The nucleotide sequence of bacteriophage T5 leucine tRNA

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Uniformly ^{32}P-labeled bacteriophage T5 leucine tRNA has been isolated by two-dimensional gel electrophoresis from phage-infected *E. coli* cells. Its nucleotide sequence has been determined by conventional techniques using TLC on cellulose for oligonucleotide fractionation:

pGGGGCUAUGCUGGAACDGmGDAGACAAUACGGCCUUAGm^{6}AUΨCCGUAGCUUAAA
UGCGUGGGAGTΨCGAGUCUCCCUAGCCCCACCAoh.

This tRNA has anticodon sequence UAG, which can presumably recognize all the four leucine-specific codons (CUN). The main feature of T5 tRNA^{Leu} is the absence of the A_{10}–C_{25} and C_{31}–Ψ_{39} pairing in the D and anticodon stems, respectively.

Bacteriophage T5 *tRNA^{Leu}* *Nucleotide sequence*

1. INTRODUCTION

It has been shown that at least 30 stable RNAs, consisting mainly of tRNA species, are coded for by bacteriophage T5 [1,2]. During the phage infection their synthesis does not interfere with rapidly diminished *E. coli* tRNA synthesis. Thus, it is possible to label phage-specific tRNAs in vivo without visible labelling of those of *E. coli* [2,3]. Subsequent isolation of individual tRNA species can be performed by 2-dimensional polyacrylamide gel electrophoresis. The fractionation pattern is shown in fig.1.

Here, we present the nucleotide sequence of bacteriophage T5 tRNA^{Leu} which corresponds to one of the most intensive spots (spot 3 [2]). It has leucine-specific anticodon UAG, and does accept this amino acid.

2. MATERIALS AND METHODS

Procedures for purifying tRNA^{Leu} and establishing its nucleotide sequence were as described [3]. Some details of RNA and oligonucleotide fractionation are presented in the figure legends.

3. RESULTS

Uniformly ^{32}P-labeled phage-specific tRNA^{Leu} was isolated from bacteriophage T5 infected *E. coli* cells [3]. A practically homogeneous preparation of this tRNA was obtained using 2-dimensional gel electrophoresis (fig.1). Its nucleotide sequence was determined essentially by conventional techniques [4]. The only difference was that TLC on cellulose was applied for the fractionation of different oligonucleotide mixtures [3]. Fig.2 shows the resolution and nucleotide sequence of the products obtained by complete digestion of tRNA^{Leu} with pancreatic and T_1 RNases. The approaches for the oligonucleotide structure determination were as in [3]. The sequence of oligonucleotides t13′ and t14 was established by analysis of the products obtained from RNase U_2 digestion of their mixture. Individual oli-

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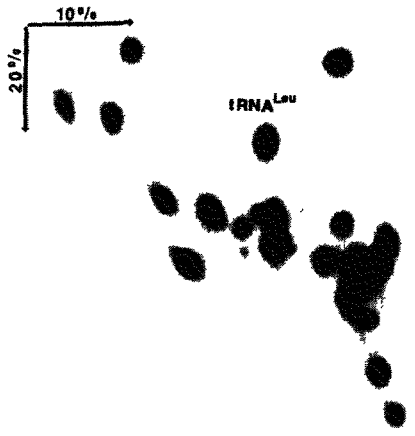

Fig.1. Two-dimensional polyacrylamide gel electrophoresis of bacteriophage T5 ^{32}P-labeled 4–5 S RNAs. Electrophoresis was in 10% polyacrylamide/7 M urea/Tris-borate (10 V/cm, 15°C) in the first dimension and in 20% polyacrylamide/4 M urea/Tris-borate (15 V/cm) in the second. The position of tRNA^{Leu} is indicated.

gonucleotides p17 and p18 were isolated from 5′- and 3′-terminal fragments of the tRNA molecule split at the anticodon with RNase T_1.

Modified nucleotides were characterized by their mobilities on 2-dimensional TLC [5] (fig.3). The extent of Gm and m^{6}A modification did not exceed 70%, and was no more than 20% in the case of D_{17}. The incomplete modification resulted in the appearance of the oligonucleotides t11, t13′, p9′ and p13′.

