Localization of Linker Histone in Chromatosomes by Cryo-Atomic Force Microscopy

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ABSTRACT Linker histones play a fundamental role in determining higher order chromatin structure as a consequence of their association with nucelosomal DNA. Yet the locations and structural consequences of linker histone binding are still enigmatic. Here, using cryo-atomic force microscopy, we show that the linker histone H5 in native chromatin and in chromatosomes reconstituted on the 5S rDNA template is located at the dyad of the nucleosome core particle, within the ''stem'' structure. Direct measurement also indicates that the length of free linker DNA between chromatosomes in native chromatin is ~30 bp, slightly shorter than that estimated from nuclease digestion assays.

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Chromatin structure is thought to be a major determinant of chromatin biology (1). The most basic structural unit of chromatin is the nucleosome, consisting of a core histone octamer (two copies of each H2A, H2B, H3, and H4), around which 146–147 basepairs (bp) of DNA is wrapped and protected from nuclease digestion (2). Linker histones (H1 and H5) bind to nucleosomal DNA, forming the chromatosome, and change a number of its properties, including protection of an additional 20 bp from nuclease digestion (3,4) and compaction of the nucleosomal complex into a 30 nm filament, whose structure remains to be fully elucidated (5).

Despite their importance and abundance in the native chromatin, localization of the linker histone within the chromatosome has proved to be technically challenging. The earliest and most commonly suggested binding site is at the dyad axis of the core particle (3,6,7); yet other sites have also been proposed (8,9). The most noted alternative model came from a study of reconstituted nucleosomes on the 5S rDNA template, which placed the globular domain of H5 (GH5) inside the DNA gyre, 65 bp away from the dyad axis (10). Yet, some have suggested that this off-dyad site might be particular to chromatosomes reconstituted with the 5S rDNA template, with an on-dyad site with other DNA (8). Recently however, a theoretical study concluded that both sites at the dyad axis and off are possible locations for GH5 binding, although the latter in this study appears to be outside the DNA gyre (11).

Atomic force microscopy (AFM) has proved to be a powerful technique to directly identify a number of features of chromatin structure (12–20), yet, to date, has not directly resolved the location of the linker histone in the chromatosome. To this end, we apply the cryo-AFM (21,22) to image purified and reconstituted chromatosomes, taking advantage of the improved structural stability and reproducibility at cryogenic temperatures.

The specimens for cryo-AFM imaging were prepared following established protocols with only minor modifications (23). Briefly, erythrocyte nuclei were first purified from freshly drawn chicken blood. After digestion with micrococcal nuclease, short chromatin fibers were released from lysed nuclei and collected for imaging. After these procedures, the typical ratio between H5 and the core histones is ~0.7:1. The chromatin fragments, lightly fixed in 0.05% glutaraldehyde, are directly adsorbed onto a cleaved mica surface pretreated with spermidine. Shown in Fig. 1 *A* is a typical large-scale image of these chromatin fragments obtained in the cryo-AFM at ~80 K using uncoated oxide sharpened silicon nitride cantilevers (Park Scientific, Sunnyvale, CA). Although the chromatosomes are adsorbed in a number of orientations, a fairly large fraction clearly shows an additional mass (''stem'') on the side of the core particle, bridging parts of the two linker DNA segments that project out from the dyad axis of the core particle. The details of this fraction are better represented in Fig. 1 *B*.

On average, the core particles in these images have dimensions (Fig. 1 *B*) surprisingly close to those of the nucleosome determined by x-ray crystallography (10.5 nm and 6 nm, respectively (24)). These measurements indicate that for this fraction, the broad side of the core particle is in direct contact with the substrate. An additional 2–3 nm vertical protrusion near the periphery of the core particles as predicted for an off-dyad binding site in (10) is not observed. The only additional topographic feature in the chromatosomes in these images compared with that expected from the crystal structure of the nucleosome is the stem structure on the side where the linker DNA projects out, suggesting that the stem is the location of the linker histone.

