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Evolutionary changes in the higher order structure of the ribosomal 5S RNA

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ABSTRACT

Comparative studies have been undertaken on the higher order structure of ribosomal 5S RNAs from diverse origins. Competitive reassociation studies show that 5S RNA from either a eukaryote or archaebacterium will form a stable ribonucleoprotein complex with the yeast ribosomal 5S RNA binding protein (YL3); in contrast, eubacterial RNAs will not compete in a similar fashion. Partial S_1 ribonuclease digestion and ethylnitrosourea reactivity were used to probe the structural differences suggested by the reconstitution experiments. The results indicate a more compact higher order structure in eukaryotic 5S RNAs as compared to eubacteria and suggest that the archaebacterial 5S RNA contains features which are common to either group. The potential significance of these results with respect to a generalized model for the tertiary structure of the ribosomal 5S RNA and to the heterogeneity in the protein components of 5S RNA-protein complexes are discussed.

INTRODUCTION

All prokaryotic ribosomes and the cytoplasmic ribosomes of eukaryotic cells contain at least one low molecular weight RNA component, the 5S rRNA. Chloroplastid ribosomes also contain this RNA species (e.g., Ref. 1) and even some mitochondria contain a 5S RNA-like molecule (2). Because of its relatively small size (approximately 120 nucleotides) and ubiquity, the 5S RNA has been an attractive model for analyses of phylogenetic relationships and numerous sequence comparisons have been undertaken. For example, Walker and Doolittle (3) recently reported an ordering of basidiomycetes based on such an approach.

Many studies on the primary and secondary structures of 5S RNAs from many origins (see Ref. 4) have suggested that the structure of this molecule has been highly conserved in the course of evolution and at least six similar estimates for a universal secondary structure have been proposed (4-9). As yet, relatively little is known about the tertiary structure although a number of different studies utilizing a variety of physical and chemical probes (e.g., Refs. 10-14) suggest that the 5S RNA has a more complex structure than

suggested by the current estimates of the secondary structure. In our own studies on the protein binding site in the eukaryotic 5S RNA-protein complex (15) we found that the RNA-protein interaction is very dependant on the higher order structure in the RNA molecule and largely or entirely independent of the nucleotide sequence. In fact, since any eukaryotic 5S RNA appears to be able to form a stable ribonucleoprotein complex with the yeast 5S RNA binding protein (15), we suggested that they all contain a common three dimensional structure. A working model for the eukaryote 5S RNA ("lollipop" structure) appears to adequately explain the important and common structural features (16).

When the numerous experimental and theoretical data on 5S RNAs from more diverse origins (i.e., eu- and archaebacteria) are examined, a number of differences are suggested. For example, when the 5S RNA from the halophilic archaebacterium H. cutirubrum was examined (17), estimates of the secondary structure based on both theoretical considerations and fragments of partial ribonuclease digestion (obtained in determining the sequence) suggested that this RNA might have a structure unlike either prokaryotic or eukaryotic species. Indeed, other 5S RNAs from archaebacteria have also been postulated to have unusual secondary structures (18-20) but each appears to be unique to the specific organism. In view of these observations we have now extended our studies on the tertiary structure of the 5S rRNAs to more diverse species. Our results indicate significant differences which appear to correlate with the nature of the RNA-protein interaction within the ribosomal 5S RNA-protein complex.

MATERIALS AND METHODS

Purification and Labeling of 5S rRNAs

Whole cell RNA was prepared by homogenizing rat liver tissue, yeast or bacterial (Escherichia coli, Bacillus subtilis, Thermus aquaticus, Solfolobus solfataricus, or Halobacterium cutirubrum) cells in 10 volumes of sodium dodecyl sulfate-containing buffer and extracting with an equal volume of phenol at 65°C (17, 21). The low molecular weight RNA components were fractioned on an 8% polyacrylamide gel slab (22) and the 5S RNAs were repurified on a 12% polyacrylamide sequencing gel (23). Each RNA was then labeled at the 3' or 5' end with either cytidine 3', 5'-[5'-^{32}P] bisphosphate using RNA ligase or adenosine 5'-triphosphate [gamma-^{32}] using polynucleotide kinase, respectively (23) and again repurified on a 12% polyacrylamide gel. In some experiments, to ensure that a native conformation was present, the

labeled RNA was heated to 70°C in appropriate buffer and slowly cooled to room temperature (24).

Competitive reassociation of 5S RNA-protein complexes

A 5S RNA-protein complex was prepared from yeast ribosomes as previously described (25) and heterologous or homologous ribonucleoprotein complexes were formed by incubating the yeast complex with labeled RNA to allow exchange of RNA components. In a typical experiment ^{32}P-labeled 5S RNA was dissolved in 20 µl of the appropriate buffer (normally 25 mM EDTA, pH 7.0) containing 5-6.0 A_{260nm} units per ml of unlabeled yeast complex and incubated at 4°C for 30-45 minutes. The complex was then characterized by electrophoresis on an 8% polyacrylamide gel (22).

Determination of Ethylnitrosourea-reactive Sites

The RNAs were treated with ethylnitrosourea and the modified phosphodiester bonds were identified essentially as described by Vlassov et al. (26). Briefly, labeled RNA (20 µg ^{32}P-labeled RNA + carrier RNA) was dissolved in 20 µl of buffer favouring either a denatured or native conformation, and reacted with 5 µl of an ethylnitrosourea-saturated ethanol solution for 2 min. at 80-90°C or 1 h at 20°C, respectively. Following alkylation, the modified bonds were cleaved with 100 mM Tris-HCl (pH 9.0) at 50°C and the resulting fragments dissolved in formamide, heated to 90°C for 1 minute and analyzed by gel electrophoresis on 12% polyacrylamide gels using chemically degraded fragments as standards for the residue number (23). Differences in ethylnitrosourea reactivity were quantitated by scanning the autoradiographs of the gels using a Gilford 250 spectrophotometer at 700nm. Less reactive sites were detected by comparing the radioactivity under native and denatured conditions.

