Identification and Characterization of an Animal $\Delta^{12}$ Fatty Acid Desaturase Gene by Heterologous Expression in Saccharomyces cerevisiae

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We have cloned a Caenorhabditis elegans cDNA encoding a $\Delta^{12}$ fatty acid desaturase and demonstrated its activity by heterologous expression in Saccharomyces cerevisiae. The predicted protein is highly homologous both to the cloned plant genes with similar function and to the published sequence of the C. elegans omega-3 fatty acid desaturase. In addition, it conforms to the structural constraints expected of a membrane-bound fatty acid desaturase including the canonical histidine-rich regions. This is the first report of a cloned animal $\Delta^{12}$ desaturase gene. Expression of this cDNA in yeast resulted in the accumulation of 16:2 and 18:2 (linoleic) acids. The increase of membrane fluidity brought about by this change in unsaturation was measured. The production of polyunsaturated fatty acids in yeast cells and the concomitant increase in membrane fluidity was correlated with a modest increase in growth rate at low temperature and with increased resistance to ethanol and oxidative stress.
ther to acyl carrier protein (ACP), to coenzyme A, or to lipid molecules (24, 26). Since desaturation reactions require one molecule of oxygen and two electrons for each reaction, desaturases can also be differentiated by the electron carrier that they require. While ferredoxin is the electron donor in the desaturation reactions catalyzed by acyl-ACP desaturases, by acyl-lipid desaturases of cyanobacteria, and by acyl-lipid desaturases in the plastids of plants (27, 28), the acyl lipid and acyl-CoA desaturases found in the endoplasmic reticulum of all eukaryotes and many bacteria use cytochrome b₅ as donor (29–31). Desaturase enzymes also show considerable selectivity both for the chain length of the substrate and for the location of existing double bonds in the fatty acyl chain (26).

Purification of fatty acid desaturases have been limited by their requirement for membrane association. One of the most fruitful approaches to examining desaturase activity has been mutational analysis. Isolation of mutants in cyanobacteria and Arabidopsis thaliana with altered fatty acid compositions has permitted the isolation of genes encoding most of the transmembrane desaturases present in this organism (32, 33). Sequence analysis of these desaturases has facilitated the cloning of a number of other desaturase genes from plants (25, 34), bacteria (35), protists (36), nematodes (37–39), and mammals (40, 41).

While most eukaryotic organisms, including mammals, can introduce a double bond into an 18-carbon fatty acid at the Δ⁹ position, mammals are incapable of inserting double bonds at the Δ¹₂ or Δ¹₅ positions. For this reason, linoleate (18:2 Δ⁹,Δ¹₂) and linolenate (18:3 Δ⁹,Δ₁₂,Δ₁₅) must be obtained from the diet and are termed essential fatty acids. These dietary fatty acids come predominantly from plant sources, since flowering plants readily desaturate at both the Δ¹₂ and the Δ¹₅ positions. Certain animals, however, including some insects and nematodes, can synthesize de novo all their component fatty acids including linoleate and linolenate. The nematode Caenorhabditis elegans can synthesize de novo a broad range of polyunsaturated fatty acids including arachidonic acid and eicosapentaenoic acids, an accomplishment not shared by either mammals or flowering plants (42, 43).

The Arabidopsis Δ¹₂ desaturase has been described (44), and a number of similar sequences have been obtained from other plants (25). However, while the activity of animal Δ¹₂ desaturation has been studied in insects (45, 46), no gene encoding an animal Δ¹₂ has been isolated. To identify and clone an animal Δ¹₂ fatty acid desaturase we used sequence information from both the cloned Arabidopsis thaliana desaturase genes and the accumulation of sequence data from C. elegans (47). In the present report, we describe the isolation of a cDNA that encodes an animal Δ¹₂ fatty acid desaturase. This protein is the first representative of the animal Δ¹₂ desaturase class. Transformed yeast expressing the enzyme contained high levels of polyunsaturated fatty acids. Physiological studies of these yeast demonstrate both that their membrane fluidity and growth characteristics are altered and that they have increased resistance to ethanol and hydrogen peroxide stress.

