A Single Point Mutation Results in a Constitutively Activated and Feedback-Resistant Chorismate Mutase of Saccharomyces cerevisiae

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The Saccharomyces cerevisiae ARO7 gene product chorismate mutase, a single-branch-point enzyme in the aromatic amino acid biosynthetic pathway, is activated by tryptophan and subject to feedback inhibition by tyrosine. The ARO7 gene was cloned on a 2.05-kilobase EcoRI fragment. Northern (RNA) analysis revealed a 0.95-kilobase poly(A)+ RNA, and DNA sequencing determined a 771-base-pair open reading frame capable of encoding a protein of 256 amino acids. In addition, three mutant alleles of ARO7 were cloned and sequenced. These encoded chorismate mutases which were unresponsive to tyrosine and tryptophan and were locked in the on state, exhibiting a 10-fold-increased basal enzyme activity. A single base pair exchange resulting in a threonine-to-isoleucine amino acid substitution in the C-terminal part of the chorismate mutase was found in all mutant strains. In contrast to other enzymes in this pathway, no significant homology between the monofunctional yeast chorismate mutase and the corresponding domains of the two bifunctional Escherichia coli enzymes was found.

In the yeast Saccharomyces cerevisiae, the biosynthesis of aromatic amino acids is regulated either at the transcriptional or at the enzyme level. At the transcriptional level, the general control system is known to regulate at least 30 structural amino acid genes in various pathways, among them most of the ARO and TRP genes. This transcriptional control responds to amino acid starvation and results in an increased transcription rate of these genes through binding of the activator protein GCN4 (35). In contrast to many bacteria, no aromatic amino acid-specific regulation is known at the transcriptional level.

At least four ARO and TRP gene products are also or exclusively regulated at the enzyme level (Fig. 1). The two isoenzymes 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase (EC 4.1.2.15) encoded by the genes ARO3 and ARO4 control the entrance of the shikimate pathway and are subject to feedback inhibition by the pathway end products phenylalanine and tyrosine, respectively (37). The TRP2 gene product anthranilate synthase (EC 4.3.1.27) and the ARO7 gene product chorismate mutase (EC 5.4.99.5) control the distribution of chorismate between the two branches of the aromatic amino acid pathway and are feedback inhibited by tryptophan and tyrosine, respectively (20, 32).

No transcriptional regulation is known for the ARO7 gene of the yeast S. cerevisiae. The ARO7 gene encodes a monofunctional chorismate mutase, a situation also found in Bacillus subtilis Marburg (21) and in Streptomyces aureofaciens (14). The yeast chorismate mutase is not only feedback inhibited by tyrosine, one of the two end products of this biosynthetic branch, but is also strongly activated by tryptophan (20), the end product of the other branch. The dual control of this enzyme by tyrosine as feedback inhibitor and tryptophan as activator is to date unique as a means of regulating enzyme activity. The monofunctional B. subtilis Marburg chorismate mutase is inhibited by prephenate but unaffected by tyrosine, phenylalanine, or tryptophan (22), and the S. aureofaciens enzyme is unregulated (14). Other investigated organisms such as Escherichia coli employ two bifunctional enzymes: a chorismate mutase-prephenate dehydratase (pheA) feedback inhibited by phenylalanine and a chorismate mutase-prephenate dehydrogenase (tyrA) feedback inhibited by tyrosine (9, 10); in both cases the N-terminal part of the bifunctional enzyme carries the chorismate mutase activity (19, 26).

In this report, we describe the cloning and nucleotide sequence comparison of the yeast ARO7 wild type and three previously described ARO7c (constitutively activated chorismate mutase) mutant alleles (20). Mutant strains carrying the ARO7c allels showed increased sensitivity to the amino acid analog 5-methyltryptophan and a 10-fold increase in basal activity of chorismate mutase. The mutant enzymes were unresponsive to tyrosine and tryptophan.