Determination of S_1 Ribonuclease-accessible Sites

Accessible single-stranded regions in the higher order structure were probed by partial digestion using S_1 ribonuclease under a variety of ionic conditions and enzyme concentrations. *In vitro* labeled RNA (25 µg of ^{32}P-labeled RNA + carrier RNA) was dissolved in 20 µl of buffer and digested with increasing concentrations of nuclease for 90 minutes at 20°C. The digestion was terminated by SDS-phenol extraction and the resulting fragments were dissolved in formamide, heated to 90°C for 1 minute and analyzed by electrophoresis on a 12% polyacylamide gel using a diethyl pyrocarbonate-aniline degraded 5S rRNA sample (23) as a standard for residue determinations.

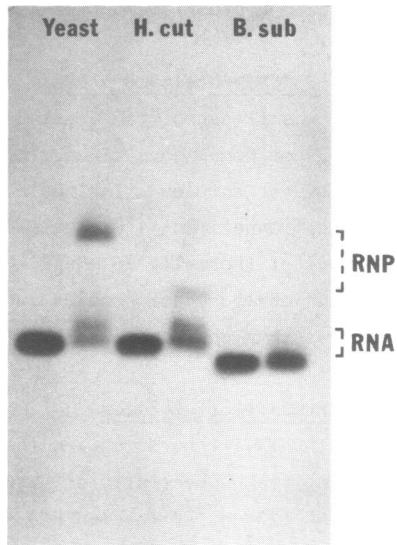


Figure 1. Formation of 5S RNA-protein complexes by competitive RNA exchange. 3' end-labeled yeast, archaebacterial (H. cutirubrum) or eubacterial (B. subtilis) 5S rRNA was incubated for 30-45 minutes in 20µl of 25 mM EDTA, pH7.0, containing 5-6 A_{260nm} units/ml of yeast 5S RNA-protein complex and applied to an 8% polyacrylamide gel. The positions of the 5S RNAs and the RNA-protein complexes are indicated; in each case an RNA sample, incubated in the absence of yeast protein, is included in the left of each pair of lanes as a control.

RESULTS

In our previous studies on the yeast 5S RNA-protein complex (15), heterologous ribonucleoprotein complexes could be formed with any eukaryotic 5S RNA despite significant differences in the nucleotide sequence. This observation, together with other data, was taken as evidence for a protein binding site based on structural domains rather than specific nucleotide sequences. Because the 5S RNA-protein complexes from bacteria are heterogeneous in their protein constituents (see Ref. 4) and show significantly more structural rearrangement during their formation (27), we decided to extend our study of heterologous complexes to include bacterial 5S RNAs. As illustrated in the example (B. subtilis) shown in Figure 1, none of the common bacterial RNAs (E. coli, B. subtilus or T. aquaticus) which we examined, competed successfully with the yeast 5S RNA to form a stable complex with the yeast 5S RNA binding protein (YL3). In contrast, the 5S RNA from an archaebacterium, H. cutirubrum, did form a ribonucleoprotein complex which was stable to electrophoretic fractionation (Fig. 1). In this

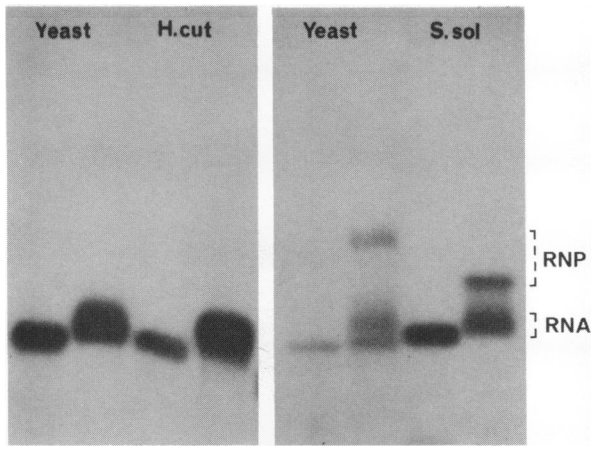


Figure 2. Comparative study on reassociated 5S RNA protein complexes containing archaebacterial 5S RNAs. 3' end-labeled yeast or archaebacterial (H. cutirubrum or S. solfataricus) 5S RNA was incubated for 30-45 minutes in 20 µl of 25 mM EDTA, pH 7.0, containing 5-6 A_{260nm} units/ml of yeast 5S RNA protein complex and applied directly to 8% polyacrylamide gel (right panels), or further incubated for 15 min. at 4°C with proteinase K (100 µg/ml) before being analyzed by gel electrophoresis (left panels). In each case an RNA sample, incubated in the absence of yeast protein is included in the left of each pair of lanes as a control.

instance, the complex migrated somewhat faster than the native yeast complex but this could have been an RNA specific difference. However, when the experiment was repeated with a second archaebacterium, Solfolobus solfataricus, essentially identical results were obtained (Figure 2) suggesting that the somewhat faster migration of the ribonucleoprotein complex is characteristic of, at least some, archaebacteria. The presence of a ribonucleoprotein complex could readily be demonstrated when the reassociated complexes were treated with protease as also illustrated in Figure 2; in all the experiments the slowest migrating band was observed to disappear.

In Figures 1 and 2, in addition to forming a complex with the RNA, the presence of the 5S RNA binding protein appeared to have a striking effect on the conformational state of the 5S RNA. With both the yeast and archaebacterial 5S RNA, much of the RNA was converted to a slower moving form. At best, only traces of this alternate form were observed with B. subtilis 5S RNA and none were observed with any of the RNAs in the absence of binding protein. It appears that a stable RNA-protein interaction leads to a distinct alternate conformational state in the RNA molecule.

