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The secondary structure of the protein L1 binding region of ribosomal 23S RNA. Homologies with putative secondary structures of the L11 mRNA and of a region of mitochondrial 16S rRNA

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ABSTRACT

An heterologous complex was formed between *E. coli* protein L1 and *P. vulgaris* 23S RNA. We determined the primary structure of the RNA region which remained associated with protein L1 after RNase digestion of this complex. We also identified the loci of this RNA region which are highly susceptible to T1, S1 and *Naja oxiana* nuclease digestions respectively. By comparison of these results with those previously obtained with the homologous regions of *E. coli* and *B. stearothermophilus* 23S RNAs, we postulate a general structure for the protein L1 binding region of bacterial 23S RNA. Both mouse and human mit 16S rRNAs and *Xenopus laevis* and *Tetrahymena* 28S rRNAs contain a sequence similar to the *E. coli* 23S rRNA region preceding the L1 binding site. The region of mit 16S rRNA which follows this sequence has a potential secondary structure bearing common features with the L1-associated region of bacterial 23S rRNA. The 5'- end region of the L11 mRNA also has several sequence potential secondary structures displaying striking homologies with the protein L1 binding region of 23S rRNA and this probably explains how protein L1 functions as a translational repressor. One of the L11 mRNA putative structures bears the features common to both the L1-associated region of bacterial 23S rRNA and the corresponding region of mit 16S rRNA.

INTRODUCTION

We recently further characterized the *E.coli* 23S RNA region associated with *E.coli* ribosomal protein L1 (1). It is now of interest to determine the secondary structure of this 23S RNA region. When looking for the secondary structure of an RNA molecule, it is very useful to identify the positions in this RNA which are preferentially cleaved by T1, S1 and *Naja oxiana* (2) nucleases. The nucleotides preferentially cut by the two former enzymes are in single-stranded regions, whereas those preferentially hydrolysed by the third are involved either in secondary or in tertiary interactions (3). Knowledge of the primary structure of this RNA molecule in different organisms is also very informative, since base-changes from one organism to another often compensate each other in order to conserve the secondary structure of the molecule. As *E.coli* protein L1 is able to bind *P.vulgaris* 23S RNA, it is of interest to

study the RNA region which remains associated to protein L1 after T1 RNase digestion of the complex.

This paper relates the determination of the nucleotide sequence of this L1-associated region of *P. vulgaris* 23S RNA, it also describes an enzymatic study of its secondary structure. Comparison of these data with those previously obtained upon studying the homologous regions of *E.coli* (1) and *B. stearothermophilus* (4) 23S RNAs allowed us to determine the general structure of the bacterial 23S RNA region associated with protein L1. Since genes of mouse and human mitochondrial 16S rRNAs have been recently sequenced (5-6), we looked for a similar structure in these RNAs. The present study also provided information very useful for identifying which nucleotide sequences from the L1-associated 23S RNA region are directly interacting with protein L1. As the L11 mRNA is expected to be the target of protein L1 translational repression (1,7,8), we looked for common structural features between this mRNA and the region of protein L1 binding on 23S RNA.

MATERIALS AND METHODS

1. Preparation of 23S RNA. The strain of *P. vulgaris* was kindly provided by Dr. Monteil. Cold 50S subunits were prepared as previously described (9) from cultures grown for 3.5 hours in a rich medium (per 1 liter : glucose 10g, yeast extract 6g, KH_2PO_4 8.5g, K_2HPO_4 14.4g and nicotinamide 1mg). ^{32}P labeled 50S subunits were extracted from cells grown for the same time, but in the minimum medium of Garen and Levinthal (10) supplemented with 1mg/l of nicotinamide and 20 to 50 mC of ^{32}P orthophosphate.

23S RNA was phenol extracted from the subunits with SSC-EDTA buffer and 1 % Na dodecylsulfate as described by Nierhaus and Dohme (11). It was then precipitated with ethanol. Remaining phenol and Na dodecylsulfate were eliminated by centrifugation through a sucrose cushion.

2. Preparation and dissociation of the ribonucleoprotein complex. 5 to 10 mg of uniformely labeled 23S RNA were complexed with 1 to 2mg of *E.coli* protein L1, in 0.35M KCl, 10mM $MgCl_2$, 10mM Tris-HCl pH 7.5 buffer at 42°C for half an hour. Protein L1 was a generous gift of Dr. H.G. Wittmann. The complex was then digested with T1 RNase at an enzyme substrate ratio between 1/10 and 1/20 (w/w), for 30 mn at 0°C. The resulting ribonucleoprotein complex was fractionated by electrophoresis on an 8 % polyacrylamide gel containing 10mM Mg^{++}, it was then dissociated by electrophoresis on a 15 % polyacrylamide gel containing 8M urea and 0.1 % Na dodecylsulfate (12). The resulting RNA subfragments

were eluted from the gel.

