TFIIIA induced DNA bending: effect of low ionic strength electrophoresis buffer conditions

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ABSTRACT

We have used a circular permutation gel shift assay to show that the 5S gene transcription factor, TFIIIA, induces a bend at the internal promoter of the *Xenopus* oocyte-type 5S gene. The degree of bending is comparable to what we have previously observed for TFIIIA induced bending of the *Xenopus* somatic-type gene [Schroth, G.P. *et al.* (1989) *Nature* 340, 487–488]. In addition, we show that TFIIIA induced DNA bending is dramatically affected by the ionic conditions used during gel electrophoresis. By modifying the conditions of the electrophoresis, we can detect two distinct conformations for the TFIIIA/DNA complex. In very low ionic strength buffers, the degree of DNA bending in the complex is estimated to be about 25 to 30 degrees, whereas in higher ionic strength buffers it is about 60 to 65 degrees. These data explain the apparent discrepancy between our results and the results of another study in which it was claimed that TFIIIA did not 'substantially' bend DNA [Zweib, C. and Brown, R.S. (1990) *Nucleic Acid Res.* 18, 583–587]. These results also demonstrate that the TFIIIA/DNA complex has a large degree of conformational flexibility. Both DNA bending and conformational flexibility are structural features which may provide a key insight into the function of TFIIIA as a positive transcription factor.

INTRODUCTION

Transcription factor IIIA is a 39 kilodalton protein which is required for the transcription of 5S genes of *Xenopus* by RNA polymerase III (1). TFIIIA binds to a 50 bp internal promoter sequence called the internal control region (ICR). The ICR spans from positions +45 to +97 within the 120 bp 5S gene (2,3). TFIIIA interacts with the ICR through a 30 kilodalton N-terminal DNA binding domain containing nine zinc fingers (4,5). In an effort to better understand the structure and conformation of the TFIIIA/DNA complex, we have characterized the complex in terms of protein-induced DNA bending. Using both a circular permutation gel shift assay and a cyclization assay, we have previously shown that TFIIIA induces a bend at the ICR of the *Xenopus* somatic-type 5S gene (6).

The structure of the TFIIIA/DNA complex has also been studied by various biochemical techniques including nuclease digestion (7–9), hydroxyl radical footprinting (10,11), methylation protection experiments (12), and circular dichroism spectroscopy (13). Phosphorus imaging electron microscopy (EM) visualization of the TFIIIA/DNA complex has also been used to show that TFIIIA bends DNA (14). The structure of single zinc fingers from other proteins has been characterized using 2-D NMR (15,16). Recently, the structure of a pair of fingers from the yeast protein SWI5 was also solved using 2-D NMR (17). Unfortunately, there is still no detailed structural information concerning the interactions of zinc fingers with DNA.

The two models which attempt to describe the interaction of the zinc fingers of TFIIIA with DNA (discussed in references 11 and 18) are fundamentally similar. In each model, TFIIIA is aligned in a collinear fashion across the ICR in the same orientation; i.e. N-terminus of the protein at the 3' end of the ICR. Both models rely heavily on the proposed 2-D NMR structures of single (15,16) and double zinc fingers (17), as well as hydroxyl radical footprinting data (10). The models differ significantly in the proposed interaction of the zinc fingers with the DNA of the ICR: i) The Berg model (18) proposes that the fingers wrap around the DNA helix following the major groove. In this model, one zinc finger interacts with approximately 3 bp of DNA (19). ii) The model proposed by Klug and co-workers (11,12) places all nine of the zinc fingers on one face of the DNA helix. Each finger interacts in subsequent major grooves, requiring the linker peptide between each finger to cross over the minor groove. According to this model, each finger interacts with about 5–6 bp of DNA. Although strikingly different in predicting the mode of interaction of the zinc fingers with the ICR, both of the models appear to account for the bending of the DNA by TFIIIA (11,18).

