

Regulation of Tyrosine Biosynthesis in *Escherichia coli* K-12: Isolation and Characterization of Operator Mutants

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Mutant strains of *Escherichia coli* have been isolated in which the synthesis of two of the enzymes involved in tyrosine biosynthesis, 3-deoxy-D-arabinoheptulosonic acid-7 phosphate synthetase (*tyr*) and chorismate mutase T-prephenate dehydrogenase, is partially constitutive. The mutations involved are closely linked to *aroF* and *tyrA*, the structural genes of these enzymes. The gene in which the mutations occur has been designated *aroK*, and the gene sequence is *aroK*, *aroF*, *tyrA*. In *aroK*⁺/*aroK* diploids, the *aroK* allele only affects the structural genes in the *cis* position. The mutant allele *aroK* is not recessive to *aroK*⁺ and *aroK*/*aroK*⁺ strains exhibit the *aroK* phenotype of resistance to 4-aminophenylalanine. It is proposed that *aroK* is an operator locus for an *aroF tyrA* operon.

The first step in the synthesis of the aromatic amino acids is the condensation of erythrose-4-phosphate and phosphoenolpyruvate to 3-deoxy-D-arabinoheptulosonic acid-7 phosphate (DAHP). In *Escherichia coli* this reaction is carried out by three isoenzymes, DAHP synthetase (*tyr*), DAHP synthetase (*phe*), and DAHP synthetase (*trp*). Both the synthesis and activity of each of these isoenzymes are controlled by the aromatic end products, tyrosine, phenylalanine, and tryptophan (7). The genes coding for these enzymes are *aroF*, *aroG*, and *aroH*, respectively.

Tyrosine is synthesized from chorismic acid, the end product of the common pathway, via three enzymatic reactions, mediated by chorismate mutase T, prephenate dehydrogenase, and transaminase A (7). The chorismate mutase T and prephenate dehydrogenase activities reside on a single protein, coded for by *tyrA* (3, 4, 13). A second chorismate mutase enzyme, chorismate mutase P, coded for by *pheA*, is involved in the biosynthesis of phenylalanine (3, 13).

The synthesis of the enzymes DAHP synthetase (*tyr*), chorismate mutase T-prephenate dehydrogenase and transaminase A is specifically controlled by tyrosine (16, 18, 19). The genes coding for the first two enzymes, *aroF* and *tyrA*, are closely linked on the chromosome, being co-transduced by bacteriophage P1kc at a high frequency (13). The structural gene for transami-

nase A has not yet been identified. Mutations in a gene *tyrR* situated at some distance on the chromosome from *aroF* and *tyrA* cause high-level constitutive synthesis of DAHP synthetase (*tyr*) and chorismate mutase T-prephenate dehydrogenase and prevent normal repression of the synthesis of transaminase A (19).

These observations and the isolation of a mutant strain of *E. coli*, which, as a result of a single mutation, has lost all DAHP synthetase (*tyr*) activity and synthesizes only low levels of chorismate mutase T-prephenate dehydrogenase (Wallace, unpublished data), support the hypothesis that the genes *aroF* and *tyrA* in *E. coli* are organized in a single operon.

Gollub and Sprinson (8) have recently described the isolation of a new class of regulator mutants in *Salmonella typhimurium* in which the mutations involved map close to both the *aroF* and *tyrA* genes and cause altered repression of the synthesis of DAHP synthetase (*tyr*) and prephenate dehydrogenase, although chorismate mutase T is reported not to be affected. On the basis of the behavior of these mutants, Gollub and Sprinson have proposed that *aroF* and *tyrA* are organized in a single operon in *S. typhimurium*.

It is the purpose of this paper to describe the isolation and characterization of a group of mutants in *E. coli* K-12 which have all the properties predicted for operator constitutive mutants of an *aroF tyrA* operon in which the formation of DAHP synthetase (*tyr*) and chorismate mu-

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tase T-prephenate dehydrogenase is coordinately controlled.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are all derivatives of *E. coli* K-12; their genotypes are listed in Table 1. The F' strain carrying F143 (KLF43/KL253) was obtained from Dr. Brooks-Low.

ϕ 80 virulent. This phage was obtained from J. Scaife, and was propagated in soft agar layers.

Chemicals. Chemicals used were obtained commercially and not further purified. D-Erythrose-4-phosphate dimethyl acetal dicyclohexylammonium salt (A grade) was obtained from Calbiochem, Los Angeles, Calif. Free erythrose-4-phosphate was prepared by the method of Ballou, Fischer, and MacDonald (2). Chorismic acid was prepared by using *A. aerogenes* 62-1 according to the method of Gibson (6). Barium prephenate was prepared by treating a solution of barium chorismate at 70 C for 1 hr and purified by chromatography on Dowex 1 resin.

