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Yeast Mutants Auxotrophic for Choline or Ethanolamine

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Three mutants of the yeast *Saccharomyces cerevisiae* which require exogenous ethanolamine or choline were isolated. The mutants map to a single locus (*cho1*) on chromosome V. The lipid composition suggests that *cho1* mutants do not synthesize phosphatidylserine under any growth conditions. If phosphatidylethanolamine or phosphatidylcholine, which are usually derived from phosphatidylserine, were synthesized from exogenous ethanolamine or choline, the mutants grew and divided relatively normally. However, mitochondrial abnormalities were evident even when ethanolamine and choline were supplied. Diploids homozygous for the *cho1* mutation were defective in sporulation. Growth on nonfermentable carbon sources was slow, and a high proportion of respiratory-deficient (petite) cells were generated in *cho1* cultures.

Mutants unable to synthesize specific molecules may be utilized to examine the roles of individual membrane components in cell growth and membrane functions. A genetic approach to the molecular requirements for membrane assembly, growth, and function is readily developed in microorganisms in which conditional mutants may be obtained. Bacterial mutants auxotrophic for various phospholipid precursors have been isolated, as have temperature-sensitive mutants with defects not readily corrected by exogenous supplementation (2, 9, 10, 14, 23, 32). These mutants provide information regarding the effects of altered lipid composition on procaryotic membrane assembly and function. Fungal mutants provide the potential for similar investigations of the roles played by specific phospholipids in the biogenesis of the complex array of eucaryotic membranous organelles. Fungal mutants autotrophic for the phospholipid precursors fatty acids (12, 13, 24, 26) and inositol (15, 27) have been described. However, in studies pertaining to membrane properties, the fungal mutants have not been exploited to the same degree as have bacterial mutants (16, 28).

This report concerns *Saccharomyces cerevisiae* mutants which require either ethanolamine or choline for growth. The mutants' properties provide some startling insights into eucaryotic cellular requirements for certain membrane phospholipids.

MATERIALS AND METHODS

Yeast strains. *S. cerevisiae* 2180-1A (**a** *gal2 SUC2*), from the Berkeley strain collection, was the parental strain from which choline-requiring mutants were derived. Strains 2180-1B (*α gal2 SUC2*) from the Berkeley collection, G599-10B (**a** *ura3 hom3 his1*) provided by John Game, and S5 (**a** *ade1*) from H. O. Halvorson were used in crosses. Strain MC13 (*α ino1-13 lys2 can1*), described by Culbertson and Henry (3), was used in crosses and as a related choline-independent (*CHO*$^+$) strain in comparative studies.

Media and growth conditions. Strains were maintained on YEPD plates (1% yeast extract, 2% peptone, 2% glucose, and 2% agar). Respiratory sufficiency was tested on YPGE plates, with 2% glycerol and 2% ethanol in place of glucose. Synthetic complete medium contained 2% glucose; 0.67% Difco Yeast Nitrogen Base without amino acids; the amino acids (in mg/liter) lysine (20), arginine (10), leucine (10), methionine (10), threonine (60), tryptophan, (10), histidine (10), adenine (10), uracil (10), and *myo*-inositol (3 mg/liter, to total 5 mg/liter with the inositol contained by Yeast Nitrogen Base); and one of the following supplements: 1 mM choline, 1 mM ethanolamine, 1 mM serine, 1 mM *N,N*-dimethylethanolamine (all from Sigma Chemical Co.), or 1 mM *N*-methylethanolamine (from Eastman Chemical Co.). Auxotrophic markers were scored on media lacking a single component of the complete medium. Inositol-less medium was prepared using Difco Vitamin-Free Yeast Base as described by Culbertson and Henry (3).

Colonies were grown on agar plates at 25 or 30°C as indicated. Liquid cultures were pregrown overnight in YEPD at 30°C. Log-phase cells were harvested by centrifugation, washed twice with synthetic medium, suspended in synthetic medium containing the various supplements at a density of 1×10^6 to 2×10^6 cells per ml, and incubated at 25 or 30°C as indicated. Optical density of cultures was determined in a Klett spectrophotometer with a red filter. Viable cells were determined by plating appropriate dilutions onto YEPD plates. To minimize aggregation of cells, the first culture dilution before plating was briefly sonicated. Cell numbers obtained by counting the colonies compared well with cell counts obtained by hemacytometer, indicating that cellular aggregation was not a significant problem in plating experiments.

