Histone–DNA Contacts in a Nucleosome Core Containing a *Xenopus* 5S rRNA Gene

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ABSTRACT: We describe histone–DNA cross-linking in a nucleosome core containing a *Xenopus borealis* somatic 5S rRNA gene. Histones H3 and H4 are cross-linked to DNA within 30 bp to either side of the dyad axis. Histones H2A/H2B and H3 are cross-linked to DNA where it enters and exits, wrapping around the histone octamer. These latter interactions extend for 80 bp to one side of the dyad axis of the nucleosome core, including the entire binding site for transcription factor TFIIIA. These extensive interactions with linker DNA might account for inhibition of TFIIIA binding and also might assist in the folding of internucleosomal DNA within the chromatin fiber.

Modification or mutation of individual histones has been found to influence the transcription of specific genes (Grunstein, 1990). Interpretation of these effects depends upon structural information concerning the position of individual histones within the nucleosome. Crystallographic analysis of nucleosome cores containing mixed DNA sequences reveals the general shape of the histone core around which DNA is wrapped (Richmond *et al.*, 1984). However, the assignment of individual histone–DNA contacts along DNA in the nucleosome core depends in large part upon results from histone–DNA cross-linking experiments (Mirzabekov *et al.*, 1977, 1982). Recently, crystallographic analysis of histone octamers has offered considerable insight into histone–histone interactions (Arents *et al.*, 1991). However, the exact organization of DNA as it is wrapped around the histone octamer and the consequences for histone structure and histone–histone interactions following association with DNA have not yet been determined.

We have examined the contribution of the core histones to the organization of DNA within a nucleosome core including part of the somatic rRNA gene of *Xenopus borealis* (Hayes *et al.*, 1990, 1991). Removal of the core histone tails using trypsin has no influence on the helical repeat of DNA within the nucleosome core or on the extent of histone–DNA interactions. Removal of histones H2A/H2B does not influence the helical repeat of DNA within the central 120 bp of DNA in the nucleosome core but reduces histone–DNA interactions outside of this region. Both of these modifications to the structure of the nucleosome core, including the *Xenopus* 5S rRNA gene, facilitate the association of transcription factor TFIIIA with the gene (Hayes & Wolffe, 1992; Lee *et al.*, 1993).

We have made use of the unique translational and rotational positioning of the histone octamer with respect to the *Xenopus* somatic 5S rRNA gene (Rhodes, 1985; Hayes *et al.*, 1990) and histone–DNA cross-linking methodologies (Levina *et al.*, 1981; Mirzabekov *et al.*, 1989) to map histone–DNA cross-links within the nucleosome core, including the 5S rRNA gene. Consistent with footprinting results (Hayes *et al.*, 1990, 1991), we provide evidence for a unique translational positioning of histone–DNA contacts, for interactions of histone H3 and histones H2A/H2B over the binding site for TFIIIA, and for histone–DNA interactions outside of the central 146 bp of DNA normally considered to interact with the core histones in the nucleosome.

MATERIALS AND METHODS

Reconstitution. DNA fragments containing the *X. borealis* 5S RNA gene were radiolabeled at a single restriction site. These included a 214-bp *Eco*RI–*Dde*I fragment from pXP-10 (Wolffe *et al.*, 1986) and 238-bp *Hpa*II–*Dde*I and 268-bp *Hpa*II–*Xba*I fragments from pXbs-1 (Peterson *et al.*, 1980). Each DNA fragment was reconstituted with histone octamers by salt dialysis using a 25-fold excess of chicken erythrocyte nucleosomal cores as a source of histones (Tatchell & van Holde, 1977). This procedure is known to result in a single predominant nucleosome positioned over the 5S RNA gene (Rhodes, 1985; Hayes *et al.*, 1990). Consistent with previous work on the influence of DNA length on histone–DNA contacts (Hayes *et al.*, 1991), no significant differences were detected in histone–DNA cross-linking using the three fragments of different length (not shown).

