

Accumulation of Palmitate in Arabidopsis Mediated by the Acyl-Acyl Carrier Protein Thioesterase FATB1¹

Peter Dörmann*, Toni A. Voelker, and John B. Ohlrogge

Max-Planck-Institut für Molekulare Pflanzenphysiologie, 14476 Golm, Germany (P.D.); Calgene LLC, Monsanto Company, 1920 Fifth Street, Davis, California 95616 (T.A.V.); and Department of Botany and Plant Pathology, Michigan State University, Michigan 48824 (J.B.O.)

The acyl-acyl carrier protein thioesterase B1 from Arabidopsis (AtFATB1) was previously shown to exhibit in vitro hydrolytic activity for long chain acyl-acyl carrier proteins (P. Dörmann, T.A. Voelker, J.B. Ohlrogge [1995] Arch Biochem Biophys 316: 612–618). In this study, we address the question of which role in fatty acid biosynthesis this enzyme plays within the plant. Over-expression of the *AtFATB1* cDNA under a seed-specific promoter resulted in accumulation of high amounts of palmitate (16:0) in seeds. RNA and protein-blot analysis in Arabidopsis and rapeseed (*Brassica napus*) showed that the endogenous AtFATB1 expression was highest in flowers and lower in leaves. All floral tissues of wild-type plants contained elevated amounts of 16:0, and in the polar lipid fraction of flowers close to 50 mol % of the fatty acids were 16:0. Therefore, flowers contain polar lipids with an unusually high amount of saturated fatty acids as compared to all other plant tissues. Antisense expression of the *AtFATB1* cDNA under the cauliflower mosaic virus 35S promoter resulted in a reduction of seed and flower 16:0 content, but no changes in leaf fatty acids. We conclude that the AtFATB1 thioesterase contributes to 16:0 production particularly in flowers, but that additional factors are involved in leaves.

Plants contain a variety of membrane and storage lipids, and in each lipid, a number of different fatty acids is found. In most tissues, fatty acids only differ with regard to their chain length and the number of double bonds, but the physiological role of the different fatty acids as well as their different combinations found in glycerol lipids is not completely understood. It is clear that the composition of membrane lipids is tightly regulated. Depending on the growth temperature, a specific ratio of saturated to unsaturated fatty acids is maintained within the membranes of the different organelles. The balance of saturated (mostly 16:0) to unsaturated fatty acids (e.g. 16:3, 18:3) in membrane lipids is important for chilling sensitivity, whereas saturated fatty acids are required for membrane stability under normal and elevated temperatures (Gibson et al., 1994; Ishizaki-Nishizawa et al., 1996). In addition to their role in membranes, saturated fatty acids, in particular 16:0 or 18:0, are elongated in some tissues to very long-chain fatty acids that serve as precursors for the biosynthesis of wax esters and other surface lipid components (Post-Beittenmiller, 1996).

Palmitate (16:0) is the predominant saturated fatty acid found in plant membrane lipids and is derived from the de novo fatty acid biosynthesis pathway inside the plastid (Ohlrogge et al., 1993). Control of fatty acid unsaturation is now understood to occur through the regulation of the activity and expression

of the plastidic $\Delta 9$ and the membrane bound desaturases (Slocombe et al., 1994; Berberich et al., 1998). However, because saturated fatty acids once exported from the plastid are not believed to be further desaturated, the control of saturated fatty acid content in plants most likely resides in the plastid. More than one enzyme is involved in the release of 16:0 from 16:0 acyl carrier protein (ACP) and its incorporation into lipids. 16:0 may be directly transferred onto glycerol-3-P inside the plastid leading to the synthesis of the so-called prokaryotic lipids. Of the two transferases involved, the second (acyl-ACP:1-acyl-glycerol-3-P acyltransferase) was found to prefer 16:0-ACP (Frentzen et al., 1983). The acyl groups of acyl-ACP can also be released by a thioesterase and subsequently exported from the plastid to the endoplasmic reticulum (eukaryotic pathway). These fatty acids are incorporated into extraplastidial lipids or are re-transported to the plastid for the biosynthesis of plastidial polar lipids. Because the prokaryotic 16:0 transferred to glycerol in the plastid is to a large extent desaturated to hexadecatrienoate (16:3) in Arabidopsis, the major proportion of 16:0 is therefore found in eukaryotic lipids and is derived from the action of one of the acyl-ACP thioesterases.

