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A Mutant of *Arabidopsis* Deficient in the Elongation of Palmitic Acid[1]

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The overall fatty acid composition of leaf lipids in a mutant of *Arabidopsis thaliana* was characterized by an increased level of 16:0 and a concomitant decrease of 18-carbon fatty acids as a consequence of a single recessive nuclear mutation at the *fab1* locus. Quantitative analysis of the fatty acid composition of individual lipids established that lipids synthesized by both the prokaryotic and eukaryotic pathways were affected by the mutation. Direct enzyme assays demonstrated that the mutant plants were deficient in the activity of 3-ketoacyl-acyl carrier protein synthase II; therefore, it is inferred that *fab1* may encode this enzyme. Labeling experiments with [^{14}C]acetate and lipase positional analysis indicated that the mutation results in a small shift in the partitioning of lipid synthesis between the prokaryotic and eukaryotic pathways. Synthesis of chloroplast lipids by the prokaryotic pathway was increased with a corresponding reduction in the eukaryotic pathway.

In all plants studied, de novo fatty acid synthesis occurs in the chloroplasts or plastids of the cell (Ohlrogge et al., 1991) by the action of a dissociable type II fatty acid synthase (Browse and Somerville, 1991). The primary product of fatty acid synthesis, 16:0-ACP, can undergo one of three competing reactions: (a) hydrolysis by acyl-ACP thioesterase, (b) elongation to 18:0-ACP (which is followed by nearly quantitative desaturation to form 18:1-ACP), or (c) transfer to lysophosphatidic acid. Therefore, the ratio of 16-carbon to 18-carbon fatty acids in the membrane lipids could be determined by the relative fluxes through these three reactions. The significance of these reactions is related to the two pathways that are available for the synthesis of membrane glycerolipids in the leaf cells of higher plants (Browse and Somerville, 1991).

In *Arabidopsis thaliana*, the "prokaryotic" pathway (Roughan and Slack, 1982), located in the chloroplast envelope, uses 18:1-ACP and 16:0-ACP for the sequential acylation of glycerol-3-P to form phosphatidic acid. The phosphatidic acid made by the prokaryotic pathway has 18:1 at the *sn*-1 position and 16:0 at the *sn*-2 position of the glycerol backbone. It is used for the synthesis of phosphatidylglycerol or is converted to diacylglycerol by a phosphatidic acid phosphatase located in the chloroplast envelope. This diacylglycerol pool acts as a precursor of the other major thylakoid lipids, i.e. monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and sulfolipid (Douce and Joyard, 1990; Browse and Somerville, 1991). In the "prokaryotic" pathway, 16:0 at the *sn*-2 position of glycerolipids may be desaturated to 16:1-*trans* (on phosphatidylglycerol) (Sparace and Mudd, 1982) or to 16:3 (on monogalactosyldiacylglycerol) (Roughan et al., 1979; Heinz and Roughan, 1983).

The "eukaryotic" pathway is initiated by the hydrolysis of 18:1-ACP and 16:0-ACP and the export of these acyl groups to the cytoplasm as CoA thioesters (Roughan and Slack, 1982). In contrast to the plastid isozymes, the acyltransferases of the ER produce phosphatidic acid that is highly enriched with 18:1 at the *sn*-2 position; 16:0, when present, is largely confined to the *sn*-1 position (Frentzen, 1990). This phosphatidic acid gives rise to phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol that are characteristic of the various extrachloroplast membranes of the cell (Moore, 1982; Datko and Mudd, 1988). In addition, the diacylglycerol moiety of phosphatidylcholine is returned to the chloroplast envelope and contributes to the synthesis of thylakoid lipids. The 16:0 acyl groups that enter the eukaryotic pathway are not further desaturated (Roughan and Slack, 1982; Browse et al., 1986b).

The contributions of the prokaryotic and eukaryotic pathways to synthesis of chloroplast membrane lipids vary among the different species of higher plants (Browse and Somerville, 1991). A quantitative analysis of lipid synthesis in wild-type *Arabidopsis* (Browse et al., 1986b) indicated that for each 1000 16:0-ACP molecules synthesized, 117 are hydrolyzed and the 16:0 is exported from the chloroplast to enter the eukaryotic pathway, 680 are elongated and desaturated to 18:1-ACP, and 193 are used in the synthesis of 18:1/16:0 phosphatidic acid, which is the precursor of the prokaryotic pathway.

In this paper, we report the characterization of an *Arabidopsis* mutant with increased 16:0 fatty acid. The combined use of biochemical and genetic approaches with *Arabidopsis* has permitted elucidation of the relationship of the level of this acyl group to overall lipid synthesis by leaf cells and a description of the effect of the mutation in altering the

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Abbreviations: ACP, acyl carrier protein; KAS, 3-ketoacyl-ACP synthase.

balance of metabolism between the prokaryotic and eukaryotic pathways.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The line of *Arabidopsis thaliana* (L.) Heynh. described here was descended from the Columbia wild type. The methods for production and selection of the mutant have been described (James and Dooner, 1990). The mutant line was backcrossed to wild type four times before being used in the experiments reported here. Plants were grown on soil, in controlled environment chambers at 22°C under continuous fluorescent illumination (150 μmol quanta m^{-2} s^{-1}).