We could not find any regulation of the ARO7 gene at the transcriptional level. Analysis of the nucleotide sequence revealed that (i) there is no consensus sequence for a binding site of the general control activator protein GCN4 in the 5′ region of the ARO7 gene, and (ii) the ARO7c phenotype was caused by an identical point mutation found in all three mutant alleles at the same locus in the C-terminal part of the protein. This resulted in a threonine-to-isoleucine substitution. It is therefore apparent that a single amino acid substitution is sufficient to activate the yeast chorismate mutase, obviating the need for tryptophan activation and locking the enzyme in the on state, in a form resistant to tyrosine inhibition.

MATERIALS AND METHODS

Strains and plasmids. All yeast strains used are derivatives of the S. cerevisiae laboratory strains X2180-1A (MATa gal2 suc2 mal CUP1) and X2180-1B (MATα gal2 suc2 mal CUP1). The RH1242 genotype is MATα aro7 leu2-2, RH558-1 (MATα gcd2-1) carries a mutation which leads to constitutive derepression of those amino acid biosynthetic genes which are subject to general control (35). The ARO7c mutant strains RH422, RH425, and RH495 were isolated as 5-methyltryptophan-supersensitive cells after mutagenesis.
FIG. 1. Regulation of the biosynthesis of aromatic amino acids in \textit{S. cerevisiae} at the protein level. Enzyme activation (+) and feedback inhibition (−) are indicated.

with \textit{N-methyl-N'-nitro-N-nitrosoguanidine} (20). Strains RH422 and RH25 were isolated from the same mutagenesis and selection experiment, whereas strain RH495 was obtained in an independent mutagenesis procedure.

\textit{E. coli} MC1061 [Δ\textit{lacF02Z4} \textit{galU} \textit{galK} \textit{strA} \textit{hsdR} \textit{Δara leu}] (7) was used for propagating plasmid DNA, and the bacteriophage M13 host \textit{E. coli} JM101 [\textit{Δlac pro thi supE} \textit{F'} \textit{traD36} \textit{lacZAM15}] (38) was used for the isolation of single-stranded DNA.

Plasmid YpAR7-1 (1) was a gift from S. G. Ball, Bethesda, Md. Vector YEp351 (16) was obtained from A. Tzagoloff. pJD207 (2) and pYactI (30) were previously described.

\textbf{Media.} YEPD complete and MV minimal media were used for the cultivation of yeasts (28). \textit{E. coli} strains were maintained on L broth and M9 minimal plates (27).

\textbf{Molecular techniques.} Preparation of plasmid DNA, restriction enzyme digestion, Southern blot hybridization, and transformation of \textit{E. coli} were done by standard procedures (25). Isolation of yeast total DNA (4), preparation of yeast poly(A)* RNA, Northern (RNA) blot hybridization (13), DNA fragment isolation, and colony hybridization of \textit{E. coli} and yeasts were done as previously described (6). For hybridization, DNA fragments were labeled by the oligolabeling technique described by Feinberg and Vogelstein (12). Yeast cells were transformed by the spheroplast method of Hinnen et al. (17) with the modifications suggested by Hsiao and Carbon (18).

\textbf{Construction of gene pools.} For each mutant strain, RH422, RH425, and RH495, a gene pool was constructed. For strain RH422, 40 \textmu g of chromosomal DNA was digested with \textit{BglII} and size fractionated by agarose gel electrophoresis. Fragments of 3.4 to 4.0 kilobases (kb) were isolated and ligated with \textit{BamHI}-digested YEp351 vector. The same amounts of chromosomal DNA from strains RH425 and RH495 were double digested with \textit{BglII} and \textit{XhoI}, fractionated to isolate fragments in the range 2.9 to 3.5 kb, and ligated with \textit{BamHI-SalI}-digested YEp351. For the isolation of the wild-type gene, the YEp13-based gene pool constructed by Nasmyth and Tatchell (29) was used.

