Biochemical and Biophysical Research Communications **272,** 263–269 (2000)
doi:10.1006/bbrc.2000.2772, available online at http://www.idealibrary.com on IDEAL®

A Palmitoyl-CoA-Specific Δ9 Fatty Acid Desaturase from *Caenorhabditis elegans*

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Received April 27, 2000

Biosynthesis of polyunsaturated fatty acids in *C. elegans* is initiated by the introduction of a double bond at the Δ9 position of a saturated fatty acid. We identified three *C. elegans* fatty acid desaturase genes related to the yeast Δ9 desaturase OLE1 and the rat stearoyl-CoA desaturase SCD1. Heterologous expression of all three genes rescues the fatty acid auxotrophy of the yeast Δ9 desaturase mutant *ole1*. Examination of the fatty acid composition of the transgenic yeast reveals striking differences in the substrate specificities of these desaturases. Two desaturases, FAT-6 and FAT-7, readily desaturate stearic acid (18:0) and show less activity on palmitic acid (16:0). In contrast, the other desaturase, FAT-5, readily desaturates palmitic acid (16:0), but shows nearly undetectable activity on the common Δ9 substrate stearic acid. This is the first report of a palmitoyl-CoA-specific membrane fatty acid desaturase. © 2000 Academic Press

Palmitoleic (16:1) and oleic (18:1) acids are major constituents of membrane phospholipids and triacylglycerol stores in animals, plants, and fungi. These organisms regulate the ratio of monounsaturated fatty acids to saturated fatty acids in cellular membranes to maintain proper fluidity (1, 2). In humans, the alteration of this ratio has been implicated in cancer, diabetes, obesity, immune disorders, as well as neurological, vascular and heart diseases (1).

Eukaryotic organisms synthesize monounsaturated fatty acids from saturated fatty acid precursors by the introduction of a double bond between carbons 9 and 10 of a saturated acyl chain. This conversion is catalyzed by fatty acyl desaturases that use molecular oxygen and reducing equivalents obtained from NAD(P)H via a short electron transport chain consisting of NAD(P)H, cytochrome b_5-reductase, and cytochrome b_5 (3). The vast majority of desaturases are endoplasmic reticulum (ER) membrane-bound diiron-oxo proteins characterized by three conserved histidine-rich motifs as well as by two long stretches of hydrophobic residues. The conserved histidine residues are important for coordinating two iron atoms at the active site on the cytosolic face of the ER while the hydrophobic residues form two membrane spanning loops that anchor the protein into the lipid bilayer (4, 5). Another type of desaturase class is the soluble acyl-acyl carrier protein (ACP) desaturases found in the stroma of plant plastids. While these are also diiron-oxo proteins, they are structurally unrelated to the ER desaturases (6).

Stearoyl-CoA desaturase (SCD) is one of the most studied and best understood membrane bound desaturases. One yeast gene, three mouse genes, two rat genes and a single human SCD gene have been cloned and characterized (7–12). The Δ9 fatty acyl desaturase gene (*OLE1*) from the yeast *Saccharomyces cerevisiae* was isolated and characterized by complementation of the *ole-1* mutant which requires unsaturated fatty acids for growth (7). This mutant is incapable of growth on unsupplemented media because it is unable to synthesize monounsaturated fatty acids. The rat SCD1 protein is 36% identical to the yeast Ole1p within a 257 AA internal region of the open reading frame. However, the N-terminal region shows no significant homologies and the yeast gene extends 113 amino acids beyond the C-terminal of the rat gene. The C-terminal region of Ole1p has been identified as a cytochrome b5 domain (13). Heterologous expression of rat SCD1 rescues the fatty acid auxotrophy of the *ole1* mutant even though the rat gene carries no cytochrome b5 domain (4). This rescue has been shown to depend on the presence of the *S. cerevisiae* microsomal cytochrome b5 gene (YSCYb5), indicating that the rat desaturase enzyme requires this electron donor and interacts successfully with yeast components of the desaturase system (13).

