

# Regulatory Elements That Control Transcription Activation and Unsaturated Fatty Acid-mediated Repression of the *Saccharomyces cerevisiae* *OLE1* Gene\*

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Jae-Yeon Choi, Joseph Stuke<sup>‡</sup>, Sue-Yun Hwang<sup>§</sup>, and Charles E. Martin<sup>¶</sup>

From the Nelson Biological Laboratory, Bureau of Biological Research, Department of Biological Sciences, Rutgers University, Busch Campus, Piscataway, New Jersey 08855-1059

In *Saccharomyces cerevisiae*, unsaturated fatty acids are formed from saturated acyl-CoA precursors by Ole1p, a  $\Delta$ -9 fatty acid desaturase. *OLE1* mRNA levels are differentially regulated by the addition of saturated or unsaturated fatty acids to the growth medium. One component of this regulation system involves the control of *OLE1* transcription. Saturated fatty acids induce a 1.6-fold increase in transcription activity, whereas a large family of unsaturated fatty acids repress *OLE1* transcription as much as 60-fold. A deletion analysis of *OLE1* promoter::lacZ fusion reporter genes identified a 111-base pair (bp) fatty acid-regulated (FAR) region approximately 580 bp upstream of the start codon that is essential for transcription activation and unsaturated fatty acid repression. Deletion of an 88-bp sequence within that region resulted in a complete loss in transcription activation and unsaturated fatty acid regulation. The 111-bp FAR element strongly activates transcription and confers unsaturated fatty acid regulation on a heterologous *CYC1* promoter test plasmid. Essential elements required for unsaturated fatty acid repression of *OLE1* were found in the 5' and 3' region of the 111-bp sequence. The FAR element-mediated activation and fatty acid repression of transcription was found to be closely tied to fatty acyl-CoA metabolism. Two fatty acid activation genes, *FAA1* and *FAA4*, were found to be essential for unsaturated fatty acid repression of *OLE1* through the FAR sequences. Disruption of either gene results in reduced levels of unsaturated fatty acid repression; disruption of both genes completely blocks the regulatory response. Acyl-CoA binding protein (ACBP) plays a role in determining the level of FAR element activated transcription. Disruption of the ACBP gene causes a >5-fold activation of *OLE1* transcription and a similar increase in *OLE1* mRNA levels. Unsaturated

fatty acid repression of *OLE1* transcription, however, is not affected by the disrupted ACBP gene. These studies show that promoter elements responsible for unsaturated fatty acid-mediated transcription repression are tightly linked to *OLE1* activation sequences and that *OLE1* transcription levels are closely tied to acyl-CoA metabolism.

Nutrient fatty acids can exert strong regulatory effects on a number of lipogenic enzymes in fungi. Medium and long chain fatty acids are readily internalized by fungi and incorporated into membrane and storage lipids. Saturated and unsaturated acids appear to differentially regulate the expression of a number of lipid biosynthetic genes, including those encoding acetyl-CoA carboxylase (2–4), fatty-acid synthase (5, 6), and fatty acid desaturase (1). The mechanisms by which cells sense fatty acids, discriminate among molecular species, and modulate gene activity in different parts of the lipid metabolic web is unclear, although it appears that multiple systems have evolved to regulate different lipogenic functions.

To identify the mechanisms that control lipid synthesis in response to extracellular acids, we examined the regulation of the  $\Delta$ -9 fatty acid desaturase, an enzyme involved in the formation of unsaturated fatty acids. The *OLE1* gene, which encodes that enzyme in *Saccharomyces*, is strongly regulated in response to extracellular fatty acids (1). This can be seen as a rapid reduction in *OLE1* mRNA levels when unsaturated acids are added to the growth medium and an increase in enzyme activity when cells are exposed to saturated fatty acids. The exogenous fatty acids that trigger these responses are rapidly incorporated into membrane lipids; thus it is reasonable to expect that the sensors and signal transducers that regulate *OLE1* in response to nutrient fatty acids may also be a part of broader controls that regulate membrane lipid composition in response to other stimuli.

Our recent studies suggest that fatty acid regulation of *OLE1* is under at least two forms of control (7). One component acts to repress transcription of the *OLE1* gene; a second acts by post-transcriptional mechanisms to further modify *OLE1* mRNA levels (7). In this paper, we examine the promoter of the desaturase gene to identify transcriptional controlling elements that respond to unsaturated fatty acids and to assess their contribution to *OLE1* expression. An essential transcription activation region, designated the FAR<sup>1</sup> element, is identified that also contains the elements required for unsaturated fatty

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<sup>‡</sup> Supported by a Charles and Johanna Busch Predoctoral Fellowship from the Bureau of Biological Research. Present address: Dept. of Food Science, Rutgers University, Cook Campus, New Brunswick, NJ 08903.

<sup>§</sup> Supported by a Charles and Johanna Busch Predoctoral Fellowship from the Bureau of Biological Research and the Anne B. and James R. Leatham grant for student research. Present address: Jackson Laboratories, Bar Harbor, ME, 04609.

<sup>¶</sup> To whom correspondence should be addressed: Nelson Biological Laboratory, Bureau of Biological Research, Dept. of Biological Sciences, Rutgers University, Busch Campus, P. O. Box 1059, Piscataway, NJ 08855-1059. Tel.: 908-445-4081; Fax: 908-445-5870.

<sup>1</sup> The abbreviations used are: FAR, fatty acid-regulated; UDt, uracil drop-out synthetic medium with tergitol; bp, base pair(s); PCR, polymerase chain reaction; kb, kilobase(s); ACBP, acyl-CoA binding protein; *FAA*, *S. cerevisiae* fatty acid activation gene (acyl-CoA synthetase); ER, endoplasmic reticulum.

TABLE I  
Yeast strains (*S. cerevisiae*)

Strain	Genotype	Source
L8-25A	$\alpha$ , <i>leu2-3, leu2-112, ura3-52, his4</i>	This laboratory
BWG1-7A	$\alpha$ , <i>leu2-3, leu2-112, ura3-52, his4-519, ade1-100</i>	L. Guarente
LPY15	$\alpha$ , <i>leu2-3, leu2-112, ura3-52, his4-519, ade1-100, hap1<math>\Delta</math>::LEU2</i>	L. Guarente
JO1-1a	$\alpha$ , <i>leu2-3, leu2-112, ura3-52, his4-519, ade1-100, hap2<math>\Delta</math>::LEU2</i>	L. Guarente
DTY-10A	$\alpha$ , <i>leu2-3, leu2-112, ura3-52, his3, ade1-100, can</i>	This laboratory
JY001	$\alpha$ , <i>leu2-3, leu2-112, ura3-52, his3, ade1-100, can, acbp<math>\Delta</math>::LEU2</i>	This laboratory
YB332	$\alpha$ , <i>ura3-52; leu2-3, 112; his3<math>\Delta</math>-200, ade2-101, lys2-801;</i>	J. Gordon
YB525	$\alpha$ , <i>ura3-52; leu2-3, 112; his3<math>\Delta</math>-200, ade2-101, lys2-801; faa1<math>\Delta</math>::HIS3; faa4<math>\Delta</math>::LYS2</i>	J. Gordon
YB492	$\alpha$ , <i>ura3-52; leu2-3, 112; his3<math>\Delta</math>-200, ade2-101, lys2-801; faa3<math>\Delta</math>::LEU2</i>	J. Gordon
YB489	$\alpha$ , <i>ura3-52; leu2-3, 112; his3<math>\Delta</math>-200, ade2-101, lys2-801; faa2<math>\Delta</math>::LEU2</i>	J. Gordon
YB513	$\alpha$ , <i>ura3-52; leu2-3, 112; his3<math>\Delta</math>-200; ade2-101; lys2-801; faa1<math>\Delta</math>::HIS3</i>	J. Gordon
YB524	$\alpha$ , <i>ura3-52; leu2-3, 112; his3<math>\Delta</math>-200; ade2-101; lys2-801; faa4<math>\Delta</math>::LYS2</i>	J. Gordon
YB526	$\alpha$ , <i>ura3-52; leu2-3, 112; his3<math>\Delta</math>-200; ade2-101; lys2-801 faa1<math>\Delta</math>::HIS3; faa2<math>\Delta</math>::LEU2, faa3<math>\Delta</math>::LEU2; faa4<math>\Delta</math>::LYS2</i>	J. Gordon

acid-mediated repression of transcription activity. There appears to be a close connection between cellular acyl-CoA metabolism and regulators that act on that region of the *OLE1* promoter.

