

Alterations in Nucleosome Core Structure in Linker Histone-depleted Chromatin*

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We have previously shown that the sequential arrangement of histone-DNA contacts is essentially the same in the nucleosomal core of sea urchin sperm nuclei, where chromatin is highly condensed and repressed, and in nuclei from lily bud sepals or yeast, where chromatin is highly active in transcription and replication and is significantly or completely depleted of histone H1. However, the difference in the strength of some histone-DNA contacts has not been understood or discussed. In this work, we demonstrate that some of these differences are due to a conformational change in the nucleosomal core. We show that the nucleosomal core in linker histone-depleted chromatin is in a different conformational state compared with the nucleosomal core in folded chromatin or in isolated core nucleosomes. This conformational state is characterized by altered strengths in the histone H4 and H2A/H2B contacts with the regions of sharply bent nucleosomal DNA around sites $+/-1$ and $+/-4$ and site $+/-5$, respectively. We demonstrate that this conformation, which we call the "stretched nucleosome," is a general feature of unfolded linker histone-depleted chromatin and may occur during chromatin activation. Our results suggest that this nucleosome structural alteration does not depend on chromatin sources and histone variants studied in this work. In addition, we show that this alteration is reversible and is caused by the stretching of linker DNA during chromatin unfolding.

The eukaryotic genome is organized as chromatin, which is a dynamic nucleoprotein complex. The basic subunit of chromatin in almost all eukaryotic cells is the nucleosome, which consists of an octamer of core histone proteins (two each of H2A, H2B, H3, and H4) and DNA wrapped in about two superhelical turns (1). Nucleosomes are connected by linker DNA associated with linker histones, which have been shown to play a key role in chromatin folding (2). In contrast, transcriptionally active chromatin is unfolded and significantly depleted of linker histones, most notably in the promoter and promoter-adjacent regions (3). Biochemical experiments have shown that histone H1 is a potential repressor of RNA polymerase I, II, and

III transcription (1). The transcriptional activity of chromatin also correlates with histone modifications that can modulate chromatin structure (4–6).

The nucleosome itself is involved in the regulation of chromatin activity (7) and may compete with *trans*-acting factors for the binding of specific DNA sequences (1, 8–13). Since the DNA of most eukaryotic cells is organized in nucleosomes, it seems likely that some structural transitions should take place in the nucleosome before or during DNA processing to allow the passage of polymerases. Well pronounced changes in nucleosome and chromatin structure have been observed in active chromatin fractions (14–16); however, the nature and mechanism of these changes are still unknown.

We have previously shown that the sequential arrangement of histone-DNA contacts in the nucleosomal core is very similar in nuclei where the chromatin is highly repressed and in nuclei where the chromatin is active (17). However, the difference observed in the strength of some histone-DNA contacts was not considered or discussed. Our present finding of a difference in the strength of some histone-DNA contacts in the nucleosomal core in linker histone-depleted chromatin compared with those contacts in nuclei where the chromatin is highly repressed or in isolated core particles suggests the existence of different conformations for the nucleosomal core. Here, we show that the strength of certain histone-DNA contacts in isolated core particles is similar to that in chromatin that is highly condensed, but is different in unfolded chromatin lacking linker histones. This difference is characterized by alterations in the histone H4 and H2A/H2B contacts with the regions of sharply bent nucleosomal DNA around sites $+/-1$ and $+/-4$ and site $+/-5$, respectively. The analysis of histone-DNA contacts in nucleosomes from different sources indicates that these alterations are a common feature of linker histone-depleted chromatin and are a result of the stretching of linker DNA. Our results suggest that this nucleosome structural alteration might occur *in vivo* in active chromatin regions depleted of linker histones.

MATERIALS AND METHODS

Preparation of Nuclei and Core Particles and Protein-DNA Cross-linking Procedure—Nuclei from sea urchin sperm, chicken erythrocytes, mouse ascites, lily bud sepals, and yeast were isolated as described previously (17, 18). Histone-DNA cross-linking, isolation of core particles, purification of cross-linked complexes, and two-dimensional gel electrophoresis of [32 P]DNA and [125 I]-histone-labeled cross-linked complexes were performed as described (17, 19).

