Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1593–1597, March 1986
Biochemistry

Identification of the binding site on 5S rRNA for the transcription factor IIIA: Proposed structure of a common binding site on 5S rRNA and on the gene

(nucleic acid–protein interaction/ribonuclease α-sarcin)

PAUL W. HUBER AND IRA G. WOOL

Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL 60637

Communicated by Josef Fried, October 31, 1985

ABSTRACT **Transcription factor IIIA interacts specifically with an internal control region of *Xenopus* 5S ribosomal RNA genes and is also a component, along with 5S rRNA, of a 7S ribonucleoprotein particle present in previtellogenic oocytes. We have determined the region of the 5S rRNA in the 7S ribonucleoprotein complex that is protected by the transcription factor from digestion with the ribonuclease α-sarcin. The binding site for factor IIIA extends from nucleotide 64 through nucleotide 116; the protected region includes two CCUGG helices separated by 11 nucleotides. The same helices occur in the factor IIIA binding site in the 5S rRNA gene and may constitute a common structural feature recognized by the protein in the gene and in the gene product.**

The transcription of *Xenopus* 5S ribosomal RNA genes requires RNA polymerase III and a minimum of three factors (1, 2). One of these factors is a protein of about 38 kDa that binds specifically to an internal region of the 5S rRNA gene (3–6). This protein, transcription factor IIIA, together with the other factors forms a stable complex that directs the accurate transcription of 5S rRNA genes (7). In addition, in immature oocytes of *Xenopus* the transcription factor is found associated with 5S rRNA in a storage particle (8–10). The 5S rRNA in these 7S ribonucleoprotein (RNP) particles is used later in oogenesis for the formation of the ribosomes of the mature oocyte (11, 12).

Factor IIIA is an unusual protein in that it binds specifically to both DNA and RNA and for that reason is of particular interest with respect to nucleic acid–protein interactions. The association of factor IIIA with 5S rRNA genes has been extensively characterized. Engelke *et al.* (3) showed that purified factor IIIA binds to a region within the 5S rRNA gene that extends from about nucleotide 45 to nucleotide 96. At the same time Bogenhagen *et al.* (4) and Sakonju *et al.* (5) delimited an intragenic control sequence by analysis of the expression of cloned deletion mutants. The domain necessary for faithful initiation of transcription encompasses nucleotides 50–83. Additional experiments with the deletion mutants established a direct correlation between the ability of altered genes to bind factor IIIA and to serve as templates for accurate initiation of transcription (6). There is good evidence then that the binding of factor IIIA to this interior promoter is required for the initiation of transcription. On the other hand, there is only limited information concerning the interaction of factor IIIA with 5S rRNA. Pieler and Erdmann (13) have shown that factor IIIA protects a set of adenosine residues in an internal loop between two helices from modification by diethyl pyrocarbonate, indicating that a specific interaction between the protein and 5S rRNA does occur. We felt that it was important to define more precisely the binding site on 5S rRNA for factor IIIA, since that would allow a comparison of the interaction of the protein with the two nucleic acids and a determination of whether factor IIIA recognizes a common feature of the structure of the DNA and RNA.

The cytotoxic ribonuclease α-sarcin can be used to determine the binding sites for proteins on RNA (14). The procedure, which is an adaptation of the "footprinting" method (15) developed to characterize the binding of proteins to DNA, had been used successfully to determine the binding sites for *Escherichia coli* ribosomal proteins on 5S rRNA (14). α-Sarcin is an uncommon ribonuclease in that it cleaves on the 3′ side of purines in both single- and double-stranded regions of RNA (16). The advantage here is that treatment with the enzyme yields a random mixture of oligonucleotides. The products of hydrolysis can be analyzed by electrophoresis on sequencing gels, and protection from the nuclease can be used to identify a protein binding site. We have used α-sarcin to determine the contact site for factor IIIA on the 5S rRNA in the 7S RNP complex found in *Xenopus* oocytes. Factor IIIA protects a specific region of 5S rRNA that encompasses nucleotides 64–116 and, most importantly, contains two CCUGG helices separated by 11 nucleotides. The 5S rRNA gene also possesses this structure, which may constitute the recognition feature for the protein on the DNA and on the RNA.

