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Genetic Mapping of *pheU*, an *Escherichia coli* Gene for Phenylalanine tRNA

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We report the genetic mapping of *pheU*, an *Escherichia coli* gene for phenylalanine tRNA. This gene was located near 94.5 min on the *E. coli* map. There are no other known tRNA or ribosomal genes in its immediate vicinity.

We have previously described the molecular cloning of *pheU*, an *Escherichia coli* gene for phenylalanine tRNA (6). Plasmid pID2, a derivative of pACYC184 carrying a 9.5-kilobase-pair *Eco*RI insert, was isolated by virtue of its ability to complement a temperature-sensitive *pheS* lesion in strain NP37 (3). For DNA sequencing, a 3.6-kilobase-pair *Sal*I fragment from pID2, containing *pheU*, was inserted into the *Sal*I site of pBR322 (6). This plasmid was designated pRK3. Here we report the genetic mapping of *pheU* near 94.5 min on the *E. coli* map. This was accomplished by the method of Greener and Hill (4). This method can be used for any *E. coli* gene cloned onto plasmids which require DNA polymerase I (*polA*) for replication. In a *polA* genetic background, selection for an antibiotic resistance encoded on the plasmid forces integration of the plasmid by homologous recombination between the insert and its corresponding region on the chromosome. The antibiotic resistance gene of the plasmid then becomes an easily selectable marker tightly linked to the cloned gene.

All strains used in this work (Table 1) have been described previously, except for strain DEV22. This strain contains a Tn*10* transposition in a *mia* gene involved in the biosynthesis of 2-methylthio-*N*6-isopentenyladenosine, a rare nucleotide in tRNA (manuscript in preparation). Whether this gene is identical to *miaA* (2) remains to be determined.

The *pheU* gene was mapped as follows. First, strain CH1330 (*polA*) was transformed with plasmid pRK3, selecting ampicillin (100 μg/ml)-resistant recombinants. These recombinants, designated CH1330::pRK3, are very rare, as expected (4). We then used strain CH1330::pRK3 as a donor in matings with strain DEV23, a spontaneous nalidixic acid-resistant derivative of strain AB1515. Time-of-entry mating, selecting ampicillin resistance, placed *pheU* between 93 and 97 min on the *E. coli* map (1). Fine genetic mapping was performed with phage P1*cl* grown on strain CH1330::pRK3 as a donor in transduction experiments. The results are shown in Table 2.

TABLE 1. Bacterial strains used

Strain	Relevant genotype	Source (reference)
CH1330	HfrC *polA argH*	C. W. Hill (4)
AB1515	F⁻ *leuB proC purE trpE rpsL*	CGSC[a] 1515
KL1057	*glyV* (suGAA/G) Δ(*tonB-trp*) F′ *trpA* (GAA211)	E. J. Murgola (5)
M2508	*metB melA*	CGSC 4626
AB2569	*proA hisG argH*	CGSC 2569
ES4	*purA*	CGSC 4431
CS8Asp/23	*metB* AspA *gltC*	CGSC 5092
NP37(pID2)	*pheS* (pACYC184::*pheU*⁺)	This laboratory (3)
NP37(pRK3)	*pheS* (pBR322::*pheU*⁺)	This laboratory (6)
DEV22	*mia*::Tn*10*	See text
DEV23	F⁻ *leuB proC purE trpE rpsL gyrA*	See text

[a] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.

These transductions were performed with phage P1*cl* grown on several independent CH1330::pRK3 isolates. This was done to make sure that pRK3 always integrates in the same position in the chromosome. This precaution was taken in view of reports that more than one gene for $tRNA^{Phe}$ may exist in *E. coli*. (M. Grunberg-Manago, personal communication). All isolates used gave consistent results, suggesting that pRK3 integration occurs at a unique site.

The data suggest the gene order *melA aspA pheU mia purA glyV* (Fig. 1). *pheU* maps in the immediate vicinity of *ampC*, a chromosomal gene for ampicillin resistance. This could lead to two possible artifacts: (i) that our

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TABLE 2. Two-factor crosses

Donor strain	Recipient strain	Marker selected	Marker scored	No. scored	Cotransduction frequency (%)
M2508	ES4	*purA*⁺	*melA*	100	3
CH1330::pRK3	ES4	Amp^r	*purA*⁺	1,079	41
CH1330::pRK3	M2508	Amp^r	*melA*⁺	297	26
CH1330::pRK3	KL1057	Amp^r	*glyV*	300	33
CH1330::pRK3	CS8Asp/23	Amp^r	*aspA*⁺	40	80
DEV22	ES4	Tc^r	*purA*⁺	200	75
DEV22	M2508	Tc^r	*melA*⁺	100	3
DEV22	KL1057	Tc^r	*glyV*	83	69

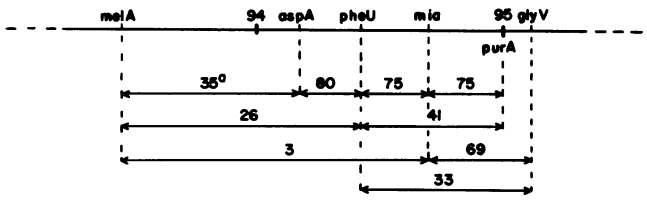

FIG. 1. Genetic map of *pheU* and neighboring markers. Numbers shown are cotransduction frequencies. a, Data of Spencer et al. (7)

CH1330::pRK3 strains were in fact spontaneous *ampC* mutants with high-level resistance to ampicillin and (ii) that pRK3 preferentially integrates via its ampicillin resistance gene at the chromosomal *ampC* gene. These artifacts were ruled out by confirming the mapping with phage P1*cl* grown on strain CH1330::pID2 (data not shown). These transductions involved selection for tetracycline resistance only, and the parent plasmid of pID2, pACYC184, does not contain an ampicillin resistance gene. In addition, control experiments indicated that no ampicillin or tetracycline recombinants can be found in transformations between strain CH1330 and the nonhybrid plasmids pBR322 and pACYC184.

DNA sequencing has revealed that *pheU* is an independent gene with its own promoter and transcription termination site (6). In view of this result it is not surprising that *pheU* does not map in the immediate vicinity of other known tRNA or ribosomal genes.

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