Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 2339–2343, March 1994
Biochemistry

A role for histones H2A/H2B in chromatin folding and transcriptional repression

(nucleosome/H3/H4 tetramer/RNA polymerase III/5S rRNA genes/analytical ultracentrifugation)

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Communicated by Jack Gorski, December 20, 1993 (received for review October 22, 1993)

ABSTRACT **Histone octamers or histone H3/H4 tetramers were reconstituted onto either closed circular plasmids containing a single *Xenopus* 5S rRNA gene or a reiterated array of *Lytechinus* 5S rRNA genes. All "reconstitutes" were found to undergo both Na^+-dependent and Mg^{2+}-dependent compaction. However, in each case, the compaction of nucleosomal templates containing H2A/H2B was much more extensive than compaction of templates containing only H3/H4 tetramers. Inclusion of 5 mM $MgCl_2$ in the transcription buffer increased the level of compaction of nucleosomal templates and led to a marked inhibition of both transcription initiation and elongation by RNA polymerase III. The inhibitory effect of Mg^{2+} was reduced significantly when DNA templates contained only H3/H4 tetramers, consistent with their lesser extent of Mg^{2+}-dependent compaction. Thus, the removal of histones H2A/H2B from nucleosomal arrays enhances gene activity, in part because of decreased levels of chromatin folding.**

The packaging of DNA into chromatin imposes several constraints to the recognition of specific sequences by transacting factors and to the movement of processive enzymes along the DNA molecule. These constraints have been most extensively explored for the transcription process (1, 2). At the level of the nucleosome, it is clear that DNA remains associated with histones during transcription of a gene *in vivo* (3–5) and that nucleosomal structures reassemble after passage of RNA polymerase II along a nucleosomal array (6–10). However, in many cases the histone composition of transcribed chromatin appears to be different than inactive chromatin. For example, histones H2A/H2B are deficient in transcriptionally active chromatin (11–13). Histones H2A/H2B also exchange more readily out of transcribed chromatin *in vivo* (14, 15). While it is not known whether changes in H2A/H2B composition precede or are a consequence of the passage of RNA polymerase through nucleosomes, it has recently been shown that their removal of these histones facilitates the accessibility of nucleosomal DNA to DNA binding proteins (16).

An additional level of constraint to the transcriptional machinery is imposed by the structural dynamics of the chromatin fiber. Several groups have begun to investigate this problem by using biochemically defined DNA templates containing promoter sequences that are reconstituted into defined nucleosomal arrays (10, 17). One of the advantages of these DNA templates is that they allow the simultaneous determination of the contributions of the core histones to higher-order chromatin folding and transcription by RNA polymerases (10). We have previously determined that salt-dependent compaction of linear and circular nucleosomal arrays can severely repress both transcription initiation and elongation by RNA polymerase III (10). These results suggest that chromatin folding may be a major factor in the inhibition of transcription observed with nucleosomal templates under standard transcription reaction conditions. In this study, we extend these observations to show that removal of histones H2A/H2B from nucleosomal arrays has a major influence both on chromatin compaction and in repression of RNA polymerase III transcription initiation and elongation.

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EXPERIMENTAL PROCEDURES

Materials. Whole chicken blood was obtained from Pel-Freez Biologicals and used as the source of histone octamers and H3/H4 tetramers. Female *Xenopus laevis* were obtained from Xenopus I (Ann Arbor, MI). Plasmid DNAs and the 208-12 DNA template were purified as described (18). The plasmids pXP10 containing the *Xenopus borealis* somatic 5S rRNA gene and E190 containing the *X. laevis* satellite I DNA gene have been described (19, 20). Radioisotopes were obtained from NEN.

Preparation of Histones. Histone octamers from adult chicken erythrocytes were purified as described (18). Histones H3/H4 were prepared from histone octamers by hydroxylapatite chromatography and with salt gradients to elute histone groups H2A/H2B and H3/H4 in a stepwise manner (21). Histones were quantitated spectrophotometrically at 230 nm. The core histone fractions contained $<<1\%$ histones H1/H5 as determined by SDS/PAGE.

Chromatin Reconstitution. Histone octamers or tetramers were reconstituted onto DNA by using the salt dialysis protocols as described (10).

