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

TOBIAS SCHMIDHEINI, PETER SPERISEN, GERHARD PARAVICINI, RALF HÜTTER, AND GERHARD BRAUS*

Institute of Microbiology, Swiss Federal Institute of Technology, Schmelzbergstrasse 7, CH-8092 Zurich, Switzerland

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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 *Eco*RI 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 (36). 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 4.1.3.27) 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 *ARO7*^{c} (constitutively activated chorismate mutase) mutant alleles (20). Mutant strains carrying the *ARO7*^{c} alleles 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 *ARO7*^{c} 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 (*MAT***a** *gal2 SUC2 mal CUP1*) and X2180-1B (*MAT*α *gal2 SUC2 mal CUP1*). The RH1242 genotype is *MAT*α *aro7 leu2-2*. RH558-1 (*MAT*α *gcd2-1*) carries a mutation which leads to constitutive derepression of those amino acid biosynthetic genes which are subject to general control (35). The *ARO7*^{c} mutant strains RH422, RH425, and RH495 were isolated as 5-methyltryptophan-supersensitive cells after mutagenesis

* Corresponding author.

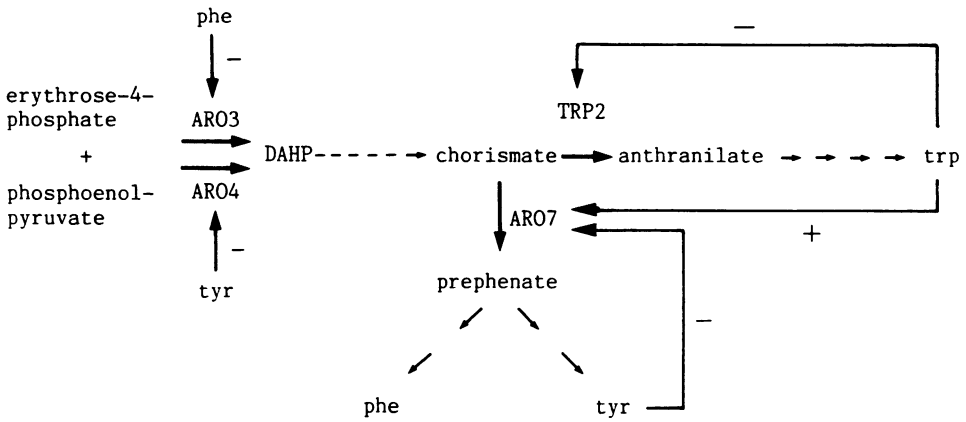

FIG. 1. Regulation of the biosynthesis of aromatic amino acids in *S. cerevisiae* at the protein level. Enzyme activation (+) and feedback inhibition (−) are indicated.

with *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.

E. coli MC1061 [Δ(*lacIPOZYA*)*X74 galU galK strA hsdR* Δ(*ara leu*)] (7) was used for propagating plasmid DNA, and the bacteriophage M13 host *E. coli* JM101 (Δ*lac pro thi supE* F' *traD36 proAB lacI*^{q}*ZΔM15*) (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. pJDB207 (2) and pYactI (30) were previously described.

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

Molecular techniques. Preparation of plasmid DNA, restriction enzyme digestion, Southern blot hybridization, and transformation of *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 *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).

Construction of gene pools. For each mutant strain, RH422, RH425, and RH495, a gene pool was constructed. For strain RH422, 40 μg of chromosomal DNA was digested with *Bgl*II and size fractionated by agarose gel electrophoresis. Fragments of 3.4 to 4.0 kilobases (kb) were isolated and ligated with *Bam*HI-digested YEp351 vector. The same amounts of chromosomal DNA from strains RH425 and RH495 were double digested with *Bgl*II and *Xho*I, fractionated to isolate fragments in the range 2.9 to 3.5 kb, and ligated with *Bam*HI-*Sal*I-digested YEp351. For the isolation of the wild-type gene, the YEp13-based gene pool constructed by Nasmyth and Tatchell (29) was used.

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.

