SACCHAROMYCES CEREVISIAE RECESSIVE SUPPRESSOR THAT CIRCUMVENTS PHOSPHATIDYLSERINE DEFICIENCY

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ABSTRACT

Phenotypic reversion of six independent *Saccharomyces cerevisiae cho1* mutants was shown to be due predominantly to mutation of an unlinked gene, *eam1*. The *eam1* gene was located very close to *ino1* on chromosome *X* by meiotic tetrad analysis. Recessive *eam1* mutations did not correct the primary *cho1* defect in phosphatidylserine synthesis but made endogenous ethanolamine available for sustained nitrogenous phospholipid synthesis. A novel biochemical contribution to nitrogenous lipid synthesis is indicated by the *eam1* mutants.

ALTHOUGH the biochemical pathways for the biosynthesis and catabolism of eukaryotic cell membrane lipids are well established, the genetic regulation of lipid metabolism is poorly understood (VAN GOLDE and VAN DEN BERGH 1977). Genetic mutants of the yeast *Saccharomyces cerevisiae*, under investigation in several laboratories, have unveiled perplexing relationships between lipid biosynthetic pathways that are not explained by biochemical studies alone (HENRY 1982).

The pathways for synthesis of the nitrogenous phospholipids, phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine in *S. cerevisiae* include both typically prokaryotic and typically eukaryotic reactions. In the absence of relevant medium supplements, yeast form phosphatidylserine by a phosphatidyltransferase reaction (CDP-diacylglycerol + serine → CMP + phosphatidylserine) that higher eukaryotes lack (STEINER and LESTER 1972; CARSON, ATKINSON and WAECHTER 1982; VAN GOLDE and VAN DEN BERGH 1977). Phosphatidylserine decarboxylation yields phosphatidylethanolamine, and subsequent methylation yields phosphatidylcholine. Exogenous choline or ethanolamine can be incorporated, via the CDP-choline or CDP-ethanolamine pathways, typical of higher eukaryotes (KENNEDY and WEISS 1956). Choline, but not ethanolamine, represses the activities that create phosphatidylethanolamine and phosphatidylcholine from phosphatidylserine (WAECHTER, STEINER and LESTER 1969; WAECHTER and LESTER 1971, 1973; CARSON, ATKINSON and WAECHTER 1982). Although phosphatidylserine normally represents 8% of total cell phospholipid, yeast can thrive without any phosphatidylserine. The yeast *cho1* mutants, deficient in phosphatidylserine synthesis, grow if exogenous ethanolamine or choline is provided (DESBOROUGH and LINDEGREN 1959; LETTS and DAWES 1979; ATKINSON *et al.* 1980; ATKINSON, FOGEL and HENRY

TABLE 1

Saccharomyces cerevisiae *strains*

Strains from which *eam1* mutants were isolated								
KA101	*MATα*	*cho1-1*	*ino1-13*	*lys2*	*can1*			
KA161-22C	*MAT***a**	*cho1-1*	*ino1-13*	*lys2*	*ade1*	*his2*	*arg4*	
KA459-8B	*MAT***a**	*cho1-2*	*his1*	*lys2*	*ade2*			
MT1	*MAT***a**	*cho1-3*	*his1*	*ade2*				
MT2	*MAT***a**	*cho1-4*	*his1*	*ade2*				
MT7	*MAT***a**	*cho1-5*	*his1*	*ade2*				
MT8	*MAT***a**	*cho1-6*	*his1*	*ade2*				
Prototype *eam1* strains								
RM401-11D	*MATα*	*cho1-2*	*eam1-1*	*ade8*	*lys2*			
SC3-6	*MAT***a**	*cho1-3*	*eam1*	*ade2*	*his1*			
Strains used in genetic analysis								
KA458-17C	*MATα*	*cho1-1*	*ade5*	*lys2*				
KA452-3C	*MAT***a**	*cho1-2*	*ade8*	*ura3*	*trp1*	*arg4*		
X2928-3D-1A	*MAT***a**	*CHO*$^+$	*ade1*	*trp1*	*ura3*	*his2*	*leu1*	*met14*
X2928-3D-1C	*MATα*	*CHO*$^+$	*ade1*	*trp1*	*ura3*	*his2*	*leu1*	*met14*
3688-5A	*MATα*	*CHO*$^+$	*his1*	*ade2*				
3688-4A	*MAT***a**	*CHO*$^+$	*his1*	*ade2*				
LK707-20A	*MAT***a**	*cho1-2*	*lys-*					
KA458-11A-E1	*MAT***a**	*cho1-1*	*eam1*	*ino1-13*	*ade5*	+	+	diploid KA480
MT813-16C	*MATα*	*cho1-6*	+	+	+	*his1*	*ade2*	

