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Regulation of Tyrosine and Phenylalanine Biosynthesis in *Escherichia coli* K-12: Properties of the *tyrR* Gene Product

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A spontaneous amber *tyrR* mutant has been isolated in which constitutive synthesis of 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP) synthetase (tyr) and DAHP synthetase (phe) is suppressible by $supC^-$, $supD^-$, $supF^-$ and $supU^-$. This finding suggests the *tyrR* gene product is a protein. Derepression of DAHP synthetase (phe) in this and in seven other spontaneous *tyrR* mutants and in four Mu-1-induced *tyrR* mutants provides further evidence for the involvement of the *tyrR* gene product in phenylalanine biosynthesis. Evidence that the *tyrR* product is a component of repressor, rather than an enzyme involved in its synthesis or modification, comes from a study of a temperature-sensitive *tyrR* mutant. This mutant is of the thermolabile type, since derepression occurs rapidly and in the presence and absence of growth.

The first reaction of aromatic biosynthesis, the condensation of erythrose-4-phosphate and phosphoenolpyruvate to form 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP), is carried out in *Escherichia coli* by three isoenzymes. The synthesis and activity of each of these isoenzymes is controlled by tyrosine, phenylalanine, and tryptophan, respectively (3, 9, 23).

In the case of tyrosine, the structural genes for DAHP synthetase (tyr) (EC 4.1.2.15) and chorismate mutase-prephenate dehydrogenase (EC 1.3.1.a) form an operon controlled by the operator gene *aroK* and the regulator gene *tyrR* (18, 33). Evidence has also been presented that the *tyrR* gene is involved in the regulation of DAHP synthetase (phe) by phenylalanine (4, 12).

The nature of the regulatory mechanism in biosynthetic systems is now being investigated intensively. The operon model of Jacob and Monod (15) has been well verified for catabolic systems by isolation of mutants and by purification and study of the appropriate repressor proteins (10, 25, 26). In the case of biosynthetic systems, amber mutants, in which constitutivity is suppressible, have now been isolated for the tryptophan (21) and methionine (19) biosynthetic systems. This indicates that the regulator genes involved code for proteins, since amber suppression is known to occur at the level of translation (7, 30).

In addition, temperature-sensitive mutants have been isolated for the tryptophan system (14) and for the arginine system (32). In the arginine mutant, the mutation seems to be of the thermolabile (TL) type rather than the temperature-sensitive synthesis (TSS) type.

Partial purification of the arginine (31) and tryptophan (35) regulator proteins has been achieved, and further studies using such preparations should elucidate the nature of the repression mechanism for these biosynthetic systems.

The study of the nature of the *tyrR* gene product and elucidation of the repression mechanism are especially interesting in view of the suggested interaction with both tyrosine and phenylalanine.

MATERIALS AND METHODS

Organisms. Strains used in this work are all derivatives of *E. coli* K-12 and are described in Table 1. Strain LS446a was obtained from L. Soll; strains CR63, K110, and T4 amber phage N58 were from C. Yanofsky.

Growth medium. The minimal medium used was half-strength 56 (56/2) described by Monod et al. (20), supplemented with 0.2% glucose, thiamine, and required amino acids. For growth of cells for cell extracts, medium 56 was used with 0.5% glucose.

Buffers. Sodium phosphate buffers used were prepared by the method of Dawson and Elliott (5).

Chemicals. Chemicals used were obtained com-

TABLE 1. *List of strains*

Strain	Sex	Genetic loci relevant to this work[a]						
		aroF	*aroG*	*aroH*	*tyrR*	*trp*	*his*	Other
AB3253	F^-	+	365	367	+	+	4	
AT2471	Hfr	+	+	+	+	+	+	*tyr4*
JP324	F^-	+	365	367	+	+	4	*pyrF40*
JP2140	F^-	+	+	+	+	A9605	29	
JP2142	F^-	+	+	+	366	+	29	
JP2144	F^-	+	+	+	366	A9605	29	
JP2199	F^-	+	+	+	367	A9605	29	
JP2200	F^-	+	+	+	368	A9605	29	
JP2201	F^-	+	+	+	369	A9605	29	
JP2202	F^-	+	+	+	370	A9605	29	
JP2203	F^-	+	+	+	371	A9605	29	
JP2204	F^-	+	+	+	372	A9605	29	
JP2205	F^-	+	+	+	373	A9605	29	
JP2206	F^-	+	+	+	368	+	29	
JP2207	F^-	+	+	+	370	+	29	
JP2208	F^-	+	+	+	370	A9605	29	*supD*$^-$
JP2209	F^-	+	+	+	370	A9605	29	*supF*$^-$
JP2210	F^-	+	+	+	370	A9605	29	*supC*$^-$
JP2211	F_{14}'*ilv*$^+$*supU*$^-$	+	+	+	370	A9605	29	*ilv*$^+$*supU*$^-$/*ilv*$^-$
JP2212	F^-	+	+	+	370	A9605	29	
JP2213	F^-	+	+	+	370	A9605	29	
JP2214	F^-	+	+	+	+	+	29	*pyrF*$^-$
JP2215	F^-	+	+	+	374	A9605	29	
JP2216	F^-	+	+	+	375	A9605	29	
JP2217	F^-	+	+	+	376	A9605	29	
JP2218	F^-	+	+	+	377	A9605	29	
JP2219	F^-	+	365	367	378	+	4	
JP2220	F^-	+	365	367	379	+	4	
JP2221	F^-	+	365	367	380	+	4	
JP2222	F^-	+	365	367	381	+	4	
JP2223	F^-	+	365	367	380	+	4	
JP2224	F^-	+	365	367	+	+	4	
JP2226	F^-	+	+	+	+	+	29	
CR63	F^-	+	+	+	+	+	+	*supD*$^-$
K110	F^-	+	+	+	+	+	+	*supF*$^-$
LS446a	F'*ilv*$^+$*supU*$^-$	+	+	+	+	A9605	29	*ilv*$^+$*supU*$^-$/*ilv*$^-$
W26	F^-	+	+	+	+	+	+	*supC*$^-$
W3110	F^-	+	+	+	+	+	+	