Initial study of plant acyl-ACP thioesterases led to the characterization of enzyme activities that were predominantly active on oleoyl-ACP with substantially less activity toward other acyl-ACPs (e.g. Ohlrogge et al., 1978; Knutzon et al., 1992). Later examination of species that produce high levels of medium-chain fatty acids in their seeds led to the discovery of seed-specific medium-chain acyl-ACP thioesterases (Pollard et al., 1991; Voelker et al.,

¹ This work was supported in part by a grant from the Alexander von Humboldt Foundation (to P.D.).

* Corresponding author; e-mail doermann@mpimp-golm.mpg.de; fax 49-331-567-8250.

1992). When DNA sequences became available for both oleoyl- and medium-chain-specific thioesterases, these sequences were found to fall into two classes. An initial surprising observation arose when an expressed sequence tag from Arabidopsis, a species that does not contain medium-chain fatty acids, was found with a cDNA sequence most highly related to the medium-chain type thioesterases. After expression in *Escherichia coli*, this cDNA was shown to encode a thioesterase active in vitro with 16:0 > 18:1^{Δ9} > 18:0 > 14:0-ACP (Dörmann et al., 1995). Further examination of a large group of thioesterase sequences and their expression in transgenic plants or *E. coli* led to the designation of the FATA and FATB classes of acyl-ACP thioesterases, which are predominantly active on oleoyl- or saturated acyl-ACP substrates, respectively (Jones et al., 1995; Voelker, 1996). The FATA class appears to be expressed ubiquitously in all plant tissues and is responsible for the major acyl-ACP thioesterase enzyme activity detected in plant extracts. Although FATB enzymes are often seed specific where they account for the production of medium-chain fatty acids in species such as California Bay or *Cuphea*, the AtFATB1 was found in leaves, roots, and siliques (Dörmann et al., 1995). We previously speculated that the AtFATB1 might be responsible for the control of 16:0 levels in Arabidopsis, however no direct evidence for such a general role of AtFATB1 was available. In this study we have examined the expression pattern of AtFATB1 in detail and constructed transgenic plants with different expression of the AtFATB1 thioesterase. These experiments allowed us to examine the in planta activity of the AtFATB1 enzyme and its acyl-ACP substrate specificity. Arabidopsis represents a species with a "normal" fatty acid pattern (i.e. accumulates predominantly 16- and 18-carbon fatty acids). Therefore, results obtained with Arabidopsis can be extended to other plants and may help to explain how plants control the balance of saturated to unsaturated fatty acids.

RESULTS

The AtFATB1 Gene Product Is Active with 16:0-ACP in Vivo

After expression in *E. coli*, the recombinant AtFATB1 thioesterase (clone TE3-2) was previously shown to be highly active with 16:0, 18:1^{Δ9}, 18:0, and

14:0-ACPs (Dörmann et al., 1995). In an attempt to elucidate its in vivo specificity, the AtFATB1 cDNA was expressed in Arabidopsis under the control of the seed-specific rapeseed napin promoter. We screened 55 transgenic T₀ lines for their 16:0 content in the T₁ seeds. Three lines with an increased amount of 16:0 were selected. The growth of the transformed plants was indistinguishable from wild type. Fatty acid analysis of batches of whole seeds revealed that the amount of 16:0 in these lines was increased severalfold from about 10.0 mol % in wild-type control plants to up to 38.6 mol % (Table I). Along with the increase in 16:0, an increase in myristate and stearate was observed. The amount of desaturated fatty acids was accordingly decreased. We also determined the amount of total fatty acids per seed, but could not find a relationship between the increased 16:0 content and alterations in fatty acid content.

AtFATB1 Transcripts Accumulate Specifically in Flowers

By western analysis, the AtFATB1 thioesterase was previously shown to be expressed in Arabidopsis leaves, roots, and whole siliques as well as in developing seeds of rapeseed (Dörmann et al., 1995). To obtain a more detailed expression pattern, we did additional northern and western analysis. Figure 1 shows the FATB expression in leaves, different developmental stages of flowers, and floral organs of rapeseed. The expression, both on RNA and protein level, increased during flower development and was high in all floral organs. The expression in floral tissues was much higher than in leaves. In accordance with the expression pattern observed in rapeseed, the AtFATB1 RNA was found to be highly expressed in Arabidopsis flowers and to a lesser extent in leaves and siliques (Fig. 2). Western analysis of different developmental stages of the reproductive tissues of Arabidopsis indicated that the FATB1 protein expression was highest in flowers.

