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Nutritional Regulation of Yeast Δ-9 Fatty Acid Desaturase Activity

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The addition of unsaturated fatty acids to cultures of *Saccharomyces cerevisiae* significantly altered the microsomal lipid composition. Supplementation with either of the naturally occurring palmitoleic (16:1) or oleic (18:1) acids caused increased levels in membrane phospholipids and reduced levels of the complementary acid. Growth in the presence of equimolar quantities of 16:1 and 18:1 acids, however, produced a fatty acid composition similar to that found in unsupplemented cell membranes. Linoleic acid (18:2) was not found in *S. cerevisiae* grown under normal conditions. It was preferentially internalized and incorporated into microsomes, however, at levels exceeding 50% of the total fatty acid species. This resulted in an almost total loss of 16:1 and a reduction of 18:1 to 25% of its normal level. The Δ-9 fatty acid desaturase, a microsomal enzyme that forms 16:1 and 18:1 from saturated acyl coenzyme A precursors, was affected by the presence of exogenous fatty acids. Enzyme activity toward the 16:0 coenzyme A substrate was elevated in microsomes from saturated-fatty-acid-supplemented cultures and sharply repressed following the addition of unsaturated fatty acids, including 18:2. Northern (RNA blot) and slot-blot analyses of mRNA encoded by the *OLE1* gene, which appears to be the structural gene for the Δ-9 desaturase, indicated that it was sharply reduced in unsaturated-fatty-acid-fed cells. These data suggest that a significant part of the regulation involves modulation of available transcripts.

Approximately 70% of the fatty acids in membrane lipids of the yeast *Saccharomyces cerevisiae* consist of palmitoleic acid (16:1) and oleic acid (18:1) (12). The remaining fatty acids are saturated, consisting primarily of palmitic acid (16:0) and lesser amounts of stearic acid (18:0) and myristic acid (14:0). Unlike most other fungi, whose most abundant unsaturated fatty acids are the di- and trienoic linoleic (18:2) and α-linolenic (18:3) species (27), *S. cerevisiae* synthesizes only monounsaturated acids when grown under normal laboratory conditions.

Unsaturated fatty acids are synthesized in fungal and animal cells by fatty acid desaturases, which are hydrophobic microsomal enzymes (3, 4, 23). The Δ-9 desaturase catalyzes the formation of the initial double bond between the 9th and 10th carbons of both palmitoyl (16:0) and stearoyl (18:0) coenzyme A (CoA) substrates to make 16:1 and 18:1. Introduction of the double bond is a complex reaction requiring the removal of electrons and hydrogens from the hydrocarbon chain of the fatty acid and the transfer of an additional two electrons from NADH to molecular oxygen via cytochrome b_5 and b_5 reductase (4, 22).

Considerable evidence has accumulated showing that the Δ-9 fatty acid desaturase in animal cells is regulated by dietary fatty acids. For example, in rat and chicken liver (18, 19, 25), the enzyme is repressed by dietary unsaturated fatty acids. The desaturase is the only component of the enzyme system that appears to be regulated, however, since cytochrome b_5 and b_5 reductase (18, 24) are maintained at constitutive levels under repressing conditions.

The unsaturated-fatty-acid-requiring KD115 mutant of *Saccharomyces cerevisiae* has been shown previously to incorporate both saturated and unsaturated acids supplied in the growth medium (8, 11, 15, 20, 28, 29). We attempted to examine whether a similar incorporation would occur with phenotypically wild-type cells and what effect the exogenous fatty acids might have on the Δ-9 desaturase activity. In this article we show that inclusion of fatty acids in the growth medium produces radical changes in microsomal membrane lipids and also affects the activity of the Δ-9 fatty acid desaturase. We also show that the level of desaturase mRNA is repressed under conditions which repress enzyme activity.

