The carboxyterminal zinc fingers of TFIIIA interact with the tip of helix V of 5S RNA in the 7S ribonucleoprotein particle

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ABSTRACT

Immature *Xenopus laevis* oocytes contain large quantities of a 7S ribonucleoprotein particle containing transcription factor IIIA (TFIIIA) and 5S RNA in a 1:1 molar ratio. We have reconstituted RNPs containing 5S RNA and either intact TFIIIA or proteolytic fragments that represent progressive C-terminal deletions of the protein. A partial trypsin digestion fragment encompassing the amino terminal seven zinc fingers of TFIIIA rebinds 5S RNA with nearly the same affinity as intact TFIIIA. We have compared the RNase protection patterns resulting from binding of intact and deleted forms of TFIIIA. RNAse protection assays using cobra venom nuclease were performed on complexes reconstituted with 5′ and 3′ end-labeled 5S RNA. Similar experiments with 3′ end-labeled 5S RNA were performed with nuclease α-sarcin. With both nucleases, nucleotides in helix V of 5S RNA show more complete protection from nuclease cleavage when the RNA is bound to intact TFIIIA than when it is bound to a 20 kDa tryptic fragment of TFIIIA lacking the C-terminal portion of the protein. These results suggest that fingers 8 and 9 of TFIIIA interact with the distal portion of helix V in the 5S RNA.

INTRODUCTION

The protein that binds to the intragenic control region (ICR) of the 5S RNA gene, transcription factor IIIA (TFIIIA), was the first eukaryotic transcription factor purified to homogeneity (1, 2, 3). The interactions of TFIIIA with the 5S RNA gene ICR have been extensively studied. Smith *et al.* (4) showed that the N-terminal portion of TFIIIA contacts the 3′ portion of the intragenic control region (ICR) and that the elongated TFIIIA protein (5) extends in a collinear fashion along the DNA leaving the C-terminus at the 5′ end of the ICR. TFIIIA was the first eukaryotic transcription factor shown to require zinc for binding to DNA (6). After the gene encoding TFIIIA was cloned and sequenced (7), Miller *et al.* (8) proposed that the DNA binding domain of the protein was organized in a series of 'Zn-fingers.' Similar 'Zn-fingers' have since been observed in many DNA binding proteins (reviewed in 9, 10). TFIIIA remains unique in that it also binds 5S RNA in a 1:1 molar ratio to form a 7S ribonucleoprotein particle (2, 3, 11).

It is still not clear how TFIIIA specifically recognizes and binds both 5S RNA and the 5S RNA gene. The overall binding site of TFIIIA on 5S RNA has been mapped by a number of groups using chemical and nuclease probes (12−16), truncated RNAs (17−19), and base substituted RNAs (20, 21). A consensus binding domain encompassing helices I, II, IV, and V of 5S RNA has emerged from these studies. Some of the models for the structure of the 7S particle have proposed that TFIIIA binds 5S RNA in the same general orientation as it does 5S DNA (13, 15, 19). Part of the appeal of this model is that the tip of helix V of 5S RNA can be drawn to resemble the tight binding site for TFIIIA in the distal portion of the intragenic control region in the 5S RNA gene (22). However, none of the studies presented to date has directly determined the orientation of TFIIIA within the 7S particle.

In this report we present two sets of experiments that provide a test of the model that TFIIIA binds 5S RNA in much the same way that it binds 5S DNA. We demonstrate that RNPs containing 5S RNA and either intact TFIIIA or TFIIIA molecules with progressive C-terminal deletions (4) can be reconstituted *in vitro*. We find that the C-terminal domains of TFIIIA are not required for 5S RNA recognition and contribute minimal binding energy to the 7S complex. Results from nuclease protection experiments performed on the same reconstituted RNPs suggest that the N-terminal 'Zn-fingers' of TFIIIA do not interact with helix V of 5S RNA.

MATERIALS AND METHODS

Materials

Restriction enzymes were purchased from New England BioLabs (Beverly Mass.). α- and γ-^{32}P labeled GTP were purchased from ICN Pharmaceuticals Inc., (Irvine, Cal.). α-^{32}P-cytidine

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3',5' bisphosphate (pCp) was obtained from New England Nuclear (Boston, Mass.). Immature and mature *X. laevis* were purchased from Xenopus I (Ann Arbor, Mi.) or Nasco (Fort Atkinson, Wi.). Papain was obtained from Boehringer Mannheim and type XIII Trypsin was from Sigma. Cobra venom nuclease and T4 RNA ligase were purchased from Pharmacia (Piscataway, NJ). α-sarcin was a kind gift from I. Wool and highly concentrated T7 RNA polymerase was a kind gift from W. Studier and J. Dunn.

