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Regulation of Phenylalanine Biosynthesis in *Escherichia coli* K-12: Control of Transcription of the *pheA* Operon

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Bacteriophage λ p*pheA-lac* was used to obtain strains of *Escherichia coli* K-12 in which *pheA* and *lacZ* are each transcribed from a separate *pheA* promoter. Mutants in which both β-galactosidase and chorismate mutase P-prephenate dehydratase (the *pheA* gene product) were derepressed were isolated, and a *trans*-acting gene (*pheR*) was identified. *pheR* was mapped at min 93 on the *E. coli* chromosome; *pheR* mutants acquired the wild-type phenotype when either F117 (which covers the 93-min region) or F116 (which covers min 59 to 65) was introduced into the cell. A rifampin resistance mutation, *rpoB366*, was found to derepress transcription of the *pheA* operon. *pheR* and *rpoB366* affected two different systems for the phenylalanine-mediated control of *pheA*. A mutation in *miaA* (*trpX*), a gene known to be involved in attenuation in the tryptophan operon, was also shown to increase transcription of the *pheA* gene.

The biosynthesis of phenylalanine from intermediary products of glucose metabolism in *Escherichia coli* is achieved via (i) a common pathway that subserves the synthesis of the aromatic amino acids and aromatic vitamins, followed by (ii) a terminal pathway for the conversion of chorismate (the final intermediate in the common pathway) to phenylalanine (Fig. 1). The regulation of phenylalanine biosynthesis is predominantly effected by control of the activity of the bifunctional enzyme chorismate mutase P-prephenate dehydratase (EC 5.4.99.5/4.2.1.51), which catalyzes the conversion of chorismate to phenylpyruvate, the first step in the terminal pathway. This enzyme, the product of the *pheA* gene, is subject both to feedback inhibition (5) and to feedback repression of synthesis. The enzyme is maximally repressed even when prototrophic strains are grown in the absence of exogenous phenylalanine; it is derepressed only threefold when a multiple aromatic auxotroph is starved for phenylalanine in batch culture (10). A 10-fold derepression was, however, demonstrated when such a strain was grown under phenylalanine limitation in a chemostat (3).

E. coli mutants altered in the regulation of phenylalanine biosynthesis have been isolated with the use of the amino acid analogs *o*- and *p*-fluorophenylalanine (FPA) (10). These mutants are constitutively derepressed for chorismate mutase P-prephenate dehydratase; the mutations are *cis*-dominant and are closely linked to the structural gene *pheA*. It was assumed that these mutations occurred in an operator locus (*pheO*; hereinafter referred to as *pheAo*, following the nomenclature suggested by Bachmann and Low [1]), which controls the expression of *pheA*. However, attempts to isolate strains with mutations in the postulated regulator gene *pheR* by this method of FPA selection were not successful.

A *pheR* mutant of *Salmonella typhimurium* has been obtained (7). This mutation is recessive in heterogenote strains carrying F116 of *E. coli* K-12, which permitted the direct inference that the gene *pheR* also exists in *E. coli* and is on the portion of the chromosome present on F116.

Recently, nucleotide sequence analysis of the leader region of *pheA* (24) suggested that the gene possesses an attenuation mechanism analogous to those described for other biosynthetic operons in *E. coli* and *S. typhimurium* (for a review, see reference 20). In vitro transcription studies of the *pheA* gene showed that 60% of transcripts initiated at the promoter terminate at the proposed attenuator site in the leader (24).

In the accompanying paper (8), we describe the construction of the bacteriophage λ p*pheA-lac* from a *pheA*::Mu d1 (*lac* Ap^r) fusion strain. We describe below the use of λ p*pheA-lac* lysogens for the successful isolation and characterization of mutants in *pheR* and present genetic evidence for the presence of two distinct and independent mechanisms for the transcriptional regulation of the *pheA* operon.

