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RAT LIVER MITOCHONDRIAL LYSINE tRNA (ANTICODON U*UU) CONTAINS A RUDIMENTARY D-ARM AND 2 HYPERMODIFIED NUCLEOTIDES IN ITS ANTICODON LOOP

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SUMMARY. A lysine tRNA (anticodon U*UU) was isolated from rat liver mitochondria and sequenced. The sequence, pCAUUGCGAm^{1}Am^{2}GCUUAGAGCm^{2}GΨUAACCUU*UU-t^{6}AAGUUAAAGUUAGAGACAACAAAUCUCCACAAUGACCA$_{OH}$, can be written in cloverleaf form. It exhibits many unorthodox features, perhaps the most striking of which is the small size of the D-arm consisting of only 9 nucleotides. The anticodon loop contains 2 hypermodified nucleotides, U*27 (probably 5-methoxycarbonylmethyluridine) and t^{6}A30 (N-[N-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl] threonine). The presence of U* in the first ("wobble") position of the anticodon probably prevents the lysine tRNA from reading asparagine (AAY) codons. t^{6}A, which is 3'-adjacent to the anticodon in most tRNAs recognizing codons starting with A, and other modified nucleosides occupy expected positions. We hypothesize that enzymes modifying the wobble position and the position 3'-adjacent to the anticodon recognize specific nucleotides in the anticodon.

The mammalian mitochondrial genetic system exhibits remarkable simplicity and economy in its decoding mechanism, gene organization, and gene expression (1,2). The complete sequence of the human mitochondrial (mt) DNA was reported recently (3). This DNA encodes the sequences of 22 mt tRNAs, which are capable of reading all codons. This is accomplished by "2-out-of-3" interaction or U·N wobble (4-6), i.e., mt tRNAs having an unmodified U residue in the first position of the anticodon read all codons of a 4-codon family. Sequence studies on Neurospora crassa mt tRNAs, in addition, have suggested the presence of a unique modified U residue (4), probably 5-methoxycarbonylmethyluridine (Martin, R.P., Sibler, A.-P., Bordonné, R., Dirheimer, G., and Agris, P.F., personal communication), in the wobble position of those mt tRNAs that are specific for the bottom 2 codons of the nonfamily boxes (7). It was of interest to determine whether a mammalian mt tRNA belonging to this class would also contain a modified U residue in the wobble position of the anticodon. As reported in this paper, sequence analysis of a lysine (codons, AAA and AAG) tRNA from rat liver mitochondria shows this indeed to be the case. Chromatographic evidence indicated the modified uridine derivative to be identical or closely related to U* found in yeast mt tRNA^{Trp} (8).

MATERIALS AND METHODS. Mitochondria were prepared from female Buffalo rats as previously described (9). To isolate mt tRNA^{Lys}, total nucleic acids were extracted in the presence of phenol from mt pellets (9,10) and fraction-

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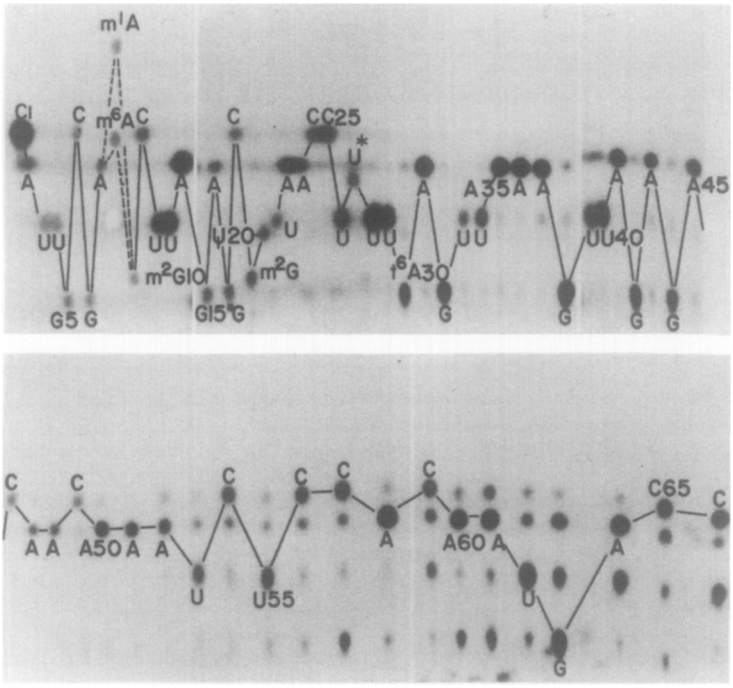

Fig. 1. PEI-cellulose thin-layer readout (12) of the nucleotide sequence of rat liver mt $tRNA^{Lys}_{U*UU}$. The solvent was 1.2 M ammonium formate, pH 3.5 (12). The autoradiograms display [5'-^{32}P]nucleoside 3',5'-bisphosphates derived from single-hit cleavages of the RNA. The sequence is read from left to right.

ated on DEAE-cellulose to obtain crude mt tRNA. Mt $tRNA^{Lys}$ 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), 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. The sequence of the tRNA was determined by a polyethyleneimine(PEI)-cellulose thin-layer readout procedure (12), with minor modifications (11,13).

