Two zinc finger proteins from *Xenopus laevis* bind the same region of 5S RNA but with different nuclease protection patterns

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ABSTRACT

Immature oocytes from *Xenopus laevis* contain a 42S ribonucleoprotein particle (RNP) containing 5S RNA, tRNA, a 43 kDa protein, and a 48 kDa protein. A particle containing 5S RNA and the 43 kDa protein (p43-5S) liberated from the 42S particle upon brief treatment with urea can be purified by anion exchange chromatography. The purified p43-5S RNA migrates as a distinct species during electrophoresis on native polyacrylamide gels. Radiolabeled 5S RNA can be incorporated into the p43-5S complex by an RNA exchange reaction. The resulting complexes containing labeled 5S RNA have a mobility on polyacrylamide gels identical to that of purified p43-5S RNPs. RNP complexes containing 5S RNA labeled at either the 5' or 3' end were probed with a variety of nucleases in order to identify residues protected by p43. Nuclease protection assays performed with α -sarcin indicate that p43 binds primarily helices I, II, IV, and V of 5S RNA. This is the same general binding site observed for TFIIIA on 5S RNA. Direct comparison of the binding sites of p43 and TFIIIA with T1 and cobra venom nucleases reveals striking differences in the protection patterns of these two proteins.

INTRODUCTION

5S RNA is complexed with several non-ribosomal proteins within the cytoplasm of immature *Xenopus laevis* oocytes. The vast majority of this 5S RNA is associated with two ribonucleoprotein particles (RNPs) referred to as 42S and 7S particles (1,2) that serve as storage complexes for 5S RNA prior to ribosome assembly later in oocyte development.

Although the 42S particle was the first non-ribosomal 5S RNA containing RNP described in immature *X. laevis* oocytes (1), detailed analysis of the interactions within the complex has lagged behind that of the 7S particle. This is due in part to the simplicity of the 7S particle which contains 5S RNA and a 40 kDa protein (transcription factor IIIA, TFIIIA) in a 1:1 molar ratio. Interest

in the 7S particle was stimulated when it was discovered that the 40 kDa protein was a positive transcription factor for 5S RNA synthesis (3–5).

Approximately 50% of the 5S RNA present in immature *Xenopus* oocytes can be found in 42S particles (1), which are relatively complex structures with four identical 15 S subunits (6,7). Each subunit contains 5S RNA, tRNA, a 43 kDa protein (p43) and a 48 kDa protein (p48) in the molar ratios of 1:3:1:2, respectively (6–8). Klotzel *et al.* (6) have shown that p48 can bind 5S RNA *in vitro*. The interaction of p48 with 5S RNA may be weak, however, since p48 and tRNA can be liberated from the complex by treatment with urea or moderate salt. In contrast, 5S RNA remains bound to the 43 kDa protein under these conditions (7). Aside from this, little information is available regarding the specific interactions of 5S RNA within the 42S particle or specifically with p43.

Although a function beyond storage of 5S RNA has not been described for p43, the deduced amino acid sequence of its cDNA (9) includes nine potential 'zinc fingers' similar to those of TFIIIA (10). In light of the degree of homology between the two proteins, it is of interest to compare the specific interactions of the two proteins with 5S RNA.

Here we report the purification and characterization of an intact p43-5S RNA complex from immature *X. laevis* oocytes. We first mapped the residues of 5S RNA protected by p43 from nuclease α -sarcin. We then directly compared the protection patterns of p43 and TFIIIA on 5S RNA with the structure specific nucleases T1 and cobra venom (CV1). Results from this comparison provided a more detailed description of the binding site of p43 on 5S RNA and revealed some striking differences between the nuclease cleavage patterns resulting from binding of the two proteins to 5S RNA.

MATERIALS AND METHODS

Materials

Restriction enzymes were purchased from New England Biolabs (Beverly, MA). α and γ -³²P labeled GTP were purchased from

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ICN Pharmaceuticals Inc. (Irvine, CA). α - ^{32}P -cytidine 3', 5' biphosphate (pCp) was obtained from New England Nuclear (Boston, MA). Immature *X. laevis* were obtained from Xenopus I (Ann Arbor, MI) or Nasco (Fort Atkinson, WI). Cobra venom nuclease (CV1) and T4 RNA ligase were purchased from Pharmacia (Piscataway, NJ). Triton X-100 was purchased from Pierce (Rockford, IL) and DEAE Sephacel from Pharmacia (Uppsala, Sweden). α -sarcin was a kind gift from I. Wool and T7 RNA polymerase was a kind gift from W. Studier and J. Dunn.

