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Nucleotide sequence of the 5'- and 3'- domains for rabbit 18S ribosomal RNA

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ABSTRACT

By direct RNA sequence analysis we have determined the primary structures of both the 5' and 3' domains for rabbit 18S ribosomal RNA. Purified 18S rRNA was labeled *in vitro* at either its 5' or 3' terminus with ^{32}P, base-specifically fragmented enzymatically and chemically, and the resulting fragments electrophoretically fractionated by size in adjacent lanes of 140 cm long polyacrylamide sequencing gels run in 90% formamide. A phylogenetic comparison of both the mammalian 5' proximal 400 residues and the 3' distal 301 nucleotides with the previously determined yeast and *Xenopus laevis* 18S rRNA sequence shows extensive conservation interspersed with tracts having little homology. Clusters of G+C rich sequences are present within the mammalian 5' domain which are entirely absent in both the *Xenopus laevis* and yeast 18S rRNAs. Most base differences and insertions within the mammalian 18S rRNA when compared with yeast or *Xenopus* rRNA result in an increase in the G+C content of these regions. We have found nucleotide sequence analysis of the ribosomal RNA directly permits detection of both cistron heterogeneities and mapping of many of the modified bases.

INTRODUCTION

In prokaryotic 16S ribosomal RNA the 5'-domain is regarded as the structural domain which organizes the proteins required to nucleate the essential reactions during ribosome assembly, while the 3'-domain is considered the functional domain which is associated with proteins involved in the protein synthetic process (1). To begin to understand the functional organization of the eukaryotic 40S ribosomal subunit with respect to messenger and tRNA binding, ribosomal subunit association, and subunit assembly, the primary structure of its 18S rRNA component must first be determined. Recently, the complete nucleotide sequences for both yeast and *Xenopus laevis* 18S rRNA have been deduced by DNA sequence analysis of the ribosomal genes (2,3). Here we report the nucleotide sequences of the 5'-and 3'-domains for rabbit 18S ribosomal RNA determined by sequence analysis of the 18S rRNA directly. Highly purified rabbit 18S rRNA was labeled *in vitro* at either its 5'-or 3'-terminus with ^{32}P and nucleotide sequences determined by rapid sequencing

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gel analysis (4,6). Use of 140 cm long polyacrylamide sequencing gels run in 90% formamide as a denaturant has allowed determination of the first 400 nucleotides from the 5'-terminus and 301 nucleotides from the 3' end. A comparison of the mammalian sequences with either yeast or *Xenopus laevis* 18S rRNAs shows that conserved regions are interspersed with tracts having little homology, suggesting that not only insertions but deletions have taken place within these domains during the evolution of the 18S ribosomal genes.

MATERIALS

Ultra-pure urea was purchased from Bio-Rad; acrylamide and bisacrylamide were obtained from Baker Chemicals; and reagent grade formamide was purchased from Fisher Scientific Co. Both calf-intestinal alkaline phosphatase (CIAP) and T_4 polynucleotide kinase were supplied by Boehringer-Mannheim and used without further purification. T_4 RNA ligase and *Neurospora crassa* endonuclease were purchased from P.L. Biochemicals, Inc. Ribonucleases T_1, T_2, and U_2 were prepared by Sankyo and obtained through Calbiochem, LaJolla, CA. *Physarum* RNase I (Phy I) prepared by the method of Pilly (7) was a kind gift from Dr. Joyce Heckman (MIT). Pyrimidine-specific enzyme from *B. cereus* was prepared as previously described (Lockard, et al., 4). Chicken liver ribonuclease was a kind gift from Dr. Philip Hieter (NIH). [γ - ^{32}P] - ATP (> 7000 Ci/mmol) was prepared from carrier-free $^{32}P_i$ (New England Nuclear, NEX-053) by the procedure of Johnson and Walseth (8). [5'- ^{32}P]C_p (2,900 Ci/mmol) was purchased from New England Nuclear, Inc. Sequencing gel electrophoresis was carried out using a Model 200 Electrophoresis Power Supply (5000-volt-200 milliamp capacity) from Northeastern Scientific Company, Boston, Mass.

