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Characterization of the *Brassica napus* Extraplastidial Linoleate Desaturase by Expression in *Saccharomyces cerevisiae*[1]

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The substrate specificity and regioselectivity of the *Brassica napus* extraplastidial linoleate desaturase (*FAD3*) was investigated in vivo in a heterologous expression system. A strain of the yeast *Saccharomyces cerevisiae* producing the plant enzyme was constructed and cultured in media containing a variety of fatty acids. The products of desaturation of these potential substrates were determined by gas chromatographic and mass spectrometric analysis of the yeast cultures. The results indicate that the enzyme has: (a) ω-3, as opposed to Δ-15 or double-bond-related regioselectivity, (b) the ability to desaturate substrates in the 16 to 22 carbon range, (c) a preference for substrates with ω-6 double bonds, but the ability to desaturate substrates with ω-6 hydroxyl groups or ω-9 or ω-5 double bonds, and (d) a relative insensitivity to double bonds proximal to the carboxyl end of the substrate.

Both ω-3 and ω-6 polyunsaturated fatty acids (PUFA) are important as structural components of membrane glycerolipids and as precursors to signaling molecules such as jasmonates in plants and eicosanoids in animals (Creelman and Mullet, 1997; Spector, 1999). Vertebrates are not capable of introducing ω-3 and ω-6 double bonds into fatty acids and consequently must obtain these PUFA from their diet. It has been suggested that the typical "western" diet, which is relatively high in ω-6 PUFA and low in ω-3 PUFA, may not supply the appropriate balance of PUFA for proper biological function (Shahidi and Wanasundara, 1998). As a result, there is interest in producing ω-3 PUFA for human and animal nutrition from various sources (Shahidi and Wanasundara, 1998), including genetically modified plants.

Generally speaking, plants carry out "ω-3" fatty acid desaturation in two compartments on two different substrate classes (Somerville and Browse, 1991; Los and Murata, 1998). In Arabidopsis plastids, the products of *Fad7* and *Fad8* desaturate both 16:2 and 18:2 esterified to various glycerolipids. Outside of the plastid and probably on the endoplasmic reticulum, the product of *Fad3* desaturates linoleic moieties esterified to the *sn-2* position of phosphatidylcholine (PC). Both reactions require molecular oxygen and an electron donor, probably ferredoxin in the plastid reaction and cytochrome (Cyt) b_5 in the extraplastidial reaction (Los and Murata, 1998; Shanklin and Cahoon, 1998). It is notable that the above plastidial and extraplastidial desaturases show high amino acid sequence similarity (e.g. 66% identity for Arabidopsis *FAD3* and *FAD7*; Yadav et al., 1993). This suggests a relatively recent evolutionary divergence and similarities in structure and function. They are thought to share a general membrane topology and His-dependent iron-binding structure with other membrane-bound desaturases (Los and Murata, 1998; Shanklin and Cahoon, 1998).

In general, information about the substrate specificities and regioselectivities of membrane-bound fatty acyl desaturases is limited (Heinz, 1993; Shanklin and Cahoon, 1998). This is largely due to difficulties in the isolation of active forms of such enzymes and their requirement for hydrophobic substrates and proteinaceous co-factors (e.g. Cyt b_5 reductase and Cyt b_5). Three classes of regioselectivity have been observed for fatty acid desaturases. The Δ-x desaturases introduce a double-bond x carbons from the carboxyl end; ω-x desaturases introduce a double-bond x carbons from the methyl end; and the so-called ν + x desaturases x carbons from an existing double bond. Data regarding the regioselectivity and substrate specificity of enzymes that introduce a double bond at the 15 position of linoleic acid are sketchy. By supplying heptanoic acid to the cyanobacterium *Synechocystis* PCC6803 cultures and allowing elongation and desaturation to occur in vivo, Higashi and Murata (1993) were able to obtain data indicating the presence of an ω-3 desaturase that acts on 17 to 19 carbon fatty acids. In higher plants, the plastid ω-3 desaturase is considered to be evolutionarily homologous to this enzyme and to have similar regioselectivity (based on the desaturation of both 16:2[7,10] and 18:2[9,12] at the ω-3 position; Somerville and Browse, 1991; Yadav et al., 1993). A related enzyme from nematodes, the *fat-1* gene product of *Caenorrhabditis elegans*, was characterized by Browse and coworkers (Spychalla et al., 1997) by expression in Arabidopsis. The results indicated that it is an ω-3 fatty acid desaturase that acts on 18 and 20 carbon fatty acids.

