TWO RECESSIVE SUPPRESSORS OF *SACCHAROMYCES CEREVISIAE CHO1* THAT ARE UNLINKED BUT FALL IN THE SAME COMPLEMENTATION GROUP

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ABSTRACT

Phenotypic reversion of ethanolamine-requiring *Saccharomyces cerevisiae cho1* mutants is predominantly due to recessive mutations at genes unlinked to the chromosome *V cho1* locus. The recessive suppressors do not correct the primary *cho1* defect in phosphatidylserine synthesis but circumvent it with a novel endogenous supply of ethanolamine. One suppressor (*eam1*) was previously mapped to chromosome *X*, and 135 suppressor isolates were identified as *eam1* alleles by complementation analysis. Additional meiotic recombination studies have identified a second genetic locus, *eam2*, that falls in the *eam1* complementation group but maps close to the centromere of chromosome *IV*. Although the normal *EAM1* and *EAM2* alleles are fully dominant over recessive mutant alleles, their dominance fails in diploids heterozygous for defects in both genes simultaneously. The unusual complementation pattern could be explained by interaction of the gene products in formation of the same enzyme.

IDENTIFICATION of the biosynthetic pathways and regulatory mechanisms controlling membrane lipid biosynthesis has been facilitated by analysis of genetic mutants in the yeast *Saccharomyces cerevisiae* (HENRY 1982; HENRY, KLIG and LOEWY 1984). One mutant, *cho1* on chromosome *V*, is unable to make phosphatidylserine (ATKINSON *et al.* 1980; ATKINSON, FOGEL and HENRY 1980). This mutant has facilitated identification of other genes that coordinately regulate nitrogenous phospholipid and phosphatidylinositol synthesis (HENRY, KLIG and LOEWY 1984).

Recessive suppressors of *cho1* implicate a connection between sphingolipid and nitrogenous phospholipid biosynthesis (ATKINSON 1983; ATKINSON 1984). Recessive suppressors, altered at the *eam1* gene on chromosome *X*, lead to production of ethanolamine at levels that endogenously feed ethanolamine-requiring *cho1* mutants (ATKINSON 1984). Degradation of excessive phytosphingosine is the proposed source of ethanolamine (ATKINSON 1983).

All recessive suppressors of *cho1* fall in the same genetic complementation group, that of the *eam1* gene (ATKINSON 1984). Meiotic genetic analysis described in this report reveals that *eam* mutants can be defective in either of two genes, unlinked to each other or to *cho1*. Despite disparate genetic location of *eam1* on chromosome *X* and of *eam2* near the centromere of chromosome

IV, recessive mutations at these loci fail to complement each other in doubly heterozygous diploids. This suggests that the two gene products interact and that a quantitative threshold of normality attained in single-mutant heterozygotes cannot be reached in doubly heterozygous diploids.

MATERIALS AND METHODS

Yeast strains: *S. cerevisiae* phosphatidylserine synthase mutants (*cho1*) and recessive suppressors meiotically mapped to chromsome *X* (*eam1*) were employed. Strains, described by ATKINSON (1984), and new to this study, are described in Table 1.

Growth media: Strains were grown on YEPD agar plates or synthetic media at 30°, as previously described (ATKINSON 1984).

Mutant isolation: New *eam* mutants were isolated as spontaneous phenotypic revertants of *cho1* mutants. Ethanolamine-independent isolates were crossed with strains of the opposite mating type bearing *cho1* mutations and complementing nutritional markers. Diploids were examined for ethanolamine requirements. Diploids that required ethanolamine were identified as bearing a recessive *cho1* suppressor, an *eam* mutation.

Genetic analysis: Diploid strains were induced to sporulate and meiotic spore tetrads analyzed as previously described (ATKINSON 1984). Crosses were designed to be homozygous mutant at *cho1*, to facilitate detection of *cho1* suppression, but heteroallelic, since homoallelic *cho1-1* diploids cannot sporulate (ATKINSON *et al.* 1980; ATKINSON 1984).

Phospholipid synthesis: Lipid synthesis was examined by ^{32}P-orthophosphate pulse-labeling in synthetic ethanolamineless medium as previously described (ATKINSON 1984).

RESULTS

Identification of an eam2 *mutant*: A clonal isolate that did not require ethanolamine was obtained from strain KA458-11A. This isolate was independent of the isolate KA458-11A-E1, shown to possess an *eam1* mutation mapping to chromosome *X* (ATKINSON 1984). The new isolate, KA458-11A-E2, was crossed with MT813-16C to form the diploid KA481 with the genetic configuration shown in Table 1. Meiotic spore tetrads obtained from KA481 are shown in Table 2. Suppression of the *cho1* ethanolamine growth requirement in this cross was not linked to *ino1* and, therefore, not due to an *eam1* gene mutation. Ethanolamine independence was not linked to *his1* and, therefore, not due to alteration of the resident *cho1-1* allele. Ethanolamine independence exhibited nonrandom segregation with respect to mating type, indicative of linkage to a centromere other than that of chromosome *III*.