Media. For the preparation of nutrient media and agars, Oxoid products were used. For synthetic media, full strength phosphate buffer mineral salts 56 of Monod et al. (11) was used, supplemented with glucose (0.5%), histidine, proline, arginine, isoleucine, leucine, and valine (at final concentrations ranging from 10^{-4} to 8×10^{-4} M) and thiamine-hydrochloride and nicotinic acid (10^{-6} M final concentration) when required. This medium is indicated in this publication as minimal medium. When aromatic end products were added, they were added in the following final concentrations: shikimic acid (10^{-6} M), L-phenylalanine and tryptophan (approximately 10^{-4} M), and L-tyrosine (10^{-3} M).

Transduction. Transductions involving phage P1kc were carried out as previously described (12).

Conjugation. The conjugation experiments were carried out as described in Adelberg and Burns (1).

Isolation of Trp⁻ strains. Mutants carrying a deletion in the *trp* region were isolated by selection for resistance to colicins V and B and to virulent ϕ 80 phage. The colicins were prepared as follows. A layer of soft agar containing 10^3 to 10^4 cells of the colicin-producing strain X178 was poured over a bottom layer of nutrient agar. After incubation for 18 hr at 37 C, the top layer was removed and residual cells were killed by exposure to chloroform vapor. A new nutrient agar layer was added, after allowing the plates to stand for some hours, a drop of a concentrated overnight culture was spread on these plates, together with a drop of a lysate of ϕ 80 virulent (titer 10^{11} particles/ml). Colonies appearing after 2 to 3 days of incubation at 37 C were purified and screened for tryptophan requirement. Approximately 5% of the colonies tested were Trp⁻.

Preparation of cell-free extracts. Cells were grown overnight in 250-ml quantities of the required medium. These cultures were then diluted to 1 liter with fresh medium, and incubation was continued until mid-exponential growth. Cells were harvested, washed with chilled 0.9% NaCl, suspended in 0.1 M phosphate buffer (pH 7.6), and disintegrated by being forced through a French press at a pressure of 20,000 psi. Cell debris was removed by centrifugation at $21,600 \times g$ for 20 min.

In initial experiments to detect those strains with elevated levels of DAHP synthetase (*tyr*) and chorismate mutase T, smaller volumes (20 to 200 ml) of the log-phase cells were washed, suspended in 0.1 M phosphate buffer (pH 7.0) to a final concentration of approximately 10^{10} cells/ml, and treated for 2×20 sec in an ultrasonic oscillator (output 3; Measuring & Scientific Equipment, Ltd.).

Assay of DAHP synthetase (EC 4.1.2.15). The method of Doy and Brown (5) was used for the assay of DAHP synthetase.

Assay of chorismate mutase. The method for the assay of chorismate mutase was based on that of Cotton and Gibson (3). Ethylenediaminetetraacetic acid (EDTA) and mercaptoethanol were added to the reaction mixture as described for the prephenate dehydrogenase assay.

Assay of transaminase A (EC 2.6.1.5). The method for assaying transaminase A was previously described (19).

Assay of prephenate dehydrogenase (EC 1.3.1.a). The method for the assay of prephenate dehydrogenase was based on that of Cotton and Gibson (3). A 0.4-ml amount of reaction mixture contained 2.0 μ moles of barium prephenate, 1.0 μ mole of nicotinamide adenine dinucleotide, 10 μ moles of tris(hydroxymethyl)amino-methane buffer (pH 7.8), 0.1 μ mole of EDTA, and 1.0 μ mole of mercaptoethanol.

Assay of 4-hydroxyphenylpyruvate. 4-Hydroxyphenylpyruvate, formed by the action of prephenate dehydrogenase, was assayed by the method of Schwinck and Adams using Millons reagent (15). Absorbancy at 490 nm was read within 1 min of adding the NaNO₂ reagent.

Protein estimation. Protein concentration in the extracts was measured by the method of Lowry et al. (10).

Specific activity. Specific activities are expressed as the number of 0.1 μ moles of substrate used or product formed in 20 min at 37 C per mg of protein.

RESULTS

Isolation of mutants. As we have described previously (19), mutant strains of *E. coli*, in which only DAHP synthetase (*tyr*) is functional, are growth inhibited by low levels (10^{-4} M) of the tyrosine analogue 4-aminophenylalanine (APA). APA is as efficient as tyrosine in its ability to repress the formation of this enzyme, but unlike tyrosine it does not function as an efficient feedback inhibitor (18).

