Specificity of Unsaturated Fatty Acid-regulated Expression of the Saccharomyces cerevisiae OLE1 Gene*

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The Saccharomyces cerevisiae OLE1 gene encodes the Δ-9 fatty acid desaturase, an enzyme which forms the monounsaturated palmitoleic (16:1) and oleic (18:1) fatty acids from palmitoyl (16:0) or stearoyl (18:0) CoA. Previous studies demonstrated that OLE1 mRNA levels and desaturase enzyme activity are repressed when either 16:1 Δ-9 and 18:1 Δ-9 are added to the growth medium (1). The polyunsaturate, linoleic acid (18:2, Δ-9,12), which is not a product of the enzyme, is also a strong repressor. The specificity of the OLE1 transcriptional regulatory sensor was examined by testing the response of OLE1 promoter-lacZ fusion reporter genes to fatty acids that differ in chain length, degree of unsaturation and double bond positions. Mono-unsaturated and polyunsaturated fatty acids that contain a Δ-9 double bond are strong repressors of reporter gene activity and native OLE1 mRNA levels. Mono-unsaturated fatty acids containing double bonds in the Δ-10, Δ-11, or Δ-5 positions showed no repression of reporter enzyme levels although they were rapidly incorporated into membrane lipids and some supported growth of an OLE1 gene disrupted strain. Although 17:1 Δ-10 does not repress OLE1 transcription, lipid analysis showed that it replaces almost all of the endogenous 16:1 Δ-9 and 18:1 Δ-9 in cellular lipids and OLE1 mRNA levels are strongly repressed. This suggests that additional systems regulate desaturase activity by post-transcriptional mechanisms that differ from the transcriptional sensor in their responses to specific fatty acids.

In Saccharomyces cerevisiae unsaturated fatty acids are formed by the Δ-9 fatty acid desaturase, which introduces a double bond between carbons 9 and 10 of palmitoyl (16:0)-1 or stearoyl (18:0)-CoA to form palmitoleic (16:1) or oleic (18:1)

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1 The abbreviations used are: xy Δ-n, fatty acyl groups containing x carbon atoms and y cis double bonds located at position n relative to the carboxyl or carbonyl end of the hydrocarbon chain; bp, base pair(s).
 MATERIALS AND METHODS

S. cerevisiae strains, growth media, and transformations—Haploid strains LL-25A (MATa, OLE1, uro3-352, his3-11, ku70, leu2-112, ura3-1, trpl-901, urd-52, his4) and L8-25A (MATa, OLEI, uro3-352, his3-11, ku70, leu2-112, ura3-1, trpl-901, urd-52, his4) were used in this study. Yeast containing the lacZ fusion plasmids p40 or pCT:OLE were grown at 30°C on uracil dropout SD medium (5) supplemented with 1% tergitol (SDT) and 1 mM of the appropriate fatty acid (obtained from Sigma or Nu-Chek Prep). All fatty acids used in this study contained double bonds in the cis configuration. Recombinant DNA manipulations using E. coli were described by Maniatis (6) and Ausubel et al. (5). Nucleic acid blots—RNA blots using total cellular yeast RNA were carried out as described previously (1). Either the L32 ribosomal subunit mRNA (7) or the yeast actin gene was used as an internal control. RNA probes were made either by the random primer method or by chemiluminescent modifications (BRL) under conditions recommended by the manufacturer.

β-Galactosidase assays—β-Galactosidase assays of cells containing plasmids with intact OLE1 promoter sequences were performed essentially as described (5). Cell densities for those assays were determined either by measurement of A600 or by hemocytometer counts. Extracts of cells from plasmids containing hybrid OLE1 upstream promoter elements and CYC1 TATA and downstream sequences were done using the procedure of Lue and Kornberg (8). Those assays were correlated with the total protein in cell extracts determined by the method of Bradford (9) using the Bio-Rad assay kit.

