Mutations in 5S DNA and 5S RNA Have Different Effects on the Binding of *Xenopus* Transcription Factor IIIA†

Qimin You, Nik Veldhoen, Florence Baudin,‡ and Paul J. Romaniuk*
Department of Biochemistry and Microbiology, University of Victoria, P.O. Box 3055, Victoria, British Columbia, Canada V8W 3P6

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ABSTRACT: The effects on TFIIIA binding affinity of a series of substitution mutations in the *Xenopus laevis* oocyte 5S RNA gene were quantified. These data indicate that TFIIIA binds specifically to 5S DNA by forming sequence-specific contacts with three discrete sites located within the classical A and C boxes and the intermediate element of the internal control region. Substitution of the nucleotide sequence at any of the three sites significantly reduces TFIIIA binding affinity, with a 100-fold reduction observed for substitutions in the box C subregion. These results are consistent with a direct interaction of TFIIIA with specific base pairs within the major groove of the DNA. A comparison of the TFIIIA binding data for the same mutations expressed in 5S RNA indicates that the protein does not make any strong sequence-specific contacts with the RNA. Although the protein footprinting sites on the 5S DNA and 5S RNA are coincident, nucleotide substitutions in 5S RNA which moderately reduce TFIIIA binding affinity do not correspond at all to the three specific TFIIIA interaction sites within the gene. The implications of these results for models which attempt to reconcile the DNA and RNA binding activities of TFIIIA by proposing a common structural motif for the two nucleic acids are discussed.

TFIIIA acts as a positive transcription factor by binding to an internal control region in the *Xenopus* 5S RNA gene, modulating the expression of these genes during oogenesis (Bogenhagen et al. 1980; Engelke et al., 1980; Sakonju et al., 1981). The metastable complex formed between TFIIIA and the 5S DNA is rapidly converted to a stable ternary initiation complex by the subsequent binding of TFIIIB and TFIIIC (Bogenhagen et al., 1982). Kinetic analysis showed that TFIIIC binds first to the TFIIIA–5S DNA complex to form a much more stable complex, and is then followed by the binding of TFIIIB and RNA polymerase III (Bieker et al., 1985). The stability of the ternary initiation complex is limited by the TFIIIB interaction (Carey et al., 1986). This ternary complex remains bound to the DNA through multiple passages by RNA polymerase (Bogenhagen et al., 1982; Wolffe et al., 1986).

In the immature oocyte, TFIIIA performs a second essential function by binding to 5S RNA in the cytoplasm, forming a ribonucleoprotein storage particle (7S RNP) that stabilizes the RNA until it is required for ribosome assembly (Pelham & Brown, 1980; Picard et al., 1980). The nucleic acid binding region of TFIIIA is organized into 9 independent repeats of 30 amino acids (Brown et al., 1985; Miller et al., 1985), each of which is coordinated through 2 conserved cysteine and histidine residues to a zinc ion to form a "finger" structure (Diakun et al., 1986; Frankel et al., 1987; Miller et al., 1985). It is of interest to determine how this zinc finger protein interacts specifically with the coding region of the 5S RNA gene, and with the transcript of this gene.

The interaction of TFIIIA with 5S RNA has been investigated in considerable detail. The thermodynamic and kinetic parameters were determined by using a nitrocellulose filter binding assay that measures the equilibrium leading to the formation of the 7S RNP (Romaniuk, 1985). The region on the 5S RNA which is protected from modification when TFIIIA is bound has been determined by using a variety of structural probes (Andersen et al., 1984; Christiansen et al., 1987; Huber & Wool, 1986; Pieler & Erdmann, 1983; Romaniuk, 1985) and encompasses roughly nucleotides 52–108. However, almost the entire 5S RNA molecule is required to maintain the unique three-dimensional shape necessary for optimal binding of TFIIIA (Andersen & Delihas, 1986; Romaniuk et al., 1987). Results from studying the effects that an extensive series of substitution mutations in the 5S RNA have on TFIIIA binding indicate that the protein relies on a combination of many weak sequence-specific interactions along with the 5S RNA structure in forming the 7S RNP complex (Baudin & Romaniuk, 1989; Baudin et al., 1991; Romaniuk, 1989; Romaniuk et al., 1987; You & Romaniuk, 1990).