Fatty Acid and Lipid Analysis

The overall fatty acid composition of leaves and other tissues was determined by heating samples (5–50 mg fresh weight) at 80°C in 1 mL of 2.5% (v/v) H_2SO_4 in methanol for 90 min in screw-capped tubes. After 1.5 mL of 0.9% NaCl solution and 0.5 mL of hexane were added, fatty acids were extracted into the organic phase by shaking, and the tubes were centrifuged at low speed to break any emulsions that had formed. Samples (1 μL) of the organic phase were analyzed by GC on a 15-m × 0.53-mm Supelcowax column (Supelco, Bellefonte, PA) using a flame ionization detector. The gas chromatograph was programmed for an initial temperature of 150°C for 3 min, followed by an increase of 15°C/min to 210°C; this final temperature was maintained for an additional 12 min.

For more detailed analyses, plant tissue was killed rapidly by immersion in liquid nitrogen and ground under liquid nitrogen in a precooled mortar and pestle. Typically, 1 g of tissue sample was transferred to a screw-capped centrifuge tube with 12 mL of chloroform/methanol/formic acid (10:10:1, by vol) and stored overnight at -20°C. This procedure precluded the formation of phosphatidylmethanol, which can be formed by the action of endogenous phospholipase D when plant tissue is extracted with methanolic solvents at or above room temperature (Roughan et al., 1978). After centrifuging, the supernatant was transferred to a new tube and the tissue pellet re-extracted with 4.4 mL of chloroform:methanol:water (5:5:1, v/v/v). The two extractions were combined and washed with 6 mL of 0.2 M H_3PO_4, 1 M KCl (Browse et al., 1986b). Lipids were recovered in the chloroform phase, dried under N_2, and redissolved in 0.5 mL of chloroform. For large tissue samples, appropriately larger volumes of extraction solvents were used.

Individual lipids were purified from the extracts either by two-dimensional TLC on silica gel G (Browse et al., 1986b) or by one-dimensional TLC on $(NH_4)_2SO_4$-impregnated silica gel G by the method of Khan and Williams (1977). For this purpose, silica gel plates (Si250) were from J.T. Baker, Inc. The $(NH_4)_2SO_4$-impregnated plates were prepared by dipping the plates in 0.15 M $(NH_4)_2SO_4$ and drying them at room temperature before activation (for 90 min at 110°C). Lipids were located by staining with I_2 or by spraying the plates with a solution of 0.001% primuline in 80% acetone, followed by visualization under UV light. To determine the fatty acid composition and the relative amounts of individual lipids, the silica gel from each lipid spot was transferred to a screw-capped tube, a known amount of 17:0 fatty acid was added as an internal standard, and fatty acid methyl esters were prepared and analyzed as described above.

KASII Assay

The leaf crude homogenate was prepared by homogenizing leaves in ice-cold 50 mM Tris-Cl buffer (pH 8.0) containing 2 mM DTT and 0.2 mM EDTA. The extract was centrifuged at 14,000*g* for 15 min at 4°C, and then ammonium sulfate was added to the supernatant to 50% saturation. After incubation for 30 min, the preparation was centrifuged at 14,000*g* for 15 min at 4°C. The supernatant was desalted on a Sephadex G-25 column and concentrated on an Amicon 30K spin column.

KASII was assayed as described by Garwin et al. (1980) with some modifications. A buffer solution of 600 mM Tris-Cl (pH 7.0), 4 mg/mL BSA, 4 mM DTT containing 10 mM ACP and malonyl transacylase (approximately 1 milliunit) in a final volume of 7.35 μL was incubated at 37°C for 10 min to allow the chemical reduction of the ACP and malonyl transacylase. Then, [2-^{14}C]malonyl-CoA (50 Ci/mol) at a final concentration of 10 μM and palmitoyl-ACP at a final concentration of 1 μM were added to the reaction mixture. The reaction was started by adding 15 μL of leaf crude homogenate containing KASII activity and incubated at 26°C for 12 min. The reaction was terminated by the addition of 400 μL of reducing agent solution containing 30% tetrahydrofuran, 0.4 M KCl, and 5 mg/mL sodium borohydride in 0.1 M K_2HPO_4. The mixture was incubated at 37°C for 15 min. Finally, 400 μL of toluene were added to the mixture, a 400-μL aliquot of the upper phase was mixed with 5 mL of Omni-Fluorescence scintillation fluid (Du Pont), and the radioactivity of the reduced product was determined by scintillation counting. Assays containing boiled enzyme preparations and assays to which the termination reagents were added before the leaf homogenate showed low levels of radioactivity in the toluene phase. The radioactivity in these control incubations were subtracted from experimental values before the rates of KASII activity were calculated. In one experiment, compounds of the toluene phase were separated by TLC on silica gel G plates with hexane:diethyl ether:acetic acid (80:20:1, v/v/v) as developing solvent (Garwin et al., 1980). A single radioactive band was identified (R_F 0.31), consistent with the production of 3-ketostearoyl-ACP in the assay, which is reduced to the 1,3-diol before extraction into the toluene (Garwin et al., 1980).

