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Repression of Aromatic Amino Acid Biosynthesis in *Escherichia coli* K-12

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Mutants of *Escherichia coli* K-12 were isolated in which the synthesis of the following, normally repressible enzymes of aromatic biosynthesis was constitutive: 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP) synthetases (phe and tyr), chorismate mutase T-prephenate dehydrogenase, and transaminase A. In the wild type, DAHP synthetase (phe) was multivalently repressed by phenylalanine plus tryptophan, whereas DAHP synthetase (tyr), chorismate mutase T-prephenate dehydrogenase, and transaminase A were repressed by tyrosine. DAHP synthetase (tyr) and chorismate mutase T-prephenate dehydrogenase were also repressed by phenylalanine in high concentration (10^{-3} M). Besides the constitutive synthesis of DAHP synthetase (phe), the mutants had the same phenotype as strains mutated in the tyrosine regulatory gene *tyrR*. The mutations causing this phenotype were cotransducible with *trpA*, *trpE*, *cysB*, and *pyrF* and mapped in the same region as *tyrR* at approximately 26 min on the chromosome. It is concluded that these mutations may be alleles of the *tyrR* gene and that synthesis of the enzymes listed above is controlled by this gene. Chorismate mutase P and prephenate dehydratase activities which are carried on a single protein were repressed by phenylalanine alone and were not controlled by *tyrR*. Formation of this protein is presumed to be controlled by a separate, unknown regulator gene. The heat-stable phenylalanine transaminase and two enzymes of the common aromatic pathway, 5-dehydroquinate synthetase and 5-dehydroquinase, were not repressible under the conditions studied and were not affected by *tyrR*. DAHP synthetase (trp) and tryptophan synthetase were repressed by tryptophan and have previously been shown to be under the control of the *trpR* regulatory gene. These enzymes also were unaffected by *tyrR*.

The pathway leading to the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, in *Escherichia coli*, is shown in Fig. 1. Two regulator genes controlling this pathway have been identified. One gene, *trpR*, regulates the formation of the enzymes of the tryptophan pathway and the tryptophan-inhibitable 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP) synthetase [DAHP synthetase (trp); references 4, 8, 15, 20]. Another gene, *tyrR*, regulates the formation of the tyrosine-inhibited DAHP synthetase [DAHP synthetase (tyr)], chorismate mutase T-prephenate dehydrogenase, and transaminase A (30).

Two observations concerning *tyrR* have led to the present report. First, a characterization of *tyrR* (including the isolation of *tyrR*$^-$ mutants) was carried out in strains lacking all DAHP synthetase activity other than DAHP synthetase (tyr) (30). The effect of *tyrR* on the phenylalanine- and tryptophan-inhibitable DAHP synthetases [DAHP synthetase (phe) and DAHP synthetase (trp)] was not reported. Since all three DAHP synthetases overlap with respect to specificity of repressing metabolites, it was considered important to determine whether *tyrR* controlled DAHP synthetases (phe) and (trp) as well as (tyr).

Second, an earlier investigation had led to the conclusion that a common regulator gene controlled synthesis of DAHP synthetases (phe) and (tyr) and chorismate mutase (3; K. D. Brown, Ph.D. Thesis, New York Univ., 1967). Evidence is presented in this paper that this common regulator gene is similar in phenotype and map location to *tyrR* and that the two genes are identical.

This paper describes the isolation and characterization of several *tyrR*$^-$ strains and continues the analysis of repression of aromatic biosynthesis commenced earlier (3, 4; K. D. Brown, Ph.D. Thesis, New York Univ., New York, 1967).

A brief summary of some of this work has appeared earlier (7).

In an accompanying paper (14*a*), Im, Davidson, and Pittard report findings substantially similar to our own on the properties of the *tyrR* locus.

MATERIALS AND METHODS

Organisms. The strains of *E. coli* used in this work are listed in Table 1. Strains W1485, 5927, A46PR8, A46PR9, and A2 were provided by C. Yanofsky. J. Pittard provided JP232, JP324, and JP568. The remaining strains were isolated in these laboratories.

Growth and cell-free extract preparation. Cells were grown on New Brunswick gyratory shakers at 37 C in medium A of Davis and Mingioli (10) supplemented as indicated. Cultures at a cell density of 5×10^8 per ml (0.2 mg of dry weight per ml) were harvested by centrifugation (0 C, 10,000 × *g*, 15 min) and washed with cold medium A minus glucose. Pellets were then resuspended in cold 0.1 M potassium phosphate buffer (*p*H 7.4) at a density of 8 mg of dry weight per ml of buffer and disrupted in a motor-driven Aminco French press (20,000 psi). After centrifugation (0 C, 20,000 × *g*, 20 min), the cell-free supernatant fluid was dialyzed for 4 hr against 400 volumes of 0.025 M potassium phosphate buffer (*p*H 7.4). Extracts were stored at −20 C. Proteins were estimated by the method of Lowry et al. (16).

Chemicals. Chorismic acid was prepared by the method of F. Gibson (12), who also provided the mutant used in this preparation, *Aerobacter aerogenes* 621. Prephenic acid was prepared by incubating chorismic acid in 0.1 M potassium phosphate buffer (*p*H 8.0) at 40 C for 12 hr. 80% conversion to prephenic acid was obtained as measured by acid conversion to phenypyruvic acid (9).

Other chemicals used were described previously (4, 5).

Estimation of indole glycerol. Indole glycerol was estimated in samples (1 ml) of culture supernatant fluids by the method used by Gibson and Yanofsky for estimating indole glycerol phosphate (14).

Estimation of anthranilic acid. Anthranilic acid was estimated in samples (1 ml) of culture supernatant fluids after acidification and extraction into ethyl acetate. The absorbancy of the ethyl acetate layer at 336 nm was read, and the anthranilic acid concentration was calculated by comparison with a control run with a known quantity of anthranilic acid.

Enzyme assays. Enzymes were assayed in duplicate in dialyzed, crude, cell-free extracts. Each experiment was repeated at least three times. In general, the duplicates varied by less than 10%, whereas the different values observed between separate experiments did not exceed 20%. Specific activities are reported as the number of micromoles of substrate used or product formed per minute per milligram of protein at 37 C.

DAHP synthetase and estimation of individual isoenzymes. DAHP synthetase (EC 4.1.2.15) and estimation of individual isoenzymes in crude, cell-free extracts were described previously (2).

5-Dehydroquinate synthetase. The methods of Srinivasan et al. (27) were used for the assay of 5-dehydroquinate synthetase.

5-Dehydroquinase. The method of Mitsuhashi and Davis (18) was used for the assay of 5-dehydroquinase (EC 4.2.1.10). The supernatant fluid of the *E. coli* W mutant 83-1 (provided by B. D. Davis) grown on limiting shikimate was used as substrate.

Tryptophan synthetase. The method of Smith and Yanofsky (26) was used for the assay of tryptophan synthetase (EC 4.2.1.20).

Chorismate mutase and prephenate dehydratase. Chorismate and prephenate dehydratase (EC 4.2.1.40) were assayed in crude, cell-free extracts by the methods of Cotton and Gibson (9). The two isoenzymes of chorismate mutase (P and T) were not separated.

Prephenate dehydrogenase. The method of Schwinck and Adams (23), modified as follows, was used for the assay of prephenate dehydrogenase (EC 1.3.1.9). The incubation time for the enzymatic reaction was reduced from 60 to 30 min, and the concentration of nicotinamide adenine dinucleotide was increased from 10^{-3} M to 2.5×10^{-3} M.

Transaminase A. The method of Wallace and Pittard (30) was used for the assay of transaminase A (EC 2.6.1.5).

