Involvement of Heme Biosynthesis in Control of Sterol Uptake by Saccharomyces cerevisiae

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Wild-type Saccharomyces cerevisiae do not accumulate exogenous sterols under aerobic conditions, and a mutant allele conferring sterol auxotrophy (erm7) could be isolated only in strains with a heme deficiency. δ-Aminolevulinic acid (ALA) fed to a heml (ALA synthetase) erg7 (2,3-oxidosqualene cyclase) sterol-auxotrophic strain of S. cerevisiae inhibited sterol uptake, and growth was negatively affected when intracellular sterol was depleted. The inhibition of sterol uptake (and growth of sterol auxotrophs) by ALA was dependent on the ability to synthesize heme from ALA. A procedure was developed which allowed selection of strains which would take up exogenous sterols but had no apparent defect in heme or ergosterol biosynthesis. One of these sterol uptake control mutants possessed an allele which allowed phenotypic expression of sterol auxotrophy in a heme-dependent background.

Biosynthetic controls in microorganisms generally maximize the use of exogenously available nutrients over those endogenously synthesized. This spares the organism the metabolic expense of forming those compounds. An anomalous situation appears to exist in the yeast Saccharomyces cerevisiae with regard to the 28-carbon membrane lipid, ergosterol. Under aerobic conditions, wild-type yeast strains synthesize large amounts of ergosterol and do not incorporate significant amounts of exogenous sterol (17). Under anaerobic conditions, sterol cannot be produced by the organism, and exogenously supplied sterol is utilized by the cells (1). The physiological advantage of this system is not apparent, because it would seem more efficient to curtail endogenous synthesis of sterol when it is available in the medium than to prevent its transport and require synthesis to occur.

Anaerobic conditions have been used to induce a sterol requirement to study the structural features of sterols needed for growth of this organism (8). Because of the difficulty of manipulating the anaerobic system, attempts have been made to develop an aerobic alternative for probing structure-function relationships in sterols. Auxotrophs which are unable to synthesize sterols offer a means of restricting sterol synthesis; however, the isolation of mutants which are tightly and unconditionally blocked in sterol synthesis and which are otherwise prototrophic has not been reported previously. An apparent requirement for a concomitant genetic defect in heme synthesis along with sterol auxotrophy has been observed by Gollub et al. (4) and in our laboratory (unpublished data). Attempts to segregate recombinant 2,3-oxidosqualene cyclase mutants from the hem (heme biosynthesis-defective) backgrounds in which they were originally isolated have been unsuccessful. The hem mutations somehow allow for the viability of these sterol-auxotrophic strains.

The first sterol auxotroph used in our laboratory, FY1, was derived from one of the first sterol-requiring mutants isolated, Ole3, shown to lack δ-aminolevulinic acid (ALA) synthetase activity (19); this locus is now referred to as heml. The heml mutant requires unsaturated fatty acid, methionine, and sterol in the heme-deficient conditions, but may also be supplemented by ALA alone to meet these requirements. Strain FY1 possesses the heml allele and grows poorly on cholesterol as its sterol supplement. A rapid-growing variant, FY3, was selected from this genetic background and was found to have acquired an additional mutation in ergosterol biosynthesis at the point of cyclization of 2,3-oxidosqualene (16).

We present here our studies on the control of sterol uptake by S. cerevisiae and the effects elicited by restoring the potential for heme synthesis by heme mutants. These studies were performed by using nutritional supplementation to manipulate the potential for heme synthesis by FY3 and other heme mutants. In addition, a genetic approach was taken by using a technique which allowed the identification of strains that accumulate increased amounts of exogenous sterol.

MATERIALS AND METHODS

Yeast strains. The yeast strains used in this study are listed in Table 1.

