The effects of disrupting 5S RNA helical structures on the binding of *Xenopus* transcription factor IIIA

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ABSTRACT

Block mutations were constructed in helical stems II, III, IV and V of *Xenopus laevis* oocyte 5S RNA. The affinities of these mutants for binding to transcription factor IIIA (TFIIIA) were determined using a nitrocellulose filter binding assay. Mutations in stems III and IV had little or no effect on the binding affinity of TFIIIA for 5S RNA. However, single mutants in stems II and V (positions 16–21, 57–62, 71–72, and 103–104) which disrupt the double helix, reduce the binding of TFIIIA by a factor of two to three fold. In contrast, double mutants (16–21/57–62, 71–72/103–104) which restore the helical structure of these stems, but with altered sequences, fully restore the TFIIIA binding affinity. The experiments reported here indicate that the double helical structures of stems II and V, but not the sequences, are required for optimal TFIIIA binding.

INTRODUCTION

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 [1–4]. In the immature oocyte, TFIIIA performs a second essential function. The protein binds to a large amount of 5S RNA in the cytoplasm, forming a ribonucleoprotein storage particle (7S RNP) that stabilizes the RNA until it is required for ribosome assembly [5, 6]. There is considerable interest in determining how TFIIIA interacts specifically with the coding region of the 5S RNA gene, and with the transcript of this gene.

TFIIIA is the canonical 'zinc finger' protein. Analysis of the cDNA sequence [7] for the protein revealed the presence of nine imperfectly repeated domains [8, 9]. Each repeating unit has approximately thirty amino acids and contains highly conserved cysteine and histidine pairs. Structural studies of TFIIIA have demonstrated that the protein contains tightly bound Zn^{2+} ions essential for its function as a transcription factor [9–11]. It has been proposed that the cysteine and histidine residues in each repeating element co-ordinate to a Zn^{2+} ion, folding the protein into a linear series of nine 'metal-binding fingers', each of which may act independently in binding to 5S DNA [9]. The tetrahedral co-ordination of cysteine and histidine side chains to a Zn^{2+} ion in each repeat has been confirmed by physical studies on TFIIIA [12, 13]. Metal-binding fingers are a structural motif common to the putative nucleic acid binding domains of a variety of eukaryotic regulatory proteins [14–16]. Protein fingers may have properties that facilitate the interaction of TFIIIA with 5S RNA [14].

The secondary structure of 5S RNA consists of double helical stems, single stranded loops, and several bulged nucleotides. Previous footprinting analyses indicated that TFIIIA protects an area including helix II/loop B, helix IV/loop E, and helix V [17–21]. The thermodynamic and kinetic parameters for the equilibrium leading to 7S RNP formation from free TFIIIA and 5S RNA have been determined using a nitrocellulose filter binding assay [19]. TFIIIA binds to *Xenopus* oocyte 5S RNA (Xlo 5S RNA) with a dissociation constant (K_d) of 1 nM. *Xenopus* somatic 5S RNA (Xls 5S RNA), which differs from of Xlo 5S RNA at six nucleotide positions within loop B, helix III and helix IV, has a three fold higher affinity for TFIIIA binding [22]. TFIIIA also binds to 5S RNA molecules from a variety of eukaryotic species with approximately equal affinity [19, 23–25]. The RNA binding activity of TFIIIA is however specific for 5S RNA, the protein having a 100-fold lower affinity for tRNA. This result suggests that TFIIIA recognizes the presence of highly conserved sequence and/or structural elements common to eukaryotic 5S RNA molecules.

In order to ascertain exactly which elements of 5S RNA structure and sequence are required for TFIIIA binding, various mutant 5S RNA molecules have been created and their affinity for TFIIIA measured. By using truncated 5S RNA mutants, it was found that nucleotides 11–108 of intact 5S RNA provide the necessary sequence and conformational information required for TFIIIA binding [22]. TFIIIA binding is more sensitive to the deletion of nucleotides from the 5′ terminus of 5S RNA as opposed to the 3′ terminus. The increased affinity of somatic vs. oocyte 5S RNA for TFIIIA is conferred by nucleotide substitutions in the 5′ half of the molecule [22]. Nucleotide substitutions within the single stranded loop regions were shown to have little or no effect on TFIIIA binding, with the exception of nucleotide substitutions in loop A which lies outside the TFIIIA footprint region [26]. This result was surprising because the

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majority of nucleotides that are highly conserved among all eukaryotic 5S RNAs are found in the single stranded loops, and would therefore be excellent candidates for the formation of sequence specific protein-RNA contacts that would explain the similar affinity that TFIIIA has for a variety of eukaryotic 5S RNAs. Deletions of bulged nucleotides present within 5S RNA have also been made and their effects on TFIIIA binding determined. Again, no obvious reduction in binding was found [27]. This result is in contrast to previous studies that demonstrated the importance of bulged nucleotides in other protein-RNA interactions [28–30].

From the results of these studies, a picture is emerging that suggests TFIIIA makes many weak sequence specific contacts in the single stranded regions of the 5S RNA. However, TFIIIA also binds specifically to the coding region of the 5S RNA gene, and several studies have suggested that the overlapping region of TFIIIA binding on the 5S RNA could co-axially stack into a continuous helix similar to the ICR on the gene [21, 26]. Since TFIIIA binds to the DNA by making sequence-specific contacts with the base pairs, it is necessary to determine whether the protein makes similar contacts with base pairs in the 5S RNA. We have therefore investigated the role that the double helical regions of *Xenopus* 5S RNA play in the specific binding of TFIIIA. Twenty-five different mutations which alter the sequence and base pairing properties of the double helical stems II, III, IV and V of 5S RNA have been constructed, and the effects of these mutations on the TFIIIA binding affinity of the RNA have been determined. In the case of stems II and V, full TFIIIA binding affinity requires the presence of the double helical conformation, but is sequence independent.