[a] Symbols: *aroF* is the structural gene for DAHP synthetase (tyr); *aroG*, the structural gene for DAHP synthetase (phe); *aroH*, the structural gene for DAHP synthetase (trp). *trpA* is the structural gene for anthranilate synthetase; *his*, any one of the structural genes for histidine biosynthesis; *pyrF*, the structural gene for orotidylic acid decarboxylase; *tyr*, any one of the structural genes for enzymes converting chorismate to tyrosine; *ilv*, any of the structural genes for the enzymes of isoleucine-valine biosynthesis. *tyrR* is a regulator gene controlling the expression of *aroF*, *tyrA* and the structural gene for transaminase A. *supD*$^-$, *supF*$^-$, and *supU*$^-$ are amber suppressors, and *supC*$^-$ is an ochre suppressor, where *sup*$^-$ designates the mutant allele having suppressor activity.

mercially and not further purified. D-Erythrose-4-phosphate dimethylacetal dicyclohexylammonium salt and phosphoenol pyruvate tricyclohexylammonium salt were obtained from Fine Chemicals of Australia, Melbourne. Free erythrose-4-phosphate was prepared by the method of Ballou et al. (1) and was adjusted to pH 5.0. Free phosphoenolpyruvate was prepared by removing the tricyclohexylammonium ion with activated Dowex 50 and was adjusted to pH 7.0.

Isolation of spontaneous FT^R mutants. For the isolation of each mutant, 0.1 ml of a washed overnight culture of strain JP2140, diluted to approximately 2×10^8 cells per ml in medium 56/2, was plated in a soft agar layer on minimal medium. A filter paper disk dipped in 10^{-2} M L-3-fluorotyrosine (FT) was placed on the plate. After incubation at 37 C for 48 h, resistant colonies in the zone of inhibition were purified on nutrient agar and checked by streaking on minimal medium with and without 10^{-4} M L-fluorotyrosine.

Mu-1 mutagenesis. The method described by

Boram and Abelson for mutagenesis with phage Mu-1 was used (2).

Propagation of Mu-1 phage. The method for propagation of Mu-1 phage described by Boram and Abelson (2) was used, except that 10^{-3} M Pb^{2+} was added to the lysate, following a suggestion by Reznikoff (F. Gibson, personal communication).

Isolation of APA^R mutants. The method used for isolation of APA^R mutants was similar to that described by Wallace and Pittard (33), except that the survivors, after mutagenesis with *N*-methyl-*N'*-nitro *N*-nitrosoguanidine and division into eight fractions and phenotypic expression, were plated on medium containing 10^{-4}, 2×10^{-4}, and 5×10^{-4} M L-4-aminophenylalanine (APA) and incubated at 42 C.

Transduction. The method used for transduction, using phage P1, was that described previously (22), except that after incubation with phage the cells were washed three times with citrate buffer (0.1 M, pH 5.5) to remove calcium ions and thereby prevent readsorption of phage to the cells.

Syntrophism tests. The method described by Gibson and Jones (8) was used to detect syntrophism.

Test for Mu-1 lysogeny. The criteria of Mu-1 immunity and release of phage were used to test for lysogeny. Immunity to Mu-1 was determined by cross-streaking against Mu-1 phage (0.1 ml of approximately 2×10^9 plaque-forming units per ml). To test for release of phage, the strain was grown for 6 to 12 h in broth, and the supernatant fluid was tested for free phage by plaque assay on strain W3110, a sensitive indicator strain.

Sensitivity to T4 amber phage, N58. T4 amber lysates were prepared by growth on a permissive (*sup*$^-$) host in broth. Sensitivity was determined by cross-streaking on nutrient agar.