METHODS

Preparation of Rabbit Reticulocyte 18S rRNA

Isolation of reticulocytes from anemic New Zealand white rabbits, preparation and deproteinization of polysomes, and isolation of non-polyadenylated RNA were as previously described (9). The 18S rRNA was purified from total poly A-minus RNA by SDS sucrose density gradient centrifugation (9). Trace ribonuclease contamination in 18S rRNA preparations was eliminated by incubating the RNA with proteinase K at 100 μg/ml in the presence of 1.0% SDS for 1 hr at 37°C, then immediately extracting with phenol as previously described (9).

Preparation of [5'-^{32}P]-Labeled and [3'-^{32}P]-Labeled 18S rRNA

Five A_{260}-units of Proteinase-K treated 18S rRNA were dissolved in 200 µl of 75 mM Tris-HCl (pH 8.3) to which calf intestinal alkaline phosphatase (CIAP) was added to a final concentration of 0.50 units/ml. After incubation at 37°C for 45 min. the reaction mixture was immediately extracted with phenol and precipitated with ethanol. 0.40 A_{260} units of dephosphorylated 18S rRNA was incubated with 4 units of T_4 polynucleotide kinase and 500 pmol of [γ-^{32}P]-ATP in a 10 µl reaction mixture containing 20 mM Tris-HCl (pH 8.5), 5 mM $MgCl_2$, and 10 mM DTT. Multiple reactions were incubated for 30 min. at 37°C, 40 µl of loading solution (99% formamide with 0.1% w/v xylene cyanol and bromophenol blue dyes and 25 mM EDTA) was then added to each and loaded onto separate lanes of a 3.5% polyacrylamide gel. For 3'-^{32}P-end-labeling, one A_{260} unit of Proteinase-K treated, but not dephosphorylated, 18S rRNA was incubated overnight with 0.5 mCi of [5'-^{32}P] Cp and T_4 RNA ligase according to the procedure of England and Uhlenbeck (10), and loaded onto separate lanes of a 3.5% polyacrylamide gel as described above. After gel electrophoresis, the ^{32}P-end-labeled 18S rRNA was localized by autoradiography, excised, recovered by electrophoretic elution, and ethanol precipitated as previously described (4).

Partial Enzymatic Digestion of ^{32}P-End-Labeled 18S rRNA For Sequence Analysis

Partial enzymatic digestion of ^{32}P-end-labeled 18S rRNA with T_1 (G) and Phy I (A+U) ribonucleases were performed in buffer containing 50 mM sodium citrate (pH 5.0), 7 M urea, 1 mM EDTA, 1.0 µg/µl carrier tRNA, 0.025% xylene cyanol and bromophenol blue, at 75°C for 10 min. (4). U_2 (A>G) RNase digestions were performed in 50 mM sodium citrate buffer (pH 3.5) at 65°C for 10 min. *B. cereus* (U+C) RNase degestions were carried out in the absence of urea at 55°C for 10 min. as previously described (4). *Neurospora crassa* endonuclease (A+U+G) digestions and controlled acid hydrolysis (H^+) were performed according to Krupp and Gross (11). Chicken liver RNase (C>U) digestion was carried out in 20 mM Tris-HCl buffer (pH 7.2) in the presence of 75% DMSO for 10 min. at 75°C.

Partial Chemical Cleavage of ^{32}P-End-Labeled 18S rRNA For Sequence Analysis

Partial chemical cleavage of ^{32}P-end-labeled 18S rRNA was performed essentially as described by Peattie (6) except the C>U and U-specific reactions were omitted with 5'-^{32}P-end-labeled RNA.

Electrophoresis of Partial Digests on Formamide Polyacrylamide Gels

Both enzymatic and chemical digestions were fractionated in adjacent

lanes of 15 and 10% polyacrylamide slab gels (0.40 mm thick x 33 cm wide x 100 or 140 cm long) run in 90% formamide as a denaturant as previously described by Lockard and Kumar (12).

