

NOTE

Fragmentary 5S rRNA Gene in the Human Mitochondrial Genome

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The human mitochondrial genome contains a 23-nucleotide sequence that is homologous to a part of the 5S rRNA's of bacteria. This homology, the structure of the likely transcript, and the location of the sequence relative to the mitochondrial rRNA genes suggest that the sequence represents a fragmentary 5S rRNA gene.

Mitochondria possess an independent system of transcription and translation and a small unique genome. This genome encodes for a few mitochondrial proteins and also for the tRNA's and rRNA's that are used in its translation system (1, 3). A number of features of mitochondrial metabolism and macromolecular synthesis resemble those of bacteria, suggesting a prokaryotic origin for the mitochondria (16). One unique feature, however, of the mitochondrial ribosomes of animal and fungal cells is the absence of a 5S RNA (5, 15). The 5S rRNA is an approximately 120-nucleotide species otherwise universally present in the large subunit of bacterial ribosomes, animal cytoplasmic ribosomes, and the ribosomes of plant cells (5, 14, 18). Among these ribosomes, the 5S RNA species possess a highly conserved sequence and structure (12, 14). The absence of a 5S species in the ribosomes of animal and fungal mitochondria, as opposed to the high degree of conservation in other ribosomes, suggests that, rather than being simply lost, the 5S function may be present in an unrecognizable form. To test this possibility, I have searched the sequence of the human mitochondrial genome for a sequence homologous to bacterial 5S rRNA's and report here that a 23-nucleotide sequence of the human mitochondrial genome is homologous with 5S RNA sequences. This homology, the probable structure of the corresponding RNA transcript, and the location of the sequence in the mitochondrial genome suggest that it is a 5S RNA gene fragment.

Figure 1 shows a portion of the sequence of the mitochondrial rRNA genes near the 3' end of the large rRNA subunit (9); the homologous sequence is underlined. The figure also shows the 5S rRNA genes of *Bacillus subtilis* (17),

Escherichia coli (6), *Halobacterium cutirubrum* (19), and human KB cells (cytoplasm) (11). The 5S sequences are aligned to show their correspondence (14, 19), and the human mitochondrial sequence has been aligned with them. Twenty-two of 23 nucleotides of the mitochondrial sequence are identical to those of the *B. subtilis* 5S rRNA. Spaces have been inserted into the sequence to align it, as is generally necessary to align the 5S species, and in consideration of the fact that the mammalian mitochondrial rRNA's are about 60% the size of their bacterial homologs (9). The overall homology between the human and *B. subtilis* sequences (including the inserted spaces) is 68%.

5S RNA species share a common structure (12-14), and the mitochondrial sequence, if transcribed (see below), shares elements of this structure as shown in Fig. 2. An important feature of this structure is the presence, in the loop, of the sequence GAAC (10, 21). This sequence is universally present in bacterial 5S rRNA's, whereas GAUC appears in animal cytoplasmic 5S rRNA's (14). An added feature is the presence of CCC in the loop; however, it can be noted that the alignment of the structure with other conserved portions of the sequence is altered relative to the universal 5S molecule.

To evaluate the likelihood that the homologous fragment arose by chance, a search was made of the entire L-strand (primary coding strand) of the genome. Each GAAC sequence was scored for the homology to 5S RNA of the flanking sequences, allowing, as in the case shown, for short deletions of the 5S sequence. This search showed that the fragment shown is unique in its homology. A second, single, similarly homologous sequence was found on the H-

KB 5'-GTCT-ACGG CCATACCACC CTGAACGCG CCGATCTCGT -CTCATCTCG GAAGTAAGC AG66TCG66C CTGGT--TAG TACTTGGATG GGAGA--CCG CCTGGGAATA CCG66TGCTG T-AGGCTT
Bs 5'--TTTGGTGG CGATAGCGAA GAGGTACAC CCGTCCCAT ACCGAAACAG GAAGTTAAGC TCTTCAGCG C-GATGGTAG TC-----GG GGGTTT-CCC CCGTGGAGAG TAGGACGCG CCAAGC--
mt 5'.....CTCA ACTTAGTATT ATACCCAC CC-A-CCCA- A--GAACA-G G--GTTTGT AGATGGCAG AGCCCG.....
Ec 5'TGCCTGGCG CGTAGCGCG GTGGTCCAC CTGACCCCAT GCCGAACCTA GAAGTGAAC GCGGTAGCG C-GATGGTAG TG-----TG GGGTCT-CCC CATGCGAGAG TAGGGAAGCT CCAGGCAT
Hc 5'-TTAAGCGG CCATAGCGT GGGGTTACT CCGTACCAT CCCGAACAG GAAGTAAGC CCGCTCGCT TCCGGT-CAG TACTGGAAGT CGAGC----C TCTGGGAAT CCG66TGCG 6CCTACT-

FIG. 1. Sequences of 5S RNA genes and the homologous sequence from the human mitochondrial genome (underlined). The sequences of the genes for *B. subtilis* (Bs; 17), *E. coli* (Ec; 6), *H. cutirubrum* (Hc; 19), and human KB (cytoplasmic) (KB; 11) rRNA's are inferred from RNA sequences. The human mitochondrial sequence (mt) extends from nucleotides 3,186 to 3,248 of reference 9, and the underlined sequence is the 3' end of the region identified as the large (16S) rRNA subunit, nucleotides 3,206 to 3,228 of reference 9. The 3'-flanking tRNA^{Leu} gene begins at nucleotide 3,230 of reference 9.

strand (located complementary to L-strand residue no. 4116 of reference 1). It, however, lacks the structure of the 5S-like fragment.