16:0 Is Enriched in Polar Lipids of Flowers

The strong differences in the AtFATB1 expression pattern observed during northern and western analysis prompted us to analyze the tissue-specific content of its presumptive product, 16:0. Fatty acid analysis of different Arabidopsis tissues revealed a

Table I. Fatty acid composition of seeds overexpressing the AtFATB1 thioesterase

Batches of 20 to 40 seeds of the wild type and of each transgenic line were analyzed.								
Line	14:0	16:0	18:0	18:1	18:2	18:3	20:1	22:1
mol %								
Wild type	0.1	10.0	3.1	12.1	32.9	23.5	16.6	1.6
TES130	0.7	38.8	6.4	6.0	27.8	13.5	6.3	0.5
TES182	0.6	26.6	5.2	6.7	31.1	17.6	11.1	1.1
TES184	0.4	33.7	5.5	7.4	24.8	18.3	9.2	0.8

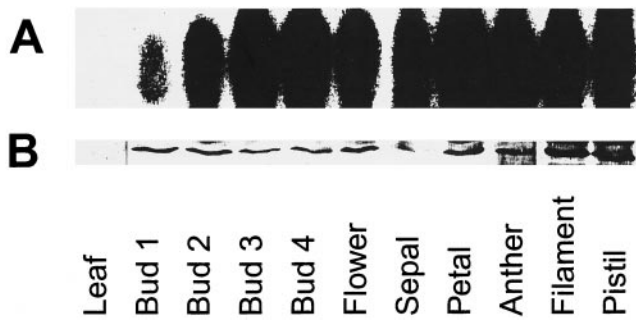


Figure 1. Expression of the FATB thioesterase in different rapeseed tissues. A, Total RNA was isolated from different rapeseed tissues and from developing flowers and 5 μ g each was loaded on an RNA gel. After blotting, the RNA was hybridized with the *AtFATB1* cDNA. B, Protein (50 μ g) was isolated from the same tissues and blotted onto a nitrocellulose membrane. The rapeseed FATB gene product was detected on the blot with anti-AtFATB protein antibodies. The developmental stages of the flower buds were: Bud 1, 1 to 5 mm long; Bud 2, 5 to 8 mm; Bud 3, 8 to 10 mm; and Bud 4, 10 to 12 mm.

tissue-dependent content of 16:0 between 11 mol % in seeds and 27 mol % in total flowers (up to 29 mol % in petals; Table II). The 16:0 content in leaves (13.6 mol %) was quite low in contrast to roots, stems, and silique walls. The amount of 16:0 found in different tissues corresponded to the expression of the *AtFATB1* gene as observed in northern and western analysis (Fig. 2). For example, *AtFATB1* expression and 16:0 content were high in flowers and all floral organs and in roots, but were low in leaves and seeds.

The high amount of 16:0 found in *Arabidopsis* flowers could be derived from any of the non-polar or polar lipid classes found in plants. We therefore analyzed the total amount of fatty acids as well as the fatty acid composition in the wax, triacylglycerol, and polar lipid fraction of whole flowers (Table III). The predominant part of fatty acids (4.20 μ mol g^{-1} FW or 79.7% of total fatty acids) was bound to polar lipids. Smaller amounts of fatty acids were found in the wax and triacylglycerol fraction. Although all three lipid classes contained elevated amounts of 16:0, the amount was highest in the polar lipid fraction (48.6%).

Alterations in *AtFATB1* Gene Expression Lead to Tissue-Specific Changes of 16:0 Content

The expression pattern of the *AtFATB1* gene in different *Arabidopsis* tissues apparently was related to the content of 16:0 in the given tissue. To address the question to which extent the *AtFATB1* thioesterase is involved in the production of 16:0, *Arabidopsis* plants were transformed with an *AtFATB1* construct in antisense orientation behind the cauliflower mosaic virus (CaMV) 35S promoter. This promoter gives rise to constitutive gene expression, and in *Arabidopsis*, it has been described to be active in most tissues, particularly in leaves, but to show comparatively

weak activity in seeds (Eccleston and Ohlrogge, 1998). A total of 50 transgenic T₁ lines was analyzed. We first screened the plants by measuring fatty acid composition of single leaves, but were unable to detect any plant with alterations in 16:0 content. By screening for alterations in seed fatty acid composition, we selected three lines that showed a reduction in seed 16:0 content from 11% to 6% (Fig. 3B). The plants of these three lines did not show any visible phenotype. These transgenic lines were submitted to fatty acid analysis in flowers, roots, and leaves. We found a considerable reduction in 16:0 in flowers, but only minor changes in roots or leaves (Fig. 3, A, C, and D).

