Volume 12 Number 10 1984 Nucleic Acids Research

Sequence and structure of a methionine transfer RNA from mosquito mitochondria

Donald T.Dubin and Chuen Chin HsuChen

Department of Microbiology, University of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, NJ 08854, USA

Received 20 February 1984; Accepted 17 April 1984

ABSTRACT

We have sequenced a methionine tRNA from mosquito mitochondria, and examined its structure using nucleases S_1 and T_1 under non-denaturing conditions. The sequence is highly homologous to a putative initiator methionine tRNA gene from Drosophila mitochondria. Its anticodon stem contains a run of three G-C base pairs that is characteristic of conventional initiator tRNAs; however, nuclease S_1 analysis suggested an anticodon loop configuration characteristic of conventional elongator tRNAs. We propose that this tRNA can assume both initiator and elongator roles.

INTRODUCTION

Initiator methionine tRNA ($tRNA_i^{met}$) has special structural features, which differ for bacterial vs eukaryotic prototypes (1,2). Mitochondria resemble bacteria in utilizing formylated $tRNA_i^{met}$ (3), and fungal mitochondria indeed contain separate genes for $tRNA_i^{met}$ (presumed to be the formylatable species) and $tRNA_m^{met}$ (see refs 1,4). Mammalian mitochondrial (mit) genomes, on the other hand, contain but a single $tRNA^{met}$ gene (5-7), whose products must presumably recognize initiating as well as internal methionine codons. We present here studies on the sequence and structure of a $tRNA^{met}$ from mosquito mitochondria; and compare it with a putative $tRNA_i^{met}$ gene recently reported for Drosophila mitochondria (8), and with mammalian mit $tRNA^{met}$.

METHODS

Procedures for purifying mit $tRNA^{met}$ from cultured mosquito (Aedes albopictus) cells, and for establishing its sequence, were as described previously (9,10). Digestion with RNases T_1 or S_1 under non-denaturing conditions was as described in refs. 11, or 12 and 13, respectively.

RESULTS and DISCUSSION

Two-dimensional gel separations of Aedes mit 4S RNA yielded a major component that proved to be $tRNA^{met}$ ("MET" of ref. 10). Ladder analysis of end

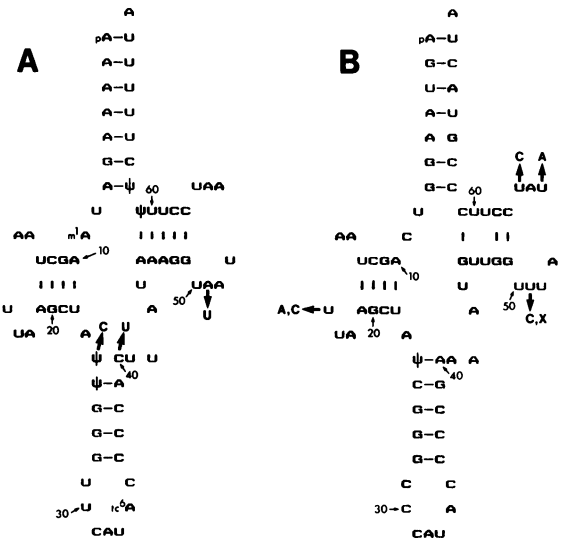

Fig. 1. Sequence of Aedes mitochondrial $tRNA^{met}$. Panel A shows the sequence of Aedes mit $tRNA^{met}$ in its presumed cloverleaf configuration (omitting terminal CCA), with differences in the homologous D. Yakuba gene sequence indicated (8). Panel B shows the bovine mit $tRNA^{met}$ sequence (B.A. Roe, personal communication; see also ref. 1) in similar format, with differences in human and/or mouse indicated ("X" designating an absent nucleotide)(5,6).

labeled samples coupled with compositional analysis of uniformly labeled samples provided an unambiguous sequence. Anomalies associated with 8 ladder positions (see ref. 9) permitted location of all modified residues, and the sequence is displayed in Fig. 1. Confirmation was provided by oligonucleotide fingerprint analysis of T_1 digests of uniformly labeled samples; seven spots were obtained, all of whose compositions were as predicted from the sequence given.