Phenylalanine transaminase. The method of Cotton (Ph.D. Thesis, Univ. of Melbourne, Melbourne, Australia, 1967) was used to measure total phenylalanine transaminase activity. This method is based on the conversion of phenylalanine to phenylpyruvate and consisted of incubating crude, cell-free extract in a 1-ml volume containing 0.5 μmole of α-ketoglutarate, 0.1 μmole of pyridoxal-5-phosphate, 5 μmoles of $MgCl_2$, and 50 μmoles of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (*p*H 8.2). The reaction mixture was incubated at 37 C for 30 min, 3.0 ml of 1 N NaOH was added, and absorbancy at 320 nm was measured immediately. The molar extinction coefficient of phenylpyruvate was taken as 17,500 (9). This total activity consists of transaminase A activity (which is heat-labile) and a heat-stable phenylalanine transaminase. Heat-stable activity was estimated by the above assay method after heat inactivation of transaminase A at 60 C for 15 min (24).

Techniques for measuring repression in E. coli. Methods for demonstrating repression by aromatic amino acids in exponentially growing cells of *E. coli* and for achieving derepression in multiple aromatic auxotrophs grown in the chemostat on limiting aromatic amino acids were previously described (4).

P1kc transduction techniques and preparation of phage stocks. Methods for P1kc transduction techniques and preparation of phage stocks were described previously (4).

Isolation of mutants sensitive to phenylalanine plus tyrosine (1-3 and 7-3). Broth-grown, exponential-phase, wild-type *E. coli* (W1485) at a cell density of 10^8 cells/ml was centrifuged, suspended in an equal volume of saline, and ultraviolet-irradiated with a dose sufficient to kill 99.9% of the cells. From the irradiated sample, 0.5 ml was inoculated into 5 ml of minimal medium containing complete multiple aromatic supplement. After incubation with shaking overnight at 37 C in the dark, this culture was fully grown. A small volume (0.1 ml) was then reinoculated into fresh minimal medium plus complete multiple aromatic supple-

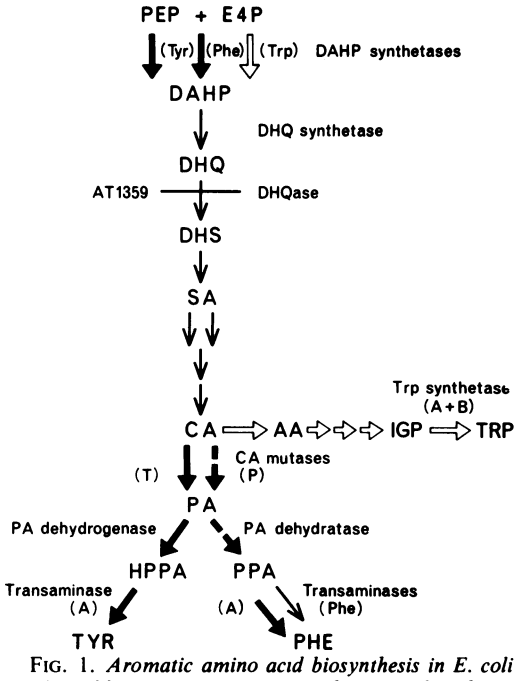

FIG. 1. *Aromatic amino acid biosynthesis in E. coli K-12. Abbreviations: E4P, erythrose-4-phosphate; PEP, phosphoenolpyruvate; DAHP, 3-deoxy-D-arabinoheptulosonic acid 7-phosphate; DHQ, 5-dehydroquinate; SA, shikimate; CA, chorismate; AA, anthranilate; IGP, indole-3-glycerol phosphate; TRP, tryptophan; PA, prephenate; PPA, phenylpyruvate; PHE, phenylalanine; HPPA, hydroxyphenylpyruvate; TYR, tyrosine. Recent reviews (11, 13, 21) describe this pathway in detail. Evidence for two shikimate kinases comes from sucrose sedimentation profiles (1), and Silbert et al. (24) have shown that both transaminase A and a heat-stable transaminase reversibly convert PPA to PHE. Symbols: enzymes specified by the aromatic regulon, thick solid arrows; enzymes specified by the tryptophan regulon, open arrows; enzymes specified by the phenylalanine regulon, broken arrows; enzymes which are either constitutive or whose regulation is uncertain, thin arrows. These groupings are discussed in the text.*

ment and grown to a density of 5×10^7 cells per ml. This culture was centrifuged, washed with minimal medium, and resuspended, and the cells were exposed to penicillin (300 units/ml) at a cell density of 10^6/ml in the presence of phenylalanine and tyrosine (20 μg of each/ml) for 6 hr. Penicillin was removed by centrifugation, and dilutions of the cells were spread on minimal agar plates. After 48 hr at 37 C, colonies were replicated on plates containing phenylalanine plus tyrosine. Colonies which failed to replicate were picked and purified.

Inhibition of growth by 4-amino-DL-phenylalanine. 4-Amino-DL-phenylalanine (APA) inhibits the growth of *E. coli* as a result of inhibition of DAHP synthetase (phe) and repression of DAHP synthetase (tyr) (25). Strains were found to vary in their sensitivity to APA, depending on their DAHP synthetase constitution. Strains which were *aroG*$^-$ *aroH*$^-$, possessing only DAHP synthetase (tyr), were sensitive to relatively low concentrations of the drug, and 10^{-4} M APA was used to inhibit them. Analogous *tyrR*$^-$ strains were resistant to 10^{-4} M APA (30). Strains possessing DAHP syn-

TABLE 1. *List of Escherichia coli strains*

Strain	Genetic loci[a]
W1485	Prototroph
1-3	*trpA*$^-$, *tyrR13*
7-3	*trpD*$^-$, *tyrR73*
AT1359	*aroD*$^-$, *pro*$^-$, *lac*$^-$, *tsx*$^-$, *gal*$^-$, *xyl*$^-$, *mtl*$^-$, *his*$^-$, *arg*$^-$, *thi*$^-$, (λ)$^-$
KB1364	*aroD*$^-$, *pro*$^-$, *lac*$^-$, *tsx*$^-$, *gal*$^-$, *xyl*$^-$, *mtl*$^-$, *his*$^-$, *arg*$^-$, *thi*$^-$, (λ)$^-$, *shi3*
JP232	*aroG*$^-$, *aroH*$^-$, *tyrR352*
YS31	*cysB*$^-$
KB3134	*pyrF*$^-$
A2	*trpA*$^-$
A46PR8[b]	*trpA*$^-$
A46PR9[b]	*trpA*$^-$
5927	*trpE*$^-$
JP324	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$, *pyrF*$^-$
JP568	*aroF*$^-$, *aroH*$^-$, *ilv*$^-$, *thi*$^-$, *his*$^-$, *trp*$^-$ (deletion)
KB3241	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$
KB3242	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$, *tyrR13*
KB3243	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^\pm$, *pro*$^-$, *tyrR352*
KB2352	*tyrR352*
KB3130	Prototroph
KB3131	*trpA*$^-$
KB3132	*tyrR13*
KB3133	*tyrR352*
KB5680	*aroF*$^-$, *aroH*$^-$, *ilv*$^-$, *thi*$^-$, *his*$^-$, *pyrF*$^-$
KB5681	*aroF*$^-$, *aroH*$^-$, *ilv*$^-$, *thi*$^-$, *his*$^-$
KB5682	*aroF*$^-$, *aroH*$^-$, *ilv*$^-$, *thi*$^-$, *his*$^-$, *tyrR13*
KB3244	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$, *cysB*$^-$
KB3245	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$, *trpE*$^-$
KB3246	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$, *trpA*$^-$
KB3247	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$, *tyrR5*
KB3248	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$, *tyrR7*
KB3249	*aroG*$^-$, *aroH*$^-$, *ilv*$^-$, *arg*$^-$, *thi*$^-$, *pro*$^-$, *tyrR12*
KB5683	*aroF*$^-$, *aroH*$^-$, *ilv*$^-$, *thi*$^-$, *his*$^-$, *tyrR352*

[a] Gene symbols: the regulatory gene *tyrR* is discussed in the text. *AroD* encodes 5-dehydroquinase, *trpA* tryptophan synthetase A, *aroF* DAHP synthetase (tyr), *aroG* DAHP synthetase (phe) and *aroH* DAHP synthetase (trp); *shi3* confers on *aroD*$^-$ auxotrophs the ability to grow at a prototrophic rate on shikimic acid as sole aromatic supplement (Brown and Doy, *manuscript in preparation*). All other symbols used are those recommended by Taylor (28).

