Published in final edited form as:

ACS Chem Biol. 2019 March 15; 14(3): 506–515. doi:10.1021/acscchembio.9b00018.

How protein binding sensitizes the nucleosome to histone H3K56 acetylation

Jaehyoun Lee, Tae-Hee Lee*

Department of Chemistry, the Pennsylvania State University, University Park, PA 16802

Abstract

The nucleosome, the fundamental gene-packing unit comprising an octameric histone protein core wrapped around by DNA, has a flexible structure that enables dynamic gene regulation mechanisms. Histone lysine acetylation at H3K56 removes a positive charge from the histone core where it interacts with the termini of the nucleosomal DNA and acts as a critical gene regulatory signal that is implicated in transcription initiation and elongation. The predominant proposal on the biophysical role of H3K56 acetylation (H3K56ac) is that weakened electrostatic interaction between DNA termini and the histone core results in facilitated opening and subsequent disassembly of the nucleosome. However, this effect alone is too weak to account for the strong coupling between H3K56ac and its regulatory outcomes. Here we utilized a semi-synthetically modified nucleosome with H3K56ac in order to address this discrepancy. Based on the results, we propose an innovative mechanism by which the charge neutralization effect of H3K56ac is significantly amplified via protein binding. We employed three-color single-molecule fluorescence resonance energy transfer (smFRET) to monitor the opening rate of nucleosomal DNA termini induced by binding of histone chaperone Nap1. We observed an elevated opening rate upon H3K56ac by 5.9 folds which is far larger than 1.5 folds as previously reported for the spontaneous opening dynamics in the absence of Nap1. Our proposed mechanism successfully reconciles this discrepancy based on that DNA opening for Nap1 binding must be larger than the average spontaneous opening. This is a novel mechanism that can explain how a small biophysical effect of histone acetylation results in a significant change in protein binding rate.

Introduction

The nucleosome is the fundamental gene-packing unit of eukaryotes. A nucleosome core particle is composed of ~147 base-pair (bp) double-stranded (ds-) DNA wrapping around an octameric histone protein core made of two copies of H2A, H2B, H3 and H4.^{1,2} A major driving force of gene packing in the nucleosome is the strong electrostatic interactions between DNA and the histone core that contains >20 % arginine and lysine residues. Nucleosomes impose physical barriers to DNA binding proteins and therefore directly regulate gene access at a molecular level. Nucleosomes have a flexible structure enabling dynamic gene regulation mechanisms that often involve post-translational histone modifications.^{3–14} Among various histone modifications, lysine acetylation is typically associated with gene activation and active transcription.^{14,15} In particular, H3K56 interacts

*Correspondence to tx118@psu.edu.

with the termini of the nucleosomal DNA and its acetylation has been critically implicated in transcription initiation and elongation.^{13,14,16} Errors in histone acetylation often result in fatal diseases such as cancers although the molecular mechanism of how histone acetylation participates in gene regulation remains elusive.^{17–20} Two dominant hypotheses are that histone acetylation removes a positive charge from the histone surface, and therefore, facilitates nucleosome disassembly and that acetylated histone recruits enzymes for cascading reactions. These proteins and protein complexes typically contain a bromodomain for acetyl-lysine binding.^{21–23} However, the reported electrostatic effects are rather small and the binding between a bromodomain-containing protein complex and acetyl-lysine is weak with a dissociation constant of $\mu\text{M} \sim \text{mM}$.^{3–6,21}

A series of results have been published on how histone acetylation at H3K56 facilitates DNA opening motion in the nucleosome termini.^{3–5} According to the results, upon H3K56 acetylation (H3K56ac) a 1.9-fold increase in the spontaneous opening rate was observed at 50 mM NaCl.^{3,24} This result suggests that we would observe a 1.9-fold increase in the rate of protein binding at the histone-DNA interface where histone H2A-H2B dimer also interacts with the DNA. Nucleosome assembly protein 1 (Nap1) is an excellent protein to test this hypothesis because its interaction with histone H2A-H2B dimer is mainly electrostatic, it does not interfere with H2A-H2B when the dimer is completely wrapped in the nucleosome, and it does not depend on chemical energy for its function (e.g. ATP hydrolysis).^{25,26} Only when the interaction between H2A-H2B and DNA is weakened and a gap is open, Nap1 would bind the dimer.²⁶ Upon binding, DNA would unwrap wider to form a stable open state that lasts for a few seconds or longer.⁵ Here we utilized this system in addition to a semi-synthetically modified nucleosome with H3K56ac in order to examine how H3K56ac affects the Nap1-induced DNA opening rate and how the result compares with the change estimated from the spontaneous opening dynamics. Our result indicates that H3K56ac increases the rate by 5.9 folds which is far larger than the estimated change. This discrepancy can be successfully reconciled based on that DNA opening for Nap1 binding must be larger than the average spontaneous opening. According to the mechanism, a small charge neutralization effect induced upon lysine acetylation can be significantly amplified by protein binding, which would eventually lead to tighter gene regulation. Furthermore, this mechanism can synergistically integrate the apparently orthogonal biophysical and biochemical effects of histone acetylation.

