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Phenylalanine and Tyrosine Biosynthesis in *Escherichia coli* K-12: Mutants Derepressed for 3-Deoxy-D-Arabinoheptulosonic Acid 7-Phosphate Synthetase (phe), 3-Deoxy-D-Arabinoheptulosonic Acid 7-Phosphate Synthetase (tyr), Chorismate Mutase T-Prephenate Dehydrogenase, and Transaminase A

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Mutant strains of *Escherichia coli* have been isolated in which the synthesis of 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP) synthetase (phe) is derepressed, in addition to those enzymes of tyrosine biosynthesis previously shown to be controlled by the gene *tyrR*. The major enzyme of the terminal pathway of phenylalanine biosynthesis chorismate mutase-prephenate dehydratase is not derepressed in these strains. Genetic analysis of the mutants shows that the mutation or mutations causing derepression map close to previously reported *tyrR* mutations. A study of one of the mutations has shown it to be recessive to the wild-type allele in a diploid strain. It is proposed that the *tyrR* gene product is involved in the regulation of the synthesis of DAHP synthetase (phe) as well as the synthesis of DAHP synthetase (tyr), chorismate mutase-prephenate dehydrogenase, and transaminase A.

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 being controlled by tyrosine, phenylalanine, and tryptophan, respectively (4, 10, 17). These amino acids also control the synthesis of one or more enzymes in each of the terminal pathways from chorismic acid to tyrosine, phenylalanine, and tryptophan (10). In the case of tyrosine biosynthesis, the structural genes for DAHP synthetase (tyr) and chorismate mutase-prephenate dehydrogenase (a key enzyme in the tyrosine pathway) form a single operon, the expression of which is controlled by a regulator gene *tyrR* (Mattern and Pittard, *in press*). In tryptophan biosynthesis, the structural gene for DAHP synthetase (trp) is separated from the structural genes of the *trp* operon; however, all three are subject to control by the regulator gene *trpR* (5, 14; Camakaris and Pittard, *in press*). The genes for DAHP synthetase (phe) and chorismate mutase-prephenate dehydratase are also separated from each other on the chromosome; although operator mutants controlling the expression of the gene for chorismate mutase-prephenate dehydratase have been described (Im and Pittard, *in press*), there have as yet been no reports of mutations affecting the synthesis of DAHP synthetase (phe). It is the purpose of this paper to describe the isolation and characterization of mutants derepressed for the synthesis of DAHP synthetase (phe). Although the method that was used to isolate these mutants was expected to select for strains derepressed for both chorismate mutase-prephenate dehydratase and DAHP synthetase (phe), chorismate mutase-prephenate dehydratase is not derepressed in these mutants. However, the observation that the tyrosine biosynthetic enzymes are also derepressed in these mutants suggests the possibility of some relationship in the control of the two pathways.

In an accompanying paper (4*a*), Brown and So-

merville present results substantially in agreement with the results presented in this paper.

MATERIALS AND METHODS

Organisms. Strains used in this work are all derivatives of *E. coli* K-12 (Table 1). Strain KLF23/KL181 was obtained from B. Low. The colicin V-producing strain X178 was obtained from J. Scaife.

Media and culture methods. Media and culture methods used in this work were described by Adelberg and Burns (1).

Buffers. Sodium phosphate buffers used were prepared by the method of Dawson and Elliott (7).

Chemicals. Chemicals used were obtained commercially and not further purified. Reagent-grade Selectacel (diethylaminoethyl cellulose) was obtained from Brown Co., Berlin, N.H. D-Erythrose-4-phosphate dimethylacetal dicyclohexyl-ammonium salt (A grade) was obtained from Calbiochem, Los Angeles, Calif. Free erythrose-4-phosphate was prepared by the

