Regulation of the nucleosome unwrapping rate controls DNA accessibility

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Received April 30, 2012; Revised June 21, 2012; Accepted July 13, 2012

ABSTRACT

Eukaryotic genomes are repetitively wrapped into nucleosomes that then regulate access of transcription and DNA repair complexes to DNA. The mechanisms that regulate extrinsic protein interactions within nucleosomes are unresolved. We demonstrate that modulation of the nucleosome unwrapping rate regulates protein binding within nucleosomes. Histone H3 acetyl-lysine 56 [H3(K56ac)] and DNA sequence within the nucleosome entry-exit region additively influence nucleosomal DNA accessibility by increasing the unwrapping rate without impacting rewrapping. These combined epigenetic and genetic factors influence transcription factor (TF) occupancy within the nucleosome by at least one order of magnitude and enhance nucleosome disassembly by the DNA mismatch repair complex, hMSH2–hMSH6. Our results combined with the observation that ~30% of Saccharomyces cerevisiae TF-binding sites reside in the nucleosome entry–exit region suggest that modulation of nucleosome unwrapping is a mechanism for regulating transcription and DNA repair.

INTRODUCTION

Eukaryotic genomes are organized into repeats of nucleosomes, which contain 147 bp of DNA wrapped ~1.65 times around an octamer of H2A, H2B, H3 and H4 histone proteins (1). Nucleosomes function to regulate DNA processing by sterically occluding transcription (2) and repair (3) complexes from nucleosomal DNA. External factors such as histone post-translational modifications (PTMs) (4), chromatin remodeling (5) and histone chaperones (6) appear to provide access to nucleosomal DNA. In addition, the inherent property of nucleosomes to spontaneously partially unwrap directly exposes buried DNA sites within the nucleosome (7–9). The equilibrium between the fully wrapped and partially wrapped nucleosome states is termed nucleosome site exposure, and conversion into a partially unwrapped nucleosome occurs many times per second (10,11). Site exposure provides DNA access for transcription factors (TFs) (8,12–15) and DNA repair complexes (16–18). However, the mechanisms that regulate nucleosome site exposure remain undetermined.

Histone PTMs (14,15,19,20) and the DNA sequence bound by the core histones (21–23) influence the nucleosome site exposure equilibrium. Histone H3 lysine 56 acetylation [H3(K56ac)], which helps regulate eukaryotic transcription, replication and repair (24–30), is located in the globular core of H3 near the nucleosome entry–exit region. Structural studies of the acetylation mimic H3(K56Q) observe minimal changes in the fully wrapped nucleosome structure (31), while replacing histone H3 with its variant, CENP-A, does significantly alter the DNA in the crystal structure (32). H3(K56ac) shifts the site exposure equilibrium toward partially unwrapped nucleosome states (20). This enhances DNA accessibility for TF binding within the nucleosome (14), while the acetyl-lysine mimic H3(K56Q) enhances nucleosome disassembly by the mismatch repair (MMR) recognition complex, hMSH2–hMSH6 (17). These results suggest that H3(K56ac) reduces DNA–histone interactions that enhance site exposure without altering the nucleosome structure in the fully wrapped state. Determining the mechanisms by which H3(K56ac) and other factors such as DNA sequence influence site exposure is central to understanding how transcription and DNA repair complexes gain access to nucleosomal DNA.

Here we examine the influence of H3(K56ac) and DNA sequence on the nucleosome unwrapping and rewrapping rates in order to determine the changes in nucleosome exposure.

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dynamics associated with changes in site exposure equilibrium. We find that H3(K56ac) and DNA sequence within the nucleosome entry–exit region separately influence the nucleosome unwrapping rate without altering the rewrapping rate. H3(K56ac) and DNA sequence additively influence the nucleosome unwrapping rate by at least an order of magnitude and result in an equivalent change in TF occupancy within the nucleosome. Furthermore, the H3(K56ac)-enhanced DNA unwrapping rate causes a parallel increase in the hMSH2–hMSH6 induced nucleosome disassembly rate. These results are consistent with the conclusion that modulation of nucleosome unwrapping by PTMs and DNA sequence is a general mechanism for regulating DNA accessibility for transcription and DNA repair.

EXPERIMENTAL PROCEDURES

DNA constructs

The 601L (8), 5SL, 5SL-dyad, 5S(1–7), 5S(28–47) and 5S(1–47) molecules for fluorescence studies were prepared by PCR from plasmid containing the 601 nucleosome positioning sequence (NPS) or the Xenopus laevis 5S rDNA NPS with a LexA-binding site (TACTGTATGAG CATA CAGTA) cloned into bases 8–27. Oligonucleotides for PCR (Supplementary Table S1) were conjugated to a S′ or internal amine with Cy3-NHS (GE Healthcare) and purified by RP-HPLC on a 218TPTM C18 (Grace/Vydac) column. The 5SX-G/C and 5SX-G/T molecules were prepared and P32 labeled as previously described (17).