Methods

DNA preparation

A nucleosomal DNA construct labeled with Cy3 and Cy5.5 was prepared by ligating oligonucleotides as previously described.²⁷ Briefly, five DNA fragments in 10 mM Tris-HCl (pH 7.5) were annealed following a standard protocol (temperature decreased from 95 °C to 5 °C for 45 minutes). The construct is derived from the 147 bp Widom 601 sequence²⁸ with a 20 nucleotides linker that contains biotin at one end (see figure S1 for the sequence). The fragment labeled with Cy3 was purchased from Integrated DNA Technologies (Coralville, IA). NHS ester functionalized Cy5.5 fluorophore (GE Healthcare Life Sciences, Pittsburgh, PA) was used to label a fragment containing a Uni-link amino modifier with a six-carbon

spacer (Integrated DNA Technologies, Coralville, IA). The annealed construct was cleaned up with a PCR purification kit (Qiagen, Valencia, CA), and ligated with T4 ligase (New England BioLabs, Ipswich, MA) at 16 °C for 16 h. The ligated construct was purified on a 2 % agarose gel. Complete ligation was verified with 10 % denaturing PAGE with 6 M (Fig. S1).

Nap1 and histone preparation

6xHis-Yeast nucleosome assembly protein 1 (Nap1) was expressed in *E. coli* and purified with Ni-NTA beads (Thermo Fisher Scientific, Waltham, MA) as reported elsewhere²⁹. *Xenopus laevis* histones H2A, H2B T112C, H3, H3K56C/C110A and H4 were prepared as reported elsewhere³⁰. Briefly, each histone expression is induced by IPTG and the inclusion body is collected and dissolved in the unfolding buffer (20 mM Tris-HCl at pH 7.4, 10 mM DTT, and 7 M Guanidine HCl). Unfolded histone was subsequently dialyzed against the Urea dialysis buffer (10 mM Tris-HCl at pH 8, 1 mM EDTA, 100 mM NaCl, and 7 M urea). Dialyzed histones are collected through a size exclusion column (GE Healthcare Bioscience, Sephacryl S-200) followed by another filtration through an ion exchange column (Tosoh Bioscience, TSKgel SP-5PW). Purified histones are lyophilized and stored at -20 °C. Chemical modifications to histones (e.g. fluorophore labeling and lysine acetylation) can be performed at this step. Equimolar H2A and H2B and separately H3 and H4 are combined in the unfolding buffer and refold against the refolding buffer (10 mM Tris-HCl at pH 7.5, 2 M NaCl, 1 mM EDTA, and 5 mM BME). The refolding reaction product is filtered through the Sephacryl S-200 (GE Healthcare Bioscience) size exclusion column to collect H2A-H2B dimer or (H3-H4)₂ tetramer. Histone H2B T112C²⁵ was dissolved in the unfolding buffer and labeled with Atto647N-maleimide (Sigma-Aldrich, St. Louis, MO) following a standard protocol. Radical-assisted *N*-vinyl-acetamide (NVA) coupling to the cysteine residue of H3K56C/C110A was carried out as reported earlier^{31,32}, which resulted in an acetylated lysine mimic H3K56_{ac}. Briefly, H3 K56C/C110A mutant at 1 mM in 200 μL total volume containing 0.2 M sodium-acetate (pH 4), 6 M guanidine-HCl, 7 mM L-glutathione, 50 mM *N*-vinyl-acetamide, 100 mM dimethyl sulfide, and 5 mM VA-044 (2,2'-[Azobis(dimethylmethyle)]bis(2-imidazoline)dihydrochloride) was incubated for 2 hours at 70 °C. The reaction mixture was dialyzed against deionized water and then lyophilized overnight for storage at -80°C. We will refer to this H3K56 acetylation mimic as H3K56_{ac}. Mass-spectrometric analyses of the acetylated histones confirmed acetylation (Fig. S2).

Nucleosome reconstitution

Nucleosomes were reconstituted by incubating DNA, H2A-H2B dimer and (H3-H4)₂ tetramer at 2.0 M NaCl followed by 5-step dialyses against 850 mM, 650 mM, 250 mM, 100 mM and 2.5 mM NaCl in a TE buffer (10 mM Tris-HCl at pH 7.5 and 0.5 mM EDTA) for 1 hour in each step. The fluorophore labeling efficiency of H2A-H2B was adjusted to 50 % by mixing labeled and unlabeled species. The efficiency was confirmed with UV-vis absorbance at 650 nm and 280 nm.

FRET measurements

Quartz microscope slides were functionalized with silane and biotin. Slides were coated with 5k Mw biotin-PEG-silane (Laysan Bio, Inc., Arab, AL) as previously published³³, and then

incubated with 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC, Avanti Polar Lipids, Alabaster, AL) to form a lipid bilayer following the manufacturer's protocol. Nucleosomes were immobilized via biotin-streptavidin conjugation. Nap1 at 600 nM was injected to the nucleosomes in 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, and 3 % glycerol in the presence of protococatechuate dioxygenase (0.02 unit/ μ l, Sigma-Aldrich), protocatechuic acid (1 mM, Sigma-Aldrich), and Trolox (1 mM, Sigma-Aldrich) for elevated photo-stability and prolonged photobleaching lifetime.

We employed a prism-coupled total internal reflection (TIR) fluorescence microscope (Fig. S3, home-built based on TE2000 from Nikon, Japan), where the FRET donor Cy3 was excited with a 532 nm laser (GCL-150-L, CrystaLaser, Reno NV). Fluorescence signals from the three fluorophores (Cy3, Atto637N and Cy5.5) were collected through a water-immersion objective (CFI Plan Apochromat, 60x/1.20, Nikon, Japan), and spectrally split into three regions with two dichroic mirrors (Chroma Technology, Bellows Falls, VT) with a cutoff wavelength of 620 nm to separate Cy3 emission from the other two, and of 690 nm to separate Atto647N emission from Cy5.5 emission. Emission filters, HQ590/70m (Chroma Technology) and HQ655LP (Chroma Technology) for Cy3 and Atto647N/Cy5.5 channels respectively, were employed to remove the laser emission and to reduce inter-channel leaks. Fluorescence signals from spatially resolved nucleosome particles on the surface in a $35 \times 100 \mu\text{m}^2$ area were integrated for 250 ms and recorded continuously with an electron multiplying CCD (EMCCD) camera (iXon+897, Andor Technology, Belfast, Ireland) with no time gap between two consecutive movie frames.