#### MATERIALS AND METHODS

**Strains, Growth Medium, and DNA Manipulations**—Strains used in this study are shown in Table I. Yeast cells containing *lacZ* fusion plasmids were grown at 30 °C on uracil dropout synthetic dextrose (8) medium supplemented with 1% tergitol (UDt) and 1 mM appropriate fatty acid (obtained from Sigma or Nu-Chek Prep). All unsaturated fatty acids used in this paper contained double bonds in the *cis* configuration. All recombinant DNA manipulations were performed according to standard methods (8, 9). Plasmid amplifications and bacterial transformations were performed using *Escherichia coli* strains HB101, DH5 $\alpha$  (Life Technologies, Inc.), or XL1-Blue (Stratagene). Yeast transformations were performed by the method of Ito *et al.* (10) or by electroporation (Life Technologies, Inc.) according to the manufacturer's protocols.

**Construction of *OLE1* Promoter:*lacZ* Fusion Deletion Series**—Plasmids used in this study are shown in Table II. Plasmid p62 was constructed by exchanging a *HindIII*/*StuI* fragment from plasmid YCP50 (11) with a DNA fragment from plasmid YEP356R (7), which contains a multiple cloning sequence fused to the protein coding region of the *E. coli lacZ* gene. Plasmid p62::–934 was constructed by the insertion of an *OLE1* 1015-bp *HindIII*/*SaI* fragment into the p62 multiple cloning site. That fragment extends from –934 base pairs upstream of the start codon through codon 27 of the *OLE1* protein region to form an in-frame fusion with the *E. coli lacZ* sequence. A nested series of *OLE1* promoter deletions were derived from that vector; these cover the region from nucleotides –934 to –142 relative to the first in-frame ATG codon. They were constructed using a combination of available restriction sites and *Bal31* digestions. All *Bal31*-generated end points were determined by DNA sequencing.

Plasmid p62::–934 $\Delta$ 88 was constructed by isolating a fragment consisting of *OLE1* nucleotides –934 to –576 and fusing it to a fragment containing bases –489 to +81. The resulting fragment was ligated into the parent p62 plasmid, yielding an *OLE1* insert with the same ends as p62::–934 but lacking 88 bases between –576 and –489. All fragments in plasmid p62 series had a 3'-end point at nucleotide +81. The promoter deletion constructs were used to transform the phenotypically wild-type *OLE1* strain, L8-25A, to uracil prototrophy.

**Construction of pCT $\Delta$  Vectors Containing *OLE1* Promoter Sequences**—Plasmids derived from vector pCT $\Delta$  are shown in Table II. Promoter test vector pCT $\Delta$  was obtained from Kornberg (12, 20). It contains a multiple cloning site fused to bases –248 through +5 of the *Saccharomyces CYC1* promoter region, which is fused in-frame to *E. coli lacZ*. The *CYC1* fragment includes two functional TATA elements, but it does not contain the *CYC1* upstream activating sequences. During the course of this study, a sequence of bases encoding the *KpnI*-*SmaI* restriction sites in the multiple cloning region of the vector was found to be a transcription-repressing element that acted independent of the inserted test sequences. Vectors in which that site was removed by restriction enzyme digestion of flanking sequences were designated as pCTm (pCT modified) vectors.

Vectors pCT714 and pCTm714 were constructed by inserting a 714-bp *HindIII*/*HpaI* fragment that extended between bases –934 and –221 of the *OLE1* promoter into the pCT $\Delta$  multiple cloning region. Vectors pCT111 and pCTm111 were similarly constructed by inserting

TABLE II  
Plasmids used in this study

Plasmid Name	<i>OLE1</i> promoter sequences inserted into plasmid
p62::(deletion series)	Numbers following :: indicate the position of the nucleotide at the 5' end of the <i>OLE1</i> promoter fragment with respect to the start codon (A of ATG is +1).
pCT 714, pCTm714	–934 → –221
pCT 714 $\Delta$ 88	–934 → –576/ $\Delta$ –489 → –221
pCTm 114	–579 → –466
pCT111, pCTm111	–576 → –466
pCTm111E10	–576 → 566/(10-bp <i>EcoRI</i> linker)/–565 → –466
pCTm100	–565 → –466
pCTm91	–556 → –466
pCTm67	–582 → –516
pCTm40	–582 → –543
pCTm25	–579 → –555
pITm714	–934 → –221
pITm111	–576 → –466

111 bp of the *OLE1* promoter region that extends from bases –576 to –466. Vector pCTm111E10 was made by inserting an *EcoRI* linker (sequence CCGAATTCGG) into the *SmaI* restriction site at the 5'-end of the 111-bp fragment of pCTm111. pCTm100 was derived from pCTm111E10 by *EcoRI* digestion to remove the 11 upstream base pairs of the *OLE1* promoter sequence.

Vectors pCTm67 and pCTm40 were constructed using synthetic paired oligonucleotides. Vector pCTm67 contains bases –582 to –516, and plasmid pCTm40 includes bases –582 and –543 of the *OLE1* promoter sequences.

Vector pCTm91 was constructed using a 91-bp fragment encompassing bases –556 to –466 of the *OLE1* promoter (derived by PCR amplification of *OLE1* promoter sequences). The base sequence of the PCR fragment was determined by DNA sequencing. Plasmid pCTm114 contains bases –579 to –466 of the *OLE1* promoter. Vector pCTm25 was prepared by digestion of vector pCTm114 at the *ApaI* restriction site within the 114-bp *OLE1* sequence and the vector *XhoI* site.

Integrating vectors pITm714 and pITm111 were constructed by removing the ARS and CEN sequences from their respective pCTm parents by *HindIII* digestion and religation.

**$\beta$ -Galactosidase Assays**—Assays of cells containing plasmids derived from *OLE1* promoter-*lacZ* fusion p62 constructs were performed as described previously (8). Cell densities for those assays were determined either by measurement at  $A_{600}$  or by hemocytometer counts. Corrections were made for light scattering by  $A_{550}$  absorption. Assays of extracts from cells containing pCT plasmids were assayed by the procedure of Buchman *et al.* (12). The latter assays were correlated with total protein in cell extracts by the method of Bradford (13) using the Bio-Rad assay kit. Two or three independent yeast transformants were assayed for each of the plasmid constructs given in Table II.  $\beta$ -Galactosidase activities reported here are the results of at least three independent experiments. Each experimental assay was performed in triplicate.