MATERIALS AND METHODS

Plasmids

The 5S RNA genes used in these experiments were constructed from a series of synthetic oligonucleotides, and introduced into pUC18 as described for the wild type *Xenopus* oocyte 5S RNA gene [22].

Synthesis of Mutant 5S RNAs

Labeled and unlabeled 5S RNAs were produced for use in binding assays by *in vitro* transcription of the constructed genes using T7 RNA polymerase and previously published procedures [22, 26].

Preparation of TFIIIA

The 7S RNP particle was isolated from the ovaries of immature *Xenopus laevis* (Xenopus I, Ann Arbor, MI) as described previously [5, 31]. Pure TFIIIA was obtained from the 7S particle by ammonium sulphate precipitation [32]. The protein pellet was resuspended in a buffer containing 50mM Hepes, KOH pH 7.5, 5mM $MgCl_2$, 500mM KCl, 1mM DTT, 2mM benzamidine and 20% glycerol (v/v) and stored at 4 °C. Protein concentrations were determined by the Bradford method (Bradford, 1976). Fractional 5S RNA binding activity of each protein preparation was determined by comparing the apparent association constant (K_a) measured for the preparation with the value of 1.3×10^9 M^{-1} determined by Scatchard analysis to be the apparent K_a obtained with 100% active TFIIIA and *Xenopus* oocyte 5S RNA [19]. Only those preparations which were greater than 90% active were used to study the binding of mutant 5S RNAs.

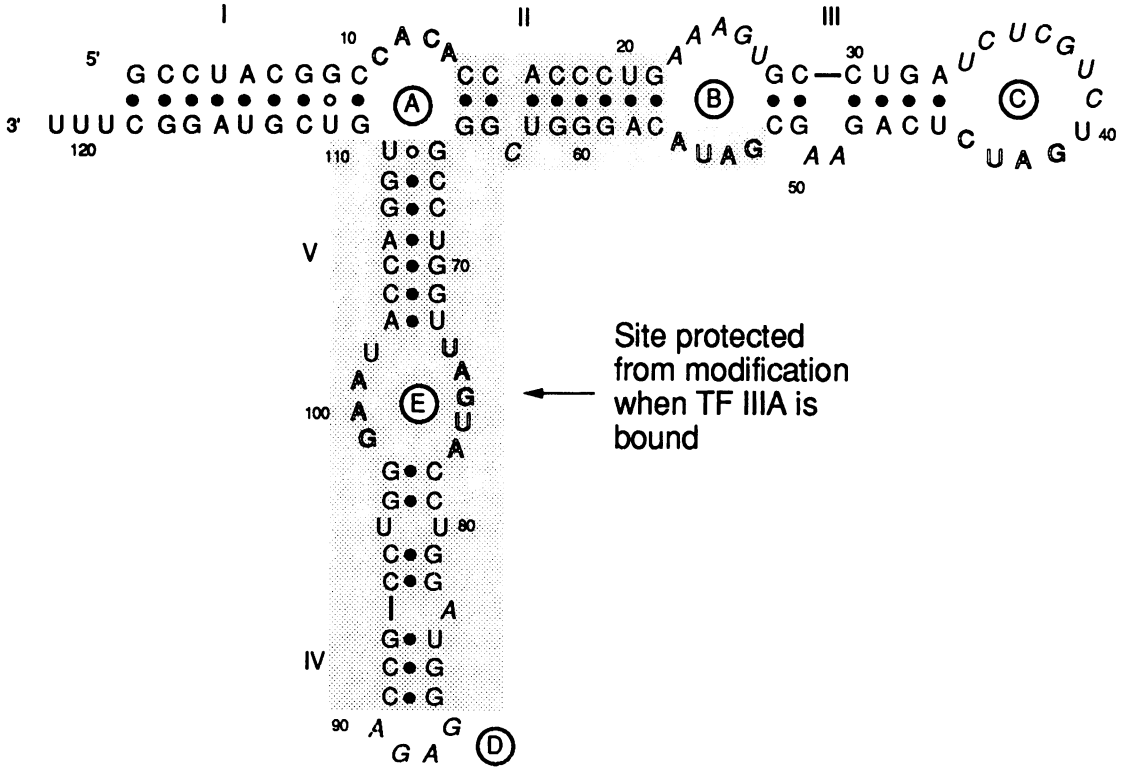

Figure 1. Secondary structure of *Xenopus laevis* oocyte 5S RNA. Shaded area represents the region protected from modification when TFIIIA is bound. Outlined nucleotides represent areas where site-specific mutations have reduced TFIIIA binding affinity by at least two-fold. Italicized nucleotides represent areas where site-specific mutations have little or no effect on TFIIIA binding affinity.

Binding Assays

The equilibrium constants for the binding of mutant 5S RNAs to TFIIIA were determined by a standard nitrocellulose filter binding assay [19].

RESULTS

Selection of mutation sites

The experiments were designed to investigate the contribution of the double stranded stems of 5S RNA to the free energy of TFIIIA binding. Figure 1 shows the secondary structure of the 5S RNA, and summarizes the results of footprinting experiments [17–21], and the effect that site-specific mutations of single stranded nucleotides had on TFIIIA binding affinity [26, 27]. Substitution of the nucleotides in stems II, III, IV, and V was accomplished by the creation of a set of 25 mutant 5S RNAs. Stem I, which is located outside of the TFIIIA binding site, has been shown by deletion mutagenesis to be unimportant for TFIIIA binding [22] and was therefore not included in this analysis.

