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Influence of sterol structure on yeast plasma membrane properties

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Fluorescence anisotropy measurements indicated that physical changes occurred in the lipids of plasma membranes of yeast sterol mutants but not in the plasma membrane of an ergosterol wild-type. Parallel experiments with model membrane liposomes verified that the physical changes in lipids observed in the sterol mutants are dependent on the sterol present and not the phospholipid composition. In addition, the physical changes in lipids observed in liposomes derived from wild-type phospholipids were eliminated by addition of ergosterol but persisted in the presence of cholesterol, cholestanol, ergostanol, or sterols from the sterol mutants. No physical changes in lipids were observed, however, in plasma membranes from a sterol auxotroph, even when the auxotroph was grown on cholesterol or cholestanol. The lack of physical changes in lipids in the sterol auxotroph may reflect the ability of the auxotroph to modify its phospholipid composition with respect to its sterol composition. These results indicate that high specificity 'sparking' sterol is not required for the regulation of overall bulk lipid properties of the plasma membrane.

Introduction

The yeast *Saccharomyces cerevisiae* requires sterol for at least two different classes of functions [1,2]. Studies with sterol auxotrophs indicate that one of these functions, the bulk membrane role, is relatively nonspecific and requires large quantities of either sterol or stanol. The second role for sterols, the high specificity 'sparking' function, is satisfied by minute amounts of sterol possessing a nuclear unsaturation at the C-5,6 position (Δ^5-sterol). This phenomenon is elucidated by the fact that sterol auxotrophs are unable to grow on cholestanol (5 μg/ml) unless minute amounts of ergosterol (10 ng/ml) are available. Although the specific role of sparking sterol is unknown, bulk membrane sterol is reported to be involved in maintaining proper membrane fluidity and integrity [3–8], and, at least in mycoplasma, seems to affect uptake of fatty acid [3]. Model systems have indicated that in order for a sterol to fulfill bulk membrane requirements it must have a 3β-hydroxyl group, a planar nucleus and an alkyl side-chain [3,5,6,8]. Within these restrictions, a large number of sterol structures are possible, many of which are found in eukaryotic organisms.

There is the tacit assumption that all naturally occurring membrane sterols are as effective as cholesterol in controlling membrane properties. However, there are several examples in which this is not the case. For instance, ergosterol and stigmasterol are less efficient than cholesterol in reducing the permeability of egg phosphatidylcholine liposomes to glycerol and erythritol [5]. In addition, at concentrations greater than 10 mol% cholesterol still aligns egg phosphatidylcholine bilayers, but ergosterol disrupts the bilayer [9].

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Abbreviation: DPH, 1,6-diphenyl-1,3,5-hexatriene.

Cholesterol has also been shown to be more effective than stigmasterol or sitosterol in liquefying dipalmitoylphosphatidylcholine monolayers [6].

It is not evident from these studies, however, that cholesterol would be preferred to other sterol structures within native membranes which may have characteristics very different from those of the simplified model systems. In order to elucidate the role of sterol structure in membrane properties and to understand how an organism may compensate for various sterol structures, we have examined the membranes and lipids of yeast with different sterol compositions in fluorescence studies. Two types of yeast strain were used for these studies: sterol mutants and sterol auxotrophs. The sterol mutants have defects late in the ergosterol biosynthetic pathway and, unlike the parental wild-type, synthesize sterols other than ergosterol which satisfy sterol requirements. The sterol auxotroph is defective in heme biosynthesis and in the early stages of ergosterol biosynthesis, and hence requires an exogenous source of sterol and unsaturated fatty acid. Use of both types of strain makes it possible to isolate membranes with a variety of sterol compositions.

In this report we present data which indicate that, although sterol mutants are unable to compensate for changes in membrane lipid properties caused by the presence of non-ergosterol sterols, a sterol auxotroph is able to make such an adjustment. This adjustment corresponds to a change in the phospholipid composition. In addition, evidence is presented which indicate that sparking sterol is not required for regulating overall bulk lipid properties of the plasma membrane.