Protein purification

TFIIIA was isolated from immature *X. laevis* oocytes as previously described (4, 23). The '30 kDa' and '20 kDa' proteolytic fragments of TFIIIA were generated by the methods described by Smith *et al.* (4) and quantified using the method of Bradford (24).

Plasmid constructs

Construction and characterization of the linker substitution mutants was described by Sands and Bogenhagen (20). Somatic 5S RNA and LS 86/97 were cloned adjacent to a truncated T7 promoter similar to that described by Romaniuk *et al.* (18). The construction took advantage of naturally occurring cleavage sites for restriction enzymes Eae I at residue 6 and Fok I at residue 93 of the *X. borealis* somatic 5S RNA gene, Xbs 201 (25). The internal Eae I−Fok I fragment was ligated to two duplex oligonucleotides. One oligonucleotide adapted the Eae I site to an Eco RI site and provided the sequence of the T7 promoter. The second oligonucleotide was ligated to the Fok I site and provided the 3' end of the 5S RNA gene adjacent to recognition sites for Dra I and Hind III. This transcription unit has four T7 specific nucleotides downstream from the transcription start site replaced by the first four nucleotides of 5S RNA. Transcription by T7 RNA polymerase begins with the first G residue of *X. borealis* somatic 5S RNA. The oligonucleotide coding for the 3' portion of 5S RNA contained a Dra I site allowing linearization of the plasmid to give an RNA with a discrete end point containing three uridine residues.

In vitro transcription

T7 transcriptions were performed in buffer containing 40 mM Tris (pH 7.6), 8 mM $MgCl_2$, 2 mM spermidine, 10mM DTT, 40 units RNAsin, 2 μg DNA, 1 μg T7 RNA polymerase and rNTPs. Reactions were incubated at 37°C for 1 hr. 5' end-labeling was performed during the T7 transcription reaction. Concentrations of ATP, UTP, and CTP were 250 μM. The concentration of GTP, the initiating nucleotide, was 20 μM. For synthesis of 5' end-labeled RNA, the transcription reaction contained 300 μCi of γ^{32}P-GTP at a final specific activity of 15 mCi/mmole. Radiolabeled RNAs were purified by native polyacrylamide gel electrophoresis in buffer containing 50 mM Tris base, 50 mM boric acid, and 1 mM EDTA (TBE). The polyacrylamide gel and running buffer contained 0.1% SDS to enhance RNA stability. Electroelution and extraction of the RNA was performed as described by Sands and Bogenhagen (20). For the synthesis of non-radiolabeled RNA, 250 μM GTP was included in the transcription reaction. Non-radiolabeled transcripts were centrifuged through pre-packed G-50 Sephadex spin columns (Boehringer Mannheim) equilibrated with 10 mM Tris (pH 7.6), 1 mM EDTA, and 0.2% SDS. Aliquots were removed and analyzed by native PAGE. Densitometry of the ethidium bromide stained gel was used to quantitate the RNA. The remaining RNA was phenol-chloroform extracted, ethanol precipitated, resuspended in 10 mM Tris (pH 7.6) and 1 mM EDTA, aliquoted, and stored at −70°C for use in 3' end-labeling reactions.

3' end-labeling reactions were performed using T4 RNA ligase essentially as described by Romaniuk and Uhlenbeck (26). 1.5 pmole 5S RNA, generated from a T7 transcription reaction, was incubated with 50 mM HEPES (pH 7.4), 1.5 mM $MgCl_2$, 3 mM DTT, 25 μM ATP, 10 μg/ml BSA, 5% DMSO, 30 pmole α-^{32}P-pCp (3,000 Ci/mmole), and 14 units of T4 RNA ligase for 16 hours at 4°C. The labeling reaction was loaded directly on a 6% polyacrylamide gel containing 0.1% SDS and processed as described for 5' end-labeled transcripts.

RNP reconstitution and mobility shift assay

5S RNA and protein concentrations are described in the figure legends. Binding reactions were carried out in 20 μl volumes at room temperature for 20 min in a reconstitution buffer containing 85 mM NH_4Cl, 1.5 mM $MgCl_2$, 2 mM DTT, 10 μM $ZnCl_2$, 10 mM HEPES (pH 7.4), 8% glycerol, 4 units RNAsin, and 0.1 mg/ml BSA. Binding reactions were loaded directly on 6% polyacrylamide (1:20, bisacrylamide:acrylamide) gel including 0.1% Triton X-100 (Surfact-Amps, Pierce). Gels were run in TBE buffer at 300 volts for 1.5 hr at room temperature.

