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Chromatin Structure Revisited

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ABSTRACT: Independently of the enormous progress in our understanding of the structure of the core particle, there remain a multitude of structural questions still to be answered. The main points discussed here can be summarized as follows: (1) The meaning of the term 'core particle' should be widened to reflect the fact that the actual length of DNA wrapped around the histone octamer in the context of the chromatin fiber may vary between ~100 and ~170 bp. (2) In the chromatosome, the linker histone forms a bridge between one terminus of the chromatosomal DNA and a point close to the dyad axis. (3) The particle that contains one molecule of HMG1 may be classified as *a bona fide* chromatosome. (4) In the extended fiber, the partition of the nucleosomal DNA into core and linker is a dynamic feature, responding to environmental influences; fiber structure-related constraints demand that linker length be beyond a certain minimal value. (5) The compact fiber structure seems to be rather irregular; the precise nature of this structure is still to be determined. Finally, the term 30-nm fiber should be dropped as a designator of the compact or condensed chromatin fiber structure.

KEY WORDS: core particle, chromatosome, linker histone, HMG1, extended chromatin fiber, condensed chromatin fiber.

I. INTRODUCTION

DNA in the eukaryotic nucleus is stably bound with small basic proteins called histones to form chromatin. Twenty-five years ago a startling discovery was made that drastically changed our view on how the chromatin fiber is organized. It was found that chromatin consists of repeating units called nucleosomes. Each nucleosome is conventionally viewed as consisting of a core particle, in which 146 bp of DNA are wrapped around an octamer of core histones, linker DNA that extends on both sides of the core particle, and a molecule of linker histone that is bound to the linker DNA (van Holde, 1988). However, it seems that even now, 25 years after the discovery of the nucleosome, there is confusion concerning the exact organization of this fundamental repeating unit of chromatin (for some recent attempts at definition see Widom, 1998a; Travers, 1999; van Holde and Zlatanova, 1999). It is the aim of this essay to critically discuss the relevant chromatin structural issues and to attempt to separate the actual situation from some preconceived or dogmatic notions. We discuss in turn each individual level of chromatin organization, starting with the core particle and ending with the condensed chromatin fiber structure.

II. THE CORE PARTICLE

When chromatin is extensively digested by micrococcal nuclease, a strong pause is observed when the DNA has been reduced to fragments 146 bp (± 2 bp) in length. The only proteins associated with the DNA at this point are an octamer of histones H2A, H2B, H3, and H4. This observa-

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tion can serve as an *operational* definition of the core particle and allows the preparation of well-defined, homogeneous protein/DNA complexes. Consequently, the structure of the core particle is now well known, with recent major contributions coming from the crystallographic analysis of the structure of the histone octamer itself (Arents and Moudrianakis, 1993) and the core particle as a whole (Luger et al., 1997). These studies have provided a wealth of knowledge about the way the histone molecules interact with each other in the protein core of the particle and about the interactions between the histone octamer and the DNA wrapped around it.

Perhaps because of these elegant studies, it has become assumed that the core particle, in itself, is an object of major physiological significance (like, say, the ribosome). We (van Holde and Zlatanova, 1999) have recently subjected this notion to critical evaluation. Based on analysis of biochemical and biophysical data published over the last 2 decades, we conclude that to regard the core particle in this way probably has little structural or physiological basis. Indeed, the actual length of the DNA wrapped around the core histone octamer varies between ~100 bp and ~170 bp, depending on the environmental conditions, and, possibly, on the postsynthetic modifications of the constituents of the particle. Strong, albeit indirect, evidence that the 146-bp definition is probably an oversimplification comes from the recent studies of the crystal structure of the core particle itself (Luger et al., 1997). It was observed that about 13 bp on each end of the "core particle" are not in contact with the histone octamer, so the length of DNA directly interacting with the octamer is only about 120 bp, or ~1.6 superhelical turns. At this point the possibility cannot be excluded that this specific conformation is due to crystal packing forces, but such an interpretation neglects a large amount of other data (van Holde and Zlatanova, 1999). At best, a particle containing 146 bp of DNA wrapped around the histone octamer can be viewed as only one possible structure in the continuum of particle structures containing DNA from ~100 to ~170 bp (Figure 1).