Materials and Methods

Culture conditions and plasma membrane isolation

Saccharomyces cerevisiae strain S288C (Berkeley stock culture collection) and two sterol biosynthetic mutants derived from S288C, JR1 and JR5 [10] (Table I), were grown overnight at 28°C to early log phase in rich medium (0.5% yeast extract, 1.0% tryptone, 2.0% dextrose). The yeast sterol auxotroph, RD5-R (Table I), was grown at 28°C to late log phase in defined media supplemented with unsaturated fatty acids and sterol as described previously [2].

TABLE I

YEAST STRAINS

Strain	Defect in sterol biosynthesis	Sterol(s) synthesized
S288C [a]		ergosterol
JR1 [a]	Δ^5 desaturase (*erg*3)	ergosta-7,22-dienol
JR5 [a]	24-methyltransferase (*erg*6)	zymosterol cholesta-5,7,24-trienol cholesta-5,7,22,24-tetraenol
RD5-R	5-aminolevulinic synthetase (*hem*1) oxidosqualene cyclase (*erg*7) Δ^5 desaturase (*erg*3)	–

[a] See Ref. 26 for complete sterol and phospholipid compositional analyses.

Yeast spheroplasts were formed using a dialyzed extracellular preparation of the enzyme lyticase from *Oerskovia xanthineolytica* as detailed previously [11]. Plasma membranes from the osmotically lysed spheroplasts were isolated on discontinuous sucrose gradients using the procedure of Bottema et al. [11].

Quantitative procedures

Protein was determined by the method of Bradford [12] using the Bio-Rad protein assay reagent with bovine gamma-globulin as the standard. Yeast cells were disrupted with glass beads and lipids were extracted with chloroform/methanol as described by Parks et al. [13]. Lipids were separated using the thin-layer chromatography (TLC) systems of Skipski and Barclay: (1) isopropyl ether/acetic acid (96 : 4, v/v) and (2) hexane/diethyl ether/acetic acid (90 : 10 : 1, v/v) [14]. Sterol quantitation was performed by gas-liquid chromatography (GLC) as described previously [13]. Phospholipids were purified using the two-dimensional TLC system of Esko and Raetz: (1) hexane/diethyl ether/acetic acid (30 : 7 : 1, v/v) and (2) hexane/diethyl ether/acetic acid (90 : 10 : 1, v/v) [15]. Quantitation of phospholipid species was performed without elution from the TLC plates by the method described by Skipski and Barclay [14]. Fatty acid compositions of the individual phospholipid species were determined

by forming methyl esters of the fatty acids with dimethylsulfoxide as described by Christie [16] and quantitating the methyl esters by GLC using the conditions perviously described [17]. All lipid analyses were done in duplicate.

Liposome preparation

Liposomes were prepared by evaporating desired lipids or lipid mixtures to dryness under nitrogen and resuspending, by vortexing and water-bath sonication, in 10 mM phosphate buffer (pH 6.8) containing fluorescent probe (1 μM) [11,17].

Fluorescence analyses

Fluorescence anisotropy was measured on a computerized spectrofluorophotometer [18], using the probe DPH [17]. Anisotropy was determined from the equation:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

where $I_{\parallel}$ and $I_{\perp}$ are the intensities of parallel and perpendicular polarized light respectively. At each temperature point, more than 1000 individual anisotropy measurements were taken. The statistical significance of each anisotropy value plotted is 0.001. Temperatures were determined within ± 0.05°C. All anisotropy experiments were performed in triplicate. The absorbance at 460 nm was less than 0.15 for all samples and the concentration of DPH was 1 μM in 10 mM phosphate buffer (pH 6.8).

Materials

DPH, bovine γ-globulin, unsaturated fatty acids and sterols were from Sigma. Sterols used for the growth of the sterol auxotroph, RD5-R, were prepurified by HPLC [19,20]. All solvents were redistilled prior to use.

