Plant Physiol. (1994) 106: 1615–1621

Cloning of a Temperature-Regulated Gene Encoding a Chloroplast ω-3 Desaturase from *Arabidopsis thaliana*[1]

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Previous genetic evidence suggested that the *fad8* and *fad7* genes of *Arabidopsis thaliana* encode chloroplast membrane-associated ω-3 desaturases. A putative *fad8* cDNA was isolated by heterologous hybridization using a gene encoding an endoplasmic reticulum-localized ω-3 desaturase (*fad3*) as a probe. The cDNA encodes a protein of 435 amino acid residues with a molecular mass of 50,134 D. Constitutive expression of the cDNA in transgenic plants of a *fad7* mutant resulted in genetic complementation of the mutation, indicating that the *fad7* and *fad8* gene products are functionally equivalent. Expression of the *fad8* cDNA in transgenic plants often resulted in the co-suppression of both the endogenous *fad7* and *fad8* genes in spite of the fact that these two genes share only about 75% nucleotide identity. In contrast to all other known plant desaturases, including *fad7*, the steady-state level of *fad8* mRNA is strongly increased in plants grown at low temperature. This suggests that the role of *fad8* is to provide increased ω-3 desaturase activity in plants that are exposed to low growth temperature. The *fad8*-1 mutation created a premature stop codon 149 amino acids from the amino-terminal end of the *fad8* open reading frame, suggesting that this mutation results in a complete loss of *fad8* activity.

The chloroplast membranes of higher plants have unusually high concentrations of trienoic fatty acids, with linolenic ($C_{18:3}$) or a combination of linolenic and hexadecatrienoic ($C_{16:3}$), making up more than 80% of the fatty acids found in this organelle (Harwood, 1982). These fatty acids are synthesized by sequential insertion of double bonds into derivatives of stearic ($C_{18:0}$) and hexadecanoic ($C_{16:0}$) acids. The formation of the first double bond in 18-carbon fatty acids is generally catalyzed within chloroplasts by one of the few soluble plant desaturases, the stearoyl-acyl carrier protein desaturase (McKeon and Stumpf, 1982). The formation of the second and third double bonds in 18-carbon fatty acids can then take place in either chloroplasts or the ER, with different membrane-bound desaturases catalyzing the formation of each subsequent double bond. In contrast, 16-carbon fatty acids are desaturated only within chloroplasts, with different membrane-bound desaturases catalyzing the formation of each double bond (reviewed by Browse and Somerville, 1991).

Most desaturases appear to be membrane proteins that have proven difficult to characterize biochemically (Schmidt and Heinz, 1990, 1993). Much of what is known about plant desaturases comes from the characterization of a series of *Arabidopsis* mutants with defects in fatty acid desaturation. Four loci (originally named *fadA*, *fadB*, *fadC*, and *fadD* but now re-named *fad4*, *fad5*, *fad6*, and *fad7*) with defects in chloroplast desaturation and two loci (*fad2* and *fad3*) with defects in the ER desaturation pathway have been described (Browse and Somerville, 1991). The possible existence of an additional chloroplast desaturase was suggested by the observation that two independently isolated *fad7* mutants displayed a mutant phenotype only when grown at temperatures above about 20°C. Since mutations that result in a temperature-sensitive phenotype are relatively rare, the probability that two independent mutations would both show a temperature-sensitive phenotype seemed unlikely. An alternative explanation was that the nearly normal levels of trienoic fatty acids in *fad7* mutant plants grown at low temperature might be due to the presence of a second, cold-induced ω-3 desaturase. Genetic evidence for this additional desaturase is presented in an accompanying paper describing the characterization of a mutation at a new locus designated *fad8* (McConn et al., 1994).

