The nucleotide sequence of a small (3S) seryl-tRNA (anticodon GCU) from beef heart mitochondria

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ABSTRACT
The primary structure of a 3S serine tRNA from beef heart mitochondria has been determined using our new two-dimensional "read-off" sequencing method (Tanaka, Y., Dyer, T.A. and Brownlee, G.G. (1980) Nucleic Acids Res. 8, 1259-1272). When arranged in the "cloverleaf" form it shows unique features since (i) it completely lacks the dihydrouridine arm, (ii) it has an extended "Ty" loop, but lacks the T and Y residues, and (iii) it has only one minor base, N6(N-threonylcarbamoyl)adenosine, next to the anticodon.

INTRODUCTION
Tanaka et al. [1] recently reported a new "read-off" two-dimensional RNA sequencing procedure based on the original method described by Stanley and Vassilenko [2] which is particularly suitable for the analysis of low molecular weight RNA. The method uses a heat catalysed degradation of RNA under controlled conditions producing on average one break per molecule. The resultant spectrum of released fragments are labelled at their 5' hydroxyl ends with $^{32}$p using T₄ polynucleotide kinase, and then fractionated in the first dimension according to size using polyacrylamide gel electrophoresis. After transfer to DEAE-cellulose thin-layers the 5' end-labelled mononucleotide is liberated as pNP by T₂ RNase digestion in situ. Finally fractionation is carried out in the second dimension using electrophoresis at pH 2.3 to separate the mononucleoside 3',5'-diphosphates. Previously the method [1] was developed using E. coli 5S RNA as a model and was applied to the sequence analysis of 5.8S ribosomal RNA from Vicia faba using 1-2 microgram quantities. Others [3-5] have also applied it or closely related methods [6] to tRNA successfully. Here we demonstrate the sensitivity of the method by applying it to submicrogram quantities (approximately 0.1 µg) of an unusual 3S serine tRNA from beef heart mitochondria although we found it necessary to modify the conditions of heat degradation of the RNA.
MATERIALS AND METHODS

The mitochondrial 3S RNA was purified as follows. Beef heart mitochondria, prepared according to Smith [7], were homogenized in 100 mM NaCl, 10 mM EDTA, 50 mM Tris-HCl pH 7.5, 0.2% SDS and pre-incubated with 0.5 mg/ml of proteinase K (chromatographically purified, BDH) at 37°C for 30 min. After phenol extraction, tRNA was purified by chromatography on DEAE-cellulose as described by Silbert et al. [8]. Mitochondrial tRNA<sub>Ser</sub> (3S) was then isolated by two-dimensional thin gel electrophoresis at pH 8.3 [9] (first dimension, 10% acrylamide 4 M urea, run under hot conditions, 60°C approx., 1500 V, 25 mA, 5-6 h; second dimension, 20% acrylamide 4M urea, 500 V, 5 mA, 40 h) using one A<sub>260</sub> unit of RNA per gel. Gels were stained in toluidine blue (0.01%) for 15 min and destained in distilled water for 30 min. The 3S RNA was then eluted from the excised gel in 0.3 ml of 0.5 M NaCl, 0.01 M EDTA, 0.1 M Tris-HCl pH 7.5 at 37°C for 4 h without crushing. The RNA was then precipitated twice by the addition of two volumes of ethanol. Samples were finally dissolved in 1-5 μl of deionized water.

Sequencing was according to Tanaka et al. [1] (detailed protocol available from G.G.B.) except that in the first step 0.1 to 0.2 μg of RNA in 1 μl deionized water was heated for 5 sec at 100°C in a sealed siliconized microcapillary. This was added to 1 μl of 2x phosphokinase buffer containing 10 μCi <sup>32</sup>P-ATP (Amersham, 3000 Ci/mMole) and 10% dimethylsulphoxide followed by 0.2 μl T<sub>4</sub> polynucleotide kinase as before [1]. After 30 min reincubation in a sealed microcapillary at 37°C 2 μl of dye mixture was added as before and 2 μl of solution used for each of the two loadings on a 12% acrylamide gel. See Brownlee [10] and ref 1 for other sequencing procedures. For minor base analyses ascending chromatography was used on cellulose T.L.C. plates (Merck, 20 x 20 cm without indicator) using solvent A, propan-2-ol/HCl/H<sub>2</sub>O (70:15:15, v/v). t<sub>6</sub>Ap was converted to <sup>32</sup>P-pto<sub>6</sub>A by T<sub>4</sub> phosphokinase labelling followed by P<sub>1</sub> nuclease digestion and purification by paper electrophoresis at pH 3.5. <sup>32</sup>P-pto<sub>6</sub>A was converted to <sup>32</sup>P-pA according to ref 11.