Mutagenesis and genetic analysis. Strain 2180-1A was treated with the mutagen ethyl methane sulfonate by the method of Lindegren et al. (18). Colonies from treated cells grown on YEPD plates were replica-plated to synthetic complete medium supplemented with 1 mM choline and to medium with no supplement. Colonies that failed to grow without added choline were isolated.

Mutant isolates were crossed to strain 2180-1B, and the diploids were induced to sporulate on acetate medium (6). Asci were digested with 10% glusulase (Endo Laboratories) and dissected by micromanipulation on a YEPD plate (5). Spore colonies were replica-plated to the various media to determine the segregation of auxotrophic requirements. Mating type was determined by spraying plates with a water suspension of cells of each mating type (5). After several hours, the formation of diploids was determined by microscopic observation of zygotes, or, in other crosses, by replica-plating to minimal medium where only diploids with complementing genetic markers could grow. Choline-requiring segregants of each mating type were selected. Isolates derived from each original mutant were crossed to each other to determine the complementation pattern.

Lipid composition analysis. Cells were labeled with [^{32}P]orthophosphate (New England Nuclear Corp.) in synthetic complete medium supplemented with ethanolamine or choline or with no supplement. The final specific activity of the phosphate in the growth medium was 0.544 mCi/mmol. The cells were grown at 30°C for five to six generations in the presence of the label. The cells were harvested in logarithmic growth at a cell density of 1×10^7 to 2×10^7 cells per ml. Labeling for additional generations produced no change in the percentage distribution of the label into lipid classes or in the specific activity of the lipid extracted, so it is assumed that a steady-state labeling condition was achieved. To determine the phospholipid composition of cells starved for ethanolamine, cultures of strain KA101 were grown for five to six generations in the presence of ^{32}P as described above. The cells were then shifted, after washing with 2 volumes of ethanolamine-deficient medium, to medium lacking ethanolamine but containing ^{32}P at the same specific activity as in the initial growth period. The cells were harvested after 6 h of additional labeling in the ethanolamine-deficient medium, and the lipid composition was determined as described below.

Lipids were extracted from spheroplasts by a single-step modification of the Folch extraction method (7). Spheroplasts were prepared by harvesting cells by centrifugation, washing twice with water, and suspending at a density of 5×10^7 to 1×10^8 cells per ml in a digestion mixture consisting of: 1.2 M glycerol, 100 mM sodium thioglycolate, 50 mM Tris-SO_4 (pH 7.5), and 0.5 mg of Zymolyase 5000 (Kirin Breweries, Takasaki, Gumma, Japan) per ml. This mixture was devised to address difficulties in cell wall removal following periods of starvation for lipid precursors. Unusually high osmotic support and sulfhydryl-reducing agent are required to remove cell walls in some circumstances and do not appear to produce damage to normal cells. After 15 min of incubation at 25°C, less than 1% osmotically insensitive whole cells remain, and all spheroplasts are maintained intact. Spheroplasts were separated from the digestion mixture by centrifugation at $1,000 \times g$ for 5 min. The supernatant was drawn off as completely as possible because the presence of glycerol in the subsequent lipid extracts distorts the chromatographic separation. Spheroplast pellets were suspended in chloroform-methanol (2:1) for 1 h and washed with 0.2 volume of water. The organic phase was drawn out, and a sample was counted in a scintillation counter to determine total labeled lipid. The remainder was dried under nitrogen and suspended in a small volume of chloroform-methanol for chromatography. The method of lipid extraction used results in rapid and efficient extraction of the major yeast phospholipids, including those of primary interest in this study: phosphatidylserine, -ethanolamine, and -choline. The method does not efficiently extract several polar lipids, including the major yeast sphingolipids, which are extractable by other methods (31). The residual polar phospholipids in our extracts remain close to the origin of the chromatography system employed and are not resolved. Since they are not of primary interest in this analysis, they were simply tabulated together and are listed as "polar lipids" in the composition analysis. They include CDP-diglyceride and residual sphingolipids (30, 31).

