Biophysical Journal Volume 72 March 1997 983-988

New and Notable

Linker Histones' Role Revisited

Ariel Prunell
Institut Jacques Monod, Centre National de la Recherche Scientifique, et Université Denis Diderot, Paris, France

A recent report in *Science* (Pruss et al., 1996) and an accompanying Research News article (Pennisi, 1996) brought into focus the prospect of asymmetrical binding of the globular domain of linker histones H1 and H5 (GH1 or GH5) to the nucleosome. The nucleosome investigated was reconstituted on a *Xenopus* 5S RNA gene sequence, and GH5 bound to it significantly off the dyad axis, partly inside the upper gyre of the DNA, in interaction with both the DNA and histone H2A. This report followed a previous observation from the same laboratory about an asymmetrical protection of entering and exiting DNAs upon digestion with micrococcal nuclease, and echoed a parallel study of the same nucleosome through a different approach, which also concluded with GH5 asymmetrical binding (Hayes, 1996).

The appeal of such an asymmetrical binding mode of the G-domain lies not so much in its unlikeliness than in the potential for the resulting H1-bound particle to be more "open" (see below) than otherwise implied by the common notion that linker histones (LHs) "seal the two turns" of the wrapped DNA by interacting with both entering and exiting DNAs at the dyad. In agreement with this notion, the crystal structure of GH5 and subsequent studies (Ramakrishnan et al., 1993; Goytisolo et al., 1996) suggest the existence of at least two DNA-binding sites in GH5 or GH1. An article by van Holde and co-workers in this issue (Ivanchenko et al., 1996) still reinforces this view. In this work, not only was the preferential binding of H1 and GH1 to supercoiled DNA confirmed, but H1 was also shown to constrain part of the supercoiling when the protein was cross-linked to the DNA before relaxation with topoisomerase I. Moreover, the data did not significantly depend on the supercoiling polarity, pointing to the existence of two (or more) duplexes in close proximity as being the cause for the binding preference, rather than the occurrence of potential cruciforms or other non-B DNA structures in negatively supercoiled DNA.

Future mapping of GH5 in other nucleosomes may eventually reveal that the above asymmetry results from DNA sequence specificity in GH5 binding. Some sequence specificity does occur in the 5S nucleosome, because only one of the two structurally equivalent binding sites (one in each gyre) is occupied, which in turn raises the possibility of cross-talk between the two sites through GH5-induced structural alteration. However, the possibility that sequence specificity alone could have lured GH5 away from its "normal" position at the dyad may appear remote in view of the existence of a cavity on the histone octamer (Arents et al., 1991), which could serve as a proper GH5 harbor (Pruss et al., 1996).

Whatever the outcome to this dilemma, LHs will continue to function, also through their two unstructured tails. The H1 and H5 N-terminal tail is short, whereas the C-terminal tail, approximately half of the molecule, is highly positively charged and mediates compaction of the chromatin fiber. Remarkably, this ability of the C-tail appears to be triggered at the single nucleosome level, as shown by the observation that this tail can bridge nucleosome entering and exiting DNAs together into a four-stranded stemlike structure (Hamiche et al., 1996b). Overall features of this stem are not expected to depend much on the exact placement of the G-domain, although an asymmetrical placement may perhaps drive the stem off the particle dyad axis. Although such a stem favors models for chromatin superstructure in which straight linkers project out from the particle (van Holde and Zlatanova, 1996), it also comes as the last step in the hierarchical folding of DNA onto the nucleosome.

Perhaps next to naked DNA only, the ultimate dynamic structure in chromatin appears to be the $(H3\text{-}H4)_2$ tetramer, which is wrapped with less than a complete DNA superhelical turn and shows a high degree of conformational flexibility (Hamiche et al., 1996a). Tetramer capping by H2A-H2B dimers, while inhibiting this flexibility, increases the wrapping to about 1.7 turns, but fails to induce a crossing of the linker DNAs, which bend away from each other because of electrostatic repulsion (Furrer et al., 1995; Hamiche et al., 1996b). The LH G-domain further increases the wrapping to almost two turns, but only the C-tail can suppress linker DNA repulsion and bridge them together (see above). This scheme suggests that, when required for function, DNA could unfold from the nucleosome through the same steps in the reverse order. At the early step of the unfolding process, the G-domain, if asymmetrically located, could more easily remain in place upon displacement of the tail (by chemical modification, mechanical constraints on the DNA, or other mechanisms). In this way, the tail alone could control access of the DNA at the dyad to nonhistone proteins or other trans-acting factors.

A correlative aspect of LH function in chromatin concerns DNA topology. A problem known as the "linking number (Lk) paradox" arose when the crystal structure disclosed in 1977 revealed a core particle wrapped with 1.75 turns of a left-handed DNA superhelix, with entering and exiting DNAs at right angles to each other. The linker DNAs were assumed to continue these trajectories, so that the full-length nucleosome appeared as a two-turn particle with two negative crossings of the DNA. Such a particle should have reduced Lk by 2, and not 1, as observed in the absence or presence of the LHs (Klug and Lutter, 1981). The quest for a solution to this paradox has stirred some controversy (Morse and Simpson, 1988; White and Bauer, 1989; Klug and Travers, 1989) before coming to a seemingly happy end, at least

Received for publication 18 December 1996 and in final form 18 December 1996.

© 1997 by the Biophysical Society

0006-3495/97/03/983/02 $2.00

in recent textbooks. But, as mentioned above, DNAs in the entrance-exit region of the nucleosome do not continue the right-angle trajectories defined in the core particle, and bend away from each other before they could cross. This leaves one negative crossing and therefore no paradox in the LH-free nucleosome. The paradox would similarly no longer exist for the LH-containing nucleosome if, as appears likely, the two duplexes in the stem remain parallel and do not wind around each other on going from one nucleosome to the next along the chromatin fiber.

