Linker Histone Tails and N-Tails of Histone H3 Are Redundant: Scanning Force Microscopy Studies of Reconstituted Fibers

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The mechanisms responsible for organizing linear arrays of nucleosomes into the three-dimensional structure of chromatin are still largely unknown. In a companion paper (Leuba, S. H., et al. 1998. *Biophys. J.* 74:2823-2829), we study the contributions of linker histone domains and the N-terminal tail of core histone H3 to extended chromatin fiber structure by scanning force microscopy imaging of mildly trypsinized fibers. Here we complement and extend these studies by scanning force microscopy imaging of selectively reconstituted chromatin fibers, which differ in subtle but distinctive ways in their histone composition. We demonstrate an absolute requirement for the globular domain of the linker histones and a structural redundancy of the tails of linker histones and of histone H3 in determining conformational stability.

INTRODUCTION

As discussed in detail in the companion paper (Leuba et al., 1998), the structure of the chromatin fiber is poorly understood, despite considerable research effort over the past two decades. In an attempt to gain further insight into the mechanisms that control the folding of a linear array of nucleosomes into three-dimensionally organized fibers, we decided to make use of the new imaging capabilities of scanning force microscopy (SFM) in combination with some more traditional biochemical approaches to study the contribution of the unstructured tails of linker histones (LHs) and of the N-terminal tail of histone H3 to fiber structure.

In the companion paper we used SFM imaging of mildly trypsinized chromatin fibers. The trypsinization data suggest that the tails of both of these histone classes participate in the formation of the three-dimensional structure of the extended chromatin fiber. However, because the cleavages of the LHs and H3 partly overlap, it is not clear how well one may distinguish the relative contributions of the tails of the LI-Is and the N-terminus of H3 in organizing the chromatin fiber. Therefore, we decided to carry out a more definitive set of experiments. The new approach relied on the imaging capabilities of SFM with reconstitution of histone H5 or its isolated globular domain (GH5) on LH-stripped chromatin fibers that contained either intact histone octamers or histone octamers that specifically lacked the N-terminal tails of histone H3. The depleted chromatin used for reconstitution contained either all core histones intact, or H2A, H2B, and H4 intact and histone H3 lacking its N-terminal tail. Chromatin specifically lacking the H3 tail has never been investigated previously; reconstitution studies directed toward understanding the role of the tails have hitherto been performed only with LH-depleted chromatin in which all core histones lacked their N-tails. The fiber structures resulting from both kinds of reconstitutions were imaged with a SEM operating in the tapping mode (Leuba et al., 1994). These images were then quantitatively characterized by extensive measurements of internucleosomal center-to-center distances. Internucleosomal angles (for a definition, see the companion paper). and fiber heights. The morphologies of the different kinds of fibers and the quantitative assessment of their structural parameters lead us to conclude that chromatin fiber structure at low ionic strength requires the presence of 1) the globular domain of the LHs, and 2) either the tails of the LHs or the N-terminal tails of histone H3. Because either the tails of the LHs or those of H3 suffice to organize fiber structure three-dimensionally. we say that they are structurally redundant.

It must be emphasized that all previous studies concerning molecular determinants of fiber structure have only looked at the process of condensation. Because of the lack of appropriate monitoring techniques, the issue of the determinants of the three-dimensional organization of the extended fiber has never before been approached.

MATERIALS AND METHODS

Preparation and fixation of chromatin

These procedures were as described in the accompanying paper (Leuba et al. 1998).

Linker histone depletion and purification of linker histones

Linker histone depletion of chromatin fibers was performed as described by Libertini and Small (1980) and Garcia-Ramirez, et al. (1990), with modifications. Briefly, 5 ml of soluble chromatin **(**4 mg/ml) in 0.35 M NaCl, 10 mM Tris-HCI (pH 8.0), were mixed with 240 mg of CM Sephadex C-25 (Pharmacia), shaken for an hour at 4°C, and the resin cake was layered onto a 5-g CM Sephadex C-25 column (2.5 cm x 7 cm) equilibrated with the same buffer. The column was washed with same buffer at 100 ml/h, until H1/H5-stripped chromatin was eluted as determined by A_{260}. Stripped chromatin was dialyzed versus 10 mM Tris-HCI (pH 7.5), 0.1 mM EDTA, and stored on ice.

Linker histones were subsequently eluted from the same column by a 100-ml linear gradient of NaCl (0.35-1.4 M) in 10mM Tris-HCl (pH 8.0), in 10-ml fractions. Eluted histones were checked for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970), dialyzed versus 10mM Tris-HCl (pH 7.5), and stored on ice. Linker histone concentration was determined by using an extinction coefficient of 2.0 mg/cm^2 at 230 nm (Renz and Day, 1976).

Purification of the globular domain of H5

GH5 was prepared as in Krylov et al. (1993), with modifications to the chromatography. Briefly, the trypsin-digested H5 was loaded onto a CM Sephadex C-25 column (0.7 cm *X* 10 cm) equilibrated with 0.1 M NaCl, 20 mM Tris-HCl (pH 8.8). The column was washed at 66 ml/h to background absorbance at 230 nm, and GH5 was eluted with a 200-ml linear gradient of 0.1-1.0 M NaCl in 20 mM Tris-HCl (pH 8.8), in 1.6-ml fractions. Aliquots were analyzed by SDS-PAGE, fractions pooled, extensively dialyzed versus 5 mM triethanolamine (TEA)-HCl (pH 7.0), and frozen in aliquots. The concentration of GH5 was determined by amino acid analysis.

Preparation of H3-tailless, linker histone-depleted chromatin fibers

Chromatin fibers were trypsinized as described by Zlatanova et al. (1995) until the LH tails and H3 N-terminus were cleaved, with the other core histones still intact, as determined by SDS-PAGE. The digested LHs were removed with CM Sephadex C-25 at 0.35 M NaCl (see above). The column-purified H3-tailless chromatin fibers were examined by SDS-PAGE and stored on ice after extensive dialysis versus 5 mM TEA-HCl (pH 7.0).

