Altered Chloroplast Structure and Function in a Mutant of Arabidopsis Deficient in Plastid Glycerol-3-Phosphate Aciyltransferase Activity

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ABSTRACT

Mutants of Arabidopsis thaliana deficient in plastid glycerol-3-phosphate acyltransferase activity have altered chloroplast membrane lipid composition. This caused an increase in the number of regions of appressed membrane per chloroplast and a decrease in the average number of thylakoid membranes in the appressed regions. The net effect was a significant decrease in the ratio of appressed to nonappressed membranes. A comparison of 77 K fluorescence emission spectra of thylakoid membranes from the mutant and wild type indicated that the ultrastructural changes were associated with an altered distribution of excitation energy transfer from antenna chlorophyll to photosystem II and photosystem I in the mutant. The changes in leaf lipid composition did not significantly affect growth or development of the mutant under standard conditions. However, at temperatures above 28°C the mutant grew slightly more rapidly than the wild type, and measurements of temperature-induced fluorescence yield enhancement suggested an increased thermal stability of the photosynthetic apparatus of the mutant. These effects are consistent with other evidence suggesting that membrane lipid composition is an important determinant of chloroplast structure but has relatively minor direct effects on the function of the membrane proteins associated with photosynthetic electron transport.

Glycerolipid synthesis in leaves of higher plants is thought to involve two biosynthetic routes designated the 'prokaryotic' and 'eu-karyotic' pathways (20). Fatty acids synthesized de novo in the chloroplasts may either enter the prokaryotic pathway in the chloroplastic envelope or be exported to the endoplasmic reticulum where they are incorporated into lipids through the eukaryotic pathway. In '16:3 plants' such as Arabidopsis thaliana, both pathways are involved in the production of chloroplast lipids. However, the majority of higher plants use the prokaryotic pathway only for the synthesis of phosphatidylglycerol, while the remaining chloroplast lipids are made by the eukaryotic pathway. This class, which includes the most advanced angiosperm genera, are designated as '18:3 plants.' Since 18:3 plants have abandoned the prokaryotic pathway for the synthesis of chloroplast glycerolipids, the question that arises concerns the role of this pathway in 16:3 plants.

We have recently described (10) the isolation and biochemical characterization of a class of mutants of Arabidopsis that lack activity for the first enzyme of the prokaryotic pathway, glycerol-3-phosphate acyltransferase, due to a single nuclear mutation at the act1 locus. This mutation effectively converts a 16:3 plant into a 18:3 plant and offers a unique opportunity to examine the functional significance of the prokaryotic pathway by comparison of isogenic mutant and wild-type plants. As a direct consequence of the deficiency in the prokaryotic pathway, the act1 mutants show specific alterations in the composition of leaf membrane lipids (10). These include a 15 to 20% reduction in phosphatidylglycerol, comparatively minor decreases in the amount of monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and sulfoquinovosyldiacylglycerol content, and minor increases in the amounts of phosphatidylcholine and phosphatidyethanolamine. The mutation also results in greatly reduced levels of 16:3 acyl groups, characteristic of prokaryotic MGD, and a corresponding increase in 18-carbon fatty acids. Since the prokaryotic pathway provides lipids specifically for chloroplast biogenesis, we have focused our investigation on the effects of the changes in membrane composition on chloroplast structure and function in the act1 mutants.

MATERIALS AND METHODS

Plant Material and Growth Conditions

All lines of Arabidopsis described here are descended from the Columbia wild type. The isolation and biochemical characterization of the independently isolated mutant lines JB25 and LK8 has been described (10). Both lines carry a recessive nuclear mutation at a locus designated act1. Before being used for experiments reported here, the lines JB25 and LK8 were backcrossed to the wild type five times and two times, respectively, and individuals with the mutant phenotype were selected from segregating populations. Unless otherwise indicated, plants were grown at 22°C under continuous fluorescent illumination (100–150 µE m−2 s−1) on a perlite:vermiculite:spaghnum mixture irrigated with mineral nutrients (12).