REFERENCES

Arents, G., R. W. Burlingame, B.-C. Wang, W. E. Love, and E. N. Moudrianakis. 1991. The nucleosomal core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left-handed superhelix. *Proc. Natl. Acad. Sci. USA.* 88:10148–10152.

Furrer, P., J. Bednar, J. Dubochet, A. Hamiche, and A. Prunell. 1995. DNA at the entry-exit of the nucleosome observed by cryoelectron microscopy. *J. Struct. Biol.* 114:177–183.

Goytisolo, F. A., S. E. Gerchman, X. Yu, C. Rees, V. Graziano, V. Ramakrishnan, and J. O. Thomas. 1996. Identification of two DNA-binding sites on the globular domain of histone H5. *EMBO J.* 15:3421–3429.

Hamiche, A., V. Carot, M. Alilat, F. De Lucia, M. F. O'Donohue, B. Révet, and A. Prunell. 1996a. Interaction of the histone $(H3-H4)_2$ tetramer of the nucleosome with positively supercoiled DNA minicircles: potential flipping of the protein from a left- to a right-handed superhelical form. *Proc. Natl. Acad. Sci. USA.* 93:7588–7593.

Hamiche, A., P. Schultz, V. Ramakrishnan, P. Oudet, and A. Prunell. 1996b. Linker histone-dependent DNA structure in linear mononucleosomes. *J. Mol. Biol.* 257:30–42.

Hayes, J. J. 1996. Site-directed cleavage of DNA by a linker histone-Fe (II) EDTA conjugate: localization of a globular domain binding site within a nucleosome. *Biochemistry.* 35: 11931–11937.

Ivanchenko, M., J. Zlatanova, and K. van Holde. 1996. Histone H1 preferentially binds to superhelical DNA molecules of higher compaction. *Biophys. J.* 72:1388–1395.

Klug, A., and L. C. Lutter. 1981. The helical periodicity of DNA on the nucleosome. *Nucleic Acids Res.* 9:4267–4283.

Klug, A., and A. A. Travers. 1989. The helical repeat of nucleosome-wrapped DNA. *Cell.* 56:10–11.

Morse, R. H., and R. T. Simpson. 1988. DNA in the nucleosome. *Cell.* 54:285–287.

Pennisi, E. 1996. Linker histones, DNA's protein custodians, gain new respect. *Science.* 274:503–504.

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.

Ramakrishnan, V., J. T. Finch, V. Graziano, P. L. Lee, and R. M. Sweet. 1993. Crystal structure of globular domain of histone H5 and its implications for nucleosome binding. *Nature.* 362:219–223.

van Holde, K., and J. Zlatanova. 1996. What determines the folding of the chromatin fiber? *Proc. Natl. Acad. Sci. USA.* 93:10548–10555.

White, J. H., and W. R. Bauer. 1989. The helical repeat of nucleosome-wrapped DNA. *Cell.* 56:9–10.

Smooth and Skeletal Muscle Single-Molecule Mechanical Experiments

Justin E. Molloy and David C. S. White
Department of Biology, University of York, York YO1 5YW, England

An increasing number of laboratories have now developed techniques for measuring the mechanical properties of single molecules (Finer et al., 1994; Molloy et al., 1995; Saito et al., 1994; Miyata et al., 1994). For studies on actomyosin, the point of making such measurements is that the force, movement, and kinetics of a single cross-bridge power stroke can be measured directly. In this issue, Guilford et al. have used an optical tweezers apparatus to measure the properties of single myosin molecules obtained from different sources. They compare the properties of smooth muscle and skeletal muscle myosins and address the question: How does smooth muscle myosin generate more force than skeletal muscle myosin, both in muscle fibers and in vitro?

The conclusions they draw from their results are straightforward to state, namely that a single molecule of phosphorylated smooth muscle myosin (SMM) develops the same force as that of skeletal muscle myosin (SkM) and has the same working stroke, but has a longer attached lifetime, resulting in a greater fraction of "pulling" cross-bridges. This finding is sufficient to explain both the whole muscle and in vitro motility observations, and suggests another round of experiments to answer more detailed questions about the SMM cross-bridge cycle. Many interesting follow-up questions immediately come to mind. For example, a recent cryoelectron microscopy study has shown that the tail of SMM, in contrast with SkM, undergoes a 35-Å movement upon release of ADP (Whittaker et al., 1995). Can this recently discovered motion be detected with the aid of optical tweezers, and is there a second phase of movement associated with the release of phosphate? A quintessential property of smooth muscle is its ability to maintain tension at a low energy cost: What is the mechanical nature of a cross-bridge in this so-called latch state? SMM is directly regulated by phosphorylation: How, and why, do small amounts of unphosphorylated SMM slow down the motility of other myosins when mixtures of both types are combined and tested in vitro? Further experiments and more detailed analysis of data will be required to answer these more subtle features of SMM cross-bridge interactions.

Optical tweezer techniques are relatively new. The limitations of the apparatus are still being uncovered, and ways of analyzing results are still being developed. Here Guilford et al. present a novel approach to analyzing single molecule mechanical data, Mean-Variance Analysis, which is derived from methods developed for patch-clamp recordings of ion channels in membranes.

Guilford et al. use the "three-bead" arrangement originally developed by Finer et al. (1994) to bring an actin filament into direct contact with a myosin molecule. This is the most commonly used method for making measurements of single actomyosin interactions, during which any individual myosin head remains attached to the actin for a small fraction of the overall time. An actin filament is suspended between two beads, each held

Received for publication 31 December 1996 and in final form 15 January 1997.

© 1997 by the Biophysical Society

0006-3495/97/03/984/03 $2.00