Of course, there must be specific details of the DNA-protein interactions that promote a strong pause in nuclease digestion at 146 bp under some conditions, but this need not have *functional* sig-

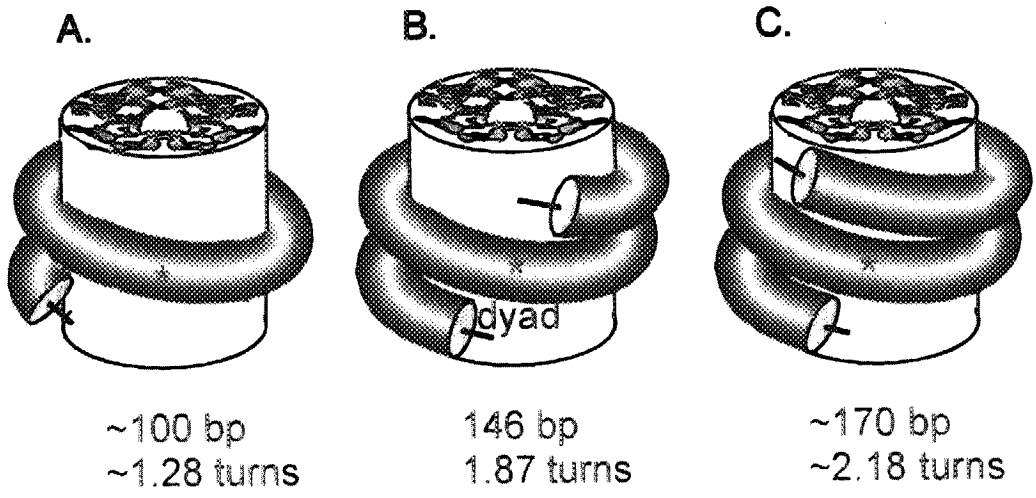

FIGURE 1. Schematic drawing of the extreme degrees of wrapping of nucleosomal DNA around the histone octamer. DNA is depicted as a tube, and the octamer as a cylinder. (A) The "minimal" stable particle contains ~100 bp wrapped around the histone octamer in ~1.28 superhelical turns. (B) The canonical core particle, with 146 bp of DNA tightly wrapped in 1.87 turns around the octamer. (C) A particle in which ~170 bp of DNA are organized by the octamer, thus forming ~2.18 superhelical turns around the octamer.

nificance. Indeed, other conditions and other nucleases give different results (van Holde and Zlatanova, 1999; Chikhirzhina, 1982).

III. THE CHROMATOSOME

When micrococcal nuclease digestion is carried out on linker histone-containing chromatin, a strong pause is observed before digestion continues to the core particle. The product of such digestion is a particle containing a histone octamer, one molecule of linker histone, and 168 bp of DNA, which has been termed the *chromatosome* (Simpson, 1978; Travers, 1999). While this operational definition seems reasonable, we would like to address two points concerning the actual structure. First, it is important to realize that it is also possible to isolate (under certain conditions) "core particles" containing 168 bp of DNA. The chromatosome, containing a molecule of a linker histone, is a distinct structural entity from such a particle (see above). The distinction lies not only in the presence or absence of linker histone, but also in the trajectory of the DNA in the two particles (Figure 2). In the 168-bp core, the DNA must closely interact with the histone octamer along its entire length; in the 168-bp chromatosome, the ends of the DNA do not interact directly with the octamer. Such a distinction, which is intuitively straightforward, has found solid experimental backing only very recently. Bednar et al. (1998) used cryo-EM to image native or reconstituted short chromatin fibers, in the presence or absence of linker histones. In the linker-histone-containing fibers, the linker segments typically leave the octamer tangentially after completing ~1.7 turns on the histone octamer and continue along this same trajectory to a zone where the two linkers come together, forming the "stem" structure observed earlier in experiments with reconstituted mononucleosomes (Hamiche et al., 1996; Prunell, 1998). It would be interesting to see further details of the H1-containing particle when the long-anticipated crystal structure of the chromatosome is resolved (Richmond et al., 1993).

