Molecular Genetics of Serine and Threonine Catabolism in Saccharomyces cerevisiae

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Manuscript received July 28, 1987
Revised copy accepted March 23, 1988

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, chal (catabolism of hydroxyamino acids), lacking this enzyme activity has been isolated. We have cloned the CHAl gene by complementation of a chal mutation. Northern analysis showed that CHAl mRNA has a size of about 1200 ribonucleotides. CHAl 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 CHAl gene is induced by serine or threonine. Under induced growth conditions haploid ilvl CHAl strains do not require isoleucine, i.e., the catabolic deaminase is able to substitute for the biosynthetic threonine deaminase encoded by the ILVl gene. We have identified a nuclear, recessive mutation, sill, that suppresses ilvl mutations by increased transcription of the CHAl gene under growth conditions leading to partial induction. The sill mutation could exert its effect by increasing the effective pools of the hydroxyamino acids. Alternatively SILI may encode a negatively acting regulatory protein for CHAl.

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 ILVl), which in a similar manner converts threonine to α-ketobutyrate, the first intermediate in isoleucine biosynthesis. The ILVl 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; Sæk 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, CHAl, that is needed for the presence of catabolic serine (threonine) deaminase in yeast.

MATERIALS AND METHODS

Materials: [α-32P]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 \textit{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 8376b, \textit{MATa} \textit{ilvl}-10 \textit{chal}-1 (Ramos and Wiame 1982) was kindly supplied by F. Ramos and J.-M. Wiame. The allele number for the \textit{ilvl} mutation, as well as the designation of the \textit{chal} mutation as \textit{chal}-1, was chosen in agreement with F. Ramos and J.-M. Wiame. Also used were strains M1-2B, \textit{MATa} \textit{ura}-32 \textit{trpl} gal2 (St. John et al. 1981) and C80-1407, \textit{MATa} \textit{bysl}. The following \textit{ilvl} strains were constructed by standard genetic procedures (Sherman, Fink and Lawrence 1974): C85-2409, \textit{MATa} \textit{ilvl}-10 \textit{chal}-1 \textit{ura}-32 \textit{trpl}; C85-2411, \textit{MATa} \textit{ilvl}-10 \textit{chal}-1 \textit{ura}-32 \textit{trpl}; C81-1667, \textit{MATa} \textit{ilvl}-100; C82-1582, \textit{MATa} \textit{ilvl}-22 \textit{trpl} arg4-16 ade2; C82-1704, \textit{MATa} \textit{ilvl}-100 \textit{bysl}; C81-1420, \textit{MATa} \textit{ilvl}-23 his4-29 ade2 and C82-1728, \textit{MATa} \textit{ilvl}-22 \textit{trpl} \textit{bysl}. Strains with suppressors of \textit{ilvl} were C82-1729, through C82-1727, \textit{MATa} \textit{ilvl}-22 \textit{trpl} arg4-16 ade2 \textit{sil}-4 through \textit{sil}-7 (derived from C82-1582) and C82-1730, \textit{MATa} \textit{ilvl}-23 \textit{sil}-8 \textit{his4}-29 \textit{ade2} (derived from C81-1420). All \textit{ilvl} alleles used in this study are non-complementing. \textit{E. coli} strain HB101 (Boyer and Roulland-Dussoix 1969) and JMM8 (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 \textit{YCP50} and \textit{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 Sam Brock 1982).

Preparation of plasmids, yeast DNA and RNA: Plasmids were prepared as a scaled-up version of the alkaline extraction procedure (Burnham 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\°C (OD\textsubscript{600} = 1-1.5) by the method of Broach et al. (1979) modified as described earlier (Holmberg et al. 1985). Enrichment for poly(A\textsuperscript{+}) RNA was not carried out.

