Isolation and Characterization of a Gene Affecting Fatty Acid Elongation in Saccharomyces cerevisiae*

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Fatty acid elongation defective mutants were isolated from Saccharomyces cerevisiae by mutagenizing strains that were defective in fatty acid synthase (FAS) activity. Cells of the fatty acid synthase-defective strains can grow when supplemented with tetradecanoic acid (14:0) due to the presence of membrane bound elongation systems that can extend the 14 carbon fatty acid to longer chain species. After mutagenesis and rescue on medium containing a mixture of 14:0, 16:0 and 18:0, cells were screened for their inability to grow on medium containing only 14:0. From 150,000 colonies, four stable isolates were identified, all of which appear to represent the same complementation group. Gas chromatography of lipid extracts from mutant elo1-1 (designated as elongation defective) cells grown with long or medium chain fatty acids indicates that it fails to efficiently elongate (12, 13, or 14) carbon fatty acids. A gene disrupted fas2::LEU2 elo1::HIS3 mutant incorporates 14–18-carbon fatty acids into membrane lipids, indicating that fatty acid transport is not affected by the mutation. Molecular cloning and sequence analysis of the ELO1 gene suggests that the encoded protein is a membrane bound polypeptide that contains at least five potential membrane spanning regions and a presumptive NADPH binding site. Analysis of the ELO1 mRNA levels indicates that the gene is expressed in cells grown on fatty acid deficient medium. It is rapidly induced in wild type cells that are supplemented with 14:0 and is repressed when cells are supplied with 16- and 18-carbon fatty acids.

Membrane glycerolipids and sphingolipids in Saccharomyces contain fatty acyl chains that range from 12 to 24 carbons in length (1). There appear to be several distinct metabolic pathways that produce long chain fatty acids. The de novo pathway generates long chain fatty acyl CoA’s within the soluble cytosolic fatty acid synthase complex, (Fasp),1 which has been reported to form products as long as the 18-carbon octadecanoic acid (18:0) (2). In addition, Saccharomyces appears to contain membrane bound enzyme systems that can elongate fatty acyl CoA’s that are derived either from Fasp or as nutrients.

The formation of long chain (18–24-carbon) fatty acids appears to require the action of several membrane-bound enzyme systems. The reaction mechanisms of a microsomal membrane elongation system has been extensively described in rat liver (3, 4). Cell fractionation and in vivo studies have also indicated that there may be multiple fatty acid elongation systems in the yeast, Saccharomyces cerevisiae (5). One is thought to be associated with the endoplasmic reticulum that is responsible for the elongation of medium chain (12:0 and 14:0) to 16- and 18-carbon species (6). A second system that converts 18-carbon fatty acids to 20-carbon species is thought to be associated with mitochondrial membranes of Saccharomyces (5). None of the enzymes for these systems have been purified to homogeneity, and no elongation specific genes have been cloned from either animal or fungal cells. Recently, a gene coding for a condensing enzyme activity has been reported to have been cloned from plants (7).

Although the primary function of the cytoplasmic fatty acid synthase complex appears to involve the formation of long chain fatty acids de novo, it also appears to have some ability to elongate shorter chain (14- and 16-carbon) acyl-CoA species. In this paper we describe a strategy for the isolation of components of membrane bound fatty acid elongation systems. These activities are revealed when fatty acid synthase activity is eliminated by disruption of one of its genes. In this paper we describe the identification and characterization of a gene that encodes a presumptive membrane bound enzyme that is involved in the elongation of 14-carbon fatty acids to 16-carbon species.

MATERIALS AND METHODS

Strains, Plasmids, and Media—S. cerevisiae strains used in this study are shown in Table I. Plasmids constructed for this study are shown in Table II. Standard yeast genetics methods were used for mating, sporulation, complementation, and construction of strains bearing the appropriate mutations (8). Cell growth conditions and growth medium have been previously described (9). All fatty acids were obtained from Nu-Chek Prep.