A 'single mutant' was created by substituting a contiguous

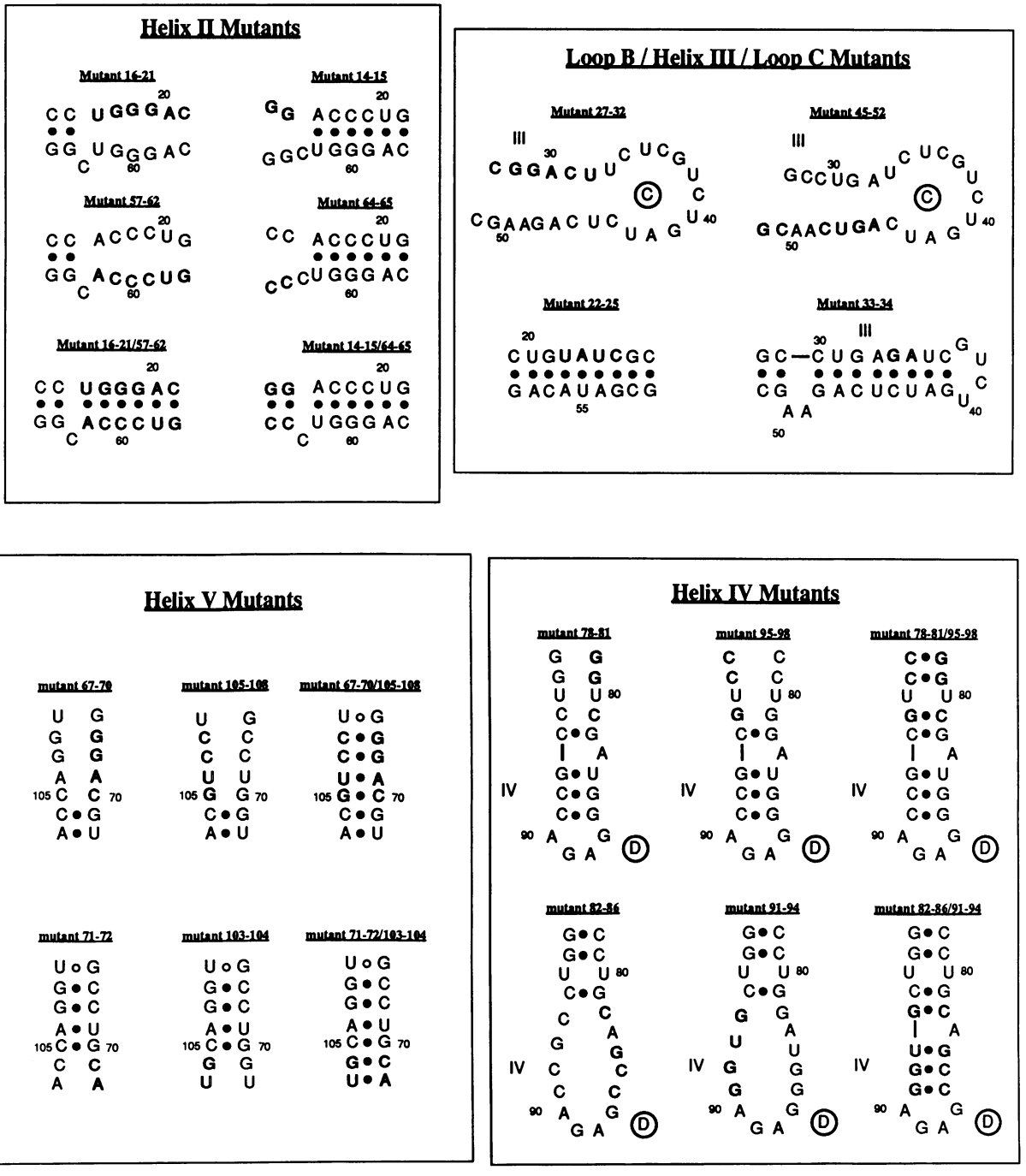

Figure 2. Stem and loop mutants used in this study. Nucleotide substitutions are indicated with outlined characters. All mutations were introduced into the intact 5S RNA molecule; only the affected region is shown for clarity.

stretch of two to four nucleotides on one strand of a double helix. A similar mutant was also created on the opposite strand of the double helical stem. The resulting mismatch in each mutant results in the formation of single stranded regions within the target helix. 'Double mutants' were obtained by combining the two single mutants, substituting compensating nucleotides on both strands of the helix that restore the helical stem structure but introduce a new sequence of base pairs (Figure 2). Three 5S RNAs with multiple mutations were created by combining the 16–21, 67–70, 95–98, 57–62 and 78–81 substitutions in several ways (Table I) to test the additive effects on TFIIIA binding affinity.

Two loops were also selected for mutagenesis. Mutant 22–25 changed the nucleotides in the 5′ half of loop B so they would Watson-Crick base pair with the nucleotides on the opposite side of the loop. This mutation created a long stem region that includes helix II, loop B, and helix III. Nucleotides 33–36 were substituted to be complementary to nucleotides 41–44 by replacing bases U and C at positions 33 and 34, respectively. The resulting mutant (Xlo 33–34) extended stem III into loop C (Figure 2). Therefore all of the single mutations studied altered both sequence and conformation, while the double mutations resulted only in changes in the sequence of the 5S RNA.