**Fatty Acid Repression/Derepression Studies**—Cells containing an *OLE1-lacZ* reporter gene were tested for unsaturated fatty acid regulation according to protocols that measure repression of activity from a derepressed state. Cells were grown overnight in uracil dropout synthetic dextrose medium supplemented with 1% UDt. These cultures were used to inoculate (at one-tenth total volume) fresh UDt medium or

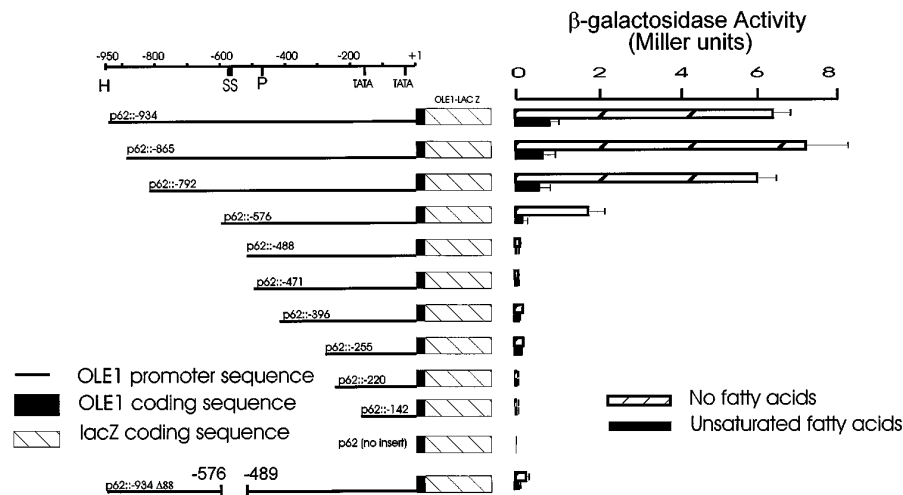


FIG. 1. Deletions of the *OLE1* promoter:*lacZ* fusion constructs and their activity in the phenotypically wild-type strain L8-25A. The upper left scale represents DNA sequences upstream of the *OLE1* protein coding region. The relative locations of two proposed TATA boxes at positions -160 (TATATA) and -30 (TATAAA), and the start codon are indicated. Restriction sites shown on the scale that were used for constructions have the following designations: H, *HindIII*; S, *SmaI*; P, *PstI*. Deletion constructs are shown in the column below the scale. The narrow line represents *OLE1* promoter sequences, the solid black bar represents the amino-terminal 27 amino acids of the *OLE1* coding sequence fused to *E. coli lacZ* (hatched bar). The number above each line indicates the position of the deletion end point with respect to the ATG start codon of the wild-type base sequence with the A of the codon designated as +1. Deletion end points were verified by sequencing as described under "Materials and Methods." The p62::934Δ88 construct represented by the bottom line contains an 88-bp deletion at the indicated position in the promoter region. Bars to the right of each diagram illustrate the  $\beta$ -galactosidase activity in Miller units (30) produced by each construct in cells grown without unsaturated fatty acids (hatched bars) and with unsaturated fatty acids (solid bars). Units of activity indicated are the average of at least three independent experiments performed on two separate transformants. Standard deviations of these values were less than 20% of the mean.

UDt medium supplemented with 0.5 mM each of the unsaturated fatty acids (16:1 and 18:1) or 1 mM of 18:2. These cultures were incubated at 30 °C with rotary shaking for 8–10 h followed by harvesting by centrifugation for analysis of  $\beta$ -galactosidase activity.

**RNA Isolation and Northern Blot Analysis**—Total yeast RNA was isolated essentially as described previously (14, 15). Equal amounts (10  $\mu$ g) of total RNA from each time point of an experiment were analyzed by Northern blots according to standard procedures for separation of RNA using 1% formaldehyde gels (8). RNA from the gels was transferred to the Zeta Probe<sup>™</sup> membrane (Bio-Rad) in 10  $\times$  SSC for 90 min at 5 inches of mercury using a Vacuum Blotter (model 785) from Bio-Rad, according to instructions from the manufacturer. Prehybridization, hybridization, and washing of membranes were performed as described in the Zeta Probe<sup>™</sup> manual. A hybridization oven (Hybaid Mini Oven MK II) was used as described in the instruction manual supplied by the manufacturer. The membrane was prehybridized at 65 °C for 10–20 min in 1 mM EDTA, 0.25 M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2, and 7% SDS. The membrane was hybridized at 65 °C overnight with  $2.5 \times 10^6$  cpm/ml. Following incubation the membrane was washed twice (30 min/wash) at 65 °C with 1 mM EDTA, 40 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2, and 5% SDS. This was followed by two 30–60-min wash cycles at 65 °C with 1 mM EDTA, 40 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2, and 1% SDS. Northern blots were quantified using a phosphorimaging analyzer (Molecular Dynamics).

**Preparation of Radiolabeled Probes**—For the detection of *OLE1* mRNA, a radiolabeled DNA probe was made using a 0.5-kb *EcoRI* fragment from the *OLE1* protein coding sequence. For an internal control of cellular mRNA levels, a 1-kb *HindIII-KpnI* fragment of the *Saccharomyces* phosphoglycerate kinase gene (*PGK1*) was isolated from plasmid pRIP1PGK obtained from S. Peltz (Robert Wood Johnson Medical School). All DNA fragments were separated by agarose gel electrophoresis in 1  $\times$  TAE and purified by Gene Clean II (Bio 101) according to manufacturer's recommendations. The purified DNA fragments were labeled to high specific activity with [ $\alpha$ -<sup>32</sup>P]dATP (DuPont NEN) by the random primer extension method (16) using a PROBE-EZE reaction kit (5 Prime  $\rightarrow$  3 Prime). Unincorporated nucleotides were removed from the sample using a Sephadex G-50 spin column (5 Prime  $\rightarrow$  3 Prime). Specific activity of labeled probes was determined by liquid scintillation counting.

**Fatty Acid Extraction and Analysis**—Total cellular fatty acids were obtained by HCl methanolysis of extensively washed cell pellets according to the modified procedure of Browse *et al.* (17) as described previously (7). Lipid fractionation and preparation of fatty acid methyl ester chromatography were performed as described previously (7). Fatty acid methyl esters were separated on a Supelcowax 10 30 m  $\times$  0.032 mm

capillary column using helium as a carrier gas in a Varian 3400CX gas chromatograph.

**Cloning and Disruption of ACBP**—A 1.6-kb DNA fragment encoding the *S. cerevisiae* acyl-CoA binding protein (ACBP) gene was cloned by PCR amplification of genomic DNA. PCR primers used for the amplification were 5'-TTCCACATGTGAAATTATGCTGG-3' (bases -853 to -831 of ACBP gene, where +1 is the A in the ATG start codon) and 5'-GTGTACTGTACCACTAGTTTGG-3' (positions +728 to +749). This fragment was cloned into PCR-script SK(+) (Stratagene). A 700-bp fragment containing the ACBP protein coding region and flanking vector 5' and 3' sequences were removed by *BglII/BclI* digestion. This was replaced with a 2-kb fragment consisting of the *Saccharomyces LEU2* gene. A 2.8-kb linear DNA fragment encompassing the *LEU2* gene and flanking ACBP gene sequences was isolated from the plasmid and used to perform a one-step gene disruption (18, 19) of the chromosomal ACBP gene in strain DTY-10A. The disruption of ACBP was confirmed by leucine prototrophy and PCR analysis of genomic DNA.

## RESULTS

**Deletion Analysis of *OLE1* Promoter::lacZ Fusion Vectors**—DNA fragments from the *OLE1* promoter consisting of a nested series of 5' deletions (starting from base -934 relative to the start codon) were placed in frame with  $\beta$ -galactosidase coding sequences in the single copy CEN plasmid (p62) and transformed into a phenotypically wild-type (*OLE1*<sup>+</sup>) strain L8-25A for analysis.  $\beta$ -Galactosidase activities for the reporter genes are shown in Fig. 1.

