Use of an unsaturated fatty acid auxotroph of *Saccharomyces cerevisiae* to modify the lipid composition and function of mitochondrial membranes

Beatrice S. Tung,* Elizabeth R. Unger,[1,*] Bernard Levin,[2,*,†] Thomas A. Brasitus,[3,†] and Godfrey S. Getz[4,*,†,]**

Departments of Pathology,* Medicine,† and Biochemistry,** The University of Chicago, Chicago, IL 60637

Abstract KD115 (ol_1), an unsaturated fatty acid auxotroph of *S. cerevisiae,* was grown in a semi-synthetic medium supplemented with 3.3×10^{-4} M palmitoleic (*cis* 16:1) or palmitelaidic (*trans* 16:1) acids. The parent strain S288C was studied as a control. The lipid composition (fatty acids, neutral lipids, and phospholipids), respiratory activity (O_2 consumption), and ultrastructure were compared in mutant yeast grown with each unsaturated fatty acid supplement. The fatty acid supplement represented 70–80% of the yeast fatty acids. Yeast grown in *trans* 16:1 contained more squalene, a higher ratio of phosphatidylethanolamine (PE) to phosphatidylcholine (PC), and had 10–20% of the respiratory activity compared to the same yeast grown in *cis* 16:1. The mitochondrial morphology of yeast in each growth supplement was notably different. The use of mixtures of *cis* and *trans* 16:1 in different proportions revealed that the PE/PC ratio, the squalene content, the respiratory defect, and the mitochondrial morphology were all similarly dependent on the fraction of *trans* 16:1 in the mixtures. As little as 10–20% of *cis* 16:1 in the mixture was sufficient to abrogate the physiological effects of *trans* 16:1 on each of the parameters noted above. The combined effects of high content of trans unsaturated fatty acid and the altered phospholipid composition seem to account for the decrease in lipid fluidity, the defective structure and function of the mitochondrial membrane.—**Tung, B. S., E. R. Unger, B. Levin, T. A. Brasitus, and G. S. Getz.** Use of an unsaturated fatty acid auxotroph of *Saccharomyces cerevisiae* to modify the lipid composition and function of mitochondrial membranes. *J. Lipid Res.* 1991. **32:** 1025–1038.

Supplementary key words palmitoleic acid • palmitelaidic acid • respiration • phospholipids • phosphatidylcholine • phosphatidylethanolamine • squalene • fluidity

The fluid lipid bilayer is widely accepted as the core of biological membranes. Amphipathic phospholipids stabilize the bilayer structure with which proteins are associated. The fatty acyl chains of phospholipids contribute the hydrophobic domain that is associated with intrinsic membrane proteins and which plays a role in the maintenance of selective permeability. The chain length and degree of unsaturation of the fatty acids influences the degree of order and "fluidity"[5] of the membrane under a variety of environmental circumstances. In addition, the polar head groups of phospholipids as well as the sterol content of membranes may have an impact on membrane fluidity (1, 2). The fluidity of the membrane permits certain enzymes and other membrane proteins to undergo the conformational and translational changes needed for the expression of their biological activity. In addition to facilitating the function of already assembled intrinsic membrane proteins, membrane physical properties may also be crucial in regulating the proper integration, stabilization and/or rates of synthesis of intrinsic membrane proteins. This has been exemplified in studies of the assembly of bacteriophage PM2 (3) and *E. coli* inner membrane (4).

This study describes the development and initial characterization of an in vivo model system that allows selective modification of the hydrophobic domain of mem-

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DSPC, distearoylphosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TLC, thin-layer chromatography; TTC, 2,3,5-triphenyl tetrazolium chloride; UFA, unsaturated fatty acid; YPD, medium containing 1% yeast extract, 2% Bactopeptone, and 1% glucose; YPDT80 or YPDT40, YPD containing 1% Tween 80 or Tween 40. Fatty acids are abbreviated by the convention, number of carbon atoms:number of double bonds.

[1]Present address: Department of Pathology, Emory University, Atlanta, GA.

[2]Present address: Department of Medical Oncology, M. D. Anderson Hospital and Tumor Institute, The University of Texas System Cancer Center, Houston, TX.

[3]Present address: Department of Medicine, University of Chicago.

[4]To whom reprint requests should be addressed at: Department of Pathology, Box 414, The University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637.

[5]The term "lipid fluidity" as applied to anisotropic bilayer lipids is used to denote the relative motional freedom of the lipid molecules or substituents thereof. A more detailed description of the sense in which the term is used is given in reference 50.

branes. The model system utilizes a respiratory competent unsaturated fatty acid auxotroph of *Saccharomyces cerevisiae,* KD115, isolated by Resnick and Mortimer (5). Because the yeast strain is unable to synthesize unsaturated fatty acids (UFA), growth in a defined medium with known UFA supplements allows manipulation of the hydrophobic environment. Several investigators (6–15) have determined the structural requirements of fatty acids that may serve as growth factors, as well as the effects of long chain unsaturated fatty acid depletion. Their studies indicate that while short chain saturated fatty acids and cyclopropane fatty acids maintain membrane fluidity when UFA are depleted, there is an absolute requirement for UFA in the development of the competent respiratory chain as well as in the coupling of oxidation and phosphorylation.

The *cis-trans* stereoisomers of unsaturated fatty acids, despite their similarity in chain length and degree of unsaturation, differ greatly in their physical properties. The naturally occurring *cis* configuration introduces a fixed "kink" in the fatty acid. This bend or kink limits the packing density of the fatty acid side chains in a membrane, reducing order and increasing fluidity in the neighborhood of the double bond (16). This is reflected by a low melting point. In contrast, the *trans* configuration closely resembles the saturated fatty acid configuration, facilitating side chain interaction and hence decreasing the fluidity of the membrane. The *trans* UFA isomer has a much higher melting point than its *cis* counterpart.

Growth of KD115 on *cis* versus *trans* isomers of the same fatty acid allows a very discrete modification of the hydrophobic environment without depletion of unsaturated fatty acids. This research utilizes the stereoisomer couple of 16:1 fatty acids (palmitoleic, *cis*; palmitelaidic, *trans*) as supplemental growth to study the effect of lipid composition alterations. The substitution of *trans* for *cis* UFA produces changes in complex lipid composition and in mitochondrial membrane structure and function. These latter changes are a major focus of this report. Further investigation to define the biochemical basis of these effects will help define the contribution of the hydrophobic environment to membrane protein-lipid interactions.

EXPERIMENTAL PROCEDURES

Materials

Lactic acid (60% syrup), amino acids, uracil, adenine, δ-aminolevulinic acid, and 2,3,5-triphenyl tetrazolium chloride (TTC), were obtained from Sigma Chemical Co. Yeast nitrogen base without amino acids, yeast extract, Bactopeptone, and agar were obtained from Difco Laboratories. Tergitol NP-40 was obtained from Union Carbide Corp. and fatty acids from Nu-Chek Prep, Inc. Tween 40 and Tween 80 were the gift from ICI Americas, Inc. Three fluorophores were used for the fluoroscence polarization measurements: 1,6-diphenyl-1,3-5-hexatriene (DPH), DL-2-(9-anthroyl) stearic acid (2-AS), and DL-12-(9-anthroyl) stearic acid (12-AS) (Aldrich Chemical Company, Milwaukee, WI or Molecular Probes Inc., Junction City, OR). ^{32}P-Orthophosphate, carrier free, [1-^{14}C]acetate Na-salt, and PCS scintillation fluid were obtained from Amersham. All other chemicals were reagent grade.

Methods

Growth and maintenance of yeast. The fatty acid auxotroph used for most experiments was KD115, a respiratory competent haploid yeast isolated from *Saccharomyces cerevisiae* strain S288C by Resnick and Mortimer (5). The genetic locus affected is ol_1, which controls fatty acid desaturase. Prototrophic strains used as controls were the parental strain S288C and revertants of KD115.

All yeast strains were maintained on YPD agar slants [1% yeast extract, 2% Bactopeptone, 1% glucose, and 1.5% agar]. For the UFA mutants, the slants contained 1% Tween 80 (YPDT80). The mutants were reisolated every 1–3 months by streaking and picking single colonies to form master plates. The master plates were replicated on YPD, YPDT80, and YPDT40 agar plates (Tween 40-1%). After 3 to 7 days growth at room temperature, the YPDT80 plates were overlaid with 0.1% TTC in 0.08 M phosphate, pH 7.0, 1.5% agar. Respiratory competent colonies were pink. Respiratory competent colonies growing only on YPDT80 were selected from the master plates for growth and storage on slants.

All yeast cultures were grown at 30°C with rotary shaking. Precultures (24 h) were inoculated from storage slants. Precultures were always grown in the same media as the main experimental culture and were used to inoculate the main culture. The main cultures were grown for six to seven generations before harvesting during late log/early stationary phase.

