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Fatty Acid Desaturase Mutants of Yeast: Growth Requirements and Electron Spin Resonance Spin-Label Distribution

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Two respiratory-sufficient and one respiratory-deficient (nuclear petite) strains of yeast Δ^9-desaturase mutants were analyzed to determine which fatty acids would serve as replacements for the naturally occurring fatty acids, 16:1 Δ^9 *cis* and 18:1 Δ^9 *cis*. The requirement can be satisfied by several fatty acids differing in double-bond position, steric configuration, chain length, and degree of unsaturation. The features common to growth-supporting fatty acids are presented and the effects of varying the carbon source and temperature are considered. In addition, we illustrate several pitfalls encountered in membrane studies which exploit lipid-requiring organisms. Since the membrane fatty acid composition of these mutants can be modified readily, electron spin resonance spectroscopy is used to compare membranes of mutant strains enriched for different fatty acids. The lipid distribution pattern of the most commonly employed electron spin resonance spin-label, 12-nitroxide stearate, was ascertained and compared to that of 18:1 Δ^9 *cis*.

Auxotrophic mutants affecting structural components provide valuable information on the relation between cellular structure and function. Prerequisite to research centering on the relevance of fatty acid composition to membrane structure, function, and biosynthesis is a knowledge of a mutant's fatty acid specificity. This study deals with the unsaturated fatty acid auxotrophs of yeast, *ole1* and *ole2* (7, 14, 21). Since these mutants are employed by several researchers interested in membrane phenomena, the present investigation aims to clarify a contradiction between earlier (21) and recent studies on KD115 (*ole1*), and elucidates the distribution of the electron spin resonance (ESR) spin-labeled fatty acid analogue, 12-nitroxide stearate (12NS), into phospholipids, free fatty acids, and neutral lipids.

MATERIALS AND METHODS

Organisms. A wild-type strain of *Saccharomyces cerevisiae*, S288C (Berkeley Collection), and three fatty acid desaturase mutants derived from it were the organisms employed in this study. Two independent mutant isolates, *ole1-1* (KD115) and *ole1-2* (KD20), are respiratory-sufficient and heteroallelic. The third mutant, *ole2* (KD46), is a nuclear petite and segregates independently of *ole1*. A general characterization of these mutants has already been presented (7, 14).

Chemicals. Stearolic acid (18:1$^{\Delta 9}$) was obtained from Lachat Chemicals, Inc., Chicago Heights, Ill. The fatty acids *cis*-Δ^5-octadecenoate (18:1 Δ^5 *cis*), *cis*-Δ^8-octadecenoate (18:1 Δ^8 *cis*), and *cis*-Δ^{12}-octadecenoate (18:1 Δ^{12} *cis*) synthesized by F. D. Gunstone were the gift of W. E. M. Lands. *Cis*-Δ^9-octadecenol (18:1 Δ^9 *cis*-ol) and *cis*-Δ^9-octadecenyl phosphate (18:1 Δ^9 *cis*-ol-PO_4) were provided by Alec D. Keith. *Cis*-Δ^9-tetradecenoate (14:1 Δ^9 *cis*) was purchased from Applied Science Laboratories, Inc., State College, Pa. 9,10-Methylene-*cis*-Δ^9-octadecenoic acid (9, 10-CH_2-18:1 Δ^9 *cis*) was obtained from H. W. Kircher. Methyl 18:1 Δ^9 *cis*, 12-acetoxy was made by reacting 5 ml of methyl ricinoleate with 100 ml of acetic anhydride at 100 C under vacuum. A $NaHCO_3$ solution was then added and the mixture was stirred overnight. Petroleum ether was used to extract the fatty acid fraction. Thin-layer chromatography with petroleum ether-diethyl ether (7:3 v/v) was employed to separate the product from unreacted materials and for purification. All other fatty acids were products of the Hormel Institute, Austin, Minn. The detergent Tergitol NP-40 was purchased from Union Carbide Corp., New York, N.Y.

The fatty acids obtained from the Hormel Institute and Applied Science Laboratories were >99% pure by their analyses. The purity of stearolic acid,

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stated as at least 95%, was found to be >99% by analytical gas-liquid chromatography of the methyl ester. The donated compounds were accepted as >98% pure.

Growth rate. Growth curves for the *ole1* mutants were obtained by inoculating Klett tubes containing 8 ml of 2×10^{-4} M fatty acid in minimal medium (0.67% yeast nitrogen base and 1% Tergitol plus either 2% glucose or 1% DL-sodium lactate) to a final concentration of about 10^6 cells/ml. Sodium lactate medium was brought to *p*H 5.8 to 6.5 with K_2HPO_4. For the petite *ole2* mutant, yeast nitrogen base was replaced by 0.3% yeast extract and the fatty acids were added at 10^{-4} M. The tubes were aerated on a rotor at constant speed in an incubator at 30 C. Klett readings (red filter) at suitable intervals were used to monitor the growth response. The correlation between Klett reading and cell number has been reported (21). A wild-type control (S288C) was grown under identical conditions for each fatty acid analyzed. Each experiment was duplicated at a later date. The distinction between growth-supporting and non-growth-supporting fatty acids was based on the above protocol.