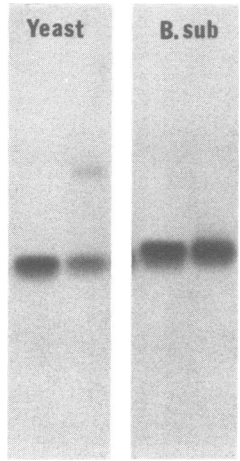


Figure 3. Formation of 5S RNA-protein complexes by competitive RNA exchanges in the presence of Mg^{2+} ions. 3' end-labeled yeast or B. subtilis 5S RNA was incubated for 30-45 minutes in 20 µl of 20 mM KCl, 5 mM $MgCl_2$, 100 mM Tris/Borate, pH 8.3, containing 5-6 A_{260nm} units/ml of yeast 5S RNA protein complex and applied to an 8% polyacrylamide gel. In each case an RNA sample, incubated in the absence of yeast protein, is included in the left of each pair of lanes as a control.

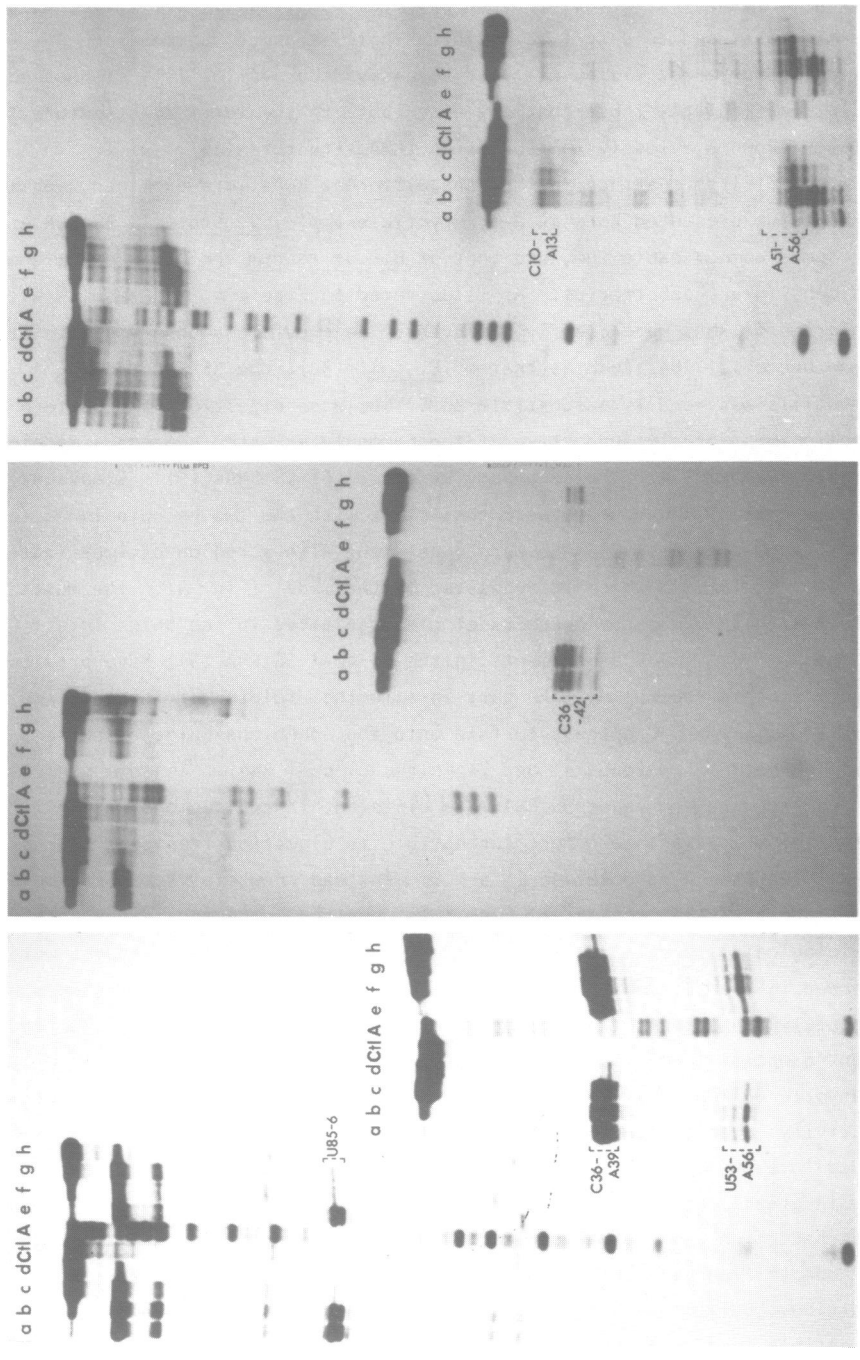
All of the reassociation experiments which are shown in Figures 1 and 2, were conducted in a low salt environment with Mg^{+2} ions, because previous studies have shown these conditions to be most favourable for the eukaryotic 5S RNA-protein complex (28). Since the conformation state appeared to be important in the reassociation of the 5S RNA into an RNP complex, further control experiments were carried out. As illustrated with B. subtilis 5S RNA in the presence of Mg^{+2} ions (Figure 3) or even when extra precaution to obtain a native conformation were taken (24,29), the eubacterial examples were still unable to effectively compete with the yeast 5S RNA to form a stable complex. Under identical conditions the yeast 5S RNA could still be reassociated into the complex although two alternate forms of the 5S RNA were no longer evident. This experiment strongly supports the suggestion that the eukaryotic and archaebacterial RNAs can take on a stable conformational state which is not possible with the eubacterial examples.

Since our experiments indicated a significant difference between the higher order structure of the eukaryotic 5S RNAs and those of the common bacteria, we undertook further studies on these differences. Because the three-dimensional structure appears to be particularly important for protein

binding (15), two different types of probe were utilized; partial S_1 nuclease digestion was used to detect accessible single stranded regions, and ethylnitrosourea-reactivity was used to assess the availability of phosphate groups, particularly those that may contribute to the tertiary structure, as has been shown previously in studies on tRNA structure (26).