Deletion of bases -934 to -576 caused an approximate 3-fold loss of reporter gene activity. Removing bases -576 through -488 caused a further 20-fold drop in reporter gene activity, indicating the presence of an activating sequence in the region near base -576. Deletions producing 5' ends between -396 and -255 exhibited small (2-fold) increases in expression corresponding to approximately one-thirtieth of the activity seen with the entire 934-bp promoter fragment. Deletions beyond base -255 produced reporter gene activities near the basal level exhibited by the plasmid that contains no *OLE1* promoter sequences. An 88-bp deletion in the putative activation region between bases -576 through -489 (Fig. 1) showed a 27-fold reduction in  $\beta$ -galactosidase activity, indicating that

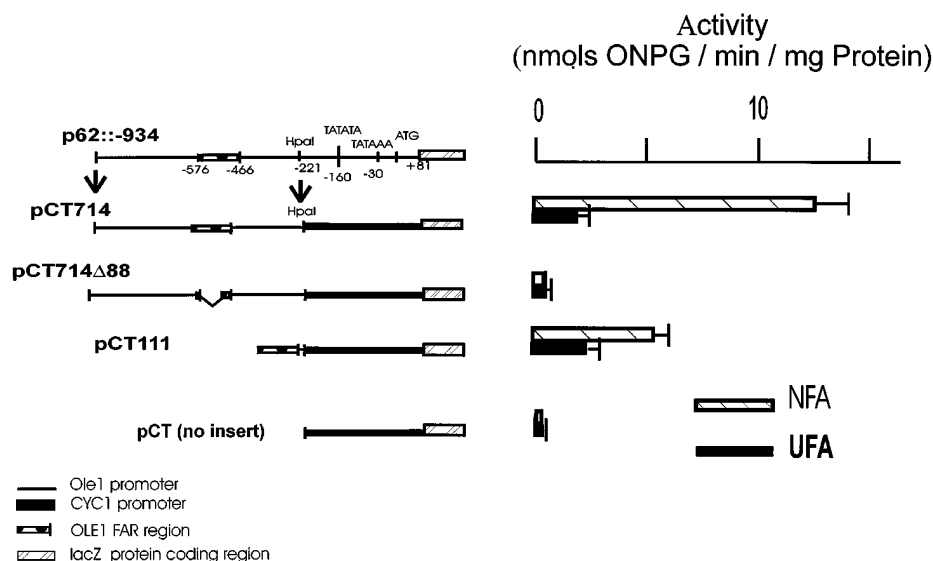


FIG. 2. Expression of reporter gene activity from heterologous pCT vectors containing *OLE1* promoter sequences. Diagrams on the left compare the *OLE1* promoter fragment in construct p62:::-934, with *OLE1* fragments inserted into heterologous constructions employing yeast *CYC1:lacZ* gene fusion plasmids. Plasmid pCT (20) contains a polylinker upstream of the *S. cerevisiae* *CYC1* promoter that lacks the *CYC1* UAS sequence. Fragments of the *OLE1* promoter were inserted into the polylinker region to test for the ability to activate transcription and confer fatty acid regulation on this test plasmid, as described under "Materials and Methods." Plasmid pCT714 contains a 714-bp fragment extending from bases -934 to a *HpaI* site at position -221, which lies upstream of the *OLE1* TATA sequences. *OLE1* promoter sequences are indicated by the black line. The left-most box placed in the *OLE1* promoter sequences indicates the position of the 111-bp FAR region shown to be essential for transcription activation and unsaturated fatty acid repression. Black bars represent *CYC1* promoter sequences; hatched boxes indicate *lacZ* coding regions. Activities of heterologous promoter test plasmids are shown to the right of the corresponding figure and are expressed in nmol of *o*-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) hydrolyzed/min/mg protein. Activities indicated are the averages of at least three independent experiments  $\pm$  S.D. for each vector. Hatched bars represent activities in cells grown without added fatty acids (NFA), shaded bars represent activities of cells grown in the presence of 0.5 mM 16:1 and 0.5 mM 18:1 (UFA).

essential transcription activation elements were located in that region.

**Fatty Acid Regulation of the Promoter Deletion Vectors—**Previous studies of a gene fusion containing the entire 934-bp promoter region fused to *lacZ*, indicated that reporter gene activity was strongly repressed by a set of mono- and polyunsaturated fatty acids (7). To identify the position of potential unsaturated fatty acid regulation regions within the promoter, parallel cultures grown in the presence or absence of a mixture of 16:1 and 18:1 were assayed for  $\beta$ -galactosidase activity (Fig. 1). These experiments were performed using standardized growth conditions that cause an 8–10-fold repression of reporter gene activity from the plasmid that contains the original 934-bp upstream *OLE1* promoter region. Higher levels of repression (up to 60-fold reductions in activity) can be produced from the same construct by continuous feeding of unsaturated fatty acids.<sup>2</sup> Reporter plasmids containing deletions from -934 to -576 showed the full range of repression normally seen under these experimental conditions. Deletions downstream from base -576, however, lost the ability to be repressed concomitantly with the loss of transcription activation.

The activity of the reporter containing the 88 bp deletion within the 934-bp fragment was reduced 2-fold in response to unsaturated fatty acids.

Tests performed on the effects of saturated fatty acids on strains containing the promoter deletion series produced a different pattern of regulation (data not shown). Supplementation of media with 0.5 mM 14:0 produced a 1.2–1.5-fold increase in activities of cells that contained plasmids p62:::-934 and p62:::-792. By contrast, reporter activity of the plasmid p62:::-576 was decreased 5-fold by the addition of 14:0. Activities of plasmids containing more extensive 5' deletions in cells incubated with 14:0 did not differ significantly from those

grown in media with no fatty acid supplements.

**A 111-bp FAR Element Contains the Primary Transcription Activation and Unsaturated Fatty Acid Regulatory Elements of *OLE1***—Because sequence elements between -576 and -489 were found to be required for transcription activation and unsaturated fatty acid regulation, a series of *OLE1* promoter fragments was tested for the ability to activate and confer unsaturated fatty acid repression on an unrelated gene. A CEN vector (designated vector pCT) that contains the basal promoter elements of the *Saccharomyces* *CYC1* gene fused to *lacZ* (20) (Fig. 2) was used for these tests. This vector lacks the *CYC1* UAS sequences and produces low levels of reporter activity when activating sequences are absent from a multiple cloning site upstream of the basal promoter region. Vector pCT contains two TATA elements of the *CYC1* gene; these have an arrangement similar to that found in elements in the *OLE1* gene.

Plasmid pCT714 contains a 714-bp *OLE1* promoter fragment that extends between bases -934 and -221 (Fig. 2). Insertion of that fragment upstream of the *CYC1* TATA region produced 66-fold higher levels of reporter gene activity, compared with the parent vector that contains no insert. Plasmid pCT714Δ88 contains the same 88-bp deletion as that in p62:::-934Δ88. That vector showed only a 2-fold increase in activity above the basal levels produced by the parent plasmid.

Reporter gene activity was also strongly activated in vector pCT111, which contains a 111-bp fragment derived from bases -576 to -466. The 5'-end of that fragment coincides with the 5'-end of the 88 bases deleted from the pCT714Δ88 vector that were found to be essential for transcription activation. Plasmid pCT111 exhibited a 28-fold increase in activity over the control plasmids that contained no *OLE1* promoter sequences.

Vectors that included either the 111-bp sequence or the intact 714-bp *OLE1* promoter sequence were repressed by the addition of unsaturated fatty acids to the growth medium (Fig.

