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Biochemistry

Histones H2A/H2B inhibit the interaction of transcription factor IIIA with the *Xenopus borealis* somatic 5S RNA gene in a nucleosome

(chromatin/transcription)

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ABSTRACT **A *Xenopus borealis* somatic 5S RNA gene was assembled with either the complete octamer of histones, $(H2A/H2B/H3/H4)_2$, or the $(H3/H4)_2$ tetramer of histones that comprises the central protein kernel of the nucleosome. Gel-mobility shifts, DNase I protection, and immunoblotting assays demonstrate that the class III transcription factor IIIA (TFIIIA) readily interacts with 5S DNA associated with the tetramer but that little or no binding is detected when 5S DNA is associated with the full octamer of histones. Thus, the presence of histones H2A and H2B in the 5S nucleosome significantly inhibits the interaction of TFIIIA with its cognate binding site within the 5S RNA gene. We propose that either the depletion of histones H2A and H2B from preexisting nucleosomes or the staged assembly of chromatin after replication in which a tetramer of histones H3/H4 associates with DNA before histones H2A/H2B will facilitate the binding of transcription factors to their cognate DNA sequences.**

The primary function of chromatin structure is to compact DNA within the nucleus. However, trans-acting factors must still gain access to particular regulatory elements in order to facilitate important processes such as replication and transcription. How this contrasting requirement for compaction and accessibility is resolved has yet to be determined. One possibility is that nucleosomes are precisely positioned with respect to DNA sequence such that trans-acting factors can still interact with regulatory elements on the nucleosome surface or in between nucleosomes. There are now many *in vivo* examples of positioned nucleosomes around key DNA regulatory elements (1). It is also clear that the organization of regulatory sequences within chromatin is important for regulation of transcription and replication, thus suggesting an active role for chromatin structure in these processes (2).

In general, the packaging of DNA in chromatin is thought to play a role in maintaining genes in a repressed state by excluding the binding of transcription factors to their cognate DNA sequences (2, 3). *In vitro* studies have shown that binding of the core histones is often sufficient to restrict DNA from interaction with transcription factors (4–10). One possible solution to the problem of how transcription factors might gain access to DNA in chromatin follows from the proposal that chromatin structure is disrupted upon replication (3). Transcription factors might associate with DNA while chromatin assembly is incomplete (8, 11). The first histones to associate with nascent DNA are H3 and H4 (12, 13). Experiments in *Xenopus* egg extracts indicate that templates associated only with histones H3 and H4 are more accessible than templates associated with a complete octamer of histones (8, 13). Other experiments suggest that depletion of histones H2A and H2B from chromatin templates increases the accessibility of RNA polymerase II to DNA (14, 15). However, there are a few examples in which trans-acting factors have been suggested to interact with regulatory elements that are already associated with a complete octamer of histones *in vitro* (16–19). In these examples, the histones, the transcription factor, and DNA form a triple complex. This interaction is unexpected since the structure of DNA in a nucleosome, and hence the sequence recognized by the trans-acting factor, is severely distorted from that observed in solution. Within the nucleosome, DNA is bent into an 80-base-pair (bp) circle (20) and the helical periodicity of DNA changes from that in solution (21).

In this work, we have examined the interaction of transcription factor IIIA (TFIIIA) with 5S DNA associated either with an intact octamer of histones or with a tetramer of histones H3 and H4. Both the octamer and the tetramer adopt the same unique rotational and translational position with respect to DNA sequence when reconstituted with the 5S RNA gene from *Xenopus borealis* (16, 22). We find that 5S DNA assembled with a tetramer of histones $(H3/H4)_2$ is as accessible to TFIIIA as the naked DNA. However, when the complete histone octamer is present on the same DNA, binding by TFIIIA is significantly inhibited. Our data support models in which the staged association of DNA with histones during chromatin assembly facilitates access of transcription factors to their cognate sequences.

