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Evidence that There Are Only Two tRNA^{Phe} Genes in *Escherichia coli*

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***pheV*, one of the genes that code for tRNA^{Phe}, was deleted from the chromosome of a strain of *Escherichia coli* K-12. As a consequence of this mutation, expression of *pheA*, the gene for chorismate mutase P-prephenate dehydratase, the first enzyme in the terminal pathway of phenylalanine biosynthesis, was derepressed. Similar derepression of *pheA* has been reported in *pheR* mutants of *E. coli* K-12 (J. Gowrishankar and J. Pittard, J. Bacteriol. 150:1130–1137, 1982). Attempts to introduce a *pheR* mutation into the Δ*pheV* strain failed under circumstances suggesting that this combination of mutations is lethal. Southern blot analysis of *pheV*$^+$ and Δ*pheV* strains indicated that there are only two tRNA^{Phe} genes in *E. coli*. It is recommended that the names *pheU* and *pheV* be retained for these genes.**

Three sources have reported the cloning of apparently different genes for tRNA^{Phe} from the *Escherichia coli* chromosome (4, 19, 21). In each case, selection for the clone carrying the tRNA gene depended on the ability of the gene when present on a multicopy plasmid to complement a thermosensitive mutant of the phenylalanyl tRNA synthetase genes *pheST*. Sequence studies showed that the coding regions of all of the putative tRNA genes are identical but there are some differences in the 5′- and 3′-flanking regions (3, 19, 21). The sequence differences between *pheU* (19) and *pheW* (21) on the one hand and *pheV* (3) on the other occur in both 5′- and 3′-flanking regions and are sufficiently extensive to establish the separate identities of the genes. The sequence differences between *pheU* and *pheW* are almost completely restricted to the 3′-flanking region, where only 11 of the reported 55 bases immediately following the coding region are identical.

By forcing integration of a plasmid containing *pheU* into the chromosome and mapping an antibiotic resistance gene on the plasmid, an approximate map position of about 94.5 min has been established for *pheU* (6). The map position of *pheV* has recently been established next to *speC* at about min 64 (2). No mapping data on *pheW* are available.

In 1982, Gowrishankar and Pittard (9) described a locus, *pheR*, which, when mutated, caused enhanced expression of the *pheA* gene. By using a closely linked transposon, Tn*10*, the locus was mapped to about min 93.5 of the *E. coli* chromosome. The *pheR* gene was cloned (10) and studies with maxicells suggested that *pheR* codes for a 19-kilodalton protein which was hypothesized to be a repressor for the *pheA* gene. One unexpected finding in these studies was that two different F′ factors, F_{117}, spanning 93 to 98 min, and F_{116}, spanning the 59- to 65-min region, both complemented *pheR* mutants. The putative *pheR* homolog on F_{116}, which by F′ analysis was shown to be within the region from 63 to 66 min (18), was termed *pheC*.

Recently, Narasaiah (G. Narasaiah, Ph.D. thesis, University of Melbourne, Melbourne, Australia, 1988) has sequenced the *pheR* gene and shown that it codes not for a protein but for tRNA^{Phe}. The implication is that *pheC* should also code for tRNA^{Phe}. The coding sequence of *pheR* is identical to that of *pheV*, *pheU*, and *pheW*, and the flanking regions strongly resemble *pheU* and *pheW*. In particular, *pheW* and *pheR* are almost identical in the 5′ regions and show a similar identity in the 3′ region, except for a cluster involving a 3-base deletion and 5 base changes (Narasaiah, Ph.D. thesis). Consequently, although current data suggest the possibility of five genes for tRNA^{Phe}, namely, *pheU*, *pheV*, *pheW*, *pheR*, and *pheC*, mapping data show that *pheV* and *pheC* are in the same region of the chromosome (between min 63 and 66), as are *pheR* and *pheU* (between min 93 and 95). Sequence data have established a difference between *pheV* on the one hand and *pheR*, *pheU*, and *pheW* on the other but have left some uncertainty about the separate identities of these latter three genes.

To resolve this dilemma, we tried to sequentially inactivate the various tRNA^{Phe} genes on the *E. coli* chromosome. That led to the conclusion that there are, in fact, only two separate genes for tRNA^{Phe}.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. All of the bacterial strains were derived from *E. coli* K-12.