Reconstitution of linker histone on linker histone-stripped chromatin fibers

Reconstitution was performed in slick microfuge tubes (PGC Scientific, Frederick, MD) by the methods of Leuba (1998). Typically, 0.75 ml of LH (~130 μg/ml) in reconstitution buffer (0.35 M NaCl, 0.1 mM EDTA, 10 mM Tris-HCl, pH 8.0) was mixed with 5 mg of CM Sephadex C-25. Then 0.1 ml (A_{260} = 20) of LH-stripped chromatin in the same buffer was added, and the mixture was dialyzed in 12,000 Da cutoff SpectraPor II (Spectrum, New Brunswick, NJ) dialysis tubing for 4 h versus 4 liters of 5 mM TEA-HCl (pH 7.0), 0.1 mM EDTA. Resin was removed by gravity filtration through empty BioSpin (BioRad, Hercules, CA) columns. Reconstituted fibers were examined by SDS-PAGE, and gels or their photographic negatives were scanned on a soft laser scanning densitometer (Zeinch, Fullerton, CA) and analyzed as described (Leuba et al., 1993).

To determine whether the added LH was actually bound to chromatin, the reconstituted sample was placed in a Centricon 100 tube (Amicon, Beverly, MA) and centrifuged for 30 mm at 1000 *X* g. Two milliliters of 5 mM Tris-HCl (pH 7.0) was added and Centricon-centrifuged for 30 mm. The washing step was repeated three times before the high-molecular-weight chromatin was resuspended in 0.5 ml of the buffer and analyzed by SDS-PAGE. Control Centricon 100 tubes containing only pure LHs were run in parallel.

Scanning force microscopy and quantitation of images

SFM and quantitation of images were carried out as described in the companion paper (Leuba et al., 1998). Quantitation was usually based on measurements of between 500 and 2000 consecutive nucleosomes on several fibers in an image. The means and the standard deviations presented in the histograms have been rounded up to reflect the number of significant digits.

A t-test for the difference between independent sample means (data from Table 1) with variances not assumed to be equal was conducted as described (Elzey, 1985).

TABLE 1 Structural parameters of the different types of chromatin fibers, determined on SFM images

Type of fiber	Molecules of H5 or GH5 per nucleosome	Center-to-center distances (nm)	Angles (degrees)	Heights* (nm)
H1/H5-containing	1.3[#]	15±5	100±40	4.0±1.3
H1/H5-stripped	0	31±10	130±40	2.2±0.6
+ H5	0.7	17±8	110±40	2.9±0.8
	1.2	17±6	100±40	4.0±0.8
+ GH5	2.0	21±14	100±40	3.5±1.9
H1/H5-stripped, H3-tailless	0	39±22	120±40	2.8±1.1
+ H5	0.4	21±9	120±40	3.3±0.8
	1.7	14±4	100±40	2.9±0.8
	3.2[§]	-	-	10±4
+ GH5	0.5	38±20	120±30	3.0±0.6
	1.2	38±21	120±40	2.7±1.0
	2.2	29±11	140±30	2.6±0.7

The structural parameters were determined as described in Materials and Methods. All fibers were glutaraldehyde-fixed and imaged on mica in air. *SFM imaging tends to decrease actual heights and can be used for comparative purposes only (see text). #Total amount of linker histones in chicken erythrocyte chromatin (Bates and Thomas, 1981). § Reconstituted fibers containing more than two molecules of H5 or GH5 per nucleosome are very bulky, with nucleosomes piling over each other. These structures are believed to result from overreconstitution, and center-to-center distances and projected angles could not be measured.

RESULTS

Preparation and biochemical characterization of reconstitution substrates and reconstituted fibers

To obtain LH-stripped fibers, long chromatin fibers were treated with CM Sephadex C-25 in the presence of 0.35 M NaCl. As seen in the scans of the electrophoretic gel patterns of the stripped material (Fig. 1 *B)*, HI and H5 were effectively removed, and the core histones remained in the equimolar amounts characteristic of native fibers (Fig. 1 *A).*

The LH-depleted chromatin fibers were reconstituted by incubating the stripped material with intact H5 or its isolated globular domain (GD) bound to CM Sephadex C-25 in 0.35 M NaCl, dialyzing the salt away, and then removing the resin. This method of reconstitution allowed proper positioning of H5 on the fibers, as judged by the recovery of the morphology and quantitative parameters of control fibers (see Figs. 2 and 3 and Table 1). By varying the input H5/chromatin DNA ratio, it was possible to reproducibly reconstitute varying amounts of LHs on the fiber. Fig. 1 *C* shows electrophoretic analysis of purified reconstituted material containing 1.2 molecules of H5 per nucleosome. Re-constitution using isolated GH5 led to fibers containing the histone pattern illustrated in Fig. 1 *D.*

To obtain chromatin fibers lacking both the LHs and the N-terminal tails of histone H3, we first performed mild trypsin digestion on native fibers (see Materials and Methods), and then stripped histones H1 and H5 by the CM Sephadex C-25 procedure. The histone composition of the resulting material is depicted in Fig. 1 *E*. As seen, the fiber was totally devoid of LHs, and no protein was present at the position of the intact H3 band; instead, bands P1' and P1, identified as H3 lacking 20 and 26 amino acid residues, respectively, from its N-terminus (Bohm et al., 1981; Bohm and Crane-Robinson, 1984), were visible between histones H2A and H4. The fact that we do not observe any other bands (in particular, the P4 and P5 bands that should result from H4 tail cleavage (Bohm and Crane-Robinson, 1984) indicates that only H3 tails have been removed. Reconstitution of H5 or GH5 on this substrate led to fibers with the histone compositions displayed in Fig. 1, *F* and G.