MATERIALS AND METHODS

DNA Fragments. Radiolabeled DNA fragments contained the *X. borealis* somatic 5S RNA gene. A 215-bp *Eco*RI/*Dde* I fragment derived from plasmid pXP-10 (23) was used for nucleosome reconstitution after radiolabeling at the *Eco*RI site. When this fragment is reconstituted with histone proteins, the axis of dyad symmetry of the resulting positioned nucleosome passes through the DNA 75 bp from the *Eco*RI site or ≈3 bp upstream of the initiation site for transcription of the 5S gene (16, 21). The *Eco*RI/*Dde* I fragment was used since it was the smallest 5S DNA fragment easily available that contained both the histone binding site (about −80 to +75) and the TFIIIA binding site (+45 to +95). All numbering of the DNA sequence is with respect to the start of transcription initiation of the 5S gene at +1.

Nucleosome Reconstitution. Nucleosome core particles were prepared as described (24). These contained DNA ≈150 bp long and only the four core histone proteins (25). Nucleosomes were reconstituted onto radiolabeled DNA fragments either by exchange with core particles (26) or by dialysis from high salt and urea with purified chicken erythrocyte histones

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Abbreviations: TFIIIA, transcription factor IIIA; ICR, internal control region.

(27, 28). In the latter, 5.0 μg of unlabeled nonspecific competitor DNA was added with the radiolabeled fragment (<0.1 μg), and protein and DNA were mixed at ratios of 0.4 and 0.6 (mass of histone/mass of DNA) in a total vol of 200 μl of high salt dialysis buffer (2 M NaCl/10 mM Tris·HCl, pH 8.0/10 mM 2-mercaptoethanol/1 mM EDTA). Samples were then dialyzed into the same buffer containing 5 M urea for 12 hr at 4°C and then into successive 90-min changes of the same buffer, but with NaCl concentrations of 1.2, 1.0, 0.8, and 0.6 M. Two further changes were carried out with 0.6 M NaCl buffer lacking urea, and the samples were exhaustively dialyzed for 12–14 hr into 10 mM Tris·HCl (pH 8.0). This procedure resulted in a maximum of a single histone octamer or tetramer being associated with any particular labeled fragment, such that one-third to one-half of the labeled fragment remained uncomplexed by histone protein. These uncomplexed DNA fragments served as an internal control for binding of TFIIIA.

TFIIIA Binding Reactions. For the quantitative mobility-shift experiments (Fig. 1), ≈5 fmol of labeled 5S DNA or 5S DNA reconstituted with a tetramer or octamer of histones was incubated in the presence of various amounts of TFIIIA (as stated in the figure legends) in 10–20 μl of binding buffer [20 mM Hepes, pH 7.4/70 mM NH_4Cl/7 mM $MgCl_2$/10 μM $ZnCl_2$/5 mM dithiothreitol/0.02% Nonidet P-40/5% (vol/vol) glycerol/20 μg of bovine serum albumin per ml] for ≈15 min at 25°C. This buffer did not affect the stability of tetramer or octamer–DNA binary complexes (data not shown). Minor variation in NH_4Cl or $MgCl_2$ content had no effect on the stability of the TFIIIA-bound complexes formed. Large scale binding reactions (Fig. 2) were as described below. Samples were then loaded directly onto 0.7% agarose gels containing 0.5× TB buffer [45 mM Tris borate (pH 8.3)] (16) while the gel was running. EDTA was omitted from all solutions to avoid denaturing TFIIIA. Electrophoresis was at 20 mA for 3 hr at 4°C.

Purification of TFIIIA. The 7S storage particle and TFIIIA were purified as described by Smith *et al.* (29). Briefly, immature ovary homogenate is fractionated on glycerol gradients, bound to DEAE-cellulose, and eluted on a salt gradient. The 7S particle fractions (1–10 mg of protein) were adjusted to 0.1 M KCl in 50 mM Hepes, pH 7.5/5 mM $MgCl_2$/1 mM dithiothreitol/10 μM $ZnCl_2$/20% glycerol (buffer A). RNase A was mixed with the 7S particle (50 μg of RNase A per mg of protein starting material), incubated for 5 min, and then mixed with an equal vol (to the total) of buffer A containing 0.1 M KCl and 10 M urea. The mixture was loaded onto a BioRex-70 column and TFIIIA was eluted with increasing concentrations of KCl. TFIIIA was eluted between 0.8 and 1 M KCl. The protein was >95% pure (30).