In oilseed crops, *FAD3* is responsible for the production of the majority of ω-3 fatty acids in seeds, α-linolenic acid in particular. Until recently, relatively little was known about the substrate specificity and regioselectivity of this

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enzyme, which is called variously the extraplastidial (or microsomal or endoplasmic reticulum) linoleate or Δ-15 or ω-3 desaturase. It has been suggested that the enzyme measures from the carboxyl end or from an existing double bond (Heinz, 1993; Griffiths et al., 1996), but analysis of hydroxy fatty acid metabolism in developing oilseeds argues against the former and favors either u + x or ω-3 regioselectivity (Reed et al., 1997).

In this paper, we describe efforts to elucidate a more complete picture of the substrate specificity and regioselectivity of the plant *FAD3*. This has been accomplished by expression of the *Brassica napus Fad3* in baker's yeast (*Saccharomyces cerevisiae*), followed by culture in media containing various fatty acid substrates and assay of the resulting desaturation products. Yeast acts as a very convenient host for such studies (Covello and Reed, 1996; Watts and Browse, 1999). It has a very simple fatty acid profile and only one major fatty acyl desaturase; it provides a eukaryotic endoplasmic reticulum, Cyt b_5, and Cyt b_5 reductase. It takes up and incorporates a wide range of fatty acids from the growth medium and the low levels of β-oxidation in *S. cerevisiae* in the presence of an appropriate carbon source allows for accumulation of both supplied precursors and any fatty acyl products formed (Kunau et al., 1987).

MATERIALS AND METHODS

Chemicals

Lesquerolic acid (20:1-OH[11c,14h]) was prepared by high-performance liquid chromatography isolation of methyl lesquerolate from trans-methylated lipids of *Lesquerella fendleri* seed (Reed et al., 1997). Other fatty acids were obtained from Nu-Chek-Prep (Elysian, MN). All fatty acids used were of known purity (typically >99%). Tergitol (type NP-40) and methanolic/HCl (3 M) were obtained from Sigma-Aldrich (Oakville, Ontario, Canada), diethylamine and acetyl chloride from Sigma-Aldrich, and pyridine from Pierce Chemical (Rockford, IL).

Yeast Strain Construction

Copy DNA of one of the extraplastidial linoleate desaturase gene family members of *Brassica napus* was amplified from the clone pBNDES3 (Arondel et al., 1992) by PCR using the oligonucleotide primers BNDES3-1 (GCCGAATTCATGGTTGTTGCTATGGAC) and BNDES3-2 (GCCGAATTCAATAGAGCTAGGAAGAAAAG) by standard methods (Ausubel et al., 1995; Covello and Reed, 1996). The PCR product was gel-purified, digested with *Eco*RI, and ligated into the centromeric yeast expression vector pSE936 containing the Gal-inducible GAL1 promoter (Elledge et al., 1991) to give the plasmid pRS131. The sequence of the insert of pRS131 was confirmed to be identical to that previously reported (Arondel et al., 1992) and in the sense orientation relative to the GAL1 promoter using a dideoxynucleotide cycle sequencing kit (DyeDeoxy, Perkin-Elmer Applied Biosystems, Foster City, CA) and a DNA sequencer (model 373, Perkin-Elmer Applied Biosystems).

The *Saccharomyces cerevisiae* strain MKP-o (*MATα can1-100 ade2-1 lys2-1 ura3-52 leu2-3, 112 his3-Δ200 trp1-Δ901*; kindly provided by Wei Xiao, University of Saskatchewan, Canada) was transformed with pRS131 by the method of Gietz et al. (1992) and selected on minimal agar plates lacking uracil (Ausubel et al., 1995) to give the strain pRS131/MKP-o.

Growth and Biochemical Analysis of Transformed Yeast

To test various substrates of the *FAD3*, the pRS131/MKP-o strain was grown in minimal medium lacking uracil and containing Gal (CM gal−ura), various fatty acids (100 mg/L unless otherwise stated), and Tergitol (Type NP-40, 0.1% [v/v]) at 20°C for 3 d and at 15°C for 3 d. The plasmid vector pSE936 in the yeast strain MKP-o was used as a control.