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 μl) 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 A_{320} 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

TABLE 1. Enzyme activities of different chromosomally encoded chorismate mutases

Strain	Genotype	Specific chorismate mutase activity (nmol/min/mg of protein)[a]				Specific indole glycerol phosphate synthase activity (nmol/min/mg of protein)[a]
		Amino acid	+0.5 mM Trp	+0.5 mM Tyr	+0.5 mM Tyr + Trp	
X2180-1A	Wild type	1.5	15.0	<0.5	13.0	1.0
RH558-1	*gcd2-1*	1.5	14.0	<0.5	12.0	3.1
RH422	*ARO7*^{c}	17.0	18.5	19.2	18.0	ND[b]
RH425	*ARO7*^{c}	15.8	16.4	16.0	17.0	ND
RH495	*ARO7*^{c}	18.2	18.6	17.4	16.5	ND

[a] Average of two independent cultivations, each measured twice. The standard deviation was <20%.
[b] ND, Not determined.

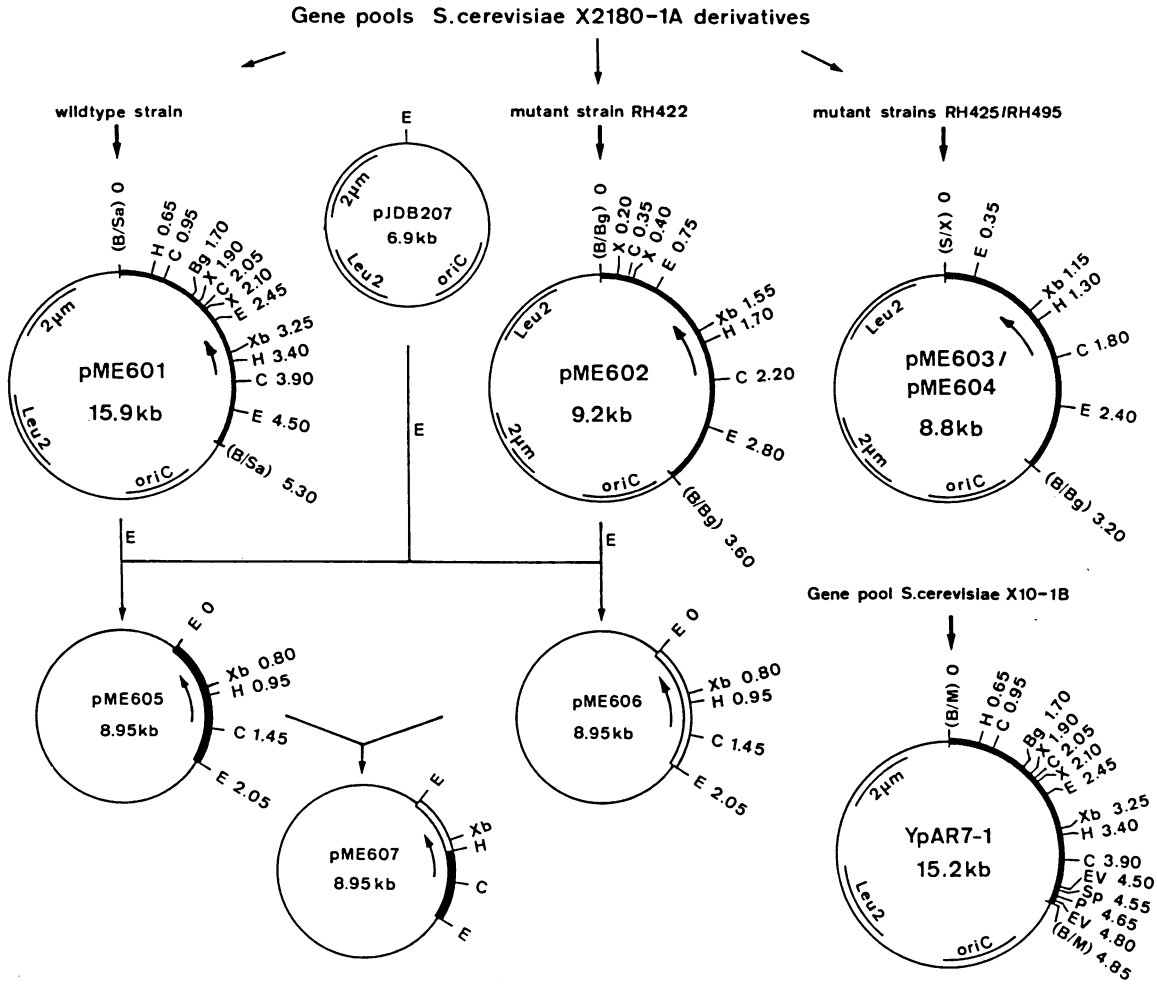

FIG. 2. Restriction maps and cloning strategy of essential plasmids. Plasmid pME601 carries the *S. cerevisiae* X2180-1A *ARO7* wild-type gene, and plasmids pME602, pME603, and pME604 carry *ARO7*^{c} mutant alleles derived from strains RH422, RH425, and RH495, respectively. Plasmids pME603 and pME604 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 *Eco*RI 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*^{c} gene. B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; M, *Mbo*I; P, *Pst*I; S, *Sal*I; Sa, *Sau*3A; Sp, *Sph*I; X, *Xho*I; Xb, *Xba*I.

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*^{c} 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 ^{32}P-labeled pBR322 and showed a 30-fold-increased chorismate mutase activity when compared with the wild-type strain (data not shown). The

TABLE 2. Chorismate mutase activities of transformed yeast strains

Strain	Plasmid	Genotype	Specific chorismate mutase activity (nmol/min/mg of protein)[a]			Activation factor	Inhibition factor
			−Amino acids	+0.5 mM Trp	+0.5 mM Tyr		