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2 Abbreviations: act1, symbol for a gene controlling the activity of plastid glycerol-3-phosphate acyltransferase; DPH, 1,6-diphenyl-l,3,5-hexatriene; F∞, initial fluorescence; Fv, variable fluorescence; Fm, maximum fluorescence; LHCP, light harvesting Chl a/b protein complex; ω; fatty acid containing n carbons and χ double bonds.
Measurements of Growth Rate

Plants were germinated at 22°C and grown as described above for 7 d. The temperature was then adjusted to that indicated in the text. Samples of four plants were harvested at 3 d intervals for the next 12 d, and their fresh weight was measured. The relative growth rate ($e^{-t}$) was determined as the slope of the natural logarithm of the average fresh weight (of 4 samples in mg) plotted against days since the temperature adjustment.

Extraction and Analysis of Chl, Proteins, and Lipids

Leaves were harvested at the rosette stage (3 weeks of age), and their fresh weight was determined prior to homogenization in cold 20 mM Tricine-KOH (pH 8.4), 5 mM MgCl₂, and 2.5 mM EDTA. Insoluble matter was removed by centrifugation at 100g for 10 min, and aliquots of the extract were used for Chl, protein, and lipid determinations. Lipids were quantified by gas chromatography using a known amount of 14:0-methyl ester as an internal standard. Fatty acid composition of total leaf lipids was determined after preparation of fatty acid methyl esters as described (7).

Isolation of Chloroplast Membranes

Chloroplast membranes were prepared by grinding leaves in cold 450 mM sorbitol, 20 mM Tricine-KOH (pH 8.4), 10 mM NaCl, 10 mM EDTA, and 0.1% BSA. The extract was filtered through Miracloth and centrifuged at 3000g for 5 min. The pellet was washed with 10 mM Hepes (pH 7.9), 10 mM NaCl, and 5 mM EDTA and resuspended in a buffer containing 300 mM sorbitol, 20 mM Hepes (pH 7.9), 10 mM NaCl, 2 mM MgCl₂, 2.5 mM EDTA, and 0.1% BSA. In the membrane preparations for SDS-PAGE the buffers lacked BSA, while MgCl₂ was omitted from the resuspension buffer in some Chl fluorescence measurements and fluorescence polarization measurements, as noted in the text.

Pigment-Protein Electrophoresis

Chloroplast membranes were isolated as described above. Pigment-protein electrophoresis was performed according to the method of Andersson et al. (1), except that the SDS (Sequanal Grade, Pierce, Rockford, IL) to Chl weight ratio was adjusted to 3.75:1.

Two-Dimensional Gel Electrophoresis

[³⁵S]Methionine (1085 Ci mmol⁻¹; 0.5 mCi ml⁻¹) was applied to leaves of 15-d-old plants. After 24 h of illumination, chloroplasts were isolated and the proteins extracted and resolved by two-dimensional electrophoresis as described (8).

Measurements of Relative Fluidity

The relative fluidity of thylakoid membranes was determined by steady state fluorescence polarization (4). DPH (3 mM stock in tetrahydrofuran) was mixed with membranes (50 μg Chl ml⁻¹) to a final concentration of 5 μM and incubated in the dark at room temperature for 40 min. The membranes were then pelleted at 3000g for 5 min and diluted in 100 mM sorbitol, 20 mM Hepes (pH 7.9), 10 mM NaCl to a final concentration of 10 μg Chl ml⁻¹ and 1 μM DPH. The measurements were carried out on an SLM 4048 spectrofluorometer (SLM Instruments, Urbana, IL) in a T-format. Excitation was provided by light at 360 nm with a half-bandwidth of 16 nm. Fluorescence was monitored at 460 nm with a half-bandwidth of 8 nm. The degree of polarization (P) was calculated by an on-line Hewlett-Packard 9825 computer.

Photosynthetic Electron Transport Measurements

Whole chain and PSI-dependent electron transport activities were assayed at 25°C in the presence of 0.1 mM NaN₃, using water and 0.5 mM N, N', N"-tetramethyl-p-phenylenediamine (reduced with 2.5 mM ascorbate) as donors, respectively, by monitoring the O₂ consumption by 0.1 mM methyl viologen in a Rank oxygen electrode. Chloroplast membranes (20–30 μg Chl) were added to the reaction mixture which contained 300 mM sorbitol, 20 mM Hepes (pH 7.9), 10 mM NaCl, 2 mM MgCl₂, 2.5 mM EDTA, 0.1% BSA, 0.1 μM gramicidin-D, and 1 mM NH₄Cl. The PSI assay also contained 1 μM DCMU to inhibit PSII activity and 10 μg mL⁻¹ superoxide dismutase. Saturating white light illumination (1200 μE m⁻² s⁻¹) was provided by a high intensity microscope lamp. PSII mediated 2,6-dichlorophenolindophenol reduction was measured at 580 nm using a Hitachi 100-60 spectrophotometer as described (25).

