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Three helical domains form a protein binding site in the 5S RNA-protein complex from eukaryotic ribosomes

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ABSTRACT

A ribosomal protein binding site in the eukaryotic 5S rRNA has been delineated by examining the effect of sequence variation and nucleotide modification on the RNA's ability to exchange into the EDTA-released, yeast ribosomal 5S RNA-protein complex. 5S RNAs of divergent sequence from a variety of eukaryotic origins could be readily exchanged into the yeast complex but RNA from bacterial origins was rejected. Nucleotide modifications in any of three analogous helical regions in eukaryotic 5S RNAs of differing origin reduced the ability of this RNA molecule to form homologous or heterologous RNA-protein complexes. Because sequence comparisons did not indicate common nucleotide sequences in the interacting helical regions, a model is suggested in which the eukaryotic 5S RNA binding protein does not simply recognize specific nucleotide sequences but interacts with three strategically oriented helical domains or functional groups within these domains. Two of the domains bear a limited sequence homology with each other and contain an unpaired nucleotide or "bulge" similar to that recently reported for one of the 5S RNA binding proteins in Escherichia coli (Peattie, D.A., Douthwaite, S., Garrett, R.A. and Noller, H.F. (1981) Proc. Natl. Acad. Sci. 78, 7331-7335). The results further indicate that the single ribosomal protein of eukaryotic 5S RNA-protein complexes interacts with the same region of the 5S rRNA molecule as do the multiple protein components in complexes of prokaryotic origin.

INTRODUCTION

Since Blobel first reported that the 5S rRNA could be released from mammalian ribosomes as a ribonucleoprotein complex (1) a variety of similar complexes have been found with ribosomes from many different organisms including wheat (2), flies (2), yeast (3), and several different bacteria (4-6). Because these complexes are easily prepared in large amounts, they, particularly that of Escherichia coli ribosomes, have been attractive models for the study of ribosome structure and interactions in general. The interaction between the 5S RNA and proteins present in the complex might be expected to be representative of the many other RNA-protein and protein-protein interactions in the ribosome. Furthermore, since the 5S RNA has been postulated to interact with tRNA (5) or the 16-18S rRNA component (7) during protein

synthesis, the complex may also offer a model for studies on RNA-RNA interactions as well.

The 5S RNA-protein complexes which have been released from eukaryotic ribosomes differ from those of prokaryotes in at least one major respect. In bacteria, the complex contains at least two (4, 5), and depending on the organism or method of preparation, three ribosomal proteins (8). In eukaryotes, only one larger protein (YL3 in yeast) has been observed in EDTA-released complexes (1-3) although 5S RNA interactions with other ribosomal proteins have been demonstrated by affinity chromatography (9, 10). Despite this difference, both physical and chemical studies suggest that all the complexes share fundamental features in the protein-RNA interaction (11). This has caused us to speculate (3) that the single larger eukaryotic 5S RNA binding protein has evolved through a fusion of genes for the multiple binding proteins in prokaryotes.

While rapid progress is being made in the elucidation of the 5S RNA structure and its protein complexes (see 2 and 12 for reviews), the protein binding sites are not fully defined and some controversy still remains. Most studies suggest that at least the primary contact sites reside in the 3'-terminal half of the 5S RNA molecule together with a small portion of the 5'-end which forms a double helix structure with the 3'-end. The important nucleotides or structures, however, are not known although a recent report by Peattie et al. (13) indicates that a "bulged" double helix represents the primary contact site for one of the 5S RNA binding proteins (L18) in E. coli. In this study we used a similar "modification exclusion" approach and rapid RNA sequencing techniques to further delineate the protein binding site in eukaryotic 5S RNA-protein complexes. The results suggest that the protein binding site is composed of three strategically located helical domains and that the protein does not simply recognize the nucleotide sequences.

MATERIALS AND METHODS

Isolation and Labeling of 5S RNAs and the 5S RNA-Protein Complex

Yeast (Saccharomyces cerevisiae, strain S288C) were grown aerobically and both the 5S RNA and 5S RNA-protein complex were isolated from 60S ribosomal subunits as previously described (3). Rat liver, wheat germ, Thermomyces lanuginosus and Escherichia coli 5S RNAs were purified from whole cell RNA extracted with SDS-phenol (3). Purified RNA was labeled in vitro at the 3' end using RNA ligase (P-L Biochemicals; Inc.) and cytidine 3', 5'-[5'-^{32}P] bisphosphate (New England Nuclear) and repurified on a 12% poly-

acrylamide sequencing gel (14).