Immunoblotting of TFIIIA Complexes. The amount of TFIIIA in complexes resolved on mobility-shift gels that is required to be observed by immunoblotting techniques was empirically determined to be ≈0.5 pmol (data not shown). Thus, reconstitutions were carried out with 5.0 μg of *Eco*RI/*Hin*dIII fragment (purified from pXP-10) that had been cleaved with *Dde* I. Nonspecific carrier DNA was omitted from these reconstituted samples. Reconstitutions were otherwise carried out as described above. Immunoblotting was as described (31) with minor modifications. Agarose gels containing TFIIIA complexes (see above) were first stained with ethidium bromide (0.4 mg/liter in 0.5× TB buffer) for 15 min, quickly photographed under UV illumination, and then soaked in 0.1% SDS in 0.5× TB buffer for an additional 20 min. The gel was then electroblotted onto a nitrocellulose filter at 250 mA for 5 min. Filters were then probed with anti-TFIIIA antiserum and visualized by standard methods.

Footprinting. Cleavage of DNA in reconstituted nucleosomes with the hydroxyl radical was accomplished as described (22). All DNase I footprinting was accomplished by gel isolation of nucleoprotein products of digestion followed by denaturing gel electrophoresis. Octamers or tetramers were assembled onto the radiolabeled DNA fragment containing the 5S RNA gene and a portion of the sample (≈0.25 pmol) was mixed with DNase I (100–500 ng), and digestion was allowed to proceed at 25°C for 1 min. The sample was then adjusted to 5% glycerol, chilled to 0°C on ice, and immediately loaded onto a running 0.7% agarose gel [45 mM Tris borate (pH 8.3)] at 4°C. Samples were electrophoresed for 4–5 hr at low current (<20 mA) and nucleoprotein complexes were identified by autoradiography of the wet gel. Labeled DNA was then isolated from these complexes and analyzed by denaturing gel electrophoresis.

RESULTS

Histones H2A and H2B Inhibit the Interaction of TFIIIA with a 5S RNA Gene Incorporated into a Nucleosome. We wished to study the structure of the triple complex between 5S DNA, the histone octamer, and TFIIIA (16). However, our experience was that the prior association of 5S DNA into a nucleosome precluded significant binding of TFIIIA. Varying the method of nucleosome reconstitution, conditions of TFIIIA binding, source of TFIIIA, or 5S DNA fragment did not improve the interaction (data not shown). We then investigated whether alterations in the structure of the histone protein component of the nucleosome, such as deficiency in histones H2A and H2B or proteolytic removal of the histone tails would affect the affinity of TFIIIA for 5S DNA in these structures.

Histone–5S DNA complexes were prepared such that a maximum of only one histone octamer or one tetramer was bound to each labeled DNA fragment. These were mixed with various amounts of purified TFIIIA in binding buffer and complexes were separated by gel electrophoresis at 4°C. As the concentration of TFIIIA increases, the free DNA band in the samples disappears and a band corresponding to a TFIIIA–DNA complex becomes evident (Fig. 1, lanes 3–8). A TFIIIA-induced shift is also observed for the tetramer–DNA complex. This shift is due to the formation of the TFIIIA–$(H3/H4)_2$–DNA triple complex (see below) and parallels the binding of TFIIIA to the free DNA. Little or no additional shift is observed in the octamer sample as a result of the

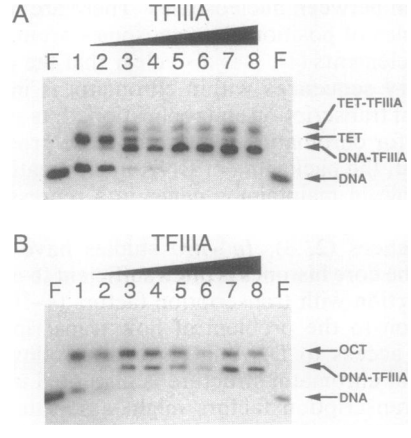

FIG. 1. (*A*) TFIIIA binds to 5S DNA previously assembled with the $(H3/H4)_2$ tetramer. Lanes: 1, 5S DNA–$(H3/H4)_2$ tetramer complex; 2–8, 5S DNA–tetramer complex and 0.5, 2, 5, 10, 20, 50, and 100 ng of TFIIIA, respectively; F, labeled DNA only. Positions of the naked DNA, TFIIIA-bound DNA, 5S tetramer (TET), and 5S tetramer–TFIIIA complexes are indicated. (*B*) TFIIIA does not bind to 5S DNA previously assembled with the histone octamer $(H2A/H2B/H3/H4)_2$. Lanes: 1, 5S–octamer complex; 2–8, 5S DNA–octamer with TFIIIA as in *A*. Positions of the naked DNA, TFIIIA-bound DNA, and 5S nucleosome complexes (OCT) are indicated.