Protein and RNP purification

42S particles were obtained from immature *X. laevis* ovaries. Ovaries were homogenized in ice cold buffer A (10 mM Tris (pH 7.6), 1 mM MgCl_2 , 2 mM dithiothreitol (DTT), 50 mM KCl and 0.1 mM phenylmethylsulfonylfluoride (PMSF)). Homogenates were spun at 3,000 rpm for 5 min in a Sorvall RT 6000 centrifuge. The supernatant was layered on 10–40% glycerol gradients in buffer A and spun at 40,000 rpm in a Beckman SW 41 rotor for 6 hr. 0.5 ml fractions were collected from the gradients and 5 μl of each fraction was added to 1 μl of 80% glycerol containing 0.05% bromophenol blue and xylene cyanol. The samples were subjected to electrophoresis on 1% agarose gels in TBE buffer (50 mM Tris base, 50 mM boric acid, 1 mM ethylenediaminetetraacetate (EDTA)) at 100 volts for 45 min. The gel was stained with ethidium bromide and fractions containing 7S particles and the 42S particles were pooled separately. Fractions enriched in the 42S particle were loaded on a DEAE Sephacel column at 5 mg protein per ml of resin. The column was washed with 5 column volumes of buffer B (100 mM KCl, 10 mM Tris (pH 7.6), 1 mM MgCl_2 , 2 mM DTT, 0.1 mM PMSF and 10% glycerol) followed by 5 column volumes of buffer B containing 150 mM KCl. The 42S particle was step eluted with buffer B containing 350 mM KCl. Fractions containing the peak of protein, determined by the dye binding method of Bradford (11), were pooled and adjusted to 6.7 M urea by addition of two volumes of 10 M urea in column buffer minus KCl. Urea treated 42S particles were loaded on the same size DEAE column and washed with 10 column volumes of buffer B containing 0.02% Triton X-100. p48 was collected in the flow through from the column. The p43-5S RNA complex was eluted from the column with a linear gradient of KCl from 100–400 mM in buffer B containing 0.02% Triton X-100. Column fractions were analyzed for the presence of p43 by SDS PAGE and the presence of the p43-5S RNA complex by 1% agarose gel electrophoresis as described for the 42S particle. The peak fractions were adjusted to approximately 100 mM KCl and loaded on another DEAE column at 15 mg protein per ml of resin. The column was washed with 3 column volumes of 100 mM KCl buffer and step eluted with 350 mM KCl buffer. Fractions containing the peak of protein were pooled. TFIIIA was prepared from 7S particles purified from immature *Xenopus laevis* ovaries as described (12, 13).

Radiolabeled 5S RNA

Uniformly labeled and 5' and 3' end-labeled 5S RNAs were generated from a *X. borealis* 5S RNA gene cloned in tandem with a truncated T7 viral promoter as described (Sands and Bogenhagen, accompanying paper). All radiolabeled RNAs were gel purified under native conditions in the presence of SDS prior to use (14).

7S particle reconstitution and p43-5S RNA exchange reaction

7S particles were reconstituted as described by Sands and Bogenhagen (in preparation). Unless otherwise stated, the reactions were performed with 5 nM 5S RNA and 20 nM TFIIIA in 20 μl volumes incubated at room temperature for 30 min.

All exchange reactions were performed with 250 nM p43-5S RNA complex (as determined by protein concentration) and 1 nM or less of radiolabeled 5S RNA in 20 μl volumes. Optimal exchange conditions were determined by a series of control reactions. The final standard buffer contained 50 mM KCl, 2 mM DTT, 1 mM CaCl_2 , 10 mM hydroxyethylpiperazine ethane sulfonic acid (HEPES) (pH 7.4), 8% glycerol and 0.1% Triton X-100. p43-5S RNA complexes containing either 5' or 3' end-labeled 5S RNA were incubated with 10,000 cpm (Cerenkov) of RNA for 45 min at 25°C. Reconstituted complexes were analyzed on 6% polyacrylamide gels containing 0.1% Triton X-100 run at 300 volts for 1.5 hr in TBE buffer.