Construction of fas2 Parental Strains for Mutagenesis—A 1.7-kb DNA fragment containing part of the FAS2 promoter and approximately 1.2 kb of its protein coding sequence (see Fig. 1a) was derived by PCR using strain DTY10A genomic DNA as a template and the following primers: DAT001 (5'-ATGTTGGTTGATTGTCGCC-3') and

Z-9-tetradecenoate,15:0, pentadecanoate; Z-9 15:1, Z-9 pentadecanoate; 16:0, hexadecanoate; 17:0, heptadecanoate; Z-9 17:1, Z-9 heptadecanoate; 18:0, octadecanoic acid; fas2Δ, a null, gene disrupted, allele of FAS2 which encodes a polypeptide component of the soluble fatty acid synthase complex; elo1Δ, a null, gene disrupted, allele of ELO1; CEN, centromere.

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1 The designations used are: Fasp, fatty acid synthase enzyme that consists of a complex containing the FAS1 and FAS2 encoded polypeptides; Elo1p, presumptive polypeptide encoded by the ELO1 gene; YPAD, yeast peptone adenine dextrose; CM, complete minimal medium containing all standard nutrients (11–14) plus 1% tergitol Nonidet P-40 to disperse fatty acids; 14/16/18 CM, complete minimal medium containing a fatty acid mixture consisting of 0.2 mM 14:0, 0.4 mM 16:0, and 0.2 mM 18:0; Z, cis-double bond; 14:0, tetradecanoic acid, 14:1,14:2, tetradecenoic acid; 16:0, hexadecanoic acid; 17:0, heptadecanoic acid; 18:0, octadecanoic acid; fas2Δ, a null, gene disrupted, allele of FAS2 which encodes a polypeptide component of the soluble fatty acid synthase complex; elo1Δ, a null, gene disrupted, allele of ELO1; CEN, centromere.
DAT002 (5'-AGATCTGAAGTTCAGCAACAGTGTCC-3').

The PCR product was ligated into the pCRII donor vector (Invitrogen), resulting in formation of pCRFA52. This plasmid was digested with CiaI/HpaI to remove 1.1 kb of the FAS2 coding region. A 2.2-kb DNA fragment of the Saccharomyces LEU2 gene was inserted into blunt ended sites of the vector. The resulting plasmid (fas2LEU) contains the LEU2 gene coding sequence in an orientation opposite to that of FAS2. Treatment with BamH1 and XhoI released a linear 2.6-kb disruption cassette. This was electroporated into cells of both mating types (DTY10a and DTY11a) to create a one-step gene disruption of FAS2 (10). The disruption was confirmed by Southern blot analysis, and the inability of those strains to grow on synthetic medium without fatty acid supplementation.

Mutagenesis and Mutant Selection—The fas2 gene disrupted strains, DTY10a2 and DTY11a2, were mutagenized according to standard protocols (11–14) with the following modifications. The cells were grown in CM liquid medium (11–14) with 1% tergalol Nonidet P-40 (CM,) supplemented with a mixture of 14:0, 16:0, and 18:0 (14/16/18 CM) (0.2 mm/0.4 mm/0.2 mm) to mid log growth phase, washed three times with sterile cold distilled water, and treated with 35 μl of ethyl methane sulfonate. This dosage produced approximately 15–20% viable cells compared with untreated cultures.

Cells were plated at densities of approximately 100–150 viable colonies/plate on 14/16/18 CM, medium. Following incubation for 3–5 days at 30°C, colonies were replica-plated via sterile velvet onto CM, medium and 1.0 mm 14:0. The selection plates were incubated 3–5 days at 30°C. Prospective elongation-deficient mutants formed colonies that failed to grow on the 14:0 containing media.

