NUCLEOTIDE SEQUENCE OF $tRNA^{Arg}_{II}$ FROM BREWER'S YEAST

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1. Introduction

Three major species of arginine-tRNA are found when brewer's yeast tRNA is fractionated by counter-current distribution with the solvent of Holley [1, 2]. The first one, $tRNA^{Arg}_{I}$, with the lowest solubility in the organic phase, can be trapped in a metastable denatured state and has been purified to an extent of 40% by Lindahl et al. [3]. The third one, $tRNA^{Arg}_{III}$, has the highest solubility in the organic phase. It has been isolated [4] and sequenced [5] in our laboratory. We present here the primary structure of the second one, $tRNA^{Arg}_{II}$, which we have obtained recently with a purification of 80% [6].

2. Experimental

Complete pancreatic and T_1 ribonuclease digests were fractionated either by chromatographic or by electrophoretic methods [7–9]. The separated oligonucleotides were sequenced by techniques published elsewhere [8, 9]. Larger oligonucleotides produced by partial T_1 ribonuclease digestion were isolated by column chromatography [10, 11] and identified on the basis of their complete hydrolysis with T_1 and pancreatic ribonucleases. The overlapping of the larger fragments permitted to establish the linear order of the nucleotides of $tRNA^{Arg}_{II}$ (fig. 1).

3. Results and discussion

The results of the analyses show that $tRNA^{Arg}_{II}$ is composed of 76 nucleotide residues including 13 minor nucleotides. The sequence of $tRNA^{Arg}_{II}$ can be written in the typical planar cloverleaf form suggested by Holley et al. [12] (fig. 3) with an aminoacyl stem seven base pairs long, two five base paired stems for the T–Ψ–C and the anticodon loops and a three base pair stem for the dihydrouracil loop. The T–Ψ–C and anticodon loops contain seven residues as in other known tRNA structures [13]. The hU loop contains 10 nucleotides. $tRNA^{Arg}_{II}$ has a pΨp 5'-terminal end like $tRNA^{Lys}$ from yeast [14] and a G–C–C–A 3' terminal end.

The first nucleotide after the amino acid stem, position 8 from the 5' terminal end, is an U or a s^4U in all sequenced tRNA's [13] with one exception: $tRNA^{His}$ which has also a s^4U in the first position after the amino acid stem but this position is the 9th from the 5' terminal end [15]. The $tRNA^{Arg}_{II}$ has an U in position 8 from the 5' terminal end.

The sequence m^1G–m^2G in positions 9 and 10 has been found previously in $tRNA^{Tyr}$ from *Torulopsis utilis* [16], in $tRNA^{Trp}$ [17] and in $tRNA^{Arg}_{III}$ from yeast [5].

As in 7 other sequenced tRNA's [13] a sequence A–A–hU is found in $tRNA^{Arg}_{II}$ which could take the tertiary structure proposed by Levitt [18] with a base pair between A_{17} and U_{48}.

All sequenced tRNA's concerned with protein biosynthesis have a sequence G–G or Gm–G in positions corresponding to positions 17 and 18 in $tRNA^{Arg}_{II}$ which follows also this general law. This sequence is followed by a hU as in all yeast tRNA's of known structure except $tRNA^{Phe}$ [19] and $tRNA^{Arg}_{III}$ [5].

The $tRNA^{Arg}_{II}$ has a m^2_2G in position 26. The extra arm has a sequence of 5 nucleotides A–G–A–hU–U.

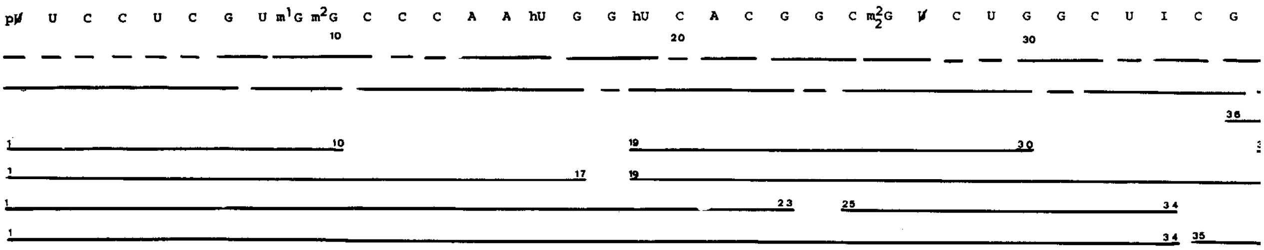


Fig. 1. The two first lines show fragments from complete pancreatic and T₁ ribonuclease digestion. The third line shows t digestion.

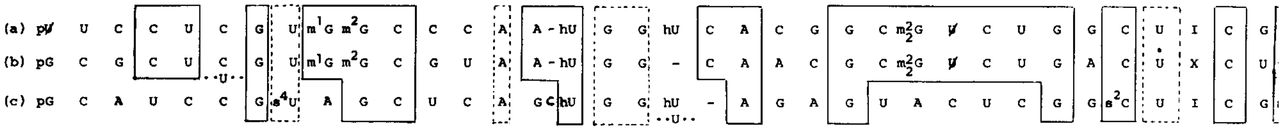


Fig. 2. Comparison of nucleotide sequences in brewer's yeast tRNA$^{Arg}_{II}$ (a), tRNA$^{Arg}_{III}$ (b) and *E. coli* tRNA$^{Arg}_{I}$. The com not taken into account for this comparison.

