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 EcoRI 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 SalI fragment from pID2, containing pheU, was inserted into the SalI 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 Tn10 transposition in a mia gene involved in the biosynthesis of 2-methylthio-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 P1c1 grown on strain CH1330:pRK3 as a donor in transduction experiments. The results are shown in Table 2.

These transductions were performed with phage P1c1 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH1330</td>
<td>HfrC polA argH</td>
<td>C. W. Hill (4)</td>
</tr>
<tr>
<td>AB1515</td>
<td>F' leuB proC purE trpE rpsL</td>
<td>CGSC 1515</td>
</tr>
<tr>
<td>KL1057</td>
<td>glyV (suGAA/G) Δ(tionB-trp) F' trpA (GAA211)</td>
<td>E. J. Morgula (5)</td>
</tr>
<tr>
<td>M2508</td>
<td>metB melA</td>
<td>CGSC 4626</td>
</tr>
<tr>
<td>AB2569</td>
<td>proA hisG argH</td>
<td>CGSC 2569</td>
</tr>
<tr>
<td>ES4</td>
<td>purA</td>
<td>CGSC 4331</td>
</tr>
<tr>
<td>CS8Asp23</td>
<td>metB AspA glyC</td>
<td>CGSC 5092</td>
</tr>
<tr>
<td>NP37(pID2)</td>
<td>pheS (pACYC184::pheU^+)</td>
<td>This laboratory (6)</td>
</tr>
<tr>
<td>NP37(pRK3)</td>
<td>pheS (pBR322::pheU^+)</td>
<td>This laboratory (6)</td>
</tr>
<tr>
<td>DEV22</td>
<td>mia::Tn10</td>
<td>See text</td>
</tr>
<tr>
<td>DEV23</td>
<td>F' leuB proC purE trpE rpsL gyrA</td>
<td>See text</td>
</tr>
</tbody>
</table>

TABLE 1. Bacterial strains used

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Recipient strain</th>
<th>Marker selected</th>
<th>Marker scored</th>
<th>No. scored</th>
<th>Cotransduction frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2508</td>
<td>ES4</td>
<td>purA^+</td>
<td>melA</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>CH1330::pRK3</td>
<td>ES4</td>
<td>Amp^+</td>
<td>purA^+</td>
<td>1,079</td>
<td>41</td>
</tr>
<tr>
<td>CH1330::pRK3</td>
<td>M2508</td>
<td>Amp^+</td>
<td>melA</td>
<td>297</td>
<td>26</td>
</tr>
<tr>
<td>CH1330::pRK3</td>
<td>KL1057</td>
<td>Amp^+</td>
<td>glyV</td>
<td>300</td>
<td>33</td>
</tr>
<tr>
<td>CH1330::pRK3</td>
<td>CS8Asp23</td>
<td>Amp^+</td>
<td>aspA^+</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>DEV22</td>
<td>ES4</td>
<td>Tc^-</td>
<td>purA^+</td>
<td>200</td>
<td>75</td>
</tr>
<tr>
<td>DEV22</td>
<td>M2508</td>
<td>Tc^-</td>
<td>melA^+</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>DEV22</td>
<td>KL1057</td>
<td>Tc^-</td>
<td>glyV</td>
<td>83</td>
<td>69</td>
</tr>
</tbody>
</table>

TABLE 2. Two-factor crosses

* Corresponding author.
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 P1cl 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.

![Genetic map of pheU and neighboring markers. Numbers shown are cotransduction frequencies. a, Data of Spencer et al. (7)](image)

We thank B. Bachmann, C. W. Hill, and E. J. Murgola for providing some of the strains used in this work and Lillian Delgado for secretarial assistance.

This work was supported by Public Health Service grants GM24576 to D.E. and GM29265 to I.S. from the National Institutes of Health. I.S. is a Sinsheimer Scholar awardee of the Alexandria and Alexander L. Sinsheimer Foundation.

**LITERATURE CITED**