Media and growth conditions. The standard growth medium used was adjusted to pH 5.5 and consisted of 0.67% yeast nitrogen base (Difco Laboratories), 0.05 M succinic acid, 2% glucose, and the following at 0.02%: methionine, uracil, histidine, tyrosine, tryptophan, phenylalanine, leucine, lysine, and adenine sulfate. Sterol was added at a concentration of 20 μg/ml from a stock solution of 4 mg/ml in Tergitol Nonidet P-40-95% ethanol (1:1, vol/vol). Unsaturated fatty acid supplementation was with either 1% Tween 80 or a mixture of oleic and palmitoleic acids (4:1, vol/vol) at 0.01% final concentration added from a 10% solution in Tergitol-ethanol. ALA was supplemented to a final concentration of 0.005% (300 μM) from a 1% stock solution in 95% ethanol. Protoporphyrin IX (PPIX) and hematin were added to a final concentration of 10 μg/ml from 1% (wt/vol) solutions in 0.01 N KOH in 50% ethanol.

For depletion of sterol, cells of strain FY3 were grown to maximal cell density in medium containing cholestanol (5α-cholestan-3β-ol) in place of sterol supplement, as described by Rodriguez et al. (12).

Cultures were grown aerobically with shaking at 30°C.
TABLE 1. Descriptions of yeast strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype-phenotype</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td>X2180-1A</td>
<td>a SUC2 mal gal2</td>
<td>Y.G.S.C.*</td>
</tr>
<tr>
<td>X2180-1B</td>
<td>a SUC2 mal gal2</td>
<td>Y.G.S.C.</td>
</tr>
<tr>
<td>FY1</td>
<td>a heml met ura</td>
<td>16</td>
</tr>
<tr>
<td>FY3</td>
<td>a heml met ura erg7</td>
<td>16</td>
</tr>
<tr>
<td>G204</td>
<td>a hem1 his4</td>
<td>18</td>
</tr>
<tr>
<td>G207</td>
<td>a hem10 his4</td>
<td>18</td>
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<tr>
<td>G210</td>
<td>a hem11 his4</td>
<td>18</td>
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<tr>
<td>G214</td>
<td>a hem13 his4</td>
<td>18</td>
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<tr>
<td>G216</td>
<td>a hem13 his4</td>
<td>18</td>
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<tr>
<td>G218</td>
<td>a hem12 ura2-33</td>
<td>T. M. Buttke and 4</td>
</tr>
<tr>
<td>GL7</td>
<td>a hem3 erg12</td>
<td></td>
</tr>
<tr>
<td>TL-Upc27</td>
<td>a SUC2 mal gal2</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Upc1</td>
<td></td>
</tr>
<tr>
<td>TY27-1</td>
<td>a erg7 Upc1</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Y.G.S.C., Yeast Genetic Stock Center, Berkeley, Calif.

Growth was measured with a Klett-Summerson photoelectric colorimeter equipped with a green filter. Klett units were found to be proportional to cell numbers by the following formula: log cells per milliliter = (log Klett + 1.86)/0.6486 in the range of 5 to 500 Klett, as verified by direct cell enumeration with a Coulter Counter.

Measurement of sterol accumulation throughout the culture cycle. Cells were grown in the media described above with sterol and Tween 80. [1-¹⁴C]Cholesterol was present at an activity of 0.01 μCi/ml. This corresponds to a specific activity of approximately 0.18 mCi/mmol. Cell samples were collected from the growing cultures by removing 5- or 10-ml portions and centrifuging them at 500 g for 10 min. Cell pellets were washed twice with 10 mM phosphate buffer (pH 7.0) containing 0.5% Tergitol Nonidet P-40 and again with buffer alone. A suspension of wild-type, unlabeled carrier cells (5 ml) was added to samples of low cell densities. The washed cell pellets were frozen, lyophilized, and extracted with chloroform after dimethyl sulfoxide treatment, as described previously (15). Free sterol and sterol esters were resolved by thin-layer chromatography (TLC) on silica gel plates by using the solvent system of Skipski et al. (14). Bands were visualized with I２ vapor, and those migrating with standards of cholesterol and cholestereryl palmitate were scraped into scintillation vials and counted in a Beckman LS 8000 scintillation counter with toluene-PPO (2,5-diphenyloxazole)-POPOP (7,4-bis-(5-phenyloxazolyl)benzene) scintillator. The amount of sterol taken up per cell was measured as counts per minute/milliliters of sample X Klett units.