Growth of cells and preparation of cell extracts. Cells were grown in minimal medium (described above). When minimal medium was supplemented with the aromatic amino acids and vitamins, they were added in the following concentrations: L-phenylalanine, 10^{-3} M; L-tryptophan, 5×10^{-4} M; L-tyrosine, 10^{-3} M; shikimic acid, 10^{-5} M; *p*-aminobenzoic acid, 4×10^{-6} M; *p*-hydroxybenzoic acid, 4×10^{-6} M; and 2,3-dihydroxybenzoic acid, 5×10^{-5} M. The cells were harvested in mid-exponential phase of growth from 200-ml cultures, washed twice in chilled 0.9% NaCl, and suspended in 0.1 M sodium phosphate buffer (pH 7.0) containing 10^{-4} M ethylenediaminetetraacetic acid. Cell breakage was achieved by ultrasonic treatment by using an MSE 500-W ultrasonic disintegrator (Measuring and Scientific Equipment, Ltd.). Cobaltous chloride was immediately added to extracts to give a final concentration of 10^{-3} M. Cell extracts were obtained by centrifugation at 20,000 $\times$ *g* for 20 min. These extracts were always assayed for enzyme activity within 1 h.

In experiments where 40- or 80-ml samples were taken, these were treated as above, except that after sonic disruption a sample was removed for protein assays and bovine serum albumin (BSA) was added to the remainder to give a total protein concentration of approximately 8 mg/ml.

Assay of DAHP synthetase. The method of Doy and Brown (6) was used for assaying DAHP synthetase, with the following modifications. Dilutions of the cell extract were made in sodium phosphate buffer (0.1 M, pH 7.0) containing 8 mg of BSA/ml and 10^{-3} M $CoCl_2$. The incubation mixture included 2 mg of BSA in sodium phosphate buffer (0.1 M, pH 6.4).

Protein estimation. Protein was estimated by the method of Lowry et al. (16).

Specific activity. One unit of specific activity is defined as 0.1 μmol of substrate used or product formed per 20 min per mg of protein at 37 C.

RESULTS

Isolation of spontaneous tyrR mutants. Since the purpose of isolating this group of mutants was primarily to identify an amber mutant, it was important that the parent strain be suppressor free. A triple isoenzyme strain with convenient markers was constructed (JP2140), and *tyrR* mutants were isolated on the basis of resistance to 3-fluorotyrosine, an analogue of tyrosine (24), by the method previously used by R. Russell in this laboratory (see Materials and Methods). Twenty-two FT^R mutants were isolated, each one from a different culture.

tyrR mutants were identified initially by cotransduction of FT^R with *trp*. Previously quoted cotransduction frequencies vary from 3 to 24% (4, 12), this difference apparently being due to genetic background. P1 transductions were carried out with a *trp*$^+$ FT^S donor (W3110) and each of the 22 mutants as recipients. *trp*$^+$ was selected and FT^R was screened as an unselected marker. The results for the eight mutants which showed cotransduction of *trp*$^+$ and FT^S are shown in Table 2. The average cotransduction frequency was 2%. To exclude the possibility that two mutations were necessary for the FT^R phenotype, P1 lysates prepared on three of the *trp*$^+$ transductants from the previous experiment were used in transductions with strain JP2140, *trp*$^+$ being selected and FT being screened as an unselected marker. The mutation linked to *trp*$^+$ does produce the FT^R phenotype (Table 3).

TABLE 2. *Cotransduction of FT^S with trp$^+$ using a trp$^+$ FT^S donor and FT^R trp$^-$ recipients: analysis of trp$^+$ transductants*

Recipient	No. scored (Trp$^+$)	No. scored as FT^S
JP2199	316	3
JP2144	120	5
JP2200	160	1
JP2201	160	4
JP2202	160	2
JP2203	160	1
JP2204	160	1
JP2205	160	4

On the basis of the chromosomal position of their lesion, these FT^R mutants appeared to be *tyrR* mutants. Strain JP2140 and the eight mutants were grown in minimal medium supplemented with the aromatic end products. The results of DAHP synthetase assays are shown in Table 4. It can be seen that synthesis of both DAHP synthetase (tyr) and DAHP synthetase (phe) is very derepressed.

Preparation of sup⁻ derivatives of FT^R mutants. *supD*⁻, *supF*⁻, and *supU*⁻ are known to suppress *his29* and *trp A9605* (29), both these mutations being present in the background of the *tyrR* mutants. *supD*⁻ and *supF*⁻ derivatives of each mutant were made by transduction using P1 (CR63) and P1 (K110), respectively, selecting for His^+ Trp^+.

Strain LS446a, from L. Soll, carries the episome F'14 *ilv*⁺ *supU*⁻, *supU*⁻ being lethal in the haploid. Sex factor and *ilv*⁺ *supU*⁻ appear to be substantially independent, in that separate curing of each was readily achieved, and transfer of *ilv*⁺ *supU*⁻ in the conjugation experiments occurred at very low frequency. Transductions using P1 (LS446a) as donor and JP2140 and the *tyrR* mutants as recipients were carried out, selection being made for the Ilv^+ His^+ Trp^+ phenotype (i.e., *ilv*⁺ *supU*⁻). Transductants were purified, checked for prototrophy, and were found to be sensitive to T4•amber phage N58 and resistant to the male-specific phage MS2. The *ilv*⁺ *supU*⁻ element seems to remain as a plasmid since the transductants can readily be cured of *ilv*⁺ *supU*⁻.