[b] A46PR8 and A46PR9 are partial revertants of the complete tryptophan auxotroph A46 (31).

thetase (phe), such as wild-type and *aroF*$^-$ *aroH*$^-$ strains, were relatively resistant to APA and were only partially inhibited by 10^{-3} M APA. However, excepting KB5683 (*see below*), analogous *tyrR*$^-$ strains could be distinguished form *tyrR*$^+$ by an increased resistance to 10^{-3} M APA.

RESULTS

Nutritional properties and accumulation products of mutants sensitive to phenylalanine plus tyrosine. Mutants 1-3 and 7-3, isolated as previously described, provided the first indication that enzymes concerned with phenylalanine and tyrosine biosynthesis share a common regulator gene (3; K. D. Brown, Ph.D. Thesis, New York Univ., New York, 1967). We found that mutant 1-3 excreted indole glycerol and mutant 7-3 excreted anthranilic acid upon growth in minimal medium (Table 2). This suggested that mutant 1-3 produced a weakly active *trpA* gene product and that mutant 7-3 produced a weakly active *trpD* gene product. This notion was supported by the fact that A46PR8 and A46PR9, isolated by Yanofsky (31) and known to produce partially active tryptophan synthetase α subunits, were inhibited in their growth by a mixture of phenylalanine and tyrosine (Table 2; only A46PR8 results are shown since A46PR9 gave very similar results).

Accumulation of intermediates before the mutant block, whether anthranilate or indole glycerol, is also inhibited by phenylalanine plus tyrosine. Tryptophan fully relieved the growth inhibition by phenylalanine plus tyrosine and prevented accumulation of intermediates (presumably by feedback inhibition and repression). Partial relief by shikimic acid suggested that growth inhibition by phenylalanine and tyrosine was caused by feedback inhibition or repression of the early aromatic pathway (probably at DAHP synthetase), or both. It was proposed (4) that the feedback inhibition and repression by phenylalanine and tyrosine at DAHP synthetase reduced the supply of aromatic intermediates, and, although this is insufficient to interfere with tryptophan production in wild-type cells, it does aggravate the limitation of tryptophan in mutant strains such as 1-3, 7-3, and A46PR8 which already have a partial block in the tryptophan pathway.

A second characteristic of mutants 1-3 and 7-3 but not of A46PR8 and A46PR9 was that several hitherto repressible enzymes of aromatic biosynthesis were present at high levels and were constitutive. It was postulated that the altered regulatory properties of mutants 1-3 and 7-3

TABLE 2. *Accumulation of indole glycerol and anthranilic acid by Escherichia coli strains*[a]

Strain	Relevant aromatic alleles	Additions to growth medium (all 10^{-4} M)	Mean generation time (hr)	Compound accumulated (μmole per ml of culture supernatant fluid)	
				Indole glycerol	Anthranilic acid
W1485	Wild	None	1.0	<0.01	0
1-3	*tyrR13 trpA*$^-$	None	1.5	0.16	0
1-3	*tyrR13 trpA*$^-$	Phe, Tyr	3.0	0.06	0
1-3	*tyrR13 trpA*$^-$	Phe, Tyr, Trp	1.1	0.01	0
7-3	*tyrR73 trpD*$^-$	None	1.6	<0.01	0.18
7-3	*tyrR73 trpD*$^-$	Phe, Tyr	3.0	<0.01	0.03
7-3	*tyrR73 trpD*$^-$	Phe, Tyr, Trp	1.0	0.01	0
A46PR8	*trpA*$^-$	None	2.0	0.31	0
A46PR8	*trpA*$^-$	Phe, Tyr	4.5	0.12	0
A46PR8	*trpA*$^-$	Phe, Tyr, Trp	1.0	0.01	0
KB3131	*trpA*$^-$	None	2.2	0.18	0
KB3131	*trpA*$^-$	Phe, Tyr	4.0	0.06	0
KB3131	*trpA*$^-$	Phe, Tyr, Trp	1.3	0.01	0
KB3132	*tyrR13*	None	1.0	<0.01	0
KB3242	*aroG*$^-$ *aroH*$^-$ *tyrR13*	None	1.0	<0.01	0
KB5682	*aroF*$^-$ *aroH*$^-$ *tyrR13*	None	1.0	<0.01	0

[a] Strains used are described in Table 1. Cultures were grown in medium A supplemented as shown. In addition, cultures of KB3242 and KB5682 were supplemented with thiamine (10 μg/ml) and their appropriate amino acid requirements (100 μg/ml each). Growth was monitored by reading absorbancies at 490 nm in a Shimadzu QV-50 spectrophotometer (1-cm light path). Samples for estimating indole glycerol and anthranilate were taken when the cell density reached 5×10^8/ml. These were centrifuged, and the accumulated compounds were measured in samples of the supernatant fluid as described in the text. The abbreviations used are explained in Fig. 1.

were caused by a second mutation in a regulatory gene provisionally identified as *tyrR* which normally controls synthesis of the affected enzymes. *TyrR* allele numbers *13* and *73* were allotted to the regulatory mutations in mutants 1-3 and 7-3, respectively. Regulatory properties of wild-type *E. coli*, mutant 1-3, and other *tyrR*$^-$ strains are described below.

The following evidence supports the double-mutation hypothesis. First, the *trpA*$^-$ allele of mutant 1-3 has been mapped (*see below*) and is readily cotransduced with a nearby marker, *cysB*$^+$, into the *cysB*$^-$ strain YS31, without concomitant transfer of the *tyrR13* allele (Table 8). The resulting *trpA*$^-$ recombinant, KB3131, exhibited properties similar to mutant 1-3 with regard to the partial tryptophan requirement, indole glycerol accumulation, and sensitivity to phenylalanine plus tyrosine (Table 2), but displayed wild-type properties with regard to regulation (Tables 4 and 5). It is noted that the generation time of this recombinant in minimal medium is slightly longer (2.2 hr) than that of mutant 1-3 (1.5 hr). Second, the *tyrR13* allele of mutant 1-3 was mapped and found to be near the tryptophan region (*see below*). This allele was cotransduced with *pyrF* and *cysB* into appropriate recipients without concomitant transfer of *trpA*$^-$. Regulatory properties of such recombinants are described below. They were wild-type with respect to *trpA*, did not excrete indole glycerol, and were not sensitive to phenylalanine plus tyrosine (Table 2). Such strains were resistant to APA in a similar fashion to strains known to possess the *tyrR*$^-$ allele (30; this paper).

Repression of enzymes of aromatic biosynthesis: DAHP synthetase (tyr). As previously reported (4), DAHP synthetase (tyr) is normally repressed in *E. coli* by 10^{-4} M tyrosine and by a combination of phenylalanine plus tyrptophan at 10^{-3} M. For comparative purposes, these results are shown in Table 3. It may be seen that limitation of the growth of the *aroD*$^-$ strains KB1364 and AT1359 in the chemostat by shikimic acid and tyrosine (in the presence of 10^{-4} M phenylalanine plus tryptophan), respectively, derepressed DAHP synthetase (tyr). Less derepression was observed when tyrosine limitation occurred in the presence of phenylalanine and tryptophan (10^{-3} M each). It was not possible from these experiments to tell whether both phenylalanine and tryptophan repressed at 10^{-3} M or whether only one of these amino acids repressed. It seemed likely, however, from a comparison with data obtained in *E. coli* W(2) that tryptophan represses DAHP synthetase (tyr) marginally, whereas phenylalanine alone represses strongly at 10^{-3} M. That this was, in fact, the case was shown when AT1359 was limited by tyrosine in the chemostat in the presence of 10^{-3} M phenylalanine and 10^{-4} M tryptophan. This resulted in approximately 10-fold repression of DAHP synthetase (tyr) (relative to fully derepressed level), whereas tyrosine