Preparation of histone octamers and LexA protein

Xenopus laevis recombinant histones were expressed and purified as previously described (33). Plasmids encoding histones H2A, H2A(K119C), H2B, H3 and H4 were generous gifts from Dr Karolin Luger (Colorado State University) and Dr Jonathan Woidom (Northwestern University). Mutations H3(C110A) and H3(K56Q) were introduced by site-directed mutagenesis (Stratagene). Histone H3(K56Ac) was prepared as previously described (14). Each of the four histones were combined at equal molar ratios, refolded and purified as previously described (14). Histone octamer (HO) was labeled with Cy5-maleamide (GE Healthcare) as previously described (33). H2A(K119C) containing histone octamer (HO) was labeled with Cy5-maleamide (GE Healthcare) as previously described (14). LexA protein was expressed and purified from pJW288 plasmid as previously described (34).

Nucleosome reconstitutions

Nucleosomes were reconstituted from DNA and purified HO by salt double dialysis and purified by sucrose gradient (14). Nucleosomes containing Cy3 labeled DNA for fluorescence studies were reconstituted with HO containing Cy5-labeled H2A(K119C). Nucleosomes containing 5SX-G/T or 5SX-G/C were reconstituted with HO containing unlabeled H2A. Nucleosomes reconstituted with 5S-Lv, SS and 5SL(147) resulted in two nucleosome positions as previously reported (35). The central positioned 5SL(147) nucleosomes used in FRET measures were purified from the depositioned nucleosomes by sucrose gradient purification.

TF binding and site accessibility equilibrium measurements

TF binding and nucleosome site accessibility equilibrium constants were measured with LexA binding to its target site buried within the nucleosome as previously described (8,14). TF binding and DNA unwrapping were detected by a reduction in FRET. LexA binding to its target site traps the nucleosome into a partially unwrapped state (8). FRET efficiency measurements were determined by the (ratio)A method (36). Fluorescence emission spectra were measured as previously described (14). We previously determined that non-specific DNA binding of LexA does not reduce the FRET efficiency and that binding of LexA to its target sequence within the nucleosome does not induce dissociation of H2A–H2B heterodimers (14).

Stopped flow nucleosome kinetics measurements

Stopped flow experiments were performed on a KinTek 2004-SF instrument at room temperature as previously described (10). Samples were excited by a XeHG arclamp with a 525 ± 22 nm excitation filter (Omega); simultaneous Cy3 and Cy5 emission was followed using a 570 ± 5 nm bandpass filter (Newport) and 680 ± 15 nm bandpass filter (Chroma), respectively. After rapid mixing, samples contained 7 nM Cy3/Cy5-labeled nucleosomes in 0.5× TE with 1, 75 or 130 mM NaCl and [LexA] varying from 0 to 50 μM. Data were smoothed by 10 point forward averaging and fit to a single exponential decay, except for unmodified nucleosomes in 0.5× TE, 1 mM NaCl, which was fit to a double exponential due to an additional slower process, which can be attributed to non-specific LexA binding (10).

Nucleosome competitive reconstitutions

Competitive reconstitutions were performed as previously described (37). Briefly, 0.6 μg of unmodified HO were combined with 0.5 μg high-affinity DNA and 2 μg low-affinity competitor DNA in 2 M NaCl, 0.5× TE, 1 mM benzamidine (BZA). Samples were placed in a 50-μl engineered dialysis chamber which was then placed in a reservoir with 2 M NaCl, 0.5× TE, 1 mM BZA. The reservoir [NaCl] was then slowly lowered to ~1 mM NaCl by pumping with 0.5× TE, 1 mM BZA over 36 h. Competitive reconstitutions were resolved by electrophoretic mobility gel shift analysis (EMSA) on a native 5% polyacrylamide gel and quantified as previously described (37).

Analysis of nucleosome position and TF-binding site databases

The fraction of TF-binding sites within the nucleosome dyad, entry exit and linker regions were determined with the reported consensus map of nucleosome positions (38) (http://refnucl.atlas.bx.psu.edu/) and TF-binding sites (39) (http://fruenkel.mit.edu/improved_map/) in S. cerevisiae. Nucleosomes with occupancy >10 and TF-binding sites with binding P-values <0.005 and no limitation on
conservation were used. For each TF-binding site we determined the number of bases between the nearest nucleosome dyad and the center of the TF-binding site rounded down to the nearest base. The random distribution simply treats every base pair in the yeast genome as the center of a potential TF-binding site while retaining the same consensus map of nucleosome positions and is therefore the probability density for the distance to the nearest nucleosome from any position. The number of sites exactly on top of a nucleosome dyad was doubled to account for the two possible ways to be any distance >0. Distributions of TF-binding sites relative to nucleosome positions were also determined with different criteria on nucleosome occupancy and TF-binding sites.

**hMSH2–hMSH6 nucleosome remodeling assay**

Nucleosome disassembly reactions were carried out at 37°C as previously described (17) with 0.25 nM of unmodified H3 or H3(K56ac) containing nucleosomes. The fraction of disassembled nucleosomes were analyzed by gel shifts on polyacrylamide gels as previously described (17) A small fraction of free DNA appears at the zero time point because we do not completely purify naked DNA away from the nucleosome and a fraction of nucleosomes fall apart during rapid mixing with hMSH2–hMSH6. We control for this by not including the zero time point in the exponential decay fit.