Chemicals, enzymes, and media. The minimal medium used was half-strength buffer 56 (16) supplemented with 0.2% glucose and appropriate growth factors. The nutrient media used were Lennox broth, nutrient broth, and nutrient agar (Oxoid Ltd.). MacConkey agar (Oxoid) was used as the indicator medium. Cells were grown for enzyme assays in repressing concentrations of aromatic end products (8).

Antibiotics were used at the following concentrations: ampicillin, 50 μg/ml; chloramphenicol, 25 μg/ml; kanamycin, 20 μg/ml; tetracycline, 5 μg/ml in minimal medium and 15 μg/ml in nutrient medium. [α-^{32}P]dATP (2,000 to 3,000

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TABLE 1. *E. coli* K-12 strains and plasmids

Strain or plasmid	Relevant genotype[a]	Origin or reference
Strains		
MM383	*deoC2 rha-5 rpsL polA12*(Ts) *thyA36 lacZ53*(λ⁻)	15
JP3142	*rpoB361* Δ*lacU169*(λ p*pheA-lac*)	
JP3317	*pheR372 trpR thi zjd-351*::Tn*10* Δ*lacU169*(λ p*pheA-lac*)	8
JP3351	*pheAO351 pheR372 zjd-351*::Tn*10 rpoB361* Δ*lacU169*	9
JP3364	*pheR374 trp ilv argG thi leu his purE met recA56 rpsL* Δ(*srl-1300*::Tn*10*)*58* Δ*lacU169*(λ p*pheA-lac*)	
JP3702	*pheR372 trp ilv argG thi his leu purE met rpsL* Δ(*zjd-351*::Tn*10*)*59* Δ*lacU169*(λ p*pheA-lac* F⁺)	
JP6802	MM383(pMU392)	This work
JP6806	MM383(pMU392 integrated)	This work
JP6812	JP6806 Δ(*pheV*)*624* Cm^s	This work
JP6813	JP6806 Δ(*pheV*)*625* Cm^s	This work
JP6814	Cm^s derivative of JP6806	This work
JP6815	Cm^s derivative of JP6806	This work
JP7327	JP6812 *zjd-351*::Tn*10*	This work
JP7342	JP6815 *zjd-351*::Tn*10 pheR372*	This work
Plasmids		
pACYC184	Cm^r Tc^r	5
pMU1150	pBR322 with ~17-kb insert of *E. coli* chromosomal DNA; *pheV*⁺ Ap^r	
pMU50	pACYC177 with ~17-kb insert of chromosomal DNA from JP3305; *pheR*⁺ Km^r Tc^r	10
pMU390	pACYC184 with 3.5-kb *Cla*I fragment from pMU1150; *pheV*⁺ Cm^r	This work
pMU391	pMU390 with the 1.0-kb *Pst*I fragment deleted; *pheV*⁺ Cm^r	This work
pMU392	pMU390 with the 1.35-kb *Pst*I-*Pst*I-*Pvu*I fragment deleted; Cm^r	This work
pMU393	pMU50 with Tn*10* removed by *Nde*I digestion and religation; *pheR*⁺ Km^r	This work
pMU394	pMU393 with the 2.0- and 0.5-kb *Eco*RV fragments deleted; Km^r	This work

[a] Abbreviations: Ap^r, ampicillin resistance; Km^r, kanamycin resistance; Tc^r, tetracycline resistance; Cm^r, chloramphenicol resistance; Cm^s, chloramphenicol sensitivity.

Ci/mmol; 10 mCi/ml) and [α-^{32}P]dCTP (2,000 to 3,000 Ci/mmol; 10 mCi/ml) were obtained from Amersham Corp. Restriction endonucleases and DNA-modifying enzymes were purchased from Boehringer Mannheim Biochemicals; Pharmacia P-L Biochemicals, Inc.; or New England Bio-Labs, Inc. Barium prephenate was from Sigma Chemical Co.

Recombinant DNA techniques. Standard recombinant DNA procedures were used essentially as described by Maniatis et al. (13).