The semi-synthetic medium used for the growth of KD115 contained (per liter): 12 g glucose, 16.6 g lactic acid (60% syrup), 10 g yeast nitrogen base without amino acids, 10 g Tergitol NP-40, 85 mg palmitoleic (*cis* 16:1) or palmitelaidic acid (*trans* 16:1) (3.3×10^{-4} M), 17 mg lysine, 18 mg arginine, 21 mg methionine, 31 mg histidine, 53 mg leucine, 353 mg threonine, 21 mg tryptophan, 25 mg adenine, 17 mg uracil, and 10 mg $FeCl_3$-$6H_2O$. The medium was buffered with 50 mM NaP_i, pH 6.1. Yeast nitrogen base (Difco) was sterilized as a 10% solution by Millipore filtration (0.45 μm) and added to the rest of the medium after it had been autoclaved. The fatty acids were initially suspended by sonication in a warm solution of Tergitol and then added to the medium.

At the time of harvesting, the frequency of reversion to UFA prototrophy was determined by plating yeast on media with or without UFA supplement. Yeast cultures containing more than 15 revertants were not used for further analysis. This level of reversion was noted only oc-

casionally. In addition, respiratory activity was checked by the TTC overlay procedure described earlier. Yeast grown on either *cis*- or *trans*-supplemented media did not form significant numbers of petites.

Isolation of mitochondria. The yeast were grown to late log/early stationary phase (1.2–2.0 × 10^8 cells/ml for KD115), harvested by centrifugation (10 min, 2,000 *g*) and washed twice by centrifugation with cold water. The yeast were resuspended in 1 ml of 0.25 M mannitol, 20 mM Tris-SO_4 (pH 7.4), 1 mM EDTA (MTE buffer) per g wet weight, and homogenized by agitation with glass beads (0.45–0.55 mm diameter). Agitation was achieved either with the Braun MSK cell homogenizer cooled with liquid CO_2, or use of a vortex mixer. Breakage was checked microscopically. The cell homogenate and washings from the beads were centrifuged (1469 g_{max}, 10 min) to remove nuclei, cell debris, and unbroken yeast. The supernatant was centrifuged (39,000 g_{max}, 20 min) to obtain the mitochondrial pellet that was resuspended in MTE and recentrifuged four times. The resulting pellet was used as mitochondria. Mitochondria obtained from mutant yeast growing in either *cis* or *trans* 16:1 were similarly enriched in succinate cytochrome C reductase (a mitochondrial enzyme marker) compared to the corresponding homogenate.

Preparation of inner and outer mitochondrial membranes. Yeast cells were converted to spheroplasts by digestion with zymolyase. The crude mitochondria were obtained as previously described (17). The crude mitochondrial pellet was swollen in 50 mM Tris-Cl (pH 7.4)–1 mM PMSF at a concentration of 1 g wet wt/ml and homogenized with a glass homogenizer for 15 strokes at 4°C. The volume was then diluted 10 times with 50 mM Tris-Cl (pH 7.4) and centrifuged at 39,000 *g* for 20 min. The process was repeated once.

The homogenized mitochondrial pellet was resuspended in 1 ml of 50 mM Tris-Cl (pH 7.4), 1 mM PMSF per g of yeast. Three ml of this suspension was layered on top of a discontinuous density gradient containing 3.5-ml steps of 0.87 M, 1.31 M, 1.60 M, and 1.89 M sucrose in 10 mM Tris-Cl (pH 7.4) and centrifuged in a SW 27.1 rotor for 3 h at 116,200 *g*.

The inner mitochondrial membrane was collected at the interface between 1.31 M and 1.60 M sucrose of the gradient. The outer mitochondrial membrane was isolated by collecting the band between 0.87 M and 1.31 M sucrose and layering it on top of 23 ml of 1.1 M sucrose 10 mM Tris-Cl, pH 7.4. After centrifugation in a Beckman Ti60 rotor at 252,000 *g* for 60 min, the outer mitochondrial membrane was collected on top of the 1.1 M sucrose. The collected membrane fractions were then diluted 5 times with 10 mM Tris-Cl (pH 7.4) and recentrifuged at 145,000 *g* for 30 min at 4°C (18). The inner and outer mitochondrial membrane pellets were resuspended in a solution of 100 mM NaCl, 50 mM Tris-Cl (pH 7.4), and 0.02% azide for fluidity measurements. Inner and outer mitochondrial membranes were characterized by assaying specific marker enzymes. Inner membrane was identified by cytochrome c oxidase (19) and outer membrane by cholinephosphotransferase (20). Liposomes were prepared from the extracted lipids of each membrane as previously described (21).

Assays

The oxygen consumption of whole cells was determined at room temperature by polarography with a Clark type electrode from Yellow Springs International. The test solution, 0.5% glucose, was estimated to contain 260 nmol O_2 per ml at saturation.

Protein concentrations were determined by the method of Lowry et al. (22). Succinate cytochrome c reductase was measured according to Rabinowitz and de Bernard (23) and cytochrome c oxidase was measured according to Cooperstein and Lazarow (19). Cholinephosphotransferase (CDP-choline:1,2-diacylglycerol cholinephosphotransferase; EC 2.7.8.2) was assayed by the method of Wilgram and Kennedy (24).

Fluorescence polarization studies (25, 26)

Steady-state fluorescence polarization studies were performed with a Perkin-Elmer 650-40 spectrofluorometer adapted for fluorescence polarization.

The methods used to load the membranes and liposomes and the quantification of the polarization of fluorescence have been described (27, 28). The results were obtained according to the modified Perrin relationship: $r = r_\infty + (r_0 - r_\infty)\,[T_c/(T_c + T_f)]$, where r is the fluorescence anisotropy; r_0 is the maximal limiting anisotropy, taken as 0.390 for DPH and 0.285 for the anthroyl probes (29); r_∞ is the limiting hindered anisotropy; T_c is the correlation time; and T_f is the mean lifetime of the excited state (30). No changes in the excited state lifetimes, as assessed by total fluorescence intensity, were demonstrated using each probe in each membrane or liposome preparations (31).

Lipid analysis

Extraction of total lipids with chloroform–methanol 2:1 was performed on a suspension of broken yeast or mitochondria as described by Letters (32). Total lipid phosphorus was determined by the method of Bartlett (33).

Phospholipids and neutral lipids were separated and purified on prewashed and preactivated thin-layer silica gel G plates (Analtech) with diethyl ether as developing solvent. Phospholipids were eluted from the silica gel with chloroform–methanol 1:9 (v/v) followed by chloroform–methanol 2:1 (v/v) and then chloroform–methanol–conc. HCl 124:65:1 (v/v/v). The last eluting solvent was neutralized prior to combination with other eluates, which were

then evaporated to dryness and redissolved in chloroform–methanol 2:1 (v/v). Neutral lipids were eluted from silica gel with diethyl ether.

Phospholipid composition was determined by two-dimensional thin-layer chromatography on silica gel H plates (34). Phospholipids were visualized with a modified acid molybdate spray (35), scraped, and the phosphorus content was determined. Neutral lipids were analyzed by one-dimensional thin-layer chromatography (36) with modifications. Spots were visualized by spraying with 10% phosphomolybdic acid in 95% ethanol. After heating the plates at 90°C for 15 min to develop the color (37), they were scanned with a Zeiss PMQII Chromatoscan at 700 nm. For counting the radioactivity of labeled lipids, the visualized individual gel spots were scraped into a scintillation counting vial. One ml of water was added followed by 9 ml of PCS counting solution.

The lipid extracts were esterified by the trans-esterification method of Stoffel, Chu, and Ahrens (38), and the fatty acid composition was determined by gas–liquid chromatography of the methyl esters in a Hewlett Packard model 5840A chromatogram with flame ionization detector. The 6-ft coiled Supelco glass column (inner diameter 2 mm) packed with 10% sp-2340 on 100/120 Supelcoport was programmed to run at temperature range 180°C–240°C increasing at 2°/min after an initial delay of 0.5 min. The peaks were identified by their retention times relative to known standards.

Analysis of hydrocarbon species

To identify the hydrocarbon species in KD115 grown in unsaturated fatty acids, hydrocarbons were separated from the other neutral lipids, both the saponifiable and nonsaponifiable, in the lipid extracts of KD115 by one-dimensional thin-layer chromatography. The bands were visualized with iodine vapor as previously described (36). The hydrocarbon band was eluted with petroleum ether and chloroform–methanol (2:1). The combined eluates were dried under N_2 and the residue was redissolved in chloroform–methanol followed by mass spectroscopic analysis. The VG 70-250 mass spectrometer was used. The mass spectrum of the hydrocarbon fraction was identified as squalene by comparison with standard and published spectra of squalene (39, 40).

Electron microscopy

Yeast were prepared for electron microscopy by a slight modification of the procedure of Zickler and Olson (41); digestion of the cell wall with zymolyase was substituted for the glusulase digestion. Thin sections were prepared on a Sorvall Porter-Blum MT2-B ultra microtome, stained with uranyl and lead, and viewed in a Siemens 101 electron microscope.