Fatty Acid Analysis—Fatty acid methyl esters were extracted from washed cell pellets by a modification of the method of Browse, McCourt, and Somerville as described previously (15, 16). Methyl esters were analyzed using a Varian 3400 CX gas chromatograph with a 30-m, 0.32-mm SupelcoWax®-10 column using helium as the carrier gas. Gas chromatography data were collected and quantified using the Shimadzu EZChrom data system.

RNA Isolation and Northern Blot Analysis—Total yeast RNA was isolated according to the methods of Schmitt et al. (17) and Schmitt et al. (18). Equal amounts (10 μg) of total RNA from each sample were resolved on a 1.0% formaldehyde gel for 2.5 h at 100 V (19). The RNA samples were then transferred to Zeta Probe® membrane (Bio-Rad) by vacuum blotting (Bio-Rad, Model 785). Pre-hybridization, hybridization with a [32P]dATP labeled specific probe, and washes to remove unbound probe were carried out according to manufacturer’s instructions. Blots were stripped and reprobed with the yeast phosphoglycerate kinase gene (PGK1) as an internal standard. Images of radiolabeled species were acquired and quantified by phosphor imaging.

Preparation of Radioabeled Probe—DNA fragments to be used as probes for Southern and Northern analyses were labeled with [α-32P]dATP (NEN) using the PROBE-EZE (5 Prime → 3 Prime, Inc., Boulder, CO) random primer labeling kit. Unincorporated nucleotides were removed using Sephadex G-50 spin columns (5 Prime → 3 Prime).

Complementation with CEN-based Library—A mutant isolate (DTY10 m2), which displayed the strongest phenotype, was backcrossed a minimum of two times to the opposite fas2 mating type. An isolate (fas2Δ; elo1-1 segregant (DTY10 m2BID) was chosen for complementation due to its good growth characteristics on 14/16/18 CM, medium and the inability to grow on 14:0 alone. Using DNA from the "A" pool of the CEN plasmid based Rose Library (20) those cells were transformed using the "yeastmaker" kit (Clontech) according to the manufacturer’s instructions. Transformants were plated onto CM, medium (Uracil) supplemented with 1.0 mw 14:0. Transformation efficiency was determined by plating aliquots of library transformed cells on 14/16/18 CM, medium (Uracil). Twenty-three colonies grew out on the selected medium out of ~60,000 cells. Six of the 23 were randomly chosen and streaked on plates. Single colonies from each of the six were used to inoculate 10 ml of CM, medium (Uracil) supplemented with the fatty acid mixture. The cultures were grown to late-log phase with shaking (250 rpm) at 30°C. Complementing plasmids were isolated from the transformants using a standard plasmid rescue protocol (21).

Subcloning—Rescued plasmids containing the presumptive ELO1 gene were characterized by restriction analysis. A plasmid (p20B6.0) containing a 6.0-kb fragment was identified that could complement the elo1–1 mutation. Restriction analysis revealed a unique ECORI restriction site within that region that produced 4.0- and 2.0-kb fragments. Neither fragment when subcloned into plasmid YCP50 could complement the mutation. The EcoRI site was used as a starting point for DNA sequencing in both directions. DNA sequencing was performed using the Sequenase II kit (U.S. Biochemical Corp.) and [35S]d-ATP (New England Nuclear) using the PROBE-EZE (5 Prime → 3 Prime, Inc., Boulder, CO) random primer labeling kit. Unincorporated nucleotides were removed using Sephadex G-50 spin columns (5 Prime → 3 Prime).

