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Specific Contributions of Histone Tails and their Acetylation to the Mechanical Stability of Nucleosomes

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The distinct contributions of histone tails and their acetylation to nucleosomal stability were examined by mechanical disruption of individual nucleosomes in a single chromatin fiber using an optical trap. Enzymatic removal of H2A/H2B tails primarily decreased the strength of histone–DNA interactions located $\sim \pm 36$ bp from the dyad axis of symmetry (off-dyad strong interactions), whereas removal of the H3/H4 tails played a greater role in regulating the total amount of DNA bound. Similarly, nucleosomes composed of histones acetylated to different degrees by the histone acetyltransferase p300 exhibited significant decreases in the off-dyad strong interactions and the total amount of DNA bound. Acetylation of H2A/H2B appears to play a particularly critical role in weakening the off-dyad strong interactions. Collectively, our results suggest that the destabilizing effects of tail acetylation may be due to elimination of specific key interactions in the nucleosome.

Keywords: histone tails; nucleosome; acetyation; optical trapping; single molecule

Introduction

DNA in the eukaryotic nucleus is hierarchically condensed and ordered in the dynamic structure of chromatin. The lowest level in this structural hierarchy is the individual nucleosome, a nucleoprotein composite of 147 base-pairs of DNA wrapped 1.65 times around a disk-shaped octamer of histone proteins.[1] Two domains of each histone protein, the N-terminal domain and the central globular domain play important structural and functional roles in the nucleosome.[2] The globular domains are critical for maintenance of the foundational architecture of the nucleosome, forming a superhelical ramp surface to accommodate DNA, with exposed arginine residues strategically placed on the ramp to interact with the DNA minor groove, where it faces the histone core, once per helical turn of DNA. The highly basic, lysine rich N-terminal "tails" have a more dynamic and complex role in chromatin structure.[3] In their unmodified state, the tails are less structured than the globular domains and are thought to interact with DNA in a charge-dependent manner. This is a flexible interaction which is thought to persist for a time even in the absence of typical globular domain-DNA-binding both *in vivo* and *in vitro*.[4,5]

Histone tail modifications, such as acetylation and methylation, have emerged as critical components of chromatin dynamics, and play an integral role in chromatin-based processes like gene regulation.[6] These and other modifications produce or destroy recognition sites, marking parts of the genome for binding by specific factors involved in transcriptional activation and repression.[7,8] Modifications may also modulate the structural contribution of histone tails to nucleosome stability and overall chromatin condensation.[9,10] Unlike methylation, acetylation results in a decrease in the overall positive charge of histone tails and is, as a result, expected to diminish the strength of binding of the tails to the negatively charged DNA. This reduction in electrostatic bond strength may in turn make both tails and nucleosomal DNA more accessible to the factors that bind them, as well as increasing the accessibility of the nucleosomal DNA to transcribing RNA

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Abbreviations used: HAT, histone acetyltransferase; RNAPs, RNA polymerases; TCA, trichloroacetic acid.

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polymerase. Experimental evidence indicates that acetylation of histone tails modulates the stability of chromatin on multiple structural levels extending from chromosomal structure to the individual nucleosome.[11] Furthermore, acetylation has been found to promote DNA independent alpha-helical structuring of the N-terminal tails that may both augment disruption of tail–DNA interactions, and create new protein–protein interaction surfaces.[12,13]

The histone acetyltransferase (HAT) p300/CBP targets histone tail lysines and influences transcriptional regulation on a number of genes,[14] including acetylation-dependent gene activation.[15,16] *In vitro*, p300/CBP acetylates a large subset of the lysine residues on the tails of all four core histones,[17] with a preference for lysines on the H3 and H4 tails in free histones, but not nucleosomal histones.[18] A great deal is known about the transcriptional outcome of p300/CBP HAT activity. Little is known, however, about the precise changes in the physical structure of the chromatin fiber or individual nucleosomes resulting from the specific acetylation patterns of HAT activities such as p300/CBP.

Histone tail removal experiments provide valuable information regarding the total contribution of tails to chromatin structure, and are, as such, a good standard of comparison for the impact of tail acetylation on nucleosome structure. The enzymatic removal of histone tail domains impairs the formation of fully condensed chromatin fibers, and weakens nucleosomal DNA-binding.[19–21] Tail removal has been shown *via* sedimentation assays to impair the ability of chromatin fibers to condense to the 30 nm fiber[20,22] and to form inter-fiber oligomers.[23] Furthermore, micrococcal nuclease assays along with sedimentation analysis and electron microscopy have all shown that under low salt conditions nucleosomal DNA unwinds by ~36 bp in tailless nucleosomes relative to intact specimens.[22,24] The mechanism by which tails exert their stabilizing effects remains largely unknown, but recent studies indicate that on chromatin fibers in solution tails interact preferentially with linker rather than nucleosomal DNA.[25]

The complexity, size, and dynamic character of chromatin present a challenge for detailed structural studies, especially in ensemble-averaged molecular populations. Much of the structural work on chromatin dynamics has utilized the linear mononucleosome or core particle as a model system. The isolated mononucleosome is a valuable model but has obvious limitations for understanding the characteristics of nucleosomes in ordered arrays.[11] Furthermore, many chromatin biophysical and biochemical techniques exploit extreme chemical or thermal conditions far from the physiological ideal to probe nucleosome structure.[19,26] Assays involving enzymatic access to nucleosomal DNA are performed under physiological solution conditions but are limited by the steric constraints and reaction conditions of the enzyme utilized.[27]

Single molecule optical trapping techniques can probe dynamic events quantitatively and directly, at the submolecular level, under physiological conditions, without the averaging and smearing effects associated with measurements taken from a large population.[28–30] Previously, using this approach, we probed the dynamic structure of individual nucleosomes by stretching nucleosomal arrays and determined that the disassembly of each nucleosome occurs in stages: a little less than half of the nucleosomal DNA is smoothly released from the histone core at low stretching force, and the remainder is released abruptly at higher force.[28] In the current study, we investigate the effects of histone tail removal and histone acetylation by p300 on DNA-binding in the nucleosome.