Labeling Experiments

Sodium [1-^{14}C]acetate (2.0 GBq/mmol, Du Pont-New England Nuclear) was diluted to 2.0 MBq/mL with water. Each pot of plants (90-cm^2 soil area) was sprayed with 0.5 mL of the diluted solution using a perfume atomizer. At various times after application of the label, leaf samples of 1 g fresh weight were harvested and killed by immersion in liquid nitrogen. The lipids were extracted and separated by TLC as described above. The lipids from one chromatographic sep-

aration were transferred to scintillation vials containing Biodegradable Counting Scintillant (Amersham), and radioactivity was determined by scintillation counting. Lipids from a second separation were transmethylated, and the fatty acid methyl esters were separated by argentation TLC (Browse et al., 1986b) using silica gel G plates that had been dipped in a solution of 5% (w/v) $AgNO_3$ in acetonitrile and dried. Individual bands were identified by autoradiography and scraped from the plates, and the radioactivity in them was determined by scintillation counting.

Lipase Positional Analysis

The fatty acid compositions at the *sn*-1 and *sn*-2 position of individual lipids were determined by lipase digestion. After TLC, lipids were extracted from the silica gel (Browse et al., 1986b). The protocol for digestion with *Rhizopus* lipase, including purification of the lyso derivatives and fatty acids, was that described by Siebertz and Heinz (1977), except that 50 mM H_3BO_3 was added to the buffer used for lipase digestion to minimize intramolecular acyl transfer on the lyso-lipids produced. Fatty acid methyl esters were formed from untreated lipids, lyso-lipids, and fatty acids as described above, and the fatty acid composition of each compound was determined by GC.

RESULTS

Genetic Analysis

The *fab1* mutant (line 1A9) was isolated by screening pedigreed M_3 seed collections from ethyl methanesulfonate-treated plants for changes in seed fatty acid composition (James and Dooner, 1990). The 1A9 line was retained because seeds of the mutant exhibited an increased amount of 16:0 and decreased amounts of 18-carbon fatty acids. An analysis of seed from M_2 sibling plants indicated that the mutant phenotype was due to a single-gene mutation (James and Dooner, 1990). In wild-type *Arabidopsis* seeds, fatty acids are present mainly in storage triacylglycerols, and the extra 16:0 in *fab1* seeds was also found in the triacylglycerol (J. Wu and J. Browse, unpublished results). Our further study showed that the mutant also contains a higher level of 16:0 and reduced levels of 18:3 in the leaf lipids (Table I). Despite the altered lipid profile, the growth and development of mutant plants at 22°C was indistinguishable from that of wild-type plants.

To confirm the genetic basis of the alteration in leaf lipid fatty acid composition, crosses were performed between the mutant and wild-type *Arabidopsis*. Leaves of the F_1 progeny showed a level of 16:0 similar to that of the wild type (Table I), indicating that the wild-type allele is dominant in leaf tissue even though the wild-type and mutant alleles are co-dominant in seeds (James and Dooner, 1991). The frequency of individuals with the mutant phenotype in the F_2 population resulting from self-fertilization of F_1 plants was also assessed by GC of leaf samples. Of 132 plants analyzed, 36 had a fatty acid composition similar to the original 1A9 line, whereas the remaining 96 individuals had leaf fatty acid compositions similar to those of the wild-type and the F_1 hybrid. This pattern of segregation shows a good fit ($\chi^2 = 0.36$, $P \geq 0.9$) to the expected 3:1 ratio of a Mendelian segregation and indicates that the altered leaf fatty acid composition is due to a single nuclear mutation at the locus *fab1*.

Biochemical Characterization

In the overall leaf fatty acid profile of the mutant, there is a 43% increase in 16:0, and the amount of 16-carbon unsaturated fatty acids is slightly increased compared to wild-type *Arabidopsis* (Table I). The mutation is also expressed to some extent in root tissue (Table I) and seeds (James and Dooner, 1990), indicating that the *fab1* lesion is probably in a constitutively expressed gene. In higher plant leaves, 16:0 is the precursor of the 16-carbon unsaturated fatty acids, including, in *Arabidopsis*, 16:1-*trans* and 16:3. Mutations at the *fadA*

Table I. *Fatty acid composition of total leaf lipids from wild-type (WT) and fab1 mutant Arabidopsis*

Homozygous (*fab1*) and heterozygous (F_1 of the cross *fab1* × WT) mutant plants were grown together with the wild type in pots (for leaf samples) or in liquid media (for root samples). Data are means ± SE ($n = 10$ for leaves and $n = 5$ for roots).

