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5S RNA Structure and Interaction with Transcription Factor A. 2. Ribonuclease Probe of the 7S Particle from *Xenopus laevis* Immature Oocytes and RNA Exchange Properties of the 7S Particle†

Janet Andersen, Nicholas Delihas,* Jay S. Hanas, and Cheng-Wen Wu

ABSTRACT: The 5S RNA complexed in the 7S particle of immature *Xenopus laevis* oocytes was ^{32}P labeled at its 3′ end and then subjected in situ to partial digestion using ribonucleases T_1, T_2, A, and V_1 in order to study the conformation of the complexed RNA and its interaction with the transcription factor A (TFIIIA). Digested samples were gel purified to retrieve 5S RNA that was still complexed with the transcription factor protein, and the cleavages in these RNAs were analyzed on sequencing gels. The RNA associated with the 7S particle is very susceptible to ribonuclease activity despite the presence of the protein. Also, the 5S RNA in the 7S particle is in a different conformation from renatured *Xenopus laevis* (Xlo) 5S RNA and appears to have less secondary structure predominantly in the stem that includes helices IV and V. A species of native Xlo 5S RNA which was isolated from 7S particle preparations under nondenaturing conditions revealed a conformation that was more similar to the 5S RNA in the 7S particle than to renatured 5S RNA. Comparison of data from partial ribonuclease digestions performed on renatured 5S RNA, on the native 5S RNA, and on the complexed 5S RNA allowed us to approximate sites of protein-induced structural change in the complexed 5S RNA, which may signal protein interaction domains. These sites include the 5′ side of helices III and V. In another approach to the study of 5S RNA–TFIIIA interactions, we have observed that incubation of ^{32}P-labeled Xlo 5S RNA with 7S particles results in the incorporation of labeled RNA into 7S particles. Heterologous eukaryotic 5S RNA can also be incorporated into 7S particles. These results suggest that a generalized eukaryotic 5S RNA structure can be recognized by TFIIIA.

The 5S ribosomal RNA is found complexed with a 37-kilodalton transcription factor protein (TFIIIA) in a 7S ribonucleoprotein (RNP) particle of the previtellogenic oocytes of *Xenopus laevis* (Xlo) (Picard & Wegnez, 1979; Pelham & Brown, 1980; Honda & Roeder, 1980). TFIIIA also binds to an intragenic transcriptional control region (which includes residues 50–83) in the 5S RNA gene to initiate transcription (Sakonju et al., 1980; 1981; Bogenhagen et al., 1980; Sakonju & Brown, 1982). Transcription of the 5S DNA in vitro is feedback inhibited by Xlo and heterologous 5S RNAs (Pelham & Brown, 1980; Gruissem & Seifart, 1982). Although the binding of TFIIIA to 5S DNA has been studied extensively by using DNase I footprinting techniques (Engelke et al., 1980; Sakonju et al., 1981; Sakonju & Brown, 1982; Hanas et al., 1983), little is known concerning the interaction of the complexed 5S RNA with TFIIIA in the 7S particle.

In this paper, we present the results of two types of experiments which approach the subject of RNA–protein interactions in the 7S particle. One method has been to probe the structure of 5S RNA in the particle with ribonucleases. By using a similar assay, Douthwaite et al. (1982) previously determined the ribonuclease cleavage sites in *Escherichia coli*

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5S RNA complexed with ribosomal proteins. The preceding paper in this issue (Andersen et al., 1984) deals with a ribonuclease digestion analysis of the structure of renatured Xlo 5S RNA and establishes the background for the study of the RNA–protein interactions in the 7S particle. It also presents a revised secondary structural model for 5S RNA which includes extended base pairing between helices IV and V. The proposed structure is used in this paper. However, references to helices IV and V are according to Delihas & Andersen (1982) in order to facilitate the discussion of these areas of the molecule.

The complexed Xlo 5S RNA was labeled at its 3′ end and probed in situ with ribonucleases so that the structure of the RNA in the 7S particle could be compared to the renatured form and to native forms that were isolated from 7S particle preparations by phenol extraction under nondenaturing conditions. Without the ability to reconstitute a 7S particle from purified TFIIIA protein and renatured 5S RNA components, partial digestions of 5′-labeled RNA were not possible.

Comparisons of cleavage data were based on noting the appearance of both enhanced and diminished cleavages at residues that showed cleavage in the digestions of protein-free 5S RNAs and on noting the appearance of new cleavages on the complexed RNA. When comparisons were made betwen the digestion of the complexed 5S RNA and those of renatured 5S RNA, certain regions of complexed Xlo 5S RNA exhibited a shift in ribonuclease sensitivity from double-strand-specific to single-strand-specific enzymes, which suggested changes in secondary structure in those regions (i.e., the stem that contains helices IV and V).

In the other aspect of this study, we find that incubation of ^{32}P-labeled Xlo 5S RNA as well as heterologous 5S RNAs with 7S particles containing unlabeled 5S RNA allows incorporation of a significant fraction of the labeled 5S RNA into the particles, as assayed by gel electrophoresis. We refer to this as an "RNA exchange" assay, and we have employed this assay to analyze the importance of 5S RNA structure in the RNA–protein interaction. A similar RNA exchange assay has been devised by Nazar & Wildeman (1983) to analyze the interaction of yeast 5S RNA with ribosomal protein L3.

Materials and Methods

Preparation of 7S Particles. The 7S particle was prepared from the ovaries of immature frogs according to the method of Pelham & Brown (1980) and was further purified as described by Hanas et al. (1983). The 7S particle preparations were routinely tested for DNA binding activity by using the DNase footprinting assay (Hanas et al., 1983).