presence of TFIIIA. A quantitative determination (32) of the fractional binding of TFIIIA in these experiments confirms that the protein binds tetramers and free DNA with approximately equal affinity (results not shown).

We have characterized each of the complexes that appears on the nucleoprotein gel shown in Fig. 1 and thus eliminated the possibility that TFIIIA binds to the octamer but does not impart any additional mobility shift in our gel system (Figs. 2 and 3). Our first approach was to mix radiolabeled DNA reconstituted with octamer or tetramer with an excess of TFIIIA (as in Fig. 1, lanes 8), treat with DNase I, and then isolate individual complexes from the nucleoprotein gel. Complexes of naked DNA with TFIIIA were resolved and excised from the wet gel (Fig. 2*A*). The labeled DNA was purified and denatured, and the cleavage pattern was analyzed on a denaturing polyacrylamide gel (Fig. 2*B*). It is clear from the autoradiograph of the nondenaturing gel that the free 5S DNA is completely bound by TFIIIA leading to a substantial mobility shift (compare Fig. 2*A*, lanes 1 and 2 with lanes 3 and 4). All of the free DNA appears to migrate at the position indicated as the lower complex. The DNase I cleavage pattern of this complex is that expected for 5S DNA with TFIIIA bound (Fig. 2*B*, lane 6) (33, 34).

As expected, the complex of a complete octamer of histones with the 5S RNA gene does not change position

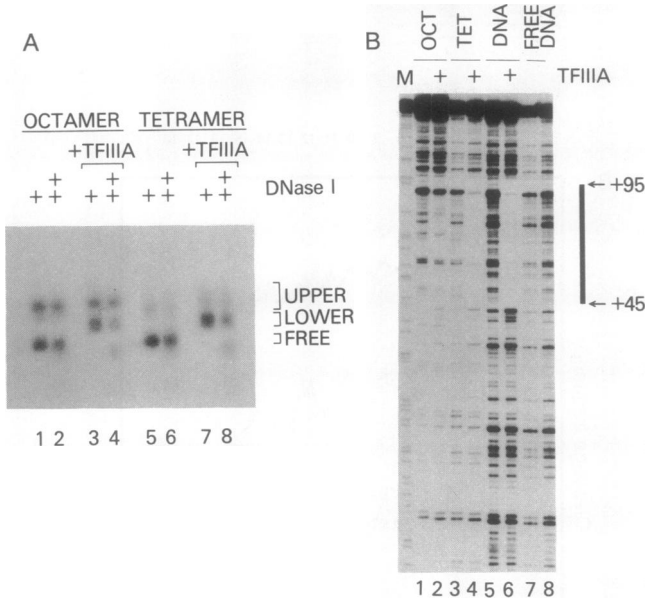

FIG. 2. Isolation of complexes between 5S DNA, TFIIIA, histone tetramer, and histone octamer after treatment with DNase I. (*A*) Preparative nondenaturing gel of complexes. Samples containing free 5S DNA and 5S DNA–histone complexes were incubated with excess TFIIIA as indicated. Samples were then treated with either 100 or 500 ng of DNase I as indicated (+ or ++). Complexes were separated on the gel and located by autoradiography. The position of the free 5S DNA band and the TFIIIA–5S DNA band (lower complex) are indicated. Both the histone–5S DNA complex and the TFIIIA–5S DNA–histone triple complex are found in the upper complex region of the gel. (*B*) DNase I footprints of the complexes resolved on the gel shown in *A*. DNase I-treated 5S DNA was recovered from the complexes on the nondenaturing gel (*A*) and cleavage products were resolved on a denaturing gel. A G-specific cleavage of the labeled 5S DNA fragment is shown (lane M). The free DNA and +TFIIIA lanes on the right are the DNase I cleavage products isolated from the free 5S DNA and TFIIIA-bound 5S DNA bands on the gel shown in *A*. Lanes 1 and 2 and lanes 3 and 4 are DNase I cleavage products from the upper complex bands containing octamer (OCT) and tetramer (TET) complexes without or with (+) TFIIIA as indicated. The TFIIIA binding site, the internal control region (ICR) situated between positions +45 and +95 of the 5S RNA gene, is indicated by the vertical bar.