Lipids were separated by the two-dimensional paper chromatography system of Steiner and Lester (30, 31), using EDTA-treated Whatman SG81 paper, dimension 1 (chloroform–methanol–30% ammonium hydroxide–water; 66:27:3:0.8), and dimension 2 (chloroform–methanol–glacial acetic acid–water; 32:4:5:1). Radioactivity was located by autoradiography with Kodak No-Screen X-ray film. Spots were cut from the chromatogram and counted in a scintillation counter. In some cases, chromatograms were sprayed with ninhydrin (4) or acid molybdate, followed by UV irradiation (8). Lipids were identified by comparison with the migration of standards purchased from GIBCO: phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, cardiolipin, and phosphatidic acid.

In some cases lipids were subjected to mild alkaline hydrolysis by the method of Ames (1), and the products were separated on Whatman no. 1 paper in the solvent methanol–98% formic acid–water (80:13:7) or the solvent phenol saturated with water–glacial acetic acid–ethanol (10:1:1.2) (29). Labeled hydrolysis products were identified by autoradiography, and unlabeled products or standards were identified by spraying with ninhydrin or molybdate.

RESULTS

Genetics. Three independent choline-requiring mutants were obtained. In each case the choline requirement was recessive in diploids and segregated as a single nuclear gene mutation (2:2 in tetrads). The three mutants failed to complement one another.

Diploids homozygous for the *cho1* mutation failed to sporulate on potassium acetate sporulation medium, as described in Materials and Methods, whether supplemented with choline or with ethanolamine. The defect in sporulation may be the result of the mitochondrial deficien-

cies described below. Because these crosses could not be sporulated, recombination analysis was not possible. However, on the basis of the complementation data, the mutants would all appear to be alleles of the same locus. In addition, a series of ethanolamine auxotrophs independently isolated by V. Letts and I. Dawes appear to be alleles of this same locus (V. Letts, personal communication).

One of the original isolates was crossed to strain 2180-1B, and a derivative was crossed to strain MC13. A derivative of this cross was backcrossed once again to MC13 to derive strain KA101 (*α cho1-1 ino1-13 lys2 can1*). This strain grows more vigorously than the original mutant isolate and, unlike the 2180 strains, tends not to form multicellular aggregates in liquid growth media.

Strain KA101, crossed to strain G599-10B, yielded meiotic linkage information which locates *cho1* on the right arm of chromosome V. Table 1 indicates the tetrad classes obtained and the recombination frequencies calculated by the formula of Perkins (22). Very likely, this is the same locus on chromosome V identified originally by C. Lindegren (personal communication) as the map location of a series of choline auxotrophs. (These mutants have been lost.)

Growth requirements of *cho1* mutants. The three original mutants grow on synthetic complete plates supplemented with either 1 mM ethanolamine, 1 mM *N*-methylethanolamine, 1 mM *N,N*-dimethylethanolamine, or 1 mM choline. In the absence of at least one of these supplements, the mutants do not grow. Further studies reported here were conducted with strain KA101, often together with strain MC13 as a closely related choline-independent (*CHO*$^+$) strain.

Figures 1 and 2 indicate growth of strain KA101 in liquid synthetic complete medium with and without the various supplements. In the absence of any supplement, strain KA101 stopped growing after approximately one doubling in optical density. Supplementation with serine did not enhance growth, whereas supplementation with ethanolamine permitted continuous growth of the mutant strain. Ethanolamine provided the most favorable supplementation for *cho1* mutants, compared to supplementation with its methylated derivatives (Fig. 2). KA101 cultures grown at 30°C in synthetic complete medium supplemented with 1 mM ethanolamine grew with a doubling time of 3 h and showed doubling times of nearly 3 h with 1 mM *N*-methylethanolamine, 3 h and 40 min with choline, and 5 h with *N,N*-dimethylethanolamine.