RNase protection assays

RNP reconstitutions were performed as described above. A 2 μl aliquot of each binding or exchange reaction was analyzed in a gel mobility shift assay as described above to confirm that the reactions were complete. α -sarcin reactions were performed with 1.5 μM enzyme at 30°C for 10 min. The reactions containing uncomplexed end-labeled 5S RNA contained 250 nM unlabeled 5S RNA purified from *Xenopus* oocytes. T1 digestions of end-labeled RNA containing 5 nM unlabeled 5S RNA and reconstituted 7S particles were performed with 0.013 units of T1; radiolabeled p43-5S RNA complexes were treated with 0.02 units of T1. The same concentrations of 5S RNA and 7S particles as previously described were treated with 0.05 units of CV1. The p43-5S RNA complexes were treated with 0.13 units of CV1. All T1 and CV1 nuclease digestions were carried out in reconstitution or exchange buffer at 25°C for 2 min. The reactions were stopped and processed as described by Sands and Bogenhagen (in preparation). 'G' ladders were generated from the respective end-labeled RNAs as described (15). The reaction products were analyzed on sequencing gels as described in the figure legends.

RESULTS

Isolation of an intact p43-5S RNA complex from the 42S particle

Denis and LeMaire (7) have shown that the 42S particle can be disrupted by treatment with urea or moderate salt, releasing a complex containing 5S RNA and p43. Mattaj *et al.* (16) have also shown that the components of the 42S particle can be completely dissociated in 1 M salt. The protein components could then be separated by precipitating p43 with salt conditions that allowed p48 to remain in solution. A detailed characterization of the interactions of p43 with 5S RNA was not performed in these studies. Since we found it difficult to redissolve p43 following this purification, we developed an alternative procedure to prepare the p43-5S RNA complex. 42S particles for this study were purified from the ovaries of immature *X. laevis* frogs. Ovaries containing exclusively previtellogenic oocytes (stage I and II, Dumont (17)) were homogenized and subjected to glycerol gradient centrifugation. The 42S particle appears to dissociate into 15S subunits under these sedimentation conditions (7). We estimate that the 42S particle proteins are at least 90%

homogeneous after this initial purification step. The RNPs were concentrated by stepwise elution from a DEAE Sephacel column, then treated with 6.7 M urea and then loaded onto a second DEAE Sephacel column. The flow through from this column contained exclusively p48 (Fig. 1). The remaining components were separated by eluting the column with a linear gradient of increasing KCl. The first peak to elute contained exclusively p43 protein and 5S RNA (Fig. 1). Free tRNA eluted later in the gradient.

The fact that 5S RNA was present in the same fractions as p43 and eluted at a lower salt concentration than free tRNA suggested that an intact p43-5S complex may have survived urea treatment and DEAE column chromatography. To confirm this we analyzed fractions containing both p43 and 5S RNA by native polyacrylamide gel electrophoresis. Peak fractions from the DEAE column containing p43 and 5S RNA (Fig. 2 upper panel, lane 3) contain a particle that stains with ethidium bromide and has a slower mobility than 5S RNA, 7S particle or the L5-5S RNA complex. Bands from the gel in Fig. 2 were excised and the components electroeluted. Proteins recovered from the eluted bands were analyzed by SDS-PAGE. Proteins present in the bands from the L5-5S, 7S and p43-5S complexes migrate with apparent molecular weights of 37 kD, 40 kD and 43 kD, respectively (Fig. 2, lower panel). Analysis of the RNA present in the p43-5S RNA complex on partially denaturing polyacrylamide gels (18) confirmed that both oocyte and somatic type 5S RNA are present (data not shown). These data indicate that the intact p43-5S RNA complex can be purified to near homogeneity from the 42S particle by urea treatment followed by DEAE-Sephacel chromatography and can survive polyacrylamide gel electrophoresis.

