

## Elo1p-Dependent Carboxy-Terminal Elongation of C14:1 $\Delta^9$ to C16:1 $\Delta^{11}$ Fatty Acids in *Saccharomyces cerevisiae*

ROGER SCHNEITER,\* VERENA TATZER, GABRIELA GOGG, ERICH LEITNER,  
AND SEPP DIETER KOHLWEIN

*Institut für Biochemie und Lebensmittelchemie, Technische Universität Graz, A-8010 Graz, Austria*

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*Saccharomyces cerevisiae* medium-chain acyl elongase (*ELO1*) mutants have previously been isolated in screens for fatty acid synthetase (FAS) mutants that fail to grow on myristic acid (C14:0)-supplemented media. Here we report that wild-type cells cultivated in myristoleic acid (C14:1 $\Delta^9$ )-supplemented media synthesized a novel unsaturated fatty acid that was identified as C16:1 $\Delta^{11}$  fatty acid by gas chromatography-mass spectroscopy. Synthesis of C16:1 $\Delta^{11}$  was dependent on a functional *ELO1* gene, indicating that Elo1p catalyzes carboxy-terminal elongation of unsaturated fatty acids ( $\alpha$ -elongation). In wild-type cells, the C16:1 $\Delta^{11}$  elongation product accounted for approximately 12% of the total fatty acids. This increased to 18% in cells that lacked a functional acyl chain desaturase (*ole1* $\Delta$  mutants) and hence were fully dependent on uptake and elongation of C14:1. The observation that *ole1* $\Delta$  mutant cells grew almost like wild type on medium supplemented with C14:1 indicated that uptake and elongation of unsaturated fatty acids were efficient. Interestingly, wild-type cells supplemented with either C14:1 or C16:1 fatty acids displayed dramatic alterations in their phospholipid composition, suggesting that the availability of acyl chains is a dominant determinant of the phospholipid class composition of cellular membranes. In particular, the relative content of the two major phospholipid classes, phosphatidylethanolamine and phosphatidylcholine, was strongly dependent on the chain length of the supplemented fatty acid. Moreover, analysis of the acyl chain composition of individual phospholipid classes in cells supplemented with C14:1 revealed that the relative degree of acyl chain saturation characteristic for each phospholipid class appeared to be conserved, despite the gross alteration in the cellular acyl chain pool. Comparison of the distribution of fatty acids that were taken up and elongated (C16:1 $\Delta^{11}$ ) to those that were endogenously synthesized by fatty acid synthetase and then desaturated by Ole1p (C16:1 $\Delta^9$ ) in individual phospholipid classes finally suggested the presence of two different pools of diacylglycerol species. These results will be discussed in terms of biosynthesis of different phospholipid classes via either the de novo or the Kennedy pathway.

The fatty acid composition of phospholipids is an important determinant of the biophysical properties of cellular membranes and as such must be subject to dynamic exchange to ensure membrane homeostasis under varying environmental conditions (10, 15). The biochemical control of fatty acid composition of different lipid classes, i.e., the regulation of the synthesis of defined lipid molecular species, however, is not well understood. *Saccharomyces cerevisiae* appears to be particularly amenable to study of this process, as both lipid and fatty acid biosynthesis are well characterized, mutants blocked in most steps of the lipid biosynthetic pathways are readily available (6, 12, 21), and the acyl chain composition of the major lipid species has recently been determined by mass spectroscopy (25).

Long-chain saturated fatty acids of 16 to 18 carbon atoms are the product of de novo synthesis by the fatty acid synthetase (FAS) (26, 35). These are desaturated between the carbon atoms in positions 9 and 10 by the single essential desaturase, Ole1p, to generate *cis*-monounsaturated C16:1 and C18:1 long-chain fatty acids (29, 30). Unsaturated fatty acids typically comprise 70 to 80% of the total fatty acids of this organism (9). Their relative composition, however, may vary considerably, depending on the strain background (13) and the

carbon source (33). Very-long-chain C24 and C26 fatty acids, on the other hand, are synthesized by a membrane-bound elongase of which two components, Elo2p and Elo3p, have recently been characterized (14, 24). Even though very-long-chain fatty acids comprise only ~1% of the cellular fatty acid pool (36), their synthesis is essential as they form part of the yeast ceramide, found in sphingolipids and in the lipid moiety of glycosylphosphatidylinositol (glycosyl-PtdIns)-anchored membrane proteins (12).