MATERIALS AND METHODS

Cell growth and microsome preparation. *S. cerevisiae* DBY-746 (*leu2-3,112 his3-1 trp1-289a ura3-52 OLE1*) was grown from overnight cultures containing 1% yeast extract, 2% peptone, 2% glucose, and 1% tergitol (YPDt). One-liter YPDt cultures containing 1 mM fatty acid supplements were inoculated at a cell density of 1×10^5 to 2×10^6 cells per ml, incubated at 30°C with moderate shaking, and harvested in late logarithmic phase (1×10^8 to 2×10^8 cells per ml) for lipid analysis (1, 2) and microsome preparation. Microsomes were isolated by a modification of the method of Daum et al. (7). Cells were pelleted, washed twice in 1.2 M sorbitol and then in 0.1M Tris-sulfate (pH 9.4)–10 mM dithiothreitol, and suspended in 20 mM potassium phosphate (pH 7.4)–1.2 M sorbitol buffer containing 0.2 mg of zymolase (100T) per ml. The spheroplasts were washed in sorbitol buffer and suspended in 0.6 M mannitol–10 mM HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.2)–0.5 mM phenylmethylsulfonyl fluoride and homogenized. Large cell debris was removed by low-speed centrifugation (3,000 × *g*) at 4°C, and mitochondria were removed by centrifugation at 12,000 × *g* for 40 min. Microsomes were then collected by centrifuging the postmitochondrial supernatant at 120,000 × *g* for 2 h at 4°C and suspended by brief homogenization in 0.1 M potassium phosphate buffer (pH 7.2). The microsomal fraction was then divided into portions, quickly frozen in dry ice-acetone, and stored under nitrogen at −70°C. Protein determinations were done by the method of Bradford (5) (Bio-Rad Laboratories assay kit). All enzymatic studies were carried out on fractions that were frozen until used and thawed only once. Results with fresh control microsomes were identical to those with microsomes

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that were frozen and stored under nitrogen for less than 1 month.

Enzyme assays. The standard assays were run in 0.1 M potassium phosphate buffer (pH 7.2) containing 1 mM NADH, 20 μM [1-^{14}C]16:0 CoA (specific activity, 20 μCi/mmol) (10, 24). Reactions were initiated by the addition of 0.3 mg of microsomal protein, and mixtures were incubated at 30°C in open glass tubes with shaking. The reactions were terminated by addition of an equal volume of 4.5 N KOH in methanol, and the mixtures were saponified for 35 to 45 min at 100°C under nitrogen. In experiments designed to follow the fate of the labeled fatty acyl CoA species, the reaction was terminated by the addition of $CHCl_3$-MeOH (1:2, vol/vol), and lipids were extracted by a modification of the method of Bligh and Dyer (1). Under those conditions, greater than 90% of the long-chain acyl CoA species were recovered in the aqueous phase of the two-phase system. The aqueous phase was then lyophilized to dryness, and the fatty acids were saponified and converted to methyl esters. The lower phase was washed with 0.1 M KCl, dried by rotary evaporation, and resuspended in $CHCl_3$-MeOH (6:1) for further analysis.

Fatty acid analysis. Fatty acids were extracted in hexane-ether (1:1) from the mixtures following acidification with HCl-methanol. Methyl esters were prepared by the BF_3-methanol procedure of Morrison and Smith (17). Samples suspended in a small volume of hexane were analyzed on a 1/8-in. stainless steel column of 10% DEGS on 80/100 mesh Chromasorb W AW at 170°C with a Hewlett Packard 5710-A gas chromatograph. Radioactive fractions were collected by using a column splitter with a 9:1 effluent vent-flame ionization detector ratio. The samples were eluted directly from the glass collection tubes into scintillation vials by three 2-ml rinses with Scintilene (Fisher Scientific) and analyzed by scintillation counting. Enzyme activity was measured by determining the ratio of relative levels of radioactive unsaturated fatty acid to radioactive saturated fatty acid per milligram of microsomal protein. The rate of desaturation was linear for up to 10 min and was proportional to the amount of microsomal fraction used.

