Volume 12 Number 20 1984 Nucleic Acids Research

Sequences of the coding and flanking regions of the large ribosomal subunit RNA gene of mosquito mitochondria

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Received 3 May 1984; Revised and Accepted 5 October 1984

ABSTRACT

We have sequenced a 1.6 kbp region of the mosquito (Aedes albopictus) mitochondrial genome containing the large ribosomal subunit ("LSU") RNA gene, and have located the ends of the gene by S_1 protection analysis and by comparison with RNA sequences. The gene is preceded by a $tRNA^{val}$ gene and followed by genes for $tRNA^{leu}_{UAG}$ (rather than $tRNA^{leu}_{UAA}$, as in mammalian mitochondria) and an extended reading frame homologous to mammalian URF1. It is approximately 1355 residues long and is very low (17%) in G+C. The 5' half is even lower in G+C (9%), and shows little apparent homology to other LSU RNA classes. The 3' half is relatively rich (26%) in G+C and has many stretches of homology to prokaryotic and mammalian mitochondrial LSU RNA.

INTRODUCTION

Analyses of ribosomal RNA from phylogenetically disparate sources have provided clues on functional and evolutionary aspects of this important class of nucleic acid (see, e.g., refs. 1-8). Earlier studies of ours have indicated that the large ribosomal subunit (LSU) RNA of mosquito (Aedes albopictus) mitochondria occupies an evolutionary extreme in several respects: it is very low in G+C content (17%) and in methylated residues (two) (ref. 1); and it is 3'-terminally polyadenylated (9). In the expectation that it would serve as a basis for further inferences on rRNA function and evolution, we have determined the sequence of a region of the Aedes mitochondrial (mit) genome that contains the LSU RNA and neighboring genes, and have localized the boundaries of the rRNA gene by S_1 protection and RNA sequencing studies.

METHODS

Procedures for growing Aedes cells and preparing mit RNA and DNA were as previously described (2,11). Clones containing mit DNA were obtained by restricting genomic DNA with HindIII or Sau3A, ligating the resulting fragments into appropriately restricted plasmid pUC9, and transforming E. coli strain JM83 (see ref. 11). Screening was performed by colony hybridization (12),

using either 5'-end labeled $tRNA^{val}$ (10) or 3'-end labeled LSU RNA. Samples were hybridized at 65° for 16h in 4X SSC, rinsed thrice at 20° in 2X SSC, and then washed twice at 65° for 30 minutes in 4X SSC; all solutions contained in addition 1mM EDTA and 0.1% SDS.

For DNA sequencing, samples were 3' end labeled using the Klenow fragment of E. coli DNA polymerase (13) and appropriate α-[^{32}P]deoxynucleoside triphosphates. In some cases, sequencing was performed on double-stranded segments labeled at one end and in some cases segments labeled at both ends were subjected to strand separation for sequencing (ref. 14, p. 180) (summarized in Fig. 1, below). The partial chemical degradation procedure of Maxam and Gilbert (15) and a modification thereof (16) were used.

LSU RNA was purified by serial centrifugation in "low" salt, and then "standard" salt, sucrose gradients (17). RNA samples were 3'-end labeled, repurified and subjected to digestion with RNases T_1 or A to release terminal oligonucleotides, as in ref. 18. RNA sequencing was performed by partial enzymatic digestion of end-labeled samples (2,19).

For S_1 nuclease protection experiments, mixtures of LSU RNA (25 ng; 0.06 pmole) and 3'-end labeled DNA segments (18-50 ng; approx. 0.07 pmole) were taken up in 20 ul of 0.04M 1,4-piperazine-diethanesulfonic acid (PIPES), pH 7.0, 0.4M NaCl, 0.001M EDTA, 80% formamide; heated for 5 min. at 68°, incubated for 17h at 37°, and diluted with 0.2 ml of ice cold "nuclease-S_1 buffer" (ref. 14, p. 208) containing varying amounts of nuclease S_1 (Boehringer-Mannheim). After 30 min. at 37°, samples were again chilled, brought to 0.5M ammonium acetate and 130 mM EDTA, extracted with phenol-chloroform, and precipitated with ethanol (following ref. 14, pp. 208-209). Rinsed pellets were dissolved in 10 ul of 1 mM Tris·HCl, pH 7.4, containing 7M urea and 1 mM EDTA, held at 80° for 3 min., chilled, and run on a sequencing gel (18,20).