Boundary Sedimentation. Boundary sedimentation velocity studies were performed as described (10), except that experiments were carried out in a Beckman Optima XL-A analytical ultracentrifuge. Scans were analyzed by the method of van Holde and Weischet (22) to obtain the integral distribution of sedimentation coefficients using the Ultrascan XL-A data analysis software program (B. Demeler, Missoula, MT).

Transcription and Sedimentation Buffers. Core buffer contained 10 mM NaHepes (pH 7.5), 2 mM dithiothreitol, 0.1 mM NaEDTA, and 5% (vol/vol) glycerol. Low Mg^{2+} buffer consisted of core buffer plus 50 mM KCl, 2 mM $MgCl_2$, and 0.5 mM each ATP, GTP, CTP, and UTP. High Mg^{2+} buffer contained the same components as low Mg^{2+} buffer, except the $MgCl_2$ concentration was 7 mM. The free Mg^{2+} concentration in low Mg^{2+} and high Mg^{2+} buffer was calculated to be 0.1 and 5 mM, respectively (10). For the sedimentation experiments, the nucleoside triphosphates in low and high Mg^{2+} buffer were replaced with 2 mM Na_5PPP_i.

***In Vitro* Transcription.** Transcription experiments were performed with extracts of *X. laevis* oocyte nuclei as described (10).

RESULTS

Micrococcal Nuclease Digestion of Octamer and Tetramer "Reconstitutes." In these studies we make use of two DNA templates. The pXP10 template is a small plasmid DNA containing a single *X. borealis* 5S RNA gene. This template previously was used to examine transcription initiation by RNA polymerase III (10, 19, 23). It does not assemble histone octamers or tetramers onto DNA with a physiological spacing (i.e., 180–200 bp); instead, histone–DNA complexes pack together as closely as possible (23). The second template, which is termed 208-12 DNA, consists of 12 tandem repeats of a *Lytechinus* 5S rRNA gene (24). Each 208-bp repeat will position a histone tetramer (25) or a histone octamer (26–28) and also contains a viable class III promoter. However, the termination signal has been deleted. This template can be assembled into nucleosomal arrays that have the correct physiological spacing. The folding properties of this template have been explored extensively (10, 18, 29, 30). It previously has been used for studies of transcription elongation by RNA polymerase III (10).

The presence of histone octamers or H3/H4 tetramers on the pXP10 and 208-12 DNA templates after reconstitution was verified by micrococcal nuclease protection. Both templates, when reconstituted with histone octamers, protected ≈146 bp of DNA after extensive micrococcal nuclease digestion (Fig. 1). In contrast, templates reconstituted with H3/H4 tetramers protected only ≈70 bp (Fig. 1). The latter result would be expected for micrococcal nuclease digestion of a DNA–H3/H4 tetramer complex (25, 31, 32). We therefore conclude that our reconstitutes have the properties expected for DNA molecules complexed with either histone octamers or H3/H4 tetramers.

Folding of Octamer and Tetramer Reconstitutes in Low and High Mg^{2+} Buffers. We have previously shown that 208-12 and pXP10 DNA templates reconstituted with histone octamers undergo compaction in both low and high Mg^{2+} buffers (10). In both cases, the extent of folding in high Mg^{2+} buffer was significantly greater than in low Mg^{2+} buffer. This Mg^{2+}-dependent increase in the level of chromatin folding was correlated with a Mg^{2+}-dependent inhibition of transcription (10). To investigate the individual roles of H2A/H2B dimers and H3/H4 tetramer in chromatin folding and transcriptional repression, we first examined whether DNA molecules reconstituted with only H3/H4 tetramers would be capable of folding in low Mg^{2+} and high Mg^{2+} buffers. Chromatin folding was indicated by a salt-dependent increase in sedimentation coefficient as determined by sedimentation velocity experiments in the analytical ultracentrifuge (10, 18, 29, 30).