MATERIALS AND METHODS

The chemicals and growth media used, many of the genetic techniques, and the methods for enzyme as-

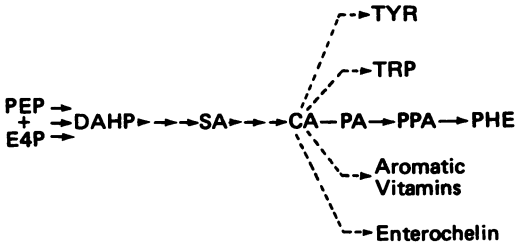

FIG. 1. Pathway of phenylalanine biosynthesis in *E. coli* and its relationship to the synthesis of the other aromatic amino acids and vitamins. PEP, Phosphoenolpyruvate; E4P, erythrose-4-phosphate; DAHP, 3-deoxy-D-arabinoheptulosonic acid-7-phosphate; SA, shikimic acid; CA, chorismic acid; PA, prephenic acid; PPA, phenylpyruvic acid. The conversion of chorismate through prephenate to phenylpyruvate is catalyzed by the bifunctional enzyme chorismate mutase P-prephenate dehydratase, a product of the *pheA* gene.

says are all described in the accompanying paper (8); λ p*pheA-lac* is also described therein. λNK370 (*b221 cI857 cI171*::Tn*10* 0 UGA261) was from N. Kleckner. Phenyl-β-D-galactoside (PG) and *o*-FPA and *p*-FPA were purchased from Sigma Chemical Co., St. Louis, Mo. Table 1 lists the strains of *E. coli* K-12 used.

Transposition of transposon Tn*10*. Random transpositions of Tn*10* into the chromosome were obtained by the method of Kleckner et al. (12).

Selection for Tet^s clones from Tn*10*-carrying strains. Quinaldic acid, $ZnCl_2$, and chlortetracycline were used in the selection for tetracycline-sensitive cells from strains carrying Tn*10*, as described by Bochner et al. (2).

PG selection for mutants with increased β-galactosidase activity. The method for selecting mutants with increased β-galactosidase activity was based on that described by Smith and Sadler (18).

Phenylalanine cross-feeding. The method used to detect cross-feeding was based on that described by Gibson and Jones (6), except that 0.01% peptone was omitted from the medium. The phenylalanine auxotroph AT2022 was used as the indicator strain in these experiments.

RESULTS

PG selection for mutants with increased β-galactosidase activity. Two related strains, JP3156 and JP3290 [both Δ*lac recA* (λ p*pheA-lac*)], were used to select for spontaneous mutants with increased β-galactosidase activity on 0.01% PG plates, as described above. Mutants were obtained at a frequency of between 10^{-4} and 10^{-5} per cell plated. They were then purified and classified into *cis*- and *trans*-acting mutants on the basis of the following property. Both JP3156 and JP3290 carry an intact *pheA* gene in addition to the *pheA-lac* fusion. Mutations in *cis* that increase β-galactosidase activity do not derepress *pheA*, whereas the *trans*-acting mutations not only increase β-galactosidase activity, but also increase the activity of chorismate mutase P-prephenate dehydratase. The derepression of chorismate mutase P-prephenate dehydratase activity can be screened by phenylalanine cross-feeding tests and then demonstrated more definitively by assays of cell extracts for enzyme activity.

The *cis*-acting mutations that were obtained with the use of λ p*pheA-lac* are not considered further in this paper. Some of the *trans*-acting mutations are described below.

Isolation and mapping of *pheR* mutants. One class of mutants obtained in the PG selection had a 10-fold derepression of β-galactosidase activity and strongly cross-fed the phenylalanine auxotroph AT2022; this class also had a 20-fold increase in the activity of perphenate dehydratase. The same mutant alleles, however, produced only 6- and 10-fold increases, respectively, in β-galactosidase and prephenate dehydratase activities in other strains of different pedigree. Two independently derived mutants (JP3297 and JP3299) were chosen for further study. The mutations causing this phenotype were tentatively assigned to the gene *pheR* and given the allele numbers *pheR372* and *pheR374*, respectively, for strains JP3297 and JP3299.

Both mutations *pheR372* and *pheR374* were recessive in F116 heterogenote strains (Table 2); this confirmed an earlier report by Gollub et al. (7), who found that a *pheR* mutation in *S. typhimurium* was recessive in heterogenote strains carrying *E. coli* F116.