The binding sites of TFIIIA on 5S DNA and 5S RNA are very similar both in size and in position. This similarity suggests that the conformations of both nucleic acids may also be similar within this region, thus explaining in part the ability of the protein to bind to both nucleic acids. It has been proposed that the TFIIIA binding site on the 5S DNA exists in an A-like double-helical conformation, based upon DNase digestion patterns, X-ray crystallography, and CD spectroscopic studies (Diakun et al., 1986; Fairall et al., 1989; Rhodes & Klug, 1986). Such a structural similarity to an RNA double helix might facilitate the binding of TFIIIA to DNA and RNA. However, not all of the evidence available supports this model (Aboul-ela et al., 1988; Becker & Wang, 1989; Gottesfeld et al., 1987).

The 5S gene promoter has a structure reminiscent of RNA polymerase III promoters in tRNA genes, and consists of box A and box C elements at the ends of the ICR with a small intermediate element in the middle of this region (Bogenhagen, 1985; Majowski et al., 1987; Pieler et al., 1985, 1987). Outside of the intermediate element, there appears to be little sequence specificity in the spacer region between box A and box C,

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‡ Present address: Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue René Descartes, Strasbourg, France.

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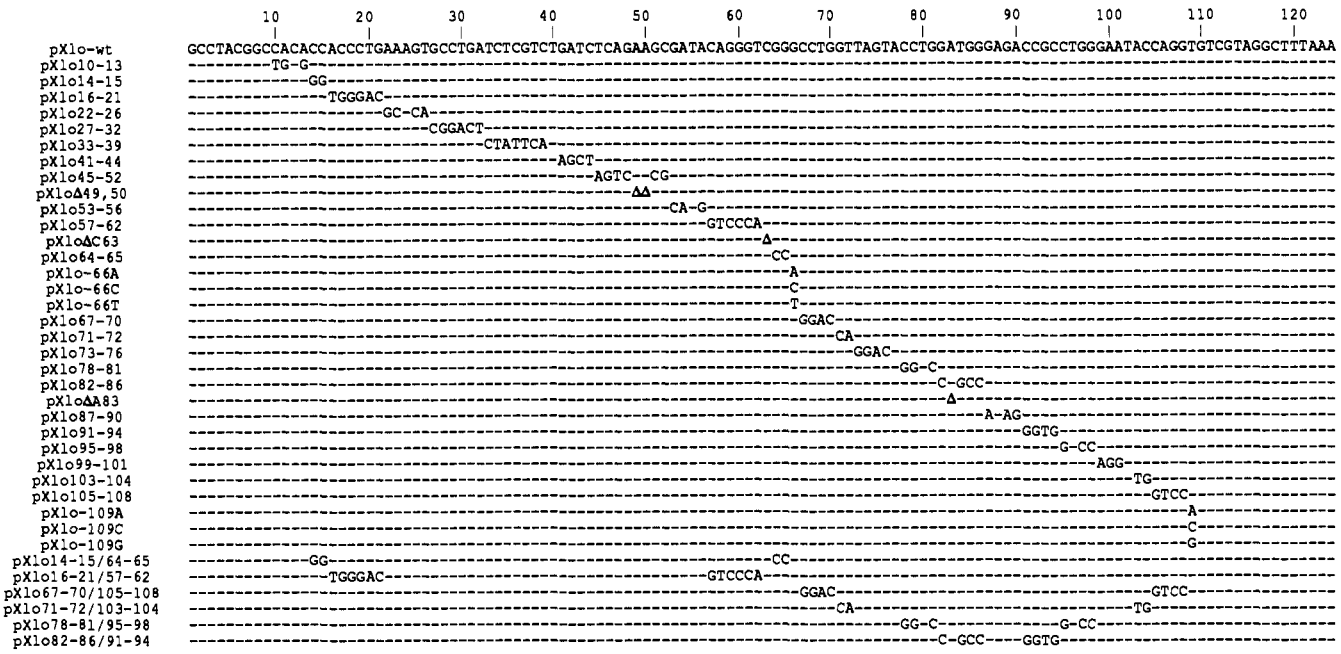

FIGURE 1: Sequence of the 5S RNA genes (noncoding strand) used in this study. For each mutant, a dash indicates that the nucleotide at that position is identical with that of pXlo-wt (wild-type *Xenopus laevis* oocyte 5S RNA gene). A (Δ) symbol indicates that the nucleotide has been deleted in the mutant.

although absolute spacing of the two boxes is critical for transcription activation (Bogenhagen, 1985; Pieler et al., 1985, 1987). The importance of these three elements in promoter function has been tested by point mutational analysis (Majowski et al., 1987; Pieler et al., 1985, 1987), but only four point mutations were tested for effects on TFIIIA binding (Pieler et al., 1987). A number of the promoter mutations may in fact influence the binding of TFIIIC alone, or both TFIIIA and TFIIIC (Majowski et al., 1987; Pieler et al., 1987). A quantitative study of the effects that mutations in the 5S DNA have on the association constant for TFIIIA binding had not been made.