Construction of elo1 Disruption (Fig. 1b)—A 2.4-kb fragment of DNA was removed by Sphl/AviI digestion from the presumptive gene coding sequence. The fragment was ligated into plasmid YCP50 after Sphl/BamHI digestion to generate plasmid YCPelo1. The 2.4-kb insert was further reduced to 2.2 kb by BstEII/EcoNI digestion to remove 200 bp of a flanking open reading frame sequence creating plasmid YCPelo1MOD. That fragment was used to retransform the mutant to confirm its ability to complement the mutation. The 2.2-kb fragment was then ligated into the BamHI/XhoI sites of pCRScriptII-SK+ (Strat-
the protein coding sequences for the indicated genes. a, disruption of FAS2. Dat001 and Dat002 indicate positions of PCR primers used to isolate the FAS2 gene fragment. b, disruption of ELO1. A, AvrI; B, BamHI; Bg, BglII; C, ClaI; E, EcoRI; H, HpaI; K, KssI; M, MluI; S, SalI; T, XhoI.

**Fig. 1. Strategy for disruption of the FAS2 and ELO1 genes.** The arrows below the wide bars indicate the direction of translation of the protein coding sequences for the indicated genes. a, disruption of FAS2. Dat001 and Dat002 indicate positions of PCR primers used to isolate the FAS2 gene fragment. b, disruption of ELO1. A, AvrI; B, BamHI; Bg, BglII; C, ClaI; E, EcoRI; H, HpaI; K, KssI; M, MluI; S, SalI; T, XhoI.

Isolation of Elongation Defective Mutants—To identify mutants that fail to elongate 14:0, mutagen-treated cells from fatty acid synthase-disrupted (fas2Δ) strains DTY10α2 and DTY11α2 were plated onto synthetic complete growth medium (14/16/18 CM), containing a mixture of 14:0, 16:0, and 18:0. Colonies were then replicated on the same medium containing only 14:0 as the fatty acid supplement. Cells with an intact elongation system would be expected to incorporate the exogenous fatty acid and convert it to longer chain species essential for growth. Therefore, cells that were elongation defective would fail to grow on medium containing 14:0 alone. Comparison of 150,000 colonies between the master and replica plates initially identified 23 presumptive elongation mutants. Secondary screening of the mutants revealed that 16 of the 23 grew slowly on 14:0 alone. These slow growing isolates were discarded. Three of the seven remaining isolates were found to revert with high frequency and were not further examined. Of the remaining four isolates, one was mating type α and three were mating type a. Crosses between the α and a strains produced diploids that phenotypically failed to complement the 14:0 growth defect, suggesting that they all contained mutations in the same gene. The mutant phenotype in all of these strains was later found to be complemented by a centromeric plasmid YCPelo1.0MOD (Table I), which contains the ELO1 gene sequence described in this paper (Fig. 2).

Mutant elo1–1 (elongation defective), designated as DTY10 m2, which showed no revertible phenotype was selected for additional back-crosses and fatty acid analysis to characterize the defect and to clone the relevant gene. In two successive back-crosses to the phenotypically wild type fas2Δ strains, a total of 24 tetrads were dissected. All 24 showed 2:2 segregation with respect to the inability to grow on 14:0. This strongly suggests that the defect is due to a single gene. Gas chromatography of fatty acids extracted from the initial isolate DTY10 m2 and the back-crossed strain used for cloning DTY10 m2–8C1D revealed that the cells could readily incorporate 14:0 but not elongate it to 16-carbon species. This indicated that an inability to transport the fatty acid across the plasma membrane was not the cause of the mutant defect.

Molecular Cloning and Sequence of the ELO1 Gene—To isolate the ELO1 gene, the elo1–1 mutant was transformed with a yeast CEN plasmid based genomic library. From a total of 60,000 transformants that grew on 14/16/18 CM, medium, 23 were identified that repaired the inability of the mutant to grow on medium supplemented with 14:0 alone. One of the complementing plasmids contained an insert of approximately 6.0 kb. Restriction digests identified a 2.2-kb fragment that could repair the elo1–1 mutation. Sequence analysis of the fragment revealed a single open reading frame of 933 bp (Fig. 2) located less than 1 kb away from the CDC6 gene on chromosome X (YJ L196C).