Determination of the TFIIIA binding affinities of mutant 5S RNAs

The affinity of each mutant 5S RNA for TFIIIA was measured using a nitrocellulose filter binding assay, in which a constant concentration of labeled 5S RNA was titrated with increasing concentrations of highly purified TFIIIA. The results of several experiments are shown in Figure 3, and the complete data are presented in Table 1. A number of single mutations in the stems resulted in a decreased TFIIIA binding affinity, while double mutations generally restored TFIIIA binding to wild type levels. As the data in Table I show, mutations which did reduce TFIIIA binding affinity had only a modest effect on the association constant. The two single mutations that extend the helix II-helix III stem either within loop B or in loop C, had virtually no effect on TFIIIA binding affinity.

Table 1. Relative Binding Data for Mutant *Xenopus* oocyte 5S rRNA Molecules

Mutant 5S RNA[a]	Relative K_a Value[b]
Stem II	
14–15	0.85±0.22
64–65	0.74±0.24
14–15/64–65	1.11±0.32
16–21	0.32±0.15
57–62	0.40±0.15
16–21/57–62	1.09±0.48
Stem III	
27–32	0.75±0.10
45–52	0.76±0.12
Stem IV	
78–81	0.88±0.01
95–98	0.78±0.02
78–81/95–98	0.86±0.01
82–86	0.81±0.30
91–94	0.96±0.18
82–86/91–94	1.21±0.35
Stem V	
67–70	0.75±0.12
105–108	0.39±0.06
67–70/105–108	0.71±0.01
71–72	0.35±0.21
103–104	0.50±0.23
71–72/103–104	1.18±0.32
loop B	
22–25	0.84±0.12
loop C	
33–34	1.09±0.21
Combination mutants	
16–21/95–98	0.15±0.03
16–21/67–70/95–98	0.16±0.05
16–21/57–62/78–81/95–98	0.63±0.08

[a] Numbers refer to those nucleotides in the wild type 5S rRNA which have been substituted (see Figure 2)

[b] Determined experimentally as the ratio: K_a(mutant)/K_a(wild type). For each mutant, the value shown is the mean of at least three independent determinations.

DISCUSSION

Since TFIIIA binds to the 5S RNA gene and the transcript of this gene, it is logical to consider whether these two nucleic acids share some common structural features which are required for the binding of TFIIIA. There are essentially two possibilities: the TFIIIA binding region of 5S DNA adopts a 5S RNA-like conformation, or the TFIIIA binding sites of 5S RNA stack into a DNA-like conformation. Although there is evidence to support both models, neither has been established unequivocally because of contradictory evidence. For example, although TFIIIA interacts primarily with the noncoding strand of 5S DNA, which is identical in sequence to 5S RNA [33], the ICR of the gene does not appear to adopt a cruciform conformation [34]. Evidence on the potential formation of an A-type helical structure in the ICR of the gene, and its functional significance is similarly contradictory [35–40]. The second model primarily is supported by the observation that there is a high degree of overlap in the TFIIIA binding sites on both DNA and RNA, the similarity being enhanced by the observation that the loop E region of the 5S RNA is helical in nature [41–43]. The similarity of the DNA and RNA binding sites would be strengthened further if helix II of 5S RNA stacks on helix V [21, 26] but there is no direct evidence to support that particular conformation of the helical arms. Both models do predict that TFIIIA binds DNA and RNA in a similar fashion and that the double stranded stems of 5S RNA therefore may be a primary target of TFIIIA binding.

The present analysis was designed to determine whether disruptions within the stems of the 5S RNA will affect the affinity of the RNA for TFIIIA. Nucleotide substitutions within the stems were selected to change both the sequence and the conformation of the RNA, both elements potentially being required for TFIIIA binding. These two different effects were then distinguished by the creation of compensating double mutations which change the primary sequence of the 5S RNA while maintaining the secondary structure conformation of the molecule.

From the data in Table 1, it is clear that disruption of stems II and V has negative effects on TFIIIA binding. This result is in agreement with the observation that these two stems are located within the TFIIIA binding site defined by footprinting techniques, and is also in agreement with results obtained from the study of TFIIIA binding to truncated RNA molecules [22]. In a previous study with linker-scanning mutations of *X. borealis* somatic 5S RNA, disruption of base pairing in stem II and stem V/loop E by the substitution of nucleotides 57–67, 67–78 and 92–105 was observed by a gel shift assay to reduce the binding of TFIIIA significantly [44].