Probing of the Protein-Binding Site by Modification Exclusion

The ribosomal protein binding site was probed using a modification exclusion procedure similar to that recently described by Peattie and Herr (15). Briefly, 3'-end labeled 5S RNA was modified using the base modification reactions of Peattie (14) with slight changes in the reaction conditions (16). Modified RNAs were allowed to exchange into the yeast RNA-protein complex by dissolving the ethanol-precipitated RNA directly in 25 mM EDTA, pH 7.0, containing 6.0 A_{260nm} units per ml of unlabeled complex and incubating at 4°C for 30-120 minutes. The complex was fractionated from free 5S RNA by electrophoresis on a 8% polyacrylamide gel, and both fractions were eluted by homogenization in SDS-containing buffer (3) followed by ethanol precipitation. Sites of modification were determined by aniline cleavage and subsequent analyses on RNA sequencing gels (14, 16); a comparison of fragments obtained from free and bound RNA fragments indicated residue modification which were partially or entirely excluded from the complex.

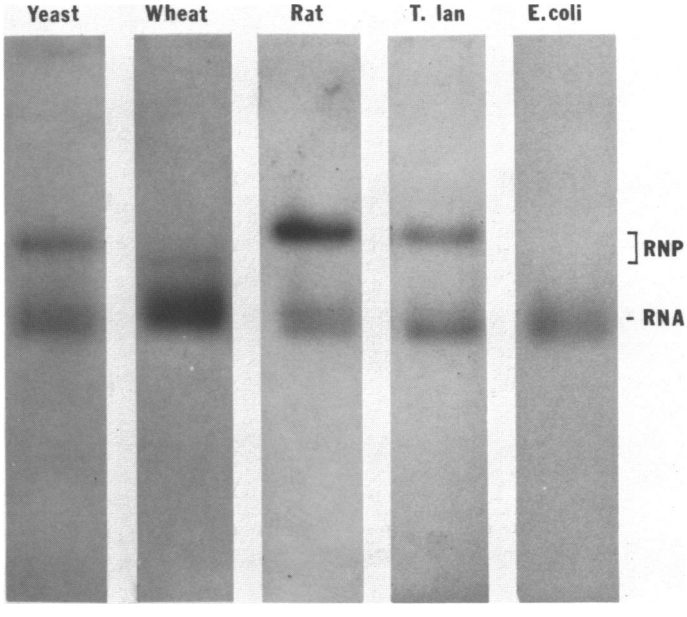
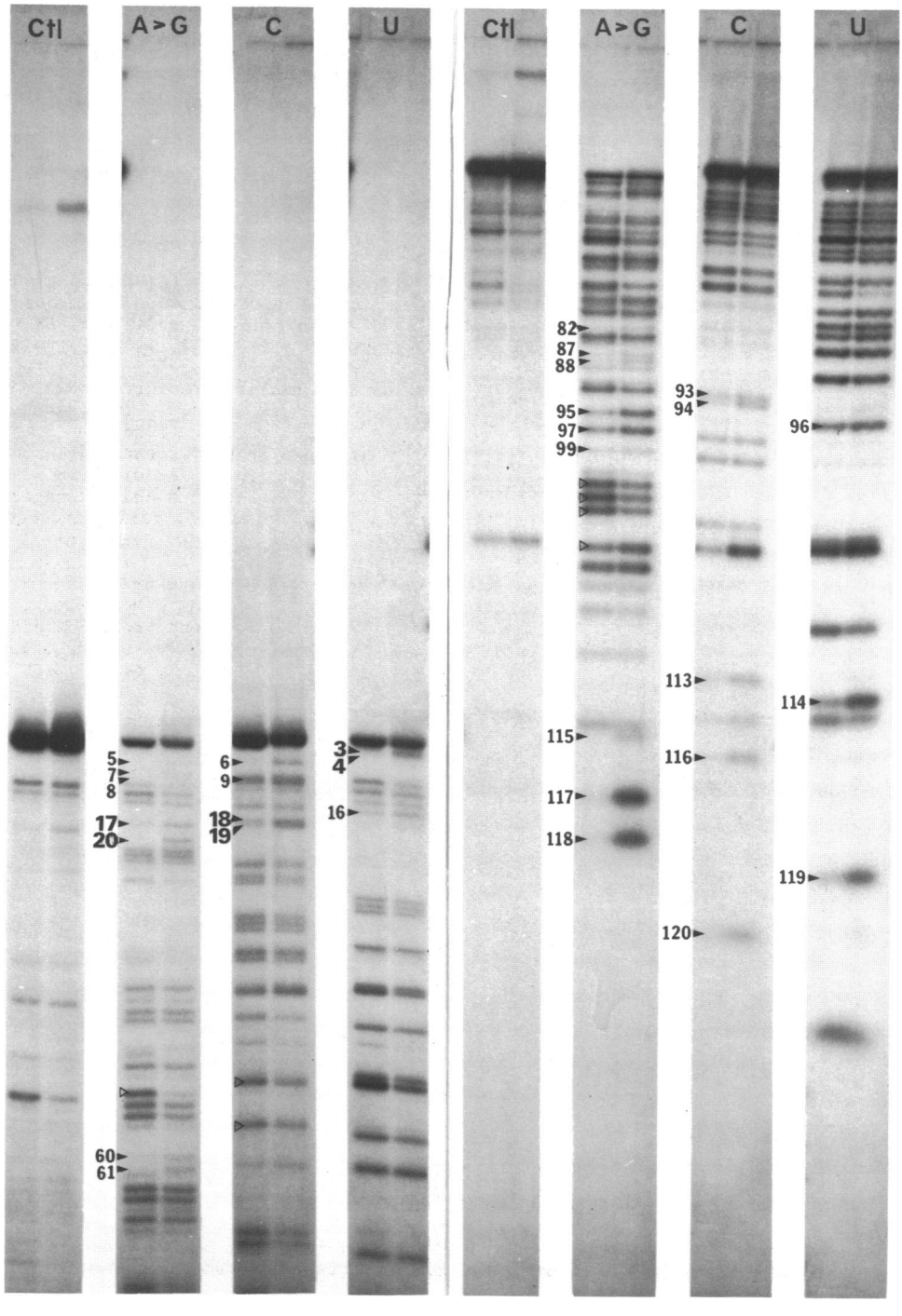


