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Distribution and Function of Genes Concerned with Aromatic Biosynthesis in *Escherichia coli*

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ABSTRACT

PITTARD, JAMES (School of Microbiology, University of Melbourne, Victoria, Australia), AND B. J. WALLACE. Distribution and function of genes concerned with aromatic biosynthesis in *Escherichia coli*. J. Bacteriol. **91**:1494–1508. 1966.—A number of mutant strains of *Escherichia coli* K-12, which are blocked in the biosynthesis of the aromatic amino acids, were examined biochemically to determine their particular enzymatic deficiencies. The mutations carried by these strains were mapped by use of the methods of conjugation and transduction. Structural genes for five of the enzymes of the common pathway leading to chorismate and for the two enzymes converting chorismate to phenylpyruvate and *p*-hydroxyphenylpyruvate, respectively, were identified. Unlike the genes of the tryptophan operon, most of these genes are distributed over widely separated regions of the chromosome.

The various enzymatic reactions by means of which the bacterium *Escherichia coli* converts erythrose-4-phosphate and phosphoenolpyruvate to chorismate are now well established (13, 25). Chorismate is a key intermediate in aromatic biosynthesis, because it is the last intermediate in the common pathway and, as such, can be converted enzymatically via a number of different pathways into the end products tyrosine, phenylalanine, tryptophan, ubiquinone, vitamin K, and folic acid (5, 6, 10, 11).

Mutant strains blocked in any of the reactions leading to chorismate show a multiple requirement for two or more of the aromatic amino acids and vitamins depending on the completeness of the block. Such mutants have been referred to as multiple aromatic auxotrophs as distinct from strains blocked in any of the specific pathways leading from chorismate to tryptophan, phenylalanine, or tyrosine, these latter strains being known as tryptophan auxotrophs or phenylalanine auxotrophs, etc.

Very little is known about the distribution on the *E. coli* chromosome of the structural genes for each of the enzymes involved in the reactions of the common pathway. The mutations carried by some aromatic mutants have been mapped (24), but, since no biochemical investigations were carried out, the functions of the mapped genes were unknown. It is the purpose of this communication to describe the chromosomal location and the specific function of five of the *aro* genes concerned with the common pathway and of one of the *phe* and one of the *tyr* genes concerned with the phenylalanine and tyrosine pathways, respectively. (For a description of symbols used, see Table 1.) Some of these mutant strains have been obtained from A. L. Taylor, some from R. Somerville, and the rest have been isolated in these laboratories.

To locate the genes on the chromosome, we have used both interrupted mating techniques and phage P1kc-mediated transductions (this phage will subsequently be referred to as P1). The data from interrupted mating experiments make it possible to assign to a gene a definite chromosomal location, and this method has been used in the construction of most chromosomal maps. The accuracy of the method, however, does not make it possible to distinguish genes that are separated by less than 1 to 2% of the *E. coli* genome. Since the *E. coli* chromosome may contain as many as 5,000 genes, mutations separated by less than 50 genes may not be separable by this method.

Phage P1 is unable to cotransduce two chromosomal markers that are separated by more than approximately 2% of the *E. coli* genome. When two markers are cotransducible, however, an estimation of the distance between them can be made by measuring the frequency with which they are separated by recombination events. This may be measured by use of a wild-type donor with a recipient that carries both mutant alleles, in which

case the frequency at which the wild-type alleles are cotransduced will vary inversely with the distance that separates them. This distance may also be measured if one mutation is present in the donor strain and the other in the recipient. In this case, the frequency with which wild-type transductants are obtained will vary directly with the distance that separates the two mutations. In general, transduction studies involving P22-*Salmonella typhimurium* systems and P1-*E. coli* systems indicate that, if two mutations occur in a single gene, then the frequency with which wild-type transductants are obtained in crosses between mutants will vary between about 5 and 0% of that obtained with wild-type donor phage, depending on the relative positions of the mutations within the gene (15, 21, 28).

In some cases in this work, where preliminary conjugation experiments indicated that a number of different mutants constituted a single genotype, only one or two members of the group were examined in interrupted mating experiments, and the group identity of the remainder was verified by transduction tests. In those cases where we have carried out time of entry experiments on mutant strains already mapped by Taylor and Thoman (24), our results do not differ significantly from theirs.

We have retained the genotype symbols used by Taylor and Thoman (24), except in two cases, in which we found that mutations to which they have given separate gene designations could be shown to be located within a single gene. These findings have raised certain problems with regard to nomenclature, which we propose to deal with as follows. In the case of mutations which Taylor and Thoman (24) have listed as occurring in two genes, *aro-C* and *aro-B*, and which we found to occur within a single gene, we have made the following changes. For reasons to be discussed later, we have retained the *aro-C* designation for both mutations and used the *aro-B* designation for one of the aromatic genes which had not previously been mapped. In the case of the two mutations which Taylor and Thoman (24) have placed in the two genes *phe-A* and *phe-B* and which we found to occur within a single gene, we have retained the *phe-A* designation for both mutations. Since we have not located any other genes concerned with phenylalanine biosynthesis, *phe-B* does not occur on our map. The specific reactions by means of which erythrose-4-phosphate and phosphoenolpyruvate are converted to chorismate and then to phenylalanine and tyrosine are shown in Fig. 1. The genetic symbols used to identify the structural genes for each of these enzymes are included in the figure.

It is to be noted that the sequence of uppercase letters used to describe different structural genes does not necessarily correspond to the sequence of enzymatic steps. For example, *aro A* is concerned with the enzyme carrying out the sixth reaction of the common pathway and not the first.

MATERIALS AND METHODS

Organisms. The strains used in this work are all derivatives of *E. coli* K-12 and are described in Table 1. The order of transfer of chromosomal genes by the Hfr strains is shown in Table 2.

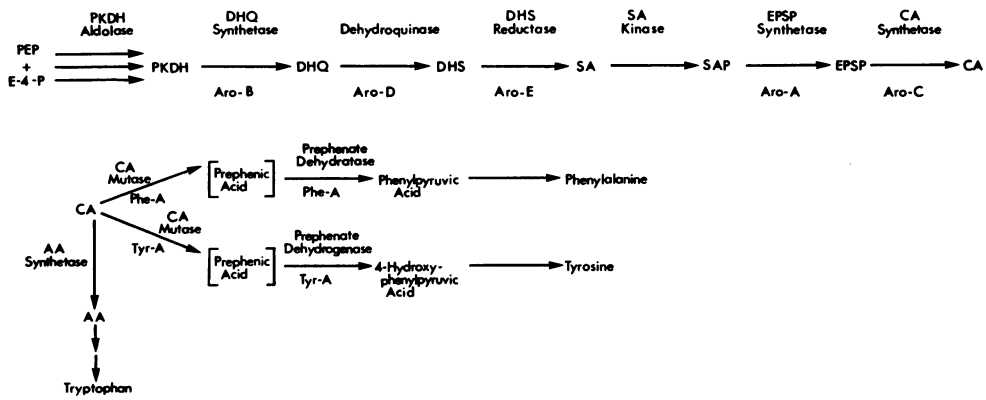

FIG. 1. *An outline of the reactions involved in the conversion of erythrose-4-phosphate and phosphoenolpyruvate to chorismate, and of chorismate to phenylalanine and tyrosine. The genetic symbols for each of the known structural genes are also included. Symbols used: PEP, phosphoenolpyruvate; E-4-P, erythrose-4-phosphate; PKDH, 7-phospho-2-keto-3-deoxy-D-arabino-heptonate; DHQ, dehydroquinate; DHS, dehydroshikimate; SA, shikimate; SAP, shikimate-5-phosphate; EPSP, 3-enolpyruvylshikimate-5-phosphate; CA, chorismate; AA, anthranilate; aro B, the structural gene for DHQ synthetase; aro D, the structural gene for dehydroquinase, etc.*