In the present study, the measurement of the effects on the

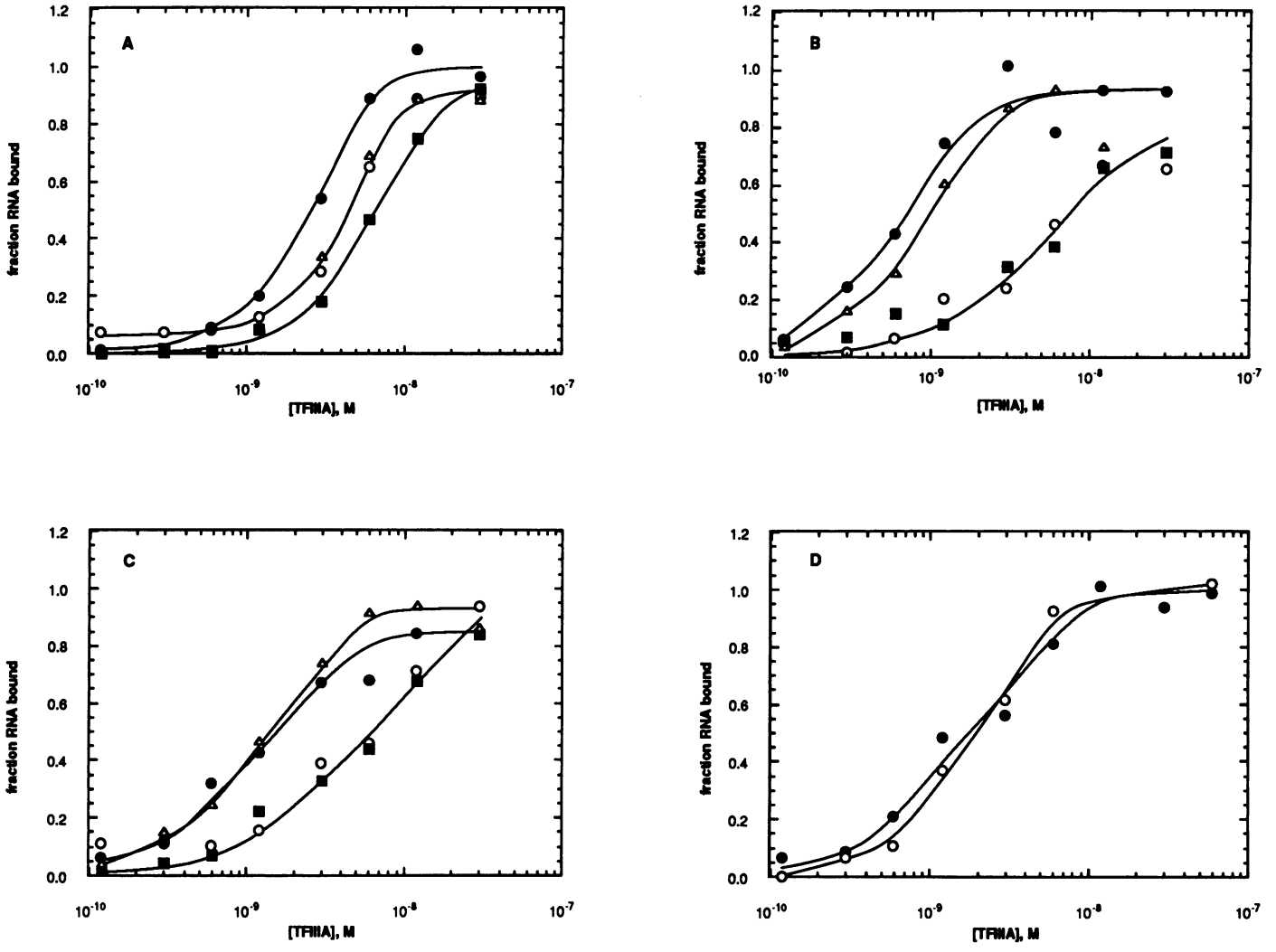

Figure 3. Nitrocellulose filter binding experiments with mutant 5S RNAs. A. (●) wild type 5S RNA, (○) 67−70 mutant, (■) 105−108 mutant, (△) 67−70/105−108 mutant. B. (●) wild type 5S RNA, (○) 16−21/95−98 mutant, (■) 16−21/67−70/78−81 mutant, (△) 16−21/57−62/78−81/95−98 mutant. C. (●) wild type 5S RNA, (○) 71−72 mutant, (■) 103−104 mutant, (△) 71−72/103−104 mutant. D. (●) wild type 5S RNA, (○) 33−34 mutant.

dissociation constant of a comprehensive set of smaller mutations, including double mutations that restore base pairing in the stems, provides a clear picture of the role of stem nucleotides in the binding of 5S RNA to TFIIIA. Substitution of nucleotides 16−21 or 57−62 within stem II reduce the TFIIIA binding affinity by a factor of 2.5−3 (Table I). The double mutant 16−21 / 57−62, which restores base pairing in stem II also fully restores TFIIIA binding. The single mutations 14−15 and 64−65 located in the same stem, along with the related double mutation, have very little effect on the binding reaction (Table 1). In contrast, replacement of the nucleotides in loop A (nucleotides 10−13) results in a three fold decrease in TFIIIA binding affinity, and an even larger effect on the competition strength of the mutant 5S RNA compared to the wild type RNA [26]. Evidently replacement of the nucleotides in loop A has a more disruptive effect on TFIIIA binding than disruption of the two base pairs in stem II which close the loop. Substitution of nucleotides 16−21 or 57−62 which disrupts 4 base pairs has a more dramatic effect on the conformation of stem II, and results in a decreased affinity for TFIIIA. The fact that the double mutant 16−21 / 57−62 fully restores TFIIIA binding indicates that the helical structure of stem II, but not its native sequence, is required for full binding affinity.

Similar results were obtained for stem V, which is also located in the TFIIIA protected area. Substitutions (71−72 and 103−104) within this stem that disrupt base pairing reduce TFIIIA binding, while a double mutation 71−72 / 103−104 which yields a stem structure with an altered sequence of base pairs restores full TFIIIA binding activity. The substitution of nucleotides 67−70 and the related double mutation 67−70 / 105−108 result in a similar reduction in TFIIIA binding affinity (Table 1), suggesting that there may be a minor sequence specific interaction between TFIIIA and these base pairs. Substitutions of highly conserved nucleotides in the neighbouring loop E region also modestly reduce TFIIIA binding affinity [26]. Studies on the solution structure of *Xenopus laevis* oocyte 5S RNA have indicated that this region adopts a sequence-specific, helical-like conformation consisting of several non-canonical base pairs [41, 42]. Nucleotide substitutions in this region disrupt this quasi-helical structure [45] and it is therefore unclear whether TFIIIA makes sequence specific or conformation specific contacts in the loop E region of the stem V-IV domain. However, the combined data from

the stem V and loop E mutants indicate that this extended stem structure is required for full TFIIIA binding affinity, with the possible formation of several weak sequence-specific contacts between the protein and the 5S RNA.

