nature structural & molecular biology

High-resolution dynamic mapping of histone-DNA interactions in a nucleosome

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The nature of the nucleosomal barrier that regulates access to the underlying DNA during many cellular processes is not fully understood. Here we present a detailed map of histone-DNA interactions along the DNA sequence to near base pair accuracy by mechanically unzipping single molecules of DNA, each containing a single nucleosome. This interaction map revealed a distinct ~5-bp periodicity that was enveloped by three broad regions of strong interactions, with the strongest occurring at the dyad and the other two about ±40-bp from the dyad. Unzipping up to the dyad allowed recovery of a canonical nucleosome upon relaxation of the DNA, but unzipping beyond the dyad resulted in removal of the histone octamer from its initial DNA sequence. These findings have important implications for how RNA polymerase and other DNA-based enzymes may gain access to DNA associated with a nucleosome.

The nucleosome is the fundamental repeating unit of eukaryotic chromatin, consisting of ~147 bp of DNA wrapped ~1.7 times around a histone octamer[1]. Nucleosomes must be stable and yet dynamic structures, both maintaining eukaryotic DNA in a condensed state and also permitting regulated access to genetic information contained therein. During many important cellular processes, DNA binding proteins must access specific genomic regions that are occluded by nucleosomes. In particular, *in vitro* studies show that RNA polymerase slows down, pauses or stalls upon encountering a nucleosome[2–7]. The resistance that RNA polymerase encounters when transcribing a chromatin template should be largely dictated by both the strengths and locations of histone-DNA interactions in the nucleosome. Therefore a detailed map of these interactions would lay an important foundation for understanding the structural details of eukaryotic transcription and how gene expression may be regulated by histone modifications, DNA sequence and nucleosome remodeling.

Analysis of the crystal structure of the nucleosome indicates that histone-DNA interactions are not uniform along the DNA[1,8]; however, experimental determination of this interaction map has proven to be challenging and is still largely controversial. Although it is well established that the overall stability of a nucleosome depends on its constituent DNA sequence and histone modifications[9–11], the way in which specific interactions in a nucleosome lead to this stability is less well understood. The mechanical nature of this problem makes it ideally suited for investigation using single-molecule manipulation approaches[12–19]. Previously, we have stretched single DNA molecules of chromatin and obtained data on the relative locations of strong histone-DNA interactions[14,17]. These data indicate the presence of three regions of strong interactions, consistent with those suggested by counting the number of apparent histone-DNA contacts seen in the nucleosome crystal structure[20]. However, subsequent single-molecule stretching experiments challenged this interpretation and suggested that force signatures from stretching experiments can be attributed to the rotation of the spool geometry of the nucleosome rather than regions of strong histone-DNA interactions[21]. These studies favor a model in which histone-DNA interactions are uniform along the DNA[22,23]. Ambiguities exist because stretching experiments cannot readily separate contributions of geometry from those of interaction strengths, nor can they quantitatively assay interaction strengths near the dyad.

Recently, we have developed a method to sequentially determine the absolute locations of histone-DNA interactions by mechanically unzipping a DNA molecule containing a nucleosome assembled with histones purified from HeLa cells[16]. However, the precision of that method was insufficient to map out all of the densely packed histone-DNA interactions in a nucleosome. In the current work, using an improved unzipping method, we have mapped the locations of the interactions to near base pair accuracy along the DNA and quantitatively assayed the strengths of these interactions. The histone-DNA interaction map, together with mechanical invasion experiments, provides a simple explanation of the pausing pattern of RNA polymerase within a nucleosome and makes testable predictions on the fate of histones during transcription.