Results and Discussion

Nucleosome stretching patterns reflect DNA release from the histone octamer surface

Here, we investigate the contributions of histone tails and their acetylation to nucleosomal stability using mechanical disruption of individual nucleosomes to directly probe histone–DNA interactions (Figure 1(a)). Briefly, a nucleosomal array assembled from purified HeLa cell histones on a DNA molecule containing 17 tandem repeats of a 208 bp 5 S positioning element (208-17) was attached at one end to the surface of a microscope coverslip and at the other end to a 0.48 μm diameter polystyrene microsphere. Nucleosomes in the array were disrupted by moving the microscope coverslip of a sample chamber at a constant rate (140 nm s^{-1}) relative to the trapped microsphere, which was kept in a fixed position. The tension (force) in the array was monitored as a function of the end-to-end displacement of the DNA (extension).

An example of data from a saturated array is shown as a force-extension relation in Figure 1(b). The corresponding amount of naked DNA (i.e. DNA not directly bound to histones) as a function of time is shown in Figure 1(c). The 17 distinctive sawtooth peaks in the force-extension relation and the 17 steps in the naked DNA *versus* time curve are signatures of the 17 positioned nucleosomes.[28]

Our mechanical disruption method releases DNA in stages from a given nucleosome. At low force (<15 pN), an average of ~65 bp of DNA is released smoothly and simultaneously from each nucleosome in the array. This is reflected by the initial smooth increase in the amount of naked DNA as stretching progresses (Figure 1(c)). At high force (>15 pN), an average of ~72 bp of DNA is independently released from each nucleosome in the array. This is reflected by each peak in the force-extension relation and by each step in the naked DNA *versus* time curve. No significant difference in disruption characteristics is observed using comparable arrays assembled without positioning elements (data not shown).

Our data are consistent with a stage-wise

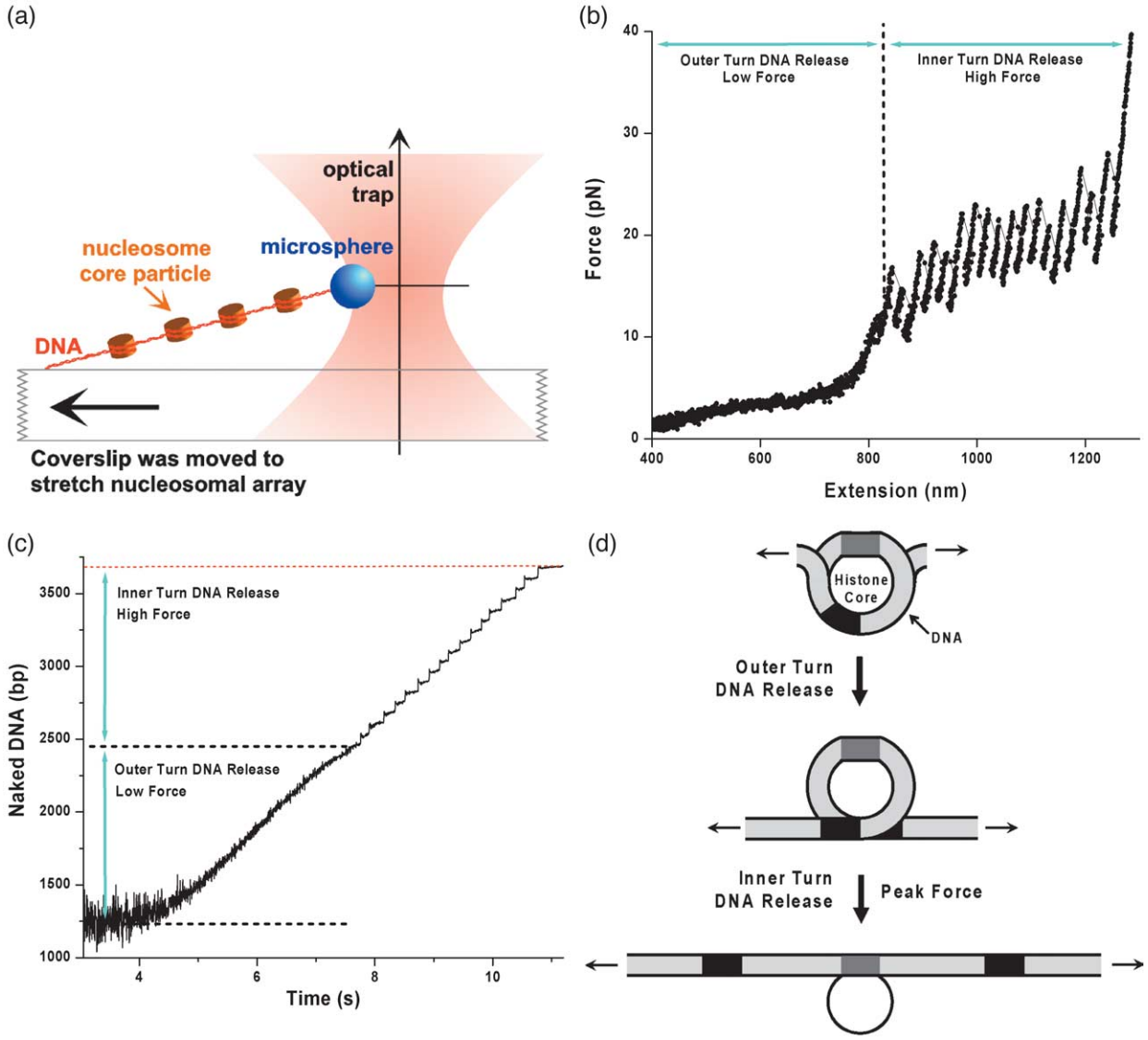

Figure 1. Hierarchical pattern of DNA release by the nucleosome observed upon stretching individual HeLa nucleosomal arrays. (a) Cartoon illustrating the experimental configuration. (b) Representative force *versus* extension plot obtained from stretching an individual nucleosomal array at constant velocity (140 nm/s). Arrays reconstituted from human core histones on the 208-17 DNA fragment display a characteristic hierarchical pattern of DNA release. (c) Data from (b) are converted to the amount of naked DNA in the tether *versus* time during the stretching process. This type of curve allows the determination of the amounts of outer and inner turn DNA released (see Materials and Methods). (d) Cartoon illustrating a model for nucleosomal disruption (see the text). The dark gray region in the nucleosomal DNA indicates the strong interactions between the DNA and histone core near the dyad, and the black regions in the nucleosomal DNA indicate the off-dyad strong interactions. The black regions thus mark the border between outer turn DNA and inner turn DNA.

disruption model (Figure 1(d)) in which entry and exit DNA is peeled symmetrically from the histone octamer.[28] The initial disruption releases the ~65 bp of DNA seen at low force. This disruption is gradual and because only low force is required to peel DNA from the protein surface, only weak protein–DNA contacts are broken. The subsequent disruption at high force involves the sudden release of the next 72 bp of inner turn DNA. This stage of disruption is sudden due to the strong interactions presumed to be near the positions ±35 bp to ±45 bp of DNA from the dyad axis, and the disruption force gives a measure of the strength of these interactions. This simultaneous breaking of symmetric bonds may be a result of the synergetic coupling between the locations of these strong interactions in a nucleosome (each about half a turn from the dyad) and geometrical constraints imposed by the end-to-end stretching of the DNA.