**MATERIALS AND METHODS**

Cloning and sequencing of a fat-2 cDNA. The National Center for Biotechnology Information’s Expressed Sequence Tag (EST) database was searched with BLAST (48), using the peptide sequences of the A. thaliana FAD2 (GenBank Accession No. L26296), FAD6 (U09503), and FAD7 (D14007) fatty acid desaturases as queries. Two partial cDNA clones identified by these searches, CEL20a7 and CEL18f3, were obtained from the C. elegans Genome Sequencing Center at Washington University School of Medicine in St. Louis. The cDNA was labeled with [α-³²P]dCTP using a random priming kit (Prime-a-Gene; Promega), and the labeled probe was used to screen the C. elegans mixed-stage lambda phage Uni-ZAP XR library (Stratagene). Positive clones were excised from the phage vector according to the manufacturer’s protocol to yield pBluescript plasmids. The clone with the longest insert, pCM18, was sequenced in both directions using dye-termination sequencing technology (Applied Biosystems). Analysis of the sequences was carried out using programs available in the Genetics Computer Group package (49), except for analysis of transmembrane domains, which was conducted with the SOSUI server at the Tokyo University of Agriculture and Technology.

Yeast expression. The plasmid pCM18 was restricted with EcoRI and Xhol to excise the cDNA, and the isolated fragment was ligated into the episomal yeast expression vector pMK195 (50) which had been digested with the same enzymes. Directional cloning of the cDNA into this vector provided for expression of the FAT-2 promoter under the control of the constitutive ADH1 promoter. The resulting construct, pMK195-fat-2, was introduced into Saccharomyces cerevisiae strain YRP685 (MATa, ura2, lys2, his4, trp1, ural3) using the lithium acetate procedure (51). Transformed cells were grown in a complete minimal media supplemented with 2% glucose but lacking uracil, since pMK195 encodes ura prototrophy.

Lipid analysis. Methods for extraction and separation of lipids, and for the analysis of fatty acid methyl esters (FAMEs) have been described (52). Briefly, cells were grown overnight in selective media in the presence of glucose. One milliliter of 2-amino-2-methyl-1-propanol, and heating overnight at 180°C (53). After cooling, the DMOX derivatives were dissolved in 4 ml of dichloromethane and analyzed by gas chromatography-mass spectrometry (GC-MS). GC-MS analysis was carried out on a 30-m × 0.2-mm AT1000 column (Alltech Associates) in a HP6890 instrument (Hewlett-Packard) operating at an ionization voltage of 70 eV with a scan range of 40–400 Da. Oven temperature at injection was 150°C, and this was increased at 5°C/min to 230°C and then held at 230°C for 10 min. In these experiments, novel fatty acids were identified by comparison of their retention times and mass spectra with authentic 16:2 and 18:2 fatty acids (NuChek-Prep, Elysian, MN). Alternatively, FAMEs were converted to fatty acid 4,4-dimethyloxazoline (DMOX) derivatives by evaporating the hexane solvent, adding 500 μl of 2-amino-2-methyl-propand, and heating overnight at 180°C (53). After cooling, the DMOX derivatives were dissolved in 4 ml of dichloromethane and...
recentrifuged at 116,000 g for 5 min. After discarding the supernatant, the cells were washed once with 1.2 M sorbitol and then suspended in a protoplasting solution of 30 mg Zymolyase 20T (Sigma), 18 ml 2M sorbitol, 4.5 ml 0.5 M potassium phosphate, pH 7.5, 0.75 ml β-mercaptoethanol, 12 μM 0.5 M EDTA, and 6.7 ml water. The suspension was incubated at 34°C for 2 h, centrifuged at 5000 g for 5 min, and the protoplasting solution discarded. The cell pellet was resuspended in a homogenization buffer consisting of 0.6 M sorbitol, 0.06 M Tris, pH 7.5, 1 mM EDTA, and 0.1% BSA. Protoplasts were disrupted with a mechanical tissue homogenizer (Tekmar). After one centrifugation of the lysate at 2500g for 10 min at 4°C, the pellet was discarded and the centrifugation was repeated. The supernatant from this second centrifugation was collected and recentrifuged at 116,000 g for 1 h. The final pellet was resuspended in the homogenization buffer before being subjected to further analysis.