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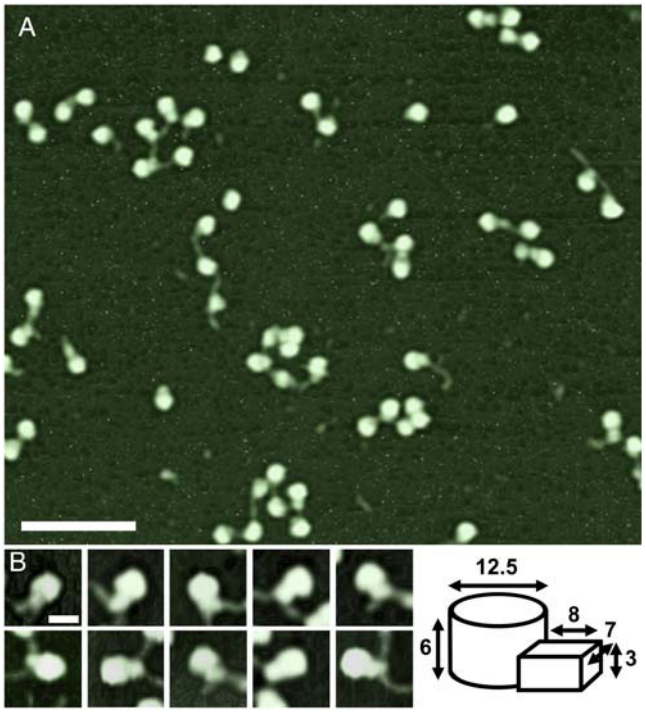

FIGURE 1 Cryo-AFM images of purified chromatin fragments from chicken erythrocytes. (*A*) At large scale, the images reveal "beads on a string", with linker DNA and nucleosomes well resolved. (*B*) Upon closer examination, a number of these particles exhibit an extra mass on the side of the core particle, bridging the two linker DNA. The dimensions of the schematic on the right are in nanometers. Scale: *A*, 100 nm; *B*, 15 nm.

To verify that this stem structure is indeed a consequence of linker histone binding, we have reconstituted the nucleosomes using purified histones and a 255 bp DNA template derived from *Xenopus borealis* 5S rDNA. The nucleosome positioning sequence contained in this template should place the dyad at position +86. Shown in Fig. 2 *A* is a collection of typical particles seen when only the purified core histones are used in the reconstitution. The dimensions of the core particles in these images are the same as those in native chromatin (Fig. 1 *B*), but no extra mass (stem) outside the core particle is observed. It should be noted that measurements of the DNA not in contact with the core histones indicate that the positioning sequence from the *Xenopus* 5S rDNA is not precise, a finding consistent with other characterizations of such positioning sequences (23,25). When purified H5 is added to already reconstituted core particles, a sizeable fraction shows an extra mass on the side of the core particle where the linker DNA appears to emerge (Fig. 2 *B*), closely resembling those in Fig. 1 *B*, with virtually identical dimensions. This stem structure is thus clearly a feature of linker histone binding and occurs regardless of any specific DNA sequence.

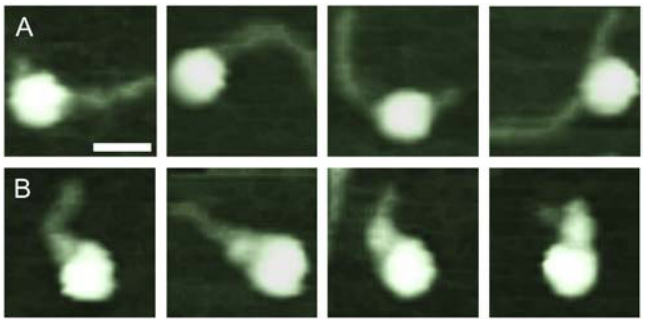

FIGURE 2 Reconstitution with a 255 bp template containing a 5S rDNA positioning sequence. (*A*) Reconstituted with purified core histones to form the core particle. (*B*) Purified H5 is added to the reconstituted core particle. Here, an extra mass on the side of the core particle is clearly seen, resembling that in Fig. 1 *B*. Scale: 15 nm.

These AFM images can also be used to estimate the protein mass contained within the stem structure (or at least provide an upper bound). Using the images of the core particle to obtain a measure of tip dilation (which is relatively small, as in previous cryo-AFM images (26)), and assuming the DNA within the stem to be essentially straight (as observed in (27,28)), the estimated protein mass is ~26 kD, reasonably close to that of H5 (20 kD). These measurements thus indicate that the stem structure contains a single copy of H5 (in addition to the linker DNA), consistent with the bridging model (7–9). However, the current resolution achieved with the cryo-AFM is not yet sufficient to directly resolve whether there is a slight asymmetry of the linker histone location with respect to the dyad, as proposed in Zhou et al. (7).