Isolation of chromosomal DNA. Chromosomal DNA was prepared by modification of the method described by A. G. Dillela and S. L. C. Woo (Focus, vol. 7, no. 2, p. 1–5, 1985). Cells from 10 ml of culture (grown overnight in nutrient broth) were suspended in STE buffer (10 mM NaCl, 20 mM Tris hydrochloride [pH 8.0], 1 mM disodium EDTA). Proteinase K was added to 100 μg/ml, sodium dodecyl sulfate was added to 0.5%, and the suspension was incubated at 50°C for 6 h. The DNA was extracted with an equal volume of a phenol-chloroform-isoamyl alcohol (25:24:1) mixture for 10 min at room temperature, followed by incubation on ice for 30 min. DNA was centrifuged at 2,000 × *g* for 20 min, the aqueous layer was collected, and NaOAc (pH 5.5) was

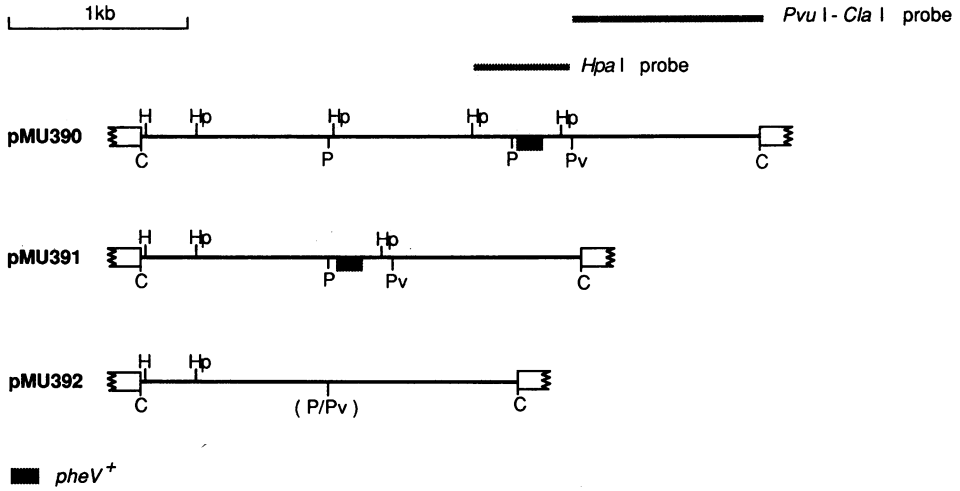


FIG. 1. Restriction endonuclease maps of *pheV*⁺ plasmid pMU390 and its derivatives. The DNA fragments used to prepare ^{32}P-labeled probes for Southern blots are shown. Restriction endonuclease cleavage sites present in the vector (pACYC184, represented by the broken boxes) are not shown. C, *Cla*I; H, *Hin*dIII; Hp, *Hpa*I; P, *Pst*I; Pv, *Pvu*I; (P/Pv), *Pst*I and *Pvu*I sites lost during construction of pMU392, which involved the use of T4 DNA polymerase to remove the protruding 3′ ends generated by these two enzymes.

added to 0.2 M. Two volumes of ethanol was gently layered on top of the DNA solution, and the DNA was spooled out with a sterile glass rod. The DNA was suspended in TE (10 mM Tris, 1 mM EDTA [pH 8.0]) and incubated for 1 h at 37°C with 100 μg of proteinase K per ml–0.5% sodium dodecyl sulfate. The phenol-chloroform extraction and ethanol precipitation steps were repeated, and the DNA was suspended in 2 ml of TE.

Southern blot hybridization. Transfer of DNA from agarose gels to Hybond-N nylon membranes and the subsequent hybridizations were performed as specified by the manufacturer (Amersham Corp.). DNA probes were ^{32}P labeled by nick translation as described by Maniatis et al. (13).

P1 *kc* transduction. P1 *kc* transductions were performed as described by Pittard (17).

Enzyme assays. β-Galactosidase activity was assayed by the method of Miller (14). Prephenate dehydratase was assayed as previously described (8), and its activity is expressed in international units per milligram of protein. Protein concentrations in cell extracts were determined by the method of Bradford (1).

RESULTS

Deletion of *pheV* from a *pheV*$^+$ plasmid. Plasmid pMU1150, which carries the *pheV* gene from *pheR374* strain JP3364, was used as the source of *pheV*$^+$. A 3.5-kilobase (kb) fragment containing *pheV*$^+$ and its chromosomal flanking sequences was subcloned from pMU1150 into pACYC184 to yield plasmid pMU390 (Fig. 1). Deletion derivatives of pMU390 which had lost either the 1-kb *Pst*I fragment (pMU391) or the 1.35-kb *Pst*I-*Pst*I-*Pvu*I fragment (pMU392) were then constructed (Fig. 1). To confirm, in accordance with the results of Caillet et al. (3), that *pheV*$^+$ was located on the 0.35-kb *Pst*I-*Pvu*I fragment present on pMU391 but missing from pMU392, both plasmids were transformed into strain JP3364. This strain, which has the *pheR374* mutation and also carries λ p*pheA-lac*, gives pale pink colonies on MacConkey agar if it contains multiple copies of the *pheV*$^+$ gene. As expected, pMU391 transformants were pale pink on MacConkey chloramphenicol plates, whereas pMU392 transformants produced red colonies.