Results

Fluorescence anisotropy of yeast plasma membranes

In fluorescence studies with the probe DPH in plasma membranes from several yeast strains, the fluorescence anisotropy decreased with higher temperatures, indicating an increase in mobility of the DPH probe in the membrane (Fig. 1). The anisotropy values at any particular temperature varied slightly with the strain. More importantly, however, the rate of decrease in anisotropy with respect to temperature was strain-dependent. A discontinuity was observed in the plasma membranes of the sterol mutants JR1 and JR5 (Fig. 1). These discontinuities, interpreted as lipid phase transitions [21–25], were not observed in the parental wild-type strain S288C (Fig. 1).

Graphs of fluorescence anisotropy measurements from plasma membranes of the sterol auxotroph RD5-R had no such discontinuities regardless of the supplemented sterol (Fig. 2). In analogy to the ergosterol-containing S288C, no discontinuity was anticipated when RD5-R was grown on ergosterol. However, the lack of a discontinuity when RD5-R was grown on cholesterol or cholestanol was surprising since, in comparison, less dramatic alterations in the structure of the sterols accumulated by JR1 and JR5 (Table I) resulted in discontinuities in the Arrhenius plots (Fig. 1). In order to define the parameters which determined this phenomenon, model lipid systems were studied.

Fluorescence anisotropy of model systems

Liposomes composed of yeast lipids were used

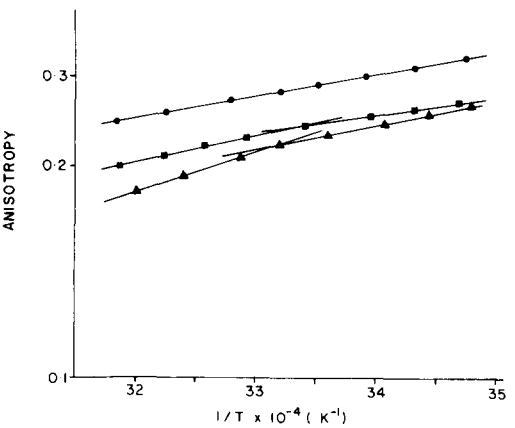

Fig. 1. Fluorescence anisotropy of sterol mutant and wild-type plasma membranes. Fluorescence anisotropy of DPH in plasma membranes from JR1 (▲), JR5 (■), and S288C (●) was measured as described in Materials and Methods from 15 to 40°C.

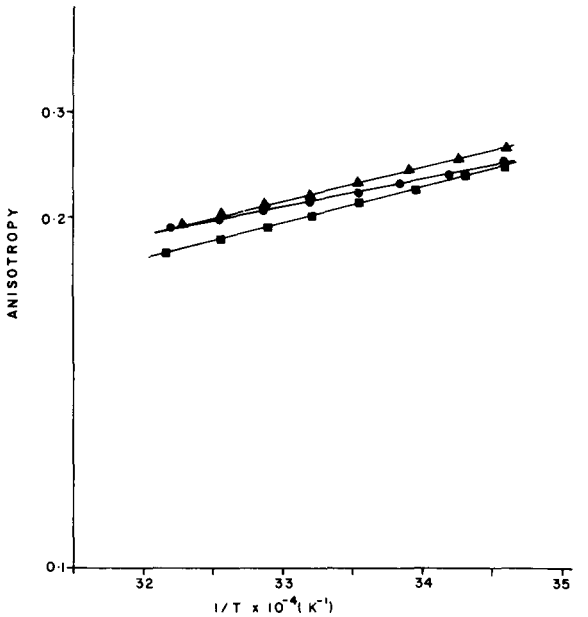

Fig. 2. Fluorescence anisotropy of sterol auxotroph plasma membranes. Fluorescence anisotropy of DPH in plasma membranes of RD5-R grown on ergosterol (●), cholesterol (▲), and cholestanol (■) was measured as described in Materials and Methods from 15 to 40°C.

