Escherichia coli B/r leuK Mutant Lacking Pseudouridine Synthase I Activity

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Escherichia coli B/r strain EB146 containing mutation leuK16 has elevated levels of enzymes involved in the synthesis of leucine, valine, isoleucine, histidine, and tryptophan (Brown et al., J. Bacteriol. 135:542–550, 1978). We show here that strain EB146 (leuK16) has properties that are similar to those of E. coli and Salmonella typhimurium hisT strains. In tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu}, from both hisT and leuK strains, positions 39 and 41 are uridine residues rather than pseudouridine residues. Furthermore, in tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} from a leuK strain, uridine residues at positions 39 and 40, respectively, are unmodified. Pseudouridine synthase 1 activity is missing in extracts of strain EB146 (leuK16), and extracts of strain EB146 (leuK16) and of a hisT strain do not complement one another in vitro. Four phenotypes of strain EB146 (leuK16), leucine excretion, wrinkled colony morphology, and elevated levels of leu and his enzymes, are complemented by a plasmid having a 1.65-kilobase DNA fragment containing the E. coli leuK12 hisT locus. These results indicate that either leuK codes for pseudouridine synthase 1 (and is thus a hisT locus in reality) or, less likely, codes for a product that affects the synthesis or activity of pseudouridine synthase 1.

Escherichia coli B/r strain EB146 containing mutation leuK16 has elevated levels of enzymes involved in the synthesis of leucine, valine, isoleucine, histidine, and tryptophan (5). Genetic experiments suggested that leuK16 mapped near gal and that it was dominant to the wild allele. Taken together, these results defined a new locus, leuK, that in some way interacted with diverse operons involved in amino acid biosynthesis. More recent unpublished experiments by E. Kline indicate that previous conclusions (5) regarding the map location and dominance characteristic of leuK are invalid. The experiments described below indicate that strain EB146 (leuK16) has a number of properties in common with hisT strains. The simplest interpretation of our results is that leuK is, in fact, a hisT locus.

tRNA was isolated from parent strain EB145 and mutant strain EB146. The profile of charged tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} species emerging from Sephacryl 4B, eluted with a reverse (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} gradient (19), is shown in Fig. 1. In our hands, tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} isoaccepting species emerged in three peaks, the first containing tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} and tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu}, the second containing tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} and tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu}, and the third containing tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} (see Table 1 for definitions of tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} isoaccepting species). In the profiles published by Hatfield (19), which are of higher resolution, tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} elutes with tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} and tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} elutes before tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu}. Figure 1 shows the results of a double-label experiment employing [\textsuperscript{3}H]leucine and [\textsuperscript{14}C]leucine charged to tRNA from the parent (EB145) and mutant (EB146) strains, respectively. For tRNA from strain EB146, the first and last peaks were shifted relative to the parent, indicating that tRNA\textsubscript{Leu}\textsuperscript{1}\textsubscript{eu} or tRNA\textsubscript{Leu}\textsuperscript{1}\textsubscript{eu} plus tRNA\textsubscript{Leu}\textsuperscript{1}\textsubscript{eu} or all three, are altered in the mutant. On the other hand, there were no differences in the elution profiles of tRNA\textsubscript{Val} or tRNA\textsubscript{Ile} species between the parent and mutant strains (J. Jones, unpublished data).

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Isoaccepting species tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu}, tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu}, and tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} were purified by a combination of the derivatization procedure of Gillam et al. (16) and two-dimensional polycrylamide gel electrophoresis (14). No differences in mobility were observed between the tRNAs of the mutant and wild-type strains on two-dimensional gels. Purified tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} species from both mutant and parent strains were sequenced by the chemical method of Peattie (29). The sequence of wild-type tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} shows gaps at positions 33, 40, and 66, corresponding to known positions of pseudouridine (psi) (35). In the sequence of tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} from the mutant, there is a band in the uridine (U) track at position 40 (Fig. 2) but not at position 33 or 66 (data not shown). No differences other than the one noted above were observed between the two sequences. These results indicate that the modification of tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} at position 40 is affected by the leuK mutation but that modifications of positions 33 and 66 are not.