In the first instance, all of the different RNAs were examined, yeast 5S RNA being presented here as a eukaryotic example, B. subtilis 5S RNA as one from a common bacterium, and that of H. cutirubrum, being an example of a halophilic archaebacterium. As illustrated in Figure 4, the results with each group of RNAs were clearly different. As previously observed with other common bacterial RNAs such as that of E. coli (30), the 5S RNA from B. subtilis was readily susceptible to S_1 nuclease digestion; the pattern of digestion was largely supportive of the commonly accepted concensus model ("wishbone model") for the secondary structure of 5S RNAs (5). Cleavages at residues C36-A39 and U85-U86 were consistent with the two hairpin loops (arms B and C) and cleavages U53-A56 were consistent with a region of imperfect base-pairing (arm B) which is suggested by the model (Fig. 5). The most anomolous feature was the absences of cleavage sites in the bulge in arm C but chemical crosslink experiments in the E. coli 5S RNA (31) have previously shown that this region may take part in a further folding of the tertiary structure (i.e. arm B appears to fold onto the anomolous bulge allowing a crosslink between residues 41 and 71 in the E. coli RNA). In some contrast, the results with the yeast 5S RNA were different in two regions; a similar portion of arm B was again most susceptible to digestion (residues A51-56) but no cleavages were observed in arm C. Instead, new cleavages were observed at residues C10-A13, consistent with this unpaired bulge and in the more physiological-like salt conditions, additional and variable cleavages were observed in the adjacent portion of arm C (G41-U50). These results were completely consistent with earlier studies on the yeast 5S RNA (32,33) and our recent proposal ("lollipop" model) for the tertiary structure of the eukaryotic 5S RNA (16). In our model we suggested, based on a diminished reactivity in phosphate residues, that the second arm (C) in Figure 5 is also folded into the tertiary structure.

In greater contrast to both groups of RNA, the 5S RNA from our halophilic archaebacterium example was strikingly more resistant to S_1 nuclease digestion. As shown in Figures 4 and 5, under both low and physiological-like salt conditions, only one region was susceptible, namely residues C36-42, the most susceptible loop in the other two RNAs. The structure in this case appears


a b c dCtl A e f g h
C10–
A13
A51–
A56
C36
-42
U85–6
C36–
A39
U53–
A56

to be more compact and unlike the 5S RNA from B. subtilis, the inaccessibility of arm C to S_1 nuclease digestion suggests that this arm may be included in the tertiary structure in a manner similar to the eukaryotic 5S RNAs.

To maintain maximum enzyme specificity, the experiments shown in Figure 3 were carried out under optimum buffer conditions at pH 4.6 in the presence of Zn^{+2} ions. Since these conditions were somewhat non-physiological the comparisons were repeated at a more physiological-like pH in the presence of Mg^{+2} ions. As previously observed with the rat 5S RNA (34) and as illustrated in Figure 6, the results were essentially identical. Once again the 5S RNA from B. subtilis contained three susceptible regions while that of a second archaebacterial example, S. solfataricus, was readily susceptible in only one region, residues U41-42 in arm C. Under both digestion conditions the conclusions reached in Figure 5, remained the same.

Because our results with S_1 nuclease digestion strongly suggested striking differences in the tertiary structure, we extensively probed the reactivity of phosphate residues using ethylnitrosourea. This technique has previously been validated in the studies on the tertiary structure of tRNA (26) and to develop the "lollipop" model for the eukaryotic 5S RNA (16). As in all of the earlier experiments (e.g. 16,26) the modifications were carried out in the presence of 20% ethanol to maximize the solubility of the ethylnitrosourea reagent and, therefore, the degree of phosphate modification. Also as in these earlier studies, preliminary experiments in which less ethylnitrosourea-saturated ethanol was added, resulted in similar but less distinct (fainter) reactivity profiles (results not shown) largely eliminating the possibility of artifactual conformational changes. Again, as shown in

Figure 4. A comparison of 3' end-labeled B. subtilis (left), H. cutirubrum (centre) and yeast (right) 5S rRNA partially degraded with S_1 nuclease and fractioned on a 12% polyacrylamide gel. Labeled RNA plus carrier (25µg) was dissolved in 20 µl of low salt (30 mM NaCl, 30 mM Na acetate, 1 mM $ZnSO_4$, 5% glycerol, pH 4.6), lanes a-d, or physiological-like (300 mM NaCl, 30 mM Na acetate, 1 mM $ZnSO_4$, 5% glycerol, pH 4.6, for B. subtilis and yeast RNA; 3.4 M KCl, 30 mM Na acetate, 1 mM $ZnSO_4$, 5% glycerol, pH 4.6, for H. cutirubrum RNA) buffer, lanes e-h. Samples in low salt buffer and 300 mM NaCl physiological-like buffer were digested with 10 units (a, e), 5 units (b, f), 2 units (c, g), or 0.2 units (d, h) of enzyme while H. cutirubrum RNA in 3.4 M KCl physiological-like buffer was digested with ten times the amount of enzyme due to enzyme inhibition from high salt concentrations. All digests were carried out at 20°C for 90 minutes and terminated by SDS-phenol extraction. The fragments were separated by electrophoresis for 18 hours (left) or 4 hours (right) at 1000 volts; the cleaved residues are identified in the margins. 3' end-labeled RNA chemically degraded at adenylic acid residues (A) and an aniline treated control (Ctl) were applied as markers for the nucleotide sequence.