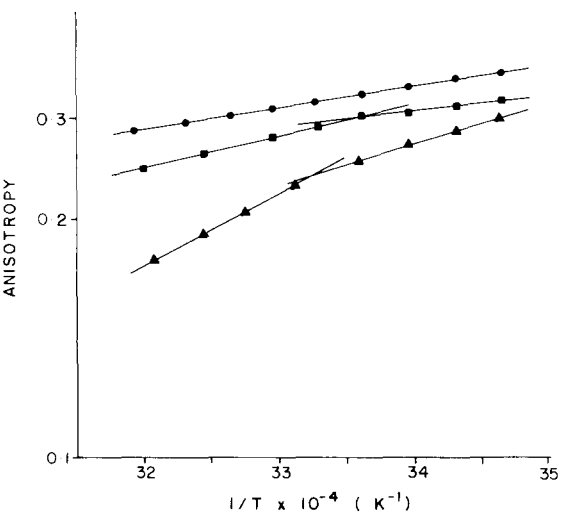

Fig. 3. Fluorescence anisotropy of lipid from the sterol mutants and wild-type. Fluorescence anisotropy of DPH in liposomes composed of total lipid extracted from JR1 (▲), JR5 (■), and S288C (●) was measured as described in Materials and Methods from 15 to 40°C.

as a model system for examination by fluorescence analysis. Initially, total yeast lipid was incorporated into liposomes in order to determine if the plasma membrane studies could be duplicated. Indeed, the fluorescence anisotropy of these liposomes gave results very similar to the plasma membranes (Fig. 3). The wild-type S288C liposomes had no discontinuity in plots of the fluorescence anisotropy, while both sterol mutants JR1 and JR5 demonstrated physical changes in membrane lipids (Fig. 3). The temperatures of the discontinuities were very close to those seen in the plasma membrane studies and overall anisotropy values were also similar.

Liposomes were then formed using purified phospholipids and nonsaponifiable sterols extracted from these organisms in the same phospholipid to sterol molar ratio as found in the plasma membranes. By mixing and matching the phospholipids and sterols of different strains, it became evident that the sterol composition, and not the phospholipid, was critical in modulating the measured membrane lipid properties (Fig. 4, Fig. 5, Table II). When liposomes were prepared with phospholipids from S288C, a physical change in lipid properties occurred which could be eliminated by the incorporation of ergosterol (Fig. 4) but not JR1 or JR5 sterols (Fig. 5). The same

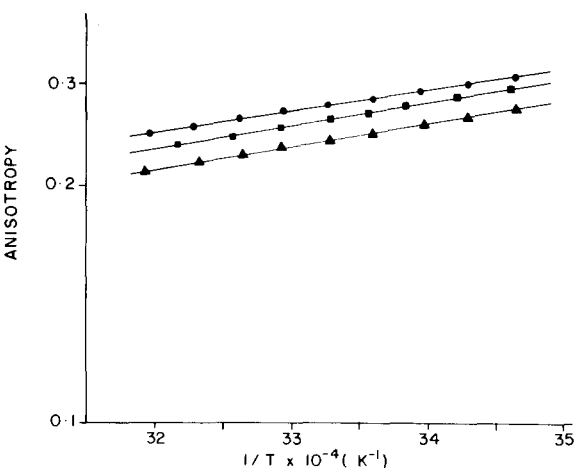

Fig. 4. Fluorescence anisotropy of liposomes containing wild-type sterol. Fluorescence anisotropy of DPH in liposomes containing S288C sterol and phospholipid from JR1 (▲), JR5 (■), and S288C (●) was measured as described in Materials and Methods from 15 to 40°C.

TABLE II

DISCONTINUITY TEMPERATURES FOR CURVES OF THE FLUORESCENCE ANISOTROPY OF MIXED LIPOSOMES

Liposomes were prepared by mixing phospholipid and sterol from different yeast strains as described in Methods. Discontinuity temperatures were calculated from fluorescence anisotropy data of DPH measurements taken as described in Methods. A detailed analysis of the sterol and phospholipid composition of the strains is presented in reference 26.

Sterol source	Phospholipid source		
	S288C	JR1	JR5
S288C	–[a]	–	–
JR1	28°C[b]	27°C	28°C
JR5	22°C	23°C	25°C

[a] No discontinuity in curve (–).

