# THE SEQUENCE OF PHENYLALANINE tRNA FROM *E. COLI*

B.G.BARRELL and F.SANGER
*MRC Laboratory of Molecular Biology, Cambridge, England*

Received 21 April 1969

Gassen and Uziel [1] have recently proposed a structure for phenylalanine tRNA from *E.coli*. We have also been studying the sequence of this tRNA but have obtained certain results that are in conflict with the above sequence.

$^{32}$P-labelled tRNA$^{Phe}$ was prepared from *E.coli* grown on $^{32}$P-phosphate [2]. Initially it was purified by making use of its specific binding to ribosomes in the presence of polyuridylic acid [3] and much of the preliminary work was done on this material. The yield of pure tRNA$^{Phe}$ was rather low with this method and in later work purification was effected using reversed phase partition chromatography [4].

Figs. 1 and 3 show the two-dimensional fractionation [2] of a ribonuclease T1 and a pancreatic ribonuclease digest of the tRNA$^{Phe}$. The sequences of the nucleotides are given in the accompanying diagrams. X appears to be the same unknown unstable nucleotide that was found in tRNA$^{Met}_{M}$ [5]. 2mt6iA appears to be the same minor component that is found in tRNA$^{Tyr}$ and is therefore tentatively identified as 2-thiomethyl-6-isopentenyl adenylic acid [6].

Digests prepared by partial digestion with ribonuclease T1, pancreatic ribonuclease and *B.subtilis* ribonuclease were also studied and fractionated on the homochromatography system [7]. The results are summarized in fig. 2 which shows the oligonucleotides that were obtained and the sequence derived from them. The sequence is deduced as the only possible one that fits our experimental results; however in certain cases, particularly in the D loop (positions 10–20), the conclusions depend on the results with only one partial digestion product (indicated by —x— in fig. 2) and there is insufficient confirmatory evidence to establish the sequence without making use of analogies with other tRNAs.

Yaniv et al. [8] have shown that in tRNA$^{Val}_{1}$ the 4-tU in position 8 can react under the influence of UV light with a C residue in position 13 to form a covalent bond. A similar reaction also occurs in tRNA$^{Phe}$ [8] and our results indicated that a stable cross linkage was formed during some of the fractionation procedures between the nucleotide A4tUAG and CUCAG. This caused some difficulty in interpreting the results.

The above sequence can be written in the "cloverleaf" form (fig. 4) and is found to conform to the common features of other tRNAs. The sequence GAA is in the anticodon position. This is the expected anticodon for the two known codons for phenylalanine, UUC and UUU. It is followed by a minor nucleotide that is probably the same as that found in tRNA$^{Tyr}$.

The sequence at the 3′ end (positions 48–76) agrees with that reported by Gassen and Uziel. They do not record the base X and there are several differences in the anticodon loop* and the D loop.

Abbreviations: tRNA$^{Phe}$, phenylalanine accepting transfer RNA; G, U, A, C, T, Ψ, the 3′-phosphates of guanosine, uridine, adenosine, cytidine, ribosylthymine and pseudouridine (5-ribosyluracil) respectively; D, dihydrouridine 3′-phosphate; 7mG, 7-methylguanosine 3′-phosphate; 4-tU, 4-thio-uridine 3′-phosphate; 2mt6iA, probably 2-thiomethyl-6-isopentenyl adenosine 3′-phosphate.

* Uziel and Gassen (Biochemistry, in press) have recently identified an anticodon sequence identical to the one we report.