RESULTS and DISCUSSION

1. Gene order and sequence.

The LSU RNA gene sequence was determined primarily using two cloned restriction fragments, designated HindIII-E and HindIII-D. Colonies containing HindIII-E hybridized to Aedes mit $tRNA^{val}$, and those containing HindIII-D to LSU RNA, under the conditions employed. The fragments proved to be adjacent and each proved to contain a portion of the LSU RNA gene; the failure of HindIII-E to hybridize to LSU RNA is apparently due to the very low G+C content of its portion of the gene (v.i.). Confirmation was provided by sequences obtained directly from genomic DNA, and from a separately cloned Sau3A

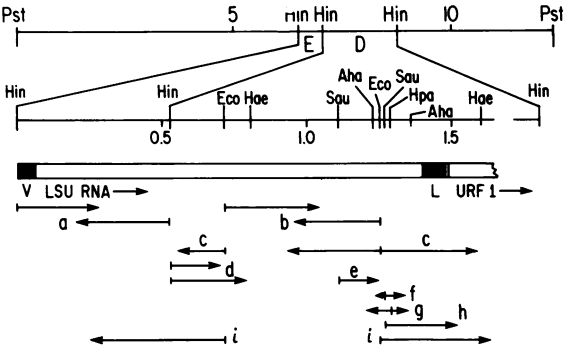

Fig. 1. Sequencing strategy. The top line represents a 12.5 kbp stretch of Aedes mit DNA and shows the HindIII sites used for the present work. The next line is an expanded version of the segments between these HindIII sites with additional relevant restriction sites indicated. Restriction enzymes denoted in the diagram by their first 3 letters are spelled out below; distance is expressed as kbp from the left-most PstI or HindIII site. We next present the gene order, and diagram the various classes of sequence determinations. These are as follows:

a. A cloned plasmid containing fragment HindIII-E was labeled after digestion with HindIII; the insert was then purified and subjected to strand separation.

b. A cloned plasmid containing HindIII-D was labeled after digestion with EcoRI; the EcoRI-EcoRI fragment was purified and subjected to strand separation.

c. As for b, except that after labeling the preparation was subjected to secondary digestion with HindIII or HaeIII, and appropriate fragments were purified.

d. The same plasmid was labeled after digestion with HindIII, and the subjected to secondary digestion with EcoRI or HaeIII and purification of appropriate fragments.

e. As for d, except that labeling was performed after Sau3A digestion, and secondary digestion was with EcoRI.

f. The HindIII-D insert was purified from the above plasmid, labeled after digestion with Sau3A plus AhaIII, and appropriate fragments were purified.

g. As for f, except that labeling was performed after digestion with HpaII plus AhaIII; or after digestion with HpaII, followed by secondary digestion with HaeIII.

h. A cloned plasmid containing a 1.2 kbp mit Sau3A fragment was labeled at the plasmid EcoRI site, subjected to digestion with PstI, and the appropriate fragment was purified.

i. EcoRI-PstI fragments of genomic DNA were labeled at the EcoRI sites and appropriate fragments were purified.

In general, two or three separate sets of sequencing reactions were performed on each class of labeled fragment.

fragment. The strategy is outlined in Fig. 1. We present in Fig. 2 the sequence determined, from the residue adjacent to the upstream HindIII site of fragment E to about the middle of the HindIII-D fragment; the sense strand is shown. The only ambiguity involves residue T1193, which in a minority of analyses yielded a slightly positive C reaction. The residue on the antisense

