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Methylated regions of hamster mitochondrial ribosomal RNA: structural and functional correlates

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ABSTRACT

The positions of post-transcriptionally methylated residues within hamster mitochondrial ribosomal RNA have been established. Comparisons with other mitochondrial rRNA, and with bacterial, eucaryotic and chloroplast rRNA show that the methylated regions i) are comprised of conserved primary sequences and/or secondary structures and ii) are situated at the subunit interface of the ribosome.

The comparative analyses also reveal that the ribose-methylated sequence UmGmU of hamster mitochondrial large ribosomal subunit (LSU^{1}) RNA lies in a universally conserved hairpin loop which contains a putative puromycin-reactive nucleotide. The "UmGmU hairpin" is within 100 nucleotides of two chloramphenicol-resistance residues of LSU RNA. We present a secondary structure for this region which is conserved in LSU RNAs. This structure allows physical juxtaposition of the three antibiotic-interacting loci and thus defines RNA components of the ribosomal-binding site for the 3'-terminus of aminoacyl-tRNA.

INTRODUCTION

An interesting feature of ribosomal RNA is the presence of post-transcriptionally methylated nucleosides. Comparisons of the minor nucleoside compositions of procaryotic (2-4), eucaryotic (5-9) and mitochondrial rRNAs (10-16) show that several of the methylated nucleosides have been conserved over a diverse phylogenetic range. Moreover, this conservation extends to the nucleotides immediately surrounding methylated residues; that is, the methylated nucleosides of rRNA are located in those regions of the molecule which demonstrate a high degree of primary sequence conservation (17,18).

Mitochondrial rRNAs have extremely simple modification patterns. 13S rRNA, the small ribosomal subunit (SSU) RNA of hamster mitochondria, contains one residue of m^5U, m^4C and m^5C and two residues of m^6_2A (10). The LSU RNA of hamster mitochondria, 17S rRNA, contains the ribose-methylated sequences GmG and UmGmU (11). In this paper we determine the nucleotide sequences of the methylated oligonucleotides from T_1 RNase and RNase A digests of hamster

mitochondrial rRNA. With these data the positions of the methylated nucleosides within 13S and 17S rRNAs can be localized by examining the primary sequences of hamster and mouse mitochondrial rRNAs (14,19). Comparisons with related sequences from other mitochondrial rRNAs (20-22) and from bacterial (23-25), eucaryotic (26,27) and chloroplast (28,29) rRNAs underscore the conserved nature of these methylated rRNA regions. In addition, we propose that the UmGmU region of 17S rRNA comprises part of the ribosomal binding site for the 3'-terminus of aminoacyl-tRNA.

Some of these results were presented briefly at the Conference on the Organization and Expression of the Mitochondrial Genome, held at Bari, Italy in June, 1980 (13).

METHODS

BHK-21 cells were grown and labeled in modified Eagle's medium supplemented with adenosine and guanosine to minimize flow of ^{3}H from [methyl-^{3}H]methionine into purine rings (11). 4 x 10^{8} Cells were incubated for 20 hours in one liter of medium containing 100 mCi of $^{32}P_i$ at 0.4 µmoles/ml and 15 mCi of [methyl-^{3}H]methionine at 7.9 µg/ml. The subsequent fractionation of cells and purification of mitochondrial rRNA, which has been described previously (11), yielded double-labeled RNA of sufficient specific activity for sequence analysis.

Methylated oligonucleotides were isolated and analysed by the sequencing procedures of Sanger and colleagues (30) and modifications thereof (31-35). T_1 RNase and RNase A digests of double-labeled RNA were fractionated by "mini-fingerprinting" (31) and the methylated oligonucleotides were identified by assaying autoradiographic fingerprint spots for the presence of tritium. For sequence determination, oligonucleotides were hydrolysed with any of the following enzymes: RNases T_1, T_2, A, U_2 and P_1, and snake venom phosphodiesterase. The P_1 RNase reaction was carried out at 37°C for 60' in 10 µl of 10 mM sodium acetate, pH 6.0, containing 5 µg of enzyme; the other RNase digestions were performed under conditions described by Brownlee (30). The reaction products were then separated by electrophoresis on filter paper at pH 3.5 (10,11), electrophoresis on DEAE-paper at pH 1.9 or pH 3.5 (30), chromatography on PEI-cellulose (33) or mini-fingerprinting (31). The identities of the reaction products were determined by co-migration with UV-absorbing standards or base composition analysis (32) and were quantitated by scintillation counting. The preparation and sequencing of 5'-endlabeled oligonucleotides has been described (34-35).

RESULTS

In vivo-labeled methylated oligonucleotides of mitochondrial rRNA

13S and 17S mitochondrial rRNAs were purified from BHK-21 cells uniformly labeled with ^{32}Pi and [methyl-^{3}H]methionine, and T_1 RNase digests of these RNAs were fractionated by mini-fingerprinting (Fig. 1). Each spot was assayed for ^{32}P, which provided a uniform nucleotide label, and ^{3}H, which provided a methyl-label. Those spots which contained significant methyl-label are listed in Table 1.