TFIIIA also interacts with 5S RNA, forming a complex known as the 7S particle (20,21). The 7S particle accumulates in large quantities in the maturing oocytes and acts as a storage form of the 5S RNA, which is utilized later in development. The structure of the 7S particle has been studied using nuclease digestion (22), phosphorus imaging EM (23), and neutron scattering (24). Based on these studies, the 7S particle has an extended, cylindrically shaped structure, with dimensions of approximately 14 nm × 6

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nm (24). An extended conformation is also consistent with the structural data available for the free, uncomplexed protein in solution. Based upon the hydrodynamic studies of Biecker and Roeder (25), TFIIIA has a highly extended and asymmetric shape. Because of its large frictional ratio, these authors proposed that TFIIIA may be extended enough to span the entire length of the ICR (>175 Angstroms).

There are, however, some indications that the conformation of TFIIIA in the protein/DNA complex may be different from that of either the free protein or the protein in the 7S particle. Recently, the conformation of TFIIIA was probed with trypsin when bound to DNA, bound to RNA as a 7S particle, and free in solution (26). The conformation of the protein was also studied by measuring the fluorescence of a derivatized version. The conclusion from this study was that the conformation of the protein either free in solution or complexed with the 5S RNA in the 7S particle was the same, but there was a distinct conformational change which occurs in the protein upon binding to DNA (26). Additionally, when both the 7S particle and the TFIIIA/DNA complex are directly visualized with phosphorus imaging EM, the TFIIIA/DNA complex appears much more compact than the 7S particle (23,14). These data may support the idea that TFIIIA in complex with the DNA of the ICR has a more compact conformation than TFIIIA in complex with the 5S RNA (6).

Since our paper describing TFIIIA induced DNA bending was published (6), a report appeared which argued that there was an 'absence of substantial bending in TFIIIA/DNA complexes' (27). The authors of this paper, Zweib and Brown, also used a circular permutation assay to monitor TFIIIA induced DNA bending. There are, however, some important differences between these studies. Zweib and Brown studied TFIIIA induced DNA bending of the oocyte-type 5S gene (27), whereas we had used the somatic-type (6). In addition, these authors used a different electrophoresis buffer system than we had used for our studies (27). In this paper, we show that the apparent contradiction between our results and the results of Zweib and Brown, is due to a difference in gel electrophoresis conditions. The difference between our results is not due to differential bending of the somatic and oocyte-type gene. Furthermore, these results demonstrate the conformational flexibility of TFIIIA/DNA complexes. Using this simple electrophoretic assay we are able to detect two distinct conformations of the TFIIIA/DNA complex: a slightly bent, more extended conformation in low ionic strength buffer conditions; and a bent, more compact, conformation in moderate ionic conditions.

MATERIALS AND METHODS

Plasmid Constructions

A 140 bp *Sau* 3A-*Eco* R1 fragment containing the *Xenopus laevis* oocyte-type 5S gene was cut out of plasmid pXlo- $\Delta 3'$ +176, and isolated on a preparative agarose gel. The fragment contains a portion of the 5S gene, from +41 to +120, (relative to the transcription start site), and about 60 bp of 3' flanking sequences, see Figure 1A. The overhangs on the ends of the fragment were filled in using Klenow enzyme (Amersham) and dNTP's. The fragment was then cloned into the *Sma* 1 site of plasmid pCY-7, a plasmid designed to study protein-induced DNA bending using a circular permutation assay (28). The fragment was cloned into pCY-7 in both possible orientations, and the two clones were named pGBP-41 and pGBP-33. The structure of the circular

permutation constructs of the oocyte-type 5S gene are nearly identical to the somatic-type gene constructs which were described previously (6). Some features of the four constructs used in these experiments, including the five restriction enzyme cut sites used to generate the circularly permuted fragments, are shown in Figure 1B.

