THE SEQUENCE OF MITOCHONDRIAL ARGININE tRNA (ANTICODON UCG) FROM A TRANSPLANTABLE RAT TUMOR, MORRIS HEPATOMA 5123D

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1. Introduction

Mammalian mitochondria contain a distinct genome of closed-circular duplex DNA of about 16.5 kb [1,2] coding for 12 S and 16 S rRNAs, 22–23 tRNAs, and a number of mitochondrial (mt) proteins [2–4]. Mt tDNA codes for unusually short mature tRNAs and apparently lacks information for leader, trailer, and intervening sequences [5]. Most mammalian mt tRNA sequences have been inferred by DNA sequencing while until recently only mt serine tRNAs (anticodon GCU) from beef heart [6], beef liver [7], and hamster BHK-21 cells [8] have been sequenced directly. As part of a project to elucidate the structural basis for differences in base composition between rat liver and hepatoma mt tRNA [9], we have recently sequenced several tRNAs from the mitochondria of a transplantable rat tumor, Morris hepatoma 5123D, including $tRNA^{Leu}_{UAG}$ [10], $tRNA^{Asp}_{GUC}$ [11], $tRNA^{Val}_{UAC}$ [12], $tRNA^{Phe}_{GAA}$ and $tRNA^{Trp}_{U*CA}$ (unpublished). Here we report the sequence of mt $tRNA^{Arg}_{UCG}$ from this tumor.

The hepatoma mt $tRNA^{Arg}$ exhibits about 86% sequence homology with the DNA-derived putative sequence of human placenta mt $tRNA^{Arg}$ [2]. Differences, including transitions and transversions, were found in acceptor stem and 'D-stem' close to the center of the cloverleaf structure and in loops I, III and IV.

The sensitive thin-layer readout procedure for RNA sequence analysis of Gupta and Randerath [13,14] enables one to identify and locate about 20 modified nucleotides in small amounts of tRNA (~0.5 μg). Information on modified nucleotides in mammalian mt tRNA can be obtained only by direct RNA sequencing. The hepatoma arginine tRNA was found 2 ψ residues (positions 29 and 36) and m^1A in position 9. The unusual occurrence of m^1A at this site has been found in all six hepatoma mt tRNAs sequenced by us.

2. Experimental

Morris hepatoma 5123D [15], kindly provided by Dr H. P. Morris, was from transplant generation 161. Materials for sequence analysis have been described previously [13,14]. To isolate $tRNA^{Arg}_{UCG}$, total nucleic acids were extracted in the presence of phenol from mt pellets [9,16] and fractionated on DEAE-cellulose to obtain crude mt tRNA. The tRNA was purified on three successive polyacrylamide gels ((i) a stacked 6%/15% gel at 4°C; (ii) an 18%, 3.5 M urea gel at 4°C; and (iii) a 20%, 7 M urea gel at 40°C; all gels were run at pH 8.3). Details of the procedure will be published elsewhere [12]. $tRNA^{Arg}_{UCG}$ was extracted from the third gel after staining with methylene blue [17] and its sequence determined by a PEI-cellulose thin-layer readout technique [13,14]. ^{32}P-labelled RNA fragments were resolved on 8%, 12%, and 20% gels and their termini analyzed in two complementary systems [13,14].

3. Results

On the first gel, mt tRNA was resolved into 10 bands, band No. 2 containing $tRNA^{Arg}_{UCG}$ (No. 1 being the fastest band). On the second gel, band No. 2 was separated into two major and two minor bands. The slower of the major bands was resolved by the third gel into one strong band and one minor band. The strong band contained pure mt $tRNA^{Arg}_{UCG}$ as shown by sequence analysis.

The sequence of hepatoma mt $tRNA^{Arg}_{UCG}$ was

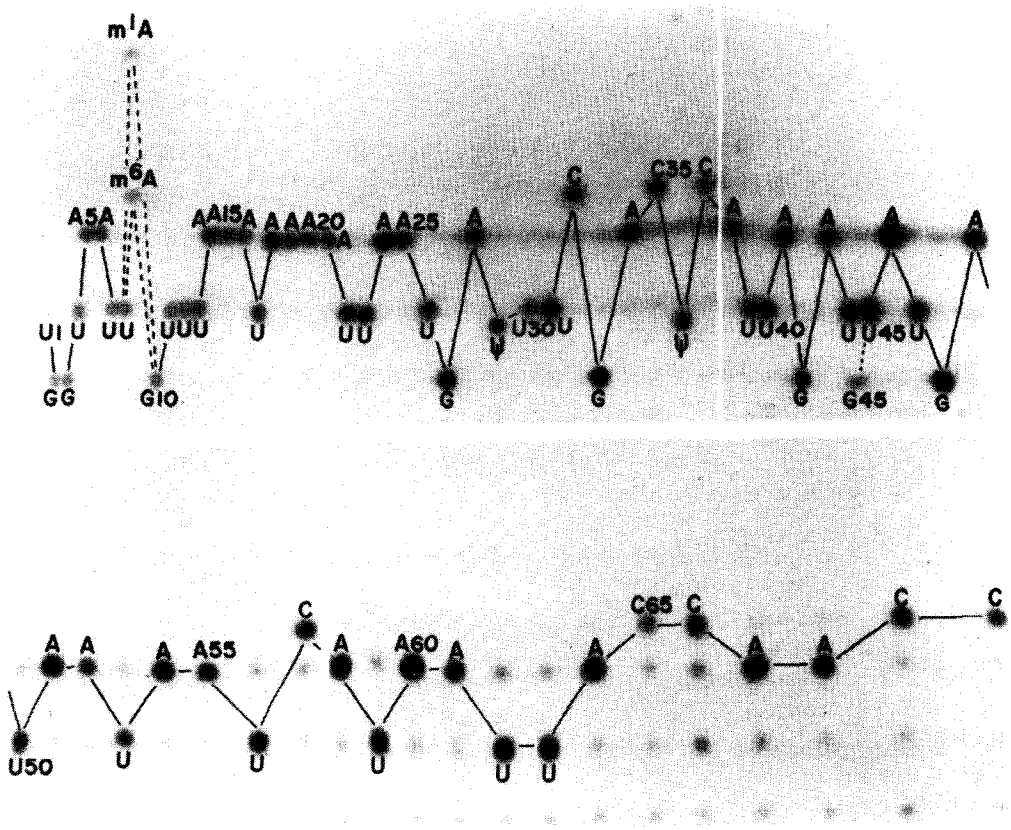
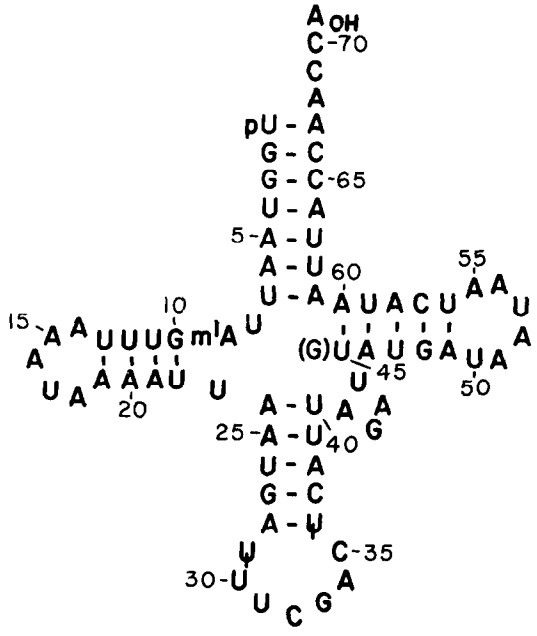