The molar yields of the tritiated spots 13S-T13 and 17S-T44 approached unity and these spots could thus be considered as radiochemically pure. The high molar yields of the other tritiated T_1-spots suggest that these are each comprised of a methylated oligonucleotide and unmodified oligonucleotide(s). 13S-T17 was resolved into two components by electrophoresis on DEAE-paper at pH 1.9: an unmethylated species, 13S-T17-I, with a base composition of $(C_2,U)G$, and a methylated species, 13S-T17-II, which yielded the sequence $m_2^6Am_2^6AAG$ upon further analysis (v.i.). It is noteworthy, but not surprising, that $m_2^6Am_2^6AAG$ migrates faster in both dimensions of the fingerprint than its unmodified congener, AAAG (see Fig. 1A). 17S-T84 lies precisely in the position expected for a GmGp dinucleotide (Fig. 1B); its high molar yield suggests the presence of some undigested GGp. The methylated components of 13S-T21 and 17S-T84 were not separated from co-migrating unmod-

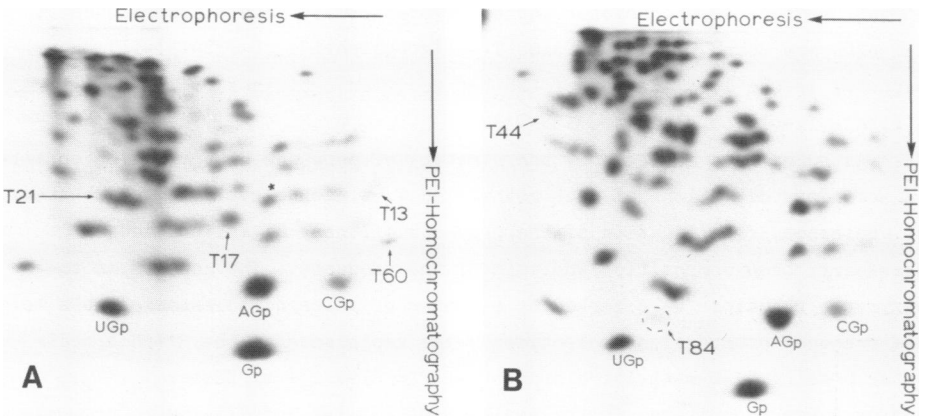

Figure 1. RNase T_1-fingerprints of double-labeled hamster mitochondrial rRNA; (A) 13S rRNA and (B) 17S rRNA. With the exception of T60, which is the unmethylated, 3'-terminal T_1-oligonucleotide of 13S rRNA (14), those spots indicated by an arrow contain methyl-label (Table 1). The unmodified spot AAAG of 13S rRNA is marked with an asterisk.

Table 1. Methylated T_1-Oligonucleotides

Methylated T_1-spot	CPM ^3H per Spot	Nucleotide Length	Apparent Molar Yield
13S-T13	237	4	1.4
13S-T17	413	4	5.3
13S-T21	153	5	9.8
17S-T44	245	11	0.90
17S-T84	275	2	3.0

The slurry corresponding to each spot of an RNase T_1-fingerprint (e.g., Fig. 1) was scraped into a scintillation vial and assayed for ^3H and ^{32}P. Aside from the spots listed, no spot contained more than 15 CPM of ^3H. The lengths of the oligonucleotides of each spot were estimated on the basis of the position of the spot in the fingerprint, together with the results of subsequent secondary analyses (see Table 2, below). Apparent molar yields were based on the amounts of ^{32}P in each spot relative to the total recovered from the fingerprint, assuming chain lengths of 956 and 1582 nucleotides, respectively, for 13S and 17S RNA (19), and the tabulated values for oligonucleotide lengths.

ified oligonucleotides. Regardless, sequence analysis of these methylated oligonucleotides did not require furthur purification (v.i.).

The compositions of the methylated T_1-oligonucleotides are displayed in the second column of Table 2. All, and only, the methylated nucleotides previously described in hamster mitochondrial rRNA are observed in these methylated oligonucleotides. In accord with the tandem arrangement of m_2^6Ap in the SSU RNAs of procaryotes and eucaryotes (3,9), both m_2^6Ap residues of 13S rRNA are found within the same T_1-oligonucleotide of 13S-T17-II. Less expected is the localization of the two modified cytidines of 13S rRNA to a single T_1-oligonucleotide, 13S-T13.