**RESULTS**

**H3(K56ac) enhances site accessibility by increasing the nucleosome unwrapping rate**

We previously reported that H3(K56ac) increases the site exposure equilibrium for LexA binding by 3-fold (14). To investigate the kinetic mechanism of H3(K56ac) on nucleosome site exposure, we used stopped flow fluorescence resonance energy transfer (FRET). FRET was used to detect the binding of a model TF, LexA, to its target sequence within the nucleosome as previously described (10). The nucleosomes contained the 147-bp high-affinity 601 NPS (40), with the LexA target sequence inserted between base pairs 8–27 (601L, Figure 1A). The NPS DNA was labeled with Cy3 at the 5′-end nearest the LexA target sequence, while Cy5 was linked to the HO part of the somatic 5S rDNA gene and has been used as a model NPS in numerous biophysical studies (43–45). This is a naturally occurring sequence containing the Xenopus borealis 5S rDNA positioning sequence (42). This is a naturally occurring sequence containing part of the somatic 5S rDNA gene and has been used as a model NPS in numerous biophysical studies (43–45).

As with the 601 sequence, we inserted the LexA target sequence between base pairs 8–27 of the 5S NPS (5SL, Figure 1A). We then reconstituted nucleosomes with the 5S rDNA labeled with Cy3 at the 5′-end nearest the LexA target sequence and HO labeled with Cy5 at H2A(K119C) (Figure 1B). H3(K56ac) and H3(K56Q) induced a slight shift in 5SL nucleosome electrophoretic mobility relative to unmodified nucleosomes (Figure 2A), as previously reported for the 601 NPS (14). To determine if the change in electrophoretic mobility was due to a position
change, we mapped the nucleosome positions on 5SL by hydroxyl radical mapping (35) using the FeBABE label as previously reported (14) and found that the H3(K56Q) did not impact the cleavage pattern (Supplementary Figure S2). Furthermore, the cleavage pattern with 5SL nucleosomes was indistinguishable from nucleosomes containing 601L (14). This indicates that the nucleosomes were well positioned within the 5S NPS and that nucleosome position was not altered by modifying H3(K56).

To determine the influence of H3(K56ac) on the SSL site exposure equilibrium, we carried out LexA titrations with unmodified, H3(K56ac) and H3(K56Q) nucleosomes containing the SSL sequence (Figure 2B–D). We determined the FRET efficiency of unmodified and modified nucleosomes at each LexA concentration in triplicate and then fit the FRET efficiency at increasing LexA concentrations to a non-cooperative binding isotherm. This analysis determines the LexA concentration at which half of the nucleosomes are bound by LexA, $S_{0.5\text{ nuc}}$. From this we use, $K_{\text{eq\_modified}}/K_{\text{eq\_unmodified}} = S_{0.5\text{ unmodified}}/S_{0.5\text{ modified}}$, to infer the relative nucleosome site exposure equilibrium (8, 14). The resulting increase in SSL site accessibility matches that observed for the 601L NPS [$K_{\text{eq\_SSL\_K56ac}}/K_{\text{eq\_SSL\_unmod}} = 1.8 \pm 0.3$ and $K_{\text{eq\_SSL\_K56Q}}/K_{\text{eq\_SSL\_unmod}} = 2.0 \pm 0.4$; Figure 2E and Table 1].

To verify that the increase in the site exposure equilibrium was due to unwrapping and not repositioning, we monitored LexA-induced nucleosome sliding by placing the Cy3 fluorophore on the 80th bp of the DNA
This results in juxtaposition of the Cy3–Cy5 FRET pair near the nucleosome dyad (Supplementary Figure S3B). Repositioning induced by LexA binding would result in a FRET change, while unwrapping will not. We do not observe any change in FRET, even at saturating concentrations of LexA (Supplementary Figure S3C). As an independent verification, we conducted hydroxyl radical mapping (35) using the FeBABE label of 5SL nucleosomes pre-incubated with saturating concentrations of LexA (1 mM). There was no observable difference between the cleavage of 5SL and 601L nucleosomes in the presence and absence of LexA (Supplementary Figure S2). The combination of the site exposure equilibrium measurements for TF binding within 5SL and 601L containing nucleosomes as well as the nucleosome position mapping strongly suggests that H3(K56ac) increases the nucleosome site exposure equilibrium independent of the underlying nucleosomal DNA sequence.