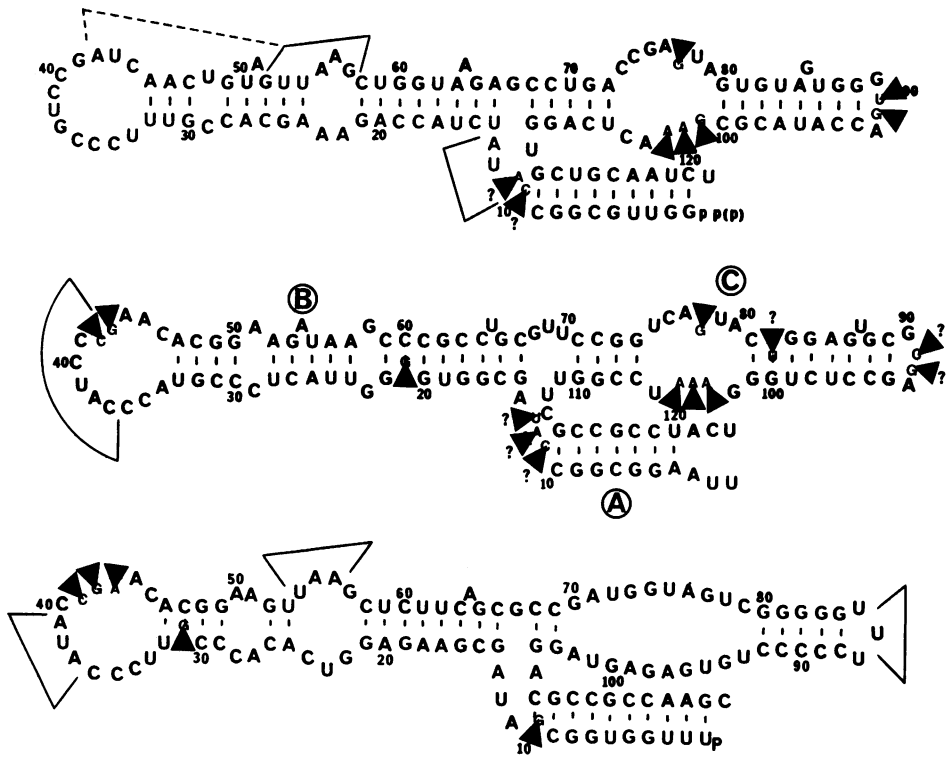


Figure 5. Summary of accessible residues in estimates of the secondary structure for the S. cerevisiae (upper), H. cutirubrum (middle), and B. subtilis (lower) 5S rRNA. The universal models are based on the base-pairing scheme of Nishikawa and Takemura (5), and the nucleotide seuqences are taken from Ref. 16, 44 and 45. The H. cutirubrum sequence includes two additional residues (G_{90} and C_{91}) which were reported by Luehrsen et al. (20) and confirmed in this study. The solid lines indicate regions which were susceptible to mild S_1 nuclease digestion under physiological-like digestion conditions. The closed triangles cover residues which showed a reduced phosphate reactivity to ethylnitrosourea under native conditions; the small question marks indicate residues where the reduction was relatively small or variable.

Figures 7 and 8, significant differences were observed between the three types of RNA. When 3' end labeled RNAs were analyzed (Fig. 7), the rat RNA (our eukaryotic example) showed reduced levels of phosphate reactivity and subsequently reduced cleavage in three regions of the molecule, residues G99-G101, A88-G89 and G75 (Table 1). A similar pattern of reduced reactivity which results in lighter bands on the gel has also been observed in the yeast and plant RNA (16). In contrast, while the H. cutirubrum molecule showed

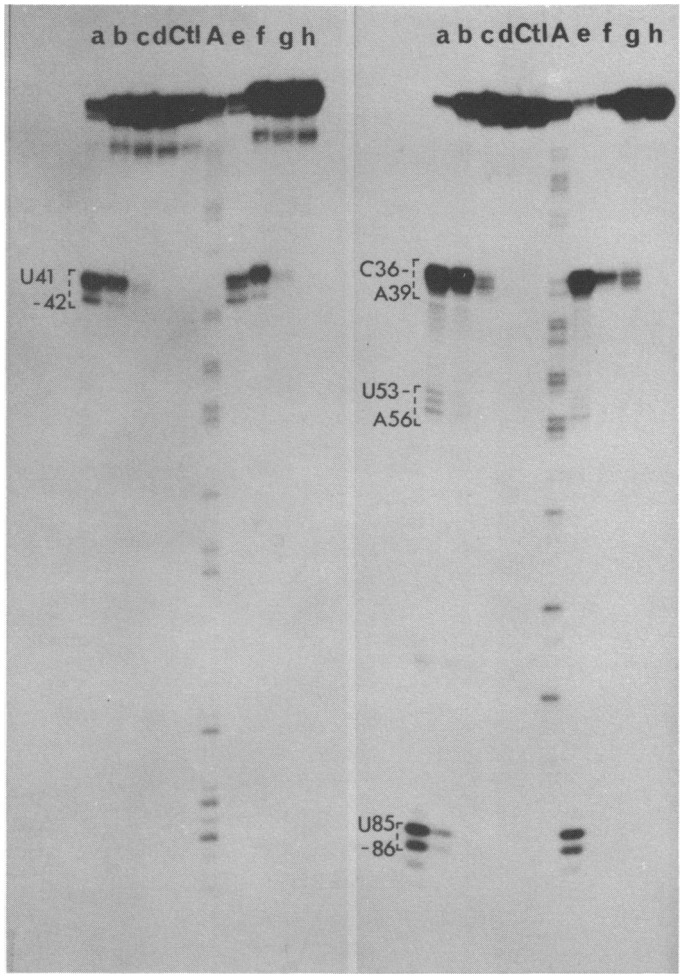


Figure 6. A comparison of 3' end-labeled S. solfataricus (left) and B. subtilis (right) 5S RNA, partially degraded with S_1 nuclease in the presence of Mg^{+2} ions. Labeled RNA plus carrier (25 µg) was dissolved in 20µl of low salt (30 mM NaCl, 30 mM Na acetate, 10 mM $MgCl_2$, 1 mM $ZnSO_4$, 5% glycerol, pH 5.5), lanes a-d; or physiological-like (300 mM NaCl, 30 mM Na acetate, 10 mM $MgCl_2$, 1 mM $ZnSO_4$, 5% glycerol, pH 5.5) buffer, lanes e-h. Digestion conditions and symbols are those described in Figure 4.