Revertants. Samples were removed from liquid cultures at stationary phase and plated on growth-supporting medium. This medium, YEPD-80, consisted of 1% yeast extract, and 2% each of peptone, dextrose, agar, and Tween 80 [polyethelene (20) sorbitan monooleate]. After 48 hr at 30 C, colonies (100 to 200 per plate) were transferred by replica plating to YEPD-80 and YEPD-40 (polyethelene sorbitan monopalmitate). Colonies deficient in desaturase activity could be distinguished by their inability to grow on YEPD-40. Revertant colonies, like wild-type, grow readily on both Tween 40 and 80 media. These criteria are diagnostic for the growth patterns of mutant strains and those of wild-type or revertant strains.

Cultures containing ~98% revertants were subjected to fatty acid analysis. These originated from cultures inoculated with *ole1-1* to 10^6 cells/ml and then grown to stationary phase. These were assayed for revertants, pelleted, and subjected to procedures for gas-liquid chromatography. Since heterogeneous rather than single colony isolates were used, our fatty acid analyses could reflect the composition of several independent revertant lines. However, the results indicate that revertant cultures represent unique cell lines. This notion stems from the observation that cultures with high levels of 16:1 Δ^9 *cis* had no appreciable 18:1 Δ^9 *cis*. Other cultures exhibited the inverse profile.

Chromatography. Gas-liquid chromatography was performed on a Varian Aerograph model 600D analytical instrument. The analytical column was 0.125 inch by 8 ft (0.3 by 244 cm) stainless steel packed with 60/80 mesh Chromosorb W (acid-washed and coated with 15% diethylene glycol succinate).

Thin-layer chromatography of the methyl esters of fatty acids isolated from cells grown on *trans*-unsaturates was performed on 10% $AgNO_3$-impregnated silica gel plates with chloroform developer. The results were compared with those obtained from cells grown on the corresponding *cis*-analogues. This procedure was used for cells which grew on 16:1 Δ^9 *trans*, 18:2 $\Delta^{9,12}$ *trans,trans*, and 18:1 Δ^9 *trans*,12-hydroxy to verify that the fatty acids were not isomerized. Cells were obtained by centrifugation and washed twice (first with 1% Tergitol in water and second with water). Pellets were then saponified in 2 N KOH and methylated in HCl-methanol (7).

Tracer analysis. The radioactive compounds used were: [*1*-^{14}C]18:1 Δ^9 *cis*, specific activity 54.1 mCi/mmole, New England Nuclear Corp., Boston, Mass. and [*9, 10*-^3H]12NS, specific activity 16 mCi/mmole, synthesized and donated by J. C. Williams. For this analysis, wild-type S288C and *ole1-1* were grown at 30 C with agitation in 40 ml of minimal medium (0.67% yeast nitrogen base, 2% glucose, 1% Tergitol) supplemented with 2×10^{-4} M fatty acid. Petite *ole2* was grown on 1% yeast extract, 2% peptone, 2% glucose, 1% Tergitol supplemented with 2×10^{-4} M fatty acid. The four fatty acids used for supplementation were: 16:1 Δ^9 *cis*, 16:1 Δ^9 *trans*, 18:1 Δ^9 *cis*, and 18:1$^{\Delta^9}$. At the time of inoculation, 1.5 μCi ^3H-12NS was added to each flask. Also, to each flask containing 18:1 Δ^9 *cis* was added 1.5 μCi [*1*-^{14}C]18:1 Δ^9 *cis*. Stationary-phase pellets were harvested by centrifugation. The pellets were washed and centrifuged once with 1% Tergitol in water and once with water, and then they were suspended in 20 ml of chloroform-methanol (2:1, v/v) and stirred overnight. Cellular debris was removed by filtering through anhydrous Na_2SO_4. The Folch procedure (3) was employed on the filtrate to obtain a lipid extract for thin-layer chromatography. The procedure for resolving the lipids into phospholipids, free fatty acids, and neutral lipids has been described (8). In a second experiment, the phospholipids were further resolved into two classes: (i) phosphatidyl choline, and (ii) phosphatidyl ethanolamine plus phosphatidyl inositol by thin-layer chromatography with chloroform-methanol-water (65:25:4).

Radioactivity was monitored on a Beckman liquid scintillation spectrometer equipped with an external quenching standard. Toluene-2,5-diphenyloxazole was the counting fluid. To correct for overlap in vials containing both ^3H and ^{14}C, [*1*-^{14}C]18:1 Δ^9 *cis* was added and the vials were recounted. Since both channels detected additional counts originating only from the extra ^{14}C, the per cent ^{14}C counts read in the ^3H channel was calculated for each vial and the original data were properly adjusted. This and the following correction normalized quenching differences among the lipid classes. Counts per minute for ^3H were corrected and converted to disintegrations per minute by adding ^3H-toluene of known specific activity to each vial after the original counts were taken and recounting to measure the instrument's efficiency for each vial.

RESULTS

Figure 1 illustrates typical growth curves on glucose for the wild-type yeast, S288C, and the two respiratory-sufficient desaturase mutants derived from it, *ole1-1* and *ole1-2*. The doubling times of the two mutants on any growth-

supporting fatty acid are nearly identical, although *ole1-2* invariably has a longer lag than its heteroallelic counterpart, *ole1-1*. From the correlation between Klett reading and cell number (21), we calculated that, in the absence of a fatty acid, the mutants undergo about 2 doublings, and about 10 in the presence of a fatty acid which satisfies the growth requirement. Representative growth curves for *ole2*, the nuclear petite, are in Fig. 2. Both Fig. 1 and 2 show mutant growth on the *trans*-unsaturate, 16:1 Δ^9 *trans*.