**DNA sequence within the nucleosome entry–exit region influences accessibility by modulating the DNA unwrapping rate**

We investigated the influence of DNA sequence changes within the 601 NPS on nucleosome unwrapping and rewrapping rates. First, we determined the LexA concentrations at which half of the nucleosomes are bound by LexA, $S_{0.5}$, for nucleosomes containing 5SL and 601L. We find at low ionic strength (0.5× TE with 1 mM NaCl) that $S_{0.5}$ of unmodified nucleosomes containing 5SL is increased relative to unmodified nucleosomes with 601L, indicating lower site exposure, $K_{eq}$ in the entry–exit region 

\[
\frac{K_{eq_{5SL-unmod}}}{K_{eq_{601L-unmod}}} = 0.6 \pm 0.1.
\]

Changing the DNA sequence from 601L to 5SL had a similar influence on the $K_{eq}$ for LexA binding within nucleosomes containing either H3(K56Q) 

\[
\frac{K_{eq_{5SL-K56Q}}}{K_{eq_{601L-K56Q}}} = 0.8 \pm 0.2
\]

or H3(K56ac) 

\[
\frac{K_{eq_{5SL-K56ac}}}{K_{eq_{601L-K56ac}}} = 0.7 \pm 0.1.
\]

We also investigated the influence of DNA sequence on $K_{eq}$ closer to physiological ionic strength. While we were unable to fully saturate LexA binding to nucleosomes containing 5SL in 130 mM NaCl, LexA binding to nucleosomes did saturate in 75 mM NaCl (Supplementary Figure S4B). We used these conditions (0.5× TE with 75 mM NaCl) to further examine the DNA sequence dependence of $K_{eq}$. Under these conditions, changing the NPS from 601L to 5SL significantly increased $S_{0.5}$, which implied that the site exposure equilibrium constant, $K_{eq}$, was reduced by ~3-fold 

\[
\frac{K_{eq_{5SL}}}{K_{eq_{601L}}} = 0.36 \pm 0.03.
\]
Table 1. Summary of the TF-binding equilibrium and nucleosome unwrapping measurements

<table>
<thead>
<tr>
<th>DNA</th>
<th>Histone</th>
<th>Na⁺ (mM)</th>
<th>$E_o$</th>
<th>$S_{0.5}$ (nM)</th>
<th>$K_{eq}$</th>
<th>$k_{eq}$</th>
<th>$k_{12}$ (s⁻¹)</th>
<th>$k_{11}$</th>
<th>$k_{21}$</th>
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</thead>
<tbody>
<tr>
<td>601L</td>
<td>unmod</td>
<td>1</td>
<td>0.68 ± 0.01</td>
<td>58 ± 6</td>
<td>–</td>
<td>7.8 ± 0.9</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>601L</td>
<td>H3(K56Q)</td>
<td>1</td>
<td>0.45 ± 0.01</td>
<td>32 ± 3</td>
<td>1.8 ± 0.4</td>
<td>15 ± 2</td>
<td>2.0 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>601L</td>
<td>H3(K56Ac)</td>
<td>1</td>
<td>0.43 ± 0.01</td>
<td>32 ± 3</td>
<td>1.8 ± 0.4</td>
<td>15 ± 1</td>
<td>1.9 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>601L</td>
<td>unmod</td>
<td>130</td>
<td>0.74 ± 0.01</td>
<td>13100 ± 500</td>
<td>74 ± 4</td>
<td>0 ± 10</td>
<td>7 ± 1</td>
<td>0.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>601L</td>
<td>H3(K56Q)</td>
<td>130</td>
<td>0.52 ± 0.01</td>
<td>5200 ± 500</td>
<td>2.5 ± 0.5</td>
<td>40 ± 10</td>
<td>3 ± 1</td>
<td>1.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>601L</td>
<td>H3(K56Ac)</td>
<td>130</td>
<td>0.50 ± 0.01</td>
<td>4000 ± 500</td>
<td>3.3 ± 0.7</td>
<td>50 ± 10</td>
<td>3 ± 1</td>
<td>0.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>5SL</td>
<td>unmod</td>
<td>1</td>
<td>0.40 ± 0.01</td>
<td>90 ± 10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5SL</td>
<td>H3(K56Q)</td>
<td>1</td>
<td>0.28 ± 0.01</td>
<td>42 ± 8</td>
<td>2.0 ± 0.5</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5SL</td>
<td>H3(K56Ac)</td>
<td>1</td>
<td>0.31 ± 0.01</td>
<td>48 ± 6</td>
<td>1.8 ± 0.3</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5SL</td>
<td>H3(C110)</td>
<td>1</td>
<td>0.42 ± 0.01</td>
<td>90 ± 10</td>
<td>1.0 ± 0.2</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>601L</td>
<td>unmod</td>
<td>75</td>
<td>0.67 ± 0.02</td>
<td>2500 ± 100</td>
<td>17 ± 3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5SL(1–147)</td>
<td>unmod</td>
<td>75</td>
<td>0.47 ± 0.02</td>
<td>6700 ± 300</td>
<td>0.36 ± 0.03</td>
<td>5.4 ± 0.7</td>
<td>0.33 ± 0.07</td>
<td>1.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>5SL(1–7)</td>
<td>unmod</td>
<td>75</td>
<td>0.48 ± 0.02</td>
<td>4600 ± 300</td>
<td>0.53 ± 0.05</td>
<td>7 ± 2</td>
<td>0.40 ± 0.1</td>
<td>1.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>5SL(28–47)</td>
<td>unmod</td>
<td>75</td>
<td>0.54 ± 0.02</td>
<td>2600 ± 200</td>
<td>0.96 ± 0.08</td>
<td>17 ± 3</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>5SL(1–47)</td>
<td>unmod</td>
<td>75</td>
<td>0.48 ± 0.02</td>
<td>5300 ± 400</td>
<td>0.46 ± 0.05</td>
<td>7 ± 1</td>
<td>0.40 ± 0.09</td>
<td>1.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>601L</td>
<td>H3(K56Q)</td>
<td>75</td>
<td>0.52 ± 0.01</td>
<td>870 ± 100</td>
<td>2.8 ± 0.4</td>
<td>50 ± 10</td>
<td>3.1 ± 0.8</td>
<td>0.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>5SL(1–7)</td>
<td>H3(K56Q)</td>
<td>75</td>
<td>0.45 ± 0.01</td>
<td>1400 ± 100</td>
<td>1.2 ± 0.2</td>
<td>20 ± 3</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