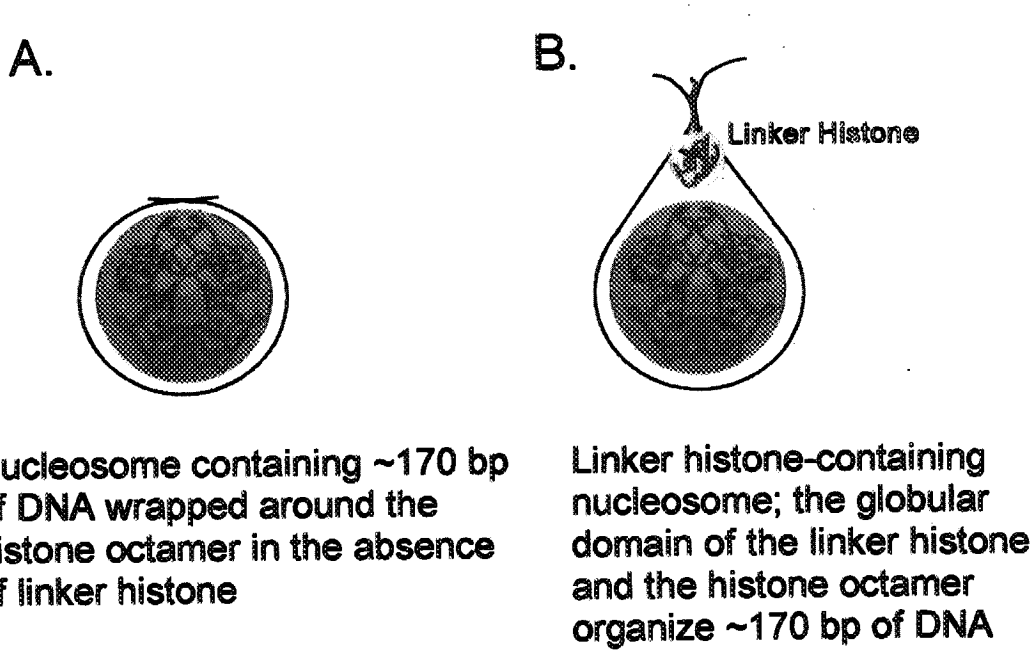

FIGURE 2. Schematic of the DNA trajectories in linker histone-containing and linker histone-lacking nucleosomal particles. Note that both types of particles contain the same length (~170 bp) of DNA, but in (A) this DNA is tightly wrapped around the histone octamer, whereas in (B) the DNA ends leave the particle tangentially, after the DNA completes 1.75 superhelical turns, to bind to the globular domain of the linker histone at some distance away. The trajectory of the DNA in such particles has only recently been directly visualized in cryo-EM (Bednar et al., 1998).

The electron-microscopic observations of individual chromatosomes in chromatin fibers may indicate that these objects are more properly to be considered "functional units" of chromatin than the core particle. It should be noted, however, that linker histones may be absent from some nucleosomes, especially in transcriptionally active chromatin regions. Thus, it seems more appropriate to regard the nucleosome, independently of the presence or absence of linker histones, as the basic unit of chromatin structure.

A remaining problem in chromatosome structure concerns the much-debated binding mode of the linker histones. As this issue has been extensively reviewed recently (Crane-Robinson, 1997; Vignali and Workman, 1998; Widom, 1998b; Travers, 1999; Belikov and Karpov, 1999), we limit the discussion to a brief description of the alternative models (Figure 3) and to the structural consequences of the mode depicted in each.

The first class of models implies binding of the globular domain at or close to the dyad axis, on the outside of the DNA gyres. The two alternative versions of this class of models assume either a symmetric binding (and hence protection of the linker DNA against nuclease digestion on both sides of the core particle) (Allan et al., 1980), or an asymmetric binding (and asymmetric protection against nuclease attack) (Travers and Muyldermans, 1996; An et al., 1998a; Zhou et al., 1998). In the asymmetric binding model, the globular domain of the linker histone makes two distinct contacts with DNA, bridging a site close to the dyad axis with a site close to the end of the chromatosomal DNA. The second class of models envisages the globular domain making contacts with only one segment of the DNA double helix, away from the dyad axis, and on the inside of the DNA gyres (Hayes and Wolffe, 1993, Pruss et al., 1996; Hayes, 1996).

In our view, the "bridging" version of the first class of models has received solid experimental evidence from crosslinking studies recently (Zhou et al., 1998). That bridging of the linker histones is asymmetric, at least in particles reconstituted on certain specific DNA fragments, is clearly seen from the asymmetric protection of linker DNA by linker histones (An et al., 1998a).

What are the structural consequences of the binding of linker histones to the nucleosome?

1. Linker histone binding fixes the length of the DNA that is wrapped around the histone octamer, thus limiting the structural variations in the core particle. Exactly how many base pairs of DNA are in direct contact with the octamer in the chromatosome and what the trajectory of the distal portions of the