MATERIALS AND METHODS

Preparation and Labeling of *Xenopus* 7S RNP Complex. Samples of the *Xenopus laevis* 7S RNP particle were generously provided by M. Carey and N. Cozzarelli (Department of Molecular Biology, University of California, Berkeley) or the complex was prepared by us according to the procedure of Pelham and Brown (9). Material in the peak obtained by centrifugation in a glycerol gradient of extracts from *X. laevis* oocytes was applied to a column of DEAE-cellulose (Whatman DE-52) and eluted with a gradient of 50–600 mM KCl. The labeling of the 7S complex was by a modification of the procedure of Andersen *et al.* (17). Approximately 2 μg of the 7S complex was incubated with cytidine 3′,5′-[5′-^{32}P]bisphosphate and RNA ligase (17) to make the 5S rRNA radioactive at the 3′ end (Fig. 1). An increase in the final concentration of serum albumin in the ligase reaction mixture to 1 mg/ml significantly increased the yield of labeled particle, perhaps because the 7S complex is unstable in the conditions used in the labeling reaction or because the 7S RNP adheres to the surface of the reaction tube (18). The radioactive 7S complex was purified by electrophoresis at 100V through a cylindrical (0.5 × 5 cm) polyacrylamide gel (8% acrylamide/0.25% *N*,*N*′-methylenebisacrylamide/100 mM Tris borate, pH 8.3/1 mM EDTA) (8). Xylene cyanol

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RNP, ribonucleoprotein; 5S rDNA, gene for 5S rRNA.

migrates with free 5S rRNA in these conditions and serves as a guide in the collection of 5S rRNA and the 7S RNP complex. Elution was into dialysis bags containing 100 mM Tris borate (pH 8.3) and serum albumin at 0.5 mg/ml. Recovery of the radioactive material was monitored with a Geiger counter. The 5S rRNA used as the control in the protection experiments was purified from the labeled, purified 7S particle by extraction with phenol/chloroform (1:1, vol/vol), chloroform, and ether. The 5S RNA was precipitated with ethanol and suspended in buffer [100 mM Tris borate (pH 8.3) containing serum albumin at 0.5 mg/ml] for digestion with α-sarcin.

Digestion with α-Sarcin. Immediately after preparation, the intact radioactive 7S RNP particle and the radioactive 5S rRNA extracted from the complex were divided into aliquots and treated with the concentration of α-sarcin designated in the legends to the figures. Digestion was for 15 min at 30°C in 100 mM Tris borate, pH 8.3/serum albumin (0.5 mg/ml). The reaction was stopped by the addition of an excess of tRNA and by extraction twice with phenol/chloroform, chloroform, and finally ether. The digests were lyophilized, suspended in 7 M urea/10 mM EDTA, and analyzed by electrophoresis in 10% or 20% polyacrylamide gels containing 7 M urea (19). Gel analysis of 5S rRNA extracted from the 7S particle but not treated with α-sarcin revealed only a small amount (<5%) of nonspecific cleavage (data not shown). Breaks, when present, were usually at or near positions 55 and 76. It is likely that this hydrolysis occurs during the ligase labeling reaction (17). The 3′ termini of *Xenopus* 5S rRNAs are heterogeneous due to variable transcription termination (20) and shortening of the nucleic acid during storage in the oocyte (21). Most 5S rRNAs are 120 nucleotides long; however, there can be five different species ranging from 118 to 122 nucleotides in the 7S particle (21, 22). Size heterogeneity can give rise to spurious bands when digests are analyzed on sequencing gels (22–24) and, hence, can cause difficulties in numbering the bands and in assigning the residues. To mitigate the problem, we collected only a small portion of the peak during preparation of the 7S complex by electrophoresis in the hope of obtaining particles containing 5S rRNA of relatively homogeneous length. Despite these precautions, extraneous bands were observed, especially in the lanes that contained the alkaline hydrolysates and the ribonuclease T_1 digests of 5S rRNA (see Fig. 2, lanes 1 and 2). For this reason, the digestion with α-sarcin of the 7S complex and of the 5S rRNA from the complex was done many times to ensure that the positions of the protected nucleotides were assigned correctly. Some somatic-type 5S rRNA is synthesized in oocytes; however, the level is comparatively low, less than 8% of the oocyte-type (25). We judge from the T_1 and α-sarcin hydrolysates of the complex that the 7S RNP particle contained only oocyte-type 5S rRNA.