TABLE 1. *Meiotic segregation of genetic markers in strain KA159*[a]

Gene pair	Parental ditypes	Non-parental ditypes	Tetratypes	Map distance
cho-can	19	8	76	60.2
cho-ura	58	4	44	32.1
cho-hom	48	1	57	29.7
cho-his	41	0	64	30.5
hom-his	102	0	6	2.8

[a] KA101 $\frac{\alpha}{\mathbf{a}}$ $\frac{can1}{+}$ $\frac{+}{ura3}$ $\frac{chol}{+}$ $\frac{+}{hom3}$ $\frac{+}{his1}$ $\frac{ino1}{+}$ $\frac{lys2}{+}$
G599-10B

Conversion tetrads: *cho4* (3.6%), *can1* (3.6%), *ura1* (0.9%), *hom1* (0.9%), *his2* (1.8%).

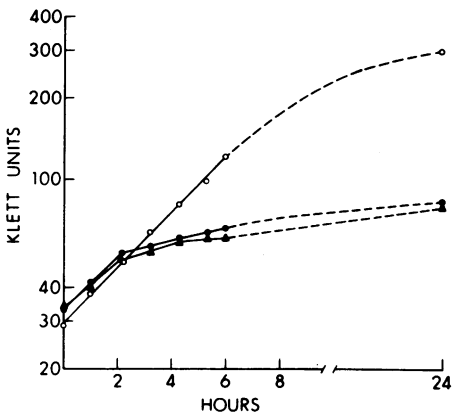

FIG. 1. *Growth requirement of the cho1 mutant. Growth in terms of culture optical density was determined in KA101 cells grown in synthetic complete medium at 30°C with no supplement (▲), with 1 mM serine (●), or with 1 mM ethanolamine (○).*

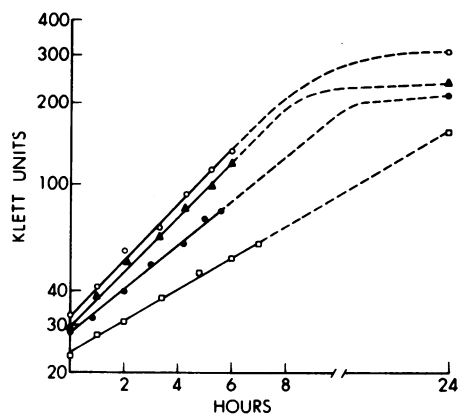

FIG. 2. *Growth rates with different supplements. Growth in terms of culture optical density was determined in KA101 cells grown in synthetic complete medium at 30°C with 1 mM ethanolamine (○), 1 mM N-methylethanolamine (▲), 1 mM N,N-dimethylethanolamine (□), or 1 mM choline (●).*

By comparison, parallel cultures of the *CHO*$^+$ strain MC13 (not shown) grew equally well, with a doubling time of 2 h, in media with or without each of the four supplements. Optimal supplementation was achieved with 1 mM concentrations of each supplement. With choline, higher concentrations did not affect growth rate or stationary culture density. Supplementation with more than 1 mM ethanolamine was less effective than 1 mM in terms of these two growth parameters. Ethanolamine is quite alkaline, and the inhibitory effects of higher concentrations were overcome by adjusting the medium pH to 5 or less.

Viability during starvation for ethanolamine, choline, or inositol. Starvation for the phospholipid precursors ethanolamine and choline resulted in the cessation of cell growth, but did not impair cell viability (Fig. 3). In KA101 cultures grown at 30°C in synthetic complete medium lacking supplements, virtually all cells retained viability and resumed growth when ethanolamine was restored after up to 10 h of growth. By contrast, KA101 cells starved of inositol undergo logarithmic viability loss comparable to that observed in strain MC13 (11).

Effect of *cho1* on respiratory sufficiency. A high proportion of respiratory-deficient (petite) cells are found in KA101 cultures. The proportion of petites is markedly affected by growth temperature. Since petites produce colonies with a different morphology from respiratory-sufficient colonies, this effect of *cho1* was first observed on agar plates. KA101 cells spread on YEPD agar plates and incubated at 25°C yield colonies that are white in color, small, and perfectly round in shape. These colonies are petite: they do not grow when replica-plated to YPGE plates containing nonfermentable carbon sources, at either 25 or 30°C. Most colonies from KA101 cells grown at 30°C are different: they are creamy in color, large, and ragged in shape. These colonies grow on YPGE plates at 30°C, but not as fast as similarly treated colonies of the *CHO*$^+$ strain MC13. Furthermore, most KA101 colonies grown at 30°C have petite sectors that fail to grow on YPGE. These sectors probably explain the ragged shape of the colonies.

