Mol Gen Genet (1990) 220:283–288

MGG

Regulation of chorismate mutase in *Saccharomyces cerevisiae*

Judy F. Brown* and Ian W. Dawes**
Dept. of Microbiology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JG, UK

Summary. The *Saccharomyces cerevisiae ARO7* gene was cloned by screening a wild-type gene bank for complementation of an *aro7* auxotrophic mutant. In vitro mutagenesis of the isolated plasmid (pJFB1) gave several transformants resistant to levels of the phenylalanine analogue 2-thienylalanine inhibitory to the wild-type transformant. Chorismate mutase assays indicated that two of the mutants (J14-26IV6 and J14-26IV9) were resistant to feedback inhibition by tyrosine displayed by wild-type strains. Analysis of the effect of other aromatic amino acids on chorismate mutase activity showed that tryptophan counteracted this inhibition. Analysis of the effect of tyrosine in the growth medium on enzyme activity indicated that the wild-type *ARO7* gene was repressed by tyrosine, a phenomenon not previously reported. Two of the 2-thienylalanine resistant mutants (J14-26IV3 and J14-26IV9) appeared to be resistant to this repression. Transcriptional analysis confirmed that the level of *ARO7* transcript decreased with increasing tyrosine concentration. In stain J14-26IV9 the *ARO7* transcript level was not affected. J14-26IV9, therefore, appears to be a double mutant, resistant to both feedback inhibition and repression by tyrosine.

Key words: Chorismate mutase – *Saccharomyces cerevisiae* – Repression

Introduction

In *Saccharomyces cerevisiae* the enzymatic pathway for biosynthesis of aromatic amino acids is branched, culminating in the formation of phenylalanine, tyrosine and tryptophan. The last biosynthetic reaction common to phenylalanine and tyrosine, the formation of prephenate from chorismate, is catalysed by the enzyme chorismate mutase, encoded by the *ARO7* gene.

In yeast and bacteria the biosynthesis of amino acids is regulated by both inhibition and repression. In yeast, however, flux through the aromatic amino acid biosynthetic pathway is reported to be controlled mainly by a system of feedback inhibition and activation of key enzymes. Chorismate mutase is subject to inhibition by tyrosine and activation by tryptophan (Lingens et al. 1966). Although the aromatic pathways are subject to general amino acid control, cloning of the *ARO7* gene (Ball et al. 1986) revealed no evidence for specific transcriptional regulation.

The work reported here, on the regulation of phenylalanine production, however, involving the independent cloning of the *ARO7* gene and isolation and analysis of regulatory mutants indicated that this gene is regulated at the transcriptional level by tyrosine. Mutant *ARO7* alleles were generated which had lost either control by inhibition or represssion or both forms of control.

* *Present address:* MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, UK
** *Present address:* Dept. of Biochemistry, University of New South Wales, PO Box 1, Kensington, NSW 2033, Australia
Offprint requests to: J.F. Brown

Materials and methods

S. cerevisiae *strains and culture conditions.* The following two strains, from this laboratory, were used: 314 (*MATα ade5*), and J14-26 (*MATa leu2-3 leu2-112 aro7 trp1 met4 lys11 ura3-52*).

Cultures were grown at 30° C in minimal medium: 20 g/l D-glucose, 1.7 g/l yeast nitrogen base (without amino acids and $(NH_4)_2SO_4$), 5 g/l ammonium sulphate, plus amino acid supplements where necessary at 50 mg each.

Plasmids and DNA. The *S. cerevisiae* gene library used was constructed by Nasmyth and Tatchell (1980) and based on the vector YEp13 (Broach et al. 1979).

The pJFB1 probe used for RNA analysis was a 3.1 kb *Stu*I-*Bam*HI pJFB1 fragment labelled with [α-^{32}P]dATP (Amersham) by nick translation using the BRL kit. The probe for rRNA comprised the vector pSP65 containing the 18 S rRNA gene, obtained from A.J.P. Brown, Dept of Genetics, University of Glasgow.

Transformation of S. cerevisiae. Yeast strains were transformed by the method of Beggs (1978).

DNA preparation. Total DNA from yeast was prepared by the method of Winston et al. (1983). Plasmid DNA was isolated according to the method of Nasmyth and Reed (1980). Small-scale plasmid preparations from *Escherichia coli* were done by the method of Birnboim and Doly (1979). Restriction enzymes were used as recommended by the supplier (Boehringer). Fragments of DNA were recovered from agarose gels by electroelution onto dialysis membrane (Smith 1980).