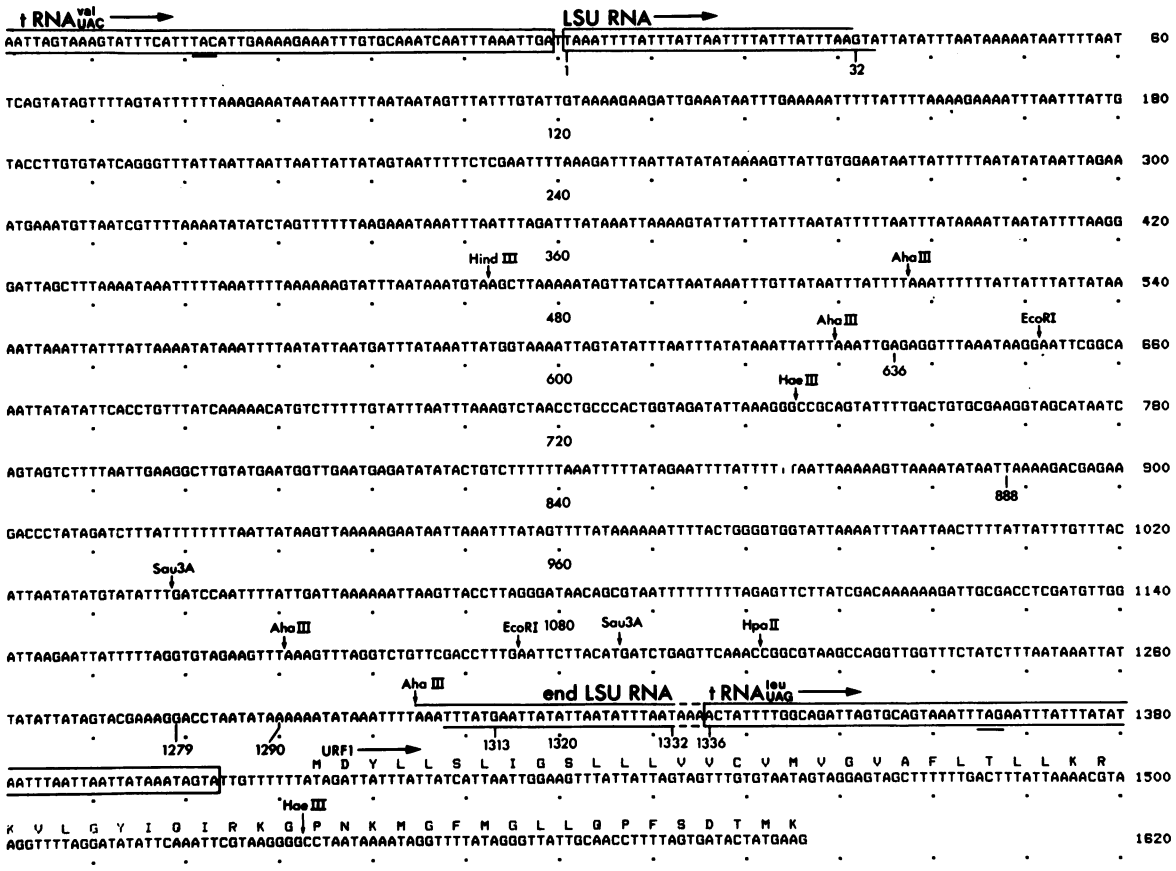

Fig. 2. The sequence of the LSU RNA gene and flanking regions. Several positions referred to in the text are noted and tRNA anticodons are underlined.

strand corresponding to G1234 of Fig. 2 yielded no chemical reaction when analyses were done on cloned DNA, presumably due to its methylation by the dcm methylase; it yielded a normal C reaction in analyses of genomic DNA.

The 5' 59 residues of the sequence correspond to residues 14 through the 3' end of Aedes mit $tRNA^{val}_{UAC}$ (HsuChen and Dubin, unpublished data).

The 5'-terminus of the LSU RNA gene was determined by comparison with 5'-end labeled LSU RNA. Essentially all label (>95%) was released as pU after RNAse P_1 digestion (19). When samples were subjected to partial enzymatic digestion with RNases A, Cl_3, U_2 or T_1 followed by ladder gel analysis (2), the following sequence was obtained:

UAAAUUUUAU UUAYUAAYYY UAYYUAYYUA AGUAYUAUAY YUAA

This localizes the 5' end of the gene to the second T after the $tRNA^{val}$ gene. To facilitate discussing the rRNA, we begin numbering at this T residue.

Determining the 3'-terminus of the gene was more difficult, due to the polyadenylation of the RNA. The most precise results were obtained by analysis of ladder gel patterns obtained after treatment of 3'-end labeled RNA with RNase T_1, or with RNase A. As illustrated in Fig. 3, complete digestion with each enzyme released complex arrays of bands, in accord with the expected heterogeneity of post-transcriptionally added poly A moieties. However, band counts indicated that the T_1-released family was 19 residues longer than the RNase A-released family. E.g., the two most abundant RNase A-released bands ran as expected for oligonucleotides $N_{35}Cp$ and $N_{36}Cp$, whereas the correspondingly abundant RNase T_1-released oligonucleotides ran as expected for $N_{54}Cp$ and $N_{55}Cp$. We infer that the last G of the gene is 19 residues upstream from the last pyrimidine; inspection of the DNA sequence near the following gene (that for $tRNA^{leu}_{UAG}$) indicates that the G in question is G1313 and the pyrimidine is T1332. Support was provided by partial enzymatic ladder sequencing (not shown), which revealed a second cluster of T_1-sensitive sites 34 residues further upstream; these are presumed to arise from G1278, G1279.