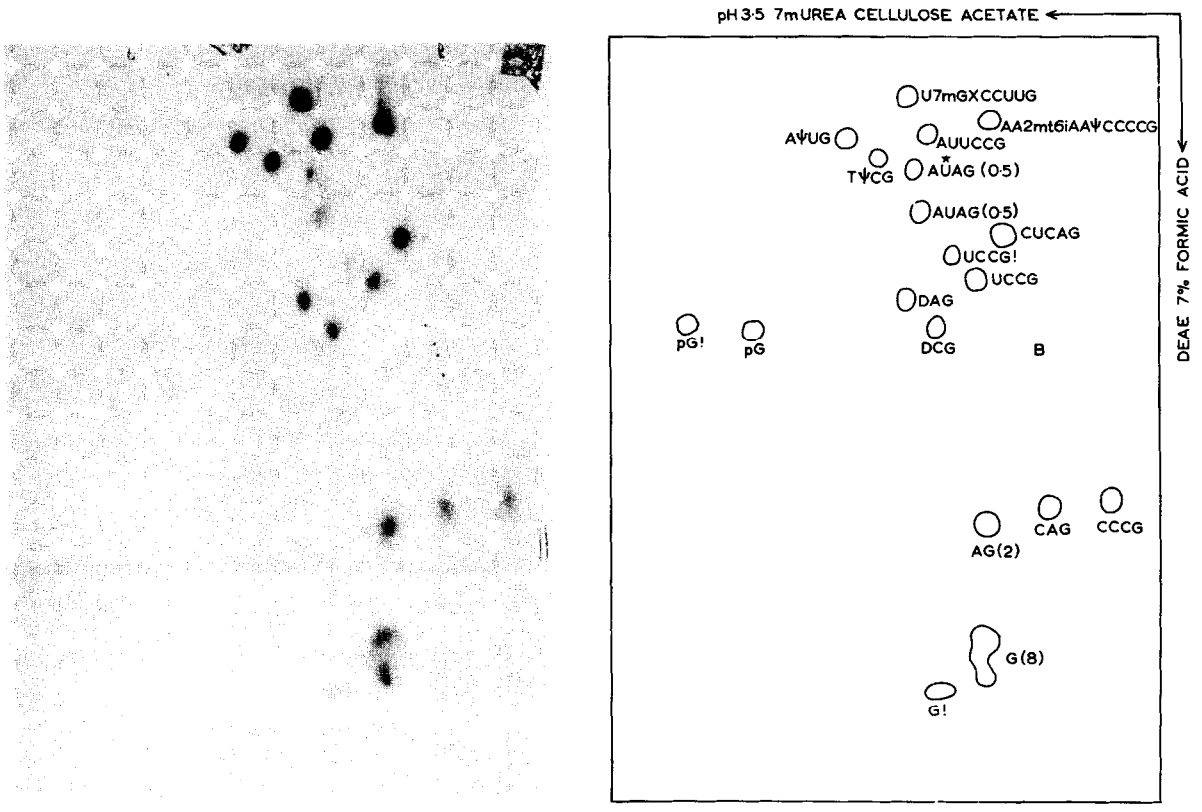


Fig. 1. A two-dimensional fractionation of a ribonuclease T1 digest of $tRNA^{Phe}$. Radioautograph and diagram showing the sequences of the nucleotides. B is the blue marker. $CACCA_{OH}$ is not included in this fingerprint. Some cyclic nucleotides are present and are indicated by "!". Molar yields of nucleotides are given in brackets. Where the nucleotides occur in one molar yield no figures are given. AŮAG is probably A4-tUAG which has been converted to AUAG while the 7% formic acid on the paper was drying. AUAG and the streak immediately above it was probably formed from A4-tUAG when the paper was wetted and electrophoresed in 7% formic acid. The streaking of the G is due to incomplete removal of the urea after electrophoresis in the first dimension.

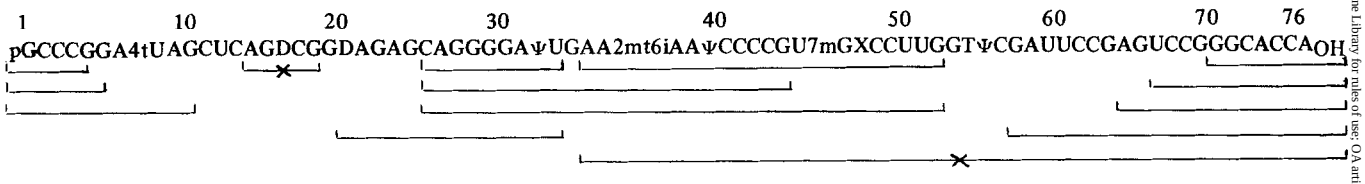


Fig. 2. Nucleotide sequence of $tRNA^{Phe}$ showing the products obtained by limited digestion with ribonuclease T1, pancreatic ribonuclease and *B.subtilis* ribonuclease. (—x—) indicates that the product was only obtained once. All the others have been isolated several times.