1980; KOVAC *et al.* 1980; NIKAWA and YAMASHITA 1981). The exceedingly low level of phosphatidylethanolamine synthesis in *cho1* mutants deprived of exogenous ethanolamine (ATKINSON, FOGEL and HENRY 1980) suggests that yeast cells do not make significant amounts of endogenous ethanolamine and can only circumvent phosphatidylserine deficiency if exogenous ethanolamine is available.

Six independent *cho1* mutants were found to generate spontaneous phenotypic revertants with unusually high frequency. These phenotypic revertants are predominantly due to recessive mutations in a gene (*eam1*) unlinked to *cho1*. The *eam1* mutants appear to make endogenous ethanolamine and, thus, circumvent requirements for either phosphatidylserine or exogenous nitrogenous bases for phosphatidylethanolamine and phosphatidylcholine synthesis.

MATERIALS AND METHODS

Yeast strains: *S. cerevisiae* phosphatidylserine synthase mutants (*cho1*) were employed. Table 1 indicates the mutants and normal (CHO$^+$) strains used. Strains with the *cho1-2* allele were derived from LAD KOVAC's strain LK707-20A (KOVAC *et al.* 1980). Strains with the *cho1-1* allele were derived from KA101 (ATKINSON *et al.* 1980). Strains with the alleles *cho1-3, cho1-4, cho1-5* and *cho1-6* were newly isolated mutants.

Mutation isolation: New *cho1* mutants were obtained from strain 3688-4A (*MAT***a**, *his1, ade2*)

obtained from SEYMOUR FOGEL. The strain was treated with ethyl methanesulfonate (LINDEGREN *et al.* 1965), colonies were grown on rich YEPD agar plates and replica-plated to synthetic medium with or without 1 mM ethanolamine. Colonies that could not grow without exogenous ethanolamine were selected.

Growth media: All cultures were incubated at 30° in these studies. Strains were maintained on YEPD agar plates (1% yeast extract, 2% peptone, 2% glucose, 1.5% agar). Synthetic complete medium contained 2% glucose, 1.5% agar, a mineral nitrogen base yielding 5 g/liter ammonium sulfate, 1 g/liter monobasic potassium phosphate, 0.5 g/liter magnesium sulfate, 0.1 g/liter calcium chloride, 500 μg/liter borate, 40 μg/liter copper sulfate, 200 μg/liter ferric choride, 400 μg/liter manganese sulfate, 100 μg/liter potassium iodide, 200 μg/liter sodium molybdate and 400 μg/liter zinc sulfate, a vitamin mixture yielding 2 μg/liter folate, 400 μg/liter niacin, 200 μg/liter *p*-aminobenzoate, 400 μg/liter thiamine hydrochloride, 200 μg/liter riboflavin and 400 μg/liter pyridoxine hydrochloride and supplements of 5 mg/liter *myo*-inositol, 300 mg/liter threonine, 40 mg/liter each leucine and lysine, 30 mg/liter each adenine, arginine and tryptophan and 20 mg/liter each histidine, methionine, tyrosine and uracil. Approximately 1 mM ethanolamine was added to agar plates by spraying with approximately 0.1 ml of a filter-sterilized 300 mM stock solution. The ethanolamine stock was neutralized to pH 7.0 with approximately 2 N HCl. Media lacking one of the supplements were used to test nutritional requirements. Sporulation medium contained 2% potassium acetate, 0.025% glucose, 0.2% yeast extract, 1.5% agar and all of the supplements listed for synthetic medium, including addition of ethanolamine. Canavanine resistance was tested on synthetic medium lacking arginine and supplemented with 6 mg/ml canavanine sulfate.