The disruptions of stems III and IV have very little effect on the TFIIIA-5S RNA interaction (Table 1). In addition, two mutants which extend Watson-Crick base pairing in this region of the 5S RNA by converting the structures of loop B and loop C (mutants 22–25 and 33–34) have no effect on TFIIIA binding. These data agree with previous observations that stem III is not protected by TFIIIA from chemical modification or RNase digestion [17, 19, 21, 46], and can be disrupted by large linker-scanning substitutions without significantly reducing TFIIIA binding [44]. The six mutations made within stem IV show little or no effect on TFIIIA binding affinity (Table 1), even though this stem is considered to be within the TFIIIA protected region. Huber *et al.* proposed that tandem CCUGG box regions within helices IV and V were critical for the binding of TFIIIA to 5S RNA [46]. The results of the present experiments do not support this suggestion. As shown in Table 1, double mutations of the CCUGG base pairs in each stem (mutants 67–70/105–108 and 78–81/95–98) which alter the sequence of these base pairs have very little effect on the binding of TFIIIA.

In previous studies, we have demonstrated that substitution of certain conserved nucleotides in loops B and C can reduce or enhance TFIIIA binding to the 5S RNA [22, 26]. In the current study, the importance of the conformation of these loops for TFIIIA was tested by introducing nucleotide substitutions which would extend Watson-Crick base pairing from the neighbouring stem through the loop. Somatic 5S RNA has a three fold higher affinity for TFIIIA than oocyte 5S RNA, primarily as a result of the three somatic-specific substitutions in loop B at positions 53, 55 and 56 [22]. This result suggested that nucleotides 53 to 56 in loop B of the 5S RNA constitute one of the elements required for optimal TFIIIA binding. Mutant 22–25 was constructed to form a double helix in loop B that links stems II and III producing an extended base paired structure. This mutation has no effect on TFIIIA binding, indicating that the conformation of loop B may not be essential for the formation of an interaction between nucleotides 53–56 and TFIIIA.

A potential interaction between TFIIIA and nucleotides 41–44 of loop C has been suggested by patterns of protection from chemical modification [21] and nucleotide substitution mutagenesis [26]. In the present study, mutant 33–34 was constructed within loop C to extend the base pairing of stem III and consequently reduce the size of the single stranded loop considerably. Binding assays indicated that this mutation has no effect on the interaction of TFIIIA with the 5S RNA (Table 1), although it has been shown previously that substitution of nucleotides 41–44 reduces the binding affinity for TFIIIA by a factor of two [26]. In the mutation studied here, substitution of nucleotides 33 and 34 allow the four nucleotides at positions 33–36 to form base pairs with nucleotides at positions 41–44 (Figure 2), an interaction that has been confirmed by solution structural studies [47]. However, this conformational change does not affect TFIIIA binding, implying that it is not the conformation, but the sequence information at positions 41–44 that is involved in the interaction between TFIIIA and loop C.

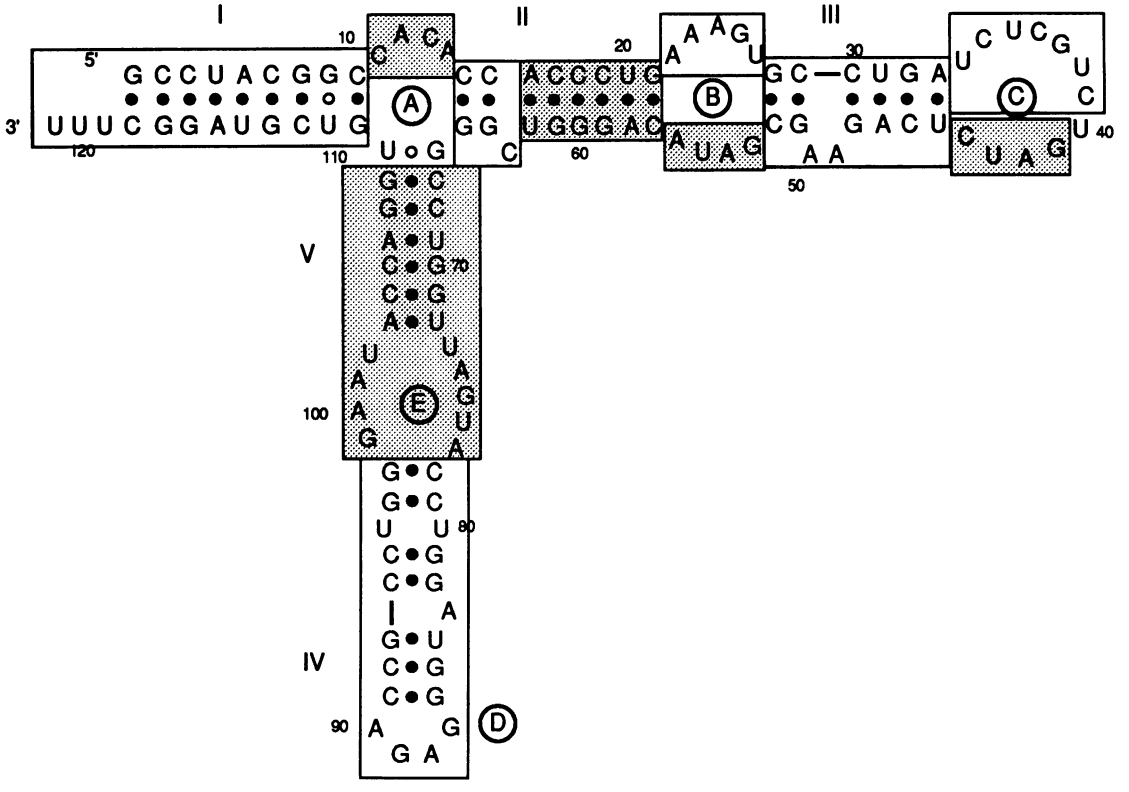

Figure 4. TFIIIA contacts on *Xenopus* oocyte 5S RNA. Shaded boxes indicate regions in which nucleotide substitution results in at least a two-fold reduction in binding affinity. Open boxes indicate regions where TFIIIA binding is essentially insensitive to nucleotide substitution.

