Chromatin Fiber Structure: Morphology, Molecular Determinants, Structural Transitions

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ABSTRACT Despite more than 20 years of research, the structure of the chromatin fiber and its molecular determinants remain enigmatic. Recent developments in high-resolution microscopic techniques, as well as the application of mathematical modeling to chromatin fiber structure, have allowed the acquisition of some new insights into the structure and its determinants. Here we present some of the newest data on the structure of the chromatin fiber in both its extended and compacted states, and bring together this new knowledge with older data in an attempt to provide a unified view of how chromatin components interact with each other to form its various conformations. The structural transitions that are believed to take place during transcriptional activation and its cessation are also discussed. It becomes obvious that despite some progress in our understanding of the fiber structure and its dynamics, huge gaps continue to exist. Bridging these gaps will require further improvements in already available techniques and the introduction of completely new approaches.

INTRODUCTION

Much is now known about the structure of the nucleosome, the fundamental repeating unit of eukaryotic chromatin (van Holde, 1988; Tsanev et al., 1992). In the core particle of the nucleosome, 146 bp of DNA is wrapped in ~1.75 left-handed superhelical turns around an octameric complex consisting of two molecules of each of core histones H2A, H2B, H3, and H4. Successive core particles are connected by stretches of linker DNA, to which the fifth major histone class, the so-called lysine-rich or linker histones, are bound. How this linear array of nucleosomal particles is organized in the nucleus remains an enigma. The first decade after the discovery of the nucleosome was flooded by specific models attempting to describe the so-called 30-nm fiber, the structure observed in vitro at physiological salt concentrations and believed to be representative of the in situ conformation (see van Holde, 1988; Tsanev et al., 1992; for references). We have recently presented evidence that the fiber has a very similar diameter, even under low-salt conditions, and suggested that the term "30-nm fiber" be abandoned as a designator for the more condensed, high-salt structure; the latter should rather be called the "condensed" or "compact" fiber (van Holde and Zlatanova, 1995) to distinguish it from the more "extended" fiber observed at lower ionic strength. Almost all proposed models for the condensed chromatin fiber have been based on the idea of regularity of structure. Recently, however, we (van Holde and Zlatanova, 1995) and others (Woodcock and Horowitz, 1995) have argued that the actual experimental evidence for a regular helical structure is far from convincing; instead, the data suggest a quite irregular, kinky conformation, with only limited regions of regularity.

Not only are the exact morphologies of the extended and condensed chromatin fibers uncertain, but very little is known about the molecular mechanisms that organize the linear array of nucleosomes into these three-dimensional fibers. That the chromatin fiber is three-dimensionally organized even at low ionic strength was originally suggested on the basis of physical studies in solution, and recently directly demonstrated by the use of scanning force microscopy (SFM) (Leuba et al., 1994, and references therein). This new technique allows imaging of biological macromolecules and structures under conditions much less harsh than those employed in standard electron microscopy (EM), because no staining or shadowing is necessary, and imaging can be performed either in air or under liquids, thus avoiding the high vacuum required in EM (Bustamante et al., 1993, 1994). Imaging native and linker-histone-depleted chromatin fibers deposited on flat mica or glass surfaces under different conditions, and creating mathematical models of such fibers allowed us to define a set of simple structural parameters that determine extended fiber morphology (Leuba et al., 1994; Yang et al., 1994). Two of these—the entry-exit angle of the DNA into and out of the core particle, and the variable length of the linker DNA—are crucial to fiber morphology and have been recognized as such by others as well (Woodcock et al., 1993). We further suggested that a major role of linker histones is to maintain the entry-exit angle (Yang et al., 1994), in accordance with the presumed location of the globular domain of linker histones at or near the entry/exit site of the DNA at the nucleosome (e.g., Boulikas et al., 1980; Allan et al., 1980). (Note that some recent studies using reconstituted mononucleosomes suggest an alternative location of the globular

Received for publication 7 August 1997 and in final form 25 October 1997.

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0006-3495/98/05/2554/13 $2.00

domain, inside the DNA gyres of the core particle (Pruss et al., 1996).)

Finally, it is clear that the conformation of the chromatin fiber must change in a reversible fashion in processes that require access of enzymes and regulatory molecules to the underlying DNA template. Obviously, processes like transcription, replication, and repair can only take place on fibers that are in an extended conformation, so there must be molecular mechanisms that would ensure transitions from the more condensed inactive conformation to those more open active structures. Upon cessation of these processes, the structure should be able to revert to its inactive compacted state.

In this article we wish to review our own recent published and unpublished data on chromatin fiber structure and to discuss, mainly on the basis of literature results, possible mechanisms of structural transitions between different conformations of the chromatin fiber. We will also touch upon some interesting questions regarding the effect of some chemical modifications of chromatin components on the topology of the DNA.

CHROMATIN FIBER STRUCTURE AT LOW IONIC STRENGTH (EXTENDED FIBER)

It has been suspected for years that it is the extended conformation of the chromatin fiber that serves as a template for transcription, replication, etc. However, the in vivo or in situ data concerning these structures are amazingly scarce. Our present knowledge on extended fiber structure comes from experiments in which isolated long chromatin fibers are solubilized in low-ionic-strength buffers and studied by biochemical or physical methods, including different types of high-resolution microscopy. These studies, however, did not lead to a unified view of the extended fiber structure. The EM data were taken as an indication of a flat zigzag conformation, as is presented in textbooks, although the original seminal papers did point out that this appearance could be the consequence of artifactual flattening on the grid of a loosely organized helix in solution (e.g., Thoma et al., 1979). Results from solution studies using physical methods could be better fitted by an open-helix model (for references, see Leuba et al., 1994).

In an attempt to resolve this apparent contradiction, we imaged chromatin fibers by SFM, using the tapping mode of operation (Bustamante et al., 1993, 1994). SFM uses a sharp tip at the end of a flexible cantilever to scan and sense the topography of a sample deposited on a flat surface (Binnig et al., 1986). In the tapping mode, the cantilever is oscillated near its resonance frequency during scanning, the effect of the sample being to reduce the amplitude of oscillation. Using the tapping mode (in contrast to the contact mode) decreases contact forces and virtually eliminates shear forces, thus reducing molecular motion and deformation occurring during contact imaging.

Chicken erythrocyte or HeLa cell chromatin fibers were imaged on glass or mica, in air, at ambient temperature and humidity, after their deposition from low ionic strength buffers. It is important to note that even when imaging is performed in air, the fibers are likely to retain at least the strongly bound and structurally essential water (Grigg et al., 1992; Hu et al., 1995). As demonstrated in Fig. 1, the fibers are loosely organized, irregular, three-dimensional arrangements of nucleosomes. The three-dimensionality can be perceived from the variation in color tones, with darker tones depicting nucleosomes that are closer to the surface, and increasingly lighter tones depicting nucleosomes that are higher above the surface. The three-dimensional nature of the extended structures can be well appreciated in surface plots seen from different inclination angles (Fig. 1).