Measurements of membrane fluidity. The relative fluidity of isolated microsomes was determined by steady state fluorescence polarization measurements of membranes containing the hydrophobic fluoroprobe DPH (1,6-diphenyl-1,3,5-hexatriene), according to McCourt et al. (57). The fatty acid content of the microsomal membrane preparations was determined by FAME analysis using a 17:0 methyl ester of known concentration as internal standard. DPH in solution of the membranes at 116,000 g for 40 min. The pellet was resuspended in 10 mM Tricine, pH 7.9, 10 mM NaCl, 100 mM sorbitol, to a final concentration of 1 μM DPH. Fluorescence polarization measurements were carried out on an SLM4800 spectrofluorometer (Spectronic Instruments) at several temperatures between 10 and 40°C. Excitation was provided by light at 360 nm with a band pass of 4 nm. The emission was collected in the T-format without monochromators using cutoff filters at 470 nm. Glan-Thompson calcite polarizers were used. The data were analyzed using the software supplied by the spectrofluorometer manufacturer. Membrane fluidity was expressed by calculating $P = (r/r_0)^2$, where $r_0$ is the theoretical limiting anisotropy in the absence of rotational motion, and $r$ is the steady state anisotropy measured in the membrane. In a fully ordered membrane, $P = 1$, and the smaller the P value, the more fluid the membrane.

Stress experiments. Transgenic yeast and control yeast transformed with the empty vector were grown aerobically at 25°C in a complete minimal (CM) medium lacking uracil and in the presence of 2% glucose. Cell growth was followed by turbidity measurements at 400 nm. Cells were harvested during the exponential phase when the optical density was between 0.1 and 1.0, corresponding to a cell density of 3 × 10⁶ to 3 × 10⁷ cells/ml. Cells were washed twice in 67 mM phosphate buffer and resuspended in the original volume prior to exposure to stress conditions. Cells were treated in 10% ethanol (v/v) or 3 mM hydrogen peroxide for 8 h. Cell viability was determined by appropriate dilution followed by plating of triplicate samples on CM agar. Colonies were counted after 2 days incubation at 28°C. Stress tolerance, expressed as percentage survivors, was determined by comparing the colony count of stressed cells to that of unstressed controls.

RESULTS

Cloning and characterization of a new fatty acid desaturase gene. A database search using Arabidopsis FAD2, FAD6, and FAD7 desaturases as queries, revealed a number of high-scoring Expressed Sequence Tags (ESTs) from C. elegans. Some of these were identical to the previously described fat-1 which encodes an ω-3 desaturase (37). However, several with high scores differed significantly from fat-1, and alignment of these sequences indicated that they originated from a single gene. Of these sequences, NCBI-57754 (D34903), NCBI-6233(M89244), NCBI-55444(D32410), NCBI-6197(M89208), and NCBI-5424(Z14917), the clone with the most sequence information was NCBI-6197(CEL18F3). This clone was obtained from the C. elegans Genome Sequencing Center. The insert from this clone was radiolabeled and used to probe approximately 50,000 plaques of a C. elegans mixed-stage cDNA library. The screen yielded 20 positive clones with the longest cDNA insert being 1.3 kb in length as judged by agarose- gel electrophoresis. One of these long clones, pCM18, was completely sequenced and found to contain a 1253-bp cDNA insert. The cDNA encoded an open reading frame for a protein predicted to consist of 376 residues, with a molecular mass of 43.3 kDa. Alignment of the predicted protein with known desaturase proteins revealed a sequence identity of 51% with FAT-1, 32% with FAD2, and 31% with FAD3; 56 amino acids were conserved in all four sequences (Fig. 1). Based on this homology to known desaturases, the protein was designated FAT-2 (fatty acid desaturase-2). Among the conserved residues were the eight histidines that occur in most membrane desaturases and have been shown to be important for desaturase activity (58). The arrangement of these residues in three histidine-rich sequence motifs with conserved spacing between the motifs is characteristic of the membrane-bound desaturases. The first motif, HXXXH starts at residue 93 of the FAT-2 sequence, the second HXXHH at residue 129, and the third HXXHH at residue 295. The FAT-2 protein also contains the sequence KAKKAQ at its carboxyl terminus, which is similar to the proposed endoplasmic reticulum (ER) retention signal KXXX common to many transmembrane ER proteins (59). This sequence analysis indicated that the pCM18 cDNA encoded a fatty acid desaturase or an enzyme with a closely related function. Since the predicted FAT-2 protein is equally similar both to the Arabidopsis FAD2 Δ₁²-desaturase and to the FAD3 ω-3 desaturase (Fig. 1), the function of FAT-2 could not be deduced from sequence analysis alone. Because the previously characterized FAT-1 is an ω-3 desaturase, it seemed likely that FAT-2 represented the C. elegans Δ₁² desaturase.