The final sequence of T5 tRNA^{Leu} was deduced from the data on the analysis of large fragments generated by partial hydrolysis of the tRNA with RNase T_1 or endonuclease S_1. Its cloverleaf structure is shown in fig.4. The leucine acceptor activity of this tRNA was proved in the experiments on aminoacylation of unlabeled tRNA as described for T5 tRNA^{Gln} [3].

4. DISCUSSION

Bacteriophage T5 transfer RNAs have been shown to have a number of differences from the generalized cloverleaf structure [6]. One of the peculiarities of T5 tRNA^{Leu} is the absence of the pairing between residues C_{10}-A_{25}, and as a result,

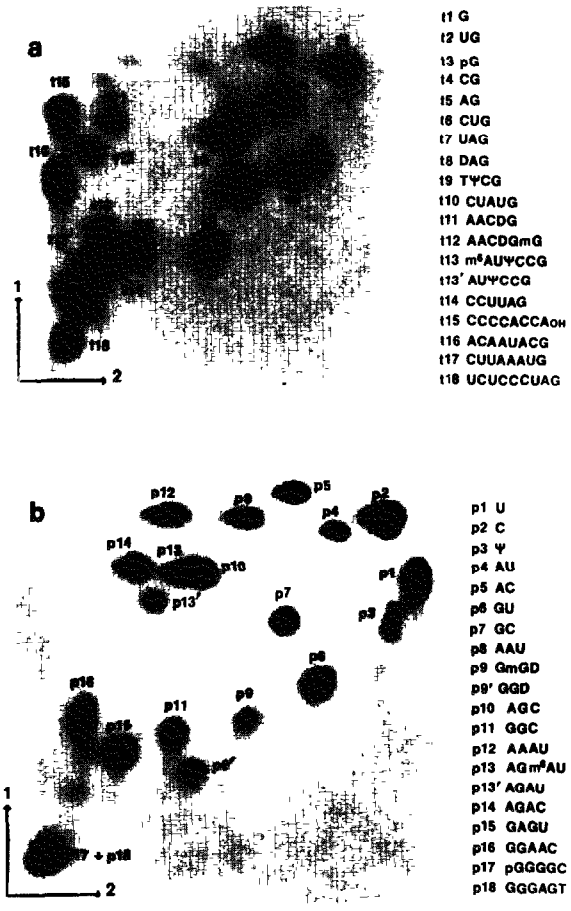

Fig.2. Two-dimensional TLC on cellulose plates (16 × 16 cm) of the complete digestion products of ^{32}P-labeled tRNA^{Leu} with RNase T_1 (a) and pancreatic RNase (b). Chromatography was in isobutyric acid/0.5 M NH_4OH (5:3, v/v, pH 3.7) in the first dimension and in *t*-butanol/0.075 M ammonium formate (pH 3.8) (1:1, v/v, pH 4.8) at 15°C in the second. Each system was developed twice in turns. The sequence of the corresponding fragments is to the right of the fingerprints.

the D stem has only 2 base pairs. A similar peculiarity was observed in the case of T5 serine tRNAs and some mitochondrial tRNAs [6]. The next unusual feature of tRNA^{Leu} is the absence of C_{31}-Ψ_{39} pairing which extends the anticodon loop up to 9 nucleotide residues. It should be mentioned that such deviations, nevertheless, do not affect its acceptor activity.

The extent of the sequence homology between T5 tRNA^{Leu} and other leucine tRNAs, including

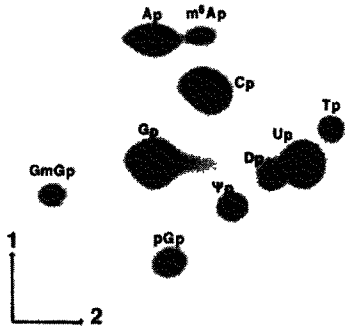

Fig.3. Two-dimensional separation of RNase T_2 digest of $tRNA^{Leu}$ by chromatography on a thin-layer cellulose plate according to [5].