X2180-1A		*ARO7*	1.5	15.0	<0.5	10.0	>3
RH1242	YpAR7-1	*ARO7*	12.0	140.0	1.9	11.7	6.3
RH1242	pME601	*ARO7*	45.0	402.0	5.2	8.9	8.7
RH1242	pME602	*ARO7*^{c}	125.0	138.0	115.0	1.1	1.1
RH1242	pME603	*ARO7*^{c}	87.0	110.0	81.8	1.3	1.1
RH1242	pME604	*ARO7*^{c}	94.0	101.0	97.9	1.1	1.0
RH1242	pME605	*ARO7*	485.0	5,022.0	36.7	10.4	13.2
RH1242	pME606	*ARO7*^{c}	122.0	134.0	137.1	1.1	0.9
RH1242	pME607	*ARO7-ARO7*^{c}	88.0	118.0	96.8	1.3	0.9

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

plasmids of both transformed yeast strains showed identical restriction patterns and were designated pME601 (Fig. 2). The 2.05-kb *Eco*RI 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 demonstrates that the cloned *ARO7* gene corresponded to a single chromosomal locus. The *ARO7* gene which has previously been cloned by Ball et al. (1) from *S. cerevisiae* X10-1B (plasmid YpAR7-1; Fig. 2) was different from our clones. The 0.95-kb *Cla*I-(*Bam*HI-*Mbo*I) fragment (positions 3.9 to 4.85) differed in restriction pattern from the corresponding region of our *ARO7* gene on pME601 (*Cla*I restriction site at position 3.9 to the ligated former *Bam*HI-*Sau*3A sites at position 5.3). Hybridization of both divergent radiolabeled fragments with *Bgl*II-restricted chromosomal DNA revealed that the short fragment of the X10-1B-derived plasmid YpAR7-1 hybridized to various fragments of the genome of the standard strain X2180-1A, whereas our clone only hybridized to one genetic locus (Fig. 3). One possible explanation could be that in strain X10-1B, but not in strain X2180-1A, a repetitive element, e.g., a Ty element, is located adjacent to the *ARO7* gene.

For the isolation of the three *ARO7*^{c} mutant alleles, gene pools constructed from strains RH422, RH425, and RH495 were transformed into *E. coli* MC1061 and hybridized with the 1.85-kb *Cla*I 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).