The genes corresponding to several of the *Arabidopsis fad* loci have recently been isolated. The *fad3* gene was isolated using a map-based approach (Arondel et al., 1992) and by T-DNA tagging (Yadav et al., 1993). T-DNA tagging was also used to isolate the *fad2* gene (Okuley et al., 1994). A clone for the *fad7* gene was first identified by chromosome walking with yeast artificial chromosomes, and subsequently a cDNA clone was identified by using the *Brassica napus fad3* gene as a hybridization probe (Iba et al., 1993). The *fad2*, *fad3*, and *fad7* genes have also been isolated from several other species by heterologous probing with the *Arabidopsis* or *Brassica* genes (Yadav et al., 1993; Hamada et al., 1994; Okuley et al.,

[1] This work was supported in part by grants from the U.S. National Science Foundation (MCB-9219356), the Japanese Ministry of Education Science and Culture (06259214 and 06804050), and the Sumitomo Foundation. S.G. and V.A. were recipients of fellowships from the Monsanto Co. and the European Molecular Biology Organization, respectively. This article is Carnegie Institution of Washington Department of Plant Biology publication 1214.

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1994; van de Loo and Somerville, 1994). In this paper we describe the isolation and characterization of the gene encoding a temperature-regulated desaturase corresponding to the *fad8* locus.

MATERIALS AND METHODS

Genetic Materials

All lines of *Arabidopsis thaliana* (L.) Heynh. described here are descended from the Columbia wild type. The plant lines JB101 (*fad7*-1 *gl1*) and SH1 (*fad7*-1 *fad8*-1) and the plasmids pVA34 and λZAP/FAD8–32 are available from the Arabidopsis Biological Resource Center at Ohio State University. The line JB101 was derived from the line JB1 (*fad7*-1) (McCourt et al., 1986) by five rounds of backcrossing to line MSU53 (*gl1*). Line LK9 (*fad7*-2) was derived by ethylmethane sulfonate mutagenesis of the wild type (Kunst, 1988). The line SH1 was isolated from the line JB1 by ethylmethane sulfonate mutagenesis (McConn et al., 1994). The transgenic *Arabidopsis* line 41–3-a was obtained from Ljerka Kunst (University of British Columbia). This line was constructed by using *Agrobacterium tumefaciens*-mediated gene transfer (Chang et al., 1994) to introduce the plasmid pBI121 (Clontech, Palo Alto, CA) into the Columbia wild type.

The plasmid pBI/fad8 was constructed in two steps. First, the cDNA insert of plasmid pVA34 (Iba et al., 1993) was excised using *Eco*RI and ligated into the *Eco*RI site of the plasmid pBS KS$^+$ in such an orientation that the 5′ end of the cDNA was closest to the *Eco*RV site in the pBS KS$^+$ polylinker. The cDNA was then excised from this plasmid, designated pBS/34, by digestion with *Eco*RV and then partial digestion of the plasmid with *Sac*I. The approximately 1.6-kb *Eco*RV-*Sac*I fragment (which contains an internal *Sac*I site) was gel purified and ligated to the *Sma*I/*Sac*I sites of the plasmid pBI121 (Clontech) to form the plasmid pBI/fad8.

A cDNA library constructed in λYES from mRNA extracted from all aerial tissues of the Columbia wild type of *Arabidopsis* was obtained from Ron Davis, Stanford University (Elledge et al., 1991). A genomic library was constructed by ligating *Sau*3A partially digested total genomic DNA from the line SH1 with *Bam*HI digested, dephosphorylated arms from the λZAP Express vector (Stratagene, La Jolla, CA). This library contained approximately 700,000 original plaques.

Plant Growth Conditions

Unless otherwise indicated in the text, plants were grown at 20 to 22°C under continuous fluorescent illumination (100–150 μmol m^{-2} s^{-1}) on a potting mixture irrigated with mineral nutrients (Browse et al., 1986a).

Screening Phage Libraries

The oligonucleotides CTCTGTCGTTCTATTTGCAC and AACCTGTGGTAAAACTCTCG were used to probe duplicate filters of the SH1 genomic library at 46°C using the following hybridization mixture: 0.6 M NaCl, 0.1% (w/v) sodium PPi, 0.2% (w/v) SDS, 125 units/mL heparin, 0.12 M Tris-HCl (pH 7.8), 8 mM EDTA, 5% (w/v) dextran sulfate. The filters were washed at room temperature (23°C) in 6× SSC. Phage were plaque purified and converted to plasmids by helper phage-mediated excision.