Ribonuclease Probe Experiments. The details of materials and methods are described in the preceding paper (Andersen et al., 1984) unless stated otherwise. The 5S RNA in the 7S particle was labeled at its 3′ end by incubating 6–12 μg of 7S particle sample with [5′-^{32}P]pCp and T_4 RNA ligase (P-L Biochemicals) at 0 °C for 16–20 h in a buffer containing 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes) (pH 7.5), 10 mM $MgCl_2$, 50 mM KCl, 3 mM dithiothreitol, 0.6 μM ATP, and bovine serum albumin at 10 μg/mL. In preliminary labelings of the 7S particle, the ligase buffer also contained 15% dimethyl sulfoxide.

Partial ribonuclease digestions were performed on newly labeled 7S particle. Digested samples were electrophoresed at 4 °C on 6% polyacrylamide gels in 50 mM tris(hydroxymethyl)aminomethane (Tris)–borate (pH 8.3) and 1 mM ethylenediaminetetraacetic acid (EDTA) (TBE) at 200 Volts for about 1.5 h until xylene cyanol FF (XC) and bromophenol blue (BP) dyes migrated to a predetermined position. With an autoradiogram as a tempate, digested 5S RNA still complexed in the RNP particle was isolated by excising bands that migrated in the predetermined 7S position and eluting the RNA in sodium dodecyl sulfate (SDS) buffer. The RNA was filtered, ethanol precipitated, and denatured in CUB 5.

The digested RNA and marker RNA were electrophoresed on sequencing gels, and the cleavage sites were analyzed from the autoradiogram of the gels.

An alternative method of analyzing 5S RNA complexed in the 7S particle involved partially digesting unlabeled 7S particle samples with ribonucleases T_1 or A. Unlabeled, digested RNA still complexed in the RNP was isolated by electrophoresis on 6% polyacrylamide gels and identified by UV shadowing. The bands that migrated in the 7S position were excised, and the digested RNA was eluted in SDS buffer and ethanol precipitated. The 5′-hydroxyls produced by ribonuclease cleavage were phosphorylated with [γ^{32}P]ATP by using polynucleotide kinase. Labeled fragments were denatured with 7 M urea in 20 mM sodium citrate (pH 5) and 1.3 mM EDTA by heating at 80 °C for 1 min. These fragments were separated by electrophoresis on 12% sequencing gels in TBE, and bands were cut by using an autoradiogram as a template. The RNA was eluted from the gel in SDS buffer, filtered, and ethanol precipitated. The redissolved RNA was divided into two aliquots. One aliquot was completely digested with 1 unit of ribonuclease T_2 at 37 °C for 30 min in 50 mM NH_4OAc (pH 4.5) and chromatographed on poly(ethylenimine) (PEI)–cellulose in 0.55 M ammonium sulfate at 4 °C (Stanley & Vassilenko, 1978; Gupta & Randerath, 1979) in order to determine the labeled end nucleotide. The other aliquot was reserved for mobility shift analysis (Pirtle et al., 1980; Silberklang et al., 1977).

Native 5S RNA was isolated from unlabeled 7S particles by phenol extraction. The deproteinized RNA was ether extracted, precipitated with ethanol, and labeled at its 3′ end as described above. The labeled samples were partially digested with ribonucleases and purified on a 12% polyacrylamide preparative gel in 50 mM Tris–borate (pH 7.5) and 1 mM $MgCl_2$ (TBM buffer). The two 5S RNA bands (n and d) that appeared on the autoradiogram were cut, and the RNA was eluted in SDS buffer, precipitated with ethanol, denatured, and analyzed on sequencing gels.

Incorporation of Heterologous 5S RNAs into 7S Particles. The incorporation of heterologous 5S RNAs into 7S particles was carried out in 20 μL of 50 mM Tris-HCl (pH 7.5), containing 35 mM KCl, and 2 mM $MgCl_2$ or 2 mM EDTA at 0 or 20 °C for 30 min–2 h with 4–6 μg of 7S particle and ^{32}P-labeled RNA. Samples were electrophoresed on 6% polyacrylamide gels containing TBE buffer, and the exchanged 7S particles were identified by the comigration of Coomassie blue staining bands with ^{32}P bands noted on the autoradiogram. The 5S RNAs used in this exchange assay were from *Phycomyces blakesleeanus*, *E. coli*, *Streptococcus cremoris*, and *Synechococcus lividus* (the sequences of these heterologous 5S RNAs have been published in the following papers, respectively: Andersen et al. (1982), Brownlee et al. (1968), Neimark et al. (1983), and Delihas et al. (1982). *E. coli* fMet-tRNA was also used in this assay.

Results

3′ Labeling and Partial Digestions. Figure 1 outlines the procedures employed to prepare and to analyze 3′-labeled Xlo 5S RNA in the 7S particle. The labeled 7S particle was then subjected to a series of partial ribonuclease digestions as described in the preceding paper (Andersen et al., 1984). The digested samples were subjected to electrophoresis on 6%