Substitution of Δ*pheV* for *pheV*$^+$ in the chromosome. Plasmid pMU392, in which *pheV* has been deleted, nevertheless contains chromosomal flanking regions on either side of the deletion that should provide sufficient homology to recombine the plasmid into the chromosome. The method used to isolate such recombinants has been described by Gay (7). Plasmid pMU392 was transformed into *polA12* strain MM383, and transformants were selected at 30°C. One of these was purified and is called strain JP6802. In this strain, in which DNA polymerase I is temperature sensitive, pACYC184 is unable to replicate at 42°C. By plating JP6802 on chloramphenicol-containing medium at 42°C, it was possible to select clones in which pMU392 had become integrated into the chromosome. This event occurred at a frequency of about 2×10^{-2}. One of these clones (JP6806) was purified and used to isolate segregants in which the *pheV*$^+$ of the chromosome had been replaced by Δ*pheV*. Strain JP6806 was grown at 42°C without chloramphenicol to allow segregants to develop in the population; chloramphenicol was then added, followed 90 min later by cycloserine. Cycloserine is bactericidal for actively growing cells which, in this case, were the nonsegregant chloramphenicol-resistant cells. Survivors were plated to rich medium and screened for chloramphenicol sensitivity. Of 1,500 colonies, 7 were chloramphenicol sensitive; they were purified and retained for further studies.

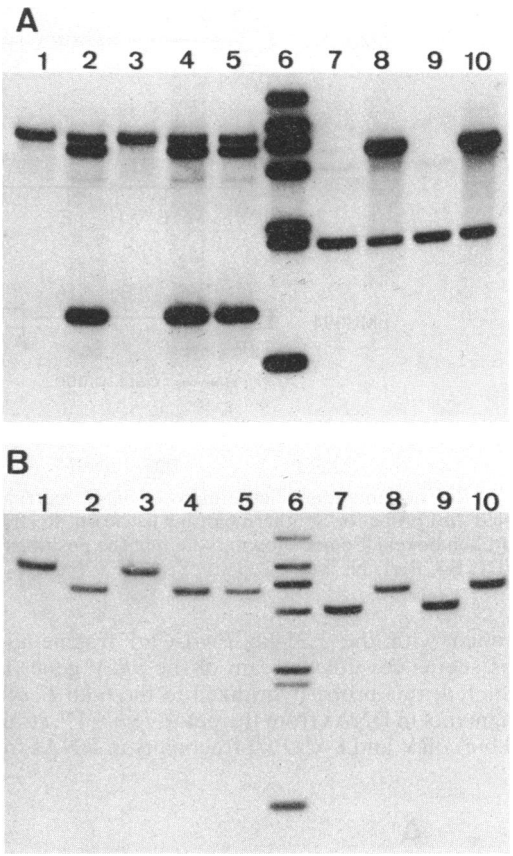


FIG. 2. Southern blot analysis of chromosomal DNAs from four chloramphenicol-sensitive segregants of JP6806. The DNAs were digested with *Pst*I (lanes 1 to 5) or *Eco*RV (lanes 7 to 10) and fractionated by electrophoresis through a 0.8% agarose gel. The DNA fragments were transferred to a Hybond-N membrane and hybridized successively with the *Hpa*I fragment (panel A) and the *Pvu*I-*Cla*I fragment (panel B) of pMU390 (Fig. 1), both of which had been labeled with ^{32}P by nick translation. Lanes: 1 and 7, JP6813; 2 and 8, JP6814; 3 and 9, JP6812; 4 and 10, JP6815; 5, MM383; 6, λ DNA digested with *Hin*dIII and end labeled with [α-^{32}P]dATP by using the Klenow fragment of *E. coli* DNA polymerase to show labeled fragments of 23.1, 9.4, 6.6, 4.4, 2.3, 2.0, and 0.6 kb.