It has been suggested that loop C of the 5S RNA may form a long range tertiary interaction with either loop D [48, 49] or loop E [50–52]. If loop C did make contact with another part of the molecule, the formation of base pairs between nucleotides 33–36 and 41–44 would break this tertiary interaction causing a major change in 5S RNA conformation. Such an alteration in conformation would almost certainly adversely affect TFIIIA binding. However, the binding affinity measured for the interaction of TFIIIA with mutant 33–34 is identical to that measured for wild type oocyte 5S RNA. This result is in agreement with conformational studies which do not support the formation of long range contacts between loops C and D in *Xenopus* oocyte 5S RNA [45, 47].

Larger reductions in TFIIIA binding affinity were observed with combination mutations which disrupt more of the secondary structure of the 5S RNA. The mutants 16–21/95–98 and 16–21/67–70/95–98 both demonstrate a greater reduction in TFIIIA binding than was observed for any of the parent single mutants (Table 1). It is interesting to note that the binding affinity of these combination mutants is roughly equivalent to the sum of the effects of each parent mutation. This result suggests that although the effects observed for each single mutant are relatively small, each nucleotide region that was substituted contributes directly to the overall free energy of TFIIIA binding. In comparison, the combination of the 16–21/57–62 and 78–81/95–98 mutations, which alters the sequence but not the base pairing of stems II and IV, results in a mutant 5S RNA that binds TFIIIA with only a slightly reduced affinity compared to the wild type 5S RNA (Table 1). This result is consistent with a view that structure, but not nucleotide sequence, is the main feature of helical stems essential for TFIIIA binding.

Exactly how do the helical stems of *Xenopus* 5S RNA participate in the binding of TFIIIA? The deep and narrow major groove of an A-type RNA double helix is generally inaccessible for interaction with functional groups on protein secondary structural domains. A more accessible region of A-type helical stems is the minor groove. The array of base pair functional groups oriented towards the minor groove provide for very little discrimination of base pair sequence [53]. Although sequence-specific protein-RNA contacts formed in the minor groove of an RNA stem have been observed in the crystal structure of a tRNA synthetase-tRNA complex [54], the apparent lack of sequence specificity in the interaction of 5S RNA stems with TFIIIA is consistent with the structural constraints of the RNA double helix. It is possible that TFIIIA contacts the sugar-phosphate backbone of the RNA stems, which would be sensitive to their conformational context far more than their sequence context. Many such potential contacts have been identified in the glutaminyl-tRNA synthetase:tRNA complex [54]. The contribution of these types of contacts to the overall high degree of specificity with which TFIIIA binds to 5S RNA would seem at first glance to be unlikely. However, the results of this and previous studies indicate that TFIIIA contacts two separate arms of the 5S RNA along the central base paired stems and neighbouring loops as indicated in Figure 4 [22, 26, 44, 55]. Although a detailed three dimensional structure of the 5S RNA is not available, a graphic model constructed on the basis of probing the solution structure of *Xenopus* oocyte 5S RNA suggests that stems II and V are co-axially stacked, but are not colinear [42]. Therefore the specific contacts on the 5S RNA for TFIIIA may be presented in a three dimensional array that is not duplicated by other RNA molecules, nor by the 5S DNA.