To investigate the specifics of TFIIIA binding to both 5S DNA and 5S RNA, we have quantified the effects that a series of identical site-directed mutations of both nucleic acids have on the binding of the protein. Sequence-specific changes within the ICR of the 5S DNA can reduce TFIIIA binding affinity dramatically, while such changes in the 5S RNA have only very modest effects on protein binding. In addition, the results of this study show that there is virtually no correlation between the nucleotides in DNA and RNA that are essential for TFIIIA binding.

Materials and Methods

Purification of TFIIIA. The purification of TFIIIA from the ovaries of immature *Xenopus laevis* and the assessment of its fractional 5S RNA binding activity were carried out as described previously (Romaniuk, 1989). Only those preparations of protein which were greater than 90% active were used to study the binding of mutant 5S DNAs.

Plasmids and 5S DNA Gene Fragments. The construction of the various mutant 5S RNA genes in the plasmid pUC18 has been described elsewhere (Baudin & Romaniuk, 1989; Romaniuk, 1989; Romaniuk et al., 1987; You & Romaniuk, 1990). Each mutant 5S RNA gene was liberated from the plasmid DNA by digestion with restriction enzymes *Eco*RI and *Hin*dIII, and subsequently labeled with ^{32}P by using a previously published method (Romaniuk, 1990).

Determination of the Equilibrium Binding of TFIIIA to 5S DNA and 5S RNA Mutants. The equilibrium constants for the binding of radioactively labeled mutant 5S DNAs to TFIIIA were determined by using a nitrocellulose filter binding assay (Romaniuk, 1990). A minimum of three independent determinations were carried out, using the wild-type oocyte 5S DNA as a control in each experiment. Data for the binding of TFIIIA to the corresponding 5S RNA mutants have been reported elsewhere (Baudin & Romaniuk, 1989; Baudin et al., 1991; Romaniuk, 1989; Romaniuk et al., 1987; You & Romaniuk, 1990).

Results

The construction of the mutations in the *Xenopus* oocyte 5S RNA gene shown in Figure 1 has been described elsewhere (Baudin & Romaniuk, 1989; Romaniuk, 1989; Romaniuk et al., 1987; You & Romaniuk, 1990). The apparent affinity constant for each mutant 5S RNA gene was determined by using a nitrocellulose filter binding assay under conditions that successfully discriminate between the specific and nonspecific DNA binding activities of TFIIIA (Baudin & Romaniuk, 1989; Romaniuk, 1990). Examples of the binding curves obtained are shown in Figure 2. A T7 promoter immediately upstream of the coding sequence was used to produce radioactively labeled 5S RNA molecules from plasmid DNA that had been digested with *Dra*I, which defines the 3′ end of the transcripts as nucleotide +121 of the gene (Romaniuk et al., 1987). The affinities of the various mutant 5S RNA molecules were determined by using a nitrocellulose filter binding assay (Romaniuk, 1985) and have been reported elsewhere (Baudin & Romaniuk, 1989; Baudin et al., 1991; Romaniuk, 1989; Romaniuk et al., 1987; You & Romaniuk, 1990). The relative TFIIIA binding affinities for the 5S DNA and 5S RNA mutants are compared in Table I.

It is striking that these mutations have a much larger effect on the affinity of 5S DNA for TFIIIA than they have on the affinity of 5S RNA for the protein. The largest effect observed on TFIIIA binding to the 5S RNA is a decrease of 8-fold in the apparent K_a value. In comparison, certain nucleotide substitutions in the 5S RNA gene result in a 100-fold reduction in the binding affinity of the DNA for TFIIIA. Clearly TFIIIA binding to DNA is much more sensitive to nucleotide