RESULTS

Highly purified 18S rRNA was dephosphorylated with alkaline phosphatase and its 5'-terminus labeled *in vitro* with ^{32}P using T_4 polynucleotide kinase and [γ-^{32}P]-ATP as detailed in METHODS. Since the extent of end-labeling usually never exceeded 4-5%, we were able to increase the specific activity of the 18S rRNA for purposes of nucleotide sequence analysis by using very high specific activity [γ-^{32}P]-ATP (> 7000 Ci/mmole) prepared by the method of Johnson and Walseth (8). Such preparations resulted in the generation of ca. 10 x 10^6 DPM per one A_{260} unit of RNA. 5'-[^{32}P]-end-labeled 18S rRNA could be conveniently purified on 3.5% acrylamide gels, then recovered after autoradiography by excision of the gel piece and electroelution of the radioactive RNA into a dialysis bag (4). 5'-end group analysis of the labeled RNA revealed greater than 95% ^{32}pU after complete digestion with nuclease P_1 and analysis by thin-layer chromatography (9). Initial sequence analysis of the 5'-[^{32}P]-end-labeled 18S rRNA was performed by base-specific partial enzymatic digestion according to the previous published protocols (Donis-Keller et al. 5; Lockard, et al. 4) except for the following improvements published recently by Krupp and Gross (11). U_2 ribonuclease was 100-fold more active and cleaved after most adenines at pH 3.5 rather than pH 5.0; partial acid hydrolysis in 10 mM sulfuric acid generated a more useful and reproducible uniform ladder than partial alkaline hydrolysis; and *Neurospora* endonuclease in 7 M urea left most C-N bonds within a sequence intact, thereby allowing discrimination of C from U residues (11). Figure 1 shows an autoradiogram of a 10% polyacrylamide sequencing gel indicating the nucleotide sequence of residues #60-189 from the 5'-terminus. More than 125 nucleotides can usually be read off on a single 100 cm long gel. Gels polymerized in 90% formamide rather than 8 M urea were much more effective in completely denaturing RNA fragments and subsequently eliminating band contraction (12). The nucleotide sequence in this figure is shown adjacent to the *Neurospora* endonuclease digest. Since *Neurospora* endonuclease generates fragments having 3' hydroxyl groups rather than 3' phosphates, bands appearing in these tracks will migrate 1 to 1.5 times more slowly than their counterparts in the adjacent lanes. Residues designated as X in the rabbit sequence represent phosphodiester linkages resistant to cleavage either en-