One of the major advantages of the SFM technique is that it is amenable to quantitative measurements. Each point in the digital image has an *X*, *Y*, and *Z* coordinate, thus allowing us to easily obtain structural data at the single-fiber level, a great advantage over the population-average information from physical methods previously employed. The three-dimensional coordinates of each nucleosome can be automatically registered by clicking with the computer mouse at the center of the top of the nucleosome; these coordinates are then fed into software programs that determine center-to-center distances of successive nucleosomes, the angles formed by lines connecting the centers of three successive nucleosomes, and fiber heights. The distributions of these parameters for native chicken erythrocyte fibers imaged on glass are presented in Fig. 2. As can be seen in Fig. 2 *A*, the center-to-center distances are distributed within the range expected on the basis of biochemical determinations, with a major peak centered around 21 nm, which corresponds to ~62 bp, in very good agreement with the value determined biochemically for these cells. Two other minor peaks are also observed in the distribution, centering around 26 nm (~76 bp) and 15 nm (44 bp), respectively. What determines the existence of these minor peaks is not clear at the moment. Our hypothesis is that the existence of discrete peaks in the distribution, superimposed on a high background, may reflect a different mutual orientation of linker histone molecules at successive nucleosomes: head to tail, head to head, or tail to tail. (Linker histones have a distinct asymmetrical three-dimensional organization consisting of unstructured basic N- and C-terminal tails, and a central structured globular domain (Hartman et al., 1977).) We are in the process of biochemically investigating this issue.

The distribution of angles is unexpectedly broad (Fig. 2 *B*), much broader than that obtained for isolated trinucleosomes (G. Zuccheri et al., unpublished results). The average value of the angle in fibers is 100 ± 40°, whereas that in trinucleosomes is 90 ± 30°. This difference may reflect some kind of longer range interactions in the fiber that are absent from isolated trinucleosomes; alternatively, it may be due to some artifacts connected to the imaging technique, for instance, different degrees of stretching upon interaction with the surface.

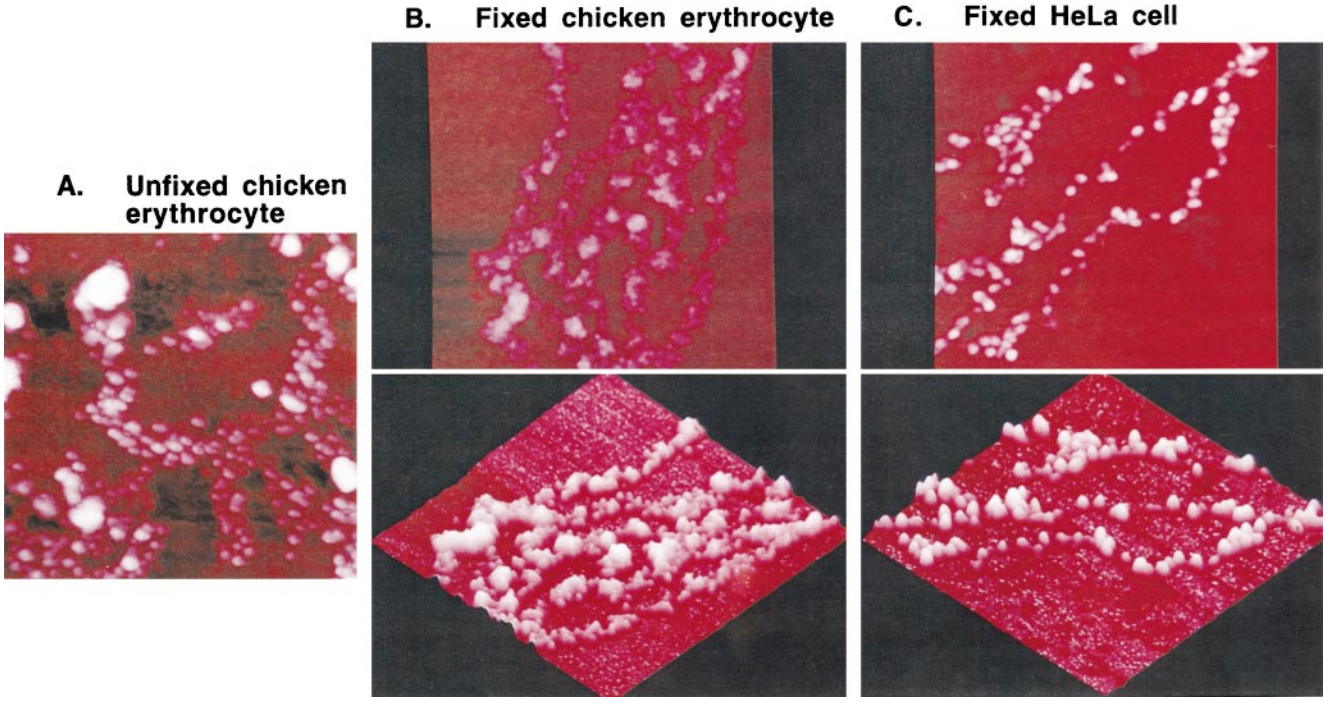

FIGURE 1 SFM images of unfixed chicken erythrocyte chromatin fibers, imaged on glass (*A*); glutaraldehyde-fixed chicken erythrocyte chromatin fibers, imaged on mica (*B*); and glutaraldehyde-fixed HeLa cell chromatin fibers, imaged on mica (*C*). To enhance the perception of three-dimensionality, fibers are also displayed in surface plots from an inclination of 45°. HeLa chromatin was prepared by H. Walia in Dr. J. Davie's laboratory.

Height measurements in the SFM are subject to a large error, due to substrate-sample and to tip-sample interactions. The nucleosome, for example, which is known from crystallography to be ~5.5 nm high, appears in the SFM as a particle of ~2.5–3-nm height. In view of this flattening, heights can only be used on a comparative basis. Fibers have an apparent average height of ~6 nm, twice that of an isolated individual nucleosome, which again points to the three-dimensional character of the low-ionic-strength fiber.

To gain some knowledge of the molecular determinants of the extended fiber structure, we have mathematically modeled this structure on the basis of some known parameters, like the dimensions of the core particle as determined by crystallography, the known twist of the DNA, etc. (Leuba et al., 1994). The morphology of such mathematically created chromatin fibers was found to crucially depend on two major parameters: the angle between the DNA entering and exiting the core particle, and the linker length. In accordance with earlier similar models (Woodcock et al., 1993), it was found that if all DNA linkers were of uniform length, a regular helix resulted, the actual fiber morphology being determined by the value of the linker length. If linkers, however, were allowed to vary in a certain physiologically relevant range, the model fiber acquired the highly irregular, kinky appearance typical of real fibers.

To evaluate the extent to which such mathematically created models resemble actual structures, we transformed the models into simulated SFM images by first projecting the computer-generated structures onto a plane (to mimic deposition on an imaging surface), and then simulating the effect of image broadening due to the finite tip dimensions according to published procedures (Keller and Franke, 1993). To make heights of nucleosomes in the calculated image agree with those actually observed, heights were compressed by a factor of 2 as the imaginary tip scanned the model. Prediction and observation agree very well (see Figure 4 in Leuba et al., 1994, and Figure 1 in van Holde and Zlatanova, 1995). This agreement indicates that, at least to a first approximation, the major molecular parameters determining extended fiber morphology are the entry/exit angle and the variability of linker lengths.

We then asked: What is the role of linker histones in the three-dimensional organization of the extended chromatin fiber? In each cell the linker histones comprise a family of closely related molecular variants, all sharing the same secondary and tertiary structure. These histones are known to interact with linker DNA at or near the entry/exit point of the core particle (Boulikas et al., 1980; Allan et al., 1980), sealing two turns of superhelical DNA around the histone octamer (for a recent study, see Hamiche et al., 1996, and Fig. 3). They are also believed to contribute, in ways still unknown, to the compaction of the chromatin fiber observed upon an increase of the ion concentration in the medium.

