THE PRIMARY STRUCTURE OF RABBIT LIVER tRNA^{Phe} AND ITS COMPARISON WITH KNOWN tRNA^{Phe} SEQUENCES

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1. Introduction

Since the nucleotide sequences of the phenylalanine transfer ribonucleic acids from yeast [1], *E. coli* [2] and wheat germ [3] have been established, it seemed of interest to determine the sequence of a mammalian tRNA^{Phe} for comparison purposes. Here we report briefly the primary structure of tRNA^{Phe} isolated from rabbit liver, and compare its nucleotide sequence to those of the other phenylalanine specific tRNA's mentioned above. Details of the sequencing work will be published elsewhere.

2. Materials and methods

Phenylalanine specific tRNA from rabbit liver was prepared as described by Petrissant et al. [4], using a three-step purification on benzoylated DEAE-cellulose and A-50 DEAE-Sephadex columns. Stripped crude tRNA from rabbit liver was chromatographed on a first BD-column eluted with a linear gradient from 0.4 M to 1.0 M NaCl (in 0.01 M $MgCl_2$–0.01 M sodium acetate buffer, pH 4.5). At the end of the gradient, elution was continued with the limit buffer containing 20% (v/v) of ethanol. Phenylalanine-tRNA was localized by the acylation test. After ethanol precipitation and chromatography of this material on a DEAE-Sephadex A-50 column, as described by Nishimura et al. [5], a peak of phenylalanine-tRNA was obtained with a specific

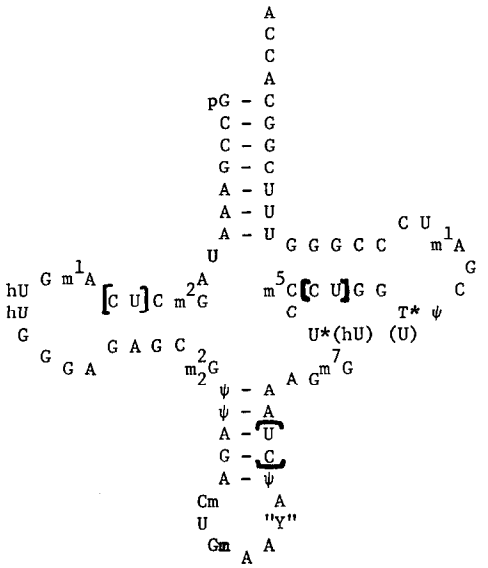

Fig. 1. Cloverleaf model of nucleotide sequence of tRNA^{Phe} from rabbit liver. Parts between square brackets have to be confirmed. For* see text.

activity of 750 pmoles [^{14}C]phenylalanine per A_{260} unit. The pooled fractions containing enriched tRNA^{Phe} were then acylated with phenylalanine (weakly labelled with [^{14}]Phe to permit the localization of the desired tRNA), using a crude yeast synthetase extract and standard aminoacylation conditions [6]. The reaction was stopped with phenol after 30 min at 37°, extracted twice with phenol, precipitated with ethanol, then re-chronatographed on benzoylated DEAE-cellulose with a linear gradient of NaCl