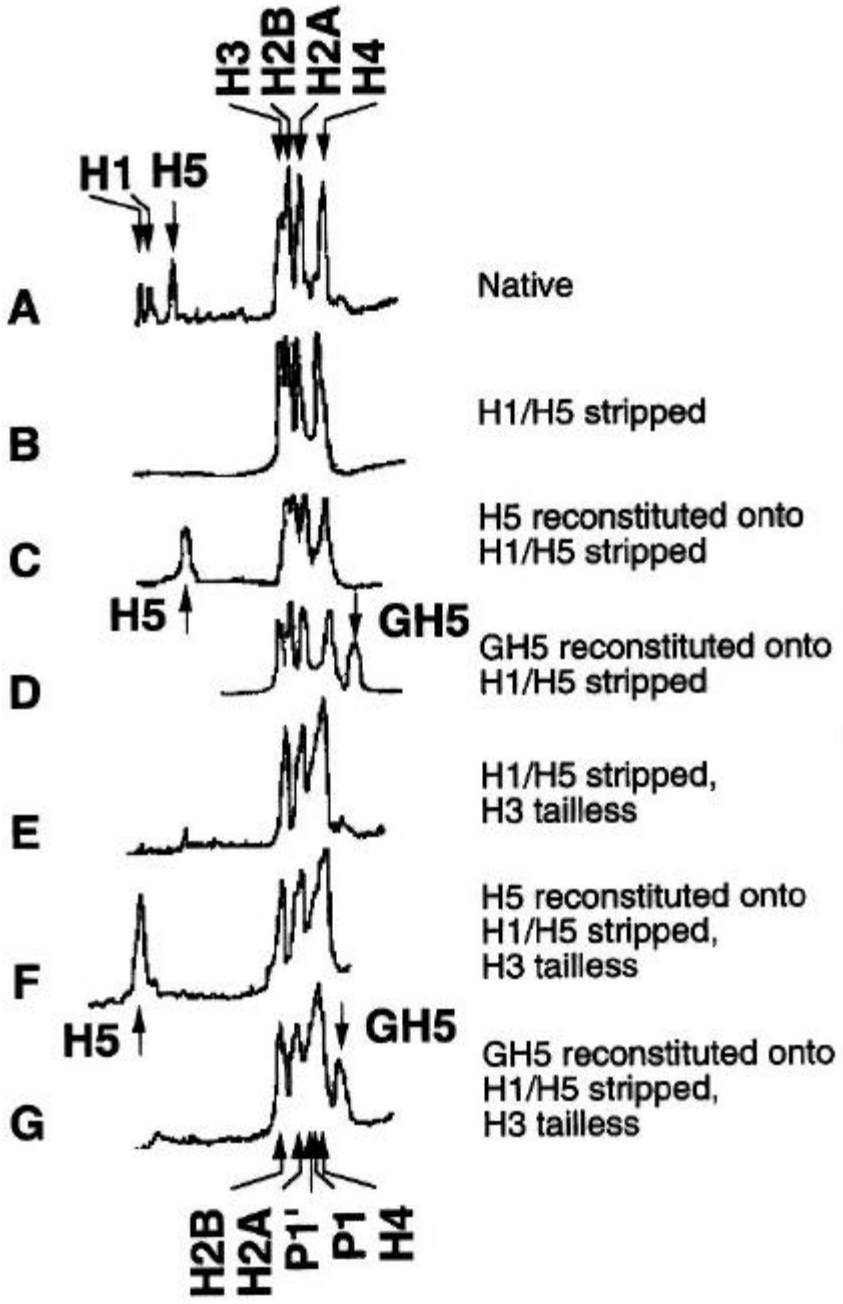

FIGURE 1 Traces of scans of electrophoretic gels of histones in the various chromatin fibers used for reconstitution and the reconstituted samples. *(A)* Histones from H1/H5-containing control fibers. *(B)* Histones from H1/H5-depleted fibers. *(C)* Histones from fibers in which H5 was reconstituted onto H1/H5-depleted fibers at a molar ratio of 1.2 molecules of H5 per nucleosome. *(D)* Histones from fibers in which GH5 was reconstituted onto H5/H5-depleted fibers (2.0 molecules of GHS per nucleosome). *(E)* Histones from LH-stripped fibers, also lacking the N-terminus of histone H3 (LH-stripped, H3-tailless chromatin fibers). (F) Histones from fibers in which H5 was reconstituted onto LH-stripped, H3-tailless chromatin fibers (1.7 molecules of H5 per nucleosome). (G) Histones from fibers in which GH5 was reconstituted onto LH-stripped, H3-tailless chromatin fibers (1.2 molecules of GH5 per nucleosome).

Structural consequences of linker histone removal

The morphology of LH-stripped chromatin fibers is illustrated in Fig. 2 *B*, and the consequences of removing these histones are quantitated in Fig. 3. First, the mean center-to-center distance increases dramatically; second, the distribution of angles is skewed toward 180°; and third, the fiber flattens. The first two changes must reflect an unpeeling of a portion of the DNA away from the nucleosome, as postulated earlier (Yang et al., 1994). Indeed, because the mean distance increases to ~30 nm, corresponding to ~88 bp, there must be a removal of ~20-30 bp beyond the "core particle" level. Such partial stripping has also been observed with mononucleosomes containing long DNAs at low ionic strength (Furrer et al., 1995; Hamiche et al., 1996) (for further discussion and references, see Zlatanova and van Holde, 1996). It seems likely that such unpeeling and extension is a consequence of strong electrostatic repulsion between long linker DNAs, once LH shielding has been lost (see Clark and Kimura, 1990).

