Molecular Genetics of Serine and Threonine Catabolism in *Saccharomyces cerevisiae*

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ABSTRACT

The catabolic L-serine (L-threonine) deaminase of *Saccharomyces cerevisiae* allows the yeast to grow on media with L-serine or L-threonine as sole nitrogen source. A mutant, *cha1* (*c*atabolism of *h*ydroxyamino *a*cids), lacking this enzyme activity has been isolated. We have cloned the *CHA1* gene by complementation of a *cha1* mutation. Northern analysis showed that *CHA1* mRNA has a size of about 1200 ribonucleotides. *CHA1* is probably the structural gene for the enzyme; it is an abundant RNA in cells grown with serine and threonine as nitrogen source, whereas it is not detected when cells are grown on ammonium or proline, *i.e.*, the transcription of the *CHA1* gene is induced by serine or threonine. Under induced growth conditions haploid *ilv1 CHA1* strains do not require isoleucine, *i.e.*, the catabolic deaminase is able to substitute for the biosynthetic threnonine deaminase encoded by the *ILV1* gene. We have identified a nuclear, recessive mutation, *sil1*, that suppresses *ilv1* mutations by increased transcription of the *CHA1* gene under growth conditions leading to partial induction. The *sil1* mutation could exert its effect by increasing the effective pools of the hydroxyamino acids. Alternatively *SIL1* may encode a negatively acting regulatory protein for *CHA1*.

THE yeast *Saccharomyces cerevisiae* is able to utilize most amino acids as sole nitrogen source. Generally, enzymes in nitrogen catabolic pathways are regulated by transcriptional induction, and in some cases also by nitrogen catabolite repression, see review by COOPER (1982). Yeast genes encoding catabolic enzymes should therefore be valuable in studies of the control of gene expression.

The hydroxyamino acids L-serine and L-threonine are readily metabolized by yeast. RAMOS and WIAME (1982) have identified a L-serine (L-threonine) deaminase (EC 4.2.1.13 or 4.2.1.16) that is responsible for the catabolism of both amino acids in *S. cerevisiae*. Measurement of enzyme activities in yeast cells grown in media with different nitrogen sources revealed the enzyme level to be controlled by the supply of serine and threonine (RAMOS and WIAME 1982). In the enzymatic reactions, serine gives pyruvate and ammonia, while threonine, with a fourfold lower maximal reaction rate, will be converted to α-ketobutyrate and ammonia. The enzyme uses pyridoxal phosphate as a cofactor. It is distinct from the biosynthetic threonine deaminase (encoded by *ILV1*), which in a similar manner converts threonine to α-ketobutyrate, the first intermediate in isoleucine biosynthesis. The *ILV1* gene has previously been cloned and sequenced (PETERSEN *et al.* 1983a; KIELLAND-BRANDT *et al.* 1984).

The occurrence of a single deaminase, responsible for the degradation of both serine and threonine, seems also to be characteristic for the liver of rats (SUDA and NAGAWA 1971) and other mammals. Two deaminases with substrate preference for serine and threonine, respectively, have been characterized in different species of the anaerobic bacterium *Clostridium* (WHITELEY and TAHARA 1966; SAGERS and CARTER 1971) and in *Escherichia coli* (SHIZUTA and TOKUSHIGE 1971; FELDMAN and DATTA 1975; ISENBERG and NEWMAN 1974; SAEKI *et al.* 1977; KIM and DATTA 1982; PETTIGREW and WAGNER 1986). The gene for the catabolic threonine deaminase of *E. coli* (*tdc*) has been cloned (GOSS and DATTA 1984, 1985).

In the present paper, we describe the cloning and transcriptional studies of a gene, *CHA1*, that is needed for the presence of catabolic serine (threonine) deaminase in yeast.

MATERIALS AND METHODS

Materials: [α-^{32}P]Deoxyadenosine-5′-triphosphate (>600 Ci/mmol) was from New England Nuclear. Restriction endonucleases and other enzymes were purchased from Boehringer Mannheim.