Further mapping of *pheR372* was achieved by using transposon Tn*10*. A Tet^s derivative of JP3297 was isolated by the method of Bochner et al. (2), and random chromosomal Tn*10* insertions were introduced by using λNK370, as described above. Strains with Tn*10* insertions near *pheR* were then selected for by preparing a P1 *kc* lysate on the pool of cells, transducing JP3151, and then selecting for tetracycline resistance and growth on 0.01% PG. One of the strains so obtained, JP3302, was subsequently shown by P1 transductions to have Tn*10* linked 95% to *pheR*.

Direct mapping of the *pheR* (Tn*10*) locus yielded results that were in conflict with the F116 data, which had implied that *pheR* was in the 59- to 65-min region of the chromosome; in view of this anomaly, the precise map location was confirmed by a variety of genetic methods. Thus, the Tn*10* (and the *pheR372* allele linked to it) in JP3302 was transferred into several Hfr strains and mapped by interrupted matings with appropriate recipient strains. These experiments established that there was only one Tet^r locus and that this was situated at about 90 to 95 min on the map, close to and clockwise from *metA* (data not shown). The Tn*10* locus was shown to

TABLE 1. List of *E. coli* K-12 strains used

Strain	Genotype[a]	Origin or reference
AT2022	*his argE3 pro pheA1*	A. L. Taylor
KLF16/KL110	F116/*argG6 metB his-1 thyA23 leu-6 recA1 rpsL104*	14
KLF17/KL132	F117/*pyrB31 thi-1 thyA25 his-1 pro-27 leu-6 thr-1 recA1 rpsL9*	14
JP3151	F^+ *purE trp his argG ilv leu metA* or *metB rpsL* Δ*lacU169* (λ p*pheA-lac*)	From CSH57 (16), involving several intermediate strains
JP3156	JP3151 *recA56 srl-1300*::Tn*10*	By P1 *kc* transduction
JP3290	F^- derivative of JP3156	From JP3156
JP3297	JP3156 *pheR372*	This work
JP3298	JP3290 *pheR373*	This work
JP3299	JP3290 *pheR374*	This work
JP3302	JP3151 *pheR372 zjd-1*::Tn*10*	This work
JP2998	*pheAo351 rpoB361* Δ*lacU169*	From JP2869 (8) by P1 *kc* transduction
JP2999	*tyrA4 rpoB361* Δ*lacU169*	8
JP3351	JP2998 *pheR372 zjd-351*::Tn*10*	By P1 *kc* transduction
JP3355	JP2999 *pheR371 zjd-351*::Tn*10*	By P1 *kc* transduction
JP3346	*aroB351 tyrA4 rpsL pheR372 zjd-351*::Tn*10 rpoB361* Δ*lacU169* (λ p*pheA-lac*)	From JP3141 (8) by P1 *kc* transductions
JP2604	*aroG aroH aroP pheP367 his argE thi rpoB352* λ^- λ^r	From JP2404 (19)
JP2982	JP2604 *argE*$^+$ *rpoB*$^+$ *thi*$^+$ Tn*10* (position not known)	By P1 *kc* transductions
JP2983	JP2982 *rpoB366*	This work
JP3281	JP2604 Δ*lacU169* (λ p*pheA-lac*)	From JP2604, involving several intermediate strains
JP3342	JP3281 *argE*$^+$ *rpoB366 pheR372 zjd-351*::Tn*10*	By P1 *kc* transductions
JP3343	JP3281 *argE*$^+$ *rpoB366 pheR*$^+$ *zjd-351*::Tn*10*	By P1 *kc* transductions
JP3344	JP3281 *argE*$^+$ *rpoB352 pheR372 zjd-351*::Tn*10*	By P1 *kc* transductions
JP3345	JP3281 *argE*$^+$ *rpoB352 pheR*$^+$ *zjd-351*::Tn*10*	P1 *kc* transductions
JP3365	*aroG aroH aroB351 pheR372 zjd-351*::Tn*10 rpsL rpoB366* Δ*lacU169* (λ p*pheA-lac*)	From JP3342, involving several intermediate strains
JP2898	JP2604 *his*$^+$ *argE pheAo*$^+$ *rpoB352*	By P1 *kc* transductions
JP2899	JP2604 *his*$^+$ *argE pheAo351 rpoB352*	By P1 *kc* transductions
JP2987	JP2604 *his*$^+$ *argE*$^+$ *pheAo351 rpoB366*	By P1 *kc* transductions
JP2988	JP2604 *his*$^+$ *argE*$^+$ *pheAo*$^+$ *rpoB366*	By P1 *kc* transductions
JP3314	*trpR thi* Δ*lacU169* (λp*pheA-lac*)	8
JP3349	JP3314 *miaA* (Tn*10*)	By P1 *kc* transduction
JP3350	JP3314 *miaA*$^+$ (Tn*10*)	By P1 *kc* transductions
JP3371	JP3314 *pheR372 zjd-351*::Tn*10 rpoB352*	By P1 *kc* transductions
JP3372	JP3314 *pheR372 zjd-351*::Tn*10 rpoB366*	By P1 *kc* transductions