Isolation of independent phenotypic revertants: Each of the seven strains from which *eam1* mutants were isolated indicated in Table 1 was streaked on YEPD plates and 20–30 clonal colonies were isolated. Each colony was regrown on YEPD and replica plated to synthetic medium lacking ethanolamine. Mutant *cho1* colonies did not grow, but papillations of phenotypic revertants appeared within 7 days. A single ethanolamine-independent papillation was selected from each *cho1* clonal colony, to ensure that independent phenotypic revertants were obtained. Each phenotypic revertant was maintained on YEPD agar plates.

Genetic analysis: Strains of opposite mating types were mated on YEPD agar plates and diploids selected by replica plating to plates lacking lysine and/or adenine. The selected diploids were replica plated to medium with and without ethanolamine to determine this growth requirement. Diploids, grown for 2 days on YEPD plates, were induced to sporulate by transferring to acetate medium with ethanolamine. Ascopores were digested with 10% Glusulase (Endo Laboratories) and spore tetrads dissected on YEPD plates.

Cross-feeding was examined by streaking the ethanolamine-requiring strain KA101 (*cho1-1*) very close to mutant colonies grown on synthetic medium lacking ethanolamine. Growth was examined after 3 days and after 1 wk.

Phospholipid synthesis was examined by 32P-orthophosphate labeling. Strains were grown overnight in liquid YEPD and transferred to synthetic complete medium lacking ethanolamine. Cultures were started at a density of 4–5 × 10^6 cells/ml. At intervals, 5-ml samples were removed, mixed with 400 μCi of ^{32}P-orthophosphate (I.C.N) and incubated 30 min. Labeled cells were harvested by centrifugation, treated with 5% (v/v) trichloracetic acid on ice and washed with water. Lipids were extracted with 1.0 ml of HANSON and LESTER's (1980) extraction mixture (15 ethanol/15 water/5 diethylether/1 pyridine) at 60° for 20 min. Extract (0.2 ml) was subject to a modified Folch wash, and the remainder was dried under air for detection of the complex sphingolipids. The 0.2-ml extract sample was mixed with 0.1 ml of water and 1.0 ml of chloroform/methanol (2:1) (modified, FOLCH, LEES and STANLEY 1957). The lower organic phase was withdrawn and dried for detection of the major phospholipids. Lipid samples were subject to chromatographic separation on Whatman SG 81 paper (STEINER and LESTER 1972). The polar sphingolipids were separated in the first dimension with 9 chloroform/7 methanol/2(4 N)ammonium hydroxide and in the second dimension with 15 chloroform/6 methanol/4 glacial acetic acid/1.6 water. The major phospholipids were separated in the first dimension with 66 chloroform/27 methanol/3 (30%) ammonium hydroxide/0.9 water and in the second dimension with 32 chloroform/4 methanol/5 glacial acetic acid/1 water. In both cases, ascending chromatography was 45 min in each dimension, with thorough drying between dimensions. Radioactive spots were located by X-ray autoradiography. Spots were cut from the chromatograms and counted in a scintillation counter.

RESULTS

New cho1 *mutants*: Four ethanolamine-requiring mutants were obtained after mutagenesis of strain 3688-4A. The new mutant strains, MT1, MT2, MT7 and MT8, were each crossed to strain KA101. The resulting diploids (*cho1-1/cho1-X*) required ethanolamine, indicating that the new mutants were defective in the *cho1* complementation group. Unlike *cho1-1/cho1-1* homoallelic diploids (ATKINSON *et al.* 1980), *cho1-1/chol1-X* diploids could sporulate on acetate medium supplemented with ethanolamine. At least 20 meiotic spore tetrads were analyzed from each cross, and only ethanolamine-requiring spore colonies were obtained, indicating that the new mutations each represent *cho1* alleles. Each mutant strain, crossed with the CHO$^+$ strain X2928-3D-1C exhibited 2:2 segregation of the ethanolamine requirement linked to *ura3* and *his1* in meiotic spore tetrads, indicating that a single nuclear mutation gave rise to the growth requirement.