RNA isolation and Northern (RNA) blots. RNA was isolated by methods described by Sherman et al. (21). Cells were harvested in mid-log phase by pouring the culture over crushed ice. The cells were suspended in LETS buffer (0.1 M LiCl, 0.01 M EDTA, 0.01 M Tris hydrochloride [pH 7.4], 0.2% sodium dodecyl sulfate [SDS], 0.1% diethyl pyrocarbonate [DEPC]), added to glass tubes containing phenol and 0.5-mm glass beads, and broken by repeated vortexing. Additional LETS buffer was added, and the mixture was centrifuged at 7,500 × *g* for 10 min. The aqueous phase was extracted twice with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), followed by one extraction with chloroform-isoamyl alcohol (24:1). LiCl was added to a final concentration of 0.5 M, and 2 volumes of absolute ethanol were added to precipitate the RNA overnight at −20°C. The precipitated material was pelleted at 17,000 × *g*, dried, and suspended in DEPC-treated sterile water. Samples were stored at −70°C.

RNA denatured in glyoxal-dimethyl sulfoxide was electrophoresed in 1.1% agarose gels in 10 mM $NaPO_4$, pH 7.5 (6, 16, 26), for 1.5 h at 100 V with constant buffer recirculation. The samples were blotted to Genescreen-Plus (Du Pont) according to the manufacturer's instructions. The blots were prehybridized for 4 h in 50% deionized formamide–20% 5× P buffer (1% bovine serum albumin, 1% polyvinylpyrrolidone [MW 40,000], 1% Ficoll [MW 400,000], 250 mM Tris hydrochloride [pH 7.5], 0.5% sodium PP_i, 5% SDS, 5 M NaCl)–20% dextran sulfate (25% solution)–denatured herring sperm DNA (1 mg/ml, final concentration). Hybridization was carried out at 42°C for 16 h. Washes were done in 2× SSPE (0.36 M NaCl, 20 mM $NaPO_4$ [pH 7.7], 2 mM EDTA)—0.1% SDS at room temperature, 2× SSPE–0.1% SDS at 65°C, and 0.1× SSPE–0.1% SDS at 65°C. Blots were probed with either a 403-base-pair (bp) *Eco*RI fragment or a 522-bp *Bst*EII fragment isolated from the cloned *OLE1* gene and labeled by the random primer extension method (9). For quantitative Northern analysis, blots were stripped of probe in boiling water and reprobed with a 536-bp *Cla*I fragment from the yeast actin gene, which served as an internal control for mRNA loading. Probed mRNA levels were determined by scanning autoradiograms with a Joyce Loebel Chromoscan 3 densitometer.

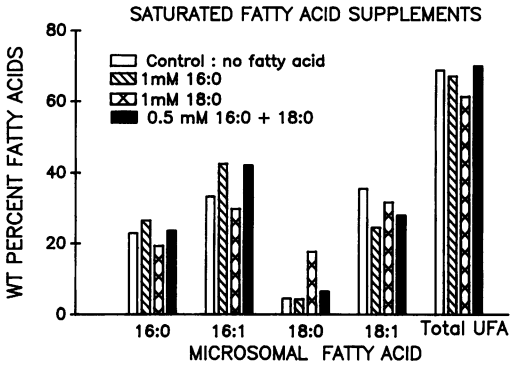

FIG. 1. Distribution of microsomal phospholipid fatty acids in cells grown on medium containing saturated fatty acids. Cells were grown on YPDt medium and the indicated 1 mM fatty acid supplement. Control cultures contained tergitol but no fatty acid. Cultures were harvested after overnight growth to late log phase. UFA, Unsaturated fatty acid. Bars indicate weight percentage (wt) of the total fatty acid species.

RESULTS

Incorporation of fatty acid supplements. Microsomal phospholipid fatty acids from logarithmic-phase strain DBY-746 grown in YPDt medium closely paralleled total cellular phospholipid compositions. In order to examine how exogenous fatty acids affect the composition of the microsomal membranes, we supplemented cultures with saturated or unsaturated fatty acids (Fig. 1 and 2).