Assay of sterol uptake. We measured the ability of yeast strains to take up sterol under aerobic conditions by growing cells to stationary phase in medium with added cholesterol. The cells were harvested and washed as described above, and the sterols were extracted after acid labilization and saponification (5). Sterols were purified by TLC as described above, and bands were scraped from plates and eluted with chloroform-methanol (4:1, vol/vol). This purified extract was then dried under N₂ and dissolved in diethyl ether before injection into a Varian 2700 gas chromatograph. Chromatograms were compared with those obtained from extracts of the same cells grown with added cholesterol to ensure that endogenously produced sterols which may coelute with cholesterol did not give false-positive results.

Isolation of sterol uptake control mutants. A culture of wild-type S. cerevisiae X2180-1A was mutagenized to approximately 10% survival with ethyl methanesulfonate by the procedure of Lindegren et al. (6). Dilutions of mutagen-exposed cells were spread onto the standard medium described above, without sterol, and solidified with 17 g of agar per liter. Plates with 100 to 500 colonies were replica inoculated by adhering the colonies onto nitrocellulose membrane disks (Millipore Corp., type HA, 9-cm diameter) and placing the filters, with the colonies facing up, onto standard medium with 0.02 μCi of [¹⁴C]cholesterol (0.39 mCi/mmol) per ml. The original master plate was stored under refrigeration. After three days of incubation at 30°C, the filters were removed from plates and alternately blotted three times on filter paper saturated with 0.5% Tergitol Nonidet P-40 solution and then on dry filter paper, to remove nonincorporated radioactivity. After the filter was wetted again, the colonies were transferred to paper filters (9.0 cm, Whatman no. 1) by pressing the nitrocellulose and paper disks together. These were then placed in a 60°C oven until the colonies dried thoroughly. The brittle nitrocellulose filter was removed, leaving the dried colonies adhered to the paper. The filter disks were then autoradiographed by exposing them to Kodak XAR-5 X-ray film for 3 days at room temperature before they were developed. Colonies showing the greatest radioactivity were then picked from the original plate and screened for sterol uptake as described above, as well as for growth on minimal medium and a nonfermentable energy source (glycerol).

Determination of sterol intermediates accumulated. Strains were inoculated into standard medium containing 0.2 μCi of [1-¹⁴C]acetate (specific activity, 55 mCi/mmol) per ml and 0.5% glucose plus the respective nutritional requirements and were grown to stationary phase. Nonsaponifiable lipids were extracted and applied to silica gel thin-layer plates adjacent to pure standards of cholesterol, lanosterol, farnesol, and squalene. These samples were chromatographed by using the solvent system cyclohexane-ethyl acetate (9:1, vol/vol). Bands were visualized by I₂ vapor. The distribution of radioactivity on plates was determined with a Packard model 7201 scanner.

Chemicals. Sterols, amino acids, hematin, ALA, and nucleotide bases were from Sigma Chemical Co. PPIX was from Calbiochem-Behring. Solvents were from Mallinckrodt, Inc., and were redistilled before use. Pure cholesterol was the gift of Henry Kircher. [4-¹⁴C]Cholesterol (specific activity, 59.4 mCi/mmol) was from New England Nuclear Corp. [1-¹⁴C]Acetate (specific activity, 55 mCi/mmol) was from ICN Pharmaceuticals. silica gel plates (F254, 0.25-mm thickness) were from E. Merck AG.

RESULTS

Growth response of hem1 and hem1 erg7 strains to ALA. The effect of ALA on the growth of strains FY1 (hem1) and FY3 (hem1 erg7) is shown in Fig. 1 and 2. The hem1 parent strain, FY1, showed growth similar to that of wild-type yeast cells (data not shown) when ALA was added, whereas growth was poor with cholesterol plus Tween 80. No growth occurred without fatty acid or heme supplementation. The erg7 derivative, FY3, grew well on cholesterol plus Tween 80 but gave a pattern of brief exponential growth followed by a period of transient growth inhibition when ALA was included in the same medium (Fig. 2). This type of growth pattern was also seen when ergosterol was used as sterol supplement (data not shown). When ALA was added to an exponentially growing culture of strain FY3, no inhibition of growth was seen (data not shown).