RNA analysis. Total yeast cellular RNA was prepared following the method of Weir-Thompson and Dawes (1984). Slot-blots were performed as follows: 15 µl 20 × SSC and 10 µl 37% (w/v) formaldehyde were added to 40 µg RNA and made up to a final volume of 50 ml. Samples were denatured by incubation at 60° C for 15 min then transferring to 0° C. 25 µl aliquots were transferred to nitrocellulose using the "Hybri-slot" apparatus (BRL) and fixed by baking at 80° C for 3 h under vacuum. Filters were hybridised with labelled DNA for 16 h according to Thomas (1980). The extent of hybridisation was estimated by densitometric scanning of autoradiographs.

In vitro mutagenesis. Plasmid DNA was mutated by treatment with *O*-methylhydroxylamine according to the method of J. Mandelstam (personal communication): 63 mg *O*-methylhydroxylamine-HCl (pH 5 with 2 M NaOH) was added to 400 µg plasmid DNA and the volume adjusted to 1 ml with H_2O. The sample was heated to 50° C for 2 h then ethanol-precipitated twice and finally redissolved in TE buffer (10 mM TRIS, 1 mM EDTA).

Chorismate mutase assay. Crude cellular extracts from *S. cerevisiae* strains were assayed for chorismate mutase activity using a method adapted by M. Edwards (personal communication) from the assay procedure developed for *E. coli* (Cotton and Gibson 1964). The assay mixture comprised 625 µl of crude extract, 25 µl 5% (w/v) BSA and 350 µl enzyme buffer. Samples were prewarmed at 30° C for 5 min and the reaction started by adding 250 µl 5 mM potassium chorismate (prepared from a solution of the barium salt in 0.01 M potassium sulphate). At zero time a 0.25 ml sample was withdrawn and added to 0.25 ml prewarmed 3 M HCl, mixed and incubated for a further 10 min at 30° C. Further samples were removed at 2 and 5 min and treated similarly. 0.25 ml of acid-treated solution was mixed with 0.75 ml 1 M NaOH and the A_{320} measured immediately. The rate of increase in A_{320} was then calculated and corrected for the control rate in the absence of added extract. This is necessary due to the spontaneous (non-enzymatic) conversion of chorismate to prephenate. The protein concentration of each of the samples was measured using the method of Lowry et al. (1951).

Results

Isolation of ARO7 *regulatory mutants by in vitro mutagenesis of the cloned gene*

The *ARO7* gene was cloned by using an *aro7* mutant of *S. cerevisiae* to screen a wild-type gene bank, based on the vector YEp13, for complementation of the *aro7* phenotype. One such transformant was isolated, *aro7* complementation being correlated with the presence of the plasmid (pJFB1). Yeast strains transformed with pJFB1 displayed a threefold increase in chorismate mutase activity compared with the wild type (data not shown). A restriction map of pJFB1 was obtained which showed a large region of homology with the independently isolated plasmid containing the *ARO7* gene (Ball et al. 1986) and the cloned insert was estimated as 5.3 kb.

DNA from pJFB1 was treated with the mutagen *O*-methylhydroxylamine which induces C-T transitions. The *ARO7* auxotrophic strain J14-26 was then tranformed with the mutated plasmid and 24 transformants were selected by their ability to grow in the absence of leucine, phenylalanine and tyrosine.

Mutants with feedback-resistant chorismate mutase should contain elevated phenylalanine and tyrosine pools and, therefore, show increased resistance to analogues of these amino acids. Nine of the transformants showed an increased resistance to the phenylalanine analogue 2-thienylalanine (50 µg/ml) compared with the wild-type transformant J14-26TR1. This phenotype was correlated with the presence of the plasmid by plasmid-loss experiments and retransformation.

Regulation of chorismate mutase activity

The four regulatory mutants which displayed the greatest increase in resistance to 2-thienylalanine (J14-26IV3, J14-26IV5, J14-26IV6 and J14-26IV9) were used along with J14-26TR1, containing the wild-type plasmid pJFB1, and the wild-type strain 314 to assay chorismate mutase activity under a variety of conditions. All data presented are the average of six separate assays.

Effect of tyrosine. Extracts of soluble protein from the above strains were assayed for chorismate mutase activity in the presence of a range of concentrations of tyrosine. The results are presented in Fig. 1.