Functional expression of FAT-2 in yeast. To determine the function of the FAT-2 protein, we expressed...
the fat-2 cDNA in S. cerevisiae, which normally produces only monounsaturated 16:1<sup>Δ9</sup> and 18:1<sup>Δ9</sup> fatty acids. The plasmid pMK195-fat-2, expressing the cDNA under control of the ADH1 promoter, was transformed into yeast cells by selection for uracil prototrophy and grown on uracil-deficient medium. As a control, the empty pMK195 vector was transformed and cultured in parallel. After 2 days of culture at 28°C the cells were harvested and FAMEs prepared. Analysis of the total fatty acids from the pMK195-fat-2-bearing strain revealed two peaks not present in the empty vector control strain. These peaks, with retention times 9.08 and 11.69 min, represented apparent desaturation products from the common yeast fatty acids 16:1<sup>Δ9</sup> and 18:1<sup>Δ9</sup> (Figs. 2a and 2b). These desaturation products were tentatively identified as 16:2<sup>Δ9,12</sup> and 18:2<sup>Δ9,12</sup> by comparison of the FAME mass spectra with those of commercial standards. The molecular ion is correct for each fatty acid; 266 for 16:2<sup>Δ9,12</sup> and 294 for 18:2<sup>Δ9,12</sup> (Figs. 2C and 2D). The identity of the desaturation products was confirmed by analysis of DMOX derivatives separated on both polar and nonpolar GC columns. The retention times of the products in these analyses were consistent with their identification as 16:2<sup>Δ9,12</sup> and 18:2<sup>Δ9,12</sup>. The mass spectrum of the 16:2 DMOX derivative exhibited a peak at m/z 530 expected for the molecular ion of the DMOX derivative and peaks at m/z 519, 236, and 248 consistent with double bonds at the Δ9 and Δ12 positions of the fatty acid. Similarly, the mass spectrum for

**FIG. 1.** Comparison of the deduced amino acid sequence of C. elegans fat-2 and fat-1 genes (fat2 and fat1), and Arabidopsis thaliana FAD2 and FAD3 (fad2 and fad3). Amino acid identities are shaded black, and conserved residues gray. The three conserved histidine-rich motifs are indicated.
the 18:2 DMOX derivative contained the expected molecular ion peak at m/z = 333 at peaks at m/z = 196, 208, 236, and 248 consistent with double bonds at Δ9 and Δ12.

The polyunsaturated 16:2 and 18:2 fatty acids accounted for 22% of the total fatty acids of yeast cells harvested during exponential growth, and increased to 46% when cultures entered stationary phase. Lipid analysis by thin layer chromatography indicated that polyunsaturated fatty acids accumulated in all of the major membrane phospholipids including phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine (data not shown).

Membrane fluidity in yeast membranes containing 16:2 and 18:2. To determine if increased levels of desaturation in yeast membranes affected fluidity, we measured membrane fluidity by fluorescence polarization, using DPH as a probe. After the measured fluorescence intensities were corrected for background fluorescence and light scattering from an unlabelled sample, the fluorescence polarization (P) was determined in membranes prepared from yeast transformed with pMK195-fat-2 and from the empty vector control strain. Polyunsaturated fatty acids in transgenic yeast microsomes used for the experiment accounted for 22% of total fatty acids. Throughout the entire temperature range used in the experiments, microsomes from cells expressing the FAT-2 desaturase showed substantially lower P values. These lower P values reflect increased rotational mobility of the DPH probe and indicate an increase in the fluidity of the membrane bilayer at every temperature. The highest P values and greatest

FIG. 2. Gas chromatography traces of wild-type yeast transformed with the empty vector (A) and transgenic yeast transformed with fat-2 (B). FAMEs of total fatty acids were identified as follows: (1) 16:0, (2) 16:1, (3) 16:2, (4) 18:0, (5) 18:1, (6) 18:2. Mass spectra of polyunsaturated fatty acids from transgenic yeast: (C) 16:2, (D) 18:2.
differential between control and FAT-2 membranes was observed at 10°C, the coldest temperature tested (Fig 3).