Incorporation of radiolabeled 5S RNA into p43-5S

Our initial attempts to reconstitute the RNP using purified p43 protein were unsuccessful due to the poor solubility of the protein under physiological salt and buffer conditions. We therefore utilized a 5S RNA exchange reaction (19) to incorporate radiolabeled RNA into purified p43-5S complexes. An exchange reaction takes advantage of the fact that free 5S RNA and 5S RNA bound to p43 reaches a dynamic equilibrium. In the presence of excess p43-5S (at least a 50-fold molar excess in our

Figure 1. Analysis of the proteins present in fractions following DEAE column chromatography. Urea treated 42S particles were loaded on a DEAE Sephacel column and bound material was eluted with a linear gradient from 100–400 mM KCl. The proteins in the fractions were analyzed on a 12% polyacrylamide SDS protein gel and stained with Coomassie brilliant blue. Lane M contains molecular weight markers of 20.1 kD (soybean trypsin inhibitor), 36.5 kD (lactate dehydrogenase), 40 kD (TFIIIA) and 55.4 kD (glutamate dehydrogenase). Lane L represents the sample loaded on the DEAE column containing the urea treated 42S particle. Lane FT contains the flow through from the column. Lanes 1–11 are the corresponding column fractions from the gradient elution of the column.

Figure 2. Analysis of the components from the DEAE column containing p43 and 5S RNA. The upper panel is an ethidium bromide stained 6% polyacrylamide gel run under conditions which allow ribonucleoprotein particles to remain intact. Lane M contains 5S RNA. Lanes 1 and 2 contain the ribosomal protein L5-5S RNA complex and the 7S particle, respectively. Lane 3 contains the peak fraction of p43 and 5S RNA from the gradient eluted DEAE column (equivalent to the sample in lanes 7–9 of Fig. 1). The lower panel is a 12% polyacrylamide SDS protein gel stained with Coomassie brilliant blue showing the proteins present in the corresponding lanes from the upper panel. The bands from the gel shown in the upper panel were excised and the components electroeluted in 0.1 × TBE plus 0.2% SDS. Lane M contains the same molecular weight markers as Fig. 1 except for the addition of a 97.4 kD protein (phosphorylase B). The apparent molecular weights of the proteins in lanes 1, 2 and 3 are 37.5, 40 and 43 kD, respectively.

Figure 3. Time course of the exchange reaction performed with the p43-5S RNA complex. Radiolabeled complexes were analyzed on 6% polyacrylamide gels containing 0.1% Triton X-100 in TBE buffer as described in Materials and Methods. The mobilities of the p43-5S RNA complex (as determined by ethidium bromide staining) and 5S RNA are indicated to the left of the autoradiogram. The lane labeled (–) contains 5S RNA internally labeled at G residues. Lanes labeled 1–60 represent the time (min) of incubation of the exchange reactions.

Figure 4. α -sarcin nuclease protection assays of p43-5S RNA complexes containing 5' and 3' end-labeled 5S RNA. Nuclease digestions were performed as described in Materials and Methods and analyzed on 20% polyacrylamide 8 M urea sequencing gels. Lanes 1 and 2 show the α -sarcin cleavage patterns of naked 5S RNA and 5S RNA complexed with p43. Lanes G contain the corresponding end-labeled 5S RNA partially cleaved with T1 nuclease under denaturing conditions. Numbers to the left of each panel represent the guanine residues cleaved during the T1 reaction. A (-) or (+) above a lane indicates the absence or presence of p43, respectively. Panel A shows the protection pattern over the 5' half of 5' end-labeled 5S RNA. Panel B shows the nucleotides protected from α -sarcin degradation over the 3' half of 3' end-labeled 5S RNA.

experiments) radiolabeled 5S RNA is incorporated into the complex. Complexes containing radiolabeled 5S RNA have a mobility on polyacrylamide gels identical to that of purified complexes stained with ethidium bromide. Initiation of the exchange reaction is quite rapid with a significant amount of 5S RNA present in the complex within one min following mixing (Fig. 3). The amount of RNA incorporated into the complex begins to plateau at approximately 30–45 min following the start of the reaction. Conditions for optimal exchange employed KCl concentrations from 0 to 50 mM in the presence of 1 mM CaCl_2 . Under these conditions greater than 90 per cent of the radiolabeled 5S RNA was incorporated into the particle.