RESULTS

Mapping of interactions with near base pair precision

The experimental configuration is sketched in **Figure 1a** (see also Methods and **Supplementary Fig. 1** online). A DNA molecule

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Received 15 August 2008; accepted 10 November 2008; published online 11 January 2009; doi:10.1038/nsmb.1526

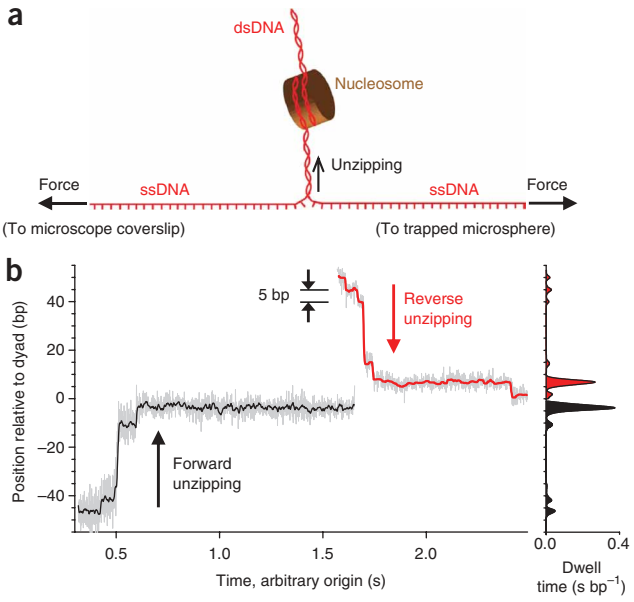

Figure 1 Nucleosome disruptions under a constant unzipping force. (**a**) Experimental configuration. A DNA molecule was mechanically unzipped through a nucleosome uniquely positioned at a 601 sequence. (**b**) Representative traces for unzipping under a constant applied force (~28 pN). Two traces are shown: one from forward unzipping (black) and one from reverse unzipping (red). Both traces were low-pass filtered from the raw traces (gray) to 60 Hz. The unzipping fork paused at specific locations, which are evident from both the traces (left) and their corresponding dwell time histograms (right).

containing a single nucleosome uniquely positioned at a 601 nucleosome-positioning sequence[24] was attached to the surface of a microscope coverslip via one of its strands and to a microsphere held in an optical trap via the other strand[16]. As the coverslip was moved away from the trapped microsphere, double-stranded DNA (dsDNA) was sequentially converted to single-stranded DNA (ssDNA) upon base pair separation. As the unzipping fork progressed through the nucleosome, it encountered resistance from histone-DNA interactions at well-defined locations and, because these interactions require dsDNA, they were sequentially disrupted. The magnitude of resistance should strongly correlate with histone-DNA affinity, and thus a histone-DNA interaction map was generated along the DNA. We improved the alignment method and showed that this technique achieved a resolution of better than 1 bp (Methods and **Supplementary Fig. 2c** online). Its accuracy and precision in determining the absolute sequence position of an interaction were both ~1.5 bp (Methods and **Supplementary Fig. 2b**).

Mapping strengths of histone-DNA interactions in a nucleosome

To quantitatively assay the strengths of the histone-DNA interactions, we unzipped through individual nucleosomal DNA molecules with a constant unzipping force of ~28 pN (Methods). Under a force clamp[25], the dwell times at different sequence positions measure the strengths of interactions at those positions, provided that disruption of each interaction follows a similar energy landscape. Thus this method allows direct mapping of the strengths of interactions. **Figure 1b** shows example traces for unzipping DNA through a nucleosome under a constant force (**Supplementary Fig. 3** online for additional traces). DNA molecules were unzipped from both directions along the DNA (referred to as 'forward' and 'reverse') (Methods and **Supplementary Fig. 3**). In both cases, the unzipping fork did not move through the nucleosomal DNA at a constant rate but instead dwelled at specific locations within the nucleosome, indicating the presence of strong interactions. In particular, these traces revealed that the fork dwelled with discrete steps spaced by ~5 bp, and the longest dwell times tended to occur near the dyad.

We generated an interaction map by averaging dwell time histogram measurements from many traces from both forward and reverse unzipping (**Fig. 2**). Several features are evident from these plots. (i) There are three broad regions of strong interactions: one located at the dyad and two approximately ±40 bp from the dyad. (ii) An ~5-bp periodicity occurred within each region of interaction. (iii) The interactions near the entry and exit DNA are particularly weak. The unzipping fork did not dwell at a 20-bp region of both entry and exit DNA, indicating that the histones are only loosely bound to the DNA. (iv) For unzipping in both the forward and reverse directions, the first two regions of interactions encountered were always detected, but not the last region. This indicates that, once the dyad region of interactions was disrupted, the nucleosome became unstable and histones dissociated from the 601 sequence. (v) The total dwell time in the nucleosome was longer in the forward direction compared with that in the reverse direction, indicating nucleosomes were more difficult to