Increased stress tolerance. There is a considerable body of literature correlating tolerance to cold, ethanol, and oxidative stress with membrane fluidity in a variety of yeast strains (15, 16, 60); it is often argued that increased membrane fluidity should increase resistance to all of these stress factors (60). The growth rate of both the experimental and the control strains were examined over a range of temperatures to determine if membrane desaturation affected cold tolerance. Growth rates and fatty acid content of transformed yeast cells either expressing the fat-2 cDNA or containing the empty vector were measured at several temperatures between 4 and 30°C. At all temperatures between 15 and 30°C, yeast cells expressing FAT-2 had growth rates identical to the control strain. However, at 12°C, the growth rate of the FAT-2 expressing yeast was substantially higher than that of the control strain (0.022/h vs 0.014/h, Table I). At 4°C, growth of both strains was too slow to measure reliably.

As a measure of resistance to ethanol stress, we measured viability under exposure to 10% ethanol. The yeast expressing FAT-2 exhibited a viability twice that of control cells when exposed to 10% ethanol for 8 h (Fig. 4A). To investigate the contribution of PUFAs to oxidative stress tolerance, we compared the ability of FAT-2 transformants and wild-type yeast cells to survive following hydrogen peroxide exposure. Yeast expressing FAT-2 survived 8 h of treatment in 3 mM hydrogen peroxide at a rate more than twice as high as those of control cells under the same conditions (Fig. 4B). These results are consistent with previous suggestions that the presence of polyunsaturated fatty acids promotes increased tolerance to ethanol and oxidative stresses (16, 60).

DISCUSSION

The fat-2 cDNA described in this paper encodes an animal Δ12 desaturase that is able to act on both 16:1 Δ⁹ and 18:1 Δ⁹ to produce polyunsaturated fatty acids. In those animals such as C. elegans, that can synthesize a wide range of polyunsaturated fatty acids, the 16:2 and 18:2 products are further desaturated and elongated to produce important cellular components, particularly eicosapentaenoic and arachidonic acids (43).

The predicted FAT-2 protein includes three histidine-rich sequences that are highly conserved among membrane bound desaturases and have been shown to be necessary for enzyme function (58). It is believed that these residues coordinate the diiron-oxo structure at the active site of the desaturases. The FAT-2 protein contains two significant hydrophobic stretches, each long enough to span the membrane twice (residues 69 to 117 and 230 to 281, in Fig. 1). In FAT-2 the position and length of these stretches relative to the conserved histidine boxes are similar to those of other membrane bound desaturases. Thus, the FAT-2 protein conforms to the model proposed by Stukey et al. (61), in which the peptide chain spans the membrane four times and exposes the three histidine clusters on the cytoplasmic side of the endoplasmic reticulum. Unlike the native yeast Δ⁹ desaturase, which has a cytochrome-like domain at its carboxyl terminus, the C. elegans FAT-2 presumably interacts with a separate cytochrome b5 to achieve its activity both in the nematode and in transgenic yeast.

Expression of the fat-2 cDNA in yeast enabled us to examine the activity of the FAT-2 protein, since S. cerevisiae contains substantial amounts of both 16:1 Δ⁹ and 18:1 Δ⁹ fatty acids in its membrane lipids. In transgenic yeast, FAT-2 recognizes both 16- and 18-carbon Δ⁹ substrates and converts up to 40% of these substrates to 16:2 Δ⁹,12 and 18:2 Δ⁹,12 (Fig. 2). In Arabidopsis, Δ¹2 desaturation is compartmentalized, with 18:1 desaturation occurring in the ER, using PC as the
principal substrate, while both 18:1 and 16:1 desaturation take place in the chloroplast with galactolipids as substrates (33, 62, 63). It has also been established that the Arabidopsis ER desaturase, FAD2, will desaturate 16:1 \( \Delta^9 \) when expressed in yeast (64).

While our work is the first characterization of a cloned animal \( \Delta^{12} \) desaturase, the enzyme activity has been studied in insects. Biochemical characterization of insect \( \Delta^{12} \) desaturases suggests that there may be differences between substrates used by plants and animals. Available evidence indicates that, unlike the plant enzymes, the cricket \( \Delta^{12} \) desaturase activity uses acyl-CoA as substrates (46). This observation raises the possibility that the C. elegans \( \Delta^{12} \) desaturase may also use acyl-CoA as a substrate.