TABLE 1. *List of strains*

Strain	Sex	Genetic loci relevant to this work[a]					
		aroG	*aroH*	*aroF*	*trp*	*tyrR*	*pyrF*
AB3253	F⁻	365[b]	367	+	+	+	+
AB3259	F⁻	+	367	363	+	+	+
AB3271	F⁻	365	367	+	+	352	+
JP170	F⁻	Δ	367	363	+	+	+
JP323	F⁻	365	367	+	−	+	+
JP544	F⁻	+	367	363	+	363	+
JP545	F⁻	+	367	363	+	365	+
JP546	F⁻	+	367	363	+	364	+
JP569	F⁻	+	367	363	Δ[c]	+	+
JP1510	F⁻	365	367	+	+	+	+
JP1511	F⁻	365	367	+	+	363	+
JP1512	F⁻	365	367	+	+	+	+
JP1513	F⁻	365	367	+	+	364	+
JP1514	F⁻	365	367	+	+	+	+
JP1515	F⁻	365	367	+	+	365	+
JP1522	F⁻	+	367	363	+	+	+
JP1523	F⁻	+	367	363	+	363	+
JP1525	F⁻	+	367	363	+	364	+
JP1527	F⁻	+	367	363	+	365	+
JP1528	F⁻	+	367	363	+	352	+
JP1540	F⁻	365	367	+	Δ	363	+
JP1543	F⁻	+	367	363	Δ	363	+
JP1555	F⁻	+	367	363	+	360	+
JP1558	F⁻	+	367	+	+	+	+
JP1559	F⁻	+	367	+	+	352	+
JP1560	F⁻	+	367	+	+	358	+
JP1562	F⁻	+	367	+	+	360	+
JP1563	F⁻	+	367	+	+	+	+
JP1564	F⁻	+	367	+	+	363	+
JP1565	F⁻	+	367	+	+	364	+
JP1566	F⁻	+	367	+	+	365	+
JP1567	F⁻	365	367	+	−	+	+
JP1568	F⁻	365	367	+	Δ	363	+
JP2033	F⁻	365	367	+	+	358	+
JP2034	F⁻	365	367	+	+	359	+
JP2035	F⁻	365	367	+	+	360	+
JP2103	F⁻	365	367	+	E365	+	−
KLF23/KL181	F123	+	+	+	+/−	+	+
KL181	F⁻	+	+	+	−	+	+

[a] Symbols: *aroG*, the structural gene for DAHP synthetase (phe); *aroH*, the structural gene for DAHP synthetase (trp); *aroF*, the structural gene for DAHP synthetase (tyr); *trp*, any one of the structural genes for enzymes converting chorismate to tryptophan; *tyrR*, regulator gene controlling the expression of *aroF*, *tyrA* (the structural gene for chorismate mutase T-prephenate dehydrogenase), and the gene for transaminase A. *PyrF* is the structural gene for orotidylic acid decarboxylase.

[b] Allele numbers were allocated in these laboratories.

[c] Symbol *trp*Δ represents a deletion of part of the tryptophan operon, isolated by the method of Gottesman and Beckwith (11). Such deletions extend into and possibly beyond the *tonB* locus.

method of Ballou, Fischer, and MacDonald (3). Chorismic acid was prepared by the method of Edwards and Jackman (9). Barium prephenate was prepared by heating a solution of chorismic acid at 70 C for 1 hr and purified by chromatography, as for chorismic acid, before it was converted to the barium salt.

Mating procedures. The conditions under which the mating experiments were carried out were described by Pittard and Wallace (18).

Isolation of mutants. The conditions under which cells were treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were those of Adelberg, Mandel, and Chen (2). Phenotypic expression of the mutants was allowed to occur by growth in complete medium for 4 hr after treatment with the mutagen.

Growth of cells and preparation of cell-free extracts. Cells were grown in minimal medium containing all of the amino acids necessary for growth. The composition of minimal medium and the concentrations of the amino acids which were added have been previously described by Adelberg and Burns (1). When minimal medium was supplemented with the aromatic amino acids, they were added in the following concentrations: DL-phenylalanine, 5×10^{-4} M; DL-tryptophan, 2×10^{-4} M; L-tyrosine, 2.5×10^{-4} M. Other growth requirements were added in the following final concentrations: Shikimic acid, 5×10^{-5} M; *p*-aminobenzoic acid, 10^{-6} M, *p*-hydroxybenzoic acid, 10^{-6} M, and 2,3-dihydroxybenzoic acid, 10^{-6} M. The cells were harvested at mid-exponential phase of growth, washed with chilled 0.9% NaCl, and suspended in 0.1 M sodium phosphate buffer (*p*H 7.0). Cell breakage was achieved by ultrasonic oscillation using a 500-w ultrasonic disintegrator (Measuring & Scientific Equipment, Ltd.) at output setting 3 for 40 sec. Cell-free extracts were obtained by centrifugation at $16,000 \times g$ for 15 min.

Test of sensitivity to APA. Unless otherwise specified, sensitivity to 4-aminophenylalanine (APA) was tested on minimal medium supplemented with 0.1 mM APA.

Assay of DAHP synthetase. The method of Doy and Brown (8) was used for assaying DAHP synthetase.

Assay of chorismate mutase and prephenate dehydratase. The methods for assay of chorismate mutase and prephenate dehydratase were based on those described by Cotton and Gibson (6). Ethylenediaminetetraacetic acid (EDTA; 0.1 μmole) was included in the incubation mixture. Mercaptoethanol was also included: 1 μmole for the assay of chorismate mutase and 20 μmoles for the assay of prephenate dehydratase.

Assay of transaminase. The method of Silbert, Jorgensen, and Lin (21), with phenylalanine as substrate, was used for assay of transaminase.