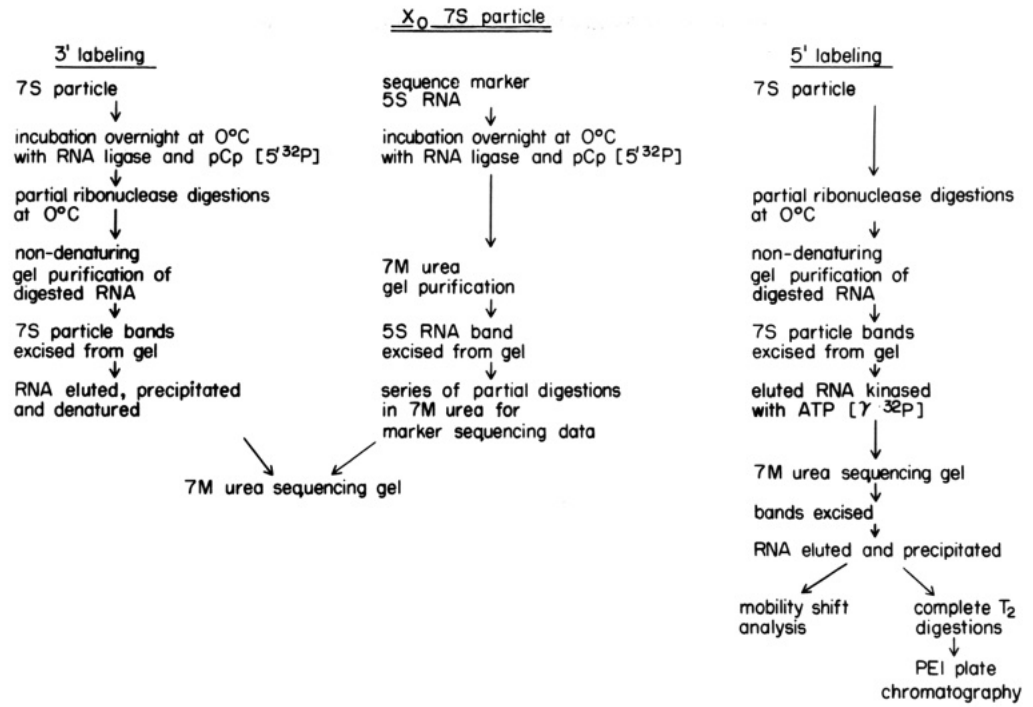

FIGURE 1: Flow chart outlining the sequence of events in the assay involving labeling, partial digestions, and gel electrophoresis of 7S particle samples and marker 5S RNA.

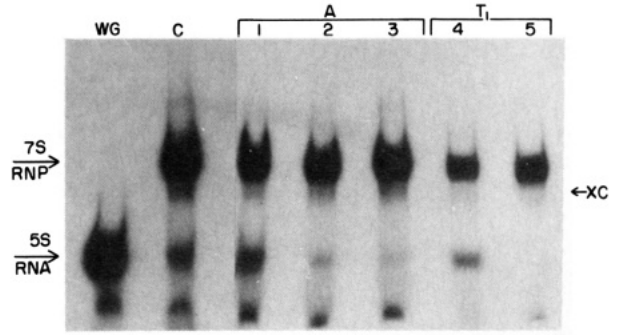

FIGURE 2: 6% polyacrylamide gel purification in TBE of 3′-labeled Xlo 7S RNP samples. The lane marked C is undigested 7S particle; lanes 1–3 are samples digested at 0 °C for 5 min with RNase A at concentrations of 1:10 000, 1:1000, and 1:500 micrograms of RNase A to micrograms of RNA, respectively; lanes 4 and 5 are samples digested with RNase T_1 at 0 °C for 30 min at concentrations of 1:100 and 1:10 units of RNase T_1 to micrograms of RNA, respectively; the lane marked WG is 3′-labeled wheat germ 5S RNA used as a position marker; XC is xylene cyanol FF used as a visual marker during electrophoresis. The fastest moving bands consist of RNA degradation fragments.

polyacrylamide gels in order to separate the intact particles containing partially digested 5S RNA from free 5S RNA and from degradation products (Figure 2). The 7S particles migrate more slowly than free 5S RNA in this gel system and can also be identified by Coomassie blue staining of the gel. For each digestion, bands that migrated in the 7S position (and bands that comigrated with marker 5S RNA) were cut from the gel. The RNA was extracted, denatured, and analyzed on sequencing gels along with RNA sequence markers.

Gel electrophoresis of 3′-labeled preparations showed a minor band of free 5S RNA which either dissociated from the particle during electrophoresis after ribonuclease digestions and/or was endogenous to the 7S particle preparations. We have termed this RNA "sample 5S RNA". Ribonuclease digestions of sample 5S RNA were not adequate for a comparison to those of complexed 5S RNA since the electrophoresis in EDTA selected for protein-free 5S RNAs of full length and these molecules proved to be more intact than those isolated from the complexed RNA.

5′ Labeling Assay. The analysis of 7S particle RNA with these RNase probe methods was complicated by the heterogeneity of oocyte 5S RNA. Doublet bands were present on sequencing gels due to the size heterogeneity at the 3′ end of the Xlo 5S RNA resulting from variability in the exact site of transcription termination (Bogenhagen & Brown, 1981). As a rule, the uppermost band was used to assign a cleavage site. To check our assignment of cleavages, an experimental procedure involving 5′ labeling of RNA fragments obtained after partial ribonuclease digestions of 7S particles was employed as outlined in Figure 1. The ^{32}P-labeled terminal nucleotide was identified for each fragment by chromatography of completely digested RNA. As shown for two examples in Figure 3, mobility shift analyses provided the terminal sequence of the labeled fragment at the cleavage site. For the examples shown in Figure 3, the ribonuclease A cleavage occurred between C_{10} and A_{11} (Figure 3A) and between C_{39} and U_{40} (Figure 3B). This method provided the exact positions of ribonuclease cleavages on the complexed RNA and verified our assignment of cleavages on 3′-labeled RNA.

Somatic 5S RNA in 7S Particles of Oocytes. Somatic 5S RNA has sequence differences from that of oocyte 5S RNA at several nucleotide positions (Ford & Brown, 1976). It is well-known that the somatic 5S RNA genes are transcribed in oocytes (Denis & Wegnez, 1977) and the somatic 5S RNA has been observed in oocyte-specific 42S storage particles (Ford & Southern, 1973). The somatic 5S RNA can be distinguished from the oocyte 5S RNA at several positions, including residues 47 (A in oocyte, G in somatic) and 56 (A in oocyte, G in somatic). 3′-Labeled samples were isolated from a 7S band and from a sample 5S RNA band on a 6% polyacrylamide gel. These 3′-labeled 5S RNAs were further purified by gel electrophoresis in 7 M urea, and each revealed three major bands. The RNA was eluted from each band, partially digested in 7 M urea with ribonucleases T_1 and U_2, and electrophoresed in 8% polyacrylamide sequencing gels (data not shown). Analysis of the ribonuclease T_1 and U_2 digestions