\textbf{DNA sequencing.} DNA was sequenced by the chain termination method of Sanger et al. (33). Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) was used instead of DNA polymerase I Klenow fragment. Specific oligonucleotide primers were produced on a DNA synthesizer (model 380B; Applied Biosystems, Foster City, Calif.). DNA sequences were analyzed by the programs MAP (34), CODON PREFERENCE (15), COMPARE (24), and PEPTIDE STRUCTURE (8) of the University of Wisconsin Genetics Computer Group Program Package, Version 5.

\textbf{Enzyme assays.} To determine chorismate mutase (EC 5.4.99.5) activity, we prepared crude extracts as described in reference 20. Crude extract (1 to 100 \textmu l) was incubated in 100 mM Tris (pH 7.6) with 1 mM barium chorismate for 10 min at 30°C in a final volume of 0.5 ml. A 0.5-ml portion of 1 M HCl was subsequently added, and the extract was further incubated for 10 min at 30°C. Finally, 4 ml of cold 1 M NaOH was added, and the \textit{A}_{280} was immediately measured. The amount of phenylpyruvate produced was determined by using a molar extinction coefficient estimate of 17,500 (1). The chorismate mutase activity was assayed without amino acid, with 0.5 mM tryptophan, or with 0.5 mM tyrosine. Protein content in crude extracts was measured by the

\begin{table}[h]
\centering
\caption{Enzyme activities of different chromosomally encoded chorismate mutases}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Strain & Genotype & Specific chorismate mutase activity (nmol/min/mg of protein)* & Specific indole glycerol phosphate synthase activity (nmol/min/mg of protein)* \\
\hline
X2180-1A & Wild type & 1.5 & 15.0 & <0.5 & 13.0 & 1.0 \\
RH558-1 & gcd2-I & 1.5 & 14.0 & <0.5 & 12.0 & 3.1 \\
RH422 & \textit{aro7} & 17.0 & 18.5 & 19.2 & 18.0 & ND \\
RH425 & \textit{aro7} & 15.8 & 16.4 & 16.0 & 17.0 & ND \\
RH495 & \textit{aro7} & 18.2 & 18.6 & 17.4 & 16.5 & ND \\
\hline
\end{tabular}
\footnotesize{\textsuperscript{a} Average of two independent cultivations, each measured twice. The standard deviation was <20\%.}
\footnotesize{\textsuperscript{b} ND, Not determined.}
\end{table}
method of Lowry et al. (23) with bovine serum albumin as the standard.

The indole glycerol phosphate synthase (EC 4.1.1.48) activity was determined as described previously (32).

RESULTS

Yeast chorismate mutase is activated by tryptophan and subject to feedback inhibition by tyrosine. Enzyme activities of the ARO7 gene product chorismate mutase were determined in the S. cerevisiae wild-type strain X2180-1A and in the regulatory mutant strain RH558-1 (gcd2-1; Table 1). The latter strain shows constitutively derepressed enzyme levels for gene products controlled at the transcriptional level through the general control activator protein GCN4. The wild-type strain and the constitutively general control-activated strain RH558-1 showed similar basal levels of chorismate mutase activity, suggesting that the S. cerevisiae chorismate mutase is not regulated by the general control system (Table 1). The TRP3 gene product indole glycerol phosphate synthase that was used as a reference enzyme, being subject to general control, was derepressed threefold in strain RH558-1. In both strains, the chorismate mutase activity could be stimulated approximately 10-fold by tryptophan and was inhibited at least 3-fold in the presence of tyrosine. In the presence of both effectors, tryptophan and tyrosine, the enzyme was in the activated state. The three mutant strains RH422, RH425, and RH495 carrying ARO7 alleles showed a 10-fold-increased chorismate mutase activity when compared with the wild-type strain. The chorismate mutase enzymes of these strains were unresponsive to tryptophan and tyrosine, suggesting that they were locked in the activated state, obviating the need for tryptophan activation and in a form resistant to tyrosine inhibition.