The growth responses of *ole1-1* (both in early and recent experiments), *ole1-2*, and the petite

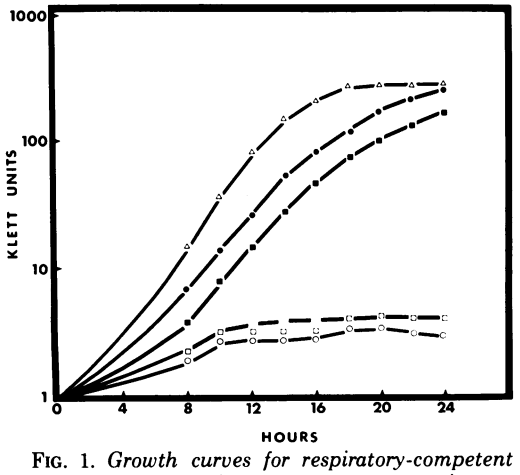

FIG. 1. *Growth curves for respiratory-competent desaturase mutants of yeast on 0.67% yeast nitrogen base plus 2% glucose. Symbols: △, wild-type S288C on 16:1 Δ^9 trans; ●, ole1-1 on 16:1 Δ^9 trans; ■, ole1-2 on 16:1 Δ^9 trans; ○, ole1-1 on minimal medium; and □, ole1-2 on minimal medium.*

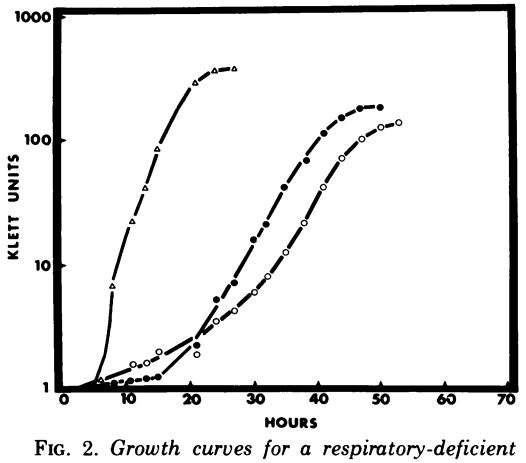

FIG. 2. *Growth curves for a respiratory-deficient desaturase mutant of yeast on 1% yeast extract-2% peptone plus 2% glucose. Symbols: △, wild-type S288C; ●, ole2 on 16:1 Δ^9 trans; and ○, ole2 on 16:1 Δ^9 cis.*

ole2 as a function of various fatty acid supplementations are summarized in Table 1. For comparison, the growth responses of several other lipid requirers were included. The *Neurospora cel* mutant and the yeast *fas1* mutant require saturated fatty acids; the *Escherichia coli* mutant and anaerobic yeast are deficient in desaturase activity. Generally, the fatty acid specificities of *ole1* and *ole2* are identical. However, a few fatty acids, supportive to less than wild-type growth levels for *ole1*, failed to support the growth of *ole2*. Whether this indicates that 10^{-4} M was not an optimal concentration of these fatty acids for *ole2* or an actual difference in the fatty acid specificity between respiratory-sufficient and petite strains is not clear. It was noted that the petite *ole2* grew less vigorously on 18:1$^{\Delta 9}$ in 0.3% yeast extract medium than in 1% yeast extract, 2% peptone medium. Thus, the discrepancies between *ole1* and *ole2* may be attributable to medium composition effects.

The growth responses of anaerobic yeast parallel those of the *ole* mutants. There is also good agreement between the *E. coli* and yeast desaturase mutants in terms of fatty acid specificity. Two exceptions, fatty acids 18:1 Δ^9 *trans* and 18:1 Δ^{11} *trans*, support only the growth of *E. coli*. The fatty acids 12:0, 14:0, and 16:0 do not support the growth of the *ole* mutants; as expected, they satisfy the growth requirements of yeast fatty acid synthetase mutants and the corresponding chain elongation mutant of *Neurospora*. The features of a growth-supporting fatty acid for yeast desaturase mutants appear to be (i) a double bond in the *cis*-configuration at either the Δ^5, Δ^6, Δ^9, or Δ^{11} position (data on Δ^5-fatty acids indicates a dependence on chain length) or (ii) a triple bond at the Δ^9 position or (iii) the Δ^9 *trans* double bond of 16:1 Δ^9 *trans*, 18:2 $\Delta^{9,12}$ *trans, trans*, and to some extent 18:1 Δ^9 *trans*, 12-hydroxy (18:1 Δ^9 *trans* did not support growth). In the studies involving yeast grown on *trans*-components, no detectable isomerization of the *trans*-compounds could be demonstrated by thin-layer chromatography.

