Commentary

New insights into unwrapping DNA from the nucleosome from a single-molecule optical tweezers method

Jeffrey J. Hayes*† and Jeffrey C. Hansen‡

*Department of Biochemistry and Biophysics, Box 712, University of Rochester Medical Center, Rochester, NY 14642; and ‡Department of Biochemistry, Mail Code 7760, University of Texas, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900

The hierarchical, multidimensional assemblies of protein–DNA structures known as the eukaryotic chromosome present both challenges and opportunities for biophysicists interested in chromatin structure (1, 2). One of the most interesting aspects of chromatin structure to come to light in recent years is the discovery that the core histone proteins alone can effectively direct the formation of multiple initial levels of this hierarchy (3). The core histones are involved in the assembly of individual nucleosomes, mediate folding of nucleosome arrays, and direct interfiber interactions important for assembly of arrays into higher-order structures (3). Secondly, the nucleosome itself has come to be viewed as a dynamic entity, undergoing transitions, which result in exposure of DNA sites contained within (2). However, the details of these dynamic transitions and the mechanism by which DNA spontaneously unravels or is forcibly unraveled from the nucleosome has not been adequately defined. An elegant application of a single-molecule optical trapping technique to the study of nucleosome structure and dynamics in a model system has provided exciting new insights into the unwinding of DNA from the nucleosomes within a nucleosomal array (4).

Because of the diversity and heterogeneity of chromatin structures, the focus of most biophysical characterization has been the primary subunit of chromatin, the nucleosome. Nucleosomes are assembled by the wrapping of two ≈80-bp superhelical turns of DNA around a central spool of proteins consisting of an octameric assembly of the four core histone proteins, H2A, H2B, H3, and H4 (Fig. 1). Apart from DNA, these proteins heterodimerize and, in the case of H3, undergo homotypic interactions to form stable H2A/H2B dimers and $(H3, H4)_2$ tetramers in solutions containing physiological ionic strengths (5, 6). Nucleosome assembly is initiated by the wrapping of 100–120 bp of DNA around the $(H3/H4)_2$ tetramer (7). Subsequently, H2A/H2B dimers bind to either side of the tetramer–DNA complex and extend the wrapping of DNA within the nucleosome up to 160 bp (5, 7). This creates a left-handed superhelical ramp of protein onto which the DNA is wrapped, essentially consisting of four histone dimers linked end-to-end: (H2A/H2B)–(H4/H3)–(H3/H4)–(H2B/H2A) (8, 9). The H3:H3 and H2B:H4 dimer–dimer interfaces are comprised of structurally similar four-helix bundles; however the latter does not remain stably associated in the absence of DNA in aqueous solutions containing physiological ionic strengths (5). Thus the H2B:H4 interface is a likely site for initial disruption of histone–histone interactions on unfolding of the nucleosome core *in vivo* (5, 10).

Wrapping the DNA onto the left-handed spiral formed by the histone fold domains requires the helix to be severely distorted into approximately two 80-bp superhelical loops (9). Given that the persistence length of DNA is about 150 bp (11), one would expect a tremendous energetic cost to bend DNA into the conformation found in the nucleosome (see ref. 12). Of course, the cost of bending nucleosomal DNA is more than offset by favorable electrostatic interactions between basic side chains on the histone proteins and the polyanionic backbone of the DNA. Histone contacts to DNA occur every 10 bp on each strand (see Fig. 1) and involve an arginine residue penetrating the minor groove; several main polypeptide chain amide interactions with two consecutive phosphates on each DNA strand and, surprisingly, substantial hydrophobic interactions with the faces of the deoxyribose sugars in the DNA (9).

As mentioned above, nucleosomes are not structurally inert entities, but rather undergo several conformational transitions that are likely to be important in facilitating interactions between transacting factors and DNA in chromatin *in vivo* (2, 13). Widom and colleagues have demonstrated that DNA binding sites in nucleosomal DNA are exposed with low probability but at sufficiently rapid rates to allow physiologically significant site accessibility (13, 14). DNA probably unwraps from the edge of the nucleosome because sites within nucleosomal DNA are transiently exposed apart from histones with a probability of about 1 in 10^3-10^5 as one moves from the periphery of the nucleosome toward the center (13, 14). Thus given the dynamic nature of this system, factors present at sufficient concentrations and having high enough affinities for naked DNA will be able to efficiently compete with histone proteins and effect significant loading of their cognate DNA elements in chromatin (13).