DNA–Protein Cross-Linking. In these experiments chemical cross-linking of histones to DNA is performed using DNA within the nucleosome core particles that has been end labeled with ^{32}P so that the position of histone–DNA contacts relative to the end of the molecule (as a reference point) can be determined. This is followed by reaction with dimethyl sulfate which methylates purine bases. The methylated product is then depurinated to an aldehyde. A Schiff base is formed between the modified DNA backbone and available lysine or histidine amino acids in the histones, which can be further stabilized by reduction with sodium borohydride (Levina *et al.*, 1981; Mirzabekov *et al.*, 1989). Very low levels of reaction are allowed so that a wide distribution of DNA–histone cross-links are generated. Exact reaction conditions are given below.

Methylation of DNA within the nucleoprotein complex was as described by Mirzabekov *et al.* (1989). This employed 10–25 mM dimethyl sulfate followed by incubation in 15 mM HEPES–NaOH, pH 7.2, and 0.1 mM EDTA, at 43 °C (resulting in partial depurination and subsequent cross-linking). The reaction was stopped by the addition of 25 mM $NaBH_4$ in 50 mM HEPES–NaOH, pH 7.2, at 0 °C, followed by dialysis into 15 mM HEPES–NaOH, pH 7.2, and 0.1 mM EDTA. We have previously shown that the 5S nucleosome core under these conditions is stable to protracted heating at

temperatures as high as 55 °C (Bashkin *et al.*, 1993). Nucleosome mobility on nondenaturing gels is dependent on the translational positioning of the histone octamer relative to the DNA fragment (Meersseman *et al.*, 1992). The cross-linking procedure itself (DMS treatment and borohydride reduction steps) does not alter the mobility of the 5S nucleosome core (not shown).

Purification of Cross-Linked Products. DNA with covalently attached proteins was ethanol precipitated in the presence of 0.5% SDS. The reaction was supplemented with 0.5 mg/mL unlabelled mixed-sequence nucleosome cores isolated from chicken erythrocytes as a carrier. The protein-tagged DNA fragments were extracted with 10 μL of phenol from a solution containing 0.25 M Tris-HCl, pH 8.0, 1 mM EDTA, and 0.25% SDS. Cross-linked DNA was recovered from the phenol layer by ethanol precipitation after dilution twice with 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and 1% SDS (TES) containing 0.15 mg/mL single-stranded random-sequence DNA fragments (made by DNase I digestion of chicken erythrocyte nuclei, with an average fragment length between 100 and 200 nucleotides) and dissolved in TES containing 9 M urea.

Two-dimensional electrophoresis was done as described (Mirzabekov *et al.*, 1989). For the electrophoresis leading to the gels and autoradiograms shown in this work, the first dimension requires the resolution of histone–DNA adducts, and the second dimension requires that of DNA, as described below. The first dimension (from left to right in the autoradiograms) requires denaturation of the histone–DNA complex by boiling in 1% SDS and 7 M urea and resolution of histone–DNA adducts on a 15% acrylamide gel containing 0.1% SDS and 7 M urea. The mobility of the DNA molecule is reduced by the cross-linked protein. Resolution in a second dimension (from top to bottom in the autoradiograms) follows removal of the histones from DNA with a protease; this allows sizing of the DNA fragments. The second dimension is again a 15% acrylamide gel containing 0.1% SDS and 7 M urea. The relative mobility of protein-bound and free DNA permits the organization of the histones relative to the end of DNA in the nucleosomal core particle to be determined.

In some of the experiments, Pronase was substituted for proteinase K. This did not influence the results. We found it essential to carefully control the power output for the first (concentrating) stage of the electrophoresis since low gel temperatures promoted 5S DNA renaturation, while high temperatures caused extensive breakdown of the residual methylated DNA bases. Assignment of spots of cross-linked DNA fragments to individual histones was based upon comparison with parallel runs of bulk cross-linked particles (Mirzabekov *et al.*, 1982; Bavykin *et al.*, 1985) and with the positions of G+A cleavage products in the gel.