TABLE 1. *List of strains*[a]

Strain no.	Previous no.	Genetic loci relevant to this work[b]							Sex
		aro A	*aro B*	*aro C*	*aro D*	*aro E*	*phe A*	*tyr A*	Other loci	
AT2022	—[d]	+	+	+	+	+	1[c]	+	*pro⁻ T6ʳ*	F⁻
AT2092	—[d]	+	+	+	+	+	2	+	*pur C⁻ T6ʳ*	F⁻
AB2861	*phe* 1/1[e]	+	+	+	+	+	351	+		F⁻
AB2859	*tyr* 2/3[e]	+	+	+	+	+	+	351		F⁻
AT2273	—[d]	+	+	+	+	+	+	352	*T6ʳ his⁻*	F⁻
AT2471	—[d]	+	+	+	+	+	+	4	*thi⁻ str-s*	♂ Hfr
AB478	—[d]	2	+	+	+	+	+	+	*pro⁻ str-s*	F⁻
AB2860		2	+	+	+	+	+	+	*pro⁻ str-r*	F⁻
AB2852		358	+	+	+	+	+	+	*pro⁻ arg⁻*	F⁻
AB2858		358	+	+	+	+	+	+	*pro⁻ arg⁻ try⁻*	F⁻
AB2853		359	+	+	+	+	+	+	*pro⁻ arg⁻*	F⁻
AB2855		361	+	+	+	+	+	+	*pro⁻ arg⁻*	F⁻
AB2851		357	+	+	+	+	+	+	*pro⁻ arg⁻ str-s*	F⁻
AB2856		357	+	+	+	+	+	+	*pro⁻ arg⁻ str-r*	F⁻
AB2829	10.5[e]	354	+	+	+	+	+	+		F⁻
AB2854		360	+	+	+	+	+	+	*pro⁻ arg⁻*	F⁻
AB2826	10.1[e]	+	351	+	+	+	+	+		F⁻
AB2847		+	351	+	+	+	+	+	*mal⁻ T6ʳ*	F⁻
AB347	—[d]	+	+	4	+	+	+	+	*thr⁻ leu⁻*	♂ Hfr
AB2830	10.6[e]	+	+	355	+	+	+	+		F⁻
AB2849		+	+	355	+	+	+	+	*T6ʳ*	F⁻
AB2850		+	+	356	+	+	+	+		F⁻
AB477	—[d]	+	+	1	+	+	+	+		F⁻
AB1360	—[d]	+	+	+	362	+	+	+	*his⁻ T6ʳ*	F⁻
AB2827	10.2[e]	+	+	+	352	+	+	+		F⁻
AB2848		+	+	+	352	+	+	+	*T6ʳ*	F⁻
AT2472	—[d]	+	+	+	+	24	+	+		♂ Hfr
AB2828	8.5[e]	+	+	+	+	353	+	+		F⁻
AB2834		+	+	+	+	353	+	+	*T6ʳ mal⁻*	F⁻
AB313		+	+	+	+	+	+	+	*thr⁻ leu⁻ thi⁻ T6ˢ*	♂ Hfr
AB311		+	+	+	+	+	+	+	*thr⁻ leu⁻ thi⁻ T6ˢ*	♂ Hfr
AB259		+	+	+	+	+	+	+	*thi⁻ str-s*	♂ Hfr

[a] The following abbreviations are used: *aro*, aromatic amino acids and vitamins; *pro*, proline; *pur*, purine; *thi*, thiamine; *arg*, arginine; *his*, histidine; *thr*, threonine; *leu*, leucine; *ilv*, isoleucine and valine; *phe*, phenylalanine; *tyr*, tyrosine; *mal*, maltose; *xyl*, xylose; *str*, streptomycin; *T6*, bacteriophage T6; (O), origin.

[b] For a description of the enzymes affected by mutations in the *aro A*, *aro B*, *aro C*, *aro D*, *aro E*, *phe A*, and *tyr A* genes, see Fig. 1.

[c] Numbers refer to allele numbers allotted to mutant strains in this laboratory and in the laboratories of E. A. Adelberg and A. L. Taylor.

[d] These strains or their derivatives are referred to in the paper of Taylor and Thoman (24). Strain AB347 carries the *aro 4* allele of strain AB444, and strain AB477 carries the *aro 1* allele of strain AB1320.

[e] These strains were provided by R. Somerville.

TABLE 2. *Order of transfer of chromosomal genes by Hfr strains**

Strain no.	Order of transfer
AB313	(O) *xyl-mal-str-pur C-his-try-pro-leu-ilv-sex factor*
AB311	(O) *his-try-pro-leu-ilv-xyl-mal-str-pur C-sex factor*
AB259	(O) *leu-pro-try-his-pur C-str-mal-xyl-ilv-sex factor*

* For abbreviations, see first footnote in Table 1.

Transducing phage P1 was obtained from N. Schwartz.

Media and culture methods. The media and culture methods used in this work have been described by Adelberg and Burns (1).

Buffers. The tris(hydroxymethyl)aminomethane (Tris)-HCl, sodium phosphate, and Veronal-HCl buffers which have been used were prepared according to the methods of Dawson et al. (8).

Mating conditions. The conditions under which mating was carried out were identical to those described by Taylor and Thoman (24), except that the diluted cells were incubated in 200-ml bottles instead of 1-liter Erlenmeyer flasks.

Nutritional tests. A 0.1-ml amount of a light suspension (approximately 10^6 cells per milliliter) of cells was added to a soft-agar layer and poured onto a minimal-medium plate supplemented with all the amino acids required for growth, except the aromatic amino acids. When the layer had set, a sterile filter-paper disc was placed on the surface of the plate, and one drop of a 1 mg/ml solution of the nutrient being tested was added to the disc. Plates were incubated for 48 hr at 37 C, and the results were read.

Interruption of mating. Samples (1.0 ml) were removed from the bottles at 2- to 5-min intervals and transferred to screw-capped tubes containing 1 ml of bacteriophage T6 [approximately 10^{10} plaque-forming units (PFU) per ml]. The cell-phage mixture was immediately agitated in a Vortex Junior mixer for 45 sec to disrupt mating pairs, and then incubated at 37 C for 10 min to allow the phage to adsorb to the T6-sensitive male parents. Phage T6 was used to kill the male parent in crosses involving the Hfr strains AB313 and AB311. Any phage-resistant mutants of the male parent were unable to grow on the selective medium which lacked amino acids necessary for their growth. When the streptomycin-sensitive Hfr strain AB259 was used, however, the exconjugant male cells were killed by the streptomycin in the medium, and phage T6 was not used.

Transduction techniques. Transduction techniques used in this work have been described (19).

Syntrophism tests. The method described by Gibson and Jones (12) was used.

Isolation of mutants. The method described by Adelberg and Meyers (3) was used. In most cases, ultraviolet light was the mutagenic agent, but, in some cases, it was *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. When the latter mutagen was used, the procedure described by Adelberg, Mandel, and Chen (2) was followed.

Preparation of cell-free extracts. Cells harvested from 18-hr cultures were washed with chilled 0.9% NaCl and then suspended in 4 ml per 1 g (wet weight) of either 0.1 M Tris (*p*H 7.8) or 0.1 M phosphate buffer (*p*H 7.6). Cell breakage was achieved by forcing cells through a French press at a pressure of 20,000 psi.

Cell-free extracts were obtained after centrifugation at 21,600 × *g* for 20 min, and were stored at −20 C.

Column chromatography of enzymes. This was carried out by use of the method described by Cotton and Gibson (5).

Assay of dehydroquinate (DHQ) synthetase. The method described by Srinivasan and Sprinson (23) was used.

