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TUMOR MITOCHONDRIAL TRANSFER RNAS: THE NUCLEOTIDE SEQUENCE OF MITOCHONDRIAL $tRNA^{Leu}_{UAG}$ FROM MORRIS HEPATOMA 5123D

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SUMMARY. A leucine tRNA (anticodon UAG) was isolated from the mitochondria of a transplantable rat tumor, Morris hepatoma 5123D, and sequenced. The sequence, pACUUU/GUAUm^{1}Am^{2}GGAUAGAAGDAAUCCAΨUGGUCUUAGm^{1}GAACCAAAAACm^{5}CUUGGUGCAACUCCAAAUAAAAGUACCA$_{OH}$, can be arranged in cloverleaf form. It exhibits several unusual features, such as the absence of the constant GG sequence in loop I, the presence of a G11·C23 base pair in the stem of this loop, the presence of UGC instead of the constant TΨC (UUC) in loop IV, a short variable arm, predominance of A·U base pairs, and the presence of m^{1}A in position 9. The other modified nucleosides (N^2-methylguanosine, dihydrouridine, pseudouridine, 1-methylguanosine, and 5-methylcytidine) occupy expected positions. In position 5, 2 nucleosides (uridine + guanosine) were found, possibly signifying a tumor-specific point mutation in mitochondrial DNA.

The fascinating subject of morphological and functional alterations of tumor mitochondria has been reviewed recently by Pedersen (1). During the past year, additional evidence has been adduced for the high susceptibility of mitochondrial (mt) DNA to chemical carcinogens (2-4), a phenomenon first reported by Wunderlich et al. (5). This and numerous other observations (1) suggest that alterations of mitochondria may play a pivotal role in the induction and maintenance of the neoplastic state. It is of interest therefore to search for structural alterations in neoplastic cells of mt DNA, mt RNAs, and proteins coded for by mt DNA. Because we had previously observed base composition differences between mt tRNA of rat liver and 2 transplantable rat tumors, Morris hepatomas 5123D and 7777 (6), we have recently initiated a project aimed at comparing sequences of mt tRNAs from rat liver and hepatomas. In this article, we report the sequence of mt $tRNA^{Leu}_{UAG}$ from Morris hepatoma 5123D.

MATERIALS AND METHODS. Morris hepatoma 5123D (7), kindly provided by Dr. H.P. Morris, was from transplant generation 161. Materials used for sequence analysis have been described previously (8,9). To isolate $tRNA^{Leu}_{UAG}$, total nucleic acids were extracted in the presence of phenol from mt pellets (6,10) and fractionated on DEAE-cellulose to obtain crude mt tRNA. Mt $tRNA^{Leu}_{UAG}$ was isolated from the latter by electrophoresis on 3 successive polyacrylamide gels ((i) a 6%/15% stacked 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; acrylamide : methylene bis(acrylamide),

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30 : 1; all gels were run at pH 8.3). The RNA was extracted from methylene blue-stained gels (11). Details of the procedure will be published elsewhere (12). The sequence of the tRNA was determined by a polyethyleneimine(PEI)-cellulose thin-layer readout procedure (8), with minor modifications (9,13).

RESULTS. Crude mt tRNA was resolved on the first gel into 10 bands; mt $tRNA^{Leu}_{UAG}$ travelled with band #6 (#1 being the fastest band). Band #6 gave one major fraction on the second gel migrating between 2 minor fractions. On the third gel, the major band from the second gel was found to be a mixture of mt $tRNA^{Phe}_{GAA}$ and mt $tRNA^{Leu}_{UAG}$, with $tRNA^{Phe}_{GAA}$ migrating ahead of $tRNA^{Leu}_{UAG}$. About 2.5 µg of mt $tRNA^{Leu}_{UAG}$ was obtained from 100 g of hepatoma tissue.