<sup>2</sup> S. Hwang and C. Martin, unpublished observations.

-584 CCGAGCCCGG GCATGTCCCg gggtttagcGG GCCCAACAAA GGCGCTTATC

-534 TGGTGGGCTT CCGTAGAAGA AAAAAAGCTG TTGACGAGC TATTTCGGGT

-484 ATCCCAGCCT TCTCTGCAGA CGCCCCAGT

FIG. 3. Nucleotide sequences associated with the FAR element responsible for transcription activation and unsaturated fatty acid regulation. Underlined sequences represent symmetric GC-rich sequences found within the 114-bp fragment of plasmid pCTm114. Bases in lower case and boldface represent the FAR region sequence required for unsaturated fatty acid-mediated repression of *OLE1* transcription in plasmid pCTm100.

2). Vector pCT111 showed an approximate 2-fold repression of activity under conditions where the reporter that contained the entire promoter was repressed 7-fold. The vector containing the 88-bp deletion within the 714-bp promoter sequence was not repressed by unsaturated fatty acid regulation. These data suggest that primary elements essential for unsaturated fatty acid regulation are contained within the 111-bp promoter sequence. A parallel set of results was also obtained in tests of these fragments in the related pCZ plasmid (20). That vector differs from the plasmid pCTA in that it contains a shorter part of the *CYC1* basal promoter region that includes only one TATA sequence (data not shown). Given the ability of the 111-bp DNA fragment to strongly activate transcription and confer fatty acid-specific repression on heterologous vectors, it was designated as the *OLE1* FAR sequence. The nucleotide sequence of this fragment and flanking sequences is shown in Fig. 3.

**Symmetric GC-rich Sequences Associated with the FAR Region**—There are three symmetric base sequences located at the distal end of the FAR region. Two identical sequences (CCCGGG) are separated by five base pairs. Another in the reverse order (GGGCCC) is located six bases downstream. During the course of this study we also determined that the pCT vector contained another CCCGGG element within the multiple cloning site; this element was situated between the inserted promoter fragments and the *CYC1* basal promoter elements. That part of the vector (derived from the pUC19 vector multiple cloning site) has been shown to be a fortuitous binding site for the general transcription factor *REB1* (12). Because the vector sequence might provide a target for an interfering transcription factor or compete with *OLE1* regulators, it was removed by restriction digest of flanking sequences. The modified vectors expressed 3–4-fold greater activity than the original vectors that contain the additional CCCGGG site (Table III). Given those results, all further tests of the FAR region were carried out using the modified pCT vector (designated as pCTm) in which that site and flanking sequences were deleted.

**Fatty Acid Regulation of Heterologous Vectors Containing Sequences Derived from the FAR Elements**—Activities of pCTm vectors containing fragments associated with the FAR region were tested to determine the minimum size required for transcription activation and unsaturated fatty acid regulation (Fig. 4). In this series of experiments, linoleic acid (18:2) was used to repress transcription. We have previously shown that it is an efficient repressor of *OLE1* expression and has the advantage that its incorporation in yeast can be accurately monitored by gas chromatography (7).

Because the previously identified 111-bp FAR region fragment contains only the GGG bases of the upstream CCCGGG sequence, vector pCTm114 was constructed to include both members of the symmetric pair. Compared with pCTm111, the activity of pCTm114 was slightly reduced, but there were no significant changes in the level of unsaturated fatty acid re-

TABLE III  
Comparison of *OLE1* promoter activities in pCT and modified pCT vectors

Plasmids were transformed into strain L8–25A and tested for  $\beta$ -galactosidase activity after growth in UDt medium with no fatty acids (NFA) or in the presence of 1 mM 18:2 (UFA). Unsaturated fatty acid-mediated repression is expressed as the ratio of activities from the plasmid in cells grown under derepressed (NFA medium) and repressed (UFA; unsaturated fatty acid supplemented) conditions. NFA/UFA ratios  $\leq 1$  indicate a loss of the regulatory response.  $\beta$ -Galactosidase activity was measured relative to soluble protein by the method of Buchman *et al.* (12) as described under "Materials and Methods." Data represent average of three or more independent experiments  $\pm$  S.D.

Vector	Supplement	Activity	NFA/UFA
<i>nmol/min/mg protein <math>\pm</math> S.D.</i>			
pCT714	NFA	13.3 $\pm$ 3.0	8.3
	UFA	1.6 $\pm$ 0.6	
pCTm714	NFA	36.9 $\pm$ 3.0	7.7
	UFA	4.8 $\pm$ 0.7	
pCT111	NFA	5.6 $\pm$ 1.2	2.3
	UFA	2.4 $\pm$ 0.6	
pCTm111	NFA	20.2 $\pm$ 3.2	1.9
	UFA	10.5 $\pm$ 2.0	
pCTA	NFA	0.2 $\pm$ 0	0.25
	UFA	0.8 $\pm$ 0.3	
pCTm	NFA	0.6 $\pm$ 0.1	0.35
	UFA	1.7 $\pm$ 0.0	

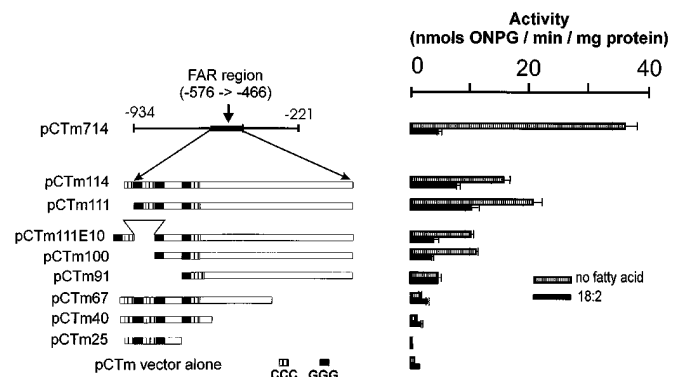


FIG. 4.  $\beta$ -galactosidase activities of L8–25A cells transformed with pCTm vectors that contain derivatives of the 111-bp FAR region in the *OLE1* promoter. The top left diagram illustrates the –934 to –221 *OLE1* promoter fragment placed in vector pCTm714 and the relative position of the FAR element with respect to the start codon. The arrows indicate the relative size and position of the 111-base fragment in an exploded view. The hatched and black boxes on the left diagrams refer to the positions of CCC and GGG bases within those fragments. Activities of reporter plasmids indicated on the right are expressed in nmol of *o*-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) hydrolyzed/min/mg of protein. Hatched bars refer to activities in cells grown under derepressed (no fatty acid conditions). Solid bars refer to activities in cells grown in presence of 1 mM 18:2. Cells were grown in 100 ml of UDt medium to a density of  $1.5 \times 10^7$ /ml with or without 1 mM 18:2 for 10 h.  $\beta$ -Galactosidase activity was measured using the cell disruption method of Buchman *et al.* (12) as described under "Materials and Methods." Three independent transformants were tested for each vector. Error bars in the figure represent standard deviations determined from a minimum of five independent experiments for each vector.

pression. Two additional vectors were constructed to test the effects of disrupting both CCCGGG sequences. A 10-bp linker was inserted into the remaining CCCGGG sequence of pCTm111 to produce pCTm111E10. That vector exhibited an approximate 50% decrease in reporter activity (compared with pCTm111) with no significant changes in the level of unsaturated fatty acid repression. Vector pCTm100 was constructed to contain only the GGG of the downstream CCCGGG site. It showed activities and level of fatty acid repression nearly identical to vector pCTm111E10. Deletion of an additional nine base pairs (pCTm91), however, resulted in a 50% reduction of derepressed activity and complete loss of fatty acid repression.