The ability of the ochre suppressor *supC*⁻ to suppress *his29* and *trpA9605* was not known. However *supC* is cotransducible with *trp* (28). P1 prepared on W26, a *trp*⁺ *supC*⁻ strain, was used in transductions with the recipients JP2140 and JP2144, selection being made for Trp^+ phenotype. Transductants were screened for His^+ and sensitivity to the T4 amber phage N58 which was known to be suppressed by *supC*⁻. The low number of His^+ $N58^S$ transductants (1 in 10 for strain JP2140; 5 in 20 for strain JP2144) suggests that *supC*⁻ suppresses *his29* but not *trpA9605*. *supC*⁻ was introduced into the other *tyrR* mutants by selection for His^+ Trp^+. The low frequency of transduction observed is consistent with this being a double selection for *trp*⁺ *supC*⁻.

Identification of an amber tyrR mutant. Two colonies of each class of *sup*⁻ transductant, for each of the eight *tyrR* mutants, were purified and screened for FT^R, together with a *trp*⁺ transductant of each *tyrR* mutant as a control.

Only one mutant, JP2202, gave rise to *sup*⁻ transductants which were sensitive to fluorotyrosine. Strain JP2202 and its *supC*⁻, *supD*⁻, *supF*⁻, and *supU*⁻ derivatives and strain JP2140, the *tyrR*⁺ control, were grown both in minimal medium plus tryptophan and in minimal medium plus aromatic end products. Cell extracts were prepared and assayed for their levels of DAHP synthetase. Synthesis of both DAHP synthetase (tyr) and DAHP synthetase (phe) is repressed almost to wild-type levels in the *supF*⁻ and *supC*⁻ derivatives and is partially repressed in the *supD*⁻ and *supU*⁻ derivatives (Table 5).

A *trp*⁺ FT^R and a *Trp*⁺ FT^S transductant of strain JP2202 were also grown, extracts were prepared, and DAHP synthetase assays were carried out to confirm that derepression in the

TABLE 3. *Cotransduction of FT^R with trp⁺ using trp⁺ FT^R donors (trp⁺ transductants of FT^R mutants) and a trp⁻ FT^S recipient: analysis of trp⁺ transductants*

Strain	P1 donor *trp*⁺ derivative of	No. scored (Trp^+)	No. scored as FT^R (unselected marker)
JP2142	JP2144	120	2
JP2206	JP2200	160	3
JP2207	JP2202	160	2

TABLE 4. *Specific activity of DAHP synthetase in cell extracts prepared from tyrR mutants and from the parent strain (JP2140) grown in minimal medium supplemented with the aromatic end products*[a]

Strain	*tyrR* allele	Sp act of DAHP synthetase[b]		
		Total	phe	tyr
JP2140	+	28	25	3
JP2199	*367*	189	75	121
JP2144	*366*	180	88	112
JP2200	*368*	167	66	116
JP2201	*369*	178	72	110
JP2202	*370*	158, 223[c]	53, 75[c]	107, 138[c]
JP2203	*371*	198	76	128
JP2204	*372*	180	64	118
JP2205	*373*	229	79	157

[a] Tryptophan, tyrosine, phenylalanine, and the aromatic vitamins were included at the concentrations stated in Materials and Methods.

[b] Activity (for units, see Materials and Methods) of DAHP synthetase (phe) was measured as residual activity in the presence of 2.5×10^{-4} M L-tyrosine, DAHP synthetase (tyr) as residual activity in the presence of 2.5×10^{-4} M L-phenylalanine, and total activity as activity in the absence of inhibitors. These concentrations inhibit over 95% of the respective activities (Wallace, unpublished results).

[c] These are the results for assays carried out on two different cell extracts.

TABLE 5. *Specific activity of DAHP synthetase in cell extracts prepared from strain JP2202, strains derived from strain JP2202 and the parent strain JP2140*

Strain	Relevant genotype		Sp act of DAHP synthetase for cells grown in					
			MM + trp[a]			MM + aro[b]		
	tyrR allele	*sup* allele	Total	phe[c]	tyr[c]	Total	phe	tyr
JP2202	*370*	+	206	80	158	158, 223[d]	53, 75	107, 138
JP2140	+	+	26, 31	20, 27	6, 4	28	25	3
JP2207	*370*	+	176	68	110	178, 234	59, 85	134, 159
JP2226	+	+	21	17	4	25, 31	19, 30	6, 5
JP2208	*370*	*supD*$^-$	53	46	12	56	40	11
JP2209	*370*	*supF*$^-$	36	27	6	35	29	8
JP2210	*370*	*supC*$^-$	30	22	7	26	24	5
JP2211	*370*	*supU*$^-$	47	34	10	25, 38	20, 29	6, 4
JP2212	*370*	+	146	58	101	187	63	116
JP2213	*370*	+	173	65	123	155	56	96

[a] MM + trp is minimal medium containing 5×10^{-4} M L-tryptophan to repress DAHP synthetase (trp) to a negligible level.