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

Cloning of the CHAI gene: The CHAI gene was cloned by complementation of the \textit{chal} mutation as follows. \textit{S. cerevisiae} strain C85-2411, \textit{MATa} \textit{ilvl} \textit{chal} \textit{ura3} \textit{trpl}, 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 Sau3A digested DNA ($288C$ background and inserted into the \textit{BamHI} 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\textsuperscript{+} 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 \textit{ILV1} gene (Petersen et al. 1983a; Kieland-Brandt et al. 1984). One other transformant was unable to grow on SDAm + Trp, but grew well with 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 \textit{Cha}\textsuperscript{+} transformant (Ramos and Wiame 1982). Total yeast DNA was prepared from the transformant and used to transform \textit{E. coli}, selecting for ampicillin resistance. The recovered plasmid was designated YCP50-Sc4-1.

RESULTS

Analysis of the \textit{CHAI} 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 \textit{chal} 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 Sam Brock 1982) of yeast DNA cleaved with different restriction enzymes was carried out using \textsuperscript{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 tetra-cycline resistance gene of YCP50 (pBR322 sequences), inserted between the \textit{BamHI} and \textit{SalI} 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 \textit{CHAI} locus, the same 3.1-kb \textit{BamHI}-\textit{SalI} fragment was inserted between the \textit{BamHI} and \textit{SalI} of the yeast integrating plasmid \textit{YIp5} (pBR322-URA3), giving plasmid \textit{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 1985).
BamHI. Four plasmid subclones were used in this study. Solid cleavage sites for 12 different restriction enzymes in the cloned region and a small portion of the pBR322 vector are given below. The two BamHI sites in parentheses designate the boundary sites.

The two BamHI sites in parentheses designate the boundary sites of the spores catabolic L-serine (L-threonine) deaminase gene. The insert and one in the pBR322 region, and the small portion of the pBR322 vector are given below. The two BamHI sites in parentheses designate the boundary sites of the yeast DNA fragment which was inserted into the BamHI site of the YCp50 vector. These two sites are not cleaved with BamHI. 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.

About 10 μg of plasmid YIp5-Sc4BS was cleaved partially with NruI (there is one NruI site in the insert and one in the pBR322 region), and the digest was used to transform C85-2411 to uracil and ura3 phenotypes. The segregation pattern of 2 Ura'Cha- tetrads obtained with 4 viable spores from the three parents had all retained the Cha- phenotype of the wild type for CHAI. In all cases, the viability of the spores was high (>90%). The number of tetrads obtained with 4 viable spores from the three crosses was 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 CHAI 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 BamHI 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 CHAI gene, the 2.4-kb HindIII-ClaI fragment that spans the BamHI site of the original insert was isolated and inserted between the HindIII and ClaI restriction sites of YCp50 to form subclone YCp50-Sc4-1. 0.1 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 the 32P-labeled plasmids (indicated below the lanes). The size of the CHAI 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 CHAI 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 CHAI and URA3 sequences.
Sc4HC (Figure 1B). Upon transformation of strain C85-2411, \( \text{MAT}^+ \text{i}v\text{l} \text{ch}a\text{l} \text{ura3} \text{trpl} \), to uracil independence a Cha\(^+\) phenotype was obtained, showing that the \( \text{CHA}1 \) gene is contained on the 2.4-kb fragment. Thus the 1.2-kb mRNA (Figure 2) corresponds in position to the \( \text{CHA}1 \) gene.

**Regulation of \( \text{CHA}1 \) mRNA levels:** To further study the effect of different nitrogen sources on the transcription of the \( \text{CHA}1 \) gene, strain X2180-1A was grown on minimal medium with either proline, serine, ammonium + Ser, or ammonium + Thr. Equal amounts of RNA (20 \( \mu \text{g} \)) from the four cultures were then probed in a Northern blot with \( ^{32}\text{P} \)-labeled \( \text{YIP5-SC4} \text{BS} \) (Figure 2B). The \( \text{URA}3 \) mRNA and the unidentified 1.4-kb transcript hybridize weakly in all RNA preparations. No hybridization to the \( \text{CHA}1 \) mRNA is detected with cells grown on medium with proline as the sole nitrogen source. Thus, the regulation of the \( \text{CHA}1 \) 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 \( \text{CHA}1 \) mRNA band is seen on medium with ammonium + Thr, in agreement with threonine being a poor inducer compared to serine (Ramos and Wiamé 1982).