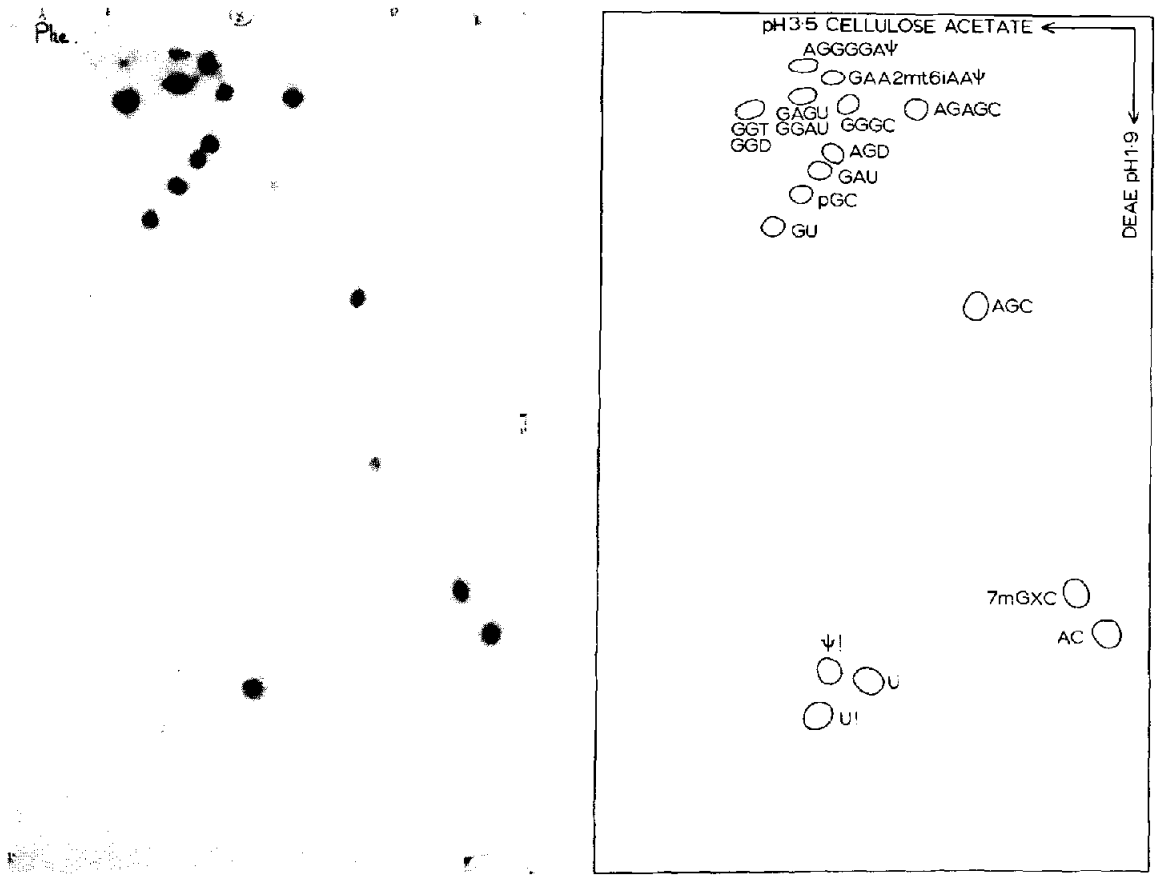


Fig. 3. A two-dimensional fractionation of a pancreatic ribonuclease digest of $tRNA^{Phe}$. Radioautograph and diagram showing the sequences of the nucleotides. C and C> are not included in this fingerprint. AGGGGAΨ is always obtained in low yield and some of the minor spots on the fingerprint are due to breakdown of this nucleotide. GGAU is also obtained in low yield and is formed from GGA4-tU after electrophoresis at acid pH.

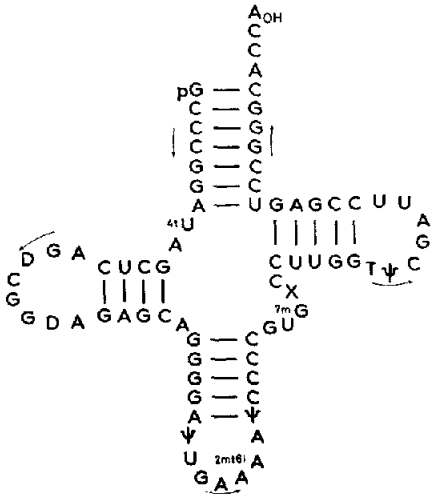


Fig. 4. The nucleotide sequence of $tRNA^{Phe}$ arranged in the "cloverleaf" pattern typical of other tRNAs.

## References

[1] H.G.Gassen and M.Uziel, Abstracts FEBS Madrid (1969) 128.
[2] F.Sanger, G.G.Brownlee and B.G.Barrell, J. Mol. Biol. 13 (1965) 373.
[3] M.Nirenberg and P.Leder, Science 145 (1964) 1399.
[4] A.D.Kelmers, G.D.Novelli and M.P.Stulberg, J. Biol. Chem. 240 (1965) 3979.
[5] S.Cory, K.A.Marcker, S.K.Dube and B.F.C.Clark, Nature 220 (1968) 1039.
[6] F.Harada, H.J.Gross, F.Kimura, S.H.Chang, S.Nishimura and U.L.Rajbhandary, Biochem. Biophys. Res. Commun. 33 (1968) 299.
[7] G.G.Brownlee, F.Sanger and B.G.Barrell, J. Mol. Biol. 34 (1968) 379.
[8] M.Yaniv, B.G.Barrell and A.Favre, to be published.