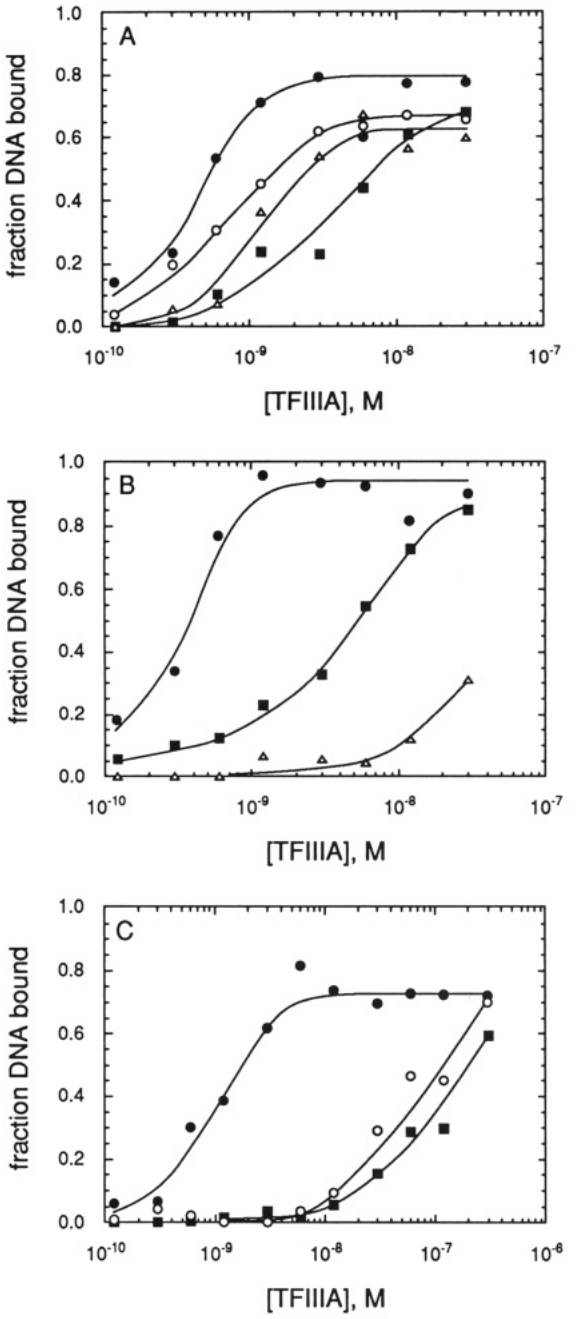

FIGURE 2: Nitrocellulose filter binding experiments with mutant 5S RNA gene. (A) Wild type (●), pXlo16-21 (○), pXlo57-62 (■), pXlo91-94 (△). (B) Wild type (●), pXlo78-81 (■), pXlo78-81/95-98 (△). (C) Wild type (●), pXlo82-86 (○), pXlo82-86/91-94 (■).

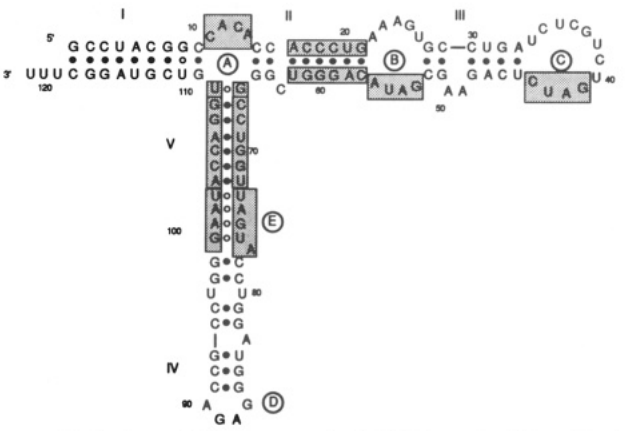

FIGURE 3: Regions of *Xenopus* oocyte 5S RNA required for optimal binding of TFIIIA. The shaded boxes indicate regions in which nucleotide substitutions result in a 2–8-fold reduction in binding affinity. Substitutions of stem nucleotides reduce TFIIIA binding affinity only when the base pairing is disrupted.