3. Fingerprint analysis of the RNA subfragments. Uniformely labeled RNA subfragments were digested with T1 and pancreatic RNases. The resulting products were fractionated by two-dimensional electrophoresis on DEAE paper (13).

4. Sequence analysis of 5'-end labeled RNA subfragments. 5'-end labeling was achieved using {γ-^{32}P} ATP (Amersham) and T4 polynucleotide kinase (14). After purification by polyacrylamide gel electrophoresis, the 5'-end labeled RNA subfragments were partially digested with T1, U2, A and *Phy* I RNases as previously described (14). The ladder was obtained by a thermal degradation in boiling water. To fractionate the digest we used the classical one dimensional electrophoresis on thin polyacrylamide gel slabs in Tris-borate buffer and the two-dimensional system as previously described (14).

5. Secondary structure study of 5'-end labeled RNA subfragments. Digestions were performed with T1, S1 and *Naja oxiana* nucleases (2). 2.5µg of carrier tRNA was added in each essay. Immediately after digestion the RNA was loaded on polyacrylamide slab gels (300 X 900 X 0.5mm) made up in Tris-borate buffer.

Digestion with T1 RNase was performed in 6µl of 10mM Tris-HCl pH 7.5 buffer containing 1 or 10mM $MgCl_2$. After a 10 min preincubation at 37°C, the hydrolysis was performed for 2-10 min at the same temperature with 0.01 U of T1 RNase. It was stopped by addition of 1µl of 400mM NaH_2PO_4 pH 3.3 (15). Digestion with S1 nuclease was performed in 6µl 50mM KCl, 1mM $ZnCl_2$, 25mM Na acetate pH 4.5 containing 10mM $MgCl_2$. After a 10 min preincubation at 37°C the digestion was performed for 2-10 min at the same temperature with 2.5 U of S1 nuclease. It was stopped by addition of 5µl 10mM ATP (15). Digestion with *Naja oxiana* RNase was carried out for 2-10 min at 0°C in 6µl 350mM KCl, 10mM $MgCl_2$, 10mM Tris-HCl pH 7.5 buffer. It was stopped by addition of 2µl 100mM EDTA.

RESULTS AND DISCUSSION

1. The region of *P.vulgaris* 23S RNA associated with *E.coli* protein L1

1.1 Preparation and dissociation of the heterologous ribonucleoprotein. An heterologous complex was formed between *P.vulgaris* 23S RNA uniformely labeled with ^{32}P, and *E.coli* protein L1. It was partially digested with T1 RNase. A resistant ribonucleoprotein complex was isolated upon fractionation of the digest on a polyacrylamide gel containing 10^{-2}M Mg^{++} (Fig. 1). This ribonucleoprotein complex was dissociated by polyacrylamide gel electrophoresis in

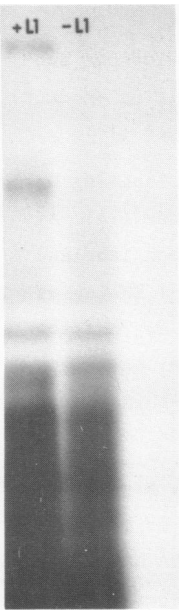


Figure 1 : Fractionation of the ribonucleoprotein released upon digestion of the complex formed between *E.coli* protein L1 and *P.vulgaris* 23S RNA.

the presence of 8M urea and 0.1 % Na dodecylsulfate. 7 RNA subfragments were released.

1.2 Sequence analysis of the RNA subfragments. Each 5'-end labeled RNA subfragment was digested with T1, U2, A and *Phy I* RNases and was thermically degraded in boiling water. The various digests were fractionated by monodimensional polyacrylamide gel electrophoresis. Inspection of the gel autoradiographs allowed identification of guanines, adenines and pyrimidines. A partial distinction between uracils and cytosines was obtained from *Phy I* RNase digests. This was confirmed by two-dimensional analysis of the products released by thermal degradation. These two methods based on sequencing gels were completed by the classical fingerprint technique. Uniformely ^{32}P labeled RNA fragments were digested with T1 and A RNases and the resulting oligonucleotides were fractionated by two-dimensional electrophoresis on paper. In this way, we determined the nucleotide sequence of each RNA subfragment and therefore of the whole *P.vulgaris* 23S RNA region associated with *E.coli* protein L1 within the T1-resistant complex (Fig. 2). Sequencing data have been seen by the referees but are not given for reason of space.