Preparation of TFIIIA

TFIIIA was prepared from the ovaries of immature *Xenopus* frogs (29). Ovaries were removed from the frogs and dounced, 20 times on ice, in homogenization buffer: 50 mM HEPES (N-Hydroxyethylpiperazine-N'-2-Ethane Sulfonic Acid), pH 7.5, 5 mM $MgCl_2$, 25 mM KCl, 0.1 mM $ZnCl_2$, 2 mM dithiothreitol (DTT), and 0.1 mM phenyl-methyl-sulfonyl-fluoride (PMSF). The homogenate was centrifuged in a microcentrifuge for 15 minutes. The supernatant was loaded directly onto glycerol step gradients. The gradients were made in SW-41 tubes and consisted of 3 ml steps of 10, 12, 15, and 30% glycerol in homogenization buffer. The glycerol gradients were centrifuged in a SW-40 rotor for 20 hours, at 39,000 rpm, 4°C. Fractions containing TFIIIA activity were loaded onto a 1 ml DEAE-Cellulose (DE52, Whatman) column equilibrated in buffer A: 20 mM HEPES, pH 7.5, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mM $ZnCl_2$, 1 mM DTT, and 0.1 mM PMSF. The column was washed with buffer A, followed by buffer A containing 220 mM KCl. The 7S ribonucleoprotein particles were eluted off of the column with buffer A containing 360 mM KCl. Fractions containing 7S particles were pooled, glycerol was added to 50%, and the fractions were stored at $-80^\circ C$. Before adding to the DNA binding reactions, the 7S particles were treated with 50 $\mu g/ml$ of RNase A and 2 mM DTT for 10 minutes to release the TFIIIA.

Binding Reactions and Gel Shift Assays

The plasmid DNA used in the TFIIIA binding reactions was prepared by the alkaline lysis method, followed by A-5m (Bio-Rad) column chromatography to remove RNA and proteins. The plasmids were cut with five restriction enzymes to generate the 550 bp circularly permuted fragments containing the TFIIIA binding site (shown in Figure 1B) and also a 4.3 kbp vector fragment. No action was taken to remove the chromosomal DNA in the plasmid preps, which we find serves as competitor DNA in the binding reactions. However, the chromosomal DNA does give rise to some non-vector bands which are visible in some of the gels shown in Figures 2 and 3.

RNase A treated 7S particles (TFIIIA) were added to the DNA in 20 mM Tris-HCl, pH 7.5, 2.5 mM $MgCl_2$, 70 mM KCl, and 1 mM DTT, and allowed to bind at room temperature for 10 minutes. Ficoll was then added up to 4%, and the samples were loaded onto polyacrylamide gels, without the addition of tracking dyes. An empirically determined amount of TFIIIA was used in each experiment, such that about 50–80% of the DNA would be bound by protein.

Gel shift assays were run on 5.5% polyacrylamide gels (29:1, acrylamide to bisacrylamide ratio) which were polymerized with 0.1% ammonium persulfate and 0.2% TEMED. All gels cast were 20 cm wide, 18 cm long, and 1.5 mm in thickness. The gels were pre-run for at least two hours, or until the conductivity was invariant with time. After pre-running, the reservoir buffer was poured off and replaced with fresh buffer. All gels were run at 150 volts, for 6–7 hours, at room temperature. Gels were stained with 1 $\mu g/ml$ ethidium bromide.

Three electrophoresis buffer systems were compared in these studies. In the first system, 1× Tris-Borate (TB), both the gel and the reservoir buffer contain 89 mM Tris and 89 mM Boric Acid, pH 8.3. In the second system, which we call 'HEPES', the gel contains 20 mM HEPES, pH 8.3, 0.1 mM EDTA, and 6% glycerol. The reservoir buffer in the 'HEPES' system contains 10 mM HEPES, pH 8.3, and 0.1 mM EDTA. The third system used is a modified version of the HEPES system, which we call the 'HEPES-plus-salt' buffer system. This gel contains 10 mM HEPES, pH 7.5, and 10 mM NaCl. The reservoir buffer contains the same buffer plus 5 mM β -mercaptoethanol (BME). The reservoir buffer was recirculated when using either of the low ionic strength HEPES gel systems, but not when using the 1× TB system. Variations of the 1× Tris-Borate system, i.e. 0.5× TB and 0.25× TB, were also used. A 123 bp DNA ladder (Bethesda Research Laboratories) was used as an electrophoresis standard. The mobilities of the TFIIIA/DNA complexes were determined by scanning the gels with a Molecular Dynamics computing densitometer (model 300A).