A second approach to establishing the 3'-end of the gene involved S_1 nuclease protection analysis. For these studies, we used either of two restriction fragments spanning the presumed rRNA-tRNA gene junction: a 337 bp EcoRI-HaeIII fragment or a 1.1 kbp EcoRI-HindIII fragment (Fig. 1); the two gave the same results. DNA, 3'end labeled at the EcoRI site, was hybridized with LSU RNA, followed by treatment with S_1 nuclease. Preliminary experiments showed that temperatures of 45° or above for either hybridization or enzyme treatment yielded no protection, presumably due to the low G+C content of the potential hybrid. However, as illustrated in Fig. 4, incubation at 37° yiel-

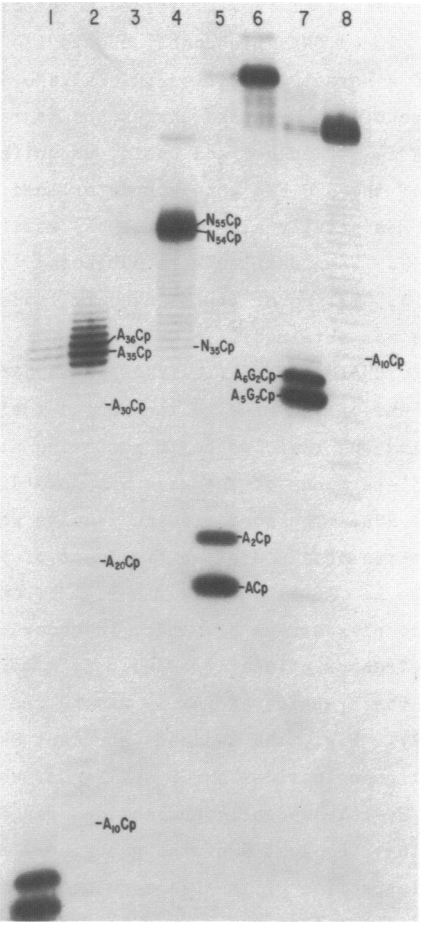

Fig. 3. Sizing of oligonucleotides released from 3'-end labeled LSU RNA by RNases A and T_1. Aliquots of RNA were digested with RNase A (10 pg/ug of RNA) or T_1 (0.8 units/ug of RNA) prior to electrophoresis through a sequencing gel. One portion of each was run for 6h at 2 kv (Lanes 1-4) and a second was run for 3h (Lanes 5-8). Lanes 1, 3, 5 and 7 show patterns for the Aedes mit SSU RNA, which provided markers as noted (2); the other Lanes show the LSU RNA patterns. Lanes 3, 4, 5 and 6 represent RNase T_1 digests and 1, 2, 7,and 8 RNase A digests. Numbering of the bands in lane 2 was based on comparison with the $A_5/A_6 \cdot G_2Cp$ markers, and numbering of the bands in Lane 4 was based on comparison with lane 2. Autoradiography was for 19h; longer exposures provided better visualization of the smaller oligonucleotides released from LSU RNA.

ded complex arrays of protected segments. Control incubations (not shown) showed that protection was dependent on the presence of LSU RNA. The protected segments were sized by running in parallel lanes samples of the same DNA