reproducible reductions in at least two of the regions, residues A103-A105 and G78, the B. subtilis 5S RNA (Fig. 7) or other eubacterial 5S RNAs (Table 1) contained no significant differences at all. Even in T. aquaticus 5S RNA, which has a comparable cluster of adenylic acid residues in this

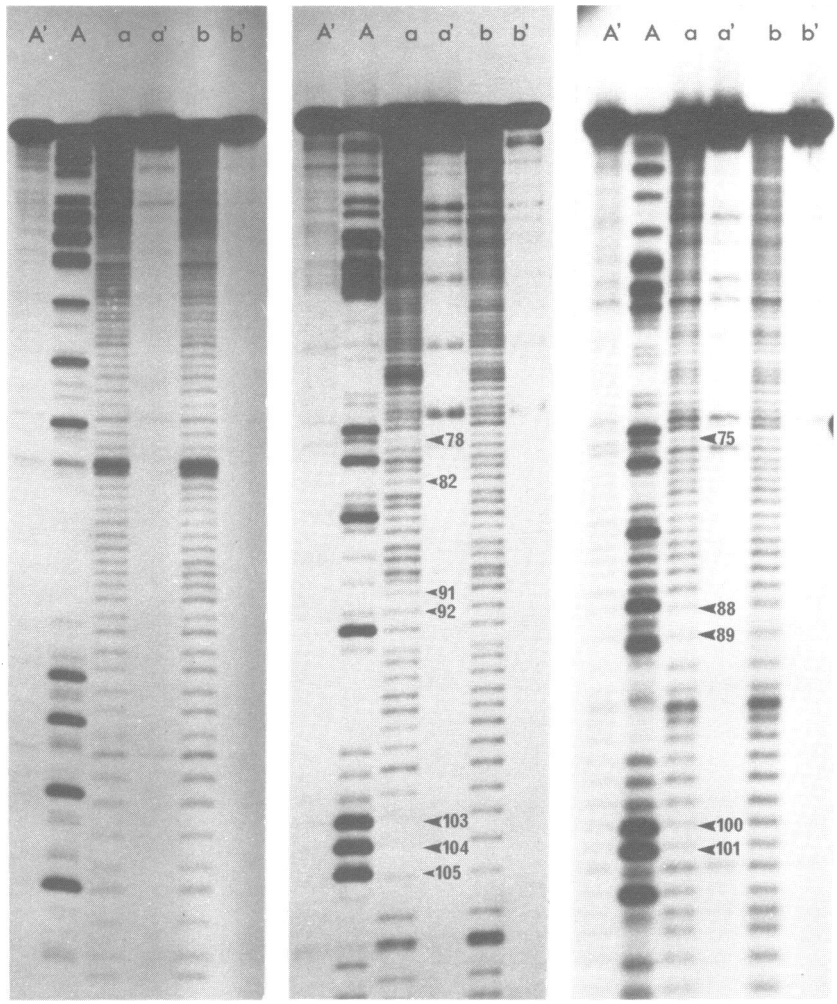


Figure 7. A comparison of phosphate alkylation in 3' end-labeled B. subtilis (left), H. cutirubrum (centre) and rat liver (right) 5S rRNA. 3' end-labeled RNA (20 µg) was dissolved in 20 µl of buffer favouring a denatured (2mM EDTA, 0.3 M Na cacodylate, pH 8.0) or native (100 mM NaCl, 20 mM $MgCl_2$, 0.3 M Na cacodylate, pH 8.0) conformation and reacted with 5 µl of an ethylnitrosourea-saturated ethanol solution for 2 minutes at 80-90°C (b) or 1 hour at 20°C (a), respectively. Electrophoresis was carried out for 4-6 hours at 1050 volts. Large arrows identify residues which showed reproducible reductions in reactivity; small arrows indicate residues where the reduction was relatively small or variable. Fragments from RNA chemically degraded at adenylic acid residues (A) and an aniline treated control (A') were applied as markers for the nucleotide sequence; 5S RNA incubated in the absence of ethylnitrosourea (a' and b') is also included to indicate nonspecific degradations.

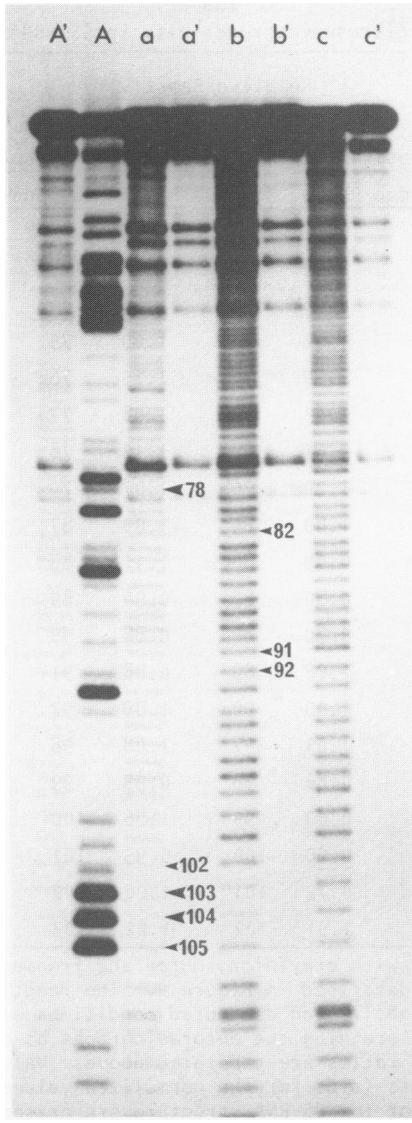


Figure 8. Relative reactivities of phosphates to ethylnitrosourea in the H. cutirubrum 5S rRNA under different salt conditions. 3'-labeled RNA was treated in 20 µl of high salt (3.4 M KCl, 20 mM $MgCl_2$, 0.3 M Na cacodylate, pH 8.0) buffer (a), lower salt buffer (100 mM NaCl, 20 mM $MgCl_2$, 0.3 M Na cacodylate, pH 8.0) buffer (b) or denaturing (2 mM EDTA, 0.3 M Na cacodylate, pH 8.0) buffer (c). Following modification, and strand scission, the fragments were analyzed by gel electrophoresis at 1000 volts for 5 hours; the symbols are as described in Figure 7.