The *cho1-2* mutation similarly proved to be altered in the *cho1* gene and, when heteroallelic with *cho1-1*, supported sporulation. Kovac's strain LK 707-20A was crossed with KA101 and yielded only ethanolamine-requiring meiotic spores. The *cho1-2* mutation segregated 2:2 in the cross with X2928-3D-1C. A spore colony from this cross, KA452-3C, was used in further genetic analysis.

Phenotypic revertants: Each of the seven *cho1* strains examined (those listed in Table 1) yielded phenotypic revertants readily. The rate of reversion was not rigorously examined, but ethanolamine-independent phenotypic revertants appeared at least 100 times more frequently on agar replicas than did adenine, histidine or lysine-independent revertants. Ethanolamine-independent revertants appeared at the same frequency as canavanine-resistant (*CAN1* to *can1*) mutations in the MT1, 2, 7 and 8 strains.

Each phenotypic revertant was crossed with a *cho1* strain of the opposite mating type, and diploids were selected on the basis of complementing lysine or adenine auxotrophic markers (*lys2, ade1, ade2, ade5* or *ade8*). Diploids that, upon subsequent testing, did not require ethanolamine were presumed to possess a dominant *SUP* suppressor or a true *CHO1*$^+$ reversion. By far, the predominant class of phenotypic revertants obtained (shown in Table 2) had a recessive mutation that when heterozygous could not support ethanolamine-independent growth of *cho1/cho1* diploids.

The 30 phenotypic revertants derived from strain KA101 (the RM1 through RM30 set) were subjected to genetic analysis. Each of these phenotypic revertants was crossed to X2928-3D-1A (CHO$^+$) and the spore tetrads were examined. In every case, including the cross with the dominant RM27 isolate, the *cho1* ethanolamine-requiring mutation reappeared in approximately one-fourth of the spores. The 30 phenotypic revertants of the RM1 through RM30 set, therefore, contain suppressor mutations that are not closely linked to *cho1*: one dominant suppressor (in RM27) and the rest recessive suppressors. The dominant suppressor was not analyzed further.

Each of the 29 RM recessive suppressor strains was crossed to KA452-3C (*cho1-2*) to examine meiotic segregation of the suppressor mutation. In this series of crosses, ethanolamine-independent growth segregated 2:2 in spore

TABLE 2

Phenotypic cho1 *revertants obtained*

Originating strain		Allele	Revertant strains	Dominant	Recessive
KA101	*MATα*	*cho1-1*	RM1 through 30	1	29
KA161-22C	*MAT***a**	*cho1-1*	SC1-1 through -19	1	18
KA459-8B	*MAT***a**	*cho1-2*	SC2-1 through -20	0	20
MT1	*MAT***a**	*cho1-3*	SC3-1 through -20	0	20
MT2	*MAT***a**	*cho1-4*	SC4-1 through -20	0	20
MT7	*MAT***a**	*cho1-5*	SC5-1 through -20	12	8
MT8	*MAT***a**	*cho1-6*	SC6-1 through -20	0	20
				14	135

Each phenotypic revertant was crossed to KA452-3C (*MAT***a** *cho1-2*) or to KA458-17C (*MATα cho1-1*). Diploids were selected on medium lacking lysine and/or adenine, and the ethanolamine requirement of the diploids was assessed.

tetrads. The genetic configuration of these diploids was *cho1-1/cho11-2*; suppressor/wild-type. Two:two segregation indicated that the recessive suppressor behaved as a single chromosomal mutation and it was able to suppress either of the *cho1-1* or *cho1-2* alleles.

Complementation analysis: The recessive suppressor derived from strain RM1 was selected as the prototype *eam1* mutant (strain RM401-11D, derived from the cross to KA452-3C). The prototype strain was mated to each of the recessive suppressor isolates derived from all other strains. (The SC strains, all *MAT***a**, are shown in Table 2.) All 106 of the SC isolates with recessive suppressors failed to complement the prototype *eam1* mutation. The strain SC3-6 was selected for testing the other recessive isolates of the RM strain set. They also proved unable to complement the SC3-6 *eam1* mutation. The genetic configuration of possible complementing and noncomplementing *eam* mutants is displayed in Figure 1. The growth properties are different from the usual pattern of complementing nutritional markers, since failure to complement permits growth without ethanolamine. The growth properties, outlined in Figure 1, are explained according to the hypothesis that *eam1* mutants accumulate phytosphingosine and that phytosphingosine degradation yields ethanolamine (ATKINSON 1983).