Preparation of Linker Histone-depleted Chromatin—Soluble chromatin from chicken erythrocyte and mouse ascites nuclei was prepared as described (20). Chromatin depleted of linker histones was prepared by extraction of linker histones using ion-exchange AG 50W-X2 resin (Bio-Rad) according to Bolund and Johns (21). Reconstituted chromatin lacking linker histones was prepared from histone octamers and DNA by stepwise salt dialysis according to Steinmetz *et al.* (22) at a histone octamer/DNA ratio 0.8:1 (w/w), keeping the DNA concentration at $A_{260} \sim 3$. The histone octamers were reconstituted from acid-extracted chicken erythrocyte core histones as described by Greyling *et al.* (23).

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Oligonucleosomal DNA used for reconstitution was prepared by phenol/chloroform extraction (24) of the soluble chromatin isolated from chicken erythrocyte nuclei.

RESULTS AND DISCUSSION

Experimental Approach—The method of covalent zero-length protein-DNA cross-linking was used to localize histone-DNA contacts in nucleosomes (19). This methodology allows the direct determination of histone-DNA contacts in intact nuclei and isolated chromatin as well as in isolated nucleosomes. Cross-linking causes the nucleosomal DNA to cleave in such a manner that only the 5'-terminal DNA fragment becomes attached to a protein molecule. The length of a cross-linked DNA fragment precisely shows the distance of a protein cross-linking site from the DNA 5'-end and can be assessed by using two systems of two-dimensional gel electrophoresis. Denaturing SDS gel electrophoresis in the first dimension is the same for both systems, where the separation of cross-linked complexes depends on the molecular weight of both DNA and proteins. The first system, called the "protein version," relies on direct chemical degradation of the DNA in the gel following separation of the cross-linked complexes in the first dimension. The released ^{125}I -labeled histones are then separated in the second dimension SDS gel according to their size. In the first dimension, the mobility of the cross-linked complex depends on the histones' molecular weight and the size of the cross-linked DNA fragment. As a result, the released histones in the gel of the second dimension are arranged as spots on the different horizontal lines. The position of these spots on a particular horizontal line indicates the location of DNA cross-linking sites for a particular histone in the nucleosome.

In the second system, called the "DNA version," the histones are digested directly in the gel by protease after separation of the cross-linked complexes in the first dimension. The released ^{32}P -labeled DNA fragments are separated according to their size in a denaturing second dimension gel. In the first dimension, the histones cross-linked to the DNA decrease the mobility of the cross-linked complexes proportionally to the histone size. As a result, after histone digestion, the released DNA fragments that are attached to the different histones fall on different diagonals in the second dimension gel. The diagonals corresponding to particular histones in the two-dimensional gel are arranged from left to right in the same order as free histones migrating from top to bottom in the one-dimensional gel. The position of the spot within each diagonal indicates the length of the DNA fragment cross-linked to a particular histone. This length can be determined by running DNA fragments of known size in the gel of the second dimension.

Histone-DNA Contacts in Nucleosomes in Condensed Chromatin and in Unfolded Chromatin—Using the histone-DNA cross-linking methodology, we have previously shown that the sequential arrangement of histone-DNA contacts is very similar in nucleosomal cores in nuclei from different sources where the chromatin is highly repressed or active in transcription and replication (17). However, we did not discuss the observed difference in strength of some histone-DNA contacts in nuclei from those sources. In this work, we qualitatively assessed the relative strength of some histone-DNA contacts according to the intensity of the corresponding signals in two-dimensional gel autoradiographs for different sources with different levels of chromatin activity and compaction: sea urchin sperm, lily bud sepals, and yeast. Chromatin in sea urchin sperm is completely inactive in RNA and DNA synthesis and is more densely packed than mitotic chromosomes (25). In contrast, the chromatin in yeast and in the dividing cells of lily bud sepals is very active in transcription and replication and is significantly or completely depleted of histone H1 (1, 17, 26–28). We found that