***ARO7* gene encodes a 0.95-kb transcript that is not subject to transcriptional regulation.** The size and direction of the *ARO7* transcript were determined by Northern analysis (Fig. 4). Poly(A)$^+$ RNA isolated from the chorismate mutase-

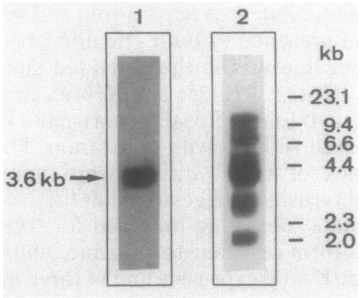

FIG. 3. Southern blot analysis of *Bgl*II-restricted genomic DNA from *S. cerevisiae* X2180-1A. The restricted DNA was divided into two aliquots and fractionated in lanes 1 and 2. Parts of the *ARO7* gene from plasmid pME601 (*Xba*I-*Eco*RI fragment from positions 3.25 to 4.50 in Fig. 2; lane 1) and from plasmid YpAR7 (*Hin*dIII-*Pst*I fragment from positions 3.40 to 4.65; lane 2) were used as radioactive probes.

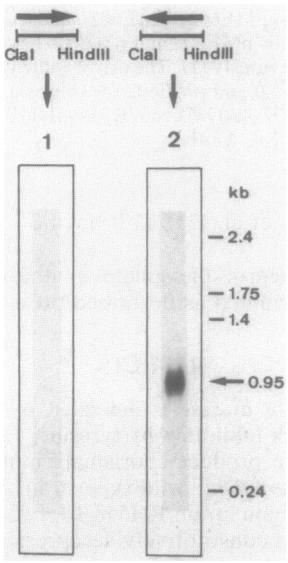

FIG. 4. Size and direction of transcription of the *ARO7* transcript. Poly(A)$^+$ RNA isolated from strain RH1242(pME605) was probed in a Northern hybridization experiment with single-stranded DNA derived from the internal *Hin*dIII-*Cla*I (positions 0.95 to 1.45 in Fig. 2) *ARO7* fragment in both orientations (arrows). Only with the probe in the direction from the *Hin*dIII restriction site toward the *Cla*I site (lane 2) was a signal for a transcript of 0.95 kb found (arrow).

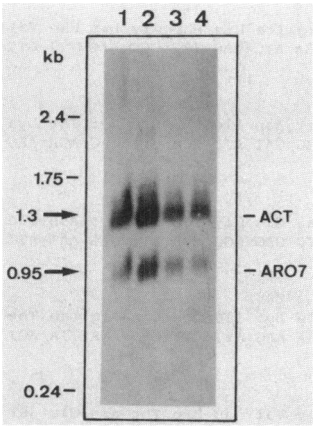

FIG. 5. Northern analysis of transcripts from the *ARO7* and the *ACT* genes. Poly(A)$^+$ RNA from the wild-type strain X2180-1A cultivated on MV medium (lane 1) or on MV medium supplemented with 50 μg of either tyrosine (lane 2) or tryptophan (lane 3) per ml and from strain RH422 carrying the *ARO7*^{c} mutant allele (lane 4) was hybridized to radiolabeled *ARO7* and *ACT* DNA (30).

overexpressing strain RH1242(pME605) was separated on a formaldehyde-agarose gel and was transferred to nylon filters. After the *Hin*dIII-*Cla*I 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 *Cla*I to *Hin*dIII (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*^{c} 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*^{c} phenotype is not due to an increased transcription rate but to a change in the chorismate mutase protein.

***ARO7* gene and three *ARO7*^{c} alleles differ in a single point mutation.** The nucleotide sequences of the 2.05-kb *Eco*RI fragments of the wild-type *ARO7* gene as well as of the three *ARO7*^{c} mutant alleles were determined. Figure 6 shows the sequencing strategy. With specific oligodeoxynucleotide primers, 99% of the four *Eco*RI 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