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Yeast, bacterial strains and plasmids: The wild-type *S. cerevisiae* strain used in this study was X2180-1A (Yeast Genetics Stock Center, Berkeley). The biosynthetic threonine deaminase and catabolic serine (threonine) deaminase deficient strain 8736b, *MAT***a** *ilv1-10 cha1-1* (RAMOS and WIAME 1982) was kindly supplied by F. RAMOS and J.-M. WIAME. The allele number for the *ilv1* mutation, as well as the designation of the *cha* mutation as *cha1-1*, was chosen in agreement with F. RAMOS and J.-M. WIAME. Also used were strains M1-2B, *MAT*α *ura3-52 trp1 gal2* (ST. JOHN *et al.* 1981) and C80-1407, *MAT*α *lys1*. The following *ilv1* strains were constructed by standard genetic procedures (SHERMAN, FINK and LAWRENCE 1974): C85-2409, *MAT*α *ilv1-10 cha1-1 ura3-52 trp1*; C85-2411, *MAT***a** *ilv1-10 cha1-1 ura3-52 trp1*; C81-1667, *MAT***a** *ilv1-100*; C82-1582, *MAT***a** *ilv1-22 trp1 arg4-16 ade2*; C82-1704, *MAT*α *ilv1-100 lys1*; C81-1420, *MAT*α *ilv1-23 his4-29 ade2* and C82-1728, *MAT*α *ilv1-22 trp1 lys1*. Strains with suppressors of *ilv1* were C82-1724 through C82-1727, *MAT***a** *ilv1-22 trp1 arg4-16 ade2 sil1-4* through *sil1-7* (derived from C82-1582) and C82-1730, *MAT*α *ilv1-23 sil1-8 his4-29 ade2* (derived from C81-1420). All *ilv1* alleles used in this study are non-complementing. *E. coli* strain HB101 (BOYER and ROULLAND-DUSSOIX 1969) and JM83 (YANISCH-PERRON, VIEIRA and MESSING 1985) were used for plasmid propagations. Plasmid pUC19 (YANISCH-PERRON, VIEIRA and MESSING 1985) was from Bethesda Research Laboratories. The yeast plasmids YCp50 and YIp5 (BOTSTEIN and DAVIS 1982) were kindly provided by M. D. ROSE and G. R. FINK, respectively.

Growth of yeast and bacteria: Two types of synthetic media for yeast were employed. Synthetic complete ammonium medium (SC) was the minimal medium described by ZIMMERMANN (1973) buffered with 10 g succinic acid and 6 g sodium hydroxide per liter (pH 5.8), and supplemented with amino acids, etc., at the concentrations described earlier (PETERSEN *et al.* 1983b). This medium contains ammonium sulfate at a final concentration of 7.6 mM and threonine and serine at concentrations of 5.0 and 3.6 mM, respectively. Synthetic ammonium minimal medium (SDAm) consisted of 0.67% Bacto Yeast Nitrogen Base without amino acids (Difco) and 2% glucose, buffered as described above. Minimal medium with threonine, serine or proline (final concentrations of 1 mg/ml, *i.e.*, 8 mM, 9 mM and 9 mM, respectively) as the nitrogen source (designated SDThr, SDSerThr, etc.) were prepared in the same manner, but using Bacto Yeast Nitrogen Base without amino acids and ammonium sulfate. When required, SD media were supplemented with one or more nutrients at the concentrations used in SC. Bacteria were handled according to standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982).

Preparation of plasmids, yeast DNA and RNA: Plasmids were prepared by a scaled-up version of the alkaline extraction procedure (BIRNBOIM and DOLY 1979), followed by CsCl equilibrium centrifugation. Crude yeast DNA was prepared according to DAVIS *et al.* (1980). Yeast RNA was prepared from exponentially growing cells at 30° (OD_{600} = 1–1.5) by the method of BROACH *et al.* (1979) modified as described earlier (HOLMBERG *et al.* 1985). Enrichment for poly(A^+) RNA was not carried out.

Northern analysis: Electrophoresis of yeast RNA (about 20 μg) was performed in 1.5% agarose-formaldehyde gels (MANIATIS, FRITSCH and SAMBROOK 1982). The RNA was transferred to nitrocellulose filters (Millipore HAWP 00010) and hybridized to ^{32}P-labeled, nick-translated DNA probes in 50% (w/v) formamide and 5 × SSPE buffer (0.9 M NaCl, 5 mM EDTA, 50 mM sodium phosphate, pH 7.7) at 42° (MANIATIS, FRITSCH and SAMBROOK 1982). Autoradiography was carried out with intensifying screens at −80° for 5–12 days using Kodak X-Omat AR film.