Figure 1. Formation of 5S RNA-protein complexes by RNA exchange. 3'-labeled yeast, wheat, thermophilic fungal (T. lanuginosus), rat liver, or bacterial (E. coli) 5S rRNA was incubated for 30-60 minutes in 25 µl of 25 mM EDTA, pH 7.0, containing 0.6 A_{260nm} units/ml of yeast 5S RNA-protein complex and applied to an 8% polyacrylamide gel. The positions of the 5S RNAs, and the RNA-protein complexes are indicated along with the origin and bromophenol blue dye marker.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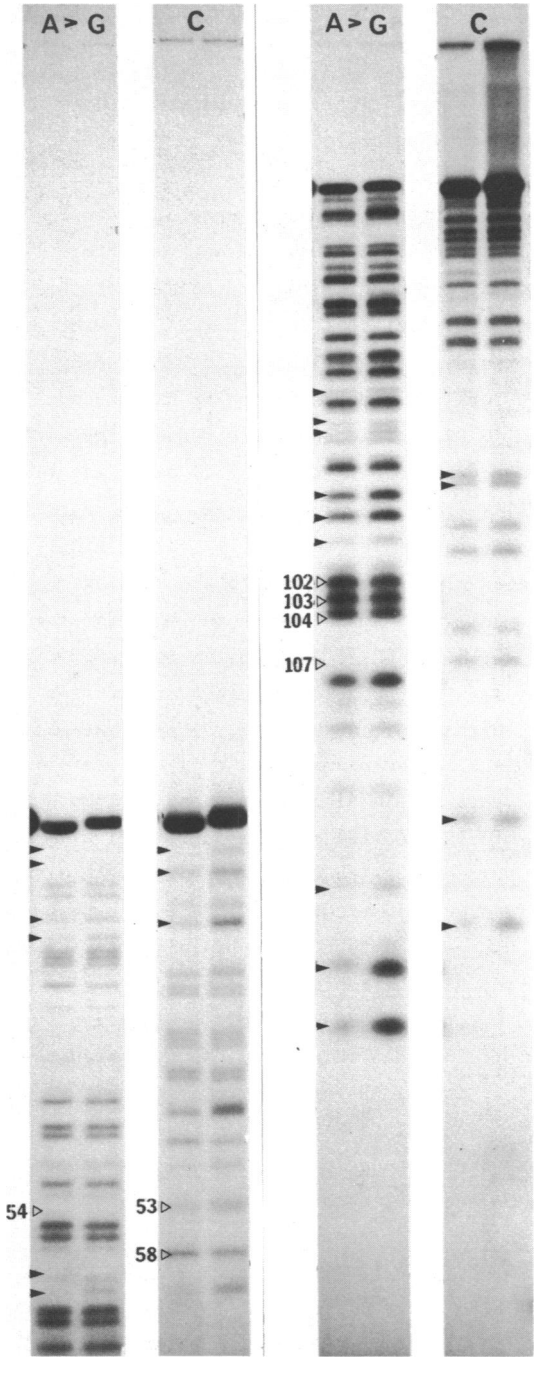