Cloning of ARO7 wild-type gene and of three mutant alleles. A YEp13-based gene pool (29) was used to transform S. cerevisiae RH1242 (aro7 leu2-2). Transformants were plated on MV minimal medium, and two colonies grew in the absence of phenylalanine, tyrosine, and leucine. Both transformants hybridized with 32P-labeled pBR322 and showed a 30-fold-increased chorismate mutase activity when compared with the wild-type strain (data not shown). The

FIG. 2. Restriction maps and cloning strategy of essential plasmids. Plasmid pME601 carries the S. cerevisiae X2180-1A ARO7 wild-type gene, and plasmids pME602, pME603, and pME604 carry ARO7 mutant alleles derived from strains RH422, RH425, and RH495, respectively. Plasmids pME603 and pME604 have identical restriction patterns. YpAR7-1 carries an ARO7 gene derived from strain X10-1B and was isolated previously (1). The arrow indicates the direction of transcription of the ARO7 gene. The 2.05-kb EcoRI fragments of pME601 (positions 2.45 to 4.50) and pME602 (positions 0.75 to 2.80) were subcloned into pJDB207 to yield pME605 and pME606. Plasmid pME607 carries a hybrid ARO7-ARO7' gene. B, BamHI; Bg, BglII; C, ClaI; E, EcoRI; EV, EcoRV; H, HindIII; M, Mbol; P, PstI; S, Salt; Sa, Sau3A; Sp, SphI; X, Xhol; Xb, XbaI.
plasmids of both transformed yeast strains showed identical restriction patterns and were designated pME601 (Fig. 2). The 2.05-kb EcoRI fragment from positions 2.45 to 4.5 was further subcloned into the vector pJDB207, resulting in pME605 (Fig. 2). After retransformation of both plasmids, pME601 and pME605, into S. cerevisiae RH1242, the average increase in enzyme level when compared with the chromosomally encoded chorismate mutase was found to be 30-fold for the YEp13-based plasmid pME601 and 300-fold for the pJDB207 derivative pME605. This difference probably reflects the different copy numbers of the two plasmids (Table 2).

![Figure 3](https://example.com/figure3.png)

**FIG. 3.** Southern blot analysis of BglII-restricted genomic DNA from *S. cerevisiae* X2180-1A. The restricted DNA was divided into two aliquots and fractionated in lanes 1 and 2. Parts of the *AR07* gene from plasmid pME601 (XbaI-EcoRI fragment from positions 3.25 to 4.50 in Fig. 2; lane 1) and from plasmid YpAR7 (HindIII-PstI fragment from positions 3.40 to 4.65; lane 2) were used as radioactive probes.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Genotype</th>
<th>Specific chorismate mutase activity (nmol/min/mg of protein)*</th>
<th>Activation factor</th>
<th>Inhibition factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>X2180-1A</td>
<td></td>
<td><strong>AR07</strong></td>
<td>1.5</td>
<td>10.0</td>
<td>&gt;3</td>
</tr>
<tr>
<td>RH242</td>
<td><strong>pME601</strong></td>
<td><strong>AR07</strong></td>
<td>12.0</td>
<td>11.7</td>
<td>6.3</td>
</tr>
<tr>
<td>RH242</td>
<td><strong>pME602</strong></td>
<td><strong>AR07</strong></td>
<td>45.0</td>
<td>8.9</td>
<td>8.7</td>
</tr>
<tr>
<td>RH242</td>
<td><strong>pME603</strong></td>
<td><strong>AR07</strong></td>
<td>125.0</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>RH242</td>
<td><strong>pME604</strong></td>
<td><strong>AR07</strong></td>
<td>87.0</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>RH242</td>
<td><strong>pME605</strong></td>
<td><strong>AR07</strong></td>
<td>94.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>RH242</td>
<td><strong>pME606</strong></td>
<td><strong>AR07</strong></td>
<td>485.0</td>
<td>10.4</td>
<td>13.2</td>
</tr>
<tr>
<td>RH242</td>
<td><strong>pME607</strong></td>
<td><strong>AR07</strong></td>
<td>122.0</td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>RH242</td>
<td><strong>pME608</strong></td>
<td><strong>AR07</strong></td>
<td>88.0</td>
<td>1.3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Average of two independent cultivations, each measured twice. The standard deviation was <20%.