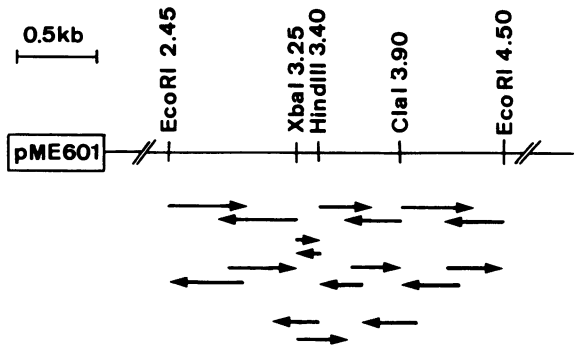

FIG. 6. Strategy for sequencing the *ARO7* gene and three *ARO7*^{c} mutant alleles. The restriction site numbering corresponds to that presented in Fig. 2. The arrows indicate the direction of sequencing and the extent of sequence obtained from individual clones.

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*^{c} sequences (Fig. 7 and 8) revealed a single cytosine-to-thymidine base pair transition at position +677 in all three *ARO7*^{c}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*^{c} 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*^{c} gene shows the ARO7^c phenotype. To verify that the ARO7^c 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*^{c} allele. A hybrid gene was constructed by replacing the *Hin*dIII-*Eco*RI 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*^{c} 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*^{c} 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*^{c} mutant alleles encode chorismate mutases that are frozen in