One class of mutant strains which are able to overcome this growth inhibition is formed by the *tyrR* mutants which synthesize DAHP synthetase (*tyr*) constitutively and which are believed to form an inactive repressor substance (19). Another possible class of APA-resistant mutants would be mutants in which the operator locus of the *aroF* gene has been altered; thus it has a reduced affinity for repressor. If the genes *aroF* and *tyrA* constitute an operon, both DAHP synthe-

TABLE 1. Description of strains

Strain	Sex	Genotype ^a							
		<i>aroF</i>	<i>aroG</i>	<i>aroH</i>	<i>aroK</i>	<i>pheA</i>	<i>tyrA</i>	<i>trp</i>	<i>tyrR</i>
JP170	F ⁻	363 ^b	Δ ^c	367	+	+	+	+	+
JP250	F ⁻	+	365	367	+	2	+	+	+
JP323	F ⁻	+	365	367	+	+	+	365	+
JP502	F ⁻	+	365	367	+	+	352	+	+
JP510	F ⁻	+	Δ	367	+	2	+	+	+
JP515	F ⁻	+	365	367	375	+	+	+	+
JP518	F ⁻	+	365	367	376	2	+	+	+
JP519	F ⁻	+	365	367	377	2	+	+	+
JP524	F ⁻	+	365	367	375	+	+	370 ^d	+
JP526	F ⁻	+	365	367	375	+	+	+	352
JP529	F ⁻	+	Δ	367	378	2	+	+	+
JP530	F ⁻	+	Δ	367	379	2	+	+	+
JP531	F ⁻	+	Δ	367	380	2	+	+	+
AB3248	F ⁻	363	365	367	+	+	+	+	+
AB3253	F ⁻	+	365	367	+	+	+	+	+
AB3278	Hfr	+	365	367	+	+	+	+	352
AT2092	F ⁻	+	+	+	+	2	+	+	+
AT2273	F ⁻	+	+	+	+	+	352	+	+
KLF43/KL253	F ⁺ (F143)	+/+	+	+	+/+	+/+	+/-	+	+
X178 ^e	F ⁺	+	+	+	+	+	+	+	+
JP2009	F ⁻	388 ^f	Δ	367	378	2	+	+	+
JP2011	F ⁻	388	Δ	367	378	+	352	+	+
JP2038	F ⁺ (F143)	+ /388	Δ	367	+ /378	+ /+	+ /352	+	+

^a Only genes concerned with aromatic biosynthesis are listed. With the exception of AT2092, AT2273, X178, and KLF43/KL253, all strains require histine, proline, arginine, thiamine, isoleucine, and valine; the strains derived from JP170 require nicotinic acid in addition. AT2092 carries *purC* and *tsx*. Genotype symbols are used to represent the following genes: *aroF*, structural gene for DAHP synthetase (*tyr*); *aroG*, structural gene for DAHP synthetase (*phe*); *aroH*, structural gene for DAHP synthetase (*trp*); *aroK*, a gene controlling the expression of *aroF* and *tyrA* and presumed to be an operator locus for the *aroF tyrA* operon; *pheA*, structural gene for chorismate mutase P-prephenate dehydratase; *tyrA*, structural gene for chorismate mutase T-prephenate dehydrogenase; *trp*, any one of the structural genes for enzymes converting chorismate to tryptophan; *tyrR*, regulator gene controlling the expression of *aroF tyrA* and the gene for transaminase A.

^b Allele numbers were allotted in this laboratory.

^c Strain JP170 was isolated as a galactose-tolerant, nicotinic acid-requiring, DAHP synthetase (*phe*)-negative mutant, from a *galE aroG⁺ nicA⁺* strain and is presumed to have a deletion removing all or part of the *gal* operon, *aroG* and *nicA* (*unpublished data*).

^d The tryptophan requirement in strain JP524 was introduced in a selection for resistance to ϕ 80 virulent and colicins V and B and is presumed to involve a deletion of part of the tryptophan operon.

^e Strain X178 possesses an episome carrying the genes *colV* and *colB* and was used in the production of these colicins.

^f Mutant allele *aroF388* alters feedback sensitivity of DAHP synthetase (*tyr*).

tase (*tyr*) and chorismate mutase T-prephenate dehydrogenase should be derepressed in such operator mutants. If, on the other hand, they are not in a single operon, mutation of the operator next to *aroF* should only cause the derepression of DAHP synthetase (*tyr*).