[a] The nomenclature for genetic symbols follows that described by Bachmann and Low (1) and for transpositional insertions that described by Kleckner et al. (13). Allele numbers are indicated where they are known. Fermentation markers are not described.

be cotransducible with *ampA* at a frequency of 33.7% and with *purA* at frequencies of 5 to 10%; *ampA* and *purA* were themselves cotransducible at a frequency of 24%. These data suggested a gene order of *pheR* (Tn*10*)-*ampA-purA* and placed *pheR* (Tn*10*) at position 93.3 to 93.5 min on the *E. coli* genetic map. The gene order was confirmed by three-factor crosses (data not shown). The relative disposition of Tn*10* with respect to *pheR*, however, could not be ascertained from these mapping experiments. There was no linkage in transduction between *pheR* (Tn*10*) and *metC*, *serA*, *thyA*, *lysA*, or *argA* (markers in the 59- to 65-min region covered by F116 [14]). On the basis of these results, we designated the site of insertion of Tn*10* as *zjd-351*::Tn*10*, in accordance with the nomenclature proposed by Kleckner et al. (13).

The F-prime F117 covers the 93- to 98-min region of the *E. coli* chromosome (14), and it was introduced into the strains JP3297 and JP3299 to determine whether it also carried the *pheR*$^+$ gene. The presence of F117 in the cells decreased the activities of β-galactosidase and prephenate dehydratase in both JP3297 and JP3299 to the fully repressed levels (Table 2).

A number of genetic tests provided unequivocal evidence for homology between F117 and the chromosomal *pheR* region. (i) When gene conversions at *pheR* were selected for (with 0.01% PG) in a *recA*$^+$ F117 heterogenote strain that had the *pheR372 zjd-351*::Tn*10* on the chromo-

TABLE 2. Effect of F116 and F117 on the specific activities of prephenate dehydratase and β-galactosidase in *pheR* strains[a]

Strain	Relevant genotype	Sp act[b]					
		In haploid strains		In *thyA*/F116 heterogenote[c]		In F117 heterogenote[c]	
		Prephenate dehydratase	β-Galactosidase	Prephenate dehydratase	β-Galactosidase	Prephenate dehydratase	β-Galactosidase
JP3290	Parent *pheR*$^+$ *recA* (λ p*pheA-lac*)	4.8	145	3	74	3.2	75
JP3297	*pheR372*	90	1,580	4.1	100	4.2	93
JP3299	*pheR374*	90	1,450	4	117	4	89
JP3298	*pheR373*	32	575	5	90	4.5	114

[a] All strains were grown in repressing concentrations of aromatic end products.

[b] Specific activities of prephenate dehydratase, anthranilate synthetase, and tryptophan synthetase are expressed n milliunits per milligram of protein. β-Galactosidase activity is expressed in units as described by Miller (16).

[c] *thyA* mutants of the strains were selected as described by Miller (16). The F-primes 116 and 117 were introduced by conjugation, with KLF16/KL110 and KLF17/KL132, respectively, as donors.

some, it was shown that all of the colonies obtained had also undergone gene conversion for the closely linked Tet^r locus. (ii) When F117 was used to mobilize the chromosome of a *pheR zjd-351*::Tn*10* strain into a *pheR*$^+$ Tet^s recipient (selecting for Leu$^+$ recombinants after early interruption), a percentage of the recombinants obtained had become *pheR* Tet^r. (iii) Lastly, when a strain carrying F117 was transduced to Tet^r with a P1 *kc* lysate prepared on JP3302, it was shown that some of the transductants carried the Tn*10* on the F-prime itself.