[b] Values represent triplicate experiments with a variance of ±0.5°C.

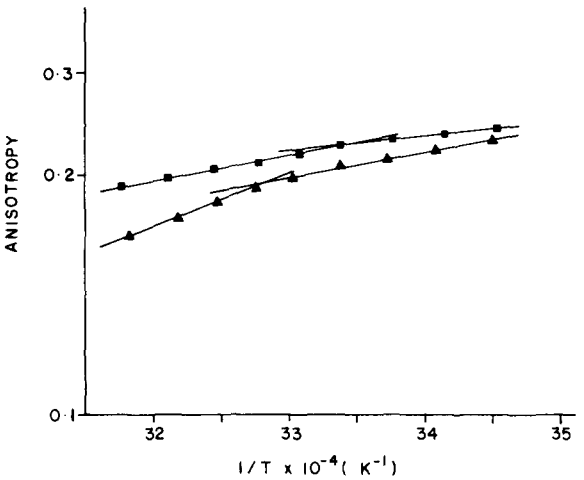

Fig. 5. Fluorescence anisotropy of mutant sterol/wild-type phospholipid liposomes. Fluorescence anisotropy of DPH in liposomes composed of wild-type (S288C) phospholipid and sterols from JR1 (▲) and JR5 (■) was measured from 15 to 40°C as described in Materials and Methods.

phenomenon was observed for phospholipids from JR1 and JR5 (i.e., physical changes in phospholipids of JR1 or JR5 were eliminated by ergosterol but not their respective sterols) (Table II).

Based on the plasma membrane experiments, these results were not unexpected. However, further studies using the phospholipids of the wild-type S288C with various purified sterols gave more anomalous results. A discontinuity was detected at 23°C in the graphs of the fluorescence anisotropy of wild-type S288C phospholipid without sterol

TABLE III

STEROL, PHOSPHOLIPID AND FATTY ACID QUANTITATION OF THE STEROL AUXOTROPH RD5-R GROWN ON DIFFERENT STEROLS

Lipids were extracted and quantitated as described in Materials and Methods. Values are relative percentage of total phospholipid headgroups (excluding 3–5% of the minor phospholipid components phosphatidylglycerol and lysophosphatidylcholine) and total phospholipid fatty acid. Abbreviations: PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidlyserine; PE, phosphatidylethanolamine; CL, cardiolipin; C14:0, myristic; C16:0, palmitic, C16:1, palmitoleic; C18:0, stearic; C18:1, oleic acid.

Sterol	PC	PI	PS	PE	CL
Ergosterol	46.5±2.5	22.3±1.9	9.3±1.8	17.7±2.1	4.2±1.6
Cholesterol	45.4±2.9	26.4±2.3	5.4±1.4	19.2±1.9	3.6±1.0
Cholestanol	47.1±3.2	28.6±2.7	5.7±1.9	14.3±1.7	4.3±1.5
	14:0	16:0	16:1	18:0	18:1
Ergosterol	6.3±1.4	42.8±2.3	11.9±1.9	5.1±1.1	33.9±2.5
Cholesterol	5.4±1.6	30.9±2.1	12.5±2.4	6.4±1.7	44.8±2.2
Cholestanol	10.9±1.5	22.6±2.0	16.4±2.4	7.5±1.9	42.6±2.6

	Sterol/phospholipid (μmol/μmol)	Sterol/protein (μmol/mg)
Ergosterol	0.072±0.015	0.0111±0.0013
Cholesterol	0.106±0.011	0.0135±0.0018
Cholestanol	0.143±0.019	0.0163±0.0022

present which was not eliminated by the incorporation of cholestanol, cholesterol, or ergostanol where discontinuities occurred at 27°C, 25°C and 29°C, respectively. Only ergosterol eliminated this discontinuity. This was of interest, since cholesterol and cholestanol prevented discontinuities in the plasma membranes of RD5-R.