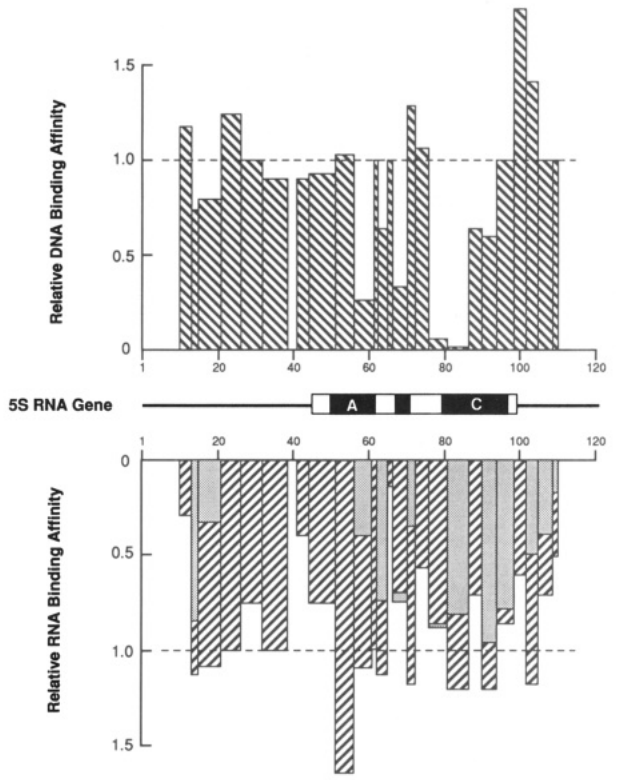

FIGURE 4: Comparison of the TFIIIA binding affinities of mutant 5S RNA genes and their corresponding 5S RNA transcripts relative to the wild-type nucleic acids. For mutations in the 5S RNA that span helical stems, the stippled bars indicate the relative TFIIIA binding affinities of single mutations which disrupt the stem structure, while the hatched bars indicate the relative TFIIIA binding affinities of compensating double mutations which maintain a stem structure with an altered base pair sequence.

sequence than is the binding of the protein to 5S RNA. This effect is even more striking if one considers that a number of the nucleotide substitutions in the 5S RNA disrupt a base-paired stem (Figure 3), while proper Watson–Crick base pairing is maintained in all of the 5S DNA mutants. Thus, compensating double mutations in the 5S RNA that maintain the base pairing within a target stem, while changing the sequence of base pairs, have little effect on TFIIIA binding affinity. Detailed analyses of the solution structures of a number of these mutant 5S RNAs have indicated that the decrease in TFIIIA binding affinity can in each case be explained by alterations in the secondary or tertiary structure of the 5S RNA (Baudin et al., 1991; Brunel et al., 1990; Romaniuk et al., 1988; Westhof et al., 1989).

Studies with linker-scanning mutations in the *Xenopus borealis* somatic 5S RNA gene indicated that mutations which disrupt 5S RNA structure and decrease TFIIIA binding do not fall within those regions of sequence important for the promoter function on the 5S DNA (Sands & Bogenhagen, 1987). Figure 4 compares the effects of substitution mutations in *X. laevis* oocyte 5S DNA and 5S RNA mapped against the three promoter elements within the ICR on the gene. It is evident from this figure that there is little correspondence between the sequence elements required for the optimal binding of TFIIIA to the two nucleic acids. However, the sequences important for the binding of TFIIIA to the 5S DNA do correspond to specific subregions within the promoter elements of the ICR. Nucleotides 57–62 of box A and nu-

Table I: Relative TFIIIA Binding Affinities for 5S DNA and 5S RNA Mutants

mutant	relative binding affinity[a]		mutant	relative binding affinity[a]	
	RNA[b]	DNA		RNA[b]	DNA
wild type	1.00	1.00	73–76	0.57 ± 0.02	1.07 ± 0.16
10–13	0.30 ± 0.01	1.18 ± 0.12	78–81	0.88 ± 0.01	0.06 ± 0.02
14–15	0.85 ± 0.22	0.74 ± 0.07	82–86	0.81 ± 0.30	0.01 ± 0.005
16–21	0.32 ± 0.15	0.80 ± 0.07	Δ83	1.00 ± 0.02	0.25 ± 0.05
22–26	1.00 ± 0.02	1.24 ± 0.15	87–90	0.71 ± 0.10	0.64 ± 0.18
27–32	0.75 ± 0.10	1.02 ± 0.15	91–94	0.96 ± 0.18	0.61 ± 0.24
33–39	1.00 ± 0.02	0.89 ± 0.09	95–98	0.78 ± 0.02	1.00 ± 0.21
41–44	0.40 ± 0.10	0.89 ± 0.11	99–101	0.61 ± 0.08	1.81 ± 0.08
45–52	0.76 ± 0.12	0.93 ± 0.07	103–104	0.50 ± 0.23	1.40 ± 0.40
Δ49,50	1.00 ± 0.02	1.00 ± 0.02	105–108	0.39 ± 0.06	0.97 ± 0.27
53–56	1.62 ± 0.12	1.03 ± 0.07	109A	0.29 ± 0.01	1.00 ± 0.09
57–62	0.40 ± 0.15	0.26 ± 0.13	109C	0.50 ● 0.03	1.00 ± 0.05
Δ63	1.00 ± 0.02	1.00 ± 0.02	109G	0.17 ± 0.05	1.00 ± 0.05
64–65	0.74 ± 0.24	0.64 ± 0.12	14–15/64–65	1.11 ± 0.32	0.70 ± 0.05
66A	0.14 ± 0.01	1.00 ± 0.08	16–21/57–62	1.09 ± 0.48	0.41 ± 0.10
66C	0.12 ± 0.01	1.00 ± 0.01	67–70/105–108	0.71 ± 0.01	0.32 ± 0.06
66T	0.27 ± 0.03	1.00 ± 0.05	71–72/103–104	1.18 ± 0.32	1.78 ± 0.58
67–70	0.75 ± 0.12	0.33 ± 0.05	78–81/95–98	0.86 ± 0.01	0.015 ± 0.005
71–72	0.35 ± 0.21	1.31 ± 0.17	82–86/91–94	1.21 ± 0.35	0.01 ± 0.001