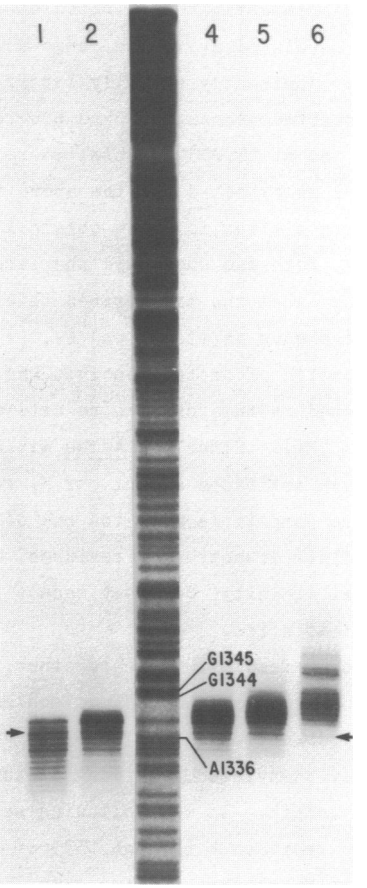

Fig. 4. S_1 protection analysis. Aliquots of the EcoRI-HindIII fragment of HindIII-D (Fig. 1) were hybridized with LSU RNA followed by treatment with varying concentrations of nuclease S_1, as described in Methods. Lanes 1, 2, 4, 5 and 6 represent aliquots treated with 10,000, 5,000, 2,000, 1,000 or 500 units/ml, respectively (a somewhat smaller sample being processed for this last reaction). Lane 3 represents a "C+T" sequencing reaction performed on a sixth aliquot of the same 3' end labeled DNA sample. We indicate bands corresponding to A1336, G1343 and G1344 of the sequence of Fig. 2 (i.e., the complements of the sequence as read from the ladder), and (by arrows) the protected bands corresponding to A1336 (see Text). The sequence ladder represents an exposure time about 10-fold that of the other lanes.

fragment, but previously subjected to partial chemical degradation as for sequencing. Lane 3 of Fig. 4 shows the "C+T" reaction for the DNA used in this run; in designating the bands, we have converted the sequence to that of the sense strand. A1336 would correspond to the theoretical protected fragment,

if the predominant LSU RNA transcripts terminated at T1332 or any of the following four A residues. Levels of S_1 in the range 500 to 2000 units/ml yielded fragments that were predominantly slightly larger than such a segment. However, the sizes of protected segments became progressively smaller with higher levels of enzyme; and at 10,000 units/ml there was a prominent band running just behind A1336, as expected for the above theoretical protected segment. These results are similar to those obtained by Van Etten et al (21) for murine mit rRNA genes, both the small and the large ribosomal subunit RNA, genes. In view of the fact that the transcribed moiety of mammalian mit small subunit RNA is quite homogeneous at its 3' end (9,21), the multiplicity of bands and the slight extension of protection into DNA corresponding to downstream genes were considered in this case to be artifacts related to 3'-terminal oligoadenylation (21). Since the Aedes mit LSU RNA is polyadenylated, it is likely that such artifacts affect our S_1 results as well. Thus, the patterns of Fig. 4 are compatible with the end of the LSU RNA gene being T1332 or one of the immediate downstream A residues, and certainly show that the end is in this general vicinity; but they cannot be taken to indicate heterogeneous transcribed moieties.

We infer from the above results, taken together, that transcription of the majority (at least) of Aedes mit LSU RNA molecules terminates at T1332 of Fig. 2 and/or immediately downstream A residues; and that these transcripts are post-transcriptionally adenylated, yielding 3'-terminal poly A tracts averaging 35 to 36 in chain length. In the following sections, we take the LSU RNA gene to correspond to residues 1 through 1335 of Fig. 2. The gene is thus about 200 residues shorter than corresponding mammalian mit genes (23-26), which of course are themselves unusually short for LSU RNAs.

Analysis of RNA by denaturing acrylamide gel electrophoresis yielded an apparent chain length of 1420 using hamster mit rRNA as size markers (ref. 2), in acceptable agreement with these inferences. The nucleotide composition of purified RNA (Table 1) was, similarly, in agreement with these inferences, and with the sequence as presented in Fig. 2.

In mammalian mit genomes (23-26) the gene following the LSU RNA gene is that for $tRNA^{leu}_{UAA}$. The Aedes mit LSU RNA gene is also followed by a $tRNA^{leu}$ gene, but, curiously, by that for the isoacceptor with a UAG, rather than a UAA, anticodon. Mammalian mit $tRNA^{leu}_{UAA}$ is unusual among mammalian mit tRNAs in that all or almost all of the invariant or semi-invariant residues of conventional tRNA (28) are conserved. In contrast, Aedes mit $tRNA^{leu}_{UAG}$ (Fig. 5) resembles the majority of mammalian mit tRNAs, and all dipteran mit tRNAs

Table 1. Nucleotide Composition of Aedes mit LSU RNA.

	From RNA	From DNA Sequence				
		"Overall"	1-1335	1-634	635-1290	1291-1335
A	39.4	40.2	38.8	42.3	34.5	53.3
G	10.8	11.1	11.4	7.4	15.9	2.2
C	6.3	5.6	5.7	1.7	9.9	0.
U/T	43.6	43.1	44.1	48.6	39.8	44.4
G+C	17.1	16.7	17.1	9.1	25.8	2.2

The RNA data are those summarized in ref. 1. The "overall" DNA composition was derived from that of the gene plus 32 post-transcriptionally added A residues. The other compositional data are designated by range of residue numbers. Values are mole %.

sequenced (e.g, refs. 10,11, 29-32), in lacking most such conserved residues.

