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Effect of Sterol Side Chains on Growth and Membrane Fatty Acid Composition of *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae GL7 cells require exogenous sterol and unsaturated fatty acid for growth. When grown in the presence of cholesterol or 7-dehydrocholesterol, the cells incorporated less saturated fatty acid into phospholipids than cells grown with ergosterol, stigmasterol, or β-sitosterol as the sterol source. This lower saturated fatty acid content was most pronounced in phosphatidylethanolamine, slightly less so in phosphatidylcholine, and least evident in phosphatidylserine and phosphatidylinositol. Growing the cells with the various sterols did not affect the ratios of individual phospholipids. The ability of strain GL7 to use 7-dehydrocholesterol as the only sterol supplement for growth was dependent upon the nature of the unsaturated fatty acids added to the growth medium. In the presence of linoleic, linolenic, or a mixture of palmitoleic and oleic acids, excellent growth was observed with either ergosterol, cholesterol, or 7-dehydrocholesterol. However, when the medium was supplemented with either oleic or petroselenic acid, the cells grew more slowly (oleic) or much more poorly (petroselenic) with 7-dehydrocholesterol than with ergosterol. A specific relationship between sterol structure and membrane fatty acid composition in yeast cells is implied.

Although *Saccharomyces cerevisiae* displays a strict sterol requirement (1) which becomes apparent under anaerobic growth conditions, the precise function of sterols in this organism has not been resolved. In animal cells, cholesterol is associated primarily with the cytoplasmic membrane, and recent studies have shown that altering the cholesterol content of cultured cells can profoundly affect membrane lipid composition (2, 3, 12), the activity of membrane-bound enzymes (31, 34, 35), and the physical properties of cell membranes (30, 33, 35). On the whole these in vivo studies tend to support the results obtained with model membranes (9, 20, 24) and agree with the supposition that one role of cholesterol is to modulate membrane fluidity.

In contrast to animal cells, yeasts and fungi contain primarily ergosterol as their membrane sterol (28). Ergosterol differs from cholesterol in having two additional double bonds (Δ^7 and Δ^{22}) and a β-oriented methyl group at C_{24}. In artificial membranes (e.g., lecithin vesicles), ergosterol is less effective than cholesterol in reducing membrane permeability (8) or increasing membrane order (32). The presence of either the Δ^{22} unsaturation, the C_{24} methyl group, or both appears to weaken van der Waals interactions between the sterol molecule and adjacent fatty acid chains. Consistent with these observations, mutants of *S. cerevisiae* deficient in C_{24} methylation accumulate C-27 sterols (5) and contain more ordered membranes than wild-type yeasts (19). Such mutants are also more permeable to small molecules (4, 17).

The requirement of anaerobic yeasts for exogenous ergosterol can, to some extent, be satisfied by other sterols (1, 15, 25, 26, 29, 38; R. Masters, Ph.D. thesis, Harvard University, Cambridge, Mass., 1963). Although variable results have been obtained, it is generally agreed that cholesterol does not support the growth of anaerobic yeasts as well as ergosterol (1, 25, 26; Masters, Ph.D. thesis, 1963). However, the addition of a β-oriented C_{24} methyl group to cholesterol improves its effectiveness as a sterol replacement for anaerobic yeast (26). Similarly, a sterol auxotroph of *S. cerevisiae* deficient in heme biosynthesis grew better with C_{24} ethyl sterols (e.g., stigmasterol and sitosterol) than with cholesterol (16). Therefore, despite their relative ineffectiveness in model membranes (7-9, 32), sterols bearing a C_{24} alkyl group are superior to cholesterol in supporting the growth of yeasts.

Recently, Gollub et al. isolated a mutant of *S. cerevisiae* defective in both heme biosynthesis and 2,3-oxidosqualene-lanosterol cyclase (13). This mutant, strain GL7, requires exogenous sterol and unsaturated fatty acid for growth, but

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in contrast to anaerobic yeasts, strain GL7 grows equally well when supplemented with either cholesterol or ergosterol (6, 18). Since these two sterols affect the properties of a given artificial membrane quite differently, it was of interest to determine (i) whether the presence or absence of alkyl groups at C_{24} of exogenous sterols had any effect on the membrane lipid composition of strain GL7 and (ii) whether the ability of the two sterol types to support growth depended on the nature of the unsaturated fatty acid supplement which the mutant requires. Our results demonstrate that in the presence of sterols bearing an alkyl group at C_{24}, strain GL7 synthesizes phospholipids containing higher levels of saturated fatty acids than cells grown with either cholesterol or 7-dehydrocholesterol. Further, for optimal growth with nonalkylated sterols, strain GL7 requires specific unsaturated fatty acids, indicating some interdependence between membrane-sterol structure and phospholipid-fatty acid composition in this organism.