These same tests failed to provide any evidence of homology between F116 and the chromosomal *pheR* region. Although this would argue against the presence of any major region of F117 on F116, it does not exclude the possibility of a small aberrant translocation which has added the *pheR* locus to the F-prime F116.

A second class of mutants obtained in the original PG selection with JP3290 had four- and sevenfold elevations in the activity of β-galactosidase and prephenate dehydratase, respectively. In one of them (JP3298) the mutation was again shown to be recessive in both F116 and F117 merodiploid strains (Table 2); on this basis, this class is also believed to represent mutations in *pheR*, with only a partial loss of repressor activity.

Role of *pheR* in the regulation of *pheA*. Growth of an *aroB pheR*$^+$ λ p*pheA-lac* lysogen under conditions of phenylalanine limitation in a chemostat was shown earlier to produce a 10- to 12-fold increase in the activity of chorismate mutase P-prephenate dehydratase and a fourfold increase in β-galactosidase activity (8). To study the physiological effect of *pheR* in the expression of the *pheA* gene, an *aroB* derivative of a *pheR* strain was constructed (JP3346) and grown in continuous culture in the chemostat under similar conditions of phenylalanine limitation. Prephenate dehydratase activity increased but only two- to threefold (Table 3). Unexpectedly, β-galactosidase activity did not change, but this may represent an instability of the enzyme itself under the conditions of phenylalanine starvation in the chemostat; when the same strain was grown at a slower rate (D, 0.05 h^{-1}) in the chemostat, the prephenate dehydratase activity remained elevated and the β-galactosidase activity fell further to 200 U. Other data which supported this interpretation were the observations that β-galactosidase activity in a *pheR*$^+$ *pheA-lac* fusion strain decreased during phenylalanine starvation in batch culture and that the activity of β-galactosidase was derepressed less in proportion to that of prephenate dehydratase when a *pheR*$^+$ λ p*pheA-lac* lysogen was grown under phenylalanine limitation in the chemostat (8). The results therefore indicated that *pheR* mutants have an altered regulation of *pheA* expression; they also suggested the presence of

TABLE 3. Specific activities of β-galactosidase and prephenate dehydratase in strains grown under phenylalanine limitation in the chemostat[a]

Strain	Relevant genotype	Sp act[b]	
		β-Galactosidase	Prephenate dehydratase
JP3346	*aroB pheR372* (λ p*pheA-lac*)	600 (600)	145 (65)
JP3365	*aroB pheR372 rpoB366* (λ p*pheA-lac*)	600 (750)	140 (105)

[a] Values in parentheses indicate the activities obtained when the strains were assayed simultaneously after growth in repressing concentrations of the aromatic end products.

[b] See Table 2, footnote *b*.

one or more additional mechanisms involved in the control of the *pheA* operon.

To examine the interaction between *pheR* and the operator of the *pheA* gene, *pheR372* was introduced into a strain carrying the *pheAo351* mutation. *pheAo351* had earlier been isolated in this laboratory (10) as a *cis*-dominant mutation that was closely linked to and constitutively derepressed the *pheA* structural gene; it was therefore postulated that it defined the operator region for a single-gene operon. Table 4 shows the prephenate dehydratase activities of a nearly isogenic set of *pheR pheAo* strains; the enzyme activity was elevated 10-fold in both the *pheR372* and *pheAo351* strains and was not further increased when the two mutations were brought together in JP3351. This indicated that mutations *pheR372* and *pheAo351* occurred in different genes involved in the same system of control affecting *pheA* expression.