Assay of dehydroquinase. The method described by Mitsuhashi and Davis (17) was used. In these assays, since no dehydroquinic acid was available, the substrate for the reaction was the filtered supernatant fluid containing the accumulation products of *E. coli* 83-1 (*see* 26).

Assay of dehydroshikimate (DHS) reductase. The method described by Yaniv and Gilvarg (27) was used. Cell-free extracts were assayed for their ability to convert shikimate to dehydroshikimate. Dehydroshikimate formed was estimated by measuring optical absorbance at 234 mμ in a Unicam SP500 spectrophotometer.

Assay of shikimate kinase. The following incubation mixture was used: shikimic acid, 2 μmoles; adenosine triphosphate (ATP), 4 μmoles; $MgCl_2$, 5 μmoles; cell-free extract, 1 to 2 mg of protein; 0.05 M Veronal buffer (*p*H 9.0) to a total volume of 2.0 ml.

Kinase activity was determined by measuring the disappearance of shikimate according to the method of Gaitonde and Gordon (9).

Estimation of shikimate-5-phosphate. This was measured by conversion to shikimate with alkaline phosphatase according to the method of Morgan, Gibson, and Gibson (18).

Assay of 3 - enolpyruvyl - shikimate - 5 - phosphate (EPSP) synthetase and chorismate synthetase. Since EPSP was not available for use as a substrate, cell-free extracts were examined for their ability to carry out the overall conversion of shikimate to anthranilate. The general conditions used for this assay have already been described by Morgan et al. (18). Shikimate was used rather than shikimate-5-phosphate as it was readily available, and all the strains being examined were tested for their ability to convert shikimate to shikimate-5-phosphate. Anthranilate was measured rather than chorismate because of the ease and sensitivity of its fluorimetric estimation. Although some of the chorismate formed from shikimate can be converted to prephenate by these extracts, this amount was kept to a minimum by preparing cell-free extracts from cells in which anthranilate synthetase was derepressed. This was achieved by growing cells in a minimal medium containing excess tyrosine and phenylalanine (2×10^{-4} M), *p*-aminobenzoic and *p*-hydroxybenzoic acids (2×10^{-6} M), and limiting indole at a concentration of 1.5×10^{-5} M.

Complementation tests. Cell-free extracts unable to carry out the overall conversion of shikimate to anthranilate were mixed in various combinations and tested for their ability to complement each other in the overall reaction. As a result of these tests, cell-free extracts could be directed into two groups (groups I and II), significant conversion of shikimate to anthranilate only being observed when extracts from both groups were present.

Identification of enzyme activities present in cell-free extracts of groups I and II. To establish which enzymatic activities were missing from extracts of groups I and II, complementation tests were carried out in which different extracts were added sequentially. In these tests, 0.2 ml of a cell-free extract from one group was added to the reaction mixture and incubated at 37 C for 40 min. The incubation mixture was then heated at 100 C for 5 min and then cooled to 37 C (step 1). Then 0.2 ml of an extract from the other group was added, and the reaction mixture was again incubated at 37 C for 40 min (step 2). The anthranilate formed was measured as described for anthranilate synthetase. Anthranilate will only be formed when the extract added first is able to convert shikimate to EPSP (i.e., possessing full activity for EPSP synthetase), and when the extract added last possesses full activity for chorismate synthetase.

Assay of anthranilate synthetase. A modification of the method described by Morgan et al. (18) was used. Chorismic acid (2.0 μmoles), $MgCl_2$ (5 μmoles), glutamine (5 μmoles), and Tris buffer, *p*H 8.2 (50

μmoles), were added per milliliter of incubation mixture.

Assay of chorismate mutase, prephenate dehydratase, and prephenate dehydrogenase. The method described by Cotton and Gibson (5) was used for all of these assays.

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

Specific activities. The specific activity of each enzyme preparation is expressed as the number of 0.1 μmole of substrate used or product formed per 20 min per mg of protein at 37 C.

RESULTS

Mutant strains were originally screened for their growth responses to shikimate, and to one or more of the end products of aromatic biosynthesis. The results of these tests are shown in Table 3.

Biochemical analysis of mutant strains blocked before shikimate. Those strains showing a complete or partial response to shikimate were tested for their ability to cross-feed each other (Table 4). These strains were then examined for their ability to carry out the second, third, and fourth reactions of the common pathway. The results of these enzyme assays are shown in Table 5. As can be seen, the assays clearly indicate three different groups of mutants, each blocked in a separate reaction. The existence of three different phenotypes amongst these mutants had already been suggested by the cross-feeding data. It is of interest that the group whose aromatic requirements can only be partially satisfied by shikimic acid, and which cross-feeds the other two groups, lacks DHS reductase activity. Similar inability of strains blocked between DHS and shikimate to grow on shikimic acid alone has already been reported by Davis (7).

Genetic analysis of mutant strains blocked before shikimate. Somerville (*personal communication*) has already shown that the mutations affecting strains AB2826, AB2827, and AB2828 occurred in genes which were carried in different transducing fragments by phage P1. We determined the

TABLE 3. *Results of growth responses of mutant strains to shikimic acid and to the end products of aromatic biosynthesis*[a]

Strain	*PHE, TYR, TRY, PAB, POB*	*PHE, TYR, TRY*	*SA*	*SA, PHE, TYR*	*PHE, TYR*	*PHE*	*TYR*
AB2847	+[b]	−	++	++	−	−	−
AB2827	++	++	++	++	+	−	−
AB1360	++	++	++	++	−	−	−
AB2834	+	−	−[c]	+	−	−	−
AT2472	+	+	−[c]	++	−	−	−
AB2829	+	+	−	−	−	−	−
AB478	++	++	−	−	−	−	−
AB2851	++	++	−	++	++	−	+
AB2852	++	++	−	++	++	−	+
AB2853	++	++	−	++	++	−	+
AB2854	++	++	−	++	++	−	+
AB2855	++	++	−	+	+	−	−
AB2830	+	−	−	−	−	−	−
AB477	++	++	−	++	+	−	−
AB2850	++	++	−	++	++	−	−
AB347	++	++	−	++	++	+	+
AT2022	++	++	−	++	++	++	−
AT2273	++	++	−	++	++	−	++
AT2471	++	++	−	++	++	−	++
AB2859	++	++	−	++	++	−	++
AT2092	++	++	−	++	++	++	−
AB2861	++	++	+[d]	++	++	++	+[d]

[a] Abbreviations used: *PHE*, phenylalanine; *TYR*, tyrosine; *TRY*, tryptophan; *PAB*, *p*-aminobenzoic acid; *POB*, *p*-hydroxybenzoic acid; SA, shikimic acid; −, no response; +, weak response; ++, strong growth response.

[b] A number of strains grow poorly on media supplemented with these five compounds. Yeast extract and, in some cases, shikimic acid will satisfy their extra requirements.

[c] Although unable to grow on shikimic acid alone, or on a mixture of phenylalanine and tyrosine, these strains grow well on shikimic acid, phenylalanine, and tyrosine.

[d] Although phenylalanine markedly stimulates growth of this organism, it grows slowly in the absence of phenylalanine.

TABLE 4. *Results of cross-feeding tests between mutant strains showing a growth response to shikimic acid*

Strains feeding	Strains being fed				
	AB2834	AT2472	AB2827	AB1360	AB2847
AB2834	−*	−	+	+	+
AT2472	−	−	+	+	+
AB2827	−	−	−	−	+
AB1360	−	−	−	−	+
AB2847	−	−	−	−	−

* Symbols: −, no cross-feeding; +, cross-feeding occurs.