Failure of eam1 *and* eam2 *complementation*: A spore colony with the new mutation (KA481-2D *Matα cho1-6 eam2 his1 ade5*) was crossed with strain KA503-7A and KA4598B (each *MAT***a** *cho1*). The resulting diploids, homozygous for *cho1* but heterozygous for *eam2*, required ethanolamine, indicating that *eam2* was a recessive mutation. Strain KA481-2D was also crossed with strain RM401-4D (*MAT***a** *cho1 eam1*). This strain contains an *eam1* mutation whose location on chromosome *X* has been verified by meiotic tetrad analysis (ATKINSON 1984). This diploid did not require ethanolamine, suggesting that *eam1* and *eam2* fall in the same complementation group.

Independent segregation of eam1 *and* eam2: Meiotic spore tetrads from strain KA498 exhibited low spore viability, unfortunately common in homozygous *cho1* crosses, but clearly independent segregation of *eam1* and *eam2*. Of four

TABLE 1

Saccharomyces cerevisiae *strains*

Strains with spontaneous *eam* mutations										
KA458-11A	*MAT***a**	*cho1-1*	*EAM+*	*ino1-13*	*ade5*					
KA458-11A-E1	*MAT***a**	*cho1-1*	*eam1*	*ino1-13*	*ade5*					
KA458-11A-E2	*MAT***a**	*cho1-1*	*eam2*	*ino1-13*	*ade5*					
KA481-2D	*MATα*	*cho1-6*	*eam2*	*his1*	*ade5*					
Strains used in genetic crosses										
MT813-16C	*MATα*	*cho1-6*	*EAM+*	*his1*	*ade2*					
KA503-7A	*MAT***a**	*cho1-3*	*EAM+*	*trp1*	*arg4*	*his1*	*his4*	*ade8*		
KA459-8B	*MAT***a**	*cho1-2*	*ade2*	*his1*	*lys2*					
Diploid strains used in mapping										
KA481										
KA458-11A-E2	*MAT***a**	*cho1-1*	*eam2*	*ino1-13*	*ade5*	+	+			
MT813-16C	*MATα*	*cho1-6*	+	+	+	*his1*	*ade2*			
KA507										
KA481-2D	*MATα*	*cho1-6*	*eam2*	*his1*	*ade5*	+	+	+	+	
KA503-7A	*MAT***a**	*cho1-3*	+	*his1*	+	*trp1*	*arg4*	*his4*	*ade8*	
KA498										
KA481-2D	*MATα*	*cho1-6*	+	*eam2*	*his1*	*ade5*	+	+	+	+
RM401-4D	*MAT***a**	*cho1-2*	*eam1*	+	+	+	*ino1-13*	*trp1*	*his4*	*ade8-18*

TABLE 2

New eam2 *mutation unlinked to* ino1

Diploid KA481	*MATa* / *MATα*	*cho1-1* / *cho1-6*	*eam2* / +	*ino1-13* / +	*ade5* / +	+ / *his1*	+ / *ade2*

Gene pair	Tetrads obtained		
	Parental	Nonparental	Tetratype
eam2:ino1	5	7	16
eam2:his1	4	2	22
eam2:MAT	3	10	9

Excluded tetrads: six were not scored for mating type. Independent segregation of *eam2* and *ino1* indicates that the new mutation is not at the known *eam1* locus. Independent segregation with regard to *his1* indicates that the new mutation is unlinked to *cho1*. Nonrandom segregation of *eam2* and *MAT*, with the frequency of tetratypes less that 67%, indicates that *eam2* is linked to a centromere.

TABLE 3

Linkage of eam2 *and* trp1

Diploid KA507	*MATα* / *MATa*	*cho1-6* / *cho1-3*	*eam2* / +	+ / *trp1*	*his1* / *his1*	*ade5* / +	+ / *arg4*	+ / *his4*	+ / *ade8*

Gene pair	Tetrads obtained		
	Parental	Nonparental	Tetratype
eam2:trp1	38	0	2

Excluded tetrads: two *trp1* to *TRP1* conversions; one *EAM2* to *EAM2/eam2* postmeiotic segregation. Meiotic linkage distance between *eam2* and *trp1* is 2.5 cM according to PERKINS' (1949) formula or 2.43 cM according to MA and MORTIMER'S (1983) formula.

tetrads with all four spores viable, two were parental (all four spore clones did not require ethanolamine), one was nonparental (two required ethanolamine, two did not), and one was tetratype (one required ethanolamine, three did not). Three tetrads with three viable spores each had one spore that required ethanolamine, placing them in either the nonparental or tetratype categories. The two mutations, although unable to complement in the KA498 diploid and clearly recessive in other diploids, segregated as nonallelic mutations.