Assay of prephenate dehydrogenase. This method was based on the method of Cotton and Gibson (6). A 0.4-ml amount of reaction mixture contained 2.0 μmoles of barium prephenate, 1.0 μmole of nicotinamide adenine dinucleotide, 10 μmoles of tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.8), 0.1 μmole of EDTA, and 1.0 μmole of mercaptoethanol.

Assay of 4-hydroxyphenylpyruvate. 4-Hydroxyphenylpyruvate was assayed as described by Schwinck and Adams using Millon's reagent (20). Absorbancy at 490 nm was read after addition of the $NaNO_2$ reagent.

Protein estimation. Protein was estimated by the method of Lowry et al. (15).

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

Preparation of colicin V. Colicin V was prepared as described by Gratia (12).

***φ*80 Virulent.** *φ*80 Virulent was obtained from J. Scaife and was propagated in broth culture, using a suitable strain, in Luria broth containing 2.5×10^{-3} M $CaCl_2$. The lysate was concentrated to a titer of above 10^{11} by ultracentrifugation.

Selection of trp deletion mutants. The method described by Gottesman and Beckwith (11) was used for the selection of colicin V, *φ*80 virulent-resistant mutants. Trp deletion mutants were isolated on minimal medium.

Curing of F' males. Acridine orange was used in the curing of F' males as described by Hirota (13).

Transduction. Transductions with phage P1 were carried out by the method of Pittard and Wallace (18).

RESULTS

As previously reported (Im and Pittard, *in press*), a strain of *E. coli* AB3259 which possesses only the single DAHP synthetase isoenzyme, DAHP synthetase (phe), is growth inhibited by 10^{-2} M *p*-fluorophenylalanine (FPA) in the presence of shikimic acid, tyrosine, and tryptophan. Some mutant strains derived from AB3259 which can grow on this medium and excrete phenylalanine have been found to be derepressed for the synthesis of the enzyme chorismate mutase-prephenate dehydratase (Im and Pittard, *in press*).

In a search for strains that may be derepressed for the synthesis of DAHP synthetase (phe) and working on the assumption that DAHP synthetase (phe) and chorismate mutase-prephenate dehydratase may be controlled by a single regulator gene, this selection for mutants of AB3259 able to grow in the presence of FPA was repeated. Those clones which grew and which cross-fed a phenylalanine auxotroph were purified and screened for the presence of altered levels of DAHP synthetase (phe).

Three strains were isolated which possessed elevated levels of DAHP synthetase (phe) when grown either in minimal medium or in minimal medium supplemented with the aromatic end products.

From the results that are shown in Table 2, it can be seen that, although the synthesis of DAHP synthetase (phe) is derepressed, contrary to expectations there is no corresponding elevation of the levels of prephenate dehydratase in these strains. Although the increase in level of DAHP synthetase (phe) activity in these mutants is only two- to threefold, the increase is consis-

TABLE 2. *Specific activities of DAHP synthetase (phe) and prephenate dehydratase*

Strain no.	DAHP synthetase (phe)		Prephanate dehydratase	
	Cells grown in minimal medium	Cells grown in minimal medium supplemented with end products[a]	Cells grown in minimal medium	Cells grown in minimal medium supplemented with end products[a]
AB3259	16	13	3	3
JP544	36	38	4	4
JP545	35	37	4	4
JP546	35	35	3	3

[a] End products include phenylalanine, tyrosine, and tryptophan.

tantly observed. The enzyme activity in the mutants still shows the same sensitivity to feedback inhibition by phenylalanine and fluorophenylalanine as does the parent strain (Fig. 1).

In addition to the change in the rate of synthesis of DAHP synthetase (phe), these strains synthesize chorismate mutase (total), prephenate dehydrogenase, and transaminase activities at increased rates, even in the presence of the aromatic amino acids (Tables 3 and 4).

Enzyme studies therefore indicate that in these strains the synthesis of at least two of the enzymes involved in tyrosine biosynthesis [namely chorismate mutase-prephenate dehydrogenase and transaminase A (DAHP synthetase (tyr) cannot be checked in these strains)] and one of the enzymes involved in phenylalanine biosynthesis [namely DAHP synthetase (phe)] are derepressed, whereas the synthesis of the major enzyme of the phenylalanine pathway, chorismate mutase-prephenate dehydratase remains unaltered.

Genetic analyses of mutant strains. Transduction studies were carried out to determine whether the mutation causing derepression of DAHP synthetase (phe) was cotransducible with the structural gene for this enzyme. Phage P1 lysates were prepared on each of the mutants and used to transduce *aroG*$^+$ into strain JP170. Transductants were purified, and six recombinants from each transduction were examined for the levels of DAHP synthetase (phe) in cells grown in minimal media supplemented with the aromatic end products. None of the transductants exhibited the elevated levels obtained in the original mutants. Furthermore, not 1 of 120 transductants was able to grow on the FPA containing media.