Isolation and characterization of the *rpoB366* mutation. Strain JP2982 carries mutations in several genes concerned with aromatic amino acid biosynthesis, rendering it extremely sensitive to low levels of *p*-FPA. In the course of attempts to isolate and characterize FPA-resistant mutants in this strain, we unexpectedly discovered that a spontaneously isolated rifampin-resistant derivative of this strain, JP2983, was also resistant to 10^{-3} M *p*-FPA. The mutation was given the allele number *rpoB366*, and after transfer by P1 *kc* transduction into wild-type strains it was seen to confer on these strains an increased ability to excrete phenylalanine. Further studies were carried out to investigate its role, if any, in the regulation and expression of the *pheA* gene. Another *rpoB* allele in our laboratory stocks that was tested, *rpoB352*, did not confer FPA resistance in JP2982, as was true also of the majority of spontaneously occurring rifampin resistance mutations. The *rpoB352* allele was used as a control to *rpoB366* in some of the experiments below.

The phenotype of *rpoB366* strains is apparently a consequence of increased activity of chorismate mutase P-prephenate dehydratase because, when compared with isogenic *rpoB*$^+$ strains, derivatives with the *rpoB366* mutation showed a two- to threefold elevation in prephenate dehydratase activity. When these derivatives were also *recA* and lysogenic for λ p*pheA-lac*, β-galactosidase activity showed a similar twofold increase (data not shown). As noted previously (8), the *recA* mutation is required to stabilize the λ p*pheA-lac* in these lysogens. The coordinate derepression of *lacZ* and *pheA* that is produced by the *rpoB366* mutation in strains in which these two genes are independently expressed from *pheAp* indicates that *rpoB366* acts at the level of transcription of the *pheA* operon.

In P1-mediated transductions, *rpoB366* was shown to be linked 40% to *argE* and 90% to *thiA*; three-factor crosses confirmed that the gene order was *argE-rpoB366-thiA* (data not shown), in accord with the established location of *rpoB* on the *E. coli* linkage map. The effect of the *rpoB* allele was dominant over the wild type in resistance to rifampin in F112 heterogenote strains; prephenate dehydratase activity was also elevated in these strains (data not shown).

The combined effects of *pheR372* and *rpoB366* were studied by the introduction of *pheR372* into isogenic *rpoB366* and *rpoB352* strains (the latter used as a control), and the results of the prephenate dehydratase assays are shown in Table 5. The mutations acted independently of one another in increasing the expression initiated from *pheAp*. This conclusion was supported by the further observation that *rpoB366*, as compared with *rpoB352*, doubled the activity of prephenate dehydratase in a strain carrying the *pheAo351* mutation (Table 5).

When an *aroB pheR rpoB366* strain lysogenic for λ p*pheA-lac* (JP3365) was grown under phenylalanine limitation in the chemostat, the prephenate dehydratase activity increased by about 40% (Table 3). The magnitude of the increase was distinctly smaller than that seen with the *aroB pheR* strain JP3346. This indicated that the *rpoB366*-mediated increase of *pheA* expression represents a second mechanism of phenylalanine-specific control of the operon; the mutation may, however, only be providing partial release from this second control mechanism. β-Galactosidase activity in JP3365 fell slightly upon starvation in the chemostat, which again was presumably a consequence of the instability exhibited by the enzyme under such conditions.

Yanofsky and Horn (22) recently described the isolation and characterization of several *rpoB* mutations that alter the efficiency of attenuation in the *trp* operon leader region. In light of that report, we too studied the effect of *rpoB366* on the expression of the *trp* operon. *rpoB366* and *rpoB352* (as control) were transduced into a *pheR trpR* strain (JP3341) in which, as described

TABLE 4. Prephenate dehydratase activity in isogenic *pheR pheAo* strains grown in repressing concentrations of aromatic end products

Strain	Relevant genotype	Prephenate dehydratase sp act (mU/mg of protein)
JP2999	*pheR*$^+$ *pheAo*$^+$	10
JP3355	*pheR372 pheAo*$^+$	95
JP2998	*pheR*$^+$ *pheAo351*	105
JP3351	*pheR372 pheAo351*	104

TABLE 5. Specific activity of prephenate dehydratase in isogenic *rpoB pheAo* strains grown in repressing concentrations of the aromatic end products