[a] Determined as the ratio of the apparent association constant for the mutant nucleic acid to the apparent association constant for the wild-type nucleic acid. Average of two or more independent determinations. [b] Data taken from Romaniuk et al. (1987), Romaniuk (1989), Baudin and Romaniuk (1989), You and Romaniuk (1990), and Baudin et al. (1991).

cleotides 67–70 of the intermediate element make similar, moderate contributions to the TFIIIA binding affinity. Nucleotides 78–86 of box C clearly are critical for TFIIIA binding, since substitutions of these nucleotides reduce the apparent K_a by as much as 2 orders of magnitude. TFIIIA has been shown to protect guanine N-7 atoms from methylation throughout the ICR, although the protection is stronger near the 3′ end (Fairall et al., 1986). Nucleotides 80–86 have been shown by point mutagenesis to be extremely important promoter elements in a TFIIIA-dependent transcription assay (Pieler et al., 1987). These results agree well with the quantitative data presented here, and taken together provide a clear picture of TFIIIA interaction sites within the promoter, and their relative contribution to the overall free energy of binding.

The results of hydroxyl radical footprinting studies of amino- and carboxyl-terminal deletions of TFIIIA on 5S DNA identified three interaction sites on the DNA at which TFIIIA fingers cluster upon binding (Vrana et al., 1988). The data in this study indicate that nucleotide substitutions within these three sites decrease the binding of TFIIIA to the 5S DNA. Thus, there is a convincing correspondence in the information on the TFIIIA interaction sites obtained by mutagenesis of the protein and the 5S DNA.

Discussion

The promoter region on *Xenopus* 5S DNA was first defined by transcription studies using 5′- and 3′-terminal deletions, and was found to span approximately nucleotides +45 to +97 (Bogenhagen et al., 1980; Sakonju et al., 1980). Subsequent DNase I footprinting studies with TFIIIA indicated that this internal control region constituted the TFIIIA binding site (Sakonju et al., 1981). Functionally important areas within the ICR were delineated by studying the transcriptional activity of a number of linker-scanning and substitution mutations, identifying the three subdomains (box A, the intermediate element, and box C) necessary for the promoter activity of the ICR (Bogenhagen, 1985; Majowski et al., 1987; Pieler et al., 1985, 1987). A combination of experimental approaches including chemical modification/selection, DNase footprinting, and template-commitment assays yielded data that indicated TFIIIA interacted most strongly with the noncoding strand of the 5S DNA within box C, and formed weaker interactions with box A and the intermediate element (Bogenhagen, 1985; Fairall et al., 1986; Majowski et al., 1987; Pieler et al., 1985, 1987; Sakonju & Brown, 1982). These studies also indicated that TFIIIC interacts with the DNA at sites located within the ICR (Majowski et al., 1987). Precise localization of TFIIIA binding interactions within the ICR resulted from hydroxyl radical footprinting on 5S DNA of sequential N-terminal and C-terminal deletions of the protein (Vrana et al., 1988). From this study, it was apparent that TFIIIA contacts specific subregions within the classical box A and box C domains, and the results confirmed that a stronger interaction between the protein and DNA occurs in the box C region.

A quantitative measurement of the contribution that specific sequences in the 5S DNA make toward the overall free energy of TFIIIA binding was not obtained from any of the studies mentioned above. Recently, a nitrocellulose filter binding assay was developed in our laboratory that accurately measures the bimolecular equilibrium leading to the formation of the binary TFIIIA–5S DNA complex from free protein and DNA (Romaniuk, 1990). Using this assay, and an extensive collection of substitution mutations in *Xenopus* oocyte 5S DNA, we have now measured the relative contribution that specific sequences in the DNA make to the binding affinity for TFIIIA. From the results reported here, it is apparent that the specific subregions within the ICR previously reported to be target sites for TFIIIA binding do indeed contain specific nucleotide sequences that contribute directly to the free energy of binary complex formation. In particular, nucleotides 57–62 of box A and nucleotides 67–70 (the intermediate element) make roughly equivalent contributions to TFIIIA binding as indicated by the observation that substitution of these sequences reduced the binding constant by a factor of 3–4. In addition, the data indicate that nucleotides 78–86 of box C represent the major TFIIIA recognition feature since substitution of these nucleotides reduces TFIIIA binding affinity by 2 orders of magnitude.