Genetic mapping: In the crosses of the RM isolates with KA452-3C, it was noted that *eam1* was linked to *ino1*. These crosses had the lethal *can1 arg4* combination, making it difficult to obtain viable four-spore tetrads needed for genetic mapping. A new *eam1* isolate was obtained from strain KA458-11A and was tested to be sure it was in the *eam1* complementation group. A suitable diploid was formed for examination of *eam1 vs. ino1* segregation. Table 3 indicates the genotype of the diploid and the meiotic spore tetrads obtained. The *eam1* mutation was unlinked to *his1* and is, therefore, not altered at the *cho1* locus. *eam1* and *ino1* were linked, with a map distance of 9.0 cM, as calculated by the PERKINS (1949) tetrad formula, or 8.8 cM as adjusted by MA and MORTIMER's (1983) empirical formula for taking multiple exchanges into account. The *eam1* gene is, therefore, on chromosome *X*, at a considerable distance from the centromere.

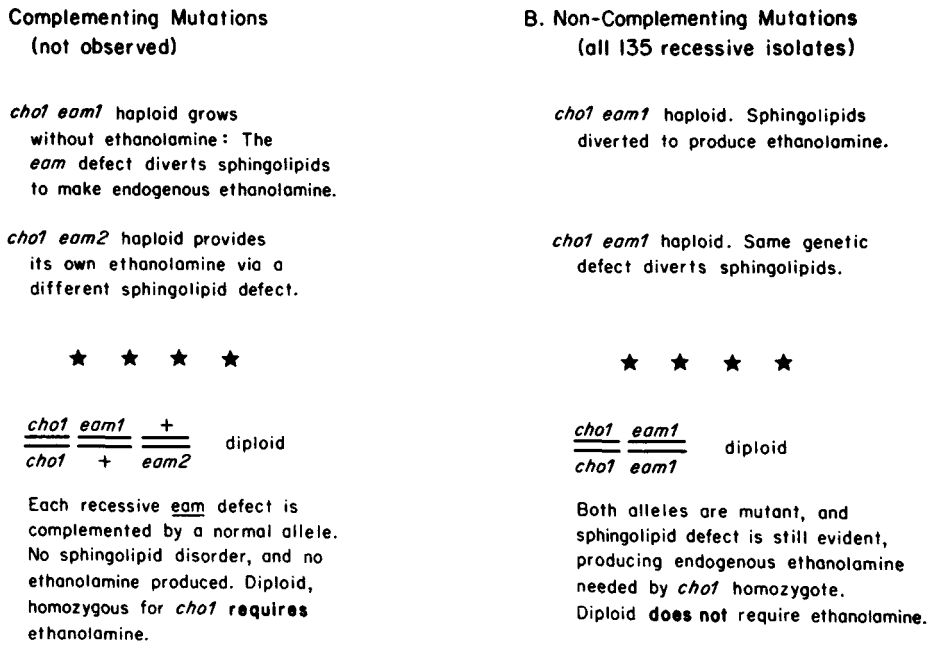

FIGURE 1.—Complementation between *eam* mutants.

Cross-feeding: The 30 ethanolamine-independent isolates of the RM set, including the one with a dominant suppressor, were grown on synthetic complete agar plates without ethanolamine for 3 days. The ethanolamine-requiring strain KA101 was streaked nearby the growing ethanolamine-independent colonies and examined after another 3 days and after 1 wk. No growth of KA101 was observed, indicating that neither *eam1* mutants nor the dominant suppressor mutant were able to cross-feed an ethanolamine-requiring strain. Although *eam1* mutants appear to provide themselves with endogenous ethanolamine (ATKINSON 1983) they do not excrete ethanolamine.