RESULTS AND DISCUSSION

Histone–DNA Cross-Links around the Dyad Axis of the Nucleosome Core. Extensive experimental evidence suggests that the histone octamer adopts a single dominant translational position on the *X. borealis* 5S RNA gene, with the dyad axis of the nucleosome core positioned around the beginning of the 5S RNA gene sequence (the start site of transcription is defined as +1; Rhodes, 1985; Hayes *et al.*, 1990, 1991). Micrococcal nuclease mapping of the boundaries of the 5S nucleosome core (J. J. Hayes and A. P. Wolffe unpublished results) reveals these to be at −70 and +79, suggesting that the dyad axis of the core will be around +10. Three helical turns of 10.7 bp/turn occur across the dyad axis of the nucleosome core around the transcriptional start of the 5S RNA gene. These cause a 2-bp discontinuity between regions of 10.0 bp/turn at the periphery of the nucleosome core (Hayes *et al.*, 1990, 1991). The minor groove faces away from the histone octamer at the dyad axis (Richmond *et al.*, 1984) and toward the octamer 0.5 and 1.5 turns to either side of the dyad axis. The minor groove of DNA faces toward the histone octamer at −7, +5, +15, and +25 with respect to the bottom strand and at −10, +2, +12, and +22 for the top strand (Hayes *et al.*, 1990, 1991).

Mirzabekov and colleagues have documented histone–DNA cross-linking around the dyad axis of nucleosome cores containing mixed sequences of DNA that are approximately 146 bp in length (Shick *et al.*, 1980; Bavykin *et al.*, 1985; Ebralidse *et al.*, 1988). Important features of nucleosomal structure determined from these studies include the presence of histone H4 cross-linking to DNA over 30 bp to either side of the nucleosomal dyad axis. Of particular importance are two sites of strong cross-linking between histone H4 and DNA at a distance of about 1.5 helical turns to either side of the dyad axis. At each of these sites a highly basic histone H4 domain is in close association with both strands of DNA (Ebralidse & Mirzabekov, 1986; Ebralidse *et al.*, 1988). It is at these sites that DNA in the nucleosome core appears to be more sharply bent (Richmond *et al.*, 1984). Within the 5S nucleosome core around the presumed dyad axis (Rhodes, 1985; Hayes *et al.*, 1990), histone H4 cross-linking is detected between −45 and +39 on the bottom DNA strand (Figure 1A, closed arrows) and between −11 and +31 on the top strand (Figure 1B, closed arrows). The bottom strand contains the coding sequence of the 5S RNA gene and the top strand the noncoding sequence. Within this region of the 5S nucleosome core, histone H4 contacts at a distance of 1.5 turns from the dyad axis, consistent with those previously described for mixed-sequence nucleosomes cores, are at +29 and −10 on the bottom strand and at +27 and −11 on the top strand (see Figure 1).

A second feature of histone–DNA interaction around the dyad axis of the nucleosome core is strong histone H3 cross-linking (Bavykin *et al.*, 1985). Within the 5S nucleosome core, histone H3 cross-linking to DNA is especially apparent between +6 and +12 on the bottom DNA strand (Figure 1A, open arrow) and at +31 on the top strand (Figure 1B, open arrow). Finally, histone H2A has been reported to be cross-linked to DNA at the dyad axis of the nucleosome core (Bavykin *et al.*, 1985) apparently through its C-terminal tail (Gushchin *et al.*, 1991). Within the 5S nucleosome core, H2A is cross-linked to 5S DNA around +9/+10 on the bottom strand (Figure 1A, asterisk) and +7/+8 with the top strand (Figure 1B, asterisk).