The accumulation of respiratory-deficient cells during logarithmic growth is shown in Fig. 4. Cultures were established from a respiratory-sufficient KA101 colony that had been grown at 30°C. Parallel cultures in YEPD medium were grown at 25 or 30°C for several days, with successive diluting to maintain log-phase cultures. At intervals, cell samples were spread on YEPD plates and incubated at 30°C, and the resultant colonies were tested for respiratory sufficiency. The proportion of respiratory-deficient cells rose in cultures grown at both temperatures. At 25°C, virtually 100% of the cultures became respiratory deficient after 4 days of growth, whereas the proportion of petites leveled off at approximately 50% in the culture growing at 30°C.

The tendency to generate petites, particularly at a low growth temperature, cosegregates with the *cho1* mutation, as illustrated in Table 2. Meiotic spore clones were grown at 30°C, and

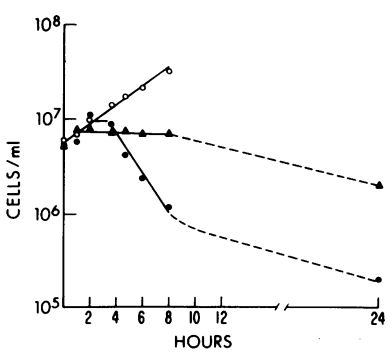

FIG. 3. *Viability of strain KA101. Cell viability was determined in KA101 cultures grown in synthetic complete medium at 30°C with inositol and ethanolamine* (○), *with no inositol* (●), *or with no ethanolamine* (△).

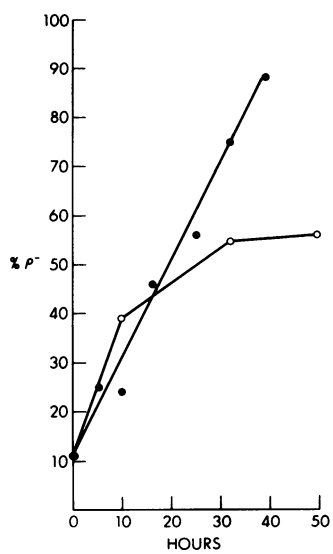

FIG. 4. *Accumulation of petites in cho1 cultures. KA101 cultures in YEPD medium were grown at either 25°C* (●) *or 30°C* (○) *for several days. Cultures were maintained in log phase, from 10^6 to 5×10^7 cells per ml, by repeated dilution with fresh YEPD medium. At intervals, cells were spread on YEPD plates and incubated at 30°C. The percentage of cells that yielded respiratory-deficient (petite or ρ⁻) colonies is shown.*

auxotrophic markers were tested by replica-plating. A suspension of cells from each spore clone was prepared and spread on YEPD agar plates, which were incubated at 25 and 30°C. The respiratory sufficiency of resultant colonies was tested on YPGE plates. The *CHO*$^+$ spore clones each yielded 90% or more respiratory-sufficient (ρ^+) colonies at both growth temperatures. All choline-requiring clones yielded 100% petite (ρ^-) colonies when grown at 25°C and up to 50% petite colonies when grown at 30°C, along with respiratory-sufficient colonies which grew on YPGE but distinctly more slowly than *CHO*$^+$ colonies.