TABLE 5. *Presence of DHQ synthetase, dehydroquinase, and DHS reductase in cell-free extracts of mutant showing a growth response to shikimic acid*

Cell-free extract prepared from strain	Specific activity of DHQ synthetase	Specific activity of dehydroquinase	Specific activity of DHS reductase
AB2834	1.16	18	<0.31
AT2472	0.74	26	0.23
AB2827	1.10	1.8	13
AB1360	0.915	2.2	13
AB2847	0.025	20	12

approximate positions of these genes on the chromosome by mating these *aro*⁻ recipients with a number of different Hfr strains. A more accurate map location was then found by carrying out interrupted mating experiments between the recipients and the particular Hfr strain that transferred the *aro*⁺ allele at the highest frequency. In the case of two of these strains, AB2826 and AB2828, the genes *aro B* (the structural gene for DHQ synthetase) and *aro E* (the structural gene for DHS reductase) both showed a fairly close linkage to *mal*. Consequently, *mal*⁻ derivatives of these strains were prepared (AB2847 and AB2834). Figures 2 and 3 show the time of entry of *aro B* and *aro E* genes relative to *mal*, with the Hfr strain AB313 as donor. The close linkage of *aro B* and *mal* was confirmed by transduction tests, and these results are expressed in Table 6.

To confirm that both AT2472 and AB2828 carried mutations in the same gene (*aro E*), transductions were carried out between these strains. The results of Table 7 show that, in transduction between P1 prepared on AB2828 and the recipient AT2472, *aro E*⁺ transductants are formed at approximately 1.7% of wild-type frequency. In the reciprocal transduction, when the recipient was AB2828 and the donor AT2472,

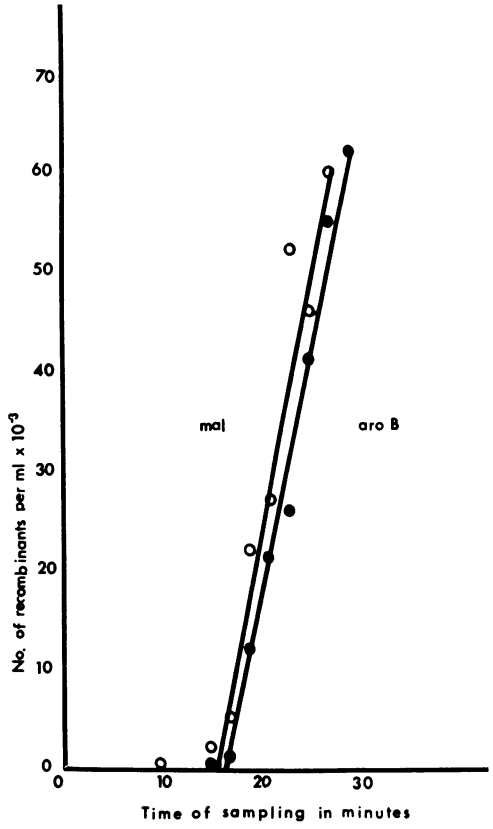

FIG. 2. *Kinetics of zygote formation when the Hfr AB313 is mated with the female AB2847.*

the transduction frequency was reduced to 8% of the wild-type frequency. Both these results are compatible with the idea that the mutations of strains AB2828 and AT2472 occur within the same gene (*aro E*). This conclusion is further supported by the biochemical data.

The *aro D* gene (the structural gene for dehydroquinase) is transferred distally to *his* by Hfr AB313, and hence as an early marker by the Hfr AB311 (*see* Table 2). In interrupted mating experiments, with the male strain AB311 as donor and either AB1360 or AB2848 as recipients, the *aro D* gene was transferred at 14 min. Since the time of entry for *his* in these experiments was 8 min, these results confirmed the earlier observations of Taylor and Thoman (24). The results of transduction tests confirming the close linkage of mutations affecting AB2848 and AB1360 are given in Table 8.

Biochemical analysis of mutant strains unable to respond to shikimic acid but requiring two or more aromatic amino acids for growth. Extracts of these strains were initially examined for their ability to

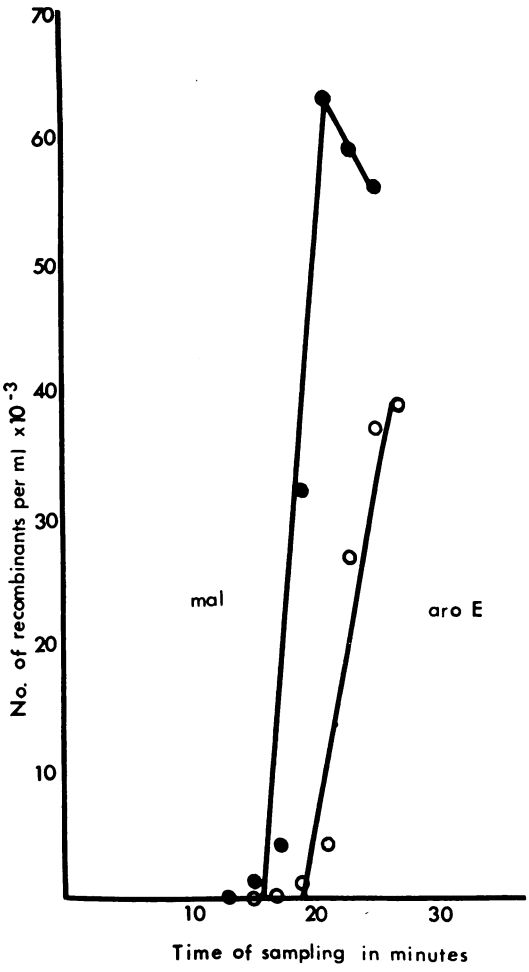

FIG. 3. *Kinetics of zygote formation when the Hfr AB313 is mated with the female AB2834.*

carry out the conversion of shikimate to anthranilate as described in Materials and Methods. The results of these assays are shown in Table 9. The specific activities for anthranilate synthetase are included to indicate those strains which could not be fully derepressed for this activity. Extracts prepared from fully derepressed cells (AB1360) and from wild-type cells (AB1171) are included as controls. In every case, extracts were prepared from cells grown in media containing 1.5×10^{-5} M indole as described in Materials and Methods.

Conversion of shikimate to shikimate-5-phosphate. All of the extracts unable to convert shikimate to anthranilate could convert shikimate to shikimate-5-phosphate. Extracts of only one strain, AB347, showed a reduced activity for this reaction, and we will return to this strain later. The results of these shikimate kinase assays are included in Table 10.

Complementation between extracts. Since these strains could be blocked in either EPSP synthetase or in chorismate synthetase, different extracts were mixed together to determine whether pairs

TABLE 6. *Testing for the cotransduction of genes aro B, aro E and mal*

Recipient*	No. of *mal*⁺ transductants	No. of *aro*⁺ transductants	No. of *aro*⁺*mal*⁺ transductants	No. of *aro*⁺ transductants that are *mal*⁺ unselected
AB2847 *aro B 351 mal 354*	215	372	88	9/40
AB2834 *aro E 353 mal 352*	267	200	0	0/40

* In both cases, the P1 phage used had been prepared on a *mal*⁺ *aro B*⁺ *aro E*⁺ donor.

TABLE 7. *Identification of aro E mutants by transduction**

Donor	Recipient	No. of *aro*⁺ transductants
AB2826 *aro C*⁺ *aro E*⁺	AB2830 *aro C 355*	ca. 8,000
AB2826 *aro C*⁺ *aro E*⁺	AT2472 *aro E 24*	ca. 8,000
AB2828 *aro E 353*	AB2830 *aro C 355*	ca. 3,600
AB2828 *aro E 353*	AT2472 *aro E 24*	60
AT2472 *aro E 24*	AB2830 *aro C 355*	ca. 2,500
AT2472 *aro E 24*	AB2828 *aro E 353*	200

* Pairs of figures represent two experiments using the same amount of donor phage (plaque-forming units per milliliter) with two different recipients. In this and subsequent tables, the numbers of *aro*⁺ transductants represent the total number of transductant colonies obtained on three plates. In those cases where the numbers obtained were very high, i.e., 8,000, numbers were estimated only to the nearest 100. Since for each test transduction a control with an unrelated recipient was always carried out, no attempt has been made to normalize these numbers to a fixed amount of plaque-forming units of input phage.