As *P.vulgaris* RNA subfragment 1 covers the entire RNA region and as the T1-resistant complex was isolated in conditions where base-paired RNA pieces

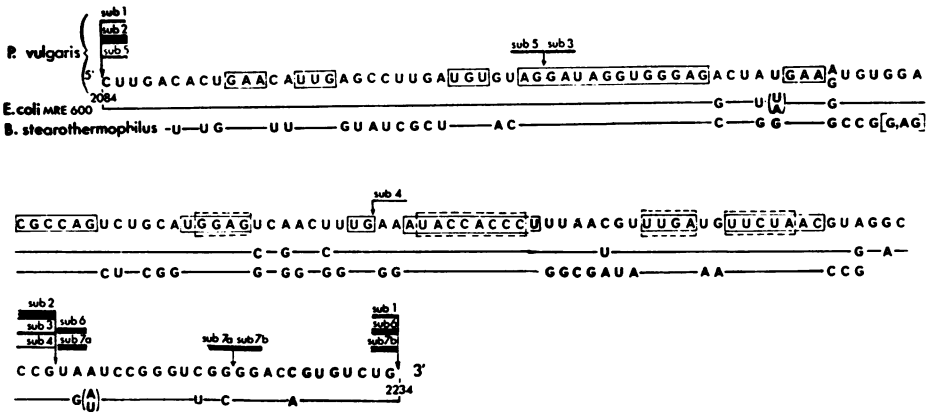


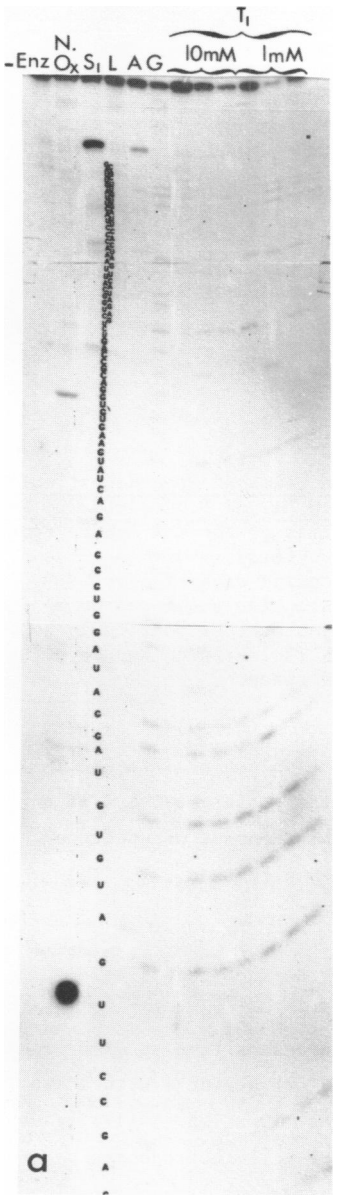
Figure 2 : RNA composition of the ribonucleoprotein released upon digestion of the complex formed between *E.coli* protein L1 and *P.vulgaris* 23S RNA. The lines above the sequence represent the extremities of the RNA subfragments, the thickness of the lines indicates their relative yields. The nucleotide sequence is compared to the corresponding ones of *E.coli* (1) and *B.stearothermophilus* (4) 23S RNAs. The oligonucleotide sequences common to the three 23S RNAs are indicated ▭, the oligonucleotides bearing an homology with the *E.coli* L11 operon (16,1) are shown ⋯. Hyphens representing phosphate linkages have been omitted for reasons of space.

remained associated, one can reasonably assume, that unless protein L1 disturbs the RNA structure, the base-pairing in isolated subfragment 1 will be similar to that of the L1-associated region. For this reason, we undertook an experimental study of RNA subfragment 1 secondary structure.

1.3 RNA subfragment 1 secondary structure

1.3.1 Experimental study. We took advantage of the fact that single-stranded RNA regions are sensitive to T1 and S1 nucleases, and regions involved in secondary or tertiary RNA-RNA interactions are sensitive to *Naja oxiana* RNase (3). Partial digestion of 5'-end labeled RNA subfragment 1 with these enzymes under conditions favouring rRNA base-pairing (10 mM Mg^{++}) (17) gave rise to fragments that we analyzed by polyacrylamide gel electrophoresis (Fig. 3). We thus identified those parts of RNA subfragment 1 which are involved in secondary or tertiary RNA-RNA interactions and those which are in single-stranded regions.