The *AtFATB1* thioesterase is involved in the release of fatty acids from ACP, which are subsequently exported from the plastid and incorporated into eukaryotic lipids. Because different leaf lipids are known to contain fatty acids derived from either the prokaryotic or the eukaryotic pathway, we considered the possibility that in the leaves of the transgenic plants, the 16:0 content of eukaryotic lipids might be different than that of the prokaryotic lipids. Therefore, the polar lipids from leaves of the three selected antisense lines were separated by thin-layer chromatography and quantified by gas-liquid chromatography. We could not find any change of 16:0 content in mono- and digalactosyldiacylglycerol, sulfolipid, phosphatidylcholine, and phosphatidylglycerol (data not shown). In particular, we did not find any reduction of 16:0 in phosphatidylethanolamine, which is a polar lipid known to contain fatty acids entirely derived from the eukaryotic pathway and which is localized outside the plastid (Fig. 3E).

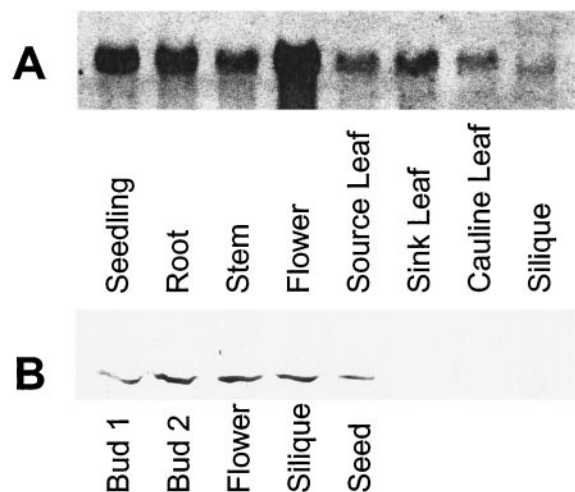


Figure 2. Expression of the *AtFATB1* thioesterase in different *Arabidopsis* tissues. A, Total RNA was isolated from different *Arabidopsis* tissues and 10 μ g was loaded in each lane. The northern blot was hybridized with the *AtFATB1* cDNA. B, Protein (40 μ g) was isolated from developing flowers, green siliques, and green seeds. After blotting to nitrocellulose, the *AtFATB1* polypeptide was detected with anti-*AtFATB1* protein antibodies. Bud 1, 0 to 1 mm long; Bud 2, >1 mm.

Table II. Fatty acid content of total lipids from different tissues of *Arabidopsis* wild-type plants

The data represent the means of three measurements. The SD was always below 10% of the given value. n.d., Not detectable.

Tissue	14:0	16:0	16:1	16:3	18:0	18:1	18:2	18:3	20:1	22:1
	<i>mol %</i>									
Total flowers	1.1	26.7	0.9	1.9	2.1	2.6	27.7	37.0	n.d.	n.d.
Green seeds	0.2	10.5	0.8	0.3	4.1	11.5	28.3	25.0	16.8	2.5
Mature seeds	0.2	11.9	0.2	n.d.	3.8	12.7	33.1	20.6	15.6	1.8
Sepals	n.d.	26.0	4.2	7.8	1.7	n.d.	17.7	42.6	n.d.	n.d.
Petals	1.2	28.9	0.8	1.0	2.6	1.4	34.3	29.6	n.d.	0.2
Stamen	2.5	26.8	1.0	0.8	2.7	7.0	21.2	38.0	n.d.	n.d.
Pistils	0.6	24.4	0.1	1.3	2.3	2.9	33.7	34.6	n.d.	1.1
Silique coats	n.d.	19.0	2.3	6.1	3.0	4.0	21.7	43.5	n.d.	0.4
Leaves	0.6	13.6	3.9	13.9	0.8	2.8	11.9	52.5	n.d.	n.d.
Roots	0.6	22.3	2.2	0.3	2.1	6.7	38.9	26.6	n.d.	0.3
Stems	n.d.	19.3	3.0	7.0	1.1	1.0	17.8	50.8	n.d.	n.d.