TABLE 3. *Repression of enzymes of aromatic biosynthesis in chemostat-grown AT1359 and KB1364*[a]

Strain	Supplement	Specific activities						
		DAHP synthetases			CA mutases (P + T)	PA dehydrogenase	PA dehydratase	Trp synthetase
		(phe)	(tyr)	(trp)				
AT1359	Complete	0.023	0	0.002	0.030	0.002	0.009	0
	Lim Phe	0.107	0.001	0.003	0.270	0.004	0.093	0.002
	Lim Trp	0.131	0.003	0.010	0.040	0.003	0.020	0.220
	Lim Tyr	0.024	0.169	0.003	0.206	0.134	0.010	0
	Phe + Trp (both 10^{-3} M) Lim Tyr	0.020	0.020	0.002	0.040	0.018	0.009	0
	Trp (10^{-3} M) Lim Tyr	0.020	0.200	0.003	0.228	0.108	0.009	0
	Phe (10^{-3} M) Lim Tyr	0.020	0.014	0.002	0.049	0.021	0.11	0
KB1364	Complete	0.018	0	0.002	0.021	0.002	0.008	0
	Lim SA	0.054	0.162	0.006	0.143	0.036	0.049	0.018

[a] Abbreviations are defined in Fig. 1. AT1359 is a multiple aromatic auxotroph blocked in dehydroquinase (Fig. 1). KB1364 is derived from AT1359 on the basis of its ability to grow rapidly on shikimic acid as a sole aromatic supplement. Experiments limiting AT1359 and KB1364 with aromatic metabolites were carried out in the chemostat, and cells were harvested after five generations. Where the limiting metabolite was an aromatic amino acid, the medium contained an excess (10^{-4} M unless otherwise indicated) of the two other aromatic amino acids. Limiting (Lim) Phe and Tyr was 2×10^{-5} M (in reservoir); Lim Trp was 5×10^{-6} M and Lim SA was 10 μg/ml. Complete supplement contained phe, tyr, and trp (10^{-4} M each). Cells with complete supplement were grown in batch culture and harvested at a cell density of 5×10^8/ml. Cultures supplemented with aromatic amino acids also contained *p*-amino-benzoic acid and *p*-hydroxybenzoic acid (10^{-6} M each). Nonaromatic amino acids required for growth (Table 1) were added to 100 μg/ml and thiamine was added to 10 μg/ml.

limitation in the presence of 10^{-4} M phenylalanine and 10^{-3} M tryptophan allowed full derepression of this enzyme (Table 3).

In the *aroF*$^+$ *aroG*$^-$ *aroH*$^-$ strains, KB3241, KB3242, and KB3243 (Table 4), only DAHP synthetase (tyr) is present, whereas, in the *aroF*$^-$ *aroG*$^+$ *aroH*$^-$ strains, KB5681, KB5682, and KB5683, only DAHP synthetase (phe) is present. All other strains listed in Tables 3 and 4 possess all three DAHP synthetases.

In the *tyrR*$^-$ *trp*$^-$ double mutants, 1-3 and 7-3, DAHP synthetase (tyr) could not be repressed by the aromatic amino acids (Table 4). The recombinant strains, KB3132, KB3242, KB5682, had received the *tyrR13* allele from mutant 1-3 by transduction without concomitant transfer of the *trpA*$^-$ allele. All three recombinants were resistant to APA. Except for KB5682, which lacks this enzyme, DAHP synthetase (tyr) was constitutive in these strains with a high specific activity similar to that seen in AT1359 grown on limiting tyrosine. The relevant *tyrR*$^+$ controls indicate the normal repressibility of DAHP synthetase (tyr) by the aromatic amino acids. KB3133, KB3243, and KB5683 received the *tyrR352* allele as an unselected marker by cotransduction with the neighboring *cysB*$^+$ or *pyrF*$^+$ marker from JP232 (a strain isolated by Wallace and Pittard; see reference 30 for a description of *tyrR352*). In the case of the first two strains, the *tyrR* character could be scored on the basis of resistance to APA. KB5683, however, showed no increase in resistance over the *tyrR*$^+$ condition in KB5681 (*see below*) and was found by screening for high, constitutive levels of prephenate dehydrogenase in several *pyrF*$^+$ transductants from the cross P1 JP232 × KB5680. Except in KB5683 (which lacks the enzyme), *tyrR352* conferred high, constitutive levels of DAHP synthetase (tyr) (*compare*

TABLE 4. *Repression of enzymes of aromatic biosynthesis in wild-type Escherichia coli and derived mutants*[a]

Strain	Aromatic alleles	Supplement	Specific activities						
			DAHP synthetases			CA mutases (P + T)	PA dehydrogenase	PA dehydratase	Trp synthetase
			(phe)	(tyr)	(trp)				
KB3130	wild	None	0.030	0.002	0.004	0.023	0.005	0.014	0
KB3130	wild	Complete	0.019	0	0.002	0.021	0.001	0.015	0
1-3	*tyrR13 trpA*$^-$	None	0.120	0.142	0.010	0.098	0.096	0.021	0.057
1-3	*tyrR13 trpA*$^-$	Complete	0.093	0.115	0.003	0.063	0.072	0.014	0.001
7-3	*tyrR73 trpD*$^-$	None	0.135	0.192	0.006	0.061	0.089	0.025	0.051
7-3	*tyrR73 trpD*$^-$	Complete	0.095	0.136	0.001	0.032	0.066	0.012	0
KB3132	*tyrR13*	None	0.152	0.179	0.004	0.053	0.063	0.006	0
KB3132	*tyrR13*	Complete	0.149	0.150	0.002	0.065	0.047	0.005	0
KB3131	*trpA*$^-$	None	0.069	0.003	0.006	0.022	0.007	0.009	0.040
KB3131	*trpA*$^-$	Complete	0.026	0	0.002	0.018	0	0.007	0
KB3133	*tyrR352*	None	0.077	0.057	0.003	0.034	0.070	0.005	0.003
KB3133	*tyrR352*	Complete	0.032	0.051	0.002	0.032	0.061	0.009	0
KB3241	*aroG*$^-$ *aroH*$^-$	None	0.002	0.018	0.001	0.025	0.018	0.016	0.004
KB3241	*aroG*$^-$ *aroH*$^-$	Complete	0	0	0	0.020	0.002	0.015	0
KB3242	*aroG*$^-$ *aroH*$^-$ *tyrR13*	None	0.003	0.094	0	0.029	0.046	0.007	0.003
KB3242	*aroG*$^-$ *aroH*$^-$ *tyrR13*	Complete	0.002	0.097	0	0.021	0.059	0.016	0
KB3243	*aroG*$^-$ *aroH*$^-$ *tyrR352*	None	0.008	0.140	0.002	0.037	0.045	0.010	0.001
KB3243	*aroG*$^-$ *aroH*$^-$ *tyrR352*	Complete	0.005	0.079	0.001	0.029	0.053	0.011	0
KB5681	*aroF*$^-$ *aroH*$^-$	None	0.069	0	0.001	0.018	0.012	0.011	0.001
KB5681	*aroF*$^-$ *aroH*$^-$	Complete	0.026	0	0	0.015	0.002	0.009	0
KB5682	*aroF*$^-$ *aroH*$^-$ *tyrR13*	None	0.082	0	0.002	0.012	0.036	0.004	0.003
KB5682	*aroF*$^-$ *aroH*$^-$ *tyrR13*	Complete	0.098	0	0.001	0.013	0.027	0.003	0.002
KB5683	*aroF*$^-$ *aroH*$^-$ *tyrR352*	None	0.046	0.002	0	0.023	0.070	0.006	0.003
KB5683	*aroF*$^-$ *aroH*$^-$ *tyrR352*	Complete	0.019	0	0.001	0.016	0.044	0.007	0

[a] Cells were grown in batch culture and harvested in exponential phase, at a cell density of 5×10^8/ml. Other details are indicated in Table 3.

tyrR13). The recombinant KB3131 which had received *trpA*$^-$ but not *tyrR13* from 1-3 was wild type with respect to repression of DAHP synthetase (tyr).