In order to test the effect of the Mg^{++} concentration on the structure of RNA subfragment 1, the partial T1 RNase digestion was performed in both 10 mM and 1 mM Mg^{++}. Figure 3 demonstrates that structural changes occur upon decrea-



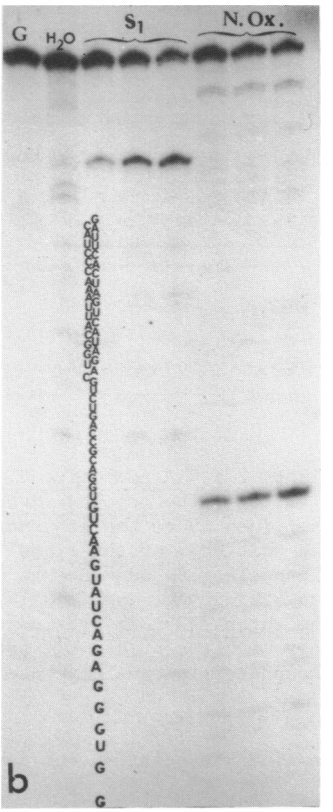


Figure 3 : Secondary structure study of RNA subfragment 1. 5'-end labeled RNA subfragment 1 was partially digested with T1 RNase in the presence of 10mM or 1 mM Mg^{++} and with S1 and *Naja oxiana* (2) nucleases, in the presence of 10mM Mg^{++}. The digests were fractionated on 20 % (a) and 15 % (b) polyacrylamide gels.

sing the divalent cation concentration and indeed that some of the guanines become more available for cleavage.

1.3.2 Model building. We looked for a base-pairing scheme that could ex-

plain the results of the sequencing gels shown in Figure 3. During this investigation we took into consideration the following observations : (i) cuts which occur at the same rate are seen on the gel as bands whose intensities are inversely proportional to the distance of the cutting position from the labeled end. (ii) Nucleotides which are not cut by T1 or S1 nucleases but are cleaved by *Naja oxiana* nuclease, can be either base-paired or involved in a tertiary interaction. The model shown in figure 6, is consistent with most of the cleavage positions observed in the presence of 10mM Mg^{++}. Some of the base-pairings proposed are rather unstable according to Tinoco *et al.* (18) . This is the case for the long series of A-U and G.U pairs in base-paired region IV. But it should be noticed that Tinoco *et al.* determined the free energies of base-pairs in the absence of divalent cations and in the presence of a large amount of monovalent cations (1M NaCl, 0.1mM EDTA, 0.01M sodium phosphate pH 7 buffer (18)). Indeed, we noticed that the guanines from regions 2099-2106 and 2183-2190 become more accessible to T1 RNase when the Mg^{++} concentration is decreased. This indicates that these stretches of RNA become single stranded. For the purposes of further discussion on the proposed structure, the various base-paired regions have been numbered I to VIII (Fig. 4).

2. The general structure of the L1-associated region of bacterial 23S RNA

2.1 Comparison of the primary structures of the L1-associated regions of *P.vulgaris*, *E.coli* and *B.stearothermophilus* 23S RNAs. The sequence of the *P.vulgaris* 23S RNA region associated with *E.coli* protein L1 differs in 12 positions from the corresponding one of *E.coli* 23S RNA. 9 of the mutations are transitions, 3 are transversions. The 44 nucleotides at the 5'-end of the RNA region are strictly conserved in the two bacterial species. Three cistronic heterogeneities were detected in the L1-associated region of *E.coli* 23S RNA, only one is observed in the case of *P.vulgaris* 23S RNA (Fig. 2).

Stanley *et al.* (4) previously observed that *B. stearothermophilus* 23S RNA can bind *E.coli* protein L1. They determined a partial sequence for the interacting RNA region. Comparison of this sequence with the corresponding ones of *E.coli* and *P.vulgaris* 23S RNAs reveals a set of common oligonucleotides (Fig. 2) Those located in the 3'-half correspond to the common sequence pattern already observed in *E.coli* and *B.stearothermophilus* 23S RNAs and in the 5'-part of the *E.coli* L11 operon (16,1). This reinforces the idea that such a pattern corresponds to a structure required for binding protein L1. Those common oligonucleotide sequences present in the 5'-half of the three 23S RNA regions should also have an important role and this point will be discussed later.