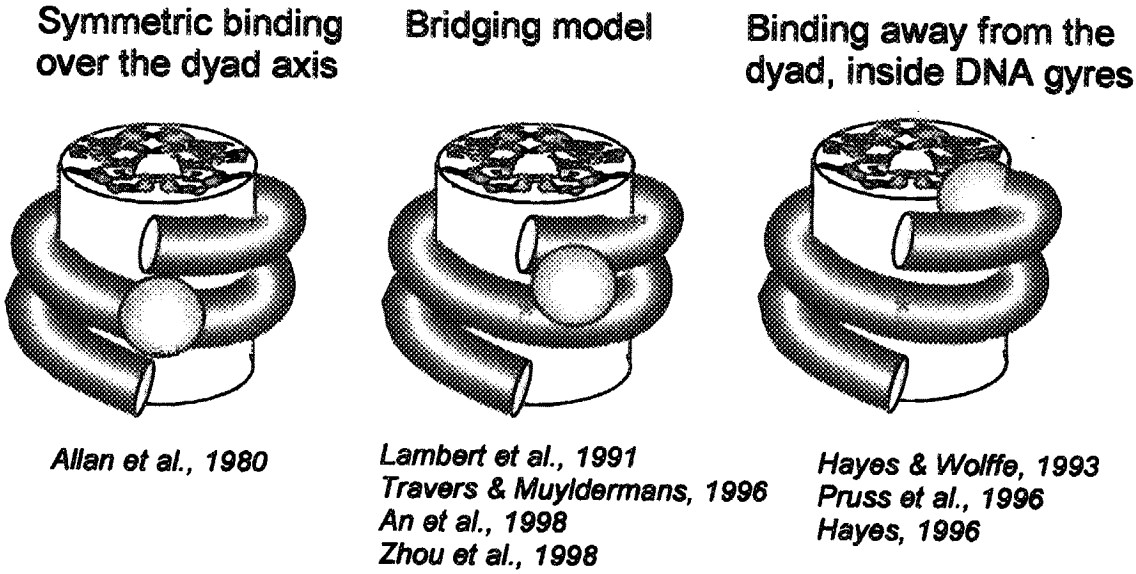

FIGURE 3. Models of linker histone binding to the chromatosome.

linker DNA is remains to be determined. In the absence of linker histone, the DNA wrapped around the core particle is free to partially unwrap. Such a partial despooling gives rise to isolated nucleosomes with less than 1.87 (146 bp in the core divided by 78 bp per superhelical turn) superhelical turns (Furrer et al., 1995), or, in the context of a fiber structure, to increased center-to-center distances between successive nucleosomes (Yang et al., 1994; Leuba et al., 1998). The absence of linker histones thus is crucial for the access of protein factors to the nucleosomally organized DNA, especially near its ends (Polach and Widom, 1995).

2. Second, and linked to the first, is the question of whether linker histone binding changes the linking number deficit associated with the organization of DNA into a core particle (for a recent in-depth discussion see Prunell, 1998). Although there has been considerable debate on this question, most data seem to indicate that ΔLk does not change after linker histone binding. This fact may be interpreted as showing that entering and exiting linker DNAs do not cross each other, but rather run in parallel in the stem structure. Such geometry should be of an important topological consequence, because it will allow binding or dissociation of linker histones to proceed with topological impunity. Bednar et al. (1998) discuss the absence of a change in ΔLk as supportive evidence for a zig-zag model (as opposed to solenoidal models) for fiber structure (see also below).
3. Linker histones are involved in fixing the angle between the incoming and outgoing DNA linkers. The average angle between successive linkers in linker histone-containing, extended chicken erythrocyte chromatin fibers is ~100°, whereas the value of this parameter is 130° in the linker histone-depleted fiber, with the distribution strongly skewed toward 180° (atomic force microscopy measurements, Zlatanova et al., 1998). Exactly how the linker histone fixes the angle is difficult to envisage mechanistically, in view of the existence of the "stem" structure, in which the incoming and outgoing DNA strands are brought together through binding to the basic unstructured C-terminal domain of the linker histone (Hamiche et al., 1996; Bednar et al., 1998).
4. Linker histone binding is absolutely necessary to three-dimensionally organize the nucleosomes in the extended chromatin fiber (at low ionic strength): in the absence of linker histones the extended chromatin fiber has the classic "beads-on-a-string" morphology, whereas in their presence this fiber is three-dimensionally organized into irregular structures (Leuba et al., 1994). Interestingly, and quite unexpectedly, the three-dimensional organization of the fiber requires the globular domain of the linker histones, and either the tails of the linker histone, or the N-terminal portion of histone H3 (Leuba et al., 1998). The structural redundancy of the tails of the linker histone and histone H3 might be explained by the need to neutralize the proximal portions of the linker DNA in the fiber, so that they can form the stem structure mentioned above (Figure 4).
5. Linker histone binding is necessary for the fiber to compact. Although some reports suggest chromatin compaction in the absence of linker histones (reviewed in Fletcher and Hansen, 1996), the relevance of these structures to the in vivo situation remains to be established (Ramakrishnan, 1997; van Holde et al., 1998). It must be pointed out that the exact structure of the fibers compacted in the absence of linker histones is also totally unknown (for a discussion see van Holde et al., 1998).