Following the $tRNA^{leu}_{UAG}$ gene are a 9-residue spacer and an extended reading frame that show good homology to sequences published earlier for Drosophila mitochondrial genomes (29). As shown in Fig. 6, our results are in agreement with the inference (29) that the reading frame corresponds to mammalian mit URF1, and support one of two possible translation initiation sites proposed for Drosophila (29,32): namely, the first ATA after the $tRNA^{leu}$ gene. The alternative site in Drosophila is an ATT triplet four amino acid residues downstream, but in the Aedes sequence this latter site unambiguously codes for leucine. It is interesting that the N-terminal octapeptide of a fungal mit URF1 counterpart (33) is highly (63%) homologous to the corresponding Aedes region (Fig. 6).

2. The rRNA-Coding Sequence.

Analysis of the rRNA gene proper revealed a remarkable dichotomy with

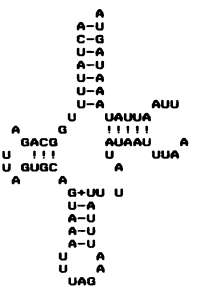

Fig. 5. Presumed secondary structure of Aedes mit $tRNA^{leu}_{UAG}$.

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                    M  E  F  I  L  S  L  IGSLLLIICVLVSVAFLTLLERKVLGYIQIRKGPNKVGLMGIPQPFCDAIKLF
DROS  ]C.......A......A.T.A.T.........
AEDES TA TTGTTTTTTATAGATTATTTATTATCATTA
                    M  D  Y  L  L  S  L  IGSLLLVVCVMVGVAFLTLLKRKVLGYIQIRKGPNKMGFMGLLQPFSDTMKLF

HUMAN                          M  P  M  ANLLLLIVPILIAMAFLMLTERKILGYMQLRKGPNVVGPYGLLQPIADAMKLF

ASPERGILLUS         M  L  Y  L  I  S  I  IEGLLVIVPALLSVAFVTVAERKTMASMGRRLGPNAVGYYGLLQAFADALKLL
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Fig. 6. The $tRNA^{leu}_{UAG}$-URF1 junction in Aedes and Drosophila mitochondrial genomes. The Aedes results are from Fig. 2 and the Drosophila from ref. 32; the ends of the tRNA genes are boxed. Drosophila nucleotides corresponding to Aedes are shown by dots. For comparison, we present the N-terminal regions of human (23) and Aspergillus nidulans (33) mitochondrial URF1.

regard to sequence conservaton. Comparisons were made mainly to E. coli 23S RNA, the most extensively studied LSU RNA and the prokaryotic prototype, and to murine mit rRNA, as a representative of mammalian mit LSU RNA. As shown in Fig. 7, below, the 3' portion of the Aedes molecule (residues 635 through 1290) contained numerous regions of primary sequence homology to these other rRNAs; in contrast, we could find little such apparent sequence conservation in the 5' portion. This difference is correlated with average nucleotide composition (Table 1): the poorly conserved portion is much lower in G and C than the conserved portion. In fact, one might have scored the 5' 60 residues as high A-T spacer if not for the RNA data.

The conserved stretches of primary structure in the 3' half of the molecule guided us in drawing secondary structure models encompassing short and long range interactions (Fig. 7) that are similar to those believed to occur in E. coli 23S RNA. In particular, we used as a framework a recently published "revised" 23S RNA model (34), which takes into account data from direct chemical studies plus comparisons with other LSU RNA classes (3-5). As shown in the figure, residues 636-843 yielded a structure, bounded by long range interaction "51", that resembles 23S RNA domain "V" (4); and residues 890-1250 yielded a structure, bounded by long range interaction "63", that is highly homologous to 23S RNA domain "VI" (4). This latter domain is especially highly conserved, and is considered to contribute to the structure of the peptidyl transferase center of ribosomes (see refs 3-6).