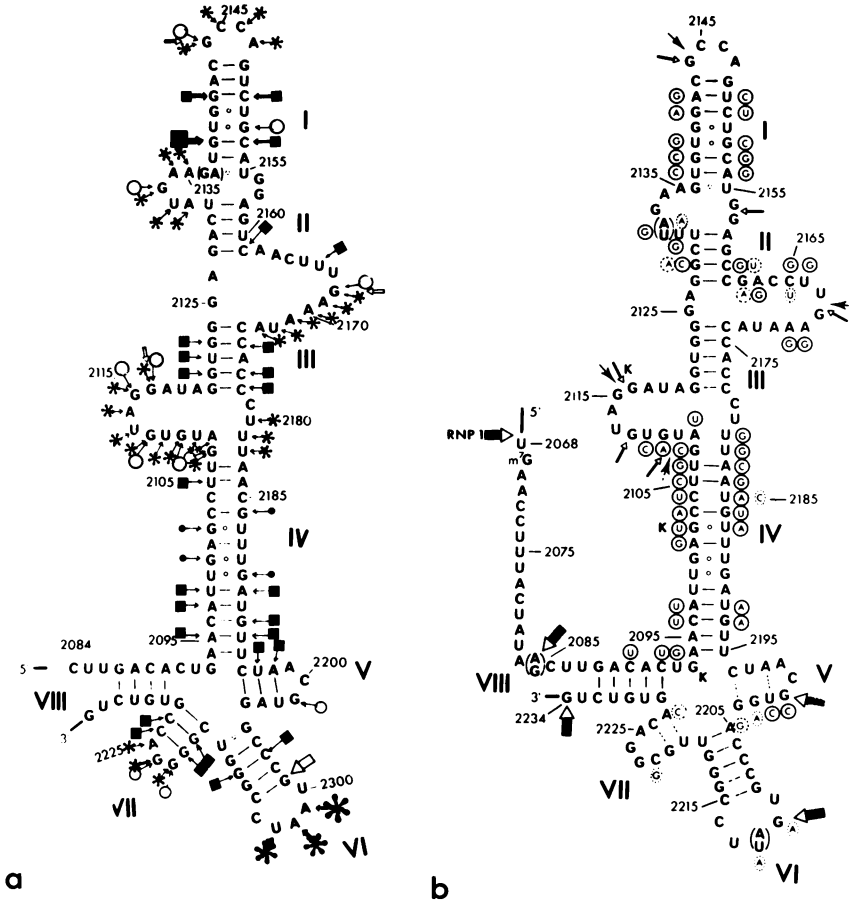


Figure 4 : (a) Possible secondary structure of *P.vulgaris* 23S RNA subfragment 1. ○→ T1 RNase, ✱— S1 nuclease, ■→ *Naja oxiana* RNase, ⇨ T1 RNase cleavage within the complex. For reason of clarity *P.vulgaris* 23S RNA nucleotides have been numbered by reference to the corresponding ones of *E.coli* 23S RNA (1).

(b) The probable structures of the *E.coli* and *B.stearothermophilus* 23S RNA regions associated with protein L1. The nucleotide sequence of the *E.coli* region (1) is given, only the base replacements in *P.vulgaris* ◌ and in *B.stearothermophilus* ◯ 23S RNAs (4) are indicated. T1 RNase cleavage position in the L1-*E.coli* 23S RNA complex ➡, in the L1 *B.stearothermophilus* 23S RNA complex ➔, sites of Kethoxal action in 50S subunits (19,20) K. The base-pairs which are unstable according to Tinoco *et al.* (18) are indicated by ··· or :: .

2.2 Comparison of their probable secondary structures. On the basis of the primary structures, most of the base-pairingsproposed in Figure 4a, for *P. vulgaris* subfragment 1, can be proposed for the corresponding regions of *E. coli* and *B.stearothermophilus* 23S RNAs (Figure 4b). Indeed, for the two latter RNA species, Stanley *et al.*(4), already proposed base-paired regions denoted I and IV. Only one difference is observed between *E.coli* and *P.vulgaris* in these putative base-paired regions : U2185 in *E.coli* is replaced by a C in *P.vulgaris*. This uracil faces a cytosine in the stem of *E.coli*. Its replacement by a cytosine does not change the stability of the postulated stem. On the other hand, several differences exist between *B.stearothermophilus* and *P.vulgaris* in these two complementary regions, but they compensate one another, so that the complementarity is conserved. Therefore, the complementarity between regions 2099-2106 and 2183-2190 which corresponds to a stable structure in the presence of 10mM Mg^{++} in *P.vulgaris* 23S RNA, is conserved throughout evolution. The corresponding base-pairing is more stable in the case of *B.stearothermophilus*. This is not surprising since this RNA must presumably retain its structure at 60°C.

In both *E.coli* and *B.stearothermophilus* 23S RNAs, compensatory base changes conserve the complementarity in the putative base-paired region II. The nucleotide sequence of the postulated base-paired region III is strictly conserved in the three RNA species. Concerning the region 2195-2234 of *E.coli* 23S RNA, the hairpin VI and base-paired region VIII are possible but the two hairpins V and VII are unstable according to Tinoco rules. Nothing can be said about the corresponding region of *B.stearothermophilus* 23S RNA since its primary structure has not been determined.