All of the above-mentioned growth studies were carried out with 2% glucose as the carbon source. Additional tests of *ole1-1* and *ole1-2* utilized 1% DL-sodium lactate, a nonfermentable substrate. With lactate as a carbon source, a larger proportion of the yeast membranes are mitochondrial in nature. Under these conditions, the fatty acid requirements did not change (Table 1). However, for wild-type S288C, growth was enhanced about 6- to 12-fold over the comparable control when the

TABLE 1. *Fatty acid growth response*[a]

Fatty acid supplement	Anaerobic yeast[b]	*Escherichia coli*[c]	*ole1-1* (KD115) Early[d]	*ole1-1* (KD115) Recent	*ole1-2* (KD20)	*ole2* (KD46)	*ole1-1* (KD115) Lactic acid	*ole1-2* (KD20) Lactic acid	*Neurospora cel*[e]	Yeast mutant *fas1*[f]
12:0				O		O			<+	<+
14:0			O	O		O			+	+
15:0									+	+
16:0			O	O		O			+	+
17:0									+	+
18:0			O	O		O			O	O
19:0									O	
20:0									O	
14:1 Δ^5 *cis*		+	O	+		+			O	
14:1 Δ^9 *cis*		+	+	+	+	+	+	+	O	O
16:1 Δ^9 *cis*		+	+	+	+	+	+	+	O	O
16:1 Δ^9 *trans*		+		+	+	+	+	+		
18:1 Δ^5 *cis*				O						
18:1 Δ^6 *cis*		+	O/−	+		+	+	+	O	
18:1 Δ^8 *cis*				O[g]						
18:1 Δ^9 *cis*	+	+	+	+	+	+	+	+	O	O
18:1 Δ^9 *trans*	O	+	O	O		O			<+	O
18:1 Δ^{11} *cis*	+	+	O/−	+		+	+	+	O	
18:1 Δ^{12} *cis*				O						
18:1 Δ^{11} *trans*		+	O	O		O				
18:1 $\Delta^{9,12}$ *cis, cis*		+	+	+	+	+	+	+	O	O
18:2 $\Delta^{9,12}$ *trans, trans*			O/−	+		+				
18:3 $\Delta^{6,9,12}$ *cis, cis, cis*				+		+				
18:3 $\Delta^{9,12,15}$ *cis, cis, cis*			+	+	+	+	+	+	O	O
20:1 Δ^5 *cis*		O	O							
20:1 Δ^{11} *cis*		+	O	+		O				
22:1 Δ^{13} *cis*		O	O	O		O				
24:1 Δ^{15} *cis*		O	O/−	O	O	O	O	O		
20:2 $\Delta^{11,14}$ *cis, cis*		+		<+		O			O	
20:3 $\Delta^{11,14,17}$ *cis, cis, cis*		+		<+		O				
20:4 $\Delta^{5,8,11,14}$ *cis, cis, cis, cis*			O	+		+				
18:1 Δ^9 *cis*-ol				O	O					
18:1 Δ^9 *cis*-ol-PO_4				O						
18:1^{-9}	+			+		+				O
18:1 Δ^9 *cis*, 12OH		+		+		O				
18:1 Δ^9 *trans*, 12OH				<+		O				
18:1 Δ^9 *cis*, 12-Acetoxy				O	O		O	O		
9,10-CH_2-18:1 Δ^9 *cis*				O						

[a] Symbols: +, essentially wild-type growth; <+, less than wild-type growth (growth rate ≥0.5 of wild-type's); O, no growth; O/−, no growth and inhibitory to wild type.

[b] Data from Bloch and co-workers (10–12; K. Bloch et al., Fed. Proc. **20**:921–927).

[c] Data from Silbert et al. (18).

[d] Data from Wisnieski et al. (20).

[e] Data from Henry and Keith (5).

[f] Data from Henry and Keith (Chem. Phys. Lipids, *in press*).

[g] Growth occurred after a 48-hr lag, with a doubling time of about 17 hr. Growth was not revertant as verified by replica plating to YEP plus Tween 80 (18:1 Δ^9 *cis*) and YEP plus Tween 40 (16:0). No colonies grew on the latter medium.

minimal medium containing lactate was supplemented with a fatty acid (Fig. 3). Wild-type growth was also facilitated by 24:1 Δ^{15} *cis*, a fatty acid which did not support the growth of mutant strains.

Table 2 presents the fatty acid composition of *ole1-1* grown on 18:3 $\Delta^{9,12,15}$ *cis, cis, cis* and on 18:1^{-9}. Growth on 2×10^{-4} M 18:3 resulted in about 72% enrichment for this fatty acid in *ole1-1* for the conditions shown and also for the other test conditions mentioned with regard to 18:1^{-9}. In some unrecorded trials, up to 85% enrichment was attained. With 18:1^{-9}, however, substantial enrichment occurred on the yeast nitrogen base medium with lactate as the carbon source, but not with

glucose. Since we occasionally noted a greater 18:1$^{\Delta 9}$ enrichment on yeast extract-peptone with glucose, we varied the concentration of the fatty acid in the complex yeast extract-peptone-glucose medium and found that 10^{-3} M 18:1$^{\Delta 9}$ provided about 53% enrichment at 30 C, and 75% at 18 C. Unexplained is the phenomenon that when cells growing on 18:1$^{\Delta 9}$ are shifted to 18 C, they clump into fairly uniform-sized aggregates (~1 mm) but continue to grow. The aggregates dissociate when the temperature is restored to 30 C. No clumping occurred with the 18:3-grown cells.