Three to five generations of growth with ALA supplementation were required to observe growth inhibition
with strain FY3. Because strain FY1 did not require three generations with ALA supplementation to attain its optimal growth rate, it did not seem likely that three generations were required for ALA to be adequately metabolized by heml mutants. Instead, we reasoned that a cellular component essential for growth was being depleted in strain FY3 during the first three generations of growth with ALA. Because sterol is the only nutritional requirement of FY3 not shared by FY1 when these strains were grown with ALA, the possibility that sterol became growth limiting for FY3 during ALA supplementation was investigated.

**Effect of ALA on sterol-depleted cells.** The addition of ALA to strain FY3 during the exponential phase had no apparent inhibitory effect, possibly because the sterol assimilated before ALA addition was enough to prevent dilution of sterol to levels below the limits of growth in the number of generations of the culture cycle which remained. Because we used cells which had already exhausted their supply of sterol, our explanation for the requisite growth before inhibition could be tested by observing whether growth inhibition occurred earlier in these cells. If sterol depletion did affect the amount of growth which occurred before inhibition, other possible explanations for growth inhibition, such as another nutrient becoming growth limiting or the accumulation of an inhibitory product after growth with ALA, would seem unlikely.

Depletion of endogenous sterol was accomplished by precycling FY3 cells on cholestanol, which cannot support the continued growth of sterol auxotrophs (12) but which allows growth to the point at which a minimum cellular sterol content is reached. Cholestanol-cycled FY3 cells showed no growth 100 h after inoculation into ALA-containing media (data not shown). Thus, depletion of sterol had the effect of preventing growth of FY3 cells in the presence of ALA, suggesting that ALA acts, as expected, by interfering with sterol uptake by FY3 cells.

**Effect of ALA on sterol uptake by FY3.** The ability of FY3 to take up exogenous sterol in the presence of ALA was examined more directly by growing cells in medium contain-
FIG. 3. Effect of ALA supplementation on sterol accumulation by strain FY3. Inocula and initial samples were identical and from a culture grown to stationary phase on the same [14C]cholesterol-containing medium used in this experiment. Cell samples were collected throughout growth, and lipids were extracted, separated by TLC, and quantitated by liquid scintillation counting. Media and symbols are the same as those described in the legend to Fig. 2, except that sterol included radiolabel. (A) Growth; (B) relative amounts of free sterol; (C) relative amounts of sterol esters, expressed as counts per minute/(milliliters of sample × Klett units).

ing [14C]cholesterol and monitoring accumulation of radioactivity into free sterol and sterol ester pools throughout growth. Both cellular sterol pools were examined because the sterol ester fraction has been found to fluctuate as a storage pool (2, 15) and could indicate the availability of sterol in the yeast cells. In the presence of ALA, growth retardation had an attendant decrease in free sterol per cell (Fig. 3). This figure also shows that the control culture completed the expected cycle of sterol ester hydrolysis and sterol esterification seen in wild-type cultures (15) but that the ALA-grown culture did not replenish the ester pool (Fig. 3C). It can also be seen that endogenous stores of sterol (sterol esters) become maximally depleted at the time growth inhibition becomes apparent.

The effect of ALA on sterol uptake was also measured when ALA was added to exponential-phase cells (Fig. 4). The growth of the culture receiving ALA paralleled the growth of the control in this experiment; however, the amount of free sterol per cell in ALA-exposed cells decreased continually from the point of ALA addition until growth stopped, while the amount of free sterol in control cells remained relatively constant. The amount of radioactive sterol appearing in sterol esters increased steadily as the stationary phase was approached by the control culture, but the ALA-supplemented culture again showed no increase in this fraction.