Phospholipid synthesis: Each of the 30 ethanolamine-independent isolates of the RM set were examined for ability to make phospholipids. The parental strain KA101 (*cho1*) does not make phosphatidylserine (ATKINSON *et al* 1980; ATKINSON, FOGEL and HENRY 1980). The dominant suppressor isolate RM27 (*cho1 SUP*) restored phosphatidylserine synthetic activity (not shown). None of the recessive isolates (*cho1 eam1*) synthesized any detectable phosphatidylserine. The time course of phospholipid synthetic rates in strain KA101 and the isogenic *eam1* isolate RM2 are shown in Figure 2. Strain KA101, with a *cho1* mutation unmasked by suppressors, makes no phosphatidyserine and rapidly ceases synthesis of the nitrogenous phospholipids phosphatidylethanolamine and phosphatidylcholine when transferred from rich YEPD medium to synthetic medium lacking ethanolamine. As phosphatidylcholine synthesis fails in strain KA101, so does synthesis of all other phospholipids and phosphosphingolipids. Under the same growth conditions, strain RM2, with an *eam1* mutation circumventing *cho1*, continues to grow and synthesize phosphatidyletha-

TABLE 3

Linkage of eam1 *and* ino1

Diploid KA480	*MATa* / *MATα*	*cho1-1* / *cho1-6*	+ / *his1*	*ade5* / +	*eam1* / +	*ino1-13* / +	+ / *ade2*
	Tetrads obtained						
Gene pair	Parental	Nonparental	Tetratype				
eam1, ino1	50	0	11				
eam1, his1	9	6	45				

Conversion tetrads:
1 *ino1* to INO^+
2 *his1* to HIS^+
1 *eam1* to EAM^+
5 either EAM^+ to *eam1* or *cho1* to CHO^+

Independent segregation of *eam1* and *his1* indicates that *eam1* is not closely linked to *cho1*, which is 30.5 cM from *his1* (ATKINSON *et al.* (1980). Meiotic linkage distance between *eam1* and *ino1* is 9.0 cM according to PERKINS' (1949) formula or 8.8 cM according to MA and MORTIMER'S (1983) formula.

noline as well as all other phospholipids. Despite the fact that strain RM2 accumulates the sphingolipid precursor phytosphingosine (ATKINSON 1983), there is no defect evident in its ability to synthesize the derived phosphophingolipids. Strain RM2 cannot make phosphatidylserine but does make all other phospholipids in the absence of exogenous ethanolamine or choline supplementation.

DISCUSSION

Characteristics of cho1 *mutants*: The first *cho1* mutant characterized (*cho1-1*, ATKINSON *et al.* 1980; ATKINSON, FOGEL and HENRY 1980) had three unusual physiological properties. Diploids homozygous for *cho1-1* would not sporulate. Haploid strains reverted at a very high rate and lost their mitochondrial genomes when grown at 24°C. By contrast, the next *cho1* mutant studied (KOVAC *et al.* 1980, designated *cho1-2*, in this report) did not cause difficulties for sporulation or mitochondrial genome maintenance. The new mutants reported here *cho1-3* through *cho1-6*) do not impair sporulation when heteroallelic with the *cho1-1* mutation; nor, in further crosses not described in this study, do they impair sporulation when homoallelic. The sporulation defect of the *cho1-1* mutant is, therefore, not characteristic of yeast defective in the *cho1* phosphatidylserine synthase gene. The new *cho1* mutants do tend to lose the mitochondrial genome when grown at 24°. Mitochondrial genome maintenance is the subject of continuing investigation.