[b] MM + aro is as described in Materials and Methods.

[c] Activity of DAHP synthetase (phe) was measured as residual activity in the presence of 2.5×10^{-4} M L-tyrosine, DAHP synthetase (tyr) as activity in the presence of 2.5×10^{-4} M L-phenylalanine.

[d] These results are for assays carried out on two different cell extracts.

FT^R mutant was due only to the mutation linked to *trp* (JP2207 and JP2226, respectively, in Table 5).

Demonstration of the presence of the tyrR allele in the sup$^-$ derivatives. P1 lysates were prepared on strain JP2208 (JP2202 *supD*$^-$), JP2209 (JP2202 *supF*$^-$), JP2210 (JP2202 *trp*$^+$ *supC*$^-$), JP2211 (JP2202 *ilv*$^+$ *supU*$^-$), and on strain JP2207 as control, and used in transductions with the *pyrF*$^-$ *tyrR*$^+$ recipient, strain JP2214. *pyrF*$^+$ transductants were selected and screened for FT^R by replica plating. Cotransduction of *tyrR* and *pyrF* with the *sup*$^-$ donors was comparable to that observed with the control lysate, the frequencies of cotransduction ranging from 2 to 9%. This indicates that the *tyrR* allele was, in fact, present and suppressed in the *sup*$^-$ derivatives of JP2202.

In addition, strain JP2211 (JP2202 *ilv*$^+$ *supU*$^-$) was cured by growth in broth, plating on nutrient agar, and testing selected clones for growth on medium with and without histidine and tryptophan (to detect loss of *supU*$^-$) and on medium containing 10^{-4} M fluorotyrosine to detect expression of the *tyrR*$^-$ phenotype. Eight segregants were detected, and these had all regained the ability to grow on medium containing fluorotyrosine. The results obtained after purification, on streaking, verified this result and indicated that these segregants had lost both *supU*$^-$ and *ilv*$^+$.

Cell extracts were prepared for two segregants (JP2212 and JP2213) and were assayed for DAHP synthetase activity. The results in Table 5 demonstrate that the *tyrR*$^-$ allele is present, confirming that the low specific activity in the *supU*$^-$ derivative was due to suppression of the *tyrR*$^-$ phenotype rather than reversion of the *tyrR* allele.

Isolation of Mu-1-induced tyrR mutants. Strain JP2140 was treated with Mu-1 as described in Materials and Methods. Thirteen individual cultures of strain JP2140, lysogenized with Mu-1, were grown to saturation, washed once in medium 56/2, and resuspended to a cell density of approximately 3×10^8 cells/ml. FT^R mutants were recovered from each of these cultures by the method used previously (Materials and Methods). One FT^R mutant was chosen from each selection, was purified, and characterized. They were all FT^R and Mu-1 lysogens by the criteria described in Materials and Methods.

tyrR mutants were identified by transduction. The donor was *trp*$^+$ *tyrR*$^+$ (W3110), and each of the thirteen mutants was used as a recipient, *trp*$^+$ transductants being selected and screened for FT^R. In four of the mutants, the mutation was linked to *trp* (Table 6). Linkage of FT^R to Mu-1 immunity and ability to release phage was also demonstrated, indicating that these mutations were generated by integration of the Mu-1 prophage within the *tyrR* gene.

These four Mu-1-induced *tyrR* mutants were grown in minimal medium plus tryptophan and in minimal medium plus aromatic end products; cell extracts were prepared, and the specific activities of DAHP synthetase determined.

TABLE 6. *Cotransduction of FT^S with trp$^+$ using a trp$^+$ FT^S donor and Mu-1-induced FT^R mutants as recipients: analysis of trp$^+$ transductants*

Recipient	No. scored	No. FT^S	Mu-1 immunity of transductants				Ability to release phage in liquid medium[a]	
			trp$^+$ FT^R		trp$^+$ FT^S			
			No. tested	No. immune	No. tested	No. immune	trp$^+$ FT^R	trp$^+$ FT^S
JP2215	160	3	6	6	3	0	+	−
JP2216	200	1	1	1	1	0	+	−
JP2217	160	3	1	1	1	0	+	−
JP2218	200	4	10	10	4	0	+	−

[a] One trp$^+$ FT^R and one trp$^+$ FT^S transductant was tested for each mutant. Each strain was grown overnight in broth and the supernatant was assayed for plaque-forming units per milliliter by plaque assay.

The four mutants have derepressed levels of both DAHP synthetase (tyr) and DAHP synthetase (phe) (Table 7).