Figure 5. Summary of the α -sarcin protection data of 5S RNA complexed with p43 with respect to the secondary structure of 5S RNA. The arrows indicate the effect of p43 on the probability of cleavage by the nuclease, as indicated. Residues not identified by arrows are not cleaved at a significant rate either in the presence or absence of p43.

α -sarcin protection assays of radiolabeled p43-5S complexes

Protection of end-labeled RNA and DNA from nuclease degradation in the presence of a specific binding protein has been used as a method to study specific protein-nucleic acid interactions. RNases are highly specific enzymes with respect to both sequence and structure. Several nucleases with different specificities are routinely used to accurately delineate the binding site of a protein on an RNA. We have used three nucleases to study the interaction of p43 with 5S RNA. We first present results obtained using the nuclease α -sarcin, which is reported to cleave at purine residues in both single and double stranded regions (20).

Figure 4 shows the protection pattern obtained when p43-5S RNA complexes containing either 5' or 3' end-labeled 5S RNA are probed with α -sarcin. p43 provides protection for only a few nucleotides in the 5' half of 5S RNA. Panel A shows several bands that have a decreased intensity in the presence of the protein. These bands correspond to nucleotides 9, 11, 21 and 23–25. Within helix II, the presence of the protein appears to enhance cleavage at residue 22. Significant protection is also observed at residues 51, 55 and 56 with a moderately reduced intensity at residue 49 (panel A). Panel B shows the protection pattern provided by p43 on 3' end-labeled RNA. Residues from 59–101 which are cleaved in the absence of protein become protected from degradation when p43 is bound to the RNA. Although α -sarcin cleaves at only a few sites between nucleotides 102–120, nucleotides 110 and 113 are protected by p43. The location of the protected nucleotides is diagrammed with reference to the secondary structure of 5S RNA in Fig. 5. Most residues protected by p43 are located in the 3' half of 5S RNA within helices I, IV and V with some protected nucleotides seen in loop B between helices II and III.

A direct comparison of the p43 binding site to that of TFIIA using structure specific nucleases

The data in Figs. 4 and 5 show that p43 interacts with the same general region of 5S RNA as do TFIIA and rat ribosomal protein L5 (helices I, II, IV and V) (21, 22). We performed additional experiments to study the protection from nucleases T1 and CV1

Figure 6. Comparison of the 5S RNA binding site for TFIIIA and p43 with the structure specific nucleases T1 and CV1. RNP reconstitutions and nuclease digestions were performed as described in Materials and Methods and analyzed on 23% polyacrylamide 8 M urea sequencing type gels. Panel A shows the protection pattern of 5' end-labeled 5S RNA alone (lanes 1), bound to TFIIIA (lanes 2) or bound to p43 (lanes 3). 'T1' and 'CV1' represent the set of experiments performed with the corresponding nuclease. The lane designated '5S' is undigested 5' end-labeled 5S RNA. 'G' represents the same RNA digested with T1 nuclease under denaturing conditions. A (-) or (+) indicate the absence or presence of protein, respectively. Panel B shows the same set of experiments described in panel A except that the radiolabeled 5S RNA used in these experiments was 3' end-labeled. The lane designations are the same as in panel A except that '5S' represents undigested 3' end-labeled 5S RNA. Positions of the guanine residues are indicated to the left of the figure.

resulting from binding of p43 to 5S RNA. At the same time, we performed parallel experiments with TFIIIA to facilitate comparison of the protection patterns of the two zinc-finger proteins.

RNase T1 cleaves at the 3' side of guanine residues in single stranded regions leaving a cyclic phosphate moiety on the 3' end of the resulting 5' oligonucleotide. CV1 is a less specific enzyme which cleaves irrespective of sequence in base paired regions (23, 24). Comparison of data obtained with one single strand and one double strand specific nuclease allows an analysis of protection patterns at a large fraction of residues in the 5' end of the 5S RNA. Figure 6 shows the patterns of cleavage due to T1 and CV1 in end-labeled 5S RNA (Lanes 1), 7S particles (Lanes 2) and p43-5S RNA complexes (Lanes 3). To review this rather complicated analysis, we will first consider the basic pattern of altered cleavages caused by binding 5S RNA to p43, and then compare this pattern to that induced by binding to TFIIIA. All of these reactions shown in Fig. 6 employed the same RNA preparations, although similar results were obtained with other batches of RNA. In Figure 6A, little or no protection by p43 is observed over the 5' portion of 5S RNA when the complexes are probed with T1 nuclease. CV1 treatment of the p43-5S complex (Lane 3, 'CV1') revealed additional protected nucleotides not observed with α -sarcin. Residues 5–10 are fully protected from nucleolytic attack. This study also shows a slight decrease in the intensity of several bands (residues 14–20), however, it is unclear whether this decrease is due to partial