Higher order structure was studied using RNases T_1 and S_1. All G residues were inaccessible to RNase T_1 under the conditions of ref. 11, in accord with the secondary structure of Fig. 1. An example of nuclease S_1 analysis is presented in Fig. 2. Of particular interest is the anticodon loop pattern, in view of findings (13) on $tRNA_I^{met}$ from E. coli and eukaryotic cytoplasm. These tRNAs exhibited restricted S_1 sensitivity in their anticodon loops, cleavage occurring only after each of the first two residues of the anticodon; this was in contrast to elongator tRNAs, whose S_1 sensitivity extended one residue further in both directions. Aedes mit $tRNA^{met}$ yielded patterns (Fig. 2) more closely resembling those found for elongator tRNAs (13).

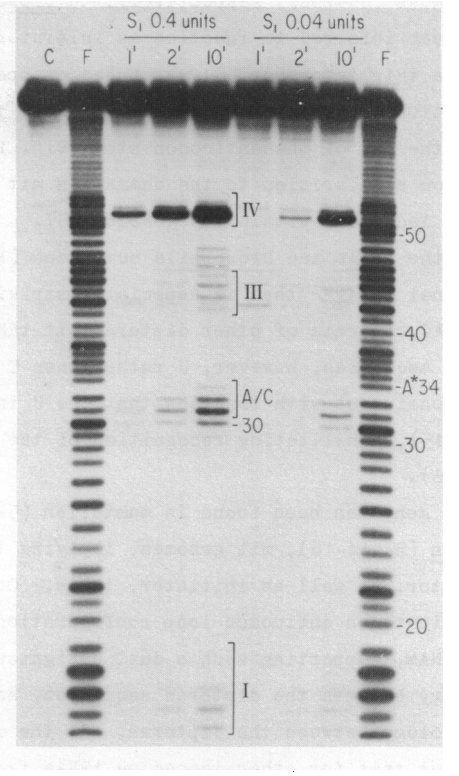

Fig. 2. S_1 nuclease digestion patterns of Aedes mitochondrial $tRNA^{met}$. 5'-end labeled samples were subjected to digestion with S_1 nuclease, 0.4 or 0.04 units per µg of RNA, for 1, 2 or 10 minutes (as noted) under the conditions of Wrede et al (13). The "C" lane represents a 10 minute mock-treated sample. "F" lanes represent formamide ladders; positions are numbered by comparison with enzymatic sequencing ladders. A*34 designates tc^6A34 (see Fig. 1); the slight "gap" 5' to this residue provides a convenient marker (see refs. 9, 13). The numbering of the S_1 bands takes into account the slower migration of S_1-released vs. formamide-released products (12, 13). "I" and "IV" indicate the regions of the loops of the corresponding arms; "III" indicates Arm III (the "extra arm"); and "A/C" designates the anticodon.

The S_1 sensitivity of the other loops of Fig. 1 is in accord with the secondary structure given, but is greater than found in earlier studies on conventional tRNA (12,13), suggesting a looser tertiary structure than occurs with conventional tRNAs. The terminal 4 or 5 base pairs of the aminoacyl stem were also sensitive to S_1, probably reflecting relatively easy "unwinding" of the run of A-U base pairs here. Similar patterns were obtained using the S_1 procedure of Wurst et al (12), and at 30°, rather than 37°.

As illustrated in Fig. 1, Aedes mit $tRNA^{met}$ is highly homologous (96%),

and moderately homologous (65-70%), respectively, to Drosophila and mammalian mit tRNAs that have been inferred to function as initiator tRNAs (5-8). For mammalian mitochondria this inference (6) was based on special features characteristic of conventional initiator tRNAs: the C 5' to the anticodon and the trio of G-C pairs at the end of the anticodon stem (Fig. 1). For Drosophila the inference relies on similarities to the mammalian mit system, involving primary sequences and locations within the genomes (8). The characteristic trio of G-C pairs in the Aedes and Drosophila sequences (Fig. 1) provides homology to conventional $tRNA_I^{met}$ that is especially striking in view of the extraordinarily low G+C contents of other dipteran mit tRNAs (8-10,14). In both dipteran $tRNA^{met}$ sequences, however, U rather than C precedes the anticodon. This is consistent with the idea that the U in question has functional significance (15), facilitating recognition of the unusual initiating quadruplet AUAA (15,16).