A third elongase, Elo1p, has been identified in a screen for *fas* $\Delta$  mutant cells that do not grow on myristic acid-supplemented media. *ole1* $\Delta$  mutant cells hence fail to elongate saturated fatty acids of 14 carbon atoms to long-chain fatty acids of 16 to 18 carbon atoms (17, 32). The substrate of Elo1p, however, is only poorly defined, and the mechanism of elongation has not yet been characterized. In the present study, we thus addressed the question whether Elo1p elongates unsaturated fatty acids by carboxy-terminal elongation ( $\alpha$ -elongation), as known from the microsomal elongation systems of mammals and plants, which catalyze the synthesis of polyunsaturated fatty acids (5, 8).

The fatty acid supplementation experiments performed in the course of this work revealed important new information about the regulation of the synthesis of lipid molecular species in yeast. Wild-type cells supplemented with either C14:1 $\Delta^9$  or C16:1 $\Delta^9$  fatty acids displayed dramatic alterations in their phospholipid composition, suggesting that the availability of acyl chains is a dominant determinant of the phospholipid class

\* Corresponding author. Mailing address: Institute of Biochemistry, Technical University Graz, Petersgasse 12, A-8010 Graz, Austria. Phone: 43-316-873-6955. Fax: 43-316-873-6952. E-mail: f548roge@mbox.tu-graz.ac.at.

composition of cellular membranes. The degree of acyl chain saturation characteristic for the different lipid classes (25, 34), however, appeared to be conserved, despite the gross alteration of the cellular acyl chain pool brought about by the fatty acid supplementation. These results will be discussed in terms of biosynthesis of different phospholipid classes via the de novo or the Kennedy pathway. In the de novo pathway, decarboxylation of phosphatidylserine (PtdSer) yields phosphatidylethanolamine (PtdEtn), which is further methylated to phosphatidylcholine (PtdCho), whereas, in the Kennedy pathway, PtdEtn and PtdCho can both be synthesized from diacylglycerol (DAG) and either CDP-ethanolamine or CDP-choline (12, 21).

#### MATERIALS AND METHODS

**Yeast strains and culture media.** The wild-type *S. cerevisiae* strain used was W303a (MATa *ade2-1 his3-11,15 leu2-3, 112 trp1-1 ura3-1*). The *elo1Δ ole1Δ* double mutant strain (MATa *elo1::HIS3 ole1::LEU2 ade2 his3 leu2 ura3*) was obtained from crossing *elo1Δ* (MATa *elo1::HIS3 ade2-1 can1-100 his3-11,15 leu2-2,112 ura3-1*) with *ole1Δ* (MATa *ole1::LEU2 ade2 his3 leu2-2 ura3*), both kindly provided by C. Martin (Rutgers University, Piscataway, N.J.). Standard yeast genetics methods were used for mating, sporulation, and construction of the double mutant strain (27). Cells were grown either in complete (yeast extract-peptone-dextrose) medium containing 1% yeast extract (Difco), 1% Bacto Peptone (Difco), and 2% glucose or in minimal (synthetic dextrose) medium containing 0.67% yeast nitrogen base without amino acids (Difco) and 2% glucose. Media supplemented with fatty acids contained 1% Brij 58 and 0.5 mM (either) C14:1 or C16:1 fatty acids (Sigma). For growth rate determination, optical density at 600 nm was monitored.

**Lipid and fatty acid analysis.** Cells were harvested in the late exponential growth phase, washed two times with 0.2% bovine serum albumin, and used immediately or stored at  $-70^{\circ}\text{C}$  before extraction of lipids. Lipids were extracted from cell homogenates by the procedure of Folch et al. (18). Phospholipid classes were separated by two-dimensional thin-layer chromatography on silica gel 60 plates (Merck, Darmstadt, Germany), using chloroform-methanol-25% ammonia (65:35:5 [vol/vol/vol]) as the first and chloroform-acetone-methanol-acetic acid-water (50:20:10:10:5 [vol/vol/vol/vol]) as the second developing solvent. Spots detected after exposure to iodine vapor were scraped off, and lipids were extracted with chloroform-methanol (1:4 [vol/vol]). Total and individual phospholipids were quantified as described by Broekhuysse (4).

Fatty acids were converted to methyl esters by  $\text{BF}_3$ -catalyzed methanolysis (23) and separated by gas-liquid chromatography (GLC) using a Hewlett-Packard HP 6890 Series GC, equipped with an HP Innovax column (15 m by 0.25 mm by 0.50  $\mu\text{m}$  in film thickness), with a temperature gradient (20 min at  $200^{\circ}\text{C}$ ,  $10^{\circ}\text{C}/\text{min}$  to  $280^{\circ}\text{C}$ , and 15 min at  $280^{\circ}\text{C}$ ). Fatty acids were identified by comparison to commercially available methyl ester standards (NuCheck, Inc., Elysian, Minn.).