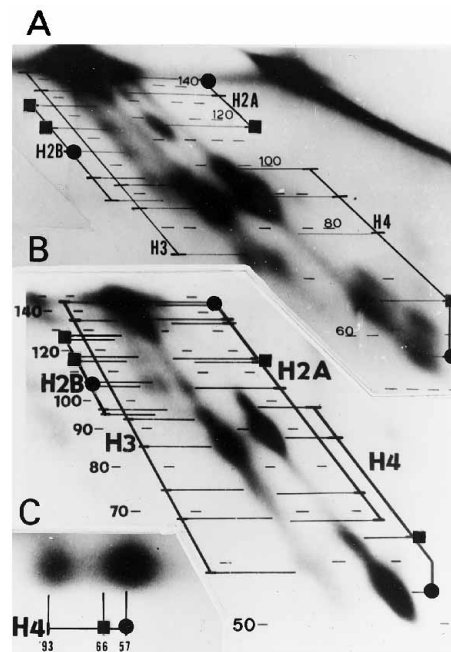


FIG. 1. Histone-DNA contacts in the nucleosomal core in nuclei from sea urchin sperm (A), lily buds (B), and yeast (C). A and B, DNA version of two-dimensional gel electrophoresis of single-stranded, ^{32}P -labeled DNA fragments cross-linked to histones. Electrophoresis in the first dimension (from left to right) was carried out in a denaturing 17% polyacrylamide gel containing 0.1% SDS and 7 M urea, where the histone-DNA cross-linked complexes were separated according to the size of both histones and DNA. After the first dimension, the histones cross-linked to DNA were digested directly in the gel, and the released DNA fragments were separated in the second dimension (from top to bottom) in a 15% polyacrylamide gel containing 7 M urea. The dashed lines show the position in the gel, and the numbers indicate the approximate length (in nucleotides) of ethidium bromide-stained DNA fragments from DNase I-digested rat liver nuclei used as markers. The precise length of the marker DNA fragments (30, 31) is shown in Fig. 2A. The positions of ^{32}P -labeled DNA fragments, cross-linked to different histones and arranged on separate diagonals, were revealed by autoradiography and are indicated by the solid lines. The extreme right diagonal (in the upper right corner) is uncross-linked DNA fragments. C, protein version of two-dimensional gel electrophoresis of ^{125}I -labeled histones cross-linked to DNA fragments. The cross-linked histone-DNA complexes were separated in the first dimension in a 15% polyacrylamide gel as described above for A and B. After the first dimension, the DNA cross-linked to the histones was chemically degraded directly in the gel, and the released ^{125}I -labeled histones were separated in the second dimension in a 15% polyacrylamide gel containing 0.1% SDS. The number at each spot indicates the size (in nucleotides) of the DNA fragments cross-linked to histones. For the sake of simplicity, only histone H4 contacts are shown in C. Solid squares indicate the histone-DNA contacts that become attenuated during chromatin unfolding compared with the contacts marked by solid circles.

the relative strength of certain histone-DNA contacts in these sources is different and correlates with a different level of chromatin compaction.

According to our previous data (17), the main cross-linking sites on the nucleosomal DNA for histone H4 are nucleotides 57, 66, and 93; for histone H2B, nucleotides 109, 119, and 129; and for histone H2A, nucleotides 121, 135, and 145 from the 5'-end of the nucleosomal DNA. The defined length of cross-linked DNA fragments in two-dimensional gel autoradiographs was measured by scanning with a computing laser densitometer (29).

In the nucleosomal core in sea urchin sperm nuclei (Fig. 1A), contacts H4(57) and H4(66) have almost equal strength, as do contacts H2B(109) and H2B(119) (numbers in parentheses indicate the distance in nucleotides from the 5'-end of one strand of core nucleosomal DNA to the particular histone contact). However, in nucleosomes from lily (Fig. 1B) and yeast (Fig. 1C)

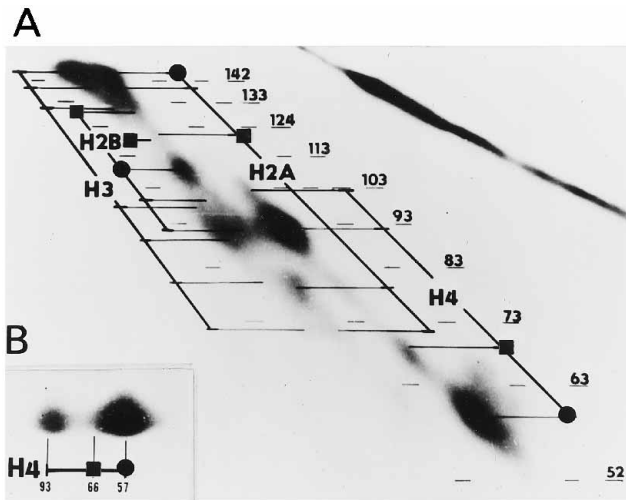


FIG. 2. **Histone-DNA contacts in chromatin depleted of linker histones.** A, DNA version of two-dimensional gel electrophoresis of single-stranded, ^{32}P -labeled DNA fragments cross-linked to histones in chromatin reconstituted from purified chicken erythrocyte histones and DNA; B, protein version of two-dimensional gel electrophoresis of ^{125}I -labeled histones cross-linked to DNA in mouse ascites chromatin. For the sake of simplicity, only histone H4 contacts are shown. For details, see the legend of Fig. 1.