Both wild-type strains displayed feedback inhibition of chorismate mutase by tyrosine (Fig. 1 a). Strain J14-26TR1 had a higher initial activity and was not inhibited to the same degree as 314, showing some activity at 5 mM tyrosine. This is expected since the transformed strain carries multiple copies of the *ARO7* gene and produced about threefold more chorismate mutase than did the wild type. The effect of tyrosine on the enzyme from the four strains representing possible regulatory mutants of *ARO7* was determined. All four strains showed inhibition by tyrosine but to different extents (Fig. 1 b). As the concentration of tyrosine increased, both J14-26IV5 and J14-26IV6 displayed a decrease in chorismate mutase activity similar to that of the unmutated strain, although initially J14-26IV6 showed increased resistance. Strains J14-26IV3 and J14-26IV9, however, appeared to have an increased resistance to inhibition by tyrosine, showing half their initial level of activity at 5 mM tyrosine compared with a decrease by a factor of approximately 10 in the other strains. These results indicate that these two strains contain mutations in the *ARO7* gene which render it resistant to feedback inhibition by tyrosine.

Effect of tryptophan. The above experiment was repeated, assaying chorismate mutase activity in the presence of a range of tryptophan concentrations. Tryptophan had virtually no effect on chorismate mutase activity, over the range of concentrations used (data not shown). This result was surprising since tryptophan had previously been reported to activate the enzyme (Jones and Fink 1983). Other groups, however, had stated that rather than stimulating chorismate mutase activity, tryptophan counteracted the inhibition by tyrosine (Miozzari et al. 1978). To resolve this contradiction, the effect of both tyrosine and tryptophan on chorismate mutase activity was studied in all six strains.

In the wild-type strain, tryptophan did overcome the inhibitory effect of tyrosine, the extent to which it counteracted inhibition increasing with tryptophan concentration (Fig. 2 a). Strain J14-26TR1 also showed this reduction in

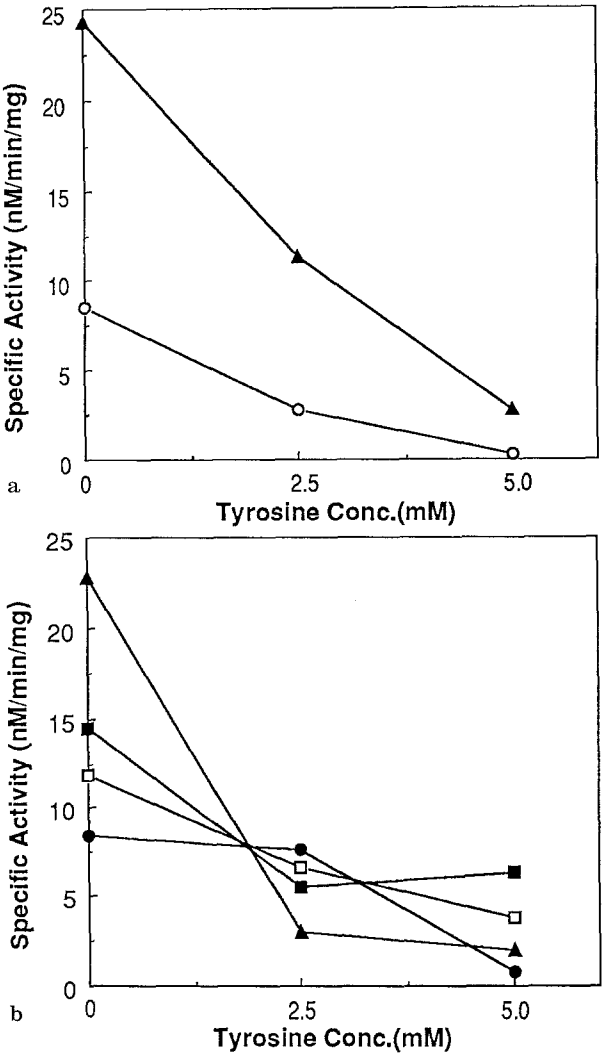

Fig. 1a and b. Levels of chorismate mutase activity displayed by yeast strains assayed in different concentrations of tyrosine. **a** ○ 314; ▲ J14-26TR1. **b** ■ J14-26IV3; ▲ J14-26IV5; ● J14-26IV6; □ J14-26IV9

tyrosine inhibition although to a lesser extent, with 5 mM tyrosine still causing inhibition even at levels of 5 mM tryptophan (Fig. 2b). In the wild-type strain 5 mM tryptophan practically abolished tyrosine inhibition and chorismate mutase activity was restored to its original level seen at 0 and 5 mM tyrosine.

Since J14-26IV9 appeared to be resistant to feedback inhibition by tyrosine, it was possible that tryptophan may, in counteracting inhibition, have led to a further increase in chorismate mutase activity. Tryptophan, however, had very little effect on chorismate mutase but when tyrosine was present there was a very slight increase in enzyme activity (Fig. 2c).