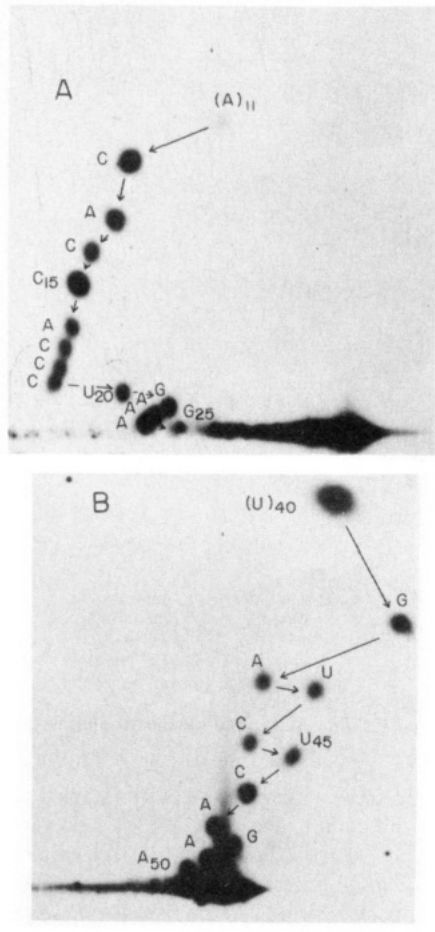

FIGURE 3: Mobility shift analyses of RNA fragments produced by partial RNase A digestions of 7S particles which were subsequently labeled at the 5′ end. The first nucleotide of each fragment (in parentheses) was determined by chromatography of completely digested RNA (data not shown). (A) 5′-Terminal sequence of the fragment where ribonuclease A cleavage has occurred between nucleotides C_{10} and A_{11}; (B) 5′-terminal sequence of the fragment where ribonuclease A cleavage has occurred between nucleotides C_{39} and U_{40}.

revealed that somatic 5S RNA is present in the 7S particle of Xlo oocytes as well as in sample 5S RNA. The presence of the minor fraction of somatic 5S RNA was not problematic to analyzing the 7S particle ribonuclease probe data.

Ribonuclease Probe of the 7S Particle. Sequencing gels showing the cleavage patterns for ribonucleases T_1, T_2, and V_1 on the protein-bound 5S RNA are shown in Figures 4 and 5B. Undigested samples (lane 1a in Figure 4 and lane 1 in Figure 5B) show cleavages that occurred prior to ribonuclease digestions. These "basal" cleavage sites have been included in our data analysis in order to present a complete picture of nucleotides assessible to hydrolysis in the complexed RNA. The basal cleavage sites of the 7S particle are different from the basal cleavage sites of renatured 5S RNA [see Andersen et al. (1984)]. Basal cleavages of the 7S particle are probably due to trace nuclease activity during 3′ labeling, whereas the basal cleavages in renatured 5S RNA occur during renaturation in the presence of Mg^{2+}. In the 5′ labeling experiments described above, the basal cleavages that are associated with the 3′-labeled control samples were not present in 5′-labeled control samples. This suggests that the basal cleavages in 7S samples did not occur during 7S particle preparation or storage.

The ribonuclease cleavages in the 7S RNP particle are summarized in Table I and Figure 6. Some ribonuclease cleavages are shown as minor cuts since they appear weak in less extensive digestions even though they appear as major cleavages in more extensive digestions. Some of these cleav-

Table I: Ribonuclease Cleavage Sites on 3′-Labeled Xlo 5S RNA Complexed in 7S Particles

nucleotide	basal[a]	RNase A	RNase T_1	RNase T_2	RNase V_1
C_{10}	+				
A_{11}				+	
C_{12}	+				
A_{13}				+	
C_{15}	(+)[b]				+
C_{18}		(+)			(+)
C_{19}		(+)			(+)
U_{20}					(+)
G_{21}					(+)
A_{22}				(+)	
A_{23}				(+)	
A_{24}				(+)	
G_{25}			(+)		
C_{28}					+
C_{29}					+
G_{31}			(+)		
U_{33}	(+)				
C_{36}				(+)	
G_{37}			(+)		
C_{39}	++	++			
U_{40}		+			
G_{41}			(+)	(+)	
A_{42}				(+)[c]	
U_{43}	(+)				
C_{44}	(+)				
C_{46}	(+)				+
A_{47}				(+)	
A_{49}				++	
A_{50}				+	
G_{51}					(+)
C_{52}	(+)				(+)
G_{53}			(+)	(+)	
A_{54}				(+)	
U_{55}		(+)			
A_{56}	(+)			+	+
C_{57}		+			
G_{61}			(+)		
U_{62}	(+)	+			
C_{63}		+			
U_{69}	(+)				
U_{73}	(+)	+			
A_{74}				(+)	
U_{76}	(+)	+			
A_{77}				(+)	
C_{78}		+			
C_{79}		+			+
U_{80}	(+)				+
G_{81}	+	+			
A_{83}	+				
U_{84}	+				
G_{86}			(+)	(+)	
G_{87}			(+)	(+)	
A_{88}				+	
G_{89}			++		
C_{91}		(+)			(+)
G_{93}		(+)	(+)		
C_{94}		(+)			
G_{97}			(+)[c]		
G_{98}			(+)[c]		
G_{99}			(+)		
A_{100}				(+)[c]	
A_{101}				(+)[c]	
U_{102}		(+)			(+)
C_{105}		(+)			(+)
A_{106}			(+)		
U_{114}		+			

[a] Cleavage sites that occur during 3′ labeling prior to partial ribonuclease digestions. [b] (+) designates minor cleavage. [c] Cleavages shown as minor since they appear as minor in less extensive digestions. These do not appear among the cleavage sites of 3′-labeled renatured Xlo 5S RNA.