The rRNA genes of the human mitochondrial genome have been sequenced (9), and this, with prior mapping utilizing DNA-RNA hybridization (2, 3), reveals the general organization of the genes. The small (12S) and large (16S) rRNA subunits are separated by a tRNA gene and flanked by tRNA's as well, i.e., tRNA-12S rRNA-tRNA-16S rRNA-tRNA. The transcription of these genes is in the order of small subunit to large subunit, and this in turn indicates that the general organization is similar to the rRNA operons of *E. coli*, i.e., 16S rRNA-tRNA-23S rRNA-5S rRNA-tRNA (20). Thus, the position of the 5S-like sequence in the mitochondrial genome, as indicated in Fig. 1, is analogous to its position in that of *E. coli*; it immediately precedes the distal tRNA in the gene cluster at the end of the large rRNA gene (18).

Although the 3'-terminal sequence of the 16S rRNA is not known, it seems likely that the mitochondrial 5S-like sequence is transcribed as a part of the large (16S) rRNA and forms the 3' end of the molecule. This can be inferred from diverse studies of the organization and transcription of the human mitochondrial rRNA's (2, 3, 22) and from the sequence determination of the 3' end of the hamster 16S rRNA (8). It is less likely that this sequence is an independent species. A 3S RNA was identified in hamster cells as a possible 5S-like species (4), but the corresponding molecule in bovine mitochondria has been shown to be an anomalously small tRNA (7). Moreover, when bovine mitochondrial RNA labeled with [γ -³²P]ATP was hybridized to a human DNA fragment (a pBR322 clone carrying the endonuclease *Mbo*I fragment 9; reference 9) that carries the distal part of the 16S rRNA gene and the adjacent tRNA gene, no small RNA was recovered other than the tRNA (E. Chen, B. Roe, and D. Nierlich, unpublished

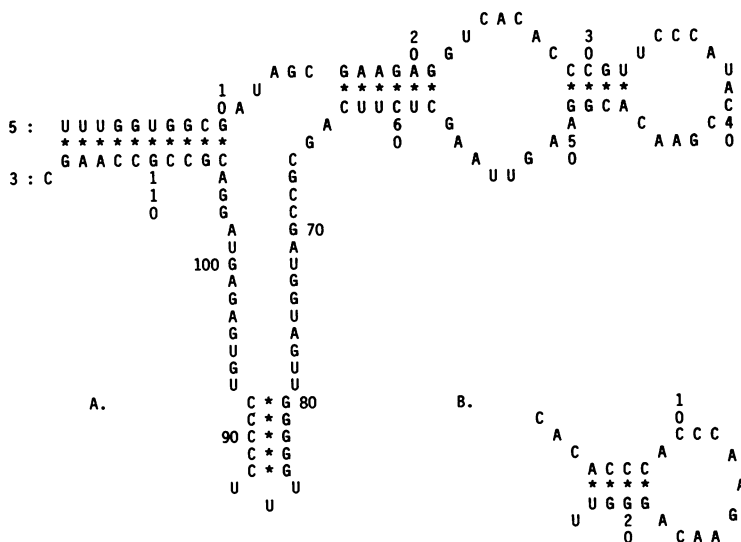


FIG. 2. Structures of (A) *B. subtilis* 5S RNA and (B) the 23-nucleotide sequence from the human mitochondrial genome. An alternative pairing of the 3'-UGGG sequence would give a 7-nucleotide loop.

data). In bacteria, the 5S rRNA is produced by processing a polycistronic transcript that includes the small and large rRNA's (18, 20). In the context of the procaryotic origin of the mitochondrion (16), it seems likely that the processing site in mammalian mitochondria was lost, and a highly truncated 5S sequence became a part of the 16S rRNA.

The sequences of the rRNA genes of murine (23) and bovine (F. Sanger, personal communication) mitochondria have also been determined. These genomes possess sequences similar to the 5S fragment present in the human. However, in neither case is the homology to *B. subtilis* so striking, and in the case of the mouse mitochondrion, the corresponding sequence contains a GAGA rather than a GAAC sequence. Among procaryotic and eucaryotic 5S species, the domains of conserved sequences have been inferred to reflect essential elements of structure and function. Thus, the apparent variability in the 5S-like sequence in the mitochondrial genomes necessitates caution in implying function. A further evaluation of the significance of this 5S-like fragment awaits the determination of additional mitochondrial rRNA sequences.