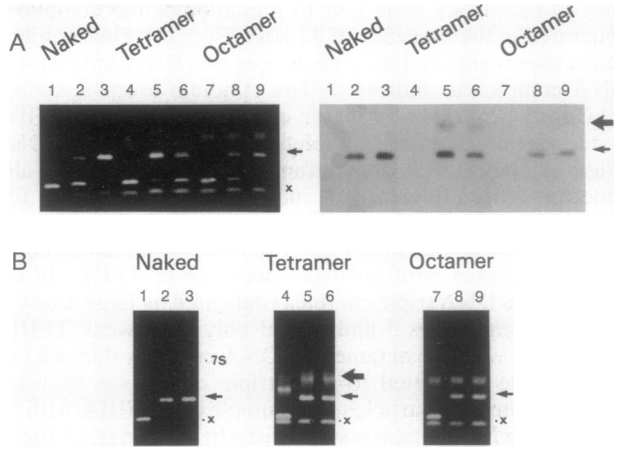

FIG. 3. Immunoblotting of TFIIIA complexes resolved on a nondenaturing gel. (*A*) (*Left*) Ethidium bromide-stained gel containing naked DNA (lanes 1–3), tetramer complexes (lanes 4–6), and octamer complexes (lanes 7–9). Lanes 1, 4, and 7 contain no TFIIIA; lanes 2, 6, and 8 contain 25 ng of TFIIIA; and lanes 3, 5, and 9 contain 100 ng of TFIIIA. The binary complex between TFIIIA and the *Eco*RI/*Dde* I fragment is indicated by the small arrow. The x indicates the small (90 bp) nonspecific DNA fragment that results from *Dde* I cleavage of the *Eco*RI/*Hin*dIII fragment and serves as an internal control. Other complexes are as described in Fig. 1. (*Right*) TFIIIA-specific immunoblot derived from the gel shown on the left. Small arrow indicates the specific TFIIIA–DNA complex, while the large arrow indicates the position of the 5S DNA–tetramer–TFIIIA triple complex. (*B*) Complexes formed with 7S particle-derived TFIIIA. The lanes of this nondenaturing gel contain naked DNA, tetramer, and octamer complexes as indicated. All samples contain 100 ng of RNase A. Lanes: 1, 4, and 7, complexes alone; 2, 3, 5, 6, 8, and 9, samples contained 200 ng of 7S particle (based on protein mass). Samples in lanes 3, 6, and 9 also contained 250 ng of nonspecific competitor DNA.

appreciably as a result of TFIIIA addition (Fig. 2*A*, lanes 1–4), while the mobility of the tetramer–5S DNA complex is clearly retarded by the presence of TFIIIA (upper complex; Fig. 2*A*, lanes 5–8). The denaturing gel of DNA in these complexes (Fig. 2*B*) indicates that TFIIIA does not bind efficiently to the 5S RNA gene when it is associated with a complete octamer of histones. The upper complex in the octamer samples (Fig. 2*A*, lanes 1–4), in the absence of TFIIIA, gives the cleavage pattern of 5S DNA in a nucleosome (Fig. 2*B*, compare lanes 1 and 5). There are only slight changes in the nucleosome footprint of the TFIIIA binding site caused by the presence of TFIIIA (Fig. 2*B*, compare lanes 1 and 2) despite the complete association of this protein with the naked DNA in the sample (Fig. 2*A*, lanes 3 and 4). Densitometric scans of these lanes confirm these observations (data not shown). These results suggest that TFIIIA interacts with 5S DNA associated with an octamer of histones only very weakly, if at all.