Isolation of temperature-sensitive tyrR mutants. These studies were carried out in a single isoenzyme strain possessing only DAHP synthetase (tyr). The method of isolation of mutants was based on the ability of the tyrosine analogue APA to act as a false corepressor (33).

The procedure for mutagenesis and selection of APA^R mutants is described in Materials and Methods. From each of the eight selections, between 160 and 320 colonies resistant to APA at 37 or 42 C were selected by replica plating to duplicate plates of the medium on which they were isolated (i.e., containing 10^{-4}, 2×10^{-4}, or 5×10^{-4} M L-APA). One plate was incubated at 37 C or 42 C and the other at 28 or 32 C. Clones that grew at the higher temperature but failed to grow at the low temperature were purified and checked carefully by streaking and by their ability to feed strain AT2471, a tyrosine auxotroph. Table 8 shows the results for the control strain AB3253 and for the four best mutants, strains JP2219 (isolated on 5×10^{-4} M APA), and JP2220, JP2221, and JP2222 (all isolated on 2×10^{-4} M APA).

The mutations conferring APA^R were shown to be in the *tyrR* region by cotransduction with *pyrF*. P1 lysates prepared on the mutants were used with the *pyrF*$^-$ recipient, JP324, *pyrF*$^+$ transductants being selected. APA^R and *pyrF*$^+$ were contransducible in each case, the average cotransduction frequency being 7%.

The *tyrR380* allele (isolated in JP2221) was chosen for further characterization. Strain JP2223, the *pyrF*$^+$ *tyrR380* transductant of strain JP324, and strain JP2224, a *pyrF*$^+$ *tyrR*$^+$ transductant of strain JP324 as control, were grown for approximately eight generations in minimal medium and in minimal medium supplemented with aromatic end products at several temperatures between 24 C and 40 C (Fig. 1a and 1b).

TABLE 7. *Specific activity of DAHP synthetase in cell extracts prepared from Mu-1-induced tyrR mutants and their parent, JP2140*

Strain	*tyrR* allele	Sp act of DAHP synthetase for cells grown in					
		MM + trp[a]			MM + aro[b]		
		Total	phe	tyr	Total	phe	tyr
JP2215	*374*	292	101	217	256	95	174
JP2216	*375*	203	72	149	296	116	191
JP2217	*376*	223	70	146	198	83	135
JP2218	*377*	260	94	181	205	85	142
JP2140	+	26, 31	20, 27	6, 4	28	25	3

[a] MM + trp is minimal medium containing 5×10^{-4} M L-tryptophan.

[b] MM + aro is minimal medium supplemented with tyrosine, phenylalanine, tryptophan, and the aromatic vitamins.

TABLE 8. *Screening of temperature-sensitive APA^R mutants and the parent, AB3253: growth characteristics and ability to feed AT 2471, a tyrosine auxotroph*

Strain	Growth[a] on medium containing APA at concentration indicated									Ability to feed AT2471	
	No APA			10^{-4} M APA			2×10^{-4} M APA				
	28	32	37	28	32	37	28	32	37	32	37
JP2219	+	+	+	±	+	+	±	+	+	−	±
JP2220	+	+	+	±	+	+	±	±	+	−	+
JP2221	+	+	+	−	−	+	−	−	+	−	+
JP2222	+	+	+	−	−	+	−	−	+	ND	ND
AB3253	+	+	+	−	−	−	−	−	−	−	−

[a] Symbols: + represents strong growth; ± represents slight growth; − represents no growth; ND, not done.

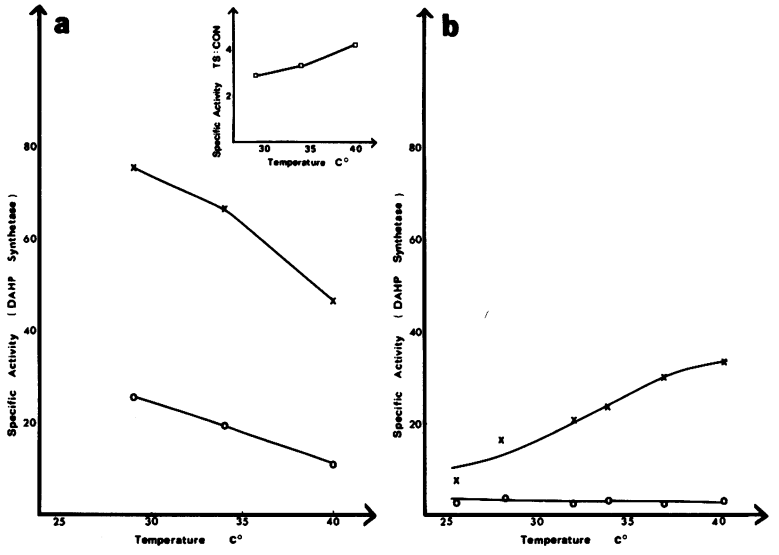

FIG. 1. (a) *Effect of temperature on rate of synthesis of DAHP synthetase (tyr) for cells grown in minimal medium for at least eight generations at 29, 34, and 40 C. Symbols: ×, JP2223; O, JP2224. Inset: Ratio of specific activities for JP2223:JP2224 against temperature (□). (b) Effect of temperature on rate of synthesis of DAHP synthetase (tyr) for cells grown in minimal medium supplemented with the aromatic end products. Cells were grown at six temperatures between 24 and 40 C. Symbols: ×, JP2223; O, JP2224.*