Potential for hemoprotein synthesis and sterol uptake. The metabolic occurrence of ALA is unique to the heme biosynthetic pathway; therefore, it seemed most likely that heme or hemoprotein was responsible for the effects incurred during ALA supplementation. Before discarding the possibility that complete metabolism of ALA to heme was not necessary to manifest the effects of ALA on sterol uptake, we examined the effect of ALA on other heme mutants. Six mutants, each blocked in one of the eight steps of heme biosynthesis (Fig. 5), were grown with and without ALA supplementation. The uptake of cholesterol was measured under these circumstances to determine whether ALA itself or another subsequent precursor was responsible for the inhibition of sterol uptake. These mutants should either have their mutant phenotype suppressed (in the heml strain) or accumulate different porphyrin intermediates upon metabolism of ALA (18). It should be noted that only one of these strains (G121) required sterol, suggesting that the other mutations are leaky to some extent, but all were negative for catalase and growth on glycerol. The intracellular heme levels were also found to be below limits of detection (18). The results of examination of sterol uptake by nine heme mutants with various heme supplements are listed in Table 2. Cholesterol uptake was seen in all of these strains without ALA and in all strains except the heml strain when ALA was present in the growth medium. ALA also had no effect on the uptake of sterol or growth of strain GL7 (hem3 [urogen I synthase−] erg2 [oxidosqualene cyclase−]), an independently isolated sterol auxotroph. Based on these data it seems unlikely that ALA itself or another intermediate of the heme biosynthetic pathway is causing the inhibition of sterol uptake.

We also observed the effect of the porphyrins, PPIX and hematin, on sterol uptake by the heme mutants and sterol auxotrophs (Table 2). These porphyrins were less effective heme supplements than ALA as they did not restore respiratory competence (ability to grow on glycerol as the energy source) or ergosterol synthesis to FY1 cells. PPIX was able to alleviate the unsaturated fatty acid requirements of FY1 and FY3 cells and allowed synthesis of sterols other than
ergosterol, presumably precursors, by FY1 (data not shown). These porphyrins also did not prevent sterol uptake by heme mutants G204, G207, G210, G121, G214, or G216. The limited effectiveness of porphyrin heme supplements, compared with ALA, was also observed previously by others (3). This evidence indicates that the efficiency with which a supplement affects cytochrome-dependent metabolism (i.e., respiration or ergosterol biosynthesis) is related to its ability to inhibit sterol uptake.

FIG. 4. Effect of ALA addition during exponential growth on sterol accumulation by strain FY3. Growth conditions and symbols are the same as those described in the legend to Fig. 3, except that cells used as inocula in this experiment were not prelabeled with [14C]cholesterol. The arrow indicates the point of ALA addition. (A) Growth; (B) relative amounts of free sterol; (C) relative amounts of steryl esters, expressed as counts per minute (milliliters of sample × Klett units).

FIG. 5. Heme biosynthetic pathway of S. cerevisiae. Heme biosynthetic intermediates are listed on the left, in the order of the reaction sequence of heme biosynthesis indicated by arrows. The enzymes catalyzing the reactions involved are listed on the right, next to their respective steps. The strain designations G204, G207, G210, G121, G216, and G214 are inserted at points corresponding to the biosynthetic defects in these strains (indicated by crosses over the arrows in the reaction sequence).

Other evidence for the idea that cytochrome-restoring levels of heme synthesis must occur to prevent sterol uptake and the growth of sterol auxotrophs comes from the isolation of a strain, derived from FY3, which was not inhibited by ALA. This strain, PFY3A, was obtained from a culture of FY3 which had reached stationary phase in ALA-containing medium. Strain PFY3A was identified by a greater accumulation of sterol in the presence of ALA, relative to other colonies of the population (data not shown). This resistant strain had acquired the additional characteristic of an inability to grow on media devoid of unsaturated fatty acid but supplemented with a low, subinhibitory concentration of ALA. FY3 grows on this type of medium. Strain PFY3A resembled FY3 in that it did not require unsaturated fatty acid when PPIX was included in the growth medium. Because unsaturated fatty acid was required by FY3 due to the lack of heme synthesis and, therefore, cytochromes, this evidence indicated that an additional defect between the steps of ALA permeation and iron insertion into PPIX had been acquired by strain PFY3A, again correlating the ability to synthesize heme with the negative effect on sterol uptake.