Column 1 is the type of the DNA construct. Column 2 is the type of histone octamer. Column 3 is the concentration of sodium. Column 4 is the average FRET efficiency without LexA. Column 5 is the half saturation concentration of LexA. Column 6 is the site exposure equilibrium relative to unmodified nucleosomes containing 601L. Column 7 is the nucleosome unwrapping rate relative to the unwrapping rate of unmodified nucleosomes containing 601L. Column 8 is the nucleosome rewrapping rate relative to unmodified nucleosomes containing 601L.

The observation that the X. borealis 5S NPS reduced the site exposure equilibrium relative to the 601 NPS was unexpected because the sea urchin 5S NPS has a significantly higher free energy for nucleosome formation (lower HO affinity) relative to the 601 positioning sequence (46). However, these results are consistent with the recent report that H2A-H2B heterodimers have a higher affinity to sea urchin 5S NPS than the 601 positioning sequence (47). To determine which region(s) of the DNA sequence is responsible for this 3-fold reduction in $K_{eq}$, we created three DNA chimeras where segments of 601L were replaced with segments from 5SL (Figure 3A). These chimeric DNAs were 5' labeled with Cy3 and reconstituted into nucleosomes with Cy5-labeled HO (Supplementary Figure S4A). We carried out LexA-binding analysis with nucleosomes containing the chimeric DNA sequences to determine the relative changes in $S_{0.5}$ to detect alterations in the site exposure equilibrium, $K_{eq}$ (Figure 3B and C; Supplementary Figure S4B). We found that most of the increase in $S_{0.5}$ and therefore reduction in $K_{eq}$ is induced by changing base pairs 1–7, located between the nucleosome entry–exit and the LexA target sequence. $K_{eq}$ increases from 0.46 ± 0.05 to 0.98 ± 0.08. Furthermore, the combined influence of changing base pairs 1–7 and 28–47 on $K_{eq}$ is similar to the influence of changing only base pairs 1–7 $K_{eq}$ increases from 0.46 ± 0.05 to 0.98 ± 0.08. This suggests that the DNA sequence located between the nucleosome entry–exit and the TF-binding site can significantly influence the site exposure equilibrium.

We performed stopped flow experiments with nucleosomes containing each of the 601L-SSL chimeric DNA to examine the mechanism by which DNA sequence modulates site accessibility (Figure 3D and Supplementary Figure S4C). The change in Cy5 fluorescence was measured at LexA concentrations between 10 and 30 μM where the rate of change of Cy5 fluorescence was independent of LexA concentration (Supplementary Figure S4D) and equal to the nucleosome unwrapping rate. We determined the nucleosome unwrapping rate, $k_{12}$, for each DNA chimera (Figure 3D and Table 1). Replacing base pairs 1–7 of 601L with 5SL significantly reduced the unwrapping rate $k_{12}$ of 601L with 5SL did not influence the unwrapping rate $k_{12}$ of 5SL. The combined change of base pairs 1–7 and 28–47 reduced the unwrapping rate $k_{12}$ of 601L. Importantly, the DNA sequence-induced changes in the unwrapping rates are nearly identical to the induced changes in site exposure equilibrium (Figure 3C and E), which implies that the changes in DNA sequence do not influence the calculated nucleosome rewrapping rates, $k_{21}$ (Figure 3F). Taken together, these results demonstrate that alterations in DNA sequence influence TF binding within nucleosomes by changing the DNA unwrapping rate without influencing the rewrapping rate.

Note that while the $K_{eq}$ measured by LexA binding increased for nucleosomes containing 5SL relative to 601L, the average FRET efficiency without LexA was lower for nucleosomes containing 5SL (0.47 ± 0.02) than 601L (0.67 ± 0.02). This contradictory result could be due to increased nucleosome unwrapping of the first few base pairs of 5SL. However, the average FRET efficiency is influenced by numerous parameters (36). In particular, the structure of a nucleosome containing the 601 positioning sequence was recently reported to contain 145 bp as compared to 147 bp for an alpha satellite DNA sequence (48). This could significantly alter the Cy3–Cy5 distance. In contrast, determining changes in the site exposure equilibrium from the $S_{0.5}$ measurements of LexA binding to
nucleosomes does not rely on the absolute average FRET efficiency. Furthermore, the agreement between the DNA sequence-induced changes in nucleosome unwrapping and the changes to the equilibrium of LexA binding to a nucleosome confirms the reliability of the LexA-binding measurements. Therefore, we used the $S_{0.5}$ measurements from LexA titrations to determine the relative change in site exposure of the LexA target sequence.