IV. THE HMG1-CONTAINING CHROMATOSOME

The high-mobility-group proteins HMG1 and HMG2 are important non-histone constituents of the eukaryotic nucleus (Bustin and Reeves, 1996). These proteins are known to bind to linker DNA, just as linker histones do (Bustin and Reeves, 1996; Zlatanova and van Holde, 1998). A ques-

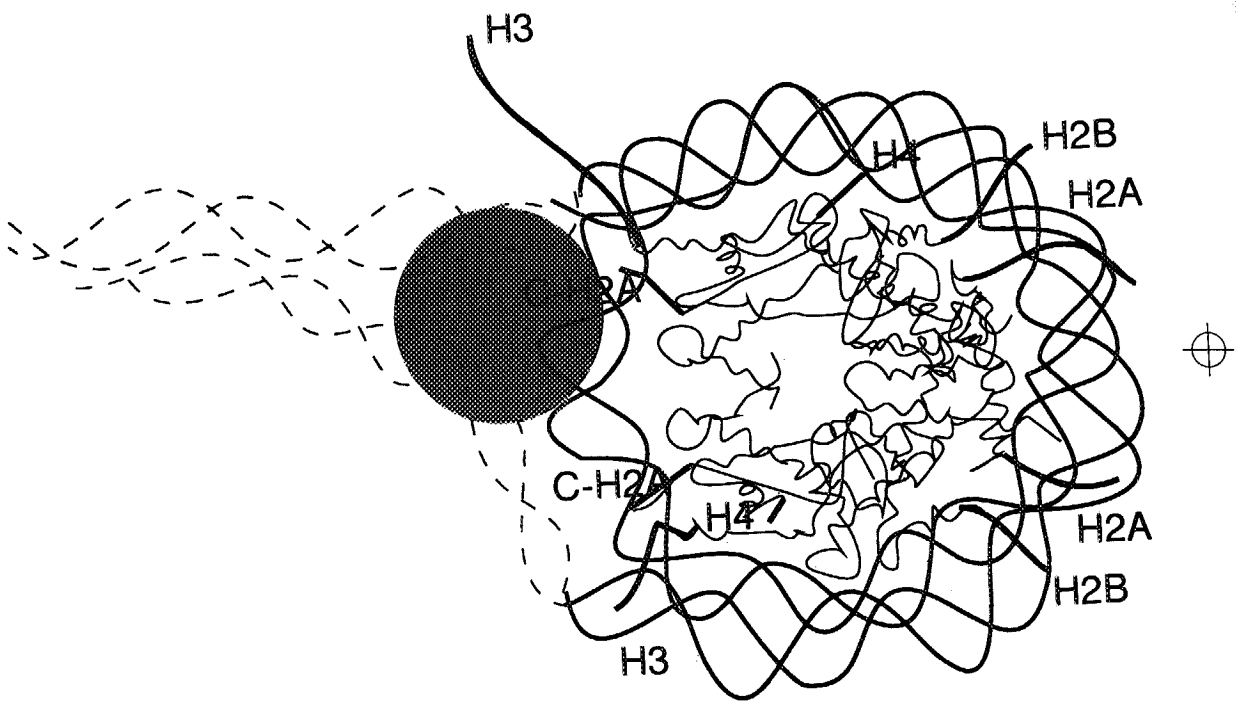

FIGURE 4. A to-scale schematic of the core particle (based on Luger et al., 1997), with adjacent stretches of linker DNA and the globular domain of the linker histone (filled circle) situated slightly off-axis (modified from Leuba et al., 1998). Note the location of the tails of the core histones (labeled thick lines), particularly the proximity of the tails of H3 to linker DNA. Such proximity allows the H3 tails to interact with linker DNA, neutralizing its charges. This interaction may be critical for the formation of the fiber structure, especially in cases when the tails of the linker histone are prevented from interacting with the linker due to postsynthetic modifications. (For the color rendering of this illustration, please refer to the end of this double issue.)

tion that has not been explicitly asked is the following: are particles containing one molecule of HMG1 (or HMG2) and no linker histones *bona fide* chromatosomes? Such particles have been obtained following MNase digestion of chromatin fibers, and also by reconstitution. The answer is, in our view, yes. First, one molecule of HMG1 bound to reconstituted mononucleosomal particles creates the chromatosome pause, protecting an additional ~20 bp from MNase digestion (Nightingale et al., 1996; Ura et al., 1996; An et al., 1998b). Second, at least in the few reconstituted particles studied, the protection is asymmetric, on one side of the nucleosome core, similar to the situation with linker histones. Interestingly, and of possible physiological relevance, the protection provided by HMG1 on the particle reconstituted on a USF/GAL4 sequence is on the side of the core opposite to the side protected by linker histones (An et al., 1998b) (Figure 5). Further studies are needed to see whether binding of HMG1 and linker histones is mutually exclusive, that is, whether the proteins compete with each other for binding to the nucleosome, where the exiting and entering DNA helices are in close spatial proximity (for further discussion see Zlatanova and van Holde, 1998). It would also be intriguing to know whether HMG1/2 can lead to the formation of the kind of "stem" structure produced by linker histones.