What happens to the extended fiber structure if the lysine-rich histones are removed? SFM images of fibers from which linker histones have been depleted show flat, beads-on-a-string filaments, of single nucleosome height (for examples of such images, see Leuba et al., 1994; Yang et al., 1994). A very intriguing change in the distribution of angles

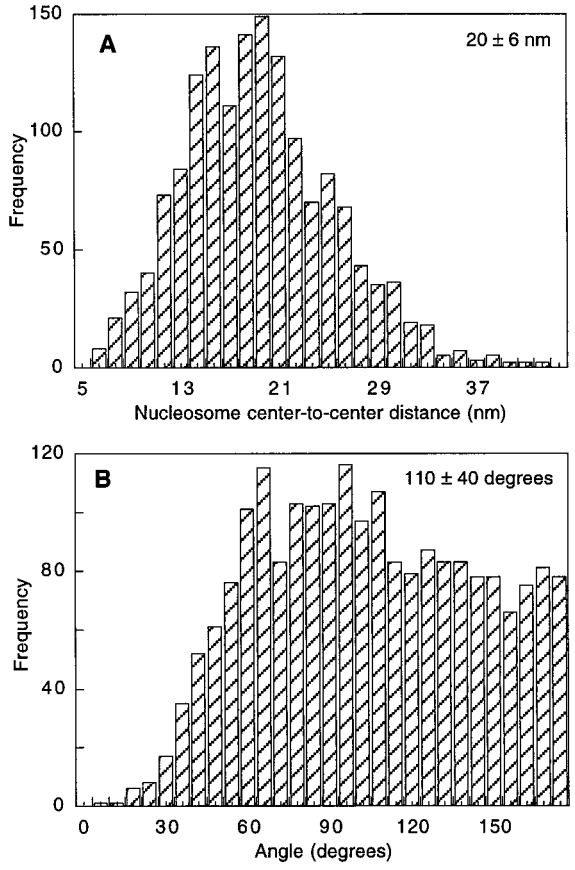

FIGURE 2 Frequency distribution histograms of center-to-center internucleosomal distances (*A*), and angles formed by lines connecting centers of three successive nucleosomes (*B*) for unfixed chicken erythrocyte chromatin fibers, imaged on glass (see Fig. 1 *A*). When the path of the fiber was not clear in these three-dimensionally organized fibers, the measurements were repeated on different days on the same images; such independent measurements have always given very similar distributions. Importantly, these distributions were significantly different from those obtained on linker histone-depleted fibers (see text).

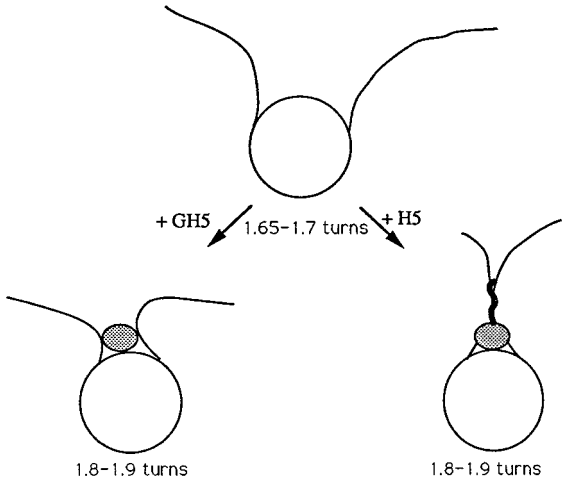

FIGURE 3 A recent model for the effect of linker histones on the path of the linker DNA entering and exiting the nucleosome. The schematic is based on cryo-EM and EM studies of Furrer et al. (1995) and Hamiche et al. (1996). The top represents the structure commonly observed for mononucleosomal particles reconstituted from a piece of DNA and core histones, in the absence of linker histones. The DNA tails are pushed apart by electrostatic repulsion and are partially peeled off the core. The addition of GH5 increases the wrapping of the DNA around the histone octamer to almost two complete turns, with the tails of the DNA still diverging. The addition of intact H5, carrying the C-terminal tail, brings the two DNA duplexes together, producing a stem structure ~30 bp in length. Salt concentration is 50 mM NaCl.

is observed upon linker histone removal. Whereas the distribution in the control, linker histone-containing fibers is a broad, ill-defined, bell-shaped curve, the distribution in the stripped fibers is strongly skewed toward large angles (Fig. 4). This change probably reflects electrostatic repulsion between linker DNA segments, forcing linkers apart. The existence of such a strong repulsion between successive linkers has recently been demonstrated on mononucleosomes reconstituted on short linear DNA fragments in the absence of linker histones (Furrer et al., 1995). The importance of overcoming this electrostatic repulsion for fiber compaction has been stressed on the basis of theoretical analysis (Clark and Kimura, 1990).

Extreme repulsion between linker DNA, in the absence of linker histones, can actually lead to removal of some DNA wrapped about the histone core. In our mathematical model, 1.75 superhelical turns of DNA in the core particle (Richmond et al., 1984) would fix the value of the angle at 90°; if the angle is allowed to vary between 0° and 180°, the number of superhelical turns will also vary from one to two complete turns around the octamer. The model structures predicted on the basis of this assumption and their transformation into SFM images lead to a flat, beads-on-a-string conformation, closely resembling that of experimental SFM images of linker histone-depleted fibers (for an example, see Figure 2 in Yang et al., 1994). Furthermore, inspection of the distributions of center-to-center internucleosomal distances for the model and for actual SFM images shows that removal of linker histones leads to increased linker lengths, which must come from a release of some DNA from the histone cores. The data unequivocally show that in the absence of linker histones, as much as one turn of the DNA can unwrap from the octamer. Earlier biophysical and biochemical data supporting this notion have recently been reviewed (Zlatanova and van Holde, 1996; see also Allan et al., 1982).

To further understand the molecular determinants of chromatin fiber structure, we have asked some additional questions. Is it the central globular domain of the linker histones, or their less organized basic N- and C-terminal tails, or both that are involved in maintaining the entry-exit angle? What is the role, if any, of the core histone tails in the three-dimensional organization of the low-ionic-strength fiber? The rationale for including the core histone tails in our search for determinants of fiber structure was based on the fact that the core histone tails do not participate in the structure of the core particle, but rather protrude (van Holde, 1988). If they were fully extended, some of the tails could