Phospholipid composition of the yeast strains

It appeared that the sterol mutants derived from S288C were unable to compensate for their unusual sterol composition, while the sterol auxotroph RD5-R was somehow able to adjust to a different sterol composition and thus avoid detectable change in membrane lipid properties. Any compensation for the sterol composition of a yeast membrane would be likely to involve changes in the phospholipids. The phospholipids of the sterol mutants JR1 and JR5 are very similar to their parental wild-type, S288C [26]. It appears that these sterol mutants are incapable of compensating for their unusual sterol patterns through gross phospholipid changes.

The phospholipid composition of the sterol auxotroph RD5-R, however, did vary with the sterol accumulated. Differences in the phospholipid composition were observed at several levels. RD5-R grown on cholesterol or cholestanol had higher sterol to phospholipid molar ratios and higher sterol to protein ratios than cultures grown on ergosterol (Table III). Changes in phospholipid species in these auxotrophic cultures were also

TABLE IV

FATTY ACID COMPOSITION OF TOTAL LIPIDS AND INDIVIDUAL PHOSPHOLIPIDS FROM THE STEROL AUXOTROPH

Fatty acid of phospholipid species from RD5-R grown on different sterols was quantitated as described in Methods. See Table III for abbreviations.

	14:0	16:0	16:1	18:0	18:1
PC					
Ergosterol	4.8±1.6	42.5±3.0	11.5±2.1	5.5±1.2	35.8±2.9
Cholesterol	7.2±1.0	32.4±2.2	12.5±1.9	4.3±1.2	43.5±2.5
Cholestanol	7.3±1.1	25.9±2.3	16.4±2.0	6.3±1.7	44.1±3.1
PI					
Ergosterol	8.2±1.1	43.9±3.4	9.7±1.0	7.7±1.2	30.4±2.7
Cholesterol	2.4±2.0	23.8±2.9	11.9±1.5	14.2±2.1	47.7±2.8
Cholestanol	6.7±1.3	14.9±1.8	16.2±1.4	12.3±1.5	49.9±3.2
PS					
Ergosterol	8.0±1.2	50.3±3.9	9.4±1.3	4.3±1.1	28.0±2.1
Cholesterol	6.1±1.3	40.1±2.2	11.1±1.4	3.6±0.9	39.1±2.9
Cholestanol	25.8±1.8	16.0±2.3	10.3±1.1	3.8±1.2	44.1±2.7
PE					
Ergosterol	7.4±1.7	40.2±2.1	17.0±1.3	1.4±0.9	34.0±2.7
Cholesterol	4.3±1.3	36.1±3.3	13.9±1.9	1.8±0.8	44.0±2.5
Cholestanol	22.9±2.3	30.6±2.8	13.4±2.3	3.0±1.1	30.1±3.1
CL					
Ergosterol	4.3±1.8	35.5±3.4	12.2±1.6	3.1±1.5	44.9±3.9
Cholesterol	7.1±1.7	24.3±2.7	11.0±2.2	5.5±0.7	52.1±2.8
Cholestanol	18.1±2.6	19.5±2.5	36.4±2.6	7.8±1.4	18.2±1.8

Sterol	% unsaturated fatty acid
Ergosterol	46
Cholesterol	57
Cholestanol	59

observed in the levels of phosphatidylinositol and phosphatidylserine. The phosphatidylserine content in ergosterol grown cells was approx. 50% higher than cells grown on cholesterol or cholestanol. The level of phosphatidylinositol increased from 22% of the total phospholipid in ergosterol grown cells to 26% and 28% in the cholesterol and cholestanol cultures respectively. Although the remaining phospholipid species fluctuated to a much lesser extent, the fatty acid composition of all phospholipids varied significantly between these cultures. Cultures grown on ergosterol had less unsaturated fatty acids, 46% of total fatty acid, compared to cultures grown on cholesterol or cholestanol which had 57% and 59%, respectively. The difference between the amount of unsaturated fatty acids found in these cultures was largely due to the increased accumulation of 18 : 1 fatty acid in cholesterol and cholestanol grown cells. Ergosterol grown cells, on the other hand, had a higher percentage of 16 : 0 fatty acid. This pattern of fatty acid distribution was maintained in most of the individual phospholipid classes (Table IV). Cholestanol grown cells accumulated more short-chain fatty acid (14 : 0) than ergosterol or cholesterol grown cells, particularly in the phosphatidylserine, phosphatidylethanolamine and cardiolipin fractions.