DISCUSSION

By expression in *E. coli*, the *AtFATB1* cDNA was previously shown to encode a thioesterase with high activity toward long-chain acyl-ACPs (Dörmann et al., 1995). Both in vitro assays and the quantification of the free fatty acids accumulating within *E. coli* demonstrated that the *AtFATB1* enzyme preferably cleaves 16:0-ACP. This pattern was quite different from the in vitro acyl-ACP thioesterase activity of *Arabidopsis* extracts and also from the *AtFATA1* cDNA clones, which are predominantly active with 18:1-ACP and only show a moderate activity with 16:0- and 18:0-ACP (e.g. Knutzon et al., 1992). Thus, the in planta role of *AtFATB1* thioesterases was unclear. To elucidate the specificity of the *AtFATB1* thioesterase in planta, we overexpressed the respective cDNA in *Arabidopsis* seeds. A major increase in the amount of 16:0 was observed (from 10%–39%), which clearly demonstrates that the *AtFATB1* enzyme is highly active in planta toward 16:0-ACP. In addition, slight increases in myristate and stearate were observed. The increase in 18:0 may be due to an increased hydrolysis of 18:0-ACP by the *AtFATB1* enzyme or by the elongation of 16:0 at the endoplasmic reticulum. In contrast to in vivo production of myristate by the enzyme expressed in *E. coli*, we detected only minor levels of myristate in the transgenic *Arabidopsis* seeds. At this point we can only speculate that this may be due to inaccessibility or a very small pool size of 14:0-ACP. Our results upon the expression of the *AtFATB1* in seeds are similar to

those obtained when a 16:0-ACP-specific thioesterase cDNA (*ChFATB1*) isolated from the medium-chain fatty acid accumulating species *Cuphea hookeriana* was expressed in canola seeds (Jones et al., 1995). The discovery of a thioesterase in *Arabidopsis* with in vivo activity for palmitoyl-ACP confirmed the hypothesis by these authors that a 16:0-ACP-specific thioesterase might be ubiquitous to all higher plant species.

The *AtFATA1* thioesterase is most active with 18:1-ACP, but also shows some activity with 16:0 and 18:0-ACP. Why then would *Arabidopsis* contain a second thioesterase producing 16:0? To address this question, we first analyzed the tissue-specific expression pattern and 16:0 content. The expression of the *FATB* RNA and protein in *Arabidopsis* and rapeseed was found to be high in developing and mature flowers and in all floral organs. Expression was comparatively low in seeds and leaves. We observed a corresponding distribution of the 16:0 content in different tissues, i.e. we found high levels of 16:0 in total flowers and flower organs, lower amounts in roots, and lowest amounts in leaves and seeds. Elevated amounts of 16:0 in different floral organs have previously been described (e.g. Matsuzaki et al., 1983; Evans et al., 1990). We could furthermore demonstrate that all floral tissues contain high amounts of 16:0, and that this fatty acid is particularly enriched in the polar lipid fraction. The polar lipid fraction of *Arabidopsis* flowers contains close to 50 mol % 16:0. This means that every second fatty acid in the polar

Table III. Lipid and fatty acid composition in *Arabidopsis* flowers

Total lipids represent the means \pm SD of two measurements. The fatty acid composition is from two measurements; SD was below 5% for all fatty acid values. n.d., Not detectable.

Lipid Fraction	Total Lipids	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:1	22:1
	$\mu\text{mol fatty acid g}^{-1}$ fresh wt					<i>mol %</i>				
Wax	0.77 ± 0.06	15.7	32.3	1.1	4.3	30.5	7.8	6.3	0.3	n.d.
TAG	0.30 ± 0.01	5.2	19.4	5.0	2.5	62.3	3.0	0.5	0.7	1.0
Polar lipids	4.20 ± 0.62	0.2	48.6	0.6	2.3	8.6	22.4	15.2	n.d.	0.6

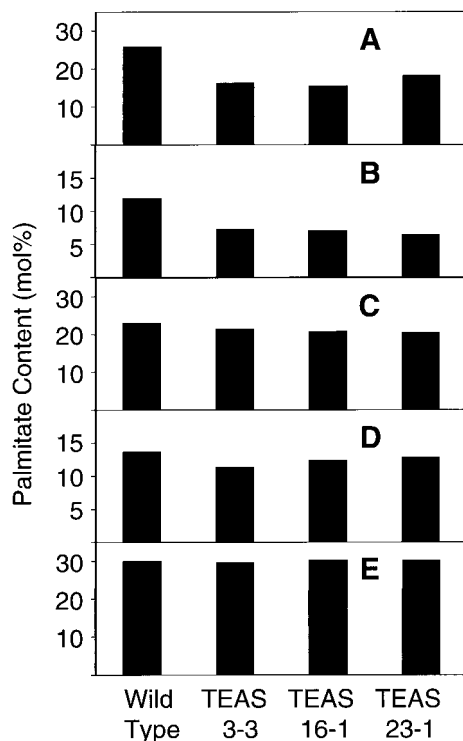


Figure 3. 16:0 content in Arabidopsis wild-type and *AtFATB1*-antisense lines. A, Flowers; B, mature seeds; C, roots; D, leaves; E, phosphatidylethanolamine of leaf tissue isolated and quantified by thin-layer chromatography and gas liquid chromatography of fatty acid methyl esters. The values represent means \pm SE of three measurements.