Fatty Acid	Leaves			Roots	
	WT	F_1 (*fab1* × WT)	*fab1*	WT	*fab1*
	mol %			*mol%*	
16:0	15.3 ± 0.11	13.6 ± 0.16	21.9 ± 0.18	27.8 ± 0.79	31.0 ± 0.81
16:1-*cis*	1.1 ± 0.04	1.0 ± 0.04	1.8 ± 0.04	0.7 ± 0.53	3.4 ± 0.82
16:1-*trans*	2.6 ± 0.04	3.2 ± 0.09	3.4 ± 0.10		
16:2	1.1 ± 0.03	1.1 ± 0.07	1.4 ± 0.04		0.7 ± 0.35
16:3	14.5 ± 0.16	15.8 ± 0.29	16.0 ± 0.32	0.2 ± 0.13	0.2 ± 0.18
18:0	0.9 ± 0.04	0.3 ± 0.06	0.8 ± 0.03	1.6 ± 0.33	1.3 ± 0.61
18:1	3.1 ± 0.13	2.7 ± 0.11	3.4 ± 0.10	5.5 ± 0.59	2.1 ± 0.76
18:2	14.9 ± 0.23	13.2 ± 0.25	12.5 ± 0.17	39.9 ± 0.67	36.4 ± 0.42
18:3	46.5 ± 0.27	48.9 ± 0.29	38.9 ± 0.38	24.3 ± 1.06	24.8 ± 1.14
Total 16-C	34.6	34.7	44.5	28.7	35.3

locus (now renamed *fad4*) and the *fadB* locus (now *fad5*) block desaturation of 16:0 to 16:1-*trans* or to 16:1-Δ7*cis* (an intermediate in 16:3 synthesis), respectively, and result in the accumulation of 16:0 in leaf tissues (Browse et al., 1985; Kunst et al., 1989). In the *fab1* leaf lipids, however, both 16:1-*trans* and 16:3 are slightly increased relative to wild type, and instead it is the 18-carbon fatty acids, particularly 18:3, that are decreased in abundance (Table I). Since the increase of 16:0 in *fab1* leaves is concomitant with a decrease of 18-carbon fatty acids, these observations suggest that the mutant might be deficient in the activity of KASII, which is normally responsible for adding two carbons to 16:0-ACP to form 3-ketostearoyl-ACP, the precursor of 18:0-ACP (Browse and Somerville, 1991).

It is important to note that the phenotype in the mutant is leaky in the sense that considerable synthesis of 18-carbon fatty acids still takes place in the mutant. This indicates either that the mutated gene still yields a protein product with partial enzyme activity or that an active isozyme encoded by a separate gene is still present in the *fab1* line, or both.

Fatty Acid Composition of Individual Lipids

The biochemical consequences of the *fab1* mutation are shown more clearly by an analysis of individual lipids extracted from leaf tissue of wild-type and mutant plants (Table II). The results obtained indicate that 16:0, as a proportion of all fatty acids, is increased in each of the major membrane glycerolipids. The increases are substantial in every case except that in monogalactosyldiacylglycerol the fatty acid is raised only from 2 to 4%. However, there is also a small increase in the level of unsaturated 16-carbon fatty acids in monogalactosyldiacylglycerol of the mutant, and these fatty acids are derived from 16:0 (Browse and Somerville, 1991). Minor amounts of 16:1 were found in phosphatidylcholine and phosphatidylethanolamine from the mutant, even though this fatty acid is present in no more than trace amounts in the same lipids from wild-type *Arabidopsis*.

The data in Table II also indicate that there are changes in the relative amounts of the three major chloroplast lipids in the mutant. In the mutant, compared with in the wild type, digalactosyldiacylglycerol is reduced from 16 to 12% of the total leaf lipids, and there is an increase in the proportion of monogalactosyldiacylglycerol.

Taken together, these observations suggest that the failure to elongate 16:0 is accompanied by a reduction in 18-carbon fatty acids in lipids derived from both the prokaryotic and eukaryotic pathways. The mutation also causes some changes in the proportions of the membrane lipids. The higher levels of 16:0 found in both phosphatidylcholine (made exclusively by the eukaryotic pathway) and phosphatidylglycerol (made mainly by the prokaryotic pathway) indicate that neither the 16:0-ACP thioesterase (an enzyme involved in exporting 16:0 to the eukaryotic pathway) nor the 16:0-ACP:lysophosphatidic acid acyltransferase (the enzyme incorporating 16:0 into prokaryotic pathway lipids) is likely to be the site of the *fab1* mutant lesion. For example, if the activity of 16:0-ACP thioesterase were increased, higher levels of 16:0 would be expected in eukaryotic pathway lipids only, whereas an increase of 16:0-ACP:lysophosphatidic acid acyltransferase activity would cause an increase of 16:0 levels in the prokaryotic pathway lipids. A more likely possibility is that the mutation reduces the activity of the KASII enzyme.