Room Temperature Chl Fluorescence

Room temperature fluorescence induction transients of isolated chloroplast membranes were measured in the presence of 10 μM DCMU (16). Membranes were diluted in resuspension buffer to a final concentration of 5 μg mL⁻¹ Chl and dark adapted for 5 min before use. The actinic light was provided by a microscope illuminator through a broadband blue optical filter (Corning 4-96), with onset of illumination controlled by an electronic shutter (Vincent Associated, Rochester, NY). Fluorescence was measured through a Corning 2-64 red filter by a photodiode placed 90° to the incident light as described (16). Transients were recorded on a Nicolet Explorer II digital oscilloscope.

Low Temperature (77 K) Chl Fluorescence

Aliquots of chloroplast membranes in resuspension buffer lacking MgCl₂ were diluted to a concentration of 10 μg Chl ml⁻¹ in 60% (v/v) glycerol, and sodium fluorescein was added as an internal standard to a final concentration of 2 mm (9). Samples were then frozen in liquid N₂ in capillary tubes (0.5 mm i.d.). Fluorescence emission spectra were acquired using an SLM 4048 spectrofluorometer. Excitation was provided by light at 440 nm with a half-bandwidth of 4 nm. Fluorescence emission was scanned in 1 nm increments from 470 to 800 nm with a half-bandwidth of 1 nm. Storage and mathematical manipulations of spectra were performed by an on-line Hewlett-Packard 9825 computer.

Gas Exchange

Photosynthetic CO₂ fixation by intact plants was measured as described previously (12).
Electron Microscopy

Rosette leaves of 3-week-old plants were fixed in 2% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h, followed by a 1 h incubation in 1% (v/v) osmium tetroxide in the same buffer. The specimens were dehydrated in a graded ethanol series and embedded in Spurr’s epoxy resin (23). Both fixation steps and dehydration were done at 4°C. Thin sections were stained with uranyl acetate and lead citrate and examined in a JEOL 100CX electron microscope. Quantitative data of chloroplast membrane profiles on electron micrographs were obtained using a map measurer from 20 chloroplast sections from two separate embeddings of wild type and mutant plant material grown at different times.

Chloroplast Copy Number

Chloroplast copy number per cell was determined in isolated protoplasts prepared as described (12). Aliquots (10–20 μL) of protoplast suspension were pipetted on microscope slides and the protoplasts were flattened by the coverslip application, so that the chloroplasts formed a monolayer within cells and could be easily counted.

RESULTS

Effects of Temperature on Relative Growth Rate

To determine the effects of the altered leaf membrane lipid composition on growth of the actl mutant line JB25, the rate of increase in fresh weight of mutant and wild-type plants grown at different temperatures in the range of 10 to 34°C was measured. The optimal growth temperature for both the mutant and the wild type was approximately 25°C, and their relative growth rates were very similar at temperatures ranging from 10 to 28°C (Fig. 1). During the initial period of exposure to temperatures greater than 30°C the mutant JB25 grew more rapidly than the wild type. However, about 6 d after the shift to high temperatures, the growth rate began to decline, and by 12 d both the mutant and wild-type plants were somewhat chlorotic. These results indicate that there are no significant deleterious effects of the actl mutation on the growth of Arabidopsis under laboratory conditions and suggest that the altered lipid composition of the actl mutants may slightly enhance the thermal tolerance of this race of Arabidopsis.