V. THE EXTENDED CHROMATIN FIBER

A. The Extended Chromatin Fiber Lacking Linker Histones

There is general agreement regarding the morphology of the linker histone-depleted fiber at low ionic strength—the fiber has a "beads-on-a-

string" appearance. There is, however, a misconception about the actual structure. The traditional view states that any given nucleosomal repeat length L_n is the sum of two constants. These are (1) the DNA around the histone octamer (we denote the length of the DNA wrapped around the histone octamer as L_o, to avoid using the ambiguous term L_c, the length of the DNA in the canonical core particle, see above), and (2) the length of the linker DNA, L_l. Actually, whereas L_n does equal $L_o + L_l$, both terms are variable within certain limits. L_o may vary between ~100 bp and ~170 bp, and L_l should vary accordingly to sum up to L_n. In the most extended, straight, beads-on-a-string appearance of the extended fiber, L_o should be ~120 bp (linkers enter and leave the particle on opposite sides, thus the DNA makes 1.5 superhelical turns at 78 bp per turn). The average value of L_n is determined by the positioning of nucleosomes during the formation (or subsequent modification) of chromatin. However, L_o and L_l may change, depending on environmental circumstances, if linker histones are removed.

It is important to realize that L_l has to be above a certain minimal value for the fiber to exist. This is especially true for the short repeat length chromatin fibers typical of lower eukaryotes, such as yeast (short repeats are also characteristic of some highly differentiated tissues in multicellular organisms, like neuronal cells, van Holde, 1988). The repeat length for *Saccharomyces cerevisiae* has long been known to be ~165 bp (Thomas and Furber, 1976; Lohr et al., 1977); more recently, a careful study of *S. pombe* chromatin reported a repeat length of 156 bp (Godde and Widom, 1992). The conventional view would tell us that in such chromatin fibers linkers should be very short, only 10 bp for the *S. pombe* case, for example. Godde and Widom (1992) have suggested that such fibers cannot form a crossed-linker three-dimensional structure, and the only way for them to form a solenoid is to coil right-handedly. A much simpler explanation would be that in such fibers the octamer organizes much less of the nucleosome DNA, leaving more in the linker.

Are there any data available that would hint at the minimal length of the linker DNA? We (Leuba et al., unpublished results) have measured in AFM images the center-to-center internucleosomal distances in chromatin fibers reconstituted from a tandemly repeated 172 bp-long nucleosome positioning sequence and core histones. The average center-to-center distance in such a fiber was found to be ~25 nm, translating into linker lengths of ~40 bp, that is, much longer than the expected value of 26 bp, if 146 bp were wrapped around the octamer. If the structure observed in the AFM is not severely distorted due to interactions of the fiber with the supporting surface, then it is reasonable to suggest that the linker should be probably rather long (not less than ~40 bp) to allow the freedom of moving the nucleosomes around to form a three-dimensionally organized, compactible structure. The consequence of this would be that only ~130 bp, or ~1.7 turns, remain wrapped about the histone core.

B. The Linker Histone-Containing Extended Chromatin Fiber

Linker histone-containing chromatin fibers at low ionic strength are extended zig-zags (Thoma et al., 1979). We have discussed the structure of this fiber on several occasions (van Holde and Zlatanova, 1996; Zlatanova et al., 1998). Here, for the sake of completeness, we only state that

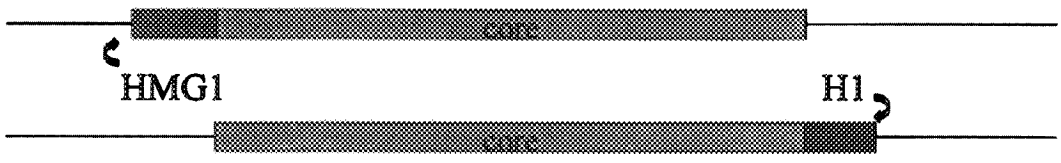

FIGURE 5. Schematic of the asymmetric protection of linker DNA against MNase digestion by H1 or HMG1. The drawing reflects data obtained on particles reconstituted from core histones and USF/Gal4 DNA, followed by addition of either linker histones (An et al., 1998a) or HMG1 (An et al., 1998b).

the linker histone-containing extended fiber is already three-dimensionally organized, with a rather loose, irregular appearance and an average diameter of ~30 nm. As discussed above, the integrity of this structure depends on the presence of the globular domain of linker histones, and either the unstructured tails of the linker histone, or the N-terminal portion of histone H3 (Leuba et al., 1998). The role of the N-terminal tail of H3 is unique, because its absence cannot be compensated for by the availability of the N-tails of the rest of the core histones. This unique role can be probably best understood in view of the crystal structure of the core particle—the N-tail of H3 is situated in such a way that it is in a unique position to interact with the linker DNA (see Figure 4).

VI. THE COMPACT CHROMATIN FIBER

The structure of the compact (condensed) chromatin fiber, as seen in physiologically relevant ionic strengths, is the least well understood. One thing has been clear for a long time, mainly on the basis of pioneering electron microscopic work (Thoma et al., 1979; De Murcia and Koller, 1981) and on sedimentation studies (Butler and Thomas, 1980): the chromatin fiber adopts a more and more compact conformation after increasing the ionic strength. However, neither the nature of the structure nor the molecular mechanisms involved in creating and maintaining it are understood.

