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A major lysine tRNA with a CUU anticodon in insect mitochondria

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ABSTRACT

We have sequenced a lysine tRNA from mosquito mitochondria that has the anticodon CUU. The preponderance of AAA lysine codons in insect mitochondrial genes, the parsimonious organization of the genomes, and the fact that this tRNA is a major component of the mosquito mitochondrial tRNA complement, lead us to suggest that the CUU anticodon recognizes AAC and AAA codons.

INTRODUCTION

Genome sequences for mouse, human and bovine mitochondria indicate that lysine is encoded in mammalian mitochondria mainly by AAA, and to a minor extent by AAC; and that both codons are translated by the product of a single tRNA gene which specifies the anticodon UUU (1-3). Analyses of the corresponding tRNAs (4,5) have shown that the 5' residue of the anticodon is modified, which modification is thought to restrict wobble to the usual U-G or U-A base-pairing in accord with the coding scheme of mammalian mitochondria (6). More recently, sequences published for *Drosophila* mit (mitochondrial) DNA indicate that in this system, too, AAA is the predominant codon for lysine (7-9); yet the only *Drosophila* mit $tRNA^{lys}$ gene thus far found is one that would specify a tRNA with the anticodon CUU (8,9). We report here the presence, in mitochondria from another dipteran source, *Aëdes*, of a major $tRNA^{lys}$ that indeed has the anticodon CUU, and whose sequence is highly homologous to the *Drosophila* mit $tRNA^{lys}_{CUU}$ genes. For comparison we present also the sequence of hamster mit $tRNA^{lys}_{U*UU}$, determined similarly.

METHODS

Mosquito mit $tRNA^{lys}_{CUU}$ was purified from *Aëdes albopictus* cells (10) using two-dimensional gel electrophoresis (11). Hamster mit $tRNA^{lys}$ was purified from BHK-21 cells (10) by electrophoresis through a 40cm long, 7% acrylamide-8M urea gel, in which it ran as a discrete band ahead of the bulk of the mit tRNA. Sequences were determined using a combination of ladder analysis of end

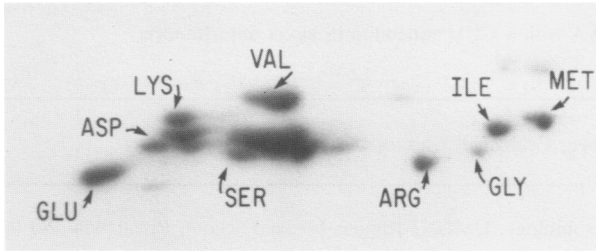

Figure 1. Acrylamide gel pattern of mosquito mit tRNA. The first dimension was leftward, the second downward (11). Identities of spots containing single species are shown.

labeled samples, and compositional analysis of samples uniformly labeled with ^{32}P and [^{3}H]methyl, as previously described (11).

RESULTS

We present in Fig. 1 a typical two-dimensional gel pattern for uniformly labeled Aëdes mit tRNA. The species that proved to be $tRNA^{lys}_{CUU}$ was recovered in amounts similar to those of other relatively plentiful tRNAs such as $tRNA^{arg}$, $tRNA^{ile}$, and $tRNA^{met}$. Fig. 2A shows the sequence determined for this tRNA, printed out in cloverleaf form (11); differences from the homologous D. yakuba gene are indicated. For comparison we show the hamster mit $tRNA^{lys}_{U*UU}$