RESULTS

Growth of KD115

A variety of long chain UFA may support the growth of KD115. The *cis* and *trans* isomers of 16:1, palmitoleic and palmitelaidic acids, respectively, were chosen for this study for two reasons. They represent a couple of stereoisomers of the same chain length and degree of unsaturation that support the growth of KD115 at almost comparable rates. Palmitoleic acid is also a major natural unsaturated fatty acid of yeast. Mutant yeast supplemented with *trans* 16:1 had a longer lag phase; however, this could be compensated for by doubling the inoculum for the *trans* 16:1 culture. Under these conditions the growth curves for KD115 with either supplement were similar. The cell density of the stationary phase culture was lower for *trans* 16:1-supplemented KD115.

Growth yield (dry weight/volume medium), which is a crude measure of the capacity of the yeast to utilize the available carbon source, was depressed about 30% in KD115 grown in *trans* 16:1. The mutant grown in *cis* 16:1 grew as efficiently on the standard minimal medium as did parental S288C with or without either UFA supplement.

TABLE 1. Fatty acid analysis of total lipids of yeast S288C and KD115[a]

	KD115-*Cis* 16:1[b]		KD115-*Trans* 16:1[b]		S288C-No UFA		S288C-*Cis* 16:1		S288C-*Trans* 16:1	
Fatty Acid	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria
C8:0	0.1 ± 0.1		0.1 ± 0.1					0.1		0.1
C10:0	0.3 ± 0.2	0.2 ± 0.1	0.9 ± 0.6	0.9	0.7	0.4	0.4	0.4	0.3	0.2
C12:0	0.6 ± 0.2	0.5 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.6	1.3	0.7	0.7	0.7	0.5
C14:0	1.3 ± 0.2	1.3 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	2.8	2.5	1.7	1.8	1.1	1.0
C16:0	17.9 ± 0.7	19.1 ± 0.6	12.6 ± 0.7	12.6 ± 1.7	19.4	20.4	17.2	18.5	8.1	8.2
C16:1	72.8 ± 0.7	72.9 ± 0.8	81.6 ± 1.2	81.7 ± 2.1	48.8	48.3	71.8	71.8	68.2	67.9
C18:0	6.1 ± 0.4	5.3 ± 0.4	2.3 ± 0.2	2.3 ± 0.2	4.7	4.2	5.4	4.5	2.7	2.5
C18:1	0.9 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	21.8	22.6	2.8	2.3	18.9	19.7

[a]Figures in table represent percent of the total fatty acids.
[b]Figures for KD115 represent the mean ± standard deviation for three separate experiments.

TABLE 2. Fatty acid composition of neutral lipids and phospholipids of yeast S288C and KD115[a]

Fatty Acid	KD115-*Cis* 16:1[b] Whole Cells		KD115-*Trans* 16:1[b] Whole Cells		S288C-No UFA Whole Cells		S288C-*Cis* 16:1 Whole Cells		S288C-*Trans* 16:1 Whole Cells	
	N.L.	P.L.	N.L.	P.L.	N.L.	P.L.	N.L.	P.L.	N.L.	P.L.
C8:0	0.5 ± 0.6		1.6 ± 1.8		0.2	0.1			0.4	
C10:0	0.4 ± 0.4	0.1 ± 0.1	0.8 ± 0.7	1.0 ± 0.4	1.6	0.2		0.1	0.4	0.1
C12:0	0.8 ± 0.7	0.2 ± 0.2	1.1 ± 1.0	1.1 ± 0.2	2.5	1.1		0.3	0.7	0.3
C14:0	2.1 ± 0.3	0.9 ± 0.9	1.4 ± 1.4	0.7 ± 0.2	4.0	2.6	2.8	1.3	2.2	0.8
C16:0	19.6 ± 4.5	20.9 ± 1.2	15.6 ± 5.3	12.4 ± 0.9	22.5	17.2	25.3	19.4	16.1	7.9
C16:1	66.6 ± 6.9	72.6 ± 0.6	71.4 ± 3.3	83.1 ± 0.9	43.6	56.9	54.3	71.2	56.8	69.9
C18:0	7.5 ± 1.8	4.9 ± 0.4	5.6 ± 2.5	1.3 ± 0.3	6.0	2.2	3.8	4.9	4.8	2.3
C18:1	2.3 ± 1.7	0.5 ± 0.2	2.6 ± 0.2	0.2 ± 0.2	19.6	19.7	13.7	2.7	18.6	18.6

[a]Figures in the table represent percent of the total fatty acids; N.L., neutral lipid; P.L., phospholipid.
[b]Figures for KD115 represent the mean ± standard deviation for three separate experiments.

Fatty acid composition

The fatty acid profiles of the total lipids of whole cells and mitochondria of parent strain S288C and UFA auxotroph KD115 are presented in **Table 1.** There were no significant differences in the fatty acid composition between whole cells and isolated mitochondria. There were increased levels of 16:1 in S288C supplemented with *cis* or *trans* 16:1 compared to unsupplemented cells. *Cis* 16:1 supplementation of S288C resulted in decreased 18:1 whereas *trans* 16:1 supplementation of S288C resulted in decreased 16:0. This outcome would be anticipated if *cis* fatty acids replace the *sn*-2 fatty acids of phospholipids, normally partly 18:1, and *trans* fatty acids replace the *sn*-1 fatty acids, normally 16:0.

The UFA auxotroph KD115 also showed considerable enrichment in the levels of 16:1 compared to wild type cells grown without fatty acid supplement. The mutant was more heavily enriched in 16:1 when the supplement was *trans* 16:1. Both *cis* and *trans* 16:1-supplemented KD115 had very low levels of 18:1. The fatty acid profiles of *cis* and *trans* 16:1-supplemented KD115 were quite similar. The one notable difference was the decreased ratio of 16:0/16:1 in *trans*-supplemented KD115. The change is quantitatively similar to the response shown by S288C supplemented with *trans* 16:1. Fatty acid analysis by gas–liquid chromatography revealed that KD115 was not able to convert the supplemented UFA isomer to its stereoisomer, i.e., *trans* 16:1-supplemented KD115 has no detectable *cis* 16:1 among its fatty acids (data not shown).

The fatty acid composition of the neutral lipids and phospholipids of each of the yeast cultures is presented in **Table 2.** Although similar analyses were made on isolated mitochondria, they are not reported in detail as they essentially mirror the fatty acid composition of the whole cells. Three differences between the fatty acid profiles of phospholipids and neutral lipids were observed: the 16:1 was at a higher level in phospholipids than in neutral lipids in all yeast examined including unsupplemented parental yeast; saturated fatty acids (14:0, 16:0, and 18:0) were higher in neutral lipids than phospholipids; and in several cases (KD115 and parental yeast supplemented with *cis* 16:1) 18:1 was relatively enriched in the neutral lipid fraction. It is notable that in the parental yeast, S288C, phospholipid 18:1 is at a low level (2.7%) when the yeast growth medium is supplemented with *cis* 16:1. The latter seems to fully substitute for the 18:1 of phospholipids in this yeast.

Lipid composition

The above results demonstrate that unsaturated fatty acid auxotrophy does allow modulation of the fatty acid composition of the yeast. How this stereoisomeric modification of the hydrophobic domain influences the lipid composition of the yeast was investigated by direct analysis of total lipid content and phospholipid and neutral lipid profiles of whole cells and isolated mitochondria. The parental prototrophic strain S288C was grown under

TABLE 3. Lipid content of yeast, S288C, and unsaturated fatty acid auxotroph KD115

	Neutral Lipid	Phospholipid	Total Lipid
		μg/mg protein	
KD115-*cis* 16:1[a]			
Whole cells	48 ± 13	55 ± 6	103 ± 16
Mitochondria	183 ± 77	206 ± 48	388 ± 85
KD115-*trans* 16:1[a]			
Whole cells	66 ± 11	82 ± 7	148 ± 6
Mitochondria	183 ± 50	367 ± 107	549 ± 133
S288C-no UFA			
Whole cells	80	87	166
Mitochondria	163	369	531
S288C-*cis* 16:1			
Whole cells	91	79	170
Mitochondria	272	378	650
S288C-*trans* 16:1			
Whole cells	98	86	184
Mitochondria	296	365	661

[a]Figures for KD115 represent the mean ± standard deviation for three separate experiments.

identical conditions and analyzed as a control. The results are presented in Tables 3–5.

Table 3 reveals that supplementation of the parental strain S288C with either *cis* or *trans* fatty acid resulted in an increased neutral lipid content without altering phospholipid content. (As shown in Table 5, the triglyceride level was increased in both whole cells and mitochondria.) In addition, the mitochondria of supplemented S288C show increased levels of sterol and free fatty acid. For the parental strain, supplementation with either stereoisomer yielded identical results.

The total lipid content (Table 3) for the UFA auxotroph KD115 with either *cis* or *trans* 16:1 supplementation was similar. Lipid content in supplemented mutant with *trans* 16:1 was somewhat higher and about equal to that of unsupplemented S288C. A higher level of both neutral lipid and phospholipid of *trans* 16:1-supplemented KD115 accounted for its somewhat higher lipid content than *cis* 16:1-supplemented KD115. However, the phospholipid content of both whole cells and isolated mitochondria of *trans* 16:1-supplemented KD115 was quite comparable to the phospholipid content of the parental strain, whether or not the latter was provided with exogenous UFA.