Here, we refer to these strong interactions as the "off-dyad strong interactions". We refer to the DNA beyond these strong interactions from the dyad as "outer turn DNA", and the DNA between these interactions and the dyad as "inner turn DNA". Therefore, there are three "signatures" that characterize the stability of a nucleosome. The first

signature is the amount of outer turn DNA, which is detected at low force, and is a measure of how tightly the outer turn DNA is wrapped around the histone core. The second signature is the amount of inner turn DNA, which measures the locations of the off-dyad strong interactions. The third signature is the peak force, which is a measure of the strength of these strong interactions. (It should be noted that although we think that peak forces measured here are indicative of bond strength, quantitative comparisons with corresponding bulk nucleosome stability measurements are non-trivial.) The latter two signatures are both detected at high force. These three signatures probe different parts or different aspects of a nucleosome structure.

Histone tail removal decreases overall affinity of histones to DNA in nucleosomes

We performed experiments to investigate the effect of histone tail removal on the stability of nucleosomes. Four types of arrays were assembled from combinations of intact histones and globular (tailless) histones. We designate these types as follows: (1) intact (all intact histones); (2) gH2A/B (globular H2A/B and intact H3/4); (3) gH3/4 (intact H2A/B and globular H3/4); and (4) gAll (all globular histones). Globular histones were obtained by subjecting native chromatin to a limiting trypsin digest (Figure 2). Purity and quality of free histones as well as histones assembled in nucleosomal arrays were assayed by SDS-PAGE (Figure 3(a)).

Our mechanical disruption experiments using

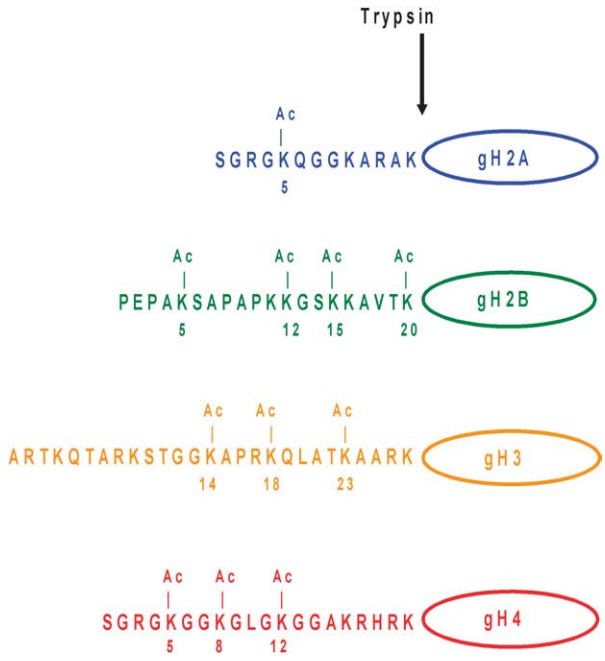

Figure 2. Schematics of histone tails (adapted from An *et al.*[49]). Here the definitions of globular histones and histone tails are based on the sites of trypsin cleavage as indicated for each histone. Ac's also indicate sites of acetylation by p300.[17]

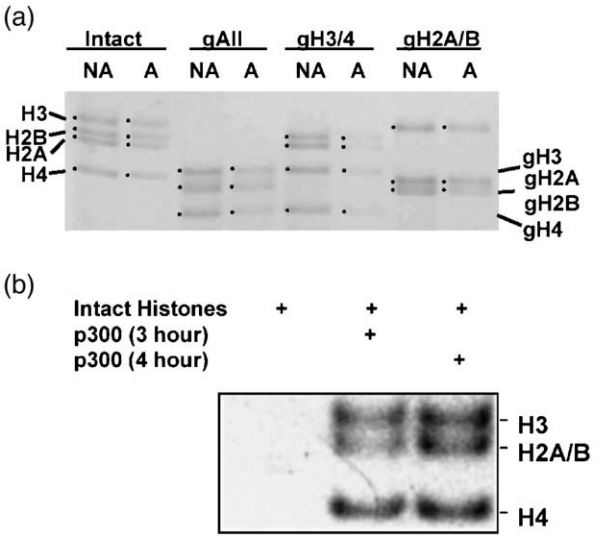

Figure 3. Biochemical preparations. (a) Preparation of core histone fractions—analysis of purified HeLa histones and chromatin arrays. Samples were precipitated in 25% (w/v) TCA and run on 20% Laemmli PAGE gels. Histones not assembled (NA) into nucleosomes and histones assembled (A) into nucleosomes are shown to be indistinguishable on this gel. (b) HAT assays of histones. *In vitro* acetylation in the presence of [H^3]-acetyl-CoA followed by electrophoresis demonstrates the differences in the quantity of acetylation of core histones by p300 at three and four-hour reaction times (lanes 2 and 3, respectively). Lane 1 shows intact histones incubated with labeled acetyl CoA in the absence of p300 for four hours.

these arrays showed distinctive contributions of different histone tails to nucleosomal stability. Representative force-extension relations are shown in Figure 4(a) with the corresponding naked DNA *versus* time curves shown in Figure 4(b). The results from a large number of similar data sets are summarized in Figures 4(c) and 6, and Table 1. These data show that removal of histone tails resulted in significant changes in the disruption signatures of nucleosomes.

Firstly, the amount of outer turn DNA was dramatically reduced by 60% after histone tail removal. This shows that histone tails govern much of the affinity of the outer turn DNA to the histone octamer. H2A/H2B and H3/H4 tails contributed to this effect collectively with the primary contribution from H3/H4 tails. Secondly, the amount of inner turn DNA was minimally affected by histone tail removal, indicating that the locations of the off-dyad strong interactions were not altered. Thirdly, the peak force for disruption was significantly reduced by histone tail removal. Peak forces were determined from the sawtooth patterns of the force *versus* extension relations of nucleosomal arrays with a similar level of saturation. For a given nucleosomal array, the peak force on the average increased as the number of nucleosomes present on the array decreased (e.g. see Figure 4(a)).