Table 1
A comparison of ethylnitrosourea reactivity in 5S rRNAs of diverse origins

Relative Reactivity									
Rat Liver		H. cutirubrum		T. aquaticus		E. coli		B. subtilis	
Residue	Ratio	Residue	Ratio	Residue	Ratio	Residue	Ratio	Residue	Ratio
76	1.05	77	0.99	70	1.00	73	1.05	73	0.90
77	0.60	78	0.46	71	1.06	74	0.96	74	0.94
78	0.97	79	1.22	72	1.00	75	0.94	75	1.16
79	1.09	80	1.80	73	1.15	76	1.00	76	1.10
80	1.05	81	1.03	74	1.08	77	1.10	77	0.90
81	0.96	82	0.61	75	1.12	78	1.13	78	1.03
82	1.04	83	1.20	76	1.07	79	1.12	79	1.11
86	1.09	89	1.09	85	1.00	87	1.00	84	0.94
87	1.08	90	0.09	86	1.11	88	1.05	85	0.91
88	0.65	91	0.80	87	0.94	89	1.22	86	1.10
89	0.80	92	0.70	88	0.88	90	1.10	87	1.14
90	1.12	93	1.08	89	0.88	91	0.96	88	1.20
91	1.11	94	1.00	90	1.09	92	1.00	89	1.00
98	0.95	101	1.03	97	1.08	98	0.92	93	0.94
99	0.69	102	0.86	98	0.95	99	0.90	94	1.10
100	0.52	103	0.60	99	1.06	100	0.91	95	1.20
101	0.74	104	0.60	100	0.95	101	0.87	96	1.07
102	1.12	105	0.72	101	1.00	102	1.12	97	0.96
103	1.05	106	1.01	102	1.02	103	0.99	98	1.08

Labeled RNA was reacted with ethylnitrosourea and fragments were separated by gel electrophoresis as described in Figure 4. The reactivity ratios between the reactivities under native and denatured conditions were calculated from degradation profiles by scanning the autoradiographs as previously reported (16) and the normalized ratios are tabulated above. Values are averages for 2-4 replicate experiments (s.d. for the normalized values = 0.072); residues from analogous regions of the 5S RNA structure are presented in each case and significant changes in the reactivity are underlined.

region, no significant differences in reactivity were observed (Table 1). Furthermore, the two regions of reduced reactivity in the H. cutirubrum 5S RNA were observed both under physiological-like high salt conditions or in the low salt conditions which were more physiological-like for the other bacteria (Figure 8). This excluded the possibility that the lack of