As the name implies, the SCDs use stearic acid (18:0) as a substrate. The other major substrate for these enzymes is palmitic acid (16:0). Expression of SCDs in

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0006-291X/00 $35.00

the yeast *ole1* mutant revealed the substrate preference of these Δ9 desaturases. Ole1p readily desaturates both 18:0 and 16:0, converting 86% of 18:0 to 18:1 and 65% of 16:0 to 16:1 (7). The rat SCD gene expressed in *ole1* was capable of desaturating 88% of 18:0 and 46% of 16:0. Recently characterized Δ9 desaturases from cabbage looper moth and the oleaginous fungus *Mortierella alpina* expressed in *ole1* showed an even greater preference for 18:0, producing 6–13 times more 18:1 than 16:1 (14, 15).

We identified three *C. elegans* open reading frames that display significant similarity to the rat and yeast Δ9 desaturases. In this report we demonstrate that all three of these desaturases complement the yeast *ole1* mutant when expressed on an episomal plasmid under control of a constitutive promoter. Monounsaturated fatty acids with double bonds at C9 are produced by all three strains. Two of the *C. elegans* Δ9 desaturases display a substrate preference typical of previously characterized stearoyl-CoA desaturases. In contrast, the third protein readily desaturates 16:0 and other medium chain fatty acids, yet converts stearic acid (18:0) to oleic acid (18:1) at a very low frequency, less than 3%. This is the first description of a palmitoyl-CoA specific desaturase with very limited activity on stearic acid.

MATERIALS AND METHODS

Identification, amplification, and cloning of C. elegans Δ9 desaturase genes. The Sanger Center *C. elegans* wormpep database (http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml) was searched using BLAST (16) with the polypeptide sequence of the rat stearoyl-CoA desaturase (SWISSPROT Accession No P07308) (17). Three open reading frames were identified: W06D12.3, VZK822l.1, and F10D2.9. The predicted coding sequence of each of these genes was amplified using either RT-PCR on total RNA from mixed stage *C. elegans*, or by PCR amplification from a *C. elegans* mixed-stage lambda phage Uni-ZAP cDNA library (Stratagene). Total RNA was prepared with TRIZOL reagent (Life Technologies) following the manufacturer's protocol. Reverse transcription-PCR was performed with the "One-Step" kit (Life Technologies). For all three genes, the upstream primer used to amplify the cDNA sequences added an EcoR1 restriction site and the downstream primer added an XhoI restriction site. After amplification the PCR products were digested with EcoR1 and XhoI and cloned directly into the episomal yeast expression vector pMK195 (18) restricted with the same enzymes. pMK195 encodes uracil prototrophy and contains a multiple cloning site for directional cloning of cDNAs to be expressed under the control of the constitutive alcohol dehydrogenase promoter. For W0D12.3 the upstream primer sequence was TCTCGGAATTCATGACTCAAATCAAAGTAGATGCG (restriction site is underlined) and the downstream primer sequence was CCCGGGCTCGAGTTATCCCAATTTGTGGAGC. For VZK822l.1 the upstream primer sequence was TCTCGGAATTCAAACAGACAGTAAAATGACGG, and the downstream primer sequence was CCCGGGCTCGAGCCCGAATGATTCAAAACAGTAC. For F10D2.9 the upstream primer sequence was TCTCGAATTCAAACGGTAAAATCACGG and the downstream primer sequence was TCGACCTCGAGTGTGGACAACCAACGCGT. Plasmids were amplified in the *E. coli* strain DH10B. The amplified cDNA was sequenced to confirm the amino acid sequence predicted by Genefinder. We assigned the genes encoding the predicted open reading frames the following names: W06D12.3: *fat-5* (GenBank Accession No. AF260242); VZK822l.1: *fat-6* (GenBank Accession No. AF260244); and F10D2.9: *fat-7* (GenBank Accession No. AF260243). Transmembrane domains were predicted by TMHMM from the Center for Biological Sequence Analysis, Technical University of Denmark (http://www.cbs.dtu.dk/services/TMHMM-1.0).