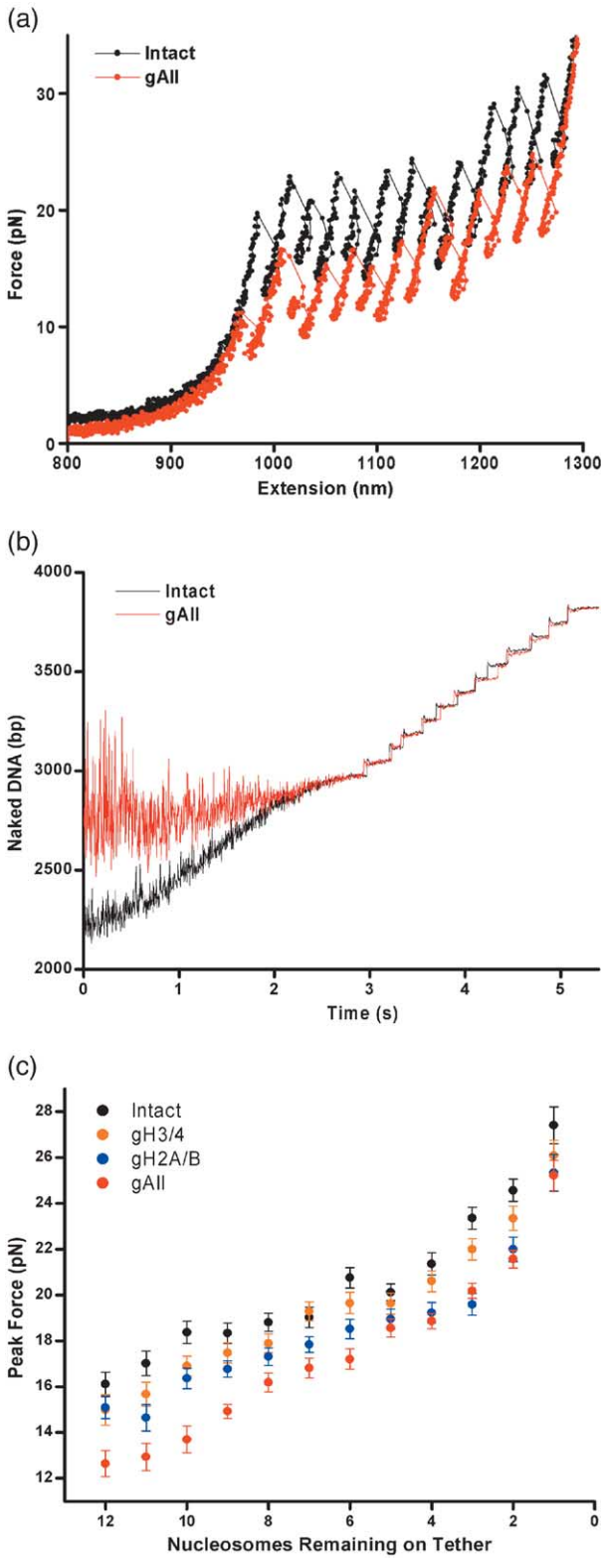

Figure 4. Stretching nucleosomal arrays assembled from tailless histones. (a) Representative force *versus* extension plots from intact (black) and tailless (red) arrays. (b) Data from (a) are converted to the amount of naked DNA *versus* time. (c) Summary data of peak force *versus* number of nucleosomes remaining on tether for histone tail removal experiments. This plot was generated by analysis of peak disruption forces from data like those shown in (a). All arrays included in this analysis contained 11–15 nucleosomes. Data are shown for the following array types: intact (black); gH3/4 (orange); gH2A/B (blue); and gAll (red). Error bars represent standard errors of the means.

This trend was expected because a nucleosomal array with N nucleosomes would be N times as likely to result in a disruption as an array with just one nucleosome under the same stretching conditions. The average peak force *versus* number of nucleosomes remaining on the tether was plotted in Figure 4(c). Removal of histone tails did not greatly alter the shape of the peak force curve but only shifted it towards lower forces. The average peak force change was the average difference between the peak force *versus* number of nucleosome curves before and after tail removal (Figure 6 and Table 1). This shows that tail removal weakens the off-dyad strong interactions. H2A/H2B and H3/H4 tails contributed to this effect collectively with the primary contribution from H2A/H2B tails.

Acetylation of histone tails decreases overall affinity of histones to DNA in nucleosomes

We performed further experiments to investigate the effects of histone acetylation on the mechanical stability of nucleosomes. Our core histones had a low level of basal acetylation, with no more than one acetyl group per H4 subunit as determined by TAU gel analysis (data not shown). To establish specific and controlled acetylation of intact core histones, we used purified recombinant p300 enzyme to acetylate free histones since p300-mediated acetylation is much more efficient with free histones than with nucleosomal histones.[18] This enzyme is known to acetylate lysines on all four core histone tails[17,18] (Figure 2), although H3 and H4 are the preferred targets with free histones.[18] Furthermore, it has been demonstrated that lysine residues on the histone tails are preferred by p300 as substrates relative to residues in the histone folds.[31]

To determine the extent of acetylation by p300 beyond the basal levels found *in vivo*, we performed quantitative histone acetyltransferase (HAT) assays. Under our acetylation conditions (see Materials and Methods), we observed a time-dependent increase in the acetylation of all four core histones (Figure 3(b)). At the three and four-hour time points, p300 added ~15 and ~19 acetyl groups per octamer, respectively. The acetylation of H4 was nearly saturated at three hours (a modest ~6% increase from three to four hours), with additional acetyl groups at the four-hour time point added primarily to H2A/H2B (increased ~67% from three to four hours) and, to a lesser extent, H3 (~27% increase) (Figure 3(b) and Table 2). These levels of acetylation are consistent with patterns previously observed.[17,32]

Our mechanical disruption experiments using these acetylated arrays demonstrated a definitive effect of acetylation on nucleosomal stability. The

Table 1. Summary of nucleosome disruption signature data (see also Figure 6)

	Outer turn DNA (bp)	Inner turn DNA (bp)	N_{DNA}	Peak force change (pN)	N_{force}
Intact	65±2	71.9±0.6	24	–	31
gH3/4	40±2	70.5±0.3	31	−0.6±0.2	39
gH2A/B	49±3	70.9±0.3	24	−1.9±0.2	34
gAll	28±2	69.0±0.2	28	−2.9±0.2	31
p300 three hour	59±1	71.4±0.2	57	−0.6±01	63
p300 four hour	50±2	71.1±0.3	28	−1.8±0.2	32

Average amounts of inner turn and outer turn DNA were determined from N_{DNA} individual nucleosomal arrays. Average changes in peak force relative to those from arrays assembled with intact histones were determined from N_{force} individual nucleosomal arrays. Uncertainties are standard errors of the means.

results from a large number of measurements are summarized in Figures 5(c) and 6, and Table 1, with representative force-extension relations shown in Figure 5(a) and the corresponding naked DNA *versus* time curves shown in Figure 5(b). The overall results resemble those obtained for histone tail removal.