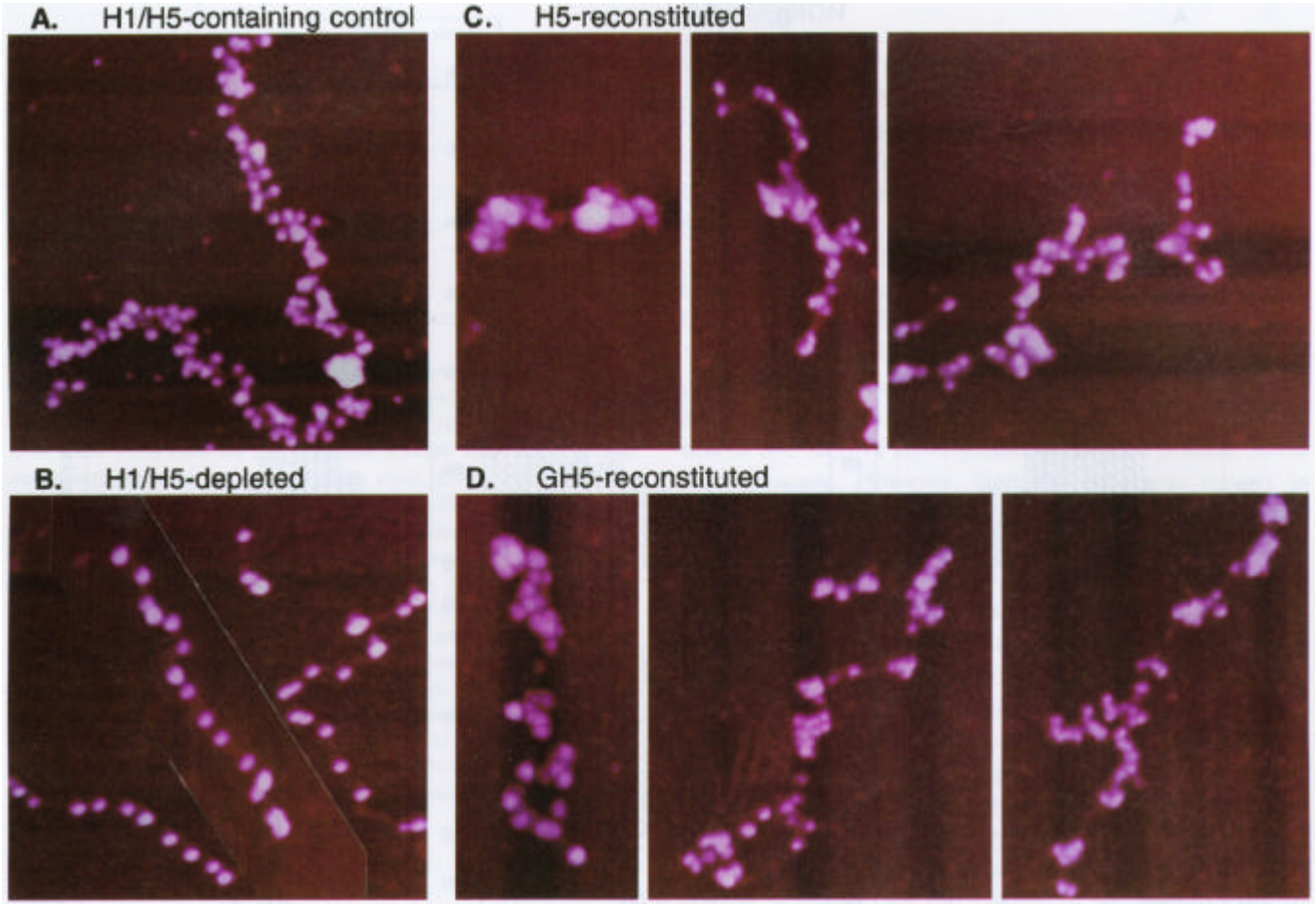

FIGURE 2 SFM images of chromatin fibers reconstituted from intact H5 or GH5 and H1/H5-stripped chicken erythrocyte chromatin fibers. All fibers were glutaraldehyde-fixed and imaged in air. *(A)* H1/H5-containing control fibers. *(B)* H1/H5-depleted fibers. (C) Fibers in which H5 was reconstituted onto H1/H5-depleted fibers at a molar ratio of 1.3 molecules of H5 per nucleosome, as determined by quantitations of electrophoretic gel patterns of the kind shown in Fig. 1. *(D)* Fibers in which GH5 was reconstituted on H1/H5-depleted fibers (two molecules of GH5 per nucleosome). Height scale is 0-15 nm *(A,* C, and *D)* and 0-7.5 nm *(B).* All images are on the same scale; the original image size was 500 nm x 500 nm.

Reconstitution of histone H5 on linker histone-depleted chromatin fibers containing intact core histones

Reconstitution of histone H5 on LH-depleted chromatin fibers containing intact core particles has previously been reported (e.g., Thoma and Koller, 1981; Allan et al., 1982). It has been shown that properly reconstituted LH can lead to a restoration of the chromatosome pause in micrococcal nuclease digestions. (The chromatosome pause is characteristic of native fibers and is absent from LH-depleted ones (Allan et al., 1980).) In addition, salt-induced condensation studies demonstrated that LH-reconstituted fibers did not differ in morphology from native ones (e.g., Thoma and Koller, 1981; Allan et al., 1986).

In accordance with these results, we demonstrated that the addition of intact histone H5 to LH-depleted chromatin fibers led to the expected transition from the flat, "beads-on-a-string" morphology of the depleted fibers to the three-dimensional structure typical of intact control fibers (Fig. 2, compare panels *A*, *B*, and *C)*. Measurements of the basic parameters of reconstituted fibers, center-to-center distances, angles, and fiber heights (Fig. 3 and Table 1) also indicated close recovery of the control fiber characteristics. In fact, control and H5-reconstituted fibers were statistically indistinguishable (see below). This result provided an essential criterion for the fidelity of reconstitution for all subsequent studies.