DAHP synthetase (phe). This enzyme was previously shown to be multivalently repressed in *tyrR*$^+$ strains by phenylalanine and tryptophan (4). Thus, when the multiple aromatic auxotroph AT1359 was limited in the chemostat by either of these amino acids, DAHP synthetase (phe) derepressed. The degree of derepression relative to the completely supplemented control was only about fivefold. This data is represented in Table 3. It should also be noted that unlike DAHP synthetase (tyr) 10^{-3} M phenylalanine did not restrict derepression of this enzyme which occurred normally on limitation of tryptophan (4).

Of the strains carrying the *tyrR13* or *tyrR73* allele (1-3, 7-3, KB3132, KB3242, and KB5682), all except KB3242 [which possesses DAHP synthetase (tyr) only] exhibited constitutive levels of DAHP synthetase (phe) activity equivalent to the derepressed levels in AT1359 starved of phenylalanine or tryptophan (Table 4). In contrast, strains KB3133 and KB5683 which had received the *tyrR352* allele by transduction from JP232 showed no change in the wild-type pattern of repression of DAHP synthetase (phe). This observation explains why KB5683 did not show increased resistance to APA compared with the *tyrR*$^+$ control KB5681. Essentially all of the assayable DAHP synthetase activity in strains KB5682 and KB5683 was inhibitable by phenylalanine (10^{-3} M) for both growth conditions shown in Table 4. Strain KB3131 which carries the *trpA*$^-$ allele of mutant 1-3 was repressed normally by the aromatic amino acids; however, as expected, growth in minimal medium (where tryptophan is limiting) caused derepression of DAHP synthetase (phe) (Table 4). The independently isolated partial *trpA*$^-$ strains, A46PR8 and A46PR9, behaved like KB3131 and are not shown.

DAHP synthetase (trp). The synthesis of this isoenzyme was previously shown to be repressed by tryptophan and to be controlled by the *trpR* gene (4, 20). Thus, DAHP synthetase (trp) was derepressed in AT1359 limited by tryptophan in the chemostat (Table 3). Repression in all *tyrR*$^-$ strains by the aromatic amino acids is normal, and it is concluded that synthesis of this isoenzyme is not affected by *tyrR* (Table 4). As expected, the tryptophan limitation which occurred when the *trp*$^-$ strains 1-3, 7-3, and KB3131 were grown in minimal medium caused derepression of DAHP synthetase (trp). Repression occurred normally when these cells were grown in the presence of the aromatic amino acids (Table 4). A46PR8 and A46PR9 (*not shown*) behaved similarly.

Chorismate mutases P and T. Total chorismate mutase activity (consisting of chorismate mutases P and T) was relatively low in wild-type cells grown in minimal medium (4; Table 4). This was presumed to be the result of repression by endogenous phenylalanine and tyrosine. Approximately 10-fold derepression occurred in KB1364 and AT1359 limited in the chemostat on shikimate and phenylalanine, respectively (Table 3). Tyrosine limitation of AT1359 in the presence of 10^{-4} M phenylalanine and tryptophan caused approximately sixfold derepression of total chorismate mutase activity. In the presence of 10^{-3} M phenylalanine, the derepression occurring with tyrosine limitation was less than twofold. This suggested that tyrosine and phenylalanine were both repressing metabolites of total chorismate mutase activity. Tryptophan limitation of AT1359 caused only marginal derepression of total chorismate mutase activity.

TyrR$^-$ strains generally displayed relatively high constitutive levels of total chorismate mutase activity (Table 4). However, in strains mutated in the structural genes for the DAHP synthetases, there appeared to be less derepression. The significance of this is not yet clear.

The two isoenzymes of chorismate mutase, P and T, were not separated in these studies. However, chorismate mutase T and prephenate dehydrogenase activities are both carried on a single protein; likewise, chorismate mutase P and prephenate dehydratase activities are on a single protein (9; R. G. H. Cotton, Ph.D. Thesis, Univ. of Melbourne, Melbourne, Australia, 1967). Thus, the repression pattern of the appropriate chorismate mutase may be inferred from that of prephenate dehydrogenase and prephenate dehydratase. The data presented here for total chorismate mutase should, therefore, be considered in conjunction with the repression data which follows for prephenate dehydrogenase and prephenate dehydratase.

Prephenate dehydrogenase. This enzyme is present in relatively small amounts in wild-type cells such as KB3130 grown in minimal medium and is repressed almost completely by growth in completely supplemented medium (Table 4). In AT1359, grown on limiting tyrosine in the presence of 10^{-4} M phenylalanine and tryptophan, however, it is derepressed approximately 90-fold above the completely supplemented control (Table 3). This indicates that tyrosine is a repressing metabolite. Shikimate limitation of KB1364 also caused derepression (>20-fold), whereas 10^{-3} M phenylalanine prevented full release from repression in AT1359 grown on lim-

iting tyrosine [*compare* total chorismate mutase (Table 3)]. This closely resembled the pattern of repression for DAHP synthetase (tyr) and indicated that, in addition to tyrosine, phenylalanine (at 10^{-3} M) is also a repressing metabolite of prephenate dehydrogenase and the associated activity, chorismate mutase T.

In *tyrR*$^-$ strains 1-3, 7-3, KB3132, KB3242, and KB5682, prephenate dehydrogenase activity was high and constitutive (Table 4). *TyrR352* strains, KB3133, KB3243, and KB5683 were also constitutive for this enzyme. In KB3131 (which had received the *trpA*$^-$ allele of 1-3), prephenate dehydrogenase was repressed normally. It is concluded that *tyrR* controls formation of prephenate dehydrogenase and, it may be inferred, chorismate mutase T. It is also inferred that all of the increase in total chorismate mutase activity seen in *tyrR*$^-$ strains is chorismate mutase T, since, as is shown in the next section, prephenate dehydratase (and hence, chorismate mutase P) is not controlled by *tyrR*.

Prephenate dehydratase. This enzyme is relatively repressed in wild-type (KB3130) cells whether grown in minimal medium or in completely supplemented medium (Table 4). The low level of prephenate dehydratase in minimal medium-grown cells is presumed to reflect repression by endogenous phenylalanine since this amino acid is the repressing metabolite for this enzyme. The latter point is demonstrated by the derepression (10-fold) which occurred when AT1359 was grown in the chemostat on limiting phenylalanine (Table 3). Limitation of KB3164 on shikimic acid also caused derepression. It may be inferred from the derepression of prephenate dehydratase (but not prephenate dehydrogenase), which occurred on phenylalanine limitation, that the increase seen in total chorismate mutase activity under these conditions was entirely chorismate mutase P. Tryptophan limitation caused about twofold derepression of prephenate dehydratase (*compare* chorismate mutase). The significance of this marginal effect is not yet clear.

In none of the *tyrR*$^-$ strains was prephenate dehydratase (and by inference, chorismate mutase P) derepressed (Table 4). This indicates that synthesis of chorismate mutase P-prephenate dehydratase may be controlled by a separate, unknown regulatory gene.

Tryptophan synthetase. It has been shown previously that this enzyme is repressed by tryptophan (8, 15). Thus, this enzyme is present in high concentration in AT1359 grown on limiting tryptophan (Table 3). Control by tryptophan was not affected by the *tyrR*$^-$ alleles (Table 4). It was noted, however, that the *trp*$^-$ alleles carried by the *tyrR*$^-$ strains 1-3 and 7-3 and the *tyrR*$^+$ derivative of 1-3, KB3131, caused tryptophan limitation when these cells were grown in minimal medium and, consequently, derepression of tryptophan synthetase [*compare* DAHP synthetase (trp)]. Repression occurred normally when these cells were grown in the presence of phenylalanine, tyrosine, and tryptophan (Table 4).