Estimation of TFIIIA Induced Bending Angle

A simplified analysis based upon the empirical method of Thompson and Landy (31) was used to estimate the degree of the bending induced by TFIIIA to the mobility of the circularly permuted TFIIIA/DNA complexes. The equation used which describes this relationship is $\mu_M/\mu_E = \cos \alpha/2$, where μ_M is the mobility of the middle positioned complex, μ_E is the mobility of the end positioned complex, and α is the bending angle. Mobility was measured in millimeters from the top of the gel. The bending angle, α , is expressed in degrees.

RESULTS

Comparison of TFIIIA Induced Bending of Somatic vs. Oocyte-type 5S gene

We wished to test whether TFIIIA differentially bends the ICR of the two major types of 5S genes, the somatic and the oocyte. The goal of these experiments was to try to demonstrate a structural difference, in terms of protein-induced DNA bending, in the complexes formed between TFIIIA and the two types of 5S genes. To study TFIIIA induced DNA bending of the two genes, we have made two very similar constructs of both types of genes in the permutation vector, pCY-7 (28). We have used the same *Sau* 3A site (at position +41 in each of the genes) as one end of the fragment. Both the oocyte and somatic-type gene fragments contain the 5S gene, from nucleotide position +41 to +120, and about 60–70 bp of 3' flanking sequences, (see Figure 1A). The two oocyte clones used in these experiments, pGBP-41 and pGBP-33, contain the oocyte-type 5S gene fragment cloned in either orientation into the permutation vector, plasmid pCY-7. When these two constructs are cut with the five enzymes used in the circular permutation assay, ten fragments are generated which are virtually identical to the fragments used in our original study on the somatic-type gene constructs: pGBP-21 and pGBP-33, (see Figure 1B and ref. 6).

Figure 2 shows the results of a 5.5% polyacrylamide gel analysis of TFIIIA/DNA complexes with these DNA fragments. The results clearly demonstrate that TFIIIA induces a bend at the internal promoter of the oocyte-type 5S gene. As expected for protein-induced DNA bending (32), the fragments which position the ICR near the end, 41A and 33E, migrate faster

Figure 1. Structure of DNA constructs used in these experiments. Panel A: A diagram of the 120 bp 5S gene, showing the position of the ICR, and the *Sau* 3A site used in the construction of the permutation clones (see materials and methods). Panel B: The two oocyte-type clones are named pGBP-41 and pGBP-33. The somatic-type clones are named pGBP-21 and pGBP-13. When the clones are cut with the five restriction enzymes shown, ten fragments are generated which are the same length but differ in the position of the ICR within the fragment. The relative position of the ICR, represented by black box, is shown for each fragment.

through the polyacrylamide gel than fragments having a more internally positioned ICR. The pattern of bending shown for these ten oocyte-type fragments is the same as the pattern we observed in the experiments with the somatic-type gene (6).

The right panel in Figure 2 shows the results of a direct comparison of the TFIIIA induced bending of the somatic and oocyte-type 5S genes on a 5.5% polyacrylamide gel. This gel compares mobility of TFIIIA bound to the five circularly permuted fragments from clone pGBP-21 (somatic) to the five fragments from clone pGBP-41 (oocyte). The complexes can be directly compared, i.e. 21A to 41A or 21C to 41C, since the relative positioning of the ICR within each fragment is the same. From this comparison, we conclude that TFIIIA induces a very similar bend in both of the major 5S genes.

Effect of Electrophoresis Buffer Conditions on TFIIIA Induced DNA Bending

After our original paper was published, a report appeared claiming that TFIIIA does not 'substantially' bend DNA (27). We noticed a striking difference between the electrophoresis buffer conditions used by Zweib and Brown (27) and our electrophoresis conditions (6). The left panel in Figure 3 shows the results of electrophoresis of TFIIIA bound to circularly permuted fragments from the two somatic-type clones, pGBP-21 and pGBP-13, under the electrophoresis buffer conditions used by Zweib and Brown. The electrophoresis conditions used for this gel are to the best of our knowledge identical to the conditions used by these authors ('HEPES' system). The analogous gels run under our original conditions (89 mM Tris-Borate) are shown in Figure 2. When the left panel in Figure 3 is compared to the gels in Figure 2, it is very clear that the mobility of the TFIIIA/DNA complexes exhibit far less dependence on the location of the binding site (i.e., less bending) in the lower ionic strength HEPES gel system, than in the 1× Tris-Borate system. We see the same decrease in the degree of bending when the oocyte-type gene is studied under these same conditions.