For the selection of operator mutants of the *aroF* gene, we made use of the high cotransduction frequency of the *aroF* and *pheA* genes (13). An overnight culture of strain AB3253 (*aroF⁺ aroG aroH*) was plated to minimal medium containing 10^{-4} M APA and incubated for 18 hr at 37 C. APA-resistant colonies were obtained at a frequency of approximately 500 per plate and

presumably consisted of *aroG⁺* and *aroH⁺* revertants, permeability mutants, *tyrR* mutants, and operator mutants. The total growth was harvested from these selection plates and inoculated into nutrient broth. When the cultures had reached mid- to late-exponential phase they were used to prepare a P1 lysate. This lysate was then used to transduce *pheA⁺* into the recipient strain JP250 *pheA aroF⁺ aroG aroH*. The PheA⁺ transductants were screened for growth inhibition by APA. Out of the 100 PheA⁺ transductants tested, 31 were APA resistant, indicating that a mutation causing APA resistance is indeed located in the *pheA-aroF* region of the chromo-

some. Some of the transductants obtained were purified and screened for the level of DAHP synthetase (*tyr*) after growth in minimal medium with or without the aromatic end products. Whereas the parent strain AB3253 had specific activities of 5.0 and 0.2 in minimal medium and minimal medium supplemented with end products, respectively, the APA-resistant transductants gave values approximately 4-fold and 50-fold higher under the same conditions. An examination of the levels of chorismate mutase and prephenate dehydrogenase in one of these transductants, JP515, showed that the synthesis of these enzymes was also derepressed.

A disadvantage of this selection method is that the measured values for chorismate mutase reflect the combined activities of the two enzymes, chorismate mutase T and chorismate mutase P, which are coded for by the *tyrA* and *pheA* genes, respectively. To be able to measure chorismate mutase T activity alone, mutant strains were isolated which lacked a functional *pheA* gene. APA-resistant colonies were isolated from strain JP250 instead of AB3253, by plating separate overnight cultures (each inoculated with a small inoculum to avoid siblings) to minimal medium containing 10^{-2} M APA and 10^{-4} M phenylalanine. Phage P1 lysates were prepared as before and were used to infect recipients lacking all three DAHP synthetase isoenzymes, i.e., *aroF aroG aroH*. *AroF*⁺ transductants were selected on minimal medium supplemented with phenylalanine. Two recipient strains were used, AB3248 and JP170. The first strain has a point mutation in the *aroG* gene; in the second strain, however, *aroG* is presumed to have been deleted in an event which removed a region of deoxyribonucleic acid extending from *gal* to *nic* (*unpublished data*). This latter strain was used to reduce the number of reversions at the *aroG* locus.

The transductants were screened for their requirement for phenylalanine and for their ability to grow on minimal medium supplemented with 10^{-2} M APA and 10^{-4} M phenylalanine. Any strains that were both Phe⁻ and APA resistant were screened for the levels of the enzymes of the tyrosine pathway: DAHP synthetase (*tyr*), chorismate mutase T, prephenate dehydrogenase. Most of the transductants showed an increased level of these enzymes. Five transductants, each obtained in a separate experiment, were chosen for detailed study: JP518, JP519, derived from AB3248, and JP529, JP530, JP531, derived from JP170.

These mutants show a slight cross-feeding of *tyrA* strains; in each case, DAHP synthetase (*tyr*) is still sensitive to feedback inhibition by tyrosine (approximately 90% inhibition at 10^{-3} M tyrosine), indicating that the feedback-sensitive site of the enzyme has not been altered.

Enzyme levels in 4-aminophenylalanine-resistant mutants. The 4-aminophenylalanine-resistant mutant strains were grown in minimal medium containing 10^{-4} M phenylalanine and in minimal medium containing all of the aromatic end products. Cell-free extracts were prepared as described above and assayed for DAHP synthetase (*tyr*), chorismate mutase T, prephenate dehydrogenase, and, in some cases, transaminase A. The results are reported in Table 2 where it can be seen that the synthesis of DAHP synthetase (*tyr*), chorismate mutase T, and prephenate dehydrogenase is derepressed in the mutants; in every case, partial repression by the aromatic end products still occurs. Transaminase A activity on the other hand is not affected in the mutants. When the values for the different enzyme activities are plotted one against the other, all three activities are seen to be coordinately expressed (Fig. 1). Since chorismate mutase T

TABLE 2. Specific activities of enzymes in parent and mutant strains grown in minimal medium containing 10^{-4} M phenylalanine (MM) and in minimal medium supplemented with the aromatic amino acids and shikimic acid (EP)^a