Repression/Derepression Studies—Cells containing an OLE1-lacZ reporter gene were used to test unsaturated fatty acid regulation in two ways. First, fatty acids were tested for their ability to repress reporter gene activity in cells in an initially derepressed state. Cells were grown overnight in a medium without unsaturated fatty acids, then washed with ice-cold distilled water. Aliquots were resuspended in 25 ml of fresh minimal medium or in a medium containing a specific unsaturated fatty acid species. The final cell density was less than 1 × 10^9/ml. After 8-12 h of growth, cells were harvested, washed in ice-cold distilled water 3 times, and then tested for β-galactosidase activity. Analogous experiments were performed on cultures grown in a medium containing no unsaturated fatty acids and those grown in repressing medium containing either 1 mM 18:1 or 18:2. Secondly, fatty acids were tested for their ability to maintain the fully repressed condition. Cells were grown in the presence of 1 mM 18:1 A-9 or 18:2 A-9,12 overnight. Low cell densities were maintained so that the cell growth rate was not saturated with unsaturated fatty acids. Cells were washed as described above, and aliquots were resuspended in minimal growth medium or media containing unsaturated fatty acids at 1 mM concentrations. After 4-6 h of growth, cells were tested for β-galactosidase activity and the results were compared with the previously described controls.

Growth and transcriptional analysis—Total cellular fatty acids were obtained by HCl-methanalysis of extensively washed cell pellets according to the procedure of Browse and Somerville (10) and direct extraction of methyl esters in hexane ether (1:1) or by saponification of washed cell pellets (11), followed by petroleum ether extraction, acidification, and re-extraction of fatty acids. Previous experiments involving addition of fatty acid standards to the cell cultures demonstrated that the washing procedure removes >98% of fatty acids not incorporated by the cells (2). Total cellular lipids were extracted from cells or from broken cell extracts by the method of Bligh and Dyer (12) as previously described (2). Phospholipids were fractionated by silicic acid chromatography as previously described (13). Transmethylation of phospholipid fatty acids was done by the method of Morrison and Smith (14). Gas liquid chromatography was performed by on-column injection of a Supelcowax 10 capillary column (0.75 mm, inner diameter, × 30 m) using a Varian 3700 gas chromatograph at 190°C using helium as a carrier gas.

Growth tests—Strain LL-18:1-14C containing the disrupted ole1::LEU2 gene was grown overnight at 30°C with rotary shaking on medium containing 1 mM 18:1 A-9. Cells were harvested by centrifugation, washed 3 times in ice-cold distilled water, and inoculated into 20 ml of SDT medium containing the appropriate 1 mM fatty acid supplement at a density of 5 × 10^6 cells/ml. Cell density was monitored by hemocytometer counts and 27 h later aliquots of the culture were pelleted in a microcentrifuge and washed 2 times with distilled water to disperse the cells before counting.

RESULTS

Two OLE1 promoter-β-galactosidase gene fusions were constructed to test the regulation of OLE1 (Fig. 1). Recombinant plasmid p40 contains a 935-bp HindIII/Sall fragment of the OLE1 promoter and 27 N-terminal codons of the protein coding sequence fused in frame to the E. coli lacZ gene in expression vector YEplp56R. At multiple copy plasmid yields high levels of reporter gene expression and was used for initial experiments to screen fatty acids. Plasmid pCT:OLE contains a HindIII/Hpa fragment of the OLE1 promoter that includes the gene activation and unsaturated fatty acid regulation sequences but does not contain the OLE1 "TATA" sequences or the transcription initiation site. That fragment was fused to the single copy CEN plasmid pCT (8) which contains the yeast CYCl TATA elements fused to β-galactosidase.

Experiments were performed on cells that were initially in one of two regulatory states. Test fatty acids were added either to cultures in which the reporter gene was derepressed (by growing cells initially without unsaturated fatty acids) or to cultures in which the reporter gene was repressed (cells grown initially in the presence of unsaturated fatty acids). Experiments on initially derepressed cells revealed that relatively long exposure times to fatty acids were required before reporter enzyme activity fell to levels representing the fully repressed state. The second method monitored the relatively rapid synthesis of the reporter enzyme during recovery from the repressed state and yielded larger ratios of derepressed over repressed activities. Cell densities must be maintained at low levels in those experiments, however, to avoid depleting the medium of unsaturated fatty acids while initially repressing the gene.

Regulation of lacZ Fusion Plasmids by Δ-9 Unsaturated Fatty Acids—Table I shows the effects of a series of mono-unsaturated and polynsaturated fatty acids on reporter gene activity in wild type strain, LL-25A, containing lacZ fusion plasmid p40 (Fig. 1b). In those experiments cells were grown in selective medium containing oleic acid (18:1 Δ-9) to repress reporter gene activity, washed extensively, and transferred to fresh medium to test for their ability to maintain repression when supplied with different fatty acids.