Effect of phenylalanine. The mutant pJFB1 plasmids were selected on the basis of their conferring increased 2-thienylalanine resistance to the host yeast strain. This was, presumably, due to the transformed strains overproducing phenylalanine. Therefore, the possibility that chorismate mutase was regulated in some way by phenylalanine was investigated. However, phenylalanine had very little effect on chorismate mutase activity over the range of concentrations used (data not shown).

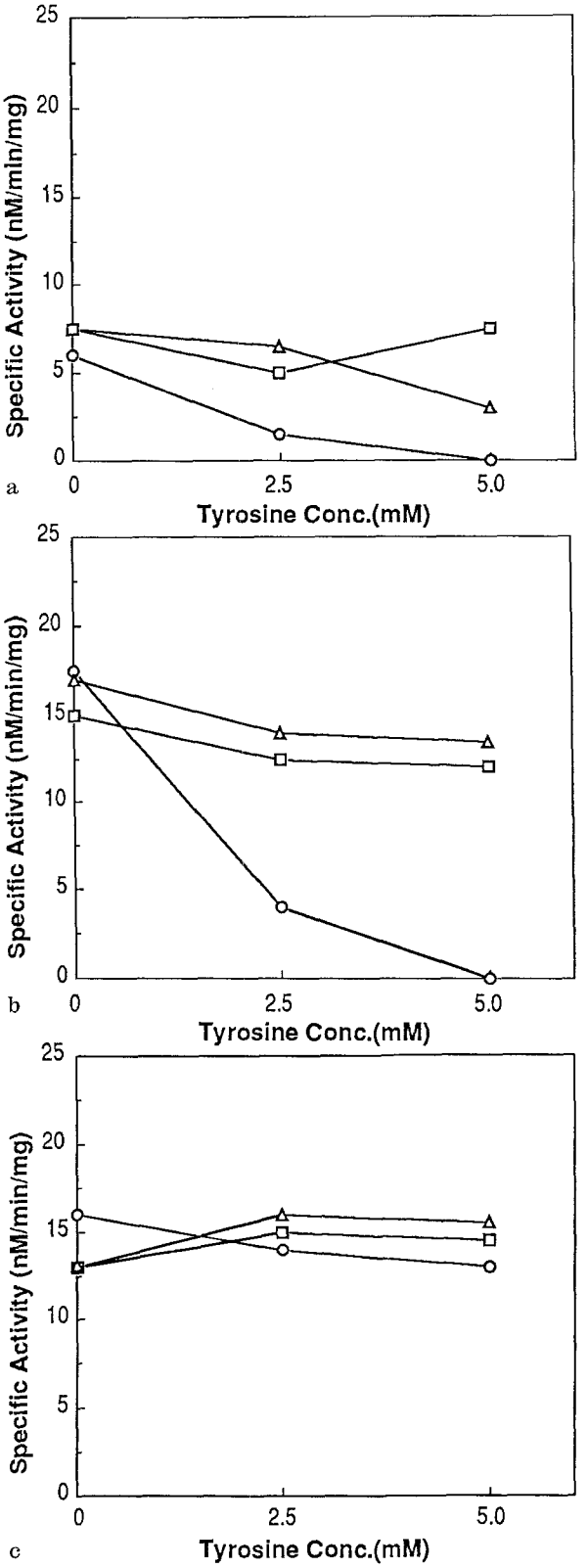

Fig. 2a–c. Levels of chorismate mutase activity displayed by yeast strains assayed in different concentrations of tyrosine and tryptophan. **a** 314, **b** J14-26TR1, **c** J14-26IV9. ○ 0 mM, △ 2.5 mM, □ 5 mM tryptophan

Regulation of ARO7 *expression in* S. cerevisiae

Expression of the *ARO7* gene was studied by assaying chorismate mutase activity in various yeast strains grown in the presence of the appropriate amino acids. These amino