Strain	DAHP synthetase (<i>tyr</i>)		Chorismate mutase T		Prephenate dehydrogenase		Transaminase A	
	MM	EP	MM	EP	MM	EP	MM	EP
JP250	10.0	<1	8.8	<1	7.5	<1	10.4	7.7
JP510	3.8	<1	4.5	<1	4.1	<1	NT ^b	NT
JP518	24.0	9.8	17.6	9.3	25.0	11.5	NT	6.3
JP519	21.4	9.6	17.6	5.4	16.6	9.2	9.6	5.6
JP529	15.5	7.1	19.5	4.0	16.5	6.2	NT	2.8
JP530	11.7	10.9	17.0	9.4	13.7	10.3	NT	3.6
JP531	33.2	13.5	32.2	13.2	26.3	17.6	NT	3.6

^a Final levels of aromatic supplements were L-tyrosine (10^{-3} M), L-phenylalanine and L-tryptophan (10^{-4} M), and shikimic acid (10^{-6} M).

^b Not tested.

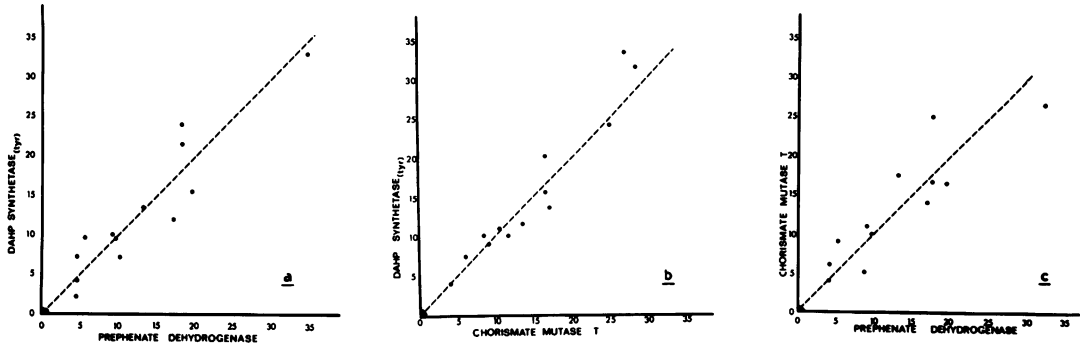


FIG. 1. Plot of specific activities of DAHP synthetase (*tyr*), prephenate dehydrogenase and chorismate mutase *T* obtained in various experiments in which parent and mutant strains were grown in either minimal media or minimal media supplemented with aromatic endproducts (see Table 2). (a) DAHP synthetase (*tyr*)-prephenate dehydrogenase, (b) DAHP synthetase (*tyr*)-chorismate mutase *T*, (c) chorismate mutase *T*-prephenate dehydrogenase.

and prephenate dehydrogenase activities are known to reside on a single protein (3, 4), the coordinated expression of these two activities was to be expected. The observation that the levels of DAHP synthetase (*tyr*) also alter in a coordinate fashion with either chorismate mutase *T* or prephenate dehydrogenase is consistent with the notion that the structural genes for DAHP synthetase (*tyr*) and chorismate mutase *T*-prephenate dehydrogenase are in the same operon.

Genetic analysis of the mutants. As a result of the selection procedure used in the isolation of the mutants, the mutations conferring resistance to 4-aminophenylalanine were known to be cotransducible with *pheA*. Further transduction analyses, however, were carried out with some of the mutants to determine more precisely the position of the mutations relative to *pheA*, *aroF*, and *tyrA*. The presumed operator gene in which these mutations have occurred has been designated *aroK*; thus, for example, although JP250 and AB3253 are *aroK*⁺, JP515 is *aroK*³⁷⁵ and JP529 is *aroK*³⁷⁸.

The results of the transduction experiments are reported in Table 3 and suggest that the relative gene order is as shown in Fig. 2.