RESULTS. Crude liver mt tRNA was resolved on the first gel into 10 bands; mt $tRNA^{Lys}_{U*UU}$ traveled with band #5 (#1 being the fastest band). This band was further separated on the second gel into 7 bands. On the third gel, band #2 from this gel gave a single band which was shown to be pure mt $tRNA^{Lys}$ by sequence analysis. About 1.5 μg of mt $tRNA^{Lys}$ was obtained from 100 g of liver tissue.

The sequence of mt $tRNA^{Lys}$ was deduced by a thin-layer readout method (12) based on the single-hit degradation principle (14). ^{32}P-labeled 5'-terminal nucleoside 3',5'-bisphosphates were identified by PEI-cellulose thin-layer chromatography in ammonium formate, pH 3.5, and ammonium sulfate solvents (12). Fig. 1 shows the ammonium formate readout encompassing positions 1 - 66 of the

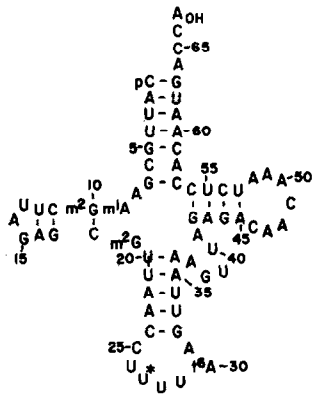

Fig. 2. The nucleotide sequence of rat liver mt $tRNA^{Lys}_{U^*UU}$ arranged in the cloverleaf form.

RNA. Single termini were obtained for all positions except for position 9 which showed m^1A + m^6A. (m^6A is formed from m^1A during the experimental manipulations due to methyl group migration (11).) The linear sequence deduced from the readout can be arranged in a cloverleaf structure as shown in Fig. 2. However, only the anticodon stem has the normal number of base pairs. Modified nucleotides were found in positions 9, 10, 19, 20, 27, and 30. This was confirmed by chromatographing the ^{32}P-labeled nucleotides in 0.55 M ammonium sulfate, as shown in part in Fig. 3. The unknown nucleotide in the anticodon wobble position (marked U* in Fig. 2) was found to migrate slightly behind pAp in formate (R_{pAp} = 0.93; R_{pUp} = 1.20) and ahead of pUp in sulfate (R_{pAp} = 2.02; R_{pUp} = 1.14). The pU*p spot was isolated from formate chromatograms, digested with nuclease P_1 as previously described (11) to pU*, which was then analyzed by cellulose thin-layer chromatography (8). The chromatographic behavior of this compound was found to be very similar or identical to that of pU* obtained from yeast mt $tRNA^{Trp}$ (8), strongly suggesting structural identity. pt^6Ap, which behaved like pGp in the formate solvent (Fig. 1), migrated between pUp and pCp in sulfate (Fig. 3).

The RNA was designated as $tRNA^{Lys}$ on the basis of its anticodon U*UU and the codon recognition rules of mammalian mt tRNAs (7).

DISCUSSION. When compared with eukaryotic cytoplasmic and prokaryotic tRNAs (15), liver mt $tRNA^{Lys}$ exhibits many unusual features. Among these, the small size of loop I and its stem (i.e., the D-arm), encompassing 9 nucleotides instead of the usual 15 - 19 (15), appears most remarkable. Except for mammalian mt $tRNA^{Ser}_{GCU}$, which completely lacks the D-arm (16-18), this is the smallest D-arm of any sequenced tRNA. Modified nucleosides within or close to

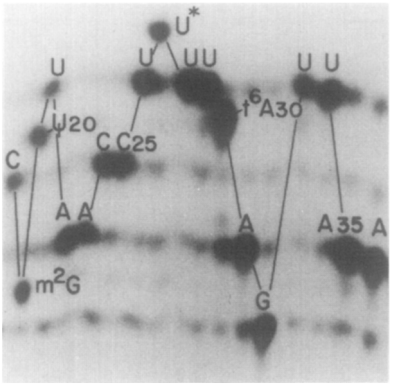

Fig. 3. PEI-cellulose thin-layer readout of the anticodon arm sequence of rat liver $tRNA^{Lys}_{U^*UU}$. The solvent was 0.55 M ammonium sulfate (12).

the D-arm (m^1A9, m^2G10, m^2G19, and Ψ20) are also found in other mammalian mt (19-21) and cytoplasmic (15) tRNAs, indicating that a normal D-arm is not required for these modifications.