Effects of High Temperature on Stability of Chloroplast Membranes

To examine the thermal tolerance of the actl mutant by additional criteria, the heat-induced changes in steady state Chl fluorescence of mutant and wild-type leaves was measured (Fig. 2). The rise in Chl fluorescence is thought to indicate an inhibition of excitation energy transfer from the LHCP antenna to PSII reaction centers, due to separation of LHCP from the PSII core (3), and is considered a sensitive indication of the photosynthetic membrane stability. The experiment was conducted by heating detached leaves from 25 to 57°C at a rate of 1°C min⁻¹, and continuously measuring the fluorescence (22). The fluorescence yield enhancement was observed at 43°C in wild-type leaves (Fig. 2), whereas the fluorescence

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Effect of temperature on the relative growth rate of wild-type and mutant Arabidopsis. The error associated with ω⁻¹ was estimated as s/Σ(x−x̄)², where s²=Σ(y−ȳ)²−[Σ(x−x̄)(y−ȳ)]²/Σ(x−x̄)²]n−2 and x and y are the means of the time and the ln of fresh weight, respectively. Where the points overlap, the error is indicated on only one side of the line for clarity.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Temperature-induced fluorescence enhancement yield of wild-type and mutant leaves. Plants were grown at 22°C. The arrows indicate estimates of threshold temperatures at which fluorescence is enhanced. Each point represents the mean ± SD (n=3).
did not rise in the mutant until about 45°C. This difference in the threshold temperature suggests greater thermal stability of the chloroplast membranes of the mutant.

The effect of temperature on the rates of photosynthetic electron transport by mutant and wild-type chloroplast membranes was measured by incubating thylakoid membranes in darkness for 10 min at various temperatures ranging from 25 to 45°C (Fig. 3). The activities of both mutant and wild-type membranes rapidly declined following incubation at temperatures above 30°C. Similar results were observed when thylakoid membranes were preincubated in darkness for various times at 40°C, then assayed for activity at 25°C (results not presented). Although the membranes of the mutant were consistently more resistant than the wild type to thermal inactivation, the difference was very small.

Membrane Fluidity

It has been suggested that the high proportion of polyunsaturated fatty acids in thylakoid membranes plays a major role in maintaining an extremely fluid matrix necessary for lateral movement of photosynthetic components (18). To determine whether the absence of 16:3 and concomitant changes in fatty acid composition in the act1 mutants changed thylakoid membrane fluidity, fluorescence polarization measurements were made on isolated chloroplast membranes containing the hydrophobic fluorophore DPH. As with similar measurements made on the membranes from other mutants with altered lipid composition (12), there were no significant differences between the polarization values obtained with thylakoid membranes from mutant line JB25 and wild type at any temperature in the range of 0 to 50°C (results not presented).

Effects of Membrane Lipid Composition on Chl and Protein Content

Under all growth conditions examined (i.e. growth at various temperatures in the range of 10–34°C and 100–200 μE m⁻² s⁻¹), leaves of the mutant JB25 exhibited a 9% reduction in Chl per unit fresh weight and a similar decrease in the amount of lipid in leaf cells (Table I). In order to determine if the reduced Chl content was due to the act1 mutation, 60 F₂ plants from a cross of WT × JB25 were scored for Chl content and lipid composition. All of the 16 plants with fatty acid composition characteristic of the mutant exhibited the reduced Chl content, whereas all of the 44 plants with normal fatty acid composition had normal Chl content. Cosegregation of the two phenotypes, and persistence of both phenotypes during five backcrosses of the mutant to the wild type, indicate that the reduction in Chl content was due to the act1 mutation. The same conclusion was reached from a similar analysis of the independent allelic mutant LK8 (results not presented).

All the Chl present in higher plants is thought to be bound to proteins of the thylakoid membrane (11). Therefore, a decrease in Chl content suggests a reduction in the amount of one or more Chl-protein complexes. The mutant exhibited a loss of about 7% of Chl a and about 13% of Chl b. Since LHCP does not accumulate in the absence of Chl b, a 13% decline in the amount of Chl b would be expected to lead to the loss of a comparable proportion of LHCP. Furthermore, assuming that LHCP normally contains equal amounts of Chl a and Chl b, most of the reduction in amount of Chl a can be attributed to reduction in amount of LHCP.

To examine whether the reduction in Chl was associated with a specific Chl-protein complex, the Chl-protein complexes from the mutant and the wild-type chloroplast membranes were separated on nondenaturing SDS-polyacrylamide gels. However, there were no apparent differences in the amount of Chl associated with any of the Chl-containing bands (results not presented). Chloroplast membrane polypeptides of the mutant and wild type were also separated by two-dimensional SDS-PAGE. No consistent differences between the two genotypes could be resolved by this approach (results not presented).