nuclei, we observed a different ratio in the intensities of the above-mentioned contacts, showing attenuation of contact H4(66) compared with contact H4(57). A similar alteration was also observed for the contacts of histone H2B in lily (Fig. 1B) and yeast (17) nuclei, showing attenuation of contacts H2B(119) and H2B(129) compared with contact H2B(109). In addition, we also found attenuation of contact H2A(121) compared with contact H2A(146) in lily (Fig. 1B) and yeast (data not shown) nuclei. It should be noted that in this work, we only did a qualitative estimation of altered contacts. We compared the relative intensity of the mentioned contacts within the same histone and within the same gel, and as shown previously, such a comparison prevents ambiguity caused by different exposures of different gels (29).

Since transcriptionally active chromatin is unfolded and significantly depleted of linker histones (1, 3), we studied the histone-DNA contacts in chromatin depleted of linker histones, using it as a hypothetical model for active chromatin. For this purpose, we used chromatin depleted of linker histones from mouse ascites cells and chromatin reconstituted from purified chicken erythrocyte core histones and DNA. Chromatin reconstituted from purified core histones and DNA was used as a control to avoid the influence of any other factors on the nucleosome structure. In both of these H1-depleted chromatin preparations, within the range of monovalent ion concentrations from 30 to 140 mM, we found the same alterations in the contacts of histones H4, H2A, and H2B with the core nucleosomal DNA (Fig. 2) as those observed in lily and yeast nuclei (Fig. 1, B and C, respectively). It should be noted that histone H2B from sea urchin sperm and lily bud sepals is much longer in size than that from chicken erythrocytes and migrates slower, above histone H3 in the SDS gel (17, 28). Since diagonals corresponding to particular histones in the two-dimensional gel are arranged from left to right in the same order as free histones migrating from top to bottom in the one-dimensional gel (19) (see "Experimental Approach"), the corresponding diagonal of histone H2B in the two-dimensional gels for these two sources is arranged to the very left (Fig. 1, A and B).

These results indicate that the conformation of the nucleosomal core in chromatin depleted of linker histones is similar to that in yeast and lily bud sepal nuclei, but is different in sea

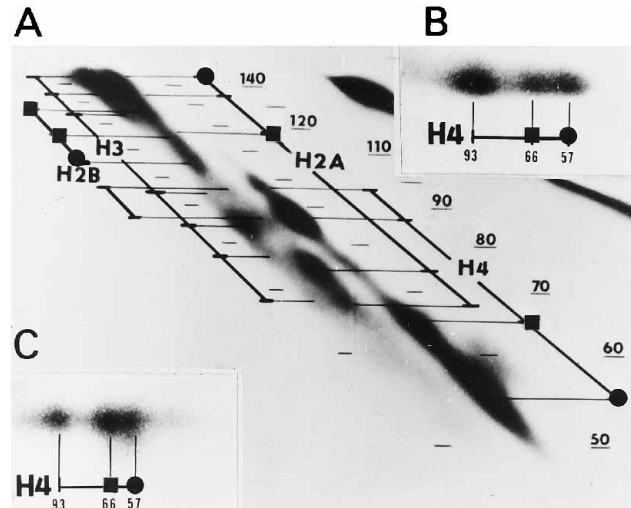


FIG. 3. **Histone-DNA contacts in core nucleosomes after isolation.** A, DNA version of two-dimensional gel electrophoresis of single-stranded, ^{32}P -labeled DNA fragments cross-linked to histones in core nucleosomes; B and C, protein version of two-dimensional gel electrophoresis of ^{125}I -labeled histone H4 cross-linked to DNA in core nucleosomes. For the sake of simplicity, only histone H4 contacts are shown. The histones in core nucleosomes were cross-linked to DNA after isolation of core particles from reconstituted chromatin lacking linker histones (A), from mouse ascites chromatin depleted of linker histones (B), and from yeast nuclei (C). For details, see the legend of Fig. 1.