Space limitations do not allow an in-depth discussion; in any event, there is not much to be said beyond what has already been presented elsewhere (van Holde and Zlatanova, 1995, 1996; Woodcock and Horowitz, 1995). To summarize, a number of researchers do not believe that there is strong evidence to support a solenoid model (van Holde and Zlatanova, 1995; Horowitz and Woodcock, 1995), or, as a matter of fact, any highly regular structure. On the other hand, other authors remain convinced that some kind of a solenoidal model is more correct (e.g., Widom, 1998a; Travers, 1999). What we can safely say on the basis of the available data is that the condensed fiber is a three-dimensionally organized, highly compact, and rather irregular arrangement of nucleosomes. As far as specifics are concerned, the nucleosomes are situated at the periphery of the fiber, and the linker DNA is most probably straight (see van Holde and Zlatanova, 1996).

While the first of these assertions seems to be generally accepted, the issue of whether the linker DNA is straight or bent has sparkled considerable controversy (reviewed in van Holde and Zlatanova, 1996; Widom, 1998a). Recently, two laboratories have addressed the issue, again with contradictory results. Butler and Thomas (1998) have reinvestigated the sedimentation behavior of isolated dinucleosomal particles as a function of salt, and now report that they observe salt-dependent compaction. The difference with their own previous results (Butler and Thomas, 1980) the authors explain by the use of more stable populations of dinucleosomes and by a more sophisticated analysis of the actual sedimentation data. On the other hand, a recent analysis of radiation-induced damage in living mammalian cells strongly supports the zig-zag (straight linker) model of the chromatin fiber (Rydberg et al., 1998).

We would add here that even if the dinucleosomes are capable of compaction (read linker DNA bending) in vitro, this does not necessarily mean that they do so in vivo, in the context of the fiber structure. Bearing in mind that the persistence length of DNA under moderate ionic strength conditions is ~150 bp (Hagerman, 1988), it is difficult to imagine that linkers much shorter than the persistence length will spontaneously bend. Bending is certainly possible if force is applied to the fiber, and such force may be generated by nucleosome/nucleosome interactions (for more information on nucleosome/nucleosome interactions see van Holde and Zlatanova, 1996).

The mechanism of chromatin fiber compaction has been debated for years. A significant recent contribution (Bednar et al., 1998) supports the accordion-like type of compaction previously suggested (for a review see van Holde and Zlatanova, 1996). Such a compaction takes place at the expense of decreasing the angle between successive linkers, which remain straight. From cryo-EM 'compaction' images the estimated mean angles change from 85° at 5 m*M* salt, through 45° at 15 m*M* salt, to 35° at 80 m*M* salt. We have modeled such a compaction process using a modification of our previous modeling program (Leuba

et al., 1994; Yang et al., 1994). In the new version, we set the number of superhelical turns at 1.75 based on the geometry of the nucleosome particle in linker histone-containing fibers (see Figure 2). The angle between successive linkers was changed independently from the number of DNA superhelical turns (these two parameters were interdependent in our previous modeling). As Figure 6 shows, reducing the angle causes compaction of the fiber of ~1.4 fold. Further compaction is achieved with even smaller angles (the present model, which uses linker lengths randomized between 51 and 73 bp, and does not incorporate the stem structures yet, cannot work for angles below 39°, because of steric hindrance of nucleosomes). Because in real chromatin the angle changes occur in the presence of bound linker histones, with conservation of the stem structures, the mechanism of collapsing the angle remains elusive. The most probable mechanism involves neutralization of the linkers beyond the stem structure, so that their mutual repulsion diminishes.