Histones H3 and H4 reconstitute onto a 5S RNA gene to assemble a tetramer $(H3/H4)_2$ in which the translational and rotational position of the central 120 bp of 5S DNA sequence are the same as in the histone octamer (ref. 22; see also Fig. 4). The addition of TFIIIA to the mixture of reconstituted tetramer and naked 5S DNA leads to a clear mobility shift for both the tetramer and naked DNA (Fig. 2*A*, lanes 7 and 8). Denaturing gel analysis of the DNase I cleavage products reveals that TFIIIA binds to the 5S RNA gene efficiently when it is complexed with a tetramer of histones (Fig. 2*B*, compare lanes 3 and 4). Densitometric analysis again confirmed these observations (data not shown).

The second approach to examine the possible interaction of TFIIIA with the octamer–5S DNA complex was to scale up our reconstitutions of tetramers and octamers associated

with the 5S RNA gene and to immunoblot the complexes generated in the presence of TFIIIA (Fig. 3*A*). Our mobility-shift experiments include a mixture of free DNA and histone–DNA complex as indicated. The ethidium bromide-stained gel (Fig. 3*A Left*) shows that in each case increasing TFIIIA concentration leads to a complete mobility shift of free DNA (Fig. 3*A*, lanes 1–3), the tetramer–5S DNA complex also generates an additional shift indicating triple complex formation (lanes 4–6), whereas the octamer shows no additional shift (lanes 7–9) (see also Figs. 1 and 2). Immunoblotting of these complexes confirmed the presence of TFIIIA in the tetramer–5S DNA triple complex (indicated by large arrow in Fig. 3*A Right*, lanes 5 and 6) and only very weak TFIIIA association with the octamer–5S DNA complex (lanes 8 and 9). Next we attempted to form triple complexes by using RNase-treated 7S particles (the complex of TFIIIA with 5S RNA) (16) with identical results (Fig. 3*B*). Naked DNA (lanes 1–3) and the tetramer complex (lanes 4 and 5) interact with TFIIIA, whereas the octamer does not (lanes 7–9). High background staining due to residual 7S particles (lanes 2 and 3) prevents use of the immunoblotting assay to determine TFIIIA association in this experiment.

We examined whether removal of the core histone tails with trypsin would facilitate the interaction of TFIIIA with the 5S RNA gene associated with a full octamer of histones. The 5S DNA associated with an octamer of intact histones or with an octamer of trypsin-treated histones was incubated with TFIIIA. In both cases, TFIIIA failed to interact with the histone–DNA complex as determined by both mobility-shift and DNase I digestion of the complexes (data not shown).

Key Contacts of TFIIIA with the ICR Are Accessible in the Tetramer but Occluded in the Octamer Complex with 5S DNA. In previous work (21), we used hydroxyl radical cleavage to document the helical periodicity of nucleosomal DNA at various positions relative to the dyad axis of the nucleosome. This reagent has several advantages over nucleases in analyzing DNA structure in nucleoprotein complexes; among these are the very small size of the probe and the fact that cleavage of DNA does not involve a competing protein–DNA interaction. Consequently, hydroxyl radical cleavage gives an accurate representation of existing contacts between DNA and protein or changes in DNA conformation. Hydroxyl radical footprinting of 5S DNA reconstituted with various molar excesses of histones H3 and H4, or of histones H2A, H2B, H3, and H4, allows the organization of the ICR when complexed with a single tetramer or octamer of histones to be assessed (Fig. 4). The tetramer organizes the central 120 bp of DNA identically to the octamer (the axis of dyad symmetry of the nucleosome is at −3 relative to the start of transcription of the 5S RNA gene; refs. 16, 21, and 22). The footprint extends to approximately +65 relative to the start of 5S RNA gene transcription (+68 relative to the dyad axis). In contrast, association of the octamer with 5S DNA leads to a footprint extending to +90. The strongest contacts between TFIIIA and the ICR occur at the 3′ end of the ICR between +80 and +93 (34, 35). This is shown as the hatched region in the ICR in Fig. 4*B*. The extended octamer footprint clearly includes the key contacts in the ICR, while these are free when 5S DNA is associated with only a tetramer.

