# THE NUCLEOTIDE SEQUENCE OF TWO LEUCINE tRNA SPECIES FROM *ESCHERICHIA COLI* K12

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## Abstract

The nucleotide sequence of two leucine tRNA species from *E. coli* K12 has been determined. Both possess a chain length of 87 nucleotides but differ in 22 positions in their primary sequence.

The specific recognition of tRNA by its cognate aminoacyl-tRNA synthetase is a crucial step in protein synthesis (1). The structural features of the tRNA molecule which confer the specificity of this recognition process are not understood (2). One approach to this problem lies in the detailed comparison of the primary sequences of a family of isoaccepting tRNA species which are all recognized by the same aminoacyl-tRNA synthetase. A good case for study is leucine tRNA in *E. coli*. There exist at least five different isoaccepting leucine tRNAs (3). The multiplicity of the $tRNA^{Leu}$ species is further extended through the occurrance of an *E. coli* amber suppressor $tRNA^{Leu}$ (4). In addition, *E. coli* leucyl-tRNA synthetase has been purified and characterized (5). From genetic and biochemical studies it is concluded to be a single enzyme. The interactions of this enzyme with the different leucine isoaccepting tRNAs have been studied in detail (6).

In this communication we report the nucleotide sequences of two leucine tRNAs from *Escherichia coli* K12. The sequence of one of these tRNAs is identical to that of a leucine tRNA from E. coli B (7).

The purification and separation of the isoaccepting leucine tRNAs labelled with [$^{32}$P]orthophosphate was accomplished by chromatography on benzoylated DEAE-cellulose (Figure 1). Leucine tRNA$_1$ and tRNA$_2$ are the

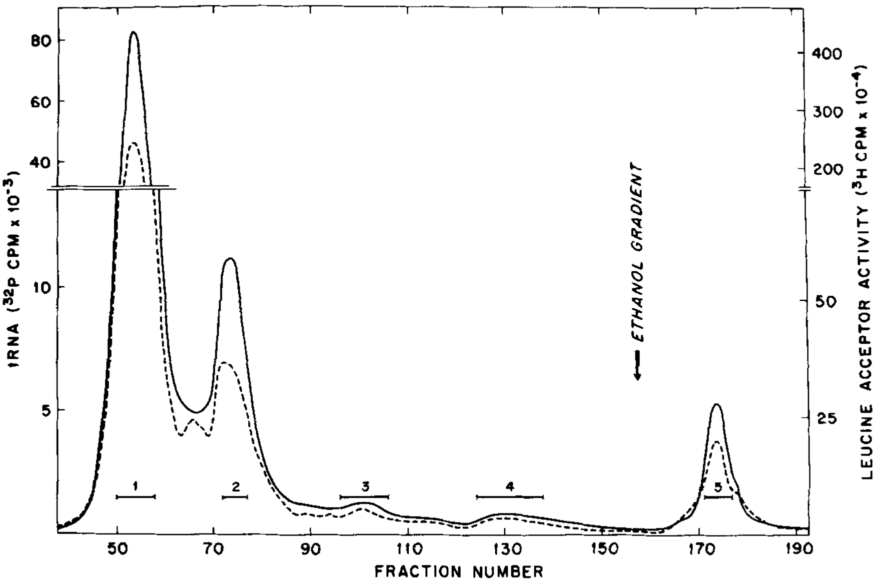


Fig. 1 Separation of [$^{32}$P]tRNA$^{Leu}$ species by chromatography on benzoylated DEAE-cellulose. (tRNA ----; leucine acceptor activity ——). E. coli K12 (strain CA244) was grown in low phosphate medium in the presence of [$^{32}$P]orthophosphate (8). After extraction of the tRNA the leucine isoacceptor tRNAs were purified by the derivatization method of Gillam et al. (9). tRNA$^{Leu}$ (1.2 x $10^9$ cpm) was applied to a BD-cellulose column (0.8 x 60 cm) previously equilibrated with 0.2 M NaCl - 0.01 M Tris-HCl (pH 7.5) - 0.01 M $MgCl_2$ - 0.04% $NaN_3$. Elution was carried out at room temperature with a linear gradient of NaCl from 0.4 to 1.7 M (420 ml total volume) followed by a linear gradient (total volume 120 ml) of ethanol from 0 to 25% (v/v) in 1.7 M NaCl. Tris-HCl, $MgCl_2$ and sodium azide were present in all solutions at the concentrations described above. A flow rate of 15 ml/hr was maintained and 2.7 ml fractions were collected. Aliquots of fractions (5 µl) were assayed for leucine acceptor activity.

major isoacceptor species. Their nucleotide sequence was determined by established procedures (10) involving complete and partial enzymic degradation of [$^{32}$P] labelled tRNA. The three minor leucine tRNA peaks contain distinct tRNA species as judged from the oligonucleotide patterns resulting from complete degradation with $T_1$ and pancreatic RNase.