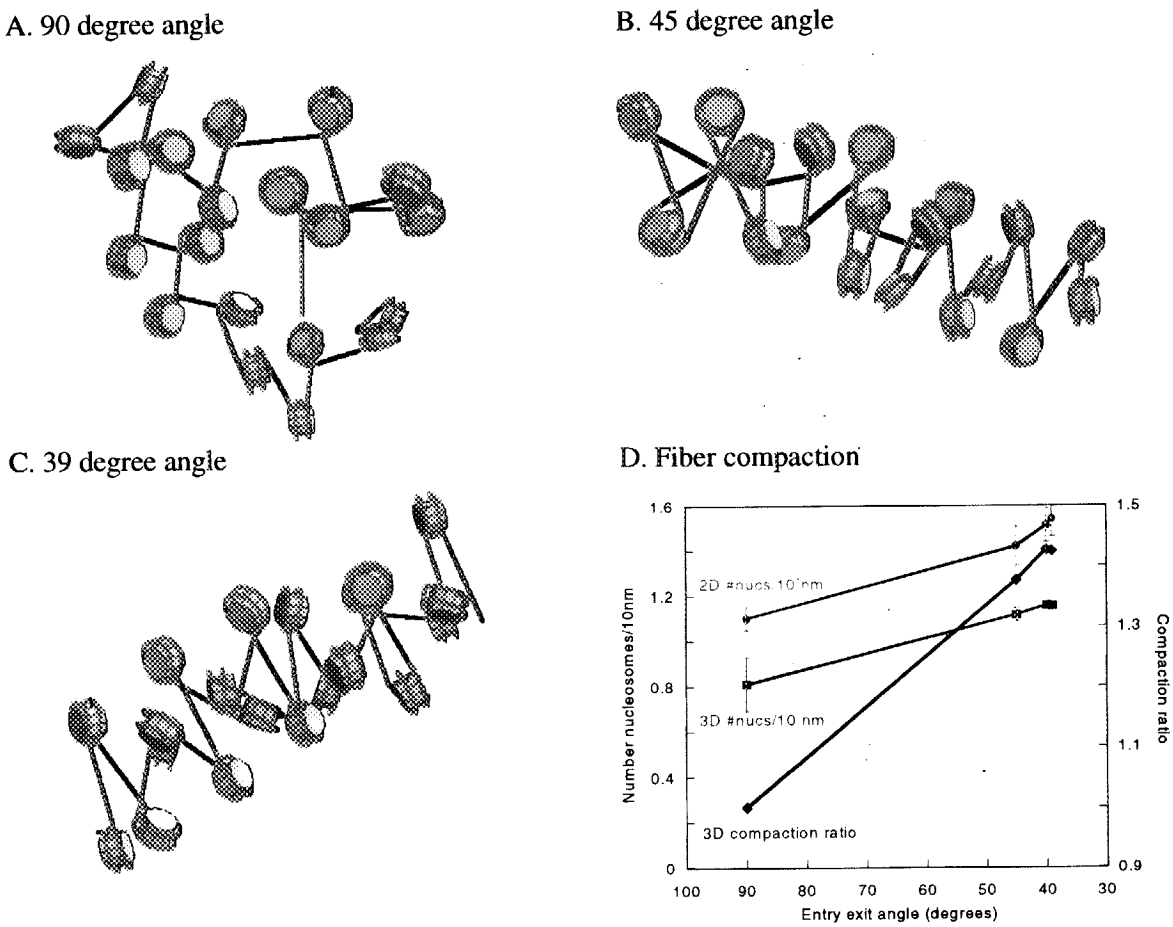

FIGURE 6. Mathematical models of chromatin fibers that contain 20 nucleosomes, with angles between successive linkers of 90° (A), 45° (B), and 39° (C). Modeling parameters were similar to those used earlier (Leuba et al., 1994; Yang et al., 1994), with the exception that the number of superhelical wraps around to histone octamer was set at 1.75 (for further information see text). The fiber compaction ratio (expressed as the ratio of the 3D-length of the fiber at 90° to the respective length at the other angles) is presented in (D), together with the number of nuclesomes/10 nm. The lines designated as 3D, and 2D, respectively, reflect measurements performed on the three-dimensional model, or after projecting this model onto a flat surface. The 2D data are in excellent agreement with earlier compaction estimates done on 3D models convoluted into AFM images (van Holde and Zlatanova, 1996). (Figure courtesy of Dr. M. Karymov.) (For the color rendering of this illustration, please refer to the end of this double issue.)

The issue of the linker histone location in the compact fiber has been highly controversial (for a detailed discussion see Zlatanova and van Holde, 1996). Recent results from cryo-EM and cryotomography (Bednar et al., 1998) indicate rather convincingly that the linker entry-exit sites, together with the bound linker histones face the fiber interior, as implied earlier from neutron-scattering data (Graziano et al., 1994). Such a location can be consistent with either solenoid or crossed-linker models.

A final point concerns the use of the term 30-nm fiber to denote the compact fiber that exists under quasiphysiological ionic conditions, and possibly in vivo. Although we have argued, on the basis of the existing experimental data, that this term be dropped (van Holde and Zlatanova, 1995), it is still in wide use. The problem stems from the fact that the diameter of the more loosely organized, extended fiber that exists at low ionic strength is also 30 nm. In view of this, the term 30-nm fiber used as a synonym for condensed fiber is meaningless.

In conclusion, despite the enormous progress in understanding the intimate structure of the 'core' particle, structural issues concerning chromatin fiber structure in its extended and condensed states are still far from resolved. There is no doubt that the recently recognized importance of chromatin structure in the regulation of processes such as transcription, replication, and repair will further stimulate research into chromatin structure at all its levels.

ACKNOWLEDGMENTS

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