Photosynthetic Characteristics

In an attempt to establish whether the wild-type lipid composition is specifically required to support maximal photosynthetic rates, measurements were made of the rates of net photosynthetic CO₂ fixation by mutant and wild-type plants and of the rates of electron transport by isolated thylakoid membranes from both genotypes. CO₂ fixation rates of JB25 and wild-type plants were 5.4 ± 0.2 and 5.5 ± 0.3 μg CO₂·mg fresh weight⁻¹·h⁻¹, respectively, when measured at saturating light intensity (300 μE m⁻² s⁻¹). The rates of electron transport by thylakoid membranes were higher in the mutant than the wild-type preparations at all light intensities, when expressed on a Chl basis (Fig. 4). The apparent increase in electron

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**Figure 3.** Effect of temperature on whole chain photosynthetic electron transport of thylakoid membranes from wild-type and mutant Arabidopsis. Activity is expressed relative to that obtained with membranes preincubated in darkness at 4°C for 10 min. The maximal rates for the mutant and wild type were 169.4 μmol O₂·mg Chl⁻¹·h⁻¹ and 156.7 μmol O₂·mg Chl⁻¹·h⁻¹, respectively. Each point represents the mean ± so (n=4).

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<table>
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<th>Measurement</th>
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<tr>
<td>Chl/fresh weight (mg/g)</td>
<td>1.53 ± 0.08</td>
<td>1.40 ± 0.05</td>
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<td>Chl a/b</td>
<td>2.93 ± 0.06</td>
<td>3.12 ± 0.08</td>
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<tr>
<td>Lipid/Chl (g/g)</td>
<td>2.48 ± 0.07</td>
<td>2.52 ± 0.01</td>
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transport rates was roughly proportional to the decreased Chl content of the mutant leaves, suggesting that it reflects a decrease in the number of antenna Chl associated with the reaction centers. The observation that PSII activity was increased to a lesser extent than PSI in the mutant suggests that there may also have been a slight reduction in the amount of PSII reaction centers in the mutant.

Chl Fluorescence Measurements

The kinetics of induction of room temperature fluorescence provides a measure of time required for the turnover of all the PSII reaction centers and reflects the number of Chl molecules associated with these reaction centers (15). The shape of the room temperature fluorescence transients obtained from JB25 and wild-type thylakoid membranes were indistinguishable (results not presented). There was also no difference in variable fluorescence values (expressed at FV/Fm) (Table II). These results considered in conjunction with the similar PSII electron transport rates of mutant and wild-type plants suggest that their PSII antennas are structurally and functionally indistinguishable.

Low temperature (77 K) Chl fluorescence emission spectra were used to compare the excitation energy distribution between Chl-containing components of thylakoid membranes isolated from mutant JB25 and wild type. In chloroplast membranes of higher plants, the component with an emission maximum at 685 nm is ascribed to LHCP of PSII, the 695 nm emission is attributed to PSII reaction center complex, and the fluorescence emitted at 734 nm originates from PSI (15). The F685/F734 ratio obtained with chloroplast membranes from the mutant was higher than the wild type (Table II) in the presence or absence of MgCl2. By normalizing to the magnitude of emission from the internal standard, fluorescein, it was apparent that the change in F685/F734 ratio is caused by lower fluorescence emission at 734 nm, accompanied by increased Chl fluorescence at 685 nm. The same result was obtained using the independently isolated mutant line LK8 (Table II). This result is consistent with decreased transfer of excitation energy from LHCP to PSI in the mutant.

When chloroplast membranes were prepared and assayed in the absence of Mg2+, the F685/F734 ratio decreased due to a reduction in thylakoid stacking and, presumably, a corresponding reduction in lateral asymmetry. In this respect, the presence or absence of Mg2+ had a much smaller effect in the mutants than in the wild type (Table II). This observation is consistent with other evidence, presented below, which indicates that there is a reduced amount of structure (e.g. stacking) in the thylakoid membranes of the mutant.