While we identified the fat-2 cDNA from EST sequences and a cDNA library, the FAT-2 protein corresponds to a predicted protein, W02A2.1 (GenPept Accession No. CAB05394), identified by the C. elegans genome sequencing project. Examination of the nema-

![FIG. 4. Stress tolerance as the percentage of survival of yeast cells. Cells were grown until early log phase, washed with 67 mM phosphate buffer and resuspended in the same buffer. Then they were treated with either (A) 10% ethanol (v/v) or (B) 3 mM hydrogen peroxide for 8 h. □, Untreated control strain; *, untreated fat-2 yeast; Δ, treated control strain; ○, treated fat-2 yeast. The error bars indicate the standard deviation of three measurements.](image)

### TABLE I

<table>
<thead>
<tr>
<th>Temperature</th>
<th>4°C</th>
<th>12°C</th>
<th>15°C</th>
<th>22°C</th>
<th>30°C</th>
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<tr>
<td><strong>Fatty acids: C</strong></td>
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<tr>
<td>16:0 + 18:0</td>
<td>17</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>16:1 + 18:1</td>
<td>81</td>
<td>77</td>
<td>80</td>
<td>61</td>
<td>62</td>
</tr>
<tr>
<td>16:2 + 18:2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>17</td>
<td>0</td>
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<tr>
<td>Growth rate (h(^{-1}))</td>
<td>—</td>
<td>—</td>
<td>0.014</td>
<td>0.022</td>
<td>0.031</td>
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Note. Cells were either with the control vector (C) or expressing fat-2, grown on complete minimal medium lacking uracil until late log phase. They were harvested and fatty acid analysis of FAMEs was carried out by gas chromatography. Growth at 4°C was too slow for accurate measurement. Numbers are the weight percent of the indicated fatty acids as a fraction of total fatty acids.
tode genome reveals that all four cloned C. elegans fatty acid desaturase genes, fat-1 (\(\omega-3\)), fat-3 (\(\Delta 6\)), fat-4 (\(\Delta 5\)), and fat-2, lie on the right arm of chromosome 4 (LGIV) (Fig. 5). The fat-3 and fat-4 genes are transcribed in the same 5' to 3' orientation with only 0.85 kb separating them (44). Their amino acid sequences are 45% identical and they share two intron/exon boundaries, indicating that these two desaturase activities could have arisen from an ancient gene duplication event. The fat-1 and fat-2 genes are also transcribed in the same 5' to 3' orientation, have similar structures of three exons and two introns, and share 51% amino acid identity. However, they are separated by approximately 5.3 kb of DNA. It is possible that these two genes also arose from an ancient gene duplication event. It has been suggested that the two mouse stearoyl CoA desaturase genes, which are clustered on chromosome 19, may have arisen by gene duplication (65).

Since expression of the C. elegans \(\Delta 12\) desaturase results in considerable synthesis of polyunsaturated fatty acids in transgenic yeast, we examined how the changes in fatty acid composition (Fig. 2, Table I) alter membrane fluidity and affect the physiology of these yeast. Transgenic yeast containing 16:2 and 18:2 exhibited a significantly more fluid membrane at all temperatures tested (Fig. 3). Many organisms, including microorganisms and plants, alter the composition of their membrane lipids to compensate for the decrease of fluidity of the lipid bilayer at low temperatures (2, 66). The homeoviscous adaptation of biological membranes is an environmentally triggered acclimation that is thought to improve membrane functionality at low temperature (9). However, the exact contribution of membrane unsaturation to low-temperature adaptation is not well understood (67). The Arabidopsis thaliana fad2 mutant, which lacks the \(\Delta 12\) desaturase activity, is unable to survive at low temperatures (68). Likewise, the Fad12 mutant of the cyanobacterium Synechocystis PCC6803, which is deficient in \(\Delta 12\) desaturase, grows more slowly than wild type at 22°C although growth at 34°C is unaffected (69). Thus, in both prokaryotic and eukaryotic organisms that contain high levels of polyunsaturated fatty acids, reductions in \(\Delta 12\) desaturation and membrane polyunsaturation compromise cell function specifically at low temperatures. When the gene encoding \(\Delta 12\) desaturase from Synechocystis PCC6803 (desA) was expressed in a cyanobacterium that normally contains only monounsaturated fatty acids (Synechococcus PCC7942), the membrane lipids of this organism became enriched with up to 25% polyunsaturated fatty acids (70, 71). This large increase in membrane unsaturation was shown to reduce low-temperature damage to the photosynthetic machinery. However, this effect was small and no improvement in the growth rate of transformed cells was reported at any temperature (70, 71). In our studies, the increased polyunsaturation of membranes in the transgenic yeast conferred a growth rate advantage to cells growing at 12°C, while no change is seen at higher temperatures (Table I). For both the prokaryote Synechococcus PCC7942 and the eukaryote S. cerevisiae, the beneficial effects of providing polyunsaturated membranes are modest and confined to the lowest temperatures within the physiological temperature range for these organisms. Taken together, these observations indicate that membrane polyunsaturation may be essential for survival or optimum growth at low temperatures, but that polyunsaturation is only one feature required, and by itself it does not confer a substantial benefit to the organism.