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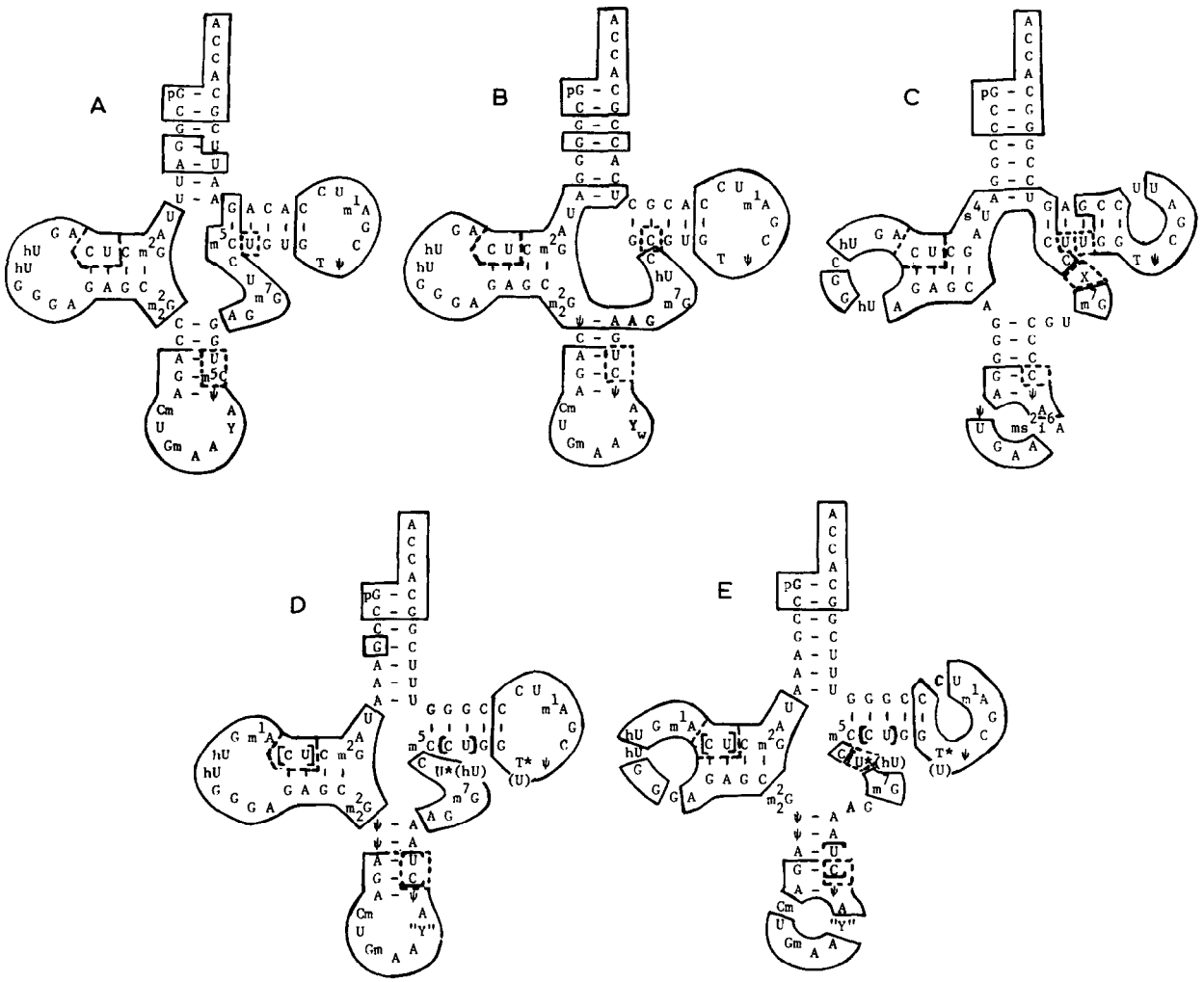

Fig. 2. Comparison of the coverleaf models of the $tRNA^{Phe}$'s of known structure. A) $tRNA^{Phe}$ (yeast): common sequences with $tRNA^{Phe}$ (rabbit liver). B) $tRNA^{Phe}$ (wheat germ): common sequences with $tRNA^{Phe}$ (rabbit liver). C) $tRNA^{Phe}$ (*E. coli*): common sequences with $tRNA^{Phe}$ (rabbit liver). D) $tRNA^{Phe}$ (rabbit liver): common sequences in eukaryotic $tRNA^{Phe}$'s. E) $tRNA^{Phe}$ (rabbit liver): common sequences in both eukaryotic and prokaryotic $tRNA^{Phe}$'s of known structure. Full boxes: common sequences. Dotted boxes: common sequences but have still to be confirmed in rabbit liver $tRNA^{Phe}$. In *E. coli* $tRNA^{Phe}$ nucleoside X could be a uridine derivative (modifications of the nucleosides are not taken into account).