Chloroplast Ultrastructure and Number

To examine the effects of changes in leaf lipid composition in the act1 mutants on chloroplast structure, a morphometric analysis was compiled from electron micrographs of thin sections of chloroplasts from the wild type and the allelic mutant lines JB25 and LK8. Even without a detailed analysis,
differences were apparent in the arrangement of chloroplast membranes between the two genotypes (Fig. 5). Although the total amount of membrane was the same in mutant and wild-type chloroplasts, the average number of thylakoid membranes per granum was decreased from 6.2 in the wild type to 3.8 in the mutant (Table III). This change was accompanied by a 65% increase in the number of grana per chloroplast. As a result, the amount of nonappressed membrane was about 30% higher in the mutants. The net effect of these changes was a decrease in the overall ratio of appressed to nonappressed membrane from 2.8 in the wild type to an average of 1.9 in the mutants. This was primarily due to an increase in the amount of membrane in contact with the stroma in the mutants. These changes in ultrastructure are illustrated in Figure 6 by an interpretive drawing which shows how the same amount of membrane is differently organized in mutant and wild-type chloroplasts. In this figure, the wild type is shown with six thylakoids per granum resulting in four stromal surfaces. By contrast, the altered organization of the same amount of membrane in the mutant results in eight exposed granal surfaces. The striking similarity of these effects in the two independently isolated act1 mutants confirms that these ultrastructural effects are specifically due to the changes in lipid composition resulting from loss of glycerol-3-phosphate acyltransferase activity.

DISCUSSION

Chloroplast Ultrastructure

The most pronounced physiological effects of the act1 mutation appeared to be differences in the arrangement of membranes within the chloroplasts. Differences in chloroplast ultrastructure were also observed in another mutant of Arabidopsis deficient in fatty acid desaturation (12). That mutant had relatively minor alterations in the organization of internal chloroplast membranes, but the chloroplasts were smaller and their number was correspondingly increased so that the total amount of membrane remained similar to that in the wild type. In the present case, the ultrastructural differences between mutant and wild type resemble those observed between sun and shade plants, respectively. In this analogy, the chloroplasts from the mutant resemble those obtained from plants grown in far red-deficient ('sun') illumination but do not exhibit the changes in stochiometry of the photosystems associated with this adaptation (14).

Because the factors which control chloroplast membrane organization are not known, it is not obvious why changes in lipid composition affect chloroplast ultrastructure. It has been suggested that membrane stacking in higher plant chloroplasts is mediated by adhesion between molecules of LHCP (24). This model is based on observations that greening or mutant plastids which are deficient in LHCP are correspondingly deficient in grana formation (2, 6), and reconstitution experiments using liposomes and purified LHCP (13, 21). However, this model does not appear to extend to the kinds of effects observed in the act1 mutants in which it is not the amount of stacking which is affected, but the number of lamellae associated with each granum. Also, a comparison of the morphometric data on the act1 mutant with a similar analysis of the LHCP-deficient chlorina mutant of barley (6) reveals major differences between the ultrastructural effects resulting from the act1 and chlorina mutants. Thus, we infer that either the lipid changes have a direct effect on ultrastructure or, more likely, there is an indirect effect of lipid composition on the amount of one or more polypeptides which are involved in mediating the formation or maintenance of certain aspects of chloroplast structure. Although we have not as yet identified any consistent differences between the polypeptide composition of chloroplast membranes of mutant and wild type, the use of more quantitative methods for analyzing the polypeptide pattern resolved by two dimensional gels may prove informative.

There seem to be two main possibilities by which altered lipid composition could lead to changes in polypeptide composition of the mutant chloroplast membranes. First, the altered lipid environment could differentially affect the rate of incorporation or turnover of some membrane proteins and, thereby, lead to a quantitative alteration in the polypeptide
Table III. Morphometric Analysis of Chloroplasts from Mutant Lines and Wild-Type Arabidopsis

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<th>JB25</th>
<th>LK8</th>
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<tr>
<td>Grana/plastid</td>
<td>54.4 ± 6.6</td>
<td>90.0 ± 7.2</td>
<td>87.6 ± 7.6</td>
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<td>Thylakoids/granum</td>
<td>6.2 ± 3.7</td>
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<td>Granal width (µm)</td>
<td>0.40 ± 0.04</td>
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<td>Stroma length (µm)</td>
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<td>Stroma/plastid (µm)</td>
<td>103.9 ± 12</td>
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<td>Appressed membrane/plastid (µm)</td>
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<td>Surface area (µm²/plastid)</td>
<td>9.9 ± 2</td>
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</table>