protection or to a slight change in the RNA conformation in that region. No significant difference was observed between the CV1 cleavage patterns of free 5S RNA and the p43-5S complex between residues 21 and 71. To assess protection in the 3' half of 5S RNA, similar experiments were performed on complexes reconstituted with 3' end-labeled 5S RNA (Fig. 6B). The free 5S RNA probed with T1 nuclease (Lane 1, 'T1') showed some cleavage at nucleotides 61, 64 and 65 in helix II with 3' end-labeled RNA. These cut sites were not observed with 5' labeled RNA. This may reflect a slight change in the structure of the RNA resulting from the differences in labeling conditions. The reason for this discrepancy is not understood. The apparent protection from T1 nuclease cutting in this region observed upon binding of p43 (lane 3) may only reflect stabilization of helix II, and is not considered significant. p43 binding produces more striking changes in cleavage in helix V. Markedly enhanced cleavages are observed at residues 85 and 86 with additional enhanced cleavages present at residues 82 and 94. This increased accessibility to a single-strand specific nuclease may reflect disruption of the RNA helix. The CV1 protection experiment shows clear protection of residues 103–111 and partial protection of 112–115 (Lane 3, 'CV1'). Binding in this region could not be evaluated using α -sarcin, since this nuclease does not cleave these residues in free 5S RNA. However, in those areas of the distal half of 5S RNA which are susceptible to cleavage by both CV1 and α -sarcin, the patterns of protection from both enzymes are generally consistent.

Figure 7. Comparison of the influence of binding of TFIIIA or p43 on the digestion of 5S RNA with nucleases T1 and V1. (A) Influence of p43 on cleavage by RNase T1. (B) Influence of TFIIIA on cleavage by RNase T1. In both panels, no attempt is made to score changes in cleavage in the vicinity of residue 64. The basal cleavage patterns of free RNA in this region do not agree for 5' and 3' labeled RNAs. The basis for this discrepancy is not understood, but may reflect differences in the incubation conditions during RNA labeling (see Materials and Methods). (C) Influence of p43 on cleavage by cobra venom nuclease V1. (D) Influence of TFIIIA on cleavage by cobra venom nuclease V1. The interpretation of the arrows in all four panels is as indicated in panel D.

The T1 and CV1 cleavage results shown in Fig. 6 are summarized in Fig. 7. Binding by p43 clearly alters nuclease cleavages within helices I, II, IV and V. TFIIIA binds to the same general regions (Figs. 6 and 7; 21, 25, 26), although we note three striking differences in the RNA cleavage patterns. First, when the two complexes are probed with CV1, both proteins protect residues 5–7. Adjacent residues 8–10, however, are protected only by p43, not by TFIIIA. Second, in helix II, TFIIIA protects residues 14–16 whereas p43 provides little if any protection of the same nucleotides. The enhanced CV1 cleavage observed in helices II and III when TFIIIA is bound to 5S RNA are not produced when p43 is bound to the RNA. Nucleotides on the 3' side of helix II appear to be more completely protected by TFIIIA than by p43. Third, an alteration in T1 RNase cleavage is observed at the base of helix V. Binding of TFIIIA results in little change in the T1 cleavage pattern in this region when compared to naked 5S RNA. In contrast, p43 appears to disrupt the base pairing in that region, resulting in enhanced T1 RNase cleavages. Destabilization of the RNA in helix V may contribute to the extensive suppression of CV1 cleavages in the p43-5S RNA complex in Fig. 6B.

DISCUSSION

Preparation of p43-5S RNA complexes containing labeled 5S RNA

Mattaj *et al.* (16) described a method whereby the 42S particle could be disrupted by high salt and processed to allow purification of the p48 protein. This method was not useful for preparation of p43, since the precipitated p43 protein is difficult to redissolve.