Fig.1. PEI-cellulose thin-layer chromatography in ammonium formate, pH 3.5 [13], of 5′-^{32}P-labelled nucleotides derived from single-hit cleavages of hepatoma mt $tRNA^{Arg}_{UCG}$.



deduced by a thin-layer readout method [13,14] based on the single-hit degradation principle [18]. ^{32}P-labelled 5′-terminal nucleotides were identified by PEI-cellulose thin-layer chromatography in two systems, ammonium formate, pH 3.5, and ammonium sulfate [13,14]. Fig.1 shows the readout in ammonium formate encompassing positions 1–70 of the tRNA. Single termini were obtained for all positions (fig.1) except positions 9 and 45 which gave m^1A + m^6A and U + G, respectively. The presence of two nucleotides in position 45 was confirmed by repeated electrophoretic and chromatographic analyses under various conditions (see above). m^6A always appears in positions of m^1A due to migration of the methyl group during the experimental manipulations [17]. The same sequence was found by chromatographing the terminal nucleotides in the sulfate solvent and

Fig.2. The sequence of Morris hepatoma 5123D mt $tRNA^{Arg}_{UCG}$ arranged in the cloverleaf form.

varying the gel-electrophoretic conditions. Due to the high A and U content of the RNA and the lack of G · C base pair clusters, no compression effects occurred on the gels. This has also been observed for the other mt tRNAs sequenced by us. The linear sequence deduced from the readout (fig.1) can be arranged in a cloverleaf structure as shown in fig.2. The RNA has been designated as $tRNA^{Arg}$ on the basis of the anticodon UCG and the codon recognition pattern of mammalian mt tRNAs [2].

4. Discussion

When compared with eukaryotic cytoplasmic and prokaryotic tRNAs [19], the hepatoma mt $tRNA^{Arg}_{UCG}$ exhibits many unusual features, such as the predominance of U (36.7%) and A (42.3%) over C (11.3%) and G (9.9%), the presence of m^1A in position 9, lack of –GG– in loop I, lack of –UUCPu– (–TψCPu–) in loop IV, small size of these loops, and lack of the constant G · C base pair adjacent to loop IV. It is not surprising therefore that the mt $tRNA^{Arg}$ bears little resemblance to other sequenced arginine tRNAs [19], including those from *Drosophila* [20] and mouse [21]. Except for the predominance of A and U residues, the hepatoma mt $tRNA^{Arg}$ also does not resemble yeast mt $tRNA^{Arg}_{ACG}$ (predicted from the DNA sequence [22]). A comparison of the hepatoma RNA sequence with the putative DNA-derived sequence of human placenta mt $tRNA^{Arg}_{UCG}$ reveals the following differences: Positions A6, U7, U17, U22, U23, G42, and A61 (fig.2) are U, A, C, C, G, A, and U, respectively, in the human tRNA and loop IV in the hepatoma tRNA contains six nucleotides (–UAAUAA–) versus three nucleotides (–UAA–) in the human tRNA. Whether some of these differences are tumor-specific needs to be explored.

The hepatoma tRNA has three modified nucleosides, one of which (m^1A9) occurs at an unusual site, while the other two (ψ29 and ψ36) have been found at these locations in many other tRNAs [19]. Whether the biosynthesis of m^1A9 in the mt tRNA requires a mitochondria-specific tRNA (adenine-1)methyltransferase distinct from the nuclear enzyme [23] is not known.

The presence of an unmodified U in the wobble position of the anticodon, very rarely found in non-mitochondrial tRNAs [19], is expected for a mt tRNA recognizing the four codons of the GCN codon family [2,24–26].

The presence of more than one nucleoside at certain sites of the sequence has been found by us also for several other mt tRNAs from Morris hepatoma 5123D (refs. 10 and 12 and unpublished experiments). The question whether this indicates tumor-specific mutations in mt DNA deserves further investigation.

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