Plant Transformations

A. tumefaciens C58 (pGV3101) containing plasmid pBI/fad8 or pBI121 was used for the transformation experiments that were carried out essentially as described (Chang et al., 1994). In brief, plants were grown at a density of five plants per pot for about 18 to 21 d until the onset of bolting. The stems (1–20 mm long) were removed with tweezers and a drop of an overnight culture of the bacteria was placed on the wound. The same operation was repeated 7 to 10 d later. The seeds produced by the five plants in each pot were combined to produce independent batches. Thus, transformants arising from seeds from different pots are known to be of independent origin, whereas transformants from the same pot may be the products of the same transformation event. The seeds were sown on agar-solidified mineral medium containing 50 μg/mL kanamycin at a density of about 2500 seeds per 150-mm Petri plate. Approximately 1 in 15,000 seeds was found to be resistant to kanamycin.

Fatty Acid Analysis

Extraction and purification of leaf lipids and gas chromatographic analysis of fatty acids were as described by Browse et al. (1986b).

DNA Sequencing

DNA sequencing was performed manually by the chain termination method or using an ABI Catalyst-8000 robot and ABI373A DNA sequenator (Applied Biosystems, Foster City, CA) to perform dye terminator sequencing. All sequences were determined on both strands.

Northern Analysis

RNA isolation and northern analysis were performed as described by Iba et al. (1993) using the 3′ noncoding region of the *fad7* or *fad8* cDNA clones as probes. Each lane contained 15 μg of total RNA. Filters were hybridized at 42°C in a solution containing 50% (v/v) formamide, 5× SSPE, 5× Denhardt's solution, 0.1% SDS, and denatured salmon sperm DNA (100 μg/mL) for 16 h. The filters were washed at 60°C in 0.5× SSC, 0.1% SDS.

RESULTS

Isolation of a Putative cDNA Clone

The use of the *fad3* gene from *B. napus* to isolate three classes of cDNAs from *Arabidopsis* by heterologous probing has been described (Iba et al., 1993). These three classes of cDNAs were designated as types I, II, and III. The type I and type II clones contained the *Arabidopsis fad3* and *fad7* genes, respectively (Iba et al., 1993). Approximately 20-fold fewer type III than *fad7* cDNAs were isolated from a cDNA library prepared from plants grown at ambient temperature. The plasmid pVA34, which contained a type III cDNA of about 1.5 kb, was chosen for further study.

The complete sequence of the cDNA insert contained within clone pVA34 was determined. This sequence is available from the GenBank data base as accession number L27158. The 1.5-kb cDNA contained a 1308-bp open reading frame that begins with the translation initiation codon ATG and ends with the stop codon TGA. The predicted protein is 435 amino acids in length and has a molecular mass of 50,134 D.

A BLASTX search of the GenBank release 73 data base revealed that the most significant homology of the predicted amino acid sequence of the cDNA in pVA34 was to the product of the *fad7* gene from *Arabidopsis* (Iba et al., 1993) and to the RCCFAD7A-1 gene of *Ricinus communis*, which is believed to encode a linoleoyl desaturase (van de Loo and Somerville, 1994). Subsequently, the same cDNA has been reported as GenBank accession number D17578 (Watahiki and Yamamoto, 1994). Figure 1A shows a comparison of the predicted amino acid sequences of the products of the new cDNA and the *Arabidopsis fad7* gene. The striking homology between the proteins encoded by these two genes and the relatively lower homology to the *fad3* gene suggested that the insert in pVA34 encodes a chloroplast ω-3 desaturase.

Functional Complementation of the *fad7* Mutation with the Type III cDNA

To determine whether the cDNA clone pVA34 encoded a chloroplast ω-3 desaturase, a genetic complementation test was conducted. To perform this test, two mutants affected in chloroplast-localized ω-3 desaturase activity were available. We chose to use the *fad7* mutant line JB101 rather than the genetically more complex *fad7 fad8* double-mutant line SH1 (McConn et al., 1994). Plasmid pBI/fad8 contains the coding sequence of the cDNA insert from the clone pVA34 under the transcriptional control of the cauliflower mosaic virus 35S promoter. The *Arabidopsis* line JB101 (*fad7 gl1*) and wild type were transformed with pBI/fad8 by *A. tumefaciens*-mediated gene transfer (Chang et al., 1994).