Firstly, for nucleosomes assembled with histones from the three and four hour acetylation reactions, the amount of the outer turn DNA was reduced by 9% and 23%, respectively. This shows that acetylation of histone tails decreases the affinity of the outer turn DNA to the histone octamer. Secondly, as expected, the amount of inner turn DNA was not affected by acetylation, indicating that the locations of the off-dyad strong interactions were not altered. Thirdly, the peak force for disruption was significantly reduced. Acetylation of histone tails by p300 did not alter the shape of the peak force *versus* nucleosome number curve but shifted it towards lower force by ∼0.6 pN and ∼1.8 pN for nucleosomes assembled with histones from the three and four-hour acetylation reactions, respectively (Figure 6, Table 1). Given that most of the additional acetyl groups added in the four hour reaction were on H2A/H2B (Table 2; Figure 3(b)), the more significant reduction in the peak force from the four hour reaction again suggests that H2A/H2B play a critical role in the off-dyad strong interactions.

In summary, we have demonstrated that distinct physical changes in chromatin structure occur in response to treatment with p300. Aside from the important role of N-terminal lysine acetylation in producing ligands for other chromatin-binding and modifying factors,[33] it is clear that specific acetylation of histone tails results in significant changes in chromatin structure. The changes in nucleosome stability observed in this study upon tail removal are consistent with previously published results obtained using other techniques to study mononucleosomes. Widom and colleagues have shown that accessibility of nucleosomal DNA to restriction endonucleases is greatly increased by removal of histone tails.[21] Previous work by van Holde and colleagues have likewise shown a significant increase in thermal instability of tailless nucleosomes.[19] By contrast, neither of these groups showed dramatic changes in mononucleosome stability upon acetylation.[27,34] However, the acetylated nucleosome samples previously tested were assembled using "hyperacetylated" histones, purified from deacetylase-blocked HeLa cells. By nature, these samples are highly heterogeneous, consisting of all possible histone modification states, which may explain other groups' inability to detect significant effects of histone acetylation. Here, we have examined the effect of acetylation by a specific cellular HAT and do see a significant effect of histone tail acetylation by p300.

Specific contributions of histone tail acetylation to nucleosome stability

Our results indicate that histone tails are critically important in stabilizing nucleosomes and that histone tail acetylation directly regulates this stabilization *via* charge neutralization. Histone tails are highly positively charged and should be strongly attracted to the negatively charged DNA.

Table 2. Summary of HAT Assay Results

	Three hour	Four hour	% Change from three to four hour	Number of K and R residues in tail
H3	2.2	2.8	27	9
H4	3.5	3.7	6	7
H3/H4	5.7	6.5	14	16
H2A/H2B	1.8	3.0	67	11
Octamer	15.0	19.0	27	54

Table showing quantification of acetylation level for each histone subunit. Absolute levels of acetylation per octamer were determined using a filter-binding assay (see Materials and Methods). Distribution of acetyl groups on histone subunits was determined by quantifying and averaging results from two gels like that shown in Figure 3(b). In addition, this Table shows the number of K and R residues for each histone tail.