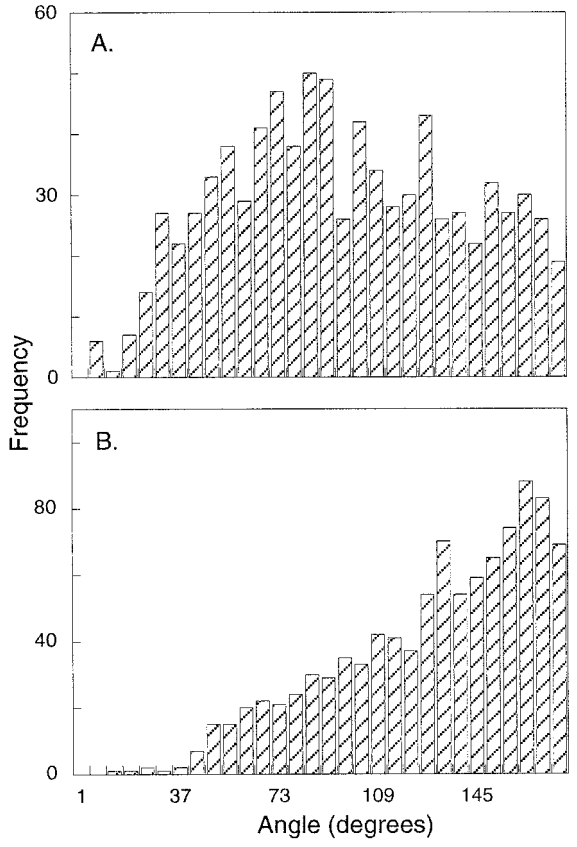

FIGURE 4 Frequency distribution histograms of angles in glutaraldehyde-fixed intact chicken erythrocyte fibers (*A*) and in linker histone-depleted fixed fibers (*B*). The maximum angle measured was 180°; for example, 90° and 270° were both taken to be 90°. Note the change in the form of the distribution curves (see text).

span rather long distances, creating opportunities for interactions with other components of the fiber: the linker DNA, the linker histones, or other nucleosomes. Indeed, the tails have been implicated in favorable nucleosome-nucleosome interactions, and have been shown to interact with linker DNA and linker histones (for further discussion and references, see van Holde and Zlatanova, 1996). In addition, they seem to be involved in the salt-induced compaction of the extended, low-ionic-strength fiber, as evidenced by changes in physical parameters of the condensed chromatin fiber upon proteolytic digestion of the tails. Allan et al. (1982) have shown that polynucleosomes that contain core histones devoid of the tails remain substantially uncondensed in high salt, even in the presence of histone H1. Similar findings were reported on reconstituted nucleosomal arrays in the absence of histone H1 (Garcia-Ramirez et al., 1992; Fletcher and Hansen, 1995).

We have approached these questions by combining the imaging capabilities of SFM with conventional biochemical approaches. We have used both trypsinization of isolated long chromatin fibers under extremely mild conditions (Zlatanova et al., 1995) and reconstitution of linker histone-depleted fibers with either intact linker histone H5 or its isolated globular domain (GH5) (Leuba et al., manuscript submitted for publication). The linker histone-depleted fibers used as substrates for reconstitution contained either all core histones intact, or H2A, H2B, and H4 intact, and histone H3 lacking its N-terminal tail (see scheme of experimental approach in Fig. 5). The fiber structures resulting from both kinds of reconstitutions were imaged in the SFM and characterized by extensive measurements of internucleosomal center-to-center distances, angles, and fiber heights.

Using fibers depleted of linker histone as the substrate for reconstitution, we demonstrated that the addition of intact histone H5 led to the expected transition from the flat, "beads-on-a-string" morphology of the depleted fibers to the three-dimensional structures typical of native fibers. The basic structural parameters of the reconstituted fibers, center-to-center internucleosomal distances (Fig. 6), angles, and fiber heights also indicated close recovery of the native fiber characteristics. These results provided an essential control for the fidelity of H5 reconstitution. When reconstitutions were performed using only the isolated GH5, both fiber morphology and the quantitative characteristics of the fibers again reverted, to a considerable degree, to those of native fibers (Fig. 6). This result indicates that when intact core histones are present, the globular domains of the linker histones are, in themselves, sufficient to stabilize the low-ionic-strength structure such that fibers exhibit three-dimensional morphology on the mica surface. In should be noted that the lack of an absolute requirement for the tails of the linker histones for the proper three-dimensional organization of the extended fiber contrasts with their being essential for further compaction of this fiber under physiological conditions (Allan et al., 1986).

Next, similar reconstitution experiments were performed on linker histone-stripped fibers that also lacked the N-terminus of histone H3. Such fibers were obtained by H1/H5 depletion of fibers that had been subjected to mild proteolysis with membrane-immobilized trypsin (Zlatanova et al., 1995). Reconstitution of intact H5 onto such H3-tailless material led to a complete recovery of native morphology and structural parameters (Fig. 6). This result was totally unexpected and indicates that the N-terminal tail of histone H3 in itself is not absolutely required for the proper three-dimensional organization of the extended fiber, despite its being essential to fiber compaction (see above).

Finally, when isolated GH5 was reconstituted on the H3-tailless, linker histone-stripped fibers, the reconstituted GH5 remained flat and showed no changes in the center-to-center distances (Fig. 6) or the angles with respect to the starting substrate for reconstitution. Thus, when both linker histone tails and H3 tails are missing, the three-dimensional structure of the extended fiber is unstable and flattens on the mica surface.

The morphologies of the different kinds of fibers and the quantitative assessment of their structural parameters lead us to conclude that the three-dimensional organization of chromatin fibers at low ionic strength requires 1) the glob-