RNase footprinting and structure mapping

RNP reconstitutions were performed as described above. An aliquot of each binding reaction was analyzed in a gel mobility shift assay. Complete binding was confirmed with the mobility shift assay or by the presence of fully protected bands. α-sarcin treatment was carried out in reconstitution buffer at 30°C for 10 min with 4 μM nuclease. Cobra venom reactions were performed with $4.5 * 10^{-4}$ units/reaction for one min. Nuclease reactions were stopped by adding 300 μl of 300 mM NaCl, 10 mM EDTA, 10 mM Tris (pH 7.6), 0.5% SDS, and 10 μg/ml tRNA. Samples were phenol chloroform extracted twice and ethanol precipitated at −70°C for 30 min. RNA was resuspended in 6 μl of 95% formamide, 0.5% SDS, 25 mM EDTA, bromophenol blue, and xylene cyanol, heated at 50°C for 20 min and analyzed on a 20% polyacrylamide, 8 M urea sequencing gel in TBE buffer. G ladders were generated with T1 nuclease under denaturing conditions as described by Donis-Keller *et al.* (27).

RESULTS

Reconstitution of RNPs containing proteolytic fragments of TFIIIA

Proteolytic fragments of TFIIIA, designated '30 kDa' and '20 kDa', first described by Smith *et al.* (4), were used to determine the orientation of TFIIIA along the 5S RNA gene. The '30 kDa' and '20 kDa' fragments are prepared by digestion of intact 7S particles with papain and trypsin, respectively (4). These two proteolytic fragments essentially represent progressive C-terminal deletions. The DNA binding domain remains intact in the '30 kDa' fragment. In contrast, the '20 kDa' tryptic fragment of TFIIIA protects only the distal two-thirds of the ICR of the 5S RNA gene (4) giving a protection pattern similar to that of a deletion mutant of the TFIIIA gene lacking the last two zinc fingers (28). Figure 1a shows a Coommassie blue stained protein gel analysis of TFIIIA and the proteolytic fragments. In our gel

system the apparent molecular weights are approximately 35 kDa and 28 kDa, respectively. In other experiments, we have compared the gel mobilities of these fragments to partial fragments of TFIIIA generated by cleavage at methionine residues with cyanogen bromide and at tryptophan residues with BNPS-skatole. This anlysis suggests that the '30 kDa' and '20kDa' fragments contain the N-terminal 285 and 230 residues of TFIIIA, respectively. Nevertheless, we refer to these fragments as '30 kDa' and '20 kDA' fragments according to the nomenclature of Smith *et al.* (4). Figure 1b shows that both truncated forms of TFIIIA can rebind 5S RNA to reconstitute RNPs with distinctive mobilities on a native polyacrylamide gel. The differences in mobility of the resulting RNPs reflect the differences in apparent molecular weight of the protein fragments. The ability to reconstitute an RNP with the '20 kDa' proteolytic fragment indicates that the entire DNA-binding domain of TFIIIA is not required for binding 5S RNA. Before proceeding with an analysis of the structure of particles reconstituted with the '20 kDa' fragment, we compared the relative affinity of intact TFIIIA and the '20 kDa' fragment for binding somatic 5S RNA in the mobility-shift assay (Fig. 2). The affinity of the '20 kDa' fragment for 5S RNA is nearly identical to that observed for intact TFIIIA; both proteins bind with an apparent K_A similar to that reported by Romaniuk (16). The '30 kDa' fragment binds 5S RNA equally well (data not shown). The fact that the '20 kDa' fragment binds 5S RNA with a relatively high affinity indicates that the C-terminal 'Zn-fingers' of TFIIIA contribute minimal binding energy to the 7S particle.

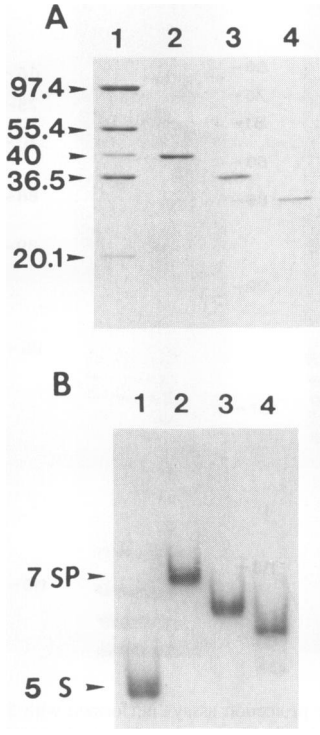

Fig. 1. Analysis of the purity and 5S RNA binding properties of intact TFIIIA and it's proteolytic fragments. Panel A shows a Coomassie blue stained 12% polyacrylamide gel of TFIIIA (Lane 2), the '30 kDa' fragment (Lane 3), and the '20 kDa' fragment (Lane 4). Lane one contains molecular weight markers 20.1 kDa (soybean trypsin inhibitor), 36.5 kDa (lactate dehydrogenase), 40 kDa (TFIIIA), 55.4 kDa (glutamate dehydrogenase), and 97.4 kDa (phosphorylase b). Panel B shows an autoradiogram of a gel mobility shift assay performed with 10nM 3′ end-labeled 5S RNA alone (lane 1), or after binding with 20 nM each of TFIIIA (lane 2), the '30 kDa' fragment (lane 3), or the '20 kDa' fragment (lane 4).