The nuclease cleavage data (Rhodes, 1985; Hayes *et al.*, 1990, 1991), taken together with our own data on histone H4, H3, and H2A cross-linking to DNA and the results of Mirzabekov and colleagues using mixed-sequence nucleosome cores (Mirzabekov *et al.*, 1982; Bavykin *et al.*, 1985), lead us to suggest that the dyad axis of the 5S nucleosome core occurs where the minor groove faces away from the histone octamer at +10 on the bottom strand and at +7 on the top strand. The distribution of major histone–DNA contacts around the dyad axis of the 5S nucleosome core is summarized in Figure 3A.

Histone–DNA Cross-Links at the Periphery of the Nucleosome Core. Micrococcal nuclease digestion of linker–histone-depleted chromatin established that strong histone–DNA contacts exist approximately 75 bp to either side of the dyad axis (Noll & Kornberg, 1977). These contacts are not clearly resolved in histone–DNA cross-linking experiments

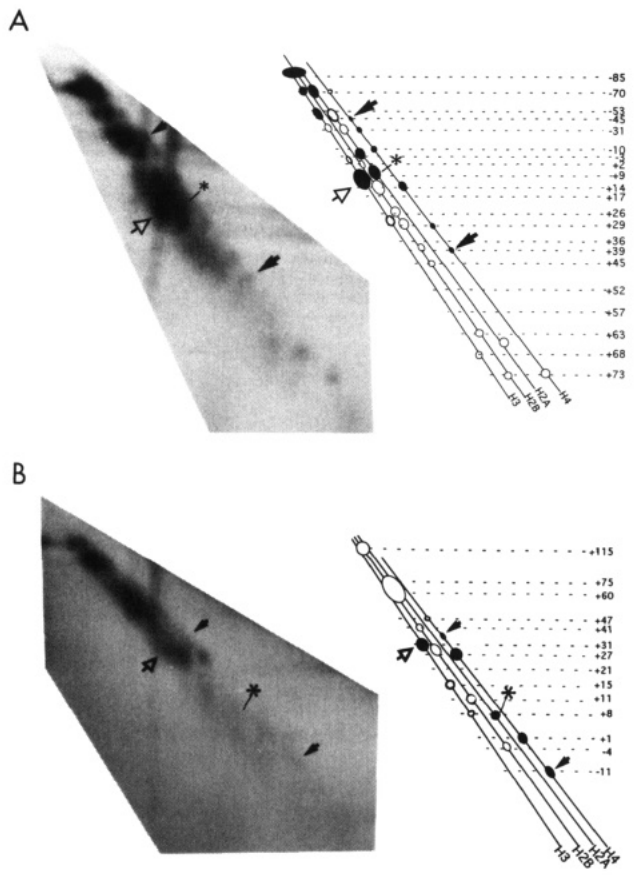

FIGURE 1: Mapping of histone–DNA cross-links around the dyad axis of the 5S nucleosome core. (A) Histone–DNA cross-links on the bottom (coding) strand of the *X. borealis* somatic 5S RNA gene. The left-hand panel shows the two-dimensional electrophoretic map adjacent to its schematic interpretation. Sites of histone–DNA cross-linking are indicated by ellipsoids on a linear projection corresponding to each histone. Closed ellipsoids indicate spots of cross-linked DNA fragments discussed in the text; open ellipsoids indicate other sites of histone–DNA cross-linking. The numbers listed vertically are markers referring to base-pair position within the *X.borealis* 5S RNA gene relative to the start site of transcription (+1). They derive from parallel G+A Maxam–Gilbert sequencing reactions. In all of the gels leading to the autoradiograms shown in the figures, the first dimension was from left to right and resolved DNA–histone adducts, whereas the second dimension was from top to bottom and resolved DNA fragments from which the histones had been removed (see Materials and Methods). The closed arrows indicate the 5′ and 3′ boundaries of histone H4 cross-linking to the 5S RNA gene around the dyad axis of the nucleosome core. The open arrow indicates major sites of histone H3 cross-linking to DNA around the dyad, and the asterisk indicates a major site of histone H2A cross-linking to DNA around the dyad. (B) Histone–DNA cross-links on the top (noncoding) strand of the *X. borealis* somatic 5S RNA gene. Representation and annotation are as described in (A).