For the isolation of the three *AR07* mutant alleles, gene pools constructed from strains RH422, RH425, and RH495 were transformed into *E. coli* MC1061 and hybridized with the 1.85-kb *Clal* fragment of pME601 (positions 2.05 to 3.90 in Fig. 2). The plasmids pME602, pME603, and pME604 were isolated from positive clones and shown to possess the same restriction pattern as pME601 (Fig. 2). The RH422 derivative pME602 was further subcloned to yield plasmid pME606. When transformed into yeasts, all new plasmids expressed a chorismate mutase which did not respond either to tryptophan activation or to tyrosine inhibition (Table 2).

**FIG. 4.** Size and direction of transcription of the *AR07* transcript. Poly(A)+ RNA isolated from strain RH1242(pME605) was probed in a Northern hybridization experiment with single-stranded DNA derived from the internal HindIII-*Clal* (positions 0.95 to 1.45 in Fig. 2) *AR07* fragment in both orientations (arrows). Only with the probe in the direction from the HindIII restriction site toward the *Clal* site (lane 2) was a signal for a transcript of 0.95 kb found (arrow).
overexpressing strain RH1242(pME605) was separated on a formaldehyde-agarose gel and was transferred to nylon filters. After the HindIII-Clai fragment (positions 0.95 to 1.45 in Fig. 2) was cloned into the vectors M13mp10 and M13mp11, radiolabeled single-stranded DNAs of both orientations were generated and then used as probes. Only the DNA derived from M13mp10 yielded a single signal, indicating a transcript of 0.95 kb transcribed in the direction Clai to HindIII (Fig. 2).

The Northern analysis shown in Fig. 5 was performed to test whether the effectors of the enzyme chorismate mutase, tryptophan and tyrosine, influence the transcription of the ARO7 gene. The yeast strain X2180-1A was cultivated in MV minimal medium and in MV medium supplemented with tryptophan and tyrosine. Poly(A)+ RNA was isolated and hybridized to radiolabeled ARO7 DNA. Radiolabeled DNA coding for actin (30) served as a reference probe. The ACT-to-ARO7 Poly(A)+ RNA ratio was similar for the wild-type cells grown under the different cultivation conditions (Fig. 5, lanes 1 to 3), suggesting that tryptophan and tyrosine regulate ARO7 gene expression only at the enzyme, not at the transcriptional level.

In addition, the Northern hybridization of strain RH422 carrying the ARO7 mutant allele (Fig. 5, lane 4) showed a similar level of ARO7 transcript when compared with the wild type (Fig. 5, lane 1). This suggests that the ARO7 phenotype is not due to an increased transcription rate but to a change in the chorismate mutase protein.

ARO7 gene and three ARO7 alleles differ in a single point mutation. The nucleotide sequences of the 2.05-kb EcoRI fragments of the wild-type ARO7 gene as well as of the three ARO7 mutant alleles were determined. Figure 6 shows the sequencing strategy. With specific oligodeoxynucleotide primers, 99% of the four EcoRI fragments were sequenced in both directions. There is one continuous open reading frame of 771 base pairs (Fig. 7) which would encode a protein of 256 amino acids commencing with the initiator methionine indicated as +1. If this methionine is accepted as the start codon, the deduced molecular weight of the chorismate mutase can be calculated as 29.75 kilodaltons. These data are in accordance with our results from a sodium dodecyl sulfate-polyacrylamide gel experiment in which we used a partially purified chorismate mutase to estimate the molecular weight as 28 kilodaltons (data not shown). Comparison of the ARO7 and the ARO7 sequences (Fig. 7 and 8) revealed a single cytosine-to-thymidine base pair transition at position +677 in all three ARO7 mutant alleles. This mutation causes a change from threonine to isoleucine in the C-terminal part of the chorismate mutase at the amino acid at position 226. In addition, the ARO7 allele isolated from strain RH495 showed a second cytosine-to-thymidine transition in the 5' region of the gene at position −482 (Fig. 7 and 8).