Cloning of the *CHA1* gene: The *CHA1* gene was cloned by complementation of the *cha1* mutation as follows. *S. cerevisiae* strain C85-2411, *MAT***a** *ilv1 cha1 ura3 trp1*, was transformed by the lithium salt method (ITO *et al.* 1983) with plasmid DNA from yeast gene pools constructed by M. D. ROSE, J. THOMAS and P. NOVICK. The pools consisted of size-fractionated DNA fragments, prepared from partially *Sau*3A digested DNA (S288C background) and inserted into the *Bam*HI site of the centromere plasmid YCp50 (pBR322-*ARS-CEN-URA3*). Plasmid DNA was used from four different pools, each of which contains inserts corresponding to about one yeast genome. Upon transformation, cells were plated on SC without uracil, and the colonies were subsequently replica-plated onto selective media. Two colonies, among a total of 7107 Ura^+ transformants, were able to grow on ammonium medium lacking isoleucine (SDAm + Trp) and grew poorly on SDThr + Trp. Both harbored a plasmid that by restriction mapping was shown to contain a yeast DNA insert with the *ILV1* gene (PETERSEN *et al.* 1983a; KIELLAND-BRANDT *et al.* 1984). One other transformant was unable to grow on SDAm + Trp, but grew well on medium with threonine as the nitrogen source whether or not isoleucine was present (SDThr + Trp or SDThr + Trp + Ile). These characteristics were as expected for a Cha^+ transformant (RAMOS and WIAME 1982). Total yeast DNA was prepared from the transformant and used to transform *E. coli*, selecting for ampicillin resistance. The recovered plasmid was designated YCp50-Sc4-1.

RESULTS

Analysis of the *CHA1* gene: A restriction endonuclease map of the 8.1-kb DNA insert in plasmid YCp50-Sc4-1 (see MATERIALS AND METHODS) is shown in Figure 1A. Retransformation of the *cha1* strain C85-2411 with this plasmid conferred ability to utilize threonine or serine as the nitrogen source. To ensure that the cloned DNA fragment derived from a contiguous segment of the yeast genome, a Southern analysis (MANIATIS, FRITSCH and SAMBROOK 1982) of yeast DNA cleaved with different restriction enzymes was carried out using ^{32}P-labeled plasmid pUC19-Sc4BS as a probe. This plasmid contains 2.8 kb of the insert in YCp50-Sc4-1, plus 0.28 kb of the tetracycline resistance gene of YCp50 (pBR322 sequences), inserted between the *Bam*HI and *Sal*I sites of pUC19 (Figure 1B). The plasmid hybridized to restriction fragments with sizes as predicted from the restriction site map in Figure 1A, showing that the cloned fragment consisted of one contiguous piece of chromosomal yeast DNA.

To show that the cloned region originated from the *CHA1* locus, the same 3.1-kb *Bam*HI-*Sal*I fragment was inserted between the *Bam*HI and *Sal*I of the yeast integrating plasmid YIp5 (pBR322-*URA3*), giving plasmid YIp5-Sc4BS (Figure 1B). Linearized yeast plasmids integrate efficiently into homologous regions of the yeast genome if they contain free yeast DNA ends (ORR-WEAVER, SZOSTAK and ROTHSTEIN

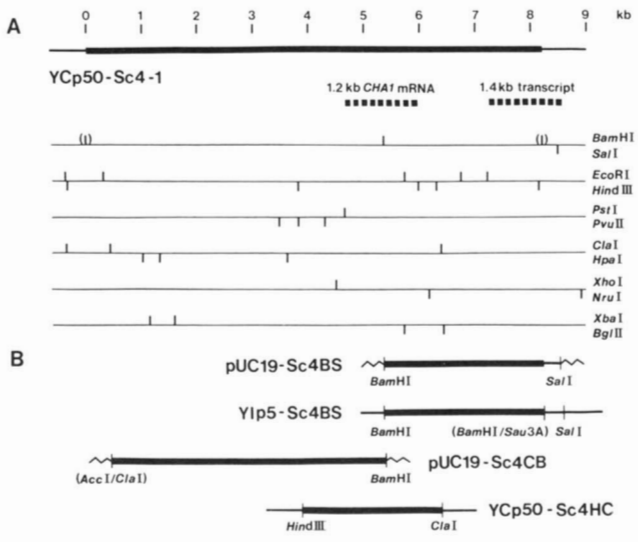

FIGURE 1.—Maps of restriction endonuclease sites, transcripts and plasmid subclones of the *CHA1* region in yeast. A, The catabolic L-serine (L-threonine) deaminase gene *CHA1* was recovered on a 8.1-kb fragment inserted in the *Bam*HI site of the yeast centromere plasmid YCp50. The approximate positions of the *CHA1* mRNA and a neighboring transcript are indicated. The cleavage sites for 12 different restriction enzymes in the cloned region and a small portion of the pBR322 vector are given below. The two *Bam*HI sites in parentheses designate the boundary sites of the yeast DNA fragment which was inserted into the *Bam*HI site of the YCp50 vector. These two sites are not cleaved with *Bam*HI. B, Four plasmid subclones were used in this study. Solid lines depict yeast DNA, thin lines indicate pBR322 DNA of the YCp50 or YIp5 vectors, and zig-zag lines pUC19 DNA.