Mapping eam2: Since *eam2* appeared to be linked to a centromere, the cross indicated as strain 507 in Table 1, with several centromere-linked markers, was analyzed. Tetrads shown in Table 3 indicated close linkage of *eam2* and *trp1* on chromosome *IV*. The distance between *eam2* and *trp1* was estimated to be 2.5 cM by the PERKINS (1949) formula or 2.43 cM as modified by the MA and MORTIMER (1983) formula.

Phospholipid synthesis: The rates of ^{32}P-orthophosphate incorporation into lipids showed that the strain KA481-2D (*MATα* cho1-6 eam2 his1 ade5), growing in synthetic medium lacking ethanolamine or choline: (1) made no detectable phosphatidylserine; (2) made normal levels of phosphatidylethanolamine,

phosphatidylcholine and phosphatidylinositol; and (3) maintained relatively high levels of phosphatidic acid. These data were identical in every detail with those reported for *eam1* mutants examined under the same conditions (ATKINSON 1984) and are not reiterated here. Unlike *eam1* mutants, the *eam2* mutant strain retained detectable levels of the methylated intermediates, phosphatidylmonomethylethanolamine and phosphatidyldimethylethanolamine, during the 30-min labeling period. The overall rate of phosphosphingolipid labeling was reduced by five-fold, compared to the labeling rates reported for a normal and an *eam1* mutant strain (ATKINSON 1984), although the pattern of labeling was similar, with 80% of the label in precursors and 20% in the mature sphingolipid ceramide-diphosphoryl, diinositol-mannose. The *eam2* mutation, like *eam1*, therefore, does not correct the primary *cho1* defect in phosphatidylserine synthesis but circumvents it with an apparent endogenous supply of ethanolamine. Sphingolipid synthesis appears to suffer a reduction but not a straightforward block.

DISCUSSION

A second recessive suppressor of *cho1*, with effects on lipid biosynthesis similar to a previously described recessive suppressor, has been described. The existence of more than one gene contributing to an as yet crudely defined biochemical phenotype is not unusual or particularly enlightening. The fact that two recessive mutations, clearly mapped to different genetic loci, fall in the same complementation group may be both disturbing to other genetic investigators and enlightening regarding the nature of *eam* mutants.

Failure of complementation between recessive mutants defective in distinct genes has been suggested in a recent analysis of *Schizophyllum commune* drug-resistant mutants (KLEIN and DEPPE 1985). In the present case, lack of complementation suggests that products of the *eam1* and *eam2* genes interact.

A plausible explanation is that *eam1* and *eam2* encode separate subunits of a single enzyme. According to this notion, both *eam1/EAM1 EAM2/EAM2* and *EAM1/EAM1 eam2/EAM2* diploids would have up to half the normal level of fully nonmutant enzyme complexes (half if the complex has only one subunit from each gene; one-quarter if the complex has two subunits from each gene). The double heterozygous diploid *eam1/EAM1 EAM2/eam2* would have much less fully normal enzyme and appear mutant rather than normal (one-fourth if only one subunit from each gene; one-sixteenth if two subunits from each gene). This explanation predicts a quantitative threshold needed to prevent the apparent endogenous generation of ethanolamine.

My current hypothesis, that the *eam* mutants have an altered ceramide synthase with reduced affinity for the substrate phytosphingosine, is compatible with the present genetic data and biochemical characterizations of *eam* mutants. The *eam1* mutants make normal levels of the phosphoinositol sphingolipids but accumulate the precursor phytosphingosine (ATKINSON 1983, 1984). The *eam2* mutants make phosphoinositol sphingolipids at a reduced rate. Both phenotypes could be explained by a crippled, but not cleanly blocked, enzyme reaction early in the sphingolipid biosynthetic pathway. The proposed "crippling"

could be due to either a defective enzyme or a defective regulatory mechanism. The failure of complementation between *eam1* and *eam2* mutants, suggesting a quantitative threshold needed for normality, argues against a regulatory mechanism being at fault in these mutants. The simpler explanation is that both mutant genes contribute directly to defects of a single enzyme. A partial defect in ceramide synthase activity could lead to marginal but growth-sustaining sphingolipid production and, at the same time, raise cellular sphingolipid precursors to a threshold level that suffers degradation, releasing enough ethanolamine to sustain nitrogenous phospholipid synthesis.

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