Cultures grown in medium containing 1 mM saturated fatty acids showed small but significant changes in microsomal phospholipid fatty acid levels. Addition of 16:0 to the medium, either singly or in combination with 18:0, caused a small increase in 16:1 and a correspondingly reduced level of 18:1. Levels of 16:0 were, however, close to those of control cells. Inclusion of 18:0, normally a minor fatty acid species, led to a slight reduction in 16:1 and a three- to fourfold increase in its own levels. The ratio of saturated to unsaturated fatty acids in the phospholipids was not significantly altered in either 16:0- or 18:0-fed cells.

Unsaturated fatty acids caused the most dramatic changes in membrane lipids (Fig. 2). Cultures fed 16:1 had twice as much of that species as controls, and 18:1 was reduced to less than half of normal levels. Conversely, 18:1-fed cultures incorporated almost double the amount of that acid, and 16:1 was reduced to less than one-third of its normal levels. Feeding cultures equal proportions of both 16:1 and 18:1 (0.5

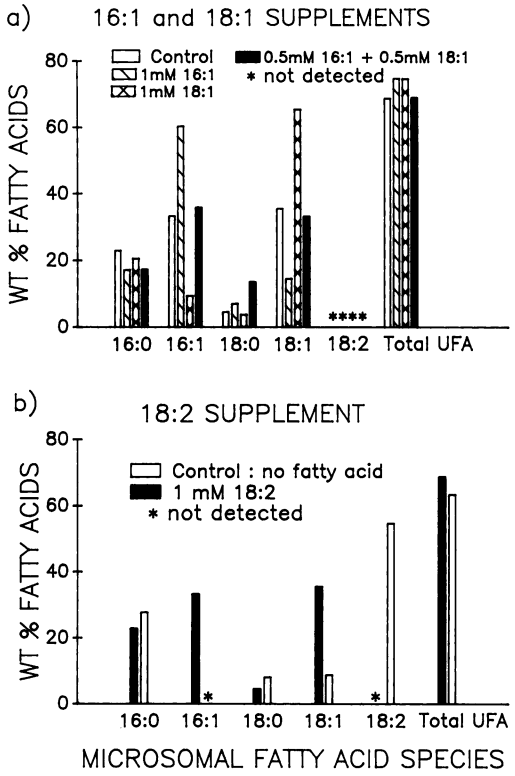

FIG. 2. Distribution of microsomal phospholipid fatty acids (a) in cells grown on medium containing 1 mM monounsaturated fatty acids and (b) in cells grown on medium containing 1 mM linoleic acid (18:2). See legend to Fig. 1 for details.

mM each) resulted in an unsaturated fatty acid composition similar to that of control cells, although 18:0 was significantly elevated and 16:0 was reduced. The incorporation of either monounsaturated species into the membranes also caused a slight increase in total phospholipid unsaturated fatty acid levels; however, equal amounts of 16:1 and 18:1 in the medium produced no significant changes.

Although 18:2 is not normally found in *S. cerevisiae*, it was readily incorporated at high levels into microsomal phospholipids (Fig. 2b). Under the growth conditions used, 18:2 constituted over 50% of the total phospholipid fatty acids, resulting in undetectable levels of 16:1, reduction of 18:1 to one-quarter of control values, and a slight reduction in total membrane unsaturated fatty acid levels.

Fatty acid desaturase activity in unsupplemented cultures. The Δ-9 desaturase activity in microsome preparations from cells grown on YPDt medium in the absence of fatty acid supplements was found to desaturate 16:0 and 18:0 CoA substrates at about equal rates, ranging from 1.8 to 2.0 nmol of product produced per mg of protein per min under standard assay conditions.