The sequence of mt $tRNA^{Leu}_{UAG}$ was deduced by a thin-layer readout method (8), as shown in Fig. 1. This readout was derived from about 1 µg of tRNA. The RNA was partially digested by a single-hit cleavage technique originally described by Stanley and Vassilenko (14), as modified by Gupta and Randerath (8,9), followed by ^{32}P-labeling, gel electrophoresis, contact transfer, and *in situ* ribonuclease T_2 digestion of the fragments (8,9). ^{32}P-labeled 5'-terminal nucleotides were identified by PEI-cellulose thin-layer chromatography in 2 solvents, 1.2 M ammonium formate, pH 3.5, (Fig. 1A) and 0.55 M ammonium sulfate (Fig. 1B), as described previously (8), except that development in the formate solvent was performed under conditions of tank saturation on PEI-cellulose sheets made in the laboratory (15). For the sulfate system, commercial sheets were used as described (8). Fig. 1 shows readouts of positions 1 - 47 in formate (A) and positions 30 - 73 in sulfate (B). Note the complete agreement for the overlapping positions (30 - 47) in the 2 solvents which was also found for the remaining positions of the sequence (not shown). Distinct single termini were obtained for all positions, except position 5 which gave a mixture of U and G, and position 9 which gave m^1A and m^6A. The presence of 2 nucleotides in position 5 was confirmed by repeated analyses in both chromatographic systems (not shown). Some m^6A always appears in positions of m^1A due to migration of the methyl group during the experimental manipulations (11). The linear sequence deduced from the readouts (Fig. 1) can be arranged in a cloverleaf structure as shown in Fig. 2. The RNA has been designated as $tRNA^{Leu}$ on the basis of the anticodon triplet UAG and the codon recognition pattern of mammalian mt tRNAs, as discussed by Barrell et al. (16).

DISCUSSION. A comparison of the hepatoma $tRNA^{Leu}$ with the prototype of prokaryotic and eukaryotic cytoplasmic tRNAs (17) indicates that the hepatoma tRNA exhibits both normal ("invariant") and unusual features. Among the latter, the following appear noteworthy: (i) Loop I has -AG- in place of the invariant -GG- sequence and has only a single pyrimidine (D19); (ii) the stem of this loop has a G11·C23 base pair instead of the usual Pyl1·Pu23 pair; (iii) loop

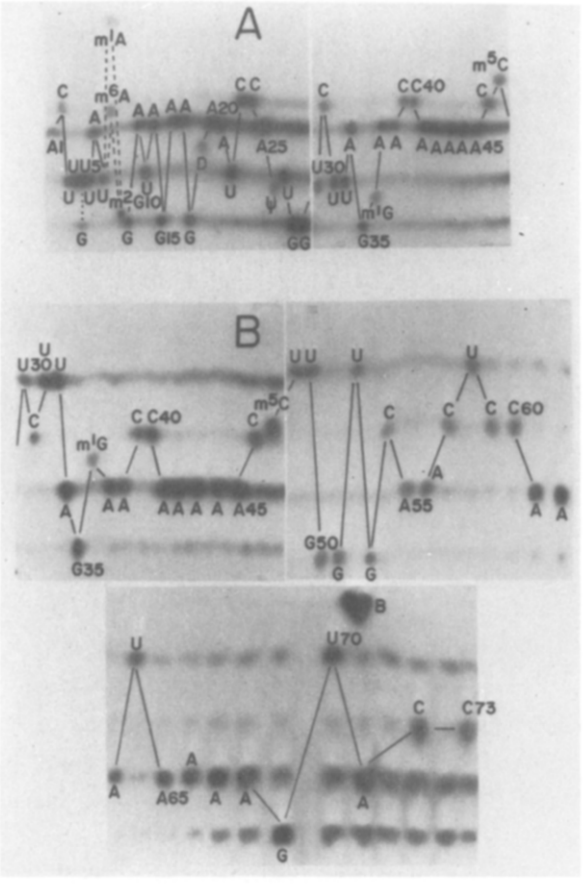

Fig. 1. PEI-cellulose thin-layer chromatography (8) of 5'-^{32}P-labeled nucleotides derived from single-hit cleavages of hepatoma mt tRNA$^{Leu}_{UAG}$. A, development in 1.2 M ammonium formate buffer, pH 3.5; B, development in unbuffered 0.55 M ammonium sulfate. The spot marked B in panel B is a background compound originating from the particular batch of [γ-^{32}P]ATP used.