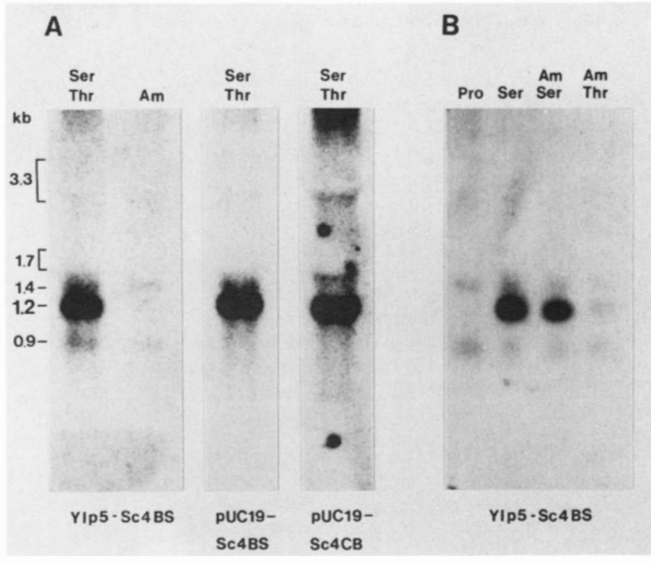

FIGURE 2.—Northern blot analysis of *CHA1* transcription. About 20 μg of RNA from yeast strain X2180-1A grown in minimal medium with different nitrogen sources (indicated above each lane) were separated by electrophoresis, blotted and probed with ^{32}P-labeled plasmids (indicated below the lanes). The size of the *CHA1* mRNA (1.2 kb) and the neighboring 1.4-kb transcript was determined from the positions of the large and small ribosomal RNAs (3.3 and 1.7 kb, respectively) and the *URA3* mRNA (0.9 kb). A, RNA from cells grown in minimal medium with either ammonium or serine and threonine as nitrogen sources was probed with the three *CHA1* plasmid subclones depicted in Figure 1B. B, Yeast RNA from cells grown in minimal media with four different combinations of nitrogen sources were probed with the plasmid YIp5-Sc4BS containing *CHA1* and *URA3* sequences.

1981). About 10 μg of plasmid YIp5-Sc4BS were cleaved partially with *Nru*I (there is one *Nru*I site in the insert and one in the pBR322 region), and the digest was used to transform C85-2411 to uracil independence. A total of 300 transformants were recovered. Thirty-six randomly chosen transformants had all retained the Cha$^-$ phenotype of the parent strain, showing that YIp5-Sc4BS does not contain an intact *CHA1* gene. Three of the transformants were further subjected to tetrad analysis after crossing to strain M1-2B, *MAT*α *ura3 trp1 gal2* (*i.e.*, wild type for *CHA1*). In all cases, the viability of the spores was high (>90%). The number of tetrads obtained with 4 viable spores from the three crosses were 17, 20 and 21. In 57 of 58 tetrads the segregation pattern of 2 Ura$^+$Cha$^-$: 2 Ura$^-$Cha$^+$ was seen. The exceptional tetrad showed 3 Cha$^+$: 1 Cha$^-$, probably as a result of a meiotic gene conversion event. These data prove that the insert originated from the *CHA1* locus.

To determine the number of transcripts that map to the cloned region in YCp50-Sc4-1, RNA was prepared from wild-type strain X2180-1A growing on either ammonium minimal medium (SDAm), or minimal medium with serine and threonine as nitrogen sources (SDSerThr). Equal amounts of the RNA preparations were analysed by Northern hybridization, using the three plasmids YIp5-Sc4BS, pUC19-Sc4BS and pUC19-Sc4CB (Figure 1B) as probes. Almost the entire fragment cloned in YCp50-Sc4-1 is covered by the three plasmids. All three plasmids hybridize strongly to a band of about 1200 nucleotides in the RNA preparation from cells grown in SDSerThr, while no hybridization is seen at that position in the RNA from ammonium grown cells with YIp5-Sc4BS as probe (Figure 2A). Evidently, the 1.2-kb transcript spans the *Bam*HI site in the insert of plasmid YCp50-Sc4-1. As expected, the YIp5-Sc4BS probe hybridizes to the *URA3* mRNA with about equal intensity in the two RNA preparations. A comparison between the intensities of the 1.2-kb RNA band and the *URA3* mRNA band shows that the former RNA is abundant. The *URA3* mRNA constitutes about 0.01% of total mRNA (BACH, LACROUTE and BOTSTEIN 1979). Hybridization is also seen to a 1.4-kb band with the plasmids YIp5-Sc4BS and pUC19-Sc4BS, but not with pUC19-Sc4CB. The approximate positions of the 1.2-kb and 1.4-kb transcripts are indicated in Figure 1A.