Figure 2. Autogradiograph of chemically modified free and protein-bound yeast 5S rRNA after aniline-induced degradation and fractionation on a 12% polyacrylamide gel. Electrophoresis was carried out at 1050 volts for 16 hours (left) or 4 hours (right). Unmodified RNA, which was exchanged into the RNA-protein complex and aniline treated, is included as a control (Ctl) to eliminate non specific cleavages. The closed arrows indicate modifications which were partially or largely excluded from the 5S RNA-protein complex; the open arrows indicate examples of unusual intensity changes which were not reproducible or present in control lanes. A, diethyl-pyrocarbonate-induced cleavages; C, 3.0 M NaCl/hydrazine-induced cleavages; U, 50% hydrazine-induced cleavages. With each reaction modified and aniline-treated free RNA was applied to the left lane and protein bound RNA was applied to the right lane. (a) A standard analysis of all residues.
(b) A repeat analysis illustrating reproducibility in nucleotide exclusion and variability in the non specific intensified or cleaved bands.

RESULTS

Exchange of 5S rRNAs into Ribonucleoprotein Complexes

As previously reported (3), the 5S RNA-protein complex from yeast ribosomes which contains one ribosomal protein (YL3) could be efficiently labeled through RNA exchange by incubating unlabeled complex in the presence of ^{32}P-labeled 5S RNA (Figure 1). In contrast, other types of RNA (e.g. tRNAs or 5.8S rRNAs) would not exchange into or form a RNA-protein complex under the same experimental conditions indicating that the natural interaction was specific. Because these earlier studies (3) indicated that this exchange was so specific and neither the yeast tRNA or 5.8S rRNA, or bacterial (Halobacterium cutirubrum) 5S rRNA would interact with the 5S RNA binding protein, we decided to more closely look at features in the 5S RNA sequence which effect 5S RNA binding. Using 5S RNAs of increasing diverse origin as a natural source of sequence difference, we examined the ability of fungal (T. lanuginosus), plant (wheat), mammalian (rat), or bacterial (E. coli) 5S rRNA to exchange into the yeast 5S RNA-protein complex (Figure 1). Surprisingly, only the bacterial 5S RNA was again unable to exchange into the complex although the degree of exchange was somewhat variable with the more diverse eukaryotic 5S RNAs. In particular, with the wheat 5S RNA, the degree of exchange was somewhat lower and the resulting complex migrated at a faster rate than the native complex from yeast cells. Apparently, despite the many sequence differences between the RNAs which were examined, only the bacterial structure is not recognized by the yeast 5S RNA binding protein. Attempts to form complexes using renatured E. coli 5S RNA (13) or other bacterial examples such as Bacillus subtilis or Thermus aquaticus (results not shown) were also unsuccessful suggesting that some fundamental structural difference between prokaryotic and eukaryotic 5S RNAs was central to the RNA-protein interaction.

Exclusion of Modified Nucleotides from Ribonucleoprotein Complexes

Because sequence comparisons did not provide an obvious explanation for the specific binding of only eukaryotic 5S RNAs (Figure 1), we attempted to identify residues which were important in the RNA-protein interaction by examining the effect of base modification. The 5S RNAs from several eukaryotes were labeled and the bases were randomly modified using standard sequencing reactions (14) so that, on average, each molecule contained fewer than one modified residue. An unmodified control was also included in each experiment to account for non-specific cleavages. When modified yeast 5S RNA was exchanged into the ribonucleoprotein complexes, the resulting band was somewhat more diffuse but an exchange nevertheless occurred with each of

