THE NUCLEOTIDE SEQUENCE OF A LEUCINE TRANSFER RNA FROM *E. COLI*

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We wish to report the nucleotide sequence of leucine transfer RNA ($tRNA_{Leu}$) from *E. coli* B. The particular $tRNA_{Leu}$ labelled with ^{32}P was first purified by electrophoresis in a polyacrylamide gel of RNA extracted from *E. coli* B grown in the presence of ^{32}P-phosphate (fig. 1). Examination of the various bands in the gel revealed that the particular leucine tRNA species was present in the slowest moving transfer RNA band (a), which in addition contains only one other, as yet, unknown tRNA. Final purification of the $tRNA_{Leu}$ was achieved by chromatography on a benzoylated DEAE cellulose column [1], the $tRNA_{Leu}$ eluting at a NaCl concentration around 0.7 M. The unknown tRNA species is eluted with 1 M NaCl containing 10% ethanol. Enzymatic digestions of the isolated $tRNA_{Leu}$ and subsequent sequence determinations of the resulting oligonucleotides were carried out as previously described in detail by us [2–4]. Fig. 2 shows a two-dimensional fractionation of a ribonuclease T_1 digest of $tRNA_{Leu}$ and an accompanying diagram giving the sequences of the nucleotides. A pancreatic ribonuclease digest was fractionated in a similar way and the sequences of the oligonucleotides determined. To overlap the end products large fragments were prepared by partial T_1 or pancreatic ribonuclease digestions and separated by homochromatography [5]. The sequences of the partial fragments were determined from their complete T_1 and pancreatic ribonuclease products. From the large number of partial digestion products a unique sequence could be deduced (fig. 3). As is the case for all other tRNAs known, the sequence can be arranged in the "clover leaf" pattern.

The $tRNA_{Leu}$ consists of 87 nucleotides and is thus the largest tRNA sequenced so far. We have not

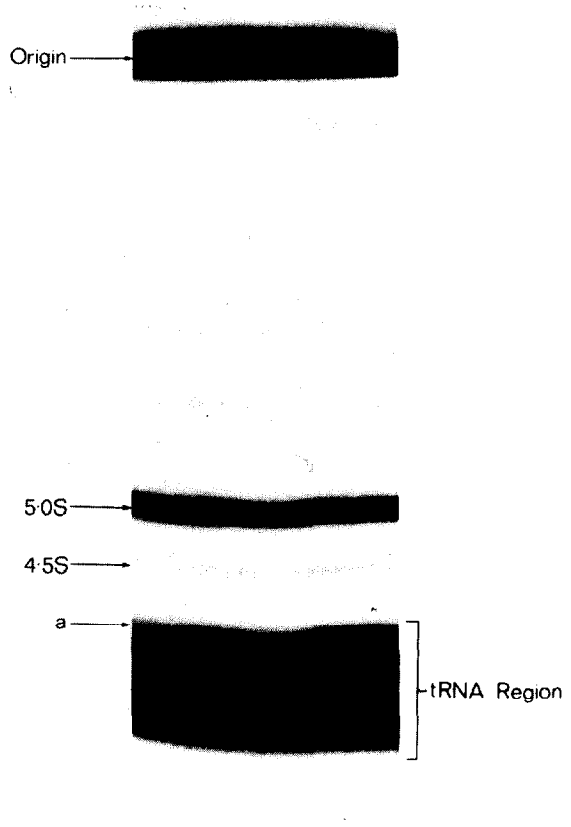

Fig. 1. Purification of $tRNA_{Leu}$ by electrophoretic fractionation on a polyacrylamide gel. The gel (9.5% acrylamine, 0.5% bisacrylamide) was prepared as described by Adams et al. [10] except that the buffer used was 0.09 M tris, boric acid pH 8.3 containing 0.025 M EDTA. RNA labelled with ^{32}P was isolated from *E. coli* B grown in the presence of ^{32}P-phosphate by phenol extraction. Electrophoresis was carried out at 40° for 16 hr at 350 V and 30 mA.