TABLE 8. *Identification of aro D mutants by transduction**

Donor	Recipient	No. of *aro*⁺ transductants
AB1360 *aro D 362*	AB2826 *aro B 351*	183
AB1360 *aro D 362*	AB2848 *aro D 352*	0
AB1360 *aro D 362*	AB2828 *aro E 353*	207

* The same amount of donor phage was used in each cross.

TABLE 9. *Ability of cell-free extracts to convert shikimate to anthranilate*

Cell-free extract prepared from strain	Micromoles ($\times 10^4$) of anthranilic acid formed per 20 min per mg of protein	Specific activity of anthranilate synthetase
AB1360*	370	4.6
AB2829	3	3.9
AB478	2	3.4
AB2851	57	2.0
AB2852	44	1.6
AB2853	85	1.3
AB2854	27	0.5
AB2855	25	1.1
AB2830	<1	8.4
AB477	12	2.6
AB2850	15	0.33
AB347	<1	1.0
AB2858	42	10.7
AB1171†	60	0.17

* AB1360, which is unable to convert DHQ to DHS, has been used as a wild-type control for the conversion of shikimate to anthranilate. Because this strain requires all the aromatic amino acids including tryptophan for growth, it was possible to derepress its anthranilate synthetase.

† Strain AB1171 has no aromatic requirements and is included as a wild-type control for those mutants in which it was not possible to fully derepress anthranilate synthetase.

of extracts could complement each other in the overall reaction. As can be seen from the results of Table 11, these tests allowed us to divide the mutants into two groups (groups I and II). Any extract from one group was able to complement an extract from the other in the overall reaction, but no complementations were observed between members of the same group. The identification of the specific reaction that was missing in the case of either group I and II mutants was determined by sequential mixing experiments as described in Materials and Methods. Complementations only occurred when group I extracts were added first in step 1, and group II extract was added for step 2 (Table 12). From these results, we concluded that group I mutants possess active EPSP synthetase and inactive chorismate synthetase, whereas extracts of group II possess active chorismate synthetase and inactive EPSP synthetase.

Reduced kinase activity of extracts of strain AB347. Even though extracts of this strain possess only one-fourth the kinase activity of wild-type strains, the fact that extracts of AB347 can complement group II extracts in sequential mixing experiments suggested that this reduction of activity does not drastically affect the formation of EPSP. That this reduced kinase activity was not due to the presence of an inhibitor in extracts of AB347 was demonstrated by mixing an extract of AB347 with an extract of strain AB2858 and measuring the combined kinase activity. The specific activity of the mixture was found to equal the mean of the two separate specific activities (Table 10).

As no kinase mutants have yet been reported in the literature, it was decided to try to determine whether the reduced kinase activity of strain AB347 was associated with its loss of chorismate synthetase activity. It seemed possible that, if the kinase gene and the chorismate synthetase gene were contiguous, either a polarity mutation in one gene or a deletion extending into both might have caused the loss of the chorismate synthetase and the reduction of the kinase activity. To test this notion the *aro*$^+$ allele from a wild-type strain was introduced into strain AB347 by transduction, recombinants being selected on minimal media not supplemented with the aromatic amino acids. A similar transduction was carried out with strain AB2830 as a recipient. This strain is also a group I mutant deficient in chorismate synthetase, but possessing full kinase activity. Two *aro*$^+$ transductants in each case were purified, and then examined for their kinase activity. The extracts prepared from the *aro*$^+$ transductants of AB347 had only one-fourth the kinase activity of the *aro*$^+$ transductants prepared from strain AB2830. From this, then, we inferred that the kinase gene and the chorismate synthetase genes are not contiguous and that the reduced kinase levels in extracts of AB347 can not be explained

TABLE 10. *Ability of cell-free extracts to convert shikimate into shikimate-5-phosphate*

Cell-free extract prepared from strain	Specific activity
AB1360*	1.9
AB2829	2.4
AB478	2.9
AB2851	3.2
AB2852	1.5
AB2853	1.6
AB2854	1.2
AB2855	1.2
AB2830	1.8
AB477	2.9
AB2850	2.3
AB347	0.7
AB2858	1.8
AB2858 and AB347	1.2

* Strain AB1360 is unable to convert DHQ into DHS, but has normal activity for the conversion of shikimate to chorismate. It has been used as a wild-type control for the shikimate kinase assay.

TABLE 11 *Ability of cell-free extracts of strains in group I and group II to convert shikimate to anthranilate in mixing experiments*

Cell-free extracts prepared from strains	Micromoles (× 10^4) of anthranilate formed in overall reaction per 20 min per mg of mixed protein		Complementation
	Value obtained in mixing experiment	Value expected if no complementation occurs*	
AB2830 (I) and AB2829 (II)	106	4	Yes
AB2830 (I) and AB478 (II)	>107	3	Yes
AB477 (I) and AB478 (II)	175	14	Yes
AB2850 (I) and AB2829 (II)	>100	18	Yes
AB2850 (I) and AB2851 (II)	160	72	Yes
AB2850 (I) and AB478 (II)	156	17	Yes
AB347 (I) and AB478 (II)	186	3	Yes
AB2830 (I) and AB2851 (II)	272	58	Yes
AB2830 (I) and AB2852 (II)	226	45	Yes
AB2830 (I) and AB2853 (II)	255	86	Yes
AB2830 (I) and AB2854 (II)	194	28	Yes
AB2830 (I) and AB2855 (II)	356	26	Yes
AB2830 (I) and AB477 (I)	5	13	No
AB2830 (I) and AB2850 (I)	5	16	No
AB347 (I) and AB2850 (I)	1	16	No
AB347 (I) and AB2830 (I)	4	2	No
AB2851 (II) and AB478 (II)	34	59	No
AB2829 (II) and AB478 (II)	1	5	No
AB2852 (II) and AB478 (II)	24	46	No
AB2853 (II) and AB478 (II)	27	87	No
AB2854 (II) and AB478 (II)	18	29	No
AB2855 (II) and AB478 (II)	14	27	No
AB2852 (II) and AB2853 (II)	32	129	No
AB2853 (II) and AB2854 (II)	8	112	No
AB2852 (II) and AB2851 (II)	41	101	No

* This value is determined by adding values obtained when each extract is incubated single (*see* Table 9).

TABLE 12. *Ability of cell-free extracts of strains in group I and group II to convert shikimate to anthranilate in sequential mixing experiments*

Cell-free extract added in step 1*	Cell-free extract added in step 2	Micromoles (× 10^4) of anthranilate formed in overall reaction per 20 min per mg of mixed protein
AB478 (II)	AB2850 (I)	2
AB2850 (I)	AB478 (II)	30
AB478 (II)	AB347 (I)	1
AB347 (I)	AB478 (II)	69
AB478 (II)	AB2830 (I)	1
AB2830 (I)	AB478 (II)	122
AB2851 (II)	AB2830 (I)	20
AB2830 (I)	AB2851 (II)	97

* For a description of steps 1 and 2, see text.

in terms of the mutation which caused the loss of activity of chorismate synthetase. The *aro*$^+$ transductants of AB347 and AB2830 were also examined for their growth rates in liquid and on solid minimal media, with and without the aromatic amino acids. Stimulation of growth rate by the aromatic amino acids was the same in both cases, indicating that the reduced level of shikimate kinase observed in cell-free extracts does not mean that the cells are starved for the aromatic amino acids. Nevertheless, experiments are in progress to determine the location of the mutation in AB347 causing this decreased specific activity for shikimate kinase.

