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REINVESTIGATION OF THE PRIMARY STRUCTURE OF BREWER'S YEAST $tRNA_3^{Arg}$

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SUMMARY

The primary structure of brewer's yeast $tRNA_3^{Arg}$ has been reinvestigated using postlabelling techniques. Several errors have been found at the 5' and 3' ends of the molecule. The sequences found previously are replaced by 5' pG-C-U-C(or U)G and U-G-A-G-U-G(or C)C-C-A_{OH}.

INTRODUCTION

Recent work on the restriction enzyme patterns of the yeast $tRNA_3^{Arg}$ gene and on the DNA sequence of the part of the gene corresponding to the 3' end of the $tRNA_3^{Arg}$ (Beckman J.S., personal communication) cast doubt on the RNA sequence previously published for the amino acid stem region of the tRNA (1-3). Therefore, we have reinvestigated the primary structure of this tRNA using postlabelling techniques.

MATERIALS AND METHODS

Brewer's yeast $tRNA_3^{Arg}$ was prepared by a modification of the original procedure (2). The RCP column chromatography was replaced by a Sepharose 4B column at pH 4.5 using a reverse ammonium sulphate gradient (4) from 2 M to 1 M at 20°C.

5' postlabelling of $tRNA_3^{Arg}$ was as follows. Dephosphorylation of $tRNA_3^{Arg}$ (0.5 mg) was done in 50 µl Tris-HCl 50 mM pH 8 containing one unit of calf intestine phosphatase (Boehringer-Mannheim) at 60° for 60 min (5). After phenol extraction and several washings the $tRNA_3^{Arg}$ was 5' labelled with $\gamma|^{32}P|$ ATP and polynucleotide kinase, and purified on 20% polyacrylamide as previously described (6).

To determine the 5' terminal sequence of the 5' end labelled $tRNAs_3^{Arg}$, the tRNA ($2 \cdot 10^5$ Cerenkov CPM) was partially digested in presence of 5-10 µg carrier tRNA in 10 µl dimethylsulfoxyde (DMSO) at 100° for 60 min. The digestion products were separated by homochromatography and the sequence deduced according to the "wandering spot" technique (6, 7).

The 3' terminal end sequence of the $tRNA_3^{Arg}$ was obtained by a different technique. The tRNA (2-5 µg) was partially digested in 10 µl DMSO at 100° for 10 min. After evaporation to dryness the digest was 5' labelled and analyzed by the "wandering spot technique". Each radioactive oligonucleotide from the homochromatography was eluted and digested with P_1 nuclease (6, 7), and the 5' $|^{32}P|$ nucleotides were characterized by thin layer chromatography (HCl, isopropanol, H_2O : 15/70/15 v/v/v).

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RESULTS

The results are shown in Fig. 1. Panel A and B show the 5' and 3' end sequences. It is clear that there are sequence heterogeneities at both ends of $tRNA_3^{Arg}$: since there are two different sets of wandering spots after nucleotide 3 ; nucleotide 4 is thus shown to be either C or U. An analogous situation exists at the other end where the 4th nucleotide from the 3' end is either G or C

Fig. 2 shows the primary structure of brewer's yeast $tRNA_3^{Arg}$. It differs from the previously reported structure (1, 3) in the amino acid stem : two dinucleotides A-G and U-G were inverted in the 3' terminal region and we found the following end sequence U-C-C-C-A instead of the previously found C-U-C-C-A sequence. In the 5' terminal region the oligonucleotides C-G and C-U-C(orU)G were also inverted.

Other studies not shown here, including partial T_1 and S_1 nucleases digestion of $tRNA_3^{Arg}$ and study of the fragments obtained confirm the sequences previously found in all other regions.

Quantitation of the radioactivity in the different spots of the homochromatography gives a ratio of 1:2 for U-G-C-C-A/U-C-C-C-A as well as for pG-C-U-C/pG-C-U-U. This could reflect the existence of two different isoacceptors

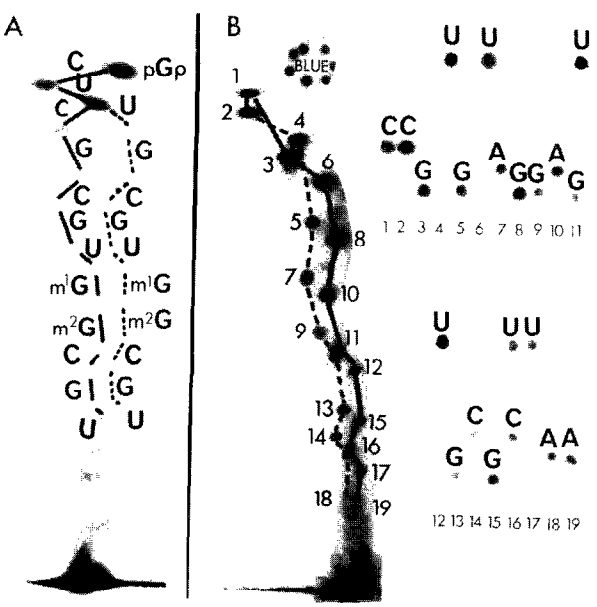

Fig. 1. Autoradiograms of homochromatography of partial digest of $tRNA_3^{Arg}$.
A : 5' end labelled $tRNA_3^{Arg}$ (see text).
B : Partial digest of $tRNA_3^{Arg}$, 5' end labelling of the resulting oligonucleotides and characterisation of the end 5' ^{32}p nucleotides on thin layer chromatography (see text). 3' end C-A is not visible : it is a very poor substrate for polynucleotide kinase labelling. Dotted lines correspond to the sequence occurring in 1/3 of the molecules.

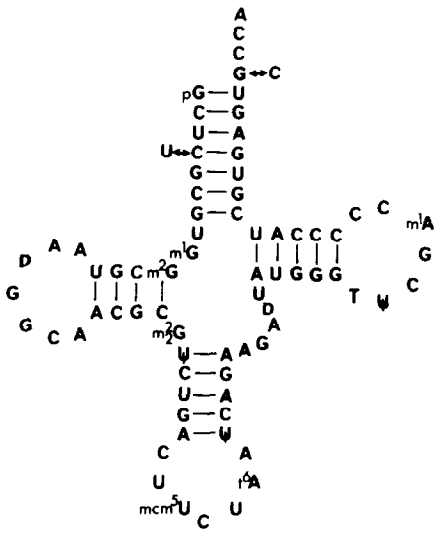

Fig. 2. Cloverleaf model of $tRNA_3^{Arg}$. The heterogeneities occurring in 1/3 of the molecules are written outside the cloverleaf model.

only in a ratio of 1:2, and not 4 isoacceptors as one could suppose from different combinations of the possibilities. To study the heterogeneities and show that there are in fact only 2 isoacceptors it would be necessary to separate them, but none of the purification techniques used are appropriate to achieve such a separation. Revision of the sequence shows that $tRNA_3^{Arg}$ has one less nucleotide in common with yeast $tRNA_2^{Arg}$ (8) and *E.coli* $tRNA_1^{Arg}$ (10). Although yeast $tRNA_3^{Arg}$ has 2 less nucleotides in common with yeast $tRNA^{Lys}$ (9) it still shows 69% homology whereas only 64% homology is found with its yeast isoacceptor $tRNA_2^{Arg}$. Hypothesis concerning the evolution of the two tRNAs from a common ancestor tRNA molecule on the basis of their sequence homologies have been discussed previously (3).

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