While that observation indicates that the 9-bp fragment contains sequences essential for fatty acid-mediated repression, deletion of a 50-bp fragment from the distal end of the FAR region (pCTm67) indicates that those sequences are also essential for activation and repression. Shorter test fragments containing 40- and 25-bp sequences encompassing the distal part of the FAR region showed a complete loss of activation and repression. They exhibit levels of activity similar to that of the parent vector with no inserted promoter sequences. Vectors containing fragments shorter than the 100-bp fragment in pCTm100 also exhibit slightly increased levels of activation in the presence of unsaturated fatty acids, similar to that observed with the vector alone. Taken together, these data indicate that both the 5'- and 3'-ends of the pCTm111 FAR region are required for maximal activation and unsaturated fatty acid-mediated repression.

To determine their response to saturated fatty acids, pCT plasmid-bearing strains were tested by supplementing cells with 14:0 (data not shown). Similar results were obtained to that seen with the 5' deletion series. Plasmid pCTm714 containing the 714-base promoter region exhibited a 1.6-fold increase in activity in cells grown in the presence of 0.5 mM 14:0. Vectors containing elements of the FAR region, including pCTm114, pCTm111, pCTm91, and pCTm67, did not show any significant increase in activity in cells incubated with the saturated fatty acid.

**Integration of pCTm111 and pCTm714 into a Chromosomal Locus Results in Increased Levels of Derepressed Activity and Unsaturated Fatty Acid-mediated Repression**—To determine if the gene activation and unsaturated fatty acid repression ef-

fects seen with the reporter genes were plasmid-specific, plasmids pCTm, pCTm111, and pCTm714 were converted to integrating vectors by cutting with *Hind*III to remove the ARS and CEN sequences. These were integrated into the *URA3* locus of strain L8-25A. Activity of the integrating vector (pITm) was increased approximately 5-fold over that of the autonomous pCTm plasmid in cells grown in unsupplemented medium (Table IV). Its activity was increased approximately 2-fold in cells grown in the presence of unsaturated fatty acids. Activity of the integrated pITm111 and pITm714 reporters was increased approximately 3-fold in unsupplemented medium, compared with their plasmid counterparts. The pITm111 reporter activity was repressed approximately 3-fold and pITm714 more than 10-fold by exposure of cells to 18:2. All three integrated reporters exhibited parallel behavior to that of their plasmid counterparts with respect to relative levels of activity and response to unsaturated fatty acid supplements.

**Mutants Defective in Fatty Acid Activation Fail to Regulate *OLE1* Transcription**—Exogenous fatty acids must be converted to acyl-CoA species to be incorporated into membrane and storage lipids. *S. cerevisiae* contains at least five genes that activate fatty acids to form acyl-CoAs. Four unlinked acyl-CoA synthetase genes designated *FAA1-4* have been recently identified and cloned (27). To test the effects of fatty acid activation on the expression of *OLE1*, combinations of null mutations in these four loci were analyzed using *OLE1* promoter::lacZ plasmids (Table V). Tests of pCTm714 reporter activity showed that strains containing either the *faa1Δ* or *faa4Δ* disrupted genes significantly reduced the level of unsaturated fatty acid repression, compared with the wild-type parent. In the wild type, 18:2 repressed levels of *OLE1* average 12% of that in cells not exposed to the fatty acid. By comparison, 18:2 repressed reporter activities in single mutants *faa1* and *faa4* were 40–42% that of unsupplemented cells. Repression was completely blocked in strain YB525, which is a double *faa1Δ,faa4Δ* mutant. Mutants *faa2Δ* and *faa3Δ* showed levels of 18:2 repression that were close to those observed in the wild-type parent. Repressed levels of reporter activity in *faa2Δ* and *faa3Δ* strains were approximately 17% of that of their corresponding derepressed levels. Reporter activity in strain YB526, which contains disruptions in all four FAA genes, also failed to be repressed, showing no difference in levels between 18:2 fed and nonfed cells. To test whether the effects of the *faa1Δ* and *faa4Δ* mutants act through the FAR region, plasmid pCTm111 was tested in strains containing the double *faa1Δ,faa4Δ* disruptions and the quadruple *faa1,2,3,4Δ* disruptions. Reporter activities failed to be repressed in both disruptant strains under conditions where activity in the wild-type parent was repressed 1.9-fold.

The presence of doubly disrupted *faa1Δ* and *faa4Δ* genes also

TABLE IV

Activities of chromosome integrated *OLE1* reporter derivatives

$\beta$ -Galactosidase activities in strain L8-25A which contains *OLE1* promoter-lacZ DNA sequences integrated into the *URA3* chromosomal locus. Assay and culture conditions are as described in Table III. NFA, fatty acid deficient medium; UFA, medium supplemented with 1 mM 18:2. Unsaturated fatty acid mediated repression is expressed as the ratio of activities from the plasmid in cells grown under derepressed (NFA medium) and repressed (UFA; unsaturated fatty acid supplemented) conditions. NFA/UFA ratios  $\leq 1$  indicate a loss of the regulatory response. Data represent average of three or more independent experiments  $\pm$  S.D.

Integrated sequence	Supplement	Activity	NFA/UFA
		<i>nmol / mg protein / min <math>\pm</math> S.D.</i>	
pITm714	NFA	92.0 $\pm$ 0.5	18.4
	UFA	5.0 $\pm$ 2.8	
pITm111	NFA	63.8 $\pm$ 11.1	3.2
	UFA	20.0 $\pm$ 5.9	
pITm	NFA	3.2 $\pm$ 0.3	0.6
	UFA	5.2 $\pm$ 1.6	

TABLE V

Activation and regulation of *OLE1* reporter activities in wild-type and FAA gene disrupted strains.

Activities from vectors containing either a 714-bp promoter fragment or the 111-bp FAR region are compared from cells grown in medium containing no fatty acids (NFA). Unsaturated fatty acid mediated repression is expressed as the ratio of activities from the plasmid in cells grown under derepressed (NFA medium) and repressed (UFA; unsaturated fatty acid supplemented) conditions. NFA/UFA ratios  $\leq 1$  indicate a loss of the regulatory response.  $\beta$ -Galactosidase assays and growth conditions were performed as described in Table III. Data represent average of three or more independent experiments  $\pm$  S.D.

Strain	Relevant genotype	Plasmid pCTm714 (714-bp promoter region)		Plasmid pCTm111 (111-bp FAR region)	
		NFA activity	NFA/UFA	NFA activity	NFA/UFA
		<i>nmol/min/mg protein <math>\pm</math> S.D.</i>	$\pm$ S.D.	<i>nmol/min/mg protein <math>\pm</math> S.D.</i>	$\pm$ S.D.
YB322	Wild type	29.4 $\pm$ 6.74	8.6 $\pm$ 0.53	20.7 $\pm$ 1.4	1.9 $\pm$ 0.4
YB513	<i>faa1Δ</i>	20.3 $\pm$ 0.25	2.6 $\pm$ 0.81		
YB489	<i>faa2Δ</i>	17.9 $\pm$ 0.75	5.7 $\pm$ 0.28		
YB492	<i>faa3Δ</i>	26.0 $\pm$ 2.3	5.9 $\pm$ 0.004		
YB524	<i>faa4Δ</i>	40.0 $\pm$ 6.3	2.5 $\pm$ 0.8		
YB525	<i>faa1,4Δ</i>	63.0 $\pm$ 11.6	1.1 $\pm$ 0.3	76.3 $\pm$ 6.4	0.9 $\pm$ 0.02
YB526	<i>faa1,2,3,4Δ</i>	57.3 $\pm$ 8.9	1.0 $\pm$ 0.03	96.1 $\pm$ 10.6	0.7 $\pm$ 0.05

TABLE VI  
Expression of reporter genes in W303 wild-type and *acbpΔ::LEU2* disrupted strains

Cells were grown and assayed for  $\beta$ -galactosidase activity according to the methods described in Table III. Data represent average of three or more independent experiments  $\pm$  S.D.