As summarized in Table 2, the strategy of sequence determination entailed secondary digestions of each methylated T_1-oligonucleotide with a battery of nucleases, fractionation of the products of these digestions and, if necessary, base composition analysis of the products. The results of the enzymatic reactions were marked by a number of idiosyncracies ascribable to the presence of modified nucleotides. The resistance of the 3'-phosphodiester bond of 2'-O-methylated nucleotides to cleavage by RNases T_1, T_2, and/or A was encountered in the analyses of 17S-T44 and 17S-T84. Curiously, the 3'-linkage of m_2^6A, although susceptible to T_2 RNase, was resistant to RNase U_2. The sequential 5'-exonucleolytic activity of spleen phosphodiesterase was abruptly arrested upon removal of the two 5'-terminal nucleotides of 17S-T44; further sequence analysis (v.i.) revealed that the succeed-

Table 2: Secondary Digestion Products of the Methylated T_1-Oligonucleotides

Methylated T_1-Oligo	T_2 RNase	RNase A	U_2 RNase	P_1 RNase	partial Spleen Phosphodiesterase	Deduced Sequence
13S-T13	m^4Cp m^5Cp Cp Gp	m^4Cp m^5Cp Cp Gp		m^4C pm^5C pC pG	intact oligo	$m^4C(m^5C,C)Gp$
13S-T17-II	$2m_2^6Ap$ Ap Gp	intact oligo	$m_2^6Am_2^6AAp$ Gp	m_2^6A pm_2^6A pA pG	intact oligo	$m_2^6Am_2^6AAGp$
13S-T21	m^5Up	Am^5Up	$(m^5U,U)Ap$	pm^5U		Am^5UUAGp
17S-T44	UmGmUp 3Up 2Ap 2Cp Gp	UmGmUp AACp Cp 3Up Gp	$(U_3,C,UmGmU)Ap$ CGp Ap	pUm 2pC pGm 2pA pG 3pU	$(UmGmU,C_2,A_2,U_3)Gp$ $(UmGmU,C_2,A_2,U_2)Gp$ $(UmGmU,C_2,A_2,U_1)Gp$	UU(UmGmU,C,U)AACGp
17S-T84	GmGp					GmGp

Digestion procedures, and the subsequent fractionation and identification of digestion products, are described in METHODS. Since the methylated components of 13S-T21 and 17S-T84 were not purified, we present only the methylated digestion products of these spots, as detected by the ^3H label. Here and elsewhere we use standard abbreviations for methylated residues; E.g., m_2^6A, N^6,N^6,-dimethyladenosine; Um, $O^{2'}$-methyluridine; also, oligo, oligonucleotide.

ing residue is 2'-O-methyluridine, evidently an improper substrate for the enzyme. Likewise, the inability of spleen phosphodiesterase to digest 13S-T13 or 13S-T17-II can be attributed to the occurence of modified nucleotides at the 5'-termini. RNase P_1, which selectively reduces the 5'-terminal residue of a T_1-oligonucleotide to a riboside, proved particularly useful for the sequence analysis of 13S-T13 and 13S-T17-II. These oligonucleotides yielded the nucleosides m^4C and m_2^6A, respectively, upon digestion with P_1.

In order to corroborate, and perhaps extend, the sequences of the methylated T_1-oligonucleotides, we fractionated RNase A digests of double-labeled mitochondrial rRNA and scanned the resultant fingerprints for spots containing ^3H. The base composition of each tritiated A-spot was estimated from its fingerprint position. T_2 RNase digestion analysis provided verification of the base composition and identified the methylated nucleoside(s) associated with each tritiated A-spot. These data, in conjunction with the sequences of the corresponding methylated T_1-oligonucleotides, allowed sequence determination of the methylated A-oligonucleotides (Table 3).

Mobility shift analysis of the UmGmU-containing T_1-oligonucleotide

To further define the sequence around UmGmU, we prepared 5'-endlabeled, UmGmU-containing T_1-oligonucleotide. Unlabeled 17S rRNA was digested to completion with both T_1 RNase and bacterial alkaline phosphatase, and the resultant oligonucleotides were 5'-phosphorylated with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP (34). 2-Dimensional fractionation of the reaction products yielded a fingerprint containing the larger (Np>5) T_1-oligonucleotides. Comparisons with in vivo-labeled fingerprints such as that of Figure 1B permitted us to identify the 5'-endlabeled UmGmU-containing

Table 3. Methylated Oligonucleotides from RNase A Digests of Hamster Mitochondrial rRNA

13S rRNA	17S rRNA
m^4Cp Gm^5Cp	UmGmUp
$m^4Cp>$ $Gm^5Cp>$	UmGmUp>
$GGm_2^6Am_2^6AAGUp$	G(GmG,G)Up
$GGGAm^5Up$	

Many of the small oligonucleotides were released by RNase A as the 2'-3'-cyclic isomer (indicated by the symbol ">"). As with 17S-T17, the m_2^6A-containing oligonucleotide migrated exceptionally fast in both dimensions of the fingerprint

oligonucleotide, which we designated 17S-TK44. An aliquot was digested exhaustively with T_2 RNase and analysed by electrophoresis on DEAE-paper; as expected, >85% of the ^{32}P migrated with the optical density marker for pUp. The sequence of 17S-TK44 was revealed by a mobility shift experiment which entailed a partial digestion of the oligonucleotide with snake venom phosphodiesterase (27) followed by 2-dimensional fractionation of the product (Fig. 2). The deduced sequence, pUUUmGmUUCAACG, agrees with our data on the *in vivo*-labeled oligonucleotide, 17S-T44.

