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Dynamics of Nucleosome Invasion by DNA Binding Proteins

Hannah S. Tims[1,2], Kaushik Gurunathan[3], Marcia Levitus[3] and Jonathan Widom[1,2,4*]

[1]*Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208-3500, USA*
[2]*Department of Chemistry, Northwestern University, Evanston, IL 60208-3500, USA*
[3]*Department of Chemistry and Biochemistry, and Biodesign Institute, Arizona State University, Tempe, AZ 85287-5601, USA*
[4]*Weizmann Institute of Science, Rehovot, Israel*

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Nucleosomes sterically occlude their wrapped DNA from interacting with many large protein complexes. How proteins gain access to nucleosomal DNA target sites *in vivo* is not known. Outer stretches of nucleosomal DNA spontaneously unwrap and rewrap with high frequency, providing rapid and efficient access to regulatory DNA target sites located there; however, rates for access to the nucleosome interior have not been measured. Here we show that for a selected high-affinity nucleosome positioning sequence, the spontaneous DNA unwrapping rate decreases dramatically with distance inside the nucleosome. The rewrapping rate also decreases, but only slightly. Our results explain the previously known strong position dependence on the equilibrium accessibility of nucleosomal DNA, which is characteristic of both selected and natural sequences. Our results point to slow nucleosome conformational fluctuations as a potential source of cell–cell variability in gene activation dynamics, and they reveal the dominant kinetic path by which multiple DNA binding proteins cooperatively invade a nucleosome.

Introduction

The large enzyme complexes that carry out replication, transcription, recombination, and DNA repair function on naked DNA substrates, yet most eukaryotic DNAs are wrapped in nucleosomes, which sterically occlude and strongly distort the DNA.[1] How these enzyme complexes gain access to their DNA substrates *in vivo* is not understood. ATP-dependent nucleosome remodeling complexes can help by moving or disassembling nucleosomes,[2] creating stretches of naked DNA on which other DNA-binding enzymes can act. What is not understood, however, is how these remodelers themselves "know" which nucleosomes to remodel. The remodelers are recruited to specific chromatin regions through the actions of other site-specific DNA binding regulatory proteins,[2–5] raising a chicken–egg question of how these latter proteins gain access to their own target sites. One potential explanation—that nucleosomes *in vivo* simply do not cover up critical regulatory target sites—is falsified at over 1000 gene promoters in yeast by the results of genomewide nucleosome mapping studies.[6–9] For example, at the well-studied *GAL10–1* locus in yeast, in many cells, a nucleosome centered over the four binding sites for

*Corresponding author. Department of Molecular Biosciences, Northwestern University, 2205 Tech Drive, Hogan 2-100, Evanston, IL 60208-3500, USA. E-mail address: j-widom@northwestern.edu.

Abbreviations used: FRET, fluorescence resonance energy transfer; FCS, fluorescence correlation spectroscopy.

the Gal4 transcription activator protein is occupied by the RSC remodeling complex, leaving the nucleosomes with only ~135 bp of wrapped DNA and potentially facilitating binding by the Gal4 protein.[10] However, many other cells in the population have full-length nucleosomes covering this region,[7] leaving all Gal4 sites sterically occluded, yet all cells in the population respond appropriately to galactose (L. Chen and J.W., unpublished).

These considerations led us to hypothesize that nucleosomes might not be inert frozen structures, as imaged by X-ray crystallography,[1] but instead might be dynamic, such that DNA wrapped in the time average might nevertheless be transiently accessible to other DNA binding proteins. Using biochemical and fluorescence resonance energy transfer (FRET)[11] assays, we and others found that nucleosomes are indeed highly dynamic, spontaneously but transiently unwrapping stretches of their DNA starting from one end,[12–17] while the rest of the nucleosome remains fixed in position along the DNA.[12,13] For both isolated nucleosomes and nucleosomes in long arrays,[18,19] the equilibrium constant for such spontaneous "site exposure" near nucleosomal DNA ends is remarkably high at ~0.01–0.1 (i.e., end stretches of nucleosomal DNA are spontaneously unwrapped 1–10% of the time), decreasing progressively with distance inside the nucleosome, down to 10^{-5}–10^{-6} for DNA sites near the middle.[14,20]