Total lipids were extracted from the leaf tissue of the transgenic and control plants and the fatty acid compositions of the extracts were measured. As previously noted (Arondel et al., 1992; Iba et al., 1993), transformation with the control plasmid pBI121 had no significant effect on leaf fatty acid composition. Four independent JB101 plants transformed with pBI/fad8 were obtained, all of which showed essentially wild-type leaf fatty acid composition in preliminary tests (results not presented).

The inheritance of the altered fatty acid composition in two of the JB101 transformants and one line of the wild type and JB101 transformed with the vector pBI121 was examined in detail. Twelve-day-old kanamycin-resistant progeny of these plants were transplanted to soil and grown for 6 to 7 d at 17°C. Five to six plants from each transgenic line were analyzed by extracting total lipids from leaf tissue and measuring the fatty acid composition (Table I). As expected, line JB101 transformed with pBI121 showed higher levels of 16:2 and 18:2 fatty acids and lower levels of 16:3 and 18:3 fatty acids than the wild-type line transformed with pBI121. In contrast, the two JB101 lines transformed with pBI/fad8 had fatty acid compositions that were very similar to those of

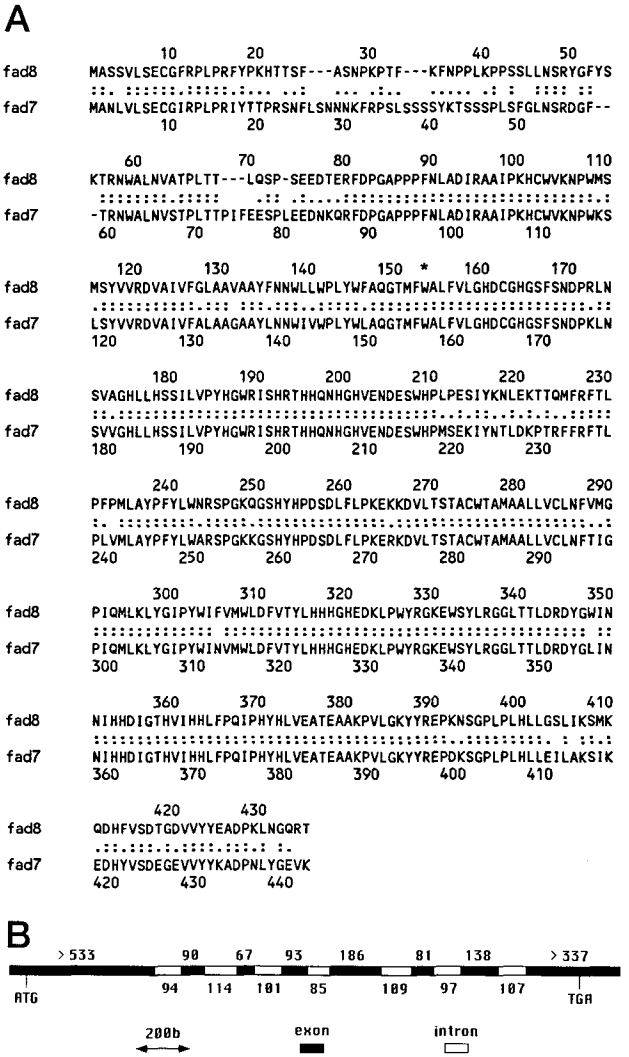

Figure 1. Structure of the *fad8* gene. A, Comparison of the predicted amino acid sequences of the *fad7* and *fad8* genes of *A. thaliana*. The asterisk indicates the site of the *fad8-1* mutation, which resulted in the conversion of the Trp codon TGG to the stop codon TAG. B, Schematic diagram of the *fad8* gene. The numbers above the line indicate the size (in bp) of exons. The numbers below the line give the size of the introns.

wild-type plants transformed with pBI121 (Table I). The amounts of 16:2 and 18:2 decreased, and the amounts of 16:3 and 18:3 showed a corresponding increase, to approximately wild-type levels. Thus, the cDNA insert from clone pVA34 functionally complemented the *fad7* mutation, indicating that it encodes a functional chloroplast ω-3 desaturase.