Comparison of relative enzyme levels revealed that β-galactosidase activity remained repressed in all cultures containing fatty acids with a Δ-9 cis double bond and a hydrocarbon.
Strain L8-25A containing plasmid p40 was grown overnight at low density in medium containing 1 mM 18:1; cells were washed and inoculated at 1/10 volume into medium containing 1 mM of the fatty acids and allowed to grow for an additional 8 h. Cells were again washed and assayed for β-galactosidase activity. β-Galactosidase units are expressed as units/Asoo (corrected for light scattering).

**Table I**

| Fatty acid supplement | β-Galactosidase activity | No fatty acid control |
|-----------------------|--------------------------|-----------------------|
| None                  | 38.9                     | 100                   |
| 18:1 Δ-9 (control)*   | 0.6                      | 1.5                   |
| 18:1 Δ-9              | 0.8                      | 1.2                   |
| 18:1 Δ-10             | 0.5                      | 1.2                   |
| 18:2 Δ-9              | 0.4                      | 1.1                   |
| 18:3 Δ-6,9,12         | 0.4                      | 1.1                   |
| 18:3 Δ-9,12,15        | 0.5                      | 1.2                   |
| 14:1 Δ-9              | 0.3                      | 0.8                   |

* Starting culture.
* Cells transferred to fresh medium containing 18:1.
* Determined in a separate series of experiments.

Strain L8-25A containing the p40 lacZ fusion plasmid grown under repressed conditions was tested according to the protocol described in Table I.

**Table II**

| Unsaturated fatty acid supplement | β-Galactosidase activity | No fatty acid control |
|-----------------------------------|--------------------------|-----------------------|
| 14:1 Δ-9                          | 0.068                    | 1.8                   |
| 18:1 Δ-9                          | 1.4                      | 3.8                   |
| 17:1 Δ-10                         | 28.1                     | 77                    |
| 18:1 Δ-5                          | 23.5                     | 64                    |
| 18:1 Δ-11                         | 23.6                     | 65.6                  |
| No fatty acid                     | 37.3                     | 100                   |

The length from 14–18 carbons. Control cultures containing no fatty acids showed approximately 65-fold greater reporter gene activity. The dienoic species, linoleic acid (18:2, Δ9,12) and two trienoic 18:3 species (Δ-6,9,12 and Δ-9,12,15) were equally strong repressors of the β-galactosidase activity in spite of differences in the positions of the second and third double bonds.

**Regulation of lacZ Fusion Plasmids by Unsaturated Fatty Acids without Δ-9 Double Bonds**—All of the fatty acids tested in the above experiments have a double bond at the Δ-9 position. To test whether a Δ-9 double bond is specifically required for repression, cells were grown under oleic acid and exhibited β-galactosidase activity comparable with or greater than that of the controls grown in the absence of fatty acids. Thus these data support the results obtained with the p40 plasmid and indicate that transcriptional control accounts for almost all of the regulation of its reporter gene activity.

**Correlation of Fatty Acid Regulation with Growth**—Experiments were carried out to determine if the ability of a fatty acid to act as a repressor is related to its ability to sustain growth of a strain with a disrupted OLE1 gene (Table IV). Cells were grown for a fixed period on each unsaturated fatty acid and then counted to determine their relative cell densities. The relative densities are compared with the control culture that contained the native 18:1 Δ-9 species which underwent approximately 6.7 doublings during the 27-h experiment. All 16–18 carbon fatty acid species tested that repress the reporter gene were found to support growth of the

**Table III**

| Supplement                  | β-Galactosidase | NFA control |
|-----------------------------|-----------------|-------------|
| No fatty acid               | 4.32            | 100         |
| 18:2 Δ-9,12                 | 0.465           | 10.7        |
| 17:1 Δ-10                   | 5.7             | 132         |
| 19:1 Δ-10                   | 4.8             | 111         |
| 18:1 Δ-11                   | 4.6             | 106         |