The hydroxyl radical footprinting studies conducted on N- and C-terminal deletions of TFIIIA identified regions within the ICR in intimate contact with specific fingers of TFIIIA (Vrana et al., 1988). Apparently, fingers 8 and 9 and a short stretch of the C-terminal domain of TFIIIA shield base pairs

42–60 of the DNA from modification by hydroxyl radical. The results of our study suggest that the sequence-specific interactions between this region of TFIIIA and the 5S DNA occur within the much smaller site of base pairs 57–62. It was suggested from the footprinting studies that fingers 5 and 6 protect the ICR across the minor groove at the intermediate element and make critical contacts with the DNA in the major groove at adjoining nucleotides including the two G residues at base pairs 70 and 71 (Vrana et al., 1988). Our data indicate that nucleotide substitutions at base pairs 67–70 of the intermediate element significantly reduce TFIIIA binding affinity, in agreement with the hydroxyl radical footprinting results. Results obtained with the N-terminal deletion mutants of TFIIIA indicated that the largest contribution to binding strength was obtained from the interaction of fingers 1 and 2 with about 17 base pairs (80–97) within box C of the ICR (Vrana et al., 1988). The hydroxyl radical footprinting data indicated that the protein appeared to be in close contact at the major groove with almost every base pair through at least one turn of the DNA double helix within this region. This area of the ICR includes six G residues demonstrated to be important for TFIIIA binding by a methylation interference assay (Sakonju & Brown, 1982). The data in Table I indicate that a strong sequence-specific interaction occurs between nucleotides 78–86 of the 5S DNA and the N-terminal fingers of TFIIIA. Thus, there is excellent agreement in the picture of relative contributions of protein and DNA regions to the overall free energy of the TFIIIA–5S DNA interaction obtained from these independent binding studies conducted with TFIIIA mutants and 5S DNA mutants. In addition, our data clearly identify which sequences within the hydroxyl radical footprint regions are likely to be in direct contact with TFIIIA.

It has been suggested based upon the results of several studies that regions of the 5S DNA adopt a conformation that is closer to an A-type double helix rather than the more classical B-type double helix normally associated with DNA (Diakun et al., 1986; Fairall et al., 1989; Rhodes & Klug, 1986). However, other studies have demonstrated that the ICR region of the 5S DNA has a B-type conformation in both the absence and presence of TFIIIA (Aboul-ela et al., 1988; Becker & Wang, 1989; Gottesfeld et al., 1987). Most recently, a carefully conducted study of the conformation of a number of model oligonucleotides by circular dichroism has demonstrated that the ICR of 5S DNA has a uniform conformation that is partway between the classical A- and B-type double helices (Fairall et al., 1989). In addition, a quantitative investigation of the hydroxyl radical footprint of TFIIIA on 5S DNA has lead to a proposal of how the fingers of TFIIIA interact with the 5S DNA (Churchill et al., 1990). In this model, alternate fingers of TFIIIA bind on one face of the DNA double helix in an equivalent manner, and with the same polarity, to the major groove, so that successive minor grooves are crossed by the linker peptides between fingers. The model is a general one, and does not currently explain the precise effects of local irregularities in protein or DNA structures. The effects of nucleotide substitutions in 5S DNA on TFIIIA binding affinity reported here are consistent with the loss of major groove hydrogen-bonding interactions between TFIIIA and 5S DNA.

The potential role of a specific DNA structure at any of the three interaction sites within the ICR cannot be addressed by our results, since in each case several neighboring nucleotides were substituted simultaneously, making it unlikely that a sequence-specific DNA conformation, if it existed in the wild-type 5S DNA, would be maintained in the mutant. However, the placement of the three interaction sites within the 5S DNA suggests that a special local DNA conformation is unlikely to be a feature of the TFIIIA–5S DNA interaction. A 5S RNA gene consists of short regions of highly conserved base pair sequences which encode the corresponding conserved sequences found in single-stranded loops of the 5S RNA, flanked by more highly variable sequences which encode the nonconserved sequences of the base-paired stems of the 5S RNA molecule. It is striking that all of the sequence-specific regions identified from our study to be important for TFIIIA binding (base pairs 57–62, 67–70, and 78–86) map to those base pairs in the gene which encode the variable sequences of base-paired stems of the 5S RNA molecule. If the TFIIIA–5S DNA interaction pattern identified from our results is a universal feature of the expression of 5S RNA genes within the eukaryotic kingdom, it is clear that the interaction sites for TFIIIA molecules would be highly variable in sequence. This observation supports the view that a special, sequence-specific conformation in the DNA is not a general requirement for TFIIIA binding to 5S RNA genes. However, it does not rule out the possibility that a special DNA conformation is required specifically for the *Xenopus* TFIIIA–5S DNA interaction.