The oligonucleotides obtained by complete enzymic degradation of leucine $tRNA_1$ are listed in Table 1. The nucleotide sequence of the entire tRNA

Table 1. Products formed by complete degradation of leucine $tRNA_1$ with

| Pancreatic RNase | | $T_1$-RNase | |
|---|---|---|---|
| 14C | pGC | 8G | CUAG |
| 9U+ψ+D | AGU | $CACCA_{OH}$ | $G^*ψG$ |
| 2AC | GGAC | 3CG | ψUAG |
| 3GC | AAGU | 2ACG | CUUCAG |
| AGC | $G^mGD$ | AAG | TψCAAG |
| AGAC | $AGG^*ψ$ | pG | UCCUUACG |
| GGC | GGAAD | 3UG | UCCCCCCCCUCG |
| 2GU | GAAGGU | DAG | $AADDG^mG$ |
| Gψ | GGGGGT | | |

as given in Figure 3 was deduced from the sequences of a number of large oligonucleotides obtained by partial degradation of the tRNA with $T_1$ RNase. This nucleotide sequence is the same as has been recently reported for a leucine tRNA from E. coli B (7).

Table 2 shows the oligonucleotides obtained from complete $T_1$ and pan-

Table 2. Products formed by complete degradation of leucine $tRNA_2$ with

| Pancreatic RNase | | $T_1$-RNase | |
|---|---|---|---|
| 12C | AAGU | 9G | UCCCG |
| 8U+ψ+D | AGGGC | $UACCA_{OH}$ | $G^*ψG$ |
| 4AC | $G^mGD$ | 2AG | CCCAAUAG |
| 2GC | 3GGU | CCG | UCCUCG |
| AAU | GGGT | ACACG | CUUACG |
| GU | GGAAD | pG | TψCAAG |
| pGC | $GAGG^*ψ$ | 3UG | CUACCUUG |
| AGAC | GAGGU | DAG | $AADDG^mG$ |
| AGU | | UAG | |

creatic RNAse digests of leucine $tRNA_2$. The nucleotide sequence of large fragments isolated from partial $T_1$ RNAse digests of leucine $tRNA_2$ and the deduced total primary structure of this tRNA is given in Figure 2. (The order of the two pancreatic oligonucleotides, GAGGU and GGU, in fragment $T_{38}$ has not yet been unambiguously determined. The ordering as shown resulted from three considerations: (i) to have a uridine in position 8 (2),

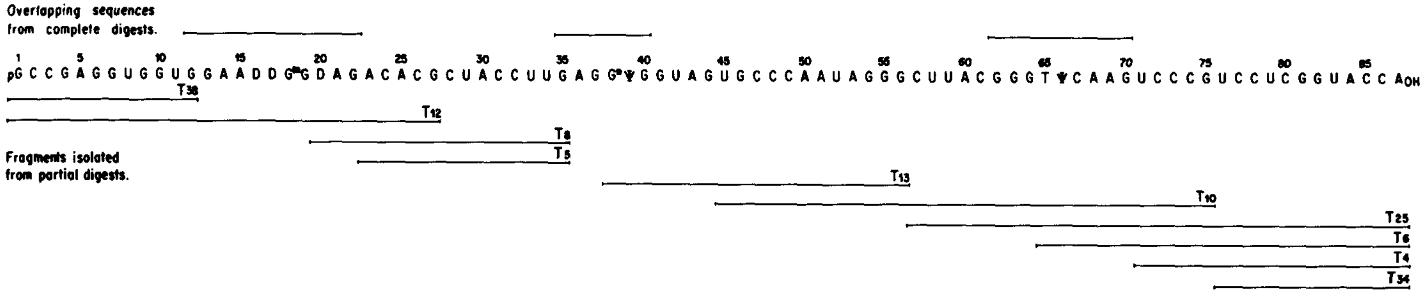


Figure 2. Large oligonucleotides produced by partial degradation of leucine $tRNA_2$. Fragments were isolated by homochromatography and further analyzed as described by Sanger et al. (10).