DISCUSSION

The nucleotide sequences around the methylated residues of hamster mitochondrial rRNA, as derived from sequences of the corresponding methylated oligonucleotides, are listed in Table 4. Recently, the complete sequence of the mouse mitochondrial ribosomal DNA has been established (19). Each of the five methylated sequences of hamster mitochondrial rRNA can be located uniquely in one site of the respective mouse mitochondrial rRNA gene (Table 4).

The m^4C, m^5C and m_2^6A residues of mitochondrial SSU RNA

We had previously determined the sequence and secondary structure of the

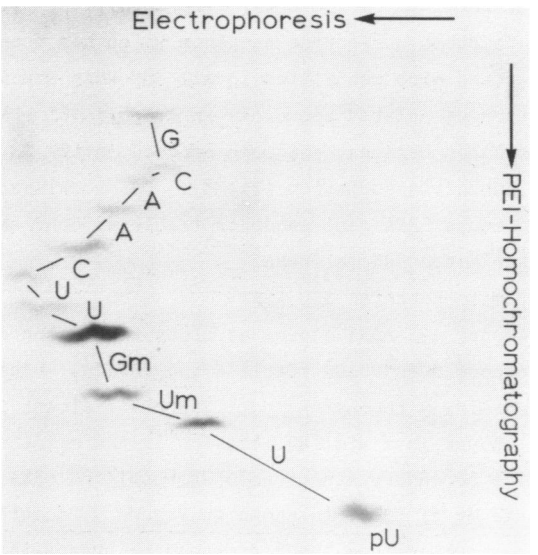

Figure 2. A mobility shift experiment revealing sequence of 17S-TK44, the 5'-endlabeled, UmGmU-containing T_1-oligonucleotide of 17S rRNA.

Table 4. Methylated Sequences of Hamster Mitochondrial rRNA

rRNA	sequences around methylated residues	positions of methylated residues	
13S	YGm^{4}C(C,m^{5}C)G	841, 842-843	88%
13S	YGGm$_2^6$Am$_2^6$AAGU	938, 939	98%
13S	YGGGAm^{5}UUAG	426	45%
17S	GUUUmGmUUCAACG	1388, 1389	88%
17S	YG(GmG,G)U	1160-1161	73%

Sequences around the methylated residues were derived from the sequences of the corresponding methylated oligonucleotides. The positions of the methylated nucleosides are indicated by nucleotide distance and percent distance from the 5'-end of the mouse mitochondrial 13S rRNA gene (chain length is 956) or 17S rRNA gene (chain length is 1582).

3'-terminal 220 nucleotides of hamster mitochondrial 13S rRNA (14). The positions of m^4C m^5C and m_2^6A within this sequence were established and comparisons with eucaryotic and procaryotic SSU RNAs demonstrated the conservation of primary and secondary structure in the vicinity of these methylated nucleosides. The tandem m_2^6A residues are located in the loop of the universal hairpin near the 3'-terminus of 13S rRNA. The methylated cytidines of 13S rRNA lie within a thirteen nucleotide sequence which has been found to be perfectly conserved and also methylated in all SSU RNAs studied (14). The kethoxal-reactivity pattern of related sequences in *E. coli* 16S rRNA indicates that both of these methylated regions, although exposed in the SSU, are obscured by the LSU during subunit association (36,38). Our localization of m^4C, m^5C and m_2^6A within the mouse rRNA gene sequence (Table 4) confirms the previous results with hamster mitochondrial rRNA.

The m^5U residue of mitochondrial SSU RNA

The m^5U of hamster 13S rRNA occurs at about the center of the molecule, as part of the sequence GGAm^{5}UUAGA (Table 4 and ref.19). The octanucleotide can also be located midway through *E. coli* 16S rRNA, albeit in unmodified form (23,24), and occurs in a similar location in the maize chloroplast 16S rRNA gene (28). The secondary structure of *E. coli* 16S rRNA in this region, as inferred by Woese *et al* (36), is shown in Figure 3, together with homologous structures constructed from the corresponding organellar rRNA sequences. Kethoxal studies have shown that in *E. coli* ribosomes this structure occurs at the 30S-50S interface (37,38). Although the *E. coli* sequence is unmodi-