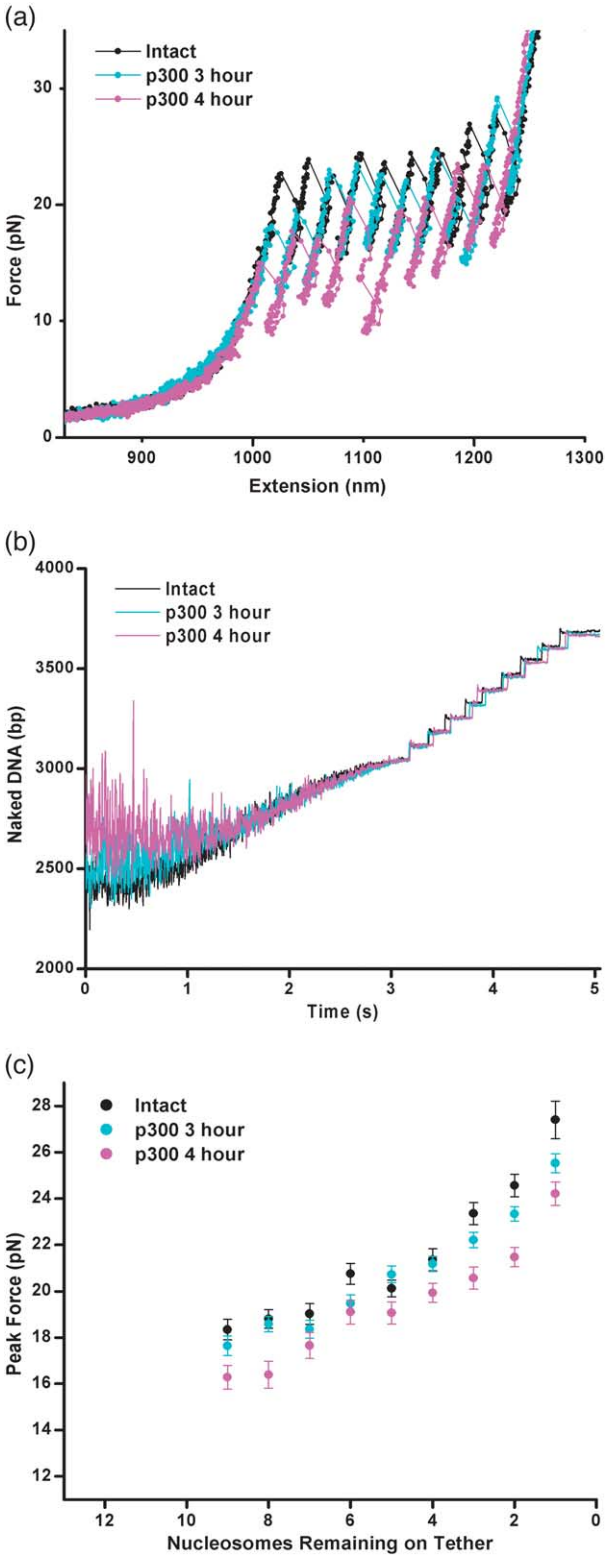

Figure 5. Stretching nucleosomal arrays assembled from acetylated histones. (a) Representative force *versus* extension plots from intact (black), three hour acetylated (cyan) and four hour acetylated (magenta) arrays. (b) Data from (a) are converted to the amount of naked DNA *versus* time. (c) Summary data of peak force *versus* number of nucleosomes remaining on tether for histone tail acetylation experiments. This plot was generated by analysis of peak disruption forces from data like those shown in (a). All arrays included in this analysis contained 8–15 nucleosomes. Data are shown for the following array types: intact (black), three-hour acetylation (cyan), and four-hour acetylation (magenta). Error bars represent standard errors of the means.

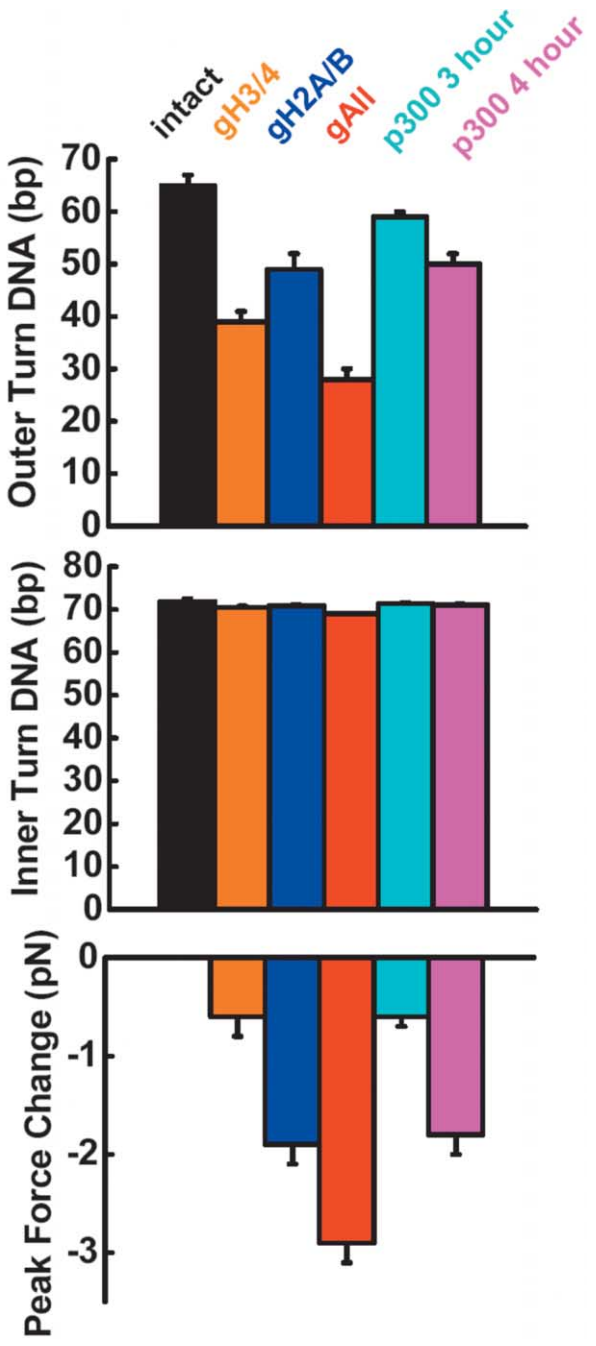

Figure 6. Summary of nucleosome disruption signature data. Error bars represent standard errors of the means.

The positive charges of histone tails are due to the lysine and arginine residues (Table 2). Removal of histone tails completely obliterates these interactions, while acetylation of histone tails only eliminates interactions at specific lysine residues.

Consistent with this, our data indicate that increasing the level of acetylation of histones from three to four hour reactions decreases nucleosome stability, and even the four hour acetylation reaction does not reduce nucleosome stability as much as complete removal of histone tails (Table 1).

Unexpectedly, neutralization of only about one lysine residue per H2A/H2B dimer and H3 subunit (Table 2) leads to the drastic changes in nucleosome stability between three and four-hour acetylations (Table 1), whereas the prior neutralization of about two residues on each causes only a moderate change. We speculate that this may be due to acetylation of one or more "critical residues" during the fourth hour, residues which make interactions with DNA which are particularly crucial to nucleosome stability. Hence, neutralization of only a single histone–DNA interaction could have strong structural consequences. Several species have been shown to acetylate tail residues in a specific order *in vivo*,[35,36] indicating a possible preferred acetylation order of HATs themselves. It could be, then, that an extra hour permits p300 to acetylate a critical residue deep in the order, causing dramatic stability changes.

Alternatively, our results may also indicate a threshold effect. Rather than a critical residue(s), there may be an acetylation threshold, beyond which there is some type of tail conformation change, such as alpha-helical structuring,[12,13] which causes a sudden decrease in stability. Charge neutralization would still play a chief role, but only in the capacity of promoting conformation change, not in eliminating histone–DNA interactions. Acetylation during the fourth hour may be sufficient to push the tails of one or more subunits over such a conformation change threshold. A threshold effect has also been observed in the acetylation-induced inhibition of higher order array structuring.[9]

Our experiments performed at different levels of acetylation provide evidence of specific contributions of acetylation of H2A/H2B *versus* H3/H4 to nucleosome stability. Comparing the levels of acetylation of the various histone tails for the three and four hour reactions shows only a 14% increase in acetylation of H3/H4, but a 67% increase for H2A/H2B (Table 2). Thus, after three hours of reaction, the rate of acetylation of H3/H4 was slowing, but that of H2A/H2B was increasing. This is consistent with H3/H4 being the preferred substrates for p300 acetylation.[18] Furthermore, noting that our globular data implicate H2A/H2B tails as primarily responsible for stabilization of the off-dyad strong interactions, we conclude that the large decrease in off-dyad interaction strength observed after the fourth hour is likely due to acetylation of H2A/H2B.