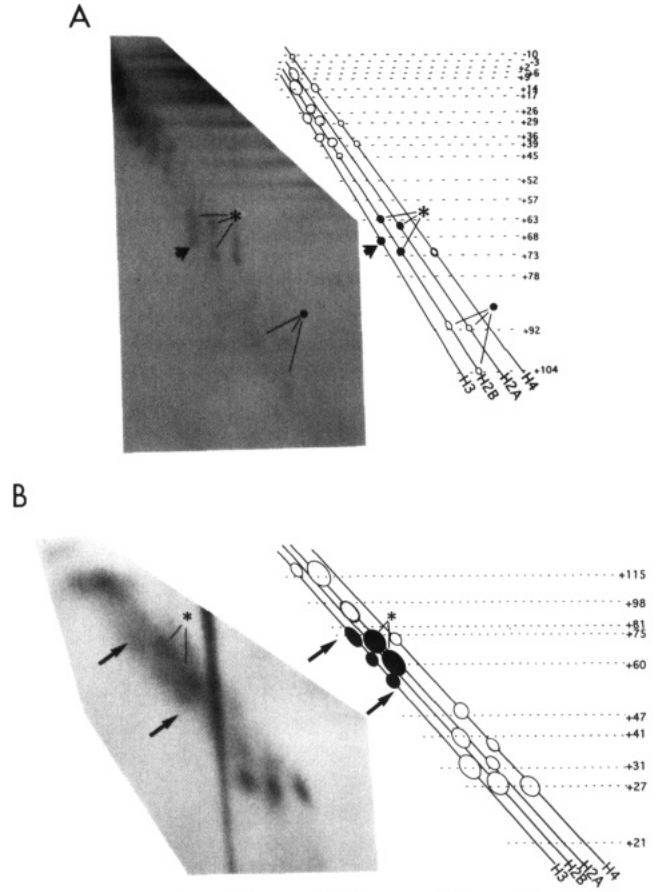

FIGURE 2: Mapping of histone–DNA cross-linking at the periphery of the 5S nucleosome core. (A) Histone–DNA cross-links on the bottom (coding) strand of the *X. borealis* somatic 5S RNA gene. The left-hand panel shows the experimental result adjacent to a schematic interpretation. Sites of histone–DNA cross-linking are indicated by ellipsoids on a linear projection corresponding to each histone. Closed ellipsoids indicate sites of cross-linking discussed in the text. The numbers listed vertically are markers referring to base-pair positions within the *X. borealis* 5S RNA gene relative to the start site of transcription (+1). They derive from parallel G+A Maxam–Gilbert sequencing reactions. The closed arrow indicates a major site of histone H3 cross-linking to DNA at the periphery of the nucleosome core. The lines drawn from the asterisk denote major sites of histone H2A and H2B cross-linking to DNA in this region. The lines drawn from the closed circle denote sites of histone H2A and H2B cross-linking around a key recognition site for transcription factor TFIIIA (between +81 and +91). (B) Histone–DNA cross-links on the top (noncoding) strand of the *X. borealis* somatic 5S RNA gene. Representation as in (A). The closed arrows denote boundaries of histone H3 cross-linking, and the lines drawn to the asterisk denote histone H2A/H2B cross-links at the periphery of the core.

using mixed-sequence nucleosomes (Shick *et al.*, 1980; Bavykin *et al.*, 1985). However, histone H3 appears to be cross-linked to one strand at the end of the particle (Belyavsky *et al.*, 1980; Bavykin *et al.*, 1990).