Preparations of the 7S RNP complex from *Xenopus* oocytes invariably contain some free 5S rRNA. The free nucleic acid, like that isolated from the particle, is predominantly oocyte-type 5S rRNA. We compared simultaneously and in identical conditions the digestion with α-sarcin of (*i*) the free 5S rRNA that is purified with the 7S RNP particle; (*ii*) the 5S rRNA extracted from the labeled 7S particle; and (*iii*) the 5S rRNA still in the 7S particle. There are two regions of decreased hydrolysis (nucleotides 23–25 and 47–59) within the free 5S rRNA relative to the 5S rRNA extracted from the complex or to the 5S rRNA bound to the protein (results not shown). Even if the free 5S rRNA is treated with phenol and chloroform to mimic the extraction procedure, the two regions resistant to hydrolysis persist. However, if free 5S rRNA and 5S rRNA extracted from the particle are denatured by heating, their digestion profiles with α-sarcin are identical (data not shown). This indicates that the difference in the digestion patterns is a consequence of some variation in the higher-order structure of the nucleic acid. The present results confirm earlier experiments in which the two forms of 5S rRNA were identified by use of either structure-specific nucleases (17) or chemical probes (13). It is important that, in the experiments designed to define the binding site for factor IIIA, the 5S rRNA that was used for control digestions was derived from the 7S complex and that none of the areas of decreased sensitivity to α-sarcin in free 5S rRNA overlap with the factor IIIA binding site.

RESULTS

It is possible to label the 3′ end of the 5S rRNA in the *Xenopus* oocyte 7S RNP particle that contains factor IIIA with cytidine 3′,5′-[5′-^{32}P]bisphosphate, using RNA ligase, thereby making it unnecessary to dissociate and reconstitute the complex (17). In fact, attempts to reform the 7S particle from its individual components *in vitro* were unsuccessful. The 7S RNP complex obtained from chromatography on DEAE-cellulose contains free 5S rRNA which also is labeled in the ligase reaction (Fig. 1, lanes 1 and 3); therefore, the radioactive 7S RNP particle was separated from the radioactive free 5S rRNA by preparative electrophoresis (Fig. 1, lane 4). A portion of the purified complex was extracted with phenol to obtain 5S rRNA for the control digestions (Fig. 1, lane 5).

After treatment with α-sarcin, the 7S RNP complex and the 5S rRNA samples were analyzed, together with digests of 5S rRNA made with ribonuclease T_1 in 7M urea and with alkali, by electrophoresis in 10% and 20% polyacrylamide gels containing 7 M urea (Fig. 2). Factor IIIA protected the nucleotides between guanosine-64 and guanosine-116 in 5S rRNA from digestion by α-sarcin (Fig. 2, lanes 4 and 6). The only purine in this sequence at which cleavage occurs both in the free nucleic acid and in the complex is guanosine-82. We do not know that factor IIIA does not make a contact with 5S rRNA beyond position 116, since the guanosine at residue 117 is resistant to hydrolysis by α-sarcin in free 5S rRNA as well as in the complex, and because the remaining bases at the 3′ end of the nucleic acid are pyrimidines which are not hydrolyzed by the nuclease. To carefully analyze the 5′ end of the molecule, we did the digestion with a lower concentration of α-sarcin (0.7 μM) to limit secondary cleavage and

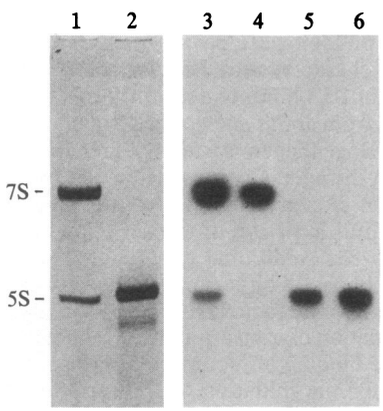

FIG. 1. Electrophoretic analysis of radioactive 5S rRNA and of the 7S RNP complex. The 7S RNP complex and the 5S rRNA were made radioactive with RNA ligase and cytidine 3′,5′[5′-^{32}P]bisphosphate (17) Lanes: 1, the preparation of 7S complex used as substrate in the ligase reaction; 2, 5S rRNA standard; 3, radioactive products (5S rRNA and 7S complex) from the ligase reaction; 4, radioactive 7S complex purified by electrophoresis; 5, purified radioactive 5S rRNA prepared from the 7S complex by extraction with phenol; 6, radioactive 5S rRNA standard. Electrophoresis was in a nondenaturing 8% polyacrylamide gel. Lanes 1 and 2 were stained with silver; lanes 3–6 are autoradiographs.