Fusion of the 5' end of the ARO7 gene and the 3' end of the ARO7 gene shows the ARO7 phenotype. To verify that the ARO7 phenotype is exclusively the result of a change in the structure of the C-terminal part of the enzyme, we fused the 5' half of the wild-type ARO7 gene with the 3' half of the mutant ARO7 allele. A hybrid gene was constructed by replacing the HindIII-EcoRI fragment of pME606 (positions 0.95 to 2.05 in Fig. 2) with the corresponding fragment of the wild-type ARO7 gene cloned in pME605. The resulting ARO7 hybrid gene on plasmid pME607 (Fig. 2) had an ARO7 wild-type promoter and encoded a hybrid protein with N-terminal sequences derived from the wild-type chorismate mutase and C-terminal sequences from the mutant enzyme. When the plasmid was transformed into strain RH1242, this fusion protein was indistinguishable from the mutant enzymes expressed by the cloned ARO7 alleles (Table 2). These data confirm that a single amino acid substitution in the C-terminal part of the chorismate mutase is sufficient to provide the cell with an enzyme that is unresponsive to regulation by tryptophan and tyrosine and is locked in the activated form.

DISCUSSION

The main finding of this study is that a single amino acid substitution in the yeast chorismate mutase results in an enzyme that is locked in the on state and is unresponsive to the effectors of the wild-type enzyme, tryptophan and tyrosine. The ARO7 wild-type gene as well as three ARO7 mutant alleles were analyzed, cloned, and sequenced.

The yeast ARO7 gene encodes a monofunctional chorismate mutase that can be activated by tryptophan and is subject to feedback inhibition by tyrosine. The ARO7 mutant alleles encode chorismate mutases that are frozen in
FIG. 7. Nucleotide sequence of the EcoRI fragment containing the ARO7 gene. +1 corresponds to the first methionine of the 771-base-pair open reading frame. The deduced amino acid sequence is shown above the nucleotide sequence. Poly(A)-d(T) regions, TATA sequences, and the putative termination-polyadenylation signal are underlined. The C-to-T transition in the ARO7 alleles of the mutant strains RH422, RH425, and RH495 resulting in a threonine-to-isoleucine substitution is indicated at position +677, and the additional transition in RH495 is shown at position -482.
the activated form, obviating the need for tryptophan analog 5-methyltryptophan (20), a false feedback inhibitor of the TRP2- and TRP3-encoded anthranilate synthase complex, which competes with the ARO7 gene product for chorismate (28, 32). Since anthranilate synthase and chorismate mutase control the distribution of chorismate at the first branch point of aromatic amino acid biosynthesis (Fig. 1), a constitutively activated chorismate mutase depletes the chorismate pool, destroys the balance between the two enzymes and the chorismate pool in the cell, and causes tryptophan starvation in the presence of the false anthranilate synthase inhibitor 5-methyltryptophan. The tryptophan feedback mechanism reduces the flux toward tryptophan in vivo to 10 to 20% of its normal capacity (28). Our in vitro data suggest that tyrosine is able to reduce the chorismate activity up to 10-fold (Table 2); no effect of phenylalanine, the other end product of this branch of aromatic amino acid biosynthesis, is known (20). Whereas the anthranilate synthase activity in the cell can be increased only up to 3-fold by the general control system at the transcriptional level, the chorismate mutase activity in the cell can be activated up to 10-fold in the presence of the specific effector tryptophan at the enzyme level.