Analysis of the lipids from the reaction mixtures revealed that most of the labeled fatty acyl CoA substrate was rapidly converted to phospholipids. After an extensive (30 min) incubation, about 54% of the total $CHCl_3$-soluble radioactivity was incorporated into phospholipids (Table 1). About two-thirds of those fatty acids were in the form of 16:1 and the remainder were present as 16:0. The presence of saturated species incorporated into the phospholipids indicated significant fatty acyltransferase activity in the microsomal fraction. About 33% of the organic-phase radioactivity was

TABLE 1. Distribution of radioactivity in microsomal membrane lipid fractions following prolonged incubation in the standard assay mixture containing [1-^{14}C]16:0 CoA[a]

Component	Radioactivity (total cpm)	
	16:0	16:1
Phospholipids	64,720	110,119
Steryl esters	13,520	9,395
Free fatty acids	98,897	6,313
Triglycerides	16,930	756

[a] Radioactivity recovered in fractionated lipids following incubation of microsomes from mid-logarithmic-phase cells grown in YPDt medium in the standard assay mixture was determined. Lipids were extracted after 30 min and fractionated by thin-layer chromatography. Each fraction was converted to methyl esters, and the ratio of radioactivity in 16:0 and 16:1 species was determined by gas-liquid chromatography. No significant radioactivity (above the control levels found with the CoA substrate) was found in 18-carbon species.

in the form of free fatty acids, which were almost exclusively 16:0, suggesting that they originated from hydrolysis of the 16:0 CoA substrate rather than lipolysis of fatty acids that were previously incorporated into membrane lipids. Lesser amounts of radioactivity were detected in the sterol ester and triglyceride fractions.

Effects of fatty acids on Δ-9 desaturase. Cultures fed saturated fatty acids had enzyme activity that ranged from 46 to 75% greater than control levels (Fig. 3). Activities were drastically reduced, however, in cultures that were fed unsaturated fatty acids either singly or in combinations. Under conditions in which there was maximal incorporation of the unsaturated fatty acids in the phospholipids, repression was complete, with undetectable levels of enzyme activity. Lower but detectable activities could be found, however, in experiments in which the calculated levels of the unsaturated fatty acids present in the cells exceeded the levels added to the growth medium (indicating that the supplements had been depleted from the medium and endogenous unsaturated fatty acid synthesis had resumed). Activity was most strongly repressed in microsomes isolated from cultures fed excess 16:1, 16:1 and 18:1 in equimolar amounts, and 18:2. A small amount of activity was detected in cells fed 18:1.

Effects of fatty acids on *OLE1* mRNA levels. Studies involving the incorporation of radioactive saturated fatty acids in an *ole1* mutant of *S. cerevisiae* had suggested previously that

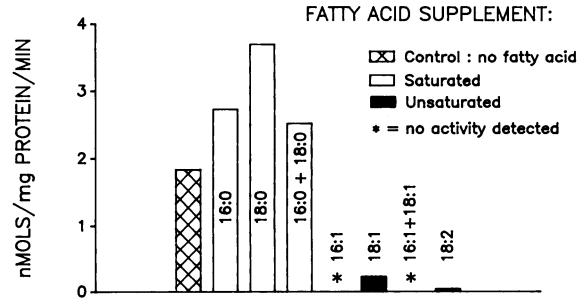

FIG. 3. Microsomal Δ-9 fatty acid desaturase activity from cultures grown on different fatty acid supplements. Assays were performed with microsomes prepared from cells grown as described in the legends to Fig. 1 and 2. Enzyme activities were measured as described in Materials and Methods with [1-^{14}C]16:0 CoA as the substrate. No enzyme activity was detected in microsomes from cells supplemented with 1 mm 16:1 or 0.5 mM 16:1 + 0.5 mM 18:1.