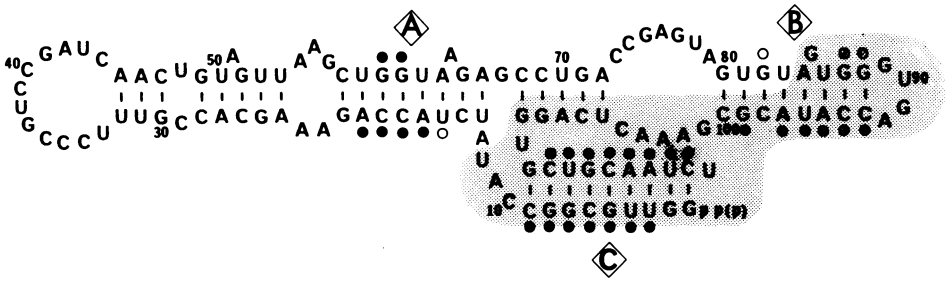
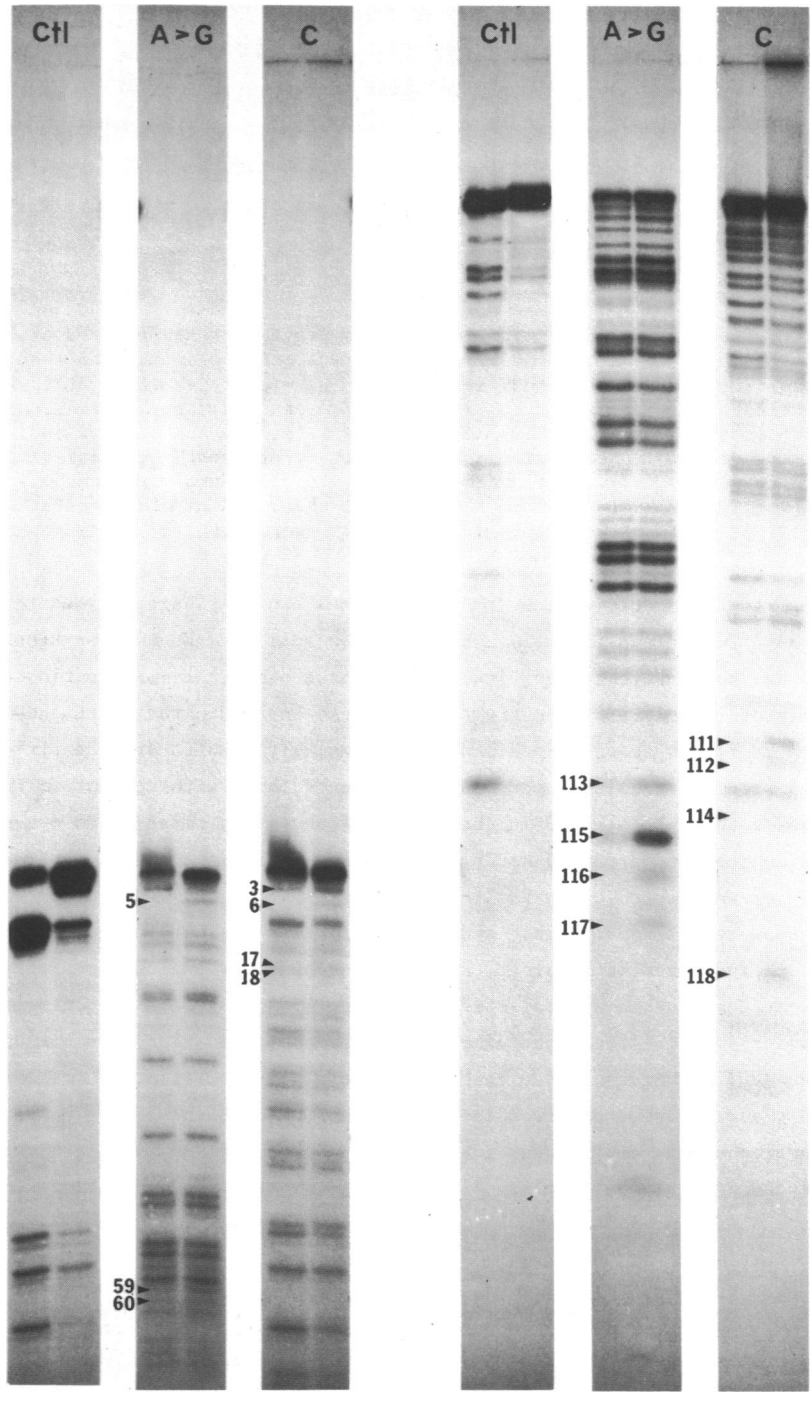


Figure 3. Exclusion of base modifications in yeast 5S rRNA from the 5S RNA-protein complex. Chemically modified 5S RNA was exchanged into the yeast 5S RNA-protein complex and the modified residues were analyzed after aniline-induced cleavage as described in Figure 2. The secondary structure is estimated according to Nishikawa and Takemura (19); the shaded area indicates the primary protein contact site as previously determined by partial ribonuclease digestion (17). The shaded circles indicate base modifications which were only slightly excluded from the complex; the solid circles indicate base modifications which were largely or completely excluded.

the base specific modifications. However, as shown in Figure 2, when the modifications were subsequently analyzed after aniline-induced cleavages, some of the bands were lighter indicating that a number of modifications were partially or largely excluded from the complex. As indicated in the additional examples from a second experiment, the exclusions which are identified in Figure 2 were reproducible over four experiments, both with respect to their position in the molecule and in the degree of exclusion relative to one another. In addition to these reduced bands, some increases in intensity (e.g. residues 102-104) or unexpected cleavages (e.g. residues 54 and 107) were also observed but these were not reproducible and frequently also present in the control lanes. As indicated in Figure 3, all of the constantly excluded modifications were located in double helical structures. For reasons which are not clear, the guanine specific reaction (dimethyl sulphate modification) was not useful in these studies because after incubation and electrophoresis, no aniline-induced cleavages were observed in either the free or bound RNA. The resulting electrophoresis profile was identical to the control lanes. Nevertheless, because the adenine specific reaction (diethyl pyrocarbonate modification) resulted in guanine modifications to a lesser extent we were able to analyze all four nucleotides.