Nature of partial repression in mutant strains. The synthesis of the tyrosine enzymes in the *tyrR* mutants previously described (19) is derepressed to higher levels than in the mutants reported in this paper and furthermore is not subject to significant repression in the presence of the aromatic end products. If the partial repression occurring in the *aroK* mutants involves residual function of the *aroK* gene, this should require functional *tyrR* gene product for expression, and the introduction of the *tyrR* allele into these strains should abolish this partial repression. Since the *tyrR* mutation is closely linked to the *trp* operon, this mutation was introduced into

strain JP515 as follows. Mutant strains resistant to ϕ 80 virulent and colicins V and B were obtained from a His⁺ revertant of JP515. Approximately 5% of these colonies required tryptophan for growth, and one of these, JP524, was used as a recipient in a cross with the *tyrR* male AB3278. Mating was interrupted after 40 and 50 min, respectively, and selection was made for Trp⁺ recombinants in minimal medium lacking histidine. Recombinants were purified and tested for the level of DAHP synthetase (*tyr*) in extracts prepared from cells grown in the presence of the aromatic end products. The results for one of these recombinants, JP526, and for the parents, JP515 and AB3278, are shown in Table 4. It can be seen that the enzyme levels in JP526 resemble those found in AB3278, i.e., the introduction of the *tyrR* allele has abolished repression. To verify that this recombinant strain did carry both the *tyrR* and the *aroK* alleles, a P1 lysate was prepared and used to transduce *pheA*⁺ into JP250 and *trp*⁺ into JP323. Eighty per cent of the *pheA*⁺ and 3% of the *trp*⁺ transductants were 4-aminophenylalanine resistant in accordance with the cotransduction frequencies of the *tyrR* and *aroK* alleles with *trp* and *pheA*, respectively. In contrast, P1 lysates prepared on other recombinants of the cross JP524 \times AB3278, which did not show increased levels of DAHP synthetase (*tyr*), yielded only 4-aminophenylalanine-resistant transductants in the PheA⁺ selection.

Examination of the phenotypes of *aroK*⁺/*aroK* diploids. Early experiments had indicated that the *aroK* mutations were dominant in diploids, as in transduction experiments in which selection was made for the 4-aminophenylalanine resistance phenotype; the same number of transductants was obtained irrespective of whether cells were plated directly to 4-aminophenylalanine containing media or whether 4 to 6 hr in the ab-

TABLE 3. Cotransduction frequencies of various *pheA*, *aroK*, *aroF*, and *tyrA* alleles^a

Donor	Recipient	<i>aroK375</i>	<i>aroK375</i>	<i>aroF363</i>	<i>aroF363</i>	<i>pheA2</i>	<i>aroK375</i>	<i>aroK378</i>
		in <i>pheA</i> ⁻ (%)	in <i>tyrA</i> ⁻ (%)	in <i>pheA</i> ⁻ (%)	in <i>tyrA</i> ⁻ (%)	in <i>tyrA</i> ⁻ (%)	in <i>aroF</i> ⁻ (%)	in <i>tyrA</i> ⁻ (%)
JP515 <i>aroK375 pheA</i> ⁺	JP250 <i>aroK</i> ⁺ <i>pheA2</i>	87						
JP515 <i>aroK375 tyrA</i> ⁺	JP502 <i>aroK</i> ⁺ <i>tyrA352</i>		86					
AB3248 <i>aroF363 pheA</i> ⁺	AT2092 <i>aroF</i> ⁺ <i>pheA2</i>			88				
AB3248 <i>aroF363 tyrA</i> ⁺	JP502 <i>aroF</i> ⁺ <i>tyrA352</i>				86			
JP250 <i>tyrA</i> ⁺ <i>pheA2</i>	JP502 <i>tyrA352 pheA</i> ⁺					50		
JP515 <i>aroK375 aroF</i> ⁺	JP170 <i>aroK</i> ⁺ <i>aroF363</i>						95	
JP529 <i>aroK378 pheA2 tyrA</i> ⁺	JP502 <i>aroK</i> ⁺ <i>pheA</i> ⁺ <i>tyrA352</i>					40		88

^a Between 100 and 200 transductants were scored for unselected markers in each case.

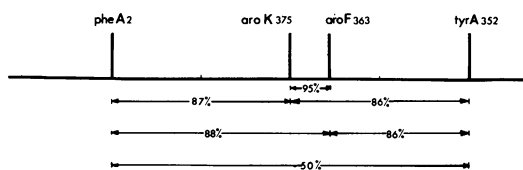


FIG. 2. Diagrammatic representation of the relative order of the mutations *pheA2*, *aroK375*, *aroF363*, and *tyrA352* obtained from transduction studies. Although not shown, *aroK378* gives identical results to *aroK375*.