To show that the 1.2-kb transcript corresponds to the *CHA1* gene, the 2.4-kb *Hin*dIII-*Cla*I fragment that spans the *Bam*HI site of the original insert was isolated and inserted between the *Hin*dIII and *Cla*I restriction sites of YCp50 to form subclone YCp50-

Sc4HC (Figure 1B). Upon transformation of strain C85-2411, *MAT***a** *ilv1 cha1 ura3 trp1*, to uracil independence a Cha$^+$ phenotype was obtained, showing that the *CHA1* gene is contained on the 2.4-kb fragment. Thus the 1.2-kb mRNA (Figure 2) corresponds in position to the *CHA1* gene.

Regulation of *CHA1* mRNA levels: To further study the effect of different nitrogen sources on the transcription of the *CHA1* gene, strain X2180-1A was grown on minimal medium with either proline, serine, ammonium + Ser, or ammonium + Thr. Equal amounts of RNA (20 μg) from the four cultures were then probed in a Northern blot with ^{32}P-labeled YIp5-Sc4BS (Figure 2B). The *URA3* mRNA and the unidentified 1.4-kb transcript hybridize weakly in all RNA preparations. No hybridization to the *CHA1* mRNA is detected with cells grown on medium with proline as the sole nitrogen source. Thus, the regulation of the *CHA1* mRNA level is not primarily through ammonium repression. Serine alone results in transcription to about the level seen with Ser + Thr (Figure 2A). The addition of ammonium to medium with serine results in a slight decrease in hybridization intensity. Only a weak *CHA1* mRNA band is seen on medium with ammonium + Thr, in agreement with threonine being a poor inducer compared to serine (RAMOS and WIAME 1982).

Suppression of *ilv1*: Haploid yeast strains carrying the *ilv1* mutation require isoleucine for growth due to a defective biosynthetic threonine deaminase, which converts threonine to α-ketobutyrate (KAKAR and WAGNER 1964). This intermediate is converted to isoleucine in four subsequent steps. RAMOS and WIAME (1982) observed that the isoleucine requirement is suppressed when *ilv1 CHA1* cells grow on medium with threonine as the nitrogen source, while this is not the case for *ilv1 cha1* double mutants. Thus, the catabolic serine (threonine) deaminase is able to substitute for the anabolic threonine deaminase in the conversion of threonine to α-ketobutyrate, explaining why *ilv1* mutations are best scored on synthetic ammonium medium lacking both isoleucine and threonine (ZIMMERMANN and GUNDELACH 1969).

We observed that different *ilv1* mutants plated on SC without isoleucine (but containing threonine) spontaneously gave rise to Ile$^+$ colonies at a frequency of 10^{-4}–10^{-5}. This was observed with four *ilv1* alleles in different genetic backgrounds (S288C or Σ1278b origin). A concomitant phenotypic loss of other genetic markers was never observed. Thus, the selected Ile$^+$ strains most likely resulted from allele-nonspecific, physiological suppression of *ilv1*, and was designated Sil$^+$ (suppression of *ilv1*). Two observations indicated that the *CHA1* gene was involved in the suppression: (1) The Sil$^+$ phenotype was dependent on exogenous threonine, since Sil$^+$ *ilv1* strains were unable to grow on SC without isoleucine and threonine. Possibly, in Sil$^+$ *ilv1* strains on SC-ile, exogenous threonine increases the intracellular threonine pool sufficiently to allow formation of α-ketobutyrate by the catabolic enzyme. (2) When *ilv1 cha1* double mutants were plated on SC without isoleucine, Ile$^+$ papillae appeared at a lower frequency (10^{-6}) than in *ilv1 CHA1* strains.