For fatty acid analysis, the cells from 50-mL cultures were collected by centrifugation and washed once with 10 mL of 1% (w/v) Tergitol and once with 10 mL of distilled water. The pellets were then saponified with 5 mL of 10% (w/v) methanolic KOH in a sealed culture tube at 80°C for 2 h. The tubes were then cooled and the non-saponifiable lipids were pre-extracted with 2× 2 mL of hexane. The methanol was neutralized (slightly acidic) with 6 M HCl and the free fatty acids were extracted with 2× 2 mL of hexane. For gas chromatography (GC) analysis, most of the fatty acids were transmethylated with methanolic/HCl (3 M) at 80°C for 1 to 2 h and then extracted with 2× 2 mL of hexane. For acid-labile (conjugated) fatty acids and for double bond positional analysis, the fatty acids were derivatized with diethylamine according to the method of Nilsson and Liljenberg (1991). GC analysis was carried out as described by Taylor et al. (1992). GC/mass spectrometry (MS) analysis of the diethylamide derivatives was performed as described by Carrier et al. (1996).

Plant Material

Seeds of field-grown *Linum usitatissimum* cv McGregor were kindly provided by Yousif Hormis (Crop Development Centre, University of Saskatchewan). AL63, an ethylmethanesulfonate-induced mutant of Arabidopsis was obtained from L. Kunst (University of British Columbia, Canada). Seeds resulting from a second backcross of this *Fad2* mutant with wild type were used for analysis.

RESULTS AND DISCUSSION

FAD3 Activity in Yeast

Figure 1 shows some examples of GC analysis of fatty acid methyl esters from yeast cultures. As expected, the pRS131/MKP-o cultures supplied with 18:2(9,12) show a peak corresponding to 18:3(9,12,15) (Fig. 1A). Typically, cultures grown at 15°C showed somewhat better desaturation product accumulation by a factor of about 2 compared with the usual 28°C growth temperature (data not shown). A similar, stronger temperature effect was previously reported for expression of the Arabidopsis extraplastidial

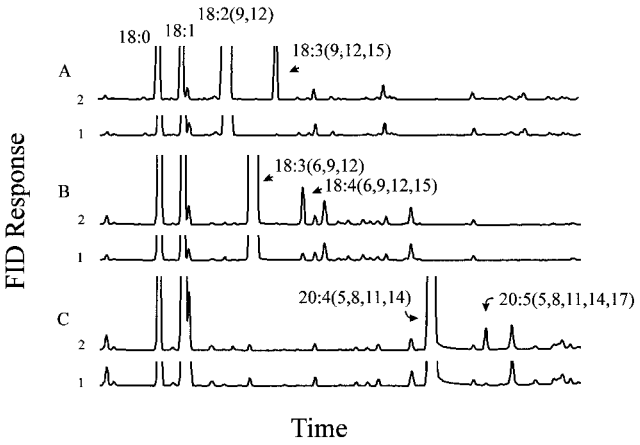

Figure 1. GC analysis of fatty acid methyl esters from yeast transformed with control plasmid pSE936 (1) and pRS131 containing *B. napus Fad3* (2) for cultures grown in media containing 18:2(9,12) (A), 18:3(6,9,12) (B), or 20:4(5,8,11,14) (C). For each pair of chromatograms, the flame ionization detection signal corresponds to the same volume of yeast culture.

oleate desaturase (Covello and Reed, 1996). For this reason, a protocol of growth for 3 d at 20°C (for relatively rapid growth) followed by 3 d at 15°C was adopted.

In an effort to check for strain- and construct-dependent differences, a strain was constructed containing the *B. napus Fad3* ligated into the 2-μm plasmid pYES2 (Invitrogen, Carlsbad, CA) containing the GAL1 promoter transformed into the yeast strain INVSC1 (Invitrogen). This strain showed levels of desaturation products similar to pRS131/MKP-o (data not shown).

Table I gives a complete listing of fatty acid substrates tested and the products of desaturation. In addition to desaturase activity, there are a number of factors that could affect the level of product accumulating in yeast cells as a fraction of total fatty acids. These include the rate of incorporation of exogenous fatty acids and their availability to the desaturase, the rate of endogenous biosynthesis of fatty acids, and the rate of breakdown of various fatty acids. Since these may vary from substrate to substrate, the data must be considered semi-quantitative. Nevertheless, they give a very useful indication of the substrate specificity and regioselectivity of the plant extraplastidial linoleate desaturase.