Barrett and Sommerville (27) employed a purification procedure similar to the one described here where 42S particles are loaded on a DEAE ion exchange column and the 48 kDa protein is eluted with a 6 M urea step. The p43 isolated in this manner is contaminated with a small amount of p48 and the question of whether it is complexed with 5S RNA was not addressed. We have modified this procedure by briefly treating concentrated 42S particles with 6.7 M urea prior to chromatography on DEAE Sephacel. This allows for the quantitative recovery of p48 in the flow through. The p43-5S RNA complex can then be eluted from the column with a linear gradient of KCl.

We used an exchange reaction to incorporate exogenous labeled 5S RNA into intact p43-5S RNA complexes. This exchange reaction allows 90% of the labeled RNA to become incorporated into the complex within 45–60 min. The labeled product of the exchange reaction has the same electrophoretic mobility as purified p43-5S RNA complexes. However, early in the reaction, more slowly migrating species are observed that may represent intermediates in the exchange in which two or more 5S RNA molecules transiently associate with a single copy of p43. A similar phenomenon is observed during an exchange reaction with 7S particles (19).

Nuclease protection studies with p43 and TFIIIA

Our nuclease protection data suggest that the p43 interacts with helices I, II, IV and V of 5S RNA. This region appears to be a common site for the interactions of a number of proteins. TFIIIA (14, 25, 26, 28–30), rat ribosomal protein L5 (22, 31) and *E. coli* ribosomal proteins L5, L18 and L25 (32) all bind the same general region of the molecule. It is of particular interest

that the binding site resembles that of TFIIIA. Joho *et al.* (9) have recently shown that the sequence of p43 contains a series of nine 'Zn-fingers' closely related to those of TFIIIA. Although the proteins are similar in a number of respects, p43 is unable to bind the 5S RNA gene (9, Sands and Bogenhagen, unpublished results). We have detected significant differences in the RNA binding properties of the two proteins in addition to this difference in their DNA binding ability. Nuclease protection experiments with 5S DNA (13) and 5S RNA (Sands and Bogenhagen, accompanying paper) indicate that the nucleic acid binding domain of TFIIIA is limited to the zinc fingers. It is possible that the domain of p43 that is not organized into 'Zn-fingers' may influence the RNA binding properties of the protein. For example, it may be that this domain causes the apparent disruption of helix V.

Both TFIIIA and p43 protect sites in all three helical stems of 5S RNA. It would appear that both proteins are closely associated with the central portion of 5S RNA located around the junction of helices I, II and IV. Although the tertiary structure of 5S RNA is not known, we have drawn the secondary structure in Fig. 7 to resemble the shape of 5S RNA predicted by the molecular model of Westhof *et al.* (33). One way to envision the binding of these 'Zn-finger' proteins to 5S RNA is that both elongated proteins may wrap around the RNA in some manner. Our data would suggest that the RNA contacts of the two proteins may be similar in some areas but different in others. The differences in nuclease protection binding could be explained if the corresponding 'Zn-fingers' of the two proteins interact with the RNA helix out of register with each other in some regions. For example, residues 8 and 9 are protected from CV1 nuclease by p43 but not by TFIIIA, while the 5' proximal portion of helix II is protected by TFIIIA, but not by p43 (Fig. 6A). We might speculate that this change in the cleavage pattern reflects differential placement of an individual zinc finger on either side of loop A. Additional experiments will be required to test this possibility.

In this study, we have presented the first detailed analysis of the interaction of p43 with individual nucleotide residues in 5S RNA. However, the sort of nuclease protection data presented in this study is limited by the large size and complexity of nuclease probes. It is likely that the use of bulky nucleases as probes may tend to overestimate the size of a protected region. This creates a general problem that apparent protection of a cleavage site may actually result from an effect at nearby residues that may alter binding of the nuclease. These effects probably contribute to isolated instances where the 'protection' patterns provided by two nucleases do not seem to agree. For example, p43 appears to protect residues G-25 and G-56 against cleavage by α -sarcin, but not against cleavage by nuclease T1. Minor discrepancies of this sort may be resolved by more detailed protection studies using smaller chemical probes. Further development of this system may provide insight into the precise interactions between 'Zn-finger' proteins and nucleic acids.

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