TABLE 4. Specific activity of DAHP synthetase (*tyr*) in *aroK*, *tyrR*, and *aroK tyrR* strains

Strain	Grown in minimal medium	Grown in minimal medium plus aromatic amino acids and vitamins
JP515 (<i>aroK375</i>)	29	12
AB3278 (<i>tyrR352</i>)	82	64
JP526 (<i>aroK375 tyrR352</i>)	57	55

sence of 4-aminophenylalanine were allowed for phenotypic expression and nuclear segregation. This dominance was confirmed when it became possible to make partial diploid strains using the F' strain KLF43/KL253 kindly supplied by B. Low. The F-merogenote F'143 which includes both the *aroF* and the *tyrA* genes was transferred by conjugation into each of the mutants and into the parent strain JP250 selecting for the Phe⁺ phenotype. Such repliconates were purified, checked for their ability to donate F'143, and tested for their sensitivity to APA. The strain JP250/F'143 was still sensitive to growth inhibi-

tion by APA, whereas the partial diploids formed with each of the mutants were APA resistant. Enzyme assays carried out on extracts of all these strains prepared from cells grown in the presence of the aromatic end products confirmed that the synthesis of DAHP synthetase (*tyr*) was still fully repressed in the partial diploid prepared from JP250 but partially derepressed in the diploids formed with the mutant strains (Table 5). In all of these experiments, samples of the cell population were taken immediately before disintegrating the cells and examined for the presence of *pheA* segregants. Between 95 and 100% of the cells in every case were Phe⁺ and good male donors of F'143.

The same high percentage of cells was still resistant to 4-aminophenylalanine, indicating that negligible gene conversion to the *aroK*⁺/*aroK*⁺ state had occurred in these cultures.

TABLE 5. Specific activities of DAHP synthetase (*tyr*) in partial diploids possessing F'143

Strain	Extracts prepared from cells grown in	
	Minimal medium	Minimal medium plus aromatic end products
JP250/F143	2.6	0.7, 0.3, ^a 0.3
JP518/F143	30	5.5
JP519/F143	14.3	5.8, 4.1, 3.7
JP529/F143	12.3	4.6, 3.7, 3.0
JP530/F143	NT ^b	6.1
JP531/F143	NT	3.7

^a Each value represents the results obtained with a different cell-free extract.

^b Not tested.

Effect of *aroK* on genes in the trans position.

To carry out this test, it was necessary to create a diploid in which one of the genes *cis* to *aroK378*, either *aroF* or *tyrA* was nonfunctional. This was done as follows. Strain JP529 is growth inhibited by tyrosine (10^{-3} M) in the presence of phenylalanine, because of the sensitivity of DAHP synthetase (*tyr*) to inhibition by tyrosine. By plating cells of JP529 to minimal medium supplemented with tyrosine (10^{-3} M) and phenylalanine (10^{-4} M), it was possible to select mutants which made a feedback-resistant DAHP synthetase (*tyr*). Strain JP2009 is such a feedback-resistant derivative of JP529, and we have designated the mutant allele *aroF388*. Strain JP2009 was then used as a recipient in a transduction in which phage P1 propagated on the tyrosine auxotroph AT2273 was used to transduce the *pheA*⁺ gene. Selection was made on minimal medium supplemented with tyrosine (10^{-3} M) so that only *pheA*⁺ transductants that had retained both the *aroK378* and the *aroF388* alleles of JP2009 would grow. These were then examined for their inheritance of the *tyrA352* allele of AT2273. Two of the 140 transductants tested were Tyr⁻ and therefore had the presumed genotype *aroK378 aroF388 tyrA352*. One of these, JP2011 was used as a recipient in a cross with the F' KLF43/KL253 strain, and selection was made for Tyr⁺ conjugatants. These were purified and checked for ability to donate F'143. The repliconate chosen for the enzyme studies has been given the strain number JP2038 and has the genotype, *aroK378, aroF388, tyrA352/F' aroK⁺ aroF⁺ tyrA⁺*. In this strain, therefore, the only functional *tyrA* gene is *cis* to *aroK⁺* and *trans* to *aroK378*. The *aroF* allele coding for a feedback-resistant form of DAHP synthetase (*tyr*) is, on the other hand, *cis* to the mutant *aroK378* allele. If *aroK378* can only function in the *cis* configuration, we would expect that extracts of JP2038 prepared from cells grown in the presence of the aromatic end products should have no prephenate dehydrogenase activity, whereas levels of DAHP synthetase activity, on the other hand, should resemble that found in extracts of JP2009 and should be feedback resistant. If *aroK378* can function in the *trans* configuration, prephenate dehydrogenase levels in JP2038 should equal those observed in JP2009 and approximately half the DAHP synthetase (*tyr*) activity should be feedback sensitive. As can be seen from Table 6, extracts of JP2038 have no detectable dehydrogenase activity and the DAHP synthetase is fully resistant to feedback inhibition. We conclude from these results that the *aroK378* allele is only able to exert an effect in the *cis* position.