During the course of these studies, we investigated the effect

Figure 2. TFIIIA induced bending of the *Xenopus* oocyte-type 5S gene, and the direct comparison between the somatic and oocyte-type genes. At left: Circular permutation gel shift assay of TFIIIA bound to fragments from the oocyte-type 5S gene clones, pGBP-41 and pGBP-33. At right: Circular permutation assay comparing TFIIIA induced DNA bending of somatic (pGBP-21) and oocyte-type (pGBP-41) 5S gene promoters. Lanes A-E are the fragments generated by cutting with the five enzymes shown in Figure 1. Lanes 'M' are a 123 bp DNA ladder molecular weight standard. Both gels are 5.5% polyacrylamide gels, in '1 × TB' buffer system (see materials and methods).

Figure 3. Effect of low ionic strength electrophoresis buffer conditions on TFIIIA induced DNA bending. At left: Circular permutation gel shift assay of TFIIIA bound to DNA fragments from the somatic-type 5S gene clones, pGBP-21 and pGBP-13, in low ionic strength 'HEPES' electrophoresis buffer system. This is a 5.5% polyacrylamide gel. Lanes are labelled as in Figure 2. At right: Circular permutation assay of TFIIIA bound to oocyte-type 5S gene fragments from clones, pGBP-41 and pGBP-33, using the 'HEPES-plus-salt' buffer conditions, (see materials and methods).

of many different buffer condition variables on TFIIIA induced bending. We found that TFIIIA significantly bends DNA in virtually all buffers tested, except in conditions of very low ionic strength. We also noted the TFIIIA/DNA complexes were much less stable in very low ionic strength buffers compared to moderate ionic strength buffers. For instance, when the gels are run in 0.5 × or 0.25 × Tris-Borate the extent of TFIIIA induced DNA bending appears identical to that shown in Figure 2. However, when the concentration is dropped to 0.1 × TB (9 mM Tris-Borate) the complexes dissociate during electrophoresis. This is consistent with our observation that the stability of the TFIIIA/DNA complexes in the HEPES buffer system was also much lower than in the 1 × TB system. We have never experienced any problems of dissociation of TFIIIA/DNA complexes in 1 × TB, even during extended gel runs (10–12 hrs). However, we found that it was difficult to get reproducible results using the HEPES system. In fact, on most gels which

Table 1. Summary of the Differences in Apparent Length (in bp) and Bending Angle for TFIIIA/DNA Complexes on both '1 × TB' and 'HEPES' Gel Systems.

	MOBILITY (bp)*		APPARENT BENDING ANGLE (Degrees)	
	TB	HEPES	TB	HEPES
Oocyte				
End, 41A	630 bp	610 bp	60–65°	25–30°
Middle, 41C	770 bp	640 bp		
Somatic				
End, 21A	660 bp	650 bp	60–65°	25–30°
Middle, 21C	790 bp	670 bp		

* The mobility of the end and middle positioned TFIIIA/DNA complexes (in base-pairs) are compared for both types of 5S genes studied in Figures 2 and 3.

we ran in low ionic conditions (buffer concentrations of less than 20 mM), the complexes dissociated during the course of the electrophoresis (6–7 hrs). We conclude that low ionic strength electrophoresis buffer conditions are generally problematic with respect to the stability of the TFIIIA/DNA complex, and we would recommend that they not be used for studying TFIIIA/DNA interactions using a gel shift assay (see note below).

We also tested the effect of glycerol in the running gel during the electrophoresis. Glycerol may have been an important parameter since the gel used in the HEPES gel system contains 6% glycerol, whereas the $1 \times$ TB gel does not. A gel containing $0.5 \times$ TB and 6% glycerol gives identical results as the same gel system without glycerol (data not shown), we therefore conclude that glycerol *per se* does not account for the differences in the migration of the complexes between the two gel systems.