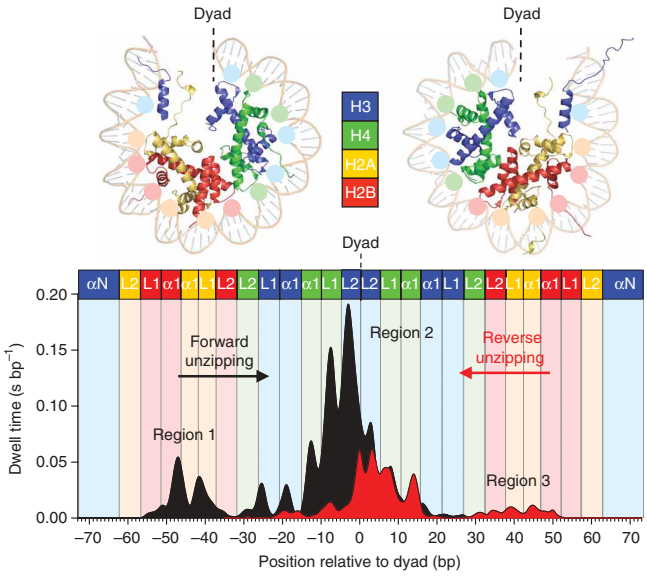

Figure 2 Histone-DNA interaction map within a nucleosome core particle. Above, the crystal structure of the nucleosome core particle[1]. Dots indicate regions where interactions between DNA and one of the core histones are likely to occur. The two halves of the nucleosome are shown separately for clarity. Below, a histone-DNA interaction map constructed from the averaged dwell time histograms of the unzipping fork at constant force (~28 pN). Individual traces were low-pass filtered to 60 Hz, and their dwell time histograms were binned to 1 bp. A total of 27 traces from the forward template and 30 traces from the reverse template were used for the construction. Each peak corresponds to an individual histone-DNA interaction, and the heights of the peaks are indicative of their relative strengths. Three regions of strong interactions are indicated: one located at the dyad (region 2) and two off-dyad locations (regions 1 and 3). Colored boxes indicate predictions from the crystal structure of where individual histone binding motifs are expected to interact with DNA. The H3 N-terminal α-helices (αN) and the histone loops (L1, L2) and α-helices (α1) that compose the L1L2 and α1α1 DNA binding sites observed in the crystal structure[1] are also indicated.

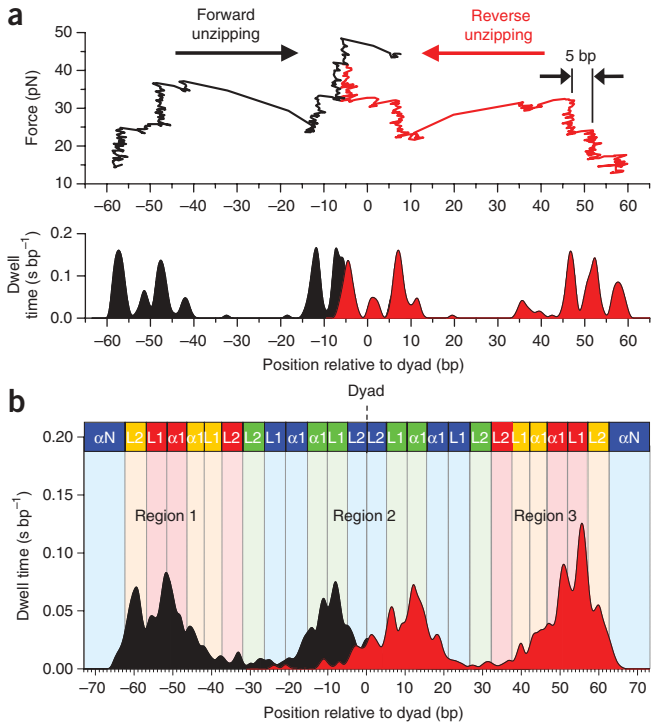

Figure 3 Nucleosome disruptions under a constant loading rate. (**a**) Representative traces for unzipping under a constant loading rate (8 pN s^{-1}). Two traces are shown: one from forward unzipping (black) and one from reverse unzipping (red). For clarity, the naked DNA signature before and after each nucleosome-disruption event is not shown. The unzipping fork again paused at specific locations, which are evident from both the traces (above) and their corresponding dwell time histograms (below). (**b**) The average dwell time histograms of the unzipping fork under a constant loading rate. Individual traces such as those shown above were low-pass filtered to 60 Hz, and their dwell time histograms were binned to 1 bp. A total of 36 traces from each direction was used for the construction. Other notations are the same as those used in **Figure 2**.