Structure of the ELO1 and Its Encoded Protein—Analysis of the upstream promoter region of the ELO1 gene revealed only one consensus TATA sequence that could serve as a basal promoter elements. This element, (sequence TATATAA) was positioned 77 bases upstream of the start codon. A sequence resembling a regulatory element identified in FAS1, FAS2, and FAS3 promoters (GCCAA) (22) is located 700 bases upstream of the start codon. Analysis of the presumptive coding sequence suggests that the ELO1 polypeptide contains 310 amino acids. It encodes a basic protein with a predicted pl of 10.2. Hydropathy analysis of Elo1p using the Kyte-Doolittle and TMpredict algorithms suggests that it is a transmembrane protein with at least five membrane spanning regions (Fig. 3). These are distributed over the entire polypeptide sequence. The initial membrane spanning region is located 64 residues from the start codon. There is no obvious amino-terminal signal sequence in the coding region. A presumptive NADPH binding site is located near the carboxyl terminus of the protein (residues 294–304). This has high identity with the NADPH binding site in the β-ketoacyl reductase domain of the Saccharomyces FAS2 gene. It also has high sequence homology to the NADPH binding domains in β-ketoacyl and enoyl reductase regions of fatty acid synthases and dehydrogenases in avians and mammals (Table III) (23–26). The protein also contains a single HXXHH motif (residues 172–176) located between predicted transmembrane sequences II and III, suggesting that the polypeptide is a subunit of a dioxy-iron cluster enzyme (27, 28). There are a series of lysine residues clustered near the carboxyl terminus of the polypeptide at positions −3, −6, −7, and −8.

Disruption of ELO1 and Analysis of the fas2Δ; elo1α Phenotype—A 2.2-kb elo1α::HIS3 cassette was used to disrupt the native ELO1 gene in wild type (DTY-10α) and fas2Δ cells as described under “Materials and Methods.” The elo1α disruptant strain, DTY001 (verified by PCR), was crossed with the DTY10 m2 fas2Δ; elo1–1 mutant to further verify that the cloned fragment contained the ELO1 gene. The resulting diploids failed to grow on medium containing only 14:0 as a supplement, indicating that the disrupted and mutant genes were not complementary. A cross of fas2Δ; elo1Δ (DTY001) with the FAS”; ELO1” (W3031B) strain was also performed to construct a FAS”; elo1Δ strain (DTY003). Dissection of 12 tetrads from this cross yielded 2 parental ditype, 7 tetratype, and 3 nonparental ditype tetrads for the LEU2 and HIS3 genes used to disrupt...
FAS2 and ELO1, respectively. This segregation pattern confirms that the ELO1 and FAS2 genes segregate independently. The independent nature of these genes is also indicated by the location of FAS2 on chromosome XVI and ELO1 on chromosome X.

Strains DTY10a2, containing the fas2 disruption, and DTY001, the doubly disrupted fas2D;elo1D strain, were tested for growth on fatty acids of different chain length and saturation (Table IV). The strain containing the fas2 disruption and a functional ELO1 gene could be rescued by a wide variety of fatty acids and fatty acid mixtures with chain lengths from 13 to 18 carbons. By contrast, cells containing both fas2 and elo1 disrupted genes were rescued by 16 carbon fatty acids and mixtures containing both 16 and 18 carbon species but did not grow on 13-, 14-, or 15-carbon saturated fatty acids. The phenotype of the fas2D;elo1D;ELO1 parent strain also grew normally on glycerol medium when supplemented with either 14:0 or the fatty acid mixture. The wild type and fas2 disrupted strains grew in 14:0 containing medium supplemented with 14:0, 16:0, or the fatty acid mixture and monitored for growth for 24 h (Fig. 6). Strains containing both the defective elo1–1 allele and the disrupted form of the gene in a fas2D background grew at rates similar to their fas2D;ELO1 parent and wild type FAS1;ELO1 parent when supplied with either 16:0 or a mixture of 14:0,16:0 and 18:0 (Fig. 6). The wild type and fas2 disrupted strains grew in 14:0 containing medium at similar rates to that observed when they were supplemented with the 14/16/18:0 mixture. When the fas2D;elo1D double disruptive strain was transferred to medium containing...
only 14:0, it grew approximately 2.5 generations before growth was arrested. The double disrupted strains did not undergo growth arrest and grew at rates comparable with the parent strains in medium containing the 16-carbon fatty acid species.