Functional assay. The *Saccharomyces cerevisiae* strain L8-14C (generously provided by Chuck Martin) contains a disruption of the yeast Δ9 desaturase gene OLE1 and requires unsaturated fatty acids for growth (7). The cells were grown in YPD medium (2% Bactopeptone, 1% yeast extract, 2% glucose) containing 0.5 mM oleic acid and 0.5 mM palmitoleic acid (NuChek Prep) as well as 1% tergitol, type NP-40 (Sigma) to solubilize the unsaturated fatty acids. The S.c. EasyComp transformation kit (Invitrogen) was used to transform constructs into strain L8-14C. The transformed cells were plated onto complete minimal medium containing 0.5 mM oleic acid, 0.5 mM palmitoleic acid and 1% tergitol, but lacking uracil. To test for genetic complementation of the *ole1* phenotype, several URA+ transformant colonies from each transformation were patched onto complete YPD plates lacking supplemental unsaturated fatty acids. After incubation at 30°C for 48 h, all of the colonies that were URA+ also grew on unsupplemented yeast media, indicating that expression of the cloned *C. elegans* desaturases rescued the *ole1* unsaturated fatty acid auxotrophy.

Fatty acid analysis. Yeast were grown in liquid YPD lacking supplemental unsaturated fatty acids for 1–2 days, and total fatty acids were extracted from pellets composed of 1–2 ml of culture. To prepare fatty acid methyl esters (FAMEs), the pellets were washed one time with water and were resuspended in 1 ml of 2.5% sulfuric acid in methanol and heated to 80°C for one hour. When the yeast were grown in the presence of exogenous fatty acids (0.2 mM, 1% tergitol), the yeast pellets were washed one time in 1% tergitol and 2 times in water before being suspended in 2.5% sulfuric acid in methanol. The resulting FAMEs were extracted in hexane. Fatty acid 4,4-dimethyloxazoline (DMOX) derivatives were prepared from the FAMEs by evaporating the hexane phase, resuspending the residue in 0.5 ml of 2-amino-2-methylpropanol, and heating overnight at 180°C (19). After cooling, the DMOX derivatives were dissolved in 4 ml of dichloromethane and washed twice with 1.5 ml of distilled water. The dichloromethane solution was evaporated under a stream of nitrogen and the residue was dissolved in hexane for injection. Analysis of FAMEs and DMOX derivatives by gas chromatography and mass spectroscopy was conducted using a Hewlett Packard 6890 series GC-MS equipped with a 30-m × 0.25-μm SP-2380 column operating at an ionization voltage of 70 eV with a scan range of 50–550 Da. Fatty acids were identified by comparison with retention times and mass spectra of FAME standards (NuChek Prep). Relative percentages of the fatty acids were calculated from peak areas. The mass spectrum (*m/z*, rel. int.) of the DMOX derivatives of the fatty acids was compared to published values (20, 21).

RESULTS

Identification and cloning of C. elegans Δ9 fatty acid desaturases. We identified three high-scoring open reading frames during a search of the *C. elegans* genomic DNA database with the rat SCD1 protein sequence. Two of the predicted proteins, VZK822l.1 and F10D2.9, displayed 49% and 48% identity with the rat SCD1 sequence respectively and the third predicted protein, W06D12.3 displayed somewhat lower identity, 43%. The primary structure of all three *C. elegans* desaturases and the rat SCD1 are similar (Fig. 1). A high degree of similarity to the rat gene is apparent,