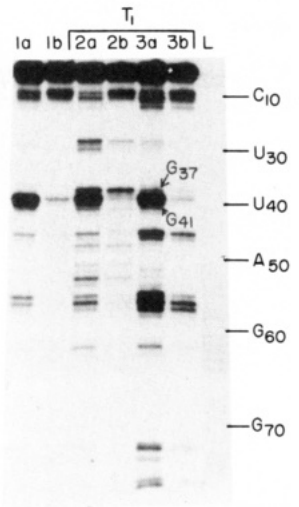

FIGURE 4: Sequencing gels showing the partial ribonuclease cleavage pattern of 3′-labeled Xlo 7S RNA particle. Arrows point to cleavages mentioned in the text. (a) 7S particle; (b) sample 5S RNA on an 8% polyacrylamide sequencing gel. (The cleavage pattern of sample 5S RNA did not prove to be adequate for comparison to that of the complexed RNA since sample 5S RNA contained a population of full-length molecules that were more intact than the complexed RNA due to gel purification in EDTA.) Lane 1 is undigested sample; lanes 2 and 3 are digestions with RNase T_1 at concentrations of 1:100 and 1:1000 units of RNase T_1 to micrograms of RNA, respectively; L marks the lane containing a faint alkaline-generated ladder which was used to help determine the position of the cleavage bands on the autoradiogram.

ages may be secondary (Douthwaite & Garrett, 1981) since only 3′-labeled 7S particle samples were probed in this assay; nevertheless, they have been included in Table I if they were not present in the ribonuclease digestions of 3′-labeled renatured 5S RNA. (Such positions are designated by footnote *c* in the table.) Figure 6 shows the position of the cleavages on the complexed Xlo 5S RNA sequence as drawn in the generalized secondary structural model for eukaryotic 5S RNA with extended base pairing between helices IV and V. The 5S RNA in 7S particles is more readily cleaved by single-strand-specific nucleases in the region of helices IV and V than is the renatured 5S RNA.

Native 5S RNAs from 7S Particle Preparations. We have also attempted to analyze the structure of native 5S RNAs from 7S particle preparations by phenol extraction of particles followed by end labeling and gel electrophoresis of the labeled RNA under nondenaturing conditions. Such isolation of native 5S RNAs reveals two electrophoretically distinct conformers of approximately equal amounts. Conformer d electrophoresed more slowly than conformer n. The ribonuclease cleavage patterns of these RNAs are shown in Figure 7. The partial ribonuclease digestions of conformer d are more similar to those of the complexed 5S RNA in the 7S particle than to those of renatured 5S RNA. Conformer d appears to have less secondary structure than conformer n especially around helix IV. Figure 7B shows the diminished V_1 cleavages at U_{73}, U_{76}, and U_{102} in conformer d as compared to conformer n. By comparing the ribonuclease digestions of the complexed 5S RNA not only with those of renatured 5S RNA but also with those of conformers n and d, we were able to identify ribonuclease cleavages that occurred only when the 5S RNA was bound to the transcription factor protein. These cleavages, then, do not reflect conformational changes that exist between the protein-free RNAs.

5S RNA Exchange in the 7S Particle. As a second method to analyze the interaction of TFIIIA with the 5S RNA, we