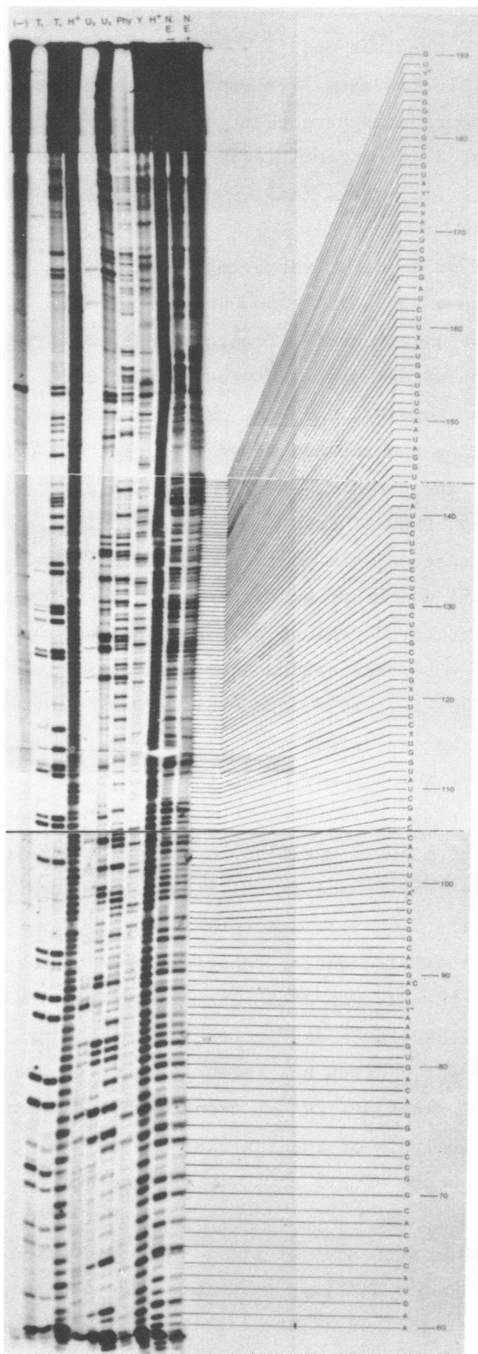

FIGURE 1. Autoradiogram of partial enzymatic digests on 5'-[^{32}P] labeled 18S rRNA electrophoresed on a 10% polyacrylamide slab gel in 90% formamide. Gel dimensions were 0.40 mm thick x 33 cm wide x 100 cm in length. Partial digests contained the following amounts of enzyme per μg of RNA. From left to right: (-) minus enzyme; (T_1)T_1 RNase, 1.25 x 10^{-3} and 1.25 x 10^{-4} Units/μg RNA; (H^+) controlled acid hydrolysis; (U_2)U_2 RNase, 0.25 x 10^{-4} and 0.25 x 10^{-5} Units/μg RNA; (Phy) Physarum nuclease I, 0.2 μL/10 μg RNA; (Y) *B. cereus* RNase, 0.003 Units/μg RNA; (H^+) controlled acid hydrolysis; (N.E.$^-$) *Neurospora crassa* endonuclease minus 7 M urea, 5 x 10^{-2} Units/μg RNA; (N.E.$^+$); *Neurospora crassa* endonuclease plus 7 M urea, 5 x 10^{-2} Units/μg RNA.

zymatically or chemically, and likely represent nucleotides with a 2'-O-methylated ribose. Y* also denotes a possible modified pyrimidine due to relatively weak enzymatic and chemical cleavages. In most instances either 2'-O-methylated nucleotides or pseudouridines have been identified at both the X and Y* positions, respectively, deduced from previously catalogued T_1 RNase digestion fragments in Novikoff (13), HeLa (14), and Xenopus (15) 18S rRNA.

Figure 2 shows an autoradiogram of a 10% polyacrylamide gel for residues #235-400 from the 5'-terminus. More than 150 nucleotides can usually be determined on a single 140 cm long gel. Tracts almost completely resistant to enzymatic digestion even at temperatures greater than 85°C in 7 M urea are evident in this region. However, both the chemical RNA sequencing procedure of Peattie (6) using the A>G and G-specific cleavage protocols for purine determination, and the use of C-specific chicken liver ribonuclease (16) in the presence of 75% DMSO at 75°C, allowed determination of most of the nucleotides in these regions, except for definitive identification of the pyrimidines (Y) #263-266 which appear likely to be C residues. Many of these sequences in highly structured regions of rabbit rRNA are entirely absent in both the previously published sequences of yeast (2) and Xenopus (3) 18S rRNA. Nucleotides #201-202, #238, and #261-272 which appear as G+C rich insertions into rabbit rRNA may extend and further stabilize helical regions already existent in both Xenopus and yeast 18S rRNA.

Figure 3 shows an autoradiogram of a 10% polyacrylamide sequencing gel of nucleotides #200-300 from the 3'-terminus. Purified 18S rRNA was labeled in vitro at its 3'-end with ^{32}P using $[^{32}P]Cp$ and T_4 RNA ligase (10), and the 3'-$[^{32}P]$-end-labeled RNA was purified on a 3.5% acrylamide gel as detailed in METHODS. All four chemical RNA sequencing reactions were usually carried out and run in parallel with the base-specific enzymatic digestions of the 3'-$[^{32}P]$-end-labeled 18S rRNA. Residues in Figures 1-3 indicating more than one nucleotide denote apparent cistron heterogeneities in the sequence. Since there are at least 200 ribosomal RNA cistrons within a mammalian cell (17), nucleotide sequence analysis of the 18S rRNA directly will likely give an average nucleotide sequence of all actively transcribed genes (18).

Figure 4 summarizes the nucleotide sequences at the 5'-and 3'-domains determined for rabbit 18S rRNA by sequence analysis of intact ^{32}P-end-labeled RNA. Nucleotides designated either as X or Y* are likely modified. The modified bases which have already been identified in T_1 RNase fragments of