As seen in **Table 4** the phospholipid composition of the parental strain S288C is unaffected by the presence of either fatty acid stereoisomer in the medium. For the UFA auxotroph KD115, the most striking response to *trans* versus *cis* 16:1 supplementation was seen in the levels of phosphatidylethanolamine (PE) and phosphatidylcholine (PC). *Trans* 16:1 supplementation of the mutant resulted in a relative decrease in PC with a reciprocal increase in PE. The phospholipid profiles for whole cells and mitochondria were comparable. The ratio of PE to PC was 0.61 (mitochondrial ratio of 0.73) in mutant yeast grown in *cis* 16:1 while that of *trans* 16:1-grown KD115 was 1.07 (mitochondrial ratio of 1.18).

The nonsaponifiable extracts of total lipids were analyzed by HPLC. Squalene, sterol, and a small amount of coenzyme Q_6 were present, **Table 5** documents an alteration in the neutral lipid profile for *trans* versus *cis* 16:1 supplementation of the UFA auxotroph. The most notable change was the higher level of hydrocarbon in *trans* 16:1-supplemented KD115 located primarily in the mitochondrial fraction. The major component (80–85%) of the hydrocarbon fraction was squalene, a precursor in ergosterol synthesis. The approximate amount of squalene is 3.1 μg/mg protein in "*cis*"-grown cells and 8.3 μg/mg protein in "*trans*"-grown cells. The presence of squalene in these fatty acid-supplemented yeasts was also confirmed by mass spectroscopy of the hydrocarbon fraction purified by TLC (data not shown).

Whole cells and mitochondria of KD115 grown in *cis* 16:1 had a lower sterol ester content than the corresponding fractions of either KD115 grown in *trans* 16:1 or parental yeast under any growth condition. KD115 supple-

TABLE 4. Phospholipid analysis of yeast, S288C, and KD115[a]

	KD115-*Cis* 16:1[b]		KD115-*Trans* 16:1[b]		S288C-No UFA		S288C-*Cis* 16:1		S288C-*Trans* 16:1	
	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria
Cardiolipin	4.3 ± 0.8	5.2 ± 1.0	7.7 ± 1.7	5.3 ± 1.0	3.4	3.3	2.6	3.2	3.6	4.1
Phosphatidic acid	3.5 ± 0.9	2.0 ± 0.4	3.6 ± 1.0	3.4 ± 0.4	3.8	6.0	5.6	4.8	4.1	5.6
Dimethylphosphatidylethanolamine	1.5 ± 0.4	1.5 ± 0.4	1.0 ± 0.7	0.8 ± 0.2	1.3	1.0	2.1	1.6	1.1	1.1
Phosphatidylserine	3.6 ± 0.6	4.5 ± 3.4	3.2 ± 1.0	5.5 ± 1.0	5.2	5.9	4.7	4.8	4.8	4.8
Lysophosphatidylethanolamine	2.0 ± 1.2	2.1 ± 1.0	0.9 ± 0.4	0.9 ± 0.6	1.2	1.5	1.1	1.4	1.0	1.0
Lysolecithin	1.3 ± 0.4	2.4 ± 1.7	0.8 ± 0.3	1.6 ± 0.7	1.4	3.0	2.0	4.0	0.5	3.6
X[c]	2.8 ± 2.5	3.0 ± 0.6	0.9 ± 1.0	2.2 ± 0.6	1.7	9.0	1.1	10.8	5.2	6.3
Phosphatidylinositol	9.4 ± 1.8	11.5 ± 1.4	8.3 ± 1.4	11.8 ± 1.6	10.7	10.1	10.7	10.0	11.2	10.3
Phosphatidylethanolamine	27.2 ± 2.1	28.7 ± 3.9	38.1 ± 1.4	37.1 ± 1.9	25.6	22.1	27.7	24.2	22.2	22.9
Phosphatidylcholine	44.5 ± 4.1	39.1 ± 2.3	35.6 ± 1.5	31.5 ± 1.7	47.1	38.2	42.4	35.1	46.2	40.1
PE/PC	0.61	0.73	1.07	1.18	0.54	0.58	0.65	0.69	0.48	0.57

[a] Figures in table are expressed as percent of total phospholipid.
[b] Figures for KD115 are mean ± standard deviation for three independent experiments.
[c] X unidentified spot, probably includes CDP-diglyceride.

TABLE 5. Neutral lipid analysis of yeast, S288C, and KD115[a]

	KD115-*Cis* 16:1[b]		KD115-*Trans* 16:1[b]		S288C-No UFA		S288C-*Cis* 16:1		S288C-*Trans* 16:1	
	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria	Whole Cells	Mitochondria
Hydrocarbon	1 ± 2	10 ± 3	13 ± 3	31 ± 4	5	11	4	0.9	6	21
Sterol ester	4 ± 0.7	14 ± 3	10 ± 2	25 ± 13	10	26	12	22	8	19
Triglyceride	14 ± 2	59 ± 24	9 ± 2	14 ± 7	20	24	31	88	43	90
Free fatty acid	7 ± 1	32 ± 17	5 ± 1	20 ± 6	7	25	7	49	10	53
1,2 Diglyceride	6 ± 1	22 ± 10	9 ± 1	24 ± 9	9	12	8	17	8	20
1,3 Diglyceride	8 ± 7	24 ± 21	10 ± 6	32 ± 26	18	40	16	39	12	43
Free sterol	6 ± 1	25 ± 10	9 ± 1	35 ± 8	11	25	13	56	10	50
Monoglyceride	2 ± 0.8	4 ± 3	2 ± 1	2 ± 1						
Total	50 ± 9	191 ± 72	68 ± 7	186 ± 89	80	163	91	271	97	296

[a]Figures in table are in terms of μg/mg protein.
[b]Results for KD115 represent the mean ± standard deviation for three independent experiments.

mented with *trans* 16:1 had a lower triglyceride content, most marked in the mitochondrial fraction, than mutant yeast grown in *cis* 16:1 or parental yeast grown with either fatty acid supplement.

Respiratory activity

Growth yield is a crude measure of the yeast's ability to fully utilize the available carbon source through oxidative phosphorylation. A reduced capacity for oxidative phosphorylation in the mutant yeast grown in *trans* 16:1 was suggested by its depressed growth yield. Whole cell oxygen consumption is a more direct measure of respiratory competence. The oxygen consumed by KD115 grown to late log/early stationary phase of growth in *cis* or *trans* 16:1 medium was determined. The parental strain S288C, and revertants of KD115 were grown under identical conditions as controls. The results are shown in **Table 6.**

It can be seen that the oxygen consumption of the UFA auxotroph KD115 was markedly affected by the nature of the UFA stereoisomer in the medium. *Trans* 16:1 supplementation reduced oxygen consumption 10-fold compared to *cis* supplementation. In contrast, the respiration of prototrophic parent S288C and revertant yeast was not affected by the UFA in the medium.

Because of the marked effect of UFA, the oxygen consumption of KD115 was determined after growth on different mixtures of the two 16:1 stereoisomers. The total fatty acid content of the growth medium was held constant in these experiments. The results are presented graphically in **Fig. 1.** Though KD115 grown in *cis* 16:1 consumed almost as much oxygen as parental yeast S288C, the highest oxygen consumption by mutant yeast was observed when the medium was supplemented with an equimolar mixture of *cis* and *trans* 16:1, though the differences were not statistically significant as the absolute respiratory rates varied somewhat between experiments. In five of eight experiments, the cells grown in the mixture had an oxygen consumption that was at least 30% higher than with a *cis* supplement alone, while in only one of eight experiments was the reverse the case. As little as 10% *cis* 16:1 in the medium was sufficient to prevent almost totally the respiratory defect observed with all-*trans* 16:1 supplementation. The unsaturated fatty acid composition of KD115 grown in mixtures of *cis* and *trans* 16:1 did not quantitatively correlate with the composition of the medium. The *cis* 16:1 was disproportionately represented (data not shown), presumably because it was more readily taken up or fixed in the complex lipids of the yeast. For example, when the medium was supplemented with a mixture containing 80% *trans* and 20% *cis* 16:1, the yeast contained only twice as much *trans* 16:1 as *cis* 16:1. Accurate quantitation could not be achieved when the fatty acid supplement contained 10% *cis* 16:1 or less, because at low proportions of the *cis* fatty acid, this component was not resolved well enough from the major peak of *trans* 16:1 to allow for quantitative assessment.

Effects of fatty acid mixtures on lipid composition

To relate the alterations in lipid composition more directly to the functional changes in respiration, the neutral lipid and phospholipid compositions were determined in KD115 grown in different mixtures of 16:1 stereoisomers. The whole cell phospholipid composition was

TABLE 6. Oxygen consumption of various yeast grown in unsaturated fatty acid supplements[a]

Yeast	Oxygen Consumption	
	Cis 16:1	*Trans* 16:1
	nmol O_2/min/10^6 cells	
KD115 (n = 5)	0.189(± 0.38)[b]	0.014(± 0.009)[b]
S288C (parent KD115)	0.245	0.236
Revertant KD115	0.245	0.264

[a]Yeast were inoculated and harvested at late log/early stationary phase as described in Methods for KD115. Oxygen consumption was measured on cells suspended in 0.5% glucose at room temperature as described in Methods.
[b] ± Standard deviation with five independent samples.