As indicated in Table I, accumulation of substrates varied widely from 1% to more than 40% of total yeast fatty acids. To aid in interpretation of the data, pRS131/MKP-o was cultured in a range of concentrations of 18:2(9,12) giving incorporation levels of 0.4% to 64% (w/w) (see Table II). The corresponding 18:3(9,12,15) accumulation indicates that desaturation in the cultures is not limited by 18:2 levels unless they are somewhere below 7% (w/w). Thus, although it is not clear that these results can be extrapolated to other substrates, it seems reasonable to assume that any that accumulate above 10% are probably not limiting, whereas substrate limitation is a possibility at lower levels, such as for 20:2(11,14) and 20:1-OH(11c,14h). In the latter cases, the accumulation of product may underestimate the activity of *FAD3* relative to other substrates.

Table I. *Conversion of exogenous fatty acids by the yeast strain pRS131/MKP-o*

See "Material and Methods" for culture conditions. Values are the means of two experiments each with duplicate cultures. For pSE936/MKP-o cultures, with the exception of 18:3(6,9,12)-supplied cultures, no significant peaks were detected at the retention time of the desaturation product. In the case of 18:3(6,9,12), the area of a peak due to substrate impurity for pSE936/MKP-o cultures was subtracted from the product peak area for pRS131/MKP-o cultures (see Fig. 1). Despite incorporation levels of 1.6% to 26.1%, ω-3 desaturation products were not detected above 0.001% for 14:0, 14:1(9), 17:0, 20:0, 20:1(5), 22:1(13), or 18:0(12h).

Substrate	Substrate Accumulation	Product	Identification[a]	Product Accumulation
	% (w/w) of total fatty acids			*% (w/w) of total fatty acids*
16:1(11)[b]	43.8	16:2(11,13)	GC/MS	0.14
16:1(9)[c]	23.3	16:2(9,13)	GC/MS	0.048
18:1(9)	57.9	18:2(9,15)	GC/MS	0.21
18:2(9,12)	48.5	18:3(9,12,15)	GC/MS	1.3
18:3(6,9,12)	46.8	18:4(6,9,12,15)	GC/MS	0.35
20:1(11)	7.3	20:2(11,17)	GC/MS	0.016
20:2(11,14)	3.4	20:3(11,14,17)	GC/MS	0.18
20:3(8,11,14)	16.3	20:4(8,11,14,17)	RT	0.10
20:4(5,8,11,14)	29.2	20:5(5,8,11,14,17)	RT	0.21
22:2(13,16)	3.7	22:3(13,16,19)	T	0.020
18:1-OH(9c,12h)	10.2	18:2-OH(9c,12h,15c)	RT	0.16
20:1-OH(11c,14h)	4.9	20:2-OH(11c,14h,17c)	RT	0.038

[a] Products were identified by GC/MS of diethylamide derivatives, comparison of retention times with standards (RT), or tentatively based on the assumption of ω-3 desaturation (T). [b] Derived from in vivo elongation of supplied 14:1(9). [c] Endogenous substrate; no fatty acid added to medium.

Table II. *Dependence of 18:3(9,12,15) accumulation in pRS131/MKP-o cultures on 18:2(9,12) supply*

Cultures were grown as indicated in the "Materials and Methods" with the addition of varying amounts of 18:2(9,12) to the medium. Values are the means of two experiments each with duplicate cultures.

Concentration of 18:2(9,12) in Culture	18:2(9,12) Accumulation	18:3(9,12,15) Accumulation
mg/L	*% (w/w) of total fatty acids*	*% (w/w) of total fatty acids*
1	0.4	0.4
10	7.3	2.4
100	63.5	1.9

Regioselectivity and Substrate Specificity of *FAD3*

In some cases, as indicated in Table I, GC/MS analysis was performed on diethylamide derivatives of fatty acids. Figure 2 shows an example of this for the product of desaturation of 16:1(9). In this case, the mass spectrum displays diagnostic ions differing by 12 D at m/z = 252 and 264, indicating the introduction of a double bond at the ω-3 position to give 16:2(9,13). Indeed, all of the data in Table I and the corresponding GC/MS data (Fig. 2 and data not shown) are consistent with the regioselectivity of the *B. napus FAD3* being described as ω-3 as opposed to the Δ-15 or $\nu + 3$ possibilities, which had been suggested previously (Heinz, 1993; Griffiths et al., 1996; Reed et al., 1997).