The double bond position of monounsaturated fatty acids was determined by GLC-mass spectrometry (GLC-MS) of dimethyl disulfide adducts of these derivatives as described previously (37), on an HP 5890 Series II Plus GC equipped with electronic pressure control, the HP Chemstation software package, and an HP 5972 mass selective detector. Injector and interface were kept at 250 and  $300^{\circ}\text{C}$ , respectively. GLC-MS analysis was performed on a capillary column, HP-5MS, 30 m by 0.25 mm by 0.25  $\mu\text{m}$  in film thickness, programmed from 150 to  $320^{\circ}\text{C}$  at  $20^{\circ}\text{C}/\text{min}$  after a 2-min hold at  $150^{\circ}\text{C}$ . Finally, the column was kept at  $320^{\circ}\text{C}$  for 10 min. All analyses were carried out in the constant-flow mode. Helium was used as carrier gas with a linear velocity of 34.1 cm/s. Aliquots of 1  $\mu\text{l}$  of the samples were injected with an HP 7673 autosampler in splitless mode. Electron impact ionization with 70-eV ionization energy was used for mass spectroscopy. Data were collected by scanning from 150 to 600 atomic mass units at 1.6 scans/s.

#### RESULTS AND DISCUSSION

**Growth of *ole1Δ* mutant cells on C14:1-supplemented media requires a functional elongase.** In our analysis of the fatty acid requirements of an *ole1Δ* mutant strain, we observed that cells lacking the desaturase displayed significant growth on C14:1-supplemented media (data not shown). Since pathways for the modification of supplemented C14:1 fatty acid have not yet been described, growth of *ole1Δ* mutant cells on C14:1 would indicate that these cells survived by incorporating C14:1 as sole unsaturated fatty acid in their membrane lipids, a possibility that we considered unlikely. We thus investigated whether the exogenously supplemented C14:1 was metabolically converted

to a longer-chain unsaturated fatty acid that, when esterified to lipid, permitted growth of an *ole1Δ* mutant strain on myristoleic acid.

*ELO1* has previously been shown to be required for the elongation of saturated medium-chain fatty acids of 14 carbon atoms in length to saturated long-chain fatty acids of 16 to 18 carbon atoms (17, 32). We thus investigated whether *Elo1p* is required for *ole1Δ* mutant cells to grow on myristoleic acid-supplemented media. To address this question, an *elo1Δ ole1Δ* double mutant strain was generated and tested for growth on media supplemented with various unsaturated fatty acids. In contrast to the two parental strains, the *elo1Δ ole1Δ* double mutant did not grow on C14:1-supplemented media, suggesting that in an *ole1Δ* mutant strain the supplemented C14:1 fatty acid is elongated to longer-chain unsaturated fatty acids and that this elongation is dependent on *Elo1p*. On C16:1-supplemented media, on the other hand, *Elo1p* function appeared to be dispensable for growth of the *ole1Δ* mutant strain, as indicated by the vigorous growth of the *elo1Δ ole1Δ* double mutant strain on palmitoleic acid-supplemented media (data not shown).

**Identification of an *Elo1p*-dependent elongation product of unsaturated fatty acids in wild-type cells.** To determine the chemical nature and relative abundance of the predicted *Elo1p*-dependent product of medium-chain unsaturated fatty acid elongation, wild-type and *ole1Δ* and *elo1Δ* mutant strains were cultivated in C14:1-supplemented media and their fatty acid profiles were analyzed. As shown in Fig. 1, a novel peak, eluting in the range of C16 fatty acid methyl esters, was detected in wild-type and *ole1Δ* mutant cells. This peak was absent from the fatty acid profile of the *elo1Δ* mutant, indicating that synthesis of this compound was directly or indirectly dependent on *Elo1p*. In wild-type cells, this elongation product comprised 12.3% of all the fatty acids. In *ole1Δ* mutant cells, uptake and elongation of C14:1 were only slightly increased, with the elongation product accounting for 18.1% of the total fatty acids (Table 1). Under these conditions, *elo1Δ* mutant cells had approximately 4.5-fold-more unsaturated than saturated fatty acids and a roughly equal ratio of acyl chains with 16 carbon atoms to those with 18 carbon atoms. In comparison, the *ole1Δ* mutant strain displayed a ratio of unsaturated to saturated fatty acids that was intermediate between those observed in wild-type cells and those observed for *elo1Δ* mutant cells. The ratio of C16 to C18 fatty acids, however, was grossly perturbed in the desaturase mutant (Table 1).

Comparison of growth rates of wild-type and *elo1Δ* and *ole1Δ* mutant cells in media supplemented with either C14:1 or C16:1 indicated that elongation of C14:1 was not growth limiting for *ole1Δ* mutant cells, suggesting that uptake and elongation of the supplemented C14:1 are rapid and efficient. *ole1Δ* mutant cells required 207 min for doubling in C14:1-supplemented media, compared to 198 min in C16:1-supplemented media (data not shown).