The 3' portion of the Aedes sequence, although much higher in G+C than the 5' half, is still significantly lower in G+C (26%) than comparable stretches of 23S RNA (54%) or mammalian mit RNA (about 40%). There is no preferential usage of G and C in putative helical, vs. single-stranded, regions. Thus many of the Aedes helices are substantially richer in A,U or G,U base pairs than are their bacterial, or mammalian mitochondrial, homologues. We have indicated in Fig. 7 several sites at which highly conserved G-C or C-G

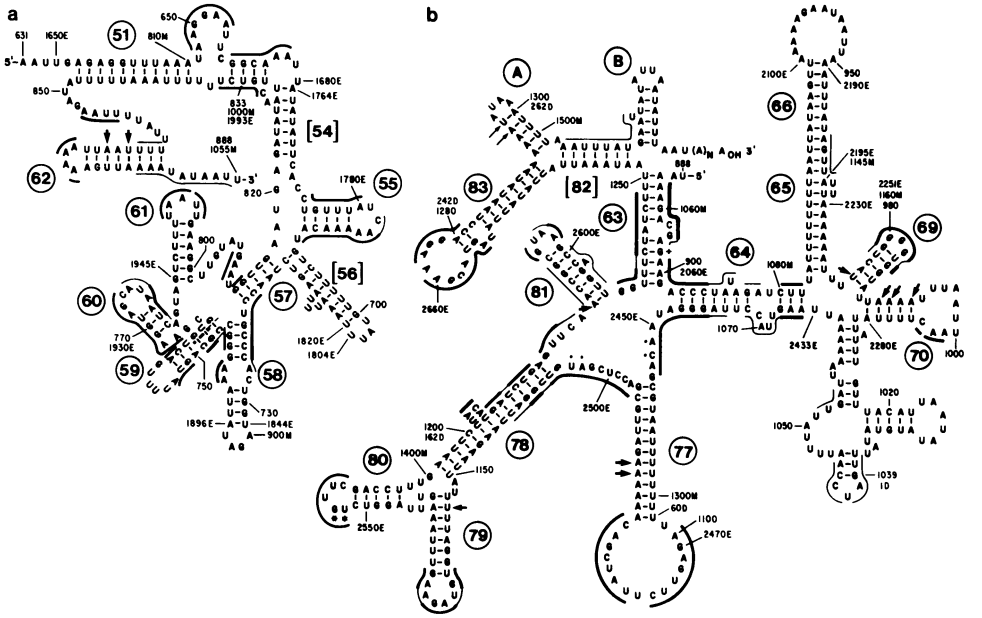

Fig. 7. Aedes mit LSU RNA secondary structure. Structures were patterned after those of E. coli 23S RNA, as described in the Text. Major features are numbered according to the system of Maly & Brimacombe (Fig. 5 of ref. 34); those supported by primary sequence homology are designated by encircled numbers, those not, by bracketed ones. The Aedes nucleotides are numbered as in Fig. 2; some corresponding numbers are also given for E. coli 23S RNA (suffix E) and murine mit LSU RNA (suffix M). D. yakuba residue numbers (suffix D) are from ref. 32, and do not reflect position in the rRNA. Heavy lines denote stretches whose primary sequence homology to E. coli and murine mit RNA aided in recognizing and substantiating secondary interactions; thin lines denote additional stretches of primary sequence homology to murine mit LSU RNA. The thick arrows indicate A-U or U-A pairs that replace highly conserved G-C or C-G pairs in other LSU RNAs; dots denote residues involved in chloramphenicol resistance in mammalian and yeast mitochondria (see ref. 35 for summary); the cross denotes the residue involved in erythromycin resistance in yeast mitochondria (8) and Staphylococcus aureus (37); and the asterisks denote methylated residues. The encircled letters in Panel B indicate putative dipteran-specific structures and the thin arrows here designate base pairs involved in compensating changes between Aedes and Drosophila. Panel A, Aedes residues 631-888; Panel B, residues 888 to the 3'-end.

pairs are replaced in the Aedes model with A-U or U-A pairs. There are many other helical stretches in the other LSU RNAs that lack conserved G,C pairs at particular sites, but that are nevertheless much higher overall in G,C pairs than corresponding Aedes stretches. For example, Aedes mit helices 54, 56, 65, 66, 77, 79 and 83 range in G+C content from 5 to 26%, compared to 20 to 77% for murine mit and 40 to 71% for E. coli. The many compensating nucleo-

tide changes involved in the Aedes helices provide strong support for their reality in both conventional, and mitochondrial, rRNA.