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LITERATURE CITED

- Anderson, S., A. T. Bankier, B. G. Barrell, M. H. L. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young. 1981. Sequence and organization of the human mitochondrial genome. *Nature (London)* **290**:457-465.
- Angerer, L., N. Davidson, W. Murphy, D. C. Lynch, and G. Attardi. 1976. An electron microscope study of the relative positions of the 4S and ribosomal RNA genes in HeLa cell mitochondrial DNA. *Cell* **9**:81-90.
- Attardi, G., P. Cantatore, E. Ching, S. Crews, R. Gelfand, C. Merkel, and D. Ojala. 1979. The organization of the genes in the human mitochondrial genome and their mode of transcription, p. 443-469. *In* D. J. Cummings, P. Borst, I. Dawid, S. M. Weissman, and C. F. Fox (ed.), *Proceedings of the ICN-UCLA Symposium on Extrachromosomal DNA*. Academic Press, Inc., New York.
- Baer, R. J., and D. T. Dublin. 1980. The sequence of a possible 5 s RNA-equivalent in hamster mitochondria. *Nucleic Acids Res.* **8**:3603-3610.
- Boynnton, J. E., N. W. Gillham, and A. M. Lambowitz. 1979. Biogenesis of chloroplast and mitochondrial ribosomes, p. 903-950. *In* G. Chambliss, G. R. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura (ed.), *Ribosomes, structure, function, and genetics*. University Park Press, Baltimore.
- Brownlee, G. G., F. Sanger, and B. G. Barrell. 1968. The sequence of 5S ribosomal ribonucleic acid. *J. Mol. Biol.* **34**:379-412.
- deBruijn, M. H. L., P. H. Schreier, I. C. Eperon, B. G. Barrell, E. Y. Chen, P. W. Armstrong, J. F. H. Wong, and B. A. Roe. 1980. A mammalian mitochondrial serine transfer RNA lacking the "dihydrouridine" loop and stem. *Nucleic Acids Res.* **8**:5213-5232.
- Dubin, D. T., K. D. Timko, and R. J. Baer. 1981. The 3' terminus of the large ribosomal subunit ("17S") RNA from hamster mitochondria is ragged and oligoadenylated. *Cell* **23**:271-278.
- Eperon, I. C., S. Anderson, and D. P. Nierlich. 1980. Distinctive sequence of human mitochondrial ribosomal RNA genes. *Nature (London)* **286**:460-467.
- Erdmann, V. A. 1976. Structure and function of 5S and 5.8S RNA. *Prog. Nucleic Acids Res. Mol. Biol.* **18**:45-90.
- Foget, B. G., and S. M. Weissman. 1967. Nucleotide sequence of KB cell 5S RNA. *Science* **158**:1695-1699.
- Fox, G. E., and C. R. Woese. 1975. 5S RNA secondary structure. *Nature (London)* **256**:505-506.
- Garrett, R. A., and H. F. Noller. 1979. Structures of complexes of 5S RNA with ribosomal proteins L5, L18 and L25 from *Escherichia coli*: identification of kethoxal-reactive sites on the 5S RNA. *J. Mol. Biol.* **132**:637-648.
- Hori, H., and S. Osawa. 1979. Evolutionary change in 5S RNA secondary structure and a phylogenetic tree of 54 5S RNA species. *Proc. Natl. Acad. Sci. U.S.A.* **76**:381-385.
- Lizardi, P. M., and D. J. L. Luck. 1971. Absence of 5S RNA component in the mitochondrial ribosomes of *Neurospora crassa*. *Nature (London) New Biol.* **229**:140-142.
- Margulis, L. 1970. *Origin of eukaryotic cells*. Yale University Press, New Haven.
- Marotta, C. A., F. Varricchio, I. Smith, S. M. Weissman, M. L. Sogan, and N. R. Pace. 1976. The primary structure of *Bacillus subtilis* and *Bacillus stearothermophilus* 5S ribonucleic acids. *J. Biol. Chem.* **251**:3122-3127.
- Monier, R. 1974. 5S RNA, p. 141-168. *In* M. Nomura, A. Tissieres, and P. Lengyel, (ed.), *Ribosomes*. Cold Spring Harbor Laboratory, New York.
- Nazar, R. N., A. T. Matheson, and G. Bellemare. 1978. Nucleotide sequence of *Halobacterium cutirubrum* ribosomal 5S ribonucleic acid. *J. Biol. Chem.* **253**:5464-5469.
- Nomura, M., E. A. Morgan, and S. R. Jaskunas. 1977. Genetics of bacterial ribosomes. *Annu. Rev. Genet.* **11**:297-347.
- Ofengand, J. 1979. The topography of tRNA binding sites on the ribosome, p. 497-529. *In* G. Chambliss, G. R. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura (ed.), *Ribosomes, structure, function and genetics*. University Park Press, Baltimore.
- Ojala, D., J. Montoya, and G. Attardi. 1981. tRNA punctuation model of RNA processing in human mitochondria. *Nature (London)* **290**:470-474.
- VanEtten, R. A., M. W. Walberg, and D. A. Clayton. 1980. Precise localization and nucleotide sequence of the two mouse mitochondrial rRNA genes and three immediately adjacent novel tRNA genes. *Cell* **22**:157-170.