disrupt when unzipped in the forward direction, probably reflecting the nonpalindromic nature of the 601 sequence.

Highlighting histone-DNA interactions near entry or exit DNA

Because the entry and exit DNA regulate the initial invasion of a nucleosome by a motor protein, we carried out experiments starting from a lower unzipping force to specifically detect interactions at those locations and then ramped up the force to allow complete unzipping through the nucleosomal DNA. We unzipped through nucleosomal DNA molecules under a constant loading rate (8 pN s^{-1}), highlighting the edge of the region first encountered[16] (Methods). **Figure 3a** shows example traces of nucleosomes unzipped from both forward and reverse directions. **Figure 3b** shows the averaged dwell time histograms measured during both forward and reverse unzipping (see **Supplementary Fig. 4** online for additional traces). Aside from the aforementioned bias in the dwell time histogram, many features are consistent with data from unzipping under a constant force. The interactions near the entry and exit DNA were more evident, still showing a clear ~5-bp periodicity. This indicates that DNA segments at least up to 60 bp from the dyad have substantial interactions with the histone core.

Features shared by nucleosomes on arbitrary DNA sequences

To determine whether the conclusions above are also valid for nucleosomes of arbitrary DNA sequence or just for the 601 sequence, we assembled nucleosomes onto a DNA segment that does not contain any known positioning elements (Methods). The assembly condition was controlled to achieve a relatively low saturation level, so that each DNA molecule had at most one nucleosome. When such nucleosomal DNA molecules were unzipped with a loading-rate clamp using the same conditions as those of **Figure 3**, we found nucleosomes at various locations on the template (**Supplementary Fig. 5** online), probably owing to the lack of any known nucleosome-positioning element on this DNA sequence. Each unzipping trace contains two