The same sequence is found in the extra arm of tRNA$^{Arg}_{III}$ from brewer's yeast [5].

The sequence G–T–Ψ–C has been found so far in all sequenced tRNA's concerned with protein biosynthesis [13]. In tRNA$^{Arg}_{II}$ it is followed by an A an a m¹A.

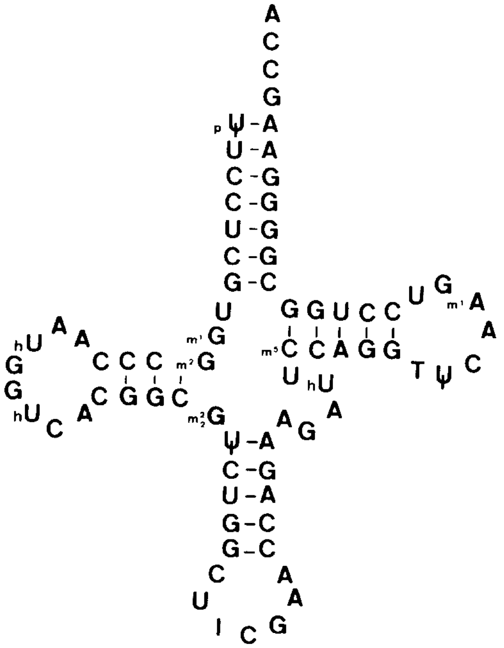


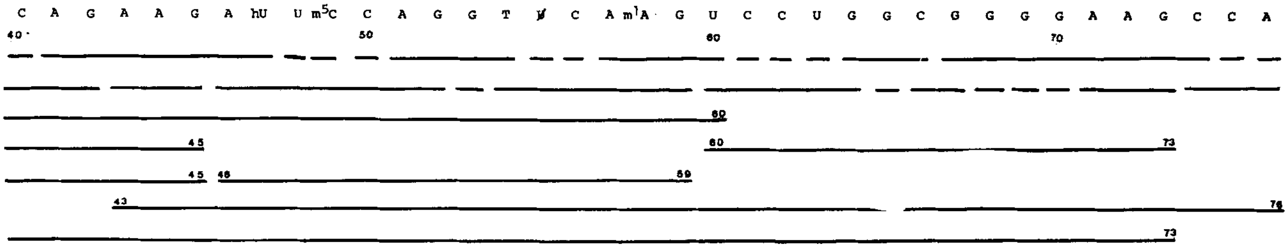
Fig. 3. Clover leaf model of the nucleotide sequence of brewer's yeast tRNA$^{Arg}_{II}$. Standard abbrevations are used for the common nucleosides. Other abbrevations are: ψ, pseudouridine; m¹G, 1-methylguanosine; m²G, 2-methylguanosine; hU, dihydrouridine; m$^2_2$G, 2-dimethylguanosine; I, inosine; m⁵C, 5-methylcytidine; T, ribosylthymine; m¹A, 1-methyladenosine.

The anticodon is I–C–G. This could correspond 1 3 of the 6 codons of arginine C–G–A, C–G–U, C–G–C [20].

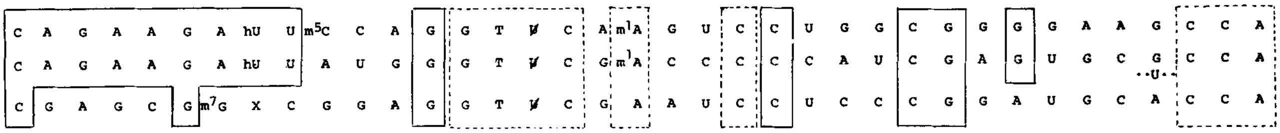
Fig. 2 shows the comparison between the structu of the tRNA^{Arg} II and III of brewer's yeast and of tRNA$^{Arg}_{I}$ from *E. coli* [21].

Between the tRNA's^{Arg} from yeast there are 49 similarities, 35 if we don't take into account the nucleotides common to all tRNA's of known structu concerned with protein synthesis [13]. The sequence 7–11, 24–30 and 40–48 are the longest common ones. They are all localized around the center of the planar clover leaf model. The tRNA's^{Arg} of yeast are recognized by the same aminoacyl-tRNA synthetase. It is possible that these sequences participate in the arginyl-tRNA synthetase recognition site. We are nov in process of studying the sequence of yeast tRNA$^{Ar}_{I}$ (the third substrate for this enzyme) to see if it also contains these common sequences.

There are very few analogies between the tRNA's of yeast and that of *E. coli*. If we don't take into account the features common to all sequenced tRNA [13] there are 17 analogies and the two longest ones are only dinucleotides. It must be emphasized that there is no cross recognition between *E. coli* tRNA^{A} and yeast arginyl tRNA synthetase or vice versa [22,



resulting from the complete digestions. The four last lines represent some of the large fragments obtained after partial T_1 ribonuclease



are enclosed with brackets. Sequences in dotted boxes are common to all tRNA's. The differences in the state of modification are

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