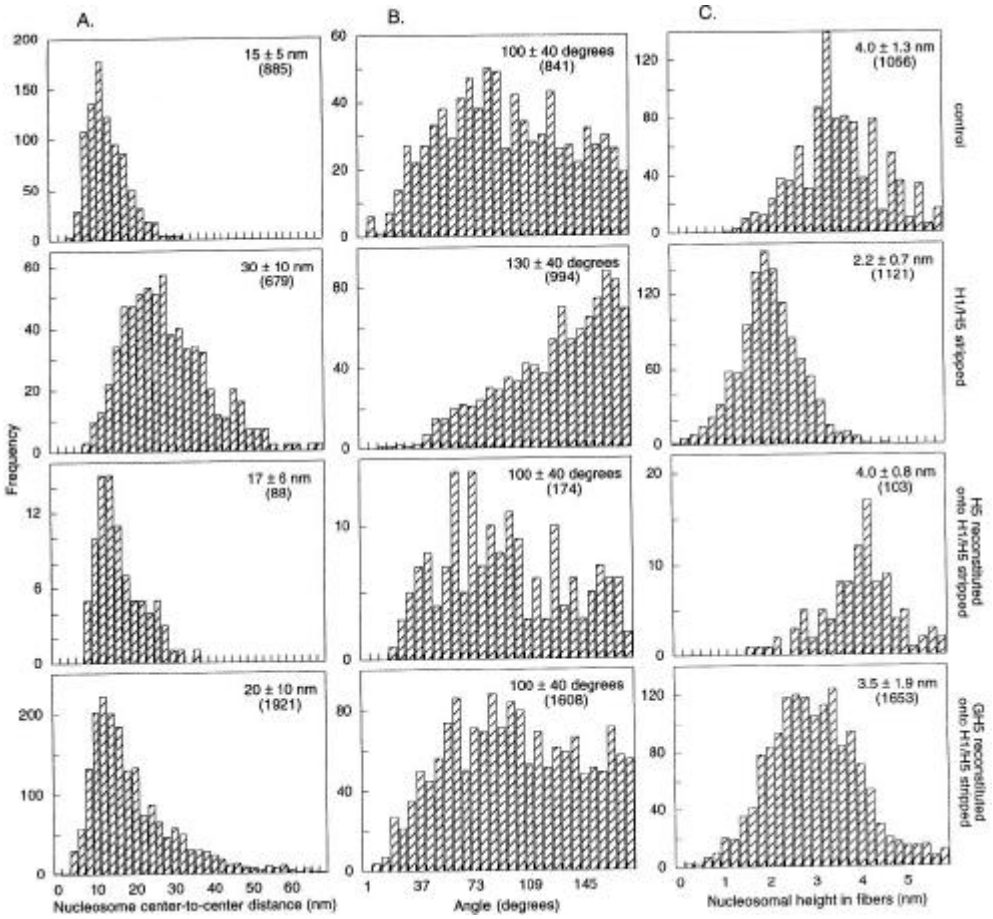

FIGURE 3 Frequency distribution histograms of center-to-center internucleosomal distances *(A)*, internucleosomal angles *(B)*, and fiber heights (C) on the four types of imaged fibers shown in Fig. 2. The numbers represent the sample means ± standard deviations. The numbers in parentheses indicate the number of data points in each histogram.

The question arises as to whether the differences and similarities between the mean values noted in Table 1 are statistically significant. Accordingly, we have performed statistical *t* tests (Elzey, *1985)* on the populations of center-to-center distances, angles, and heights reported in Table 1. Where we would expect similarity, we observe such, as indicated by low *t* ratios. We observe differences (indicated by high *t* ratios) in those cases where we would expect them on the basis of significant differences in the protein content of the reconstitutes. An example in which the means of these measurements of control, H1/H5-containing fibers are compared with those of fibers reconstituted with different levels of H5 is shown graphically in Fig. 4. The minimum *t* ratios plotted in Fig. 4 all correspond to reconstituted fibers that have a near-native complement of LHs (~1.3 per nucleosome; Bates and Thomas, 1981). A ratio of *t* 2 indicates there is no statistically significant difference *(95%* confidence level) between the two indicated means.

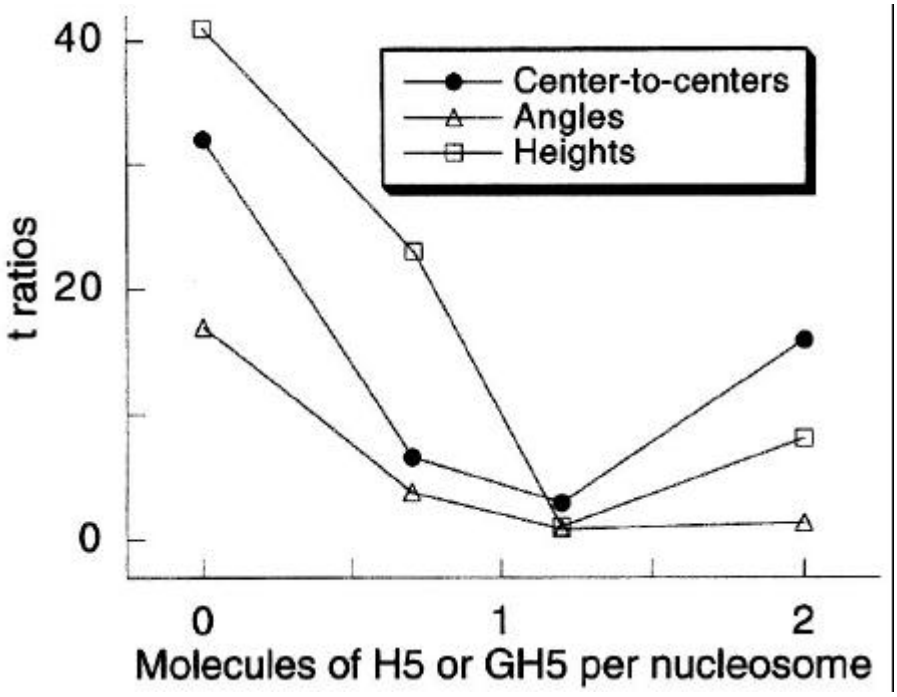

FIGURE 4 Example of *t* ratios for the population of control H1/H5 containing chromatin fibers compared with the populations of LH-stripped chromatin fibers reconstituted with 0, 0.7 H5, 1.2 H5, and 2.0 GH5 (data from Table 1). A t-test for the difference between independent sample means with variances not assumed to be equal (Elzey, 1985) was performed on the populations of center-to-center distances, angles, and heights, comparing control chromatin to the above-noted reconstituted fibers. The t-test indicated that, for instance, there was no statistical difference (at the 95% confidence level) between the sample means for control H1/H5-containing fibers and fibers reconstituted with 1.2 molecules of H5 per nucleosome, the values being 0.9 and 0.73 for the angles, and heights, respectively. The other two pairwise comparisons, in which no statistically valid differences were revealed, were between the control fibers and the two GH5 (molecules per nucleosome) reconstituted on H1/H5-stripped material, and between the H1/H5-stripped, H3-tailless reconstitution substrate and the 1.2 GH5 reconstituted on that substrate. All other pairwise comparisons revealed that the population means differed significantly, as discussed in the text.

Reconstitution of GH5 on linker histone-depleted chromatin fibers containing intact core histones

In the next series of experiments, reconstitutions were performed using only the isolated globular domain of histone H5. Significantly, the extended beads-on-a-string structure of the starting LH-stripped material coiled up, restoring, to a considerable degree, the appearance of the control fiber (Fig. 2 *D).* The quantitative characteristics of the GH5-reconstituted fibers (Fig. 3 and Table 1) show that 1) the average center-to-center distance decreased from ~30 nm in the stripped fiber to ~20 nm; 2) the average angle also decreased, from ~130° in the stripped fiber to ~100° in the GH5-reconstituted fiber. What is more important, the shape of the distribution of angles changed from the highly skewed one characteristic of the stripped fiber to that of the control fiber. Finally, the average apparent fiber height and the distribution of heights in the GH5-reconstituted fiber were intermediate between those of control and stripped fibers. Thus, when intact core histones are present, the globular domain of the LHs is, in itself, sufficient to partially stabilize the low-ionic-strength three-dimensional structure, such that it does not flatten upon deposition on the mica surface. These results are entirely consistent with the observations in the companion paper on the effects of removing linker histone tails by proteolysis.