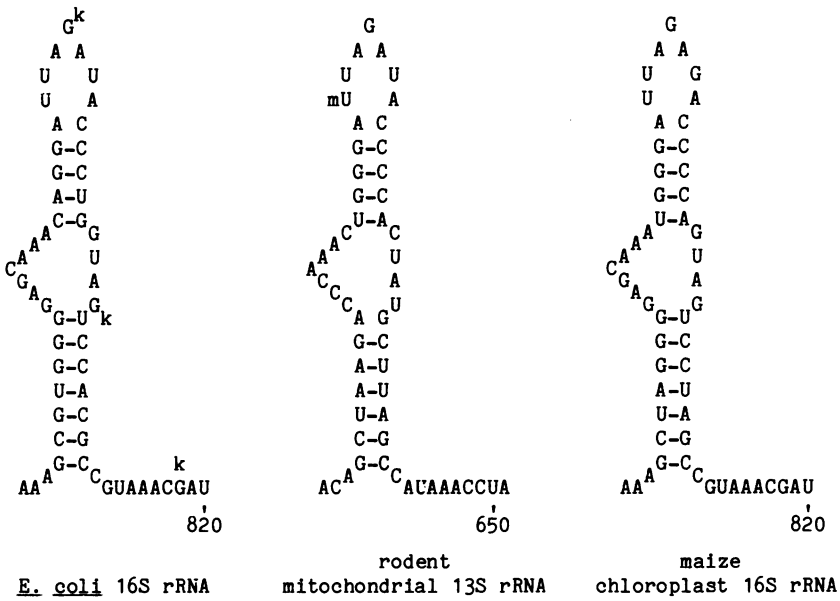

Figure 3. The conserved secondary structure of the m^5U region of 13S rRNA. The rodent mitochondrial sequence was derived from hamster (Table 4) and mouse (19) mitochondrial sequences. The E. coli 16S rRNA secondary structure is from Woese et al (39) and the organellar rRNA structures were derived by comparison with it. Kethoxal-reactive guanines are marked with a "k". To facilitate reference to the original papers, here and in Figures 4 and 5 the original numbering systems are used. Note that these systems do not necessarily begin counting at the 5'-terminal residue of transcripts: hence the differences from Table 4. Also, note that 16S RNA residue 820 of ref. 39 corresponds to residue 819 of refs. 23 and 24.

fied, a T_1-oligonucleotide derived from the loop, AUUAGp, is universal among procaryotes ("oligomer 33", ref. 17; also see 40,41), and in at least one organism (Rhodopseudomonas spheroides) the first U is modified (42). In this connection it is interesting to note the claim that plant mitochondria have arisen from purple photosynthetic bacteria (43), a group that includes R. spheroides (also see ref. 44).

The universal UmGmU hairpin of LSU RNAs

We have proposed that the UmGmU sequence of hamster mitochondrial 17S rRNA is homologous to the universal UmGmΨ sequence of eucaryotic 28S rRNA and to a UmG sequence of fungal mitochondrial and E. coli LSU RNAs (11). A recent study revealed the presence of GmU in mycoplasma 23S rRNA (4), and indeed every LSU RNA examined to date contains either the UmGmΨ sequence or

one of its undermodified congeners: UmGmU, UmG or GmU. The present work supports this idea and permits us to extend it. The UmGmU sequence of hamster mitochondrial 17S rRNA is located at a distance of about 12% from the 3'-end of the molecule (Table 4). The nucleotide residues immediately flanking it can form a stable hairpin comprised of a five base-pair stem and a five residue, methylated loop (Fig. 4). Similar hairpins can be constructed from homologous sequences located at a distance of 10-15% from the 3'-ends of E. coli (25), yeast mitochondrial (22), human mitochondrial (nucleotides 3034-3048 of ref. 21) and rat mitochondrial (nucleotides 1495-1509 of ref. 20) LSU RNAs; in each case, the loop sequence UGUUC has been strictly conserved. Also, the sequence of the highly conserved, UmGmΨ-containing T_1-oligonucleotide of vertebrate 28S rRNA (27) has the potential to form an analogous hairpin structure (Fig. 4). Recently the position of the UmGmΨ has been localized to within the 3'-terminal 20% of 28S rRNA (45).

The UmG of E. coli 23S rRNA occurs in the T_1-oligonucleotide CUmG (3). Its position in the molecule cannot be established on the basis of its sequence alone because GCUG occurs 22 times in 23S rRNA. However, a GCUG sequence lies within the E. coli 23S rRNA hairpin depicted in Figure 4, in precisely the position which would render the UmG moiety homologous to the UmGm sequence of mammalian mitochondrial and vertebrate LSU rRNAs. Likewise, we propose that the putative UmG of yeast mitochondrial LSU RNA (11,16) is also located in a homologous hairpin (Fig. 4). We also believe that a

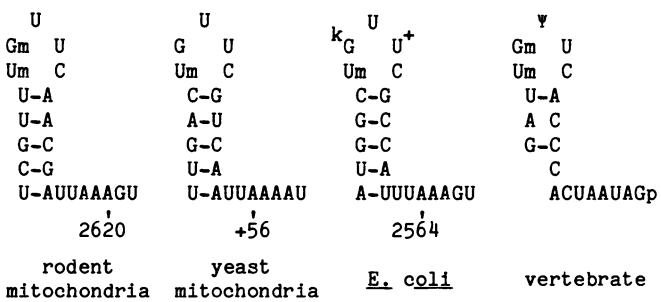

Figure 4. The universal UmGmU hairpin of LSU RNA. The rodent mitochondrial hairpin was derived from hamster (Table 3) and mouse (19) sequences; the yeast mitochondrial hairpin was derived from the sequence of Dujon (22); the E. coli hairpin from the sequence of Brosius et al (25); and the vertebrate hairpin from the sequence of a T_1 oligonucleotide found in human, rat, mouse, and chicken 28S RNA (27). Kethoxal-reactive and puromycin-reactive nucleotides are marked with "k" and "+", respectively.

ribose-methylated T_1-oligonucleotide reported (46) for Neurospora crassa mitochondrial LSU RNA, (A,C,U)Gp, will prove to be ACUmGp since the corresponding GACUG sequence is found in the UmG hairpin of yeast mitochondrial LSU RNA (Fig. 4).