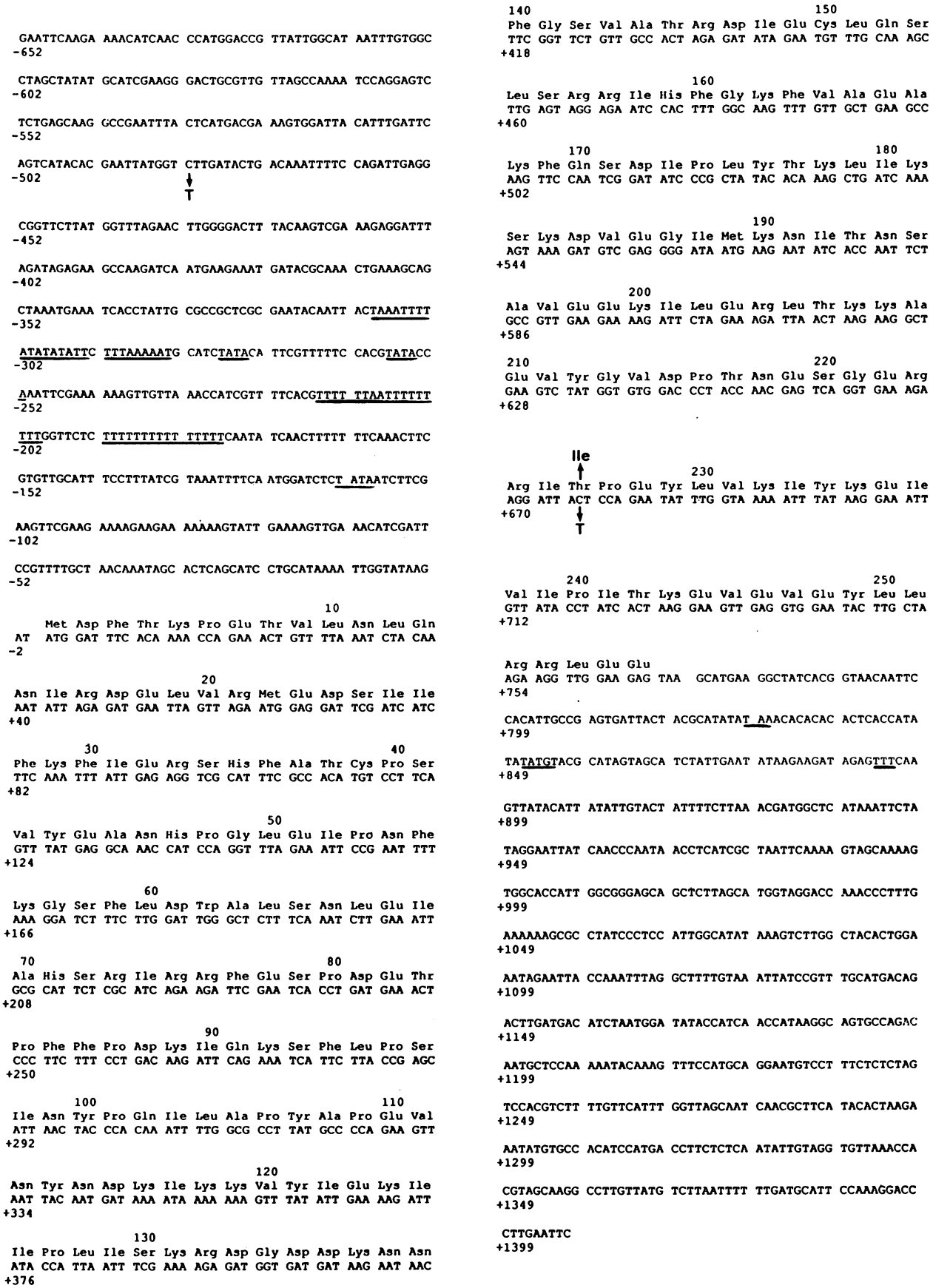

FIG. 7. Nucleotide sequence of the *Eco*RI fragment containing the *ARO7* gene. +1 corresponds to the first methionine of the 771-base-pair open reading frame. The deduced amino acid sequence is shown above the nucleotide sequence. Poly(dA-dT) regions, TATA sequences, and the putative termination-polyadenylation signal are underlined. The C-to-T transition in the *ARO7*^{c} 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.

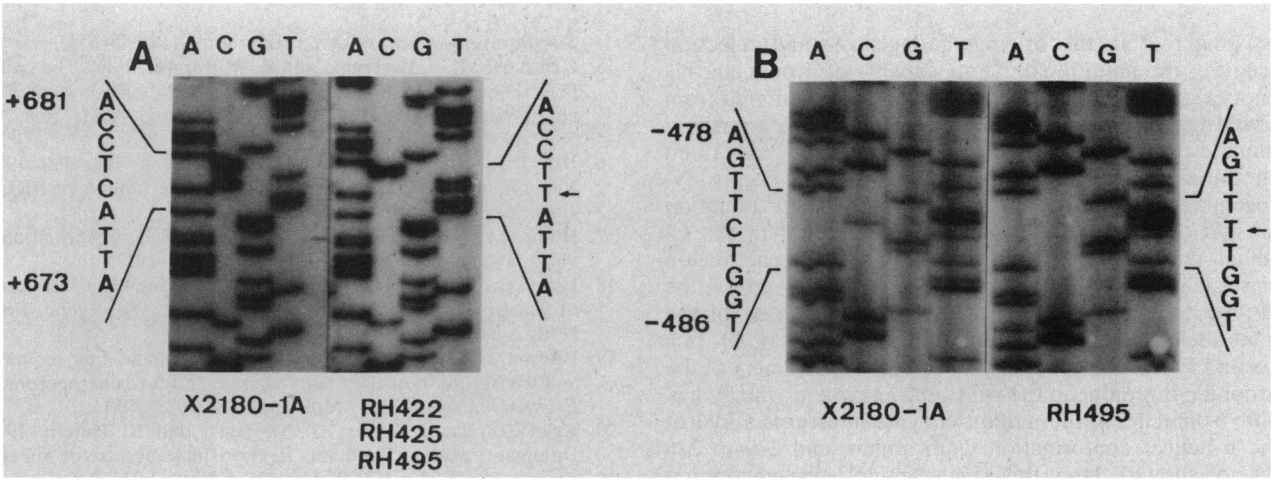

FIG. 8. Part of the nucleotide sequence of the *ARO7* wild-type gene and *ARO7*^{c} mutant alleles. When compared with the wild-type *ARO7* gene, all three *ARO7*^{c} alleles of strains RH422, RH425, and RH495 showed a transition from C to T at position +677 (A). In the *ARO7*^{c} allele derived from strain RH495, thereis an additional transition at position −482 (B).

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*^{c} 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*^{c} 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*^{c} mutant alleles were mutated at the same locus,

suggesting that in the original mutagenesis and selection procedure, the mutational events capable of producing this phenotype are very limited. The *ARO7*^{c} 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 Chou and Fasman 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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