Therefore, the existence of the base-paired regions I to IV and VI and VIII is strongly supported by phylogenetic data. In addition, these base-paired regions fit well with the positions of T1 RNase cleavages within the L1-*E.coli* 23S RNA complex and within the L1-*B.stearothermophilus* 23S RNA complex (Fig. 4b). They can also explain previous results obtained upon modification of *E.coli* RNA subfragment 1 with soluble carbodiimide, a reagent which modifies non base-paired uridines (21). Thus the long hairpin interrupted by a series of loops and terminated by a branched structure, which is represented in Figure 4b, is likely to correspond to the general structure of the bacterial L1-associated region of 23S RNA. Figure 6a shows that parts of this hairpin have a nucleotide sequence highly conserved throughout bacterial evolution. The conserved segments likely have an important role. Some of them should serve to bind protein L1, others might bind protein L9. Indeed,

the 23S RNA region associated with protein L1 in the synthetic complex was released associated with both proteins L1 and L9 upon digestion of 50S subunits (22). Finally, those which are more accessible in the subunits like the large bulge loop on the 5'-side of the hairpin whose guanine 2116 is attacked by kethoxal (19,20) might serve to bind ribosome substrates.

In *E.coli* MRE 600 23S RNA, heterogeneity has been found at position 2084, where the guanine can be substituted by an adenine (1). In this case a larger ribonucleoprotein (denoted 1) was obtained, which contained the 16 nucleotides preceding C 2084. Since these 16 nucleotides do not possess internal complementarity, they should constitute a single-stranded region of *E.coli* 23S RNA. It is noticeable that these 16 nucleotides contain a 7 methyl guanine (1).

3. Is there in mouse and man mitochondrial 16S rRNAs a region homologous to the L1-associated region of bacterial 23S RNA ? As genes of the large ribosomal 16S rRNAs of mouse and human mitochondria have been recently sequenced (5,6) it was important to look if these RNAs contain a region similar to the L1-associated region of bacterial 23S rRNA. Etten *et al.* and Eperon *et al.* found that only a few sequences of *E.coli* 23S rRNA are strongly conserved in mouse and man mit 16S rRNA, respectively. We observed that the sequence at the 5'-end of the L1-associated region is one of them (Fig. 5). The homologous region of mouse mit 16S rRNA is located between positions 2282 and 2301 in the DNA structure, that of man mit 16S rRNA between positions 2721 and 2739. We would like to mention that a similar region is also found in the cytoplasmic 28S rRNAs of *Xenopus laevis* and *Tetrahymena* (23) (Fig. 5). Therefore, the sequence of the *E.coli* 23S rRNA region which preceds the L1-binding site and which contains a m^7G is conserved throughout ribosomes evolution from bacteria

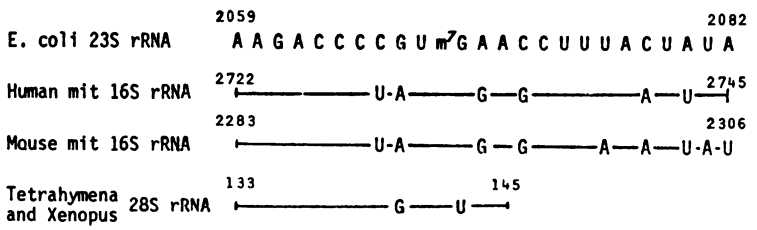


Figure 5 : Existence in human and mouse mit 16S rRNAs and in *Tetrahymena* and *Xenopus laevis* 28S rRNAs of a sequence similar to that of the *E.coli* 23S rRNA region preceding the L1 binding region. The sequence of mouse mit 16S rRNA has been established by Van Etten *et al.* (6) that of human mit 16S rRNA by Eperon *et al.* (5) and that of *Xenopus laevis* and *Tetrahymena* 28S rRNA by Gourse and Gerbi (23).

to eukaryotes. This region probably has an important role. As the nucleotides around the m^7G are free of base-pairing in *E.coli*, they might serve to bind ribosome substrates. And, this might be related to the fact that protein L1 together with proteins L11 and L16 are required for aminoacyl-tRNA binding (24). It is remarkable that protein L1 precisely controls its own synthesis and that of protein L11 (7).

The sequences located after positions 2300 and 2738 in mouse and man mit 16S rRNAs, respectively, and after position 2075 in bacterial 23S rRNA do not display strong homologies in their primary structures. Nevertheless, their sequence potential structures have the same general shape (Fig. 6b,c) : a long hairpin interrupted by loops, the large loop on the 3'-side of the hairpin bearing in all RNAs the sequence Py-Pu-Pu-C-X-X-X-X-Pu-Pu-A-Py (where X can be any nucleotide). Conservation of the sequence of this loop in both bacteria and mitochondria is highly unlikely to occur by chance : the probability to find such a sequence in a 100 nucleotide long RNA fragment (the length of the two mitochondrial hairpins) is low : $\frac{100}{2^6 \times 4^2} = 0.1$ and obviously, the probability to find it in several hairpins of this size and more precisely in a loop located on the 3'-side of these hairpins is extremely low. Invariant nucleotides are also observed at three other positions of the bacterial and mitochondrial RNA structures (Fig. 6d). Such evolutionary conserved nucleotides should have an important role. This might be to bind protein L1 in the bacterial RNA and an L1-like protein in mitochondrial RNA. According to the important role of protein L1 for bacterial ribosome function (24), the existence of an L1-like protein in mitochondrial ribosomes is quite likely.