However, many issues regarding the dynamics of the association of DNA with core histone remain unresolved. Moreover, the response of nucleosomal DNA to external stresses has not been adequately studied. This latter issue is of critical importance because nucleosomes are disassembled and invaded by many different DNA-dependent processive enzymes (2, 15–17). The new work by Brower-Toland *et al.* (4) addresses this issue directly *in vitro*. A key to their success is the use of a well defined nucleosomal array model system reconstituted from purified core histones and a DNA template consisting of tandemly repeated nucleosome positioning sequences (3, 18). By carefully applying tension to the ends of the oligonucleosomal structure using an

Brower-Towland *et al.* were able to detect the stepwise uncoiling of *individual* nucleosomes within the array.

See companion article on page 1960.

†To whom reprint requests should be addressed. E-mail: jjhs@mail.rochester.edu.

www.pnas.org/cgi/doi/10.1073/pnas.062034799

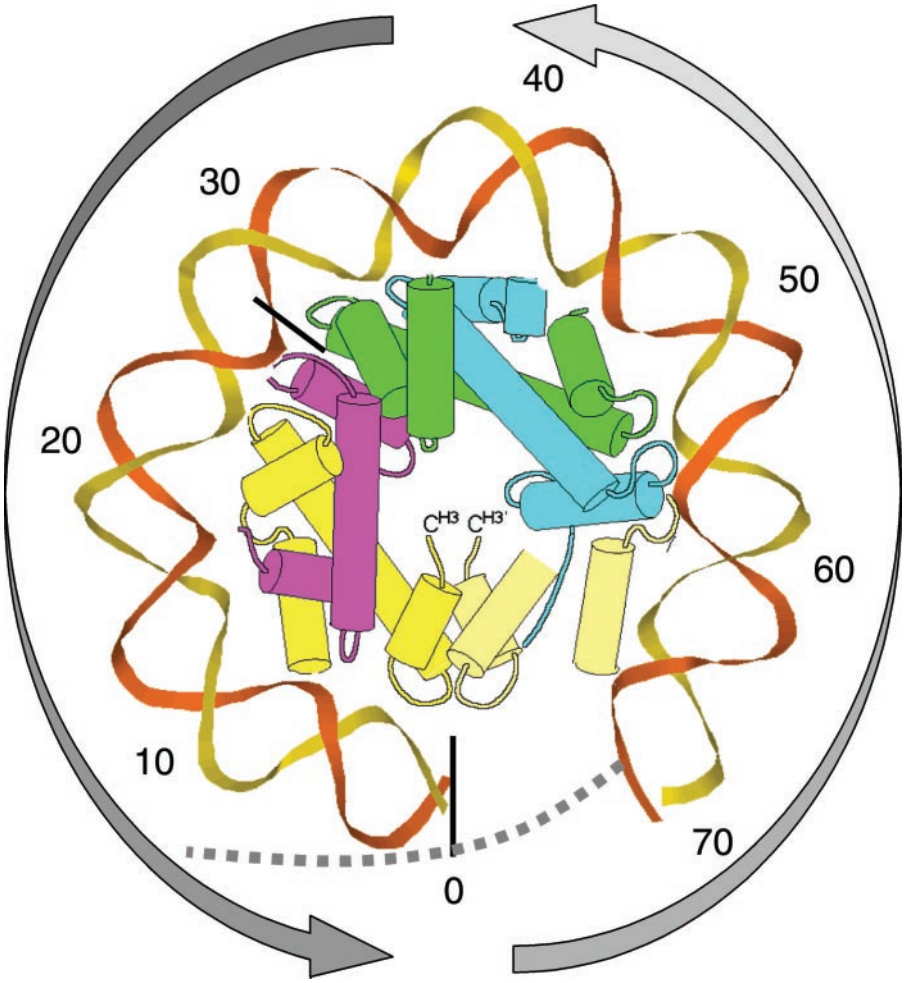

Fig. 1. Unwrapping histone–DNA contacts within the nucleosome. Cartoon with core histones H2A, H2B, H3, and H4 shown as blue, green, yellow, and magenta, respectively, with α-helices as columns. DNA in the nucleosome is shown (ribbons, ref. 9) with approximate distance in base pairs from the dyad (center, bold vertical line) of the nucleosome indicated. Note that only the top four polypeptides and top turn of DNA in the top half of the symmetrical structure are shown for clarity. A small amount of H3 (light yellow) emanating from the bottom half of the structure is shown as well. The H3:H3 and H4:H2B interfaces between dimers are indicated as vertical and angled bold lines, respectively. Gray dashed line indicates a possible path of DNA after exiting the nucleosome. The stretches of DNA unwrapped in the first and second transitions in the study by Brower-Toland *et al.* are indicated by the light and dark gray curved arrows, respectively. The figure is based on refs. 8 and 9.