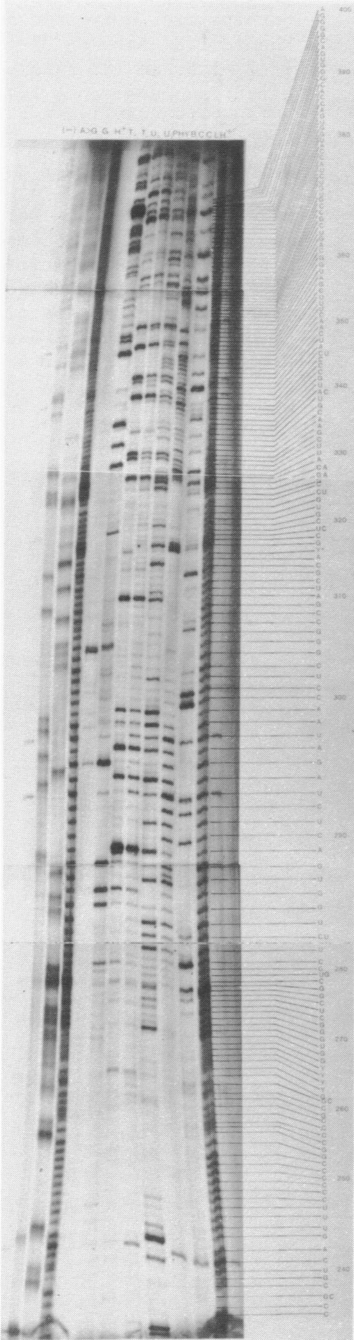

FIGURE 2. Autoradiogram of both partial enzymatic and chemical digests on 5'-[^{32}P]-labeled 18S rRNA electrophoresed on a 10% polyacrylamide slab gel in 90% formamide. Gel dimensions were 0.40 mm thick x 33 cm wide x 140 cm in length. Partial enzymatic digestion conditions were the same as in Figure 1 with the inclusion of chicken liver RNase (CL), 0.5 μL/10 μg. A>G and G represent purine-specific chemical cleavages of Peattie (6).

FIGURE 3. Autoradiogram of both partial enzymatic and chemical digests on 3'-[^{32}P]-labeled 18S rRNA electrophoresed on a 10% polyacrylamide slab gel in 90% formamide. Gel dimensions were originally 0.40 mm thick x 33 cm wide x 140 cm in length. Partial enzymatic and chemical digestions were identical to Figure 2 with the inclusion of pyrimidine-specific chemical cleavages C>U and U (6).