lipids of flowers is saturated, which may impact the fluidity of the corresponding membranes. Furthermore, the correlation between *AtFATB1* expression and 16:0 content in the different tissues indicates that the high amount of 16:0 found in flowers might be a result of *AtFATB1* activity.

To substantiate this hypothesis, we constructed transgenic plants with a reduced *AtFATB1* expression by introducing a CaMV 35S promoter antisense construct. The plants were screened by GLC for alterations in 16:0, the presumed product of the *AtFATB1* enzyme. Because the *AtFATB1* signals in northern and western blots were quite weak in the wild type and in the selected antisense lines, it was not possible to quantify the reduction in mRNA level or protein (data not shown). Because the thioesterase activity with 16:0-ACP in leaf tissue is very low and presumably represents the enzymatic activity of both the *FATB1* and the *FATA1* protein, we did not consider that enzyme assays would give any further information on the down regulation of the *FATB1* activity. For these reasons, we cannot make any conclusions on the residual activity of the *FATB1* enzyme, which might still be present in the different antisense lines.

In the transgenic lines, we found a strong reduction of 16:0 in flowers and seeds, but only minor changes in roots and leaves. The decrease of 16:0 in flower and seed lipids clearly demonstrates, that at least in these tissues, the *AtFATB1* enzyme is actively involved in the biosynthesis of 16:0 (Kinney, 1996). The strong reduction (from 11–6 mol %) found in seeds is somewhat unexpected considering that the expression of the antisense construct was driven by the CaMV 35S promoter, which is not highly active in plant seeds (Eccleston and Ohlrogge, 1998). Seeds are rich in triacylglycerol, which contains fatty acids entirely derived from the eukaryotic lipid biosynthetic pathway. Since the *AtFATB1* thioesterase supposedly is one of the enzymes that channel acyl groups into the eukaryotic pathway, a strong impact of changes in its activity on the 16:0 content of extraplasmid lipids such as triacylglycerol would be expected.

Although the 35S promoter gives rise to strong expression in leaves, we did not find any difference in the 16:0 content of total fatty acids in leaves of the transgenic plants. In addition, we analyzed the fatty acid content of each of the polar leaf lipids and could not find a difference in 16:0. Even more puzzling, the 16:0 content of one of the major extraplasmid lipids (phosphatidylethanolamine) remained constant. Because we saw a clear antisense effect on 16:0 in flowers and seeds of the very same transgenic lines (Fig. 3), we assume that in leaves, the *AtFATB1* enzyme is not the major controller of flux into 16:0. Possibly, the reduction in *FATB1* gene expression was strong enough to reduce the amount of 16:0 in flowers and seeds, but the residual *FATB1* enzyme activity might still be high enough to sustain 16:0 production in other tissues. Furthermore, alternative pathways, such as the *AtFATA1* thioesterase activity with 16:0-ACP, may contribute to the control of 16:0 production for the incorporation into extraplasmid leaf lipids. Over-expression of this enzyme in seeds has been shown to increase palmitic and stearic acid levels (Hitz et al., 1992). By overexpressing the Bay 12:0-specific thioesterase behind the CaMV 35S promoter in transgenic rapeseed plants, it was shown that laurate was produced by chloroplasts, but did not accumulate in leaf lipids. This result was attributed to the degradation of laurate by β -oxidation (Eccleston et al., 1996). However, because 16:0 represent a “normal” membrane fatty acid, it seems less likely that β -oxidation plays a major role in the maintenance of the 16:0 content in Arabidopsis leaves.

In conclusion, we have shown that the *AtFATB1* thioesterase reveals a tissue-specific expression pattern in accordance with the 16:0 content. The *AtFATB1* enzyme is involved in the production of the 16:0 in flowers and seeds. Finding a second thioesterase (*AtFATB1*) specific for 16:0 production and very high amounts of this fatty acid in polar lipids of flowers, leaves us with the question of whether there is a specific physiological role for 16:0 in flowers.