Genetic analysis of mutant strains unable to respond to shikimic acid but requiring two or more aromatic amino acids for growth. On the basis of transduction tests, these mutant strains could be divided into two groups which were identical to the groups formed on the basis of the enzymatic

studies. Transduction between members of a group gave *aro*$^+$ transductants at low frequencies (approximately 1% of wild-type frequency), whereas transduction between representatives of different groups gave *aro*$^+$ transductants at wild-type frequencies.

Group I mutants. The results of an interrupted mating experiment between one member of group I, AB2849, and the Hfr AB313 are shown in Fig. 4. This gene affecting chorismate synthetase activity we have termed *aro C*. Transduction experiments demonstrating that all members of group I carry mutations in the *aro C* gene are shown in Table 13. On the basis of interrupted mating experiments, Taylor and Thoman (24) placed the mutations affecting AB347 and AB477, which are present in their strains AB444 and AB1320, approximately 3.5 min apart on the chromosome, and designated them *aro C* and *aro B*, respectively. However, in this study, *aro*$^+$ transductants were formed at less than 1% wild-type frequency in crosses between these mutants (Table 13). Furthermore, in other transduction experiments, we have been able to show that the *aro C1* allele of AB477 and the *aro C4* allele of AB347 are both cotransduced with *pur C* at frequencies of 38 and 36%, respectively. Because of this latter result we have retained the *aro C* designation rather than the *aro B* for these mutations.

Group II mutants. The mutations carried by two group II mutants, AB478 and AB2856, and affecting EPSP synthetase were mapped in interrupted mating experiments using the male

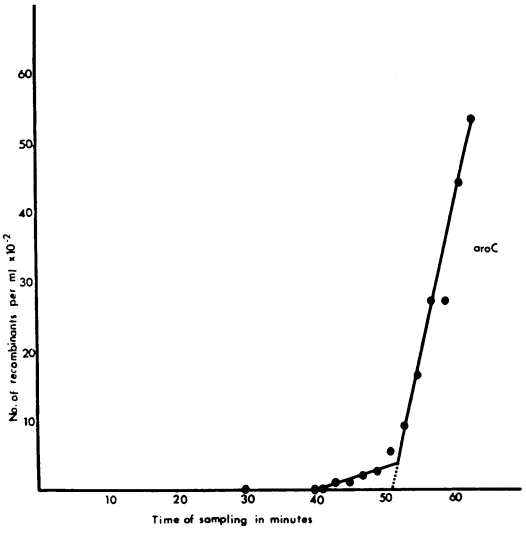

FIG. 4. *Kinetics of zygote formation when the Hfr AB313 is mated with the female AB2849.*

TABLE 13. *Identification of aro C mutants by transduction**

Donor	Recipient	No. of *aro*$^+$ transductants
AB2826 *aro C*$^+$ *aro A*$^+$	AB347 *aro C 4*	ca. 6,000
AB2826 *aro C*$^+$ *aro A*$^+$	AB2829 *aro A 354*	ca. 6,000
AB2830 *aro C 355*	AB347 *aro C 4*	155
AB2830 *aro C 355*	AB2829 *aro A 354*	ca. 7,000
AB2850 *aro C 356*	AB347 *aro C 4*	73
AB2850 *aro C 356*	AB2829 *aro A 354*	ca. 7,000
AB477 *aro C 1*	AB347 *aro C 4*	30
AB477 *aro C 1*	AB2829 *aro A 354*	ca. 11,000

* Pairs of figures represent two experiments in which the same amount of donor phage (plaque-forming units per milliliter) was used with two different recipients.

strain AB259. In both cases, the time of entry of this gene, *aro A*, was 30 min, which is in agreement with the results of Taylor and Thoman (24). Transduction experiments demonstrating that all members of group II carry mutations in the *aro A* gene are given in Tables 14 and 15.

Biochemical analysis of mutant strains blocked between chorismate and phenylalanine. Three mutants of this class were examined. Of these, AT2022 and AT2092 were both obtained from Dr. Taylor, and AB2861, from Dr. Somerville. Cotton and Gibson (5) have shown in *Aerobacter aerogenes* and in *E. coli* W that there are two chorismate mutase enzymes that are separable when cell-free extracts are chromatographed on diethylaminoethyl (DEAE)-cellulose columns. They have also shown that prephenate dehydrogenase activity is associated with one peak and prephenate dehydratase activity with the other. We found a similar situation in *E. coli* K-12, and the chromatographic separation of these enzymes from cell-free extracts of a wild-type strain is shown in Fig. 5. Figure 6 shows a similar experiment involving extracts from the phenylalanine auxotroph AT2022. It can be seen that one of the chorismate mutase peaks and its corresponding dehydratase activity are missing from extracts of the phenylalanine auxotroph. Extracts of the phenylalanine auxotroph strain AT2092 show a similar loss of prephenate dehydratase and its corresponding chorismate mutase peak (Cotton, *personal communication*). On the other hand, extracts of mutant AB2861, although having no prephenate dehydratase activity, still possess two peaks of chorismate mutase activity.

Genetic analysis of mutant strains blocked between chorismate and phenylalanine. Again on the

basis of interrupted mating data, Taylor and Thoman (24) have referred to the mutation of AT2022 as occurring in the *phe B* gene, and that of AT2092 as occurring in the *phe A* gene; they have placed these genes approximately 6.5 min apart on the *E. coli* chromosome. We found that, in transduction tests between these two mutants, *phe*$^+$ transductants were formed at less than 0.25% of the frequency with a wild-type donor. In these tests, we used AT2022 as the recipient in all crosses and compared the frequency of *pro*$^+$ and *phe*$^+$ transductants. On the basis of these results, we concluded that the mutations can not be separated by 6.5 min but, in fact, occur within the same gene. The results of these experiments and of a transduction involving the phenylalanine auxotroph AB2861 are shown in Table 16. The relatively high ratio of *pro*$^+$ to *phe*$^+$ obtained in the wild-type cross was unexpected. Phenylalanine auxotrophs, however, grow slowly in the absence of phenylalanine because of the chemical conversion of prephenate to phenylpyruvate. It is possible that the background growth of the *phe*$^-$ recipient prevented some of the *phe*$^+$ transductants from forming colonies.

TABLE 14. *Identification of aro A mutants by transduction**

Donor	Recipient	No. of *aro*$^+$ transductants
AT2471 *aro D*$^+$ *aro A*$^+$	AB1360 *aro D 362*	980
AT2471 *aro D*$^+$ *aro A*$^+$	AB478 *aro A 2*	1,350
AB2852 *aro A 358*	AB1360 *aro D 362*	530
AB2852 *aro A 358*	AB478 *aro A 2*	7
AB2853 *aro A 359*	AB1360 *aro D 362*	167
AB2853 *aro A 359*	AB478 *aro A 2*	12
AB1360 *aro A*$^+$ *aro C*$^+$	AB2830 *aro C 355*	2,150
AB1360 *aro A*$^+$ *aro C*$^+$	AB2852 *aro A 358*	1,760
AB2855 *aro A 361*	AB2830 *aro C 355*	690
AB2855 *aro A 361*	AB478 *aro A 2*	1
AB2851 *aro A 357*	AB2830 *aro C 355*	1,300
AB2851 *aro A 357*	AB478 *aro A 2*	4
AB2854 *aro A 360*	AB2830 *aro C 355*	ca. 3,600
AB2854 *aro 4 360*	AB478 *aro A 2*	4
AB2829 *aro A 354*	AB2830 *aro C 355*	ca. 8,700
AB2829 *aro A 354*	AB478 *aro A 2*	186
AB478 *aro A 2*	AB2830 *aro C 355*	ca. 3,200
AB478 *aro A 2*	AB2852 *aro A 358*	0

* Pairs of figures represent two experiments in which the same amount of donor phage (plaque-forming units per milliliter) was used with two different recipients. In every pair of crosses, one cross, in which either AB1360 or AB2830 is the recipient, serves as a control to measure the transducing ability of the donor phage.