We have also investigated the effect of adding various salts to the HEPES system in an attempt to ascertain the buffer parameters which are important for maintaining the bent conformation of the TFIIIA/DNA complex. The right panel in Figure 3 shows that when 10 mM NaCl is added to a modified version of the HEPES gel system, the conformation of the complex appears more bent than in the HEPES system without the added salt. The addition of the salt has clearly changed the conformation of the TFIIIA/DNA complexes in the HEPES gel system. It should also be noted that the 'HEPES-plus-salt' gel system contains 5 mM BME in the reservoir buffer; we have found this to have a significant stabilizing effect on the TFIIIA/DNA complexes in low ionic strength buffers.

To quantitate the difference in mobility of the TFIIIA/DNA complexes under the two electrophoresis conditions ($1 \times$ TB and HEPES), the gels shown in Figure 2 and 3 were analyzed using a scanning densitometer, and the mobility of the complexes was measured relative to a 123 bp DNA ladder molecular weight standard. The apparent lengths (in bp) of the end positioned TFIIIA/DNA complexes (21A and 41A) and the middle positioned complexes (21C and 41C) are given in Table 1. For both the oocyte and the somatic-type 5S genes, the TFIIIA/DNA complexes migrate more slowly on the gels which contain the $1 \times$ TB buffer. The slower migration of the complexes in $1 \times$ TB, when compared to the HEPES buffer, is indicative of a more bent conformation (32).

Table 1 also gives the estimated values of the apparent bending angle for both the oocyte and somatic-type genes under the two different electrophoresis conditions (see methods). The degree of bending of both the oocyte and somatic-type genes shows a marked effect on the buffer system used for the circular permutation assay. Both types of TFIIIA/DNA complexes have an apparent degree of bending of about $60-65^\circ$ on the $1 \times$ TB buffered gels, however the bending decreases to about $25-30^\circ$ when studied on gels using the HEPES buffer system. This is consistent with the results of Zweib and Brown, who estimated that the TFIIIA induced DNA bending angle was about 30° (27). The values for the bending angles in Table 1 represents the combined data of several different gels, and therefore are given as a range.

DISCUSSION

Protein-induced DNA bending is a common structural feature of many protein/DNA complexes (reviewed in 33). The ability of a protein to change the conformation of its cognate DNA binding site is presumed to be important in facilitating the function of that protein. Recently, in a very elegant experiment, Goodman

and Nash (34) were able to functionally replace a protein-induced DNA bend at a recombination site with both an intrinsically bent piece of DNA and a different DNA binding site that was bent by another protein. In this case, the 'specific' protein-induced DNA bend could be functionally replaced by other completely different 'bending modules' (34). In some cases, it seems that the function of the DNA binding protein may solely be to bend DNA into a particular conformation or shape. DNA bending proteins may function in facilitating the formation of subsequent complexes, or generally promoting the action of other DNA processing proteins. Protein-induced DNA bending and/or intrinsically bent DNA have been found to be functionally important in transcription (35,36), replication (37,38), and recombination (34).

In this paper we have further described TFIIIA induced DNA bending of the internal promoter of *Xenopus* 5S genes. Furthermore, we have demonstrated that the zinc finger DNA binding domain of TFIIIA is flexible enough to accommodate either of two differently bent conformations of the TFIIIA/DNA complex. The conformation detected in very low salt (< 20 mM HEPES buffer) is much less bent than the conformation seen in more concentrated buffers. The low salt structure was the conformation studied by Zweib and Brown (27), and gave results which appeared to contradict our original observations (6). Unfortunately, these authors apparently never attempted to reproduce our buffer conditions in their work, even though the comparison to our work was discussed. We would like to emphasize that simply by adding 10 mM NaCl to the low ionic strength HEPES gel system, TFIIIA induces a much larger bend at the internal promoter of the 5S gene (See Figure 3). There is no question that under even moderate buffer conditions, TFIIIA induces a bend in the ICR of both the oocyte and somatic-type 5S genes.