MATERIALS AND METHODS

S. cerevisiae, strain GL7 (*erg12 heme3* [13]), was kindly supplied by D. B. Sprinson. The mutant was grown on a synthetic medium supplemented with methionine (13). Sterols were added to the medium as 0.2% solutions in either Tween 80–ethanol (1:4, wt/wt) or Brij 58–ethanol (1:4, wt/wt) to a final concentration of 10 μg/ml. Unsaturated fatty acids were included in the sterol–Brij 58 solutions to a final concentration of 100 μg per ml of medium. Cultures were grown aerobically at 30°C, and growth was monitored by measuring absorbance at 540 nm. For fatty acid analysis, cells from the log phase of growth were harvested, washed twice with distilled water, and freeze-dried. Dried cells were suspended in methanol and heated under reflux for 1 h. Two volumes of chloroform were added, and the cells were left to stand overnight. After the addition of 0.1 M KCl (0.2 volume of the total volume of $CHCl_3$-CH_3OH), the chloroform phase was separated and concentrated under vacuum before thin-layer chromatography. For analysis of total phospholipids, lipids were separated on Silica Gel G thin-layer plates developed with petroleum ether-diethyl ether-acetic acid (70:30:2, vol/vol/vol; 29). Individual phospholipids were separated with the solvent system of Skipski et al. (36). Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were well separated in this system, but phosphatidylserine (PS) and phosphatidylinositol (PI) were not. Lipids were visualized either with iodine or with molybdate spray reagent (10). Fatty acids were transesterified directly from the silica gel with 10% boron trichloride-methanol by heating at 80°C for 30 min. The fatty acid methyl esters were extracted into pentane and analyzed on a Perkin-Elmer gas-liquid chromatograph (model 900) equipped with a 6-ft (about 180-cm) column of 10% SP 2330 on 100/120 Chromosorb W AW. Analyses were routinely run isothermally at 170°C.

The phospholipid composition of strain GL7 was determined by growing cells in the presence of [^{32}P]P_i (2 μCi/ml) for seven generations. After treatment with trichloroacetic acid (5% final concentration), phospholipids were extracted from lyophilized cells as described above, but in this case separation of the individual phospholipids was performed by two-dimensional chromatography (40). Phospholipids were localized by autoradiography, and areas corresponding to the labeled lipids were scraped into scintillation vials containing water (Cerenkov effect).

Cholesterol, 7-dehydrocholesterol, stigmasterol, and ergosterol were obtained from Sigma Chemical Co. (St. Louis, Mo.) and recrystallized before use. Sitosterol (>97%) was purchased from Applied Science Laboratories (State College, Pa.) and was used without further purification. Detergents and unsaturated fatty acids were obtained from Sigma. Boron trichloride in methanol is the product of Applied Science, and [^{32}P]orthophosphoric acid was obtained from New England Nuclear Corp. (Boston, Mass.). The gas-liquid chromatography column packing was from Supelco.

RESULTS

Fatty acid composition of total phospholipids. With Tween 80 as a source of unsaturated fatty acid, strain GL7 was able to use either ergosterol or cholesterol as a sterol supplement. Total phospholipids from cells grown with cholesterol contained significantly lower amounts of saturated fatty acids than phospholipids from ergosterol-grown cells (Table 1). Although Tween 80 contains predominantly esterified oleic acid, myristoleic and palmitoleic acids are also present (23), and these are readily incorporated by the mutant (Table 1). In subsequent experiments, Brij 58, a nonhydrolyzable detergent, was substituted for Tween 80 and either oleic acid or palmitoleic acid was added as the single unsaturated fatty acid supplement. Under these conditions, sitosterol and stigmasterol supported growth of strain GL7 almost as well as ergosterol, whereas cholesterol was somewhat less effective (Fig. 1A). Phospholipid-fatty acid analysis revealed the presence of only the unsaturated fatty acid added to the medium (Table 1). Whether oleic or palmitoleic acid was the exogenous fatty acid source, cholesterol-grown cells always contained lower amounts of saturated fatty acids in their phospholipids than did cells grown with ergosterol, sitosterol, or stigmasterol.