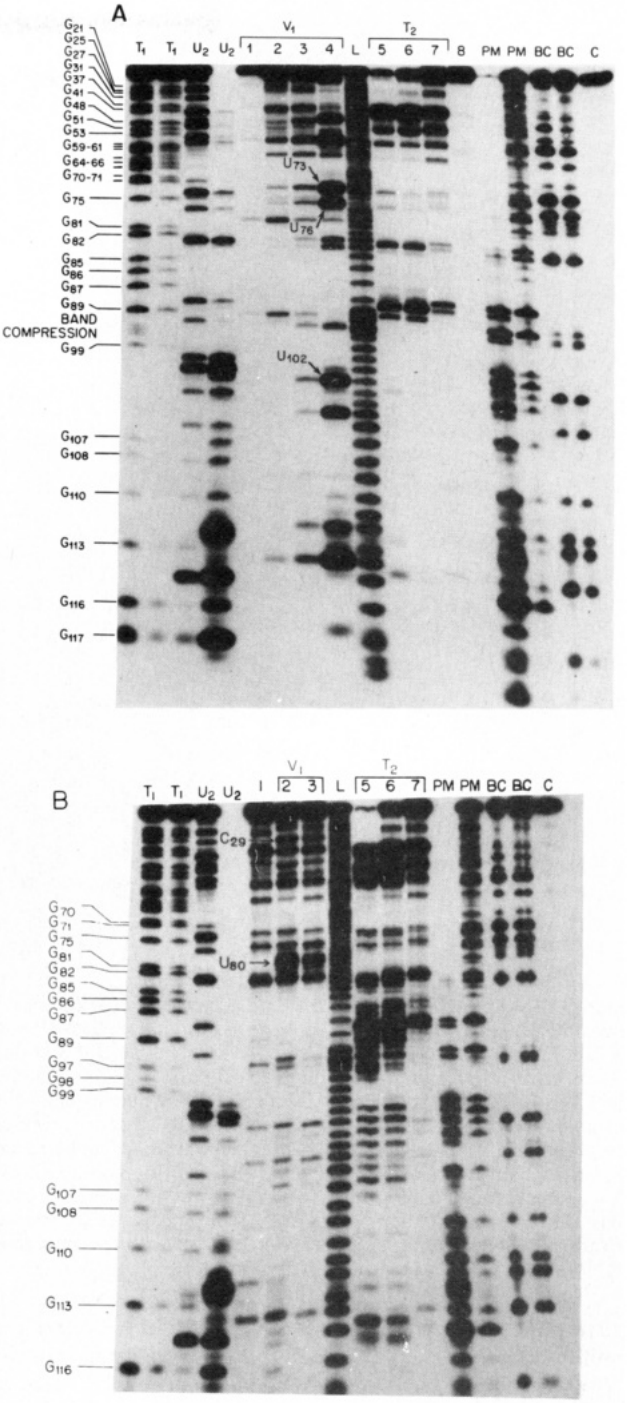

FIGURE 5: 12% polyacrylamide sequencing gels showing the pattern of digestions of (A) 3′-labeled renatured Xlo 5S RNA as shown in the preceding paper (Andersen et al., 1984): lanes 1–4 are digestions with RNase V_1 at concentrations of 1:9, 1:17, 1:43, and 1:86 units of V_1 to micrograms of RNA, respectively; lanes 5–7 are digestions with RNase T_2 at concentrations of 1:50, 1:100, and 1:1000 units of T_2 to micrograms of RNA, respectively; lane 8 is an undigested, renatured sample. Lanes marked T_1, U_2, PM, and BC are partial digestions with RNases T_1, U_2, Phy M, and BC, respectively, in 7 M urea; the lane marked C is undigested, unrenatured control; the lane marked L is an alkaline ladder. (B) 3′-Labeled 7S particle samples: lane 1 is undigested 7S particle; lanes 2 and 3 are samples digested with RNase V_1 at concentrations of 1:43 and 1:86 units of RNase V_1 to micrograms of RNA, respectively; lanes 5–7 are samples digested with RNase T_2 at concentrations of 1:50, 1:100, and 1:1000 units of RNase T_2 to micrograms of RNA, respectively; lanes marked T_1, U_2, PM, and BC are 7 M urea partial digestions with RNases T_1, U_2, Phy M, and BC, respectively; the lane marked C is undigested, denatured 5S RNA that was incubated at 50 °C for 15 min in CUB 5 buffer; L is as described in (A).

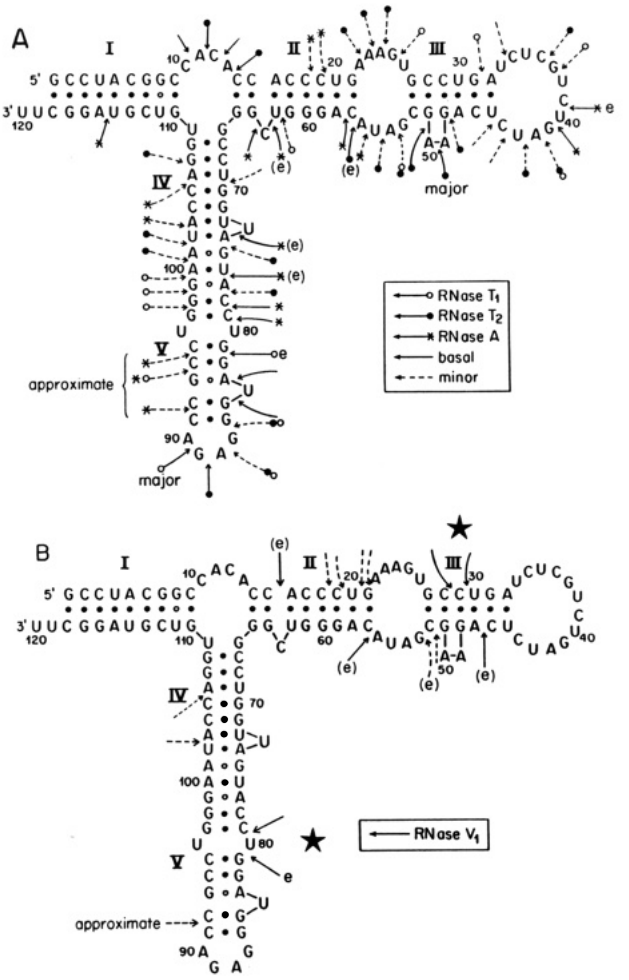

FIGURE 6: Xlo 5S RNA sequence drawn according to the consensus secondary model with extended base pairing between helices IV and V [see Figure 8 in the preceding paper (Andersen et al., 1984)]. (A) Arrows mark the single-strand-specific ribonuclease cleavages found in 3′-labeled 7S RNP particles. The insert in the figure explains the symbols used to distinguish between RNases T_1, T_2, and A. Minor cleavages are distinguished by dashed line arrows, "e" denotes enhancement of basal cleavages (see text). (B) Arrows mark the RNase V_1 cleavages found in 3′-labeled 7S RNP particles. Stars have been placed to designate putative protein interaction domains as determined by the presence of additional RNase V_1 cleavage sites in the complexed RNA.