It appears that the sterol auxotroph RD5-R is able to modify its phopholipid fatty acid composition with respect to its sterol composition. The sterols used to supplement RD5 in this study are unmodified by the organism [2], so its sterol composition consists solely of the exogenously supplied sterol species. This ability to compensate for the sterol composition may prevent changes in the physical properties of membrane lipids. The sterol mutants JR1 and JR5 do not appear to have this flexibility because they do not selectively synthesize fatty acids. By contrast sterol auxotrophs, which must have an exogenous source of unsaturated fatty acids, may accumulate fatty acids discriminately to control membrane properties.

Discussion

It is evident that changing the naturally occurring sterol composition of a given membrane may have very profound effects. This has been clearly established in yeast by the use of sterol biosynthetic mutants which synthesize sterols other than ergosterol. Such mutants have membrane properties very different from those of wild-type yeast. It has been shown by fluorescence anisotropy studies of mitochondria and Arrhenius kinetics of the mitochondrial enzyme kynureine hydroxylase that phase transitions occur in the sterol mutant membranes which do not occur in the wild-type membrane [17]. For two other yeast mitochondrial enzymes, cytochrome oxidase and sterol methyltransferase, the transition temperature is lower in the sterol mutants [27]. Moreover, ESR studies indicate that the membranes of yeast sterol mutants are more rigid than membranes of yeast wild-types, suggesting greater membrane order in the mutants [28]. These sterol mutants are also more sensitive to ethanol and detergents [29].

Data (presented here) from fluorescence anisotropy measurements of model systems and plasma membranes, indicate that the sterols present in the membranes of sterol mutants are unable to prevent changes in membrane lipid properties. Results from our studies with the wild-type reveal that the ergosterol present in its plasma membrane is responsible for the absence of membrane lipid changes. The sterols present in the mutants do not prevent membrane lipid changes even when mixed with wild-type phospholipids. While it has been previously demonstrated that cholesterol eliminates membrane lipid changes in a variety of model systems [5,6], cholesterol failed to prevent the membrane lipid changes of wild-type yeast phospholipids. This suggests that the phospholipid composition of yeast is better suited for ergosterol than cholesterol.

In the plasma membranes of the sterol auxotroph RD5-R, however, cholesterol did prevent lipid alterations over the physiological temperature range 15 to 40°C. Unlike the sterol mutants (JR1 and JR5), the sterol auxotroph (RD5-R) was capable of significantly altering its phospholipid fatty acid composition. By compensating for the sterol composition possibly through fatty acid or specific phospholipid changes, the sterol auxotroph appears to be able to grow on a variety of sterols and still maintain suitable membrane lipid properties. Similar effects of changing sterol content on phospholipid composition have also been observed in

yeast [30] and mammalian cells [31].

It is apparent that sparking levels of ergosterol (Δ^5-sterol) are not required for modulating overall bulk lipid properties of the plasma membrane. This was demonstrated by the fact that plasma membranes from cholestanol-cycled RD5-R (i.e., possess insufficient Δ^5-sterol for further growth in the presence of cholestanol) [1] showed no lipid perturbations during fluorescent studies. Since the auxotrophic membrane also appears to function adequately with cholesterol, it is interesting to question why yeast evolved to synthesize ergosterol. Because of the C-28 methyl group on the side-chain, ergosterol requires approximately 12 more ATP equivalents to biosynthesize than cholesterol [32]. It is possible that yeast developed a particular phospholipid composition first and then acquired a sterol to function best with that composition. The converse situation is also possible. Alternatively, yeast may have developed a need for a C-28 methyl by virtue of specificity of the C-5,6 desaturase enzyme, to ensure production of Δ^5-sterol to fulfill the sparking requirement [2].

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