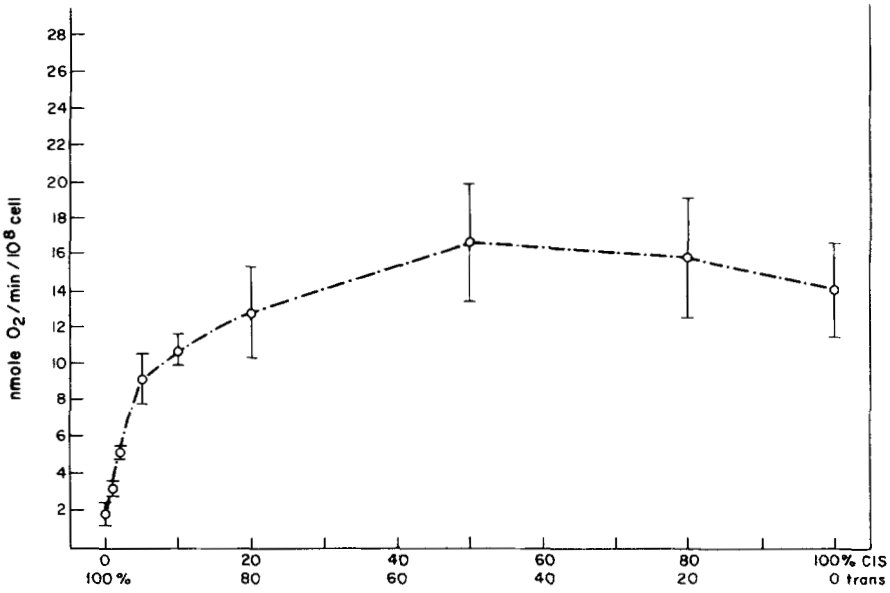

Fig. 1. KD115 were grown to late log phase (1.5–2.0 $\times 10^8$ cells/ml). Different proportions of *cis* and *trans* 16:1 were used as the fatty acid supplement, always maintaining the total fatty acid supplement at a constant concentration. The respiratory activity (O_2 consumption) determined from three to six independent experiments (mean ± SD).

assessed after long-term growth of the cultures with [^{32}P]orthophosphate and [1-^{14}C]acetate to label all lipids including neutral lipids. The lipids were extracted and separated as described in the Methods section for direct chemical analysis, but in this case the percent radioactivity in each fraction was analyzed as a measure of relative mass. The results obtained with this method for KD115 cultures supplemented with 100% *cis* or *trans* 16:1 indicated that the percentage radioactivity provided a faithful assessment of the lipid composition obtained by direct chemical analysis.

A graphic representation of the percentage of PC and PE versus the percentage of *cis* and *trans* 16:1 in the medium (**Fig. 2**) emphasizes the relationship between the proportion of these phospholipids and the fatty acid supplement provided. As the *cis* isomer is introduced to the predominantly *trans* medium, PC increases as PE falls. For neutral lipids, introducing *cis* isomer reduces sterol

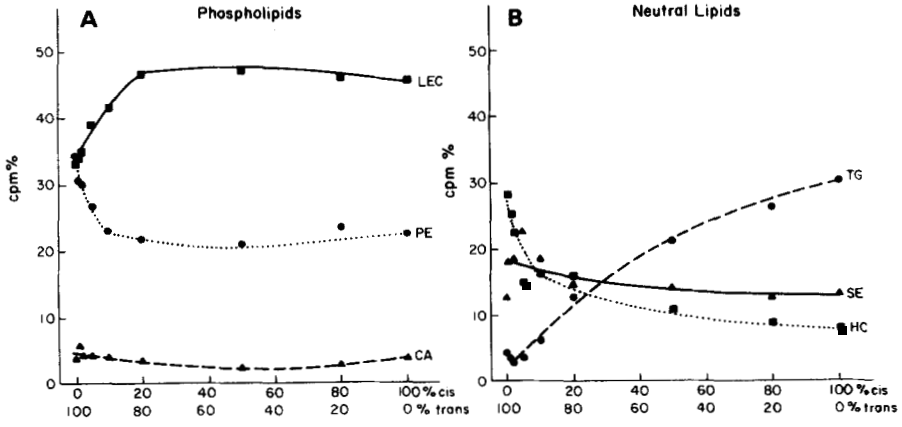

Fig. 2. KD115 were grown as described in the legend to Fig. 1, except [^{32}P]orthophosphate was added to the growth medium. A: Phospholipid composition was determined from the proportional distribution of radioactivity (^{32}P) present in the individual phospholipids after separation by two-dimensional thin-layer chromatography. Only phosphatidylethanolamine (PE), phosphatidylcholine (PC) or lecithin (LEC), and cardiolipin (CA) are featured in the figure. All other phospholipids behaved like cardiolipin. Only phosphatidylethanolamine and phosphatidylcholine revealed systematic variation with different fatty acid mixtures. B: Neutral lipid composition was determined from the proportional distribution of radioactivity derived from [1-^{14}C]acetate that was present throughout yeast growth. Neutral lipids were separated by one-dimensional thin-layer chromatography. Only the major neutral lipids are shown in the figure; triglyceride (TG), sterol ester (SE), and hydrocarbon (HC, mainly squalene).

TABLE 7. Yeast membrane (M) and liposome (L) fluorescence polarization studies

Probe	Fraction	Number of Measurements[a]	Anisotropy,[b] r at 25°C
DPH	*cis*-inner M.	8	0.291 ± 0.001[c]
DPH	*trans*-inner M.	8	0.300 ± 0.002[c,***]
DPH	*cis*-inner L.	8	0.196 ± 0.005[c]
DPH	*trans*-inner L.	8	0.212 ± 0.006[c,*]
DPH	*cis*-outer M.	4	0.200 ± 0.010
DPH	*trans*-outer M.	4	0.205 ± 0.009
DPH	*cis*-outer L.	4	0.157 ± 0.001
DPH	*trans*-outer L.	4	0.178 ± 0.005**
2-AS	*cis*-inner M.	8	0.193 ± 0.006[d]
2-AS	*trans*-inner M.	8	0.199 ± 0.005[d]
2-AS	*cis*-outer M.	4	0.182 ± 0.003
2-AS	*trans*-outer M.	4	0.184 ± 0.002
12-AS	*cis*-inner M.	8	0.155 ± 0.005[c]
12-AS	*trans*-inner M.	8	0.162 ± 0.007[c]
12-AS	*cis*-outer M.	4	0.125 ± 0.002
12-AS	*trans*-outer M.	4	0.128 ± 0.002

[a]Two independent membrane preparations were each studied, either in quadruplicate or duplicate.
[b]Values represent means ± SEM; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; *trans* compared to *cis* values in each group.
[c]$P < 0.001$; [d]$P < 0.001$; inner compared to outer values for each group.

ester and squalene and results in a steady increase in triglyceride content (Fig. 2). For each of the lipid components plotted, with the exception of triglyceride content, the response to the *trans* stereoisomer is largely abolished by the addition of 10–20% *cis* fatty acid to the medium. The profile of changes in PE, PC, and squalene, especially the phospholipids, simulates the pattern of changes seen in respiratory activity as a function of the fatty acid mixtures provided in the medium (Fig. 1).

Membrane fluidity

Inner and outer mitochondrial membranes were isolated from the UFA auxotroph KD115 grown with *cis* and *trans* 16:1 supplementation. The lipid fluidity of their membranes and respective liposomes were assessed by steady-state fluorescence polarization techniques using three fluorophores, which differ in a number of respects. DPH molecules are rod-shaped (42), localize deep in the lipid bilayer, and are aligned relatively parallel to the phospholipid acyl chains (43). The anthroyloxy fatty acid probes (2-AS and 12-AS) assume a more spherical shape in bilayers than DPH and localize at various depths in the bilayers [2-AS localizes in the bilayer closer to the aqueous interface than 12-AS (44)]. The results are summarized in **Table 7.** Membrane lipid fluidity, as assessed by r values of DPH, 2-AS, and 12-AS, were found to be significantly lower in inner relative to outer mitochondrial membrane. Each of the membranes prepared from KD115 grown with *trans* 16:1 fatty acid also showed lower lipid fluidity than the corresponding membranes from yeast grown with *cis* fatty acid. Furthermore, values of r of intact membranes invariably exceeded those of the corresponding liposomes; consistent with the well-known effect of proteins on membrane fluidity (21).