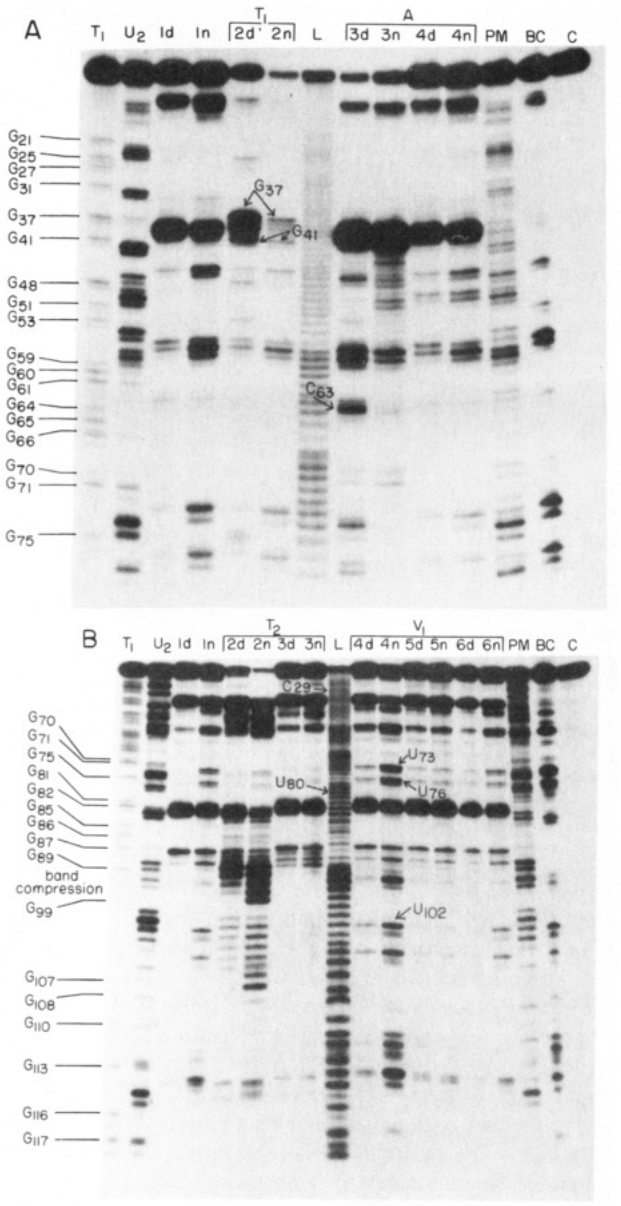

FIGURE 7: Sequencing gels showing the partial ribonuclease cleavage pattern of 3′-labeled conformers d and n after nondenaturing gel purification. Arrows point to RNase cleavages mentioned in the text. (A) Conformers d and n on an 8% polyacrylamide sequencing gel: lane 1 is undigested sample; lane 2 is digestion with RNase T_1 at 0 °C for 30 min at concentrations of 1:100 units of RNase T_1 to micrograms of RNA; lanes 3 and 4 are digestions with RNase A at 0 °C for 10 min at concentrations of 1:1000 and 1:10000 micrograms of RNase A to micrograms of RNA, respectively; lanes marked L, C, T_1, U_2, PM, and BC are as described in Figure 5. (B) Conformers d and n on a 12% polyacrylamide sequencing gel: lane 1 is undigested sample; lanes 2 and 3 are digestions with RNase T_2 at 0 °C for 30 min at concentrations of 1:100 and 1:1000 units of RNase T_2 to micrograms of RNA, respectively; lanes 4–6 are digestions with RNase V_1 at 0 °C for 20 min at concentrations of 1:43, 1:86, and 1:170 units of RNase V_1 to micrograms of RNA, respectively; lanes marked L, C, T_1, U_2, PM, and BC are as described in Figure 5.

have utilized a gel electrophoresis assay that measures the ability of labeled 5S RNA to become incorporated into the 7S particle upon incubation. The term exchange is used to denote the comigration of ^{32}P-labeled 5S RNA with the 7S particle during polyacrylamide gel electrophoresis. This interaction may be the result of binding of ^{32}P-labeled 5S RNA with free transcription factor protein A during the association and dissociation of the 7S particle in vitro but does not preclude the binding of ^{32}P-labeled 5S RNA to free protein A endogenous to the 7S particle preparations. The RNA binding or exchange reaction is illustrated in Figure 8. When a trace quantity of labeled 5S RNA is incubated with a preparation of the 7S particle in the presence of Mg^{2+}, a major fraction (70–80%) of the ^{32}P-labeled Xlo RNA comigrates in native polyacrylamide gels with the Coomassie blue stained band of 7S RNP. The comigration of the ^{32}P-labeled band with the Coomassie blue band shows that RNA incorporation has taken place and not the addition of labeled RNA to the RNP. Less exchange of Xlo 5S RNA occurs in EDTA than in Mg^{2+}.

In order to analyze the dependence of this interaction on 5S RNA structure, we have employed several heterologous 5S RNA molecules in this assay. In the presence of Mg^{2+}, we find that the heterologous eukaryotic 5S RNA from *Phycomyces blakesleeanus* [for the sequence, see Andersen et al. (1982)] exchanges at a significant level (approximately 20%), but eubacterial 5S RNA will exchange only at a low level (approximately 4%)(data not shown).

Significant exchange of eubacterial 5S RNAs was observed when these 5S RNAs were incubated with 7S particles in the presence of EDTA (Figure 9). In 2 mM EDTA, ~16% of

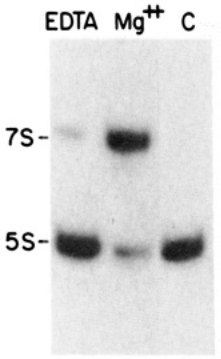

FIGURE 8: Autoradiogram of a 6% polyacrylamide gel showing the mobility of 3′-labeled Xlo 5S RNA in TBE buffer after incubation with unlabeled 7S particle at 20 °C for 2 h: EDTA = RNA exchange incubation in buffer with 2 mM EDTA; Mg^{2+} = RNA exchange incubation in buffer with 2 mM Mg^{2+}; C = 3′-labeled Xlo 5S RNA and unlabeled 7S particle in buffer with 2 mM Mg^{2+} but without incubation. Each lane stained with Coomassie blue at the 7S position (data not shown).

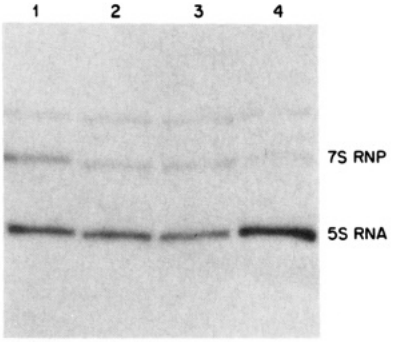

FIGURE 9: Autoradiogram of a 6% polyacrylamide gel showing the migration of ^{32}P-labeled Xlo and heterologous 5S RNAs after incubation with 7S particle in buffer containing 2 mM EDTA. Exchange incubations were performed at 20 °C for 30 min. Incubations were with (1) ^{32}P-labeled Xlo 5S RNA, (2 and 3) ^{32}P-labeled *E. coli* 5S RNA, or (4) ^{32}P-labeled *Synechococcus lividus* 5S RNA. The band migrating slower than the 7S RNP particle band has been analyzed and found to be an aggregate of 7S particles. The appearance of aggregates in 7S particle preparations was variable.

^{32}P-labeled *E. coli* 5S RNA exchanges. Thus, in the presence of EDTA, the efficiency of the exchange with eubacterial 5S RNA is higher, but the interaction of homologous Xlo 5S RNA is reduced (to ~45% or less). This suggests that the specificity of the 7S particle exchange interaction is altered and is less discriminating in the presence of EDTA. *E. coli* [^{32}P]fMet-tRNA will not exchange either in Mg^{2+} or in EDTA.