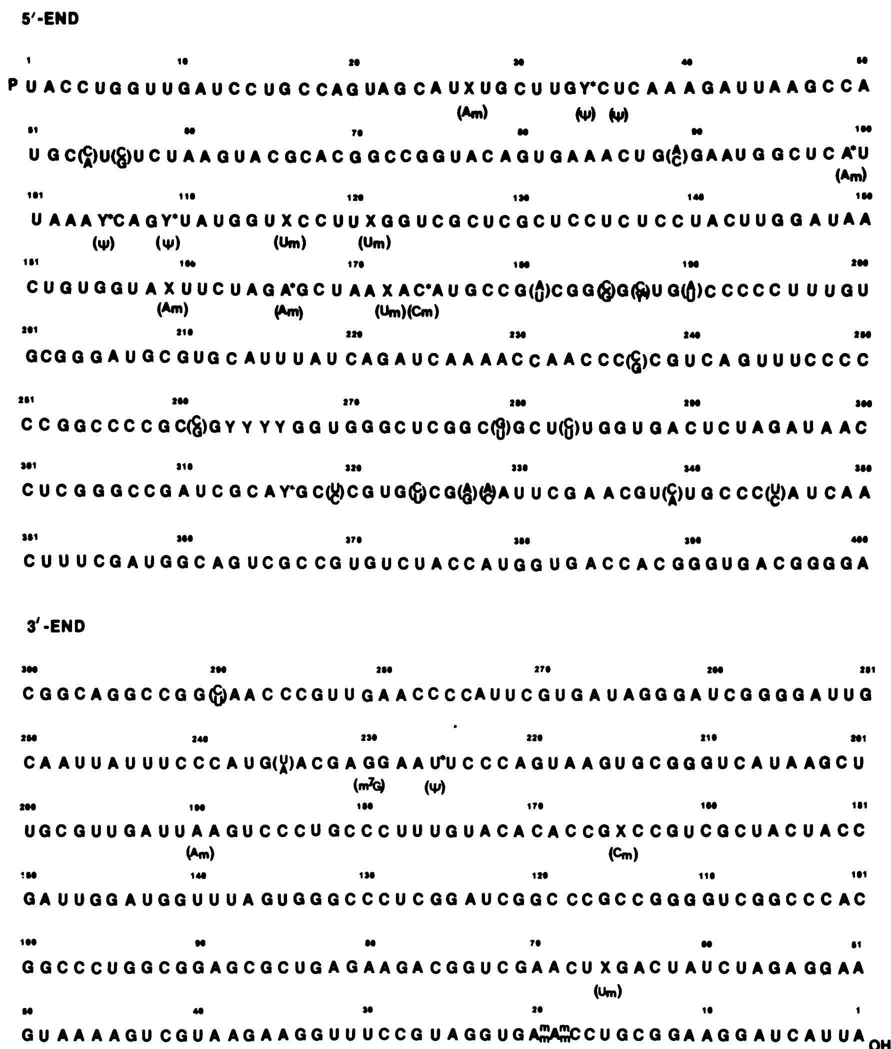

FIGURE 4. Nucleotide sequence at the 5'-and 3'-domains of rabbit 18S ribosomal RNA. X-denotes a phosphodiester linkage resistant to chemical and enzymatic cleavage, and Y* denotes a possible modified pyrimidine. Known modified bases at thes positions in other 18S rRNAs are indicated parenthetically below.

Novikoff, HeLa, and <u>Xenopus</u> 18S rRNA (13-15) having an identical sequence are indicated parenthetically below.

DISCUSSION

A phylogenetic comparison of both the 5'-and 3'-domains of rabbit 18S rRNA with both Xenopus laevis and yeast nucleotide sequences is shown in Figure 5. The pattern of sequence homology between the mammalian and the amphibian and yeast 18S rRNA shows conserved regions which are interspersed with tracts having little or no homology. The rabbit 5'-sequence compared with yeast shows seven major clusters of putative insertions (U_{120} - G_{123}; C_{179}, G_{180}, G_{187}; G_{201}, C_{202}, U_{207}, C_{209}; C_{235} - C_{238}; A_{242} - U_{244}, C_{248} - C_{252}; G_{259} - G_{272}; G_{280}, C_{281}; U_{297}, G_{309}, A_{310}, A_{315}, Y_{316}) resulting in a 33 nucleotide increase in the size of this region compared with yeast. Most of these insertions as well as the 75 base differences between the rabbit and yeast sequences result in a 19% increase in the overall G+C content of this domain. A 9% evolutionary increase in the total G+C content for 18S rRNA has already been noted between Xenopus and yeast (3).

The rabbit 5'-sequence when compared with Xenopus reveals a striking twelve nucleotide G-rich insert (#261-272) also absent in yeast. Recently, using the experimentally established secondary structure model for E. coli 16S rRNA as a basis, models have been derived for both yeast and Xenopus 18S rRNA by search for both primary structural homology and for compensating base changes in the putative helical regions (19). These models support the concept that the secondary structure of ribosomal RNA may have been extensively conserved in evolution. It is interesting that both this 12 nucleotide insert (#261-272) as well as 26 of the 44 insertions into this domain, occur within a putative helical stem proposed by Brimacombe and coworkers (19) between residues #222-245 of the yeast sequence. Since this region was found to be very resistant to enzymatic cleavage during sequence analysis, as shown in Figure 2, it is possible that insertion of these G+C rich sequences extends and stabilizes a helix or helices already present within lower eukaryotic rRNA. The significance of such insertions within this region of the 18S rRNA with regard to both the structure and function of the 40S subunit awaits further investigation. It is noteworthy that most of the sequences absent from Xenopus but still present in both rabbit and yeast rRNA (#240-241; #245-247; #254-257) also reside within this highly mutable region. These apparent deletions in Xenopus must have recently occurred after the divergence of mammals and amphibians from a common ancestor.