Reconstitution of histone H5 on H3-tailless, linker histone-stripped chromatin fibers

The experiments with trypsinized chromatin fibers (see companion paper) suggested that the N-terminal tails of histone H3 might be an important determinant in the three-dimensional organization of nucleosomes in the extended fiber. To study this issue in a more definitive type of assay, we repeated the reconstitution experiments described above, but instead of using LH-stripped fibers as the reconstitution substrate, we utilized stripped fibers that also lacked the N-terminus of histone H3 (Fig. 1). Such H3-tailless, LH-depleted fibers appeared flat (Fig. *5 B),* the center-to-center distances displayed a broad distribution around 40 nm, and the angles were distributed in a way closely resembling the distribution in H1/H5-stripped fibers (Fig. 6 and Table 1). Reconstitution of intact H5 on such H3-tailless material (see Fig. 1 *F)* led to a complete recovery of control fiber morphology (Fig. *5 C),* and of the distributions of center-to-center distances and angles characteristic of control fibers (Fig. 6 and Table 1). This result was totally unexpected; it indicates that the N-terminus of histone H3 in itself is not absolutely required for the proper three-dimensional organization of the extended fiber, despite its indispensibility to fiber compaction (see Introduction of accompanying paper).

Reconstitution of GH5 on H3-tailless, linker histone-stripped chromatin fibers

The final set of reconstitution experiments was designed to examine fiber structure in the absence of the tails of both the LHs and histone H3. To that end, isolated GH5 was reconstituted on stripped chromatin in which H3 tails had also been cleaved. The results of this reconstitution are shown in Figs. 5 and 6; GH5/H3-tailless reconstituted fibers remained flat, and did not change in the distributions of either the center-to-center distances or angles from those in the starting reconstitution substrate. In other words, no structural effect of the presence of GH5 in these fibers could be perceived. However, controls carried out to check for the presence of GH5 in the reconstituted material (Fig. 1 G) clearly showed that GH5 had been successfully reconstituted, in agreement with earlier reports that the core histone tails are not required for proper positioning of LHs at the nucleosome (Allan et al., 1982). Thus, when both LH tails and H3 tails are missing, the three-dimensional structure is unstable and flattens on the mica surface.

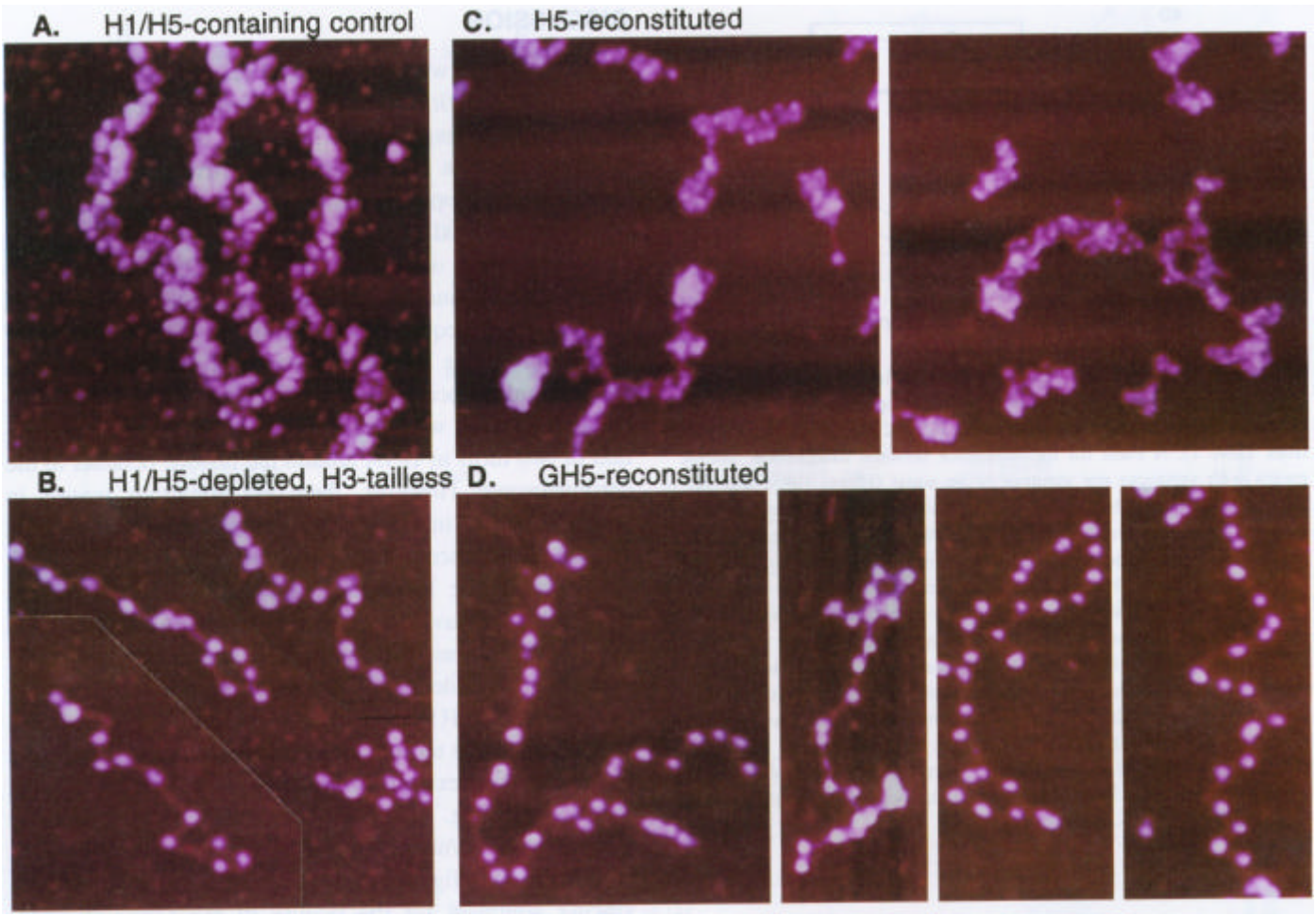

FIGURE 5 SFM images of chromatin fibers reconstituted from intact H5 or GH5 and H1/H5-stripped, H3-tailless chicken erythrocyte chromatin fibers. *(A)* H1/H5-containing control fibers. *(B)* H1/H5-depleted fibers, also lacking the N-terminus of histone H3. (C) Fibers in which H5 was reconstituted on the H1/H5-stripped, H3-tailless material, at a molar ratio of 1.7 molecules of H5 per nucleosome. *(D)* Fibers in which GH5 was reconstituted on the H1/H5-stripped, H3-tailless material, at a molar ratio of 1.2 molecules of GH5 per nucleosome. The height scale is 0-15 nm *(A* and C) and 0-7.5 nm *(B* and *D).* All images are on the same scale; the original image size was 500 nm *X* 500 nm.