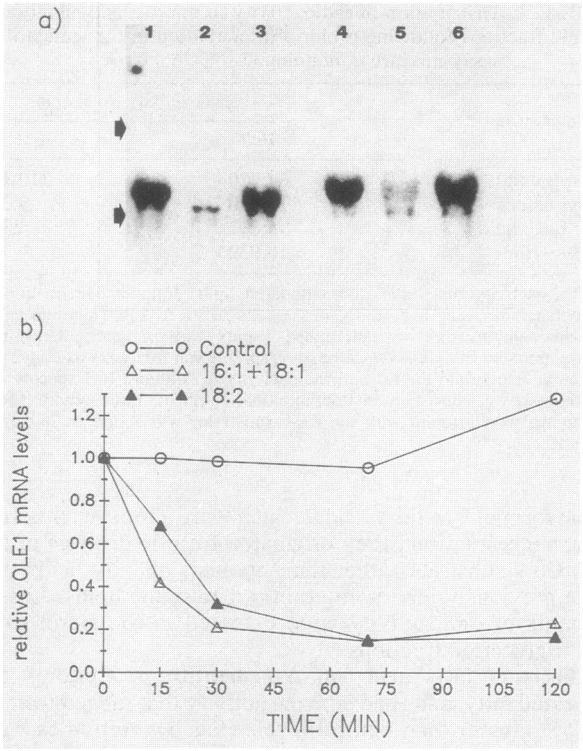

FIG. 4. (a) RNA blot from cells (4×10^7 cells per ml) grown in YPDt shifted to medium containing either 16:0 or 16:1. Total RNA was isolated 4 and 10 h after the shift and probed with an *Eco*RI fragment from the *OLE1* gene. Lanes: 1 to 3, 4 h postshift; 5 and 6, 10 h postshift; 1 and 4, control (no fatty acid); 2 and 5, 16:1 fed; 3 and 6 16:0 fed. RNA was loaded at 15 μg per lane. The arrows indicate the positions of the large and small rRNA bands identified by ethidium bromide fluorescence. (b) Quantitative Northern analysis of *OLE1* mRNA levels following addition of 1 mM 18:2 or a combination of 0.5 mM 16:1 and 0.5 mM 18:1 to logarithmic-phase cells. No unsaturated fatty acids were added to the control culture. Blots were hybridized with a 522-bp *Bst*EII fragment within the coding sequence of the *OLE1* gene and autoradiographed on pre-flashed X-ray film. The filters were then stripped and reprobed with a 563-bp *Cla*I fragment from the coding sequence of the yeast actin gene, which served as an internal control for mRNA loading. *OLE1* mRNA levels were calculated from relative intensity of the actin mRNA band from each fraction and then normalized to control *OLE1* levels prior to the addition of the fatty acids.

the mutant was defective in the structural gene for the Δ-9 desaturase (15). We recently cloned and characterized the *OLE1* gene (23a), and analysis of the sequence further indicates that it is the structural gene for the Δ-9 enzyme.

In order to test the effects of unsaturated fatty acid feeding on Δ-9 desaturase mRNA levels, RNA isolated from fatty acid-fed cells was examined by Northern blot analysis. The gene encodes an abundant 1.7- to 2.0-kb transcript in unsupplemented cells. Little difference was observed in mRNA levels in cells grown on YPDt medium or 16:0-fed cells either 4 or 10 h after addition of the fatty acid to the culture (Fig. 4a). Message levels were sharply reduced at both times, however, in cells fed 16:1. In order to examine the kinetics of unsaturated fatty acid repression on *OLE1* mRNA levels, we performed quantitative RNA blot analysis of cells fed either 18:2 or a combination of 16:1 and 18:1. Densitometry scanning of blot autoradiograms with the yeast actin gene mRNA levels used as a standard showed that *OLE1* mRNA levels dropped rapidly, resulting in a 10-fold decrease by 30 min after the addition of unsaturated acids (Fig. 4b).