The pertinent sequence of Chlamydomonas chloroplast LSU RNA, nucleotides 2061-2073 of ref. 29 (cf ref. 22), cannot form the proposed hairpin. However, the sequence in this region may be uncertain because it contains an unidentified residue (nucleotide 2071) and the only two deletions (relative to E. coli 23S rRNA) in the 190 nucleotide sequence derived for the chloroplast rRNA. It would be of interest to examine the methylation pattern of chloroplast 23S rRNA, and perhaps to reexamine its primary sequence.

In sum, these observations suggest that the "UmGmU hairpin" is a universal feature of LSU RNAs and, as such, can be expected to play a vital role during protein synthesis. Recent studies of LSU-binding antibiotics provide clues to the nature of this role. Greenwell et al (47) have been able to affinity label E. coli ribosomes specifically at the A' site with a reactive puromycin derivative. The reactive alkylating group of this substance, which is presumably analogous to the 3'-penultimate cytosine of aminoacyl-tRNA, forms a covalent bond with 23S rRNA at the pyrimidine residue Y in the sequence GUYCG (48). This sequence is found four times in 23S rRNA (Y positions found at nucleotides 964, 2555, 2605 and 2690, counting from the 5'-end; cf ref. 25); interestingly, one of these potential puromycin-reactive sites occurs in the loop of the E. coli UmG hairpin (Fig. 4). Herr and Noller have shown that the guanine residue of the 23S rRNA T_1-oligonucleotide CUmG is sensitive to kethoxal treatment of E. coli 50S subunits but remains unreactive during similar treatment of the 70S ribosome (49). This indicates that the UmG hairpin of 23S rRNA lies at the 30S-50S subunit interface, where the puromycin-binding site would be expected.

Dujon has ascribed the chloramphenicol resistance of two yeast strains to single base substitutions in the mitochondrial LSU RNA at positions corresponding to nucleotides 2447 and 2503 of E. coli 23S rRNA (22,25). Chloramphenicol inhibits protein synthesis by competitively interfering with the binding of the 3'-terminal two or three nucleotides of aminoacyl-tRNA (50). Therefore, in the active ribosome, the two chloramphenicol-resistance sites and the puromycin-reactive site, although fairly well separated in the primary sequence of LSU RNA, probably converge to participate in the binding of the 3'-terminus of aminoacyl-tRNA. To begin to define the molecular conformation of this portion of the peptidyltransferase center we have examined

possible secondary structure of LSU RNA in the region. Previously the comparative approach had proven successful for uncovering secondary interactions of rRNA (14,39,51). In addition to the yeast mitochondrial sequence referred to above and of course that for E. coli 23S rRNA, extensive sequence data for regions flanking putative "UmGmU hairpins" are available for mouse, rat and human mitochondrial LSU RNA (19-21). By aligning all these regions and searching for local complementarities that are conserved despite divergence of primary sequence, we were able to generate a common secondary structure, as shown for E. coli and mouse mitochondrial LSU RNAs in Figure 5. As noted above, the sequence presented by Allet and Rochaix for chloroplast 23S rRNA (29) cannot form the "UmGmU hairpin ; nevertheless the four other secondary interactions illustrated in Figure 5 can all be constructed from that sequence. In any case, it can be seen that the proposed common secondary structure is compact by virtue of extensive hairpin formation, in good accord