Another noteworthy facet of the Aedes secondary structure model is the high conservation of hairpin 80. Analogy to mammalian mit LSU RNA (7) and to 23S RNA (4) indicates that this is the site of the sole methylated subsequence of Aedes mit LSU RNA, Um•Gm•U (1). The loop of hairpin 69, the site of the only other methylated subsequence in mammalian mit LSU RNA (7), is also conserved, suggesting that its failure to be methylated in the Aedes system is a function of enzyme, rather than substrate, availability. We note also that the erythromycin sensitivity locus of both the Aedes and the mammalian mitochondrial LSU RNAs falls nicely into an extension of helix 63, whereas in bacterial (3-5), eukaryotic (6) and fungal mitochondrial (36,38) LSU RNA it falls just beyond this helix. Perhaps this apparent difference is related to the fact that mammalian mit ribosomes constitute an exception (see ref. 39) to the generalization that a G in this position confers erythromycin resistance whereas an unmodified A confers sensitivity (37,38). Examination of insect mit ribosomes for erythromycin sensitivity would be of considerable interest.

The Aedes model of Fig. 7 differs from that of E. coli 23S RNA in that structures 52, 53, 67, 68, and 71 through 76 of the latter are absent, and the loops of structures 56 and 58 are markedly shortened; mammalian mit LSU RNA resembles the Aedes mitochondrial in this regard. When the corresponding subsequences (Table 2) are excluded from consideration, the Aedes mit LSU RNA stretch of Fig. 7 shows 51% primary sequence homology to E. coli, and 64% homology to murine mitochondrial, LSU RNAs.

D. yakuba mit DNA sequences are available for regions corresponding to

Table 2. Low Homology Subsequences in Conserved Regions of LSU RNA.

Residue Number (structure)		
Aedes mit	Murine mit	E. coli
-	826-830	1679-1763 (52,53)
-	868-874	1805-1819 (56)
733-736	900-903	1845-1895 (58)
-	1088-1091	2092-2093 (65)
-	-	2101-2187 (67)
-	1146-1148	2196-2227 (68)
1011-1034	1192-1238	2282-2432 (71-76)

Except for the pair Aedes 733-736:Mouse 900-903, the tabulated stretches were excluded from the homology calculations cited in the Text. The numbers in parentheses (last column) indicate secondary structures in the 23S RNA model of Maly & Brimacombe (34) that contain the designated residues.

Table 3. Homology Relationships for Aedes and Drosophila Mit LSU RNA.

Aedes	Drosophila	Insertions	Deletions	Homology
1-29	296-324	0	0	93%
30-68	325-360	1	4	54%
69-140	361-432	0	0	93%
1039-1290	1-252	0	0	94%
1291-1335	253-296	1	2	76%

Aedes residue numbers are from the present work and Drosophila numbers are from refs. 29 and 32. Insertions and deletions refer to manipulations done on the Aedes sequence to maximize homology.

our rRNA residues 1 through 140, and 1039 to 1334 (refs. 29,32). The two sequences are quite similar over these stretches (average homology 88%) and the Aedes rRNA gene sequence is thus likely to be representative of insect, or at least dipteran, mit LSU RNA as a class. However, as is true for the homology relationships among the 3' portions of the Aedes mit, the murine mit, and the E. coli sequences (Fig. 7), the Aedes-Drosophila homology is patchy. There are three stretches of 93-94% homology and two of 54 and 76% homology, as summarized in Table 3. It is interesting that secondary structure "A" (Fig. 7), although corresponding to no structure in mammalian mit and E. coli sequences and occuring in a region of relatively poor Aedes-Drosophila primary sequence homology, is conserved in Drosophila by virtue of four compensating base changes (thin arrows in Fig. 7). This suggests that structure A indeed exists in dipteran mit LSU RNA. The putative Aedes mit structure "B" (Fig. 7) occurs where a conserved "quasi-attentuator" hairpin is found in mammalian mit LSU RNA genes (27), and a similar structure can be drawn for Drosophila mit LSU RNA (32). However, the dipteran mit structures lack the high G,C stems characteristic of the mammalian mit hairpins in question, and their homology to the mammalian structures is dubious.

There is an inverse correlation between chain length and G+C content of rRNA on the one hand, and relative amount and complexity of ribosomal proteins on the other (39). Nothing is known about dipteran mitochondrial ribosomes, but the present results lead us to expect that their protein complement, like their RNA, will prove to be most unusual, and different even from those of mammalian mit ribosomes.

ACKNOWLEDGMENTS

This work was supported by grant GM-14957 from the National Institutes of Health. R.M.K. is a predoctoral trainee under Institutional National Research

Service Award CA-09069. We thank K. Timko and L. Moon-McDermott for expert technical assistance and J. Klausneyer for drawing the structure diagrams.

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