Co-suppression of *fad7* and *fad8* Expression

Surprisingly, most of the wild-type plants that were transformed with pBI/fad8 showed a significant alteration in fatty acid composition. Thirteen independent wild-type plants (from a total of 28) that were transformed with pBI/fad8 showed fatty acid compositions that resembled those of

Table I. *Leaf fatty acid composition of transgenic lines*

Plants were grown at 17°C. The values are the means ± SD (n = 5).

Genotype	Fatty Acid							
	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
	mol%							
JB101 (pBIfad8) No. 18c	17.0 ± 2.0	4.0 ± 0.2	0.7 ± 0.2	21.0 ± 3.0	0.5 ± 0.1	2.2 ± 0.2	10.8 ± 0.6	42.6 ± 0.4
JB101 (pBIfad8) No. 23a	18.7 ± 0.7	4.3 ± 0.3	0.8 ± 0.1	19.0 ± 1.0	0.8 ± 0.2	2.2 ± 0.2	10.0 ± 0.2	42.0 ± 2.0
WT (pBIfad8) No. 20a	15.8 ± 0.7	4.8 ± 0.4	15.7 ± 0.7	2.6 ± 0.9	0.4 ± 0.1	3.4 ± 0.3	34.0 ± 2.0	21.0 ± 2.0
WT (pBIfad8) No. 26b	16.5 ± 0.6	5.0 ± 0.2	15.6 ± 0.6	1.9 ± 0.4	0.7 ± 0.1	3.2 ± 0.2	37.0 ± 1.0	20.1 ± 1.0
SH1 (pBI121)	18.0 ± 3.0	4.9 ± 0.2	8.0 ± 1.0	6.0 ± 1.0	0.6 ± 0.2	2.6 ± 0.6	33.0 ± 3.0	24.0 ± 3.0
JB101 (pBI121)	18.5 ± 0.5	4.1 ± 0.3	6.5 ± 0.4	11.8 ± 0.4	0.5 ± 0.1	2.5 ± 0.3	15.6 ± 0.7	38.8 ± 0.4
WT (pBI121)	17.0 ± 2.0	4.5 ± 0.1	1.0 ± 0.3	21.0 ± 1.0	0.7 ± 0.2	1.9 ± 0.2	11.0 ± 0.5	41.3 ± 0.9

plants containing the *fad7* mutation or both the *fad7* and *fad8* mutations. Two lines (Nos. 20 and 26) with reduced levels of unsaturation were chosen for further study. Transgenic progeny from these two lines were selected, and the fatty acid composition was measured (Table I). Each of these plants showed levels of 16:2 and 18:2 that were much higher, and levels of 16:3 and 18:3 that were correspondingly lower, than the levels of these fatty acids found in wild-type plants. In fact, the level of 16:2 found in these plants was even higher, and the level of 16:3 was even lower, than the levels of these fatty acids found in a line (SH1) carrying mutations in both the *fad7* and *fad8* genes (Table I). This result suggests that introduction of the pVA34 cDNA resulted in co-suppression (Napoli et al., 1990; van der Krol et al., 1990) of both the endogenous *fad8* and *fad7* genes.

Genomic Sequence

Two clones containing the putative *fad8* gene were isolated from a genomic library of line SH1 (*fad7 fad8 gl1*) by screening the library with gene-specific oligonucleotides based on the sequence of the cDNA in pVA34. The nucleotide sequence corresponding to the coding region from one of these clones, λZAP/FAD8–32, was determined and is available from the GenBank data base under accession number U08216. Comparison of the genomic and cDNA sequences revealed that the gene contains eight exons, ranging from 67 to greater than 533 nucleotides in length, and seven introns, ranging from 85 to 114 nucleotides in length (Fig. 1B). The structure of the *fad8* gene is very similar to that of the *fad3* and *fad7* genes (Iba et al., 1993; Nishiuchi et al., 1994), indicating that the three genes have only recently diverged from a common ancestral gene.