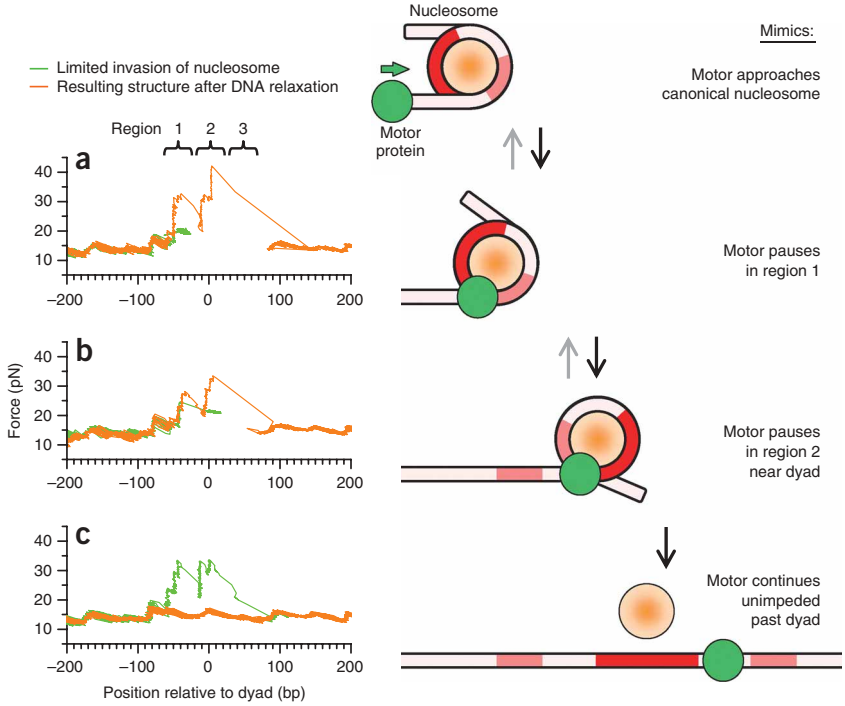

Figure 4 Mechanical unzipping (left) to mimic motor enzyme progression into a nucleosome (right). (**a**) DNA was unzipped with a loading-rate clamp (8 pN s^{-1}) until the unzipping force reached ~20 pN, which typically occurred within the first region of interactions (green curve). The unzipping force was then held at this force for 10 s, resulting in a horizontal force line due to the hopping of the unzipping fork among different positions within the first region. These steps mimic a motor invasion into the first region of interactions and subsequent pausing within the region (right). The tension in the DNA was then relaxed for ~3 s, and the state of the nucleosome was determined by unzipping a second time (orange curve). (**b**) Similar to the experiment in **a**, except that the unzipping force was held at ~21 pN immediately after the unzipping fork entered the dyad region of interactions. These steps mimic motor invasion into the dyad region of interactions before pausing (right). (**c**) Similar to the experiment in **b**, except that DNA was unzipped past the dyad region of interactions. This mimics motor invasion past the dyad (right).

major regions of strong interaction, with the second region presumably located near the dyad. These nucleosomes possessed essentially identical characteristics to those of the 601 sequence, except that their peak forces within each region were typically smaller by a few pico-Newtons, reflecting weaker interactions of histone with nonpositioning DNA sequences. The key features remained essentially identical: the presence of three regions of strong interactions, with the strongest at the dyad; the 5-bp periodicity; and the loss of nucleosome stability upon dyad disruption. These results indicate that the conclusions of this work are not restricted to nucleosomes on the 601 sequence but are general to nucleosomes on any sequence.

Mechanical invasion of a nucleosome

To mimic invasion by a motor protein as it progresses into a nucleosome, we carried out three sets of mechanical invasion experiments (**Fig. 4**). In the first set, unzipping was allowed to proceed into and then held within the first region of strong interactions, before the DNA was relaxed to allow rezipping (**Fig. 4a**). The state of the nucleosome was subsequently examined by unzipping through the entire 601 sequence. Most of the traces examined in this way (75%) showed a canonical nucleosome structure at the 601 sequence. The remaining 25% showed altered structures, probably resulting from incomplete re-annealing of the DNA in the presence of histones (**Supplementary Fig. 6** online). In the second set, unzipping was allowed to proceed into and then held within the dyad region of interactions, before the DNA was relaxed to allow rezipping (**Fig. 4b**). Most of the resulting structures (70%) again resembled a canonical nucleosome at the 601 sequence. In the third set, unzipping was allowed to proceed past the dyad region of interactions, before the DNA was relaxed to allow rezipping (**Fig. 4c**). Subsequently, all traces showed force signatures indistinguishable from those of the naked 601 sequence, indicating complete removal of the histone octamer from the 601 sequence. These results indicate that motor enzymes may be capable of accessing nearly half of the underlying DNA without resulting in histone dissociation.

DISCUSSION

Histone-DNA interaction map of a nucleosome

This study presents a high-resolution quantitative map of histone-DNA interactions in a nucleosome. It not only provides a direct measure of the locations of interactions to near base pair resolution, but also quantitatively assays the strengths of these interactions. The overall features of the interaction map are not specific to the 601 sequence but are shared by DNA of arbitrary sequence (**Supplementary Fig. 5**).

The histone-DNA interaction map reveals the existence of three regions of strong interactions. This is the most direct evidence that the histone-DNA interactions within a nucleosome are not uniform: the strongest region of interactions is located at the dyad and another two regions of strong interactions lie approximately ±40 bp from the dyad. The locations of all three regions are strongly correlated with those estimated from the crystal structure of the nucleosome[8,20]. The central region is clearly the strongest, and this observation explains why nucleosome stability has been shown to be most sensitive to DNA sequence near the dyad[26]. The locations of the off-dyad regions are also consistent with findings from our previous nucleosome-stretching measurements[14,17]. This also indicates that, in the single-molecule stretching experiments, nucleosome spool geometry may not contribute substantially to force signatures or contribute in a way that coincides with the effects due to the two regions of off-dyad interactions.