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REFERENCES

1. Sakonju, S., Bogenhagen, D.F. and Brown, D.D. (1980) Cell **19**, 13–25.
2. Sakonju, S., Brown, D.D., Engelke, D., Ng, S.-Y., Shastry, B.S. and Roeder, R.G. (1981) Cell **23**, 665–669.
3. Bogenhagen, D.F., Sakonju, S. and Brown, D.D. (1980) Cell **19**, 27–35.
4. Engelke, D.R., Ng, S.-Y., Shastry, B.S. and Roeder, R.G. (1980) Cell **19**, 717–728.
5. Picard, B. and Wegnez, M. (1979) Proc. Natl. Acad. Sci. USA **76**, 241–245.
6. Pelham, H.R.B. and Brown, D.D. (1980) Proc. Natl. Acad. Sci. USA **77**, 4170–4174.
7. Ginsberg, A.M., King, B.O. and Roeder, R.G. (1984) Cell **39**, 479–489.
8. Brown, R.S., Sander, C. and Argos, P. (1985) FEBS Lett. **186**, 271–274.
9. Miller, J., McLachlan, A.D. and Klug, A. (1985) EMBO J. **4**, 1609–1614.
10. Hanas, J.S., Hazuda, D.J., Bogenhagen, D.F., Wu, F.Y.-H. and Wu, C.-W. (1983) J. Biol. Chem. **258**, 14120–14125.
11. Shang, Z., Liao, Y.-D., Wu, F.Y.-H. and Wu, C.-W. (1989) Biochemistry **28**, 9790–9795.
12. Diakun, G.P., Fairall, L. and Klug, A. (1986) Nature **324**, 698–699.
13. Frankel, A.D., Berg, J.M. and Pabo, C.O. (1987) Proc. Natl. Acad. Sci. USA **84**, 4841–4845.
14. Berg, J.M. (1986) Science **232**, 485–487.
15. Vincent, A. (1986) Nucl. Acids Res. **14**, 4385–4381.
16. Evans, R.M. and Hollenberg, S.M. (1988) Cell **52**, 1–3.
17. Pieler, T. and Erdmann, V.A. (1983) FEBS Letters **157**, 283–287.
18. Andersen, J., Delihas, N., Hanas, J.S. and Wu, C.-W. (1984) Biochemistry **23**, 5759–5766.
19. Romaniuk, P.J. (1985) Nucl. Acids Res. **13**, 5369–5387.
20. Huber, P.W. and Wool, I.G. (1986) J. Biol. Chem. **261**, 3002–3005.
21. Christiansen, J., Brown, R.S., Sproat, B.S. and Garrett, R.A. (1987) EMBO J. **6**, 453–460.
22. Romaniuk, P.J., Leal de Stevenson, I. and Wong, H.-H.A. (1987) Nucl. Acids Res. **15**, 2737–2755.
23. Pieler, T., Erdmann, V.A. and Appel, B. (1984) Nucl. Acids Res. **12**, 8393–8406.
24. Hanas, J.S., Bogenhagen, D.F. and Wu, C.-W. (1984) Nucl. Acids Res. **12**, 2745–2758.
25. Andersen, J. and Delihas, N. (1986) J. Biol. Chem. **261**, 2912–2917.
26. Romaniuk, P.J. (1989) Biochemistry **28**, 1388–1395.
27. Baudin, F. and Romaniuk, P.J. (1989) Nucl. Acids Res. **17**, 2043–2056.
28. Romaniuk, P.J., Lowary, P., Wu, H.-N., Stormo, G. and Uhlenbeck, O.C. (1987) Biochemistry **26**, 1563–1568.
29. Peattie, D.A., Douthwaite, S., Garrett, R.A. and Noller, H.F. (1981) Proc. Natl. Acad. Sci. USA **78**, 7331–7335.
30. Mougel, M., Eyermann, F., Westhof, E., Romby, P., Expert-Bezancon, A., Ebel, J.-P., Ehresmann, B. and Ehresmann, C. (1987) J. Mol. Biol. **198**, 91–107.
31. Hanas, J.S., Bogenhagen, D.F. and Wu, C.-W. (1983) Proc. Natl. Acad. Sci. USA **80**, 2142–2145.
32. Shang, Z., Windsor, W.T., Liao, Y.-D. and Wu, C.-W. (1988) Anal. Biochem. **168**, 156–163.
33. Sakonju, S. and Brown, D.D. (1982) Cell **31**, 395–405.
34. Reynolds, W.F. and Gottesfeld, J.M. (1985) Proc. Natl. Acad. Sci. USA **82**, 4018–4022.
35. Gottesfeld, J.M., Blanco, J. and Tennant, L.L. (1987) Nature **329**, 460–462.
36. Aboul-ela, F., Varani, G., Walker, G.T. and Tinoco, I., Jr.,. (1988) Nucl. Acids Res. **16**, 3559–3572.
37. Becker, M.M. and Wang, Z. (1989) J. Biol. Chem. **264**, 4163–4167.
38. Fairall, L., Martin, S. and Rhodes, D. (1989) EMBO J. **8**, 1809–1817.
39. McCall, M., Brown, T., Hunter, W.N. and Kennard, O. (1986) Nature **322**, 661–664.
40. Fairall, L., Martin, S. and Rhodes, D. (1989) EMBO J. **8**, 1809–1817.
41. Romaniuk, P.J., Leal de Stevenson, I., Ehresmann, C., Romby, P. and Ehresmann, B. (1988) Nucl. Acids Res. **16**, 2295–2312.
42. Westhof, E., Romby, P., Romaniuk, P.J., Ebel, J.-P., Ehresmann, C. and Ehresmann, B. (1989) J. Mol. Biol. **207**, 417–431.
43. Varani, G., Wimberly, B. and Tinoco, I.J. (1989) Biochemistry **28**, 7760–7772.

44. Sands, M.S. and Bogenhagen, D.F. (1987) Mol. Cell. Biol. **7**, 3985–3993.
45. Stevenson, I.L., Baudin, F., Brunel, C., Romby, P., Ehresmann, C., Ehresmann, B. and Romaniuk, P.J. (1990) *submitted*
46. Huber, P.W. and Wool, I.G. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 1593–1597.
47. Brunel, C., Romby, P., Ehresmann, C., Romaniuk, P.J., Ehresmann, B. and Westhof, E. (1990) J. Mol. Biol. **in press**,
48. Toots, I., Misselwitz, R., Bohm, S., Welfle, H., Villems, R. and Saarma, M. (1982) Nucl. Acids Res. **10**, 3381–3389.
49. McDougall, J. and Nazar, R.N. (1983) J. Biol. Chem. **258**, 5256–5259.
50. GÜringer, H.U., Bertram, S. and Wagner, R. (1986) Nucl. Acids Res. **14**, 7473–7485.
51. Hancock, J. and Wagner, R. (1982) Nucl. Acids Res. **10**, 1257–1269.
52. Pieler, T. and Erdmann, V.A. (1982) Proc. Natl. Acad. Sci. USA **79**, 4599–4603.
53. Seeman, N.C., Rosenberg, J.M. and Rich, A. (1976) Proc. Natl. Acad. Sci. USA **73**, 804–808.
54. Rould, M.A., Perona, J.J., SÜll, D. and Steitz, T.A. (1989) Science **1135**, 1135–1142.
55. Baudin, F., Romby, P., Romaniuk, P.J., Ehresmann, B. and Ehresmann, C. (1989) Nucl. Acids Res. **17**, 10035–10046.