TABLE 6. Specific activities of DAHP synthetase (*tyr*) and prephenate dehydrogenase in extracts from cells grown in the presence of aromatic end products

Strain	Genotype	DAHP Synthetase (<i>tyr</i>)	Prephenate dehydrogenase
JP529	<i>aroK378 aroF⁺ tyrA⁺</i>	7.1	6.2
JP529/ F143	<i>aroK378 aroF⁺ tyrA⁺ / aroK⁺ aroF⁺ tyrA⁺</i>	3.0	3.3
JP2009	<i>aroK378 aroF388^a tyrA⁻</i>	2.2 ^b	4.5
JP2011	<i>aroK378 aroF388 tyrA352</i>	3.0 ^b	0
JP250	<i>aroK⁺ aroF⁺ tyrA⁺</i>	0	<1
JP2038	<i>aroK378 aroF388 tyrA352 / aroK⁺ aroF⁺ tyrA⁺</i>	2.8 ^b	<0.4

^a *aroF388* mutation alters feedback sensitivity of DAHP synthetase (*tyr*).

^b This DAHP synthetase activity is not sensitive to inhibition by 2×10^{-4} M L-tyrosine, which causes 90% inhibition of wild-type DAHP synthetase (*tyr*).

DISCUSSION

The properties of the *aroK* mutants described in this paper are (i) resistance to growth inhibition by the tyrosine analogue 4-amino-phenylalanine, (ii) close linkage of the *aroK* mutations to the structural genes *aroF* and *tyrA*, (iii) coordinate derepression of the synthesis of DAHP-synthetase (*tyr*) and chorismate mutase T-prephenate dehydrogenase, (iv) that all partial diploids with an *aroK/aroK⁺* genotype exhibit an AroK⁻ phenotype, (v) that one *aroK* mutant allele has been shown to affect only the genes in the *cis* position.

These properties strongly suggest that the genes *aroF* and *tyrA* are organized in one operon for which *aroK* is the operator.

The fact that the regulation of the synthesis of transaminase A, which catalyses the last step in the tyrosine biosynthetic pathway, is not altered in the mutant strains, suggests that the structural gene for this enzyme is not part of the *aroF tyrA* operon.

By using a different selection method, Gollub and Sprinson have isolated mutants of *S. typhimurium* which they believe to have mutations in an operator locus of an *aroF tyrA* operon (8). The essential differences between their mutants and the strains reported here are the following. (i) Although derepressed in minimal medium, the synthesis of the enzymes in their mutants is fully repressed in media containing 5.5×10^{-4} M tyrosine. (ii) Whereas the synthesis of DAHP synthetase (*tyr*) and prephenate dehydrogenase is derepressed, the level of chorismate mutase T is unaltered.

The synthesis of the tyrosine biosynthetic enzymes in the *E. coli* mutants is still partially repressible by tyrosine, as is the case with the operator mutants of the tryptophan operon (9). The introduction of the *tyrR* mutation, however, prevents this partial repression, suggesting that it represents residual binding capacity of the operator for the repressor.

Although the results indicate that, in the trans position, *aroK*⁺ is not dominant over *aroK* in so much as *aroK*⁺/*aroK* cells do not exhibit an *AroK*⁺ phenotype, a comparison of Table 2 and Table 5 shows that DAHP synthetase (*tyr*) levels in *aroK*⁺/*aroK* cells grown in the presence of end products are approximately one half those obtained in the haploid *aroK* strains. This difference does not appear to be caused by either gene conversion or segregation. It would not be predicted in terms of the simplest model of operator function, and we currently have no explanation for these findings.

Attempts to implicate the tyrosine specific activating enzyme in the repression of DAHP synthetase (*tyr*) have to date been unsuccessful (14, 17). It appears therefore that the effective corepressor for the *aroF tyrA* operon may be tyrosine itself. The regulator gene *tyrR* has been shown to code for a product required for normal repression (19) and may in fact code for an aporepressor.

Although *pheA* is located at approximately the same distance from *aroK* as is *tyrA*, the expression of this gene is not regulated by tyrosine. In *tyrR* mutants as well as in an *aroK* mutant that is also *pheA*⁺ (JP515), the synthesis of chorismate mutase P-prephenate dehydratase is not derepressed. Similarly, mutations affecting a presumed operator locus of *pheA* cause marked derepression of the synthesis of chorismate mutase P-prephenate dehydratase, without affecting the rate of synthesis of the gene product of *tyrA* (Im and Pittard, *J. Bacteriol.*, *in press*).

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