Since the excision events that produced these chloramphenicol-sensitive strains could leave either *pheV*$^+$ or Δ*pheV* on the chromosome, depending on where the crossover event occurred, the technique of Southern (20) was used to identify the strains deleted for *pheV*. Chromosomal DNA was prepared from each of these strains and from strain MM383, cut with *Pst*I or *Eco*RV, run on gels, and probed with the nick-translated 487-base-pair *Hpa*I-*Hpa*I fragment carrying the *pheV*$^+$ gene. This probe should hybridize to a 1-kb *Pst*I fragment containing a sequence immediately upstream of the *pheV* gene and to the 6-kb *Pst*I and *Eco*RV fragments containing the *pheV* gene itself (Fig. 1) (2, 4). The expected fragments were present in DNAs from strains MM383, JP6814, and JP6815 but not in DNAs from strains JP6812 and JP6813 (Fig. 2A). These data indicate that strains JP6814 and JP6815, which cannot be distinguished from control strain MM383, are *pheV*$^+$, while strains JP6812 and JP6813 are Δ*pheV*. This conclusion was supported by data obtained when the blot shown in Fig. 2A was washed

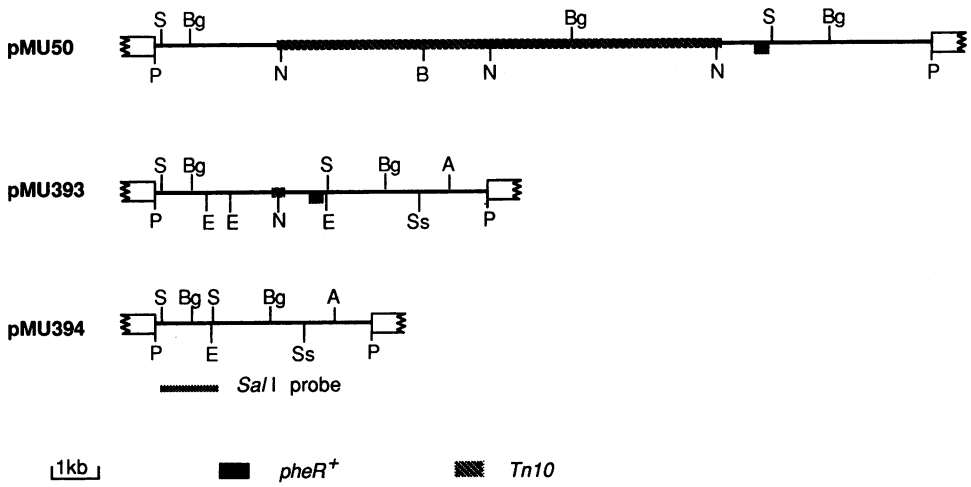
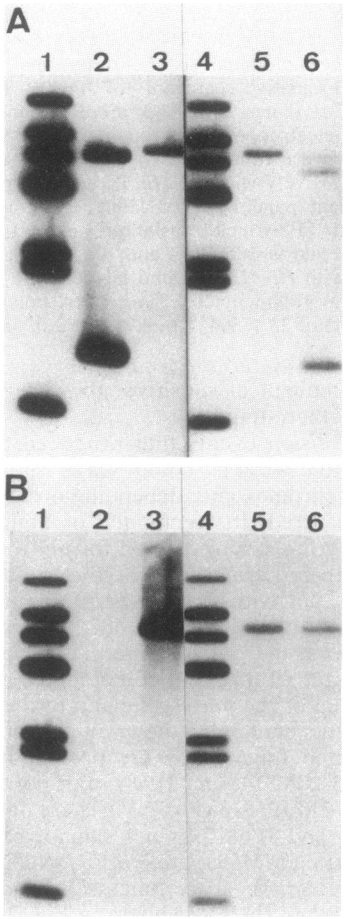


FIG. 3. Restriction endonuclease maps of $pheR^+$-carrying plasmid pMU50 and its derivatives. The DNA fragment used to prepare the ^{32}P-labeled *Sal*I probe for Southern blotting is shown. Restriction endonuclease cleavage sites present in the vector (pACYC177, represented by the broken boxes) are not shown. Note that the positions of the *Bgl*I and *Sal*I sites reported previously (10) have been amended. A, *Ava*I; B, *Bam*HI; Bg, *Bgl*I; N, *Nde*I; P, *Pst*I; S, *Sal*I; Ss, *Sst*II.

and probed with the 1.25-kb *Pvu*I-*Cla*I fragment, which contains sequences downstream of the *pheV* gene (Fig. 1). As predicted, this probe hybridized to the 6-kb *Eco*RV and *Pst*I fragments in DNAs from the putative $pheV^+$ strains and to 4.5-kb *Eco*RV and 8-kb *Pst*I fragments in DNAs from the putative Δ*pheV* strains (Fig. 2B). The increased size of the *Pst*I fragment seen in DNAs from Δ*pheV* strains is due to fusion of two fragments as a consequence of filling of the *Pst*I site during construction of pMU392. The three remaining chloramphenicol-sensitive strains were $pheV^+$ (data not shown).