FRET data analysis

Time trajectories of Cy3, Atto647N and Cy5.5 fluorescence intensities were computed from the EMCCD movies taken as described in the previous section. Inter-channel leaks in the fluorescence intensities were corrected. With the leak parameters, α , β , γ , and δ that were measured independently and the leak-corrected intensities, I_3 , I_{647N} , and $I_{5.5}$ for Cy3, Atto647N, and Cy5.5, respectively, were computed by solving the following system of linear equations.^{5,27}

$$\begin{aligned} I_3' &= (1 - \alpha - \delta)I_3 \\ I_{647N}' &= \alpha I_3 + (1 - \beta)I_{647N} + \gamma I_{5.5} \\ I_{5.5}' &= \delta I_3 + \beta I_{647N} + (1 - \gamma)I_{5.5} \end{aligned}$$

, where α , β , γ , and δ are leak ratios for I_3 to Atto647N channel, I_{647N} to Cy5.5 channel, $I_{5.5}$ to Atto647N channel, and I_3 to Cy5.5 channel, respectively. I_3' , I_{647N}' , and $I_{5.5}'$ are the fluorescence intensities that are not leak-corrected. As previously reported and briefly depicted in figure 1, we inspected the three fluorescence intensities to categorize the nucleosome showing (1) DNA unwrapping before dimer disruption and (2) dimer disruption before DNA unwrapping. We selected group (1) nucleosomes for further analysis on the initial DNA opening dynamics with a hidden Markov (HMM) model.

HMM analysis

FRET_{5,5} was computed with a formula $I_{5,5} / (I_3 + I_{5,5})$ for hidden Markov model (HMM) analysis in order to extract the kinetics of DNA unwrapping as described previously³⁴. A four-state model (HF, MF, LF1 and LF0 for high-, mid-, low1- and low0-FRET) was used because it is the minimum number of states that properly modeled the data according to our visual inspection and well supported by the FRET transition histograms shown in figure 2A. The high-FRET (HF) state is the intact nucleosomal state and the mid-FRET (MF) state is the first intermediate state with the DNA termini partially unwrapped. The kinetic rate constant from HF to MF (Fig. 2B, Table S1) was obtained from 3 sets of data (each $n > 30$). The standard deviation was taken as the error of the HMM analysis.

Results

Nap1 binding accompanies nucleosome termini opening

We monitored the DNA unwrapping and histone H2A-H2B disruption dynamics simultaneously with three-color single-molecule FRET (smFRET) after injecting 600 nM Nap1 (Fig. 1). We followed the time trajectories of the fluorescence intensities of Cy3, Atto647N and Cy5.5 and selected the nucleosomes that show a sign of DNA unwrapping (upper path in Fig. 1A) prior to H2A-H2B dimer disruption (lower path in Fig. 1A). We previously reported that the initial steps of nucleosome disassembly are heterogeneous and that approximately 40 % of nucleosomes show DNA unwrapping prior to dimer disruption.⁵ We carried out further analysis on this 40 % of the population. The initial DNA unwrapping forms an open state accompanying a high-FRET_{5,5} to mid-FRET_{5,5} transition regardless of the Atto647N fluorescence level (Fig. 2).⁵ As no FRET transition is observed in the absence of Nap1, this transition must represent the initial DNA unwrapping due to Nap1 binding. A crystal structure of a Nap1-H2A-H2B complex reveals that Nap1 interacts with both H2A and H2B where they interact with DNA.³⁵ Recent cryo-EM structures of nucleosomes with an unwrapped DNA terminus shows that large-scale DNA unwrapping can happen spontaneously that will allow for Nap1 to compete against DNA and bind H2A-H2B.³⁶ We also identified two other FRET_{5,5} states (LF1 and LF0 in figure 2) that convolve further DNA unwrapping and dimer motion. We analyzed the transition rates among all these four FRET_{5,5} states based on hidden Markov modeling and assigned the rate of the high-FRET to mid-FRET transition to the rate of the initial DNA unwrapping (Fig. 2 and Table S1). We will refer to this initial DNA unwrapping as Nap1-binding induced nucleosome opening or Nap1-induced opening and the rate constant as $k_{binding}$ (Fig. 2B).

Nap1-induced nucleosome opening rate is increased upon H3K56 acetylation

We compared the rates of the initial DNA opening with and without acetylation at H3K56 (Fig. 2B). This acetylation is coupled to transcription initiation and elongation.¹⁴ The result indicates that H3K56ac increases the Nap1-induced nucleosome-opening rate ($= k_{binding}$) by 5.9 ± 1.8 folds. An increased opening rate supports the hypothesis that H3K56ac facilitates transcription factor binding and transcription elongation through the nucleosome. It has been reported that H3K56ac does not affect the binding constant of Nap1 to (H3-H4)₂.²⁶ Therefore, the increased opening rate is not due to direct recognition of the acetyl group by Nap1. It has been published that the spontaneous DNA opening rate on a millisecond-

timescale increases upon H3K56 acetylation.^{3,24} In the published results, the opening rates in the unacetylated and acetylated cases are 0.28 ± 0.02 /ms and 0.15 ± 0.02 /ms, respectively, and the closing rates are 0.36 ± 0.02 /ms and 0.35 ± 0.04 /ms, respectively. The 1.9 ± 0.3 -fold increase in the opening rate is far less than the 5.9-fold increase in k_{binding} . Given that the spontaneous motion is at a millisecond-timescale and the Nap1-induced opening is at a second-timescale, the change in the total dwell time of the open state (1.5 ± 0.2 -fold increase) is more relevant to the change in k_{binding} . This value represents the ratio between the probabilities of finding the nucleosome in an open conformation at any random moment. Assuming that Nap1 can bind only in an open conformation, this value should represent the change in k_{binding} . Therefore, we will compare the 5.9-fold increase in k_{binding} with the 1.5-fold change in the spontaneous opening dynamics for the following analyses.