Nuclease protection assays of reconstituted RNPs

Nuclease protection assays were performed with RNPs reconstituted by binding either TFIIIA or the proteolytic fragments to end-labeled somatic 5S RNA. To facilitate end-labeling of the RNA, we constructed a somatic 5S RNA gene in which transcription is directed by a truncated T7 promoter similar to that described by Romaniuk *et al.* (18). Transcription of the Dra I-linearized plasmid initiates at the first nucleotide of the 5S RNA gene and leaves a homogeneous 3′ end with three U residues. 5S RNA synthesized by the T7 RNA polymerase can either be 3′ end-labeled by the technique described by Romaniuk and Uhlenbeck (26) or can be 5′ end-labeled by including γ-^{32}p-GTP in the transcription reaction. When the end-labeled RNA was mixed with protein to reconstitute RNPs, a portion of each binding reaction was subjected to native gel electrophoresis to confirm complete binding. The remaining portion of the binding reaction was then mixed with nuclease to analyze the protection from nuclease afforded by the protein.

RNases generally are very specific enzymes with respect to sequence and secondary structure and yield much less information regarding protein-RNA interactions than can be obtained from a DNase footprinting assay. Therefore, several nucleases are usually employed to determine the binding site of a protein on RNA. In preliminary experiments, we screened several nucleases to identify reagents that yield different cleavage patterns with the RNPs reconstituted with proteolyzed TFIIIA compared to intact TFIIIA. All of the data accumulated in this general survey

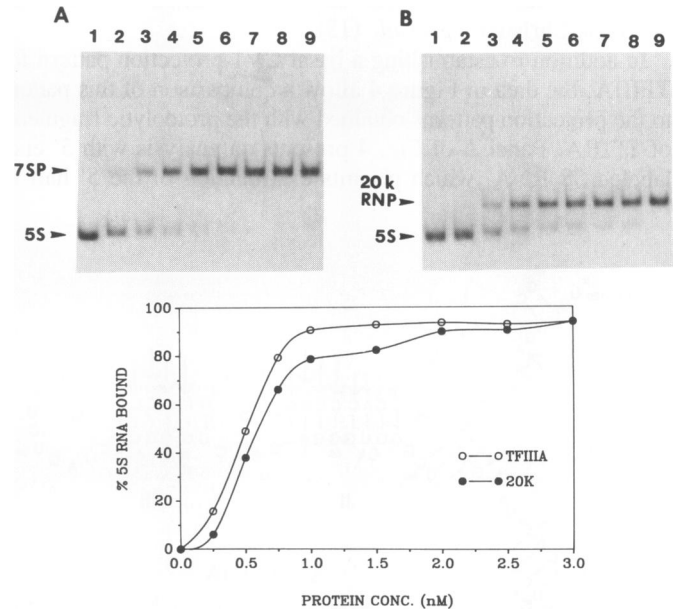

Fig. 2. Binding titrations of TFIIIA and the '20 kDa' fragment with somatic 5S RNA. Reconstitutions were carried out as described in Materials and Methods. All binding reactions contained 0.1 nM 3′ end-labeled RNA. The protein concentrations ranged from 0 to 3.0 nM. Reconstituted complexes were analyzed in a gel mobility shift assay and detected by autoradiography. The resulting autoradiogram was scanned to quantify the free and bound 5S RNA. Open circles represent binding of TFIIIA and closed circles represent binding of the '20 kDa' fragment.

indicated that only subtle differences could be detected between the protection patterns resulting from binding intact TFIIIA or the '30 kDa' or '20 kDa' fragments. We present results obtained with two of these nucleases, α-sarcin and cobra venom nuclease (CV1). α-sarcin is used since Huber and Wool (15) have shown that it can be used to visualize clear protection by TFIIIA of cleavage sites in the distal half of 5S RNA. CV1 cleaves independently of sequence in base-paired regions of RNA to yield 3'-OH and 5'-phosphoryl termini (29). We have found that CV1 nuclease is a very useful probe for studying interactions of TFIIIA with 5S RNA, since it is generally agreed that TFIIIA binds to base paired regions of 5S RNA.

The general pattern of protection obtained using CV1 to probe 7S particles reconstituted with intact TFIIIA is shown in Figure 3 with reference to the secondary structure model for 5S RNA. The cleavage sites depicted in Figure 3 are derived from the data shown in Figure 4. CV1 cuts at a large number of sites in 5S RNA to yield a protection pattern consistent with that obtained by Huber and Wool (15) with α-sarcin.