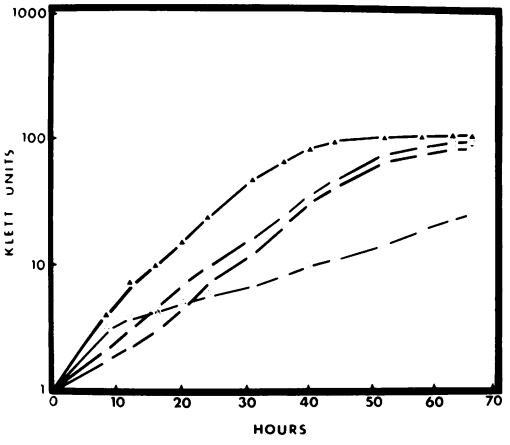

FIG. 3. *Growth curves for respiratory-competent desaturase mutants of yeast on 0.67% yeast nitrogen base plus 1% lactate. Symbols: △, wild-type S288C on minimal medium; ▲, wild-type S288C on 14:1 Δ^9 cis; □, ole1-2 on 14:1 Δ^9 cis; ○, ole1-1 on 14:1 Δ^9 cis.*

Also shown in Table 2 are the fatty acid compositions of several revertants. None demonstrated completely normal desaturase activity: revertants produced either 16:1 Δ^9 *cis* (rev$_1$) or 18:1 Δ^9 *cis* (rev$_{2,3,4}$), though wild-type S288C from which the mutants were derived produces about 24% 16:1 and about 50% 18:1 when grown on minimal medium. Thus, the revertants probably represent the effects of suppressors or second-site mutations. These alternatives will be resolved by genetic analysis.

Table 3 shows the lipid distribution pattern of ^{3}H-12NS in *ole1-1*, petite *ole2*, and wild-type S288C. Both *ole1-1* and wild-type vary in the distribution of 12NS with fatty acid supplementation in the same way, whereas petite *ole2* exhibits a different pattern. The similarity between *ole1-1* and wild-type does not extend to the distribution of ^{14}C-18:1 Δ^9 *cis*. Here, the two desaturase mutants, *ole1-1* and petite *ole2*, are more similar as expected on the basis of their mutual requirement for unsaturated fatty acids. The distribution of 12NS into the major phospholipid classes of yeast (phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol) grown on several fatty acid supplements is recorded in Table 4. Now, wild-type and *ole1-1* show a similar distribution pattern for 12NS and 18:1 Δ^9 *cis*; *ole2* shows a unique distribution. Tables 3 and 4 demonstrate that 12NS is incorporated into the phospholipids, free fatty acids, and neutral lipids of a respiratory-com-

TABLE 2. *Fatty acid composition of ole1-1, ole1-1 revertants, and wild-type S288C*

Strain	Fatty acid supplement[a]	Growth conditions[b]	Per cent fatty acid composition						
			16:0	16:1	18:0	18:1	18:2	18:3	18:1$^{\Delta 9}$
ole1-1	2×10^{-4} M 18:3	30, YEP glucose	20.98	0.39	6.22	0.85	0	71.56	0
ole1-1	2×10^{-4} M 18:3	18, YEP glucose	19.62	0.98	4.98	1.72	0.27	72.43	0
ole1-1	10^{-3} M 18:1$^{\Delta 9}$	30, YEP glucose	38.21	1.65	5.23	1.82	0	0	53.09
ole1-1	10^{-3} M 18:1$^{\Delta 9}$	18, YEP glucose	18.30	1.09	3.05	1.81	0	0	75.75
ole1-1	2×10^{-4} M 18:1$^{\Delta 9}$	30, YNB lactate	28.73	2.75	8.97	2.11	1.53	0	55.91
ole1-1	2×10^{-4} M 18:1$^{\Delta 9}$	18, YNB lactate	28.82	3.65	12.29	2.04	2.03	0	51.17
ole1-1	2×10^{-4} M 18:1$^{\Delta 9}$	30, YNB glucose	64.74	7.76	12.38	6.42	0	0	8.68
ole1-1	2×10^{-4} M 18:1$^{\Delta 9}$	30, YEP glucose	57.22	3.50	10.22	3.90	1.83	0	23.33
ole1-1 rev$_1$	2×10^{-4} M 18:3	18, YNB glucose	12.05	58.63	3.48	4.52	0	21.32	0
ole1-1 rev$_2$	2×10^{-4} M 18:3	18, YNB lactate	35.20	0	6.12	26.82	0	31.86	0
ole1-1 rev$_3$	2×10^{-4} M 18:1$^{\Delta 9}$	30, YNB glucose	32.16	1.54	7.70	51.33	3.63	0	3.64
ole1-1 rev$_4$	2×10^{-4} M 18:1$^{\Delta 9}$	30, YEP glucose	14.18	4.13	3.33	69.37	2.26	0	6.73
S288C	None	30, YNB glucose	15.92	24.06	8.83	50.17	1.02	0	0
S288C (7)[c]	Tween 80	30, YEP glucose	15.0	11.9	8.3	64.4	0	0	0

[a] 18:3 is 18:3 Δ^{9,12,15} *cis,cis,cis* and 18:1$^{\Delta 9}$ is stearolic acid.

[b] YEP, 1% yeast extract, 2% peptone; YNB, 0.67% yeast nitrogen base. Either 2% glucose or 1% lactate was the carbon source. Growth was at 30 C or at 18 C after limited growth at 30 C to about 10 Klett units.