Table II. *Fatty acid composition of leaf lipids from wild-type (WT) and fab1 mutant Arabidopsis*

Lipid	Percentage of Total Polar Lipids[a]	Fatty Acid Composition							
		16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
Phosphatidylcholine									
WT	16.9 ± 0.99	21.8	–[b]	–	–	2.9	6.7	33.3	35.4
fab1	16.0 ± 0.35	33.5	3.3	–	1.2	2.5	2.5	23.4	34.4
Phosphatidylethanolamine									
WT	10.9 ± 0.52	30.9	–	–	–	3.3	3.6	36.0	26.1
fab1	11.1 ± 0.72	35.2	1.8	–	0.9	1.9	5.1	27.0	28.2
Phosphatidylinositol									
WT	2.8 ± 0.15	36.2	–	–	–	4.7	3.0	30.8	25.3
fab1	3.1 ± 0.40	44.6	–	–	1.8	2.5	2.7	19.6	28.8
Sulfoquinovosyldiacylglycerol									
WT	3.7 ± 0.47	38.6	–	–	–	3.6	2.4	19.1	36.3
fab1	3.6 ± 0.64	48.5	2.2	–	–	1.9	2.2	12.9	32.4
Phosphatidylglycerol									
WT	10.7 ± 0.82	29.7	23.1[c]	–	–	1.9	6.6	9.5	29.2
fab1	11.8 ± 0.81	46.1	21.7[c]	–	–	1.6	5.1	5.9	19.6
Digalactosyldiacylglycerol									
WT	16.1 ± 0.32	13.7	–	–	3.2	1.3	1.4	5.2	74.8
fab1	11.8 ± 0.09	26.7	2.2	–	3.9	0.6	1.6	4.4	57.8
Monogalactosyldiacylglycerol									
WT	39.1 ± 0.27	1.6	1.1	1.9	33.4	0.3	1.1	3.1	57.3
fab1	42.4 ± 0.36	4.2	2.3	2.1	35.6	0.1	0.6	2.3	52.6

[a] Mean ± SE (n = 4). [b] Dashes indicate acyl group was not detected. [c] Δ3-*trans* isomer.

Table III. *KASII activity from wild-type (WT) and fab1 mutant Arabidopsis*

Experiment	WT	*fab1*
	pmol product mg^{-1} protein min^{-1}	
1	35.2	17.7
2	35.3	25.5
3	59.7	34.1
4	49.1	42.7
5	72.2	40.5
6	31.7	19.6
7	80.3	43.3
Mean	51.9	31.9
SE (n = 7)	7.3	4.1

Assay of the KASII

The indirect evidence presented above suggests that the *fab1* gene controls the activity of KASII located in plastids. To confirm this conclusion, we measured the enzyme activity by directly assaying the enzyme from leaves of both wild-type and mutant plants (Table III). The conditions used provided an assay that was linear with time (up to at least 15 min) and with increasing protein concentration (up to 20 μg of protein/assay) (data not shown). The results from different enzyme preparations showed some variability both in the absolute rates of KASII activity and in the ratio between the rates measured in preparations from wild-type and mutant tissues. Nevertheless, the data from seven independent experiments (total of 23 assays each for wild type and mutant) showed a clear, statistically significant difference between the rate of KASII activity in *fab1* mutant extracts and that in extracts from wild-type *Arabidopsis* (Table III). The ratios of protein/fresh weight and protein/Chl for the mutant were indistinguishable from wild type (data not shown), and protein recoveries from the enzyme preparation procedure were also similar for the two plant lines. Therefore, we believe that the data shown in Table III reflect a real in vivo reduction in KASII activity in the mutant. This provides direct evidence that a biochemical lesion in the *fab1* line is indeed a reduction in KASII activity.

Labeling of Leaf Lipids in Vivo

The fatty acid analyses of individual leaf lipids (Table II) reflect the steady-state lipid composition of cellular membranes. These data show changes, relative to wild type, in the proportions of individual lipids in leaves of the mutant. This finding points toward the possibility that there are changes in lipid metabolism in response to the mutant lesion as seen in other fatty acid mutants of *Arabidopsis* (Browse et al., 1989; Kunst et al., 1989; Miquel and Browse, 1992). To define these changes more accurately and to provide further information concerning the kinetics of 16:0 synthesis in the mutant and wild type, we labeled plants with [^{14}C]acetate and then followed redistribution of radioactivity in lipid acyl groups during the subsequent 144 h.

The kinetics of labeling in the wild type were similar to those found previously (Browse et al., 1986b) and are consistent with the parallel operation of the prokaryotic and eukaryotic pathways of lipid synthesis. We demonstrated previously (Browse et al., 1986b) that the major initial products of the prokaryotic and eukaryotic pathways in *Arabidopsis* are monogalactosyldiacylglycerol and phosphatidylcholine, respectively. These contain more than 70% of the total radioactivity soon after the start of the experiment in both wild type and the *fab1* mutant (Fig. 1, top). Other phospholipids synthesized by the prokaryotic pathway (phosphatidylglycerol) and the eukaryotic pathway (phosphatidylethanolamine) also contain label at these short times (Fig. 1, bottom). The label found in monogalactosyldiacylglycerol soon after the start of the experiment is derived from the prokaryotic pathway, whereas the increased amount of label in monogalactosyldiacylglycerol at longer times reflects synthesis of this lipid from phosphatidylcholine via the eukaryotic pathway (Browse et al., 1986b). On this basis, the data for *fab1* in Figure 1 show an increase, compared with the wild type, in monogalactosyldiacylglycerol synthesis by the prokaryotic pathway and a corresponding decrease in synthesis of phosphatidylcholine. However, there is still some synthesis of monogalactosyldiacylglycerol by the eukaryotic pathway, since there is an increase of label in monogalactosyldiacylglycerol at longer times. By the end of the experiments, phosphatidylcholine in the mutant leaves contains the same amount of label as does the wild type, indicating that fewer chloroplast lipids were synthesized from phosphatidylcholine through the eukaryotic pathway in the mutant. Both the synthesis and turnover of [^{14}C]phosphatidylglycerol