There are some discrepancies between the nuclease protection patterns we have observed for TFIIIA and results published by other groups. Some of these differences may result from the fact that we have used somatic-type 5S RNA while other groups have studied oocyte-type RNA or may reflect different technical methods. For example, in a previous study of nuclease CV1 protection by TFIIIA, Christiansen *et al.* (13) did not note the distinctive pattern of protection we have consistently observed in the 5' half of helix II (residues 14−21). However, these authors used primer extension methods that did not provide a good view of the 5' end of 5S RNA and the RNA preparations they employed contained a large fraction of RNA with basal cleavages in loop c, which would have prevented primer extension into helix II. We consider that our use of directly end-labeled RNA provides a clearer view of the 5' end of 5S RNA than the work of Christiansen *et al.* (13).

In addition to establishing a basal CV1 protection pattern for TFIIIA, the data in Figure 4 allow a comparison of this pattern to the protection patterns obtained with the proteolytic fragments of TFIIIA. Panel A of Fig. 4 presents an analysis with 5' end-labeled 5S RNA, which permits examination of the 5' half of the RNA. The most remarkable change in the CV1 cleavage pattern is a protection from CV1 nuclease at nucleotides 14−16 in helix II. All three forms of TFIIIA produce an identical protection in this region and result in an identical set of enhanced cleavages in the adjacent portion of helix II (residues 17−21) and in helix III. These observations suggest that the C-terminal domain of TFIIIA does not contact this portion of 5S RNA.

Protection patterns in the 3' half of 5S RNA are studied using RNPs reconstituted with 3' labeled RNAs in Fig. 4, panels B and C. These two panels represent similar samples subjected to electrophoresis for different periods of time to resolve products of cleavage in different portions of 5S RNA. Cleavage patterns observed in the three RNPs are identical in helix IV (residues 104−112 and residues between 66 and 70). Residues near helix V (residues 77−79 and 92−94) show partial protection when 5S RNA is bound to TFIIIA and the '30 kDa' fragment. The same residues show reduced protection when the RNA is bound to the '20 kDa' fragment. Four separate CV1 protection experiments were performed and the autoradiograms were scanned to determine the relative protection of the bands. In each case, in the presence of the '20 kDa' fragment, the residues bordering helix V have a decreased protection relative to bands in helix IV. The '20 kDa' fragment also appears to induce

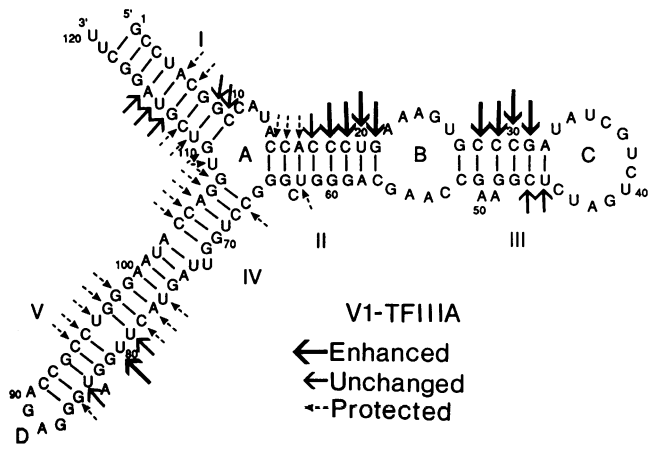

Fig. 3. Summary of the CV1 nuclease protection pattern observed for 5S RNA complexed with intact TFIIIA. The secondary structure of *X. borealis* somatic 5S RNA is drawn according to Anderson *et al.* (33) and Christiansen *et al.* (13).