***Elo1p* is required for carboxy-terminal elongation of unsaturated fatty acids.** The position of the double bond in the elongation product was determined by GLC-MS analysis of fatty acid dimethyl disulfide adducts, as previously described (37). Fragmentation of C16:1 $\Delta^7$  into two main products of *m/z* 117 and 245 allowed unequivocal identification of the *Elo1p*-dependent products as being a C16:1 $\Delta^{11}$  fatty acid, indicating that *Elo1p* is required for  $\alpha$ -elongation of C14:1 $\Delta^9$  to C16:1 $\Delta^{11}$  (Fig. 2). Furthermore, fragmentation of the *Elo1p*-dependent product was identical to that of a chemically synthesized C16:1 $\Delta^{11}$  fatty acid. In contrast, GLC-MS analysis of an authentic C16:1 $\Delta^9$  fatty acid methyl ester yielded major products

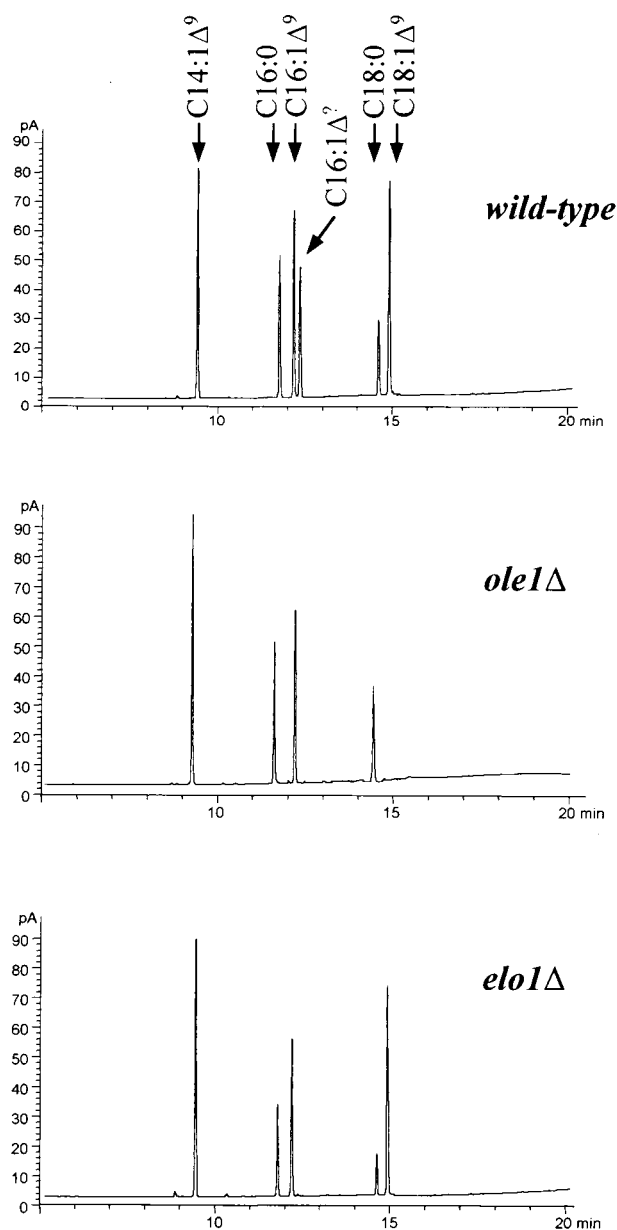


FIG. 1. Fatty acid profiles of wild-type and *elo1Δ* and *ole1Δ* mutant cells cultivated in C14:1 $\Delta^9$ -supplemented media. Wild-type and *ole1Δ* and *elo1Δ* mutant cells were cultivated in C14:1 $\Delta^9$ -supplemented minimal media to late exponential growth phase, cells were harvested, lipids were extracted, and fatty acid methyl esters were analyzed by gas chromatography as described in Materials and Methods. Positions of saturated and unsaturated fatty acids in the chromatograms are indicated. C16:1 $\Delta^7$  indicates the *ELO1*-dependent product.

at  $m/z$  217 and 145, consistent with the predicted fragmentation properties of C16:1 $\Delta^9$  fatty acid (data not shown).