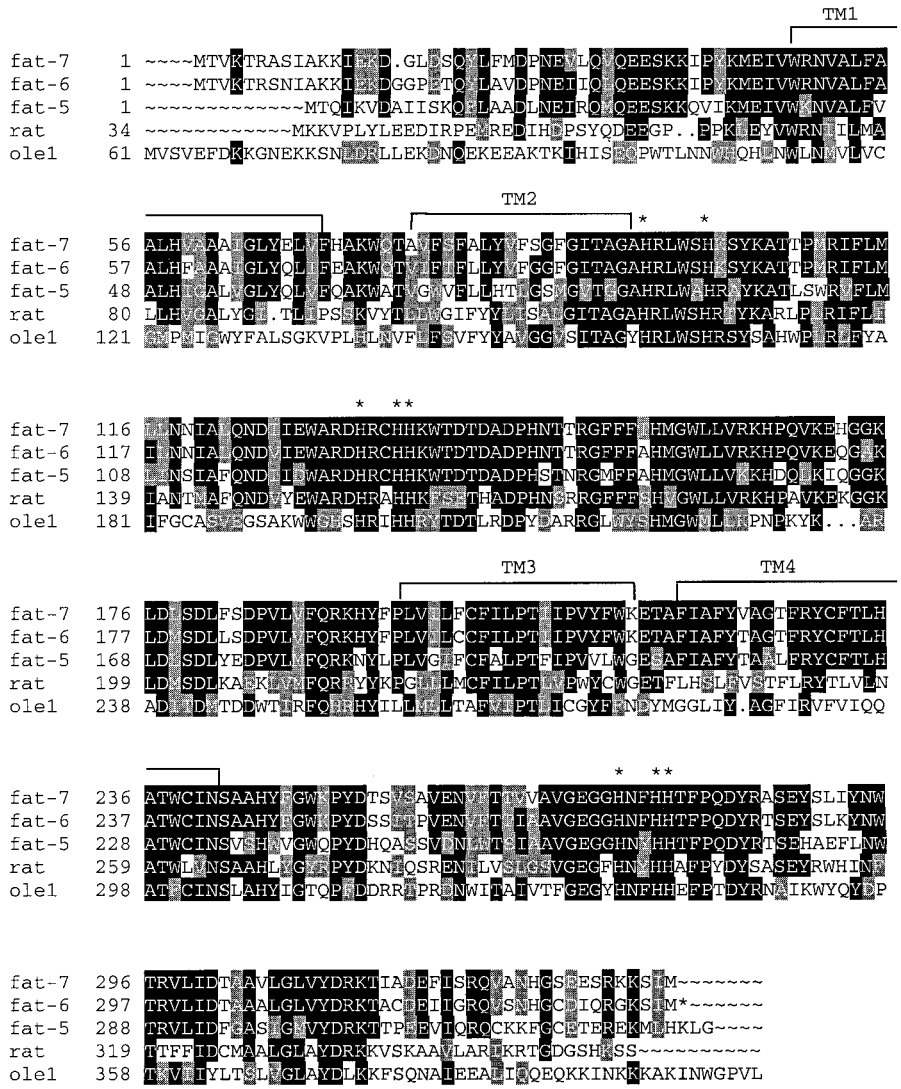

FIG. 1. Comparison of the three *C. elegans* Δ9 desaturase predicted proteins with portions of the rat SCD1 and the yeast Ole1p sequences. Trans-membrane domains as predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM-1.0/) are shown by bars drawn above the sequence and the conserved histidine residues are indicated with asterisks.

especially in the areas near each of the histidine-rich regions that are predicted to be essential for coordinating the iron moieties at the active site in all members of the membrane desaturase superfamily. The putative desaturases also contain four stretches of hydrophobic residues that are predicted to form two membrane traversing loops, each consisting of two transmembrane segments (Fig. 1). The gene products displayed a somewhat lower amino acid identity with the yeast Δ9 desaturase Ole1p (27–33% identity). This protein contains an extra 60 AA on the N-terminal and an extra 113 C-terminal AA responsible for encoding a cytochrome b5 domain. A cytochrome b5 domain is not present on the mammalian SCD proteins, nor is it encoded by any of the three *C. elegans* SCD-like genes.

C. elegans can synthesize a range of fatty acids using only saturated and monounsaturated fatty acids obtained from its diet of *E. coli*. This organism can also be grown axenically with no dietary fatty acids, indicating that the same fatty acids can be made by *de novo* synthesis (22). Four other desaturase genes in *C. elegans* have been isolated and characterized by expression in yeast or Arabidopsis. These include the Δ12 desaturase (23), the ω3 desaturase (24), the Δ5 desaturase (25, 26), and the Δ6 desaturase (27). Each desaturase appears to be present in only one copy. It is not clear why there are three Δ9-like desaturases in *C. elegans*. The most abundant fatty acid in *C. elegans* phospholipids and triacylglycerols is 18:1Δ11, *cis*-vaccenic acid (28). One possibility for producing the *cis*-vaccenic acid is via a Δ11-specific 18:0 desaturase. Such an enzyme has been described in cabbage looper moth (*Trichoplusia ni*), but this desaturase is expressed only in the adult pheromone gland where it is