The use of longer DNA fragments in our experiments than previously examined by Mirzabekov and colleagues allows for the first time an accurate assessment of histone–DNA cross-linking at the periphery of the structure. At one boundary of the 5S nucleosome core, histone H3 is cross-linked with the bottom strand at +68 (Figure 2A, closed arrow) and with the top strand between +55 and +75 (Figure 2B, closed arrows). On the bottom strand the minor groove faces the octamer at +65 and +75 and on the top strand at +62 and +72. We can therefore extend the interpretation of Mirzabekov and colleagues to conclude that histone H3 can be cross-linked to both strands of DNA at one boundary of the 5S nucleosome core. At the other end of the 5S nucleosome core, strong H3 cross-linking to DNA is apparent at −70 (bottom strand) (Figure 1A). Histone H2A/H2B cross-linking to DNA in this region is also apparent. Although we can discriminate easily between histone H3 and histone H4 contacts, at the level of resolution achieved in our experiments it is not possible to discriminate in this region of the gel between sites of individual histone H2A or H2B cross-linking to DNA. At the dyad axis and toward the 3′ boundary of the nucleosome core, it is possible to clearly discriminate between DNA cross-linking to either histone H2A or H2B. These sites of cross-linking to histone H3 are in good agreement with the limits of the 5S nucleosome core at −70 and +77 as defined by micrococcal release (J. J. Hayes and A. P. Wolffe, unpublished results). Histones H2A and H2B are also cross-linked to

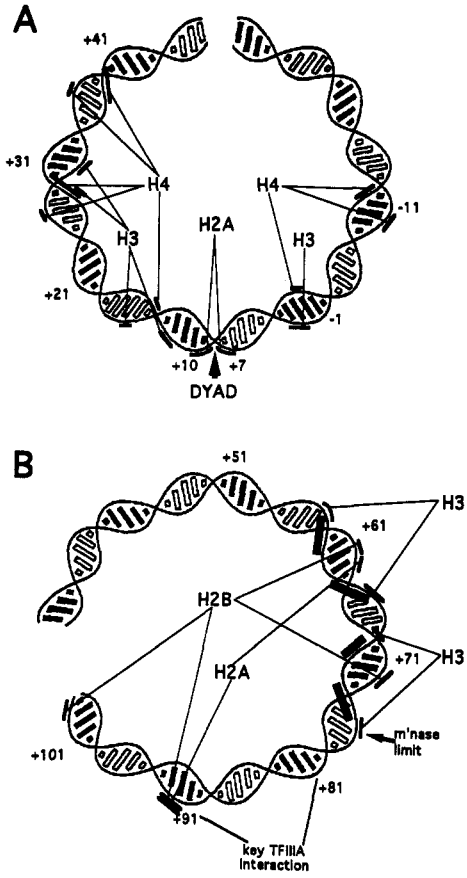

FIGURE 3: Model for histone–DNA contacts in the 5S nucleosome core. (A) Histone–DNA contacts around the dyad axis. DNA is shown in an 80-bp circle; base pairs are indicated by open and closed retangular blocks (Calladine & Drew, 1986). The numbers indicate base-pair positions within the *X. borealis* somatic 5S RNA gene relative to the start site of transcription (+1). The dyad axis is indicated. Histone–DNA cross-links are indicated by closed bars. (B) Histone-DNA contacts at the 3′ boundary of the 5S nucleosome core. The numbers indicate base-pair positions within the X. *borealis* somatic 5S RNA gene. Histone H2A and H2B contacts to the coding strand are indicated by small bars and those to the noncoding strand by large boxes. Histone H3 contacts are indicated. The major kinetic barrier to micrococcal nuclease digestion caused by the nucleosome core is indicated, as is the key TFIIIA recognition element.

DNA on the bottom strand between +63 and +73 (Figure 2A, asterisk) and on the top strand between +60 and +75 (Figure 2B, asterisk). These cross-links around the +77 micrococcal nuclease limit of digestion of the 5S nucleosome core are summarized in Figure 3B. Cross-linking of histone H4 to DNA also appears to occur at both termini of the nucleosome core.