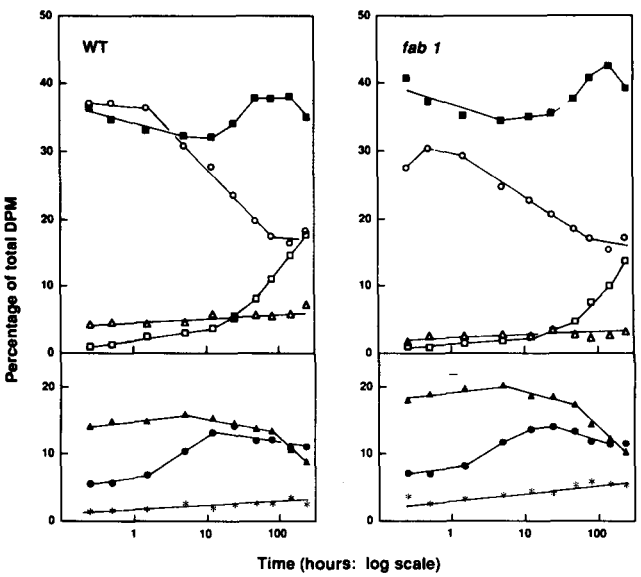

Figure 1. Redistribution of radioactivity among the polar leaf lipids of wild-type (WT) and *fab1* mutant *Arabidopsis*. Plants were incubated in the light and labeled with [^{14}C]acetate at zero time. ■, Monogalactosyldiacylglycerol; ○, phosphatidylcholine; □, digalactosyldiacylglycerol; △, sulfoquinovosyldiacylglycerol; ▲, phosphatidylglycerol; ●, phosphatidylethanolamine; *, phosphatidylinositol.

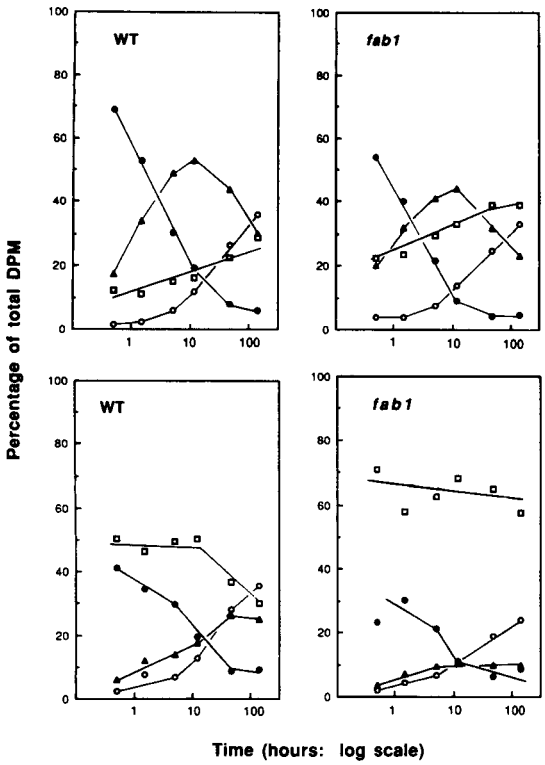

Figure 2. Redistribution of radioactivity among fatty acids of phosphatidylcholine (top) and phosphatidylglycerol (bottom) from leaves of wild-type (WT) and *fab1* mutant *Arabidopsis*. Lipids from [^{14}C]acetate-labeling experiment described in the legend to Figure 1 were derivatized using H_2SO_4 in methanol, and the fatty acid methyl esters were separated by argentation TLC. □, Saturated; ●, monoenoic; △, dienoic; ○, trienoic.

appear to be slightly increased in the *fab1* mutant. Radioactivity accumulates in digalactosyldiacylglycerol only toward the end of the experiment, indicating that digalactosyldiacylglycerol is synthesized mainly by the eukaryotic pathway in the *fab1* mutant, as it is in the wild type, but the proportion of digalactosyldiacylglycerol synthesis is reduced in the mutant compared with wild type. These relatively subtle changes in labeling pattern indicate that the flux through the prokaryotic pathway is increased in the mutant while there is a concomitant decrease in flux through the eukaryotic pathway.