Comparison of the genomic exon sequences from the mutant line SH1 with the wild-type cDNA sequence in pVA34 revealed the existence of a single nucleotide difference between the two genes. This mutation in the genomic sequence resulted in the transition of a G/C bp to an A/T bp at nucleotide 446 of the open reading frame. This mutation converted the codon TGG, which encodes Trp, to the stop codon TAG, resulting in premature termination of the open reading frame (Fig. 1A). This mutation also created a new recognition sequence for the restriction endonuclease *Mae*I. This provided a convenient test for the presence of the mutation. Two oligonucleotides (with sequences TCAAATTCAATCCACCAC and CCAGCCATGGTAAGGGACCA) were used to amplify a 0.56-kb genomic DNA fragment spanning the site of the mutation from wild type, JB101(*fad7*), and SH1 (*fad7 fad8*) lines via PCR. The PCR products from all three of these lines were digested with the restriction endonuclease *Mae*I and run on an agarose gel. The *Mae*I-digested PCR products from the wild-type and JB101 lines showed single bands of 0.56 kb, whereas the *Mae*I-digested PCR product from line SH1 had two bands of approximately 0.21 and 0.35 kb in size (data not shown). This result indicates that genomic DNA from line SH1, but not from the wild-type or JB101 lines, contained the mutation that resulted in the creation of a new *Mae*I recognition site. The PCR product from line JB101 was also sequenced in the region where the mutation occurred. This sequencing data confirmed that the line JB101 contains the wild-type DNA sequence in this region. Thus, we conclude that the insert in pVA34 corresponds to the *fad8* gene.

Regulation of *fad8* Expression

Biochemical characterization of the mutant line SH1 suggested that the desaturase activity encoded for by the *fad8* gene is induced by growth at low temperature (McConn et al., 1994). To determine whether transcriptional regulation might play a role in the low temperature-induced expression of *fad8* activity, the level of steady-state *fad8* mRNA was measured in samples from plants grown at different temperatures. A northern blot containing RNA prepared from wild type and two independent *fad7* mutant lines (JB101 and LK9) grown at either 20 or 30°C was probed with a DNA fragment from the 3′ end of the *fad8* cDNA. For comparison, the same blot was also reprobed with the 3′ end of the *fad7* cDNA. The *fad8* probe hybridized to a band of about 1.8 kb in the RNA samples from wild-type plants grown at 20°C or on blots from plants grown at 30°C that had been shifted to 20°C for 24 h (Fig. 2B). By contrast, *fad8* mRNA was not detectable in the RNA samples from plants grown at 30°C or on blots from plants grown at 20°C and then shifted for 24 h to 30°C (Fig. 2B). This result indicates that at least part of the low temperature-induced increase in the activity of the *fad8* gene is the result of an increase in the steady-state level of *fad8* mRNA in plants grown at low temperatures. As noted previously (Iba et al., 1993), there was no significant effect of growth temperature on the amount of *fad7* mRNA in wild-

