

Effects of Histone Acetylation on the Equilibrium Accessibility of Nucleosomal DNA Target Sites

J. D. Anderson¹, P. T. Lowary¹ and J. Widom^{1,2*}

¹*Department of Biochemistry
Molecular Biology, and Cell
Biology, Northwestern
University, Evanston
IL 60208-3500, USA*

²*Department of Chemistry
Northwestern University
Evanston, IL 60208, USA*

Posttranslational acetylation of the conserved core histone N-terminal tail domains is linked to gene activation, but the molecular mechanisms involved are not known. In an earlier study we showed that removing the tail domains altogether by trypsin proteolysis (which leaves nucleosomes nevertheless intact) leads to 1.5 to 14-fold increases in the dynamic equilibrium accessibility of nucleosomal DNA target sites. These observations suggested that, by modestly increasing the equilibrium accessibility of buried DNA target sites, histone acetylation could result in an increased occupancy by regulatory proteins, ultimately increasing the probability of transcription initiation. Here, we extend these observations to a more natural system involving intact but hyperacetylated nucleosomes. We find that histone hyperacetylation leads to 1.1 to 1.8-fold increases in position-dependent equilibrium constants for exposure of nucleosomal DNA target sites, with an average increase of 1.4(±0.1)-fold. The mechanistic and biological implications of these results are discussed.

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*Corresponding author

Introduction

Each of the four core histone proteins of the nucleosome have conserved N-terminal tail domains that are known to play important roles in gene regulation, although the mechanisms involved are not established. Recent studies have established an unequivocal link between posttranslational acetylation of these domains and gene activation.^{1–6} Among the several (non-exclusive) mechanisms that have been proposed for how tail domain acetylation may contribute to gene activation *in vivo*, one mechanism supposes that a primary effect of tail domain acetylation is to alter the structure or dynamics of nucleosomes so as to enhance the ability of site specific regulatory proteins to bind to DNA target sites inside nucleosomes.^{7–12}

The site exposure model for the dynamic behavior of nucleosomes^{12–14} provides a framework for quantitative analysis and understanding of the interactions of proteins with nucleosomal DNA target sites. Let N represent a nucleosome in its native (most prevalent) conformation. The site exposure model supposes the existence of a transient state S

of the nucleosome, at equilibrium with N, in which the nucleosome has transiently exposed some of its DNA, such that a DNA target site that previously was inaccessible is now freely accessible to a binding protein R or nuclease E. These can bind to make a complex RS or ES, the latter of which can go on to activate catalysis to yield products E and P. Real nucleosomes *in vitro* behave as though such site exposure processes are occurring constantly yet transiently, in a rapid pre-equilibrium. Site exposure is non-dissociative: one side of the DNA remains bound while the other side is exposed.^{12,13} It occurs *via* a transient partial uncoiling of the DNA, not by nucleosome translocation (J.D.A. & J.W., unpublished results). As a consequence of this intrinsic property of site exposure, even sites that in the time average are buried within the middle of the nucleosome are nevertheless constantly but transiently accessible. The equilibrium constants for site exposure K_{eq}^{conf} (the equilibrium fraction of the time that nucleosomal DNA target sites are freely accessible, as though they were naked DNA) depend on the position of the target site inside the nucleosome, decreasing from the end of the nucleosomal DNA inward toward the middle. The apparent equilibrium affinity of proteins binding to nucleosomal target sites, and, equivalently, the observed rate constants for diges-

E-mail address of the corresponding author:
j-widom@northwestern.edu

tion of nucleosomal DNA by restriction endonucleases or non-specific exonucleases, are reduced from their values on naked DNA by a factor equal to the position-dependent value of K_{eq}^{conf} , thus providing multiple independent but equivalent assays for measurement of K_{eq}^{conf} .

In a recent study we utilized these equilibrium binding and restriction enzyme digestion kinetics assays to examine whether and to what extent the tail domains contribute to determining the quantitative values of K_{eq}^{conf} at positions throughout the nucleosome.¹² We measured the position-dependent values K_{eq}^{conf} for native nucleosomes and for nucleosomes from which all tail domains have been removed by trypsin proteolysis, a widely used model system thought to mimic the possible effects of histone acetylation (see references 12 and 15 for more discussion of this point). We found that complete removal of the tail domains leads to 1.5 to 14-fold increases in position-dependent equilibrium constants for site exposure. The present study extends this work on tailless nucleosomes to a more-natural model system using hyperacetylated nucleosomes isolated from HeLa cells grown in butyrate to inhibit natural deacetylases. We quantify the extent to which histone hyperacetylation affects equilibrium constants for site exposure at positions throughout the nucleosome.