On the basis of the transduction data and the enzymatic analyses, we concluded that the phenylalanine auxotrophs AT2092, AT2022, and AB2861 possess mutations affecting the same gene. We propose, therefore, that the *phe B* locus of Taylor and Thoman (24) should be deleted from the map until another gene concerned with phenylalanine biosynthesis is discovered. In interrupted mating experiments with the male AB313 and the female AT2092, the *phe A* gene was transferred at 30 min and *his* at 40 min, confirming the results of Taylor and Thoman (24).

Biochemical analysis of mutant strains blocked between chorismate and tyrosine. We examined only three strains blocked in tyrosine biosynthesis: AT2471, AT2273, and AB2859. When extracts of AT2471 were chromatographed as already described for the phenylalanine auxotrophs, the chorismate mutase peak with its corresponding prephenate dehydrogenase activity was found to be missing (Fig. 7). We obtained the same results with extracts of strain AT2273 and of strain AB2859.

Genetic analysis of mutant strains blocked between chorismate and tyrosine. The results of transduction tests between these three *tyr*$^-$ strains are presented in Table 17. Interrupted mating experiments which were carried out between the male AB313 and the female AT2273 showed that the *tyr A* gene was transferred at 30 min and *his* at 40 min, in agreement with the results of Taylor and Thoman (24).

Since interrupted mating experiments indicated

TABLE 15. *Identification of aro A mutants by transduction*

Donor	Recipient	No of *aro*$^+$ transductants	No. of *arg*$^+$ transductants
AT2472 *aro A*$^+$ *arg*$^+$	AB2852 *aro A 358 arg 3*	1,030	1,540
AB478 *aro A 2 arg*$^+$	AB2852 *aro A 358 arg 3*	10	ca. 2,400
AB478 *aro A 2 arg*$^+$	AB2855 *aro A 361 arg 3*	15	ca. 2,370
AB478 *aro A 2 arg*$^+$	AB2851 *aro A 357 arg 3*	0	ca. 2,500
AB478 *aro A 2 arg*$^+$	AB2853 *aro A 359 arg 3*	4	ca. 2,500
AB478 *aro A 2 arg*$^+$	AB2854 *aro A 360 arg 3*	0	ca. 2,500

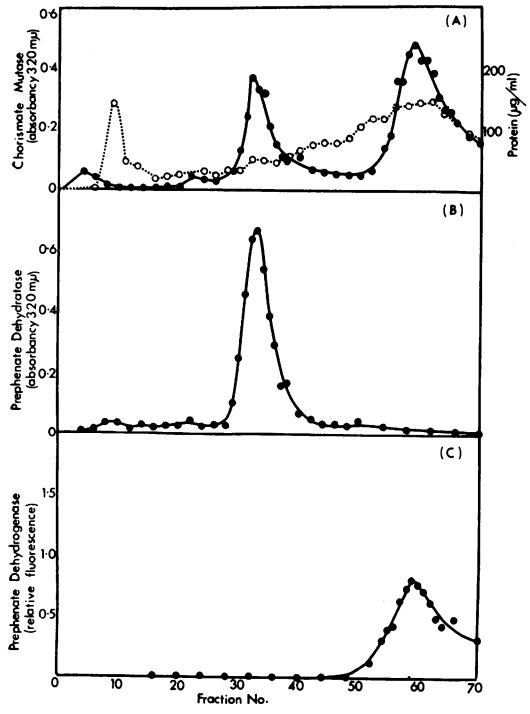

FIG. 5. *Chromatography of a cell-free extract of strain AB1360 on a DEAE cellulose column. (A) Chorismate mutase activity (●), protein (○); (B) prephenate dehydratase activity; (C) prephenate dehydrogenase activity.*

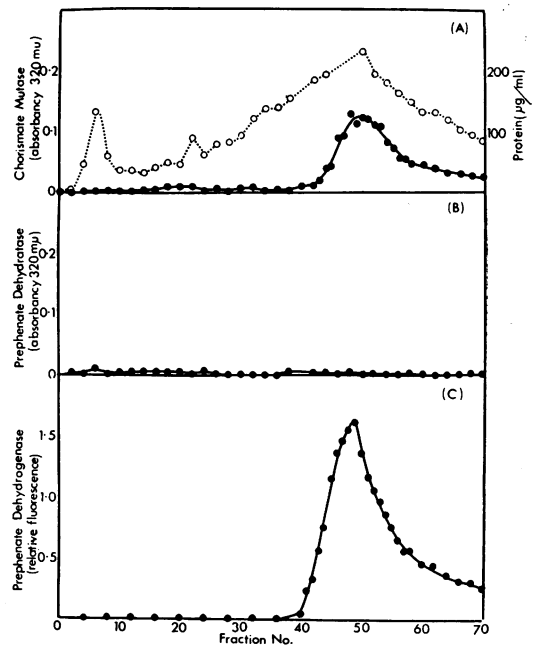

FIG. 6. *Chromatography of a cell-free extract of the phenylalanine auxotroph AT2022 on a DEAE cellulose column. (A) Chorismate mutase activity (●), protein (○); (B) prephenate dehydratase activity; (C) prephenate dehydrogenase activity.*

that the *phe A* and *tyr A* genes were relatively close together on the chromosome, transduction experiments were carried out to see whether the two genes were cotransducible. Phage P1 was prepared on the tyrosine auxotroph AT2273 and used to transduce the *phe*$^+$ gene into strain AT2092. Transductants were selected on minimal media supplemented with tyrosine but no phenylalanine. The *phe*$^+$ transductants were then streaked to minimal media with and without tyrosine to see whether any had inherited the *tyr*$^-$ allele. Approximately 50% of these *phe*$^+$ transductants failed to grow in the absence of tyrosine. In the reciprocal cross in which AT2092 was the donor and AT2273 the recipient, 40% of the *tyr*$^+$ transductants inherited the *phe*$^-$ allele and were unable to grow in the absence of phenylalanine. We conclude therefore that the *tyr A* and the *phe A* genes are cotransducible by phage P1.

DISCUSSION

These results indicate that most of the genes concerned with aromatic biosynthesis are widely separated on the *E. coli* chromosome, unlike those concerned with the specific pathway of tryptophan biosynthesis which form a single operon. In some cases, the data that we have obtained from transduction tests lead us to different conclusions from those of Taylor and Thoman (24), and we would like to propose the following explanation for the different results.

Taylor and Thoman mated the Hfr strain AB313 with two different females, AT2022 and

TABLE 16. *Identification of phe A mutants by transduction*

Donor	Recipient	No. of *phe*$^+$ transductants	No. of *pro*$^+$ transductants
Wild-type *pro*$^+$ *phe A*$^+$	AT2022 *pro 2 phe A1*	400	ca. 4,000
AT2092 *pro*$^+$ *phe A2*	AT2022 *pro 2 phe A1*	0	ca. 4,000
AB2861 *pro*$^+$ *phe A 351*	AT2022 *pro 2 phe A1*	5	1,010

AT2092. Both of these recipient strains carried mutant alleles for genes concerned with histidine and phenylalanine biosynthesis. Strain AT2092 carried the *his 1* and *phe A* alleles, and strain AT2022 carried the *his 4* and *phe B* alleles. In a number of interrupted mating experiments involving AB313 and AT2092, the wild-type alleles corresponding to *his 1* and *phe A* (*his* 1^+ and *phe* A^+) were transferred at 40.5 and 29 min, respectively. In a number of experiments involving the same male strain and the recipient AT2022, the wild-type alleles corresponding to *his 4* and *phe B* were transferred at 46 and 35 min, respectively. Because the difference of 6.5 min between the times of entry of the *his* 1^+ and *his* 4^+ alleles was unexpected, these results were tested by crossing both recipient strains with another Hfr, strain AB311. The order in which various markers are transferred by both these Hfr strains can be seen in Table 2. Strain AB311 transferred the *his* 1^+ allele and the *his* 4^+ allele at 6 min; i.e., the 6.5-min difference between these markers had now disappeared. Unfortunately, strain AB311 transfers the *phe* A^+ and *phe* B^+ allele as distal markers, and it was not possible to check the difference in times of entry observed for *phe* A^+ and *phe* B^+. On the basis of these results, Taylor and Thoman have proposed that (i) the *his* 1^+ and *his* 4^+ alleles are normally less than 1 min apart, but in Hfr AB313 the *his 4* allele has undergone a transposition, and (ii) that the two mutations affecting phenylalanine biosynthesis occur in two distinct genes separated by approximately 6 min on the chromosome.