The effect of chain length can be seen in the series of feedings of 18:2(9,12), 20:2(11,14), and 22:2(13,16). While all three of these substrate were desaturated, 22:2(13,16) was an apparently poor substrate. Activity was also observed for the substrates 16:1(9) and 16:1(11) but giving a lower level of accumulation than 18:1(9).

The presence of a double bond at the ω-6 position also has a strong effect, as seen in the comparison of the 18:1, 18:2, and 18:3 feedings with the 20:1, 20:2, 20:3, and 20:4 feedings. These comparisons also indicate that the presence of double bonds proximal to the carboxyl group does not have a strong effect. Of particular interest is the 18:3(6,9,12) experiment. Previously, it was found that linseed microsomes were capable of desaturating 18:2(9,12), but not γ-linolenic acid (18:3[6,9,12]) (Griffiths et al., 1996). In the yeast expression system described herein, the *B. napus* desaturase appears to catalyze both reactions (Fig. 1B; Table I). The explanation for this difference is not clear; it could be a species difference or it could be related to factors affecting the availability of substrate and the accumulation of products in the two systems.

Unusual Fatty Acid Substrates

Engeseth and Stymne (1996) showed that plant desaturases were capable of desaturating oxygenated fatty acids. This is particularly relevant in the case of a genus in the family *Brassicaceae* called *Lesquerella,* whose members produce unsaturated hydroxy fatty acids in their seeds. These include ricinoleic acid (18:1-OH[9c,12h]), lesquerolic acid [20:1-OH(11c,14h)], densipolic [18:2-OH(9c,12h,15c)], and auricolic acid [20:2-OH(11c,14h,17c)] (Hayes et al., 1995; Reed et al., 1997). In vivo radiolabeling experiments indicate that in various *Lesquerella* species, densipolic and auricolic acids are synthesized by desaturation of ricinoleic and lesquerolic acids, respectively (Engeseth and Stymne, 1996; Reed et al., 1997). In this study, analysis of yeast cultures expressing the *B. napus Fad3* and grown in the presence of ricinoleic and lesquerolic acids (see Table I) confirm the in planta radiolabeling studies and indicate that both fatty acids are desaturated by the plant extraplastidial linoleate desaturase.

Our results for 20 carbon substrates that are not found in the *Brassicaceae* are interesting. In the yeast expression system, the *B. napus* desaturase was capable of desaturation of dihomo-γ-linolenic acid (20:3) and arachidonic acid (20:4; see Fig. 1C). However, it has been reported that when Arabidopsis leaves were sprayed with 20:3 or 20:4, no ω-3 desaturation was detected, despite the fact that transgenic plants expressing a nematode (*Caenorhabditis elegans*) ω-3 desaturase converted these fatty acids (Spychalla et al., 1997). The differences between the yeast and Arabidopsis experiments may simply be quantitative but, under some conditions at least, the plant *Fad3* product appears to be capable of desaturating the 20-carbon ω-6 fatty acids.

In Planta Desaturation

When cultures were grown without exogenous fatty acid or with oleate in the medium, a fatty acid identified by GC/MS as 18:2(9,15) accumulated. Similarly, a small amount of 16:2(9,13) appeared (see Table I). Presumably,

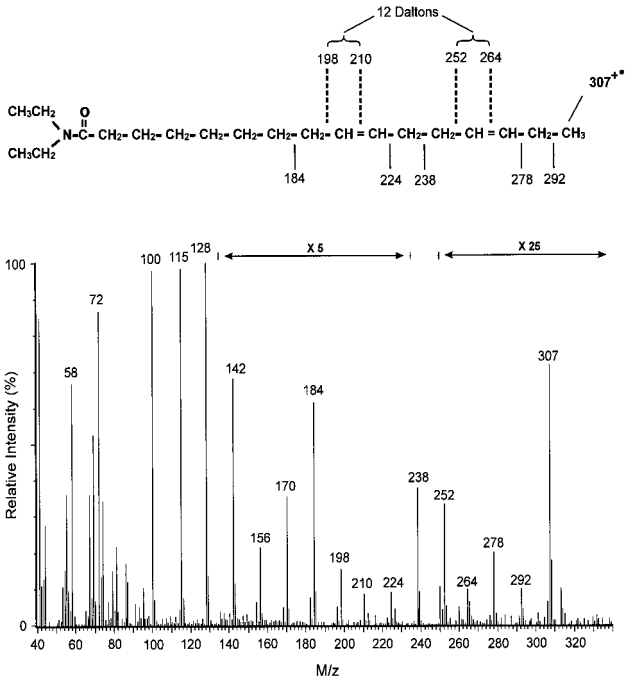

Figure 2. MS identification of 16:2(9,13). Mass spectrum of a compound separated by GC from a transmethylated pRS131/MKP-o culture grown in the presence of 16:1(9). See "Materials and Methods" for details.