TABLE 2. *Cosegregation of cho1 and generation of petites* (ρ^-)[a]

Tetrad	Spore	*cho*	*lys*	*ino*	*ade*	Mating type	Phenotype of colonies at 25°C	Phenotype of colonies at 30°C
1	A	−	+	−	+	α	ρ^-	ρ^-/ρ^+
	B	−	+	+	−	**a**	ρ^-	ρ^-/ρ^+
	C	+	−	+	−	α	ρ^+	ρ^+
	D	+	−	−	+	**a**	ρ^+	ρ^+
2	A	−	−	−	−	**a**	ρ^-	ρ^-/ρ^+
	B	+	+	−	−	**a**	ρ^+	ρ^+
	C	+	+	+	+	α	ρ^+	ρ^+
	D	−	−	+	+	α	ρ^-	ρ^-/ρ^+
3	A	+	+	+	−	**a**	ρ^+	ρ^+
	B	+	−	−	−	α	ρ^+	ρ^+
	C	−	+	+	+	α	ρ^-	ρ^-/ρ^+
	D	−	−	−	+	**a**	ρ^-	ρ^-/ρ^+

[a] Genotype of the parental diploid:

KA101 α *cho1* *ino1* *lys2* *can1* +
KA102: S5 **a** + + + + *ade1*

Phospholipid composition. The steady-state phospholipid composition of strain KA101 was examined under different growth conditions. Table 3 indicates lipid compositions determined by [^{32}P]orthophosphate labeling over five to six generations of growth in synthetic media with and without ethanolamine or choline. The composition of strain MC13 grown with ethanolamine is shown beside that of strain KA101 grown with ethanolamine or with choline. Also shown is the composition after several hours of continued labeling in the absence of any supplement. In all KA101 extracts, no phosphatidylserine label was detected above background levels. In these experiments, background counts were 50 cpm and the total lipid label in each chromatogram was 5×10^4 cpm. Phosphatidylserine labeling was therefore less than 0.1% of total lipid label in KA101 cells. Compared to the normal level of approximately 8%, observed in MC13 cells, the *cho1* mutant contains less than 1/80 the normal amount of phosphatidylserine. Hydrolysis of lipids from KA101 failed to produce detectable glycerophosphorylserine, and no ninhydrin-reacting material could be detected on chromatograms in the position where phosphatidylserine migrates. The only ninhydrin-positive phospholipid from strain KA101 was phosphatidylethanolamine.

The absence of phosphatidylserine is but one difference between the lipid compositions of *cho1* and *CHO*$^+$ strains. The proportion of phosphatidylinositol is nearly 50% greater in KA101

TABLE 3. *Phospholipid composition*[a]

Strain	Medium supplement	PL	PI	PS	PC	PE	PMME	PDME	CL	PA	Total nmol of lipid P per 10^7 cells
KA101	Ethanolamine (1 mM)	4.6	32.8	—[b]	41.7	15.0	1.0	1.1	1.2	2.6	13.7
KA101	Choline (1 mM)	3.2	29.4	—	57.8	4.8	—	—	1.8	3.0	13.3
MC13α	Ethanolamine (1 mM)	4.9	22.7	8.1	31.8	23.3	1.9	2.5	1.6	3.2	13.8
KA101[c]	Pregrowth: ethanolamine (1 mM) 6-h final growth: no supplement	6.3	42.3	—	43.3	2.0	—	—	2.7	2.5	12.9

[a] Lipid abbreviations: PL, polar lipids; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidyldimethylethanolamine; CL, cardiolipin; PA, phosphatidic acid. Numbers given in the body of the table are percentages of total lipid phosphorus determined for each lipid.

[b] —, No label detected above background.

[c] During the ethanolamine starvation experiment, ^{32}P was maintained at a uniform specific activity before and after transfer to ethanolamine-deficient medium as described in the text.

cells than in MC13 cells. Additionally, there is a greater proportion of phosphatidylcholine and less phosphatidylethanolamine. The proportions of these two lipids is abnormal even in cells grown with ethanolamine, and even more so in cells grown with choline. The phosphatidylethanolamine content drops to about 5% of total phospholipid over five to six generations of growth with choline, and does not drop further with longer growth under these conditions. The lipid composition detected after several hours of deprivation of both supplements (Table 3) is nearly the same as the highly unusual composition of cells growing with choline. The net effect upon composition in unsupplemented cells may be seen in Table 3.