In addition to the *pheV*-specific fragments described above, the DNAs of all of the Cm^s strains examined contained a 7-kb *Pst*I fragment and a 2-kb *Eco*RV fragment which hybridized to the *Hpa*I but not the *Pvu*I-*Cla*I probe. Since the *Hpa*I probe contains the coding sequence for $tRNA^{Phe}$, the most likely explanation for this observation is that the 7-kb *Pst*I fragments and 2-kb *Eco*RV fragments contain a second $tRNA^{Phe}$ gene. To determine whether this gene is *pheR* (10), another probe (*Sal*I probe) was prepared from a region flanking *pheR* but not including the coding sequence of *pheR* (Fig. 3). This probe and the *Hpa*I probe were then used with *Pst*I digests of chromosomal DNAs from $pheV^+$-and Δ*pheV* strains and with *Pst*I digests of DNAs from the $pheV^+$ (pMU390) and $pheR^+$ (pMU393) plasmids. The results are shown in Fig. 4; the band which remained in the *pheV*-deleted strain lit up with both probes and presumably carried *pheR*.

Comparison of the restriction map of $pheR^+$ plasmid pMU393 with the published map of $pheU^+$ plasmid pID2 (19) indicated that these two plasmids probably carry the same *Pst*I insert. This notion was supported by examination of the *E. coli* chromosome map (11) in the region between min 91.8 and 96.0, which revealed that the only possible location for either gene is within the same *Pst*I fragment at about min 94

FIG. 4. Southern blot analysis of DNA to identify the second $tRNA^{Phe}$ gene. Chromosomal DNAs from Δ*pheV* (JP6812) and $pheV^+$ (JP6814) strains and $pheV^+$ (pMU390) and $pheR^+$ (pMU393) plasmids were cut with *Pst*I and fractionated by electrophoresis through a 0.8% agarose gel. The DNA fragments were transferred to a Hybond-N membrane and hybridized successively with the *Hpa*I fragment of pMU390 (A) and the *Sal*I fragment of pMU394 (B), both of which had been labeled with ^{32}P by nick translation. Lanes: 1 and 4, λ DNA digested with *Hin*dIII and end labeled with [α-^{32}P]dATP; 2, pMU390; 3, pMU393; 5, JP6812; 6, JP6814.