**Table IV**

| unsaturated fatty acid supplement | 18:1 Δ-9 cell density (12 h) | 18:1 Δ-9 cell density (27 h) |
|-----------------------------------|------------------------------|------------------------------|
| None                              | 2.2                          | 0.3                          |
| 18:1 Δ-9                          | 100                          | 100                          |
| 16:1 Δ-9                          | 98                           | 95                           |
| 14:1 Δ-9                          | 47                           | 67.6                         |
| 18:2 Δ-9,12                       | 121                          | 85                           |
| 18:3 Δ-6,9,12                     | 92                           | 85                           |
| 18:3 Δ-9,12,15                    | 78                           | 84                           |
| 17:1 Δ-10                         | 128                          | 62.5                         |
| 19:1 Δ-10                         | 2                            | 3.6*                         |
| 18:1 Δ-5                          | 45                           | 16.8                         |
| 18:1 Δ-11                         | 62                           | 41.4                         |
| 20:1 Δ-11                         | 3.5                          | 2.2                          |
| 20:1 Δ-13                         | 1.9                          | 0.5                          |

* No budding cells at 24 h; cultures failed to grow further when incubation was continued for 72 h.
ole1::LEU2 gene-disrupted strain at levels comparable with those observed when 18:1 Δ-9 was the supplement. 14:1 Δ-9 promoted growth leading to about half the density of the other species (approximately 6 generations). Fatty acids that had no effect or minimal effects on regulation, such as 17:1 Δ-10 and 18:1 Δ-11, also sustained growth at levels similar to that found with medium containing the 14:1 Δ-9 species. Petroselonic acid (18:1 Δ-5) promoted growth at a slightly lower rate than the Δ-9-containing species. The longer chain, non-repressing fatty acids 19:1 Δ-10, 20:1 Δ-11, and 20:1 Δ-13 did not support significant growth in tests that extended for 72–96 h.

Incorporation of Exogenous Fatty Acids into Membrane Lipids of OLE1 Cells—Since transcriptionally non-repressing fatty acids such as 17:1 Δ-10 and 18:1 Δ-11 can repair the growth requirements of the ole1 gene disrupted strain they must enter the cells and be incorporated into membrane lipids. Their inability to repress OLE1 transcription could be due to the fact that those fatty acids are selectively excluded from strains containing a functional desaturase or that they are modified to another species upon entry into the cell. To examine that possibility, OLE1 cultures were analyzed to determine if non-repressing fatty acids were incorporated into cellular total lipids and phospholipid fractions. Cultures of L8-25A (relevant genotype OLE1) were grown under derepressed conditions overnight and then transferred into minimal medium or medium containing those fatty acids for 12 h. Gas chromatograms of total lipid fatty acids from washed cells are shown in Fig. 2, and their relative levels are shown in Table V.

Cells grown on minimal medium (Fig. 2a) contained the normal distribution of fatty acids in which 16:1 and 18:1 comprise greater than 75% of the total fatty acids. Fig. 2b indicates the fatty acid distribution when 17:1 Δ-10 is added to the medium. Surprisingly, 17:1 Δ-10 is the dominant species, accounting for almost 80% of the total cellular fatty acids. Since that fatty acid does not have a significant effect on reporter gene transcription we expected the desaturase to be active at normal, derepressed levels. This would be indicated by high levels of the 16:1 and 18:1 enzyme products in the lipid fraction. Those fatty acid levels were in fact strongly depressed (compare their abundance with 16:0 and 18:0), indicating that in the presence of 17:1 Δ-10, desaturase enzyme activity is sharply reduced. In separate experiments the same distribution of fatty acids was found in the phospholipid fraction of 17:1 Δ-10 fed cells, as determined by analysis of lipid fractions separated by silicic acid chromatography. 17:1 Δ-10 comprised approximately 72% of phospholipid fatty acids in that test, while 16:1 and 18:1 represented approximately 5.5% of the total (data not shown).

Analysis of cells fed 18:1 Δ-11 (Table V) indicated that it was incorporated at moderate levels in cellular lipids. Unlike the effects seen with 17:1 Δ-10 fed cultures, however, cellular 16:1 and 18:1 levels were not severely reduced (indicating that the desaturase activity was not repressed in the presence of that fatty acid). Due to overlapping peaks of the endogenous 18:1 Δ-9 and the fed 18:1 Δ-11, it was not possible to accurately quantify their relative levels in lipids from cultures fed only the Δ-11 species. To further examine the incorporation of the 18:1 Δ-11, a second series of experiments was done in which cells were fed a mixture of 18:2 Δ-9,12 and 18:1 Δ-11 (Fig. 2c). The 18:2 should repress the production of endogenous 16:1 and 18:1 formed by the desaturase, thus revealing the presence of the incorporated Δ-11 species (Fig. 2c). An approximate 3:1 mixture of the 18:2 and 18:1 species was added to the media and analysis of the lipids revealed that the two fed species comprised approximately 70% of the total fatty acids in that ratio. The retention time of the 18:1 peak also corresponds to that of the Δ-11 species although a shoulder on the chromatogram suggests that a small amount of endogenous 18:1 may also be present. The sharp loss of 16:1 clearly indicates that the desaturase activity was strongly repressed under those growth conditions.