Purified tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} from the parent and mutant were analyzed by procedures that give both the nucleotide sequence and the identity of modified bases (17, 18). In tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} from the mutant but not the parent, the U at position 39 is unmodified (Fig. 3). In all other respects, the sequences from the mutant and parent were identical, corresponding to the sequence derived by the Sanger procedure (4). Purified tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} from the mutant and parent were analyzed in a similar fashion. In tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} from the mutant, neither of the Us at positions 39 and 41 was modified to psi as they were in the parent, but the U at position 66 was normally modified (data not shown).

To summarize, for three different tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} isoaccepting species analyzed, tRNAs from the mutant lacked psi residues within the 3′ side of the anticodon loop (at positions 39, 40, or 41) but contained psi residues within the TψC loop and, for tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu}, within the 3′ side of the anticodon loop. Some preliminary experiments indicate that the leuK mutation affects psi modification within tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} in the same way as for tRNA\textsubscript{Leu}\textsuperscript{1}\textsuperscript{eu} (L. Searles, unpublished data).
The pleiotropic effects of the leuK mutation upon amino acid biosynthetic operons (5) and the effect of the leuK mutation upon the U-ψ modification in tRNA^{Leu} species invite comparison with hisT mutations in Salmonella typhimurium and E. coli. Mutations in hisT result in elevated expression not only of the his operon (32), but also of several other amino acid biosynthetic operons, including the leu operon (10, 23, 30). hisT is the structural gene for pseudouridine synthase I, an enzyme that converts U to ψ within the anticodon loop of several tRNAs, including tRNA_{Leu}^{L}. In fact, the ψ modification pattern of tRNA_{Leu}^{L} from a hisT strain (39 and 41 unmodified; 66 modified) is just that reported here for tRNA^{Leu} from a leuK strain.

This comparison prompted us to measure pseudouridine synthase I in parent, hisT, and leuK strains by using the 3H release assay developed by Cortese et al. (10). The basis of this assay is that in the conversion of U to ψ, a hydrogen is released from the carbon atom at position 5 of the pyrimidine ring. tRNA from a leuK strain acted as a substrate for a pseudouridine synthase activity that was present in crude extracts of wild-type S. typhimurium, E. coli K-12, and E. coli B/r (Table 2; experiment 1). Extracts prepared from hisT strains of S. typhimurium and E. coli did not catalyze release of 3H from this substrate (Table 2, experiment 2), suggesting that the activity observed in experiment 1 was due to pseudouridine synthase I activity. An extract from mutant strain EB146 (leuK16) behaved like extracts from hisT strains, i.e., it did not catalyze 3H release from the tRNA substrate (Table 2, experiment 2). The lack of activity in extracts prepared from strain EB146 (leuK16) was not the result of a diffusible inhibitor because such extracts did not inhibit the activity present in wild-type extracts (Table 2, experiment 4). Extracts prepared from leuK and hisT strains did not complement one another in vitro (Table 2, experiment 3).

![FIG. 1. Sepharose 4B chromatography of tRNAs from strains EB145 (○) and EB146 (leuK) (●). Strains were grown and tRNA was isolated as described by Zobay (37). Conditions for tRNA charging are described by Blank and Soll (3). An extract of an arabinose-negative gal-typical S. typhimurium strain containing tRNA synthetases was prepared as described by Muench and Berg (27). Samples of 500 μg of tRNA were charged with 1 μCi of [3H]leucine (400 Ci/mmol) (leuK tRNA) or 2 μCi of [14C]leucine (200 mCi/mmol) (parent tRNA). The two samples were mixed, applied to the column, and eluted as described by Hatfield (19).](http://jb.asm.org/)

The following analysis points up the similarities between leuK and hisT mutations. The two loci differ in their map position: hisT is about 40% linked to purF by P1 transduction (7, 31), whereas leuK is reportedly linked to gal (5). Upon reexamining the linkage of leuK to gal, E. Kline (personal communication) observed the following. Almost all Gal+ transductants from a cross between phage P1 grown on EB146 (gal-leuK) and a gal recipient indeed excreted leucine (a phenotype associated with leuK). However, the same result was obtained from a control cross between phage P1 grown on EB145 (gal+ leuK) and a gal recipient, indicating that the leucine excretion observed in these crosses is not due to cotransduction of leuK and gal (E. Kline, personal communication).