To see if comparable modifications were excluded with eukaryotic 5S RNAs of diverse origin, we repeated our experiment with modified 5S RNA from a thermophilic fungus (T. lanuginosus) and rat liver. As indicated by the arrows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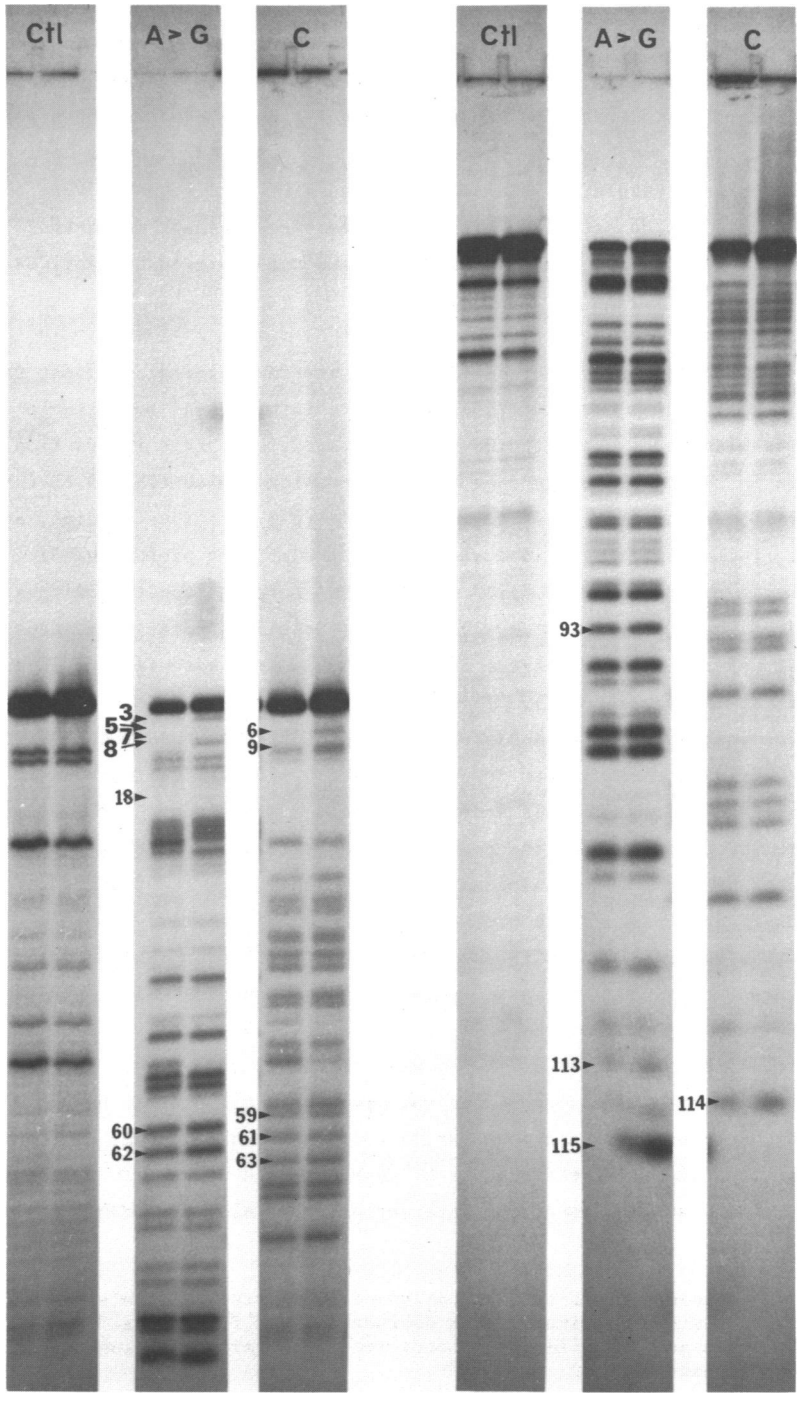
in Figures 4 and 5, a number of modifications were again partially excluded from the complex, although the number and degree of exclusion was lower. Each of these excluded modifications were observed in at least three separate experiments. As indicated in Figure 6, all of the excluded residues were in the same helical regions in which exclusion was observed with the native 5S RNA-protein complex (Figure 3). In some instances intensified bands were present, as observed with the native complex, but these were present in control lanes as well, indicating non specific cleavages and not modified nucleotides.