All six of the *cho1* mutants examined yield apparent revertants approximately 100 times more frequently than apparent *ade2, his1, lys2* or *ino1* revertants in the same strain. In strains that were canavanine sensitive, $CAN1^+$ to *can1* mutants were detected as frequently as were apparent *cho1* revertants. Genetic analysis of the apparent *cho1* revertants revealed that ethanolamine-independent growth was due, predominantly, to recessive mutation in a single gene,

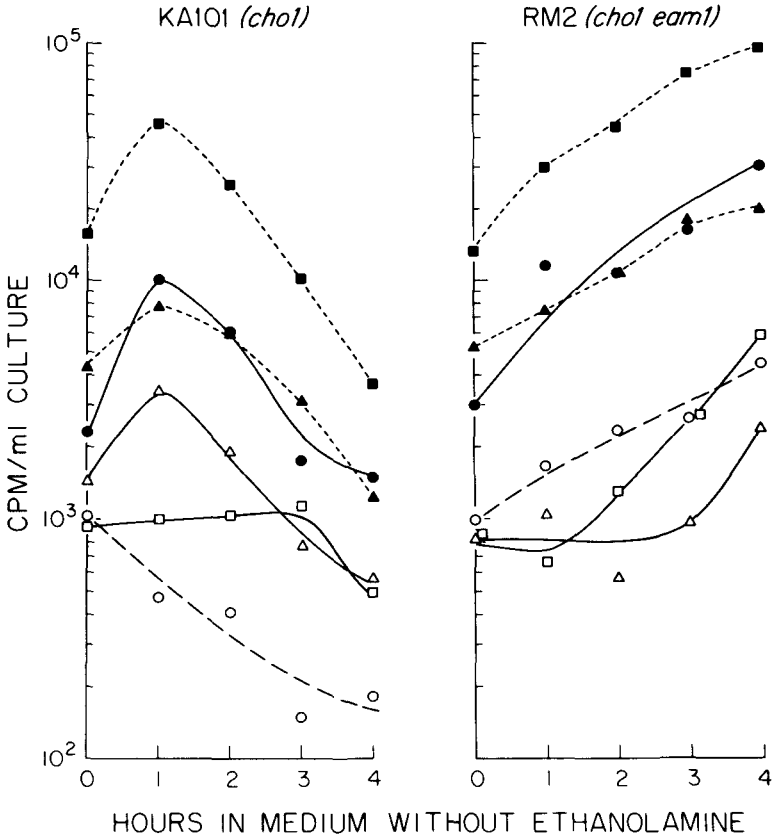

FIGURE 2.—Phospholipid synthesis. Strain KA101 (*cho1*) and its derivative RM2 (*cho1 eam1*) were grown in YEPD medium and transferred to synthetic medium without ethanolamine. Rates of phospholipid synthesis were determined by pulse labeling 30 min with ^{32}P-phosphate. Lipid species detected are phosphatidylinositol (●), phosphatidylethanolamine (○), phosphatidylcholine (△), phosphatidic acid (□), the mature sphingolipid ceramide-diinositol, diphosphoryl-mannose (▲) and its precursors, a poorly resolved mixture of inositol-containing phosphosphingolipids (■). No phosphatidylserine or CDP-diglycerides were detectable.

eam1. The unusually high frequency of apparent *cho1* reversion is not due to *cho1* to *CHO1*$^+$ reversion but to *EAM1*$^+$ to *eam1* mutation. The frequency of *eam1* mutation compares very closely to the frequency of *can1* mutation and is, therefore, not unusually high.

Genetic analysis of eam1: Complementation analysis of 149 independent apparent *cho1* revertants revealed that 135 revertants carried a recessive mutation in the *eam1* complementation group. Meiotic spore tetrad analysis of two different crosses showed that the *eam1* genetic locus is very close to the *ino1* locus on chromosome *X*. The distance between *eam1* and *ino1* is 9.0 cM, calculated according to the PERKINS (1949) formula, or 8.8 cM, as adjusted by the MA and MORTIMER (1983) formula. The *eam1* locus is not physically related to the *cho1* locus on chromosome *V* (ATKINSON *et al.* 1980).

The ethanolamine-independent growth phenotype of all *eam1* mutants ex-

amined segregated 2:2 in spore tetrads from diploids with two different *cho1* alleles (*cho1-1* and *cho1-2* in one series of crosses and *cho1-1* and *cho1-6* in the mapping cross). Suppression of the *cho1* ethanolamine requirement by *eam1* is, therefore, not allele specific.