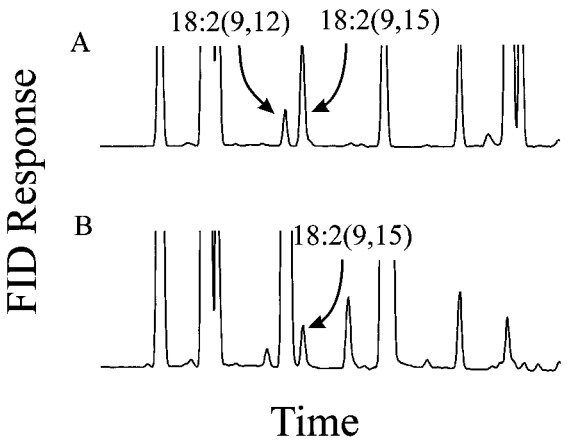

Figure 3. GC analysis of fatty acid methyl esters from seeds of the AL63 (*Fad2*) mutant of Arabidopsis (A) and flax (B). FID, Flame ionization detection.

this results from desaturation of endogenous 16:1(9) and 18:1(9). In this regard, it is interesting to note the previous detection of fatty acids in leaves of a *Fad6* (plastidial ω-6 desaturase) mutant of Arabidopsis that were tentatively identified as 16:2(9,13) and 18:2(9,15) (Browse et al., 1989). Since the majority of leaf lipids in Arabidopsis are synthesized by the prokaryotic pathway (in the plastids), this suggests that the plastid ω-3 desaturase has some activity on mono-unsaturates. To investigate the possibility of a similar capability of the plant *Fad3* product, analysis was performed on the predominantly eukaryotic-pathway-derived seed lipids of two plants: AL63, a *Fad2* mutant line of Arabidopsis (L. Kunst, personal communication), which contains low levels of 18:2(9,12), and flaxseed (cv MacGregor), which contains a very active extraplastidial ω-3 desaturase. When fatty acids from these seeds were analyzed, AL63 and flaxseed were found to contain approximately 1% and 0.1% (w/w) 18:2(9,15), respectively (see Fig. 3). While it is possible in these plants that the 18:2(9,15) is a product of the plastidial ω-3 desaturase, it appears that, in planta, *FAD3* has some activity on mono-unsaturates.

CONCLUSIONS

In conclusion, based on heterologous expression in *S. cerevisiae*, the *B. napus* extraplastidial linoleate desaturase has: (a) ω-3 regioselectivity; (b) the ability to desaturate substrates in the 16 to 22 carbon range; (c) a preference for substrates with ω-6 double bonds, but the ability to desaturate substrates with ω-6 hydroxyl groups or ω-9 or ω-5 double bonds; and (d) a relative insensitivity to double bonds proximal to the carboxyl end of the substrate.

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LITERATURE CITED

Arondel A, Lemieux B, Hwang I, Gibson S, Goodman HM, Somerville CR (1992) Map-based cloning of a gene controlling omega-3 fatty acid desaturation in *Arabidopsis*. Science **258:** 1353–1355

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, Albright LM, Coen DM, Varki A, eds (1995) Current Protocols in Molecular Biology. John Wiley & Sons, New York

Browse J, Kunst L, Anderson S, Hugly S, Somerville C (1989) A mutant of *Arabidopsis* deficient in the chloroplast 16:1/18:1 desaturase. Plant Physiol **90:** 522–529

Carrier DJ, Cunningham JE, Hogge LR, Taylor DC, Dunstan DI (1996) Gas chromatography-mass spectrometry characterization of some fatty acids from white and interior spruce. J Chromatogr A **715:** 317–324

Covello PS, Reed DW (1996) Functional expression of the extraplastidial *Arabidopsis thaliana* oleate desaturase gene (*FAD2*) in *Saccharomyces cerevisiae*. Plant Physiol **111:** 223–226