Modeling open and closed states of the nucleosome

In order to account for this large discrepancy, we propose a mechanism by which Nap1 binding amplifies the small effect of H3K56ac on the spontaneous opening dynamics. To formulate the mechanism, we first need to model the open and closed states of the nucleosome. The size of the spontaneously open gap is determined mainly by two conflicting forces. The first is the electrostatic attraction between the histone core and DNA that can be modeled with a Coulomb potential. The second is the bending potential of DNA that can be modeled as in Eq. S1 (supplemental information) according to a worm-like chain (WLC) model.^{37–39} Subsequently, the total potential energy of the DNA termini in a nucleosome as a function of the opening gap or simply opening, r (see Fig. 3), is given in Eq. S1.

In Eq. S1, DNA bending angle at each base-step is set to be constant. The distribution function of the average distance between DNA and histone, r , would follow a standard Boltzmann distribution function. (Eq. 1)

$$p(r) = A \cdot \exp \left[- \left(- \frac{nm}{4\pi\epsilon_r\epsilon_0} \cdot \frac{1}{r} + \kappa(r - r_0)^2 \right) / k_B T \right] \quad (\text{Eq. 1})$$

, where A is a normalization constant, k_B is the Boltzmann constant and T is the measurement temperature (= 298 K).

The electrostatic attraction between the charges on DNA and histone is computed as a sum Coulomb potential between two sets of point charges. A histone H3 α N helix where K56 is located contains total 4 positive charges at R49, R52, R53 and K56. Therefore, we set $m = 4$. Assuming that only the immediately proximal region of DNA would interact with the histone H3 α N helix, n is set to be 6 (Fig. 3). We approximated that the distances between the DNA negative charges and H3 R49, R52, R53 and K56 vary within a small range so that $\langle \frac{1}{r} \rangle \approx \frac{1}{\langle r \rangle}$, which is a reasonable approximation according to the structure shown in figure 3.

The distance between the fluorophore labeling locations in the closed state is 3.0 nm according to a crystal structure (between the green and orange residues in Fig. 3A, PDB ID: 1kx5). The FRET value reported in the closed state is 0.77 ± 0.02 , corresponding to 4.4 ± 0.1 nm (FRET radius $R_0 = 5.4$ nm between Cy3 and Cy5).³ This discrepancy is common due to tight packing in a crystal and highly non-physiological buffer conditions for crystallization. This difference of 1.4 ± 0.1 nm should then be added to the DNA-histone gap estimated from the same crystal structure. The average distance r between H3 α N helix and the DNA charges measured among the colored charge pairs (red and blue) in figure 3 is 1.2 nm, resulting in the estimated open gap in the closed state, $r_{closed} = 2.6 \pm 0.1$ nm. The open state FRET efficiency is 0.61 ± 0.09 , which converts to 5.0 ± 0.3 nm.³ These results lead to a difference of 0.6 ± 0.3 nm in the opening between the closed and open states. Therefore, r in the open state, or $r_{open} = 3.2 \pm 0.3$ nm. This result is in very good agreement with a recently published open conformation of the nucleosome monitored with cryo-EM.³⁶ The estimated distance between the H3 α N helix and the DNA terminus in the reported model (PDB ID: 6esh) is 3.30 nm as illustrated in Fig. 3B which agrees very well with the r_{open} value of 3.2 ± 0.3 nm.

The estimated opening between DNA and histone is 2.6 nm in the closed state which is not sufficiently large to have the permittivity of bulk water. The relative permittivity of a water layer of this thickness can be 20~60 depending on how rigidly the water molecules are bound.^{40,41} When DNA unwraps, the opening becomes larger at 3.2 nm. The fact that the closed and open conformations are observed at a millisecond time resolution strongly suggests that they belong to two states separated by an energy barrier. Otherwise, the two conformations should be two points on a single potential energy surface that would not result in observably stable states at a millisecond resolution. For this size of a complex (10 – 1000 kDa), <1 nm thermal motions within $\sim 3k_B T$ potential variation should be faster by orders of magnitude. This analysis led us to a conclusion that the transition barrier between the closed and open states is likely due to a large amount of solvent flux in and out of the opening gap, which should lead to transitions in the permittivity of the water layer. The fact that we observed only one stable open state leads us to another conclusion that the open state permittivity would not increase any more by opening the gap further. At this range of opening, the bending potential dominates the total energy. Consequently, the nucleosome structure favors further opening if the electrostatic attraction between DNA and histone becomes weaker as a result. Therefore, we set the permittivity in the open state to be 77 that is the bulk water permittivity at 50 mM NaCl and 298 K.⁴²