Transaminase A. This enzyme catalyzes the reversible conversion of *p*-hydroxyphenylpyruvate to tyrosine and phenylpyruvate to phenylalanine (24). Transaminase A is heat-labile, thus allowing its activity on phenylalanine-phenylpyruvate to be distinguished from the heat-stable phenylalanine transaminase in crude extracts. It was repressed in *tyrR*$^+$ strains by tyrosine. This was confirmed by the derepression of transaminase A activity (six- to eightfold) when AT1359 was grown on limiting tyrosine (Table 5). Phenylalanine and tryptophan at 10^{-4} M did not prevent the derepression on limiting tyrosine, nor did they restrict it when present at 10^{-3} M [contrast DAHP synthetase (tyr) and chorismate mutase T-prephenate dehydrogenase].

In all *tyrR*$^-$ strains, control by repression was absent (Table 5), leading to high constitutive levels of transaminase A in these strains.

Heat-stable phenylalanine transaminase. There was no significant derepression of heat-stable phenylalanine transaminase under any of the growth conditions listed in Table 5, including phenylalanine limitation of AT1359 in the chemostat. It is concluded that this enzyme is either not repressible (i.e., is constitutive) or the optimal conditions for derepression were not attained in these experiments.

5-Dehydroquinate synthetase and 5-dehydroquinase. 5-Dehydroquinate synthetase and 5-dehydroquinase, the second and third enzymes, respectively, of the common aromatic pathways are relatively insensitive to repression by the aromatic amino acids in *E. coli* (4). Their levels in wild-type W1485 and in the *tyrR*$^-$ strain 1-3, grown in minimal medium, were only slightly higher than in cells grown in the presence of all three aromatic amino acids (Table 6). Similarly, there was only slight derepression (less than twofold) in multiple aromatic auxotrophs grown in the chemostat on limiting aromatic amino acids (4). It is concluded that the *tyrR* gene has no effect on the synthesis of these enzymes and that these enzymes are either constitutive or that the optimal conditions for their derepression have not yet been found.

Table 7 summarizes the most significant repression data obtained from the chemostat experiments with AT1359 and from the study of

TABLE 5. *Repression of phenylalanine and tyrosine transaminases in E. coli K-12*[a]

Strain	Aromatic alleles	Supplement	Transaminase specific activities		
			Tyrosine (transaminase A)[b]	Phenylalanine as substrate[c]	
				Heat-labile (transaminase A)	Heat-stable
AT1359	*aroD*⁻	Complete	0.022	0.016	0.007
		Lim Phe	0.023	0.013	0.008
		Lim Trp	0.034	0.014	0.008
		Lim Tyr	0.133	0.088	0.011
		Phe + Trp (both 10^{-3} M) Lim Tyr	0.107	0.070	0.009
		Trp (10^{-3} M) Tyr	0.122	0.081	0.010
		Phe (10^{-3} M) Lim Tyr	0.138	0.097	0.013
KB1364	*aroD*⁻ *shi*⁺	Complete	0.026	0.017	0.007
KB1364	*aroD*⁻ *shi*⁺	Lim SA	0.149	0.105	0.008
1-3	*tyrR13 trpA*⁻	None	0.082	0.047	0.013
1-3	*tyrR13 trpA*⁻	Complete	0.073	0.043	0.012
7-3	*tyrR73 trpD*⁻	None	0.067	0.040	0.009
7-3	*tyrR73 trpD*⁻	Complete	0.060	0.033	0.010
KB3130	wild	None	0.049	0.029	0.010
KB3130	wild	Complete	0.020	0.012	0.009
KB3132	*tyrR13*	None	0.079	0.044	0.010
KB3132	*tyrR13*	Complete	0.075	0.038	0.010
KB3131	*trpA*⁻	None	0.050	0.031	0.007
KB3131	*trpA*⁻	Complete	0.019	0.011	0.007
KB3133	*tyrR352*	None	0.054	0.023	0.008
KB3133	*tyrR352*	Complete	0.053	0.029	0.009
KB3241	*aroG*⁻ *aroH*⁻	None	0.054	0.025	0.007
KB3241	*aroG*⁻ *aroH*⁻	Complete	0.021	0.009	0.007
KB3242	*aroG*⁻ *aroH*⁻ *tyrR13*	None	0.068	0.031	0.006
KB3242	*aroG*⁻ *aroH*⁻ *tyrR13*	Complete	0.073	0.029	0.007
KB3243	*aroG*⁻ *aroH*⁻ *tyrR352*	None	0.061	0.037	0.009
KB3243	*aroG*⁻ *aroH*⁻ *tyrR352*	Complete	0.052	0.030	0.012

[a] Abbreviations and experimental procedure for AT1359 and KB1364 are indicated in Table 3, whereas procedures for strains other than these are indicated in Table 4.

[b] Transaminase A activity on tyrosine was measured in unheated extracts.

[c] Total phenylalanine transaminase activity was measured in unheated extracts, and the heat-labile (transaminase A) component was estimated by subtracting the heat-stable activity remaining in extracts heated 15 min at 60 C.

tyrR⁻ strains. Two major new points emerge from this summary. First, *tyrR* controls the synthesis of DAHP synthetases (phe) and (tyr), chorismate mutase T-prephenate dehydrogenase, and transaminase A. Apart from a failure to affect the repressibility of DAHP synthetase (phe), the *tyrR352* allele of Pittard and Wallace exhibited a similar phenotype to *tyr-R13*. Although the same regulatory gene, *tyr-R*, controls the synthesis of these enzymes, they differ markedly in their control by repressing metabolites. DAHP synthetase (phe) is repressed multivalently by phenylalanine and tryptophan. DAHP synthetase (tyr) and chorismate mutase T-prephenate dehydrogenase are repressed by low concentrations of tyrosine and by high concentrations of phenylalanine. Transaminase A is repressed by tyrosine alone. Second, chorismate mutase P-prephenate dehydratase is markedly repressed by phenylalanine but is not controlled by *tyrR*. It is postulated that a separate regulatory gene exists for these activities.

Genetic mapping of tyrR. The finding of Pittard and Im that an allele which conferred similar regulatory properties to *tyrR* alleles *13* and *73* was linked to the tryptophan operon (*personal communication*) enabled the separation of the *tyrR13* and *trpA*⁻ alleles of the double mutant, 1-3, by cotransduction with nearby markers, and their mapping.

The transduction analysis for these alleles is shown in Table 8.

Of the markers tested, *pyrF* was closest to *tyrR13* (39% cotransduction). *CysB* (31%), *trpA*

(16%), and *trpE* (24%) were also cotransducible with *tyrR13*. Another marker in this general area, *pabB* (30 min), was not cotransducible with *tyrR13*. *PyrF* was 50 to 53% cotransduced with *cysB* and 25 to 27% cotransduced with *trpA*. Data indicates that *pyrF* is closer than *tyrR13* to *cysB* and *trpA* and that *tyrR13* must lie to the right of *pyrF* at about 26 min on the chromosome time scale of Taylor (28). Figure 2 shows the relationship of *tyrR* to nearby markers and to genes of aromatic amino acid metabolism.

The *tyrR352* allele of the donor strain KB2352 had the following cotransduction frequencies with *pyrF*, 41%; *cysB*, 33%; *trpA*, 24%; *trpE*, 21%. This indicated a location for *tyrR352* approximately the same as that for *tyrR13*. In contrast, when JP232 was used as the source of donor phage carrying *tyrR352*, the cotransduction frequencies of this allele with *pyrF*, *cysB*, *trpA*, and *trpE* were reduced by approximately half. This suggested that JP232 carried gene(s) restricting cotransduction in this region. This restriction was eliminated when *tyrR352* was transferred into KB2352 or other strains, the largely wild-type genetic backgrounds of which were isogenic with KB3132 (the donor strain for *tyrR13*).