DISCUSSION

Previous studies and those illustrated in Figure 1 indicate that the yeast 5S RNA binding protein (YL3) is able to interact and form stable complexes with many eukaryotic 5S RNAs. Nevertheless, this interaction is specific in that the protein does not form complexes with tRNA, 5.8S rRNA or bacterial 5S rRNAs (17). As indicated in Figure 3, previous studies, using partial ribonuclease digestion, showed that the primary protein binding site resides in the 3'-terminal half of the 5S RNA molecule together with a small portion of the 5'-end (17). However, reconstitution studies indicated that other structural features of the 5S RNA molecule were essential, at least for the initial interaction (17). The present results are completely consistent with these earlier results and strongly suggest that the additional structural feature which is essential to complex formation is a helical region (binding region A in Fig. 3). If this additional region is included, the nucleotide sequence which constitutes the protein contact site for the single protein in eukaryotes is completely analogous to the regions which are thought to contact the two or three prokaryotic protein components in bacterial complexes (12). Again, this is consistent with our notion that the larger eukaryotic protein constitutes a fusion of the prokaryotic protein sequences (3).

Our results with heterologous 5S RNA-protein complexes raise an important point regarding the nature of the actual protein binding site. 5S RNAs from yeast, the thermophilic fungus, and rat liver were all able to exchange into the yeast complex, and, although variable in degree and number, nucleotide modifications in comparable regions of each molecule were excluded from the complex. This suggests that the interactions were also comparable in each

Figure 4. Autoradiograph of chemically modified, free and protein-bound rat liver 5S rRNA after aniline-induced degradation and fractionation on a 12% polyacrylamide gel. Modifications and electrophoresis conditions are those described in Figure 2.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case although perhaps not as extensive with heterologous complexes. It is of interest to note further that, on average, in both the homologous and heterologous complexes, the degree to which modifications were excluded was greater for region C (interaction between the 3' and 5'-ends) than for the other two helical domains. One possibility is that the protein-RNA interaction occurs primarily through region C and perhaps the additional interactions add stability and specificity to the overall complex. This is supported by our previous studies using partial ribonuclease digestion (17) which also primarily implicated region C.

A detailed comparison of the nucleotide sequences in each binding region (Figure 7) raises two interesting features. Among the three binding sites a limited sequence homology was observed in only two cases (B and C) and only in region C (interaction between the 3' and 5'-ends) were homologous residues excluded in all the complexes which were examined. Secondly, changes in the E. coli 5S RNA sequence alter the spatial arrangement between the homologous residues. The fact that equivalent residues for region C were excluded in all complexes adds further support to the notion that this may constitute a primary site of interaction. On the otherhand, the fact that there were no excluded homologous residues in the other sites, suggests that one of the important features in the interaction is the presence of helical domains (A-C in Fig. 3) which are strategically oriented relative to one another, or of specific functional groups which are precisely oriented in these helical structures. Recently, a similar study on the interaction of E. coli 5S RNA with one of its binding proteins (EL18) indicated that a helical region in the E. coli 5S RNA equivalent to binding site A in yeast was the primary contact site (13). If the three equivalent protein sequences are fused in the eukaryotic complex, each may recognize a different helical region in the 5S RNA molecule, explaining the need for three separate domains in the eukaryotic binding site.

The reason why prokaryotic 5S RNAs will not exchange into the eukaryotic complex is not obvious, but it is attractive to speculate that perhaps essential functional groups or the three helical domains themselves are not properly oriented because of extra nucleotides or changes in the sequence. The sequence comparison in Figure 7 may be supportive of this because the spatial arrangement of homologous nucleotides is only altered in the E. coli sequence.

Figure 5. Autoradiograph of chemically modified, free and protein-bound T. lanuginosus 5S rRNA after aniline-induced degradation and fractionation on a 12% polyacrylamide gel. Modifications and electrophoresis conditions are those described in Figure 2.