Although 19:1 Δ-10 did not repress transcription of the OLE1 reporter gene and was incapable of supporting growth of the disruption strain, that fatty acid was readily incorporated into cellular lipids (Fig. 2d) and comprised about 16% of the total fatty acids. Endogenous 16:1 and 18:1 were slightly reduced under those conditions, resulting in total cellular unsaturated fatty acid levels that were similar to those found in cells grown with no fatty acids in the culture medium.

**Effect of Fatty Acids on OLE1 mRNA Levels**—OLE1 mRNA levels were measured to determine the response to repressing and non-repressing fatty acids. In those experiments cells were grown under derepressed conditions to mid-logarithmic phase, shifted to fatty acid containing medium, and allowed to grow for an additional 4 h before harvesting and RNA isolation. Previous studies had shown that OLE1 mRNA is reduced to low levels within 15 min under repressing conditions (1). OLE1 mRNA levels were compared with ribosomal
subunit L32 mRNA as an internal control for loading.

Fig. 3 shows that OLE1 transcript levels in cultures grown in 18:1 A-11 were similar to those in control cultures containing no fatty acids. Cultures grown under the same conditions in the presence of 18:2 A-9,12 and 17:1 A-10, however, contained sharply reduced OLE1 mRNAs that were approximately 1/20 the levels found under derepressed conditions, indicating that both species strongly repress available OLE1 message.

**DISCUSSION**

The studies of OLE1 gene expression reported in this paper are designed to dissect the mechanisms of unsaturated fatty acid regulation in yeast. A variety of unsaturated fatty acids have been fed to yeast cells to determine and correlate their individual effects on: (a) OLE1 native mRNA levels, (b) OLE1 transcription per se (as measured by the activity of a reporter gene under the control of the OLE1 promoter, and (c) the relative activity of the Δ-9 desaturase enzyme as determined by the level of its products (16:1 and 18:1) in cellular lipids.

We have previously reported that the addition of unsaturated fatty acids to the growth medium strongly represses Δ-9 desaturase (OLE1) mRNA levels. The data presented here show that one component of that regulatory response occurs at the level of transcription since β-galactosidase gene fusions containing only OLE1 upstream promoter sequences are strongly repressed by certain unsaturated fatty acids. Although the transcriptional regulation is triggered by a range of fatty acids that vary with respect to chain length and number of double bonds, it has a highly specific requirement for a double bond in the Δ-9 position. This suggests that this regulatory mode is initiated by the binding of a fatty acid to a protein sensor rather than as a response to changes in the physical characteristics of the membrane lipid bilayer caused by the increased availability of unsaturated fatty acids. The sensor protein must apparently recognize the carboxyl (or carbonyl group) of the hydrocarbon chain and the Δ-9 double bond, since it is insensitive to chain length and additional double bonds distal to that position. It appears to tolerate some differences in the structure of the chain between carbons 1 and 9, however, since 18:3 A-6,9,12 is a highly effective repressor in spite of the presence of a double bond at the Δ-6 position.

This specificity of the transcriptional sensor was surprising in light of previous studies (4) which demonstrated that a much wider range of Δ-9 and non-Δ-9 double bond-containing unsaturated fatty acids satisfy the growth requirements of OLE1 mutant strains. Data presented here suggest that the transcriptional regulation of OLE1 is one component of a more complex system that regulates desaturase activity and controls the composition of unsaturated fatty acids in membrane lipids. This is particularly evident from experiments that show that 17:1 A-10, which does not regulate transcription, strongly represses OLE1 mRNA levels and desaturase activity. The absence of OLE1 message in those cells in the presence of continued transcriptional activity points to the existence of a post-transcriptional mechanism that may control mRNA stability. The identification of 17:1 A-10 as a specific stimulus for post-transcriptional regulation of the OLE1 gene will be useful in defining the characteristics of that system.