In previous studies of the *fad5* (formerly *fadB*) mutant of *Arabidopsis* (a line that is deficient in desaturation of 16:0 on prokaryotic monogalactosyldiacylglycerol), we found that 1-acyl,2-16:0 molecular species of monogalactosyldiacylglycerol were preferentially degraded (Kunst et al., 1989). To investigate the possibility that 16:0 acyl groups might be preferentially turned over from some glycerolipids in the mutant plants, we analyzed the distribution of radioactivity among the fatty acids from each lipid during the course of the labeling experiment. The results for phosphatidylcholine (a major product of the eukaryotic pathway) and phosphatidylglycerol (synthesized predominantly by the prokaryotic pathway) are shown in Figure 2. For both of these lipids, the *fab1* mutant shows increased label, relative to wild type, in saturated fatty acids (predominantly 16:0) at the shortest labeling time. The differential between *fab1* and wild type in [^{14}C]16:0 is maintained throughout the experiment. Data for the fatty acids from other lipids shown in Figure 1 demonstrated a similar pattern, with the lipid from *fab1* plants showing an increase in the proportion of [^{14}C]16:0 at the beginning of the experiment and throughout the long chase period (results not shown). Broadly speaking, therefore, our data give no indication that any of the lipids from *fab1* leaves are turned over more rapidly as a result of the presence of increased proportions of 16:0 acyl groups.

Lipase Positional Analysis

A more accurate estimate of the contribution of each pathway to the synthesis of chloroplast lipids can be made by positional analysis of the lipid fatty acids using a position-specific lipase (Siebertz and Heinz, 1977). Several lines of evidence (Roughan and Slack, 1982; Browse et al., 1986b; Browse and Somerville, 1991) indicate that the chain length of the fatty acid at the *sn*-2 position is an accurate predictor of whether a particular molecule has been synthesized by the prokaryotic (16-carbon fatty acids at *sn*-2) or eukaryotic (18-carbon fatty acids at *sn*-2) pathway.

The fatty acid composition of lyso derivatives obtained after lipase digestion of lipids from wild-type and mutant plants are shown in Table IV. The composition of these lyso derivatives reflects the fatty acid composition at *sn*-2 of the parent glycerolipids. The data in Table IV indicate that the proportion of total leaf monogalactosyldiacylglycerol made by the eukaryotic pathway was 19.8% (sum of 18-carbon fatty acids) in the mutant, as compared with 26.3% in the wild type, a decrease of 25%. On a similar basis, the proportions of digalactosyldiacylglycerol and sulfolipid synthesis that occur via the eukaryotic pathway are also decreased (Table IV).

DISCUSSION

The genetic approach has been extremely useful in studies of many metabolic pathways, including the pathways of lipid biosynthesis in higher plants (Browse and Somerville, 1991). Previous investigations of six classes of *Arabidopsis* mutants with specific alterations in the fatty acid composition of their leaf lipids have contributed to our knowledge of the biochemistry and regulation of membrane lipid synthesis in higher plants (Browse et al., 1985, 1986a, 1989, 1993; Kunst et al., 1988; Miquel and Browse, 1992). In this report, we describe the characterization of an *Arabidopsis* mutant with increased levels of 16:0 fatty acids in lipids derived from both the prokaryotic and eukaryotic pathways.

Our results show that the *fab1* mutant contains higher levels of 16:0 in lipids derived from both the prokaryotic and eukaryotic pathways and that these higher levels are associated with reductions in 18-carbon fatty acids. Therefore, this mutant differs from both the *fad4* mutant, in which 16:0 is increased in chloroplast phosphatidylglycerol, and the *fad5* mutant, in which 16:0 is increased primarily in monogalactosyldiacylglycerol synthesized through the prokaryotic path-

Table IV. *Fatty acid composition at the sn-2 position of glycerolipids from leaves of wild type (WT) and fab1 mutant Arabidopsis*
16:X represents the sum of 16:1 plus 16:2 plus 16:3.

Lipid (lyso derivative)	Fatty Acid Composition						Total 16-C Fatty Acid
	16:0	16:X	18:0	18:1	18:2	18:3	
			mol %				*mol%*
Phosphatidylcholine							
WT	4.9	-[a]	0.9	7.8	49.2	37.4	4.9
fab1	3.3	4.3	0.6	4.5	39.7	47.6	7.6
Phosphatidylethanolamine							
WT	2.1	-	0.4	2.3	61.0	34.1	2.1
fab1	1.2	2.6	0.3	1.2	49.9	44.9	3.8
Phosphatidylinositol							
WT	6.4	-	1.1	2.4	47.1	43.0	6.4
fab1	7.5	0.9	-	1.9	40.3	49.5	8.3
Sulfoquinovosyldiacylglycerol							
WT	56.4	-	2.0	3.2	11.1	27.3	56.4
fab1	63.1	-	1.1	1.2	8.4	26.3	63.1
Digalactosyldiacylglycerol							
WT	10.1	6.3	0.3	0.7	3.3	79.2	16.4
fab1	13.4	9.5	0.2	0.6	2.8	73.3	22.9
Phosphatidylglycerol							
WT	34.3	50.7	0.5	1.9	6.3	6.1	85.1
fab1	40.1	50.2	-	0.2	4.3	5.2	90.3
Monogalactosyldiacylglycerol							
WT	1.1	72.6	0.1	0.3	1.4	24.5	73.7
fab1	1.4	78.8	0.1	0.2	0.7	18.7	80.2

[a] Dashes indicate acyl group was not detected.

way (Browse et al., 1985; Kunst et al., 1989). From our initial observations, we inferred that the mutant is partially deficient in activity of KASII.