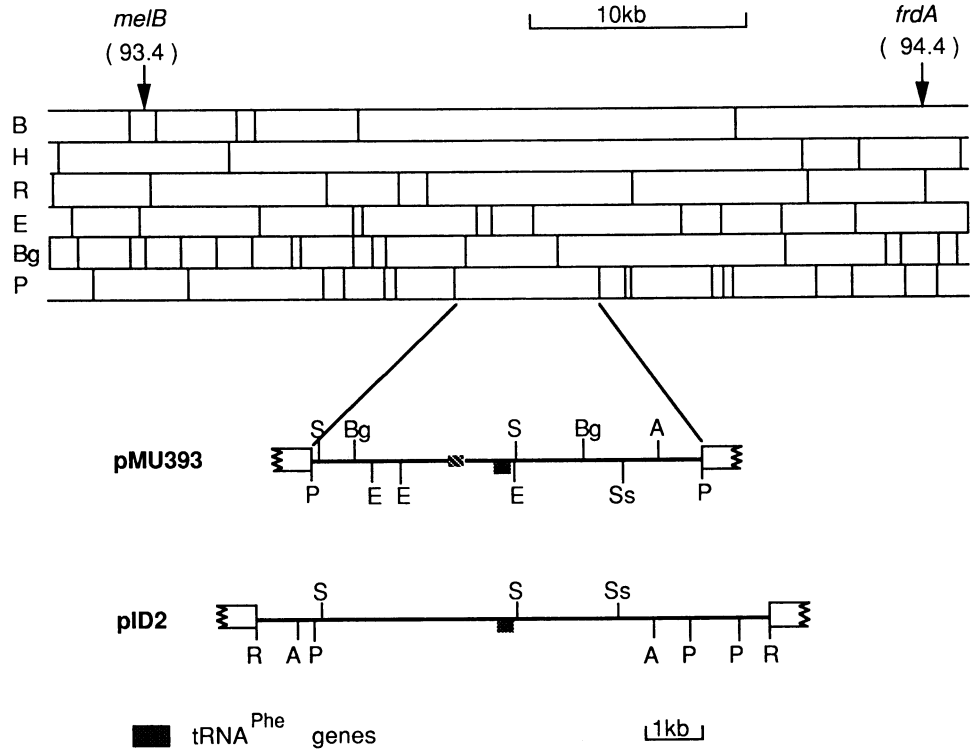


FIG. 5. Comparison of the restriction endonuclease maps of the *pheR*$^+$ (pMU393) and pheU$^+$ (pID2; from reference 19) plasmids and the region of the *E. coli* chromosome from min 93.4 to 94.4 (redrawn from reference 11; map coordinates, ~4425 to ~4462 kb). Restriction endonuclease cleavage sites present in the vector (pACYC184, represented by the broken boxes) are not shown. The locations of *Bgl*I and *Eco*RV cleavage sites in pID2 DNA were not determined. The orientation of the 3.6-kb-*Sal*I fragment in pID2 has been reversed from that shown in reference 19. A, *Ava*I; B, *Bam*HI; Bg, *Bgl*I; E, *Eco*RV; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I; Ss, *Sst*II.

(Fig. 5). This is in agreement with the hybridization data of Komine et al. (12), who placed *pheU* on Kohara's clones 647, 648, and 649.

Derepression of prephenate dehydratase in a Δ*pheV* strain. Mutations in the *pheR* gene result in derepression of the *pheA* gene (9). To see whether deletion of *pheV* would have the same effect, one of each of the putative *pheV*$^+$ and Δ*pheV* derivatives of JP6806 was grown in minimal medium supplemented with phenylalanine. Cell extracts prepared from the cultures were assayed for prephenate dehydratase activity. Strain JP6812, with the apparent *pheV* deletion, was derepressed for prephenate dehydratase (Table 2). *pheV*$^+$ strain JP6815 had the same specific activity as parent strain JP6806. A *pheR372* derivative of strain JP6815 (JP3742) was also derepressed and had a significantly higher level of prephenate dehydratase activity than the Δ*pheV* strain.

TABLE 2. Effects of mutations in *pheV* and *pheR* on specific activities of prephenate dehydratase in strain MM383 and its derivatives

Strain	Relevant genotype	Prephenate dehydratase sp act (mU/mg of protein)
MM383	*pheV*$^+$ *pheR*$^+$	16
JP6806	*pheV*$^+$ *pheR*$^+$	14
JP6812	Δ*pheV pheR*$^+$	41
JP7327	Δ*pheV pheR*$^+$ *zjd-351*::Tn*10*	32
JP6815	*pheV*$^+$ *pheR*$^+$	13
JP7342	*pheV*$^+$ *pheR372 zjd-351*::Tn*10*	86
JP3317[a]	*pheV*$^+$ *pheR372*	57

[a] Not a derivative of MM383.

Attempt to make a Δ*pheV pheR* double mutant. Strain JP3351 has Tn*10* closely linked to the *pheR372* allele, and a P1 lysate prepared on JP3351 generally cotransduces tetracycline resistance and the PheR$^-$ phenotype at a frequency of about 95%. A P1 lysate prepared on JP3351 was used to transduce tetracycline resistance into the two Δ*pheV* strains and also into two *pheV*$^+$ segregants as controls. The frequencies with which tetracycline-resistant transductants were obtained were approximately 5×10^{-7}/PFU and 10^{-5}/PFU for the Δ*pheV* and *pheV*$^+$ strains, respectively. Thus, the number of tetracycline-resistant transductants in each of the crosses involving the Δ*pheV* strains was less than 10% of that obtained with the two *pheV*$^+$ recipients. Five tetracycline-resistant transductants were purified from each cross and used to prepare new P1 lysates. These were used to transduce tetracycline resistance into *pheR*$^+$ λ p*pheA-lac* strain JP3142. The colonies produced on MacConkey agar using lysates prepared on the *pheV*$^+$ transductants were red, whereas those from the Δ*pheV* transductants were pale pink. This difference in λ p*pheA-lac* expression was confirmed by using a β-galactosidase assay (data not shown). Thus, lysates prepared on the *pheV*$^+$ transductants retained the close linkage between Tn*10* and *pheR372*, whereas those prepared on Δ*pheV* transductants could no longer cotransduce Tn*10* and *pheR372*. In fact, as we were able to demonstrate with a transduction experiment using *pheR372* λ p*pheA-lac* strain JP3702 as the recipient, a lysate prepared on tetracycline-resistant Δ*pheV* strain JP7327 was able to cotransduce Tn*10* and *pheR*$^+$ with a frequency of 90% (18 of 20 pale pink transductant colonies on MacConkey agar). The simplest explanation for all of these observations is that a