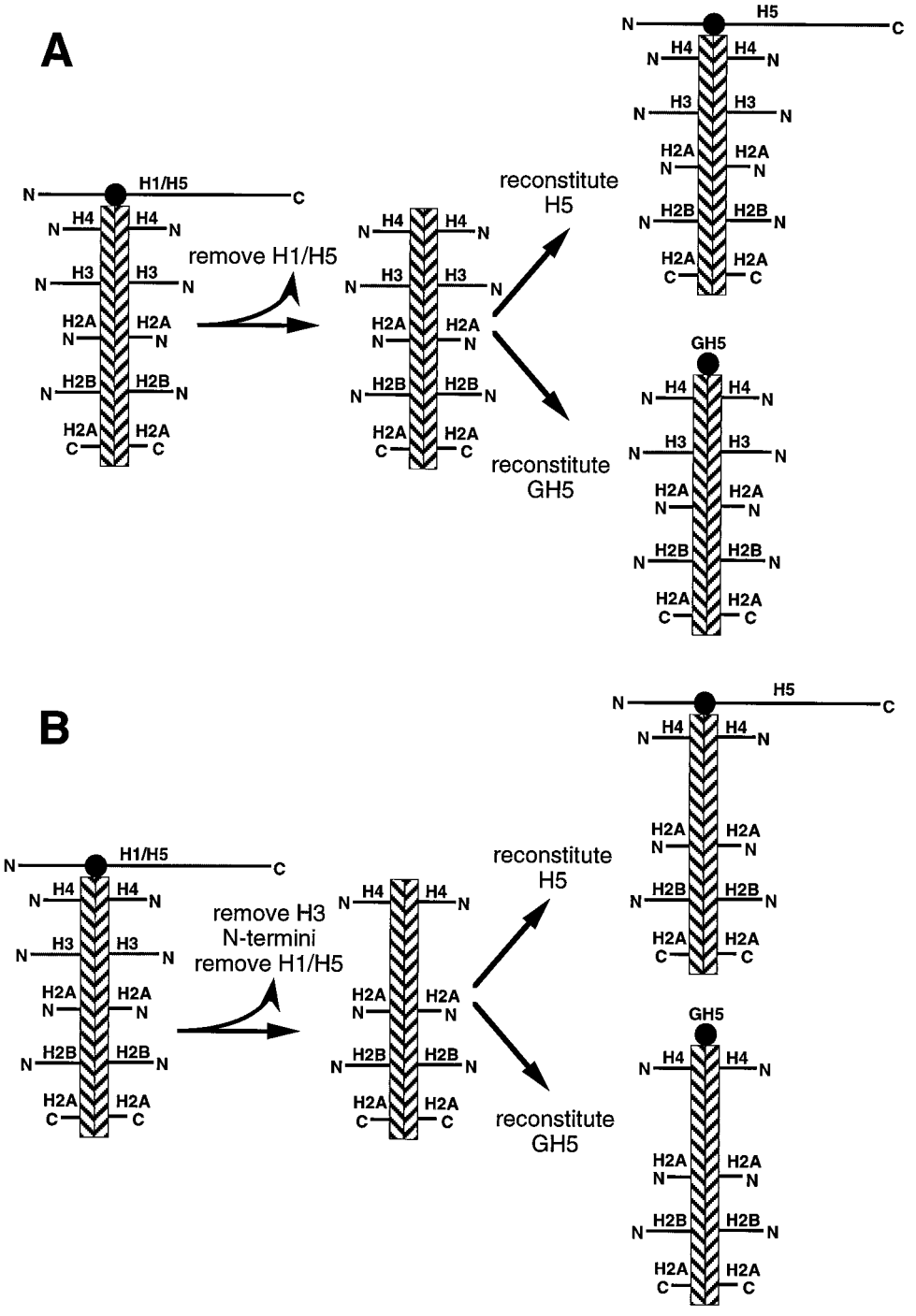

FIGURE 5 Experimental approach for the reconstitution experiments performed to study the contribution of the individual structural domains of the linker histones and of the N-terminal tail of histone H3 to the stability of the three-dimensional conformation of the low-ionic-strength fiber, as studied by SFM imaging. The scheme depicts a side view of each nucleosome in the fiber, with the tails of the core histones protruding from the core particle structure; the structural domains of linker histones are represented by a line, a filled circle, and another longer line (N-terminus, globular domain, and C-terminus, respectively). All histone tails are depicted as devoid of any secondary structure, as maximally extended polypeptide chains, with 0.362 nm per amino acid residue (Corey and Pauling, 1953). Reconstitution was carried out using linker histone-stripped chicken erythrocyte chromatin fibers (*A*) or linker histone-stripped fibers, which also lacked the N-terminal tail of histone H3 (*B*), as the starting substrate.

ular domain of the linker histones and 2) either the tails of the linker histones or the N-terminus of histone H3. If the tails of both of these histones are missing, fibers exhibit flat morphologies in the SFM, with the normal constraint on the angle being lost. The role of the N-terminal tail of histone H3 seems to be unique, because the tails of the other core histones cannot substitute for it in stabilizing the three-dimensional arrangement of nucleosomes in the extended

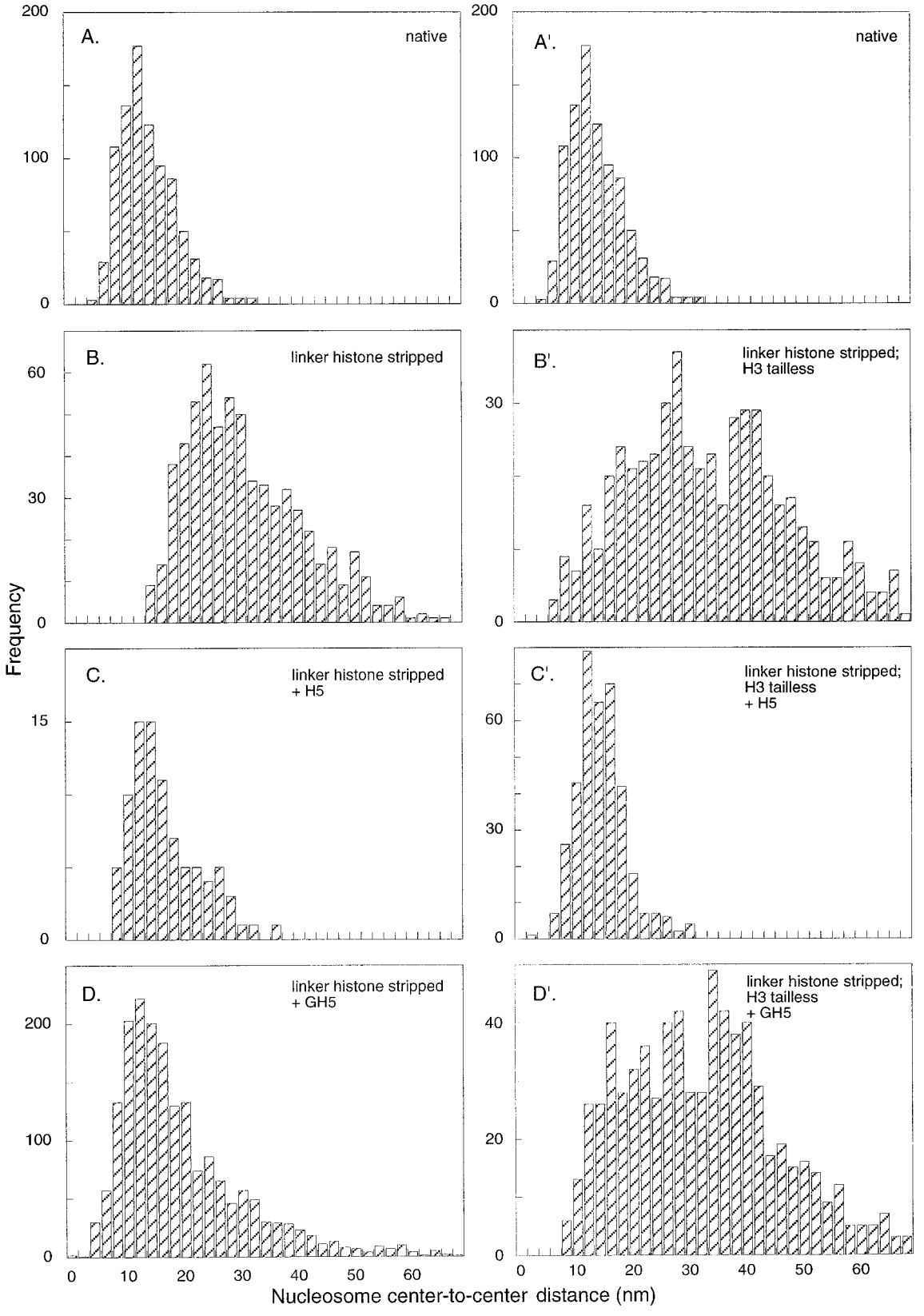

FIGURE 6 Frequency distribution histograms of center-to-center internucleosomal distances on the different types of fibers described in Fig. 5. (*A* and *A′*) Native chicken erythrocyte chromatin fibers. (*B* and *B′*) Fibers used as reconstitution substrates, H1/H5-stripped fibers and H1/H5-stripped fibers also missing the N-terminal tails of histone H3, respectively. (*C* and *C′*) Fibers in *B* and *B′*, reconstituted with intact histone H5 at a molar ratio of 1.3 molecules of H5 per nucleosome. (*D* and *D′*) Fibers in *B* and *B′*, reconstituted with isolated globular domain on histone H5 at a molar ratio of ~2 molecules of GH5 per nucleosome. Note that the center-to-center distances in the H1/H5-containing control fibers were measured to be ~15 nm (44 bp), rather than the 20 nm expected on the basis of the experimentally determined linker length in this tissue. This difference between observed and expected distances is due to glutaraldehyde fixation, because when measurements were performed on unfixed material (see Fig. 2 *A*), the value obtained was ~20 nm.

chromatin fiber. On the basis of these results and data in the literature, we propose the unified view illustrated in Fig. 7 for the roles played by the different domains of the linker histones and the N-terminal tail of histone H3 in chromatin fiber structure. The globular domain of the linker histones is required to seal two turns of DNA around the histone octamer. This closure allows the linker histone C-terminal domain to bind to both linkers simultaneously, creating the kind of stem structure recently described in mononucleosomal particles (Hamiche et al., 1996). The actual angle between successive nucleosomes will be determined, in such a scenario, by linker divergence at the distal end of the stem structure (see Fig. 3).