We found that the ability to produce polyunsaturated fatty acids offered a more significant advantage to yeast cells subjected to two other environmental stress factors, ethanol and hydrogen peroxide. The viability of transgenic yeast expressing FAT-2 was twice that of control cells when exposed to either 10% ethanol or 3 mM hydrogen peroxide. Although S. cerevisiae

\[\text{FIG. 5. (A) Relative location of C. elegans } \Delta^4 \text{ (fat-3), } \Delta^5 \text{ (fat-4), } \omega^3 \text{ (fat-1), and } \Delta^{12} \text{ (fat-2) desaturase genes on chromosome IV. Approximate map locations are 3.03 for fat-4, 3.08 for fat-3, 5.52 for fat-2: fat-2 and fat-1 are separated by approximately 5.3 kb. (B) Structure of fat-1 and fat-2 on their respective YAC (Y67H2) and cosmid (W02A2). Introns are shaded.}\]
is considered to be an ethanol-tolerant species, ethanol does inhibit cell growth, viability, solute accumulation, and proton fluxes at concentrations above the threshold of tolerance (11). Ethanol stress is known to produce changes in the composition of the yeast plasma membrane including the levels and chain length of unsaturated fatty acids, resulting in modification of membrane fluidity, and it has been suggested that these changes are specific responses that ameliorate the effect of ethanol (72). However, attempts to test the possible correlation between membrane fatty acid composition or fluidity and ethanol tolerance have produced contradictory results (12, 13, 15, 17). These studies are complicated by the fact that comparisons were often made across different yeast strains or species which can be expected to differ in many characteristics. By contrast, our results were obtained by comparing control and transgenic cells that are isoegenic except for the fat-2 cDNA. They show a distinct increase in viability for the transgenic cells containing polyunsaturated fatty acids and in this respect are consistent with previous studies in which yeast cells were grown in the presence of 18:2 (73) or were expressing a plant desaturase (64).

A number of mechanisms for ethanol tolerance have been proposed (15, 16), including hypotheses in which an increase in plasma membrane fluidity allows continued efficient operation of ATPases and other integral membrane proteins. If increased membrane fluidity is required for ethanol tolerance, then the consistent higher fluidity observed in yeast expressing FAT-2 (Fig. 3) would be expected to improve cell viability under conditions where cells are transferred directly to a high ethanol concentration (Fig. 4A). The extent to which polyunsaturated membranes confer ethanol tolerance under fermentative growth remains to be determined.

The increased tolerance to oxidative stress of yeast expressing FAT-2 (Fig. 4B) might also involve fluidity changes within the plasma membrane or endomembranes of the cell. However, in general, tolerance to oxidative stress is known to involve enzyme-based detoxification and free-radical scavenging mechanisms that have been described from many different organisms (72, 74–77). Typically, these mechanisms are strongly induced by mild oxidative stress. Because polyunsaturated fatty acids are considerably more susceptible to aerobic peroxidation and free-radical formation than monounsaturated or saturated fatty acids, it is likely that yeast cells expressing FAT-2 experience a mild, constitutive level of oxidative stress under normal culture conditions. It is possible, therefore, that polyunsaturated lipids provide increased protection against oxidative stress through the induction of endogenous tolerance mechanisms.

We have shown that yeast overexpressing the FAT-2 Δ12 desaturase are capable of producing large amounts of polyunsaturated fatty acids which increase membrane fluidity and simultaneously confer greater tolerance to ethanol and hydrogen peroxide. The identification of the fat-2 gene may help the isolation of mutants at the fat-2 locus in C. elegans. These mutants will provide new opportunities to elucidate the role of polyunsaturated fatty acids and compounds derived from them in the development and biology of this organism.

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