The TFIIIA footprint on 5S RNA extends from nucleotides 53 to 90, and formation of the correct conformation in this binding site requires the complementary sequence to this footprint (Andersen & Delihas, 1986; Andersen et al., 1984; Christiansen et al., 1987; Huber & Wool, 1986; Pieler & Erdmann, 1983; Romaniuk, 1985; Romaniuk et al., 1987). Thus, there is a striking correspondence in the location of the TFIIIA binding regions on 5S RNA and the 5S RNA gene. A number of techniques have been employed to determine which features of the RNA are important to the TFIIIA–5S RNA interaction, including gel shift assays, chemical cross-linking, chemical modifications, and filter binding assays. Results obtained from the measurement of TFIIIA binding affinities of site-specific mutants of 5S RNA indicate that the major determinants for protein binding are primarily structural (Baudin & Romaniuk, 1989; Baudin et al., 1991; Romaniuk, 1989; Romaniuk et al., 1987; You & Romaniuk, 1990). Virtually all of the mutations which significantly reduce TFIIIA binding affinity disrupt the structure of the 5S RNA around the junction of the three helical domains (Figure 3). Chemical cross-links detected between TFIIIA and 5S RNA are also clustered in this region of the RNA molecule (Baudin et al., 1989). It is therefore apparent that the secondary and tertiary structures of the 5S RNA form the essential recognition features for TFIIIA, and there is currently no evidence to indicate that TFIIIA forms any strong sequence-specific contacts with nucleotides on the RNA.

Conclusions

Several models that have been proposed in attempts to explain how TFIIIA could interact specifically with both 5S DNA and 5S RNA suggest that TFIIIA makes identical contacts within similar conformational contexts on both nucleic acids. The data presented in this study allow an examination of this concept and indicate that such models are unnecessary. It is apparent that TFIIIA binds specifically to 5S DNA by forming sequence-specific contacts with three discrete regions within the coding part of the gene. Substitution of the sequence at any of the sites significantly reduces TFIIIA binding affinity, by as much as 100-fold in the case of the box C subregion. Although these experiments cannot address the question of whether a special conformation within the 5S DNA is one of the requirements for optimal TFIIIA binding, the

placement of the target sequences within highly variable sequences of the 5S RNA gene does suggest that a special conformation is not an evolutionarily conserved feature of TFIIIA–5S DNA interactions in general. In comparison, TFIIIA does not make any strong sequence-specific contacts with the 5S RNA. Although the protein binding sites on the 5S DNA and 5S RNA are coincident, those regions of the 5S RNA that have been identified as being moderately involved in TFIIIA binding do not correspond at all to the three specific TFIIIA interaction regions within the 5S DNA. It is evident that substitution of nucleotides 57–62 and 67–70 in the 5S RNA lowers TFIIIA binding affinity but the effect of these mutations results from the disruption of base-paired stems. Incorporation of compensating double mutations (e.g., 16–21/57–62) that restore the local base pairing in the 5S RNA results in the recovery of full TFIIIA binding affinity. In contrast, the decreased protein binding activity of the 57–62 mutation in the 5S DNA cannot be compensated for by the substitution of base pairs 16–21, thus illustrating another fundamental difference in the role of sequence vs structure in the interaction of DNA and RNA with TFIIIA. Finally, substitution of nucleotides 78–86 in the 5S RNA leads to a disruption of a helical stem, but has virtually no effect on TFIIIA binding. Substitution of these nucleotides in the 5S DNA results in a very large reduction in the affinity for TFIIIA.

Our results suggest that the DNA and RNA binding activities of TFIIIA are distinct, even though there is apparently a single nucleic acid binding site on the protein. It is possible that the two activities have evolved separately from an ancestor TFIIIA molecule which exhibited general nucleic acid binding properties. This concept predicts that fingers (or regions within any one finger) were optimized for binding either to 5S DNA or to 5S RNA. Directed mutagenesis studies of the TFIIIA protein, combined with detailed binding studies of the mutants to DNA and RNA, should provide useful information on this point.

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