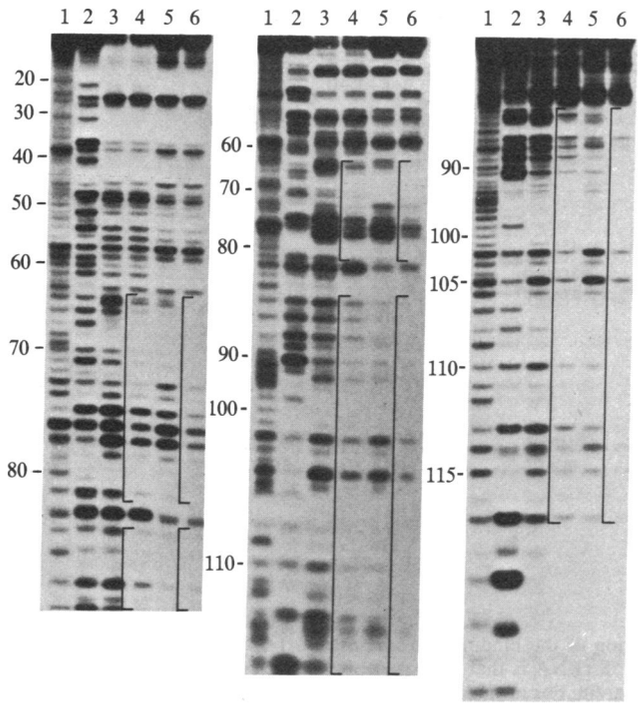

FIG. 2. Protection of 5S rRNA from digestion with α-sarcin by transcription factor IIIA. Immediately after preparation, the radioactive 7S RNP complex was digested with α-sarcin for 15 min at 30°C. The radioactive 5S rRNA that served as a control was extracted from the 7S RNP and treated with α-sarcin in conditions identical to the digestion of the complex. The digests were analyzed by electrophoresis in 10% (*Left* and *Center*) or 20% (*Right*) polyacrylamide gels containing 7 M urea. Lanes: 1, alkaline hydrolysate of 5S rRNA; 2, ribonuclease T_1 digest of 5S rRNA; 3, digest of 5S rRNA with 6.9 μM α-sarcin; 4, digest of the 7S RNP complex with 6.9 μM α-sarcin; 5, digest of 5S rRNA with 1.4 μM α-sarcin; 6, digest of the 7S RNP complex with 1.4 μM α-sarcin. The digestion with ribonuclease T_1 was in 7M urea; hydrolysis with α-sarcin was in 100 mM Tris borate buffer (pH 8.3). Brackets enclose regions of 5S rRNA protected by factor IIIA. Due to the heterogeneity of the 3′ end of *Xenopus* 5S rRNA, additional bands are present in the digests. These are most obvious in lanes 1 and 2. The assignment of residues was obtained from more than 20 replications of the experiments. The region at the 5′ end (nucleotides 1–35) was analyzed more carefully in separate experiments by using lower concentrations of α-sarcin for the digestion and by extending the time of electrophoresis.

we extended the time of electrophoresis to improve the separation in this region of the gel; factor IIIA does not protect that portion of 5S rRNA from digestion by α-sarcin (results not shown). Thus, the region of 5S rRNA protected by factor IIIA includes all of helices IV and V and small portions of I and II (Fig. 3). The results agree with the earlier observation that modification with diethyl pyrocarbonate of adenosine residues 74, 77, 90, 100, 101, and 103 of *Xenopus* 5S rRNA is decreased in the presence of factor IIIA (13). However, the chemical probe can reveal protection in single-stranded regions of the molecule only, which seriously limits the useful information one obtains. Since most purine residues are susceptible to hydrolysis by α-sarcin, protection from the nuclease yields a more precise definition of the binding site; it is especially important that it could be shown with α-sarcin that the helical regions IV and V are also part of the binding domain. Another advantage of the present procedure over ordinary enzyme protection or chemical modification protocols is that there is little chance of rearrangement of the protein on the nucleic acid, since in the conditions of the experiment there is on the average only one cleavage per molecule and hence a minimum of secondary digestion.