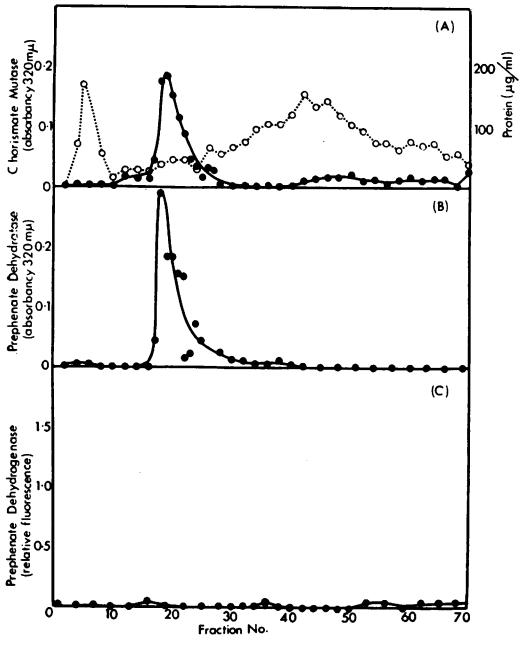

FIG. 7. *Chromatography of a cell-free extract of the tyrosine auxotroph AT2411 on a DEAE cellulose column. (A) Chorismate mutase activity (●), protein (○); (B) prephenate dehydratase activity; (C) prephenate dehydrogenase activity.*

As we have already shown, transduction tests demonstrate that the *phe A* and the *phe B* mutations occur, in fact, in the same gene. In other words, the 6-min difference in the times of entry of *phe* A^+ and *phe* B^+ found in the crosses with AB313 has to be accounted for by a hypothesis which will also explain the observed difference in the times of entry of *his* 1^+ and *his* 4^+ alleles. In the crosses involving AT2092 and AT2022, selection was also made for the early markers *xyl*$^+$ or *mal*$^+$, and the times of entry obtained for these markers indicated that each one was entering either recipient at approximately the same time. Therefore, we cannot explain the 6-min difference by postulating that the initation of chromosome transfer is delayed by 6 min when strain AT2092 is the recipient. What we do propose, however, is that recipient strains are able to affect the rate at which the chromosome is transferred from the male donor, and that in matings involving the recipient AT2022 this rate is depressed. To explain the identical time of entry for early markers in these crosses, it is necessary to postulate that this alteration of rates of transfer affects distal markers much more than it does proximal ones. This hypothesis will also explain the differences in times of entry for the alleles that we have referred to as *aro* $C1^+$ and *aro* $C4^+$. We have at present no direct evidence in support of this hypothesis, but, in view of our results, it seems more acceptable than the transposition postulated by Taylor and Thoman.

We have not examined any of our mutant

TABLE 17. *Identification of tyr A mutants by transduction*

Donor	Recipient	No. of *tyr*$^+$ transductants	No. of *his*$^+$ transductants
AB2826 *tyr*$^+$ *his*$^+$	AT2273 *tyr A 352 his*$^-$	ca. 2,500	ca. 5,000
AB2859 *tyr A 351 his*$^+$	AT2273 *tyr A 352 his*$^-$	420	ca. 7,500
AT2471 *tyr A his*$^+$	AT2273 *tyr A 352 his*$^-$	3	2,820

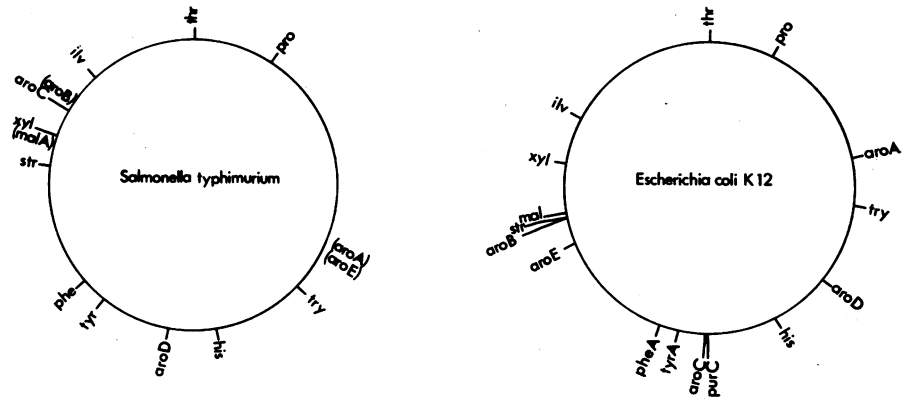

FIG. 8. *Distribution of aromatic genes on the chromosome of Salmonella typhimurium and Escherichia coli K-12. The map of S. typhimurium is based on the publication of Sanderson and Demerec (20). The functions of the different aromatic genes have not been described, and the gene symbols used, e.g., aro A etc., need not correspond to similar gene designations on the E. coli map. The parentheses around a symbol signify that the position is only known approximately. The map of E. coli K-12 is constructed from our own data and that of Taylor and Thoman (24). A description of the functions of the various genes is to be found in Fig. 1 and in the text. The relative order of phe A and tyr A has not yet been determined.*

strains for their ability to carry out the first reaction of the common pathway. Since it has been shown in *E. coli* W and in *E. coli* K-12 that there are probably three isoenzymes able to carry out this reaction (4, 14, 22), the isolation of mutants blocked in the first reaction would not be expected after the selection technique that was used.

Sanderson and Demerec (20) have recently published a genetic map of *Salmonella typhimurium*, in which they listed five aromatic loci: *aro A*, *aro B*, *aro C*, *aro D*, and *aro E*. Since they present neither genetic nor biochemical evidence in support of separate gene designations for *aro A* and *aro E* on the one hand and *aro B* and *aro C* on the other (in each case the pair of genes being separated by less than 1 min on the chromosome), it is not possible to examine their claim that the distribution and function of the aromatic loci on the chromosomes of both *E. coli* and *S. typhimurium* are probably both the same. If each genetic locus that they refer to controls the structure of a different enzyme, then it appears as though divergence may exist between the two organisms as far as these loci are concerned. A comparison of the *S. typhimurium* map and the *E. coli* map is shown in Fig. 8.

ADDENDUM IN PROOF

We have recently isolated a mutant strain which, although able to grow on minimal media alone, has a strict requirement for tyrosine in the presence of phenylalanine and tryptophan. Either dehydroquinic acid or shikimic acid can replace tyrosine as a growth factor. It is probable that this mutant has lost the tyrosine sensitive isoenzyme which converts erythrose-4-PO_4 and phosphoenolpyruvate to PKDH.

This new gene, for which we propose the designation *aro-F*, is cotransducible with the *phe A* gene at a frequency of 50% and with the *tyr A* gene at a frequency of 60%. Hence, two genes controlling two of the first specific reactions after the branch point and one of the genes involved in the first specific reaction of the common pathway are situated very close together on the chromosome. The significance of this arrangement for the regulation of this pathway is currently under investigation.

ACKNOWLEDGMENTS

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