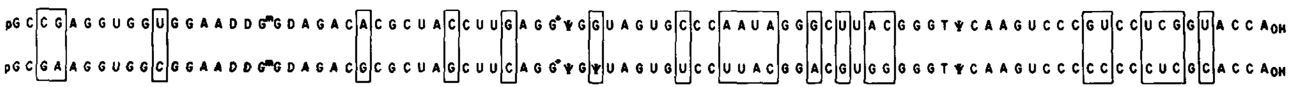

Figure 4. Comparison of the nucleotide sequences of E. coli leucine $tRNA_1$ (bottom) and leucine $tRNA_2$ (top). Differences in the sequences are indicated by empty brackets.

(ii) to allow maximum base pairing in the clover-leaf model and (iii) to give the minimum deviation from leucine $tRNA_1$.)

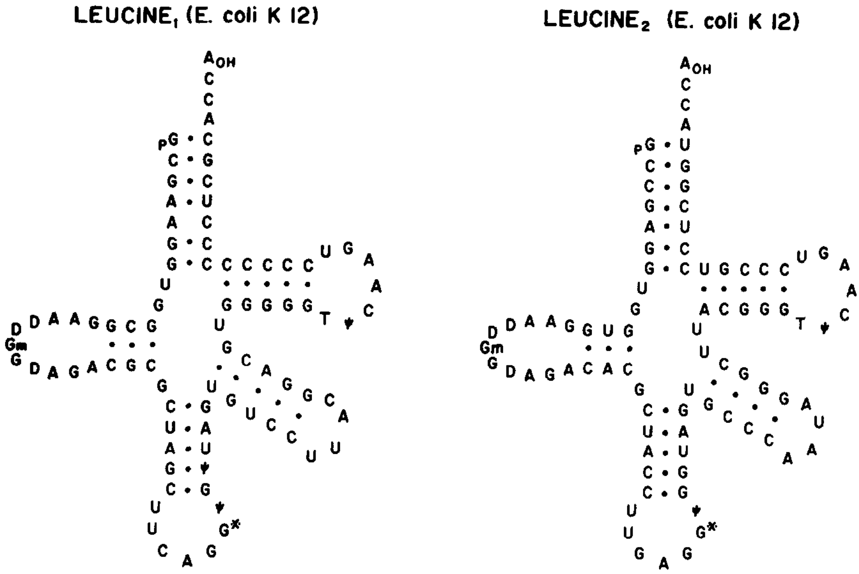


Figure 3. Clover leaf model of the nucleotide sequence of two E. coli $tRNA^{Leu}$ species. Abbreviations: A, C, G, U; Adenylic, cytidylic, guanylic and uridylic acids, respectively; $G^m$, 2'-O-methylguanosine; $G^*$, uncharacterized guanylic and derivative; ψ, pseudouridine; T, ribothymidine.

Both tRNAs possess the same chain length of 87 nucleotides. From the anticodon sequences the codon response is expected to be CUG for leucine $tRNA_1$ and CUU and CUC for leucine $tRNA_2$. This has been observed in studies with unlabelled tRNAs from the same strain (11). The modified nucleoside adjacent to the 3' end of the anticodon in both tRNAs is an unknown guanosine derivative. There are indications that it may be an acylated G. We have not detected thiouridine in these two tRNAs. However, this result needs confirmation with unlabelled tRNA, since identification of this nucleotide in $[^{32}P]$ labelled tRNA is difficult (12). As seen in Fig.3 there are 22 nucleotide differences between the sequences of the two leucine tRNAs. It is striking that any change of a nucleotide in a base-paired region also involves the change of the other base in the pair. The loss of base pairs in double helical regions probably results in a less stable structure and possibly non-functional tRNA as has been found in mutant tyrosine tRNA species (13). The nucleotide sequence of the dihydrouridine loop is the same in both tRNAs. Whether this is part of a recognition site (cf. 14) may be more clearly answered when the sequences of the remaining leucine isoacceptors are known. Despite the many nucleotide changes in the primary structures of leucine $tRNA_1$ and $tRNA_2$ there are only minor differences in their interaction with *E. coli* leucyl-tRNA synthetase (6).

Acknowledgements

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