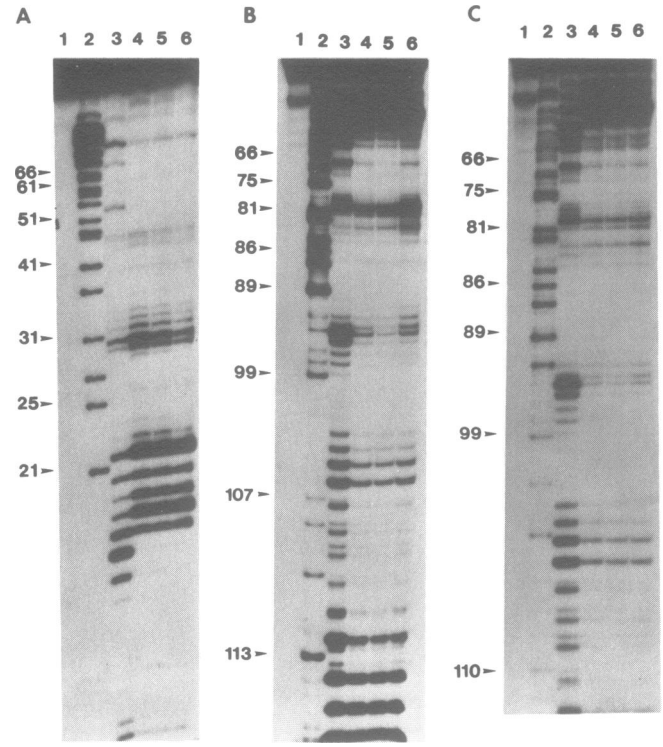

Fig. 4. CV1 nuclease protection assays performed with 5' and 3' end-labeled 5S RNA and TFIIIA, the '30 kDa', and the '20 kDa' proteolytic fragments. Panel A shows the protection pattern with 5' end-labeled somatic 5S RNA. Lanes 1−6 contain 10 nM total 5S RNA. Lane 1 contains labeled RNA alone. Lane 2 contains RNA treated with T1 nuclease as previously described to generate a 'G-ladder'. Lanes 3−6 contain RNP preparations treated with CV1 as described in Materials and Methods. Lane 3 contains no 5S RNA binding protein. Lane 4 contains 20 nM TFIIIA, lane 5 contains 20 nM of the '30 kDa' fragment, and lane 6 contain 20 nM of the '20 kDa' fragment. Panels B and C show CV1 nuclease protection assays performed with 3' end-labeled 5S RNA. Panels B and C represent similar reactions performed on separate batches of reconstituted RNPs, but differ in that electrophoresis was continued for 6 and 8.5 hrs, respectively. Lane designations are as described in Figure 4A.

additional cleavage at approximately residue 83. Such induced hypersensitivity sites in DNAse protection experiments are often interpreted as the result of an edge-effect at the boundary of a protein binding site.

Our results with CV1 nuclease protection can be summarized as follows. There is a remarkable similarity in the patterns of protection afforded by all three forms of TFIIIA. The most significant difference was a subtle decrease in the protection near residue 82 of 5S RNA in helix V, where the '20 kDa' form of TFIIIA provides reduced protection. This region has been included in the binding site for intact TFIIIA defined by Huber and Wool (15) using the nuclease α-sarcin. Therefore, we attempted to compare the detailed pattern of protection of 3′ end-labeled 5S RNA from α-sarcin in RNPs reconstituted with the proteolytic fragments of TFIIIA. α-sarcin is a ribonuclease which cleaves RNA at purine residues regardless of secondary structure (30).

Figure 5 shows that the binding of TFIIIA to 3′ end-labeled 5S RNA produces a pattern of protection from α-sarcin degradation similar to that observed by Huber and Wool (15). TFIIIA binding induces changes in nuclease cleavages in helices I, II, IV and V. We have seen no significant differences in the protection afforded by the '30 kDa' fragment as compared to that of intact TFIIIA. In addition, residues in helices I, II, and IV show the same pattern of protection in RNPs reconstituted with all three forms of TFIIIA. However, the pattern of protection provided by the '20 kDa' fragment differs from that provided by intact TFIIIA or the '30 kDa' fragment between nucleotides 80−88 in helix V. The most noticeable difference is that the '20 kDa' protein fails to protect a hypersensitive site in free 5S RNA at approximately residue 83. Several other weak cleavage sites surrounding residue 83 that are protected from α-sarcin degradation by intact TFIIIA or the '30 kDa' fragment are poorly protected by the '20 kDa' protein.