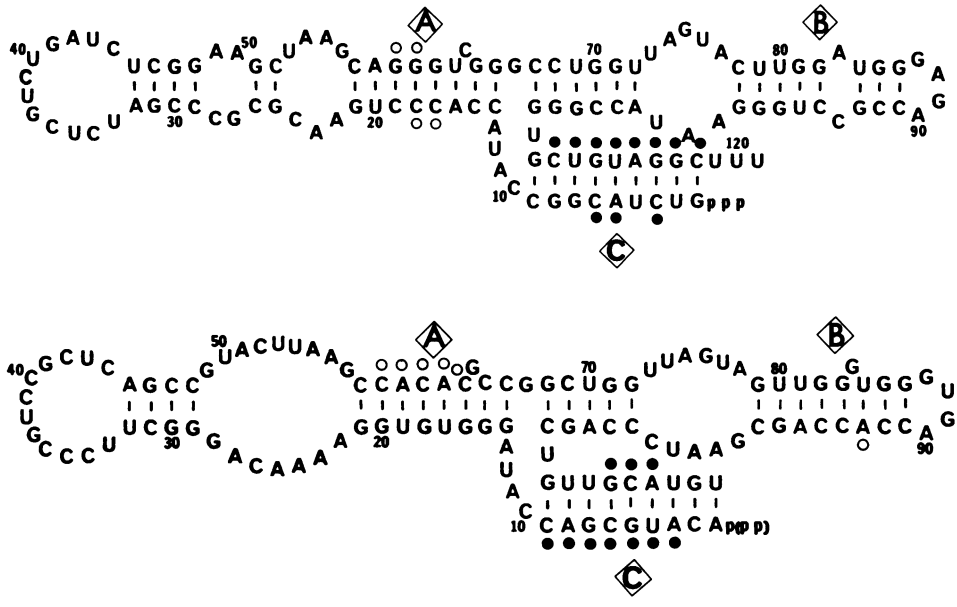

Figure 6. Exclusion of base modifications in rat liver (upper) or T. lanuginosus (lower) 5S rRNA from the 5S RNA-protein complex. Chemically modified 5S RNA was exchanged into the yeast 5S RNA-protein complex and the modified residues were analyzed after aniline-induced cleavage as described in Figure 4 and 5. The shaded circles indicate base modifications which were only slightly excluded from the complex; the solid circles indicate base modifications which were largely or completely excluded.

In their recent publication, Peattie et al. (13) also noted that the double helix, which constitutes a RNA-protein contact site for EL18, contains a "bulge", and speculated that this nucleotide may underlie a specific recognition or interaction. In their study, a modification in this residue was partially excluded from the complex. An equivalent bulge (residue 64) is found in the eukaryotic RNAs but, in our studies, this particular modification was not excluded from the eukaryotic complex. Nevertheless, it is interesting to note that a second of the three helical domains (B) also contains a "bulge" (residue 84), making these two contact sites even more similar. Since modifications in either "bulge" were not excluded, it seems more likely that these alter the helical structure in some recognizable way rather than contribute to the interaction directly. A recently proposed model for the tertiary structure of the eukaryotic 5S RNA (24) may offer an explanation. In addition to the "bulges", the two helical regions also share a limited

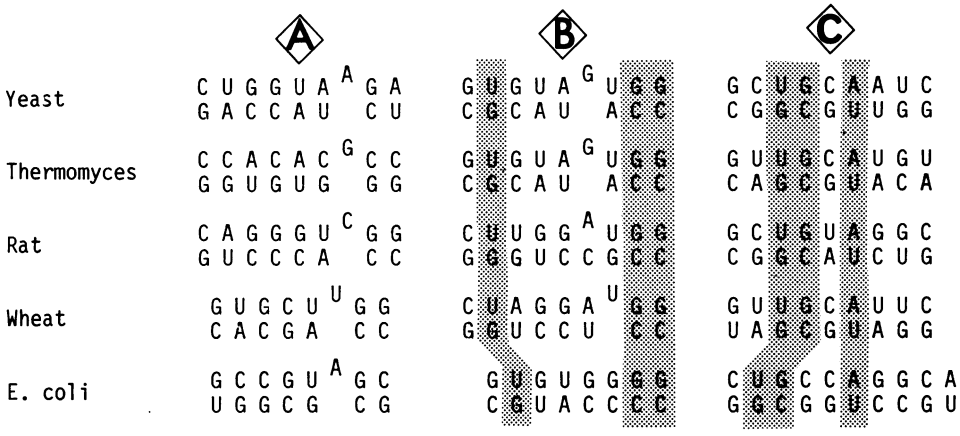

Figure 7. Comparison of analogous helical domains in the protein binding site of 5S RNAs from diverse origins. The shaded areas indicate homologous residues; the nucleotide sequences for the yeast, thermophilic fungus, rat, wheat, and E. coli 5S RNA sequence were taken from ref. 19-23, respectively.

sequence homology. In yeast, for example, the structure

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...GGU...
   III
...ACCAU...
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is shared by both and modification to all of these residues were partially restricted. It would appear, therefore, that these two contact sites are equivalent in several respects.

Because the contact sites are double stranded regions and their sequences are not strongly conserved, it is attractive to postulate that the RNA binding sites in the eukaryotic 5S RNA binding protein may be related to those in DNA binding proteins, such as histones, and that fundamental features in nucleic acid-protein interactions have been conserved for both RNA and DNA. We are currently examining the YL3 protein in an effort to answer this question.

ACKNOWLEDGEMENTS

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