Genetic studies on sterol uptake control. The fact that sterol uptake occurred when sterol was not required (in the presence of a leaky heme mutation or under semianaerobic conditions (7)) and was inhibited when heme synthesis but not sterol synthesis could occur (FY3 with ALA) indicated that a system of sterol uptake control exists which responds
TABLE 2. Effect of ALA and porphyrin heme supplements on heme mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Respiration*</th>
<th>Effect of supplements on:</th>
<th>Growth inhibition*</th>
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<tr>
<td></td>
<td>ALA</td>
<td>PPIX</td>
<td>Hematin</td>
</tr>
<tr>
<td>G204</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>G207</td>
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<td>G216</td>
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<td>-</td>
<td>-</td>
</tr>
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<td>FY3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GL7</td>
<td>-</td>
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</table>

* Assayed during growth on glycerol as energy source. All cultures were negative with no supplement.

** Assayed by gas chromatographic analysis with the wild type as the negative control and cholesterol as the exogenous sterol. All cultures were positive with no supplement.

*** Assayed turbidometrically. –, No noticeable difference in growth curves; +, noticeable difference in growth curves.

**** Assayed by[^14C]cholesterol incorporation. I, Inhibited relative to control culture.

ND, Not determined.

to heme synthesis. On this basis, we assumed that it should be possible to obtain mutants which are uncoupled in this response and which take up sterol concurrent with heme and ergosterol synthesis. By using the autoradiographic technique described above, we screened for the uptake of sterol colonies from a mutagenized culture of wild-type yeast cells which had grown on media containing [^14C]cholesterol. Mutants were found which would take up exogenously supplied cholesterol, whereas the unmutagenized parent strain would not. The strains were then screened for the ability to grow on minimal medium and on media containing glycerol as the sole energy source. Strain TL-Upc27 is one strain obtained in this manner and one whose phenotype was examined in more detail to determine whether other distinguishing traits accompanied its ability to accumulate exogenous sterol.

Defects in sterol biosynthesis can be revealed by chromatographic analysis of nonsaponifiable lipids (9). Because no requirement for sterol was found in strain TL-Upc27, any defect in sterol biosynthesis would necessarily have been either late in the ergosterol biosynthetic pathway or leaky and occurring in an early step. Strain TL-Upc27 accumulated radioactivity from [1-[^14C]acetate into nonsaponifiable lipids which migrated with the same Rf as those of the parent, wild-type strain in a TLC system which separated sterol precursors (Fig. 6). This was an indication that defects in the early portion of the sterol biosynthetic pathway were not present in strain TL-Upc27. Gas chromatographic analysis also revealed no differences in the nonsaponifiable lipids from strain TL-Upc27 and those of the wild-type strain (data not shown), whereas mutants defective in late steps of the ergosterol biosynthetic pathway were readily detected in this manner.

These data indicate that strain TL-Upc27 differed from the wild type in its ability to accumulate exogenous sterol or inability to exclude it. Because our data indicate that heme biosynthesis is involved in this process and because crosses between wild-type and FY3 yielded viable erg7 spores only in a heml background, we presumed that a defective sterol uptake control mechanism, such as that of strain TL-Upc27, may allow the viability of erg7 spores in a HEM1 genetic background. This prediction was tested by screening for HEM1 erg7 strains among haploids produced by sporulation of a diploid heterozygous for heml, erg7, and upc1 (sterol uptake control) alleles. This diploid was obtained by mating

FIG. 6. Sterol intermediates accumulated by strain TL-Upc27 and wild-type parent X2180-1A. Radiochromatogram scans of nonsaponifiable lipids from (A) strain X2180-1A and (B) strain TL-Upc27 grown in medium containing [^14C]acetate (as described in the text). Direction of solvent migration is from left to right.
strains TL-Upc27 and FY3. Screening was done by testing viable haploid meiotic progeny for their ability to grow without lipid supplements, with ALA in place of lipid supplements, with ergosterol as the only lipid supplement, or with ergosterol and unsaturated fatty acids. Segregants were obtained which required only sterol supplementation for growth.