DISCUSSION

A question we wanted to resolve was whether the structure of the contact region for factor IIIA on 5S rRNA is the same or different than that on the 5S rRNA gene (5S rDNA). The binding site for factor IIIA on 5S rDNA, determined by protection from hydrolysis by DNase I, is within a control region near the center of the gene (i.e., it includes residues 45–96; ref. 3), whereas the binding site on 5S rRNA encompasses most of the 3′ half of the molecule (nucleotides 64–116). It is important to bear in mind that the region of a nucleic acid protected from nuclease digestion by a binding protein may well be larger than the structure of the actual contact site. Indeed, data from several types of experiments indicate that the strongest contacts between factor IIIA and 5S rDNA are located within the 3′ end of the internal control region. Factor IIIA still binds to the 5S rRNA gene even when the gene is truncated by deletions to residue 74 from the 5′ side, or to residue 83 from the 3′ side, although binding was weaker, especially in the former experiments (6). This segment (nucleotides 74–83) constitutes a minimal essential sequence for the binding of factor IIIA, although other portions of the control region are necessary to achieve tight binding and the initiation of transcription (4, 5). Sakonju and Brown (27) identified, by chemical modification of 5S rDNA, the purine and phosphate residues that are critical for association with factor IIIA. The critical purine residues are a cluster of eight guanosine residues (at positions 70, 71, 81, 82, 85, 86, 87, and 89) on the noncoding strand and a single nucleotide (position 91) on the coding strand; the phosphate groups needed for interaction with factor IIIA are on nucleotides 70–72 and 80–90 on the noncoding strand. Finally, binding experiments with proteolytic fragments of factor IIIA reinforce the idea that the association of the protein with the 5S rRNA gene occurs principally on the 3′ side of the control region (18). Mild treatment with protease generates three fragments from factor IIIA. A 10-kDa structural domain from one end of the protein is required for initiation of transcription but cannot bind to the gene. On the other hand, a 20-kDa peptide derived from the other end of the protein binds tightly and specifically to the 3′ side of the control region (residues 63–93); thus, the fragment binds to all the nucleotides in the region where the essential DNA–protein contacts are located. It would appear then that one domain of factor IIIA (the 20-kDa peptide) functions chiefly to anchor the protein to the gene while a second domain (the 10-kDa peptide) controls the initiation of transcription. Together, these results establish that the most important contact points are found within a region extending roughly from position 70 through 90.

There are aspects of these results that are particularly pertinent to this discussion: the residues in the factor IIIA contact site on the gene, with only one exception, are in the noncoding strand which, of course, has the same sequence as the 5S rRNA; and the residues essential for binding to the rDNA are found in the protected region on 5S rRNA. It is important also that the stoichiometry of the binding of factor IIIA to 5S rRNA (8) and to the gene (18, 28) is most likely 1:1. These observations suggest that the same molecular interactions might occur in the binding of factor IIIA to 5S rRNA and to 5S rDNA. However, what makes it so difficult to assume that the protein makes the same contacts with RNA and DNA is that the nucleic acids are likely to have very different structures. The factor IIIA binding site on 5S rRNA cannot be arranged in the form of a Watson–Crick double helix. The domain has an internal loop, a hairpin, a bulged nucleotide, and non-Watson–Crick base pairs. What is more, the DNA is likely to be in the B configuration, whereas the RNA will resemble the A form; these structures are very different.

Nonetheless, we propose a contact site on the RNA and on the gene that is the same (Fig. 3). We suggest that factor IIIA

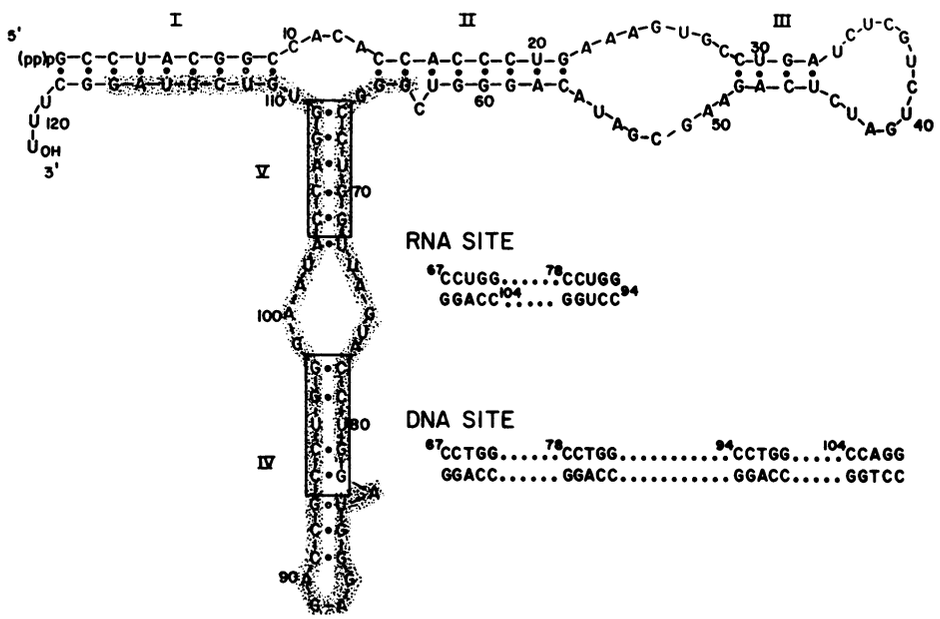

FIG. 3. The secondary structure of *Xenopus* 5S rRNA with a designation of the binding site for factor IIIA as determined by nuclease protection analysis using α-sarcin. The secondary structure for eukaryotic 5S rRNA is that proposed by Garrett *et al.* (26). The shaded area designates the nucleotides protected by factor IIIA against digestion with α-sarcin; boxes enclose the two helices that we propose are recognized by factor IIIA.