To determine whether the leuK16 mutation is linked to purF, the following crosses were performed. E. coli K-12 strain CV875 (purF) was transduced to prototrophy with P1 phage grown on E. coli B strain EB146 (purF+ leuK16). Transductants were scored for leucine excretion by an auxanographic test (9). None of 170 transductants analyzed excreted leucine (Table 3). Another cross was performed with recipient E. coli B strain CV878 (purF::Tn10) and phage grown on E. coli B strain EB146 (leuK16); prototrophic transductants were scored for both leucine excretion and

![FIG. 2. Partial nucleotide sequence of tRNA_{Leu}^{L} from (a) strain EB145 (parent) and (b) strain EB146 (leuK16). Purified species were end-labeled with 3P using RNA ligase (6) and sequenced by the chemical method of Peattie (29). Samples were applied to a 20% acrylamide-0.67% bisacrylamide-8 M urea gel.](http://jb.asm.org/)

<table>
<thead>
<tr>
<th>tRNA^{Leu} species</th>
<th>Elution from Sepharose 4B</th>
<th>Anticodon (3'-5')</th>
<th>Codon(s)</th>
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<tr>
<td>1</td>
<td>4</td>
<td>GAC</td>
<td>CUG</td>
</tr>
<tr>
<td>2</td>
<td>1 or 2</td>
<td>GAU</td>
<td>CU, CU, CU</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>GAG</td>
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<tr>
<td>4</td>
<td>5</td>
<td>AAU</td>
<td>UU, UUG</td>
</tr>
<tr>
<td>5</td>
<td>1 or 2</td>
<td>AAC</td>
<td>UUG</td>
</tr>
</tbody>
</table>

* Species designation refers to order of elution from an RPC-5 column.
* Figure 1 and reference 19.
* References 3, 4, 11, 13, 20, 28, and 35; L. Searles, unpublished data.
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FIG. 3. Partial nucleotide sequence of tRNA\textsuperscript{343} from strains EB145 (A) and EB146 (leuK) (B). Purified samples were analyzed by the rapid read-out sequencing method of Gupta and Randerath (17) as modified by Gupta et al. (18). The asterisk marks the difference in sequence between the parent and mutant tRNAs.

wrinkled colony morphology. We observed that strain EB146 (leuK16), like hisT-containing strains of S. typhimurium, had a wrinkled colony morphology when grown on plates containing 2% glucose and 2% gluconate. Again, no linkage of purF to leuK16 was found among 300 transductants tested. In addition, we did not observe linkage of purF to leuK16 in a transduction involving phage grown on a purF::Tn10 donor and a leuK16 recipient when selection was made for tetracycline resistance associated with Tn10 (Table 3).

To summarize, the assignment of leuK to a location near gal (5) is incorrect. Our transduction experiments do not define the location of leuK. If leuK is a hisT locus, then our inability to detect linkage to purF suggests that these loci are not closely linked in E. coli B. However, the possibility remains that the two loci are linked, but that we did not observe linkage because of factors relating to interspecies crosses.

To determine whether phenotypes associated with the leuK16 mutation were complemented by a wild-type hisT locus, E. coli B strain EB146 (leuK16) was transformed separately with plasmids p300 and pNU61, containing the hisT locus from E. coli K-12 on 2.3- and 1.65-kilobase fragments, respectively (25). The resulting transformants had normal colony morphology, did not excrete leucine, and had normal (low) levels of β-isopropylmalate dehydrogenase (leuB product) and histidinol phosphatase (hisB product) (Table 4). These results strongly suggest that hisT complements the leuK16 mutation.

The simplest interpretation of the biochemical and genetic complementation experiments described here is that leuK16 is a hisT mutation. Other more complicated possibilities can be imagined. For example, the wild-type leuK gene may code for a product that regulates the synthesis or activity of pseudouridine synthase I. The fact that an E. coli B leuK mutation is complemented by an E. coli K-12 hisT gene may reflect a difference in regulation between the two organisms or may be a result of high plasmid copy number.

The remaining discussion, relating to the expression of hisT mutations in different organisms, assumes that leuK16 is a hisT mutation. In S. typhimurium, a hisT mutation causes marked elevation of his operon expression (32) and modest elevation in the expression of the leu and ilv operons (10, 30). In E. coli K-12, a hisT mutation has a substantial effect upon the his operon but only a small effect upon leu and ilv operons (22). These results may be compared with those for E. coli B/r containing leuK16: the leu operon is most highly derepressed, with the his, ilv, and trp operons being substantially elevated (5). Note that both column chromatographic profiles of charged tRNAs and enzyme assays suggest that pseudouridine synthase I activity is totally absent in each of the three strains compared (10, 22). Another phenotype relevant to this discussion is growth rate. S. typhimurium (23) and E. coli K-12 (7) hisT strains grow more slowly than the parent, but this is not the case for E. coli B/r strain EB146 (5).