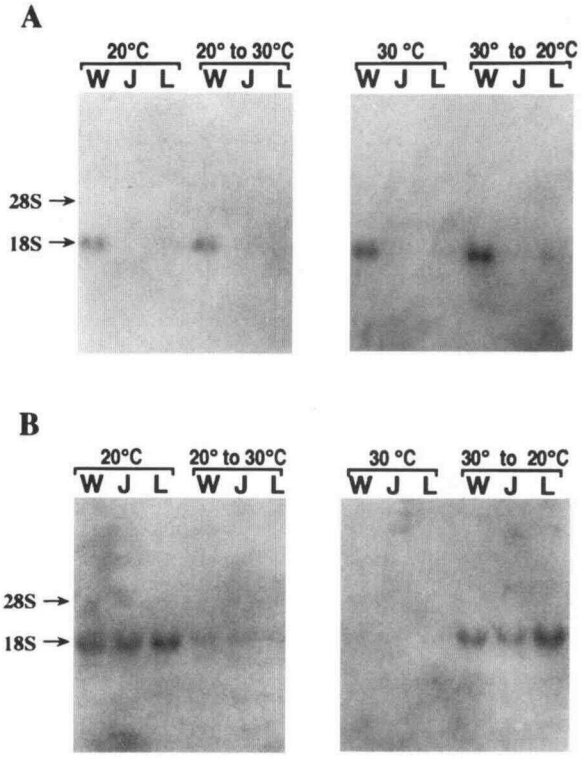

Figure 2. Effect of temperature and genotype on mRNA levels of *fad7* and *fad8* genes. RNA was extracted from wild-type (W), JB101 (J), or LK9 (L) plants that had been grown at 20 or 30°C or shifted from one temperature to the other for 24 h. A was probed with the 3′ region of the *fad7* cDNA. B was probed with the 3′ region of the *fad8* gene. The position of migration of the 18S and 28S rRNAs is shown by the arrows.

type plants and *fad7* mRNA does not accumulate in either JB101 or LK9 (Fig. 2A).

DISCUSSION

The *fad7* and *fad8* genes of *Arabidopsis* have been characterized by genetic criteria as encoding chloroplast-localized ω-3 desaturases. Putative clones for both of these genes, provisionally designated as type II and type III clones, were identified by heterologous screening using the *B. napus fad3* gene as a probe (Iba et at., 1993). The type II cDNA was previously identified as corresponding to the *fad7* gene because it was genetically mapped to the site of the *fad7* mutation (Iba et al., 1993), whereas the *fad8* mutation is not closely linked to the *fad7* gene (McConn et al., 1994). The type III cDNA can genetically complement the *fad7* mutation, indicating that it encodes an enzymatically equivalent chloroplast ω-3 desaturase. However, the type III cDNA clone could not be readily mapped to the *fad8* locus because the *fad8* mutation only has a phenotype in the presence of a *fad7* mutation. Additional evidence that the type III cDNA corresponds to the *fad8* gene is based on the finding that there is a mutation in the type III genomic gene from the line SH1 (*fad7*-1, *fad8*-1) that is not present in the progenitor line JB101(*fad7*-1). The observation that the accumulation of mRNA for the type III desaturase is regulated by temperature, whereas that of the type II gene is not, is also consistent with the genetic evidence indicating that the *fad8* gene is only expressed at low temperature (McConn et al., 1994)

The available biochemical evidence suggests that the protein encoded by the *fad8* gene is localized to the chloroplast (McConn et al., 1994). However, the amino-terminal region of the *fad8* open reading frame does not exhibit characteristic features of a chloroplast transit peptide, such as a high concentration of the hydroxylated amino acids Ser and Thr (von Heijne et al., 1989). This lack of a typical chloroplast transit peptide may be related to the fact that chloroplast desaturases are believed to be integral membrane proteins that are most likely localized to either the thylakoid or inner envelope membranes. Although proteins that are localized to the thylakoids have typical chloroplast transit peptides, neither of the two genes known to encode proteins localized to the chloroplast inner envelope contains a typical chloroplast transit peptide (Dreses-Werringloer et al., 1991; Willey et al., 1991).

An interesting observation regarding the phenomenon of co-suppression (Napoli et al., 1990; van der Krol et al., 1990) was made during the course of experiments designed to produce plant lines that overexpress the *fad8* desaturase. About half of the wild-type plants transformed with the construct pBI/fad8, which contains the full-length *fad8* cDNA expressed under the control of the cauliflower mosaic virus 35S promoter in a sense orientation, showed decreased rather than increased levels of chloroplast ω-3 desaturase activity, presumably as the result of co-suppression of the endogenous *fad8* gene (Table I). In fact, several of these transgenic lines had even lower levels of chloroplast ω-3 desaturase activity than the plant line SH1, which is homozygous for both the *fad7*-1 and *fad8*-1 mutations (Table I). This indicates that the pBI/fad8 construct co-suppresses the activities of both of the endogenous *fad7* and *fad8* genes. A similar phenomenon has been previously reported in petunia in which the chalcone synthesis genes *CHS-A* and *CHS-J*, which are 86% identical at the nucleotide level (Koes et al., 1989), were both co-suppressed by the introduction of a chimeric 35S/*CHS-A* construct. However, the *fad7* and *fad8* genes, which are 76% identical at the nucleotide level, currently represent the most divergent pair of genes that have been reported to co-suppress each other. No co-suppression was observed in the four transgenic JB101 lines. This could simply reflect the fact that a comparatively small number of transgenic lines were examined or may be related in some way to the fact that *fad7* mRNA does not accumulate in JB101.