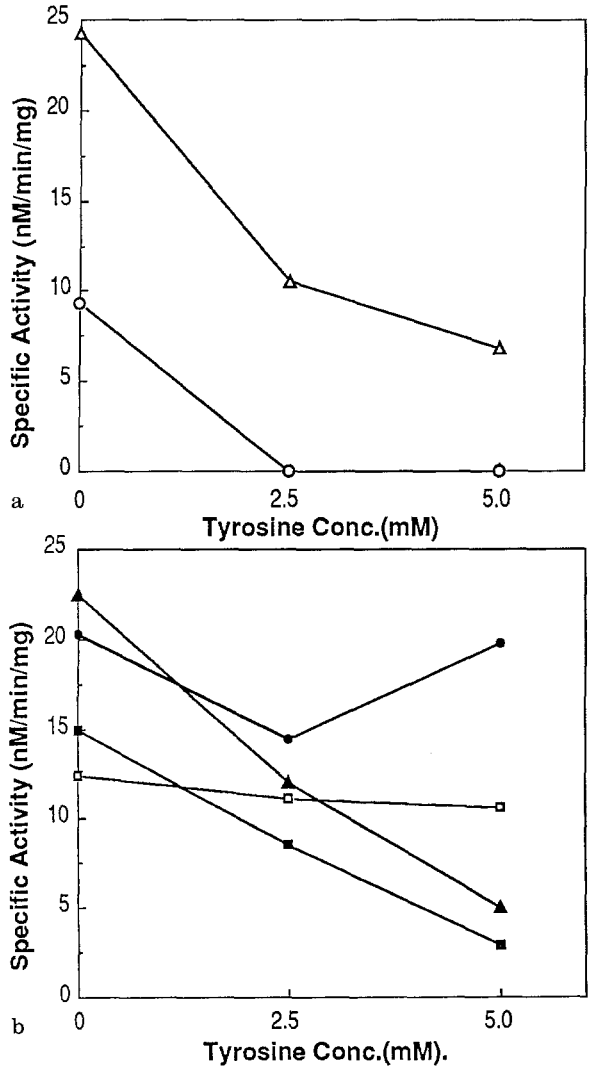

Fig. 3a and b. Levels of chorismate mutase activity displayed by yeast strains grown in different concentrations of tyrosine. **a** ○ 314; △ J14-26TR1. **b** ■ J14-26IV3; ▲ J14-26IV5; ● J14-26IV6; □ J14-26IV9

acids were removed prior to assay, therefore any alteration in chorismate mutase activity was due to a change in the amount of enzyme produced. Data presented are the average of six separate assays.

Effect of tyrosine. Strains 314, J14-26TR1, J14-26IV3, 5, 6 and 9 were grown in minimal medium containing different concentrations of tyrosine, plus appropriate amino acid supplements. The cells were washed several times to remove exogenous amino acids before assaying for chorismate mutase. As the concentration of tyrosine in the growth medium was increased, there was a corresponding decrease in chorismate activity (Fig. 3a). Therefore tyrosine was probably repressing transcription of the *ARO7* gene. Overexpression of *ARO7*, in J14-26TR1, led to the production of higher levels of enzyme at all tyrosine concentrations but repression was still seen. The mutated strains J14-26IV3 and J14-26IV5 seem to be repressed by tyrosine in a similar manner to the unmutated strain (Fig. 3b). J14-26IV6 and J14-26IV9, however, appeared to be resistant to this effect, showing no appreciable decrease in activity with increasing tyrosine concentration in the growth medium.

Effect of tryptophan. Chorismate mutase activity was assayed in the three strains, 314, J14-26TR1 and J14-26IV9,