DNA bending energy was computed according to the model using the following equation,

$$E_{bending} = k_B T \frac{a}{2l_{total}} \theta_{total}^2 \approx \kappa (r - r_0)^2, \text{ where } a, \text{ the persistence length of DNA is 48 nm, } l_{total},$$

the sum of the bending segment lengths is 6 bp or 2.04 nm (Fig. 3, from the hinge to the half way of the DNA exit region).^{37,38} With the above conditions, we iteratively adjusted the relative permittivity (ϵ_r) of the closed state, spring constant (κ) and r_0 so that the κ value satisfies the above equation at the maximum population point in the closed state and that the maximum points in the r distributions in Eq. 1 for the open and closed states match the measured r_{open} and r_{closed} . With $r_{closed} = 2.6$ nm and $r_{open} = 3.2$ nm, a range of 33 to 37 for ϵ_r of the closed state, a range of 12.5 to 15.0 pN/nm for κ and a range of 3.42 to 3.50 nm for

r_0 were obtained. These r_0 values result in the θ_{total} value of 0.40 – 44 radians or 24° – 26° total bending in the closed state (see figure 3 for the structural model). Within the full range of $r_{closed} = 2.6 \pm 0.1$ nm and $r_{open} = 3.2 \pm 0.3$ nm, we were able to find a set of solutions with a spring constant as low as 9.0 pN/nm with $r_{closed} = 2.7$ nm and $r_{open} = 3.4$ nm, resulting in 3.4 – 3.5 nm for r_0 and 40 – 41 for ϵ_r , and as high as 44 pN/nm with $r_{closed} = 2.5$ nm and $r_{open} = 2.8$ nm, resulting in 3.0 nm for r_0 and 21 – 24 for ϵ_r . In summary, 9.0 – 44 pN/nm for κ leads to ϵ_r and r_0 values that match our experimental observations according to the model in Eqs. 1 and S1. For convenience, we will use the parameters found with the average $r_{closed} = 2.6$ nm and $r_{open} = 3.2$ nm for further analyses. The population distributions of the nucleosome as a function of r in the closed and open states are plotted in figure 4A.

H3K56ac shifts the opening gap distribution in both closed and open states

Based on the values optimized as described above, the opening gap distributions in the closed and open states in the H3K56 acetylated case were computed with m decreased by 1 in Eq. 1 (i.e. $m = 4 - 1 = 3$). The distributions are shown in figure 4B. The distributions of the open state in the unacetylated (black) and acetylated (blue) cases are compared side by side in figure 4B. They are normalized to represent their population densities relative to each other (i.e. 1.5-fold larger area under the blue curve than under the black curve). Therefore, the ratio between the two integrated areas is the ratio between the probabilities of finding an open conformation at any random moment. This ratio should directly represent the ratio between the binding rates of a protein that can bind only to an open conformation. As the ratio is 1.5 ± 0.2 , one can anticipate that the binding rate in the acetylated case would increase by 1.5 ± 0.2 folds.

Nap1 binding amplifies the change induced by H3K56ac

The Nap1-induced DNA opening rate increases by 5.9 ± 1.8 folds upon H3K56 acetylation that is far more than what we anticipated from the above analysis. Moreover, the timescale of Nap1 binding is 4~5 orders of magnitude slower than that of spontaneous DNA opening at a few ms timescale. In order to reconcile these discrepancies, we assume that Nap1 binding requires opening of the DNA-histone interface larger than the average spontaneous opening. This is a reasonable assumption given that the size of Nap1 (48 kDa, PDB ID: 2AYU) is large and its acidic surface in the stable dimer format is buried in a pocket.⁴³ The maximum rate of Nap1 binding attempts should then be the spontaneous opening rate multiplied by the population fraction that has an opening larger than the required opening (i.e. shaded areas in figure 4B). Assuming a constant success rate of binding, the ratio between the two shaded areas in figure 4B is subsequently the ratio of Nap1 binding rate between the unacetylated and acetylated cases. The ratio becomes 5.9 at 4.25 ± 0.05 nm opening (note: the uncertainty is given by the resolution of estimation, which is 0.05 nm) that is 1.05 nm further opening from the average r_{open} in the unacetylated case. The rate of opening that reaches 4.25 nm or longer in the unacetylated and acetylated cases are 1.1 and 5.8 /s, respectively, which are the maximum attempt rates of Nap1 binding. Comparing these values to the observed rates, we obtain 55 and 51 times of spontaneous opening events before one successful binding event in the unacetylated and acetylated cases, respectively. These values correspond to 1.8 and 1.9 % of success rates for binding. The similarity between these two values further supports our mechanism by confirming that the strength of

Nap1 binding to H2A-H2B does not depend on H3K56 acetylation. In conclusion, a simple and reasonable assumption that Nap1 binding would require a larger opening than the average spontaneous opening results in a novel mechanism by which protein binding significantly amplifies the charge neutralization effect of histone acetylation (figure 5). The effect becomes stronger with a larger protein requiring a larger opening as shown in figure 4B (see the increasing “n-fold increase” as a function of opening).

Discussions

Histone lysine acetylation neutralizes a positive charge and consequently weakens the interaction between DNA and histone. This biophysical effect should contribute to facilitating nucleosome disassembly during transcription initiation and elongation. However, this mechanism is based on a weak effect and therefore cannot account for the reported strong outcomes of acetylation.^{13,14,16} In our observation, the 5.9-fold increase upon H3K56 acetylation in the rate of Nap1-induced DNA opening is far larger than the mere 1.5-fold change in the spontaneous opening dynamics. In order to reconcile this discrepancy, we propose that cascading biochemical reactions upon histone acetylation are triggered by a protein whose binding requires a large DNA opening that is rare during spontaneous nucleosomal motions. By requiring these rare events of opening, the charge neutralization effect of histone acetylation would be much amplified. This is because large-opening events make up the tailing edge of a Gaussian distribution whose slight shift in the center would make a big impact on the chance of accessing these rare events (Figs. 4B and 5). Histone acetylation at H3K56 removes a positive charge, which weakens the interaction between the histone core and DNA near the termini, shifting the distribution of the termini opening to a slightly longer distance. The shift is toward 0.4 nm further opening according to our measurements and analyses. This slight change, however, can result in a big difference in the population density with a large opening. For instance, the rate of DNA opening by 1.05 nm further from the average opening is 1.1 /s in the unacetylated case while it increases to 5.8 /s in the acetylated case (Fig. 5). This >5-fold difference is much more significant than the mere 1.5-fold change in the spontaneous opening dynamics. These opening rates combined with the binding success rates should yield the binding rates. Subsequently, in cases where protein binding is non-specific (e.g. Nap1) and therefore the binding rate remains constant upon acetylation, the change in the opening rates should directly represent the change in the binding rate. Our analysis indicates that the success rates of Nap1 binding in the unacetylated and acetylated cases are 1.8 and 1.9 %, respectively, further validating our mechanism. If the protein contains a bromodomain, the change would be even more amplified by a higher success rate of binding upon acetylation. This is up to our knowledge the first mechanism that can synergistically integrate the biophysical and biochemical roles of histone acetylation in gene regulation. In either case, our mechanism significantly amplifies the charge neutralization effect of histone acetylation via enzyme or factor binding. DNA-binding proteins often contain many lysine residues that can be acetylated. Acetylation must weaken the interaction between these proteins and DNA, whose effect can also be amplified by the proposed mechanism.