Since mutations in a gene *tyrR* had already been shown to cause derepression of DAHP synthetase (tyr), chorismate mutase-prephenate dehydrogenase and transaminase A (23), attempts were made to determine (i) whether the mutations in these new strains mapped in the vicinity of *tyrR* and (ii) whether they had any effect on the synthesis of DAHP synthetase (tyr). Since the gene *tyrR* was known to be cotransducible with *trp* at frequencies of about 5% (Wallace, *unpublished data*), the following experiments were carried out.

P1 phage lysates were prepared on strains JP544, JP545, and JP546 and were used to transduce *trp*$^+$ into the *trp*$^-$ recipient JP323, which possesses only the single DAHP synthetase isoenzyme, DAHP synthetase (tyr). The *trp*$^+$ transductants were tested for resistance to 0.1 mM APA, this being the analogue previously used to isolate *tyrR* mutants (23). In each case, approximately 5% of transductants were found to be APA resistant.

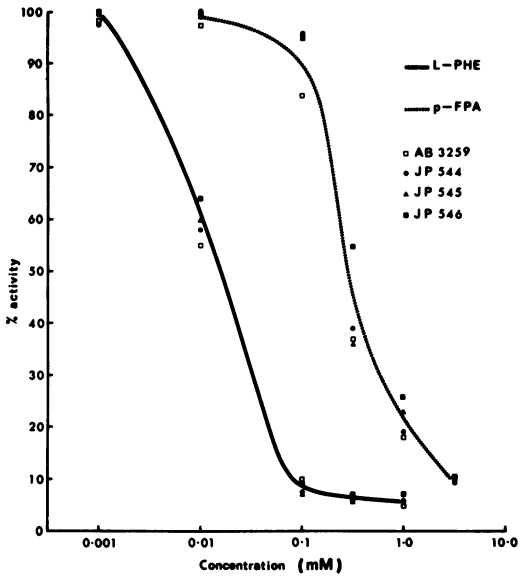

FIG. 1. *Inhibition of DAHP synthetase (phe) by L-phenylalanine and the phenylalanine analogue, p-fluorophenylalanine.*

TABLE 3. *Specific activity of enzymes in cell-free extracts prepared from cells grown in minimal medium*

Strain no.	Chorismate mutase (P + T)	Prephenate dehydratase	Prephenate dehydrogenase	Transaminase A
AB3259	0.5	3	1.7	9.0
JP544	19.5	4	11.0	11.0
JP545	19.0	4	11.3	10.0
JP546	16.5	3	11.3	11.0

Cell-free extracts were prepared from both the APA-resistant and the APA-sensitive transductants selected from each transduction. The levels of various enzymes in these extracts are shown in Table 5. It can be seen that DAHP synthetase (tyr), chorismate mutase (P + T), prephenate dehydrogenase, and transaminase A are derepressed in the APA-resistant transductants but not in the APA-sensitive ones. These results indicated the presence of *tyrR* type mutations in these APA-resistant transductants. These mutations have been designated *tyrR363*, *tyrR364* and *tyrR365*, respectively, in strains JP544, JP546 and JP545.

To demonstrate that these transductants had also received a mutation which causes derepression of DAHP synthetase (phe), it was necessary to either (i) introduce the *aroG*$^+$ allele into these strains or (ii) transfer the regulatory mutation back into an *aroG*$^+$ strain. By transferring the F genote F_{16} (16) into these transductants, it was possible to convert them to donors which in turn were able to transfer chromosomal markers to an *aroG*$^+$ strain. The male derivatives of JP1511, JP1515, and JP1513 were crossed with the recipient JP569 (*trp* deletion *aroG*$^+$ *aroF363 aroH367*). Selection was made for Trp$^+$ recombinants, and, since previous experiments had shown that, when transferred by conjugation, *tyrR* and *trp* were coinherited at a frequency of almost 100% (Im, *unpublished data*), only one Trp$^+$ recombinant from each cross was purified and checked for the repressibility of synthesis of DAHP synthetase (phe). The results are presented in Table 6, JP1523 being the strain carrying the mutation originally present in JP544, and JP1527 and JP1525 being derived from JP545 and JP546, respectively. Strain JP1522 is a Trp$^+$ recombinant obtained in a cross in which the donor was an F_{16} derivative of the APA-sensitive transductant JP1510. It is clear from Table 6 that the original *trp*$^+$ transductants, JP1511, JP1515, and JP1513, all possess mutations which cause derepression of the synthesis of DAHP synthetase (phe) and which map either in or very close to the *tyrR* gene.