**Suppression of \( \text{i}v\text{l} \):** Haploid yeast strains carrying the \( \text{i}v\text{l} \) mutation require isoleucine for growth due to a defective biosynthetic threonine deaminase, which converts threonine to \( \alpha \)-ketobutyrate (Kakar and Wagner 1964). This intermediate is converted to isoleucine in four subsequent steps. Ramos and Wiamé (1982) observed that the isoleucine requirement is suppressed when \( \text{i}v\text{l} \) \( \text{CHA}1 \) cells grow on medium with threonine as the nitrogen source, while this is not the case for \( \text{i}v\text{l} \text{cha}1 \) double mutants. Thus, the catabolic serine (threonine) deaminase is able to substitute for the anabolic threonine deaminase in the conversion of threonine to \( \alpha \)-ketobutyrate, explaining why \( \text{i}v\text{l} \) mutations are best scored on synthetic ammonium medium lacking both isoleucine and threonine (Zimmermann and Gundelach 1969).

We observed that different \( \text{i}v\text{l} \) mutants plated on SC without isoleucine (but containing threonine) spontaneously gave rise to Ile\(^+\) colonies at a frequency of \( 10^{-4} \)–\( 10^{-6} \). This was observed with four \( \text{i}v\text{l} \) alleles in different genetic backgrounds (S288C or \( \Sigma \)1278b 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 \( \text{i}v\text{l} \), and was designated \( \text{sill} \) (suppression of \( \text{i}v\text{l} \)). Two observations indicated that the \( \text{CHA}1 \) gene was involved in the suppression: (1) The \( \text{sill} \) phenotype was dependent on exogenous threonine, since \( \text{sill} \) \( \text{i}v\text{l} \) strains were unable to grow on SC without isoleucine and threonine. Possibly, in \( \text{sill} \) \( \text{i}v\text{l} \) strains on SC, exogenous threonine increases the intracellular threonine pool sufficiently to allow formation of \( \alpha \)-ketobutyrate by the catabolic enzyme. (2) When \( \text{i}v\text{l} \text{cha}1 \) double mutants were plated on SC without isoleucine, Ile\(^+\) papillae appeared at a lower frequency (\( 10^{-6} \)) than in \( \text{i}v\text{l} \) \( \text{CHA}1 \) strains.

**Genetic analysis of \( \text{sill} \) mutants:** To analyse whether the \( \text{sill} \) phenotype was caused by a mutation in a single chromosomal gene, four \( \text{sill} \) colonies were isolated from C82-1582, \( \text{MAT}^+ \text{i}v\text{l}-22 \text{trpl} \text{arg4-16} \text{ade2} \), and two in strain C81-1667, \( \text{MAT}^+ \text{i}v\text{l}-100 \). The \( \text{i}v\text{l} \) parents and the \( \text{sill} \) strains were crossed to C80-1407, \( \text{MAT}^a \text{lys1} \), and the resulting diploids analyzed by tetrad analysis. In all crosses (5–13 tetrads analyzed in each), \( \text{i}v\text{l} \) segregated 2+ : 2− as expected (scored by inability of \( \text{i}v\text{l} \) spores to grow on SC without isoleucine and threonine). In each of the six \( \text{sill} \) crosses, about half of the \( \text{i}v\text{l} \) 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 \( \text{sill} \) phenotype is due to a single gene unlinked to \( \text{i}v\text{l} \).

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

A complementation analysis of the \( \text{sill} \) mutations was carried out by crossing strain C82-1582 and its four \( \text{sill} \) derivatives to C81-1420, \( \text{MAT}^a \text{i}v\text{l}-23 \text{his4-29} \text{ade2} \) and eight \( \text{sill} \) isolates of this strain. The parent diploid and the twelve \( \text{sill} \times \text{sill} \) diploids did not grow, or grew only poorly, on SC without isoleucine, again demonstrating the recessive character of \( \text{sill} \) mutations. In contrast, all the 32 \( \text{sill} \times \text{sill} \) diploids grew well on this medium. Thus the \( \text{sill} \) mutations were noncomplementing, suggesting that they are mutations in one gene. The same result was obtained when the C82-1582 \( \text{sill} \) derivatives were crossed to \( \text{sill} \) isolates from C82-1728. The mutation conferring the \( \text{sill} \) phenotype has been denoted \( \text{sill} \).