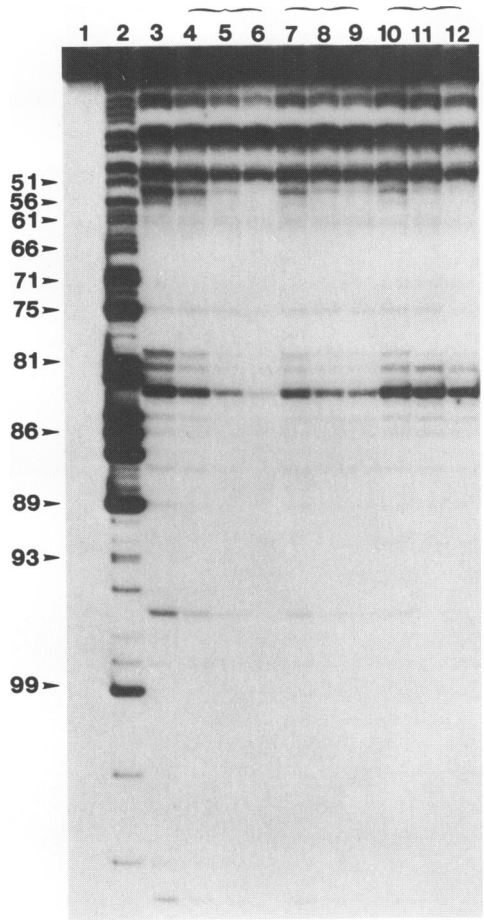

Fig. 5. α-sarcin footprinting reactions performed with reconstituted RNPs containing 3′ end-labeled 5S RNA and either TFIIIA, '30K' fragment, or '20K' fragment. All reactions contained 0.1 nM 3′ end-labeled somatic 5S RNA. Lane 1 contains 5S RNA alone. Lane 2 is a 'G-ladder' prepared by treatment of 3′ end-labeled 5S RNA with T1 nuclease under denaturing conditions. Lanes 3−12 are treated as described in Materials and Methods with α-sarcin. Lane 3 shows the cleavage pattern obtained with free 5S RNA. Lanes 4−6 contain 2 nM, 10 nM, and 20 nM TFIIIA, respectively; lanes 7−9 contain 2 nM, 10 nM, and 20 nM '30K' protein, respectively, and lanes 10−12 contain 2 nM, 10 nM, and 20 nM '20K' protein, respectively.

DISCUSSION

In this paper, we have compared the 5S RNA binding properties of two proteolytic fragments of TFIIIA to those of the intact protein. The '30 kDa' and '20 kDa' fragments used in this study are the same fragments used by Smith *et al.* (4) to aid in defining the mode of binding of TFIIIA to 5S DNA. These two fragments essentially represent progressive C-terminal deletions of TFIIIA. Both fragments were found to rebind efficiently to 5S RNA (Figs. 1 and 2). It is particulary impressive that the '20 kDa' fragment, which contains only the 7 amino-terminal zinc fingers, binds 5S RNA with an affinity comparable to that of intact TFIIIA. This result suggests that the same N-terminal tight binding domain involved in binding 5S DNA is also a tight binding domain for RNA.

Nuclease studies with reconstituted RNPs containing the truncated TFIIIA molecules and end-labeled RNA provide direct evidence that the C-terminal portion of the binding domain interacts with helix V of 5S RNA. Changes in the cleavage patterns in helix V of 5S RNA bound to the '20 kDa' fragment compared to the intact protein result from removal of the C-terminal 'Zn-fingers'. Results obtained with two nucleases complement each other in that they both show marked changes in and around helix V. In this analysis, it is important to emphasize that we observed no significant changes in cleavage patterns in any other region of the molecule when 5S RNA is bound to TFIIIA, the '30 kDa' fragment, or the '20 kDa' fragment.

The results obtained by nuclease treatment of RNPs reconstituted with truncated forms of TFIIIA contrast with the predictions of models in which TFIIIA is thought to bind in a similar orientation to 5S RNA and 5S DNA (13). If fingers 1 through 4 of TFIIIA were to bind to helices V and IV of 5S RNA as they bind to the distal part of the control region of the 5S RNA gene, we might have expected differences in the nuclease cleavage patterns in helix II for intact TFIIIA and the '20 kDa' trypsin fragment. The footprint of the '20 kDa' fragment of TFIIIA on 5S DNA ends near residue 67 of the gene (4). In the RNA version of the control region, helix II would lie outside of the binding site for the '20 kDa' fragment. However, our results show an identical protection pattern in helix II for intact TFIIIA and the '20 kDa' fragment (Fig. 4). The most significant differences in nuclease cleavage patterns that we observed appear to reflect reduced protection by the '20kDa' fragment near residue 83 of the RNA helix V.

Our data suggests that the C-terminal 'Zn-fingers' of TFIIIA interact with helix V of 5S RNA. However, these results cannot begin to suggest a comprehensive model for the structure of the 7S particle in the absence of a well-defined model for the tertiary structure of 5S RNA. The recent publication of a computer model for the tertiary structure of 5S RNA (31) suggests that helices II, III, IV and V lie in a nearly collinear arrangement similar to that proposed by Christiansen *et al.* (13). Nevertheless, this structure is still distinctly different from that of duplex DNA. At present, we can only suggest that simple models in which TFIIIA interacts in a similar fashion with helical regions of DNA and RNA are not likely to be correct. We have synthesized derivatives of 5S RNA containing extensive deletions of the tip of helix V that retain the ability to bind TFIIIA with high affinity (Bogenhagen, in preparation). These results provide further evidence that the tip of helix V does not constitute an RNA analogue to the tight binding site for TFIIIA at the distal portion of the intragenic control region in the 5S RNA gene. Available data indicates that the integrity of all three helical stalks in 5S RNA must be maintained for TFIIIA to bind. Nucleotide deletions and base substitutions in helix I completely abolish TFIIIA binding (17, 19–21). Similar modifications to helix II have the same effect (18, 20, 21). As noted above, our data indicates that both fragments of TFIIIA as well as the intact protein provide an identical, clearcut pattern of protection in helix II (Figure 4). In addition, recent crosslinking data suggests that tight interactions between TFIIIA and 5S RNA occur primarily in helix II (32). The N-terminus of TFIIIA containing the high affinity RNA binding domain, may be responsible for these interactions. Although these data suggest a possible orientation of TFIIIA on 5S RNA, the exact conformation of TFIIIA in the 7S particle will require information obtained from higher resolution physical techniques.