Correlation of mechanical data with nucleosome structure

The structure of the nucleosome places significant constraints on which histone tails are capable of binding to outer and inner turn DNA. Our results indicate structural targets of interaction for each tail with nucleosomal DNA and provide an indication of the relative strengths of these interactions. From the crystal structure of the nucleosome, when viewed along the DNA superhelical axis, the N-terminal histone tails are seen to emerge from the core histones at intervals of ~1/8 revolution (~45°)[1,37] (Figure 7(a)). The H3 tails are the longest, and emerge between the gyres of the DNA superhelix at $\sim \pm 45°$ from the dyad closest to the DNA entry and exit points. The H4 tails are significantly shorter than the H3 tails and emerge at $\sim \pm 90°$ from the dyad outside the DNA superhelical gyres closest to the inner turn DNA. The H2B tails are the second longest and emerge at $\sim \pm 135°$ from the dyad between the superhelical gyres of the DNA. The H2A tails are the shortest

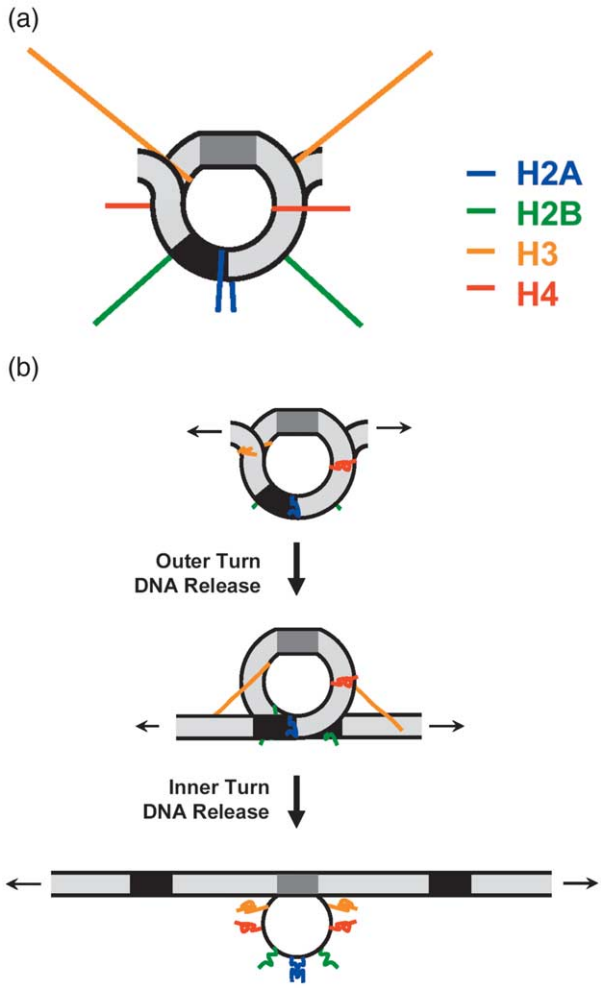

Figure 7. Model of contributions of tails to nucleosomal stability. (a) Locations and relative lengths of histone tails in the nucleosome (adapted from illustrations by Wolffe & Hayes[38]). Tails are shown fully extended for the purpose of length comparison. (b) A model of the contributions of histone tails to nucleosome stability during the nucleosome disruption process (see the text for explanation).

and emerge outside the DNA superhelical gyres at $\sim \pm 180^{\circ}$ from the dyad.

These structural data indicate that the affinity of the outer turn DNA to histone octamer should be primarily regulated by H3/H4 tails because they locate near the entry/exit DNA. Furthermore, because the H4 tails are shorter than the H3 tails, and positioned farther from the entry/exit points, it is reasonable to speculate that the H3 tails might play the primary role in this function. H2B tails should also play some role in the outer turn DNA-binding because of their lengths and locations. It is also evident that the strengths of the off-dyad strong interactions are expected to be primarily regulated by the H2A/H2B tails.

The results of our mechanical measurements complement these structural interpretations and shed important light on the locations and strengths of the interactions of tails with nucleosomal DNA. Our single molecule data are entirely consistent with the structural information, although our experiments currently do not separate contributions from H3 and H4, or H2A and H2B tails. Interestingly, our data also show that H3/H4 tails exert some influence on the off-dyad strong interactions, indicating that H3 and/or H4 tails may remain attached to the DNA while the nucleosomal DNA is peeled off symmetrically up to the off-dyad strong interactions.

Based on the correlation of the nucleosome structure with our mechanical data, we propose a model for the contributions of histone tails and their acetylation to nucleosomal stability (Figure 7(b)). In the relaxed state of a nucleosome, H3/H4 tails help to stabilize the entry and exit portions of the nucleosomal DNA. This stabilization is enhanced by the additional contribution from the H2B tails. As the DNA is peeled away from the surface of the histone octamer, H3 tails remain associated with the DNA. The peeling continues until the off-dyad strong interactions are encountered. These interactions are primarily stabilized by H2A/H2B tails, but are further enhanced by H3 tails in an extended conformation. The subsequent disruption of the off-dyad strong interactions releases interactions of the H2B, H2A, and H3 tails with DNA.

Implications of histone acetylation for transcription

Nucleosomes present obstacles for access to nucleosomal DNA or histone tails by the transcription machinery. RNA polymerases (RNAPs), transcription factors, and nucleosome remodeling machines must access the DNA associated with nucleosomes. The decrease in overall DNA-binding we have observed upon acetylation would produce a significant increase in DNA entry–exit angle in nucleosomes on an array. Since increasing the entry–exit angle would result in an increase in array length, an obvious consequence of change in angle is chromatin decondensation, which also provides increased accessibility for regulatory factors. This is in keeping with the results of hydrodynamic studies, in which tailless and acetylated arrays cannot adopt the maximally folded 55 S conformation.[10,20] In addition, some transcription regulatory factors also bind to specifically acetylated histone tails in nucleosomes (e.g. Brd2 binds to H4 acetylated at lysine 12 through it's bromodomain).[33] The purported dual functions of histone acetylation (charge neutralization and factor recruitment) may work in concert: modification of histone tails leads to charge neutralization, exposing the tails for factor recruitment.