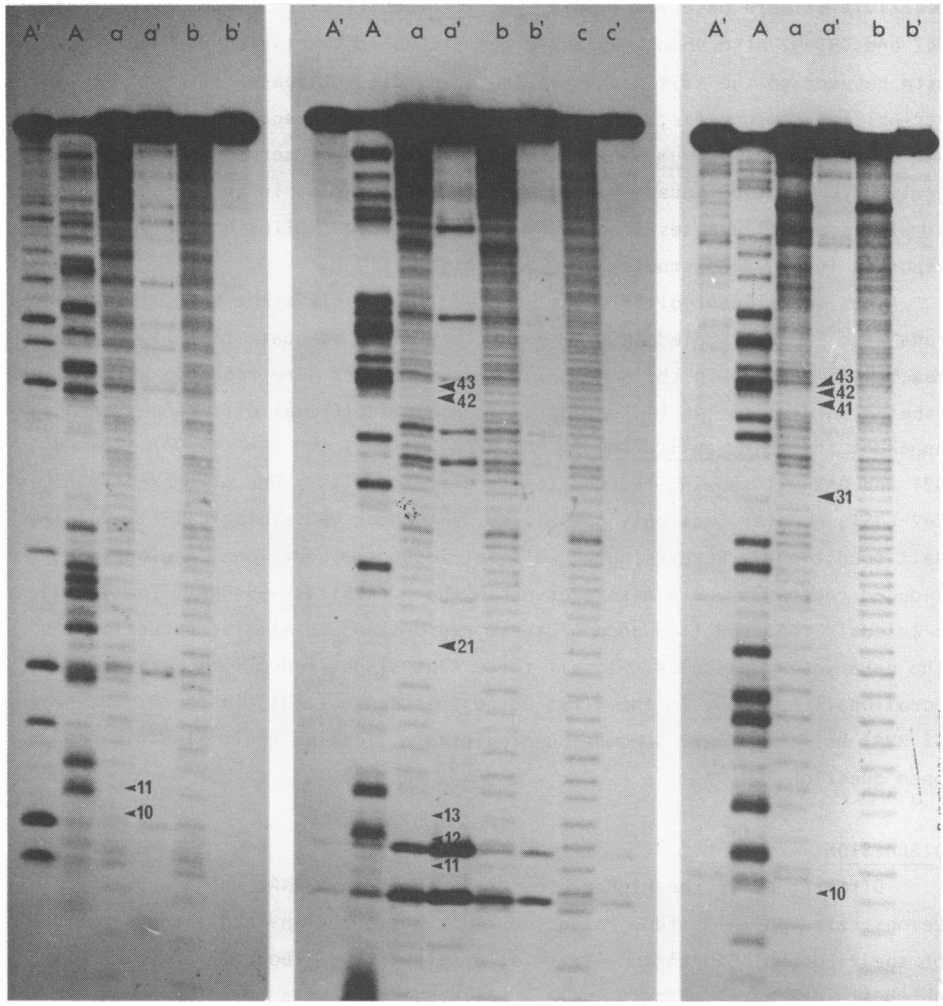


Figure 9. A comparison of phosphate alkylation in 5' end-labeled B. subtilis (left), H. cutirubrum (centre) and S. cerevisiae (right) 5S rRNA. 5' end-labeled RNA was treated with ethylnitrosourea and analyzed by gel electrophoresis as described in Figs. 7 and 8. Fragments from RNA chemically degraded at adenylic acid residues (A) and an aniline treated control (A') were applied as markers for the nucleotide sequence; 5S RNA incubated in the absence of ethylnitrosourea (a',b' or c') is also included to indicate nonspecific degradations.

differences in reactivity in the eubacterial 5S RNAs was simply the result of differences in the incubation buffers. Even when the E. coli 5S RNA was specifically renatured to ensure a native conformation (24), no reduced

reactivities were observed (Table 1). In H. cutirubrum, two other regions, U82 and C91-92 also showed variable reductions in reactivity particularly with respect to the salt concentration (Fig. 8); the reason for this variability was unclear. Occasionally an increased reactivity, for example A80 in the H. cutirubrum 5S RNA (Table 1), was also observed; these could be truly more reactive because of a special configuration in the molecule or simply very labile sites in the 5S RNA. Similar anomolies have also been reported in previous studies of tRNA (26).

With significant differences observed in the different RNAs, long gel runs with 3' and labeled RNAs were not considered adequate to fully evaluate reactivity changes in the 5' end so the experiments were repeated using 5' end labeled molecules. As indicated in Figure 9, additional differences were again apparent in the H. cutirubrum 5S RNA with at least two new regions (residues G21 and C42-43) showing reduced levels of reactivity. One of these (residues C42-G43), however, was only clearly evident under "physiological-like" (high) salt conditions. Surprisingly, while at least two additional regions of reduced reactivity were also present in the B. subtilis 5S RNA (Fig. 9), as previously reported (16) none of these regions showed similar reductions in the eukaryotic molecules. In all three RNAs slight reductions were also occasionally present in the first bulge (residues C11-U13 in the H. cutirubrum 5S RNA) but these were somewhat unpredictable, leaving their significance an open question.

DISCUSSION

Differences in the higher order structure of 5S RNAs which were demonstrated in this study raise two important questions about the structure of the ribosomal 5S RNA and its relationship to the ribosomal proteins with which it interacts. First, while a universal consensus for a Y-shaped secondary structure ("wishbone" model) has been generally accepted by most workers (see Ref. 4), it appears that an overal consensus for the tertiary structure, at least for the free molecule, is probably not appropriate. Two working models have already been suggested for the tertiary structure. In E. coli, in view of a chemical crosslinking (31) and other data, one of the two arms of the Y-shaped secondary structure has been estimated to fold over onto the other arm (35,36). As indicated earlier, this is consistent with the S_1 nuclease cleavage sites in arm C (Fig. 3) of the B. subtilis 5S RNA (residues U85-U86). In our estimate for the eukaryotic 5S RNA (16), both arms (B and C) overlap to form a loop or "lollipop"-like structure. This

satisfactorily explains the disappearance of the cleavage sites in arm C and the reduced reactivities to ethylnitrosourea. We further suggest in this study that the tertiary structure of the H. cutirubrum 5S RNA is more similar to the eukaryotic structure except that additional interactions (e.g., hydrogen bonds with phosphate groups) such as those found in the B. subtilis 5S RNA draw the structure tighter. This could result in the increased resistance to S_1 nuclease digestion and the faster migrating ribonucleoprotein complex which we observed in our study. Whatever the case, our study clearly underlines differences in the tertiary structure and suggests that, in terms of a universal model, the approach taken by Fox and Woese to defining the secondary structure (i.e., the definition of a minimum structure) may equally be appropriate for the tertiary structure. Under this scheme the eubacterial structure would be the minimum most open tertiary structure, while that of archaebacteria, at least H. cutirubrum, would be the most compact.

A related issue which this study raises, is the question of a universal structure within the active ribosome which might be expected if the 5S RNA has a universal role in ribosome function. Previous comparisons may offer an explanation for the more open structure in 5S RNAs from eubacteria. Those studies showed that while the E. coli 5S RNA-protein complex contains three separate and smaller RNA binding proteins (37), that of H. cutirubrum contains two larger protein species (38) and the eukaryotic complex contains a single protein component (39) of a molecular weight which is approximately equal to the total protein content of the other complexes (25). As a result, a fusion of ribosomal protein sequences has been speculated and protein sequence comparisons offer some support for this idea (25,40). The binding of the prokaryotic proteins has also been shown to take place in a co-ordinated fashion (41), and recent studies on the actual protein binding sites in the 5S RNAs indicate that the proteins probably recognize helical regions (15,42) and that there are three such sites in the 5S RNA molecule (15). Accordingly, taking all of these observations together, it is attractive to postulate that in most bacteria each of the three protein components may interact primarily with a different helical region and that co-ordinate interactions between the proteins and respective binding sites bring about a more closed structure in the complex or ribosome. In contrast, in the eukaryotic complex the RNA binding sites within the protein may be fixed and, as a result, the RNA structure itself is initially a more closed structure and less susceptible to major rearrangements during protein binding. Physical and chemical studies (27,43) which indicated a lower degree of conformational change in eukaryotes

during complex formation are also consistent with this notion. Further studies on the interactions and related conformational changes will be required to test this possibility. The present studies, nevertheless, clearly show differences in the higher order structures of 5S RNAs of diverse origin and underline the potential usefulness of higher structure analyses as a more sensitive measure of phylogenetic relationships.

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