To determine the substrate specificity of the elongation process that is operating on unsaturated fatty acids, wild-type and *ole1Δ* and *elo1Δ* mutant cells were cultivated in C16:1 $\Delta^9$ -supplemented media and their fatty acid profiles were analyzed. A novel peak in the C18:1 region of the chromatogram was apparent in lipid extracts from wild-type cells. This peak was not detected in lipid extracts from *elo1Δ* mutant cells, suggesting that its synthesis depends on the presence of a functional elongase. Determination of the double bond position in this elongation product by GLC-MS analysis of fatty acid dimethyl

disulfide adducts allowed unequivocal identification of the product as C18:1 $\Delta^{11}$  (data not shown). As shown in Table 1, elongation efficiency of C16:1 *in vivo* is approximately 12-fold lower than that of C14:1. Only 1% of C18:1 $\Delta^{11}$  was detected in wild-type cells grown in the presence of C16:1 $\Delta^9$ , compared to 12.3% of C16:1 $\Delta^{11}$  that was synthesized by the elongation of C14:1 (Table 1). The notion that C16:1 is a poorer substrate for Elo1p-dependent elongation is also apparent from the fact that only 0.5% of C18:1 $\Delta^{13}$  was detected in cells supplemented with C14:1, in which case elongation of C16:1 $\Delta^{11}$  by a second cycle generates a C18:1 $\Delta^{13}$  fatty acid.

The fatty acid profile of cells supplemented with C16:1 $\Delta^9$  (Table 1) furthermore revealed that all three strains appeared to maintain a ratio of unsaturated to saturated fatty acids of between 2 and 3. The ratio of C16 to C18 fatty acids, however, was grossly disturbed in the two mutants. Under these conditions, the endogenous synthesis of C18:1 $\Delta^9$  appeared to be strongly repressed in the *elo1Δ* mutant but not in the wild type, suggesting that Ole1p activity is tightly downregulated by C16:1 $\Delta^9$  (3), but not by C14:1 $\Delta^9$ , and that this repression is somewhat relieved by the presence of functional Elo1p.

**Effects of altered fatty acid profile on the phospholipid composition.** Next, we wished to determine whether an altered fatty acid composition, generated by cultivation of the yeast strains in the presence of  $\Delta^9$ -unsaturated fatty acids, affected the phospholipid composition of these cells. For this purpose, phospholipids of wild-type and *ole1Δ* and *elo1Δ* mutant cells cultivated in either C14:1 $\Delta^9$ - or C16:1 $\Delta^9$ -supplemented media were analyzed. As shown in Table 2, phospholipid compositions of wild-type and mutant cells deviated greatly from each other and were strongly dependent on the chain length of the unsaturated fatty acid supplied. The most notable observations were as follows. (i) In wild-type cells, the relative content of the two major phospholipid classes, PtdEtn and PtdCho, was strongly dependent on the type of unsaturated fatty acid supplemented. While PtdEtn was the most abundant phospholipid (74.5%) in wild-type cells supplemented with C14:1, PtdEtn levels were reduced to 38.9% when cells were supplemented with C16:1. Concomitantly, PtdCho increased from 17.9% in C14:1-supplemented cells to 48.7% in cells supplemented with C16:1. This tendency toward an increased content of PtdEtn at the expense of PtdCho in cells supplemented with C14:1 was also observed for the *ole1Δ* mutant. (ii) Furthermore, in *ole1Δ* mutant cells grown in C14:1-supplemented media, the relative content of phosphatidic acid (PtdOH) was greatly elevated ( $\sim$ 10-fold compared to the wild type), suggesting that the unfavorable fatty acid composition of these cells results in increased steady-state levels of PtdOH, possibly as a result of an altered phospholipid turnover. (iii) In all three strains, the relative content of PtdIns was lower in C14:1-supplemented cells than in C16:1-supplemented cells. This chain-length-dependent decrease of PtdIns was most pronounced (eight-fold) in the *elo1Δ* mutant. (iv) PtdSer levels appeared to be least affected by chain length alterations or mutations in *ELO1* and *OLE1*.

On one hand, this analysis illustrates the flexibility of the cellular phospholipid composition; on the other hand, it demonstrates that the phospholipid composition is greatly affected by the availability of acyl chains. These data suggest, in fact, that acyl chain supply is a dominant determinant of the phospholipid composition of a cellular membrane, suggesting that the lipid head group composition is more modulatory. This notion is supported by biophysical data showing that the introduction of a single double bond decreases the melting temperature of dipalmitoyl-PtdCho by 46°C. Head group exchange, on the other hand, appears to affect the phase behavior in a

TABLE 1. Comparison of fatty acid composition of total lipids from wild-type and *ole1Δ* and *elo1Δ* mutant cells cultivated in C14:1Δ<sup>9</sup>- and C16:1Δ<sup>9</sup>-supplemented media<sup>a</sup>