optical trapping method, they were able to detect the stepwise uncoiling of *individual* nucleosomes within the array. These studies showed that protein–DNA contacts in the exterior ½ turns within the nucleosome (about ± 70 to ± 40 bp from the nucleosome dyad; see Fig. 1) appear to be roughly energetically equivalent as the DNA uncoils or peels away from these sites in a continual fashion. Interestingly, DNA contacts about ± 40 bp from the nucleosomal dyad appear to represent an energetic barrier to further uncoiling and a transition point past which peeling occurs readily up to positions just near the nucleosomal dyad. This transition point may serve to facilitate nucleosome invasion after the external 30–35 bp of DNA have been peeled away by the action of a polymerase, for example.

Amazingly, even after all but about 10 bp of DNA has been pulled away from the core histone surface, relaxation of the stress results in rather efficient reformation of the nucleosomes. The authors provide evidence that this is due to the maintenance of interactions of about a helical turn or so of DNA with core histones across the dyad axis of the nucleosomes. Several aspects of this result are interesting.

First, when the DNA is completely uncoiled in the physiological conditions used in the experiment it is likely that the H2A/H2B dimers dissociate from the interface with the H3/H4 tetramer, although it is possible that they maintain some interactions with the DNA perhaps via the histone tail domains. On recoiling, the dimers apparently rebind (although definitive evidence of dimer rebinding needs to provided) to form canonical nucleosomes, a feat not easily accomplished in physiological salts without appropriate histone chaperones or chromatin assembly factors (1, 2). This finding suggests that controlled, gradual release and rebinding of DNA, as is likely to occur as a result of the mechanical forces generated by a processive enzyme such as RNA polymerase, provides a mechanism for nucleosome dissolution and reformation of native nucleosomes (16).

Second, a possible function of the unique histone–DNA interactions in the vicinity of the dyad axis is suggested by these studies. Although most DNA within the nucleosome has an average helical periodicity of 10.0 bp per turn, the central ≈2 helical turns is known have a helical periodicity of about 10.7 bp per turn (7, 19). Moreover, the DNA traversing this region has the shallowest curvature of any DNA in the nucleosome (9). Given that the average helical periodicity of DNA in solution is ≈10.6 bp (1) the central one to two turns of DNA in the nucleosome are clearly the most naked-DNA-like in the entire complex. Nonetheless, this region makes multiple energetically important contacts to core histones H3 and H4. The results of Brower-Toland *et al.* suggest that the histone–DNA contacts in the center of the nucleosome provide a nucleation point for wrapping DNA around the rest of the structure (4). This is especially interesting in light of results in which the displacement and translocation of the histone octamer by RNA polymerase seems to occur rapidly after the polymerase invades histone–DNA contacts near the nucleosomal dyad (15).

Another interesting issue is the finding that some DNA unwrapping in each "half" of the nucleosome appears to occur simultaneously, possibly implying some allostery or cooperativity is operative during unwrapping (10). Specifically, the mechanical unwrapping DNA from positions +40 to about +5 bp from the dyad within an individual nucleosome seems to occur concomitantly with the unraveling of the DNA from positions −40 to −5 within the same nucleosome (ref. 4; Fig. 1). Such communication may be mediated by the core histone tail domains in a manner similar to that proposed to occur on H1 binding (10). Relatedly, one wonders whether some "order" exists in the unwrapping of individual nucleosomes or whether the process is purely stochastic. For example, it would be interesting to see whether nucleosomes closest to the end of the array or those next to already-unraveled structures have a higher probability of unraveling than more interior nucleosomes or those with fully neighbors.

Interestingly, previous work employing a similar technique did not define such discrete, reversible single nucleosome transitions in the force-extension curves for pulling chromatin fibers (20, 21). There are several likely reasons for this. First, Cui and Bustamante used native linker histone-containing chromatin fibers isolated from chicken erythrocytes, as opposed to the nucleosomal arrays reconstituted with purified core histones used by Brower-Toland *et al.* (4). Linker histones stabilize nucleosomal DNA association with the histone octamer (1), which may serve to dampen or mask intranucleosomal transitions. Additionally, linker histones may interfere with the ability of nucleosomes to reform on relaxation, leading to the irreversibility of nucleosome reassociation observed by Cui and Bustamante (20). Similarly, the lack of reversibility in nucleosome folding observed in a similar application of this technique employing extract-assembled nucleosomal arrays may be due to ancillary proteins present in the chromatin assembly extracts used or possibly due to the lack of properly spaced nucleosomes in the assembled nucleosomal arrays (21). Nonetheless, these studies provide an important proof-of-principle and suggest that such techniques may be used to study the energetics of internucleosomal interactions (see below).