In minimal medium, the specific activity in the control decreased markedly with increasing temperature. This may mean that the enzyme itself is temperature sensitive; alternatively it may reflect some effect of temperature or growth rate on protein synthesis. That the specific activity in the mutant also decreases is probably a reflection of the same phenomenon. To eliminate all effects other than that of the two different *tyrR* alleles, the ratio of specific activities for JP2223:JP2224 is plotted in the inset. The specific activity of the mutant is 4.7 times that of the control at 40 C and 2.7 times that of the control at 28 C, consistent with slight temperature sensitivity of repression.

The results for cells grown in end products are much more readily interpreted. In the mutant, derepression increases with temperature. The specific activity of the mutant is 14 times the control at 40 C and 3 times the control at 24 C.

Effect of temperature-shift. In the first experiment strain JP2223 was grown at 26 C for about seven generations and was then shifted to 40 C in medium containing the aromatic end products. Synthesis of DAHP synthetase (tyr) becomes derepressed very soon after the temperature-shift (Fig. 2). This result suggests the mutation is probably of the TL type, since one would expect mutants of the TSS type to require more than a generation before derepression in response to a temperature shift is observed (17, 27).

An experiment to test the nature of the temperature-sensitive phenotype more directly was carried out. Strain JP2223 was grown at 26 C in minimal medium supplemented with the aromatic end products, was centrifuged, washed with sterile medium 56, and suspended in fresh medium lacking glucose but otherwise the same as above. This was divided into samples A, B, and C. Glucose was added to A and B; culture A was grown at 26 C; B and C were grown at 40 C. After 60 min glucose was added to culture C, and flasks B and C were returned to 26 C. The results in Fig. 3 show derepression in both B and C but not in the control A; that is derepression follows heating carried out both in the absence and presence of growth. The *tyrR 380* product is therefore of the TL type.

DISCUSSION

Isolation of a *tyrR* mutant which has a constitutive phenotype suppressed by the amber suppressors *supD*$^-$, *supF*$^-$, and *supU*$^-$ and by the ochre suppressor *supC*$^-$ demonstrates that the *tyrR* gene product is a protein, since amber suppression occurs at the level of translation (7, 30). Repression appears to be completely restored by the *supF*$^-$ and *supC*$^-$ alleles, which both suppress by causing the insertion of tyrosine. The fact that there is no significant difference between the two is inter-

esting since the amber suppressor *supF*⁻ has an estimated efficiency of approximately 51 to 55%, and the ochre suppressor *supC*⁻ has an estimated efficiency of 1 to 16% (7, 30, 34). The lack of any measurable difference is in contrast to the results obtained by Morse and Yanofsky (21) in their studies with amber mutants of the *trpR* gene. The results obtained may suggest either that synthesis of the repressor is itself under repressor control or, alternatively, that repressor is present in at least fivefold excess in the wildtype. *supD*⁻, which causes insertion of serine, and *supU*⁻, which causes insertion of glutamine, both restore repression to almost wildtype levels. It would therefore appear that the nature of the particular amino acid in this position of the molecule is not particularly critical, although translation of the region beyond this codon is essential for repressor activity.

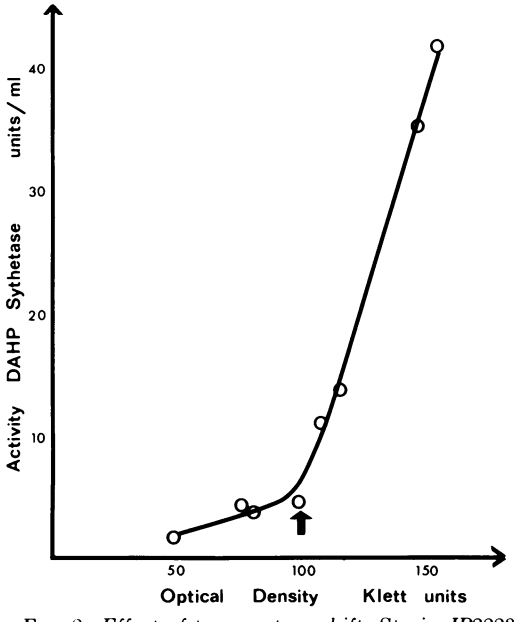

FIG. 2. *Effect of temperature shift. Strain JP2223 was grown in minimal medium supplemented with the aromatic end products at 26 C and shifted when the turbidity of the culture reached a Klett reading of 100 to 40 C as indicated by the arrow. Samples (80 ml) were taken and treated as described in Materials and Methods.*