**DNA sequence and modification of H3(K56) additively regulate TF binding within nucleosomes**

Our observation that DNA sequence does not alter the influence of either H3(K56ac) or H3(K56Q) on nucleosome site exposure equilibrium suggests that the influence of DNA sequence is independent of the modification state of H3(K56). To test this hypothesis, we prepared Cy3–Cy5-labeled nucleosomes that contained the DNA chimera 5S(1–7) and the acetyl-lysine mimic H3(K56Q). We found that the influence of replacing the first 7 bp of 601L with 5SL did not alter the impact of H3(K56Q) on the LexA concentration to bind half of the nucleosomes, $S_{0.5}$, and therefore the site exposure equilibrium, $K_{eq}$ 

$$K_{eq} = \frac{K_{eq,5S(1–7)-K56Q}}{K_{eq,601L-K56Q}} = 0.36 \pm 0.06$$

$$K_{eq,5S(1–7)-unmod}/K_{eq,601L-unmod} = 0.36 \pm 0.03; \text{ Figure 3B and C}. \text{ We then determined that the influence of replacing the first 7 bp of 601L with 5SL on the unwrapping rate was unaffected by H3(K56Q) [}K_{12,5S(1–7)-K56Q}/K_{12,601L-K56Q} = 0.4 \pm 0.1 \text{ and } K_{12,5S(1–7)-unmod}/K_{12,601L-unmod} = 0.33 \pm 0.07; \text{ Figure 3D and E}]. \text{ These results are consistent with the conclusion that H3(K56) and the DNA sequence influence nucleosome unwrapping rate and ultimately TF binding independently. This implies that the DNA sequence and the modification of H3(K56) may additively combine to significantly influence TF binding. In fact, the unwrapping rate of nucleosomes modified at H3(K56) within the 601L NPS is 10±2 times larger than...
unmodified nucleosomes within the 5SL NPS. This difference enhances TF binding by 8 ± 2 times.

The 601 positioning sequence has a higher HO binding affinity but lower nucleosome unwrapping equilibrium than a 5S positioning sequence

The observation that substitution of the first 7 bp of the 601 NPS with the *X. borealis* 5S NPS reduces the site exposure equilibrium was unexpected. To further investigate this, we determined the difference in free energies for nucleosome formation between the 601-5S chimeras with competitive reconstitutions (49). This method determines the free energy difference, ΔΔG_{nuc}, for nucleosome formation between distinct DNA sequences. Nucleosomes are reconstituted with a fluorophore-labeled NPS in the presence of low-affinity competitor DNA and HO. The naked DNA and HO establish a dynamic equilibrium with nucleosomes during the reconstitution by gradual salt dialysis. The equilibrium constant, Keq, is then determined by an EMSA (Figure 4A) and the free energy for nucleosome formation relative to a reference DNA sequence is determined from:

$$\Delta G_{\text{nuc}} = \Delta G_{\text{nuc}}^{\text{ref}} - \Delta G_{\text{nuc}}^{\text{ref}} = -k_B T \ln(K_{\text{eq}}/K_{\text{eq,ref}})$$. We then compared this to the difference in free energy for the nucleosome to remain fully wrapped (Figure 4), which we determined from site exposure equilibrium measurements ($\Delta G_{\text{wrap}}^{\text{nuc}} = k_B T \ln(K_{\text{eq}}/K_{\text{eq,ref}})$).

We find that substitution of the first 7 bp of the *X. borealis* 5S sequence into the 601L sequence reduced the nucleosome formation free energy ($\Delta G_{\text{nuc,5S}}^{\text{601L}} - \Delta G_{\text{nuc,601L}}^{\text{601L}} = -0.5 ± 0.3\text{kcal/mol}$). This observation is in agreement with the reduced site exposure equilibrium induced by this chimeric NPS ($\Delta G_{\text{wrap,5S}}^{\text{601L}} - \Delta G_{\text{wrap,601L}}^{\text{601L}} = -0.35 ± 0.05\text{kcal/mol}$). The insertion of 5SL base pairs 28–47 into the 601L sequence increased the ΔG_{\text{nuc,5S}}^{\text{601L}} (ΔG_{\text{nuc,601L}}^{\text{601L}} = 0.3 ± 0.2kcal/mol), but did not impact the site exposure free energy ($\Delta G_{\text{wrap,5S}}^{\text{601L}} - \Delta G_{\text{wrap,601L}}^{\text{601L}} = -0.03 ± 0.05\text{kcal/mol}$). The insertion of both 5SL base pairs 1–7 and 28–47 into the 60L sequence displayed a free energy change equal to the sum of the free energy changes of the separate substitutions ($\Delta G_{\text{5SL,60L}}^{\text{1–7}} - \Delta G_{\text{5SL,60L}}^{\text{1,7}} = -0.2 ± 0.2\text{kcal/mol}$).