In contrast to the genes TRP2 and TRP3 (5, 39), the ARO7 gene is not derepressed by the general control system, and we did not find a consensus GCN4 protein-binding site (36) in the 5' region of the gene (Fig. 7). The 5' region of the ARO7 gene possesses only the usual putative promoter elements of an unregulated, constitutively expressed yeast gene; poly(dA-dT) stretches as possible constitutive upstream elements at positions −310 to −284, −216 to −200, and −192 to −178 and putative TATA boxes at −278 to −275, −258 to −255, and −113 to −110 (36). The ARO7 mRNA levels did not change either in the presence of tyrosine or tryptophan or in a constitutive ARO7 strain. These data suggest that the ARO7 gene is not regulated at the transcriptional level. Among the structural genes encoding aromatic amino acid biosynthetic genes, only the ARO7 and the TRP1 genes (5) are known to be constitutively transcribed. The constitutive transcription of the ARO7 gene is unique, if one considers that chorismate mutase catalyzes an important regulatory reaction at one of the branch points in the aromatic amino acid biosynthetic pathway. Besides the TRP2 and TRP3 genes, other comparable genes such as ARO3 and ARO4 (encoding two isoenzymes that control the entrance to the shikimate pathway) are also regulated at the transcriptional level by the general control system and are additionally regulated by feedback inhibition at the enzyme level. It remains to be answered why the cell regulates most of the genes of the aromatic amino acid biosynthetic pathway by the general control system but does not regulate the ARO7 gene, although its gene product catalyzes a regulatory important reaction.

The coding region of the ARO7 gene comprises a 771-base-pair open reading frame, which would encode a polypeptide of 256 amino acids with a calculated molecular size of 29.75 kilodaltons. These data are supported by Northern analysis which revealed a poly(A)+ RNA of 0.95 kilobases (Fig. 4) and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a 28-kilodalton protein corresponding to the chorismate mutase activity (data not shown). Surprisingly, no homology was found in the deduced amino acid sequence between the yeast chorismate mutase and the N-terminal chorismate mutase activities of the two bifunctional enzymes of E. coli (19, 26), whereas other yeast enzymes such as the ARO3, ARO4, and TRP4 gene products share significant homology with their E. coli counterparts. The codon bias index of 0.26 of the yeast ARO7 gene, calculated by the method of Bennetzen and Hall (3), suggests that the ARO7 gene is, like all TRP genes in yeasts, expressed only at low levels. In the 3' region downstream of the open reading frame, a Zaret and Sherman termination consensus sequence (40) is located at position +769 TAA...+851 TATGT...68% AT)...+893 TTT (underlined in Fig. 6).

As there seems to be no regulation of the ARO7 gene at the transcriptional level, the complex regulation of the gene product gains additional interest. The study of the ARO7 mutant alleles seemed especially suitable for assembling more information about the regulation of the monofunctional yeast chorismate mutase. These mutant alleles exploit the rare phenotype of a constitutively activated enzyme frozen in the on state in a form unresponsive to the effectors of the wild-type enzyme, tyrosine and tryptophan. The mutation therefore destroys the function of switching the enzyme down to the basal level or even lower in the presence of tyrosine when it is no longer required. Surprisingly, all three ARO7 mutant alleles were mutated at the same loci,
suggesting that in the original mutagenesis and selection procedure, the mutational events capable of producing this phenotype are very limited. The AROT alleles differed from the wild-type gene only in a single C-G-to-T-A transition within the coding region (Fig. 8). Such transitions have often been found after mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (11, 31). The observed point mutation results in a threonine-to-isoleucine substitution in the C-terminal part of the chorismate mutase. A gene fusion consisting of the wild-type promoter, the 5′ part of the wild-type coding sequence, and the 3′ part of one of the mutant alleles exhibits the mutant phenotype (Table 2). In a C. H. Pasman secondary plot (8), the replacement of the hydrophilic threonine in the wild-type enzyme by the hydrophobic isoleucine in the mutant enzyme interrupts a hydrophilic α-helical conformation from amino acid 220 to 226 (data not shown). How this single amino acid substitution affects the conformation of the whole enzyme to yield a constitutively activated and feedback-resistant chorismate mutase requires further investigation.

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LITERATURE CITED

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