[c] Distribution is based on per cent incorporation of [*1-*^{14}*C-*] acetate.

TABLE 3. *Distribution of [9,10-^{3}H]12-nitroxide stearate and [1-^{14}C]18:1 Δ^9 cis into yeast lipids*

Fatty acid supplement	Tracer	Yeast strain	Lipid class[a]	dpm	Per cent distribution
16:1 Δ^9 *trans*	^{3}H-12NS	*ole2*	PL	19,253	19.8
			FFA	35,044	36.0
			NL	43,111	44.3
18:1$^{\equiv 9}$	^{3}H-12NS	*ole2*	PL	16,098	20.5
			FFA	12,322	15.7
			NL	50,069	63.8
18:1 Δ^9 *cis*	^{3}H-12NS	*ole2*	PL	88,055	52.3
			FFA	12,611	7.5
			NL	67,721	40.2
16:1 Δ^9 *cis*	^{3}H-12NS	*ole1-1*	PL	2,293	14.9
			FFA	3,003	19.5
			NL	10,086	65.6
16:1 Δ^9 *trans*	^{3}H-12NS	*ole1-1*	PL	2,150	11.1
			FFA	2,931	15.1
			NL	14,364	73.9
18:1$^{\equiv 9}$	^{3}H-12NS	*ole1-1*	PL	2,281	10.4
			FFA	3,035	13.9
			NL	16,541	75.7
18:1 Δ^9 *cis*	^{3}H-12NS	*ole1-1*	PL	5,605	25.5
			FFA	2,143	10.3
			NL	14,095	64.2
16:1 Δ^9 *cis*	^{3}H-12NS	S288C	PL	3,183	16.0
			FFA	2,393	12.0
			NL	14,340	72.0
16:1 Δ^9 *trans*	^{3}H-12NS	S288C	PL	2,968	14.8
			FFA	2,884	14.4
			NL	14,178	70.8
18:1 ^{9}	^{3}H-12NS	S288C	PL	1,549	13.6
			FFA	1,706	14.9
			NL	8,164	71.5
18:1 Δ^9 *cis*	^{3}H-12NS	S288C	PL	4,476	22.7
			FFA	2,610	13.2
			NL	12,618	64.0
18:1 Δ^9 *cis*	^{14}C-18:1 Δ^9 *cis*	*ole2*	PL	473,096 cpm[b]	70.6
			FFA	6,644 cpm	1.0
			NL	190,592 cpm	28.4
18:1 Δ^9 *cis*	^{14}C-18:1 Δ^9 *cis*	*ole1-1*	PL	31,695 cpm	69.4
			FFA	2,398 cpm	5.3
			NL	11,581 cpm	25.4
18:1 Δ^9 *cis*	^{14}C-18:1 Δ^9*cis*	S288C	PL	28,503 cpm	44.6
			FFA	3,478 cpm	5.4
			NL	31,932 cpm	50.0

[a] PL, phospholipid; FFA, free fatty acid; and NL, neutral lipid.
[b] Counts per minute.

petent desaturase yeast mutant, a nuclear petite desaturase mutant, and a wild-type yeast. Although the respiratory-competent strain and wild-type generally show the same distribution of 12NS, the data indicate that 12NS is incorporated by cells as a unique fatty acid compared to 18:1 Δ^9 *cis*. Therefore, its distribution pattern may not be comparable to any other fatty acid.

DISCUSSION

In an earlier attempt to determine the specificity of the fatty acid requirement for the yeast desaturase mutant *ole1-1* (KD115), we assayed several unsaturated and branched-chain fatty acids for their ability to support growth (21). At that time, we found growth occurred only with fatty acids containing a Δ^9

TABLE 4. *Distribution of [9,10-^{3}H]12-nitroxide stearate and [^{14}C]18:1 Δ^9 cis into phospholipids*

Fatty acid supplement	Tracer	Yeast strain	Lipid class	dpm	Per cent distribution
16:1 Δ^9 *cis*	^{3}H-12NS	*ole2*	PC[a]	4670	83
			PE+PI	963	17
16:1 Δ^9 *trans*	^{3}H-12NS	*ole2*	PC	1,737	70
			PE+PI	761	30
18:2$^{\Delta 9}$	^{3}H-12NS	*ole2*	PC	2,197	82
			PE+PI	499	18
18:1 Δ^9 *cis*	^{3}H-12NS	*ole2*	PC	8,083	93
			PE+PI	602	7
16:1 Δ^9 *cis*	^{3}H-12NS	*ole1-1*	PC	1,173	58
			PE+PI	841	42
16:1 Δ^9 *trans*	^{3}H-12NS	*ole1-1*	PC	1,080	58
			PE+PI	794	42
18:1$^{\Delta 9}$	^{3}H-12NS	*ole1-1*	PC	703	48
			PE+PI	757	52
18:1 Δ^9 *cis*	^{3}H-12NS	*ole1-1*	PC	1,303	37
			PE+PI	2,193	63
16:1 Δ^9 *cis*	^{3}H-12NS	S288C	PC	2,339	57
			PE+PI	1,770	43
16:1 Δ^9 *trans*	^{3}H-12NS	S288C	PC	1,330	68
			PE+PI	613	32
18:1$^{\Delta 9}$	^{3}H-12NS	S288C	PC	1,230	49
			PE+PI	1,296	51
18:1 Δ^9 *cis*	^{3}H-12NS	S288C	PC	1,260	46
			PE+PI	1,513	54
18:1 Δ^9 *cis*	[*1*-^{14}C]18:1 Δ^9 *cis*	*ole2*	PC	71,106 cpm[b]	99
			PE+PI	385 cpm	1
18:1 Δ^9 *cis*	[*1*-^{14}C]18:1 Δ^9 *cis*	*ole1-1*	PC	5,272 cpm	49
			PE+PI	5,568 cpm	51
18:1 Δ^9 *cis*	[*1*-^{14}C]18:1 Δ^9 *cis*	S288C	PC	1,799 cpm	50
			PE+PI	1,819 cpm	50