The differences in the fluidity of mitochondrial membranes and mitochondrial lipids derived from cells grown in *cis* or *trans* 16:1 fatty acid were not as large as might have been anticipated. Could this be accounted for by adaptive changes in lipid composition? The present data show that membranes prepared from cells grown with *trans* 16:1 contained larger amounts of PE and squalene (Tables 4 and 5) and more rigid fatty acyl chains than membranes from *cis* 16:1-grown cells. It was, therefore, of interest to examine whether liposomes prepared from dipalmitoylphosphati-

TABLE 8. DPH studies on model liposomes

Preparation	Determined Mean % Composition	Number of Measurements[a]	r at 25°C	r at 45°C
DPPC	100%	8	0.238 ± 0.003	0.120 + 0.003
DSPC	100%	6	0.245 + 0.003	
DPPE[b]				
DPPC + 5% Chol	97/4	6	0.227 + 0.008	0.141 + 0.007[c]
DPPC + 10% Chol	91/9	6	0.226 + 0.010	0.171 + 0.009[c]
DPPC + 5% Sq.	96/5	8	0.213 + 0.003[d]	
DPPC + 10% Sq.	91/9	8	0.170 + 0.002[d]	
DPPC + DPPE	56/44	8	0.278 + 0.002[d]	
DPPC + DPPE + 5% Sq.	53/43/4	8	0.252 + 0.002[e]	
DPPC + DPPE + 10% Sq.	54/37/9	8	0.223 + 0.003[e]	

[a]Two independent liposome preparations were each studied, either in triplicate or quadruplicate.
[b]Could not prepare liposomes, therefore, no values were obtained.
[c]$P < 0.05$ or less compared to DPPC values at 45°C.
[d]$P < 0.05$ or less compared to DPPC values at 25°C.
[e]$P < 0.05$ or less compared to DPPC + DPPE values at 25°C.

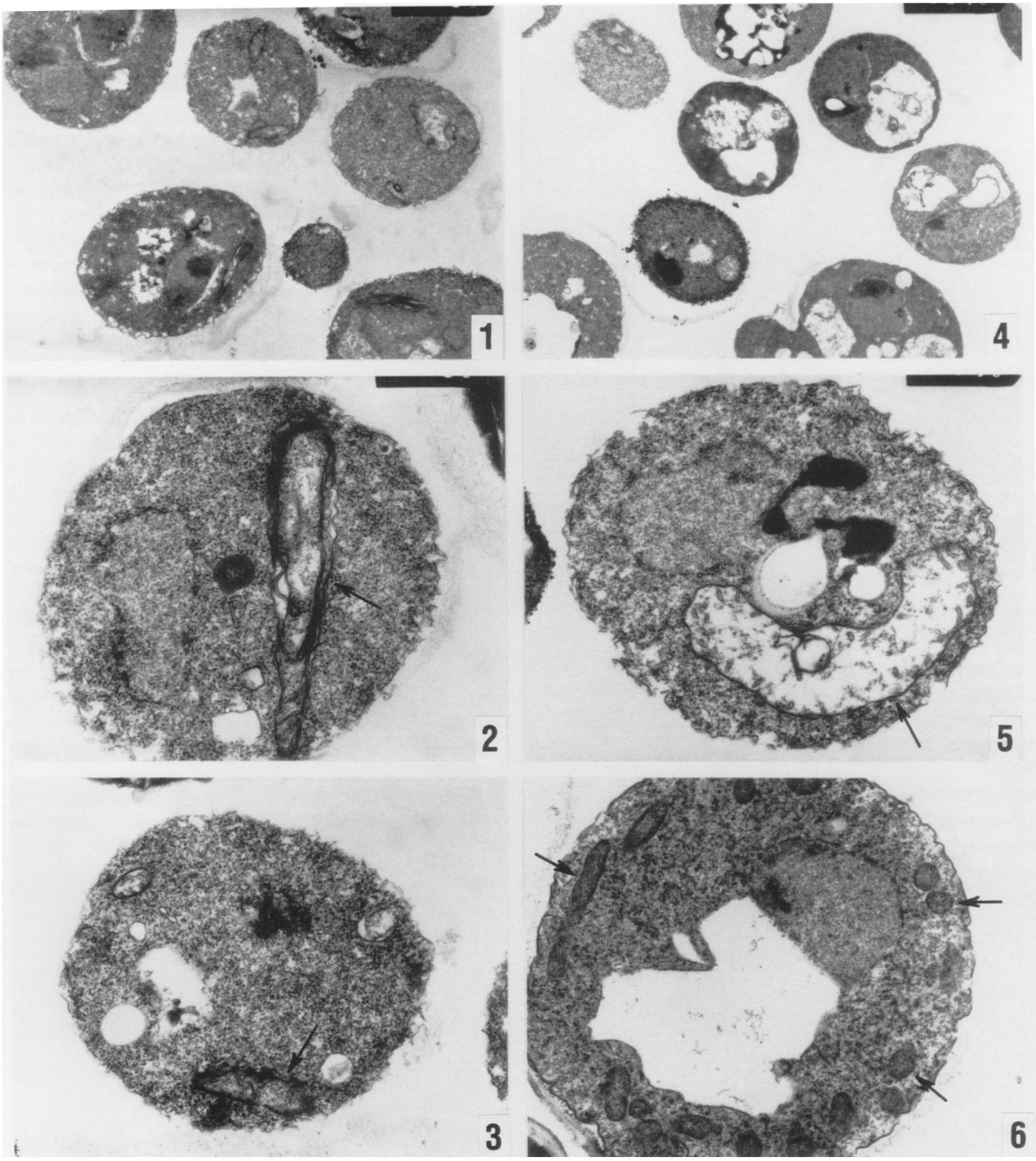

Fig. 3. Electron micrographs were prepared as described in the Experimental Procedures section. Yeast cells; digestion of the cell wall with zymolase; Magnification 6,000× and 14,000×. (1–3) KD115 grown with *trans* 16:1 supplement; (4, 5) KD115 grown with *cis* 16:1 supplement; (6) D273/10B grown without UFA supplement; arrows indicate mitochondria.

dylcholine (DPPC), dipalmitoylphosphatidylethanolamine (DPPE), distearoylphosphatidylcholine (DSPC) with and without additional cholesterol and/or squalene would model changes in fluidity observed in liposomes prepared from total lipid extracts of yeast membranes.

Liposomes prepared from DPPC were more fluid than those synthesized from DSPC and mixtures of DPPC and DPPE (**Table 8**). Liposomes could not be prepared with DPPE alone. The addition of cholesterol at either 5% or 10% did not affect the fluidity of liposomes prepared from

DPPC at 25°C. In contrast, at the phase transition temperature, 45°C, 10% of added cholesterol decreased liposome fluidity compared to 5% cholesterol. On the other hand, the addition of squalene had an opposite effect, increasing the fluidity of liposomes, prepared either with DPPC or with mixtures of DPPC and DPPE, particularly when present at 10% by weight. Alteration in the lipid composition of membranes appeared to play a major role in determining their fluidity.

Mitochondrial morphology

The morphologic consequences of *trans* fatty acid substitution in membranes was investigated by performing electron microscopy on whole cells of the UFA auxotroph KD115 grown in either *cis* or *trans* 16:1. Wild type strain D273 was also examined. Representative electron micrographs are presented in **Fig. 3.**

The mitochondria of *cis* 16:1-grown KD115 were enlarged with an electron lucent matrix. The large size may be due to swelling, fusion of mitochondria, or lack of division of mitochondria. Cristae of unusual configuration were evident. The mitochondrial boundary membrane was not continuously intact. Several mitochondria contained various circular and irregular membrane inclusions giving the appearance that the large mitochondria were actually surrounding regions of cytoplasm.

The mitochondria of *trans* 16:1-grown KD115 were also large, elongated, and few in number. The mitochondria were not swollen and their matrix was more electron opaque. Most strikingly, the boundary layer of these mitochondria appeared to form multiple parallel layers resembling, in some respects, the membrane whorls of myelin figures. Such membrane configurations were also included within the substance of the mitochondria. Orthodox cristae were not clearly discernable.

In preliminary experiments, the effect of growth in mixtures of *cis* and *trans* 16:1 stereoisomers on the morphology of mitochondria was explored. Again, as little as 10% *cis* 16:1 added to a medium with predominantly *trans* 16:1 was sufficient to prevent the development of mitochondrial profiles typical of these observed in mutant yeast grown in 100% *trans* 16:1 medium. The addition of fatty acid to the medium in which the parental yeast was grown had no effect on the morphology of their mitochondria (data not shown).

DISCUSSION

The use of the unsaturated fatty acid auxotroph of yeast, KD115, has provided the opportunity for selective modification of the fatty acyl moieties of the complex lipids of cellular membranes. In these studies the effect of supplementation with two stereoisomers of 16:1 on fatty acyl components of the membrane and its resulting fluidity were compared. The *cis* isomer, palmitoleic acid, is normally a major fatty acid of yeast (about 50%). However, when it and its *trans* stereoisomer, palmitelaidic acid, are provided at relatively high concentration, each constitutes 70–80% of the total fatty acid of neutral lipid and phospholipid. Thus, using these supplements for the auxotrophic mutant results in a highly homogeneous fatty acid composition. These two stereoisomers differ not only in their configuration but also in their physical properties. At physiological temperature, *cis* 16:1 is a highly fluid fatty acid, whose conformation precludes a very tight packing density of the hydrophobic membrane domain. On the other hand, the *trans* 16:1, which is less fluid, permits close packing in the hydrophobic domain more similar to a saturated fatty acid.