TABLE 1

Fatty Acid Composition of L8-14C (*ole1*) Yeast Transformed with the *C. elegans* Δ9 Desaturases FAT-5, FAT-6, and FAT-7 as Compared to the Rat SCD1 and Yeast Ole1p Δ9-Desaturases Expressed in the Same Strain

Enzyme expressed	Fatty acid composition[1] (%)				% conversion of 16:0	% conversion of 18:0	18:1/16:1 ratio
	16:0	16:1	18:0	18:1			
FAT-5	28.8 ± 3.1	42.2 ± 3.3	9.7 ± 0.83	0.28 ± 0.08	59.5 ± 1.6	2.7 ± 0.6	0.007 ± 0.002
FAT-6	56.2 ± 2.3	4.1 ± 0.37	6.9 ± 0.30	23.8 ± 1.3	6.8 ± 0.7	77.6 ± 1.2	5.8 ± 0.39
FAT-7	32.2 ± 9.2	8.7 ± 1.9	3.2 ± 0.60	48.7 ± 10.8	22.5 ± 8.5	93.6 ± 2.0	5.7 ± 1.2
Rat SCD1[2]	32.0 ± 1.5	28.1 ± 0.95	4.7 ± 0.08	33.9 ± 2.3	46.8 ± 0.3	87.9 ± 0.5	1.21 ± 0.12
Ole1p[2]	21.9	41.7	4.7	29.8	65	87	0.71

[1] FAT-5, FAT-6, and FAT-7 values represent the relative fatty acid compositions as a percentage of total fatty acid content determined by GC peak area for five independent trials (±SD).
[2] Data from Stukey *et al.* (1990).

necessary for producing pheromone precursors (29). It is not possible to predict, by sequence comparisons alone, if one of the *C. elegans* SCD-like genes encodes a Δ11 desaturase. The *C. elegans* desaturases show nearly equal identities to the cabbage looper moth Δ9 and Δ11 desaturases (data not shown). Alternatively, *cis*-vaccenic acid might be produced by elongation of 16:1Δ9 (palmitoleic acid), as in bacteria and yeast (30). In order to determine if any of the predicted proteins function as Δ9 desaturases or Δ11 desaturases, we expressed them in a yeast strain in which the Δ9 desaturase gene *ole1* has been disrupted.

Functional characterization. The yeast strain L8-14C carries the disruption allele *ole1*Δ*::LEU2* and has limited and finite growth potential (4–5 divisions) unless the growth medium is supplemented with unsaturated fatty acids (7). We transformed the L8-14C strain with an episomal plasmid containing each of the three *C. elegans* Δ9 desaturase-like open reading frames under control of the constitutive alcohol dehydrogenase promoter. We found that, unlike L8-14C, all three of the transformed strains were able to grow on rich media (YPD) as well as complete minimal media (-uracil) in the absence of any supplementary unsaturated fatty acids. We concluded that expression of any of the three *C. elegans* Δ9-like desaturase genes conferred upon the yeast the ability to produce unsaturated fatty acids. In order to determine the identity of unsaturated fatty acids produced by each of the *C. elegans* enzymes, we analyzed the fatty acid composition of the transgenic yeast strains.