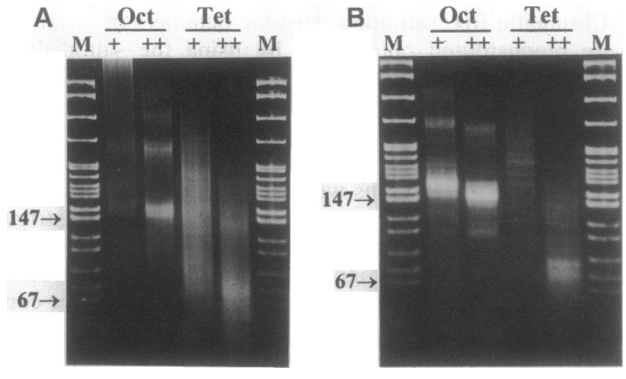

FIG. 1. Micrococcal nuclease digestion of octamer and tetramer reconstitutes. Either histone octamers (Oct) or H3/H4 tetramers (Tet) were reconstituted at $r = 1.0$ onto circular pXP10 (*A*) or linear 208-12 (*B*) DNA molecules as described. Each reconstitute was digested with micrococcal nuclease (0.08 unit per μg of DNA) for either 5 min (lanes +) or 12 min (lanes ++) at 37°C. The DNA and $CaCl_2$ concentrations in the reaction mixture were 60 μg/ml and 1.25 mM, respectively. Aliquots (0.6 μg) of each digest were deproteinized in 1% SDS (30 min; 37°C) and electrophoresed on a 5% native polyacrylamide gel, shown is the photograph obtained from the ethidium-stained gel. Lanes M, pBR322/*Msp* I size standards. Position of 67- and 147-bp bands are indicated to the left of each gel.

Fig. 2 shows the influence of salts on the sedimentation coefficient distributions of linear 208-12 octamer and tetramer reconstitutes. The 208-12 DNA templates were reconstituted with 1.0 mol of histones per mol of 208-bp DNA ($r = 1.0$). Based on the distributions obtained in core buffer (Fig. 2, ● and ○) and values established previously (18, 30, 33), this produced preparations of octamer and tetramer reconstitutes in which ≈10% of the templates were saturated with 12 octamers or tetramers (cumulative fraction, ≥0.9), and 90% of the templates contained 9–11 octamers or tetramers (cumulative fraction, <0.9). Relative to the $s_{20,w}$ values obtained in core buffer, the octamer reconstitutes showed 15–30% increases in $s_{20,w}$ in low Mg^{2+} buffer and 25–60% increases in $s_{20,w}$ in high Mg^{2+} buffer. These $s_{20,w}$ increases were consistent with those obtained previously for saturated and slightly subsaturated 208-12 nucleosomal arrays under similar ionic conditions (10, 18, 30). The H3/H4 tetramer reconstitutes also showed increases in $s_{20,w}$ in low and high Mg^{2+} buffers, although in each case the magnitude of the $s_{20,w}$ increase was only approximately half that seen for octamer reconstitutes (Fig. 2). Thus, linear arrays of H3/H4 tetramers appear capable of salt-dependent compaction, albeit at significantly reduced levels compared to octamer arrays. Whereas most of the tetramer reconstitutes showed salt-dependent increases in $s_{20,w}$ in high Mg^{2+} buffer, under these conditions ≈10% of the H3/H4 tetramer reconstitutes sedimented at ≈11 S (Fig. 2, arrow), which is the sedimentation coefficient of free 208-12 DNA (18). This indicates that H3/H4 tetramers quantitatively dissociate from a small fraction of the linear 208-12 templates in high Mg^{2+} buffer. In contrast, the octamer arrays did not dissociate in high Mg^{2+} buffer (Fig. 2), consistent with previous results (10).

We next tested the folding capabilities of circular H3/H4 tetramer arrays. For these experiments, either histone octamers or H3/H4 tetramers were reconstituted onto a preparation of pXP10 plasmid DNA that was ≈60% closed circular (and highly negatively supercoiled) and ≈40% open circular (nicked). Reconstitutes initially were analyzed by

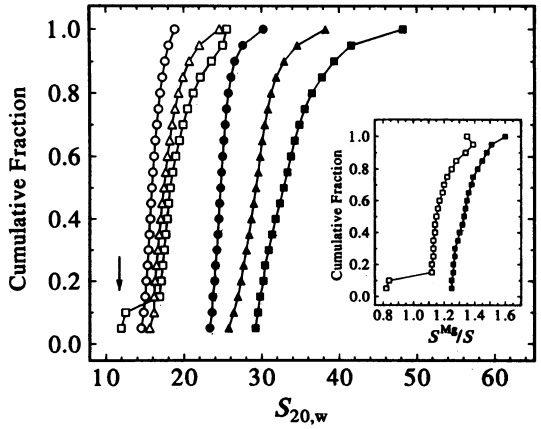

FIG. 2. Sedimentation velocity analysis of linear 208-12 reconstitutes in core, low Mg^{2+}, and high Mg^{2+} buffers. The $r = 1.0$ 208-12 tetramer (open symbols) and octamer (solid symbols) reconstitutes from Fig. 1 were sedimented in core (○, ●), low Mg^{2+} (△, ▲), and high Mg^{2+} (□, ■) buffers as described. Shown are the integral distribution of $s_{20,w}$. (*Inset*) Ratio of $s_{20,w}$ in high Mg^{2+} buffer relative to $s_{20,w}$ in core buffer (s^{Mg}/s) over the entire $s_{20,w}$ distribution.