In contrast, the ΔΔG_{\text{wrap}} induced by changing both base pairs 1–7 and 28–47 ($\Delta G_{\text{wrap,5SL,60L}}^{\text{1–7}} - \Delta G_{\text{wrap,601L}}^{\text{601L}} = -0.43 ± 0.06\text{kcal/mol}$) and between 601L and 5SL(1–147) ($\Delta G_{\text{wrap,5SL,60L}}^{\text{1–147}} - \Delta G_{\text{wrap,601L}}^{\text{601L}} = -0.57 ± 0.03\text{kcal/mol}$) is similar to the free energy difference induced by base pairs 1–7 alone. These comparisons are consistent with the conclusion that the influence of base pairs 1–7, 28–47 and 48–147 on the nucleosome stabilityfree energy is additive but that only base pairs 1–7 influence the exposure of the LexA TF-binding site. Taken as a whole these results suggest that changes in the nucleosomal DNA sequence can separately tune TF-binding site exposure as well as overall nucleosome stability.

**TF-binding sites (30%) reside in the entry–exit regions of *S. cerevisiae* nucleosome positions**

Our observation that H3(K56ac) and DNA sequence have a combined influence on TF binding within nucleosomes

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**Figure 4.** Relation between DNA–histone binding and DNA unwrapping free energies. (A) Cy3 fluorescence image of native PAGE analysis of competitive reconstitutions performed in triplicate for unmodified HO with the DNA chimeras used for site exposure measures. (B) Change in free energy of nucleosome formation (ΔΔG_{nuc}, light gray) relative to 601L as determined by competitive reconstitution from the gel in (A), and the change in nucleosome free energy for wrapping (ΔΔG_{wrap}, dark gray) relative to 601L as determined from site accessibility measures (Figure 3B and C). The error bars for ΔΔG_{nuc} are the SD of the three independent measurements for each nucleosome type. (C) Table of the change in free energy values in kcal/mol for nucleosome formation of each DNA species relative to *Lumbriculus variegatus* 5S NPS (ΔΔG_{nuc,5S}), for nucleosome formation of each DNA species relative to 601L DNA (ΔΔG_{nuc,601L}) and for nucleosome wrapping for each DNA species relative to 601L DNA (ΔΔG_{wrap,601L}).
sustains that the regulation of DNA unwrapping could be a major regulator of TF occupancy. To investigate if TF-binding sites are poised for this regulatory mechanism in vivo, we determined the fraction of TF-binding sites that reside within S. cerevisiae nucleosome entry–exit regions. We examined the consensus maps of S. cerevisiae nucleosome occupancy (38) and the map of TF-binding sites (39) to determine the distance from the center of a TF-binding site to the dyad center of the nearest nucleosome (Figure 5A). The nucleosome dyad and entry–exit regions were defined to be 0–36 and 37–74 bp from the nucleosome dyad center, respectively. We found that 31 and 33% of TF-binding sites are within the dyad and entry–exit regions, respectively (Figure 5B).

Approximately 36% of TF-binding sites are located in linker DNA between nucleosomes. Variation in the criteria for TF and nucleosome occupancy modestly altered these results (Supplementary Figure S5). We compared the observed distance distribution of TF-binding sites from the nearest nucleosome dyad to the distance distribution of every position in the genome to the nearest nucleosome dyad (Figure 5A, black line). The expected fractions of TF-binding sites within the dyad, entry–exit and linker DNA regions for randomly positioned nucleosome are 36, 37 and 22%, respectively (Figure 5B). These observations suggest that while TF-binding sites appear somewhat biased toward being in linker DNA between nucleosomes (50–52), there is rather little suppression of TF-binding sites within the nucleosome; thus a significant fraction of TF-binding sites are located within nucleosome entry–exit regions and hence poised to be regulated through nucleosome unwrapping. Furthermore, these observations are consistent with the report that within S. cerevisiae the positions of TF-binding sites at gene promoters are correlated with the nucleosome entry–exit region for nucleosomes containing the histone variant H2A.Z (53).

**DISCUSSION**

Site exposure is an inherent property of the nucleosome that appears to provide transcription (8,12–15) and DNA
repair (16–18) complexes direct access to DNA. Here, we demonstrate that H3(K56ac) and DNA sequence influence TF occupancy by altering the nucleosome unwrapping rate, but not the rewrapping rate. We find that DNA sequence and H3(K56ac) additively influence the nucleosome unwrapping rate, which allows these two factors to function together to enhance or counteract their influence on TF occupancy (Figure 7). We observe 3-fold changes in TF occupancy induced by either H3(K56ac) or DNA sequence alone and a 10-fold effect in combination. These results, in combination with the observation that 30% of S. cerevisiae TF-binding sites are located within nucleosome entry–exit regions, suggests that the modulation of the nucleosome unwrapping rate could be a mechanism for regulating TF occupancy in vivo.