The fatty acid composition of two of the strains, L8-14C/*fat-6* (VZK822l.1) and L8-14C/*fat-7* (F10D2.9), were similar (Table 1). Both strains produced 16:1 and 18:1, although the ectopically expressed desaturases preferably desaturated the 18-carbon substrate stearic acid. Yeast expressing *fat-6* and *fat-7* converted 78% and 94% of the stearic acid (18:0) to oleic acid (18:1), and 7% and 23% of the 16:0 to 16:1. This preference is also apparent in the rat SCD1 (4), the cabbage looper moth Δ9 desaturase (14), and two recently characterized *Mortierella alpina* Δ9 desaturases (15) when expressed in L8-14C. The cabbage moth Δ9 desaturase under control of the *ole1* promoter produced six times more 18:1 than 16:1, while the rat gene converted 88% of 18:0 to 18:1 and 47% of 16:0 to 16:1 when expressed in the same strain. The *ole1* gene expressed on an episomal plasmid in the L8-14C strain displayed a higher activity on 16:0, converting 65% of 16:0 to 16:1 and 87% of 18:0 to 18:1 (4).

To confirm that the desaturations occurred at the Δ9-position, DMOX derivatives of yeast fatty acids were analyzed by GC-mass spectrometry. DMOX derivatives have mass spectra that are more easily interpreted than the spectra of methyl esters and permit unambiguous determination of double-bond locations in polyunsaturated fatty acids (20, 21). The mass spectra of the 18:1 molecule produced by *ole1* yeast expressing either *fat-6* or *fat-7* revealed that the molecule produced is indeed the Δ9 isomer, with the 12 a.m.u. separated spectral peaks occurring at 196 and 208.

The strain expressing the third *C. elegans* Δ9 desaturase, L8-14C/*fat-5* (W06D12.3), displayed a novel fatty acid composition. This gene product showed a high activity on 16:0, converting an average of 60% (±1.6%, n = 5) of palmitic acid (16:0) to palmitoleic acid (16:1) (Table 1). Analysis of DMOX derivatives confirmed that the double bond of the 16:1 molecule is present at the Δ9 position, as revealed by the 12 a.m.u. separated spectral peaks at 196 and 208. Only a very small amount of 18:1Δ9 can be detected in these yeast, less than 0.3% of the total yeast fatty acids which represents less than 3% conversion of stearic acid to oleic acid. A second 18:1 peak is also present at about 3 times the abundance of the 18:1Δ9 peak, and spectral analysis of the DMOX derivative of this fatty acid reveal the 12 a.m.u. separated peaks at 224 and 236, which represents a double bond at the Δ11 position. This fatty acid, 18:1Δ11, is produced naturally in yeast by the elongation of 16:1Δ9.

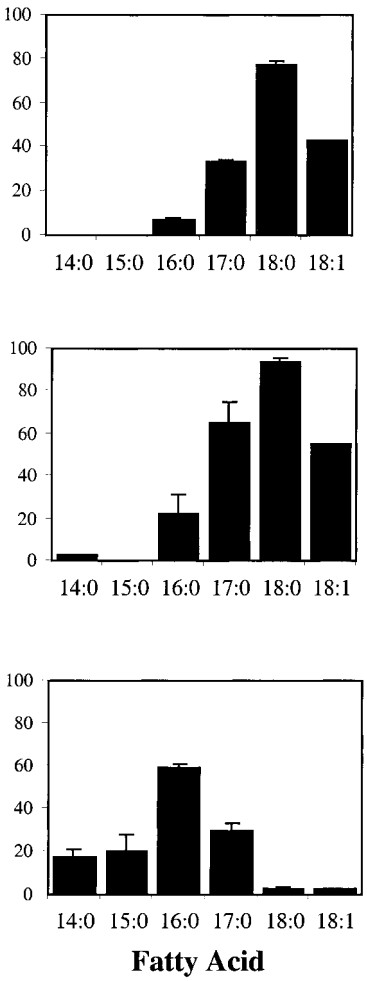

FIG. 2. Δ9 desaturation of fatty acids by transgenic *ole1* yeast expressing the *C. elegans* Δ9 desaturases. Values represent the mean conversion (%) of 2–5 trials (±SD), except for 18:1Δ11 (*trans*), which was measured in only one trial.