One wonders whether some "order" exists in the unwrapping of individual nucleosomes or whether the process is purely stochastic.

Several interesting questions arise from the single-fiber work that should be addressable by such single-molecule optical "tweezers" techniques. First, in the salt conditions used the study by Brower-Toland *et al.* (4), the array initially will be folded (3). Thus, to what extent do internucleosomal interactions play a role in the observed force-extension curves? In the absence of linker histones these interactions should be weak, but should be experimentally accessible. Previous single-fiber stretching studies suggest that at least some of the energetics of chromatin folding can be detected by this approach (20, 22). Relatedly, what do the force-extension curves look like when the core histones lack their N-terminal tail domains, which mediate interactions responsible for condensation of the chromatin fiber? Finally, to what extent does the binding of linker histone H1 to the reconstituted array (23) stabilize both folding of the model chromatin fiber and the wrapping DNA within the nucleosome? The work by Brower-Toland *et al.* paves the way for new studies of individual nucleosome dynamics and stability within physiologically relevant nucleosomal arrays and chromatin fibers.

We thank Dr. D. Pruss for assistance with artwork. This work was supported by National Institutes of Health Grant RO1GM52426 (to J.J.H.)

1. Van Holde, K. E. (1989) *Chromatin* (Springer Verlag, New York).
2. Wolffe, A. P. (1998) *Chromatin Structure and Function* (Academic, San Diego), 3rd Ed.
3. Hansen, J. C. (2002) *Annu. Rev. Biophys. Biomol. Struct.* **31,** in press.
4. Brower-Toland, B. D., Smith, C. L., Yeh, R. C., Lis, J. T., Peterson, C. L. & Wang, M. D. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 1960–1965.
5. Eickbusch, T. H. & Moudrianakis, E. N. (1978) *Biochemistry* **17,** 4955–4967.
6. Karantza,V., Freire, E. & Moudrianakis, E. N. (1996) *Biochemistry* **35,** 2037–2046.
7. Hayes, J. J., Clark, D. J. & Wolffe, A. P. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6829–6833.
8. Arents, G., Burlingame, R. W., Wang, B. W., Love, W. E. & Moudrianakis, E. N. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10148–10152.
9. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. (1997) *Nature (London)* **389,** 251–259.
10. Wolffe, A. P. & Hayes, J. J. (1999) *Nucleic Acids Res.* **27,** 711–720.
11. Shore, D., Langowski, J. & Baldwin, R. L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4833.
12. Camerini-Otero, D. & Felsenfeld, G. (1977) *Nucleic Acids Res.* **4,** 1159.
13. Polach, K. J., Lowary, P. T. & Widom, J. (1995) *J. Mol. Biol.* **298,** 211–223.
14. Anderson, J. D. & Widom, J. (2000) *J. Mol. Biol.* **296,** 979–987.
15. Studitsky, V. M., Clark, D. J. & Felsenfeld, G. (1995) *Cell* **83,** 19–27.
16. Studitsky, V. M., Kassavetis, G. A., Geiduschek, E. P. & Felsenfeld, G. (1997) *Science* **278,** 1960–1963.
17. Eggleston, S., O'Neill, T., Bradbury, E. M. & Kowalczykowki, S. (1995) *J. Biol. Chem.* **270,** 2024–2031.
18. Simpson, R. T., Thoma, F. & Brubaker, J. M. (1985) *Cell* **42,** 799–808.
19. Hayes, J. J., Tullius, T. D. & Wolffe, A. P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7405–7409.
20. Cui, Y. & Bustamante, C. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 127–132.
21. Bennink, M. L., Leuba, S. H., Leno, G. H., Zlatanova, J., de Grooth, B. G. & Greve, J. (2001) *Nat. Struct. Biol.* **8,** 608–610.
22. Katritch, V., Bustamante, C. & Olson W. K. (2000) *J Mol. Biol.* **295,** 29–40.
23. Carruthers, L. M, Bednar, J., Woodcock, C. L. & Hansen, J. C. (1998) *Biochemistry* **37,** 14776–14787.