DISCUSSION

Previous studies on unsaturated-fatty-acid-requiring mutants of *S. cerevisiae* have shown that these cells can incorporate and use a variety of unsaturated fatty acids (12), although growth varied markedly with the supplement. This study shows that wild-type yeast strains also preferentially incorporate large amounts of exogenous unsaturated fatty acids into their membranes. Addition of a single fatty acid species caused striking changes in phospholipid fatty acid compositions, while equal amounts of the two naturally occurring unsaturated species produced a fatty acid composition resembling that of unsupplemented cells. Although the cells incorporated large amounts of unsaturated acids from the medium, the total phospholipid unsaturated fatty acid levels remained relatively constant, suggesting that the cells closely regulate the ratio of unsaturated to saturated acids in membrane lipids. Incorporation also seems to be selective, in that we did not observe significant increases in the triglyceride content of supplemented cells, suggesting that the cells incorporated the fed species as needed rather than rapidly accumulating them into reserves.

The addition of 16:1 and 18:1 together or individually strongly repressed the Δ-9 fatty acid desaturase activity, even though cells fed either species alone were unable to make the complementay fatty acid. Surprisingly, the dienoic acid, 18:2, which is not synthesized in these strains, caused equally severe repression and the most radical changes in the membrane lipid composition.

A small stimulation of Δ-9 desaturase activity was caused by adding a saturated species to the medium, although there was little change in membrane lipid compositions. By contrast, 16:0 and 18:0 supplementation has been reported to strongly repress acetyl CoA carboxylase, which is the first step in saturated fatty acid biosynthesis (13, 14).

It seems unlikely that the lowered levels of desaturase enzyme activity are the result of inhibition caused by the presence of increased product in the membrane lipids, since activity was also strongly repressed by 18:2, which is formed by a different enzyme. Repression of the desaturase activity by unsaturated fatty acids, however, might be triggered either by the response of the pre-existing desaturase in the membrane to changes in its physical or fluid environment (caused by the insertion of the exogenous acids) or by an independent mechanism that responds to the presence of exogenous fatty acids and controls either the level of synthesis or the stability of the desaturase protein.

The idea that fluidity changes in the microsomal membrane exert major control over enzyme activity seems to be less likely in this case for several reasons. First, preferential incorporation of either 16:1 or 18:1 resulted in strong repression of the enzyme activity. Increased levels of 16:1 in the membrane could be interpreted as "fluidizing," compared with the normal lipid composition; however, incorporation of high levels of 18:1 and the reduction of 16:1 would have to be regarded as having a comparatively "rigidifying" effect on the membrane. Second, the incorporation of both 16:1 and 18:1 together also caused a strong repression of the enzyme but resulted in fatty acyl compositions almost identical to those found in cells growing on unsupplemented medium. We cannot rule out the possibility, however, that exogenous fatty acids might be paired on phospholipids in combinations that produce a different molecular species

distribution (and consequently a different fluid state) than that produced by endogenous unsaturated fatty acids. Given the limited number of fatty acid species in yeast cells compared with other eucaryotes, however, this seems unlikely.

An alternative regulatory stimulus that does not act at the level of the membrane may reside in the fatty acids supplied in the growth medium. Previous studies on fatty acid biosynthesis in *S. cerevisiae* have shown that acetyl CoA carboxylase, the first regulated step in saturated fatty acid biosynthesis, is sensitive to the presence of exogenous saturated fatty acids (13, 14). Evidence from mutants defective in acyl CoA synthetase activity suggested that the saturated fatty acids in the growth medium had to be converted to an acyl CoA derivative in order to repress the activity of the acetyl CoA carboxylase enzyme. A similar mechanism of regulation could involve either internalized unsaturated free fatty acids or a more soluble unsaturated acyl CoA derivative of the internalized acids as the regulatory stimulus. The existence of a soluble effector in the form of an unsaturated acyl CoA-binding protein or a free unsaturated-fatty-acid-binding protein would provide a mechanism for transcriptional or translational control of desaturase production. The RNA blots shown here indicate that a significant degree of control is invoked by modification of mRNA levels.

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