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REFERENCES

1. Engelke, D. R., S. Ng, B. S. Shastry, R. G. Roeder. 1980. Cell **19**, 717–728.
2. Pelham, H.R., and D. D. Brown. 1980. Proc. Natl. Acad. Sci. USA **77**, 4170–4174.
3. Picard, B., and M. Wegnez. 1979. Proc. Natl. Acad. Sci. USA **76**, 241–245.
4. Smith D. R., I. R. Jackson, and D. D. Brown. 1984. Cell **37**, 645–652.
5. Bieker, J. J., and R. G. Roeder. 1984. J. Biol. Chem. **259**, 6158–6164.
6. Hanas, J.S., D.J. Hazuda, D.F. Bogenhagen, F. Y.-H. Wu and C.-W. Wu. 1983. J. Biol. Chem. **258**, 14120–14125.
7. Ginsberg, A. M., B. O. King, and R. G. Roeder. 1984. Cell **39**, 479–489.
8. Miller, J., A. D. McLachlan, and A. Klug. 1985. EMBO J. **4**, 1609–1614.
9. Klug, A., and D. Rhodes. 1987. TIBS **12**, 404–409.
10. Berg, J. M. 1990. J. Biol. Chem. **265**, 6513–6516.
11. Honda, B. M. and R.G. Roeder. 1980. Cell **22**, 119–126.
12. Pieler, T., and V. A. Erdman. 1983. FEBS Lett. **157**, 283–287.
13. Christiansen, J., R. S. Brown, B. S. Sproat, R. A. Garrett. 1987. EMBO J. **6**, 453–460.
14. Andersen, J. A., N. Delihas, J. S. Hanas, and C. W. Wu. 1984b. Biochemistry **23**, 5759–5766.
15. Huber, P. W., and I. G. Wool. 1986. Proc. Natl. Acad. Sci. USA **83**, 1593–1597.
16. Romaniuk, P. J. 1985. Nucleic Acids Res. **13**, 5369–5387.
17. Andersen, J., and N. Delihas. 1986. J. Biol. Chem. **261**, 2912–2917.
18. Romaniuk, P. J., I. L. de Stevenson, and H.-H. A. Wong. 1987. Nucleic Acids Res. **15**, 2737–2755.
19. Pieler, T., U. Guddat, S. L. Oei, and V. A. Erdmann. 1986. Nucleic Acids Res. **14**, 6313–6326.
20. Sands, M. S., and D. F. Bogenhagen. 1987. Mol. Cell. Biol. **7**, 3985–3993.
21. You, Q. and P.J. Romaniuk, 1990. Nucleic Acids Res. **18**, 5055–5062.
22. Sakonju, S. and D.D. Brown. 1982. Cell **31**, 395–405.
23. Hanas, J. S., D. F. Bogenhagen, and C. W. Wu. 1983. Proc. Natl. Acad. Sci. USA **80**, 2142–2145.
24. Bradford, M. M. 1976. Anal. Biochem. **72**, 248–254.
25. Bogenhagen, D. F., S. Sakonju and D. D. Brown. 1980. Cell **19**, 27–35.
26. Romaniuk, P.J. and O. C. Uhlenbeck. 1983. Meth. Enzym. **100**, 52–59.
27. Donis-Keller, H., A. M. Maxam, W. Gilbert. 1977. Nucleic Acids Res. **4**, 2527–2538.
28. Vrana, K.E., M.E.A. Churchill, T.D. Tullius, and D.D. Brown. 1988. Mol. Cell. Biol. **8**, 1684–1696.
29. Lockard, R. E. and A. Kumar. 1981. Nucleic Acids Res. **9**, 5125–5140.
30. Endo, Y., P. W. Huber, I. G. Wool. 1983. J. Biol. Chem. **258**, 2662–2667.
31. Westhof, E., P. Romby, P. J. Romaniuk, J.-P. Ebel, C. Ehresmann, and B. Ehresmann. 1989. J. Mol. Biol. **207**, 417–431.
32. Baudin, F., P. Romby, P. J. Romaniuk, B. Ehresmann, C. Ehresmann. 1989. Nucleic Acids Res. **17**, 10035–10046.
33. Andersen, J. A., N. Delihas, J. S. Hanas, and C. W. Wu. 1984a. Biochemistry **23**, 5752–5759.