Fatty acid	Composition (mol%) in medium					
	C14:1Δ <sup>9</sup> supplemented			C16:1Δ <sup>9</sup> supplemented		
	Wild type	<i>ole1Δ</i>	<i>elo1Δ</i>	Wild type	<i>ole1Δ</i>	<i>elo1Δ</i>
C14:0	ND	ND	0.9 ± 0.1	0.5 ± 0.1	1.3 ± 0.1	3.1 ± 0.3
C14:1	21.4 ± 1.6	58.1 ± 4.3	38.7 ± 2.9	ND	ND	ND
C16:0	13.5 ± 0.9	14.3 ± 2.1	10.4 ± 0.8	14.3 ± 1.2	22.8 ± 1.9	22.7 ± 2.1
C16:1Δ <sup>9</sup>	17.5 ± 1.2	ND	18.6 ± 2.1	62.0 ± 4.6	69.7 ± 5.3	62.0 ± 4.4
C16:1Δ <sup>11</sup>	12.3 ± 1.1	18.1 ± 1.3	ND	ND	ND	ND
C18:0	7.6 ± 0.6	6.2 ± 0.4	4.9 ± 0.3	5.8 ± 0.4	4.6 ± 0.6	7.1 ± 0.6
C18:1Δ <sup>9</sup>	20.1 ± 1.4	ND	24.3 ± 1.8	10.8 ± 0.9	ND	ND
C18:1Δ <sup>11</sup>	ND	ND	ND	1.0 ± 0.9	ND	ND
C18:1Δ <sup>13</sup>	0.5 ± 0.1	0.5 ± 0.1	ND	ND	ND	ND
C26:0	5.9 ± 0.4	2.7 ± 0.3	1.8 ± 0.2	5.1 ± 0.4	1.5 ± 0.2	5.2 ± 0.4
Ratio						
Unsat./sat.	2.7	3.3	4.5	2.9	2.3	1.9
C16:x/C18:x	1.5	4.8	1.0	4.3	20.1	11.9

<sup>a</sup> Cells were grown in C14:1Δ<sup>9</sup>- or C16:1Δ<sup>9</sup>-supplemented media to late exponential growth phase. Lipids were extracted and subjected to alkaline hydrolysis, and fatty acids were converted to methyl esters by BF<sub>3</sub>-catalyzed esterification. Fatty acid methyl esters were separated by gas chromatography as described in Materials and Methods. Ratios of unsaturated to saturated fatty acids (Unsat./sat.) and that of 16-carbon-atom-chain-length to 18-carbon-atom-chain-length fatty acids are indicated. Values represent means ± standard deviations of three independent determinations. ND, not detected.

degree that is more comparable to that exerted by a two-carbon increase in acyl chain length (11). While lipid phase properties are certainly not the sole parameters *in vivo*, additional variables such as charge, size, and polarity of the lipid head group; the intrinsic curvature of a lipid species; and even superlattice formation may all significantly affect the head group composition of cellular lipid bilayers (2, 28, 31).

**Analysis of the distribution of the elongation product in individual phospholipid classes.** Having analyzed the dependency of the phospholipid class composition on the acyl chain length of the supplemented unsaturated fatty acid, we tested whether incorporation of the C16:1Δ<sup>11</sup> elongation product displayed a preference for certain phospholipid classes. Therefore, the fatty acid composition of individual phospholipid classes, isolated from wild-type and *ole1Δ* and *elo1Δ* mutant cells cultivated in media supplemented with C14:1Δ<sup>9</sup>, was determined. As shown in Table 3, this analysis revealed that, in *ole1Δ* mutant cells, the C16:1Δ<sup>11</sup> elongation product was incorporated in all of the phospholipid classes, with the highest relative content in PtdCho (18.8%) and PtdEtn (15.8%), compared to PtdSer (8.7%) and PtdIns (6.1%). This enrichment

of unsaturated C16:1Δ<sup>11</sup> in order from PtdIns and PtdSer via PtdEtn to PtdCho parallels the general increase in the content of unsaturated acyl chains from PtdIns via PtdSer and PtdEtn to PtdCho observed both in C14:1-supplemented cells and in wild-type cells grown in rich medium (25, 34), an observation which suggests that the relative degree of acyl chain saturation of the different phospholipid classes is maintained even under conditions where the endogenous acyl coenzyme A pool is grossly altered.

The ratio of C16:1Δ<sup>9</sup> to C16:1Δ<sup>11</sup> in the different phospholipid classes, however, varied markedly. In wild-type cells, PtdSer and PtdEtn contained approximately fourfold-more C16:1Δ<sup>9</sup> than C16:1Δ<sup>11</sup>. In PtdCho and PtdIns, on the other hand, the ratio of C16:1Δ<sup>9</sup> to C16:1Δ<sup>11</sup> was close to 1. This apparent acyl chain preference of different phospholipid classes is particularly noteworthy in light of the fact that both PtdSer and PtdIns are synthesized from a common precursor, CDP-DAG. The observation that PtdEtn has a C16:1Δ<sup>9</sup>-to-C16:1Δ<sup>11</sup> ratio similar to that of PtdSer suggests that the bulk of PtdEtn is synthesized by decarboxylation of PtdSer (i.e., the *de novo* pathway). In contrast, the similarity in the C16:1Δ<sup>9</sup>-to-