We also noticed that the relative amount of 18-carbon fatty acids compared to 16C fatty acids is greatly reduced in the L8-14C/*fat-5* strain, as compared to L8-14C expressing other Δ9 desaturases. Whereas 16 C to 18 C fatty acids are generally present in a 3:2 ratio in most strains, in L8-14C/*fat-5* they are present in a 6:1 ratio (Table 1). It appears that the yeast elongation activity is regulated by the amount of 18:0 present in the cells. When the 18:0 is not converted to 18:1, the yeast maintains this amount of 18:0 at 5–10% of the total fatty acids, even though, in the absence of 18:1, the total amount of 18-carbon fatty is much lower than in wild type. Strain L8-14C supplemented only with 16:1 display a similar fatty acid composition (7).

We tested a number of medium and long-chain saturated and unsaturated fatty acids in order to further investigate the substrate specificities of the *C. elegans* Δ9 desaturases (Fig. 2). The saturated fatty acids 14:0, 15:0, and 17:0 are present in small amounts in *S. cerevisiae*. We examined whether these fatty acids are desaturated in the transgenic strains carrying the *C. elegans* Δ9 desaturases. In addition, we propagated the yeast in the presence of several other fatty acids by adding 18:1 Δ11 (*trans*), 18:1 Δ11 (*cis*) and 20:0 fatty acids to the growth media in the presence of 1% tergitol. We found that FAT-5 was capable of desaturating the medium chain fatty acids 14:0 and 15:0, but showed very little activity on fatty acids longer than 16 carbons. In contrast, FAT-6 and FAT-7 did not desaturate 14:0 or 15:0, but did show considerable activity on 17:0 and 18:1 Δ11 (*trans*). Desaturation of 18:1 Δ11 (*trans*) results in the formation of 18:2 Δ9 (*cis*) Δ11 (*trans*), conjugated linoleic acid (CLA). None of the *C. elegans* desaturases showed any activity on 18:1 Δ11 (*cis*) or 20:0.

DISCUSSION

We identified three SCD-like genes in the recently completed *C. elegans* genomic sequence and tested their functional activity by expression in yeast. Using the yeast strain L8-14C, in which the Δ9 desaturase gene *ole1* is disrupted, we found that all three of the *C. elegans* SCD-like genes rescue the unsaturated fatty acid auxotrophy of this strain. However, upon analysis of the fatty acid composition of the transgenic *ole1* strains, we discovered that one gene, *fat-5*, does not encode a stearoyl-CoA desaturase. When expressed in yeast, this enzyme does not desaturate stearic acid (18:0), and instead catalyzes the insertion of a double bond at the Δ9 position of palmitic acid (16:0). In contrast, the other two SCD-like genes, *fat-6* and *fat-7*, encode enzymes that show typical SCD activity, desaturating both 18:0 and 16:0, but with a preference for 18:0. Further examination demonstrated that the *C. elegans* FAT-5 is specialized for medium chain saturated fatty acids (14:0–16:0) while FAT-6 and FAT-7 desaturases prefer longer chain fatty acids (16:0–18:0), as well as the Δ11 (*trans*) isomer of 18:1. As more and more SCDs are identified in rapidly progressing sequencing projects, these results demonstrate the importance of functional tests of enzyme activity and substrate specificity.

A Δ9 desaturase specific for 16:0 has also been isolated from the cat's claw vine *Doxantha unguis-cati* (31). The structure of this and other soluble acyl-ACP Δ9 desaturases of plant plastids is very different from the structure of the acyl-lipid or acyl-CoA desaturases more typical of eukaryotes. The crystal structure of the related castor Δ9-18:0-ACP desaturase has been determined, and structural predictions and site-specific mutagenesis studies have been used to identify eight residues located near the bottom of the substrate-binding pocket that help to determine the chain-length specificity of the enzymes (32). Only one of these eight residues is altered in the cat's

claw 16:0-ACP desaturase (Leu118 → Trp). When this alteration was introduced into the castor Δ9-18:0-ACP desaturase by site-specific mutagenesis, its specificity was changed to prefer 16:0 substrate (31). Presumably the bulky side chain of tryptophan reduces the depth of the substrate pocket to favor binding of 16:0-ACP over the longer substrate, 18:0-ACP. A similar reduction (relative to the 18:0 desaturases) in the acyl-CoA binding pocket of FAT-5 might explain this enzyme's specificity. Since structural data are not available for the membrane bound desaturases, it is not known which portion of the protein is responsible for recognizing and binding substrate. Therefore, we cannot predict which residues in FAT-5 are responsible for the specificity of this enzyme.