TABLE 4. *Specific activity of enzymes in cell-free extracts prepared from cells grown in minimal medium supplemented with phenylalanine, tyrosine, and tryptophan*

Strain no.	Chorismate mutase (P + T)	Prephenate dehydratase	Prephenate dehydrogenase	Transaminase A
AB3259	3.0	3	0.5	6.0
JP544	14.0	4	9.0	11.0
JP545	7.0	4	11.2	11.0
JP546	8.5	3	13.0	11.5

More accurate mapping was accomplished by transduction, using the three markers *trpE*, *pyrF*, and *tyrR* (conjugation studies with an F′ strain X178 having already indicated that *tyrR* mapped clockwise from the *trp* operon). P1 lysates were grown on the *trpE*$^+$ *pyrF*$^+$ *tyrR*$^-$ strains AB3271, JP2033, and JP2034. Strains AB3271, JP2033, and JP2034 had all been isolated as APA-resistant mutants in a strain possessing only the tyrosine-sensitive isoenzyme DAHP synthetase (tyr) (23). Whereas strain AB3271 exhibits high levels of DAHP synthetase (tyr) in both the presence and absence of the aromatic amino acids, JP2033 and JP2034 only exhibit high levels in the absence of the aromatic amino acids (B. J. Wallace, *unpublished data*). These lysates were used to transduce the *trpE*$^-$, *pyrF*$^-$, *tyrR*$^+$ recipient JP2103. *Trp*$^+$ (Table 7) and *Pyr*$^+$ (Table 8) transductants were selected on minimal medium lacking tryptophan and uracil, respectively. The results in Tables 7 and 8 show that the *tyrR* alleles, *tyrR362*, *tyrR358*, and *tyrR360*, are all cotransducible with *trpE* at a frequency of about 4% and with *pyrF* at a frequency of about 13%, and that the gene order is *trpE pyrF tyrR*. The experiment was repeated using a P1 lysate prepared on JP544. The results shown in Tables 7 and 8 indicate that the mutation in JP544, which confers resistance to APA and which we have called tyrR363, also shows very similar cotransduction frequencies with *trpE* and *pyrF*. The cotransduction frequencies of *tyrR* with *trp* and *pyrF* are low compared to those obtained by K. Brown (*personal communication*) and by R. Russell in this laboratory. This appears to be due to an as yet unresolved difference in the genetic background of the P1 donors used in each case.

Effects of tyrR mutations on DAHP synthetase in double isoenzyme strains possessing DAHP synthetase (phe) and DAHP synthetase (tyr). To study the effect of *tyrR* mutations on both DAHP synthetase (tyr) and DAHP synthetase (phe) in double isoenzyme strains, the *aroG*$^+$ allele was separately introduced by P1 transduction into *tyrR* mutants possessing only DAHP synthetase (tyr) (AB3271, JP2033, JP2035, JP1511, JP1513, and JP1515). Since, the growth of *tyrR* mutants possessing only DAHP synthetase (tyr) is still repressed by tyrosine (23), it is possible to select for *aroG*$^+$ transductants on minimal medium supplemented with tyrosine.

Transductants were purified and examined for the levels of DAHP synthetase (tyr) and DAHP synthetase (phe) in cells grown in minimal medium supplemented with the aromatic end prod-

TABLE 5. *Specific activity of enzymes in cell free extracts prepared from the 4-aminophenylalanine (APA)-sensitive and the APA-resistant transductants*[a]

Strain	APA	DAHP synthetase (tyr)		Chorismate mutase (P + T)		Prephenate dehydratase		Transaminase A
		MM	EP	MM	EP	MM	EP	EP
JP1510	S	8.5	0.5	16	8	3	3	4.5
JP1511	R	16.0	10.0	33	12	4	3	6.5
JP1514	S	—	<0.5	—	6	—	4	4.0
JP1515	R	21.0	9.0	36	18	3	4	6.0
JP1512	S	—	1.0	—	9	—	4	4.0
JP1513	R	34.0	20.0	46	35	3	3	7.5

[a] JP1510 and JP1511 were *trp*$^+$ transductants selected in the transduction experiments in which the donor of *trp*$^+$ was JP544. Similarly, for JP1512 and JP1513, the donor was JP546; for JP1514 and JP1515, the donor was JP545. Abbreviations: MM, minimal medium; EP, minimal medium supplemented with phenylalanine, tyrosine, tryptophan, and shikimic acid; —, not tested.