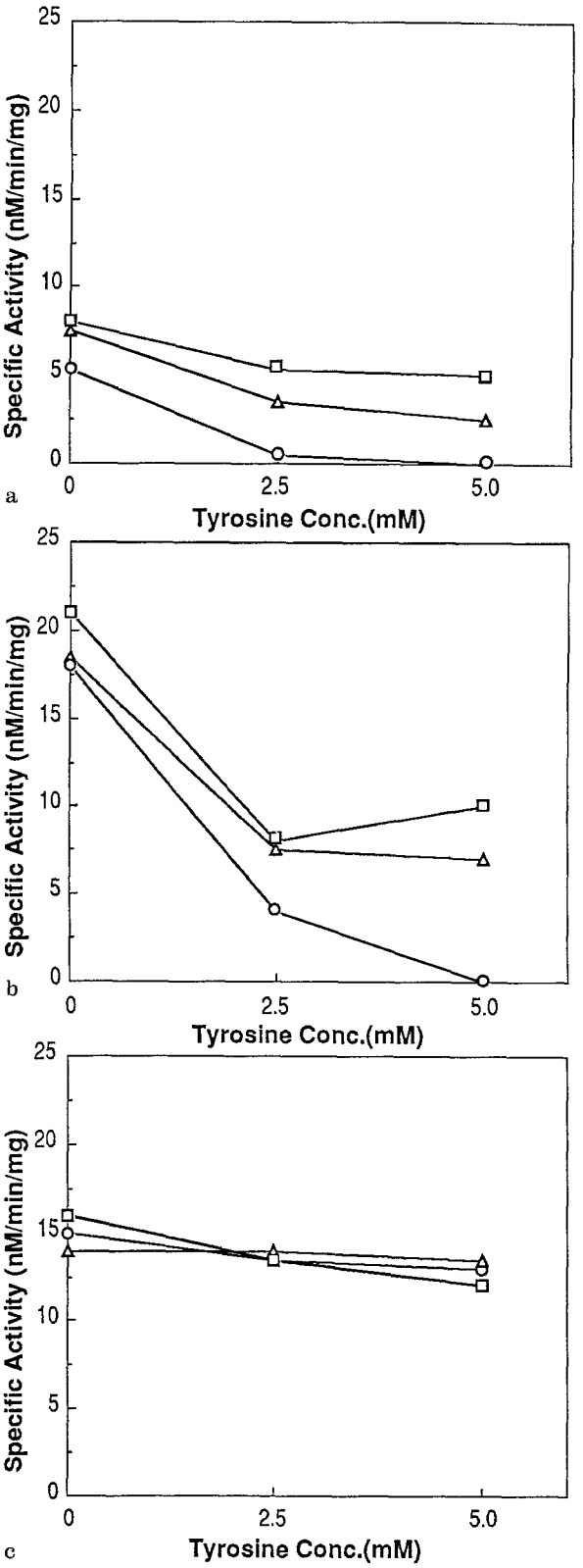

Fig. 4a–c. Levels of chorismate mutase activity displayed by yeast strains grown in different concentrations of tyrosine and tryptophan. **a** 314, **b** J14-26TR1, **c** J14-26IV9. ○ 0 mM, △ 2.5 mM, □ 5 mM tryptophan

grown in various concentrations of tryptophan. Tryptophan caused a very slight increase in chorismate mutase activity in all three strains. Lingens et al. (1967) described an induction of *ARO7* expression but this could not be confirmed by later work (Kradolfer 1977).

Table 1. Levels of *ARO7* mRNA relative to rRNA

Tyrosine concentration (mM)	Strain	
	J14-26TR1	J14-26IV9
0	1.00	2.12
2.5	0.62	1.93
5.0	0.07	3.60

Data are the average of three measurements and are standardised relative to the value obtained for J14-26TR1 under conditions of no additional tyrosine

Table 2. Amount of pJFB1 DNA within the cell relative to total DNA

Tyrosine concentration (mM)	Strain	
	J14-26TR1	J14-26IV9
0	1.00	0.92
2.5	1.25	0.75
5.0	1.35	0.60

Results are standardised relative to the value obtained with J14-26TR1 under conditions of no additional tyrosine. The data presented are the average of three separate aliquots of each sample

Since tryptophan had been shown to counteract inhibition by tyrosine, the same three strains were grown in the presence of both tyrosine and tryptophan to determine whether tryptophan also reduced tyrosine repression (Fig. 4). With strain 314, tryptophan had little effect on the extent of tyrosine repression, although higher enzyme activity was noticed with increasing tyrosine concentration (Fig. 4a). Strain J14-26TR1 showed similar results (Fig. 4b) while J14-26IV9 still appeared to be resistant to the repression effect (Fig. 4c). The latter also showed no increase in activity with increased levels of tryptophan.

Effect of phenylalanine. Chorismate mutase was assayed over a range of phenylalanine concentrations (0 to 5 mM) in the growth medium but no significant change in *ARO7* expression was observed (data not shown).

Transcriptional analysis of the ARO7 *gene in* S. cerevisiae *strains J14-26TR1 and J14-26IV9*

Although the aim of these experiments was to isolate mutants resistant to feedback inhibition by tyrosine, the analysis of those obtained revealed information not only on the regulation of chorismate mutase activity but also on the control of *ARO7* expression. As well as chorismate mutase being inhibited by tyrosine, its synthesis appears to be repressed by tyrosine. *ARO7* mRNA levels were therefore studied in the wild-type condition and with the repression-resistant gene.

Total RNA from strains J14-26TR1 and J14-26IV9, grown in a range of tyrosine concentrations, was analysed by slot-blot hybridisation using the labelled pJFB1 probe. A second control filter was probed with labelled pSP65 plasmid DNA which hybridises with 18 S RNA. Transcription of the 18 S rRNA should not be affected by changing the concentration of tyrosine present in the medium. The intensities of the various bands were measured by densitometry (Table 1).