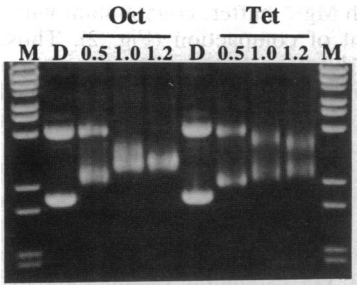

FIG. 3. Native agarose gel electrophoresis of circular pXP10 reconstitutes. pXP10 DNA (lanes D) was reconstituted with either histone octamers (Oct) or H3/H4 tetramers (Tet) at the indicated r values. Aliquots (0.6 μg) were then electrophoresed on a 1% native agarose gel as described (10). Lanes M, λ/*Bst*EII size markers.

native agarose gel electrophoresis (Fig. 3). When reconstituted at r = 0.5, both the H3/H4 tetramers and histone octamers associated exclusively with the negatively supercoiled DNA, as indicated by the decreased mobility of only the closed circular DNA band in 1.0% agarose gels. This demonstrates that the H3/H4 tetramer has a marked preference for negatively supercoiled DNA. At r = 1.0, both the closed circular tetramer and octamer reconstitutes were approaching saturation with histones, as evidenced by the similar mobilities of the respective closed circular bands at r = 1.0 and 1.2 (Fig. 3). In contrast, the open circular molecules, which showed increased mobilities after binding histones, were underloaded with histones at r = 1.0. The reasons for the differential electrophoretic behavior of the open circular and closed circular reconstitutes in 1.0% agarose gels are unclear and will require quantitative analysis such as that described recently for linear chromatin molecules (34).

Sedimentation analysis indicated that the folding of the r = 1.0 pXP10 octamer and tetramer reconstitutes closely resembled that of the 208-12 reconstitutes. Relative to the $s_{20,w}$ obtained in core buffer, the $s_{20,w}$ of the closed circular octamer reconstitutes (cumulative fraction, >0.4) increased by 20–40% in low Mg^{2+} buffer and by 40–60% in high Mg^{2+} buffer (Fig. 4). In contrast, the $s_{20,w}$ of the closed circular H3/H4 tetramer reconstitutes increased by only 15% in low Mg^{2+} buffer and by 25% in high Mg^{2+} buffer. A similar situation was observed for the more slowly sedimenting open circular reconstitutes (cumulative fraction, <0.4); in high Mg^{2+} buffer, the $s_{20,w}$ of the open circular octamer reconstitutes increased by 20%, compared to only 10% for the open circular tetramer reconstitutes (Fig. 4).

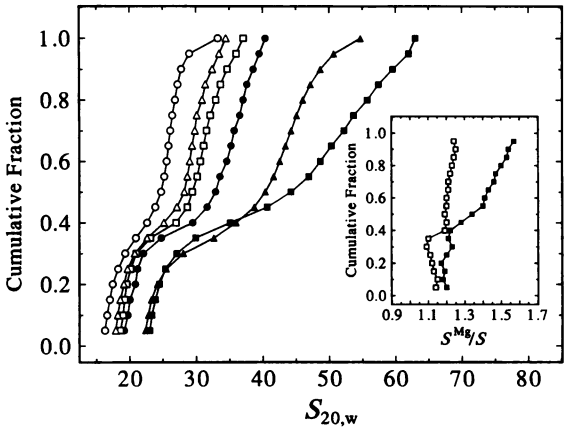

FIG. 4. Sedimentation velocity analysis of pXP10 reconstitutes in core, low Mg^{2+}, and high Mg^{2+} buffers. The r = 1.0 pXP10 octamer (solid symbols) and tetramer (open symbols) reconstitutes from Fig. 1 were sedimented in core (○, ●), low Mg^{2+} (△, ▲), and high Mg^{2+} (□, ■) buffers as described. Shown are the integral distributions of $s_{20,w}$. (*Inset*) Ratio of $s_{20,w}$ in high Mg^{2+} buffer relative to $s_{20,w}$ in core buffer (s^{Mg}/s) over the entire $s_{20,w}$ distribution.