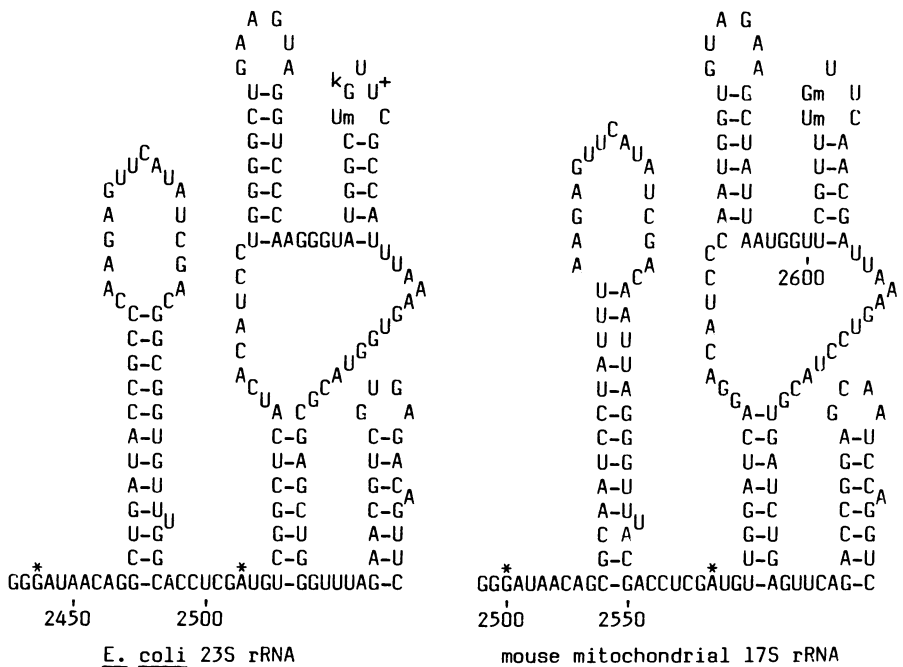

Figure 5. Secondary structure of LSU RNA encompassing the binding sites for the 3'-terminus of aminoacyl-tRNA. Asterisks indicate sites homologous to the chloramphenicol-resistance sites determined for yeast mitochondrial LSU RNA (22). "K" and "+" indicate kethoxal- and puromycin-reactive sites as determined for E. coli 23S RNA (cf Text).

with the idea that puromycin and chloramphenical binding sites are indeed close to one another in the ribosome.

Vertebrate 28S rRNA contains in molar quantities not only the UmGmΨ sequence discussed above but also a UmGmU trinucleotide (8,9,11). Our argument that mitochondrial UmGmU is homologous to eucaryotic UmGmΨ was based on kinetics of methylation, the phylogenetic distribution of UmGmΨ, and the general absence of pseudouridine in mitochondrial rRNA (11). The structural homology between the UmGmU region of 17S RNA and the UmGmΨ-containing T_1-oligonucleotide of 28S RNA (Fig. 4) provides further support for this proposal. The presence of UmGmU in vertebrate 28S rRNA thus remains a curiosity which cannot be explained satisfactorily at present. Perhaps its synthesis is a secondary, and biologically insignificant, activity of the methylase(s) responsible for the production of UmGmΨ. More interestingly, one could speculate that the vertebrate UmGmU sequence may represent a structure in the ribosomal P' site which is functionally analogous to the "UmGm hairpin" in the A' site.

The GmG sequence of mitochondrial LSU RNA

GmG of hamster mitochondrial 17S rRNA can be localized to a position 73% of the distance from the 5'-terminus (Table 4). The surrounding sequence UGGGGUGACCUC does not have an obvious counterpart within the published LSU RNA sequences of *E. coli*, maize chloroplast or yeast mitochondria. This raises the possibility that our localization of GmG may have been misled by a sequence change between hamster and mouse mitochondrial LSU RNAs. However, if our localization is accurate, then a partially homologous sequence UGGUCGGACAUC can be found 79% from the 5'-end of *E. coli* 23S rRNA (nucleotides 2302-2313 of ref. 25) Furthermore, the kethoxal-sensitivity pattern of this sequence indicates that it lies on the 50S particle at the 30S-50S interface (49). The proposed homology is attractive because it preserves the generalization that all of the methylated regions of rodent mitochondrial rRNA are situated at the ribosomal subunit interface. Certainly their conserved nature, and the genetic investment required for their synthesis, suggest that the post-transcriptional modifications perform important functions. Thus it is not surprising that the methylated regions of rodent mitochondrial rRNA are found at the ribosomal subunit interface, where central processes of protein synthesis occur.