Lipid biosynthesis in eam1 *mutants*: A comparison of a *cho1* mutant and a spontaneous *cho1 eam1* derivative showed that the *eam1* mutation does not correct the primary *cho1* defect in phosphatidylserine synthesis. In the absence of exogenous nitrogenous bases, the *cho1 eam1* strain synthesized phosphatidylethanolamine at a rate that increased with culture growth. The *eam1* mutants make endogenous ethanolamine available for synthesis of phosphatidylethanolamine. Modest amounts of phosphatidylcholine are made, but the rate of phosphatidylcholine labeling is much less than that of *cho1* cells supplemented with 1 mM exogenous ethanolamine (ATKINSON, FOGEL and HENRY 1980). Possibly only limiting amounts of ethanolamine are made available in *eam1* mutants, and little phosphatidylethanolamine is converted to phosphatidylcholine. The *eam1* mutants do not cross-feed *cho1* mutants, indicating that excessive amounts of ethanolamine are not made and excreted. Also different from exogenously supplemented *cho1* cells is the high level of phosphatidic acid detected. Phosphatidic acid could simply represent accumulation of precursor that is not used in phosphatidylserine formation. Alternatively, phosphatidic acid could be elevated, in a physiological response, to replace the missing phosphatidylserine in *eam1* mutants. Supplemented or starved *cho1* cells do not accumulate phosphatidic acid, so this response is peculiar to *eam1* mutants.

In a preliminary report, excessive phytosphingosine levels in the *cho1 eam1* strain RM2 were described, and the hypothesis was raised that degradation of phytosphingosine produced ethanolamine (ATKINSON 1983). Sphingolipid degradation in rat brain liberates ethanolamine or phosphorylethanolamine that can be incorporated into phosphatidylethanolamine (STOFFEL and HENNING 1968; STOFFEL, STICHT and LEKIM 1968; STOFFEL and ASSMANN 1970). The biochemical aberration in *eam1* mutants, which leads to phytosphingosine accumulation, is the subject of further investigation. Two hypotheses were raised (ATKINSON 1983) to explain excessive phytosphingosine in *eam1* mutants. A straightforward block in sphingolipid biosynthesis could lead to accumulation of the precursor phytosphingosine. Alternatively, the phytosphingosine found in *eam1* mutants could be a degradation product, reflecting excessive turnover of sphingolipids and other cell surface materials. The present study eliminates the possibility that later steps in sphingolipid synthesis are completely blocked. The *eam1* mutant strain synthesizes the final inositol-containing phosphosphingolipids at a high rate. If the initial labeling time point for strain KA101 (*cho1*) is taken as an indication of normal rates of phosphosphingolipid synthesis, then the strain RM2 (*cho1 eam1*) clearly makes these lipids at normal rates. The excessive phytosphingosine found in strain RM2 is not accumulated because of failure to form the derived mature sphingolipids, nor does it drive excessive synthesis of the phosphosphingolipids. The disturbance leading to phytosphingosine accumulation, and subsequent release of ethanolamine, could be a subtle

change in sphingolipid biosynthesis. For instance, a mutant ceramide synthase, with reduced affinity for phytosphingosine, could lead to phytosphingosine accumulation. Excessive phytosphingosine could drive the crippled reaction, leading to formation of normal levels of the mature sphingolipids. The alternative hypothesis, that phytosphingosine is a degradation product reflecting excessive sphingolipid turnover, seems unlikely in light of this study. Excessive turnover would lead to net depletion of sphingolipids, unless matched by a higher rate of synthesis. The rates of phosphosphingolipid synthesis detected (Figure 2) were normal.

Novel source of ethanolamine: Normal yeast do not make substantial amounts of endogenous free ethanolamine. The *cho1* mutants isolated by many investigators require exogenous ethanolamine or choline for sustained growth. When grown with only choline supplementation, the *cho1-1* mutant does make trace amounts of phosphatidylethanolamine, suggesting a normal very minor ethanolamine source (ATKINSON, FOGEL and HENRY 1980). The *eam1* mutants may exaggerate the contribution of the normal minor source, resulting in growth-supporting endogenous ethanolamine production. The *eam1* mutants point to a novel mechanism for growth-sustaining levels of nitrogenous phospholipid synthesis in yeast.

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