TABLE 6. *Specific activity of enzymes in cell-free extracts prepared from Trp$^+$ recombinants grown in minimal medium supplemented with phenylalanine, tyrosine, and tryptophan*

Donor strain	Recipient	Trp$^+$ recombinants	DAHP synthetase (phe)	Chorismate mutase (P + T)	Prephenate dehydrogenase	Prephenate dehydratase	Transaminase A
F_{16}JP1510 (*tyrR*$^+$)	JP569	JP1522	16	3	0.5	2	4.0
F_{16}JP1511 (*tyrR363*)	JP569	JP1523	45	11	9.0	2	6.0
F_{16}JP1515 (*tyrR365*)	JP569	JP1527	47	12	11.0	2	6.5
F_{16}JP1513 (*tyrR364*)	JP569	JP1525	49	16	18.0	2	7.0

TABLE 7. *Mapping of tyrR by transduction, using trpE$^+$ pyrF$^+$ tyrR$^-$ donors and a trpE$^-$ pyrF$^-$ tyrR$^+$ recipient (JP2103): analysis of Trp$^+$ transductants*

P1 donor	*tyrR* allele	No. scored	Per cent of transductants that score as					
			Pyr$^+$ APA^s	Pyr$^+$ APA^R	Pyr$^-$ APA^s	Pyr$^-$APA^R	Pyr$^+$ (total)	APA^R (total)
AB3271	352	619	41	4	55	0.3	44	4
JP2033	358	280	35	5	60	1	39	6
JP2034	359	360	39	2	59	0.3	41	3
JP544	363	624	41	3	56	0.2	44	4

ucts. It can be seen in Table 9 that, whereas *tyrR363*, *tyrR364*, and *tyrR365* cause derepression of the synthesis of both isoenzymes, *tyrR352*, *tyrR358*, and *tyrR360*, although causing derepression of the synthesis of DAHP synthetase (tyr), appear to depress the synthesis of DAHP synthetase (phe). More convincing results of the effect of these *tyrR* mutants on the synthesis of DAHP synthetase (phe) is shown in Table 10, where it can be seen that the levels of this isoenzyme in the *tyrR*$^-$ strains are reduced to half of that of the wild-type strain AB3259. It should be noted that AB3271 (*tyrR352*), JP2033 (*tyrR358*), and JP2035 (*tyrR360*) were isolated as APA-resistant mutants of AB3253, a single isoenzyme strain possessing only DAHP synthetase (tyr). On the other hand, *tyrR363*, *tyrR364*, and *tyrR365* originated from JP544, JP546, and JP545 which were selected as *p*-FPA-resistant mutants of AB3259, a single isoenzyme strain possessing only DAHP synthetase (phe). Thus, it appears that the method of selection may favor particular types of mutant.

Dominance studies. Merodiploids have been constructed to study the dominance relationships between mutant (*tyrR*$^-$) and wild-type (*tyrR*$^+$) alleles. To date, only one of these strains has been extensively studied, and the results are reported below.

Strain KLF23/KL181 was used as donor of

TABLE 8. *Mapping of tyrR by transduction, using trpE⁺ pyrF⁺ tyrR⁻ donors and a trpE⁻ pyrF⁺ tyrR⁺ recipient (JP2103): analysis of Pyr⁺ transductants*

P1 donor	*tyrR* allele	No. scored	Per cent of transductants that score as					
			Trp⁺ APA[s]	Trp⁺ APA[R]	Trp⁻ APA[s]	Trp⁺ APA[R]	Trp⁺ (total)	APA[R] (total)
AB3271	352	197	61	11	24	4	72	15
JP2033	358	80	58	5	35	3	63	8
JP2034	359	104	58	7	32	4	64	11
JP544	363	520	67	3	46	4	70	7

TABLE 9. *Specific activities of enzymes in strains possessing both DAHP synthetase (phe) and DAHP synthetase (tyr)*

Strain	*tyrR*	Growth medium[a]	DAHP synthetase[b]			Prephenate dehydrogenase
			(tyr)	(phe)	Total	
JP1558	*tyrR⁺*	MM	4.2	13.0	15.6	3.8
JP1558	*tyrR⁺*	EP	0.5	7.8	9.2	0.5
JP1559	*tyrR352*	MM	12.8	6.8	17.2	16.1
JP1559	*tyrR352*	EP	10.7	4.0	13.1	12.8
JP1560	*tyrR358*	MM	14.3	11.2	25.5	18.2
JP1560	*tyrR358*	EP	0.1	5.4	5.6	<0.5
JP1562	*tyrR360*	MM	15.7	12.5	25.2	16.1
JP1562	*tyrR360*	EP	0.5	5.0	5.6	0.5
JP1563	*tyrR⁺*	EP	0.5	10.0	9.5	0.5
JP1564	*tyrR363*	EP	14.5	30.4	44.3	12.9
JP1565	*tyrR364*	EP	18.8	27.0	46.0	20.4
JP1566	*tyrR365*	EP	11.8	33.0	43.5	12.8

[a] Symbols: MM, minimal medium; EP, minimal medium supplemented with phenylalanine, tyrosine, and tryptophan.