Strain TY27-1 was one of the strains found in this analysis to show the Hem\(^+\) Erg phenotype. Strain TY27-1 was found to incorporate \(^{14}\)Cacetate into nonsaponifiable lipids with the same \(R_f\) in TLC as those lipids obtained from FY3 grown in the presence of ALA (Fig. 7). This was expected of a

\[\text{HEM1 erg7 strain because ALA suppresses the effect of the hem1 mutation of FY3 but not the erg7 defect.}\]

Because this phenotype was never obtained from a cross between FY3 and a wild-type strain, we assumed that the \text{upc1}\ allele of strain TL-Upc27 was present in TY27-1 and permitted its viability, as the \text{hem1} allele had allowed the
viability of FY3. To confirm this, TY27-1 was crossed with the wild-type strain, X2180-1B, and tetrad analysis was performed by testing the lipid requirements and sterol uptake capacity of haploid segregants (Table 3). This analysis showed that only strains requiring sterol as the sole lipid supplement or strains without any lipid requirement were obtained, indicating that only the erg7 auxotrophic marker of strain FY3 was inherited by TY27-1. It was also found that 10 of the 20 strains with no lipid requirement displayed the Upc phenotype, thereby showing that the proposed upc1 allele was present in TY27-1, segregated independently of the erg7 allele and in a 2:2 ratio.

Although there was generally poor viability of the Erg spores (only 4 of 10 expected upc1 erg7 spores), the data are consistent with the hypothesis that the upc1 allele must be present for viability of Erg spores. The best examples of this are tetrads 3, 7, 8, and 9, in which both of the expected upc1 alleles would be accounted for, recombination occurred to produce one or two Upc (Erg<sup>+</sup>) spores, and corresponding numbers of the predicted erg7 spores were not viable.

**DISCUSSION**

The uptake of sterol by *S. cerevisiae* occurs under conditions which are known to limit sterol synthesis, such as anaerobic conditions and defects in the ergosterol biosynthetic pathway (1, 7, 13, 17). Why sterols are not taken up under other circumstances to spare the metabolic expense of synthesizing them is not clear. If exogenous sterol enters the cells only when synthesis is precluded, it would seem possible that there is no active control of sterol exclusion by yeast cells at all, but rather that sterol is excluded merely by physical displacement by the sterol synthesized endogenously. This would mean that endogenous sterol has an advantage in entering the membranes and that sterol uptake is dependent on the level of endogenous production. Our experiments, presented here, with a mutant strain incapable of synthesizing its sterol indicate that sterol may be excluded by a mechanism independent of sterol synthesis but dependent on heme synthesis. This information seems to obscure any physiological advantage of the mechanism which excludes exogenous sterol, because it suggests that control is even less directly related to the availability of sterol in the cell.

A model of sterol uptake control involving heme is consistent with some of the observations of sterol uptake and sterol auxotrophy in *S. cerevisiae*. Under anaerobic conditions, in which sterol uptake must occur to permit growth, heme synthesis would be precluded by the oxygen requirements of coproporphyrinogen decarboxylation (10) and protoporphyrinogen oxidation (11). A heme-dependent system of control over sterol uptake might also be expected to limit the number of possible genetic backgrounds from which sterol auxotrophs can be isolated. As was mentioned above, tight, unconditional sterol-auxotrophic mutants have previously been isolated only in *hem* backgrounds. There is good evidence, however, that enzymes of the early steps of ergosterol biosynthesis (i.e., before squalene synthesis) are vital to the cell for supplying metabolic intermediates used in systems other than sterol synthesis, thereby making a complete loss of these functions lethal to the cell (13).

Because relatively little is known about the mechanism of sterol uptake by yeast cells, data on sterol uptake control is still rather inconclusive. Most evidence suggests that control or at least sterol exclusion may result from a displacement effect exerted by sterol already in the membrane. The discovery of mutants which have no apparent defect in sterol biosynthesis or heme biosynthesis but which take up sterol under circumstances in which wild-type strains do not, as well as the further characterization of the sterol uptake process, may provide a means of determining what processes are involved in relating heme synthesis, sterol synthesis, and sterol uptake. These studies are currently in progress.

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**LITERATURE CITED**