DISCUSSION

The growth and phospholipid composition data tend to support the hypothesis that the *cho1* defect lies in phosphatidylserine synthesis. The *cho1* mutants grow if supplemented with ethanolamine or one of its methylated derivatives. The original mutant selection was done in the presence of choline in the expectation that the choline auxotrophs isolated would be similar to the *Neurospora* choline auxotrophs, which are defective in the methyl transferases (15, 25) that catalyze the conversion of phosphatidylethanolamine to phosphatidylcholine. However, the fact that ethanolamine is as effective in supporting growth as choline is makes this expectation unlikely. The growth characteristics of the yeast *cho1* mutants are consistent with a defect in the conversion of phosphatidylserine to phosphatidylethanolamine. However, the phospholipid composition data do not support this hypothesis. An *Escherichia coli* mutant defective in this step accumulates phosphatidylserine (9). The yeast mutants described here are completely deficient in this lipid. The finding that phosphatidylserine is absent in the *cho1* mutant raises the possibility that the defect lies in the synthesis of phosphatidylserine. Obviously, a clear understanding of the nature of the defect will require rigorous biochemical analysis. These studies are in progress. Whatever the true nature of the defect, it is clear that growth of the mutant depends upon the utilization of exogenous choline, ethanolamine, or one of its methylated derivates. In yeast, these precursors have been shown to be directly incorporated into phospholipids (33–35) via the pathways originally described by Kennedy and Weiss (17).

A striking conclusion that may be drawn from properties of the yeast *cho1* mutants is that the presence of detectable phosphatidylserine is not required for continuing vegetative growth and division of this eucaryotic organism. Bacterial mutants with a temperature-sensitive defect in the phosphatidylserine synthetase are unable to grow at the nonpermissive temperature (review by Raetz [23]). However, these bacteria lack an alternative route, like the pathway described by Kennedy and Weiss (17), to synthesize phosphatidylethanolamine. Furthermore, they do not make phosphatidylcholine. Thus, comparable mutant defects in yeast and bacteria result in different phospholipid deficiencies and indicate different lipid requirements for growth. Bacteria require phosphatidylserine synthesis, either itself or as a precursor to phosphatidylethanolamine. The results obtained with the *cho1* mutants show that in yeast phosphatidylserine is not strictly required, nor are normal levels of phosphatidylethanolamine.

Phosphatidylcholine synthesis and accumulation are required for growth of the *cho1* mutant. Since none of the supplementation conditions employed permits synthesis of phosphatidylethanolamine but not phosphatidylcholine, it cannot be concluded that phosphatidylcholine is specifically required for all yeast growth. Phosphatidylethanolamine is largely dispensable, as shown by the mutants' lipid composition while growing with choline supplementation (Table 3). Although phosphatidylcholine does not appear to be dispensable, interruption of its synthesis and accumulation does not lead to cell death (Fig. 3). In contrast, starvation for the phospholipid precursor, inositol, results in drastic viability loss in yeast inositol mutants (Fig. 3; 11).

The *cho1* mutants exhibit several properties that are different from CHO^+ strains and are presumed to be due to phospholipid abnormalities. In comparison with the CHO^+ strain MC13, the *cho1* strain KA101 grows more slowly even with optimal supplementation, grows poorly on nonfermentable carbon sources, and displays a cold-sensitive tendency to lose mitochondrial function completely, and its homozygous diploids fail to sporulate. All of these characteristics could be due to defects of the mitochondrial membranes. Mitochondrial membranes may be more sensitive to compositional changes of the type found in *cho1* mutants than are the functions of other membranes that are required for growth and division. There are many phospholipid alterations evident in *cho1* cells. Those responsible for mitochondrial abnormalities are not yet resolved. Possibilities include a specific requirement for phosphatidylserine. Alternatively, abnormalities caused by differences in the lipids synthesized by the Kennedy pathway could lead to physiological difficulties. The fatty acid compositions of phosphatidylcholines made

by the de novo and Kennedy pathways differ (34). These alterations, or more subtle changes in the balance of the different cellular phospholipids, could be the cause of observed mitochondrial deficiencies in *cho1* mutants.

The *cho1* defect in phosphatidylserine synthesis and the resultant abnormal pattern of phospholipid metabolism make these mutants ideal subjects for manipulating the relative proportions of membrane phospholipids and for examining the roles of these lipids in the functions of eucaryotic membranes.

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