Why are the tails of histone H3 structurally redundant to the tails of linker histones? The answer can be sought in the crystallographic analysis of the structure of the histone octamer (Arents et al., 1991; Arents and Moudrianakis, 1993) and the recently published crystal structure of the nucleosome core particle (Luger et al., 1997). The structural data indicate that only the N-termini of H3 are situated in a manner to allow extensive interaction with the linker DNA close to the entry-exit point. The H3 tails, when maximally extended, can reach over a significant portion of the linker DNA, so as to neutralize its charges, even at times when the tails of the linker histones may not be interacting with the linker DNA.

CHROMATIN FIBER STRUCTURE AT HIGH IONIC STRENGTH (CONDENSED FIBER)

As stated in the Introduction, the morphology of the condensed chromatin fiber (which is believed to represent the physiologically relevant structure for at least the inactive portions of the genome) is not yet resolved. Almost all proposed models for this structure present some sort of regular helices; however, the actual experimental data suggest a quite irregular, kinky, and/or blobby conformation, with only limited regions of regularity (van Holde and Zlatanova, 1995; Woodcock and Horowitz, 1995).

We have attempted to acquire some knowledge of the condensed fiber structure by SFM imaging. Because of technical limitations, we could not, for the time being, image under buffers of different ionic composition and strength. Imaging in air of unfixed fibers solubilized at low ionic strength and then brought to higher salt also turned out to be impossible, because even relatively low salt concentrations (~10 mM NaCl) would cause dissociation of the fiber into its DNA and histone components, probably because of interactions with the substrate. The need to wash the deposited sample with water before actual imaging could also be a source of artifacts, because changes in the milieu change fiber conformation. These technical difficulties made us turn to the more conventional way of high-

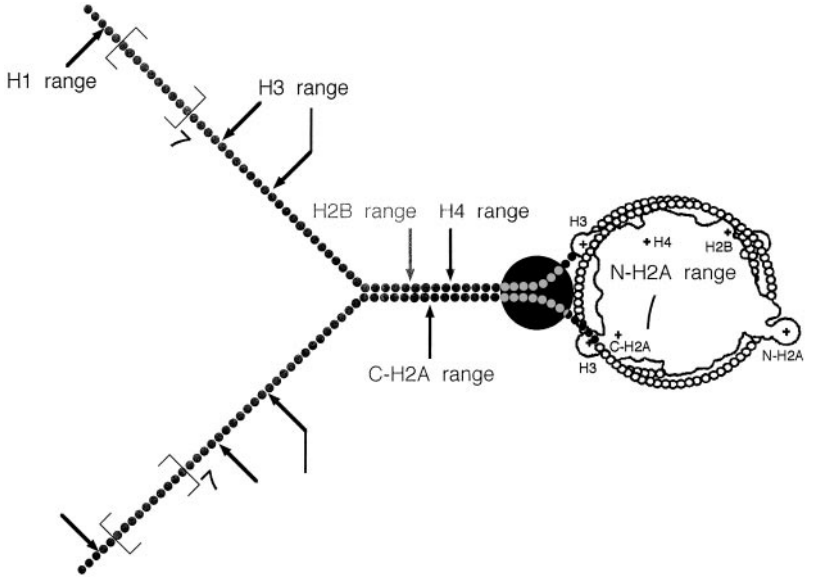

FIGURE 7 A speculative model for the role of linker histones and core histone termini in defining extended chromatin fiber structure. An outline of the core particle structure (plate 2C of van Holde et al., 1995) depicts the histone octamer, with open circles showing the path of the DNA around it, extending into the linker stem structure proposed by Hamiche et al. (1996) (*filled circles*). In the structure of the histone octamer (Arents et al., 1991), the core histone N-termini are not present: missing are 22 N-terminal residues of H4, 42 residues of H3, 35 residues from H2B, 14 residues of H2A, and 20 C-terminal residues of H2A. The "origins" from which these missing histone terminal domains exit the core are marked by + signs. This assignment has recently been confirmed by the crystallographic analysis of the entire nucleosome core particle (Luger et al., 1997). The globular domain of the linker histone (*large black circle*) is placed at the entry/exit point of the nucleosome, close to the nucleosome dyad. The maximum possible length of the C-terminal tail of the linker histone was taken to be 120 amino acid residues. The distances that the termini of all histones could span in the direction of the linker DNA are shown by arrows. The figure is meant to emphasize that of all the core histone tails, only those of H3 are of sufficient length and so positioned as to interact with a significant portion of the linker DNA. In fact, because our experiments utilize chromatin fibers from which 26 (or 20) amino acid residues are missing from the N-terminal domain of histone H3, it is probably this portion of the molecule that is essential for fiber stability. It should be noted that the linker DNA lengths shown here are much longer than any known linker; they are so extended only to show the possible range of the H1 C-terminal tail.

resolution imaging of salt-induced fiber conformations, similar to that used in EM (e.g., Thoma et al., 1979). Long chromatin fibers were first brought to a series of solutions of the desired ionic composition and strength, then fixed with glutaraldehyde, dialyzed versus buffers containing no salt, and then deposited on mica and imaged. Series of representative images of chicken erythrocyte chromatin fibers, fixed at different concentrations of NaCl or $MgCl_2$, are presented in Figs. 8 and 9. As expected, increasing the salt concentration leads to increasing compaction of the fiber. The appearance of the fibers is very irregular and blobby, and individual nucleosomes are no longer discernible, even at ~40 mM NaCl or ~0.2 mM $MgCl_2$.

This blobby appearance is remarkably similar to negatively stained EM images of condensed fibers which, together with sedimentation and biochemical analysis of (intermediately) micrococcal nuclease-digested fibers, led to the "superbead" model for condensed fiber structure (e.g., Renz et al., 1976; reviewed in Zentgraf et al., 1981). Because the SFM imaging technique, when used in air, may still be prone to artifacts, a final conclusion as to the salt-condensed fiber structure must await imaging in liquid.

STRUCTURAL TRANSITIONS BETWEEN EXTENDED AND CONDENSED CHROMATIN FIBER CONFORMATIONS

It is obvious that in vivo the chromatin fiber undergoes reversible structural transitions between its different conformations. Our understanding of these transitions is surprisingly poor, even when they are studied under in vitro conditions on isolated long chromatin fibers. We have recently reviewed this topic in detail (van Holde and Zlatanova, 1996), so here we will only mention the main points concerning these transitions. We will also briefly discuss literature data dealing with the highly controversial issue of the effect of histone acetylation, a postsynthetic modification that is believed to be connected to transcription activity, on chromatin DNA topology.