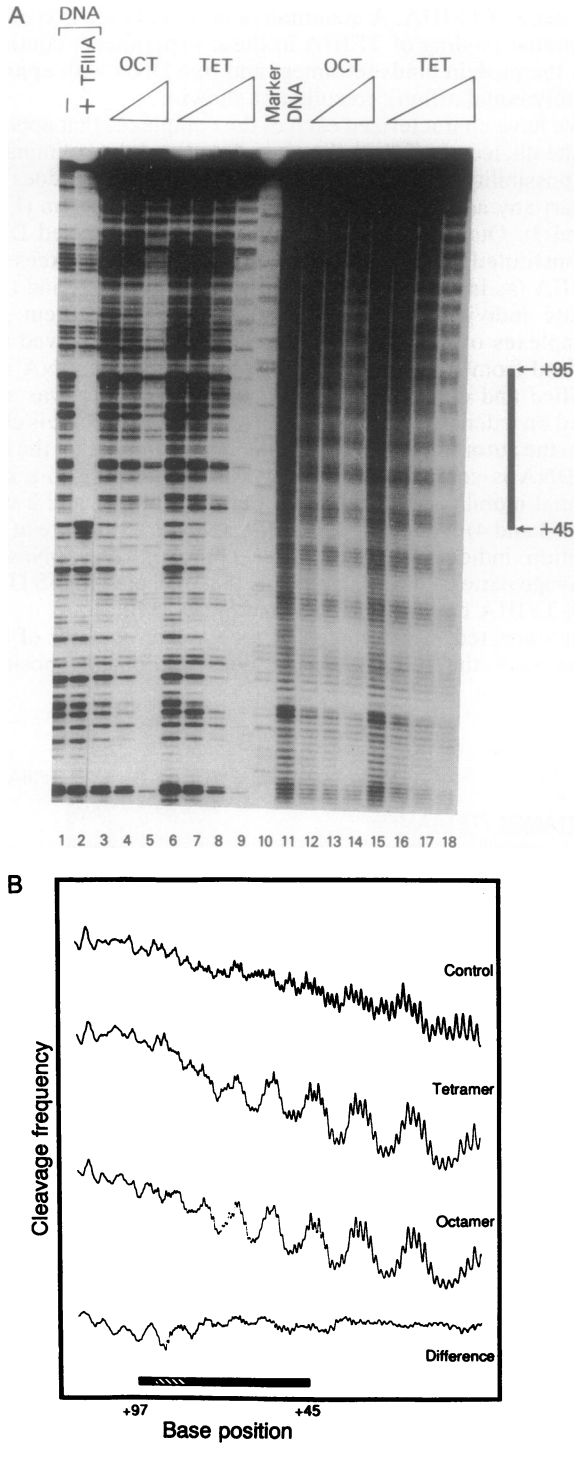

FIG. 4. DNase I and hydroxyl radical footprinting of 5S DNA assembled with the histone octamer (OCT) or the histone tetramer (TET). (*A*) Denaturing gel of cleavage products. The DNase I cleavage patterns of naked 5S DNA and 5S DNA bound by TFIIIA are shown for reference. The 5S DNA was reconstituted with increasing amounts of octamers or tetramers as indicated. Lanes: 1–8, samples probed with DNase I; 11–18, samples probed with hydroxyl radical. The marker lane is as described in Fig. 2*B*. The TFIIIA binding site is indicated by a vertical bar. (*B*) Densitometer scans of selected lanes of the gel shown in *A*. The control, tetramer, and octamer scans correspond to lanes 11, 12, and 16, respectively. A plot of the difference between the tetramer and the octamer scans is also shown. The locations of the TFIIIA binding site and the most important region for contacts to DNA are indicated by a solid horizontal bar and a hatched box, respectively.