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REFERENCES

1. Abbreviations: SSU, small ribosomal subunit; LSU, large ribosomal subunit.
2. Dubin, D.T. and Gunalp, A. (1967) Biochim. Biophys. Acta 134, 106-123.
3 Fellner, P. and Sanger, F. (1968) Nature 219, 236-238.
4. HsuChen, C.-C. and Dubin, D.T. (1980) J. Bacteriol., in press.
5. Lane, B.G. (1965) Biochem. 4, 212-219.
6. Klootwijk, J. and Planta, R.J. (1973) Eur. J. Biochem. 39, 325-333.
7. Maden, B.E.H. and Tartof, K. (1974) 90, 51-64.
8. Hashimoto, S., Sakai, M. and Muramatsu, M. (1975) Biochem. 14, 1956-1964.
9. Khan, M.S.N., Salim, M. and Maden B.E.H. (1978) Biochem. J. 169, 531-542.
10. Dubin, D.T., Taylor, R.H. and Davenport, L.W. (1978) Nuc. Acids Res. 5, 4385-4397.
11. Dubin, D.T. and Taylor R.H. (1978) J. Mol. Biol. 121, 523-540.
12. Dubin, D.T., Baer, R.J., Davenport, L.W., Taylor, R.H and Timko, K. (1979) in "Transmethylation," Usdin, E., Borchardt, R.T. and Creveling, C.R., eds. Elsevier/North Holland, New York, pgs. 389-398.
13. Dubin, D.T. and Baer R.J. (1980) in "The Organization and Expression of the Mitochondrial Genome," Kroon, A.M. and Saccone, C., eds., Elsevier/North Holland, New York, in press.
14. Baer, R. and Dubin D.T. (1980) Nuc. Acids Res., in press.
15. Lambowitz, A.M. and Luck, D.J.L. (1975) J. Mol. Biol. 96, 207-214.
16. Klootwijk, J., Klein, I. and Grivell L.A. (1975) J. Mol. Biol. 97, 337-350.
17. Woese C.R., Fox, G.E., Zablen, L., Uchida, T., Bonen, L., Pechman, K., Lewis, B.J. and Stahl, D. (1975) Nature 254, 83-86.
18. Brand, R.C. and Gerbi, S.A. (1979) Nuc. Acids Res. 7, 1497-1511.
19. Van Etten, R.A., Walberg, M.W. and Clayton, D.A. (1980) Cell, in press.
20. Saccone, C., Cantatore, P., Pepe, G., Holtrop, M., Gallerani, R., Quagliariello, C., Gadaleta, G. and Kroon, A.M. (1980) in "The Organization and Expression of the Mitochondrial Genome," Kroon, A.M. and Saccone, C., eds., Elsevier/North Holland, New York, in press.
21. Eperon, I.C., Anderson, S. and Nierlich, D.P. (1980) Nature 286, 460-467.
22. Dujon, B. (1980) Cell 20, 185-197.
23. Brosius, J., Palmer, M.L., Kennedy, P.J. and Noller, H.F. (1978) Proc. Natl. Acad. Sci. USA 75, 4801-4805.
24. Carbon, P., Ehresmann, C., Ehresmann, B. and Ebel, J.P. (1979) Eur. J. Biochem. 100, 399-410.
25. Brosius, J., Dull, T.J. and Noller, H.F. (1980) Proc. Natl. Acad. Sci. USA 77, 201-204.
26. Rubtsov, P.M., and Skryabin, K.G. (1979) Dokl. Akad. Nauk USSR 248, 760-762.
27. Eladari, M.-E., Hampe, A. and Galibert, F. (1977) Nuc. Acids Res. 4, 1759-1767.

28. Schwarz, Zs. and Kossel, H. (1980) Nature 283, 739-742.
29. Allet, B. and Rochaix, J.D. (1979) Cell 18, 55-60.
30. Brownlee, G.G. (1972) "Determination of Sequences in RNA." American Elsevier New York.
31. Volckaert, G., Min Jou, W and Fiers, W. (1976) Anal. Biochem. 72, 433-446
32. Volckaert, G. and Fiers, W. (1977) Anal. Biochem. 83, 222-227.
33. Volckaert, G. and Fiers, W. (1977) Anal. Biochem. 83, 228-239.
34. Frisby, F. (1977) Nuc. Acids Res. 4, 2975-2996.
35. Silberklang, M., Gillum, A.M. and RajBhandary U.L. (1977) Nuc. Acids Res. 4, 4091-4108.
36. Santer, M. and Shane, S. (1977) J. Bacteriol. 130, 900-910.
37. Chapman, N.M. and Noller, H.F. (1977) J. Mol. Biol. 109, 131-149.
38. Herr, W., Chapman, N.M. and Noller, H.F. (1979) J. Mol. Biol. 130, 433-449.
39. Woese, C R., Magrum, L.J., Gupta, R., Siegel, R.B., Stahl, D.A. Kop, J., Crawford, N., Brosius, J., Gutell, R., Hogan, J.J. and Noller, H.F. (1980) Nuc. Acids Res. 8, 2275-2293.
40. Fox, G.E., Magrum, L.J., Balch, W.E., Wolfe, R S. and Woese, C.R. (1977) Proc. Natl. Acad. Sci. USA 74, 4537-4541.
41. Bonen, L., Doolittle, W.F. and Fox, G.E. (1979) Can. J. Biochem. 57, 879-888.
42. Zablen, L. and Woese, C.R. (1975) J. Mol. Evol. 5, 25-34.
43. Fox, G.E., Stackebrandt, E., Hespell, R.B., Gibson, J., Maniloff, J. Dyer, T.A., Wolfe, R.S., Balch, W.E., Tanner, R.S., Magrum, L.J., Zablen, L.B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B.J., Stahl, D.A., Luehrsen, K.R., Chen, K.N. and Woese, C.R. (1980) Science 209, 457-463.
44. Dickerson, R.E., Timkovich, R. and Almassy, R.J. (1976) J. Mol. Biol. 100, 473-491.
45. Maden, B.E.H. and Reeder, R.H. (1979) Nuc. Acids Res. 6, 817-830.
46. Lambowitz, A.M. and Luck, D.J.L. (1976) J. Biol. Chem. 251, 3081-3095.
47. Greenwell, P., Harris, R.J. and Symons R.H. (1974) Eur J. Biochem. 49, 539-555.
48. Eckermann, D.J. and Symons, R.H. (1978) Eur. J. Biochem. 82, 225-234.
49. Herr, W. and Noller, H.F. (1979) J. Mol. Biol. 130, 421-432.
50. Nierhaus, D. and Nierhaus, K.H. (1973) Proc. Natl. Acad. Sci. USA 70, 2224-2228.
51. Fox, G.E. and Woese, C.R. (1975) Nature 256, 505-507.