urchin sperm nuclei. Histone H1 may not be present in significant amounts in lily bud sepals or may not be present at all in yeast (17, 28, 32–35). Since H1-depleted chromatin under the ionic conditions used in this work is unfolded (36) and the strength of studied histone-DNA contacts in the nucleosomal core in chromatin depleted of linker histones is similar to that observed in lily and yeast nuclei, a significant portion of chromatin in yeast and lily nuclei, prepared as described (17), might also be unfolded. In addition, it was also shown that in contrast to most eukaryotes, at least 40% of the yeast genome is actively transcribed (26) and that all yeast chromatin is equally susceptible to DNase I (27), suggesting that a considerable part of the yeast genome is not tightly packaged.

It has been shown that the stoichiometry of histone H1 per nucleosome varies with the source (37) and that histone H1 is less abundant in active chromatin fractions (1, 3). It was suggested that the lack of histone H1 may affect the local state of compaction in chromatin (38). Our results show that the level of chromatin compaction correlates with the alterations in histone-DNA contacts in the nucleosomal core. This suggests that the changes in the level of compaction leads to gross structural changes in the nucleosome.

Nucleosome Structural Alterations in Unfolded Chromatin Are Reversible and Do Not Depend on Chromatin Sources and Histone Variants—Since the same histone-DNA contacts in the nucleosomal core of nuclei from different sources differed in intensity, it was essential to check whether this difference was inherent in the nucleosomal cores themselves or was somehow dependent on the chromatin structure. For this purpose, we mapped the histone-DNA contacts in core particles, cross-linked after their isolation from reconstituted chicken erythrocyte chromatin lacking linker histones (Fig. 3A), from mouse ascites chromatin depleted of linker histones (Fig. 3B), and from yeast nuclei (Fig. 3C). We found that in core particles isolated from these sources, the relative intensities of studied H4, H2A, and H2B contacts were similar to those observed in nuclei from sea urchin sperm, become chromatin is highly condensed and repressed (Fig. 1A).

We have shown previously that histone H1 forms contacts

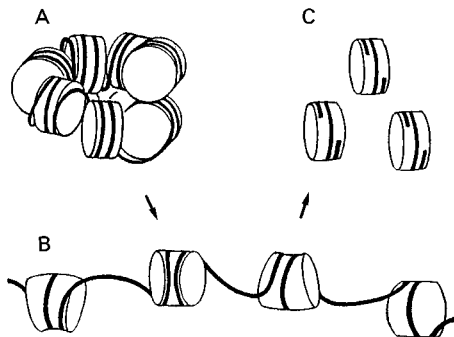


FIG. 4. Effect of linker DNA on the conformational state of the nucleosomal core. **A**, hypothetical solenoidal model of 30-nm chromatin fiber (41–43) with a nucleosomal repeat of ~200 base pairs. The linker DNA is shown supercoiled as a continuation of core nucleosomal DNA as suggested (40–43). **B**, formation of stretched nucleosomes in unfolded chromatin depleted of linker histones. The stretching of the linker DNA causes the deformation (shown by the changed form) of nucleosomal cores. This conformational state is characterized by alterations in the strength of some histone-DNA contacts. **C**, isolated nucleosomal core particles. Removal of the linker DNA causes the stretched nucleosomes to revert to the conformational state similar to that found in nuclei (**A**).

not only with linker DNA, but also with the DNA of the nucleosomal core (39, 40). It could be assumed that the observed attenuation of the histone H2A-, H2B-, and H4-DNA contacts in the nucleosomal core in H1-depleted chromatin might be due to the disruption of histone H1 contacts with the nucleosomal core. However, since the contacts of histones H2A, H2B, and H4 with core nucleosomal DNA were restored after removal of linker DNA (during core particle isolation from H1-depleted chromatin), this indicates that the strength of certain histone-DNA contacts in the nucleosomal core in chromatin is affected by linker DNA, but not by the contacts of histone H1 with the nucleosomal core.