Genetic analysis of Sil$^+$ mutants: To analyse whether the Sil$^+$ phenotype was caused by a mutation in a single chromosomal gene, four Sil$^+$ colonies were isolated from C82-1582, *MAT***a** *ilv1-22 trp1 arg4-16 ade2*, and two in strain C81-1667, *MAT***a** *ilv1-100*. The *ilv1* parents and the Sil$^+$ strains were crossed to C80-1407, *MAT*α *lys1*, and the resulting diploids analyzed by tetrad analysis. In all crosses (5–13 tetrads analyzed in each), *ilv1* segregated 2+:2− as expected (scored by inability of *ilv1* spores to grow on SC without isoleucine and threonine). In each of the six Sil$^+$ crosses, about half of the *ilv1* spores were able to grow on SC without isoleucine (but containing threonine), while this was not observed with the parents, consistent with the assumption that the Sil$^+$ phenotype is due to a single gene unlinked to *ilv1*.

To further analyze the genetic behavior of *sil* mutants, the four C82-1582 derivatives were brought into an *ilv1* homozygous background by crossing to C82-1704, *MAT*α *ilv1-100 lys1*. The four diploids were unable to grow on SC without isoleucine, or they grew only poorly, showing that the *sil* mutations behaved as recessive markers. The results of tetrad analysis are shown in Table 1. The segregation for Sil$^+$ is 2:2, revealing a single Mendelian gene, in agreement with the analysis described above. Furthermore, when *trp1* was used as a marker for first meiotic division segregation, the percentage of tetratype was not significantly below 67%, i.e. *sil* was not significantly centromere linked (HAWTHORNE and MORTIMER 1960).

A complementation analysis of the *sil* mutations was carried out by crossing strain C82-1582 and its four Sil$^+$ derivatives to C81-1420, *MAT*α *ilv1-23 his4-29 ade2* and eight Sil$^+$ isolates of this strain. The parent diploid and the twelve Sil$^+$ × Sil$^-$ diploids did not grow, or grew only poorly, on SC without isoleucine, again demonstrating the recessive character of *sil* mutations. In contrast, all the 32 Sil$^+$ × Sil$^+$ diploids grew well on this medium. Thus the *sil* mutations were noncomplementing, suggesting that they are mutations in one gene. The same result was obtained when the C82-1582 Sil$^+$ derivatives were crossed to Sil$^+$ isolates from C82-1728. The mutation conferring the Sil$^+$ phenotype has been denoted *sil1*.

The *sil1* mutation is not linked to *CHA1*: The relationship between *sil1* and the *CHA1* gene was investigated by tetrad analysis: C82-1724, *MAT***a** *ilv1-22 trp1 arg4-16 ade2 sil1-4*, was crossed to C85-2409, *MAT*α *ilv1-10 ura3-52 trp1 cha1*. Among thirty tetrads,

TABLE 1

Tetrad analysis of *sil1* in an *ilv1 CHA1* homozygous background

Cross with	Total No. of tetrads	No. of tetrads with Sil$^+$:Sil$^-$ segregation				No. of tetrads Sil/Trp		
		3:1	2:2	1:3	4:0	PD[a]	NPD	T
C82-1582	10	0	0	0	10			
C82-1724 (*sil1-4*)	9	1	7	1	0	0	3	4
C82-1725 (*sil1-5*)	9	0	9	0	0	0	1	5
C82-1726 (*sil1-6*)	10	0	10	0	0	4	1	2
C82-1727 (*sil1-7*)	30	0	30	0	0	5	6	19

The *ilv1* strain C82-1582, *MAT***a** *ilv1-22 trp1 arg4-16 ade2*, and four Sil$^+$ derivatives (C82-1724 through C82-1727) were crossed to the *ilv1* strain C82-1704, *MAT*α *ilv1-100 lys1*, and the resulting diploids were sporulated and dissected.

[a] PD = parental ditype, NPD = nonparental ditype, T = tetratype.

all showed the expected 2:2 segregation for *cha1* (scored by the inability of *cha1* spores to grow on SDThrSer supplemented with tryptophan, arginine, adenine and uracil). The Sil$^+$ phenotype (growth on SC-ile) was observed only in *CHA1* spores. In total, 32 of the 60 *CHA1* spores had a Sil$^+$ phenotype, showing that *sil1* and *cha1* segregated independently. Absence of linkage of the two genes was also seen when the segregation of *sil1* in individual tetrads was considered: 7 tetrads showed 2:2 segregation for Sil$^+$:Sil$^-$, 18 tetrads gave a 1:3 segregation and 5 were 0:4. These segregations were interpreted to be parental ditype, tetratype and nonparental ditype, respectively.