Fig. 7 shows gas chromatograms of fatty acid methyl esters from the fas2Δ;elo1Δ strain grown on the 14/16/18-carbon fatty acid mixture. Cells grown on the fatty acid mixture contained approximately 39% 14-carbon, 52% 16-carbon, and 7% 18-carbon fatty acids. After exposure to 14:0 for 24 h 87% of the fatty acids were 14-carbon species in the form of 14:0 and 14:1. The presence of high levels 14:1 in that strain indicates that the 14:0 fatty acid supplement was internalized in the cells, because that species is produced by the cytoplasmic Δ-9 fatty acid desaturase.

To further confirm the inability of the fas2Δ;elo1Δ strain to elongate 14:0, fatty acid replacement experiments were done to compare the behavior of the fas2Δ;ELO1 parent strain with the doubly disrupted fas2Δ;elo1Δ strain. Strains fas2Δ;ELO1 and fas2Δ;elo1Δ were grown for 48 h on medium containing 14:0/15:0 and 17:0. This resulted in the replacement of the 16- and 18-carbon fatty acids with 15- and 17-carbon species (Fig. 8, a and c). Cells were then washed and shifted to medium supple-

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**TABLE III**

| Comparison of putative NADPH binding domain in Elo1p with similar domains in fatty acid synthases and other NADPH enzymes |
|---------------------------------------------------------------|
| **Elo1p putative NADPH domain (294–304)**                     |
| a-Subunit of yeast fatty acid synthase β-ketoacyl reductase   |
| EVYKRGASGK                                                   |
| EVDFWGASRTK                                                   |
| Human fatty acid synthase predicted enoyl reductase domain    |
| VFTTVGSAEKR                                                   |
| Rat fatty acid synthase enoyl reductase NADPH binding site    |
| VFTTVGSAEKR                                                   |
| Chicken fatty acid synthase enoyl reductase domain            |
| VFATVGSAEKR                                                   |
| Goose fatty acid synthase enoyl reductase domain              |
| FVTVGSAEKR                                                    |
| β-Subunit of yeast fatty acid synthase enoyl reductase domain |
| FGSGFGSSADDT                                                   |
| Horse alcohol dehydrogenase                                   |
| FSTGYGSSAVKV                                                  |

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**Fig. 3.** Hydropathy analysis of the ELO1 protein coding sequence using the Kyte-Doolittle (32) (a) and the TMpredict program (b). The TM predict algorithm is based on the statistical analysis of a database of naturally occurring transmembrane proteins (33). Roman numerals in each panel indicate presumptive membrane spanning regions of the polypeptide chain.
In this paper we describe the isolation and initial characterization of a gene encoding an protein that affects fatty acid elongation. In yeast, the cytoplasmic fatty acid synthase appears to have some ability to extend fatty acyl CoA species. This can mask the activity of other chain elongation systems. The strongest response to fatty acid nutrients occurs transiently when FAS";ELO1" or fas2Δ;ELO1" cells are transferred to medium containing only 14:0 (Figs. 9 and 10, a, b, d, and e). ELO1 mRNA levels are induced approximately 3–5-fold over a 60-min period following the shift in growth medium. This level decayed to the original levels by 4 h. The increase in ELO1 mRNA levels appears to occur in parallel with the accumulation of 14-carbon fatty acids into cellular lipids (Fig. 10, b and e), and its decline occurs when the 14-carbon species have reached steady state levels. This dramatic increase in ELO1 expression was not seen when the cells were fed 16:0 (Fig. 10, c and f).