(0.8 M–1 M) and of ethanol (0–15%) in 0.01 M $MgCl_2$, 0.01 M acetate buffer pH 4.2. A single peak of pure phenylalanine $tRNA^{Phe}$ (1600–1700 pmoles [^{14}C]Phe per A_{260} unit) was eluted at a concentration of about 9% ethanol. The subsequent sequence analysis confirmed the homogeneity of the tRNA, since any contaminating oligonucleotides would be found either in the T_1 or pancreatic RNAases digests.

The structural investigations for the determination of the primary sequence and the conditions for complete and partial hydrolysis with either T_1 or pancreatic RNAase were as previously described [7].

3. Results

Fig. 1 shows the nucleotide sequence of rabbit liver $tRNA^{Phe}$ depicted in the cloverleaf form. It contains 76 nucleotides, including several minor nucleosides: m^2G, hU, m^1A, m_2^2G, Ψ, Cm, Gm, "Y", m^7G, m^5C

and T. This tRNA is the first to be found carrying m^1A in the hU loop. The positions U* and T* are incompletely modified; in these cases the alternative nucleosides which were found in minor proportion are indicated in the adjacent parentheses.

The U in position 30 from the 3′ end of the rabbit liver $tRNA^{Phe}$ was found to be replaced by dihydrouridine in a molar ratio of U/hU equal to 6. On the other hand rabbit liver $tRNA^{Phe}$ is characterized by an incomplete modification into ribothymine of the U residue in position 23 from the 3′ end. We found 1/3 U and 2/3 T in our purified tRNA; this is not surprising, since several authors have described crude tRNA preparations with amounts of T less than 1 [8]. More recently, it has been reported from two laboratories that T-Ψ is replaced by A-Ψ in $tRNA_F^{Met}$ from different sources [9, 10].

A fluorescent nucleoside "Y" is also present in rabbit liver $tRNA^{Phe}$. It has an ultra violet absorption spectrum similar to that of Y from yeast but we cannot yet say whether it is identical with either Y, peroxy-Y, or hydroxy-Y, as described by Nakanishi et al. [11] for different mammalian tRNA preparations. "Y", like Y and peroxy-Y in yeast $tRNA^{Phe}$ and wheat germ $tRNA^{Phe}$ respectively, is located adjacent to the 3′ end of the anticodon.

The presence of related fluorescent nucleosides in this position is only one of many similarities between the $tRNA^{Phe}$ species of known structure. Fig. 2 shows the sequences which are common to the $tRNA^{Phe}$'s of known sequence (without taking into account the minor modifications of different nucleosides). It can be seen that striking similarities exist within the class of eukaryotic $tRNA^{Phe}$'s: they are identical in 5 regions and differ significantly in only 3 regions located in the anticodon, GTΨC and CCA stems. The pronounced similarities between corresponding regions of the cloverleaf forms of the eukaryotic $tRNA^{Phe}$'s suggest great similarities in their tertiary structure. This is in good agreement with the fact that the intercharge-ability between eukaryotic enzymes and their corresponding tRNA's is very easy, the crossed systems generally work as well as the homologous ones [12]. In our case, $tRNA^{Phe}$ from rabbit liver is easily aminoacylated with the yeast enzyme [13]. There are much more differences between the eukaryotic $tRNA^{Phe}$'s and $tRNA^{Phe}$ from *E. coli*, the only prokaryotic $tRNA^{Phe}$ of known structure. This can be related with the fact that $tRNA^{Phe}$ from *E. coli* is charged to a much lower extent by the yeast enzyme than the eukaryotic tRNA's [12]. Nevertheless it must be emphasized that there are still large sequence analogies between the eukaryotic $tRNA^{Phe}$'s and the prokaryotic $tRNA^{Phe}$: essentially the whole hU stem, the terminal region of the amino acid stem and a part of the extra loop. It is interesting to point out that the knowledge of the structure of a new sequence of $tRNA^{Phe}$, that of rabbit liver, does not modify the features common to the tRNA's charged by the phenylalanyl-tRNA synthetase from yeast previously described [14, 15].

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