***sil1* causes increased amount of *CHA1* transcript:** With the working hypothesis that *sil1* causes increased expression of *CHA1*, its effect on the amount of *CHA1* messenger was investigated. The *ilv1* strain C82-1582 and its *sil1* derivative C82-1724 (*sil1-4*) as well as C82-1730 (*sil1-8*) were grown on SC medium. Total yeast RNA was isolated and equal amounts probed with YIp5-Sc4BS in a Northern hybridization (Figure 3). Hybridization to the *CHA1* mRNA is observed in C82-1582 (*ilv1 sil1*) with an intensity comparable to that of the 0.9-kb *URA3* mRNA and the 1.4-kb RNA. In the photographic reproduction presented in Figure 3, the *URA3* band is difficult to see in some lanes, but was observed in the original autoradiogram. When comparison is made to Figure 2B, it appears that the *CHA1* mRNA is repressed on SC relative to SDSer + ammonium. The reason for this is unknown, but it may be due to the higher amount of serine in SDSer + ammonium or there could be competition for uptake between serine and other amino acids. Stronger *CHA1* mRNA bands are seen in the two *sil1* strains, but not at the level seen in SDSerThr grown yeast. Thus, in the wild type, a low level of *CHA1* mRNA is seen in synthetic complete ammonium medium (which contains serine and threonine), and the level is increased by the *sil1* mutation.

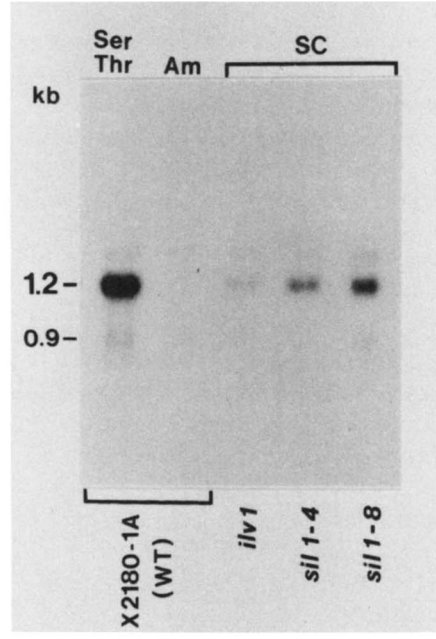

FIGURE 3.—Northern analysis of *sil1* strains. Total yeast RNA (20 μg) from the *ilv1* strain C82-1582 and from the two *sil1* strains C82-1726 (*ilv1 sil1-4*) and C82-1730 (*ilv1 sil1-8*) grown in synthetic complete medium was probed with the *cha1* plasmid subclone YIp5-Sc4BS. Equal amounts of RNA from strain X2180-1A in minimal medium with serine and threonine, or with ammonium, were probed for comparison.

DISCUSSION

In this paper we have described the molecular cloning of the *CHA1* gene of *S. cerevisiae*. The gene is needed for the presence of the catabolic L-serine (L-threonine) deaminase, which provides the yeast with the ability to produce ammonium from the two hydroxyamino acids and thereby to use them as sole nitrogen source (RAMOS and WIAME 1982). The *CHA1* gene, which is contained on a 2.4-kb *Hin*dIII-*Cla*I fragment, may well be the structural gene for the enzyme.

The length of the *CHA1* mRNA was determined to be about 1200 ribonucleotides. If we assume that

200 nucleotides are untranslated, the molecular weight of the *CHA1* gene product should be about 38,000. Catabolic hydroxyamino acid deaminases have been purified from different organisms but, to our knowledge, not from yeast. In rat liver, the molecular weight of crystalline pure L-serine (L-threonine) deaminase was determined to be 64,000 by sedimentation equilibrium centrifugation (SUDA and NAKAGAWA 1971), and in *Clostridium acidiurici* the molecular weight of the enzyme was 72,000, as determined by gel filtration (SAGERS and CARTER 1971). In *E. coli*, the biodegradative threonine deaminase exists as a tetramer of four identical subunit polypeptides with molecular weights of 38,000–39,000 (SAEKI *et al.* 1977; KIM and DATTA 1982; WHANGER *et al.* 1968). The subunit of the *Salmonella typhimurium* enzyme has a slightly smaller size of 36,000 (KIM and DATTA 1982). Assuming a dimeric configuration of the purified deaminases from rat liver and Clostridium, the known catabolic serine or threonine deaminase subunits have molecular weights of 32,000–39,000, in agreement with the estimated size of the *CHA1* gene product in yeast.