Structural changes in chromatin compaction

A major unresolved question is whether linker DNA bends during compaction. In vitro data come from measurements of diffusion coefficients of dinucleosomal particles, sedimentation coefficients of oligonucleosomes of various lengths, and EM and cryo-EM imaging of such structures, all as a function of salt (for references, see van Holde and Zlatanova, 1996). An independent experimental approach probed for linker DNA conformation in situ, by monitoring the distribution of thymine dimers in core and linker DNA, after irradiation of nuclei (Pehrson, 1989, 1995). Although results from these various techniques and laboratories are not wholly consistent, the preponderance of data indicates that linker DNA remains extended, even at high salt concentrations, and is probably straight in the condensed fiber.

We believe that a major change in fiber structure that occurs during compaction involves a collapse of the angle ϕ made between the lines drawn between the centers of three consecutive nucleosomes. Indirect evidence for such a change comes from sedimentation studies of oligonucleosomes (e.g., Butler and Thomas, 1980): the observed variation in the sedimentation coefficient of trinucleosomes with salt may be easily accounted for by a change in the angle ϕ (see van Holde and Zlatanova, 1996). Direct measurements on trinucleosomes imaged by cryo-EM at different salt concentrations (Bednar et al., 1995) strongly support such a notion. Finally, computer-generated mathematical

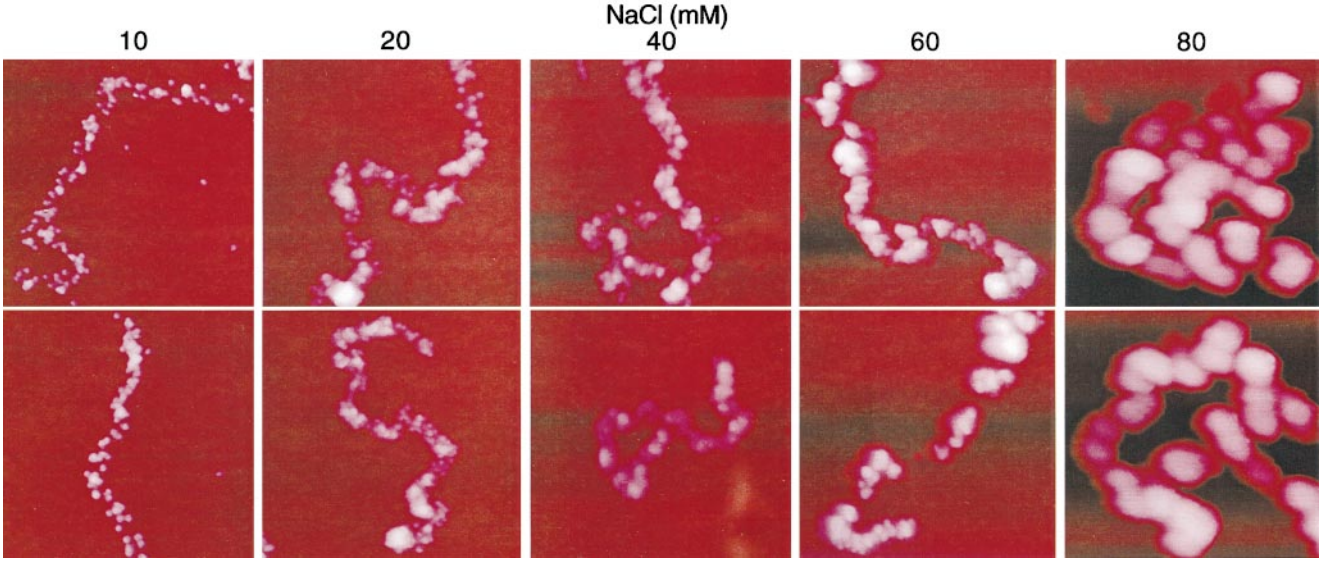

FIGURE 8 NaCl-induced compaction of the extended chromatin fiber, as visualized by SFM imaging. Chicken erythrocyte fibers, glutaraldehyde-fixed at the respective salt concentrations, were dialyzed versus 5 mM triethanolamine-HCl, pH 7.0, and deposited on freshly cleaved mica from this solution. Imaging is in air. Salt concentrations are marked above each panel. Images were taken in collaboration with Dr. G. Yang.

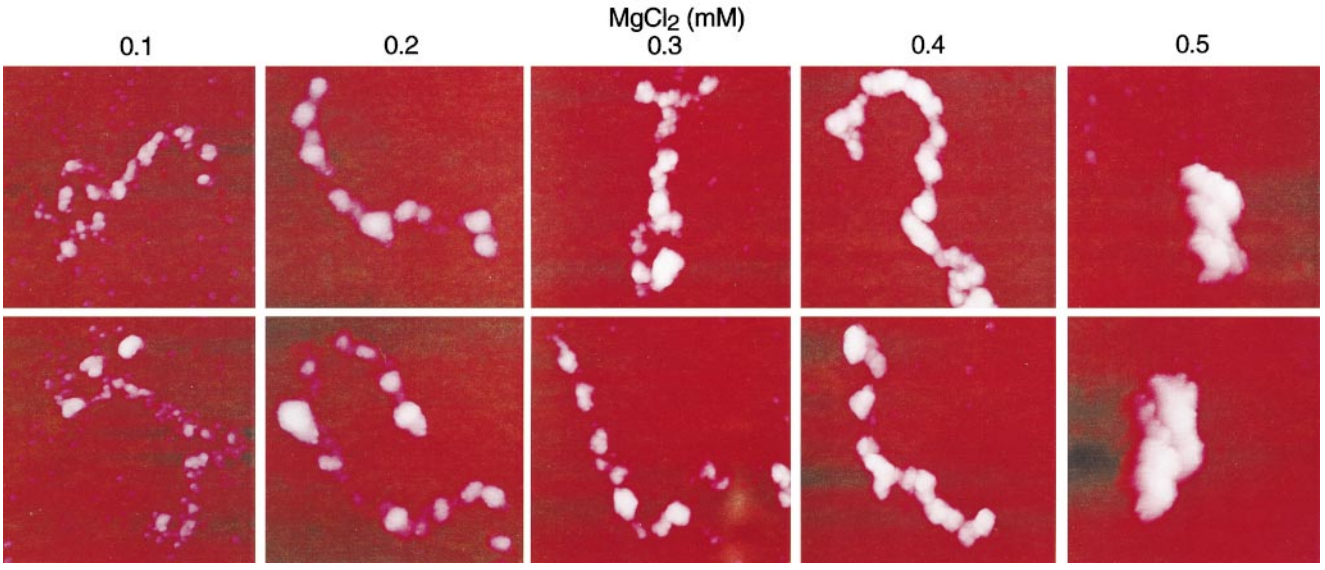

FIGURE 9 $MgCl_2$-induced compaction of the extended chromatin fiber, as visualized by SFM imaging. For further details, see legend to Fig. 8.

models show that the reduction in the angle value does lead to some compaction. Because the degree of compaction is far from the physiologically relevant one, it is clear that other factors are also involved. Although nucleosome-nucleosome interactions can be postulated as one such factor, evidence for their participation is scanty. That such interactions may exist is suggested by some in vitro studies of isolated core particles or by in vitro chromatin reconstitution experiments (for more details and references, see van Holde and Zlatanova, 1996). In our view, however, the physiological relevance of nucleosome-nucleosome interactions remains elusive, because no direct evidence for their existence in chromatin fibers has been presented.