MATERIALS AND METHODS

Plant Material

Arabidopsis (ecotype Col-2) plants were grown at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 20°C under a 16-h light/8-h dark photoperiod. Rapeseed (*Brassica napus* cv Quantum) plants were grown in the greenhouse.

RNA and Protein Analysis

RNA was isolated from Arabidopsis or rapeseed tissues according to Logemann et al. (1987) and Jones et al. (1995), respectively. RNA was blotted to nylon membranes and hybridized to the *AtFATB1* cDNA (*Sall*-*Not*I fragment of clone TE3-2; Dörmann et al., 1995) using standard RNA protocols (Sambrook et al., 1989). Protein from Arabidopsis Col-2 or from rapeseed tissues was isolated and used for western-blot experiments with immuno-purified polyclonal anti-*AtFATB1* antibodies as described previously (Dörmann et al., 1995).

Lipid Analysis

Polar lipid classes from leaves were separated by thin-layer chromatography with acetone:toluene:water (91:30:8, v/v). Flower lipids were separated in hexane:diethyl ether:formic acid (80:20:2, v/v) into a polar lipid fraction, triacylglycerol, and waxes. Lipids separated by thin-layer chromatography or total lipids isolated from different tissues were quantified by fatty acid methylation with 1 N HCl in methanol and gas-liquid chromatography as described in Rossak et al. (1997).

Transgenic Arabidopsis Plants with Altered *AtFATB1* Expression

The coding region (including the chloroplast targeting peptide) of the *AtFATB1* cDNA was amplified by PCR from plasmid TE3-2 (Dörmann et al., 1995) using the two primers JO133 (5'-ATCTAGAGTCGACCTCCTCGTCATG-GTGGCC) and JO128 (5'-AAGCTTCTCGAGGTAGTAG-CAGATATAGTT). The resulting blunt-end fragment was ligated into the *Eco*RV site of pBluescriptIIKS+ in anti-sense orientation to the β -galactosidase promoter, as verified by sequencing. The *Xba*I-*Kpn*I fragment containing the *AtFATB1* insert was ligated into the binary vector pBINAR-Hyg (von Schaeven, 1989; Becker, 1990).

The *AtFATB1* cDNA derived from plasmid TE3-2 was ligated behind the seed-specific napin promoter for seed-specific overexpression (S. Boddupali, personal communication).

The *AtFATB1* constructs were transferred into Arabidopsis plants via *Agrobacterium*-mediated vacuum infiltration (Bechtold et al., 1993; Bent et al., 1994).

ACKNOWLEDGMENTS

We are grateful to Christoph Benning (Michigan State University) for providing space, resources, and advice for

this project. We thank Sekhar Boddupali (Monsanto) for providing transgenic Arabidopsis plants overexpressing the *AtFATB1* cDNA under the control of the napin promoter. We would like to thank Deborah Hawkins and Chingying Li (Calgene LLC) for the lipid class analysis of Arabidopsis flowers.

Received November 18, 1999; accepted March 5, 2000.

LITERATURE CITED

- Bechtold N, Ellis J, Pelletier G (1993) In planta *Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *CR Acad Sci III* **316**: 1194–1199
- Becker D (1990) Binary vectors which allow the exchange of plant selectable markers and reporter genes. *Nucleic Acid Res* **18**: 203
- Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, Giraudat J, Leung J, Staskawicz BJ (1994) RPS2 of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. *Science* **265**: 1856–1860
- Berberich T, Harada M, Sugawara K, Kodama H, Iba K, Kusano T (1998) Two maize genes encoding ω -3 fatty acid desaturase and their differential expression to temperature. *Plant Mol Biol* **36**: 297–306
- Dörmann P, Voelker T, Ohlrogge JB (1995) Cloning and expression in *Escherichia coli* of a novel thioesterase from *Arabidopsis thaliana* specific for long-chain acyl-acyl carrier proteins. *Arch Biochem Biophys* **316**: 612–618
- Eccleston VS, Cranmer AM, Voelker TA, Ohlrogge JB (1996) Medium-chain fatty acid biosynthesis and utilization in *Brassica napus* plants expressing lauroyl-acyl carrier protein thioesterase. *Planta* **198**: 46–53
- Eccleston VS, Ohlrogge JB (1998) Expression of lauroyl-acyl carrier protein thioesterase in *Brassica napus* seeds induces pathways for both fatty acid oxidation and biosynthesis and implies a set point for triacylglycerol accumulation. *Plant Cell* **10**: 613–622
- Evans DE, Sang JP, Cominos X, Rothnie NE, Knox RB (1990) A study of phospholipids and galactolipids in pollen of two lines of *Brassica napus* L. (rapeseed) with different ratios of linoleic to linolenic acid. *Plant Physiol* **92**: 418–424
- Frentzen M, Heinz E, McKeon TA, Stumpf PK (1983) Specificities and selectivities of glycerol-3-phosphate acyltransferase and monoacylglycerol-3-phosphate acyltransferase from pea and spinach chloroplasts. *Eur J Biochem* **129**: 629–636
- Gibson S, Falcone DL, Browse J, Somerville C (1994) Use of transgenic plants and mutants to study the regulation and function of lipid composition. *Plant Cell Environ* **17**: 627–637
- Hitz W, Yadav N, Kinney A, Perez-Grau L (1993) Genetic modification of the amounts of saturated and polyene fatty acids in the triacylglyceride of oilseeds. *Am Chem Soc* **206**: 158
- Ishizaki-Nishizawa O, Fujii T, Azuma M, Sekiguchi K, Murata N, Ohtani T, Toguri T (1996) Low-temperature