Chromatin in sea urchin sperm is highly condensed and tightly packaged in nuclei (Fig. 4A) (25) such that the linker DNA might not affect the histone-DNA contacts in core nucleosomes. In contrast, in chromatin depleted of linker histones, which appears at low monovalent cation concentrations (up to ~40 mM) as a beads-on-a-string filament (Fig. 4B) (36), some histone-DNA contacts are altered in strength compared with those in nuclei where chromatin is highly condensed. However, after removal of linker DNA, for example during core particle isolation (Fig. 4C), the mentioned contacts become as strong (Fig. 3) as in highly condensed chromatin in sea urchin sperm nuclei (Fig. 1A). Since the strength of the described contacts in nuclei (Fig. 1A) where chromatin is highly condensed and the linker DNA is tightly packaged (Fig. 4A) is the same as in isolated core particles (Fig. 3) where the linker DNA is absent (Fig. 4C), we can consider the removal of linker DNA equivalent to condensation in terms of its influence on the observed histone-DNA contacts in the nucleosome core. This suggests that some of the described contacts that exist in highly repressed and condensed chromatin may be distorted in chromatin from which histone H1 was released (for example during chromatin activation) but restored again after chromatin folding. This also suggests that the described alterations are reversible and depend on the specific arrangement of the linker DNA, which could be conditioned by linker histones.

Internucleosomal contacts existing in folded chromatin are disrupted both in chromatin depleted of linker histones and in isolated core particles. However, nucleosomes in chromatin depleted of linker histones are still connected by linker DNA. We suggest that the nucleosomes in this unfolded chromatin might undergo deformation due to the stretching of the linker

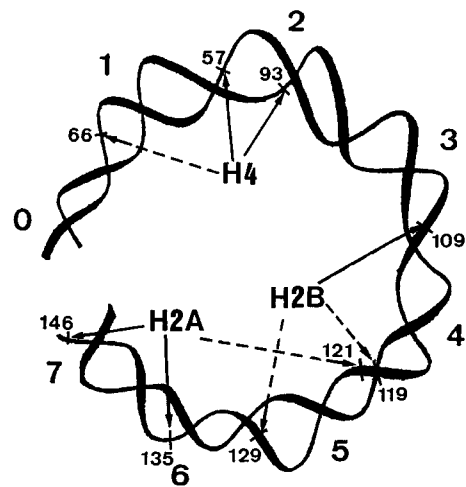


FIG. 5. Arrangement of histone-DNA contacts on half of the core nucleosomal DNA for histones H2A, H2B, and H4. The dashed arrows indicate attenuated contacts that occur during chromatin unfolding (see "Results and Discussion"). The smaller numbers at the sites of histone-DNA contacts indicate the distance in nucleotides from the 5'-end of core nucleosomal DNA. The larger numbers on the outside of the helix represent the number of DNA double helix turns from the dyad axis (indicated by position 0) (44).

DNA (Fig. 4B). This deformation could cause the observed alterations in the histone H4 and H2A/H2B contacts with the sharply bent regions of nucleosomal DNA, around sites ± 1 and ± 4 and site ± 5 , respectively (Fig. 5). We suggest that these alterations occurring in the nucleosomal cores as linker DNA becomes extended are concomitant with chromatin unfolding (Fig. 4B). The fact that the altered contacts are restored again in the isolated nucleosomes (Fig. 3) indicates that the alterations in histone-DNA contacts observed in the nucleosomal core of linker histone-depleted chromatin are not caused by the disruption of internucleosomal contacts in folded chromatin.

It should be pointed out that the described alterations in the histone-DNA contacts in chromatin depleted of linker histones (Fig. 2) were found over the entire range of ionic strengths from 30 to 140 mM, including physiological ionic strength. This result is in good agreement with an earlier observation that H1-depleted chromatin at physiological ionic strength appears as irregular "clumps" with no fiber morphology, unlike linker histone-containing chromatin, which folds up into a fiber under these conditions (36). It also indicates that the linker DNA cannot be organized and that chromatin cannot be properly folded without linker histones. Our results show that this lack of folding might affect the nucleosome structure by altering histone-DNA interactions.

In addition to the described contacts H4(66), H2A(121), H2B(119), and H2B(129), we also observed alterations in other histone-DNA contacts, but the mechanism of their alterations is different.¹ For example, the contact of histone H2A with nucleotide 77 (dyad axis) is also altered. This contact is absent in nuclei (Fig. 1) and heavily attenuated in H1-depleted chromatin (Fig. 2), but appears very strong in isolated core particles (Fig. 3). However, as we have shown recently, the alteration of this contact occurs due to the rearrangement of the histone H2A C-terminal domain and is not related to chromatin folding (29). In this particular work, we consider only those histone-DNA contacts in which the alterations in intensity correlate with chromatin folding.