The AFM images also provide a unique opportunity to directly measure the length of protein-free DNA between the chromatosomes, which is often inferred using electrophoresis of nuclease digested fragments. From a sample obtained

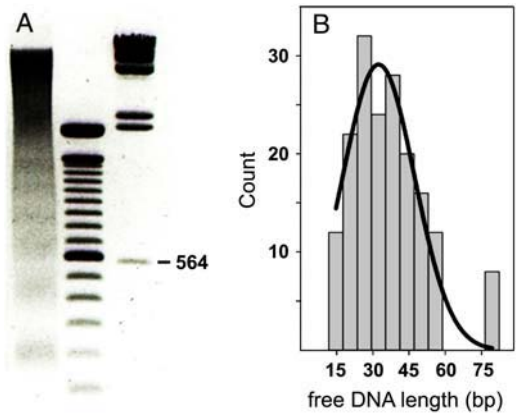

FIGURE 3 Analysis of total DNA in the chromatosome. (*A*) DNA ladder (*left lane*) analysis using nuclease digestion and agarose gel. Using regression fitting, the repeat length is 232 bp. (*Middle and right lanes*) 100 bp ladder standard and *Hin*dIII λ-DNA digest, respectively. (*B*) Directly measured linker DNA length. The fitted peak is at 37 bp.

by light nuclease treatment, the repeat length inferred from DNA ladder analysis is 232 bp (Fig. 3 *A*), whereas the directly measured mean length for the linker DNA is 37 bp (Fig. 3 *B*). The total length of DNA contained in the chromatosome is then 195 bp, which is ~30 bp longer than that found with nuclease protection assay (3,4), suggesting that ~30 bp DNA within the stem may not be protected from nuclease digestion. The ''free'' linker DNA is, thus, relatively short, which should have direct implications on feasible models of the higher order folding of the chromatin.

In conclusion, cryo-AFM has directly resolved the location of the linker histone in the chromatosome with both purified native chromatin and reconstituted chromatosomes. The linker histone is found to bind outside of the nucleosome core particle at a location near the dyad. These findings provide the most direct support for the bridging model of the chromatosome.