Other unusual features, some of which appear to be quite common for mammalian mt tRNAs, however (3,16-18), include the C6/A58 mismatch - never found in any tRNA - and, in addition, the lack of U8, the invariant -GG- sequence in loop I, and the invariant G·C base pair at the base of loop IV. m^1A9 has been found also in 13 other rat mt tRNAs sequenced by us ((19-21) and unpublished results) and therefore appears to represent a specific feature of mammalian mt tRNAs. In view of its numerous unusual features, especially the rudimentary D-arm, the mt $tRNA^{Lys}$ may be incapable of assuming an L-shaped structure resembling that of yeast $tRNA^{Phe}$ (22) unless the lack of internal tertiary interactions is offset by external stabilizing forces contributed by the hydrophobic mt environment.

The results reported in this paper, in conjunction with results reported earlier (19-21), indicate that specific rules for codon recognition by yeast and Neurospora crassa mt tRNAs (4,6,8) apply to rat mt tRNAs as well. In particular, an unmodified U is found in the first (wobble) position of the anticodon of mt tRNAs recognizing codons belonging to the family boxes and ending in N, while a modified U (U*), probably 5-methoxycarbonylmethyluridine (Martin, R.P., Sibler, A.-P., Bordonné, R., Dirheimer, G., and Agris, P.F., personal communication), occupies the wobble position of mt tRNAs recognizing nonfamily codons ending in a purine. (U* was found also in rat liver and hepatoma mt $tRNA^{Trp}_{U^*CA}$ (our unpublished results) and a related compound, 2-thio-5-methoxycarbonylmethyluridine, was found in the wobble position of cytoplasmic $tRNA_3^{Lys}$ from rabbit liver (23).) Thus, the anticodons U*AA, U*CA, U*UU, U*UC, and

U*UG of mammalian mt tRNAs are likely to have this modification, while the anticodons UAC, UCC, UCG, UAG, UGU, UGG, UGA, and UGC are not modified. It is appropriate to ask whether the enzyme(s) catalyzing the formation of U* recognize the anticodon sequences themselves or some other features of the mt tRNAs. Inspection of the human mt tRNA sequences (inferred from the DNA sequence (3)) fails to reveal any common structural features potentially providing recognition sites for the enzyme(s) responsible for U modification in the wobble position. Therefore, it appears likely that it is the anticodon sequence itself which is recognized by the enzyme(s). If this hypothesis is correct, this modification would (i) require U in the second or A in the third position and (ii) be prevented by G in the second position of the anticodon. Similarly, queuosine (Q), a highly modified derivative of G (24), occurs in the wobble position only if the 3'-adjacent nucleotide is U, i.e., in tRNAs recognizing NAY codons.

It appears likely that the anticodon sequence itself is also responsible for the modification of the nucleotide immediately 3'-adjacent to it as t^6A (or its N^6-methyl derivative, mt^6A) is found in almost all tRNAs recognizing codons starting with A (15). This modification probably is necessary for the stabilization and correct formation of the A·U pair between the first base of the codon and the third base of the anticodon (24). One may postulate therefore that the 2 hypermodified nucleotides, U* and t^6A, act together to assure the fidelity of interaction between mt $tRNA^{Lys}$ anticodon and the lysine codons AAA and AAG in mt mRNA.

As shown here and elsewhere ((19-21) and our unpublished results), most modified nucleotides in mammalian mt tRNAs are located in the same positions as in cytoplasmic tRNAs. On the basis of these results, and taking into account the highly unusual primary structures of mammalian mt tRNAs, we hypothesize that most modifying enzymes recognize secondary/tertiary structure elements of mammalian tRNAs rather than specific nucleotide sequences. However, those enzymes that modify the wobble nucleotide and the nucleotide 3'-adjacent to the anticodon, respectively, appear to recognize specific nucleotides within the anticodon triplet.

In spite of vast differences in primary structure of isocoding, isoaccepting tRNAs from mammalian mitochondria, mammalian cytoplasm, and mitochondria of lower eukaryotes such as yeast and Neurospora crassa, the modification patterns of these tRNAs have been highly conserved. It may be postulated therefore that posttranscriptional modifications may be more critical for proper functioning of tRNA than some features of primary structure.

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