In the 5'-domain the rabbit sequence was found to be 86% homologous with Xenopus and 69% homologous with yeast. A much more striking homology is evident in the 3'-domain with a 97% homology between rabbit and Xenopus

```
5'

         1                                                50
XENOPUS  pUACCUGGUUGAUCCUGCCAGUAGCAUAUGCUUGUCUCAAAGAUUAAGCCA
RABBIT   pUACCUGGUUGAUCCUGCCAGUAGCAUAUGCUUGUCUCAAAGAUUAAGCCA
YEAST    pUAUCUGGUUGAUCCUGCCAGUAGCAUAUGCUUGUCUCAAAGAUUAAGCCA   (insertion: U)

         51                                              100
XENOPUS  UGCACGUGUAAGUACGCACGGCCGGUACAGUGAAACUGCGAAUGGCUCAU
RABBIT   UGC(C/A)U(C/G)UCUAAGUACGCACGGCCGGUACAGUGAAACUG(A/C)GAAUGGCUCAU
YEAST    UGCAUGUCUAAGUAUAUAAGC UACAGUGAAACUGCGAAUGGCUCAU   (insertion: AAUUUA)

         101                                             150
XENOPUS  UAAAUCAGUUAUGGUUCCUUUGAUCGCUCCAUCUG-U--UACUUGGAUAA
RABBIT   UAAAUCAGUUAUGGUUCCUUUGGUCGCUCGCUCCUCUCCUACUUGGAUAA
YEAST    UAAAUCAGUUAUCGUUUAU....UUGAUAGUUCCUUUACUACAUGGAUAA   (insertion: U)

         151                                             200
XENOPUS  CUGUGGUAAUUCUAGAGCUAAUACAUGCCGACGGCGCUGACCCCC....A   (insertion: A)
RABBIT   CUGUGGUAAUUCUAGAGCUAAUACAUGCCG(A/U)CGG(C/G)G(C/Y)UG(A/U)CCCCCUUUGU
YEAST    CCGUGGUAAUUCUAGAGCUAAUACAUGC--UUAAAA-UCUCACCCUUUGA   (insertion: G)

         201                                             250
XENOPUS  --GGGAUGCGUGCAUUUAUCAGACCAAAACCAAUCCG-G--GG----CCC
RABBIT   GCGGGAUGCGUGCAUUUAUCAGAUCAAAACCAACCC(C/G)CGUCAGUUUCCCC
YEAST    --AGGA-G-AUGUAUUUAUUAGAUAAAAAUCAAU----GUC---UUC---   (insertion: A)

         251                                             300
XENOPUS  CCG----CGC-------------CCCGGCCGCUUUGGUGACUCUAGAUAAC
RABBIT   CCGGCCCCGC(C/G)GYYYYGGUGGGCUCGGC(G/U)GCU(C/U)UGGUGACUCUAGAUAAC
YEAST    --GGACUC---------------UUUGAUU--CAUAAUAACUUUUCG-AAU

         301                                             350
XENOPUS  CUCGGGCCGAUCGCACGUCCGUG CGACAUUCGGAUGUCUGCCCUAUCAA   (insertions: CC; GGC/C G/A A; AU)
RABBIT   CUCGGGCCGAUCGCAYGC(U/C)CGUG(C/U)CG(A/G)(A/C)AUUCGAACGU(C/A)UGCCC(U/C)AUCAA
YEAST    CGCAGGCC--UUGU--GCUGGCGAUGGCAUUCAAAUUUCUGCCCUAUCAA   (insertions: U; UU)

         351                                             400
XENOPUS  CUUUCGAUGGUACUUUCUGCGCCUACCAUGGUGACCACGGGUAACGGGGA
RABBIT   CUUUCGAUGGCAGUCGCCGUGUCUACCAUGGUGACCACGGGUGACGGGGA
YEAST    CUUUCGAUGGUAGGAUAGUGGCCUACCAUGGUUUCAACGGGUAAUACGGG

         300                                             251
XENOPUS  GACAGGUCGGUAACCCGCUGAACCCCGUUCGUGAUAGGGAUCGGGGAUUG   (insertions: G G)
RABBIT   GGCAGGCCGG(U/C)AACCCGUUGAACCCCAUUCGUGAUAGGGAUCGGGGAUUG
YEAST    GAGAGGCUGGUAAUCUUGUGAAACUCCGUCGUGCUGGGGAUAGAGCAUUG   (insertions: U U)

         250                                             201
XENOPUS  CAAUUAUUUCCCAUGAACGAGGAAUUCCCAGUAAGUGCGGGUCAUAAGCU
RABBIT   CAAUUAUUUCCCAUG(U/A)ACGAGGAAUUCCCAGUAAGUGCGGGUCAUAAGCU
YEAST    UAAUUAUUGCUCUUCAACGAGGAAUUCCUAGUAAGCGCAAGUCAUCAGCU

         200                                             151
XENOPUS  CGCGUUGAUUAAGUCCCUGCCCUUUGUACACACCGCCCGUCGCUACUACC
RABBIT   UGCGUUGAUUAAGUCCCUGCCCUUUGUACACACCGCCCGUCGCUACUACC
YEAST    UGCGUUGAUUACGUCCCUGCCCUUUGUACACACCGCCCGUCGCUAGUACC