DISCUSSION

The major conclusion of this paper is that TFIIIA will form a triple complex with *X. borealis* 5S DNA associated with a histone tetramer, but that the assembly of a complete octamer on 5S DNA significantly inhibits TFIIIA from binding to the ICR. Our results extend those of Rhodes (16) and Gottesfeld (4), who studied the association of TFIIIA with a nucleosome containing 5S DNA, albeit from two different species of *Xenopus*. Rhodes concluded that TFIIIA could recognize its binding site when the *X. borealis* 5S RNA gene is in a nucleosome, while Gottesfeld concluded that TFIIIA could

not recognize its binding site in a nucleosome containing the *Xenopus laevis* 5S gene. We demonstrate that the efficiency of binding of TFIIIA depends on the type and stoichiometry of histones associated with the 5S RNA gene and that association of a *X. borealis* 5S gene with a complete histone octamer significantly inhibits TFIIIA binding. Differences between our experiment and that of Rhodes (16) include our extensive use of purified TFIIIA rather than crude 7S storage particles treated with RNase (36). The 7S storage particles contain other protein complexes that fractionate with the TFIIIA/5S RNA particles (29). These proteins or free RNA may facilitate the binding of TFIIIA to 5S DNA in the nucleosome, perhaps by promoting the dissociation of histones H2A and H2B (37). However, our preparations of RNase-treated 7S particles in which RNA is clearly still present (Fig. 3*B*) behave identically to purified TFIIIA. Furthermore, in all our footprinting experiments, we have isolated individual complexes from nondenaturing gels, thus eliminating the possibility that mixtures of binary complexes could give the appearance of triple complex formation.

Why might trans-acting factors such as TFIIIA bind well to DNA organized with the $(H3/H4)_2$ tetramer but not with the octamer? We (22) and others (27) have shown that significantly less DNA is in contact with histones in the tetramer structure. Moreover, in the case of the nucleosome on the *X. borealis* somatic 5S gene, the additional DNA sequence that is in contact with the octamer contains the most important contacts for TFIIIA binding to DNA. Another difference between the tetramer and the octamer is the stability of the interaction with DNA. Thermal denaturation experiments have shown that the tetramer stabilizes much less DNA than the octamer (38). Thus, transcription factors such as TFIIIA might be able to compete much more effectively for a common binding site with the tetramer than with the octamer.

Relationship to Functional Studies and Physiological Significance. The consequences of chromatin assembly for 5S RNA gene transcription are perhaps the most thoroughly documented for any system. Early studies demonstrated that the prior addition of histones to naked DNA prevented transcription (39–41). More recent work in which the assembly of chromatin is carefully documented suggests that the efficiency of nucleosome assembly has to be very high before transcription is inhibited (9, 42, 43). In two instances, the assembly of a nucleosome over the *X. borealis* somatic 5S RNA gene was shown to correlate with the repressed state (5, 42). These results are consistent with our observation that TFIIIA will not interact with the 5S RNA gene in a nucleosome. Similar conclusions have been reached with the promoters of genes transcribed by RNA polymerase II (2, 6, 7, 10). However, it should be noted that whether or not a nucleosome is positioned on 5S DNA *in vivo* is not resolved (44).

Chromatin structure and transcription complexes are disrupted by DNA replication (11, 23). Each cell division event leads to competition between chromatin structural proteins and transcription factors for binding to DNA and a new opportunity for reestablishing or altering the state of gene expression (45). *Xenopus* cell-free extracts reproduce this competition *in vitro* (13, 43). In both *Xenopus* and mammalian cell-free extracts, chromatin assembly on replicating DNA occurs in stages, with a tetramer of histones H3 and H4 first rapidly associating with the newly replicated DNA (12, 43, 46). Next, histones H2A and H2B are assembled into the nascent chromatin to form ordered arrays of nucleosomes. This reproduces the chromatin assembly process believed to occur *in vivo* (47, 48). Once fully assembled into chromatin in these extracts, the *Xenopus* 5S RNA gene is refractory to the binding of transcription factors (8, 43). Thus, the core histones alone can be sufficient to establish a repressed state for 5S transcription such that the subsequent presence of transcription factors will not activate expression (8, 9). However, partially assembled chromatin is less refractory to 5S gene activation. DNA bound by only the $(H3/H4)_2$ tetramer can still be activated by class III factors (8, 13, 49). Thus, chromatin assembly is staged to present a window of opportunity for access of transcription factors to the promoter elements of genes. The failure of transcription factors to bind at this time would lead to the establishment of a state of repression following the sequestration of histones H2A and H2B and, later, of H1 (3, 11).

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