undefined.

In these initial experiments, we stressed *Dunaliella* by exposing it to low temperature and measured the response in different membranes of the cell. The extent and timing of the changes in lipid composition were statistically significant and confirm the potential of *Dunaliella* as a model for regulatory studies.

The low-temperature-induced changes in lipid composition occurred throughout a period of approximately 96 h, during which there was no cell division. When cell division finally resumed, the 12°C lipid pattern appeared to be fully established, inasmuch as there was no further change for at least another 100 h, except for a slight additional decrease in the Chl content and a sizable rise in the level of carotenoids (D. V. Lynch and G. A. Thompson, Jr., unpublished observations).

It became obvious early in the study that attention must be given to changes in cell structure brought on by low temperature stress. Morphometric analysis of cells grown at high and low

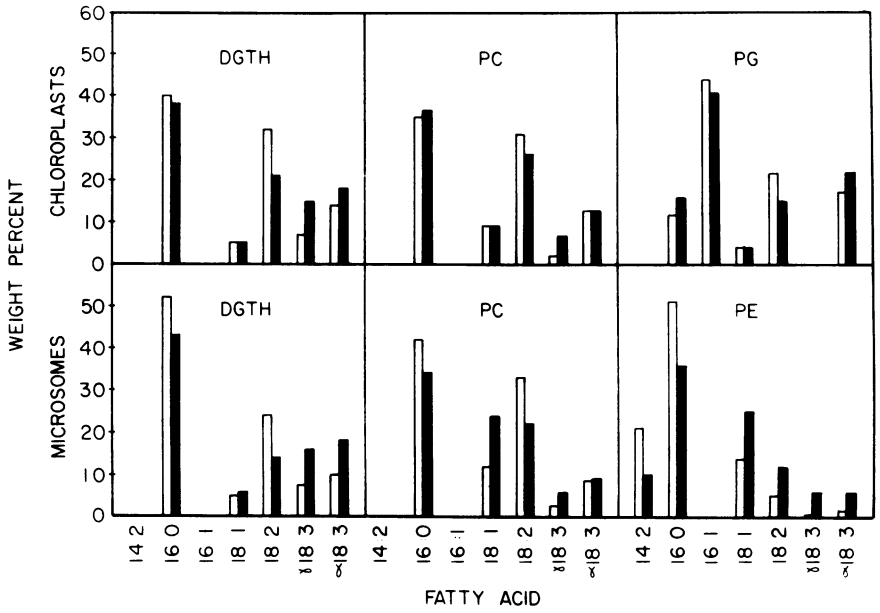

FIG. 4. Fatty acid composition of the major PL and DGTH from chloroplasts and microsomes isolated from 30°C-grown cells (open bars) and 12°C-grown cells (shaded bars).

Table V. *Fatty Acid Composition of DGDG, MGDG, and SL from Chloroplasts*

Growth temperature regimes are described in text. Values expressed as mean ± SD of four to seven analyses from two separate experiments.

Fatty Acid	30°C	30°C→12° C (12 h)	30°C→12° C (60 h)	12°C
		DGDG		
16:0	29.3 ± 0.6	31.1 ± 2.1	27.4 ± 0.4	26.3 ± 2.1
16:2	5.0 ± 0.6	7.1 ± 0.2	6.0 ± 0.1	3.2 ± 0.3
16:3 (isomer)[a]	12.3 ± 1.4	12.4 ± 2.0	15.9 ± 1.0	16.8 ± 1.4
16:4 (+18:1)	4.0 ± 0.3	5.9 ± 0.4	5.9 ± 0.2	8.5 ± 1.7
18:2	9.5 ± 1.5	10.2 ± 0.1	6.5 ± 0.2	3.7 ± 0.4
18:3 (α)	38.6 ± 1.4	31.9 ± 0.7	37.7 ± 0.9	40.8 ± 2.3
		MGDG		
16:0	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	tr
16:2	0.6 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0
16:3	1.8 ± 0.5	2.4 ± 0.4	2.5 ± 0.5	2.6 ± 0.1
16:3 (isomer)	1.0 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	0
16:4	47.8 ± 0.7	46.0 ± 2.1	44.8 ± 0.5	47.4 ± 1.6
18:2	2.8 ± 0.7	3.9 ± 0.5	1.7 ± 0.2	0.8 ± 0.1
18:3 (α)	45.8 ± 1.3	44.3 ± 1.1	50.0 ± 0.7	48.9 ± 2.0
		SL		
16:0	68.1 ± 2.2	73.7 ± 4.4	70.2 ± 3.0	64.6 ± 2.0
18:1	3.4 ± 0.8	2.6 ± 0.3	3.1 ± 0.1	3.8 ± 0.9
18:2	4.5 ± 0.3	3.6 ± 0.3	3.0 ± 0.3	1.9 ± 0.2
18:3 (α)	22.6 ± 0.6	17.5 ± 4.9	26.1 ± 2.2	29.4 ± 2.1