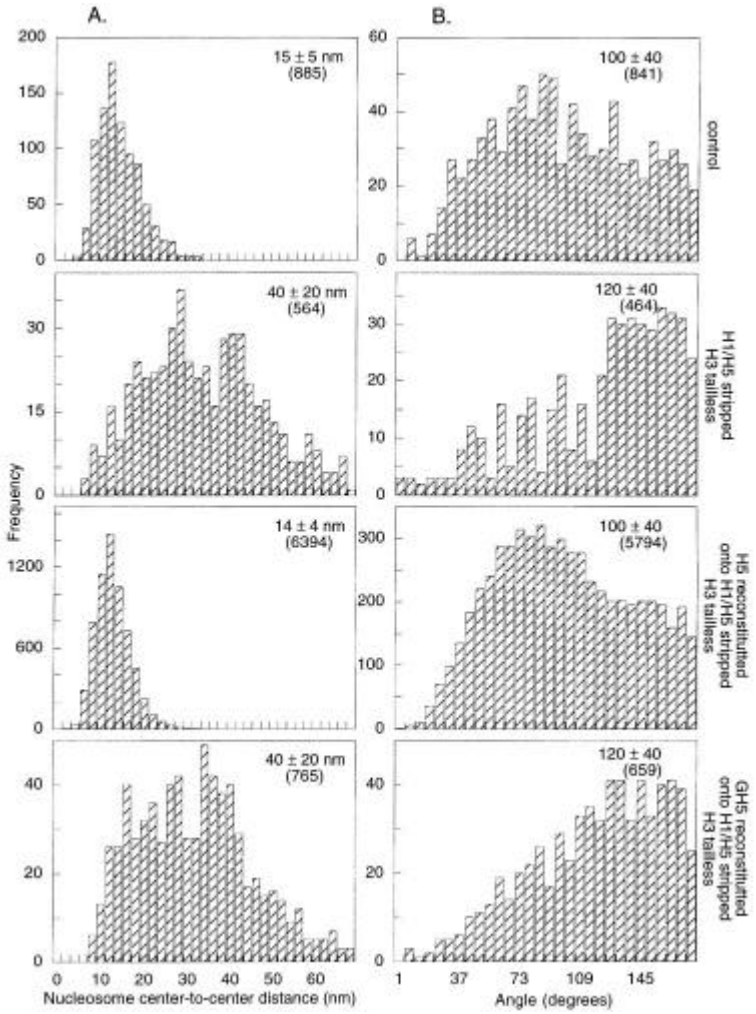

FIGURE 6 Frequency distribution histograms of center-to-center internucleosomal distances *(A)* and internucleosome angles *(B)* on the four types of imaged fibers shown in Fig. 5. For further details, see legend to Fig. 3.

DISCUSSION

In these studies, we have obtained reconstituted chromatin fibers of well-defined but subtly differing histone compositions. As in all experiments involving reconstituting some components on a partially depleted structure, one has to make sure of proper disposition of the reconstituted component in the final assembly.

Reconstitution of LHs on LH-stripped fibers or reconstituted arrays of nucleosomes has been used extensively in the past. One frequently cited criterion for the fidelity of reconstitution has been the recovery of the chromatosome pause in micrococcal nuclease digestion patterns (e.g., Allan et al., 1980), which reflects the H1-mediated stabilization of two turns of DNA around the histone octamer in the chromatosome. However, there are numerous reports in which a "pause" in digestion is observed at or near 168 bp, even in the absence of LH (see van Holde, 1988, chapter 6, for a discussion; Bavykin et al., 1990). In view of this ambiguity, we have not used this biochemical assay as a criterion for proper reconstitution. However, we feel confident about the fidelity of reconstitution, because intact H5 reconstituted on Hl/H5-stripped chromatin fibers conferred on such fibers the morphology and the structural parameters of soluble fibers extracted from nuclei (see Figs. 2 and 3 and Table 1). In Fig. 4, the *t* ratio reaches its minimum at precisely the LH/nucleosome ratio found in native chicken chromatin. This figure provides, we believe, the most convincing argument for the fidelity of reconstitution, in as much as structural parameters of the fibers are used as criteria.

The major conclusion emerging from the data is that the three-dimensional organization of the low-salt, extended chromatin fibers requires 1) the globular domain of the LHs, and 2) either the tails of the LHs or the N-terminal domain of histone H3. If the tails of both histones are missing, fibers exhibit flat morphology in the SFM, and the normal constraints on the angle seem to be lost. Consistent with these conclusions, complete removal of LHs leads to the same kind of flat, extended structures.

At first glance, it would seem that the results of the trypsin digestion studies (see accompanying paper) are inconsistent with the above. We find that the zigzag conformation of the flattened fibers is still maintained after extended digestion of LHs and H3. However, there is an important distinction between these and the reconstitution studies. In the latter case, we are dealing with chromatin fibers in which the tails of the LH are completely lacking, whereas in the former, the tails have been cleaved but, for the most part, may still be present on linker DNA. Tails or portions thereof that remain bound to linker DNA may still serve to partially neutralize DNA charges and thereby allow two linkers to approach one another. The presence of the globular domain of the LHs will, in this case, further constrain the entry/exit angle, leading to the zigzag appearance of the trypsinized fiber.

As this work shows, the N-terminal domain of H3 and the H 1 termini are redundant in stabilizing the three-dimensional organization of the extended fiber, provided that the globular domain of LHs remains bound. If the globular domain is released, then the stability of the structure is compromised, independently of whether N-H3 is still available for binding. If LH tails are not present, the tail of H3 is essential for the structure, and its absence cannot be compensated for by the tails of the other core histones. Although the N-terminal domains of the other histones might play other roles in determining fiber structure, the present data say nothing about this.