**The \( \text{sill} \) mutation is not linked to \( \text{CHA}1 \):** The relationship between \( \text{sill} \) and the \( \text{CHA}1 \) gene was investigated by tetrad analysis: C82-1724, \( \text{MAT}^a \text{i}v\text{l}-22 \text{trpl} \text{arg4-16} \text{ade2 sill-4} \), was crossed to C85-2409, \( \text{MAT}^a \text{i}v\text{l}-10 \text{ura3-52 trpl cha1} \). Among thirty tetrads,
all showed the expected 2:2 segregation for \(chal\) (scored by the inability of \(chal\) spores to grow on SDThrSer supplemented with tryptophan, arginine, adenine and uracil). The \(Sil^+\) phenotype (growth on SC-ile) was observed only in \(CHAI\) spores. In total, 32 of the 60 \(CHAI\) spores had a \(Sil^+\) phenotype, showing that \(silI\) and \(chal\) segregated independently. Absence of linkage of the two genes was also seen when the segregation of \(silI\) in individual tetrads was considered: 7 tetrads showed 2:2 segregation for \(Sil^+:Sil^-\), 18 tetrads gave a 1:3 segregation and 3 were 0:4. These segregations were interpreted to be parental ditype, tetratype and nonparental ditype, respectively.

**\(silI\) causes increased amount of \(CHAI\) transcript:**
With the working hypothesis that \(silI\) causes increased expression of \(CHAI\), its effect on the amount of \(CHAI\) messenger was investigated. The \(ilvl\) strain C82-1582 and its \(silI\) derivative C82-1724 (\(silI-4\)) as well as C82-1730 (\(silI-8\)) 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 \(CHAI\) mRNA is observed in C82-1582 (\(ilvl\) \(silI\)) 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 \(CHAI\) 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 \(CHAI\) mRNA bands are seen in the two \(silI\) strains, but not at the level seen in SDSerThr grown yeast. Thus, in the wild type, a low level of \(CHAI\) mRNA is seen in synthetic complete ammonium medium (which contains serine and threonine), and the level is increased by the \(silI\) mutation.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Cross with</th>
<th>Total No. of tetrads</th>
<th>No. of tetrads with (Sil^+:Sil^-) segregation</th>
<th>No. of tetrads (Sil/Trp)</th>
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<tr>
<td></td>
<td>3:1</td>
<td>2:2</td>
<td>1:3</td>
</tr>
<tr>
<td>C82-1582</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C82-1724 ((silI-4))</td>
<td>9</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>C82-1725 ((silI-5))</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>C82-1726 ((silI-6))</td>
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<td>0</td>
<td>10</td>
</tr>
<tr>
<td>C82-1727 ((silI-7))</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
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</table>

The \(ilvl\) strain C82-1582, MAT\(a\) \(ilvl-22\) trpl arg4-16 ade2, and four \(Sil^+\) derivatives (C82-1724 through C82-1727) were crossed to the \(ilvl\) strain C82-1704, MAT\(a\) \(ilvl-100\) bsl1, and the resulting diploids were sporulated and dissected.

\(^*\) PD = parental ditype, NPD = nonparental ditype, T = tetratype.

**DISCUSSION**

In this paper we have described the molecular cloning of the \(CHAI\) gene of \(S.\ cer\) ves\(iae\). 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 Wiam 1982). The \(CHAI\) gene, which is contained on a 2.4-kb \(HindIII\)-\(ClaI\) fragment, may well be the structural gene for the enzyme.

The length of the \(CHAI\) mRNA was determined to be about 1200 ribonucleotides. If we assume that...
200 nucleotides are untranslated, the molecular weight of the \textit{CHA1} gene product should be about 38,000. Catabolic hydroxylaminoo 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 centfriguation (Suda and Nakagawa 1971), and in \textit{Clostridium aciducitrici} the molecular weight of the enzyme was 72,000, as determined by gel filtration (Sagers and Carter 1971). In \textit{E. coli}, the biodegradative threonine deaminase exists as a tetramer of four identical subunit polypeptides with molecular weights of 38,000–39,000 (Saeiki \textit{et al.} 1977; Kim and Datta 1982; Whanger \textit{et al.} 1968). The subunit of the \textit{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 \textit{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 \textit{CHA1} gene product in yeast.