The Northern analysis showed that the steady-state level of the *CHA1* mRNA is strongly regulated. The *CHA1* mRNA is abundant in yeast grown with serine (or serine and threonine) as sole nitrogen source. In contrast, the *CHA1* mRNA was not detected in RNA from cells growing in minimal medium with ammonium as the sole nitrogen source. The strong regulation agrees with a two orders of magnitude regulation of enzyme activity under similar growth conditions (RAMOS and WIAME 1982). This agreement supports the assumption that *CHA1* is the structural gene for the deaminase. Since the *CHA1* mRNA is not detected when proline serves as sole nitrogen source, it appears that the transcription of the *CHA1* gene is regulated mainly through serine and threonine induction, rather than by ammonium repression, with serine being the more effective inducer. However, the addition of ammonium to media with serine decreases the *CHA1* mRNA hybridization signal. Again, this is in agreement with the study of RAMOS and WIAME (1982), who measured a 2–3-fold increase in deaminase activity when proline was used instead of ammonium as the nitrogen source, or when nitrogen catabolite repression was relieved by the *gdhCR* (*ure2*) mutation. We favor the idea that the effect of ammonium on *CHA1* is indirect and due to a decrease in the effective, intracellular pools of the hydroxyamino acids, possibly brought about by repression of the general amino acid permease (GRENSON, HOU and CRABEEL 1970).

The regulation of the *CHA1* gene has features in common with other nitrogen catabolic genes in yeast. Arginase (encoded by *CAR1*) and ornithine aminotransferase (*CAR2*), catalyzing the first and second step in the catabolic pathway of arginine, are regulated transcriptionally by induction with arginine (MIDDELHOVEN 1970; WHITNEY and MAGASANIK 1973; SUMRADA and COOPER 1982; JAUNIAUX *et al.* 1982). In addition, the enzymes are subject to nitrogen catabolite repression when cells are grown in the presence of readily used nitrogen sources. Several *trans*-acting regulatory mutations have been characterized (MESSENGUY and DUBOIS 1983; MESSENGUY, DUBOIS and DESCAMPS 1986). In proline degradation, proline oxidase (*PUT1*) and pyrroline 5-carboxylate dehydrogenase (*PUT2*) are inducible with proline as the inducer, but do not appear to be under nitrogen repression (BRANDRISS and MAGASANIK 1979a, b; BRANDRISS 1983; WANG and BRANDRISS 1986). The degradation of allantoin involves several genes that are inducible by allophanate, with the *DAL81* gene product acting as a common positive regulator (COOPER and LAWTHER 1973; WHITNEY, COOPER and MAGASANIK 1973; CHISHOLM and COOPER 1982).

Under induced growth conditions, the catabolic deaminase may substitute for the biosynthetic threonine deaminase in forming α-ketobutyrate from threonine, the first step in isoleucine biosynthesis. This ability was used in an attempt to isolate mutants with increased levels of the catabolic enzyme by plating haploid *ilv1* strains on synthetic complete ammonium medium lacking isoleucine. Since this medium contains serine and threonine, the transcription of *CHA1* is partially induced, but to a level that is insufficient to suppress *ilv1* mutations. All the isolated suppressor mutations were found to belong to one complementation group, designated *sil1*. The *sil1* mutation appears spontaneously under selective conditions at a high frequency (10^{-4}–10^{-5}). The *sil1* gene was found to be unlinked to *CHA1*. Different *ilv1* suppressor mutations have been studied earlier. KAKAR (1963) has described both a dominant and a recessive type of allele nonspecific *ilv1* suppressors. The recessive suppressor permitted the utilization of threonine as a substitute for isoleucine for growth. Physiological suppressors of *ilv1* have also been noted by GUNDELACH (1973). The present study showed increased levels of *CHA1* mRNA in *sil1* strains, as compared to wild-type cells. Thus, *sil1* acts by increasing the steady-state levels of *CHA1* mRNA. The mechanism for this effect is not known. The *sil1* mutation could cause an increase in the effective pools of serine and threonine, so that *CHA1* is sufficiently induced (*e.g.*, by serine) allowing α-ketobutyrate to be formed from threonine, which leads to the suppression of *ilv1*. We find it improbable that *sil1* should cause increased biosynthesis of threonine, since the suppression of *ilv1* requires exogenous threonine. Increased pools of serine and threonine could be brought about by enhanced uptake of these amino acids into the cell. An alternative way to explain

the increased transcription of *CHA1* caused by *sil1* is that the wild type *SIL1* gene codes for a negatively acting regulatory element of *CHA1*.

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