Much of the interest in fatty acid desaturation is the result of a proposed correlation between the high degree of membrane lipid polyunsaturation and tolerance to low and freezing temperatures. In this context, the finding that the accumulation of mRNA for the *fad8* gene is temperature regulated is of particular interest. RNA isolated from plants grown at 20°C contained levels of *fad8* mRNA that were detectable by northern analysis, whereas RNA isolated from plants grown at 30°C had no detectable *fad8* mRNA. These differences in steady-state RNA levels could be the result of alterations in

either the rate of *fad8* transcription or in the stability of the *fad8* mRNA. Further analysis will be required to distinguish between these two possibilities. Although temperature-regulated expression of a cyanobacterial desaturase has been previously reported (Los et al., 1993; Wada et al., 1993), to our knowledge this is the first report of the regulation of a higher plant desaturase gene by temperature.

Catalytic hydrogenation of membranes of whole cyanobacterial cells resulted in large increases in the amount of transcript for a fatty acid desaturase (Vigh et al., 1993). These elegant studies are interpreted as evidence for a mechanism that can regulate the amount of desaturase transcript in response to changes in the fluidity of the membrane. A similar mechanism does not appear to regulate expression of the *fad8* gene. This was evident from the fact that the expression of the *fad8* gene was not significantly affected by the large reduction in chloroplast membrane trienoic fatty acids caused by the *fad7* mutations (Fig. 2). Therefore, the enhanced expression of the *fad8* gene at low temperature may reflect the existence of a mechanism that responds to temperature per se rather than to the physical properties of the membrane.

Because the effect of the *fad8* mutation on chloroplast fatty acid composition is relatively minor in wild-type plants grown at normal temperature (McConn et al., 1994), the physiological role of the *fad8* gene is not yet clear. The finding that a constitutively expressed *fad8* cDNA can functionally complement a mutation at the *fad7* locus indicates that the *fad7* and *fad8* gene products carry out the same chemical reaction. The *fad8*-1 mutation resulted in the formation of a stop codon in the *fad8* open reading frame, which is expected to eliminate translation of approximately two-thirds of the Fad8 protein. Because the truncated Fad8 protein almost certainly lacks any desaturase activity and plants that are homozygous for the *fad8*-1 mutation are viable, a functional *fad8* gene appears not to be required for normal plant growth. A possible function for the *fad8* gene is suggested by the finding that steady-state levels of *fad8* mRNA are increased by growth at low temperatures. The deleterious effects of low temperature on mutants of *Arabidopsis* with reduced levels of polyunsaturation is direct evidence that fatty acid composition is a component of low temperature fitness (Hugly and Somerville, 1992; Miquel et al., 1993). The importance of trienoic fatty acid composition in conditioning cold tolerance was also recently demonstrated in transgenic tobacco plants that have increased levels of trienoic fatty acids (Kodama et al., 1994). The fact that plants heterozygous for the *fad7* mutation show a reduction in chloroplast ω-3 fatty acids (Browse et al., 1986a) implies that the *fad7* gene is not feedback regulated and has an activity level that is barely sufficient to maintain normal levels of ω-3 desaturase activity in plants grown at constant, moderate temperatures. Therefore, the function of the *fad8* desaturase might be to provide a rapid increase in chloroplast ω-3 desaturase activity following a sudden cold spell, particularly in rapidly growing tissues where rates of fatty acid desaturation may not match rates of fatty acid synthesis.

ACKNOWLEDGMENTS

We thank Ljerka Kunst for providing line 41-3-a and Ron Davis for providing a cDNA library.

Received June 17, 1994; accepted September 19, 1994.

Copyright Clearance Center: 0032-0889/94/106/1615/07.

The GenBank accession numbers for the sequences reported in this article are L27158 (*fad8* cDNA) and U08216 (*fad8*-1 genomic sequence).

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