Why does the N-terminal domain of histone H3 play a unique part in structurally complementing the tails of the LHs? An earlier report suggested that the tails of the core histones could be replaced by extraneous basic polypeptides in forming the condensed structure (Allan et al., 1982). If the function of the N-tails of H3 is to provide electrostatic shielding of the DNA charge in the linker, these tails must be able to accomplish this in a way that the tails of the other core histones cannot, because the other core histone tails, although present, are not able to compensate for this missing H3 tail. In fact, there is abundant evidence that H3 may interact with linker DNA in a unique way. For example, it has been shown that H3 is the only core histone that can be cross-linked to linker DNA (Belyavsky et al., 1980; Karpov et al., 1982). Using a radiolabeling procedure to selectively label lysine residues that interact with DNA, Hill and Thomas (1990) have also shown that the N-terminus of H3 could make substantial contacts with the linker in extended chromatin. The same procedure, when applied to H2A and H4, failed to reveal substantial contacts with linker DNA. The only other core histone interacting with the linker was H2B, and H2B from the particular chromatin source used in that work (sea urchin sperm) is considerably longer than its somatic counterpart; whether H2B from “normal” chromatin would also interact with linker DNA is not clear at this point.

The structural basis for the preferential interaction of the N-terminus of H3 with linker DNA can be understood from the crystal structure of the histone octamer (Arents et al., 1991; Arents and Moudrianakis, 1993) and that of the core particle (Luger et al., 1997). In the structure of the histone octamer, the N-terminal regions of all four histones and the C-terminal region of H2A are not imaged, but it is still possible to identify the positions at which these regions start to unfold beyond the octamer surface. The “origins” of the N-tails of only H3 are situated in such a manner as to allow interaction of these tails with the linker DNA close to the entry-exit point. This unique position of the H3 tails is seen even better in the crystal structure of the core particle (Luger et al., 1997). In this structure, considerable portions of the N-terminal tails of the two molecules of H3 have been positioned. Although the exact location of the histone tails, especially those going beyond the overall confines of the complex, may differ in the core particle crystal and in the chromatosome where linker DNA is available for interaction, it is clear that the N-terminal tails of H3 are the most likely candidates for such interactions. A drawing representing our tentative view of the structure of the chromatosome, based on the core particle structure and on the results from this and other studies (see Zlatanova and van Holde, 1996; and An et al., 1998), is displayed in Fig. 7. Thus it is our contention that the integrity of chromatin fiber structure depends upon the neutralization of linker DNA, and that this can be accomplished (because of steric restrictions) only by the tails of the linker histones and the N-terminal tails of H3.

Does the redundancy of the tails have any functional significance? We note that there may be nonoverlapping in vivo situations in which the interactions of one or the other of the tails with the DNA will be weakened. For example, LHs can be phosphorylated, primarily in the unstructured tails; conversely, some regions in interphase chromatin exhibit hyperacetylation of core histone tails. Perhaps the redundancy allows the basic structure of the chromatin fiber to be preserved in the face of either of these threats to its integrity. Excessive unfolding, which could occur if only one set of determinants were present, might lead to serious misfunctioning of nuclear processes. SFM analysis of dynamic changes in chromatin structure accompanying chemical or environmental modifications must await future technical developments, which should eventually allow imaging of chromatin under liquid, thus improving sample preservation still further, and allowing studies of conformational changes in real time.

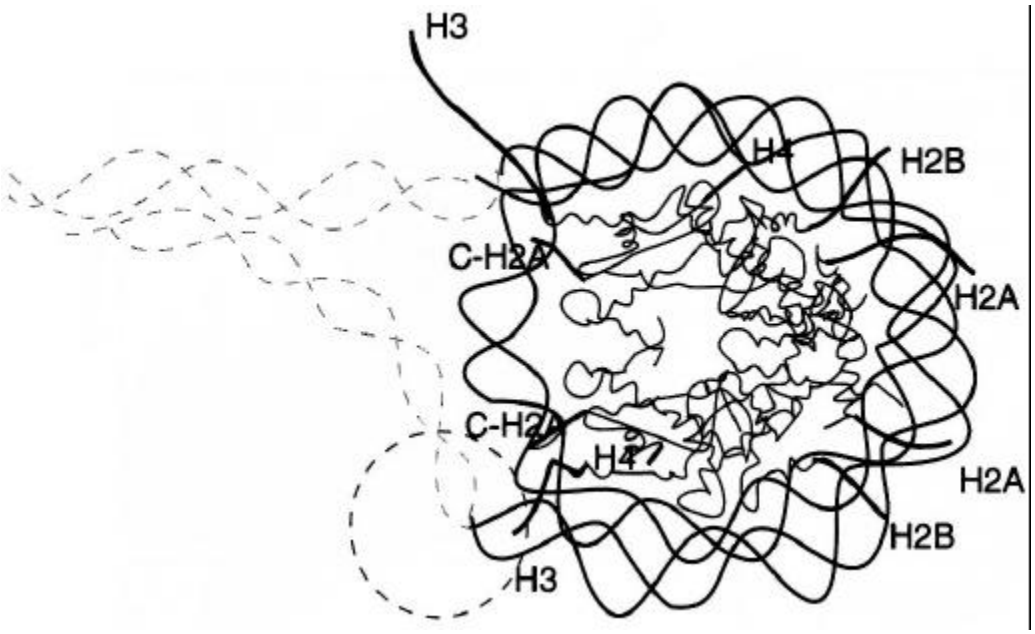

FIGURE 7 A to-scale schematic of the core particle, based on the crystal structure (Luger et al., 1997) with additional elements (GD of LH and adjacent stretches of linker DNA), representing our view of the structure of the chromatosome. The structure of the core particle is a sketched outline of figure 1 *A* from Luger et at. (1997), with line traces of the paths of the DNA gyres and the backbones of the histone molecules in the octamer. The gray circle outside of the particle is the globular domain of histone H5 drawn to scale and is situated slightly off-axis, as proposed by Travers and Muyldermans (1996), and more recently by us (An et al., 1998). The symmetrically located dashed circle represents the alternative off-axis position of the GD. It should be noted that the exact location of the GD is not important for the present model; a central over-the-axis location is equally compatible with the proposed interactions. Linker DNA is arbitrarily drawn as dashed double helices going out of the core particle. Note the location of the tails of the core histones *(labeled thick lines),* particularly the proximity of those of histone H3 to linker DNA.

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