Transcription Initiation from Octamer and Tetramer Reconstitutes in Low and High Mg^{2+} Buffers. The r = 1.0 pXP10 reconstitutes described above were used to examine whether initiation of transcription from H3/H4 tetramer arrays would be sensitive to free Mg^{2+} in the same manner as the octamer reconstitutes. As an internal control, the pXP10 reconstitutes were mixed with a naked DNA template encoding a satellite 1 transcript. In all cases, the level of satellite transcription was the same in low and high Mg^{2+} buffer (Fig. 5). The r = 1.0 octamer reconstitutes were >95% repressed in low Mg^{2+} buffer and completely repressed in high Mg^{2+} buffer (Fig. 5, lanes 2 and 3, respectively). Predigestion of the octamer reconstitutes with *Eco*RV, which cleaves within histone-free but not histone-occupied 5S promoters (23), completely abolished the small amount of 5S transcription signal present in low Mg^{2+} buffer (unpublished results). Thus, only the small fraction of 5S promoters that were free of histone octamers were capable of supporting transcription initiation in these experiments. These data demonstrate that most of the transcriptional repression observed in low Mg^{2+} buffer resulted from promoter occupancy by histone octamers. However, in those cases in which the promoter was free of histones, the increased folding that occurs in high Mg^{2+} buffer (Fig. 4) also led to repression of transcription initiation.

Although some histone-specific inhibition of transcription initiation was observed from the r = 1.0 H3/H4 tetramer reconstitutes (Fig. 5, compare lane 1 with lanes 4 and 5), the level of inhibition was substantially less than the inhibition observed from octamer reconstitutes. In addition, the transcription signal from the tetramer reconstitutes did not show the Mg^{2+}-dependent decrease observed for the octamer reconstitutes; if anything, the signal increased slightly (Fig. 5, compare lanes 4 and 5). The lack of a Mg^{2+}-dependent decrease in transcription is consistent with the markedly reduced levels of Mg^{2+}-dependent compaction of the tetramer reconstitutes. The slight increase could be due to increased instability of the tetramer–DNA complex in high Mg^{2+} buffer (see Fig. 2). Unlike the octamer reconstitutes, a significant amount of transcription was detected after predigestion of the tetramer reconstitutes with *Eco*RV (unpub-

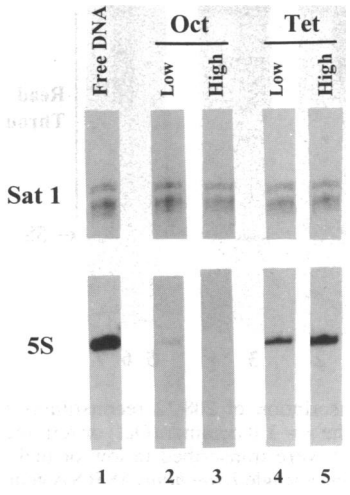

FIG. 5. Transcription of pXP10 reconstitutes in low and high Mg^{2+} buffers. The r = 1.0 pXP10 octamer (Oct) or tetramer (Tet) reconstitutes from Fig. 1 were mixed with naked satellite 1 DNA and transcribed in either low or high Mg^{2+} buffer as described. The satellite 1 (Sat 1) DNA transcripts resulted from a 12-hr exposure and the 5S RNA gene transcripts resulted from a 4-hr exposure of the same gel.

lished results). This indicates that when bound over the gene, H3/H4 tetramers are less effective than histone octamers at preventing access of transcription factors to 5S DNA. In summary, the data shown in Fig. 5 demonstrate that reconstitution of 5S DNA with H3/H4 tetramers leads to less repression of transcription initiation than reconstitution of 5S DNA with histone octamers. This appears to result from both enhanced accessibility of the histone-occupied promoter to transcription factors and substantially reduced levels of folding in both low and high Mg^{2+} buffer.