recognizes two CCUGG helices separated by 11 nucleotides. We note that identification of this putative 5-base-pair repeating structure was only likely to have come from an examination of the binding site for factor IIIA on the RNA. The contact site on 5S rRNA would be in helices IV and V (Fig. 3):

$$^{67}\text{CCUGG} \quad \ldots\ldots \quad ^{78}\text{CCUGG}$$
$$\text{GGACC}^{104} \quad \ldots\ldots \quad \text{GGUCC}^{94}$$

The relevant region on the 5S rRNA gene would have the following structure:

$$^{67}\text{CCTGG} \ldots\ldots {}^{78}\text{CCTGG} \ldots\ldots\ldots\ldots {}^{94}\text{CCTGG} \ldots\ldots {}^{104}\text{CCAGG}$$
$$\text{GGACC} \ldots\ldots \text{GGACC} \ldots\ldots\ldots\ldots \text{GGACC} \ldots\ldots \text{GGTCC}$$

We propose that factor IIIA binds only to the CCTGG base pairs at positions 67–71 and 78–82, since that would accord with the finding that deletions beyond position 94 do not affect binding of the factor, or the DNase I footprint, or initiation of transcription (4–6). These two CCTGG base pairs are one turn of the helix apart. Moreover, this is exactly the segment of the intragenic control region important for binding. It may be significant, then, that the repeat beginning at position 94 is not on the same side of the helix as the one at position 78 and that in the final repeat that begins at position 104 there is an adenosine in the noncoding strand rather than a thymidine. This latter change might weaken or abolish recognition. The proposal accords with the finding that one molecule of the factor binds to the gene (18, 28). If, however, the stoichiometry is two, as has been suggested (29), then the second molecule of the factor might bind to the CCTGG base pairs at positions 94–98 and 104–108, although that would be in conflict with the deletion data discussed above. If a single molecule of factor IIIA is sufficient to initiate transcription on the gene, as seems most likely, then the second site (positions 94–98 and 104–108), which might be weaker, could facilitate transcription by serving as a loading region or might serve as a holding domain for the factor prior to packaging into the 7S complex with the 5S rRNA transcript.

The proposal requires the assumption that factor IIIA induces a transition in the DNA from the B to the A form so as to create a structure comparable to that in 5S rRNA. The complementary change (A to B) in the structure of the RNA cannot occur, since the 2′ hydroxyl group on the ribosyl moiety prohibits the transition. It is noteworthy that the two CCTGG sequences at positions 67 and78 are separated by 11 bases, which is the approximate number per turn of the helix for DNA in the A rather than the B configuration (30). Thus, these sequences will be on the same side of the helix in the A form of DNA. Moreover, this repeated sequence is rich in guanosine and cytidine, a composition that favors the A conformation (31); indeed, to date, all DNA domains known to have an A-type structure contain at least two consecutive guanosines (32). Diffraction studies of crystals of the iodinated tetramer d(ICCGG) (33) and of the octamers d(GGCCGGCC) (32) and d(GGGGCCCC) (34) have shown an A-type conformation. Finally, studies on the interaction of factor IIIA with the 5S rRNA gene revealed a small, yet specific, change in the linking number that could reflect a reduction in the pitch of the helix that occurs in the transition of DNA from the B to the A form (35, 36). It is possible, therefore, either that factor IIIA induces a B-to-A transition in the binding site on 5S rDNA or that, because of the sequence of bases there, this locality is ordinarily in the A configuration.