[b] Activity of DAHP synthetase (tyr) was measured as the residual activity when phenylalanine was included in the incubation mixture to inhibit the activity of DAHP synthetase (phe). Similarly, DAHP synthetase (phe) was measured as the residual activity when tyrosine was included in the incubation mixture. Total activity was that measured in the absence of either of the two inhibitors. The final concentration of phenylalanine or tyrosine in the incubation mixture was 2.5×10^{-4} M. This concentration of phenylalanine or tyrosine inhibits over 95% of the activity of DAHP synthetase (phe) or DAHP synthetase (tyr), respectively (Wallace, *unpublished data*).

the F123 factor which covers the *trp* region of the *E. coli* chromosome. The *trpΔ tyrR363* recipient JP1540 was a single isoenzyme strain possessing only DAHP synthetase (tyr). Merodiploids (F-*trp⁺*/*trp⁻*) were selected in an interrupted mating experiment on minimal medium not containing tryptophan. Uracil was not included in the medium, as a counterselection against the male. The ability to transfer *trp⁺* into a *trp⁻ recA⁻* recipient KL181 and the ability to give rise to *trp⁻* segregants were taken as criteria for merodiploidy. One of these merodiploids was examined for levels of DAHP synthetase (tyr) and prephenate dehydrogenase. The results are shown in Table 11. It can be seen that, although the synthesis of DAHP synthetase (tyr) and prephenate dehydrogenase are constitutive in JP1540, the synthesis of these enzymes is repressible in the merodiploid F123/JP1540. As expected, this merodiploid was APA-sensitive.

The experiment was repeated using the *trp⁻ tyrR363* recipient JP1543 which is a single isoenzyme strain possessing only DAHP synthetase (phe). The synthesis of this isoenzyme was also found to be repressible (Table 12).

To study dominance relationships in a merodiploid of the type F-*tyrR363*/*tyrR⁺*, it was necessary to pick up the *tyrR363* mutation on the F123 factor. Use was made of the APA-sensitive merodiploid F123/JP1540 (F-*trp⁺*, *tyrR⁺*/*trpΔ*

TABLE 10. *Effect of tyrR352 and tyrR360 on DAHP synthetase (phe) in single isoenzyme strains*

Strain	*tyrR*	Growth medium[a]	Specific activity	
			DAHP synthetase (phe)	Prephenate dehydrogenase
JP1528	*tyrR352*	MM	15.0	10.0
JP1528	*tyrR352*	EP	6.0, 5.0, 5.5[b]	9.0
JP1555	*tryR360*	MM	16.0	10.0
JP1555	*tyrR360*	EP	7.0	0.5
AB3259	*tyrR*$^+$	MM	16.0	1.7
AB3259	*tyrR*$^+$	EP	13.0	0.5

[a] Symbols: MM, minimal medium; EP, minimal medium supplemented with phenylalanine, tyrosine, and tryptophan.
[b] Each figure represents the result of a separate experiment.

TABLE 11. *Repressibility of DAHP synthetase (tyr) and prephenate dehydrogenase in the merodiploid F123/JP1540*

Strain	Sex	Specific activity[a]	
		DAHP synthetase (tyr)	Prephenate dehydrogenase
JP1540	F$^-$	15.3, 16.4[a]	9.2, 11.8
F123/JP1540	F′	0.1, 0.7	<0.5, 0.5
Segregant	F$^-$	20.9	9.0

[a] In extracts prepared from cells grown in minimal medium supplemented with tyrosine, tryptophan, phenylalanine, and shikimic acid.
[b] Each figure represents the result of a separate experiment.

tyrR363) described above. In a population of such cells, derivatives of the type F-*trp*$^+$ *tyrR363*/*trp*Δ *tyrR363* which occur due to gene conversion may be selected as APA-resistant mutants. Such derivatives were isolated on minimal medium supplemented with 5 mM APA. The levels of DAHP synthetase (tyr) and prephenate dehydrogenase in two of these derivatives are shown in Table 13 where it can be seen that these enzymes are derepressed. If the F factor indeed carries the *tyrR363* mutation, it is expected to show the following properties. (i) When it is transferred into a *tyrR*$^+$ single isoenzyme strain possessing only DAHP synthetase (tyr), the resulting merodiploids (F-*tyrR363*/*tyrR*$^+$) should be APA sensitive; (ii) when it is transferred back into the *tyrR363* single isoenzyme strain possessing only DAHP synthetase (tyr), the resulting merodiploids (F-*tyrR363*/-*tyrR363*) should be APA resistant. The results presented in Table 14 confirm these predictions and prove that *tyrR363* is trans-recessive. We are currently in the process of testing all of the other *tyrR* mutants in the same manner.

DISCUSSION

Mutant strains of *E. coli* K-12 have been isolated in which the synthesis of DAHP synthetase (phe) is constitutive. The mutations responsible for derepression are not of the operator type since they have been shown to map at a position on the chromosome remote from *aroG*, the structural gene for DAHP synthetase (phe). In these mutant strains, the synthesis of the three enzymes previously shown to be controlled by the *tyrR* gene, namely DAHP synthetase (tyr), chorismate mutase-prephenate dehydrogenase, and transaminase is also derepressed.