Spontaneous nucleosomal site exposure is thought to facilitate the ability of RNA polymerase and other processive enzymes to elongate through a nucleosome.[16,21–23] In addition, it may play a role in photolyase-mediated repair of DNA, which occurs more quickly than can be explained by known ATP-dependent remodeling activities,[24] and it may contribute to genomewide transcriptional regulation[25,26] through nucleosome-induced cooperativity.[27–30]

In order for such intrinsic nucleosome dynamics to contribute to the real abilities of gene regulatory proteins to gain access to their DNA target sites, it is necessary that site exposure occurs both with an acceptably high probability (equilibrium constant) and with an acceptably high rate. In an initial study,[16] we analyzed the dynamics of the ends of wrapped nucleosomal DNA and found that nucleosomes indeed spontaneously open up (partially unwrap their DNA) with a remarkably high frequency, ~4 times per second (i.e., DNA remains fully wrapped for only ~250 ms before spontaneously unwrapping). Once unwrapped, this open state lasts for ~10–50 ms before the DNA spontaneously rewraps. Thus, for regulatory DNA target sites located at short distances inside a nucleosome, the rate of spontaneous nucleosome site exposure is sufficiently great so as to plausibly allow regulatory proteins to find and bind to these sites *in vivo*.

What happens, however, when a critical regulatory binding site is located further inside a nucleosome, where its equilibrium accessibility is not 1–10% but orders of magnitude lower? This question has not been systematically investigated. Moreover, the isolated data that do exist are complicated by problems of heterogeneous nucleosome positioning and unexpected nucleosome disassembly,[31] or by blinking of FRET dyes[32,33] (see Koopmans *et al.*[34]). On simple thermodynamic grounds, the greatly reduced equilibrium accessibility measured in our earlier work[14,20] must reflect a decreased unwrapping rate, an increased rewrapping rate, or both. Here, we utilize two independent complementary approaches—stopped-flow FRET and FRET fluorescence correlation spectroscopy (FCS)—to measure the rates of nucleosome unwrapping and rewrapping for differing DNA sites from the end of the nucleosomal DNA inward toward the middle. Our results establish that spontaneous access to sites further inside a nucleosome occurs with greatly reduced rate and lead to new conclusions about the dynamics and mechanisms with which proteins can gain access to nucleosomal DNA target sites *in vivo*.

Results

Coupled protein binding/FRET assay for position-dependent site exposure

To investigate the position-dependent kinetics of nucleosome site exposure, we take advantage of the steric occlusion of wrapped nucleosomal DNA by coupling site exposure to the binding of a site-specific DNA binding protein. For convenience, we use the LexA repressor protein of *Escherichia coli*. We construct homogeneously positioned nucleosomes that contain somewhere within their wrapped DNA a specific target site for LexA, such that LexA would "like" to bind to its target site but cannot because the site is sterically occluded inside the nucleosome. The nucleosomes are specifically labeled with a FRET donor dye (Cy3) placed at the DNA 5′ end distal to the LexA target site and with a fluorescence acceptor dye (Cy5) attached to a unique engineered cysteine residue (H3 V35C C110A)[12] located nearby in a space on the histone core (Fig. 1a). We estimate the expected donor/acceptor distance at ~2 nm, in comparison to an ~6-nm characteristic distance for energy transfer for this dye pair; thus, we expect to, and indeed do, observe a high FRET efficiency when the DNA is fully wrapped.[12,16] A second copy of the acceptor, present in the other symmetry-related copy of H3, is much farther away and thus contributes little to the FRET signal (Fig. 1b). Spontaneous unwrapping of the nucleosomal DNA increases the distance between the unwrapped