In subsequent experiments, ergosterol and 7-dehydrocholesterol, two sterols which have identical ring unsaturations and differ only in their aliphatic side chains, were added. Mixtures of palmitoleic and oleic acids, the normal unsaturated fatty acids of yeasts (23), were added as unsaturated fatty acid supplements. They were provided in a ratio of 1:4 (16:1/18:1) in order to simulate the cellular fatty acid composition of

TABLE 1. *Fatty acid composition of phospholipids isolated from S. cerevisiae strain GL7 grown with various sterols*

Experiment[a]	Sterol supplement	Phospholipid-fatty acid composition[b]						% Saturated fatty acids[c]
		14:0	14:1	16:0	16:1	18:0	18:1	
I	Ergosterol	6.2	2.9	28.0	14.1	4.5	42.8	40.1
	Cholesterol	4.2	1.4	19.6	12.5	4.3	57.3	28.7
II	Ergosterol	5.4		19.8		2.3	71.5	28.4
	Cholesterol	3.6		15.8		2.8	77.2	22.8
	Sitosterol	5.4		18.6		1.6	73.7	26.4
	Stigmasterol	6.0		20.7		2.0	70.6	29.4
III	Ergosterol	4.1		18.2		3.3	73.8	25.6
	Cholesterol	2.4		13.9		3.0	80.4	19.3
	Stigmasterol	5.0		18.9		2.6	71.9	26.5
IV	Ergosterol	3.3		24.1	65.5	6.4		34.4
	Cholesterol	3.5		18.6	71.0	6.0		29.0
	Sitosterol	4.2		26.2	65.6	3.1		34.4
	Stigmasterol	4.8		26.5	62.2	4.9		37.8
V	Ergosterol	4.8		26.2	32.1	3.6	30.8	37.1
	7-Dehydrocholesterol	3.8		19.1	33.2	4.7	36.5	30.2

[a] The unsaturated fatty acid supplements in each experiment were: I, Tween 80; II and III, oleic acid; IV, palmitoleic acid; V, palmitoleic and oleic acids (1:4).

[b] Given as percentage of total fatty acids.

[c] All phospholipids contained about 1% 12:0.

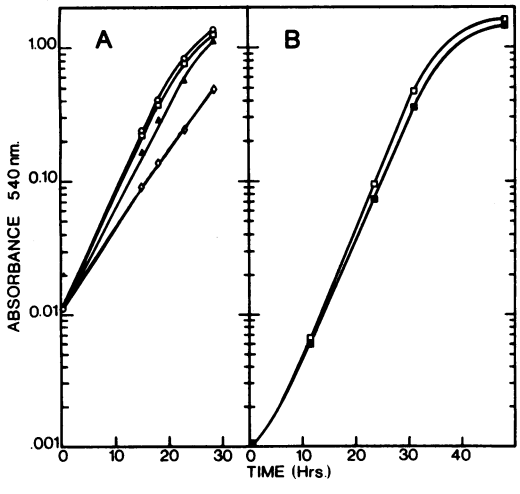

FIG. 1. *Growth of S. cerevisiae strain GL7 in the presence of (A) oleic acid or (B) a mixture of palmitoleic acids and oleic acids (1:4). Various sterol supplements were added to the medium. Symbols:* (○) *sitosterol;* (◇) *cholesterol;* (■) *7-dehydrocholesterol;* (□) *ergosterol;* (△) *stigmasterol.*

wild-type yeast cells (23). Under these conditions, 7-dehydrocholesterol supported growth almost as well as ergosterol (Fig. 1B), and yeast cells grown with 7-dehydrocholesterol incorporated lower amounts of saturated fatty acids into phospholipids than did ergosterol-grown cells (Table 1).