The choice of KASII as the putative target of the mutation rather than 3-ketoacyl-ACP reductase, 3-hydroxyacyl-ACP dehydratase, or enoyl-ACP reductase was based on the observation that these three additional enzymes of the fatty acid synthesis cycle probably also act in the synthesis of 16:0-ACP, whereas KASII is thought to be specific for the further elongation of 16:0-ACP (Shimikata and Stumpf, 1982). The defect in KASII in the mutant was confirmed by direct assay of the enzyme. The results obtained (Table III) indicate the *fab1* mutant contains only 65% of the KASII activity found in wild-type *Arabidopsis*. Such a result is entirely consistent with the observation that 18-carbon fatty acids still predominate in the mutant. Thus, in the mutant, relative to wild type, the elongation of 16:0 to 18:0 is reduced by 16%, and the fluxes of 16:0 into both the prokaryotic and eukaryotic pathways are substantially increased. As a result, both chloroplast and extrachloroplast membranes contain more saturated fatty acid. The saturated fatty acids were increased by 46% in phosphatidylcholine from the mutant compared with that from wild-type plants, and there were 82 and 57% increases in the 16:0 proportion of digalactosyldiacylglycerol and phosphatidylglycerol, respectively. However, these changes in the degree of the membrane-lipid unsaturation had no obvious effect on the growth rate or vigor of the mutant under our standard growth conditions (22°C, 150 μmol quanta m^{-2} s^{-1} continuous fluorescent illumination, 50–70% RH).

The mutation in *fab1* plants alters the proportions of individual lipids found in leaves of the *fab1* plants relative to the wild type, as shown in Table II. In the *fab1* mutant compared to wild type, the proportion of monogalactosyldiacylglycerol in the total lipids increases by about 9%, and the proportion of digalactosyldiacylglycerol decreases by 27%. In addition, the pattern of lipid synthesis is affected. The ^{14}C-labeling experiment (Fig. 1) and positional analysis of acyl groups in leaf lipids (Table IV) both indicate that in leaves of the mutant there is a significant reduction, as compared with wild type, in the amount of monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and sulfolipid synthesis by the eukaryotic pathway. The data in Table IV show reductions of 2.5% for monogalactosyldiacylglycerol, 8% for digalactosyldiacylglycerol, and 15% for sulfolipid. Previous studies of the *fad2* (Miquel and Browse, 1992), *fad5* (Kunst et al., 1989), *fad6* (Browse et al., 1989), and *act1* (Kunst et al., 1988) mutants of *Arabidopsis* have provided evidence that lipid metabolism is regulated to ameliorate the consequences of each mutant lesion by altering the flux through the two pathways of lipid synthesis. However, it is not clear what factors mediate the partitioning of lipid synthesis between the two pathways. In the case of the *fab1* mutant, the increased synthesis of chloroplast lipids via the prokaryotic pathway would appear to do little to reduce the level of lipid saturation except inasmuch as 16:0 on position *sn*-2 of monogalactosyldiacylglycerol can be desaturated to 16:3.

It is known that there are three isozymes of KAS that are expressed concurrently in the plastid (Jaworski et al., 1989). KASIII appears to initiate the synthesis of the acyl chain,

KASI elongates the acyl chain to 16:0-ACP, and KASII is specifically required for the elongation of 16:0-ACP to 18:0-ACP. Our results show clearly that the activity of KASII can determine the degree of unsaturation and the ratio of 16-carbon fatty acids to 18-carbon fatty acids in plant leaf membranes. In addition, we can infer that the ratio of 16:0-ACP to 18:1-ACP is a factor in determining the partitioning of lipid synthesis between the prokaryotic and eukaryotic pathways.

One striking observation from the lipid analyses reported here relates to the increased proportion of 16:0 found in phosphatidylglycerol from *fab1* plants. In *fab1* plants, the proportion of fatty acids with melting points above 20°C (16:0 plus 16:1-*trans* plus 18:0) in phosphatidylglycerol is 69% compared with a value in wild type of 55%. The significance of this finding lies in the fact that surveys of many plant species (Murata et al., 1982; Murata and Yamaya, 1984; Roughan, 1985) have consistently found that plants containing more than 60% of these high melting point fatty acids in the leaf phosphatidylglycerol are invariably subject to damage at temperatures in the range 0° to 10°C, i.e. they are chilling-sensitive plants. The isolation and characterization of the *fab1* mutant provides a new means to investigate the hypothesis that phosphatidylglycerol molecular species containing high melting point fatty acids are the primary determinant of chilling sensitivity in plants.

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