Low ionic strength electrophoresis buffer systems have been used before to demonstrate protein-induced DNA bending for other proteins including the *E. Coli* cAMP receptor protein (27) and the Lex A repressor (39). However, neither of these proteins are zinc finger proteins or interact with a binding site as long as the ICR. The ICR is about 50 bp (over 175 Angstroms) long, and may be the longest DNA binding site of any known sequence specific DNA binding protein. The flexibility of DNA is sensitive to the ionic conditions of the solution. From studies on the DNA persistence length, there is a threshold of flexibility in many DNA molecules which occurs around 10 mM Na^+ ion concentration. Above 10 mM Na^+ , DNA is generally quite flexible, but below 10 mM Na^+ most DNA molecules become much more rigid (40). This is presumably due to salt effects on the charge repulsion of the DNA phosphate backbone. This may help to rationalize the two conformations we have described in this paper, since the total Na^+ in the HEPES gel system is less than 10 mM. In the low ionic strength buffer system, the ICR may become more rigid and less flexible or bendable. This would make it more difficult for TFIIIA to induce a bend in the ICR. However, in more moderate buffer conditions the DNA probably is more flexible, which allows TFIIIA to induce a bend in the internal promoter. Interestingly, the zinc finger DNA binding domain of TFIIIA has the conformational polymorphism to accommodate the more extended low salt conformation.

Both DNA bending and conformational flexibility may be significant features of the TFIIIA/DNA complex and may provide insight into the function of the protein. Recent work in yeast has shown that TFIIIA is required for accurate assembly of the transcription complex onto the 5S gene, but is not needed for

transcription initiation (41). In both yeast and *Xenopus*, RNA polymerase III transcription complexes form with the same sequential binding of factors: First, TFIID binds to the ICR, which is subsequently followed by the binding of TFIIC and TFIIB (42). In the yeast system, it is possible to strip off the TFIID and TFIIC with a high salt or heparin wash, and isolate the TFIIB/DNA complexes on a gel filtration column. The purified TFIIB/DNA complexes are competent for multiple rounds of transcription initiation by yeast polymerase III (41). The implication of these experiments is that TFIIB is the only true transcription initiation factor for RNA polymerase III transcription of 5S genes, and that TFIID and TFIIC are formally transcription complex assembly factors.

We suggest that the function of TFIID is simply to bend the DNA of the ICR into a particular geometric configuration, which is subsequently recognized by TFIIC (and eventually TFIIB). This would explain the lack of any other well established biochemical activity for TFIID in transcription. The TFIID bending 'activity' may play a structural role in facilitating the formation of RNA polymerase III transcription complexes onto the 5S gene. Of course, the detailed mechanism for 5S gene transcription complex formation may involve many key steps. For instance, our model does not explain the significance of the 10 kilodalton C-terminal domain of TFIID, which does not contain zinc fingers and is not needed for DNA binding, but is required for transcription complex formation. As yet, the role of the C-terminal domain has not been clearly defined in terms of facilitating the formation of the active transcription complex. The C-terminal domain may simply be a determinant in the interaction between TFIIC and the bent TFIID/DNA complex (43).

If we assume that a bent conformation is an important feature of the TFIID/DNA complex which is recognized by TFIIC, then any investigation of these interactions should be conducted in moderate ionic strength conditions. The low salt TFIID/DNA complex structure described in this paper may not be suitable for TFIIC binding, since TFIIC could have varying affinities for the TFIID/DNA complex depending upon the conformation of the complex. In order to optimize the interactions between the components, more than one type of electrophoresis buffer system should be considered in a study of 5S gene:TFIID:C complex formation.

The conformational flexibility in the TFIID/DNA complexes which we have demonstrated in this paper, may help to reconcile the published structural data available for free TFIID and TFIID complexed with either RNA or DNA. The low salt TFIID/DNA conformation described in this work may have an extended structure like that of the free protein (25) or the 7S particle (23,24). In contrast, the bent conformation observed in higher salt, may be more compact than either the 7S particle or the free protein, which is suggested by several lines of experimentation (6,14,26). Obviously further studies are needed to determine the size and shape of the TFIID/DNA complex, as well as more detailed studies which will be important for ascertaining the exact mode of interaction of zinc fingers with DNA (12,11,18,19).

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