Although the fatty acid composition of the prototrophic parental yeast S288C is also modified when this yeast is grown in media containing relatively high concentrations of *cis* or *trans* 16:1 it never achieves the same degree of fatty acid homogeneity observed in the mutant strain. When provided as an exogenous supplement, the 16:1 fatty acid was less prominent among the total fatty acids of parental yeast compared to mutant yeast. In addition, the 16:1 fatty acid in the parental yeast grown in *trans* 16:1 supplement was probably a mixture of *cis* and *trans* 16:1 isomers. The parental yeast retains the capacity to synthesize *cis* 16:1 regardless of the supplement provided, but the mutant yeast can neither synthesize 16:1 fatty acid nor convert *trans* 16:1 to *cis* 16:1. Though the supplementation of parental yeast, S288C, with either *cis* or *trans* 16:1 had little impact on the growth or function of these cells, they nevertheless sensed the difference between the two stereoisomers. When additional *cis* 16:1 was provided, there was a compensatory reduction in the amount of 18:1 fatty acid in the phospholipids. When *trans* 16:1 was furnished, the phospholipid C18:1 was maintained at a high level, while there was a compensatory reduction in the saturated fatty acid 16:0 (Table 2).

The major finding of this study is that this selective alteration of the hydrophobic environment has pleomorphic effects particularly influencing the mitochondria. The data presented suggest that these pleomorphic changes in mitochondrial structure and function are all closely related consequences of either the alterations in fatty acid composition or the associated lipid compositional modifications. *Trans* supplementation decreases the respiratory competence of the yeast, alters the phospholipid and neutral lipid composition, and causes structural alterations in the mitochondrial membranes (Tables 4 and 5; Figs. 2 and 3). The use of various mixtures of *cis* and *trans* 16:1 as growth supplements has permitted us to relate some of the alterations in lipid composition to the respiratory activity of yeast. The changes in phosphatidylcholine, phosphatidylethanolamine, and squalene, mitochondrial mor-

phology and respiratory-competence all respond to the unsaturated fatty acid stereoisomer composition of the medium with the same concentration dependence (Figs. 1 and 2). In all cases, 10–20% of *cis* 16:1 in the medium is sufficient to almost totally prevent the changes wrought by the provision of only *trans* 16:1 to the mutant yeast. The limited heterogeneity of the fatty acid chains in mutant yeast results in an increased lipid-lipid interaction and a "tighter" packing density (1). The introduction of a relatively small amount of *cis* 16:1 could serve to reduce the unusual homogeneity of the hydrophobic domain and abolish many of the effects of *trans* 16:1 supplementation. The respiratory membranes containing a very high proportion of *cis* 16:1 do not function quite as well as wild type yeast. Such membranes also seem to be relatively tightly packed. The lack of similar changes in the parent strain is probably the result of the lower level of substitution achieved and the greater heterogeneity of its fatty acids. It also suggests that *trans* 16:1 fatty acid is not directly toxic to the yeast.

The decrease in membrane fluidity associated with rich *trans* 16:1 substitution could account for the rigid mitochondrial membranes observed under the electron microscope. This may be attributable not only to the high level of *trans* 16:1 present in these membranes but also to the alteration in the relative proportion of PE and PC. The head group of PC is more bulky than that of PE. The molecular area of PC in monolayer is larger than that of PE with equivalent fatty acid components (45). Thus the fatty acid and phospholipid components together comprise a highly compact bilayer. Fluorescence polarization studies (Tables 7 and 8) confirm the contribution of both fatty acids and phospholipids to the reduced membrane fluidity observed in mutant yeast enriched in *trans* 16:1. The severe constraint conditioned by the desaturation defect and the singular nature of the unsaturated fatty acid source greatly limit the plasticity of the fatty acid components of these complex lipids. Hence the major adaptation possible with this yeast fatty acid auxotroph depends upon the capacity to modify the complex lipid composition.

Although the yeast auxotroph supplemented with *trans* 16:1 contained relatively rigid fatty acid chains as well as a higher ratio of PE to PC, the measurements of fluidity revealed only modest differences between the membranes of *cis* and *trans* fatty acid-supplemented mutant yeast. This is probably attributable to compensatory changes in other lipids. Modest variations in fluidity were also noted in studies of a double mutant fatty acid auxotroph of yeast supplemented with a variety of saturated and unsaturated fatty acids (46). However, the changes in the profile of neutral and phospholipids of this double mutant were not examined. In our study, membranes prepared from cells grown with *trans* 16:1 supplements possessed a high content of squalene as well as higher ratios of phospholipid/protein than membranes prepared from cells grown in *cis* 16:1. The ratio of protein/lipid has been shown to influence membrane fluidity (21). The increase in squalene, the precursor of sterols including ergosterol, and smaller increase in sterol and sterol ester may well reflect adaptations by KD115 grown in *trans* supplement to maintain the limited fluidity of membranes enriched in *trans* 16:1 fatty acid. The effects of squalene on the fluidity of membrane lipids is substantiated by the model liposome experiments (Table 8), in which it is shown that increasing squalene concentration increases the fluidity of DPPC and DPPE liposomes. Sterols have been shown to serve this role in model systems and in sterol mutants (47–50). In other systems, other strategies appear to have been used to maintain the physical properties of membranes. In the L-cell sterol mutant, the reduction in sterol content of the mutant membranes appears to be balanced by a compensatory increase in the membrane content of PC enriched in *cis* unsaturated fatty acid (26), a form of compensation not fully possible in our desaturase-deficient yeast mutant.

The ratio of total products derived from mevalonate (sterol, esterified sterol, and squalene) relative to phospholipid is held reasonably constant, in both mutant and parent wild type mitochondria, supplemented with either *cis* or *trans* fatty acids (i.e., 0.19–0.23, calculated from data presented in Tables 3 and 5). However, the proportion of these products in the form of squalene (hydrocarbon fraction) is higher in yeast grown in *trans* 16:1 fatty acid, especially in mutant yeast grown under these conditions. The mechanisms that account for these phenomena are not clear, and need to be investigated further. These mechanisms could be relevant to the effects of *trans* fatty acids in the diet of higher eukaryotes.

We are grateful to the late Ms. Barbara Stevens and to Dr. Hewson Swift for the electron microscopy, and to Dr. J. Tonsgard and Messrs. L. Lusk and L. E. Frazier for help with the fatty acid gas chromatography. We thank Dr. J. Fried for his assistance with infrared and mass spectroscopy, and Ms. Karen Sande for her assistance with the preparation of tables. We also thank Dr. L. Olson for critical reading of the manuscript. This work was supported by Public Health Service grants GM-18858, GM-0093, and HL-04442.

Manuscript received 24 January 1991 and in revised form 2 April 1991.

REFERENCES

1. Baldassare, J. J., and D. F. Silbert. 1979. Membrane phospholipid metabolism in response to sterol depletion. Compensatory compositional changes which maintain 3-O-methylglucose transport. *J. Biol. Chem.* **254:** 10078–10083.
2. Rintoul, D. A., S. M. Chou, and D. F. Silbert. 1979. Physical characterization of sterol-depleted LM-cell plasma membranes. *J. Biol. Chem.* **254:** 10070–10077.