TABLE 6. *Specific activities of 5-dehydroquinate synthetase and 5-dehydroquinase in wild-type Escherichia coli (W1485) and tyrR strain, 1-3*[a]

Strain	Supplement	Specific activities	
		5-Dehydroquinate synthetase	5-Dehydroquinase
W1485	None	0.027	0.100
W1485	Complete	0.020	0.093
1-3	None	0.025	0.112
1-3	Complete	0.020	0.092

[a] Experimental procedure is described in Table 4. Complete medium is described in Table 3.

The possibility has not been completely excluded that the *tyrR13* phenotype is the result of two mutations, one affecting the gene mutated in *tyrR352* [and hence repression of DAHP synthetase (tyr), chorismate mutase T-prephenate dehydrogenase, and transaminase A] and an unknown regulatory gene for DAHP synthetase (phe). The second mutation, however, would have to be extremely close to *tyrR* since eight randomly chosen $cysB^+$ $tyrR^-$ transductants (from the cross P1 1-3 × YS31) and 16 $pyrF^+$ $tyrR^-$ transductants (from the cross P1 1-3 × KB3134) all showed the *tyrR13* phenotype (*compare*, KB3132; Brown, *unpublished data*).

The $trpA^-$ allele of 1-3 maps as expected, being 37% cotransducible with *cysB* and 26% cotransducible with *pyrF*. These frequencies are similar to those obtained for the independently isolated $trpA^-$ allele of strains A2. None of the $trpA^-$ $pyrF^+$ transductants from the cross P1 1-3 × JP324 were $tyrR^-$. This confirmed that *tyrR13* lies well to the right of *pyrF*.

In addition to *tyrR* alleles *13* and *352*, four further *tyrR* alleles have been mapped. One is the *tyrR73* allele of 7-3, whereas three (carried by KB3247, KB3248, and KB3249) were obtained by spontaneous mutation of strain KB3241 to APA resistance. This selection technique was essentially similar to that used by Wallace and Pittard to obtain *tyrR352* (30). The latter three were allotted *tyrR* allele numbers *5*, *7*, and *12*. All four alleles confer the *tyrR13* phenotype, that is, constitutive synthesis of DAHP synthetase (phe) as well as DAHP synthetase (tyr), chorismate mutase T-prephenate dehydrogenase, and transaminase A. Cotransduction frequencies of these four alleles with

TABLE 7. *Summary of repression data*

Strain	Allele	Supplement	Derepression[a]					
			DAHP synthetases		CA mutases (P + T)	PA dehydrogenase	PA dehydratase	Transaminase A
			(phe)	(tyr)				
KB3130	Wild	Complete	13	0	8	1	16	14
KB3132	*tyrR13*	Complete	100	78	24	35	5	54
KB3133	*tyrR352*	Complete	22	27	12	45	9	38
AT1359	$aroD^-$	Lim phe	72	0	100	3	100	16
		Lim trp	88	1	15	2	22	24
		Lim tyr	16	100	76	100	10	96
		10^{-3} M phe Lim tyr	13	7	18	16	12	100

[a] Per cent of maximal specific activity observed. Maximal specific activities (= 100%) are taken from Tables 3-5. They were: DAHP synthetase (phe) 0.149; DAHP synthetase (tyr) 0.169; CA mutases (P + T) 0.270; PA dehydrogenase 0.134; PA dehydratase 0.093; transaminase A 0.138. Abbreviations as in Fig. 1.

TABLE 8. *Cotransduction frequencies with phage P1 in tyrR region*[a]

Donor	Recipient	Alleles		Per cent cotransduction
		Selected (no.)	Unselected (no.)	
1-3	JP324	*pyrF*$^+$ (116)	*trpA*$^-$ (30)	26
1-3	YS31	*cysB*$^+$ (348)	*trpA*$^-$ (127)	37
A2	JP324	*pyrF*$^+$ (168)	*trpA*$^-$ (42)	25
JP324	A2	*trpA*$^+$ (125)	*pyrF*$^-$ (34)	27
A2	YS31	*cysB*$^+$ (174)	*trpA*$^-$ (61)	35
JP324	YS31	*cysB*$^+$ (134)	*pyrF*$^-$ (71)	53
YS31	A2	*trpA*$^+$ (165)	*cysB*$^-$ (68)	41
YS31	JP324	*pyrF*$^+$ (114)	*cysB*$^-$ (57)	50
1-3	KB3244	*cysB*$^+$ (116)	*trpA*$^-$ (43)	37
KB3132	JP324	*pyrF*$^+$ (290)	*tyrR13* (114)	39
KB3132	KB3244	*cysB*$^+$ (232)	*tyrR13* (73)	31
KB3132	KB3245	*trpE*$^+$ (375)	*tyrR13* (89)	24
KB3132	KB3246	*trpA*$^+$ (162)	*tyrR13* (26)	16
KB2352	JP324	*pyrF*$^+$ (232)	*tyrR352* (94)	41
KB2352	KB3244	*cysB*$^+$ (464)	*tyrR352* (153)	33
KB2352	KB3245	*trpE*$^+$ (232)	*tyrR352* (56)	24
KB2352	KB3246	*trpA*$^+$ (232)	*tyrR352* (49)	21

[a] Transducing phage was P1kc. The donor and recipient strains are described in Table 1. The prototrophic transductants were scored for *tyrR*$^-$ by replication onto plates containing 10^{-4} M 4-amino-DL-phenylalanine which inhibited the growth of *tyrR*$^+$ strains.

cysB and *pyrF* did not differ significantly from those shown in Table 8 for *tyrR13* and *tyrR352*.

DISCUSSION

Characterization of the two mutations carried in each of strains 1-3 and 7-3 has assisted the genetic and physiological analysis of repression of aromatic biosynthesis in *E. coli*. One of the mutations (*trpA*$^-$ in 1-3 and *trpD*$^-$ in 7-3) conferred partial tryptophan auxotrophy. The other mutation, *tyrR*$^-$, rendered constitutive all of the enzymes normally repressed by tyrosine [namely, DAHP synthetase (tyr), chorismate mutase T-prephenate dehydrogenase, and transaminase A] as well as one normally multivalently repressed by phenylalanine plus tryptophan [DAHP synthetase (phe)].

One can only speculate on the selection pressure which operated to favor the *tyrR*$^-$ mutation in strains 1-3 and 7-3 selected for sensitivity to a mixture of phenylalanine plus tyrosine. One possible explanation follows. We know that partial tryptophan auxotrophs grow only slowly in minimal medium and are sensitive to phenylalanine plus tyrosine (Table 2). A *tyrR*$^-$ mutation in such strains could cause faster growth in minimal medium by increasing the flow of aromatic intermediates through the defective tryptophan pathway as a result of derepression of DAHP synthetase. This suggestion is supported by the observation that the double mutant 1-3 (*trpA*$^-$ *tyrR*$^-$) grows faster in minimal medium than a *trpA*$^-$ *tyrR*$^+$ derivative, KB3131 (Table 2). Such

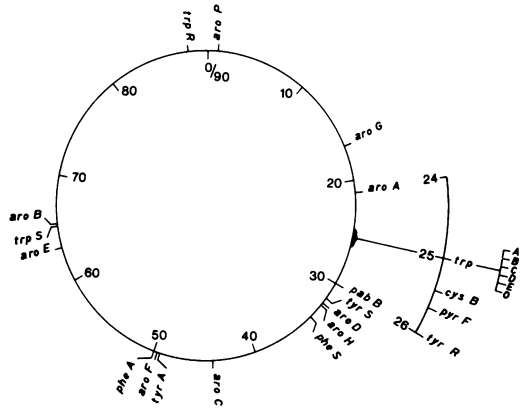

FIG. 2. *Map of E. coli chromosome showing location of tyrR and other markers. The location of tyrS, the gene specifying the tyrosyl-transfer ribonucleic acid synthetase, is shown at 32 min [after Schlesinger and Nester (22)]. Other known loci concerned with aromatic amino acid metabolism are also shown. Apart from aroP which specifies the general aromatic transport system (5, 6) the gene symbols used are those recommended by Taylor (28).*

a hypothesis would explain why the selection procedure for 1-3 and 7-3 (which involved plating onto minimal medium after penicillin selection in phenylalanine plus tyrosine medium) favors the selection of double *trp*$^-$ *tyrR*$^-$ mutants over single *trp*$^-$ mutants. The *trpA*$^-$ mutation of the double mutant would protect it during the penicillin selection step (since phenylalanine

plus tyrosine inhibit growth), whereas the *tyrR*$^-$ mutation would accelerate growth on minimal medium. Single *tyrR*$^-$ mutants would not be selected, since their growth would not be inhibited by phenylalanine plus tyrosine and hence they would be killed in the penicillin selection step.