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REFERENCES and FOOTNOTES

1. van Holde, K. 1989. Chromatin. Springer-Verlag, Berlin.
2. Kornberg, R. D. 1977. Structure of chromatin. *Annu. Rev. Biochem.* 46:931–954.
3. Simpson, R. T. 1978. Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. *Biochemistry.* 17:5524–5531.
4. Noll, M., and R. D. Kornberg. 1977. Action of micrococcal nuclease on chromatin and the location of histone H1. *J. Mol. Biol.* 109:393–404.
5. Robinson, P. J., and D. Rhodes. 2006. Structure of the '30nm' chromatin fibre: a key role for the linker histone. *Curr. Opin. Struct. Biol.* 16:336–343.
6. Staynov, D. Z., and C. Crane-Robinson. 1988. Footprinting of linker histones H5 and H1 on the nucleosome. *EMBO J.* 7:3685–3691.
7. Zhou, Y. B., S. E. Gerchman, V. Ramakrishnan, A. Travers, and S. Muyldermans. 1998. Position and orientation of the globular domain of linker histone H5 on the nucleosome. *Nature.* 395:402–405.
8. Travers, A. 1999. The location of the linker histone on the nucleosome. *Trends Biochem. Sci.* 24:4–7.
9. Thomas, J. O. 1999. Histone H1: location and role. *Curr. Opin. Cell Biol.* 11:312–317.
10. Pruss, D., B. Bartholomew, J. Persinger, J. Hayes, G. Arents, E. N. Moudrianakis, and A. P. Wolffe. 1996. An asymmetric model for the nucleosome: a binding site for linker histones inside the DNA gyres. *Science.* 274:614–617.
11. Fan, L., and V. A. Roberts. 2006. Complex of linker histone H5 with the nucleosome and its implications for chromatin packing. *Proc. Natl. Acad. Sci. USA.* 103:8384–8389.
12. Lindsay, S. M., L. A. Nagahara, T. Thundat, U. Knipping, R. L. Rill, B. Drake, C. B. Prater, A. L. Weisenhorn, S. A. Gould, and P. K. Hansma. 1989. STM and AFM images of nucleosome DNA under water. *J. Biomol. Struct. Dyn.* 7:279–287.
13. Lal, R., and S. A. John. 1994. Biological applications of atomic-force microscopy. *Am. J. Physiol.* 266:C1–C21.
14. Fritzsche, W., and E. Henderson. 1996. Scanning force microscopy reveals ellipsoid shape of chicken erythrocyte nucleosomes. *Biophys. J.* 71:2222–2226.
15. Leuba, S. H., C. Bustamante, K. van Holde, and J. Zlatanova. 1998. Linker histone tails and N-tails of histone H3 are redundant: scanning force microscopy studies of reconstituted fibers. *Biophys. J.* 74:2830–2839.
16. Yodh, J. G., N. Woodbury, L. S. Shlyakhtenko, Y. L. Lyubchenko, and D. Lohr. 2002. Mapping nucleosome locations on the 208–12 by AFM provides clear evidence for cooperativity in array occupation. *Biochemistry.* 41:3565–3574.
17. Kepert, J. F., K. F. Toth, M. Caudron, N. Mucke, J. Langowski, and K. Rippe. 2003. Conformation of reconstituted mononucleosomes and effect of linker histone H1 binding studied by scanning force microscopy. *Biophys. J.* 85:4012–4022.
18. Nikova, D. N., L. H. Pope, M. L. Bennink, K. A. van Leijenhorst-Groener, K. van der Werf, and J. Greve. 2004. Unexpected binding motifs for subnucleosomal particles revealed by atomic force microscopy. *Biophys. J.* 87:4135–4145.
19. Wang, H., R. Bash, J. G. Yodh, G. Hager, S. M. Lindsay, and D. Lohr. 2004. Using atomic force microscopy to study nucleosome remodeling on individual nucleosomal arrays in situ. *Biophys. J.* 87:1964–1971.
20. Hansma, H. G., K. Kasuya, and E. Oroudjev. 2004. Atomic force microscopy imaging and pulling of nucleic acids. *Curr. Opin. Struct. Biol.* 14:380–385.
21. Han, W., J. Mou, J. Sheng, J. Yang, and Z. Shao. 1995. Cryo atomic force microscopy: a new approach for biological imaging at high resolution. *Biochemistry.* 34:8215–8220.
22. Sheng, S., Y. Gao, A. S. Khromov, A. V. Somlyo, A. P. Somlyo, and Z. Shao. 2003. Cryo-atomic force microscopy of unphosphorylated and thiophosphorylated single muscle myosin molecules. *J. Biol. Chem.* 278:39892–39896.
23. Panetta, G., M. Buttinelli, A. Flaus, T. J. Richmond, and D. Rhodes. 1998. Differential nucleosome positioning on *Xenopus* oocyte and somatic 5 S RNA genes determines both TFIIIA and H1 binding: a mechanism for selective H1 repression. *J. Mol. Biol.* 282:683–697.
24. Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent, and T. J. Richmond. 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature.* 389:251–260.
25. An, W., K. van Holde, and J. Zlatanova. 1998. Linker histone protection of chromatosomes reconstituted on 5S rDNA from *Xenopus borealis*: a reinvestigation. *Nucleic Acids Res.* 26:4042–4047.
26. Sheng, S., D. M. Czajkowsky, and Z. Shao. 1999. AFM tips: how sharp are they? *J. Microsc.* 169:1–5.
27. Hamiche, A., P. Schultz, V. Ramakrishnan, P. Oudet, and A. Prunell. 1996. Linker histone-dependent DNA structure in linear mononucleosomes. *J. Mol. Biol.* 257:30–42.
28. Bednar, J., R. A. Horowitz, S. A. Grigoryev, L. M. Carruthers, J. C. Hansen, A. J. Koster, and C. L. Woodcock. 1998. Nucleosomes, linker DNA, and linker histone form a unique structural motif that directs the higher-order folding and compaction of chromatin. *Proc. Natl. Acad. Sci. USA.* 95:14173–14178.