Only one $tRNA^{met}$ gene has been found in mammalian (5-7), and only one thus far in Drosophila (8, 14-16), mit genomes, implying that the gene products serve as elongator, as well as initiator, tRNAs. Our S_1 patterns for the Aedes $tRNA^{met}$ indicate an anticodon loop configuration resembling that of standard elongator tRNAs, supporting such a dual assignment.

The high homology between the dipteran sequences, and the lesser but still substantial homology between the dipteran, and the mammalian, mit $tRNA^{met}$ classes, exceed that for other isocoding tRNAs from these systems (see, e.g., ref. 14), in line with the high evolutionary conservation of $tRNA_i^{met}$ (1,2). The few differences between Drosophila and Aedes sequences (Fig. 1) are worth noting. A distinctive feature of the Aedes sequence is the U,C "mismatch" at the base of the anticodon stem. The Drosophila sequence also contains a mismatch here, but with bases reversed; perhaps a 4 base-pair anticodon stem is not merely permitted for dipteran mit $tRNA^{met}$, but is favored. The other Aedes-Drosophila difference, at position 60, corresponds to one of the few variable positions of the mammalian mit $tRNA^{met}$ family (Fig.1). Comparison of the dipteran $tRNA^{met}$ sequences with those of other classes of $tRNA_i^{met}$ or $tRNA_m^{met}$ (1) revealed relatively poor homology (generally <50%) and no trend towards prokaryotic prototypes. Thus, as for other dipteran mit tRNAs (9,10), the present results provide few clues on evolutionary origins.

The modification status of Aedes $tRNA^{met}$ is rather different from that of bovine mit $tRNA^{met}$ (Fig. 1), resembling that of other Aedes mit tRNAs (9,10). The role of these modifications is not known; conceivably, one or a combination of them may serve to distinguish formylatable from nonformylatable tRNA molecules. Experiments are underway to explore this question.

ACKNOWLEDGMENTS

This work was supported by NIH Grant GM 28100. We thank Mrs. K. Timko for her expert technical assistance.

REFERENCES

1. Gauss, D. H. and Sprinzl, M. (1983) Nucl. Acids Res. 11, r1-r53 and r55-r103.
2. Heckman, J. E., Hecker, L. I., Schwartzbach, S. D., Barnett, W. E., Baumstark, B., and RajBhandary, U. L. (1978) Cell 13, 83-95.
3. Smith, A. E. and Marcker, K. A. (1968) J. Mol. Biol. 38, 241-243.
4. Köchel, H. G., Lazarus, C. M., Basak, N., and Küntzel, H. (1981) Cell 23, 625-633.
5. Anderson, S., Bankier, A.T., Barrell, B.C., de Bruijn, M.H.L., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R. and Young, I.G. (1981) Nature 290, 457-465.
6. Anderson, S., de Bruijn, M. H. L., Coulson, A. R., Eperon, I. C., Sanger, F. and Young, I. G. (1982) J. Mol. Biol. 156, 683-717.
7. Bibb, M. J., Van Etten, R. A., Wright, C. T., Walberg, M. W. and Clayton, D. A. (1981) Cell 26, 167-180.
8. Clary, D. O., Goddard, J. M., Martin, S. C. Fauron, C. M-R. and Wolstenholme, D. R. (1982) Nucl. Acids Res. 10, 6619-6637.
9. HsuChen, C-C., Cleaves, G. R., and Dubin, D. T. (1983) Plasmid 10, 55-65.
10. HsuChen, C-C., Cleaves, G. R., and Dubin, D. T. (1983) Nucl. Acids. Res., 11, 8659-8662.
11. Pavlakis, G. N., Lockard, R. E., Vamvakopoulos, N., Rieser, L., RajBhandary, U. L. and Vournakis, J. N., (1980) Cell 19, 91-102.
12. Wurst, R. M., Vournakis, J. N., and Maxam, A. M. (1978) Biochemistry 17, 4493-4499.
13. Wrede, P., Woo, N. H., and Rich, A. (1979) Proc. Natl. Acad. Sci. USA, 76, 3289-3293.
14. Clary, D. O., Wahleithner, J. A. and Wolstenholme, D. R. (1983) Nucl. Acids Res. 11, 2411-2425.
15. Clary, D. O., and Wolstenholme, D. R. (1983) Nucl. Acids Res. 11, 4211-4227.
16. de Bruijn, M. H. L. (1983) Nature 304, 234-241.