The analysis of repression of aromatic amino acid biosynthesis to date indicates three regulons. The term regulon is used to describe a group of genes controlling related functions and regulated together by the same macromolecular repressor substance (17). The groups of enzymes specified by these three regulons, together with those enzymes whose regulation is uncertain, are shown in Fig. 1 and are discussed below.

Aromatic regulon. This regulon specifies the five enzymatic activities controlled by the *tyrR* gene. Of these, four [namely, DAHP synthetase (tyr), chorismate mutase T-prephenate dehydrogenase, and transaminase A] are repressed by tyrosine. Except in the case of transaminase A, repression is not entirely specific to tyrosine and high concentrations (10^{-3} M) of phenylalanine also repress. It is rather surprising that 10^{-3} M phenylalanine should repress these enzymes more effectively than 10^{-4} M phenylalanine, since, at both concentrations, both of the known phenylalanine transport systems are saturated (5); hence, the intracellular pool of phenylalanine would be expected to be similar for both exogenous concentrations. *AroF* and *tyrA*, the structural genes for DAHP synthetase (tyr) and chorismate mutase T-prephenate dehydrogenase, respectively, are thought to be contiguous and may form an operon (19; Fig. 2). The location of the structural gene for transaminase A is unknown. The absence of coordinate repression with DAHP synthetase (tyr) and prephenate dehydrogenase by tyrosine and the different response of transaminase A to repression by 10^{-3} M phenylalanine suggests that this gene is not part of the tyrosine operon.

The fifth enzyme controlled by *tyrR*, DAHP synthetase (phe), requires two amino acids for repression, phenylalanine and tryptophan. DAHP synthetase (phe) is coded by a structural gene, *aroG*, which maps far from *aroF* and *tyrA* (Fig. 2).

The finding that *tyrR* controls enzymes which can be repressed independently of each other by tyrosine [DAHP synthetase (tyr), chorismate mutase T-prephenate dehydrogenase, transaminase A], by 10^{-3} M phenylalanine [DAHP synthetase (tyr), chorismate mutase T-prephenate dehydrogenase] and by phenylalanine plus tryptophan [DAHP synthetase (phe)] implies that *tyrR* codes for an aporepressor which can interact with all three aromatic amino acids to produce three distinct repressors, each with a different spectrum of action. First, tyrosine interacts with the aporepressor so that *aroF*, *tyrA*, and the transaminase A operon are repressed but not *aroG*. Second, a high phenylalanine concentration alters the aporepressor structure to produce an active repressor for *aroF* and *tyrA* but not the transaminase A operon or *aroG*. Third, phenylalanine plus tryptophan change the structure of the aporepressor so that *aroG* is repressed but not *aroF*, *tyrA*, and the transaminase A structural gene.

It is postulated that the constitutive synthesis of all enzymes of the aromatic regulon by the *tyrR13* class of regulatory mutants results from the formation by these mutants of an aporepressor which is no longer activated by any of the aromatic amino acids. In *tyrR352* strains, the aporepressor is no longer activated by tyrosine but can still be activated by phenylalanine plus tryptophan. This would explain the marked difference in repressibility of DAHP synthetase (phe) conferred by the *tyrR13* and *tyrR352* alleles. From their otherwise similar phenotypes and similar map locations, it seems probable that *tyrR13* and *tyrR352* are different alleles of the same regulatory gene. It would be premature, however, to conclude that this is definitely the case until appropriate complementation tests between the two alleles have been performed. The designation *tyrR* for both classes of allele is, therefore, provisional, pending the outcome of the complementation tests.

The aromatic regulon differs from other regulons comprising multiple unlinked operons such as the arginine regulon. All of the operons of the latter respond in unison to the same repressing metabolite, whereas the aromatic regulon responds only in part to a particular repressing metabolite. The aromatic regulon is derepressed in unison only in the *tyrR13* class of mutants or when growth of a multiple aromatic auxotroph is limited by shikimic acid. Repression in unison is observed only when cells are grown in the presence of all three aromatic amino acids.

The finding that a common regulatory gene, *tyrR*, controls the synthesis of two of the DAHP synthetases emphasizes the importance of checking the effect of genetic and metabolic changes on the other interdependent elements of the pathway. This may be relevant to the characterization of regulatory genes for the three aspartate kinases and other enzymes of the branched aspartate pathway. One of the aspartate kinases is repressed multivalently, whereas the other two are each repressed by a single amino acid, end product (29).

Phenylalanine regulon. In contrast to DAHP synthetase (phe), the multifunctional protein, chorismate mutase P-prephenate dehydratase, is repressed by phenylalanine alone and is not under the control of *tyrR*. It is suggested that the structural gene for this protein, *pheA*, is controlled by an unknown regulator gene, thus, forming a separate regulon.

Chorismate mutase P-prephenate dehydratase derepressed about 10-fold in the multiple aromatic auxotroph AT1359 grown in the chemostat on limiting phenylalanine. Pittard and Gibson have reported (21) that these enzymatic activities are only marginally repressed by phenylalanine. Only threefold derepression was observed in multiple aromatic auxotrophs starved of phenylalanine in batch culture (21). This disparity indicates that the chemostat may be superior to the batch technique in achieving derepression of this enzyme. Unlike the steady state of continued growth and protein synthesis under limiting conditions obtained in the chemostat, limitation (and hence derepression) in batch culture occurs only in the closing stages of growth when the repressing amino acid runs out. The observation (21) that mutants thought to be operator constitutive for *pheA* showed 10-fold derepression of chorismate mutase P-prephenase dehydratase also suggests that the conditions of batch culture used by these authors were suboptimal for derepression.

Tryptophan regulon. The tryptophan operon and *aroH* [the structural gene for DAHP synthetase (trp)] were previously shown to form a regulon controlled by the tryptophan regulator gene *trpR* (4, 8, 15, 20).

Regulation of the common aromatic pathway and phenylalanine transaminase. There is little information on repression of enzymes of the common aromatic pathway after DAHP synthetase. The second and third enzymes of the pathway, 5-dehydroquinate synthetase and 5-dehydroquinase, derepressed only marginally (less than twofold) when single aromatic amino acids or shikimate limited the growth of multiple aromatic auxotrophs in the chemostat (4). Their levels were unaffected by mutation of *tyrR* (Table 6). Similar observations were made for the heat-stable phenylalanine transaminase. It is concluded either that these three enzymes are constitutive or that the optimal conditions for their derepression have not yet been found.

It should be noted that Mitsuhashi and Davis (19) reported that a strain of *E. coli* W, blocked in 5-dehydro-shikimate reductase (83-2), had about 10 times more 5-dehydroquinase activity than the wild type. This observation remains unexplained and indicates that further work is required before a definite conclusion can be reached on the repressibility of 5-dehydroquinase in *E. coli*.

Gibson and Pittard have reported that the fifth enzyme of the common pathway, shikimate kinase, is derepressed approximately fivefold in strains possessing only DAHP synthetase (trp) and in *tyrR*$^-$ strains possessing only DAHP synthetase (tyr) (13). Limitation of a multiple aromatic auxotroph in batch cultures by either tyrosine, tryptophan, or shikimate caused slight derepression (13). Gibson and Pittard point out that it is difficult to interpret these results without further work, particularly as there are probably two shikimate kinases in *E. coli* (1).

Repression of the remaining three enzymes of the common pathway has not been studied in *E. coli*.

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