The amount of *ARO7* mRNA produced by J14-26TR1 decreased with increasing tyrosine concentration, the amount of transcript present at 5 mM tyrosine being approximately 14-fold lower than the level produced under conditions of no added tyrosine. The levels of rRNA did not show any corresponding decrease in intensity, remaining at a constant level in both strains. This indicates that tyrosine was possibly repressing the expression of the *ARO7* gene although post-transcriptional regulation cannot be ruled out entirely. The strain J14-26IV9, containing the mutated plasmid, showed a slight increase in the transcript at 5 mM tyrosine, and this strain is, therefore, resistant to repression by tyrosine.

When the levels of *ARO7* mRNA produced by each strain were compared in conditions of no added tyrosine it was observed that strain J14-26IV9 was producing approximately twice as much transcript as J14-26TR1, indicating that there was an increase in the expression of the gene in the mutated strain. This was confirmed by Northern analysis (data not shown).

Analysis of plasmid copy number

Although the concentration of *ARO7* mRNA decreased with increasing tyrosine concentration, this was not conclusive proof that tyrosine was repressing transcription of the *ARO7* gene. The *ARO7* gene was present on an episomal plasmid and hence the level of transcript within the cell is not just a function of the rate of transcription of the gene but also of the number of copies present.

Total DNA was prepared from strain J14-26TR1 grown in minimal medium (plus appropriate supplements) containing a range of concentrations of tyrosine. The ratio of plasmid to chromosomal DNA was determined (Table 2) from which it can be seen that the number of copies of pJFB1 did not decrease with increasing tyrosine concentration.

Fluctuations in plasmid copy number could also be responsible for the phenotype of strain J14-26IV9. The above experiment was repeated, therefore, using DNA from strain J14-26IV9. No increase in plasmid DNA relative to total DNA was noted (Table 2). When relative amounts of pJFB1 present in each strain under conditions of no added tyrosine were compared, the two values were similar, indicating that the initial copy number was similar in each case. At 5 mM tyrosine, however, there was a twofold difference which, if not due to discrepancies during preparation, implied an increase in chorismate mutase mRNA in J14-26IV9. This is supported by the results of Table 1.

Discussion

Random in vitro mutagenesis of plasmid DNA with *O*-methylhydroxylamine has proven successful in obtaining regulatory mutations in the *ARO7* gene. By mutating pJFB1 and selecting for transformants with increased resistance to the phenylalanine analogue 2-thienylalanine we have obtained multicopy vectors expressing chorismate mutase, the activity and/or expression of which is deregulated.

The phenotypes of the four strains studied can be summarised as follows: J14-26IV3 showed a partial resistance to feedback inhibition by tyrosine while J14-26IV6 appeared to have increased resistance to tyrosine repression. J14-26IV5 displayed characteristics similar to the unmutated J14-26TR1, being subject to both inhibition and re-

pression by tyrosine. The mutant strain J14-26IV9 appeared to be resistant to both repression and feedback inhibition by tyrosine, indicating that there may be at least two separate mutations within the plasmid pJFB1, one in the upstream regulatory sequences and the other in the *ARO7* structural gene. More information, however, is required before these two mutations can be separated and analysed independently. Once the transcription initiation site has been elucidated it may be possible to isolate the mutated upstream controlling regions, link them to the wild-type *ARO7* structural gene sequences and observe if the resulting construct still shows resistance to repression but produces a protein sensitive to feedback inhibition. Alternatively, the existence of two separate mutations could also be proved by linking the mutated structural gene to wild-type upstream sequences and showing that there is inhibition without repression.

Although strain J14-26IV9 is insensitive to feedback inhibition and repression by tyrosine, it only secreted 0.1 mg/ml phenylalanine into the medium, indicating that deregulation of chorismate mutase alone may not alter the flux of the pathway sufficiently to achieve significant overproduction of phenylalanine. To obtain overproducing strains adequate for commercial purposes it will be necessary to deregulate other key enzymes in the pathway.

Although tyrosine-mediated repression of chorismate mutase has been well documented in *E. coli* (Camakaris and Pittard 1983; Hudson and Davidson 1984) it has not been reported in *S. cerevisiae*. Transcriptional control has been implicated in the derepression of tryptophan biosynthetic enzymes in response to tryptophan starvation (Miozzari et al. 1977). Derepression of these enzymes also occurred, however, in response to starvation for unrelated metabolites such as arginine and histidine and, therefore, it was concluded to be not a tryptophan-specific response but elicited by the general amino acid control system. This raises questions of whether *ARO7* is under general amino acid control and if the observed repression by excess tyrosine is associated with it.