         150                                             101
XENOPUS  GAUUGGAUGGUUUAGUGGGUCCUCGGAUCGGCCCGCCGGGGUCGG-CCAC   (insertions: A; C)
RABBIT   GAUUGGAUGGUUUAGUGGGCCCUCGGAUCGGCCCGCCGGGGUCGGCCCAC
YEAST    GAUUGAAUGGCUUAGUGGGCCUCAGGAUCG-CUUG-AGGGG--GGC----   (insertions: A; U; A; AA)

         100                                              51
XENOPUS  GGCCCUGGCGGAGCGCCGAGAAGACGAUCAAACUUGACUAUCUAGAGGAA
RABBIT   GGCCCUGGCGGAGCGCUGAGAAGACGGUCGAACUUGACUAUCUAGAGGAA
YEAST    AACUCCAUCUCAGAGCGGAGAAUUUGGACAAACUUGGUCAUUUGGAGGAA

         50                                                1
XENOPUS  GUAAAAGUCGUAACAAGGUUUCCGUAGGUGAACCUGCGGAAGGAUCAUUA(OH)
RABBIT   GUAAAAGUCGUAAGAAGGUUUCCGUAGGUGAACCUGCGGAAGGAUCAUUA(OH)
YEAST    CUAAAAGUCGUAACAAGGUUUCCGUAGGUGAACCUGCGGAAGGAUCAUUA(OH)
                                                            3'
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FIGURE 5. Comparison of the rabbit 18S rRNA nucleotide sequence at the 5'- and 3'-domains with both _Xenopus laevis_ and yeast 18S rRNA.

and 78% between rabbit and yeast. The greater sequence conservation of the 3'-domain in eukaryotic 18S rRNA may reflect its likely importance in mRNA and tRNA binding, and the function of the 40S subunit during protein synthesis as has been shown for prokaryotic 16S rRNA (1). Previous alignments between the E. coli 16S sequence and both the D. melanogaster (20) and B. mori (21) 3'-terminal sequences have indicated very strong homology at nucleotides #1-45 and #160-195. Recent studies have shown that the 5'-nucleotide of the anticodon of E. coli N-Acetyl-$tRNA^{Val}$ and N-Acetyl-$tRNA^{Ser}$ can be conveniently cross-linked with high efficiency to 16S rRNA by ultraviolet irradiation when the tRNA occupies the ribosomal P site (22). The specific nucleotide in the 16S rRNA involved in the cross-link has recently been identified as C_{1400} (23) corresponding to C_{167} from the 3'-terminus in Figure 4 within the highly conserved region #160-195 of eukaryotic 18S rRNA, suggesting the likelihood of similar tRNA:rRNA interactions in eukaryotes.

Finally, the methods described here for ^{32}P-end-labeling of large RNA molecules to high specific activity and the use of thin 140 cm long polyacrylamide gels for determination of nucleotide sequences 300-400 residues from the ^{32}P-end-labeled terminus, are general and can be used for sequence analysis of most comparably large and highly structured RNA molecules. Methods for extending sequence analysis beyond 400 nucleotides from a ^{32}P-end-labeled terminus are currently under investigation. The use of 90% formamide rather than 8 M urea as a denaturant, is much more effective in denaturing RNA fragments and substantially reducing band contraction in the sequencing gels. It has become evident from this work that nucleotide sequence analysis of the rRNA directly not only will allow detection of apparent cistron heterogeneities, but also will allow mapping of most of the modified basis with knowledge of their occurrence in previously catalogued T_1 RNase digestion fragments (13-15).

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