Transcription Elongation Through Octamer and Tetramer Reconstitutes in Low and High Mg^{2+} Buffers. The ability of RNA polymerase III to elongate through octamer and tetramer reconstitutes was examined in low and high Mg^{2+} buffer. The principle of this experiment is to make use of a terminator-less *Lytechinus* 5S rRNA gene, which is tandemly repeated 12 times in the 208-12 DNA template. Transcription of the 208-12 DNA in a *Xenopus* oocyte nuclear extract under low Mg^{2+} conditions produced a series of large (up to 2500 nt) transcripts (Fig. 6, lane 6). These are designated read-through transcripts and result from initiation at one of the 12 promoters followed by elongation to the end of the DNA template. These RNA species represent bona fide transcripts initiated from the 5S promoters; no transcription was observed from an analogous template, 172-12, composed of 12 tandem repeats of a deletion mutant of *Lytechinus* 5S DNA lacking key internal promoter elements (lane 5). A naked *Lytechinus* 5S gene (lane 7) was included in all reactions as an internal control. Although somewhat variable, the difference in transcription of the naked 5S gene in low and high Mg^{2+} buffer was not significant.

Transcription of $r = 1.0$ octamer reconstitutes in low Mg^{2+} buffer produced significant amounts of read-through transcripts (Fig. 6, lane 1). This indicates that histone octamers did not prevent elongation by RNA polymerase III, even though the DNA was uniformly assembled into arrays of nucleosomal structures, and the array was partially folded in low Mg^{2+} conditions (Fig. 2). However, a major inhibition of transcription elongation of octamer reconstitutes was observed in high Mg^{2+} buffer, concomitant with a significantly greater extent of compaction (Fig. 2). Thus, as observed previously (10), the decreased transcriptional elongation through octamer reconstitutes observed in high Mg^{2+} buffer was correlated with Mg^{2+}-dependent compaction of the nucleosomal arrays.

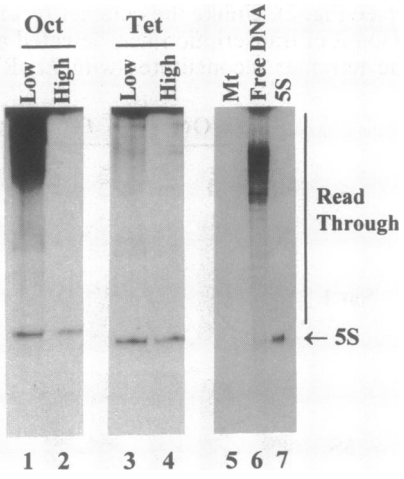

FIG. 6. Transcription of 208-12 reconstitutes in low and high Mg^{2+} buffers. The $r = 1.0$ octamer (Oct) or tetramer (Tet) reconstitutes from Fig. 1 were transcribed in low or high Mg^{2+} buffer. A plasmid containing a single *Lytechinus* 5S RNA gene was included in the reaction mixture as a histone-free control. Transcripts from the 208-12 template are indicated as read through, while transcripts from the 5S RNA gene are labeled as 5S. Lanes: 1 and 2, results with the octamer reconstitutes after a 24-hr exposure; 3 and 4, results with the tetramer reconstitutes after a 4-hr exposure. Also shown are transcripts from several histone-free DNA templates: the 172-12 promoter mutant (lane 5), the 208-12 DNA template (lane 6), and the *Lytechinus* 5S RNA gene alone (lane 7).

We next examined the ability of RNA polymerase III to elongate through $r = 1.0$ 208-12 tetramer reconstitutes. Direct comparisons indicated that significantly more read-through transcripts were produced from the tetramer reconstitutes than from the corresponding octamer reconstitutes in low Mg^{2+} buffer (Fig. 6, lanes 1 and 3 at identical exposures). In addition, the Mg^{2+}-dependent reduction in the amount of transcripts produced from the tetramer reconstitutes did not approach the virtually complete reduction observed for the octamer reconstitutes (Fig. 6, compare lanes 1 and 2 with lanes 3 and 4). We conclude that the tetramer reconstitutes do not repress transcriptional elongation as effectively as the octamer reconstitutes in high Mg^{2+} buffer, consistent with their lesser degree of Mg^{2+}-dependent compaction (Fig. 2).