strain with a *pheR* Δ*pheV* genotype is nonviable and that when a Δ*pheV* strain was used as the recipient with a lysate from a Tn*10* *pheR* strain the only tetracycline-resistant transductants that could be obtained were those that had retained the chromosomal *pheR*$^+$ as a result of a crossover between Tn*10* and *pheR*. Such an interpretation is compatible with the results of the hybridization experiments, which indicated the presence of only two tRNA^{Phe} genes, corresponding to *pheV* and *pheR*.

DISCUSSION

The data from the Southern blots indicate that there are only two tRNA^{Phe} genes in *E. coli*. This conclusion is supported by the failure to isolate *pheR372* recombinants in a strain with gene *pheV* already deleted. The mapping data that are available are also compatible with the notion that *pheV* and *pheC* are the same, as are *pheR* and *pheU*. Moreover, recently obtained data show that the sequence of the *pheC* gene is identical to that of the *pheV* gene (Narasaiah, Ph.D. thesis; 3). We cannot explain the basis of the difference in the reported flanking region sequences of *pheR*, *pheU*, and *pheW*, but there are two possibilities; i.e., that these apparent differences are the result of sequencing errors or that they represent DNA sequence differences in the different host strains from which these clones were generated.

An examination of restriction maps of *pheU* and *pheR* indicate that they are located at min 94 on the map of Kohara and co-workers (11); *pheV* has been localized to min 64 (2).

Since *pheU* and *pheV* were the first two genes to be described, we recommend that the term *pheU* be retained for the gene at min 94, that *pheV* be retained for the gene at min 64, and that *pheR* be deleted from the *E. coli* genetic map.

Recently, Komine et al. (12) used Kohara's miniset library of the whole *E. coli* K-12 genome (11) to locate and identify all of the tRNA genes in that genome. To do so, they isolated, purified, and then characterized tRNAs from UV-irradiated bacteria separately infected with each of the 476 clones. When appropriate, final identification of tRNA genes was made by sequencing DNAs from relevant regions of the clones. Komine et al. (12) found only two tRNA^{Phe} genes, one at 3,124 kb or 64 min and the other at 4,443 kb or 94 min. The DNA sequence of the gene at 64 min corresponded to that of *pheV* (3), but the sequence of the gene at 94 min differed in the 3′-flanking region from those of *pheU* (19) and *pheW* (21). Although Komine et al. pointed out that they may have missed very weakly expressed tRNA genes or those located in the small regions not covered by the clone library, they nevertheless concluded that there are only two tRNA^{Phe} genes in *E. coli* K-12. Our results, obtained by a quite different approach, provide independent support for this conclusion.

Our results also indicate that two copies of the tRNA^{Phe} genes are necessary and sufficient for reduced transcriptional expression of *pheA* in phenylalanine-replete medium. This accounts for the observations that (i) *pheA* expression is derepressed in *pheU* (that is, *pheR* [9]) and *pheV* mutants, (ii) both F117 and F116 reverse the derepression of *pheA* expression in *pheU* mutants (9), and (iii) *pheU* derivatives with a tandem chromosomal duplication of the 59- to 65-min region also show reversal of *pheA* derepression (J. Gowrishankar, R. Lang, and J. Pittard, unpublished data). Increasing the gene copy number beyond two has only a marginal effect in further reducing *pheA* expression (10). Presumably, each of the tRNA^{Phe} genes is expressed constitutively, and the concentration of tRNA^{Phe} in a strain with only one gene copy is insufficient to exert full attenuation control on *pheA* transcription (18), although it is sufficient for apparently normal growth in both rich and minimal media. The greater level of derepression seen in *pheU* mutants than in *pheV* mutants (Table 2) presumably reflects a difference between the contributions these two genes make to the intracellular pool of tRNA^{Phe}. This could be due to a difference in the efficiencies of transcription or RNA processing or may be a consequence of the fact that *pheU* is half as far as *pheV* from *oriC* and therefore the former is present at a higher relative gene dosage in exponentially growing cultures.

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