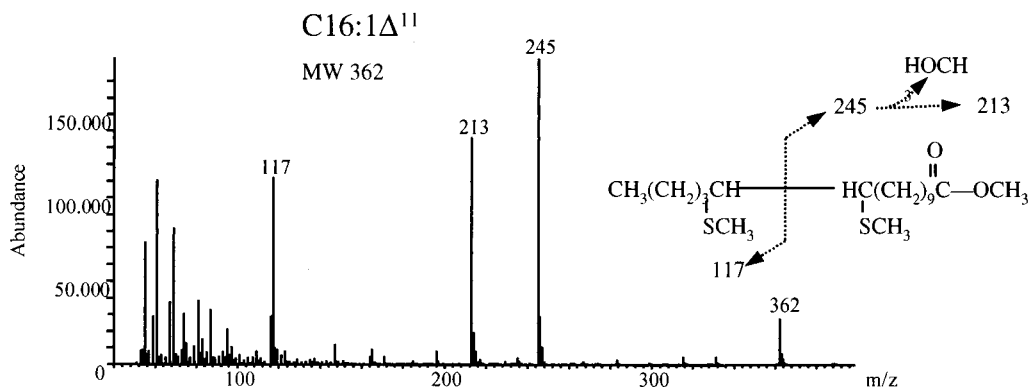


FIG. 2. Characterization of the double bond position present in the *ELO1*-dependent product of C14:1Δ<sup>9</sup>, C16:1Δ<sup>11</sup>. Dimethyl disulfide adducts of fatty acid methyl esters were prepared and analyzed as described in Materials and Methods.

TABLE 2. Comparison of the phospholipid composition of wild-type and *ole1Δ* and *elo1Δ* mutant cells cultivated in C14:1Δ<sup>9</sup>- and C16:1Δ<sup>9</sup>-supplemented media<sup>a</sup>

Phospholipid	Composition (mol%) of cell type in medium					
	Wild type		<i>ole1Δ</i>		<i>elo1Δ</i>	
	C14:1Δ <sup>9</sup>	C16:1Δ <sup>9</sup>	C14:1Δ <sup>9</sup>	C16:1Δ <sup>9</sup>	C14:1Δ <sup>9</sup>	C16:1Δ <sup>9</sup>
PtdSer	1.3 ± 0.1	3.1 ± 0.4	5.9 ± 0.9	1.6 ± 0.1	2.1 ± 0.2	1.3 ± 0.4
PtdEtn	74.5 ± 4.6	38.9 ± 2.2	36.7 ± 1.8	29.3 ± 3.7	42.6 ± 0.3	21.8 ± 4.2
PtdCho	17.9 ± 3.6	48.7 ± 1.8	27.0 ± 2.7	51.3 ± 0.8	50.4 ± 0.9	45.9 ± 6.2
PtdIns	3.7 ± 0.6	6.0 ± 0.4	8.2 ± 3.9	11.5 ± 0.7	2.6 ± 0.6	20.2 ± 2.3
PtdOH	1.3 ± 0.3	0.5 ± 0.1	14.2 ± 1.0	3.8 ± 2.9	0.6 ± 0.1	5.3 ± 1.4
CL <sup>b</sup>	1.4 ± 0.3	2.9 ± 0.6	7.9 ± 2.0	2.6 ± 0.8	0.6 ± 0.2	5.5 ± 2.9

<sup>a</sup> Cells were grown in either C14:1Δ<sup>9</sup>- or C16:1Δ<sup>9</sup>-supplemented media to late exponential growth phase. Lipids were extracted, and individual phospholipid classes were separated by two-dimensional thin-layer chromatography and quantified as described in Materials and Methods. Values represent means ± standard deviations of three independent determinations.

<sup>b</sup> CL, cardiolipin.

C16:1Δ<sup>11</sup> ratio of PtdCho and PtdIns suggests that, in this case, the bulk of PtdCho is synthesized through the Kennedy (CDP-choline) pathway, possibly by reutilizing molecular species of DAG that are produced from the turnover of PtdIns. Turnover of PtdIns is required for the maturation of sphingolipids, in which the synthesis of inositolphosphorylceramide from ceramide and that of mannosyl-diinositolphosphorylceramide from mannosylinositol-phosphorylceramide each consumes PtdIns and yields DAG (1). Interestingly, a recent study of the molecular species of PtdCho synthesized in hepatocytes either through the de novo methylation pathway or through the Kennedy pathway revealed that different molecular species of PtdCho are produced by the two pathways, with saturated species predominating in the Kennedy pathway and arachidonic-acid-containing species predominating in the methylation pathway (16). Remarkably, these two classes of PtdCho species appear to be functionally distinct, since overexpression of the PtdEtn *N*-methyltransferase-2 in conditional mutants of the CDP-choline pathway does not rescue the temperature-sensitive growth defect of these cells (19). A functional distinction of PtdCho synthesized by the de novo pathway from that formed by the Kennedy pathway is also evident in yeast, where attenuation of PtdCho synthesis via the Kennedy pathway, but not via the methylation pathway, can rescue the temperature-sensitive growth phenotype of *sec14* mutants (7, 20, 22).