- resistance of higher plants is significantly enhanced by a nonspecific cyanobacterial desaturase. *Nat Biotechnol* **14**: 1003–1006
- Jones A, Davies HM, Voelker TA** (1995) Palmitoyl-acyl carrier protein (ACP) thioesterase and the evolutionary origin of plant acyl-ACP thioesterase. *Plant Cell* **7**: 359–371
- Kinney AJ** (1996) Development of genetically engineered soybean oils for food applications. *J Food Lipids* **3**: 273–292
- Knutzon DS, Bleibaum JL, Nelsen J, Kridl JC, Thompson GA** (1992) Isolation and characterization of two safflower oleoyl-acyl carrier protein thioesterase cDNA clones. *Plant Physiol* **100**: 1751–1758
- Logemann J, Schell J, Willmitzer L** (1987) Improved method for the isolation of RNA from plant tissues. *Anal Biochem* **163**: 16–20
- Matsuzaki T, Koiwai A, Kawashima N** (1983) Total fatty acid and polar lipid content in developing flower of *Nicotiana tabacum*. *Plant Cell Physiol* **4**: 199–206
- Ohlrogge J, Shine W, Stumpf P** (1978) Fat metabolism in higher plants. *Arch Biochem Biophys* **189**: 382–391
- Ohlrogge JB, Jaworski JG, Post-Beittenmiller D** (1993) De novo fatty acid biosynthesis. In TS Moore Jr, ed, *Lipid Metabolism in Plants*. CRC Press, Boca Raton, FL, pp 3–32
- Pollard MR, Anderson L, Fan C, Hawkins DJ, Davies HM** (1991) A specific acyl-ACP thioesterase implicated in medium-chain fatty acid production in immature cotyledons of *Umbellularia californica*. *Arch Biochem Biophys* **284**: 306–312
- Post-Beittenmiller D** (1996) Biochemistry and molecular biology of wax production in plants. *Annu Rev Plant Physiol Plant Mol Biol* **47**: 405–430
- Rossak M, Schäfer A, Xu N, Gage DA, Benning C** (1997) Accumulation of sulfoquinovosyl-*O*-dihydroxyacetone in a sulfolipid-deficient mutant of *Rhodobacter sphaeroides* inactivated in *sqdC*. *Arch Biochem Biophys* **340**: 219–230
- Sambrook J, Fritsch EF, Maniatis T** (1989) *Molecular Cloning: A Laboratory Manual*, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Slocombe SP, Piffanelli P, Fairbairn D, Bowra S, Hatzopoulos P, Tsiantis M, Murphy DJ** (1994) Temporal and tissue-specific regulation of a *Brassica napus* stearyl-acyl carrier protein desaturase gene. *Plant Physiol* **104**: 1167–1176
- Voelker TA** (1996) Plant Acyl-ACP thioesterases: chain-length determining enzymes in plant fatty acid biosynthesis. In JK Stelow, ed, *Genetic Engineering*, Vol 18. Plenum Press, New York, pp 111–133
- Voelker TA, Worrell AC, Anderson L, Bleibaum J, Fan C, Hawkins DJ, Radke SE, Davies HM** (1992) Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. *Science* **257**: 72–74
- von Schaeven A** (1989) Untersuchung zur ER vermittelten subzellulären Kompartimentierung fremder Proteine in höheren Pflanzen. PhD thesis, Freie Universität Berlin, Berlin