Although the accurate success rates of binding and the precise distance changes of the nucleosome dynamics are not the foci of this work, it is important to test the robustness of

our conclusions against variations in the key parameters. We varied the key parameters in order to examine if there is any circumstance where the uncertainty in the result becomes so large that our mechanism cannot be validated. A large uncertainty in our analysis may originate from the estimated number of negative charges on DNA (m) that effectively interact with the positive charges on the histone H3 α N helix. Accurate estimation of n is not feasible because the distributions of the ions within this region under our buffer conditions are unknown. Obtaining numerical solutions from exact modeling of the nucleosome structure and the buffer environment with all the ions and water molecules may be feasible, but it is unlikely that such modeling would result in a single solution with the global minimum energy with one fixed ionic environment. We estimated $n = 6$. This is our estimated maximum number of DNA charges based on a crystal structure (PDB ID 1kx5, Fig. 3) that can be in direct contact with the histone charges. We also tested $n = 4 - 12$ and found that the overall trend of the result and the conclusions are not affected although the relative permittivity value changes within 27 – 59 and subsequently the opening distance vary slightly within a $\pm 0.1 - \pm 0.2$ nm range.

Another source of uncertainty is the bulk water permittivity ($= 77$) assumed for the open conformation.⁴² Accurate estimation of this value is not feasible because it is unknown how rigidly the water molecules are bound at the DNA-histone interface in the open conformation under our experimental conditions. Our rationale based on only one stable open state should be reasonable in drawing that the open state permittivity would not increase any more by opening the gap further. If it would, the nucleosome would favor a further open conformation to become more stable by relieving the bending stress because the total energy is dominated by the bending potential at this opening range (see the near Gaussian distributions in figure 4B). We also tested this value down to 37 and found that the overall trend of the results is not affected although the closed state permittivity need adjusted accordingly. Subsequently the opening distance varies slightly but not more than by ± 0.2 nm.

We estimated a spring constant for bending that is derived from a standard DNA bending model based on a WLC model^{37,38} resulting in

$$E_{bending} = k_B T \sum_i^N \frac{a}{2l} \theta^2 = k_B T \frac{a}{2l_{total}} \theta_{total}^2 = k_B T \frac{a}{2l_{total}} \arcsin^2 \left(\frac{r - r_0}{l_{total}} \right) \approx \kappa (r - r_0)^2, \text{ where } a \text{ is the}$$

persistence length of double stranded DNA and l is the bending segment length. We used $l_{total} = 6$ bp or 2.04 nm which is the distance between the hinge and the half way to the end (Fig. 3). This estimation assumes that in the 6-bp bending region each base-step bending angle is identical and that $\arcsin(\theta) \approx \theta$. The first assumption is reasonable because no other constraint than the electrostatic attraction from the H3 α N helix is applicable to this DNA region and the α N helix positive charges are spread over a long stretch of the helix (3 of the 4 total helical turns, Fig. 3). The second assumption is reasonable in that at the total bending angle 26° the error ($= [\arcsin(\theta) - \theta] / \arcsin(\theta)$) is 3.7 %, which is far below the measurement error and other uncertainties.

We examined the impact of varying the linear spring constant equivalence, or κ , on the results. We found that a value of κ smaller than 9 pN/nm results in the $E_{electrostatic}$ term dominating the total energy with no $p(r)$ maximum around the measured value. A value

larger than 44 pN/nm requires the value of $r_0 - r_{closed}$ smaller than the lower limit (i.e. $r_{open} - r_{closed}$). The range 12.5 – 15.0 pN/nm obtained with the average r_{closed} and r_{open} is reasonable considering these limits.

Our analyses and measurements provide a strong support to our proposal that the charge neutralization effect of histone acetylation is significantly amplified by protein binding that requires large opening. Furthermore, this novel mechanism can synergistically integrate the biophysical effect of histone acetylation with a biochemical mechanism and can explain how the nucleosome is sensitized to histone acetylation via enzyme or factor binding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This work was supported by NIH grant GM123164.