**RESULTS**

**Growth of fas2Δ::ELO1" and fas2Δ::elo1Δ in CM medium with different fatty acids**

Cultures were inoculated with approximately 10^4 cells/ml and grown at 30°C with shaking in a water bath for 48 h. Cultures were inspected visually for growth. NG, no observable growth; ++, slow/poor growth; slight turbidity <10^6 cells/ml; ++, moderate growth; ≥10^6 cells/ml; ++++, stationary phase growth, equivalent to wild type; ≥2 × 10^9 cells/ml.

| Fatty Acid           | fas2Δ::ELO1" | fas2Δ::elo1Δ |
|----------------------|--------------|-------------|
| 12:0 (0.7 mM)        | NG           | NG          |
| 13:0 (0.7 mM)        | + +          | NG          |
| 14:0 (0.7 mM)        | + +          | NG          |
| 15:0 (0.7 mM)        | + +          | NG          |
| 16:0 (0.7 mM)        | + +          | +++         |
| 17:0 (0.7 mM)        | + +          | + +         |
| 18:0 (0.7 mM)        | + +          | + +         |
| 14:1 (0.7 mM)        | NG           | NG          |
| 14:0/14:1 (0.5/0.5 mM) | NG         | NG          |
| 15:0/15:0 (0.5/0.5 mM) | NG         | NG          |
| 16:0/16:1 (0.5/0.5 mM) | NG         | NG          |
| 17:0/17:0 (0.5/0.5 mM) | NG         | NG          |
| 18:0/18:0 (0.5/0.5 mM) | NG         | NG          |

+ ng, no observable growth; +, slow/poor grower; slight turbidity <10^6 cells/ml; ++, moderate growth; ≥10^6 cells/ml; ++++, stationary phase growth; equivalent to wild type; ≥2 × 10^9 cells/ml.

**Growth on glycerol as a carbon source (plates)**

Cells were streaked onto CMt glycerol medium and scored for growth (+) or no growth (−) after incubation at 30°C for 5 days.

| Yeast strain | CMt glycerol NFA | CMt glycerol 14/0 | CMt glycerol 14/16/18 |
|--------------|------------------|-------------------|----------------------|
| DTY10AFAS";ELO1" | + + +            | + +               | + + +                |
| DTY10afas2Δ;ELO1" | − − +            | − +               | − + +                |
| DTY003 FAS";elo1Δ" | + + +            | + +               | + + +                |

**DISCUSSION**

In this paper we describe the isolation and initial characterization of a gene encoding an protein that affects fatty acid elongation. In yeast, the cytoplasmic fatty acid synthase appears to have some ability to extend fatty acyl CoA species. This can mask the activity of other chain elongation systems. Disruption of fatty acid synthase makes the cells dependent on the elongation enzymes to extend medium chain fatty acids to...
form essential long chain species. This allowed us to identify by mutation genes that are components of these complex systems. Several lines of evidence suggest that ELO1p is an enzymatic component of a fatty acid elongation system: 1) cells deficient in ELO1p and FAS2p cannot grow on fatty acids shorter than 16:0, 2) fed 14:0 is incorporated into those cells and modified by desaturation but is not elongated, 3) ELO1 mRNA levels respond to fatty acid nutrients in a chain length-dependent manner with the presumptive substrate, 14:0, eliciting the highest level of expression, and 4) motifs are present in the protein sequence that correspond to NADPH binding sites found in enoyl and β-keto reductase domains of fatty synthases over a broad range of species. The primary activity of ELO1p appears to involve the highly specific elongation of 14:0 to 16:0. Given that cells containing disrupted forms of both this enzyme and fatty acid synthase can elongate 16:0 to 18:0 and 15:0 to 17:0 indicates that the ELO1p elongation system has a high degree of chain length specificity and that there are additional enzyme systems that catalyze the elongation of longer chain fatty acids.