There are very few reports of regulation of gene expression in any of the other genes for aromatic amino acid biosynthesis. The DAHP synthase isoenzymes are not repressed by any of the amino acids, either singly or in combination, nor is dehydroquinate dehydratase (Doy and Cooper 1966). Lingens et al. (1966) have described an induction of chorismate mutase by tryptophan but this could not be confirmed by this work or that of others (Kradolfer et al. 1977; Ball et al. 1986). Prephenate dehydratase is, however, reported to be repressed by phenylalanine while prephenate dehydrogenase is induced by phenylalanine but not repressed by tyrosine (Lingens et al. 1967). These effects are of the order of 2- to 3-fold whereas feedback inhibition can be a 100-fold effect. The latter has, therefore, always been cited as the main mode of control. The results of this work, however, indicate that repression may be more important in the regulation of aromatic amino acid biosynthesis than previously thought.

The concentration of the internal tyrosine pool of the yeast cell is approximately 0.7 mM (Jones and Fink 1982). The tyrosine concentrations which evoked this repression response may, therefore, be slightly above the normal physiological levels encountered by the cell growing in the absence of added tyrosine. In the commercial production of phenylalanine, however, high levels of tyrosine may be encountered due to tyrosine supplementation of auxotrophic strains. To achieve the maximum yield of phenylalanine, therefore, it will be important to use yeast strains which not only overexpress chorismate mutase, but which are insensitive to both feedback inhibition and repression by tyrosine.

Acknowledgements. This work was supported in part by G.D. Searle & Co. and by the Science and Engineering Research Council. We thank Dr. B.L.A. Carter for helpful discussion.

References

Ball SG, Wickner RB, Cottarel G, Schau M, Titaux G (1986) Molecular cloning and characterisation of *ARO7-OSM2*, a single yeast gene necessary for chorismate mutase activity and growth in hypertonic medium. Mol Gen Genet 205:326–330

Beggs JD (1978) Transformation of yeast by a replicating hybrid plasmid. Nature 275:104–108

Birnboim HC, Doly J (1970) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513–1523

Broach JR, Backman K (1978) Transformation in yeast: development of hybrid cloning vector and isolation of the *CAN*1 gene. Gene 8:121–133

Camakars H, Pittard J (1983) In: Herrman KM, Somerville RL (eds) Amino acids; Biosynthesis and genetic regulation. Addison-Wesley, Reading, Mass, pp 339–350

Cotton RGH, Gibson F (1964) The biosynthesis of phenylalanine and tyrosine enzymes converting chorismic acid into prephenic acid and their relationships to prephenate dehydratase and prephenate dehydrogenase. Biochim Biophys Acta 100:76–88

Hudson GS, Davidson BE (1984) Nuclei acid sequence and transcription of the phenylalanine and tyrosine operons of *E. coli* K12. J Mol Biol 180:1023–1051

Jones EW, Fink GR (1982) In: Strathearn JN, Jones EW, Broach JR (eds) The molecular biology of the yeast *Saccharomyces cerevisiae*. Cold Spring Harbor, New York

Kradolfer P, Zeyer J, Miozzari R, Hutter R (1977) Dominant regulatory mutants in chorismate mutase of *Saccharomyces cerevisiae*. FEMS Microbiol Lett 2:211–216

Lingens F, Goebel W, Uesseler H (1966) Regulation der Biosynthese der aromatischen Aminosäuren in *Saccharomyces cerevisiae*, 1. Hemmung der Enzymaktivitäten. Biochem Z 346:356–367

Lingens F, Goebel W, Uesseler H (1967) Regulation der Biosynthese der aromatischen Aminosäuren, 2. Repression, Induktion und Aktivierung. Eur J Biochem 1:363–374

Lowry OH, Rosebrough NJ, Farr AC, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Maniatis T, Fritsch TF, Sambrook J (1982) Molecular cloning; a laboratory manual. Cold Spring Harbor, New York

Miozzari G, Wiederberger P, Hutter R (1978) Tryptophan biosynthesis in *Saccharomyces cerevisiae*: Control of flux through the pathway. J Bact 134:48–59

Nasmyth KA, Tatchell K (1980) The structure of transposable yeast mating type loci. Cell 19:753–764

Smith HO (1980) Recovery of DNA from gels. Methods Enzymol 65:371–380

Thomas PS (1980) Hybridisation of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77:5201–5205

Weir-Thompson EM, Dawes IW (1984) Developmental changes in translatable RNA species associated with meiosis and spore formation in Saccharomyces cerevisiae. Mol Cell Biol 4:695–702

Winston F, Chumley F, Fink JR (1983) Eviction and transplacement of mutant genes in yeast. Methods Enzymol 101:211–228

Communicated by B.J. Kilbey

Received May 2, 1989