RESULTS

(1) Sequence of beef heart mitochondrial tRNA<sub>Ser</sub>

Figure 1 shows an autoradiogram of the two-dimensional sequencing procedure applied to the 3S mitochondrial tRNA<sub>Ser</sub>. Despite a certain degree of over-degradation, it was possible to read the sequence from residue 2 to residue 55. Some difficulty was present in the length of the "run" between
Figure 1. Two-dimensional sequencing of beef heart mitochondrial tRNA$_{\text{Ser}}$

First dimension: (top to bottom) 12% acrylamide 7M urea gel electrophoresis for either 8 h (left) or 5 h (right) on a 60 cm gel at 1800V. Second dimension (left to right): DEAE-cellulose TL electrophoresis at pH 2.3.
A2-6 and C34-36, but this was resolved by aligning the ladder of the first dimension with the two-dimensional pattern. Another problem was the length of the G "run" at residue G51. The sequencing gel (Fig. 1) suggested an extra G residue (viz: a U-G-G-G-C sequence). The origin of this ambiguity was not clear but was resolved by classical two-dimensional fingerprint analysis of the pancreatic ribonuclease digest of the in vitro 32P-labelled seryl-tRNA compared to that of E. coli tRNA^Phe [12,13]. This clearly showed the presence of G-G-C rather than G-G-G-C in seryl-tRNA. The sequencing method fails to give the immediate 3' terminal residues so that wandering spot analysis of 32P-labelled tRNA was used to establish the sequence of residues 51-63 (see Fig. 2 and Methods). Spot a (Fig. 1) ran slightly slower than G on the sequencing gel suggesting the presence of a minor base. This was identified as t^6A (N-threonylcarbamoyl A) by comparison of P1-nuclease digested spot a (to convert it to pX) with an authentic sample of 32P-labelled-p^6A on chromatography in solvent A (see methods) and DEAE-cellulose TL electrophoresis at pH 3.5. To exclude the possibility of any other minor bases co-electrophoresing with any of the four major nucleotides in the sequencing system (Fig. 1), all spots were separately eluted, treated with P1-nuclease and analysed by cellulose thin-layer chromatography in solvent A.

DISCUSSION

Figure 2 shows the seryl tRNA arranged in the traditional "cloverleaf" form. It has both features in common and features different from the generalized tRNA structure [14]. Thus the features in common are U8, U19, the terminal C-C-A0OH and the number of residues in the amino-acid stem (seven base pairs) and in the anticodon loop (seven nucleotides). The differences are: (i) the usual sequence T-Ψ-C-G(A) in loop III (corresponding to loop IV in other tRNAs) is replaced by the sequence U-C-U-A(A).

This loop shows an unusual eight nucleotides (instead of seven) with two purine residues (A46 and G47) in place of the single semi-invariant pyrimidine; (ii) the stem of the third arm is substituted by an A-U base pair instead of a G-C base pair in the generalized tRNA structure; (iii) the anticodon stem shows four base pairs instead of five. However, a non Watson-Crick interaction between residues A13-A29 would extend it to five.

Some of these structural features observed here for the seryl-tRNA are common also to several other mitochondrial tRNAs. For example, the sequence
Figure 2. Sequence of beef heart mitochondrial seryl-tRNA written in "cloverleaf" form. Invariant and semi-invariant bases are respectively circled or in parentheses [14]. The novel features are represented by squares (see text).

T-Ψ is absent in N. crassa trNA_{Met} [15] and in tRNA_{Thr} [16]. An anticodon stem with four standard base pairs is also present in N. crassa tRNA_{Val} and in tRNA_{Trp} [16], and like other mitochondrial tRNAs the seryl-tRNA has a low number of minor bases.

We can speculate about the possible three-dimensional structure of the 3S RNA by attempting to "fit" it to the established structure of yeast tRNA_{Phe} [17]. Clearly the third arm and the amino acid stem could form a continuous helix as it does in tRNA_{Phe}. We note a possible Watson-Crick base pairing between A_{46}-U_{45}-A_{44} and U_{8}-A_{9}-U_{10}. Another possible interaction is that between residues G_{11}-C_{12} and C_{32}-G_{31}. If these interactions occur, the molecule could assume an "L" shape as occurs in tRNA_{Phe}, although only a detailed structural analysis could prove this.

Finally, the sequence reported here completely agrees with the indepen-
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dently determined DNA sequence (M.H.L. de Bruijn, accompanying manuscript) except that the C-C-A is a post-transcriptional modification. There only remains the minor discrepancy that 5-methylcytosine was detected in half molar yields although we failed to locate it in this report. Perhaps the different tissue sources (heart versus liver) could explain this discrepancy.

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REFERENCES