The differences described above may be due to one or a combination of the following possibilities.

(i) The his, leu, ilv, and trp operons of E. coli K-12 and S. typhimurium are controlled by transcription attenuation (21).
The structure of the control regions of the relevant operons may be different in different organisms. This is not true for the his operons of E. coli K-12 and S. typhimurium (which differ by two nucleotides) (2, 12), but it is certainly the case for the leu operons of these two organisms (18 of 84 nucleotides within the leader regions differ) (34). For E. coli B/r, there is no relevant nucleotide sequence information. Conceivably, the number, position, or identity of control codons may be different in E. coli B/r.

(ii) There may be strain-related differences in the structures of some tRNA species. Available information (15) suggests that both sequence and modification differences exist but are few.

(iii) Regulation by transcription attenuation is known to be affected by factors influencing both transcription and translation. For example, mutations affecting the structure of RNA polymerase cause altered expression of the trp operon (36). It is not unlikely that some component(s) of the transcription or translation machinery in these strains differ. Certainly, such differences could explain the growth rate and regulatory patterns observed.

### TABLE 3. Transduction crossovers performed to determine the map position of leuKα

| Phase P1 vir grown on E. coli B strain® | | | % of transductants that: |
|----------------------------------------|---------------------------------|--------|-------------------------|--------|
| EB146 (leuK16) | E. coli K-12 | Strain CV875 | purF::Tn10 | purF+ | 0 (170) |
| EB145 | E. coli K-12 | Strain CV875 | purF::Tn10 | purF+ | 0 (40) |
| EB146 (leuK16) | E. coli B/r | Strain CV878 | purF::Tn10 | purF+ | 0 (232) |
| EB145 | E. coli B/r | Strain CV878 | purF::Tn10 | purF+ | 0 (54) |
| CV877 (purF::Tn10) | E. coli B/r | Strain EB146 | leuK16 | tet | 100 (43) |
| CV877 (purF::Tn10) | E. coli B/r | Strain EB145 | leuK+ | tet | 0 (35) |

® Transductions were carried out by procedures described by Miller (26) in a minimal medium (9) containing 0.2% glucose. Selection for tetracycline resistance was on L-plates (26) containing 25 μg of tetracycline per ml. Problems of restriction in transductions between strains B/r and K-12 were reduced by incubating the recipient at 55°C for 30 min before performing the transduction or by using strains lacking restriction systems.

® Genotypes of strains were as follows. Strain EB146: dau-5 rpsL (mal+ λ) from E. coli K-12 leuK16. Strain CV875: F- rpsL lac gal-1,2 T1' T7' hsdR hsdM purF77::Tn10. Strain CV878: hsdR11 met-100 (mal+ λ) from E. coli K-12 gal-151 purF77::Tn10. Strain CV879: purF77::Tn10 dau-5 rpsL (mal+ λ) from E. coli K-12. Strain CV875 was constructed by transducing E. coli K-12 strain EG247 (F' hsdR hsdM rpsL lac gal-1,2 T1' T7') with phage P1 vir grown on E. coli K-12 strain NK6035 [Δ[λ(gpt-lac)5 purF77::Tn10 relA1 spoT1 thi-1 λ+]) and selecting for tetracycline resistance. Strain CV877 was derived from E. coli B strain WA837 [hsdR11 gal-151 met-100 (mal+ λ) from E. coli K-12] by transduction with phage P1 vir grown on E. coli K-12 strain NK6035 and selection for tetracycline resistance. Strain CV878 is a purF77::Tn10 derivative of strain EB145 obtained by transduction with phage P1 vir grown on strain CV877.

leuKα causes overproduction and excretion of leucine, a phenotype that was scored by an auxanographic test described by Calvo et al. (9). Plates for measuring the wrinkled colony morphology contained in addition to 2% glucose, 2% glucose which accentuated the phenotype. The number in parentheses is the total number of colonies analyzed.
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LITERATURE CITED