References

1. Davey CA, Sargent DF, Luger K, Maeder AW, Richmond TJ. (2002) Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 Å resolution. *J Mol Biol* 319, 1097–1113. [PubMed: 12079350]
2. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389, 251–60. [PubMed: 9305837]
3. Kim J, Lee J, Lee TH. (2015) Lysine Acetylation Facilitates Spontaneous DNA Dynamics in the Nucleosome. *J Phys Chem B* 119, 15001–15005. [PubMed: 26575591]
4. Kim J, Wei S, Lee J, Yue H, Lee TH. (2016) Single-Molecule Observation Reveals Spontaneous Protein Dynamics in the Nucleosome. *J Phys Chem B*, 120, 8925–8931. [PubMed: 27487198]
5. Lee J, Lee TH. (2017) Single-Molecule Investigations on Histone H2A-H2B Dynamics in the Nucleosome. *Biochemistry* 56, 977–985. [PubMed: 28128545]
6. Lee JY, Wei S, Lee TH. (2011) Effects of histone acetylation by Piccolo NuA4 on the structure of a nucleosome and the interactions between two nucleosomes. *J Biol Chem* 286, 11099–11109. [PubMed: 21282115]
7. Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K. (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet* 40, 897–903. [PubMed: 18552846]
8. Agaloti T, Chen G, Thanos D. (2002) Deciphering the transcriptional histone acetylation code for a human gene. *Cell* 111, 381–392. [PubMed: 12419248]
9. Dhall A, Wei S, Fierz B, Woodcock CL, Lee TH, Chatterjee C. (2014) Sumoylated human histone H4 prevents chromatin compaction by inhibiting long-range internucleosomal interactions. *J Biol Chem* 289, 33827–33837. [PubMed: 25294883]
10. Berger SL. (2002) Histone modifications in transcriptional regulation. *Curr Opin Genet Dev* 12, 142–148. [PubMed: 11893486]
11. Rothbart SB, Strahl BD. (2014) Interpreting the language of histone and DNA modifications. *Biochim Biophys Acta* 1839, 627–643. [PubMed: 24631868]
12. Suganuma T, Workman JL. (2011) Signals and combinatorial functions of histone modifications. *Annu Rev Biochem* 80, 473–499. [PubMed: 21529160]
13. Li Q, Zhou H, Wurtele H, Davies B, Horazdovsky B, Verreault A, Zhang Z. (2008) Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* 134, 244–255. [PubMed: 18662540]

14. Suganuma T, Workman JL. (2018) Chromatin and Metabolism. *Annual Review of Biochemistry* 87, 27–49.
15. Struhl K (1998) Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev* 12, 599–606. [PubMed: 9499396]
16. Chen CC, Carson JJ, Feser J, Tamburini B, Zabarónick S, Linger J, Tyler JK. (2008) Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. *Cell* 134, 231–243. [PubMed: 18662539]
17. Petrij F, Giles RH, Dauwerse HG, Saris JJ, Hennekam RC, Masuno M, Tommerup N, van Ommen GJ, Goodman RH, Peters DJ, Breuning MH (1995) Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* 376, 348–351. [PubMed: 7630403]
18. Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr., Evans RM. (1998) Role of the histone deacetylase complex in acute promyelocytic leukaemia. *Nature* 391, 811–814. [PubMed: 9486654]
19. Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, Fanelli M, Ruthardt M, Ferrara FF, Zamir I, Seiser C, Grignani F, Lazar MA, Minucci S, Pelicci PG. (1998) Fusion proteins of the retinoic acid receptor- α recruit histone deacetylase in promyelocytic leukaemia. *Nature* 391, 815–818. [PubMed: 9486655]
20. Goodman RH, Smolik S. (2000) CBP/p300 in cell growth, transformation, and development. *Genes Dev* 14, 1553–1577. [PubMed: 10887150]
21. Zeng L, Zhou MM. (2002) Bromodomain: an acetyl-lysine binding domain. *FEBS Lett* 513, 124–128. [PubMed: 11911891]
22. Horn PJ, Peterson CL. (2001) The bromodomain: a regulator of ATP-dependent chromatin remodeling? *Front Biosci* 6, 1019–1023.
23. Loyola A, Almouzni G. (2004) Bromodomains in living cells participate in deciphering the histone code. *Trends Cell Biol* 14, 279–281. [PubMed: 15183183]
24. Wei S, Falk SJ, Black BE, Lee TH. (2015) A novel hybrid single molecule approach reveals spontaneous DNA motion in the nucleosome. *Nucleic Acids Res* 43, 111–121.
25. D'Arcy S, Martin KW, Panchenko T, Chen X, Bergeron S, Stargell LA, Black BE, Luger K. (2013) Chaperone Nap1 shields histone surfaces used in a nucleosome and can put H2A-H2B in an unconventional tetrameric form. *Mol Cell* 51, 662–677. [PubMed: 23973327]
26. Andrews AJ, Chen X, Zevin A, Stargell LA, Luger K. (2010) The histone chaperone Nap1 promotes nucleosome assembly by eliminating nonnucleosomal histone DNA interactions. *Mol Cell* 37, 834–842. [PubMed: 20347425]
27. Lee JY, Lee J, Yue H, Lee TH. (2015) Dynamics of nucleosome assembly and effects of DNA methylation. *J Biol Chem* 290, 4291–4303. [PubMed: 25550164]
28. Lowary PT, Widom J. (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J Mol Biol* 276, 19–42. [PubMed: 9514715]
29. Lee JY, Lee TH. (2012) Effects of DNA methylation on the structure of nucleosomes. *J Am Chem Soc* 134, 173–175. [PubMed: 22148575]
30. Luger K, Rechsteiner TJ, Richmond TJ. (1999) Preparation of nucleosome core particle from recombinant histones. *Methods Enzymol* 304, 3–19. [PubMed: 10372352]
31. Hoyle CE, Bowman CN. (2010) Thiol-ene click chemistry. *Angew Chem Int Ed Engl* 49, 1540–1573. [PubMed: 20166107]
32. Li F, Allahverdi A, Yang R, Lua GB, Zhang X, Cao Y, Korolev N, Nordenskiöld L, Liu CF. (2011) A direct method for site-specific protein acetylation. *Angew Chem Int Ed Engl* 50, 9611–9614. [PubMed: 21922615]
33. Yue H, Fang H, Wei S, Hayes JJ, Lee TH. (2016) Single-Molecule Studies of the Linker Histone H1 Binding to DNA and the Nucleosome. *Biochemistry* 55, 2069–2077. [PubMed: 27010485]
34. Lee TH. (2009) Extracting kinetics information from single-molecule fluorescence resonance energy transfer data using hidden markov models. *J Phys Chem B* 113, 11535–11542. [PubMed: 19630372]
35. Aguilar-Gurrieri C, Larabi A, Vinayachandran V, Patel NA, Yen K, Reja R, Ebong IO, Schoehn G, Robinson CV, Pugh BF, Panne D. (2016) Structural evidence for Nap1-dependent H2A-H2B deposition and nucleosome assembly. *EMBO J* 35, 1465–1482. [PubMed: 27225933]