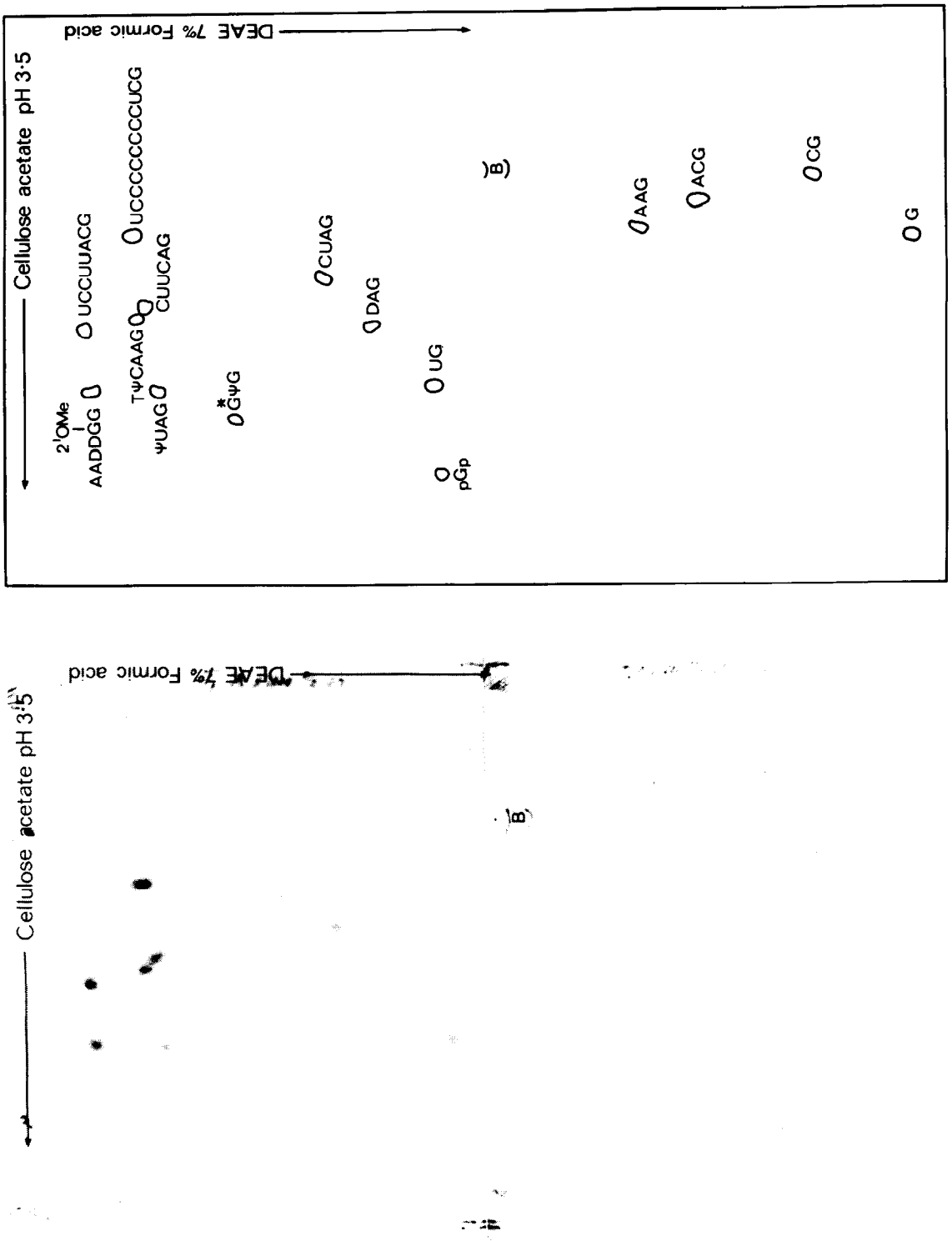

Fig. 2. A two-dimensional fractionation of a ribonuclease T_1 digest of $tRNA_{Leu}$. B refers to the position of the blue marker.

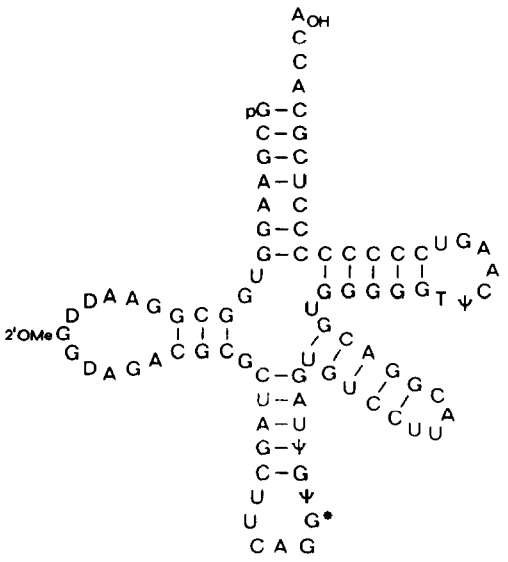

Fig. 3. The nucleotide sequence of $tRNA_{Leu}$ arranged in the "clover leaf" pattern. Standard abbreviations are used for the common nucleotides. Other abbreviations are: D, dihydrouridylic acid; ψ, pseudo-uridylic acid; T, ribothymidylic acid; 2'O Me G, 2'-O-methyl guanylic acid; G* is most likely either 1 or 2 methyl guanylic acid.

detected 4-thiouridine in this species. However, the possibility that it is converted into uridine during our isolation procedure cannot be excluded. Such a conversion was, in fact, often noted during our sequence studies on the two methionine tRNAs [2–4]. All tRNAs sequenced so far have a G in position 15 measured from 3'-end and a C in the corner between the TψC stem and the "finger". The base-pairing of these two nucleotides is a feature of the model proposed by Levitt [6]. It is of the greatest interest that $tRNA_{Leu}$ in the corresponding positions has an A and a U respectively. A similar finding has also been reported for *E. coli* tryptophan tRNA [7]. This coordinate base-change found in two different tRNAs therefore supports the model proposed by Levitt.

Finally, in a recent investigation we have demonstrated that this particular leucine tRNA species is the one being modified after T4 phage infection [8, 9].

Acknowledgement

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