Creelman RA, Mullet JE (1997) Oligosaccharides, brassinolides, and jasmonates: nontraditional regulators of plant growth, development, and gene expression. Plant Cell **9:** 1211–1223

Elledge SJ, Mulligan JT, Ramer SW, Spottswood M, Davis RW (1991) lambdaYes: a multifunctional cDNA expression vector for the isolation of genes by complementation of yeast and *Escherichia coli* mutations. Proc Natl Acad Sci USA **88:** 1731–1735

Engeseth N, Stymne S (1996) Desaturation of oxygenated fatty acids in *Lesquerella* and other oil seeds. Planta **198:** 238–245

Gietz D, St. Jean A, Woods RA, Schiestl RH (1992) Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res **20:** 1425

Griffiths G, Brechany EY, Jackson FM, Christie WW, Stymne S, Stobart AK (1996) Distribution and biosynthesis of stearidonic acid in leaves of *Borago officinalis*. Phytochemistry **43:** 381–386

Hayes DG, Kleiman R, Phillips BS (1995) The triglyceride composition, structure and presence of estolides in the oils of *Lesquerella* and related species. J Am Oil Chem Soc **72:** 559–569

Heinz E (1993) Biosynthesis of polyunsaturated fatty acids. *In* TS Moore Jr, ed, Lipid Metabolism in Plants. CRC Press, Boca Raton, FL, pp 33–89

Higashi S, Murata N (1993) An in vivo study of substrate specificities of acyl-lipid desaturases and acyltransferases in lipid synthesis in *Synechocystis* PCC6803. Plant Physiol **102:** 1275–1278

Kunau W-H, Kionka C, Ledebur A, Mateblowski M, Moreno de la Garza M, Schultz-Borchard U, Thieringer R, Veenhuis M (1987) β-Oxidation systems in eukaryotic organisms. *In* HD Fahimi, H Sies, eds, Peroxisomes in Biology and Medicine. Springer-Verlag, Berlin, pp 128–140

Los DA, Murata N (1998) Structure and expression of fatty acid desaturases. Biochim Biophys Acta **1394:** 3–15

Nilsson R, Liljenberg C (1991) The determination of double bond positions in polyunsaturated fatty acids: gas chromatography/mass spectrometry of the diethylamide derivative. Phytochem Anal **2:** 253–259

Reed DW, Taylor DC, Covello PS (1997) Metabolism of hydroxy fatty acids in developing seeds in the genera *Lesquerella* (Brassicaceae) and *Linum* (Linaceae). Plant Physiol **114:** 63–68

Shahidi F, Wanasundara UN (1998) Omega-3 fatty acid concentrates: nutritional aspects and production technologies. Trends Food Sci Technol **9:** 230–240

Shanklin J, Cahoon EB (1998) Desaturation and related modifications of fatty acids. Annu Rev Plant Physiol Plant Mol Biol **49:** 611–641

Somerville C, Browse J (1991) Plant lipids: metabolism, mutants, and membranes. Science **252:** 80–87

Spector AA (1999) Essentiality of fatty acids. J Am Oil Chem Soc **34:** S1–S3

Spychalla JP, Kinney AJ, Browse J (1997) Identification of an animal omega-3 fatty acid desaturase by heterologous expression in *Arabidopsis*. Proc Natl Acad Sci USA **94:** 1142–1147

Taylor DC, Barton DL, Rioux KP, MacKenzie SL, Reed DW, Underhill EW, Pomeroy MK, Weber N (1992) Biosynthesis of acyl lipids containing very-long chain fatty acids in microspore-derived and zygotic embryos of *Brassica napus* L. cv Reston. Plant Physiol **99:** 1609–1618

Watts JL, Browse J (1999) Isolation and characterization of a delta-5 fatty acid desaturase from *Caenorhabditis elegans*. Arch Biochem Biophys **362:** 175–182

Yadav NS, Wierzbicki A, Aegerter M, Caster CS, Pérez-Grau L, Kinney AJ, Hitz WD, Booth Jr JR, Schweiger B, Stecca KL, Allen SM, Blackwell M, Reiter RS, Carlson TJ, Russell SH, Feldmann KA, Pierce J, Browse J (1993) Cloning of higher plant omega-3 fatty acid desaturases. Plant Physiol **103:** 467–476