[a] Because of its slightly longer retention time but the same mol wt, this is thought to be a different isomer from the other 16:3 shown.

temperature confirmed a number of sizable differences, including a 2.8-fold increase in cell volume at 12°C. Growth at low temperature also led to (a) a slight increase in the quantity of chloroplast membranes and (b) a dramatic increase in the content of ER-like membranes. These changes explain the increase in PL, especially phosphatidylethanolamine, in total lipid extracts of 12°C-grown cells. Evidence suggesting a pronounced expansion of ER following exposure to low temperature has been reported for a variety of higher plants, including alfalfa, black locust, rye and others (28). By utilizing recently developed stereological techniques of the type employed in the present study, it would now be feasible to determine whether and to what extent a proliferation of ER is a characteristic response of plants to low temperature stress. In theory, an increase in ER would automatically elevate the cells' content of microsomal fatty acid desaturases, possibly contributing to the observed higher level of lipid unsaturation.

A more precise evaluation of the cell's response to low temperature can be gained by analyzing individual membrane types. Because it naturally has no cell wall, *Dunaliella* is ideally suited for cell fractionation studies. Using highly enriched preparations of chloroplasts and microsomal membranes, we were able to characterize the structural lipid composition of these organelles. Significant differences, along the expected lines, were found (Table III–V). Those lipids that were common to both compartments, *i.e.* phosphatidylcholine and DGTH, exhibited organelle-specific differences in their fatty acid complements (Fig. 4). Furthermore, it was possible to detect distinct lipid changes in each compartment as the cells adapted to chilling. Most of the phospholipid-bound fatty acids experienced a sizable increase in unsaturation (Table IV; Fig. 4). Increased unsaturation is well-known to have a fluidizing effect on the parent lipid (11). The temperature-induced lipid changes occurred noticeably faster in microsomal membranes than in chloroplasts (Table IV), presumably because the enzymes catalyzing fatty acid desaturation are situated in the microsomes (25, 28). Studying the detailed temporal patterns of change in PL fatty acid unsaturation of both chloroplasts and microsomes (and in other organelles) promises to be a useful tool in understanding intracellular lipid dynamics.

The GL content of 12°C-grown cells was 20% greater than that of 30°C-grown cells (Table I). This is in close agreement with the 22% increase in chloroplast membrane as determined by morphometry (Fig. 2B).

The ratio of MGDG to DGDG decreased from approximately 3 in chloroplasts from 30°C-grown cells to 2 in chloroplasts from 12°C-grown cells. Physical chemical studies of purified GL have determined that the phase transition temperatures of hydrogenated DGDG is about 30°C lower than that of equivalent MGDG (4). The more bulky digalactosyl group appears to have a strong fluidizing effect not shown by the monogalactosyl moiety. One might therefore expect that the decreasing ratio of MGDG:DGDG

that we observed at low temperature might contribute significantly to the fluidization of the 12°C membrane.

The fatty acid composition of each GL class did not change greatly during low temperature acclimation (Table V). The striking differences in fatty acid composition between MGDG and DGDG remained in the 12°C-grown cells despite a significant fall in the MGDG to DGDG ratio. If this fall indicates an enhanced enzymic conversion of MGDG to DGDG, considerable retailoring of the constituent fatty acids must have also taken place.

The ability to isolate relatively homogeneous cell fractions containing distinctive lipid components is an important first step towards understanding membrane lipid dynamics in the plant cell. The second, and more formidable challenge, will be to correlate the substantial lipid metabolic activities of the chloroplast with those of the ER. These studies are now underway in our laboratory. Based on the preliminary evidence described above that the two synthetic compartments respond somewhat differently to low temperature, the continued use of temperature stress as a way of identifying their respective contributions seems warranted.

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