Cotransduction studies using the markers *trpE*, *pyrF*, and *tyrR* favor the conclusion that one of these strains, JP544, possesses a single mutation in the *tyrR* gene, causing all of the observed changes. However, there is as yet no conclusive genetic evidence eliminating the possibility of a second mutation closely linked to the *tyrR* gene and affecting only the synthesis of DAHP synthetase (phe). We are in the process

TABLE 12. *Repressibility of DAHP synthetase (phe) and prephenate dehydrogenase in the merodiploid F123/JP1543*

Strain	Sex	Specific activity[a]	
		DAHP synthetase (phe)	Prephenate dehydrogenase
JP1543	F$^-$	32.5	11.5
F123/JP1543	F′	12.0, 13.1[b]	<0.5, <0.5
Segregant	F$^-$	42.0	8.5

[a] In extracts prepared from cells grown in minimal medium supplemented with tyrosine, tryptophan, phenylalanine, and shikimic acid.
[b] Each figure represents the result of a separate experiment.

TABLE 13. *Specific activities of DAHP synthetase (tyr) and prephenate dehydrogenase in merodiploids of the type F-tyrR363/tyrR363*

Merodiploid	Sex	Specific activity[a]	
		DAHP synthetase (tyr)	Prephenate dehydrogenase
F-*trp*$^+$, *tyrR363*/*trp*Δ *tyrR363* (1)	F′	36.0	14.5
F-*trp*$^+$, *tyrR363*/*trp*Δ *tyrR363* (2)	F′	30.0	18.4

[a] In extracts prepared from cells grown in minimal medium supplemented with tyrosine, tryptophan, phenylalanine, and shikimic acid.

TABLE 14. *Test for the presence of tyrR363 on the F123 factor*

Donor	Recipient[a]	Resulting merodiploid[b]	Sensitivity to APA[c] (0.1 mM)	
			Sensitive	Resistant
F-*trp*$^+$, *tyrR363*/*trp*Δ, *tyrR363*	JP1567 (trp$^-$ tyrR$^+$)	F-*trp*$^+$, *tyrR363*/*trp*$^-$ *tyrR*$^+$	38/38	0/38
F-*trp*$^+$, *tyrR363*/*trp*Δ, *tyrR363*	JP1568 (*trp*Δ *tyrR363*)	F-*trp*$^+$, *tyrR363*/*trp*Δ *tyrR363*	0/40	40/40

[a] JP1567 and JP1568 are *ilv*$^+$ derivatives of JP323 and JP1540, respectively, prepared by transduction.

[b] Merodiploids were selected on minimal medium not containing tryptophan, isoleucine, and valine, in interrupted mating experiments.

[c] 4-Aminophenylalanine.

of setting up complementation studies to check this possibility.

Biochemical studies also strongly support the hypothesis that a single mutation in *tyrR* is sufficient to explain these results. All the mutants isolated on the basis of *p*-FPA resistance on medium containing tryptophan, tyrosine, and shikimic acid have derepressed levels of DAHP synthetase (phe), DAHP synthetase (tyr), and the tyrosine biosynthetic enzymes. In addition, three *tyrR* mutants, isolated on the basis of APA resistance (23), also exhibit altered repression of the synthesis of DAHP synthetase (phe), although in this case there is over-repression rather than derepression.

Wallace and Pittard (23) postulated that *tyrR* codes for a protein aporepressor which, when combined with tyrosine, causes repression of DAHP synthetase (tyr) and the enzymes of the terminal pathway of tyrosine biosynthesis. In view of the present findings, it is now postulated that the aporepressor can (i) combine with tyrosine to control the regulon consisting of the *aroF tyrA* operon and the structural gene for transaminase A (Mattern and Pittard, *in press*) and (ii) combine with phenylalanine and repress the synthesis of DAHP synthetase (phe). The aporepressor must, therefore, have affinity for both tyrosine and phenylalanine. This is consistent with the variation in characteristics of the *tyrR* mutants, depending on the analogue and medium used in mutant isolation. APA is an analogue of tyrosine (19), and derepression of the tyrosine biosynthetic enzymes in APA-resistant mutants (23) suggests that the postulated aporepressor has lost affinity for tyrosine. On the other hand, *p*-FPA is an analogue of both phenylalanine and tyrosine (19). The *tyrR* mutations in strains isolated on *p*-FPA cause derepression of DAHP synthetase (phe) in addition to the derepression of the tyrosine biosynthetic enzymes. Hence, in these mutants, the postulated aporepressor must have lost affinity for both amino acids and for APA.

The demonstration that the wild-type allele is dominant over the mutant allele *tyrR363* is consistent with a system of negative control and with the hypothesis that *tyrR* is the gene which codes for an aporepressor.

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