Wild type

![Wild type diagram]

Mutant

![Mutant diagram]

**Figure 6.** Schematic illustration of the ultrastructural differences between chloroplast membranes from wild-type and the act1 mutants.

composition of the membranes. This could account for the reduction in Chl content. Alternatively, the altered lipid composition may, in principle, decrease the activity of one or more reactions associated with photosynthetic electron transport leading to a change in the distribution of excitation energy between the two photosystems. Thus, ultrastructural changes and the associated changes in polypeptide composition could reflect an adaptive response, of the kind observed in sun and shade plants (14), designed to bring about balanced energy distribution between the photosystems. We consider the latter possibility least likely because there does not appear to be a significant effect on the ratio of PSI to PSII activity in the mutant.

**Chloroplast Membrane Function**

When expressed on a Chl basis, the mutant had a higher rate of electron transport than the wild type. After correction for an 8.5% reduction in Chl content, the rates of whole chain electron transport were indistinguishable in the two genotypes. We also could not detect any changes in room temperature fluorescence between mutant and wild-type thylakoid membranes (Table II). These results suggest that the photosynthetic capacity of the mutant and the wild type is essentially equivalent. The only significant difference between mutant and wild type was an increase in \( F_{685} \) and a decrease in \( F_{734} \). Reduced fluorescence yield at long wavelengths may be caused by changes within the PSI antennae pigment bed, or it may be attributed to a decrease in energy spillover from PSII to PSI (15). We favor the latter concept because we observed no significant changes in PSI activity (after correction for the reduction in Chl content), and because it is consistent with the observed increase in 77K fluorescence at 685 nm which otherwise requires additional explanation. A simple explanation for these effects is that the alterations in structural organization of the chloroplast membranes brought about by compositional changes of the membranes, reduces the spillover from PSII to PSI.

**Lipid Composition and Enhanced Thermal Tolerance**

In spite of substantial changes in leaf lipid metabolism and membrane lipid composition the mutant line JB25 exhibits a growth rate which is comparable or greater than that of the wild type. This observation is consistent with the results obtained for two other *Arabidopsis* mutants deficient in fatty acid desaturation of chloroplast membranes (12; L Kunst, J Browse, CR Somerville, unpublished data). In each of these cases, a reduction in the degree of lipid desaturation is correlated with an enhanced growth rate at high temperatures. In several other species, the acclimation to growth at high temperatures is usually accompanied by an increase in the threshold temperature at which Chl fluorescence enhancement occurs (3, 5, 22). The mechanisms responsible for this adaptive response are not known, but the adaptation is associated with changes in membrane lipid desaturation. A comparison of the act1 mutant and wild type by this criterion also indicated slightly increased thermal tolerance of the mutant. These results are consistent with the concept that the fatty acid composition of chloroplast membranes may be a component of the thermal adaptation response characterized in species such as *Nerium oleander* (19).

**Why do 16:3 Plants Persist?**

Since a single mutation can lead to the loss of the prokaryotic pathway, it seems legitimate to pose the question as to why some species retain the pathway while others do not. Under the conditions we have examined, there are no appar-
ent physiological disadvantages associated with the loss of the prokaryotic pathway. On the contrary, preliminary evidence suggests that the mutants may have slightly enhanced thermal tolerance. This is consistent with the observation that growth of the 16:3 plant Atriplex lentiformis at elevated temperature leads to the complete loss of the 16:3 fatty acyl group (17). Although this was observed before the two pathway hypothesis of glycerolipid synthesis was formulated (20), the implication is that flux through the prokaryotic pathway is suppressed in this organism at an elevated temperature. It is striking, in this respect, that the loss of the prokaryotic pathway in the act1 mutant is also associated with enhanced thermal tolerance of chloroplast membranes. Extrapolating from this coincidence, we consider it possible that the presence of two pathways for lipid biosynthesis may provide a useful mechanism for regulating the physical properties of the chloroplast membranes in response to environmental conditions. Because of our lack of knowledge about the mechanisms by which plants regulate the degree of fatty acid unsaturation, it is currently possible to contrast the mechanism of regulation with that employed by 183 species under similar circumstances.

ACKNOWLEDGMENTS

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LITERATURE CITED