Bogenhagen (37) has constructed a series of substitution mutants of *Xenopus* somatic-type 5S rDNA that contain linker replacements of nucleotides within the center of the intragenic control region. He has identified two sequences at the boundaries of this region that serve as recognition sites for factor IIIA. One of these corresponds to the CCTGG sequence at positions 78–82 in the oocyte 5S rRNA gene; however, because of a cytidine-to-thymidine substitution,

the somatic sequence is CTTGG. This sequence is a high-affinity binding site for factor IIIA, in agreement with earlier data (4, 5, 18, 27). The second sequence important for recognition, which exhibits dyad symmetry with respect to the first, is CCAAG at positions 52–56. Although the factor does not bind tightly to this 5′ domain, the interaction is indispensable to the initiation of transcription. Using a series of the substitution mutants, Bogenhagen (37) has found that the replacement of nucleotides within a 21-base-pair segment of the intragenic control region does not impair factor binding or efficiency of transcription as long as the spacing between these two domains (nucleotides 52–56 and 78–82) is exactly maintained. Thus, this portion of the internal control region can accommodate a great deal of sequence variability, suggesting that much of this segment does not make a critical contact with factor IIIA. However, it is important that for the substitution mutants which were actively transcribed, the putative recognition sequence we propose at positions 67–71 remained intact and in register with respect to the sequence at positions 78–82. (An exception is a mutant containing a thymidine-to-cytidine substitution at position 69.) There may be three domains within *Xenopus* 5S rDNA important for factor IIIA activity: residues 52–56, 67–71, and 78–82. Although the 5′ residues 52–56 are necessary for the formation of an active transcription complex, they are not part of the factor IIIA binding site on the RNA. This may reflect a difference in how the protein interacts with DNA as compared to RNA; however, it is more likely that the 5′ domain affects initiation of transcription, perhaps by facilitating binding of factors IIIB and IIIC, rather than being essential to anchor factor IIIA to the gene. In support of the latter interpretation are the earlier findings that deletion of nucleotides 52–56 does not abolish binding of factor IIIA (6), whereas it does eliminate initiation of transcription (5), and that none of the critical contact nucleotides identified by chemical modification are in this segment (27).

There is indirect evidence that factor IIIA has a single site for the binding of 5S rDNA and 5S rRNA. Hazuda *et al.* (38) have determined that the 20-kDa proteolytic fragment of factor IIIA, which binds to the 3′ side of the internal control region, can be isolated from a protease digest of the 7S RNP particle as a complex with 5S rRNA. It is significant that 5S rRNA must be removed from the fragment to obtain binding to 5S rDNA. Finally, Pelham and Brown (9) have reported that *Xenopus* 5S rRNA can inhibit transcription of 5S rRNA genes, presumably by competing for binding to factor IIIA. In this connection *E. coli* 5S rRNA, which lacks the two CCUGG helices found in the *Xenopus* nucleic acid, does not compete with the 5S rRNA gene for binding to factor IIIA (39).

We postulate that the sequence CCUGG is critical for the binding of factor IIIA to 5S rRNA and to the gene. In *Xenopus* somatic 5S rDNA the cytidine at position 79 is changed to thymidine so that the relevant helix has the sequence CTTGG. Since the binding of factor IIIA to the somatic gene appears stronger than to the oocyte gene (27, 40) and is certainly not appreciably reduced, that cytidine is not absolutely essential. Thus, it is possible that factor IIIA can be accommodated on the gene despite modest changes in the canonical structure of the two helices. The greater affinity of factor IIIA for somatic 5S rDNA has been attributed to changes elsewhere than at position 79 [e.g., of residues 53 and 55 (27, 40)]; what is not certain is whether the change in affinity is of a magnitude sufficient to be of physiological significance, since oocyte and somatic 5S rRNA genes are transcribed with the same efficiency (40).

The proposal that factor IIIA binds to two CCUGG helices in 5S rRNA and 5S rDNA has a good deal to recommend it: parsimony, plausability, and that it can be disproved experimentally. For example, the hypothesis makes a strong, testable prediction: that deletions in the gene beyond position 94 will not affect binding of factor IIIA to 5S rDNA or initiation of transcription (which is already known to be the case), but that it will abolish binding to 5S rRNA (which remains to be tested).

We are grateful to M. Carey and N. Cozzarelli for providing us with the initial samples of *Xenopus* 7S complex and for advice and helpful discussions. We thank J. Andersen and N. Delihas for instruction in labeling the 7S complex, W. Taylor and K. Vrana for assistance in preparing the particle, and V. Paz for help in carrying out the experiments. The structure of the binding site for factor IIIA was suggested by C. Cantor. The work was supported by Grant GM33702 from the National Institutes of Health.