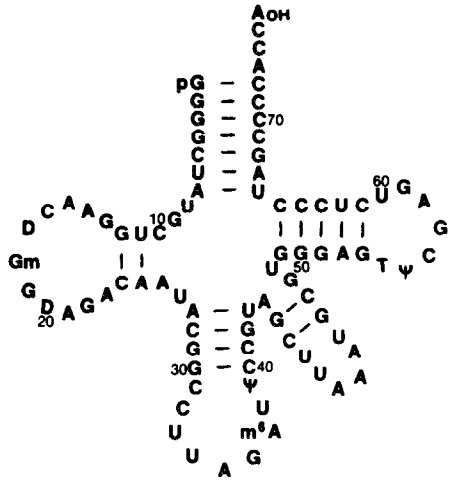

Fig.4. Cloverleaf structure of bacteriophage T5 $tRNA^{Leu}$. The numbering system of nucleotides is according to the numbering of $tRNA^{Phe}$ from yeast [6].

those from *E. coli* and phage T4, varies within the range 50–65% (variable loops are not considered in this comparison). A surprisingly high degree of homology of T5 $tRNA^{Leu}$ is shown with bean (78%) and maize (77%) chloroplast leucine tRNAs, especially in the acceptor and anticodon stems. Whether this similarity reflects any evolutionary regularities or such high sequence homology is quite accidental remains as yet unclear.

Like T5 and mitochondrial tRNAs, corresponding to amino acids with 4-codon families, T5 $tRNA^{Leu}$ has unmodified uridine in the first position of the anticodon (U_{34}). This is distinct from the situation in tRNAs from prokaryotes and from eukaryotic cytoplasm in which a U residue at the first position is always modified [6], the exceptions being 2 chloroplast leucine tRNAs and yeast $tRNA^{Leu}$ [6]. It is generally accepted that any modification in the wobble position affects tRNA decoding properties. In the case of tRNAs belonging to 4-codon families, the absence of U modification in the first anticodon position leads to the appearance of the ability of these tRNAs to recognize all 4 possible codons [7–9]. Although we did not study the decoding properties of T5 $tRNA^{Leu}$, it is reasonable to suppose that it is also able to read all the 4 leucine codons (CUN), but apparently with different efficiency [10]. T5 $tRNA^{Leu}_{UAG}$ should preferentially recognize the CUA codon, which is rarely used in the *E. coli* genetic code, and the corresponding isoacceptor *E. coli* tRNA is represented by the minor fraction [10]. It has been previously shown that most phage T4 tRNAs have anticodons specific for rarely occurring *E. coli* codons which at the same time are frequently used in the T4 genome. On the strength of this fact, phage-specific tRNAs were supposed to optimize the translation of phage mRNAs [11]. Unfortunately, lack of information on the codon usage does not permit us to clarify this question in the case of bacteriophage T5.

REFERENCES

[1] Hunt, C., Desai, S.M., Vaughan, J. and Weiss, S.B. (1980) J. Biol. Chem. 255, 3164–3173.
[2] Kazantsev, S.I., Tchernov, A.P., Shlyapnikov, M.G., Kryukov, V.M. and Bayev, A.A. (1979) Dokl. Akad. Nauk SSR 247, 744–748.
[3] Shlyapnikov, M.G., Kaliman, A.V., Kazantsev, S.I., Kryukov, V.M. and Bayev, A.A. (1984) Biochim. Biophys. Acta 782, 313–319.
[4] Barrell, B.G. (1971) in: Procedures in Nucleic Acid Research, vol.2 (Cantoni, G.L. and Davies, D.R. eds) pp.751–779, Harper and Row, New York.
[5] Nishimura, S. (1972) Prog. Nucleic Acid Res. Mol. Biol. 12, 48–85.
[6] Sprinzl, M. and Gauss, D.H. (1984) Nucleic Acids Res. 12, r1–r131.
[7] Heckman, J.E., Sarnoff, J., Alzner-DeWeerd, B., Yin, S. and RajBhandary, U.L. (1980) Proc. Natl. Acad. Sci. USA 77, 3159–3163.

[8] Barrel, B.G., Anderson, S., Bankier, A.T., De Bruijn, M.H.L., Chen, E., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R. and Young, I.G. (1980) Proc. Natl. Acad. Sci. USA 77, 3164–3166.

[9] Bonitz, S.G., Berlani, R., Coruzzi, G., Li, M., Macino, G., Nobrega, F.G., Nobrega, M.P., Thalenfeld, B.E. and Tzagoloff, A. (1980) Proc. Natl. Acad. Sci. USA 77, 3167–3170.

[10] Ikemura, T. (1981) J. Mol. Biol. 146, 1–21.

[11] Schmidt, F. and Apirion, D. (1983) in: Bacteriophage T4 (Mathews, C.K. et al. eds) pp.208–217, Washington, DC.