We observed a 5-bp periodicity in the interaction map, whereas before this work a 10-bp periodicity would have been expected. The crystal structure of the nucleosome shows that specific DNA-histone contacts are made each time the DNA minor groove faces the histone octamer surface, leading to binding sites spaced at ~10 bp[1]. Closer inspection shows that interactions from the two strands of the dsDNA completely stagger with each other and alternate between the two strands along the sequence at every 5 bp. However, in crystal structure analyses, the histone interaction with each minor groove of the DNA has been treated as a single binding site[1,20,27]. This is reasonable, as disruption of a histone interaction with one of the DNA strands at a minor groove may result in a concurrent disruption of a histone interaction with the other strand. Before our experiments, we had anticipated that a 10-bp periodicity would be observed. The fact that we have actually observed a 5-bp periodicity indicates that the histone interactions with two strands of DNA at its minor groove are decoupled, and can thus be disrupted sequentially instead of simultaneously.

The interactions near the exit and entrance DNA were found to be particularly weak, although they maintain the 5-bp periodicity. We propose that these weak interactions permit spontaneous peeling of DNA ends from the octamer surface, as observed by equilibrium accessibility assays[28,29].

Implications for transcription

Although RNA polymerases are known to be powerful molecular motors[30,31], the presence of a nucleosome still presents a major obstacle[2–7]. The mechanical unzipping experiments described here resemble the action of RNA polymerase, which opens up a transcription bubble and unzips the downstream DNA while advancing into a nucleosome (**Fig. 4**, right). The histone-DNA interaction map (**Fig. 2**) has important implications for how RNA polymerases may gain access to DNA associated with a nucleosome. RNA polymerase is expected to initially proceed smoothly but pause when it encounters the off-dyad interactions. Disruption of these interactions permits it to proceed toward the dyad. The polymerase will then pause most strongly within the dyad region of interactions. Once it overcomes the dyad interactions, it will proceed through the rest of the nucleosomal DNA with minimal resistance. The interaction map also predicts that the 601-positioned nucleosome acts as a polar barrier: transcription in the forward direction is less efficient than in the reverse direction. It is likely that asymmetries of this sort exist in eukaryotic genomes, and they may have functional importance for normal gene expression where positioned nucleosomes reside at key positions transited by RNA polymerase (Pol) II[32]. Notably, many of these predictions have been verified by biochemical studies of Pol II or Pol III transcription through nucleosomes[2–7].

Although the interaction map also suggests that transcription pausing may show a finer, ~5-bp periodic pattern, an ~10-bp periodicity has been observed[5,6,33,34]. Although this periodicity has been attributed to nucleosome restriction of RNA polymerase rotation coupled with DNA loop formation, this work offers a simpler explanation. The ~10-bp periodicity in transcription pausing may be due to RNA polymerase cooperatively disrupting a pair of interactions located at each minor groove of DNA.

Although the pausing pattern of RNA polymerase is dictated by both the mechanical barriers it encounters and its own motor properties, similarities between the dwell time in the histone-DNA interaction map (**Fig. 2**) and the polymerase pausing pattern within a nucleosome suggest that the barriers encountered by the polymerase are a major determinant of its pausing behavior. Thus, this

explanation of the pausing pattern within a nucleosome provides a simpler explanation than existing models[3,5,33]. The consistency of the histone-DNA interaction map with biochemical assays of the RNA polymerase pausing pattern is an indication that this map may also be used to predict how other motor enzymes pass through nucleosomes.

The results from nucleosome-invasion experiments yield testable predictions regarding the fate of nucleosomes during transcription. If RNA polymerase backtracks before the dyad, histones will not dissociate from the DNA but will tend to reform a canonical nucleosome at the same location (**Supplementary Discussion** online), perhaps encouraging further backtracking of the polymerase. Once the RNA polymerase passes the dyad, histones will most likely be removed from their original locations.