4. The nucleotides of the L1-associated region of bacterial 23S rRNA which are in close contact with the protein. In preceding paragraphs we determined the probable secondary structure of the L1-associated region of 23S rRNA. It is now of interest to determine which areas in this structure are in close contact with the protein. Comparison of T1 RNase digestion products from subfragment 1 and from the L1-23S RNA complex in both *E.coli* and *P.vulgaris* shows that the guanines in the bulge and hairpin loops of the long hairpin are strongly buried in the presence of protein L1. This is not the case for the guanines in the hairpin loops of the branched structure (Fig. 4a,b). Therefore, protein L1 is in close contact with the long hairpin, but not with the branched structure. This latter should be maintained within the ribonucleoprotein by RNA-RNA interactions.

As mentioned above, the base-pairing is very poor in some parts of this hairpin : the regions 2099-2106 and 2183-2190 become single-stranded upon

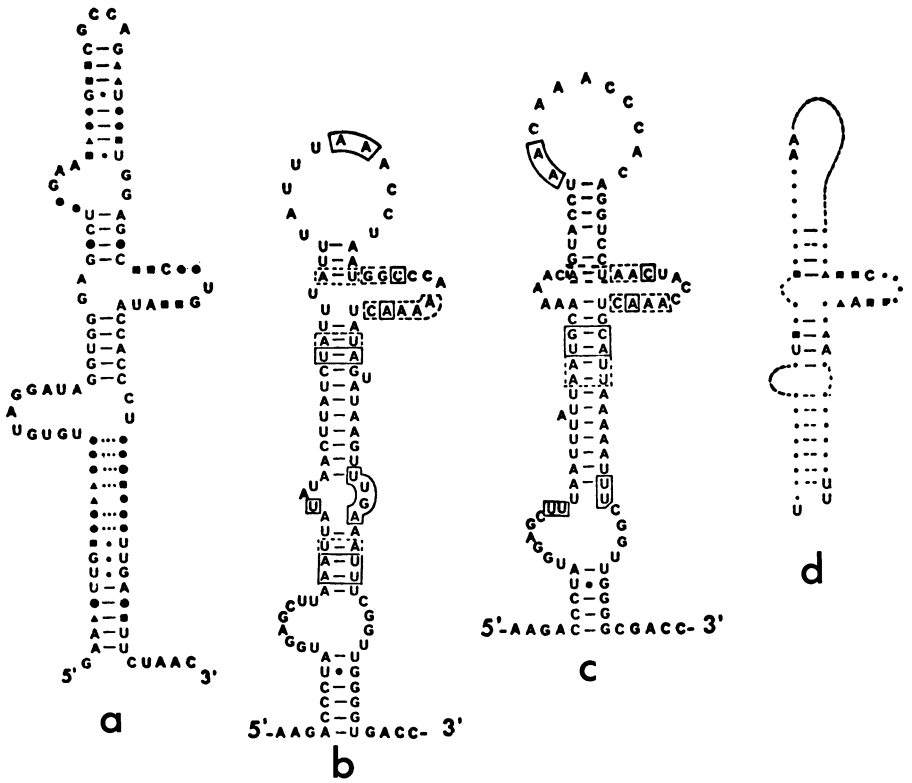
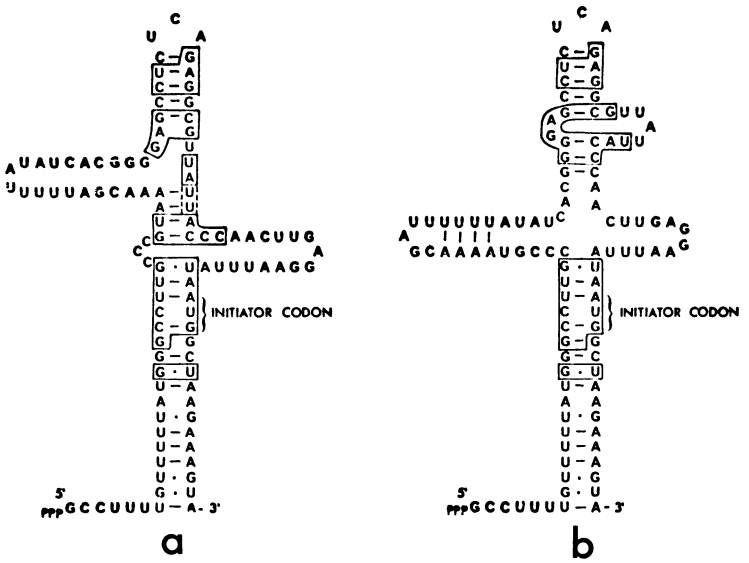