Strain	Plasmid	Activity	Disruptant/wild type activity
		nmol/min/mg protein	
DTY-10A (wild-type)	p62::–934	206 $\pm$ 1.5	
JY001 ( <i>acbpΔ::LEU2</i> )	p62::–934	740 $\pm$ 54.4	3.6
DTY-10A (wild-type)	pCTm 714	74.0 $\pm$ 5.8	
JY001 ( <i>acbpΔ::LEU2</i> )	pCTm 714	374 $\pm$ 23.2	5.0
DTY-10A (wild-type)	pCTm111	49.6 $\pm$ 3.7	
JY001 ( <i>acbpΔ::LEU2</i> )	pCTm111	222 $\pm$ 38.4	4.5

appears to have a strong effect on the activation levels of the reporter genes. Derepressed activities of both pCTm714 and pCTm111 in the double disruptant were increased 2–3-fold over the wild-type parent.

**Acyl-CoA Binding Protein Affects the Level of *OLE1* Transcription and Cellular Fatty Acyl Composition**—The connection between acyl-CoA synthase activity and unsaturated fatty acid repression suggested that the ACBP could be a component of the molecular sensor that acts in the fatty acid-mediated regulation of *OLE1*. Almost all of the cell's fatty acyl-CoA species are bound to this ubiquitous 10-kDa protein that binds medium and long chain acyl-CoA species with high specificity and high affinity. Furthermore, ACBP does not bind CoA, nonesterified fatty acids, or acyl carnitines (26).

To assess the effects of this protein on *OLE1* regulation, the gene was cloned by PCR and disrupted by replacing sequences that flank the coding region of the protein with the *Saccharomyces LEU2* gene. Surprisingly, no visible phenotype other than a slight retardation in growth was observed in the disruptant strains. The effect of the disruption, however, was to produce an approximate 3–4-fold increase in transcription activity of reporter plasmids that contain the entire *OLE1* upstream region (p62::–934 and pCTm 714) and an approximate 5-fold increase in transcription activity with the pCTm111 vector containing the 111-bp FAR region (Table VI).

The effect of the ACBP gene disruption on transcription had a concomitant effect on *OLE1* mRNA levels (Fig. 5). Quantitative phosphor image analysis, using the *Saccharomyces PGK1* gene as an internal standard, revealed that *OLE1* mRNA levels in the disruptant strain were 5.5-fold higher than in its wild-type parent grown in fatty acid-deficient medium. Surprisingly, disruption of the ACBP gene had no effect on relative levels of unsaturated fatty acid repressed *OLE1* transcription (Fig. 5). ACBP disruption also appears to have profound effects on cellular fatty acid composition. The increased levels of *OLE1* transcript seemed to result in a net increase in desaturase activity relative to saturated fatty acid biosynthesis. Analysis of total cellular fatty acids revealed a striking increase in 14–16 carbon species and an increase in the ratio of unsaturated to saturated fatty acids. Due to increased desaturation of 14:0 and 16:0, 14:1 levels increased to 5% wild type from less than 1% and 16:1 levels increased from 41.7–59.2% in the *acbpΔ::LEU2* disruptant. Levels of the 18 carbon species were reduced in the disruptant, primarily as a function of the reduction in 18:1–21% (down from 29%) of the total fatty acid mass (Table VII).

**Participation of Hap1p in FAR Region Activated Transcription**—Endoplasmic reticulum desaturases are thought to require heme-dependent electron transfer as part of their reaction mechanism. We have recently found that Ole1p has a cytochrome *b<sub>5</sub>*-like heme binding domain at its carboxyl termi-

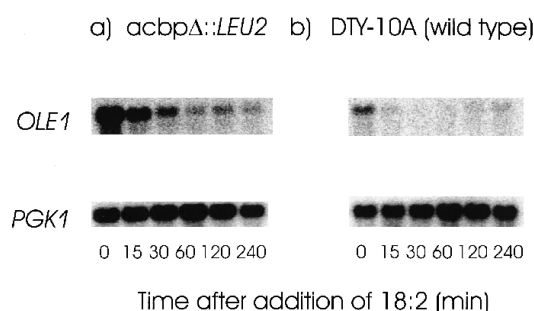


FIG. 5. Northern blot analysis of *OLE1* expression in DTY10A (wild type) and JY001 (*acbpΔ::LEU2*) under derepressed and 18:2 repressed conditions. Repression of *OLE1* mRNA following addition of unsaturated fatty acids to 1) strain JY001, which contains a disrupted ACBP gene, and 2) wild type. Cells were grown to a density of  $2 \times 10^7$ /ml at 30 °C in UDt medium, as described under "Materials and Methods." At time 0, 1 mM 18:2 was added to the growth medium. Total RNA was extracted by the hot phenol extraction procedure as described at intervals indicated in the figure. RNA blots were probed with an *OLE1* specific probe. After phosphor image analysis, blots were stripped and reprobed with the *Saccharomyces PGK1* gene as an internal standard. Phosphor images of the resulting blots are shown in the figure.

nus.<sup>3</sup> Given that the Hap1p and Hap2/3/4p transcription factors are components of regulatory systems that govern synthesis of heme proteins in *Saccharomyces*, we used the *OLE1* reporter genes to test whether these transcription factors were essential for activation and unsaturated fatty acid mediated regulation of *OLE1* (25). Plasmids p62::–934 and pCTm111 tested in the *hap1Δ::LEU2* gene disrupted strain and its wild-type parent (Table VIII).  $\beta$ -Galactosidase activities from the 934- and 111-bp promoter sequences were reduced by 40 and 30%, respectively, in the *hap1* disrupted strain, compared with wild type. By contrast, a *hap2Δ::LEU2* disruptant showed no reduction in activity over the congenic wild-type strain.

The absence of the Hap1p transcription factor appeared to have no significant effect on fatty acid regulation of the reporter gene. Under the standard assay conditions, activity in the *hap1Δ::LEU2* disruptant strain grown in the presence of unsaturated fatty acids was reduced to approximately 20% of that found in cells grown in fatty acid-deficient medium (data not shown). Given the effects of increased *OLE1* transcription on fatty acyl composition seen in the *acbpΔ::LEU2* disruption strain, it was thought that complementary changes might be produced by the reduced level of *OLE1* transcription in the *hap1Δ::LEU2* disrupted strain. Analysis of the disruptant and its parent strain revealed no significant differences in either the fatty acyl composition or the total cellular fatty acid content of the two strains (data not shown).

## DISCUSSION

*Saccharomyces*, like other eukaryotes, maintains a balanced ratio of unsaturated and saturated fatty acids in its membrane lipids under a wide range of physiological conditions. This requires the coordinated regulation of fatty acid synthesis, which produces saturated fatty acids, and fatty acid desaturation, which converts most of the saturated acids to unsaturated species. The maintenance of fatty acyl composition appears to be important in controlling the properties of both cellular membranes and storage lipids. The  $\Delta$ -9 fatty acid desaturase is a critical component of this system and is a highly regulated activity that responds to both nutrient and physiological controls (7). A major question concerns how cells monitor the availability of fatty acid precursors and the acyl composition of glycerolipids to regulate the activities of the desaturase and

<sup>3</sup> Mitchell and Martin, submitted for publication.