Strain	Relevant genotype	Prephenate dehydratase sp act (mU/mg of protein)
JP3342	*pheR372 rpoB366*	112
JP3343	*pheR*$^+$ *rpoB366*	13
JP3344	*pheR372 rpoB352*	52
JP3345	*pheR*$^+$ *rpoB352*	7
JP2987	*pheAo351 rpoB366*	130
JP2988	*pheAo*$^+$ *rpoB366*	13
JP2899	*pheAo351 rpoB352*	72
JP2898	*pheAo*$^+$ *rpoB352*	7

in the accompanying paper (8), the *lacZ* gene is under *trp* operon control and *trpA* is expressed from *pheAp*. The results of the enzyme assays (Table 6) showed that *rpoB366* but not *rpoB352* increased the activity of anthranilate synthetase and β-galactosidase (both expressed from *trpEp*) by amounts comparable to those obtained with the *miaA* mutation (see below). We conclude, therefore, that *rpoB366* diminishes attenuation control in the *trp* operon.

Mutations in *miaA*. *miaA* (*trpX*) was originally isolated by Yanofsky and Soll (21) as a mutation that relieved transcription termination at the attenuator site of the *trp* operon. It has subsequently been shown to affect the modification of $tRNA^{Trp}$, $tRNA^{Phe}$, and $tRNA^{Tyr}$, the *miaA* gene product being in some way necessary for the methyl-thio modification of isopentenyl adenine (ms^2i^6-A) at one position in the anti-codon loop of each of these three tRNA species (4). *miaA* has also been reported to produce a two-fold increase in prephenate dehydratase activity (24).

To study whether *miaA* also acts at the level of transcription of the *pheA* operon, the mutation was transduced, with the aid of the Tn*10* linked to it (4), into JP3314, which carries the *trp-lacZ* and *pheAp-trpA* fusions. The incoming *miaA* mutation was screened by checking for increased β-galactosidase activity, and then the prephenate dehydratase and tryptophan synthetase levels were determined in isogenic *miaA* and *miaA*$^+$ strains. *miaA* increased chorismate mutase P-prephenate dehydratase activity (Table 6). That this effect was the result of an increased rate of transcription of the *pheA* gene is shown by the fact that *trpA* expression from *pheAp* was also coordinately derepressed in this strain.

DISCUSSION

***pheA-lac* fusions used to obtain regulatory mutants of the *pheA* operon.** As described above, λ p*pheA-lac* was useful not only in selecting for regulatory mutants but also in differentiating the mutations affecting genes acting in *cis* from those acting in *trans*. It was also of use in confirming the futility of the FPA resistance selection attempts for *pheR* mutants. Thus, of 250 mutants resistant to 10^{-3} M *o*-FPA obtained from a λ p*pheA-lac* lysogen, approximately 20% were identified as *pheA* regulatory mutants on the basis of phenylalanine cross-feeding tests; none, however, showed any increase in β-galactosidase activity, indicating that none of these mutations acted in *trans* on the *pheA* operon. As *pheR* mutants themselves are significantly resistant to *o*-FPA and *p*-FPA, the basis for this bias against *pheR* during FPA resistance selection remains unexplained.

F116 effect on *pheR*. The direct mapping data on *pheR372 zjd-351*::Tn*10* unquestionably located the gene position at 93.3 to 93.5 min on the linkage map. There are three possible explanations for the observation that the *pheR* mutations were recessive in F116 heterogenotes. (i) F116 carries an extragenic suppressor. This is an

TABLE 6. Enzyme specific activities in isogenic *rpoB* and isogenic *miaA* strains[a]

Strain	Genotype[b]	Sp act[c]			
		Anthranilate synthetase	Tryptophan synthetase	Prephenate dehydratase	β-Galactosidase
JP3371	*rpoB352 pheR372*	4.0	21.2	7	750
JP3372	*rpoB366 pheR372*	12.1	41.0	13	1,400
JP3349	*miaA*	22.0	7	15	1,500
JP3350	*miaA*$^+$	4.2	4.5	7	980

[a] All strains were grown in repressing concentrations of the aromatic end products.

[b] All strains were *trpR* and lysogenic for λ p*pheA-lac*. The latter is integrated into the chromosome by *trp'CBA'* homology, so that the genes for anthranilate synthetase and β-galactosidase are expressed from *trpEp* and tryptophan synthetase and prephenate dehydratase activities represent expression from two separate *pheA* promoters (8).