We found that FAT-6 and FAT-7 are capable of desaturating trans-vaccenic acid. This activity has also been observed in rat liver microsomes and in mice (33, 34). Since the *trans* double bond does not introduce a kink in the hydrocarbon chain in the manner of a *cis* double bond, presumably the linear *trans*-vaccenic acid can fit into the substrate binding region of SCDs due to the shape similarity to stearic acid (18:0). Desaturation of 18:1 Δ11 (*trans*) results in the formation of 18:2 Δ9 (*cis*), Δ11 (*trans*), conjugated linoleic acid (CLA). This fatty acid occurs naturally in food, and was first isolated and identified from grilled ground beef extracts that exhibited anticarcinogenic activity (35). The effect of CLA on body fat content, lean body mass, and glucose intolerance suggests that CLA plays a role in lipid and carbohydrate metabolism. Indeed, some isomers of CLA have been shown to modify membrane fatty acid composition by decreasing the activity of stearoyl-CoA desaturase in mice (36).

At present we do not know the specific roles of each of the three *C. elegans* Δ9 desaturase genes. We suggest that the palmitoyl-CoA desaturase FAT-5 plays a special role in the synthesis of cis-vaccenic acid in the nematode triacylglycerol stores or membrane phospholipids. Unlike mammals, *C. elegans* posses a Δ12 desaturase which catalyzes the first step in the conversion of monounsaturated fatty acids to polyunsaturated fatty acids (23). Since oleic acid (18:1Δ9) is the main precursor for the *C. elegans* Δ12 desaturase, it may be important to accumulate another monounsaturated fatty acid that is not a substrate for this enzyme in order to preserve the optimal ratio of saturated/monounsaturated/polyunsaturated fatty acids in membranes. The mouse and yeast Δ9 desaturase genes are highly regulated at the level of transcription. The mouse genes SCD1 and SCD2 are expressed in different tissues and are regulated differently in response to dietary influences (11) and during adipocyte differentiation (37). Regulatory elements required for the repression of SCD1 and SCD2 transcription by polyunsaturated fatty acids and sterols have been identified (38, 39). The 60-base-pair polyunsaturated fatty acid (PUFA)-response element is thought to be necessary for regulation by sterol regulatory element binding proteins (SREBPs) as well as by a PUFA binding protein (1). However, the two mouse SCD genes are regulated differently by the antidiabetic thiazolidinediones during preadipocyte differentiation (37). SCD1 transcription is repressed in response to these drugs, while no detectable change in transcription of SCD2 occurs. Interestingly, comparison of the fatty acid composition of the treated and untreated preadipocytes reveals that the thiazolidinediones selectively decrease the desaturation of 16:0 to 16:1, but do not affect the conversion of 18:0 to 18:1, suggesting an important role for the mouse SCD1 in the desaturation of palmitic acid (16:0). Transcription of the yeast *OLE1* gene is activated by stearic acid (18:0) and repressed by unsaturated fatty acids (40). Promoter elements required for this activation and repression by fatty acids have been identified (41) but do not show similarity to the mouse PUFA response element. We failed to identify sequences similar to either the mouse PUFA response element or the yeast fatty acid regulated region in the *fat-5*, *fat-6*, or *fat-7* promoters. However, we believe it is likely that these genes are regulated by dietary influences and perhaps in a stage-specific or tissue specific manner. Investigations of the expression patterns and promoter analysis of these genes will be necessary to test this hypothesis.

ACKNOWLEDGMENTS

We thank Drs. Chuck Martin, Doug Knipple, and Howard Grimes for providing plasmids and yeast strains. We also thank Dr. Jim Wallis for helpful comments on the manuscript. This work was supported in part by U.S. Department of Agriculture grant USDA-NRICGP97-35301-4426 to J.B., NSF postdoctoral fellowship DBI-9804125 to J.W., and the Agricultural Research Center, Washington State University.

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