The Northern analysis showed that the steady-state level of the \textit{CHA1} mRNA is strongly regulated. The \textit{CHA1} mRNA is abundant in yeast grown with serine (or serine and threonine) as sole nitrogen source. In contrast, the \textit{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 agrees with the study of Ramos \textit{et al.} (1982). This agreement supports the assumption that \textit{CHA1} is the structural gene for the deaminase. Since the \textit{CHA1} mRNA is not detected when proline serves as sole nitrogen source, it appears that the transcription of the \textit{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 \textit{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 \textit{gdhCR} (\textit{ure2}) mutation. We favor the idea that the effect of ammonium on \textit{CHA1} is indirect and due to a decrease in the effective, intracellular pools of the hydroxylamino acids, possibly brought about by repression of the general amino acid permease (Grenson, Hou and Crabbeel 1970).

The regulation of the \textit{CHA1} gene has features in common with other nitrogen catabolic genes in yeast. Arginase (encoded by \textit{CAR1}) and ornithine aminotransferase (\textit{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 1975; Sumrada and Cooper 1982; Jauniaux \textit{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 \textit{trans-}acting regulatory mutations have been characterized (Messenguy and Dubois 1983; Messenguy, Dubois and Descamps 1985). In proline degradation, proline oxidase (\textit{PUT1}) and pyrroline 5-carboxylate dehydrogenase (\textit{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 \textit{DAL81} gene product acting as a common positive regulator (Doherty 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 \textit{a}-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 \textit{ilv1} strains on synthetic complete ammonium medium lacking isoleucine. Since this medium contains serine and threonine, the transcription of \textit{CHA1} is partially induced, but to a level that is insufficient to suppress \textit{ilv1} mutations. All the isolated suppressor mutations were found to belong to one complementation group, designated \textit{sil}. The \textit{sil} mutation appears spontaneously under selective conditions at a high frequency (10^{-4}–10^{-5}). The \textit{sil} gene was found to be unlinked to \textit{CHA1}. Different \textit{ilv1} suppressor mutations have been studied earlier. Kakar (1963) has described both a dominant and a recessive type of allele nonspecific \textit{ilv1} suppressors. The recessive suppressor permitted the utilization of threonine as a substitute for isoleucine for growth. Physiological suppressors of \textit{ilv1} have also been noted by Gundelach (1973). The present study showed increased levels of \textit{CHA1} mRNA in \textit{sil} strains, as compared to wild-type cells. Thus, \textit{sil} acts by increasing the steady-state levels of \textit{CHA1} mRNA. The mechanism for this effect is not known. The \textit{sil} mutation could cause an increase in the effective pools of serine and threonine, so that \textit{CHA1} is sufficiently induced (\textit{e.g.}, by serine) allowing \textit{a}-ketobutyrate to be formed from threonine, which leads to the suppression of \textit{ilv1}. We find it improbable that \textit{sil} should cause increased biosynthesis of threonine, since the suppression of \textit{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 \textit{CHAI} caused by \textit{sill} is that the wild type \textit{SILL} gene codes for a negatively acting regulatory element of \textit{CHAI}.

We are grateful to Fernando Ramos and Jean-Marie Wiame for supplying the \textit{chaj} mutant, and for critical comments on this manuscript. The generous supply of yeast gene pools by Mark Rose is greatly appreciated. Richard Egel is acknowledged for his critical comments on the manuscript. We thank Lotte Vaa, Kirsten Schultz and Gertte Bank for excellent technical assistance and Claus Gjermansen for help and discussions. Nina Rasmussen and Ann-Sofi Steinholz were very helpful in the preparation of the figures. We thank Diter von Wettstein for his support and critical reading of the manuscript. This work was supported by grant 16-3956.H from the Danish Technical Research Council and grant 135/001-84154 from Tekoloalgrådet to Diter von Wettstein.

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Communicating editor: E. W. Jones