3. Tsukagoshi, N., R. Schafer, and R. M. Franklin. 1977. Structure and synthesis of a lipid-containing bacteriophage. Effects of lipids containing *cis* and *trans* fatty acids on the reconstitution of bacteriophage PM2. *Eur. J. Biochem.* **73:** 469–476.
4. Ito, K., T. Sato, and T. Yura. 1977. Synthesis and assembly of the membrane proteins in *E. coli*. *Cell.* **11:** 551–559.
5. Resnick, M. A., and R. K. Mortimer. 1966. Unsaturated fatty acid mutants of *Saccharomyces cerevisiae*. *J. Bacteriol.* **92:** 597–600.
6. Proudlock, J. W., J. M. Haslam, and A. W. Linnane. 1971. Biogenesis of mitochondria 19. The effects of unsaturated fatty acid depletion on the lipid composition and energy metabolism of a fatty acid desaturase mutant of *Saccharomyces cerevisiae*. *J. Bioenerg.* **2:** 327–350.
7. Haslam, J. M., J. W. Proudlock, and A. W. Linnane. 1971. Biogenesis of mitochondria 20. The effect of altered membrane lipid composition on mitochondrial oxidative phosphorylation in *Saccharomyces cerevisiae*. *J. Bioenerg.* **2:** 351–370.
8. Haslam, J. M., T. W. Spithill, A. W. Linnane, and J. B. Chappell. 1973. Biogenesis of mitochondria. The effects of altered membrane lipid composition on cation transport by mitochondria of *Saccharomyces cerevisiae*. *Biochem. J.* **134:** 949–957.
9. Marzuki, S., G. S. Cobon, P. D. Crowfoot, and A. W. Linnane. 1975. Biogenesis of mitochondria. The effect of membrane unsaturated fatty acid content on the activity and assembly of the yeast mitochondrial protein-synthesizing system. *Arch. Biochem. Biophys.* **169:** 591–600.
10. Marzuki, S., G. S. Cobon, J. M. Haslam, and A. W. Linnane. 1975. Biogenesis of mitochondria. The effects of altered steady-state membrane lipid composition on mitochondrial-energy metabolism in *Saccharomyces cerevisiae*. *Arch. Biochem. Biophys.* **169:** 577–590.
11. Watson, K., R. L. Houghton, E. Bertoli, and D. E. Griffiths. 1975. Membrane-lipid unsaturation and mitochondrial function in *Saccharomyces cerevisiae*. *Biochem. J.* **146:** 409–416.
12. Walenga, R. W., and W. E. M. Lands. 1975. Effectiveness of various unsaturated fatty acids in supporting growth and respiration in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **250:** 9121–9129.
13. Walenga, R. W., and W. E. M. Lands. 1975. Requirements for unsaturated fatty acids for the induction of respiration in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **250:** 9130–9136.
14. Astin, A. M., and J. M. Haslam. 1977. The effects of altered membrane sterol composition on oxidative phosphorylation in a haem mutant of *Saccharomyces cerevisiae*. *Biochem. J.* **166:** 287–298.
15. Astin, A. M., J. M. Haslam, and R. A. Woods. 1977. The manipulation of cellular cytochrome and lipid composition in a haem mutant of *Saccharomyces cerevisiae*. *Biochem. J.* **166:** 275–285.
16. Seelig, J., and A. Seelig. 1980. Lipid conformation in model membranes and biological membranes. *Q. Rev. Biophys.* **13:** 19–61.
17. Daum, G., P. C. Bohni, and G. Schatz. 1982. Import of proteins into mitochondria: cytochrome b_2 and cytochrome c peroxidase are located in the intermembrane space of yeast mitochondria. *J. Biol. Chem.* **257:** 13028–13033.
18. Blanchardie, P., J. P. Carde, and C. Cassagne. 1977. Occurrence and biosynthesis of very long chain fatty acids and alkanes in plasmalemma-enriched fractions from *Saccharomyces cerevisiae*. *Biol. Cell.* **30:** 127–136.
19. Cooperstein, S. J., and A. Lazarow. 1951. A micro spectrophotometric method for the determination of cytochrome oxidase. *J. Biol. Chem.* **189:** 665–670.
20. Kuchler, K., G. Daum, and F. Paltauf. 1986. Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in *Saccharomyces cerevisiae*. *J. Bacteriol.* **165:** 901–910.
21. Brasitus, T. A., D. Schachter, and D. Mamouneas. 1979. Functional interaction of lipids and proteins in rat intestinal microvillus membranes. *Biochemistry.* **18:** 4136–4144.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
23. Rabinowitz, M., and B. De Bernard. 1957. Studies on the electron transport system. X. Preparation and spectral properties of a particulate DPNH and succinate cytochrome c reductase from heart muscle. *Biochim. Biophys. Acta.* **26:** 22–29.
24. Wilgram, G. F., and E. P. Kennedy. 1963. Intracellular distribution of some enzymes catalyzing reactions in the biosynthesis of complex lipids. *J. Biol. Chem.* **238:** 2615–2619.
25. Brasitus, T. A., P. K. Dudeja, and R. Dahlya. 1985. Premalignant alterations in the lipid composition and fluidity of colonic brush border membranes of rats administered 1,2 dimethyl-hydrazine. *J. Clin. Invest.* **77:** 831–840.
26. Brasitus, T. A., and P. K. Dudeja. 1985. Alterations in the physical state and composition of brush border membrane lipids of rat enterocytes during differentiation. *Arch. Biochem. Biophys.* **240:** 483–488.
27. Brasitus, T. A., and D. Schachter. 1980. Lipid dynamics and lipid-protein interactions in rat enterocytes basolateral and microvillus membranes. *Biochemistry.* **19:** 2763–2769.
28. Brasitus, T. A., A. R. Tall, and D. Schachter. 1980. Thermotropic transitions in rat intestinal plasma membranes studied by differential scanning calorimetry and fluorescence polarization. *Biochemistry.* **19:** 1256–1261.
29. Schachter, D., and M. Shinitzky. 1977. Fluorescence polarization studies of rat intestinal microvillus membranes. *J. Clin. Invest.* **59:** 536–548.
30. Heyn, M. P. 1979. Determination of lipid order parameters and rotational correlation times from fluorescence depolarization experiments. *FEBS Lett.* **108:** 359–364.
31. Lakowicz, J. R., F. G. Prendergast, and D. Hogen. 1979. Differential polarized phase fluorometric investigations of diphenylhexatriene in lipid bilayers. Quantitation of hindered depolarizing rotations. *Biochemistry.* **18:** 508–519.
32. Letters, R. 1966. Phospholipids of yeast. II. Extraction, isolation and characterization of yeast phospholipids. *Biochim. Biophys. Acta.* **116:** 489–499.
33. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
34. Getz, G. S., S. Jakovcic, J. Haywood, J. Frank, and M. Rabinowitz. 1970. A two-dimensional thin-layer chromatographic system for phospholipid separation. The analysis of yeast phospholipids. *Biochim. Biophys. Acta.* **218:** 441–452.
35. Vaskovsky, V. E., and E. Y. Kostetsky. 1968. Modified spray for the detection of phospholipids on thin-layer chromatograms. *J. Lipid Res.* **9:** 396.
36. Skipski, V. P., J. J. Good, M. Barclay, and R. B. Reggio. 1968. Quantitative analysis of simple lipid classes by thin-layer chromatography. *Biochim. Biophys. Acta.* **152:** 10–19.
37. Stahl, E., and Jork, H. 1968. XIX. Direct evaluation of the chromatogram spectrophotometer. Zeiss Information, vol. 68. *In* Thin-Layer Chromatography. E. Stahl, editor. Aca-

demic Press, New York. 52–62.
38. Stoffel, W., F. Chu, and E. H. Ahrens. 1959. Analysis of long-chain fatty acids by gas-liquid chromatography. Micromethod for preparation of methyl esters. *Anal. Chem.* **31:** 307–308.
39. McLafferty, F. W., and D. B. Stautter. 1989. The Wiley/NBS Registry of Mass Spectral Data. Wiley-Interscience, New York. **5:** 4724.
40. Cornu, A., and R. Massot. 1979. Compilation of Mass Spectral Data. Second edition. Heyden & Son Ltd., London. Vol. I: 201A.
41. Zickler, D., and L. W. Olson. 1975. The synaptonemal complex and the spindle plaque during meiosis in yeast. *Chromosoma.* **50:** 1–23.
42. Aldrich, M. P., and J. M. Vanderkool. 1976. Temperature dependence of 1,6-diphenyl-1,3,5-hexatriene fluorescence in phospholipid artificial membranes. *Biochemistry.* **15:** 1257–1261.
43. Thulborn, K. R., L. M. Tilley, W. H. Sawyer, and E. Treloar. 1979. The use of n-(9-anthroyloxy) fatty acids to determine fluidity and polarity gradients in phospholipid bilayers. *Biochim. Biophys. Acta.* **558:** 166–178.
44. Bashford, C. L., C. G. Morgan, and G. K. Radda. 1976. Measurement and interpretation of fluorescence polarisations in phospholipid dispersions. *Biochim. Biophys. Acta.* **426:** 157–172.
45. Phillips, M. C., and D. Chapman. 1968. Monolayer characteristics of saturated 1,2-diacyl phosphatidylcholines and phosphatidylethanolamines at the air-water interface. *Biochim. Biophys Acta.* **163:** 301–313.
46. Esfahani, M., E. M. Kucirka, F. X. Timmons, S. Tyagi, A. E. Lord, Jr., and S. A. Henry. 1981. Effect of exogenous fatty acids on growth, membrane fluidity, and phospholipid fatty acid composition in yeast. *J. Supramol. Struct.* **15:** 119–128.
47. Chapman, D. 1973. Physical chemistry of phospholipids. *In* Form and Function of Phospholipids. B. B. A. Library, Vol. 3. G. B. Ansell, J. N. Hawthorne, and R. M. C. Dawson, editors. Elsevier Scientific Publishing Company, New York. 117–142.
48. Ladbrooke, B. D., and D. Chapman. 1969. Thermal analysis of lipids, proteins and biological membranes. A review and summary of some recent studies. *Chem. Phys. Lipids.* **3:** 304–367.
49. Welti, R., D. A. Rintoul, F. Goodsaid-Zalduordo, S. Felder, and D. F. Silbert. 1981. Gel phase phospholipid in the plasma membrane of sterol-depleted mouse LM cells. Analysis by fluorescence polarization and X-ray diffraction. *J. Biol. Chem.* **256:** 7528–7535.
50. Schachter, D. 1984. Fluidity and function of hepatocyte plasma membranes. *Hepatology.* **4:** 140–151.