[a] PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; and PI, phosphatidyl inositol.
[b] Counts per minute.

cis double bond (Table 1). However, in subsequent experiments with fatty acids obtained mainly from the Hormel Institute, the results differed significantly. Several other double-bond positions were found adequate for growth. Faced with this contradiction, we undertook a more comprehensive study to determine which fatty acids would satisfy *ole1-1*, its allelic counterpart *ole1-2*, and the nonallelic *ole2*, a nuclear petite; no essential differences were detected (Table 1). All three mutants responded similarly to each fatty acid tested. Furthermore, no growth inhibition of wild-type was observed for any fatty acid employed in the present investigation.

We believe that the earlier *ole1-1* data do not reflect manipulative or experimental errors, but rather mirror the presence of toxic impurities or oxidative cleavage products (e.g., short chain peroxides) in some of the fatty acids employed. This belief stems from the observation that the Hormel counterparts of several fatty acids previously found to inhibit wild-type growth do not exhibit this property. To test our hypothesis, we selected for re-evaluation several fatty acids used in the earlier studies. The criteria for selection were (i) failure to support mutant growth and inhibition of wild-type growth response in previous experiments (21) and (ii) mutant growth and no inhibition of wild-type growth with equivalent Hormel counterparts. No growth occurred. Furthermore, when samples were removed and plated to growth-supporting medium, no surviving cells were detected. These observations confirm the toxicity hypothesis and render improbable the alternate notion that the original *ole1-1* isolate carried an incidental permeability barrier or other metabolic restriction which was subsequently lost by mutation or

suppression.

The specificity of the fatty acid requirement of several biosynthetic mutants is shown in Table 1. The exact basis of any organism's specificity, however, is still unknown. It may result from (i) certain physical properties of the fatty acid or its acyl derivatives, (ii) aspects of membrane structure, function, or biosynthesis, (iii) the range in specificity of various enzymes, such as permeases and acylases, or (iv) some combination of the above factors. Current data on the yeast desaturase mutants indicate that fatty acid specificity is unrelated to respiratory competence. Thus, *ole2*, a petite mutant, and the two respiratory-sufficient mutants, *ole1-1* and *ole1-2*, responded similarly to the various fatty acid supplements. Moreover, among respiratory-sufficient strains, the results from growth studies on 1% lactate (a nonfermentable substrate) paralleled those on 2% glucose.

Our recent studies further establish that the *cis*-configuration is not an essential structural requirement for yeast (Fig. 1 and 2, Table 1). Several *trans*-unsaturates were growth-supporting, but the 18-carbon *trans*-fatty acid, 18:1 Δ^9 *trans* (melting point 45 C) was not. Esfahani, Barnes, and Wakil (2), employing the *E. coli* desaturase auxotroph, reported that at 37 C, 18:1 Δ^9 *trans* supports growth and is incorporated into phospholipids at levels higher than those attained with 18:1 Δ^9 *cis*. With 18:1 Δ^9 *trans*, a shift to 27 C depressed viability and caused cell lysis. Their observations suggest a correlation between the optimal growth temperature of an organism (yeast, about 30 C; *E. coli*, about 37 C) and the melting points of the fatty acids supportive to the organisms's growth. Such a correlation would account for the difference between yeast and *E. coli* in ability to grow on 18:1 Δ^9 *trans*. Our results with yeast are essentially consistent with this melting point hypothesis. One exception is 18:$1^{\equiv 9}$. Although this acetylenic fatty acid has a melting point of about 45 C, it supports vigorous growth. Likewise, 18:$1^{\equiv 9}$ satisfies the need for unsaturated fatty acids in anaerobic yeast (11). These results may reflect an intrinsic difference between the melting points of phospholipids containing 18:$1^{\equiv 9}$ and those containing 18:1 Δ^9 *trans*. Although structurally similar to saturated fatty acids, both 18:$1^{\equiv 9}$ and 18:1 Δ^9 *trans* failed to satisfy the growth requirement of the yeast saturated fatty acid auxotroph, *fas1* (Table 1).

