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Telling Left from Right in Breathing Nucleosomes

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About three-quarters of the human genome are sequestered by nucleosomes, DNA spools with a protein core. They dictate a wide range of biological processes, including gene regulation, recombination, replication, and chromosome condensation. Nucleosomes are dynamical structures and temporarily expose portions of their wrapped DNA through spontaneous unspooling from either end through a process called site exposure or nucleosome breathing. This had been shown already in 1995 by measuring the accessibility of restriction sites inside nucleosomal DNA to the corresponding enzymes (1) and later by performing fluorescence resonance energy transfer (FRET) experiments in which pairs of dyes were placed at strategic positions inside nucleosomes (see, e.g., (2)). Such experiments taught us that nucleosomes can be very different from each other as a result of the sequence-dependent mechanical properties of their wrapped DNA (3). Nucleosomes not only show a wide range of stabilities (depending on DNA sequence), but also many behave highly asymmetrically, with one end being much more stably wrapped than the other.

Well-positioned nucleosomes with large degrees of asymmetry might be biologically relevant. For example, by averaging over all transcription start sites in *Caenorhabditis elegans*, one learns that these sites feature positioned nucleosomes. There is evidence that these well-positioned nucleosomes are more common for those genes that do not have core promoter elements (e.g., TATA boxes) (4), suggesting that their function lies in demarcating the transcription start sites. Moreover, these nucleosomes are (on average) highly asymmetric. Asymmetric nucleosomes, as shown in (5), can act as polar barriers for RNA polymerases, making transcription in one direction more efficient than in the other. An understanding of the dynamic behavior of nucleosomes is thus highly relevant, but the above-mentioned experiments can only detect nucleosome breathing at one given basepair at a time. What has been missing is a method that allows us to "see" a breathing nucleosome as a whole and, at the same time, also to distinguish between the two DNA ends of a nucleosome.

In this issue of *Biophysical Journal*, Pollack and coworkers (6) manage to achieve just this in an impressive tour de force. They perform small angle x-ray scattering on a solution of nucleosomes, all of which contain the same basepair sequence, specifically the Widom 601 positioning sequence. The Pollack group has perfected this method using contrast matching between the solvent and the protein core. That way, they "see" only the DNA, whereas the histone proteins that form the core of the complex remain invisible. Different states of unwrapping cause different scattering profiles, and what is eventually detected is the ensemble average stemming from all nucleosomes in their different unwrapping states. To find those different unwrapping states and their probabilities, they create an ensemble of theoretical unwrapping states that leads to the right average scattering profile (ensemble optimization method).

Most remarkably, the authors even manage to distinguish the two ends of their nucleosomes. This is possible because the unwrapped DNA portions of the nucleosomes have different basepair sequences, therefore different elastic properties and thus different conformational fluctuations. To learn what type of fluctuation should be expected, the free DNA portions in the model nucleosomes were allowed to fluctuate by using a sequence-dependent elastic DNA model called cgDNA. This is a coarse-grained DNA model that represents DNA by the positions and orientations of its nucleobases, which are modeled as rigid plates.

The authors find that the 601 nucleosome shows highly asymmetric fluctuations, something that earlier experiments with restriction enzymes had already suggested (7) and that had also been observed by mechanical micromanipulation experiments in combination with FRET (8). In the new experiment, however, we can see for the first time the whole ensemble of nucleosomes in their various unwrapping states, especially a partially unwrapped asymmetric

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state, and how this ensemble of configurations changes with the ionic strength of the solution.

The 601 sequence is an artificial sequence that was found in a large pool of random DNA molecules when searching for molecules with high affinities to be wrapped into nucleosomes (9). The importance of the 601 nucleosome lies in the fact that virtually all micromanipulation experiments with single nucleosomes or reconstituted chromatin fibers use this specific nucleosome (3). The authors also look at a natural nucleosome positioning sequence, the 5S rDNA sequence, and find that the corresponding nucleosome behaves differently from the 601; in particular, it does not show an intermediate state but rather unwraps in an all-or-none fashion as the salt concentration is increased.

Each experimental method might influence the behavior of the nucleosomes. For instance, recent computer simulations (10) suggest that the incorporation of restriction sites into the 601 nucleosome in (7) had a huge impact on its DNA accessibility and, in fact, that experiment does not teach us much about the behavior of the 601 nucleosome but that of its modified version, called 601.2. Concerning the current study, one might wonder to what extent, if any, the contrast-matching procedure might influence the stability and dynamics of the nucleosomes. It will thus be exciting to compare the findings with other studies that can observe whole nucleosomes. A new possibility is to catch nucleosomes in the act of breathing by rapidly freezing them. Such an electron cryomicroscopy study was published this year, also using the 601 nucleosome, but the method does not (yet) distinguish between the two ends of the nucleosome (11). That study allowed to observe directly that strongly unwrapped nucleosomes (e.g., by 40 basepairs) typically have lost one of their H2A-H2B dimers. On the other hand, the contrast variation used in the current study renders such dimer loss invisible; the authors instead had to complement their study by performing additional FRET experiments to detect the dimer loss.

To summarize, this exciting study shows for the first time directly that nucleosomes unwrap asymmetrically as a result of the basepair sequence involved. Together with other established and new emerging experimental methods and computer simulations, the time is ripe to make huge progress in understanding the immensely complex sequence-dependent dynamic behavior of nucleosomes.

REFERENCES

1. Polach, K. J., and J. Widom. 1995. Mechanism of protein access to specific DNA sequences in chromatin: a dynamic equilibrium model for gene regulation. *J. Mol. Biol.* 254:130–149.
2. Koopmans, W. J., R. Buning, …, J. van Noort. 2009. spFRET using alternating excitation and FCS reveals progressive DNA unwrapping in nucleosomes. *Biophys. J.* 97:195–204.
3. Eslami-Mossallam, B., H. Schiessel, and J. van Noort. 2016. Nucleosome dynamics: sequence matters. *Adv. Colloid Interface Sci.* 232:101–113.
4. Dreos, R., G. Ambrosini, and P. Bucher. 2016. Influence of rotational nucleosome positioning on transcription start site selection in animal promoters. *PLoS Comput. Biol.* 12:e1005144.
5. Bondarenko, V. A., L. M. Steele, …, V. M. Studitsky. 2006. Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. *Mol. Cell.* 24:469–479.
6. Mauney, A. W., J. M. Tokuda, …, L. Pollack. 2018. Local DNA sequence controls asymmetry of DNA unwrapping from nucleosome core particles. *Biophys. J.* 114:773–781.
7. Anderson, J. D., and J. Widom. 2000. Sequence and position-dependence of the equilibrium accessibility of nucleosomal DNA target sites. *J. Mol. Biol.* 296:979–987.
8. Ngo, T. T., Q. Zhang, …, T. Ha. 2015. Asymmetric unwrapping of nucleosomes under tension directed by DNA local flexibility. *Cell.* 160:1135–1144.
9. Lowary, P. T., and J. Widom. 1998. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J. Mol. Biol.* 276:19–42.
10. Culkin, J., L. de Bruin, …, H. Schiessel. 2017. The role of DNA sequence in nucleosome breathing. *Eur. Phys. J. E Soft Matter.* 40:106.
11. Bilokapic, S., M. Strauss, and M. Halic. 2018. Histone octamer rearranges to adapt to DNA unwrapping. *Nat. Struct. Mol. Biol.* 25:101–108.