IV has -UGCA- rather than the normal -UUCPu- (-TΨCPu-) sequence; (iv) the stems are rich in A·U pairs; (v) in contrast to all other known leucine tRNAs, the extra arm is short; and (vi) position 9 is m^{1}A, a nucleoside normally found only in loops I and IV (17). In general, the hepatoma mt tRNA$^{Leu}_{UAG}$ bears little resemblance to the 16 other known leucine tRNAs (17), including cytoplasmic tRNA$^{Leu}_{MmAA}$ from the same tumor (11) and mouse L-cell mt tRNA$^{Leu}_{UAA}$ whose structure has been inferred from DNA sequence analysis (18).

It is noteworthy that the only other known tRNAs active in protein synthesis having a -UGC- sequence in loop IV (N. crassa initiator mt tRNA^{Met} (19) and N. crassa mt tRNA^{Thr} (20)) also have -AGD- in loop I. Heckman et al.

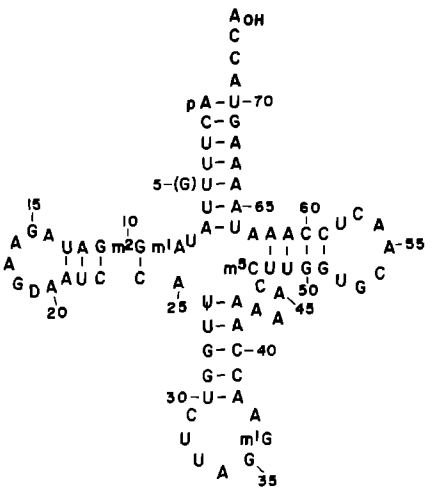

Fig. 2. The sequence of Morris hepatoma 5123D mt $tRNA^{Leu}_{UAG}$ arranged in clover-leaf form.

(19) have speculated that loops I and IV of N. crassa initiator mt $tRNA^{Met}$ are bound by tertiary interactions between these sequences, i.e., $\begin{matrix}-UGC- \text{ (loop IV)} \\ -AGD- \text{ (loop I)}\end{matrix}$, in analogy to the tertiary base pairs detected by X-ray crystallography in yeast $tRNA^{Phe}$, i.e., $\begin{matrix}-T\Psi C- \text{ (loop IV)} \\ -GGG- \text{ (loop I)}\end{matrix}$ (21). Our observation that the presence of A rather than G in position 17 of mt $tRNA^{Leu}_{UAG}$ is accompanied by the substitution of G53 for the usual U (Ψ) would be consistent with this speculation, suggesting specific tertiary interactions between loops I and IV in hepatoma mt $tRNA^{Leu}_{UAG}$. Thus, this RNA may conceivably be capable of assuming an L-shaped structure resembling that of yeast $tRNA^{Phe}$ (21).

The presence of m^1A in position 9 in the sequence $-Um^1A(m^2)G-$ has been observed also in 5 other hepatoma mt tRNAs sequenced by us, i.e., $tRNA^{Val}_{UAC}$ (12), $tRNA^{Asp}_{GUC}$, $tRNA^{Phe}_{GAA}$, $tRNA^{Arg}_{UCG}$, and $tRNA^{Trp}_{UCA}$ (unpublished), and thus appears to be a specific feature of these (and perhaps other) mammalian mt tRNAs. This positively charged nucleoside may conceivably participate in tRNA - synthetase binding as it is present in a region of the tRNA known to interact with synthetase (22). The 5 other modified nucleosides occur in normal positions. In view of the unusual sequence of the RNA, this suggests that the modifying enzymes primarily recognize secondary/tertiary structure elements of the RNA rather than specific nucleotide sequences.

The presence of an unmodified U in the wobble position of the anticodon, very rarely found in other tRNAs (17), is expected for a mt tRNA recognizing the 4 codons of the CUN codon family (16,20,23,24).

As observed for other mammalian mt tRNAs (12,25), the sequence of mt $tRNA^{Leu}_{UAG}$ offers the possibility of alternative base pairings, e.g., positions 2 - 8 with positions 12 - 18, positions 38 - 42 with positions 48 - 52, and positions 49 - 54 with positions 69 - 74 (the latter blocking the CCA-end). Whether such alternative base pairing occurs in vivo (thus having functional significance) needs further investigation.

The existence of more than 1 nucleotide at a certain site of the sequence (position 5, see Figs. 1 and 2) has been found by us in sequencing work on several other mt tRNAs from Morris hepatoma 5123D (ref. (12) and unpublished results). We are currently investigating whether this somewhat surprising phenomenon reflects tumor-specific mutations in mt DNA.

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