The facts that the amber *tyrR* mutant is derepressed for the synthesis of DAHP synthetase (phe) in addition to DAHP synthetase (tyr), and that synthesis of both enzymes becomes repressed in concert in the presence of *sup*⁻ alleles, are strong evidence for the dual role of the *tyrR* gene product in tyrosine and phenylalanine biosynthesis. The derepression of synthesis of both enzymes in the other seven spontaneous *tyrR* mutants also supports this conclusion, as does the derepression in the four Mu-1-induced mutants. Since it is believed that these mutants were generated by integration of the Mu-1 prophage within the *tyrR* gene, it is

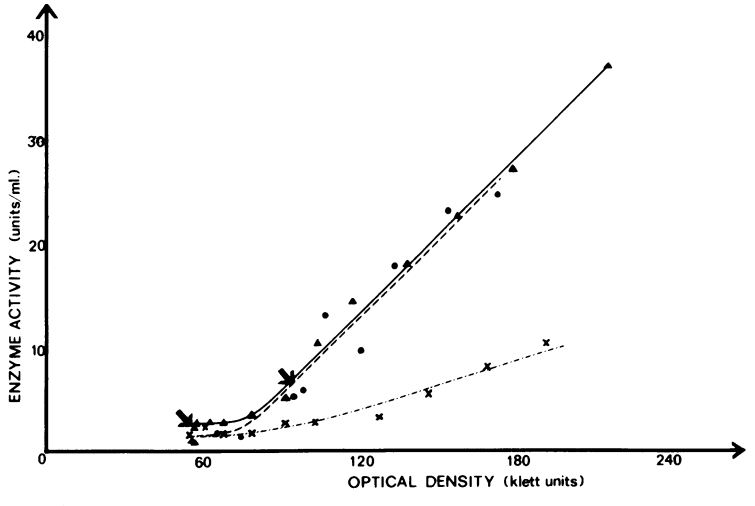

FIG. 3. *Effect of shift from 26 to 40 C, and subsequent shift from 40 to 26 C on DAHP synthetase activity. JP2223 was grown in minimal medium supplemented with aromatic end products to a Klett reading of 60, was washed, and suspended in the same medium except lacking glucose. Glucose was added to A (×) and B (●) but not to C (▲). The control, A, was grown at 26 C; cultures B and C were grown at 40 C for 60 min, then glucose was added to C, and both were returned to 26 C where indicated by the arrows. Samples (40 ml) were taken throughout the experiment and enzyme activities were determined.*

most unlikely that their derepressed phenotype is due to more than a single mutation. Also, Im and Pittard (13) present evidence, based on diploid studies, for the involvement of *tyrR* in phenylalanine regulation.

The fact that it was possible to isolate Mu-1-induced *tyrR* mutants and an amber *tyrR* mutant is suggestive evidence that the *tyrR* gene product is a nonessential protein with a specialized regulatory role. These mutations might be expected to cause such an alteration in the gene product that it would be non-functional. The fact that the four Mu-1-induced mutants and the amber mutant have the same high derepressed levels of enzyme synthesis is consistent with this notion.

Isolation of a temperature-sensitive *tyrR* mutant of the TL type is consistent with the *tyrR* gene product being a component of repressor or, alternatively, some enzyme involved in its modification. However, the absence of a substantial lag before derepression occurs after temperature shock makes it unlikely that the *tyrR* gene product is an enzyme modifying some metabolically stable cell component such as a transfer ribonucleic acid (tRNA) species. Experiments with this mutant are in progress to see if either tyrosine or phenylalanine affect the thermal stability of the *tyrR* (TL) repressor. If an effect were to be demonstrated, it would suggest that tyrosine or phenylalanine, respectively, produce a structural change in the repressor (27).

That tyrosine rather than charged $tRNA^{Tyr}$ is the corepressor in tyrosine regulation in *E. coli* seems likely from the work of Ravel et al. (24), which demonstrated that certain analogues which were not activated by tyrosyl-tRNA synthetase were, nevertheless, able to repress the synthesis of DAHP synthetase (tyr). Heinonen et al. (11), in a more extensive study of the regulation of DAHP synthetase (tyr) in *S. typhimurium*, showed that charging of $tRNA^{Tyr}$ seems to be unnecessary for repression. This conclusion was based on the repressed levels of tyrosine biosynthetic enzymes in a *tyrS* mutant when the growth rate was restricted by limitation of charged $tRNA^{Tyr}$ and, in addition, the ability of analogues that are not activated to mediate repression. Their demonstration of increased derepression of DAHP synthetase (tyr) brought about by introduction of a *tyrS* mutation into a *tyrR* mutant is of great interest in view of its possible implication of some involvement of tyrosyl-tRNA synthetase in enzyme regulation. However, any direct involvement as an element of the repressing complex appears to have been excluded.

In conclusion, the *tyrR* gene product is a nonessential protein with a specialized role in repression; present evidence suggests it is a component of repressor and probably interacts with both tyrosine and phenylalanine. As yet, there is no evidence to distinguish between a transcriptional or a translational model for regulation in this system.

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