36. Bilokapic S, Strauss M, Halic M. (2018) Histone octamer rearranges to adapt to DNA unwrapping. *Nat Struct Mol Biol* 25, 101–108. [PubMed: 29323273]
37. Wiggins PA, Nelson PC. (2006) Generalized theory of semiflexible polymers. *Phys Rev E Stat Nonlin Soft Matter Phys* 73, 031906–031918. [PubMed: 16605557]
38. Vologodskii A, Frank-Kamenetskii MD. (2013) Strong bending of the DNA double helix. *Nucleic Acids Res* 41, 6785–6792. [PubMed: 23677618]
39. You H, Iino R, Watanabe R, Noji H. (2012) Winding single-molecule double-stranded DNA on a nanometer-sized reel. *Nucleic Acids Res* 40, 151–156.
40. Cherepanov DA, Feniouk BA, Junge W, Mulikidjanian AY. (2003) Low dielectric permittivity of water at the membrane interface: effect on the energy coupling mechanism in biological membranes. *Biophys J* 85, 1307–1316. [PubMed: 12885673]
41. Stobbe H, Peschel G. (1997) Experimental determination of the static permittivity of extremely thin liquid layers of water dependent on their thickness. *Colloid and Polymer Science* 275, 162–169.
42. Buchner R, Heftner GT, May PM. (1999) Dielectric Relaxation of Aqueous NaCl Solutions. *The Journal of Physical Chemistry A* 103, 1–9.
43. Park YJ, Luger K. (2006) The structure of nucleosome assembly protein 1. *Proc Natl Acad Sci U S A* 103, 1248–1253. [PubMed: 16432217]

Figure 1. Experimental setup and smFRET measurements to investigate DNA termini opening induced by Nap1 binding. (A) Three-color smFRET setup to investigate DNA opening dynamics induced by Nap1 binding. (B) Representative fluorescence intensity traces of the two pathways of the initial nucleosome disassembly steps catalyzed by Nap1 as shown in (A).

Figure 2.

The rate of DNA opening induced by Nap1 increases by 5.9 folds upon H3K56 acetylation. (A) FRET_{5,5} transition histograms show transitions among four states (HF, MF, LF1 and LF0) in the unacetylated (WT) and acetylated (H3K56ac) cases. (B) The kinetic rate constants among the four FRET_{5,5} states are shown in the chart (see Table S1 for the values). The four FRET efficiencies are shown in the upper table. The rates of initial DNA opening induced by Nap1 binding, $k_{binding}$, in the unacetylated (WT) and acetylated (H3K56ac) cases are shown in the lower table, revealing 5.9-fold increase upon H3K56 acetylation.

Figure 3.

Structural model for the nucleosome termini opening dynamics. (A) A magnified region where the H3 αN helix interacts with the DNA in the closed conformation (PDB ID: 1kx5). The blue-colored residues on the H3 αN helix are the four basic residues (R49, R52, R53 and K56) interacting with the DNA termini region (6 residues) colored red. The green colored residue is where Cy3 was labeled and the orange colored residue is where Atto647N was labeled in the previous report to probe spontaneous opening dynamics. (B) The nucleosome terminal region in an open conformation that is based on a cryo-EM structure

(PDB ID: 6esh). The average distance between the 4 blue residues in the H3 α N helix and the blue region of the DNA terminus is 3.30 nm. (C) The model parameters in Eqs. 1 and S1 depicted in the structure shown in A.

Figure 4.

Distributions of DNA opening gap in the open and closed states in the unacetylated (WT) and acetylated (H3K56ac) cases. (A) DNA opening gap distributions of the open (black) and closed (gray) states in the unacetylated nucleosome. These curves are from Eq. 1 with $\epsilon_r = 77$ (or 37 for closed state), $n = 4$, $m = 6$ and $\kappa = 12.5$ pN/nm. (B) Normalized DNA opening gap distributions of the open (black) and closed (gray) states in the unacetylated case (WT) and the open (blue) and closed (sky blue) states in the acetylated case (H3K56ac). The

curves are from Eq. 1 with $\epsilon_r = 77$ (or 37 for closed state), $n = 6$, $m = 4$ (or 3 for acetylated cases) and $\kappa = 12.5$ pN/nm.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 5.

The proposed mechanism of how enzyme binding sensitizes the nucleosome to H3K56ac. Acetylation at H3K56 shifts the DNA opening gap distribution from the gray to the blue distribution in the chart. The mechanism requires a large nucleosome opening for Nap1 binding. This requirement amplifies the ratio of the two population fractions for Nap1 binding (shaded areas at the edge of the two distributions) to 5.9 from 1.5 that is the ratio of the entire open state populations in the unacetylated and acetylated cases (the entire dotted areas under the two distributions).