¹ I. M. Gavin, S. I. Usachenko, and S. G. Bavykin, manuscript in preparation.

Our methodology allows us to observe only the histone-DNA contacts involving mostly histidines and some lysines (16, 45); however, alterations might also occur with other histone-DNA or histone-histone contacts. We refer to the changes we observe as a structural transition of the nucleosomal core, yielding a new conformational state that we call a "stretched nucleosome." We suggest that this conformational state is caused by the deformation of the nucleosomal core due to the stretching of linker DNA in the absence of linker histones during chromatin unfolding. Sea urchin sperm, compared with chicken erythrocytes, mouse ascites, and yeast, contains several variants of histone H2B with a considerably extended N-terminal domain containing an additional cluster of lysine and arginine residues (46). Lily, as well as other plants, also contains several variants of histones H2A and H2B that are much longer than those from the other sources studied in this work (28, 47–49). Our observation that the stretched nucleosome appears in unfolded chromatin from different sources, containing different histone variants with different lengths of N- and C-terminal domains, suggests that this conformational state does not depend on chromatin sources and histone variants.

We have shown previously that the primary organization (linear sequential arrangement of histone-DNA contacts along nucleosomal DNA) of core nucleosomes in all three higher eukaryote kingdoms is very similar (17). In this work, we demonstrate that the new conformational state of the stretched nucleosome is uniform for all species studied and is a common feature for nucleosomes in unfolded chromatin depleted of linker histones.

Possible Functional Role of Stretched Nucleosomes—The cross-linking domain of histone H4, ¹⁶KRHR¹⁹, which contacts the region of sharply bent nucleosomal DNA (45) and which forms all the mentioned contacts H4(57), H4(66), and H4(93), plays a role in the regulation of gene expression and replication in yeast (50). A single amino acid substitution of any residue in this domain dramatically decreases the ability of yeast to mate and increases the duration of S phase (51–53). In addition, this domain contains sites for post-translational modifications: Lys-16, which is the site of acetylation and His-18, which is the site of phosphorylation (49). Our results suggest that alterations in the contacts of this domain with nucleosomal DNA during chromatin unfolding might also be involved in the regulation of gene activity.

It has been shown that transcription causes nucleosomes to become DNase I-sensitive and that chromatin exhibits a "half-nucleosomal" cleavage periodicity, which was interpreted as nucleosome "splitting" (15). It has also been demonstrated that in transcriptionally active chromatin, destabilized nucleosomes ("lexosomes") contain some additional non-histone proteins (14). Our experiments with chromatin reconstituted from purified histones and DNA suggest that the conformational state of a stretched nucleosome does not require the presence of other specific proteins. However, we do not exclude the possibility that additional non-histone proteins are necessary to bring chromatin and nucleosomes to this state *in vivo*.

Since histone H1 is present in highly repressed and condensed chromatin but is observed to be less abundant in active chromatin fractions (1, 3), one can assume that it could be released during chromatin activation before transcription (54–56). Our results suggest that the unfolding of the chromatin fiber after removal of histone H1 will cause some conformational changes within nucleosomes characterized by a significant alteration of some histone-DNA contacts (Fig. 5). These changes might destabilize the nucleosomes, affect their interaction with *trans*-acting factors, and facilitate DNA processing by polymerases.

Currently, the interaction of nucleosomes with *trans*-acting factors is described by two models (9, 11, 12). One model implies an interaction of some factors with nucleosomes, forming a ternary complex. The other model suggests the replacement of nucleosomes by transcription factors during replication. Regarding the ternary complex model, the results represented in this work suggest that the alterations in histone-DNA contacts during chromatin unfolding may play a role in the interaction of some *trans*-acting factors with nucleosomes.

A number of experiments have been done in which the interaction of linker histones, *trans*-acting factors, and RNA polymerases has been studied with single nucleosomes reconstituted on short specific sequences (10, 13, 57). The results presented in this work show that the conformation of the nucleosomal core in single nucleosomes is different compared with that in nucleosomes in linker histone-depleted chromatin. In addition, as we have recently shown, the flexible histone H2A C-terminal domain is also rearranged in isolated nucleosomes (29). Therefore, from a structural/functional viewpoint, it would be more appropriate to study the interaction of the mentioned proteins with nucleosomes in linker histone-depleted chromatin rather than with single nucleosomes.

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