Fatty acid compositions of individual phospholipids. Since individual phospholipids of wild-type *S. cerevisiae* differ somewhat in their fatty acid composition (37), it was of interest to determine whether the sterol-induced changes in fatty acid composition occurred in each phospholipid type. In agreement with previous reports (37), we found the anionic phospholipids (PS and PI) contained higher levels of saturated fatty acids than PE and PC (Table 2). When strain GL7 was grown with 7-dehydrocholesterol instead of ergosterol, the reduction of saturated fatty acids observed in total phospholipids (Table 1) was found to occur primarily in PE and PC and to a much lesser extent in PS and PI (Table 2). Similar results were obtained with cells supplemented with oleic acid (data not shown). Thus, the sterol-induced fatty acid changes did not require specific unsaturated supplements, nor were they related to differences in sterol uptake. In both cases the added sterol was recovered from the cells in roughly equal amounts (0.21 and 0.26% [dry weight] for 7-dehydrocholesterol and ergosterol, respectively). An analogous comparison was made between cholesterol and sitosterol. Again, PC and PE of cells grown with cholesterol contained less saturated fatty acids than did sitosterol-grown cells (Table 2). The reduction of saturated fatty acids

TABLE 2. *Fatty acid composition of individual phospholipids isolated from S. cerevisiae strain GL7 grown with various sterols*

Sterol supplement[a]	Phospholipid analyzed	Fatty acid composition[b]					% Saturated fatty acids[c]
		14:0	16:0	16:1	18:0	18:1	
Ergosterol	PS + PI	3.9 (1.6)	31.7 (4.5)	27.2 (5.2)	8.9 (0.7)	27.3 (7.0)	44.5 (5.5)
	PE	3.0 (1.7)	24.9 (3.3)	38.4 (4.4)		33.5 (2.4)	27.9 (3.6)
	PC	5.0 (1.6)	18.6 (3.5)	44.3 (9.4)	3.8 (1.1)	27.4 (5.6)	27.4 (3.9)
7-Dehydrocholesterol	PS + PI	3.0 (0.9)	28.3 (5.6)	25.4 (9.8)	9.9 (1.8)	32.9 (4.2)	41.2 (7.9)
	PE	2.0 (0.8)	13.0 (1.8)	46.1 (5.1)		38.7 (3.6)	15.0 (1.5)
	PC	2.6 (0.4)	11.9 (2.4)	49.5 (6.9)	3.0 (1.4)	32.6 (5.5)	17.5 (2.7)
Cholesterol	PE	2.0	18.1	37.9	2.0	39.0	22.1
	PC	3.1	15.3	38.7	4.8	38.0	23.2
Sitosterol	PE	2.9	28.2	27.0	1.8	39.0	33.9
	PC	6.0	20.9	41.0	3.7	28.4	30.6

[a] The unsaturated fatty acid supplements were palmitoleic acid and oleic acid (1:4).
[b] Given as percentage of total fatty acids; results are mean of three experiments. Standard deviations are given in parentheses.
[c] All phospholipids contained 1 to 2% 12:0.

was always slightly greater in PE than in PC. Clearly, the fatty acid changes are not specific for the C_{24} methyl group, and the Δ^{22} double bond is not responsible for the higher levels of saturated fatty acids in cells grown with C_{24} alkylated sterols.

When cells were grown in the presence of $^{32}P_i$, the relative ^{32}P concentrations were: PC, 47%; PE, 17%; PS, 12%; PI, 20%; and an unidentified component, <5%. The proportions of radioactive phospholipids were the same whether the sterol supplement for strain GL7 was ergosterol or 7-dehydrocholesterol (data not shown).

Effects of various unsaturated fatty acid supplements on the growth of strain GL7. In Fig. 2 are shown growth curves of cells supplied with either oleic acid, petroselenic acid ($18:1^{\Delta 6}$), α-linolenic acid, or γ-linolenic acid. When ergosterol was the sterol source, cells grew best on α- and γ-linolenic acid and somewhat less well on the monounsaturated acids (oleic and petroselenic). These results are in agreement with previous observations on the relative growth-promoting efficiencies of multiple and single unsaturated fatty acids for *S. cerevisiae* (39). In the presence of 7-dehydrocholesterol, the two linolenic acids also afforded excellent growth (Fig. 2B), but oleate was substantially less efficient and petroselenate was quite ineffective (Fig. 2A). The poor response to petroselenate cannot be attributed to the position of the double bond (Δ^6) per se, since γ-linolenate ($\Delta^{6, 9, 12}$) supported growth nearly as well as α-linoleate ($\Delta^{9, 12, 15}$). Linoleic acid ($18:2^{\Delta 9, 12}$) was also tested as an unsaturated fatty acid source for strain GL7 in the presence of both ergosterol and 7-dehydrocholesterol. The results (not shown) did not differ significantly from those obtained with the isomeric linolenates.