Figure 6 : Comparison between the L1-associated region of bacterial 23S rRNA and putative homologous regions of human and mouse mitochondrial 16S rRNAs.
a) Nucleotides of the L1-associated region of 23S rRNA conserved in bacterial evolution. The nucleotides conserved in *E.coli*, *P.vulgaris* and *B.stearothermophilus* are indicated, at the places where mutations occurred ■ indicates that the nucleotide remained a purine, ▲ a pyrimidine, ● was any nucleotide.
b) Putative secondary structure of the region of mouse mit 16S rRNA located between nucleotides 2283-2386 in the mit DNA structure established by Van Etten *et al.* (6). This structure is compared to that of the L1-associated region of 23S rRNA ▭ identical nucleotides at similar positions, ⁚⁚⁚ replacement of the transition type.
c) Putative secondary structure of the region of human mit 16S rRNA located between nucleotides 2721 and 2823 in the mit DNA structure established by Eperon *et al.* (5). Same symbols as in b.
d) Features common to the structure of L1-associated region of bacterial 23S rRNA (Fig. 6a) and to the putative structures of human and mouse 16S rRNAs shown in Fig. 6b and 6c.

decreasing Mg^{++} concentration. This should also occur when the temperature is increased to 42°C during complex formation. So that we have two alternatives : either protein L1 favours refolding of the poorly base-paired regions or, on

the contrary it prevents this folding by interacting with one of the single-strand. The second hypothesis would be in agreement with data from the literature. Guanines 2093, 2102 and 2116 are attacked by kethoxal within the *E.coli* 50S subunit (19,20) which means firstly that these guanines are not base-paired and secondly that their contact with protein L1 is not sufficient to prevent attack by the small molecule of kethoxal.

5. Comparison between the binding region of protein L1 on 23S rRNA and the putative binding site of this protein on L11 mRNA. Ribosomal proteins S4, S7,



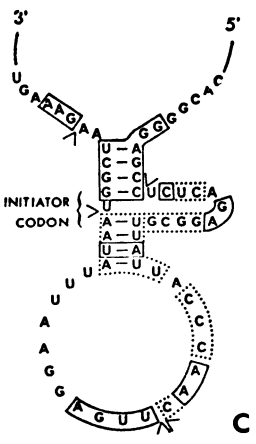


Figure 7 : Putative secondary structures of the region of *E.coli* L11 mRNA containing the initiator A-U-G. The primary structure of this RNA has been determined by Post *et al.* (15). The structures proposed are compared to that of the L1-associated region of *E.coli* 23S rRNA. ▭ Existence of identical nucleotide at similar positions in the two structures. ▭ Replacement of the transition type. > Existence of an additional nucleotide in the 23S rRNA region. Model c displays most of the features common to both the L1-associated region of bacterial 23S rRNA and the homologous region of mit 16S rRNA (Fig. 6d).

S8, L1, L4 and L10 were found to be translational repressors (7,8,25-28). Yates *et al.* (7) proposed that this repression proceeds by a direct binding of these proteins on the initiation sites of translation on the mRNAs. We already observed the existence of an homology between the nucleotide sequence at the 5'-end of the *E.coli* L11 operon and the sequence of the L1-associated region of *E.coli* 23S rRNA (1). Nomura *et al.* (29) observed homologies in both the primary and secondary structures of the binding regions of proteins S4 and S7 on *E.coli* 16S rRNA and the region of the mRNAs where these proteins act as repressor. We looked for such homologies between 23S rRNA and L11 mRNA. From the sequence of the L11 mRNA region containing the initiator A-U-G (16) several models of secondary structure can be built, which display stiking homologies with the L1 binding region of 23S rRNA (Fig. 7). It is noticeable that one of these models (Fig. 7c) bears most of the features common to both the L1 binding region of bacterial 23S rRNA and the homologous region of mitochondrial 16S rRNA. So that these common features may well be those required for binding protein L1. A direct analysis of the mRNA region associated with protein L1 would be very informative in this respect.

The observed homologies reinforced the idea that regulation by the repressor r-proteins is based on competition between rRNA and mRNA for these regulatory r-proteins (7).

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