Discussion

Protein-Induced Conformational Changes in Xlo 5S RNA. A comparison of the ribonuclease cleavage patterns of the complexed 5S RNA to those of renatured 5S RNA and of native conformers n and d reveals new RNase V_1 cleavage positions at C_{28} and C_{29} in helix III, and at C_{79} and U_{80} in helix V that occur only when the RNA is complexed with protein TFIIIA (compare Figure 5B to Figures 5A and 7B). The partial digestions of 3′-labeled renatured 5S RNA show a minor secondary RNase V_1 cleavage at U_{80} only. In another study (Douthwaite et al., 1982), new and stimulated RNase V_1 cleavages were found within putative ribosomal protein binding domains when eubacterial 5S RNAs complexed with ribosomal proteins were probed with ribonucleases. In complexed Xlo 5S RNA, the new V_1 cleavages at C_{28}, C_{29}, C_{79}, and U_{80} reflect a conformational change in the 5S RNA induced by TFIIIA binding and may indicate areas of protein interactions. These areas have been designated with a star in Figure 6B.

Structure of the Complexed 5S RNA. The accessibility of parts of the stem that includes helices IV and V to single-strand-specific ribonucleases shows an unfolding of helices IV and V in the 5S RNA complexed in the 7S particle. The ribonuclease A cleavages that occur at residues U_{73} and U_{76} with concomitant loss of ribonuclease V_1 cleavage at these sites in the complexed RNA highlight this phenomenon.

The RNase V_1 digestions of conformer d show minor cleavages at positions U_{73}, U_{76}, and U_{102} (see Figure 7B). There are definitive RNase V_1 cleavages at these positions in the digestions of conformer n and of renatured Xlo 5S RNA. However, positions U_{73} and U_{76} which exhibit definitive RNase A cleavages in the digestions of the complexed RNA show only minor cleavage by RNase A in the digestions of conformer d. Also, the RNase T_1 cleavages found at residues G_{97-99} in the digestions of the complexed 5S RNA are not found in the digestions of conformer d. Therefore, conformer d does not exhibit the degree of reduced secondary structure in the stem that encompasses helices IV and V that the complexed RNA exhibits.

There are ribonuclease V_1 cleavage sites that occur in the digestions of renatured 5S RNA that are not present in the digestions of the complexed RNA (i.e., U_{30}, A_{32}, C_{36}, and A_{83}). However, these are not present in the digestions of conformer d either, and therefore, lack of cleavage in the complexed 5S RNA probably does not reflect protection of these residues by TFIIIA.

Partial ribonuclease digestions of the 5S RNA associated with the 7S particle show that a large portion of the RNA is accessible to ribonucleases. There is a suggestion of minor protection from ribonuclease cleavage at positions G_{37}, G_{41}, and G_{87} in less extensive digestions. These three positions are in the vicinity of the new RNase V_1 cleavage sites. Other than these residues, there were no other observed sites protected from single-strand-specific ribonuclease on the complexed RNA. We must consider the possibility that significant RNA exchange took place during the ribonuclease digestions which thus made it difficult to define protection sites.

If one purpose of the storage particle were to inhibit RNase degradation of the 5S RNA, the RNA may have been relatively resistant to ribonuclease degradation. However, our results suggest that the RNA associated with the 7S particle is readily accessible to ribonucleases in vitro. If RNA exchange occurs in vivo, then the 7S particle would not function to protect the RNA but perhaps to maintain a conformation of the RNA for subsequent ribosomal protein interactions.

The looped-out position C_{63} in helix II, which is protected from single-strand-specific ribonuclease cleavage is renatured 5S RNA, is accessible to single-stranded ribonuclease A cleavage in the complexed RNA. This looped-out position corresponds to A_{66} in *E. coli* 5S RNA, which has been implicated in the binding of ribosomal protein L18 (Peattie et al., 1981). Helix II of the eukaryotic 5S RNA from yeast has been included in the L3 ribosomal protein binding domain (Nazar & Wildeman, 1983). Perhaps the TFIIIA protein maintains the Xlo 5S RNA in a conformation for Xlo ribosomal protein L3 to bind during ribosomal assembly.

Recently, a 7S RNP particle from mature *Xenopus laevis* oocytes has been isolated and characterized by diethyl procarbonate modification studies (Pieler & Erdmann, 1983). Several adenine residues around helix V on the complexed RNA show minor protection from chemical modification. Position A_{47} was accessible to chemical modification only on

the complexed 5S RNA, which suggests structural rearrangement of helix III.

5S RNA Exchange in the 7S Particle. The ability of the heterologous eukaryotic 5S RNA from *Phycomyces blakesleeanus* to exchange into the particle suggests that protein TFIIIA can recognize and interact with a generalized eukaryotic 5S RNA structure. The percentage sequence homology of *Phycomyces* 5S RNA to Xlo 5S RNA is only 63%. Nucleotide sequences that can fold in 5S RNA higher order structure appear to constitute the predominant requirement for a stable interaction with TFIIIA as opposed to a recognition of particular blocks of sequences specific to Xlo 5S RNA. However, nucleotides highly conserved in eukaryotic 5S RNAs may also play a role in recognition and binding. Only a minor fraction of eubacterial 5S RNAs exchanges in the presence of Mg^{2+}. This weak interaction probably reflects inherent structural differences between eubacterial and eukaryotic 5S RNAs (Delihas & Andersen, 1982).

The presence of EDTA during incubation appears to decrease the specificity of the exchange interaction since eubacterial 5S RNAs incorporate at a 3–4-fold greater level in EDTA compared with incorporation in Mg^{2+}. However, since tRNA does not exchange in the presence of EDTA, the specific recognition of a generalized 5S RNA structure appears to still prevail during the exchange in EDTA.

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