Functionally, decreased DNA-binding and chromatin decondensation both serve to increase factor access to protein and nucleic acid moieties in chromatin.[38,39] Decreasing the force required to disrupt off-dyad strong interactions on the nucleosome might facilitate the transit of RNAPs and ATP-dependent remodelers through nucleosomal DNA as well. If the unmodified nucleosome presents a simple physical barrier to a transcribing RNAP, then any reduction in nucleosomal DNA-binding affinity, such as that induced by acetylation would directly enhance RNAP transit through the off-dyad strong interactions.

In conclusion, we have presented results indicating that acetylation of histone tails, aside from its well-appreciated role in creating ligands for bromodomain-containing proteins,[40] also has significant effects on nucleosome structure, possibly through charge-neutralization of critical residues. Not only does acetylation decrease the resistance of the nucleosome to mechanical unfolding, but it also decreases the average amount of DNA stably bound to histone protein. Judging from the effect of complete tail removal, acetylation impacts on a significant fraction of the total contribution of histone tails to nucleosome structure. These effects may contribute to the formation of "permissive" chromatin structures by increasing the accessibility of nucleosomal DNA to binding proteins and decreasing the energy required to transcribe through nucleosomes.

Materials and Methods

Protein purification

Recombinant His_6-tagged human p300 was overexpressed in insect cells using a baculoviral system, and purified as described.[18,32] Human core histones were prepared from washed HeLa S3 nuclear pellets.[41] Prior to histone extraction, one batch of washed nuclear pellet was treated with 6.7 μg/ml TCPK-treated trypsin for 18 hours at 4 °C to selectively remove exposed N-terminal tails from intact chromatin before purification. Tryptic activity was then selectively inhibited with a tenfold excess of soybean trypsin inhibitor. Further fractionation of intact and tailless histone heterodimers was accomplished by hydroxylapatite chromatography as previously described by Simon & Felsenfeld.[42] To insure that no tryptic activity remained in the tailless histone fractions after purification, all proteins in assembled

arrays were shown to have the correct molecular mass by electrophoretic analysis. These results indicate that the populations of various tailless nucleosomal arrays utilized in the single molecule experiments are identical except for their tail deletion states.

Nucleic acids

The protocol for the DNA template preparation is identical with that used for our previous studies.[28] Briefly, the 208–17 DNA fragment was obtained from the pCP681 plasmid (kindly provided by C. Peterson) by restriction digest and was subsequently end-labeled with biotin- and digoxigenin labeled dNTPs by Klenow fill-in reaction.

Histone acetylation

Bulk *in vitro* acetylation of human histones and histone fractions with recombinant p300 was performed using previously described reaction conditions.[32] Extent of acetylation by p300 was controlled by reaction time. Precise determination of the quantities of acetylation produced on the various purified histone proteins was accomplished using a combination of filter binding assays and fluorography. In the filter-binding assays,[32] the product of a HAT reaction using [^{3}H]acetyl-CoA (Dupont NEN) as substrate was incubated in 25% (w/v) trichloroacetic acid (TCA) for one hour, and aspirated through a filter, which was then washed with 5% TCA and acetone, and scintillation counted. In parallel, the product of a HAT reaction was quantified by electrophoretically separating on 20% acrylamide Laemmli PAGE gels followed by fluorography for labeled protein detection, and densitometry. Results from the filter-binding assays and densitometry along with input protein and acetyl CoA concentrations were used to determine the number of acetyl groups added under these reaction conditions.

Determination of basal histone acetylation level was performed by Triton-acetate-urea gel analysis as described by Lennox & Cohen.[43]

Chromatin assembly

Chromatin arrays were assembled on DNA templates by dialysis through a NaCl gradient (2.0 M–0.6 M over 18 hours at 4 °C) using a core histone:5 S positioning element molar ratio of 1.75 : 1. The quality of histone proteins in arrays was determined by denaturing gel electrophoretic analysis of TCA-precipitated array proteins. Digestion of arrays with EcoRI,[44] which cleaves the DNA molecule between each 5 S monomer, confirmed that the proper spacing was induced by the positioning element. This assay was also used to determine the level of saturation of the DNA with nucleosomes. In this assay, 250 ng of arrays were digested with 7 units of EcoRI for one hour at 37 °C. Reactions were quenched by addition of EDTA (to 2.5 mM) and glycerol (to 6% (w/v)) and subsequently electrophoresed on a pre-chilled 5% native polyacrylamide gel. DNAs were visualized by ethidium staining.

Optical trapping data acquisition and analysis

The experimental setup is essentially identical with those used in previous work.[28,45–48] Sample preparation and velocity clamp experiments were performed as previously reported.[28,48] All single-molecule experiments were performed in buffer containing 10 mM Tris (pH 8.0), 1 mM EDTA, 100 mM NaCl, 1.5 mM $MgCl_2$, 0.02% (v/v) Tween 20, and 0.01% (w/v) milk protein. Our instrument can resolve relative displacements of ~1 nm. However, the determination of DNA extension has an uncertainty of $\sim\pm33$ nm for a 4000 bp long tether, corresponding to a tether length uncertainty of $\sim\pm100$ bp. This gives $\sim\pm4$ bp of uncertainty in the determination of the total amount of DNA bound per nucleosome. To facilitate the comparison of different force-extension curves, these curves are shifted along the extension axis to coincide with the very high force portion (>30 pN) of the corresponding naked DNA curve.

The number of nucleosomes in an array corresponds to the number of sawtooth peaks in its force-extension curve (e.g. Figure 1(b)), or equivalently to the number of steps in its corresponding naked DNA *versus* time graph (e.g. Figure 1(c)). The amounts of outer and inner turn DNA released are calculated from a naked DNA *versus* time graph based on the following procedure.[28,48] At the beginning of the stretch process when the force is below ~3 pN, there is a phase during which the amount of naked DNA remains constant, as indicated by the initial plateau in the naked DNA *versus* time curve (see the lower broken line in Figure 1(c)). The length at this point indicates the initial quantity of naked DNA in the tether. As the stretch continues, DNA is gradually released from all nucleosomes up to the beginning of the first sudden rise (step) in the amount of naked DNA (see the middle broken line in Figure 1(c)). The difference in these amounts of naked DNA divided by the number of nucleosomes on the array gives the amount of outer turn DNA bound per nucleosome. The amount of inner turn DNA released per nucleosome is determined by the average step size in the naked DNA *versus* time curve. This same method of analysis was used for nucleosomal arrays assembled from unmodified and modified histones.

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