DISCUSSION

Transcriptional repression by the core histones has been associated with both the wrapping of DNA into nucleosomes (35–42) and higher-order folding of the nucleosomal array (10). Previous studies have found that removal of histones H2A/H2B promotes the accessibility of trans-acting factors to 5S DNA in both nucleosomes (16, 43) and chromatin (44) and leads to increased 5S RNA gene transcription (23, 45). However, these studies did not take into account the possible influence of removal of histones H2A/H2B on the higher-order folding of nucleosomal arrays. In this work we have begun to approach this problem.

Roles of H2A/H2B Dimers in Chromatin Folding and Nucleosome Stability. The >60% increase in $s_{20,w}$ observed in high Mg^{2+} buffer for the small fraction of $r = 1.0$ 208-12 octamer reconstitutes that were saturated with 12 nucleosomes (Fig. 2) results from formation of a maximally folded solenoid-like structure (10). The bulk of the $r = 1.0$ 208-12 octamer reconstitutes in high Mg^{2+} buffer were unable to form the maximally folded structure because of their subsaturation (P. S. Schwarz and J.C.H., unpublished data). Instead, they fold into intermediate 30–40 S structures that are stabilized by local nucleosome–nucleosome interactions (29, 30). Similar mechanisms presumably are responsible for compaction of the circular pXP10 octamer reconstitutes. Our results indicate that the absence of histones H2A/H2B significantly reduces the extent of salt-dependent compaction of both the 208-12 and pXP10 reconstitutes (Figs. 2 and 4). Thus, H2A/H2B dimers appear to be required to achieve maximal levels of chromatin folding.

Each histone tetramer organizes 120 bp of DNA (25, 46) and each histone octamer organizes at least 160 bp of DNA (47, 48). Thus, contacts as far as 80 bp from the dyad axis of the nucleosome core are mediated by histones H2A/H2B. It is therefore not surprising that chromatin deficient in H2A/H2B does not compact completely. The basic amino acids of the H2A/H2B dimers may be necessary to help neutralize the phosphodiester backbone of DNA (49) or to guide the path of the DNA that links nucleosomal structures. In addition, the reduced folding of H3/H4 tetramer reconstitutes could reflect the absence of internucleosomal H2A/H2B interactions (50), possibly those mediated by the core histone tails (29).

The stability of the interaction of the histone octamer with DNA also depends on H2A/H2B. A small fraction of the 208-12 H3/H4 tetramer reconstitutes dissociate into free DNA in high Mg^{2+} buffer. This behavior also has been observed with octamer reconstitutes, but only at higher salt concentrations (18, 30, 33). Thus, the tendency of the H3/H4

tetramer–DNA complex to dissociate under physiological salt conditions appears to be greater than that of the nucleosome. This instability must be taken into account when considering the transcriptional properties of tetramer reconstitutes.

Roles of H2A/H2B Dimers in Transcriptional Repression. Numerous experiments have suggested a dynamic role for H2A/H2B in the transcription process *in vivo*. For example, histones H2A/H2B exchange out of chromatin *in vivo* (14). This exchange is facilitated by the transcription process (15). Transcribed chromatin is deficient in histones H2A/H2B (11–13). These changes may contribute to the appearance of altered nucleosomal structures on transcribed regions (51, 52). While these *in vivo* results implicate H2A/H2B deficiency as one of the characteristics of transcriptionally active chromatin, they do not provide mechanistic information about how the loss of H2A/H2B facilitates transcription. In our *in vitro* model system studies, the level of transcriptional repression of octamer reconstitutes in standard (i.e., high Mg^{2+}) transcription buffer was much more pronounced than that observed for the tetramer reconstitutes (Figs. 4 and 5). This is consistent both with a role for histone octamers in transcriptional repression and with a role for H2A/H2B deficiency in the relief of transcriptional repression. Part of the effect of H2A/H2B deficiency can be traced to increased transcription factor accessibility of the 5S promoter when complexed with a H3/H4 tetramer rather than a histone octamer (Fig. 5; refs. 16 and 43). This serves to enhance transcription initiation. However, it is also clear that deficiency in H2A/H2B disrupts the ability of nucleosomal arrays to fold into the higher-order structures that are repressive to both transcription initiation and elongation. Thus, we propose that one of the consequences of depletion of H2A/H2B from active chromatin is to stabilize a more extended chromatin structure that will impose less impediment to RNA polymerases.

We thank P. Schwarz for excellent technical assistance. This work was supported by National Institutes of Health Grant GM 45916 (to J.C.H.).

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