The existence of several membrane bound fatty acid elongation systems in Saccharomyces have been reported based on mutant analysis and enzymatic studies of isolated cell fractions. Yeast mutants with defective acetyl-CoA carboxylase cannot synthesize long chain fatty acids de novo but were capable of elongating 13:0 and 15:0 supplements to longer chain species, suggesting that this elongation activity was not dependent on malonyl-CoA (6, 29). Multiple malonyl-CoA-dependent elongation reactions have also been reported in isolated mitochondrial fractions (5). These include an ATP-dependent activity that acts on endogenous substrates and activities that extend 18:0- and 20:0-CoAs. Subfractionation experiments showed that all three activities are primarily associated with the outer membrane fraction.

The enzymatic properties of the rat liver microsomal elongation complex have been most extensively described in studies...
by Cinti and co-workers (4). Difficulties in purifying these intrinsic membrane enzymes to homogeneity, however, has hindered the isolation and extensive biochemical characterization of these systems. Those studies indicated that the membrane bound elongation systems catalyze similar reactions to those of the soluble fatty acid synthase. Unlike the animal, avian, and yeast cytoplasmic fatty acid synthases, which consist of very large polypeptides with multiple, modular enzyme activities, the membrane bound elongases appear to be made up of individual proteins, each catalyzing a different part of the elongation cycle. Elo1p also appears to be a membrane bound protein, and its length appears to be sufficient to encode only a single enzyme in the elongation complex.

Several independent hydropathy analyses of the ELO1 coding sequence indicate that it is a membrane protein with at least five bilayer spanning sequences. Given the distribution and extent of these sequences, it appears that only a small part of the protein is exposed to either membrane surface. The presence of an NADPH binding motif at the carboxyl terminus of ELO1p suggests that it may act as a type of reductase in the elongation cycle. Surprisingly, the coding sequence also contains HXXHH motifs between predicted membrane spanning regions II and III that are characteristic of dioxy iron cluster proteins. These unusual enzymes contain coordinately linked iron atoms to the histidine sites that do not require a heme moiety. They include the large family of plant, animal, and fungal fatty acid desaturases in addition to reductases such as ribonucleotide reductase. The location of the HXXHH motifs with respect to the presumptive NADPH binding site presents a difficulty with respect to understanding the topology of Elo1p. If the two sites are authentic for their functions, the proposed topology of the enzyme would place the two domains on the opposite surfaces of the membrane, requiring electrons from NADPH to be transferred to the iron center across the membrane bilayer. Given that possibility, it seems more likely that there is either one less or one more membrane spanning region between those two motifs. Region III appears to be sufficiently wide to encompass two membrane spanning sequences and could be a likely candidate for an additional.
transmembrane segment that would place both functional motifs on the same membrane surface.

The presence of clustered lysine residues on the carboxyl terminus of the predicted polypeptide are also intriguing. Cytosolic carboxyl-terminal dilysine motifs have been implicated in the efficient retrieval of Type I transmembrane proteins from the cis-Golgi to the endoplasmic reticulum in mammalian cells and in yeast (30, 31). This retrieval mechanism has been shown to have high specificity for proteins with two lysine residues at $-3$ and $-4$ or $-3$ and $-5$ with respect to the carboxyl terminus, although the particular combination of residues in ELO1 ($-3$, $-6$, $-7$, and $-8$) have not been experimentally excluded as a retrieval marker. Associated with this clustering of lysine residues are two asparagine residues at the carboxyl terminus. These unusual distributions of residues may play some role in the maintenance, targeting, or retrieval of proteins assembled in a complex on a cytoplasmic membrane surface.

The work presented in this paper paves the way for the identification of other components of membrane bound fatty acid elongation enzymes. Ultimately, these advances should provide essential information concerning the assembly, organization, and function of these complex enzyme systems.

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