[c] See Table 2, footnote *b*.

unlikely explanation, as the F116 effect was noted by us with all three of our independent *pheR* mutations (including one with partial loss of repressor activity) and also by Gollub et al. with their *pheR* mutation in *S. typhimurium* (7). (ii) The *pheR* phenotype is produced by the interaction of mutations in two separate genes, and the presence of a functional gene product from either can make a strain *pheR*$^+$. In that case it might be further postulated that F116 carries one of these functional genes and that the corresponding gene on the chromosome carries a silent mutation in all of the strains we examined. The complexity of this explanation, as well as our current inability to conceive of a molecular model for such gene-product interaction, also makes this possibility unlikely. (iii) The third possibility, which appears to be the most likely explanation, is that F116 carries the *pheR* gene itself. It may be that in some strains of *E. coli* K-12 other than those which we tested, the *pheR* gene is located in the 59- to 65-min region (homologous to the map location of *pheR* in *S. typhimurium* [7]) and that the parent Hfr from which F116 was derived, AB312, was one such strain, or it may be that the gene *pheR* has undergone an aberrant translocation from its normal site on the chromosome onto F116. Our experimental results failed to demonstrate any homology between F116 and the chromosomal *pheR* region, but the tests were genetic in nature and not sensitive enough to exclude the presence of small regions of identity. The natural occurrence of inverted repeat sequences about the *argF* gene segment, rendering that gene transposable, has been reported recently (9, 23), and it is possible that *pheR* too may be situated on a transposable segment of the *E. coli* chromosome. We are presently in the process of trying to distinguish among these various possibilities.

***pheR* and *rpoB366* mutations.** Our results from the chemostat starvation studies indicated that *pheR* and *rpoB366* defined two independent mechanisms by which intracellular phenylalanine concentration feeds back to repress the expression of the gene for chorismate mutase P-prephenate dehydratase. Both mechanisms were concerned with transcriptional control of the *pheA* operon. *pheR* mutants showed a 10- to 20-fold derepression of chorismate mutase P-prephenate dehydratase, the exact magnitude appearing to depend on strain background, whereas *rpoB366* mutants showed a two- to threefold increase in the activity of this enzyme. It is most likely that these are the only two mechanisms involved in the control of *pheA* and that the residual derepression seen during phenylalanine starvation of the *pheR rpoB366* double mutant in the chemostat is merely a reflection of the incomplete effect of the *rpoB366* mutation. However, the existence of an additional, albeit minor, mechanism of regulation cannot be ruled out.

The mechanisms by which *pheR* and *rpoB366* control the expression of *pheA* can reasonably be conjectured at present, but their verification would necessarily depend on more detailed studies at the molecular level. The fact that both *pheR* and *pheAo351* define a single mechanism of control would indicate that these represent mutations in a gene coding for an aporepressor molecule and the operator of *pheA*, respectively; this then would be the classical operator/repressor mechanism of transcriptional control (11).

There is substantial circumstantial evidence for the presence of a mechanism of attenuation control of the *pheA* operon. As mentioned earlier, the most compelling evidence comes from DNA sequencing studies of the *pheA* leader and from results of in vitro transcription of the *pheA* gene (24). The effect of *miaA* on the transcription of *pheA* lends support to this idea. In another study, McCray and Hermann (15) showed that Fe^{3+} starvation produced a fivefold increase in prephenate dehydratase activity; and iron starvation was earlier (17) shown to affect the methyl-thio modification of isopentenyl adenine (ms^2i^6-A) in several tRNA species, including $tRNA^{Phe}$. This is the same modification that is lost in *miaA* mutants (4).

The effect of the *rpoB366* mutation on *pheA* fits very well with an attenuation model of control of the operon. Thus, *rpoB366* would be analogous to the Rif^r termination relief mutations of the *trp* operon described by Yanofsky and Horn (22), and it probably alters the β subunit of RNA polymerase to reduce the efficiency of termination at the attenuator site of the *pheA* gene. The fact that *rpoB366* increased the expression of the *trp* operon lends further support to a common mechanism underlying attenuation control in these two operons.

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