As illustrated in Table 2, *ole1-1* cells grown on 18:$1^{\equiv 9}$ at 30 C have less of this fatty acid than when grown at 18 C. This result seems unexpected since the phase transition for the membrane lipids of 18:$1^{\equiv 9}$-enriched cells occurs at around 25 C (*unpublished data*). Apparently, the cell at 18 C, to maximize membrane fluidity, merely incorporates 18:$1^{\equiv 9}$ at the expense of its own saturates, 16:0 and 18:0, characterized by even higher melting points. The observation that cells grown on 18:$1^{\equiv 9}$ clump at 18 C may also be related to lipid-phase transitions. Arrhenius plots of the ESR motion parameter, τ_c, versus temperature indicate a phase transition at about 25 C for mutants grown on 18:$1^{\equiv 9}$. By growing cells on this fatty acid at 30 C and then shifting to various temperatures from 18 to 30 C, the highest temperature at which clumping occurs may correspond to 25 C. If this correlation can be extended to mutants grown on other fatty acids, a simple method for measuring lipid-phase transitions in this organism may be established. Comparisons between membranes of cells at temperatures above and below this transition provide clues to the role of fatty acids in membranes. Experiments designed to monitor membrane alterations in these mutants as a function of fatty acid supplement and growth temperature are in progress.

ESR spectroscopy is frequently used to monitor and analyze physical alterations of membrane components. A fatty acid analogue, 12NS, is an ESR spin-label designed to transmit signals reflecting information about the physical features of its immediate molecular environment. It has been shown that 12NS is incorporated into the phospholipids, free fatty acids, and neutral lipids of *Neurospora crassa* during growth and can be found in these lipid classes in the isolated mitochondria (8). The distribution of 12NS was determined by signal content. Since the signal is destroyed by *Neurospora* and isolated mitochondria at easily measurable rates (6, 8), some uncertainty attaches to the actual distribution of the 12NS molecule. However, by using a radioactive form of this spin-label, we have determined that it is incorporated into the lipids of a respiratory-competent yeast desaturase mutant, a petite desaturase mutant, and a wild-type strain. But the distribution pattern did not resemble that of the naturally occurring fatty acid 18:1 Δ^9 *cis*. Consequently, ESR spectra from cells spin-labeled in vivo with 12NS may reflect the physical features of a lipid environment which is largely nonmembranous. Nevertheless, with proper controls, ESR and spin-labeled fatty acids are valuable tools for probing certain physical aspects of membrane components.

The yeast desaturase mutants are assignable to a broader category of lipid-requiring organisms. Falling into this category are the desaturase mutants of yeast (7, 14, 21) and *E. coli* (18, 19), and the chain elongation mutants of yeast (16, 17; S. A. Henry and A. D. Keith, Chem. Phys. Lipids, *in press*) and *Neurospora* (5, 9, 13). Included also are several strains of *Mycoplasma* (4, 15) and certain conditional lipid requirers exemplified by yeast grown anaerobically (1, 10–12; K. Block et. al., Fed. Proc., **20**:921–927) or in biotin-deficient medium (20). Our experiences with the yeast desaturase mutants emphasize that several critical factors must be considered in utilizing these or similar mutants for membrane studies. Unless certain precautions are taken, the data obtained may readily lead to inaccurate or over-simplified conclusions.

We have already demonstrated the significant effect of toxic impurities in fatty acids used in growth studies even though the compounds were judged to be >99% pure. Other factors that might influence the results obtained with these organisms relate to the evaluation of growth studies. The term "no growth" must be qualified. If a compound fails to support growth, it does not necessarily imply that a certain fatty acid cannot support growth at some other concentration, temperature, etc. For example, *ole1-1* grew on the fatty acid 18:2 $\Delta^{9,12}$ *cis*, *cis* at 10^{-4} M with a doubling time of 1.4 hr. But, at 10^{-3} M, the doubling time was increased to >24 hr. Essentially identical results were obtained with 14:1 Δ^{9} *cis*. On the other hand, 10^{-3} M 18:1 Δ^{9} *cis* was optimal; 10^{-5} M led to a 24-hr doubling time (21). Recently, when tubes containing *ole1-1* on the fatty acids 18:1 Δ^{5} *cis*, 18:1 Δ^{8} *cis*, and 18:1 Δ^{12} *cis* (YNB and 2% glucose) were incubated for 48 hr beyond the normal 24-hr test period, a weak growth response was recorded and verified on 18:1 Δ^{8} *cis*. However, the response in Table 1 is given as negative based on the 24-hr test period.

A misconception common to investigations of lipid-requiring organisms concerns fatty acid enrichment. Maximum enrichment for a particular fatty acid may not be assumed merely on the basis of a mutant's growth curve which mimics wild type. All *ole1-1* growth curves on 18:1$^{\Delta 9}$ subjected to the growth conditions in Table 2 approximated wild type; however, maximum enrichment for 18:1$^{\Delta 9}$ depended not on concentration and temperature alone, but also on carbon source and medium composition.

Finally, the complexities of investigating the role of fatty acids in biomembranes are typified by the observation that 24:1 Δ^{15} *cis* though incompatible with growth of desaturase mutants still facilitated an increase in wild-type growth response on lactate medium. This result may indicate some "sparing effect" whereby the incorporation of 24:1 Δ^{15} *cis* into noncritical lipid positions (e.g., into neutral lipids) releases endogenous unsaturates for incorporation elsewhere. The broader observation that unsaturates significantly enhance the growth of wild-type on lactate medium implies that fatty acid desaturation is growth-limiting for aerobic yeast. It is likely that the rate of mitochondria formation depends on the availability of unsaturated fatty acids.

Hopefully, an awareness of the pitfalls encountered in biomembrane research will minimize their occurrence and facilitate critical evaluation.

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