TABLE VII  
Total lipid fatty acid compositions of DTY10A (wild-type) and JY001 (*acbpΔ::LEU2*)

Cells were grown in 40 ml of SDT medium at 30 °C to  $2 \times 10^7$  cells/ml and lipids were extracted according to procedures described under "Materials and Methods." Data represent average wild-type percent  $\pm$  S.D. of each fatty acid species from three independent experiments.

Strain	14:0	14:1	16:0	16:1	18:0	18:1	%sfa	%ufa
DTY-10A	3.6 $\pm$ 0.4	0.9 $\pm$ 0.1	17.0 $\pm$ 3.7	45.8 $\pm$ 4.3	3.6 $\pm$ 1.0	29.2 $\pm$ 0.6	24.1 $\pm$ 4.2	75.9 $\pm$ 4.2
JY001	3.4 $\pm$ 0.4	5.6 $\pm$ 0.2	7.5 $\pm$ 0.4	60.0 $\pm$ 2.0	2.4 $\pm$ 0.3	21.2 $\pm$ 1.6	13.3 $\pm$ 0.2	86.7 $\pm$ 0.4

TABLE VIII

Comparison of reporter gene activities in isogenic HAP1, *hap1::LEU2*, and *hap2::LEU2* strains

Reporter gene activities from plasmids p62::–934 and pCT111 in the *hap1Δ::LEU2* (LPY15) and *hap2Δ::LEU2* (JO1–1a) mutant strains compared with that of the wild-type parent (BWG1–7A). Cells were grown and assayed for  $\beta$ -galactosidase activity according to the methods described in Figure 1. All assays were done in triplicate. Values are given as an average of six independent experiments  $\pm$  S.D. (p62::–934) or an average from two independent experiments  $\pm$  S.D. (pCT111).

Strain/plasmid/construct	$\beta$ -Galactosidase activity	HAP1 activity
	miller units	%
HAP1/p62	16.7 $\pm$ 2.5	100
<i>hap1</i> /p62	6.6 $\pm$ 2.9	39.8
<i>hap2</i> /p62	16.3 $\pm$ 3.2	97.7
HAP1/pCT111	4.6 $\pm$ 0.15	100
<i>hap1</i> /pCT111	1.4 $\pm$ 0.25	30.4

other lipogenic enzymes. Recent evidence from this laboratory suggests that the regulation of unsaturated fatty acid formation involves a diverse array of controls.

This analysis of the *OLE1* promoter indicates that the formation of unsaturated fatty acids is strongly regulated at the level of transcription by nutrient fatty acids. This appears to be a major component of the previously observed elevation of *OLE1* enzyme activity by saturated fatty acids and its repression in response to unsaturated acids (1). Essential transcription activation and unsaturated FAR elements appear to be located in a short 111-bp region located approximately 500 bp upstream of the *OLE1* transcribed region. That fragment is sufficient to activate and confer unsaturated fatty acid repression on an unrelated gene that contains only the basal promoter elements and no upstream transcription activating sequences. Saturated fatty acids, however, do not activate vectors containing only the FAR region elements, suggesting that sequences that respond to those stimuli lie in another region of the promoter. Taken together these observations suggest that there are at least two independent systems that regulate *OLE1* transcription in response to fatty acids.

The identification of transcription factors that act through the FAR region is critical to our understanding of the mechanisms of fatty acid-mediated repression. The data presented here indicate that a specific 9-bp sequence at the distal end of the 100-bp FAR fragment is essential for fatty acid-mediated repression and plays some role in activation (Figs. 3 and 4). A region at the opposite end of that fragment is essential for activation. If the latter sequences are targets for transcription-activating DNA binding proteins, then they must also play a role in FAR region-mediated unsaturated fatty acid repression.

The data presented here are consistent with either of two models for control of *OLE1* transcription (21). One is that repression is mediated by an unsaturated fatty acid-responsive DNA binding protein. When activated, it competes with transcription factors for binding to FAR element sequences. A second model is that repression is triggered by an unsaturated fatty acid-activated auxiliary transcription factor that interacts with DNA binding proteins occupying the FAR region.

Associated with the essential regulatory sequence at the 5' boundary of the FAR region is a series of symmetric GC-rich elements. These include a pair of CCCGGG sequences followed

by an inverse GGGGCC sequence within a 30-bp region. A homologous pair of CG-rich sequences was found in the transcription-activating region of the promoter of the sterol biosynthetic gene, *ERG11* (22). That gene encodes a microsomal cytochrome P-450 enzyme responsible for the demethylation of lanosterol. The significance of the CG-rich sequences is further reinforced by the occurrence of homologous paired sequences in the upstream noncoding region of the *ERG3* gene, which encodes the sterol C-5 desaturase (23, 24). All three genes encode intrinsic enzymes that act at the endoplasmic reticulum surface. The existence of homologues to the *OLE1* sequences in another lipid biosynthetic pathway suggests they may be involved with a type of transcriptional control for lipogenesis that has yet to be identified.

The effect of the combined *faa1*, *faa4* disruption in blocking repression of *OLE1* transcription is intriguing and suggests that repression is related to the availability of acyl-CoA species formed from the exogenous fatty acids. The two genes that affect the repression of *OLE1*, (*FAA1* and *FAA4*), account for approximately 99% of the cellular 14:0 CoA and 16:0 CoA synthetase activities in wild-type cells grown in glucose medium (29). Furthermore, both genes are responsible for almost all of the activation of imported 14:0 and 16:0. It is not clear, however, whether they are also the primary activators for unsaturated species such as 16:1, 18:1, or 18:2. Disruption of *FAA1* and *FAA4* results in a striking reduction in the incorporation of these fatty acids into glycerolipids and a sharp increase in cellular fatty acids (27). One possibility is that the *OLE1* regulatory circuit responds to the size or the composition of the intracellular acyl-CoA pool generated by the two synthetases. Alternatively, *Faa1p* and *Faa4p* may be involved in the intracellular transport of exogenous fatty acids or in their partitioning to cellular locations that are accessible to the regulatory sensor.

The acyl-CoA binding protein appears to play a significant role in *OLE1* expression. Disruption of this abundant and highly specific binding protein increases *OLE1* transcription greater than 5-fold, which accurately correlates with a 5-fold increase in *OLE1* mRNA levels. Although disruption of the ACBP gene does not produce a significant phenotype with respect to growth, it appears to cause an increase in the levels of unsaturated fatty acids relative to saturated species in total cellular lipids. One interpretation of this response might be that the regulatory sensor that detects available substrate for the desaturase responds to saturated ACBP-bound acyl-CoAs. The absence of ACBP-bound substrate may elicit a cellular response ordinarily used to monitor levels of available saturated substrates for the enzyme. In the absence of this signal, *OLE1* expression is increased to compensate for the perceived reduction in substrate. If the actual acyl-CoA levels are not rate-limiting, this increase in *OLE1* expression could have the concomitant result of increasing levels of cellular unsaturated fatty acids.

Hap1p (28) appears to be one of several transcription activators that recognize FAR region elements. Analysis of reporter gene activity in Hap1p-deficient cells indicates that it is responsible for more than half of the *OLE1* transcription activity in wild-type cells grown under fermentative conditions. Disrup-



tion of *HAP1*, however, does not appear to affect either the balance of cellular saturated and unsaturated species or the relative levels of fatty acids found in those cells. This indicates that, in the absence of HAP1p, other transcription factors activate the *OLE1* FAR element to produce sufficient mRNA to maintain normal membrane fatty acyl lipid composition. We are currently attempting to identify these unknown activation and repressor elements by the isolation of regulation defective mutants.

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