Interestingly, the supplemented C14:1 was not detected in

PtdSer species of wild-type and *elo1Δ* mutant cells but was incorporated to 14.6% in PtdSer of *ole1Δ* cells (Table 3). This apparent exclusion of C14:1 from PtdSer in desaturation-competent cells is remarkable and may reflect the preference and/or restricted accessibility of PtdSer synthase for/to certain molecular species of CDP-DAG (12). In *ole1Δ* cells, on the other hand, this species preference appears to have been overcome, possibly due to an acute requirement of the cell to increase the degree of lipid unsaturation (Table 1).

**Conclusions.** Taken together, the present study provides evidence that the yeast *ELO1* gene is required for carboxy-terminal elongation of unsaturated fatty acids. Since the two very-long-chain elongases, Elo2p and Elo3p, are closely related to Elo1p, with more than 52% identity and 72% homology (24), it appears reasonable to suggest that these operate by a similar mechanism. The second important observation made in this study is that the exogenous addition of unsaturated fatty acids results in a dramatic alteration in the phospholipid composition, suggesting that acyl chain supply is a dominant parameter of the lipid composition. Altering the cellular acyl chain pool, however, appears not to affect the degree of acyl chain saturation characteristic of each phospholipid class (25, 34). The third and possibly most interesting observation is that the C16:1Δ<sup>9</sup>-to-C16:1Δ<sup>11</sup> ratio of individual phospholipid classes may be used to trace the biosynthetic origin of the corresponding lipid species. Experiments to validate this approach utiliz-

TABLE 3. Fatty acid composition of individual phospholipid classes from wild-type and *ole1Δ* and *elo1Δ* mutant cells cultivated in C14:1Δ<sup>9</sup>-supplemented media<sup>a</sup>

Fatty acid	Composition (mol%) of phospholipid from cell type											
	PtdSer			PtdEtn			PtdCho			PtdIns		
	Wild type	<i>ole1Δ</i>	<i>elo1Δ</i>	Wild type	<i>ole1Δ</i>	<i>elo1Δ</i>	Wild type	<i>ole1Δ</i>	<i>elo1Δ</i>	Wild type	<i>ole1Δ</i>	<i>elo1Δ</i>
14:1Δ <sup>9</sup>	ND	14.6	ND	8.0	36.6	20.1	24.5	42.8	37.2	11.1	27.8	7.4
16:0	42.3	72.2	50.8	18.5	44.4	19.6	17.0	22.7	15.3	27.3	37.5	31.3
16:1Δ <sup>9</sup>	12.2	ND	4.9	30.6	ND	27.8	17.6	ND	21.3	6.9	ND	6.1
16:1Δ <sup>11</sup>	2.7	8.7	ND	7.9	15.8	ND	15.8	18.8	ND	5.8	6.1	ND
18:0	3.2	4.5	4.2	1.7	2.5	1.3	9.0	15.8	7.8	26.4	28.0	32.7
18:1Δ <sup>9</sup>	39.6	ND	40.0	33.3	ND	31.2	16.1	ND	18.4	21.9	ND	22.1
Acyl chain saturation	45.5	76.7	55.0	21.0	46.9	20.9	26.0	38.5	23.1	53.7	65.5	64.0
16:x	57.2	80.9	55.7	57.0	60.2	47.4	50.4	41.5	36.6	40.0	43.6	37.4

<sup>a</sup> Cells were grown in C14:1Δ<sup>9</sup>-supplemented media to late exponential growth phase. Lipids were extracted, individual phospholipid classes were separated by two-dimensional thin-layer chromatography and subjected to alkaline hydrolysis, and fatty acids were converted to methyl esters by BF<sub>3</sub>-catalyzed methanolysis. Fatty acid methyl esters were separated by gas chromatography as described in Materials and Methods. Values represent means of two determinations with standard deviations between experiments of less than 10%. The degree of acyl chain saturation and that of acyl chains 16 carbon atoms in length (C16:x) are indicated. ND, not detected.

ing mutants in the de novo pathway and those defective in the Kennedy pathway are currently being performed.

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