The observation that the H3(K56ac) and DNA sequence influence the nucleosome unwrapping rates but not the rewrapping rates suggests that they influence the free energy of the fully wrapped state while not affecting the free energies of the unwrapped and the transition states. This could be caused by a reduction in DNA–histone binding. The influence of H3(K56ac) removes a negative charge, which could disrupt water-mediated hydrogen bonding near K56 as is observed for H3(K56Q) (31). The first 7 bp of the 601 DNA sequence contain a GG dinucleotide that is in phase with the GC/GG dinucleotides, which are important for the strong positioning. However, this predicts that the first 7 bp of the 601 sequence should be more tightly wrapped than the 5S sequence, which is not what we observe. Instead, the DNA sequence may alter the precise DNA structure near the entry–exit region as is observed for nucleosomes containing the 601 and alpha satellite DNA molecules (48). This could alter direct and water-mediated hydrogen bonding in the entry–exit region and influence the free energy of the fully wrapped nucleosome state. In contrast, it appears the DNA bending involved in nucleosome rewrapping is not significantly different between the 601 and 5S sequences since the rewrapping rate is unaffected.

Our conclusion that the nucleosome rewrapping rate is not influenced by DNA sequence and H3(K56ac) relies on our observation that the relative changes in the rate of unwrapping and the LexA-binding equilibrium are identical. This observation implies that the combined influence of $k_{21}$, $k_{23}$ and $k_{32}$ (Figure 1C) does not alter the LexA-binding equilibrium to nucleosomes. If the rewrapping rate, $k_{21}$, were to change, $k_{23}$ and $k_{32}$ would need to change to exactly compensate. Instead, the simplest explanation is that these rates are not influenced by H3(K56ac) and DNA sequence. However, further studies that directly measure $k_{21}$, $k_{23}$ and $k_{32}$ are required to confirm this interpretation.

Our studies provide additional insight into the influence of DNA sequence on site exposure equilibrium. A previous study showed introduction of a polyA track placed at the first 16 bp within a 601-like sequence...
required to determine if additional histone PTMs in the nucleosome entry–exit regulate the DNA unwrapping rate to control protein binding within nucleosomes.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online: Supplementary Table 1 and Supplementary Figures 1–6.

ACKNOWLEDGEMENTS
The authors wish to thank Jonathan Widom and Karolin Luger for the *X. laevis* histone and LexA expression vectors and Karin Musier-Forsyth for access to a Typhoon Trio fluorescence scanner and a fluorescence plate reader.

FUNDING
American Heart Association Predoctoral Fellowship [0815460D to J.A.N.]; [10PRE3150036 to A.M.M.]; OSUCCC and the James Pelotonia Fellowship (to J.A.N.); National Institutes of Health (NIH) [CA067007 and GM080176 to R.F.]; [GM083055 to M.G.P. and J.J.O.]; Career Award in the Basic Biomedical Sciences from the Burroughs Wellcome Fund (to M.G.P.) and National Science Foundation [MCB0845695 to J.J.O.]; [DMR1105458 to R.B.]. Funding for open access charge: NIH [GM083055].

Conflict of interest statement. None declared.

REFERENCES

Figure 7. Kinetic model of H3(K56Ac) and DNA sequence modulation of nucleosome unwrapping rate. (A and B) Nucleosomes containing TF-binding sites at two different loci within the genome (nucleosome 1 with entry–exit in blue, nucleosome 2 with entry–exit in magenta). The DNA sequence between the entry–exit and TF-binding site influences the inherent nucleosome unwrapping rate to regulate TF binding within each nucleosome. Acetylation/de-acetylation of H3 lysine 56 at nucleosome 1 enhances/suppresses the DNA unwrapping rate to adjust TF occupancy. This influences both the TF occupancy within nucleosome 1 but also TF occupancy relative to nucleosome 2.

increased nucleosome site accessibility ~1.5-fold (22). Here we find that changing only the first 7 bases of the 601 DNA sequence to the *X. borealis* 5S sequence decreases the rate of DNA unwrapping by 2.5-fold. In contrast, the central 80 bp of the 601 NPS are largely responsible for the enhanced binding free energy relative to the 5S sequence (55). These results, in combination with ΔΔG measurements, indicate that while 601 was selected for optimal nucleosome stability (40), it is not optimized to suppress partial DNA unwrapping at the entry–exit region.

In vivo, nucleosomes are embedded within chromatin where higher order compaction could impact the influence of nucleosome unwrapping/rewrapping kinetics on TF occupancy. Recent studies of nucleosome unwrapping/rewrapping fluctuations indicate that higher order chromatin compaction does not significantly impact TF occupancy within the nucleosome but does impact occupancy in linker DNA (56,57). While this suggests that the influence of H3(K56ac) and DNA sequence on TF occupancy within the nucleosome will occur in the context of chromatin, additional studies are required to investigate the influence of chromatin higher order structure.

Other histone PTMs in the nucleosome entry–exit region could function like H3(K56ac) to enhance site accessibility by altering the nucleosome unwrapping rate. H4(K77ac) and H4(K79ac), which are located at the DNA–histone interface 40–45 bp within the nucleosome, enhanced TF binding 2-fold to their site between base pairs 8–27 within the nucleosome (15). Furthermore, histone H3 PTMs at P38 (58), Y41 (59) and T45 (60,61) reside at the nucleosome entry–exit. Further studies are