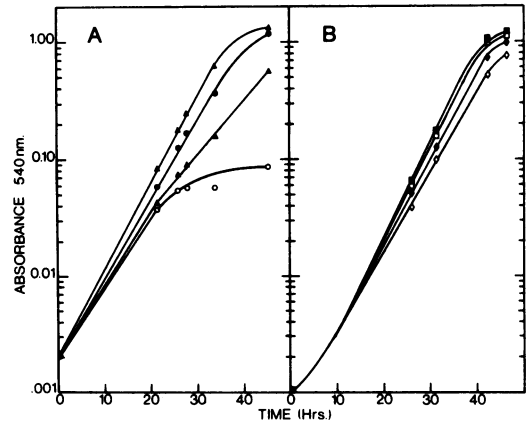

FIG. 2. *Growth of S. cerevisiae strain GL7 in the presence of ergosterol (closed symbols) or 7-dehydrocholesterol (open symbols). Various unsaturated fatty acids were added to the medium. A:* (△, ▲) *oleic acid;* (○, ●) *petroselenic acid. B:* (□, ■) *α-linolenic acid;* (◇, ◆) *γ-linolenic acid.*

DISCUSSION

Previously, the sterol requirement of *S. cerevisiae* has been examined by growing the cells anaerobically (1, 15, 25, 26, 29, 38; Masters, Ph.D. thesis, 1963). The conflicting results obtained were attributed primarily to the extreme difficulty in excluding traces of oxygen from the culture (26). Because of a defect in 2,3-oxidosqualene-lanosterol cyclization and a heme deficiency (13), strain GL7 is unable to synthesize

either sterols or unsaturated fatty acids. Since the mutant readily incorporates various sterols without changing sterol structure (6, 18), this organism has been useful for studying sterol structure-function relationships. For the same reason, it has been possible to examine the effect of sterol replacement on the membrane composition of this simple eucaryotic cell. It appears that the identity of the sterol side chain exerts some control on the fatty acid composition of phospholipids in the yeast membranes. Our results with this organism show that the presence or absence of a C_{24} alkyl group in the sterol side chain has a marked influence upon the fatty acid pattern found in the cellular phospholipids. Growth in the presence of sterols lacking a C_{24} alkyl group reduces the relative amounts of saturated fatty acids in neutral phospholipids and, to a lesser extent, in anionic phospholipids. This reduction is not due to a difference in the ratios of individual phospholipids synthesized, nor is it related to differences in the amount of sterols incorporated. We cannot exclude the possibility that the various sterols are localized within different membranes in the yeast cell. Studies are in progress to determine whether there is a relationship between sterol structure and intracellular membrane localization. (These fatty acid changes do not represent an effect of sterol on unsaturated fatty acid uptake since sterol-induced fatty acid changes were also observed when strain GL7 was supplemented with heme in place of unsaturated fatty acids; T. M. Buttke and R. Reynolds, unpublished data.)

The direction of the observed fatty acid changes are in line with existing information. In model membranes, sterols containing an alkyl group at C_{24}, such as ergosterol or sitosterol, have relatively small effects on membrane order (8, 33). Indeed, at high concentrations ergosterol actually disorganizes the bilayer (33). (Whether this is true for model membranes regardless of lipid composition is not known. Lecithin liposomes have been used extensively for studying ergosterol effects [8, 32].) Similar effects have been observed in vivo. Mutants of *S. cerevisiae* deficient in C_{24} methyltransferase, and therefore unable to alkylate the sterol side chain (5), contain more ordered membranes than those of wild-type yeasts (19). A disordering effect is thus attributable to the C_{24} alkyl group. Increased membrane fluidity in a specific region (probably the interior) of the bilayer is thereby implied. By the same token, the increased levels of unsaturated fatty acids incorporated into the phospholipids of cells grown on 7-dehydrocholesterol instead of ergosterol may reflect an attempt by the yeast to increase membrane fluidity, compensating for the absence of the bulky, and therefore disordering, C_{24} methyl group.

Our finding that sitosterol and stigmasterol substitute reasonably well for ergosterol emphasizes the relatively nonspecific nature of the sterol requirement in yeast, as long as the aliphatic side chain contains a bulky alkyl group. Furthermore, whereas the C_{24} methyl group of ergosterol is β-oriented, sitosterol and stigmasterol contain α-oriented ethyl groups at C_{24}. Thus the growth of strain GL7 is not significantly affected by either the size of the alkyl group or its specific orientation.