When a single histone tetramer is reconstituted onto the 5S RNA gene, histones H3 and H4 do not appear to contact DNA in these regions (Hayes *et al.*, 1991); hence, it seems probable that histone H2A/H2B participation is necessary to bring the terminal DNA segments into proximity with the major tissue histone H3/H4 binding sites around the dyad. These contacts may also be a consequence of interaction of the histone H3 and H4 N-terminal tails with DNA, as it enters and exits, wrapping around the histone octamer. Thus contacts of DNA with the histone dimers, H2A/H2B, may be necessary to allow histone H3 to make contact with DNA where it begins wrapping around the nucleosome core.

Histone–DNA Cross-Links over the Binding Site for TFIIIA. DNase I cleavage of the 5S nucleosome core reveals that DNA is rotationally organized on the surface of the histone octamer at least until +75 relative to the start site of 5S RNA gene transcription (+1) (Rhodes, 1985; Hayes *et al.*, 1990). These contacts are more limited that those revealed using hydroxyl radical, probably because of the potentially disruptive action of DNase I in displacing relatively weak histone–DNA contacts (Hayes *et al.*, 1990; Hayes & Clemens, 1992). Hydroxyl radical cleavage shows that DNA is rotationally organized on the histone octamer as far as +100 (Hayes *et al.*, 1990, 1991), a distance of approximately 90 bp from the dyad axis. This result would imply that histone–DNA contacts might exist over as much as 180 bp of DNA. That such extensive interaction may occur has been previously suggested following analysis of histone–DNA contacts in linker DNA (Karpov *et al.*, 1982; Bavykin *et al.*, 1990) and micrococcal nuclease digestion analysis of chromatin depleted of linker histones (Todd & Garrard, 1977; Albright *et al.*, 1980).

Transcription factor TFIIIA has key interactions with a 5S RNA gene over one helical turn of DNA between +81 and +91 (Hayes & Clemens, 1992; Hayes & Tullius, 1992). This region is responsible for contacting the three N-terminal zinc fingers of the protein; these contacts are responsible for 90% of the binding energy of the protein (Liao *et al.*, 1992). TFIIIA will not interact with a 5S nucleosome core assembled with unmodified core histones, presumably because the region from +81 to +91 is in contact with histones (Hayes & Wolffe, 1992; Lee *et al.*, 1993). In fact, within the 5S nucleosome, histones H2A and H2B are cross-linked to 5S DNA between +91 and +99 on one of the DNA strands (Figure 2A, closed circle). It is these contacts that presumably inhibit TFIIIA interaction with 5S DNA in this nucleosome core (Hayes & Wolffe, 1992; see Figure 3B).

It has been suggested that the extended core histone–DNA contacts are a consequence of a superhelical arrangement of linker DNA between nucleosomes (Bavykin *et al.*, 1990). Indeed, short internucleosomal DNA has been shown to readily fold under the ionic conditions used in these experiments in the absence of histone H1 (Hansen *et al.*, 1989; Yao *et al.*, 1991). Our results suggest that a large part of this compaction in internucleosomal DNA length could be due to the interaction of this DNA with a single histone octamer.

CONCLUSIONS

We have mapped the cross-linking of histones to DNA following reconstitution of a single histone octamer with short DNA fragments containing the *X. borealis* somatic 5S RNA gene. The histone octamer assumes a single major translational position with respect to DNA sequence with the dyad axis located between +7 and +10 relative to the start site of 5S RNA gene transcription (+1). Histones H3 and H4 make contact with DNA within 30 bp to either side of the dyad axis. At the periphery of the nucleosome core, histones H3 and H2A/H2B make contact with DNA on both strands. The translational positioning of the histone octamer relative to the 5S RNA gene brings the binding site for TFIIIA into contact with the core histones. In particular, histone H3 and histones H2A/H2B are cross-linked to this binding site between +91 and +99. These cross-links are more than 80 bp away from the dyad axis of the nucleosome core and suggest that considerable portion of internucleosomal DNA may have some interaction with the core histones. Future studies will explore this possibility and examine the consequences of histone depletion (Hayes & Wolffe, 1992) or histone modification (Lee *et al.*, 1993) on histone–DNA cross-linking and transcription factor accessibility to DNA in the nucleosome.

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