METHODS

Nucleosomal DNA templates. We prepared nucleosomal DNA templates using methods similar to those previously described[16]. Briefly, each DNA construct consisted of two separate segments (**Supplementary Fig. 1a**). An ~1.1-kbp anchoring segment was prepared by PCR from plasmid pRL574 (ref. 35) using a digoxigenin-labeled primer and then digested with BstXI (NEB) to produce a ligatable overhang. Each unzipping segment was prepared by PCR using a biotin-labeled primer, and then digested with BstXI and dephosphorylated using calf intestinal phosphatase (CIP; NEB) to introduce a nick into the final DNA template. Nucleosomes were assembled from purified HeLa histones onto the unzipping fragment by a well-established salt-dialysis method[36]. The two segments were joined by ligation immediately before use. This produced the complete template that was labeled with a single dig tag on one end and a biotin tag located 7 bp after the nick in one DNA strand.

We prepared the forward 601 unzipping segment (0.8 kbp) by PCR from plasmid 601 (ref. 24) as described previously[16]. The reverse template is nearly identical to the forward template, except that the reverse unzipping segment was flipped so that the unzipping fork would approach the nucleosome from the opposite direction. To achieve this, the reverse segment was produced using different primers, such that the ligatable overhang produced through BstXI digestion and the nick introduced via CIP were located on the end opposite to that of the forward segment. The unzipping segment that does not contain any known nucleosome-positioning element (~0.8 kbp) was prepared by PCR from plasmid pBR322 (NEB).

Hairpin DNA templates. We prepared three different hairpin templates from the forward template (without nucleosomes) by truncating the unzipping segment at precise locations using restriction enzymes and ligating the same hairpin onto the end in each case. The lengths of the unzipping templates are indicated in **Supplementary Figure 2b**.

Unzipping under constant force. For experiments involving unzipping through a nucleosome under a constant force, we started the unzipping with a loading-rate clamp (8 pN s^{-1}) until the desired force of ~28 pN was reached within a nucleosome. The unzipping force was then held constant by feedback control of the coverslip position[25]. This force is much stronger than the sequence-dependent unzipping force of the naked 601 sequence (13–16 pN), minimizing the dwell time contribution due solely to DNA base-pairing interactions, but is weak enough to allow sufficient dwell time at each DNA sequence position for detection. Upon reaching the end of the 601 sequence, the unzipping was continued under a loading-rate clamp (8 pN s^{-1}). Unzipping before and after the 601 segment under a constant loading rate generated distinct unzipping signatures that could be used for data alignment (see below).

Unzipping under constant loading rate. An optical trapping setup was used to unzip a single DNA molecule by moving the microscope coverslip horizontally away from the optical trap (**Supplementary Fig. 1b**). As barriers to fork progression were encountered, a computer-controlled feedback loop increased the applied load linearly with time (8 pN s^{-1}) as necessary to overcome those barriers. Whenever the unzipping fork stopped, for example, at an interaction, the unzipping force was ramped up linearly with time until the interaction was disrupted[37]. When two interactions occurred in close vicinity, upon the disruption of the first interaction the force was unable to relax back to the baseline before being ramped up again for the second interaction, subjecting this subsequent interaction to a higher initial force. Therefore, for each region of interactions, the dwell time histogram highlighted the edge of the region first encountered. Another feature of this method was the display of the distinctive force signature for a nucleosome, allowing for ease of identification of the nucleosome structure[16] (compare traces in **Supplementary Fig. 3** with **Supplementary Fig. 4**).

Data collection and alignment. Data were low-pass filtered to 5 kHz, digitized at ~12 kHz and later filtered to 60 Hz. Previously, to improve the positional precision and accuracy, the experimental curves were aligned to the theoretical curve by cross-correlation of a region immediately preceding the nucleosome disruption[16]. In the current work, we further improved the precision and accuracy of the data by an additional cross-correlation of a region immediately following the nucleosome disruption. To account for minor instrumental drift, trapping-bead size variations and DNA linker variations, the alignment allowed for a small additive shift (<5 bp) and multiplicative linear stretch (<2%) using algorithms similar to those previously described[38].

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

We thank members of the Wang laboratory and B. Brower-Toland for critical reading of the manuscript, J. Jin for helpful advice with biochemical preparations and D.S. Johnson for helpful discussions on instrumentation. We wish to acknowledge support from the US National Institutes of Health (GM059849 to M.D.W.; GM25232 to J.T.L.), the Keck Foundation (to M.D.W.), the Cornell Nanobiotechnology Center (to M.D.W. and J.T.L.) and the Molecular Biophysics Training Grant Traineeship (to M.A.H.).

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