Sitosterol- (side chain saturated) and ergosterol-grown cells have the same, relatively more saturated, fatty acid composition, suggesting that the C_{22} double bond is not essential for the disordering effect of ergosterol. On the other hand, cells grown on stigmasterol (Δ^{22} double bond) contained slightly higher levels of saturated fatty acids than cells grown with sitosterol (Table 1). Therefore, the presence of a C_{22} double bond, although not essential, may enhance the disordering effects of stigmasterol and ergosterol. The apparently additive effects of a C_{24} alkyl group and a Δ^{22} double bond are in agreement with the finding of Nes et al. (26) that brassicasterol (C_{24}-αCH_3; Δ^{22} double bond) was slightly more effective than 24β-methylcholesterol and much more effective than cholesterol in supporting the growth of anaerobic yeasts.

The importance of relatively fluid membranes for optimal growth of strain GL7 is further illustrated by the following observations. In the presence of ergosterol, strain GL7 grew very well regardless of the unsaturated fatty acid provided. With 7-dehydrocholesterol, however, the mutant grew well only when supplemented with di- or trienoic fatty acids. Growth was slower with oleic acid and ceased after 25 h with petroselenic acid. These two unsaturated fatty acids have relatively high melting temperatures (12 and 30°C, respectively) and would not be expected to raise membrane fluidity as much as linoleic or linolenic acids. It is also interesting that with 7-dehydrocholesterol as a sterol source, a mixture of palmitoleic and oleic acids caused better growth than either monounsaturated acid alone. In previous, related studies on the sterol requirements of yeast, either oleic acid (16) or Tween 80 (1, 25, 26) was the source of unsaturated fatty acids. As our results demonstrate, the choice of the unsaturated fatty acid supplement is an important factor in the growth response of yeast to various sterols.

Observations related to ours have been obtained with the protozoan *Tetrahymena*. Normally, *Tetrahymena* synthesizes tetrahymanol, a pentacyclic triterpenoid, instead of sterol (22). Exogenous cholesterol inhibits the synthesis of

tetrahymanol and after conversion to cholesta-5,7-22-triene-3β-ol, is incorporated into the protozoan cell membranes replacing tetrahymanol (21). Ergosterol, however, which has the same 5,7,22-triene system, is incorporated into *Tetrahymena* membranes intact (21). Replacement of tetrahymanol with ergosterol causes the synthesis of more saturated phospholipids, especially PE (11, 27). These changes in fatty acid composition are similar to the changes observed in strain GL7. Studies with *Tetrahymena* comparing the fatty acid compositions of cholesterol-grown and ergosterol-grown protozoa have not been reported.

Finally, we would like to raise the question of why the yeast synthesizes a sterol containing the side chain of ergosterol. Alkylated sterols are typically found in simple microbial eucaryotes as well as in plants (25), both of which are subject to wide variations in temperature. If the effect of the alkyl group (and/or C_{22} double bond) is to increase membrane disorder (fluidity), then this extra degree of fluidity may permit yeast cells to grow well at relatively low (25 to 28°C) temperatures. This may be especially important in *S. cerevisiae* which synthesizes only monoenoic acids and therefore cannot raise membrane fluidity by producing more highly unsaturated acids. Fluidizing effects of the ergosterol side chain may similarly allow yeast cells to grow in the presence of relatively high concentrations (4 to 5%) of ethanol. Yeast grown anaerobically with ergosterol have been reported to be more resistant to ethanol than yeast cells grown with cholesterol, and this increased resistance appeared to be related to the membrane lipid composition (38). Similarly, mutants of *S. cerevisiae* deficient in C_{24} methyltransferase are more sensitive to ethanol than wild-type yeast (14). We have confirmed these observations in strain GL7 and find that ergosterol-grown cells are more resistant to ethanol than cells grown with 7-dehydrocholesterol (Buttke, unpublished data).

The results reported here rationalize to some degree the benefits yeast cells derive from the ergosterol side chain. It also deserves mention that ergosterol, the sterol that yeast cells ordinarily produce, enables this organism to grow optimally with a variety of unsaturated fatty acid sources. By contrast, yeast cells using sterols containing the normal cholesterol side chain grow optimally only under more selective conditions.

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