1. Shastry, B. S., Ng, S.-Y. & Roeder, R. G. (1982) *J. Biol. Chem.* **257,** 12979–12986.
2. Lassar, A. B., Martin, P. L. & Roeder, R. G. (1983) *Science* **222,** 740–748.
3. Engelke, D. R., Ng, S.-Y., Shastry, B. S. & Roeder, R. G. (1980) *Cell* **19,** 717–728.
4. Bogenhagen, D. F., Sakonju, S. & Brown, D. D. (1980) *Cell* **19,** 27–35.
5. Sakonju, S., Bogenhagen, D. F. & Brown, D. D. (1980) *Cell* **19,** 13–25.
6. Sakonju, S., Brown, D. D., Engelke, D. R., Ng, S.-Y., Shastry, B. S. & Roeder, R. G. (1981) *Cell* **23,** 665–669.
7. Bogenhagen, D. F., Wormington, W. M. & Brown, D. D. (1982) *Cell* **28,** 413–421.
8. Picard, B. & Wegnez, M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 241–245.
9. Pelham, H. R. B. & Brown, D. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4170–4174.
10. Honda, B. M. & Roeder, R. G. (1980) *Cell* **22,** 119–126.
11. Mairy, M. & Denis, H. (1971) *Dev. Biol.* **24,** 143–165.
12. Wegnez, M. & Denis, H. (1973) *Biochimie* **55,** 1129–1135.
13. Pieler, T. & Erdmann, V. A. (1983) *FEBS Lett.* **157,** 283–287.
14. Huber, P. W. & Wool, I. G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 322–326.
15. Galas, D. J. & Schmitz, A. (1978) *Nucleic Acids Res.* **5,** 3157–3170.
16. Endo, Y., Huber, P. W. & Wool, I. G. (1983) *J. Biol. Chem.* **258,** 2662–2667.
17. Andersen, J., Delihas, N., Hanas, J. S. & Wu, C.-W. (1984) *Biochemistry* **23,** 5759–5766.
18. Smith, D. R., Jackson, I. J. & Brown, D. D. (1984) *Cell* **37,** 645–652.
19. Donis-Keller, H., Maxam, A. M. & Gilbert, W. (1977) *Nucleic Acids Res.* **4,** 2527–2538.
20. Bogenhagen, D. F. & Brown, D. D. (1981) *Cell* **24,** 261–270.
21. Denis, H. & Wegnez, M. (1973) *Biochimie* **55,** 1137–1151.
22. Andersen, J., Delihas, N., Hanas, J. S. & Wu, C.-W. (1984) *Biochemistry* **23,** 5752–5759.
23. Peattie, D. A., Douthwaite, S., Garrett, R. A. & Noller, H. F. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7331–7335.
24. Montoya, J., Ojala, D. & Attardi, G. (1981) *Nature (London)* **290,** 465–470.
25. Ford, P. J. & Southern, E. M. (1973) *Nature (London) New Biol.* **241,** 7–12.
26. Garrett, R. A., Douthwaite, S. & Noller, H. F. (1981) *Trends Biochem. Sci. (Pers. Ed.)* **6,** 137–139.
27. Sakonju, S. & Brown, D. D. (1982) *Cell* **31,** 395–405.
28. Bieker, J. J. & Roeder, R. G. (1984) *J. Biol. Chem.* **259,** 6158–6164.
29. Hanas, J. S., Bogenhagen, D. F. & Wu, C.-W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2142–2145.
30. Peck, L. J. & Wang, J. C. (1981) *Nature (London)* **292,** 375–378.
31. Arnott, S. & Selsing, E. (1974) *J. Mol. Biol.* **88,** 551–552.
32. Wang, A. H.-J., Fujii, S., van Boom, J. H. & Rich, A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3968–3972.
33. Conner, B. N., Takano, T., Tanaka, S., Itakura, K. & Dickerson, R. E. (1982) *Nature (London)* **295,** 294–299.
34. McCall, M., Brown, T. & Kennard, O. (1985) *J. Mol. Biol.* **183,** 385–396.
35. Reynolds, W. F. & Gottesfeld, J. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1862–1866.
36. Hanas, J. S., Bogenhagen, D. F. & Wu, C.-W. (1984) *Nucleic Acids Res.* **12,** 1265–1276.
37. Bogenhagen, D. F. (1985) *J. Biol. Chem.* **260,** 6466–6471.
38. Hazuda, D., Hanas, J. & Wu, C.-W. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44,** 1772.
39. Hanas, J. S., Bogenhagen, D. F. & Wu, C.-W. (1984) *Nucleic Acids Res.* **12,** 2745–2758.
40. Wormington, W. M., Bogenhagen, D. F., Jordan, E. & Brown, D. D. (1981) *Cell* **24,** 809–817.