The exact role of linker histones in fiber compaction is another unresolved issue. That these histones are needed for the fiber to attain its proper conformation, at both low and high ionic strengths, has been known for years. But the way in which the linker histones and their individual structural domains contribute to fiber structure is still not known. Even the location of these histones at the nucleosome and in the fiber is a matter of debate. What we can assert is that the globular domain is needed to stabilize the structure of the nucleosomal particle, and that the C-terminal tail is required for compaction, probably by interacting with linker DNA and neutralizing its negative charges.

Finally, several lines of experimental evidence converge to point to the importance of the unstructured tails of the core histones in fiber structure and compaction. Above, we have described our recent reconstitution experiments, indicating a role of the N-terminal tail of H3 in stabilizing extended fiber structure. Participation of this tail in the formation and/or stabilization of the compacted fiber has been suggested earlier on the basis of physical studies of trypsinized condensed chromatin fibers.

Chromatin unfolding during transcription

The compacted state of the chromatin fiber cannot possibly serve as a template for transcription, because it interferes with the accessibility of the underlying DNA template to regulatory molecules and the very bulky transcriptional machinery. That transcriptionally active chromatin is in a more "open" conformation has been suggested on the basis of numerous nuclease digestion experiments and sedimentation studies. Moreover, a number of chemical and/or compositional changes have been described that are characteristic for transcriptionally active chromatin. Among these, two deserve special attention: 1) depletion of linker histones and 2) acetylation of core histone tails.

Linker histones seem to be present on transcribed or transcribable genes, but in reduced amounts (Zlatanova and van Holde, 1992). In some cases gene activation may be accompanied by a change in the way linker histones interact with DNA, rather than by histone removal. It has been suggested that the histone may remain bound to the DNA via its tails, whereas the globular domain may lose its DNA contacts (Nacheva et al., 1989). From what we know about the contribution of these histones to fiber structure, reduction in their amount or in their strength of binding may be expected for active gene chromatin.

The core histones H2A, H2B, H3, and H4 all contain highly conserved sites for lysine acetylation. These sites reside exclusively in the unstructured N-terminal tails of these histones (see van Holde, 1988, for details). As early as 1964, Vincent Allfrey suggested that this modification might be specifically associated with transcribable regions in chromatin (Allfrey et al., 1964). An enormous number of studies over the subsequent years have tended to support this idea (for recent reviews, see Loidl, 1994; Sternglanz, 1996; Brownell and Allis, 1996). Very recent work has

begun to clarify some important points; for example, it is now clear that specific acetyltransferases are associated with the complexes of proteins involved in transcriptional initiation (for example, Brownell et al., 1996). However, it seems likely that the effect of acetylation is not restricted to initiation sites. For example, it has been shown that the whole region of the β-globin gene cluster that is nuclease-sensitive is also hyperacetylated (Hebbes et al., 1992, 1994).

What has remained elusive, however, is the mechanism underlying this correlation between acetylation and transcribability. It may involve a number of possibilities, such as acetylation-mediated reduction in the strength of binding of core histone tails to DNA, a decrease in the affinity of linker histone binding, and/or changes in DNA topology (for a discussion on the first two mechanisms, see van Holde and Zlatanova, 1996; for an overview of the third possibility, see below).

Does the level of histone acetylation affect chromatin topology?

There are a number of reasons to suspect, a priori, that changes in chromatin topology might be involved in acetylation-mediated gene activation. Modifications in superhelical torsion have frequently been postulated to be potential effectors of transcription, by destabilizing nucleosomal arrays. Such modifications might be accomplished either by changes in the wrapping of DNA about individual nucleosomes, or by changes in histone interaction with linker DNA—the latter being a function of the core histone tails and/or the linker histones.

In recent years, a number of investigators have approached this question by comparing the linking number change associated with the formation of minichromosomes from circular DNAs and either normal or hyperacetylated histones. It has long been known that the change in linking number per nucleosome ($\Delta L_k/n$, where n is the number of nucleosomes) is about -1.0 when histones with a "normal" level of acetylation are used for reconstitution of oligonucleosomes on circular DNA templates (Simpson et al., 1985; Norton et al., 1989). In a number of studies, experimenters have employed core histones raised to the high levels of acetylation believed to be associated with transcriptionally active chromatin. Both in vitro and in vivo techniques for incorporating these into minichromosomes have been used. Unfortunately, the results have been remarkably inconsistent. Both the in vitro studies of Norton et al. (1989, 1990) and the in vivo experiments of Thomsen et al. (1991) indicated that reconstitution with hyperacetylated core histones produced a significantly less negative $\Delta L_k/n$ than was obtained with control histones. This would seem to correspond to a winding of DNA by acetylation. Bauer et al. (1994) have suggested that this effect does not arise from changes in either the length or the twist of DNA associated with the core particle, but rather from acetylation-mediated distortion of the shape of the nucleosome in such a way as to decrease the number of times the DNA winds around the particle.

However, there are reported results in direct contradiction to those of Norton et al. (1989, 1990). An in vivo study of Krajewski and Luchnik (1991) seems to find the opposite effect. In another very carefully controlled in vivo study, Lutter et al. (1992) see essentially no change in $\Delta L_k/n$, even if high levels of acetylation are used.

The reasons for these marked discrepancies are by no means clear. There are, as pointed out by Lutter et al. (1992), a number of differences between the experimental systems that may make direct comparison questionable. Thus, for example, the minichromosomes made by Norton et al. clearly lack linker histones or nonhistone proteins; the SV40 chromatin assembled in the experiments of Lutter et al. has been shown to have them. The differences in protein composition, however, seem not to be the explanation, because Lutter et al. demonstrate that removal of H1 (and most nonhistone proteins) by a 0.45 M salt wash has very little effect on supercoiling. In fact, a small unwinding effect of linker histones on naked DNA (Ivanchenko et al., 1996a,b) and a H5-induced further DNA wrapping around the histone core (Zivanovic et al., 1990; Hamiche et al., 1996) would both lead to a decrease in negative superhelicity upon H1 removal, contrary to the small increase observed by Lutter et al. (1992). An alternative possible explanation for the results of Norton et al. (1989) might be connected to a preferential loss of nucleosomes from the acetylated minichromosomes during relaxation (the number of nucleosomes was determined on both the acetylated and unacetylated templates before, but not after the relaxation). At the moment, we must regard the inconsistency as a puzzle, which we hope will be resolved by future experiments.

In summary, recent evidence seems to be converging on a model in which both linker histones and the N-terminal tails of core histones play roles in regulating the accessibility of DNA in chromatin for transcriptional factors and machinery. The observation that both the linker histones and the acetylatable tails of core histone are involved in stabilization of the chromatin fiber suggests a synergism between these elements in preparing the fiber for transcription.

We thank Dr. G. Yang (University of Oregon, Eugene) and Dr. J. Davie and H. Walia (University of Manitoba, Winnipeg, Canada) for experimental collaboration. We also appreciate the use of the scanning force microscope (Nanoscope III; Digital Instruments, Santa Barbara, CA) in the laboratory of Dr. C. Bustamante.

This paper was a research contribution presented at the DIMACS/MBBC/PMMB Workshop on DNA Topology, Rutgers University, April 1997.

This work was supported by a National Institutes of Health grant (GM50276) to KvH and JZ, and a National Institutes of Health Postdoctoral Fellowship 1F32GM16600 to SHL.

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