Additional controls may also exist that affect the activity or the stability of the desaturase enzyme. These are suggested by the pattern of fatty acid integration in wild type and OLE1 mutant cultures that were fed 18:1, Δ-11. In the wild type cells OLE1 transcription and mRNA levels are unaffected by that species, yet the integration of the non-repressing fatty acid into membrane lipids results in a reduction of endogenous 16:1 and 18:1 levels suggesting that the activity of the enzyme is modulated by the presence of the exogenously supplied acid. The ability of cells to maintain balanced ratios of saturated and unsaturated fatty acids under widely differing conditions give further evidence that there are finely tuned controls that regulate membrane fatty acid composition. It is somewhat surprising that 17:1 A-10 triggers a strong post-transcriptional repression of the gene, whereas 18:1 A-11 and 19:1 A-10 do not. This may indicate that 17:1 A-10 repression is triggered by the physical characteristics of membrane lipids containing that species rather than by the specific recognition of the fatty acid. The intermediate chain length of the 17-carbon species may mimic the normal distribution of 16:1 and 18:1 in maintaining appropriate membrane fluid properties whereas the longer chain fatty acids may have a rigidifying effect on the phospholipid bilayer, triggering the need for additional 16:1 and 18:1. The regulatory sensor that detects the properties of a “normal” membrane would then be responsible for initiating the destabilization of the OLE1 message.

The question arises as to where in the cell the sensors for these regulatory systems reside and what form of fatty acid triggers each response. Sensory elements that are associated with the endoplasmic reticulum, for example, could be expected to be part of a system that regulates the composition and fluid properties of the lipid bilayer, while elements that are soluble cytoplasmic proteins might represent a simpler form of metabolic control. Since exogenous fatty acids transported into the cell are presumably converted to CoA derivatives, it is possible that they could be the regulatory stimulus for a cytoplasmic sensor. Transport of unsaturated fatty acids into the cell would presumably increase the proportion of unsaturated species in the long chain acyl-CoA pool, providing the regulatory stimulus. In the simplest case the transcriptional regulatory sensor might be a domain of a soluble unsaturated acyl-CoA-binding protein that can be transported to the nucleus and act directly on the OLE1 transcription apparatus. This is analogous to transcriptional regulators that are responsive to steroid hormones such as the glucocorticoid receptor (15, 16). Fatty acid-binding proteins have been recently identified in nearly all mammalian tissues and are generally found as abundant cytosolic proteins (17). Studies of the binding affinities of the two most well characterized proteins L-FABP and L-FABP indicate that they bind a wide degree of saturated and unsaturated fatty acids as well as other hydrophobic ligands (18). This suggests that they lack the necessary specificity found with the OLE1 transcriptional sensor but do not rule out the existence of other acyl-binding proteins that have a high specificity for an unsaturated species.

It is equally plausible to suggest that sensors for transcriptional and/or post-transcriptional mechanisms may act at the level of the membrane and recognize fatty acids that are
acylated to glycerolipids. These might function to maintain the balance of saturated and unsaturated fatty acids in membrane lipids. If the transcriptional regulatory sensor identified here is an integral membrane protein, other proteins would be required to complete the regulatory circuit. Post-transcriptional sensors, however, could act locally at the endoplasmic reticulum to regulate the translation or stability of the OLE1 message or by modulation of enzyme activity.

The regulation of the Δ9 enzyme in yeast has similarities to its regulation in liver and adipocytes (21–23). Liver desaturase mRNA levels have been shown to be regulated in response to a variety of dietary lipids. In fact, a wide variety of organisms ranging from microbes to mammalian cells incorporate exogenous fatty acids into cellular lipids (23, 24), and specific mechanisms have evolved to transport these molecules across the plasma membrane (19, 20). This appears to be part of a system generally used by cells to bypass the energetically expensive synthesis of fatty acids (which make up a large part of the cellular mass) by preferentially importing saturated and unsaturated fatty acids from the growth medium. Evidence presented in this paper indicates that the transcriptional regulatory sensor identified here is an integral membrane protein, other proteins would act locally at the endoplasmic reticulum to regulate the translation or stability of the OLE1 message or by modulation of enzyme activity.

The existence of multiple sensors and regulatory paths for the OLE1 gene that exert a graduated degree of control on desaturase activity appear to parallel other membrane lipid biosynthetic enzymes. Similar multiple levels of control have been described for 3-hydroxy-3-methylglutaryl-coenzyme A reductase, and these have been cited as evidence of its key regulatory role in sterol metabolism (25). Dissection of the components of these regulatory circuits should yield information concerning the mechanisms that balance the synthesis of membrane lipid species.

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