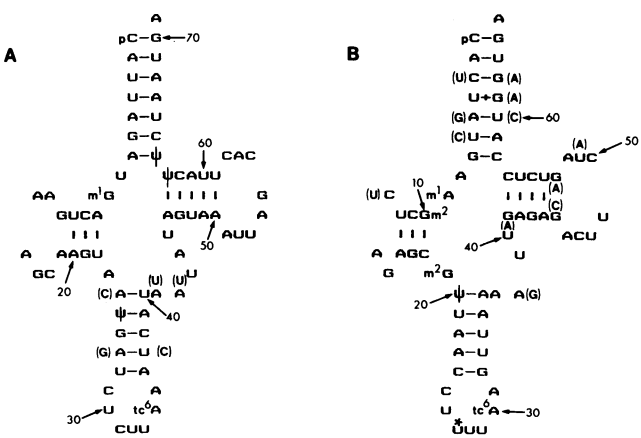

Figure 2. Mosquito mit $tRNA^{lys}_{CUU}$ (A) and hamster mit $tRNA^{lys}_{U*UU}$ (B). 3'-terminal CCA residues are not included. For the mosquito sequence, we indicate in parentheses differences in the D. yakuba mit $tRNA^{lys}_{CUU}$ gene (8), and for the hamster sequence, differences in the rat mit tRNA sequence (4).

sequence (Fig. 2B), determined in similar manner. U*27 of this latter sequence yielded anomalous reactions in chemical sequencing: strongly positive "A", weak "C", and negative "G" and "U" reactions. We designate this residue as a modified U (and hence the tRNA as $tRNA_{U^*UU}^{lys}$) in part because of good overall sequence homology with $tRNA_{U^*UU}^{lys}$ from other mammalian mitochondria; in addition, analysis of a similarly migrating tRNA from mouse (L-cell) mitochondria yielded the same ladder anomaly and the same sequence otherwise as reported (3) for the mouse mit $tRNA^{lys}$ gene. In contrast, there was no anomaly associated with the corresponding residue, C31, of the mosquito tRNA, nor were any modified C's detected in compositional analyses.

DISCUSSION

The homology between hamster mit $tRNA_{U^*UU}^{lys}$ and corresponding mouse, rat, cow and human tRNAs is fairly good for mammalian mit tRNA, ranging from 69% to 86%; in particular, the hamster tRNA has the severely truncated D arm that characterizes these others. The conservation of modification status (Fig. 2B) is also as observed for other mammalian mit isoacceptors (11).

Our most important finding is the occurrence in mosquito mitochondria of a major lysine tRNA with an unmodified CUU anticodon. This RNA is 93-94% homologous to putative $tRNA_{CUU}^{lys}$ genes in Drosophila mitochondria (8,9), indicating that the latter genes are active and specify major tRNAs. Wobble base pairing (12), even with the relaxed constraints found in mitochondria (6), prescribes that such tRNAs recognize only AAC; and indeed this is the case for a $tRNA_{CUU}^{lys}$ from rabbit cytoplasm (13). However, AAA constitutes over 90% of the lysine codons in Drosophila mit DNA, and its translation would then require a second major lysine tRNA, with (by analogy with mammalian mitochondria) the anticodon U*UU. This latter would be expected to recognize AAC as well as AAA, and parsimony arguments lead us to favor the alternative idea: that dipteran mit $tRNA_{CUU}^{lys}$ recognizes both AAC and AAA. There is a likely precedent for this in mammalian mitochondria, where a single $tRNA_{CAU}^{met}$ (14) apparently serves to translate internal AUG and AUA (1-3), converting AUA from an isoleucine to a methionine codon. Mammalian (B.A. Roe, personal communication) and Aëdes (our unpublished data) mit $tRNA_{CAU}^{met}$ have unmodified anticodons, resembling Aëdes mit $tRNA_{CUU}^{lys}$ in this regard. E. coli (15) and spinach chloroplasts (16) contain $tRNA_{C^*AU}^{ile}$ species, in which modification of the C residue restricts recognition to AUA, and there is evidence (U.L. RajBhandary, personal communication) that the same mechanism effects conventional decoding of AUA in N. crassa mitochondria (17). It is possible that in those protein

synthetic systems which "mistranslate" AUA to methionine [which currently include metazoan and yeast (18) mitochondria], U* and unmodified C in the wobble position of tRNAs are, in general, informationally equivalent.

The ancestry of dipteran mit $tRNA^{lys}_{CUU}$ is obscure. The dipteran "protosequence" (19) shows <40% homology to protosequences constructed from any of the groups represented in ref. 5, including mammalian mit $tRNA^{lys}_{U*UU}$ and bacterial or eukaryotic cytoplasmic $tRNA^{lys}_{CUU}$.

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