Results and Discussion

Nucleosome reconstitution and purification

The present studies utilize a family of DNA constructs that are derived from a strong non-natural nucleosome positioning sequence (601) isolated in an earlier SELEX experiment.¹⁶ Construct 601.2 (Figure 1(a)¹⁷) introduces a number of nucleotide substitutions into 601 so as to create sites for different restriction enzymes at locations along the entire nucleosomal length. The free energy of interaction of 601.2 with histone octamer in nucleosome reconstitution, the location of the single strongly preferred nucleosome position on this template, and the position-dependent equilibrium constants for site exposure along the nucleosome length have previously been reported.¹⁷

Hyperacetylated HeLa histones (Figure 1(b) and (c)) were purified from cells grown in culture using standard butyrate treatment protocols (to inhibit deacetylases), with an additional step of size-exclusion chromatography. Acid urea gel electrophoresis (Figure 1(c)) confirms that the histones extracted from the butyrate-treated cells are enriched for the most highly acetylated fractions as described.^{18,19}

Nucleosomes were reconstituted from purified DNA and histones by salt gradient dialysis, and purified by sucrose gradient ultracentrifugation. Examples of typical gradient profiles are shown in

Figure 2(a). Subsequent reanalysis of the purified nucleosomes by sucrose gradient ultracentrifugation (Figure 2(a)) or native gel electrophoresis (Figure 2(b)) shows them to be largely free of contaminating naked DNA. The small amount of contaminating DNA that remains (and any additional naked DNA that may be created when the nucleosomes are brought to digestion conditions) will not contribute to the observed kinetics because it is digested to completion within the first timepoint, which is omitted from the analysis (see below and Materials and Methods).

The native gels show that each of the reconstituted nucleosome samples migrate predominantly as single bands, consistent with a single preferred nucleosome position. (Note that multiple nucleosome positions, when these exist, can be detected and resolved by native gel electrophoresis even with DNA as short as 146 bp.^{20,21}) Direct mapping data and other results that imply a single strongly preferred nucleosome position on construct 601.2 reconstituted with chicken erythrocyte histones are discussed in reference 17. Additional data confirming this interpretation for the present nucleosomes reconstituted with control or hyperacetylated HeLa histones are discussed below.

Restriction enzyme digestion kinetics assay for position-dependent K_{eq}^{conf}

We used the restriction enzyme digestion kinetics assay¹²⁻¹⁴ to measure relative values of K_{eq}^{conf} at target sites throughout the nucleosome. The goal of the present study is to compare K_{eq}^{conf} values at sites throughout the nucleosome for nucleosomes prepared from control (i.e. not hyperacetylated) histones *versus* nucleosomes prepared from hyperacetylated histones. Consequently, rather than measuring absolute values of K_{eq}^{conf} , we measure relative values obtained from the ratio of digestion rates on pairs of nucleosomal samples that differ only in the acetylation states of their histones.

Parallel digestions of control and hyperacetylated nucleosomes were carried out in identical solution conditions. Aliquots were removed as a function of time and quenched. Samples were analyzed *via* denaturing polyacrylamide gel electrophoresis. Representative results of one such experiment, probing the Hha I recognition sequence spanning base-pairs 91-94 from the 5' end of 601.2 (base-pairs 76-79 from the edge of the nucleosome), are illustrated in Figure 3(a)-(c) for naked DNA, control HeLa nucleosomes and hyperacetylated HeLa nucleosomes, respectively. Note that the digestion on naked DNA utilized a 20,000-fold lower enzyme concentration.

Results for all assays are summarized quantitatively in Table 1. Hyperacetylation of the histone tail domains increases K_{eq}^{conf} by 1.1 to 1.8-fold, with an average increase of 1.4(\pm 0.1)-fold.

Additional studies (not shown) were carried out to directly compare K_{eq}^{conf} at a subset of sites in

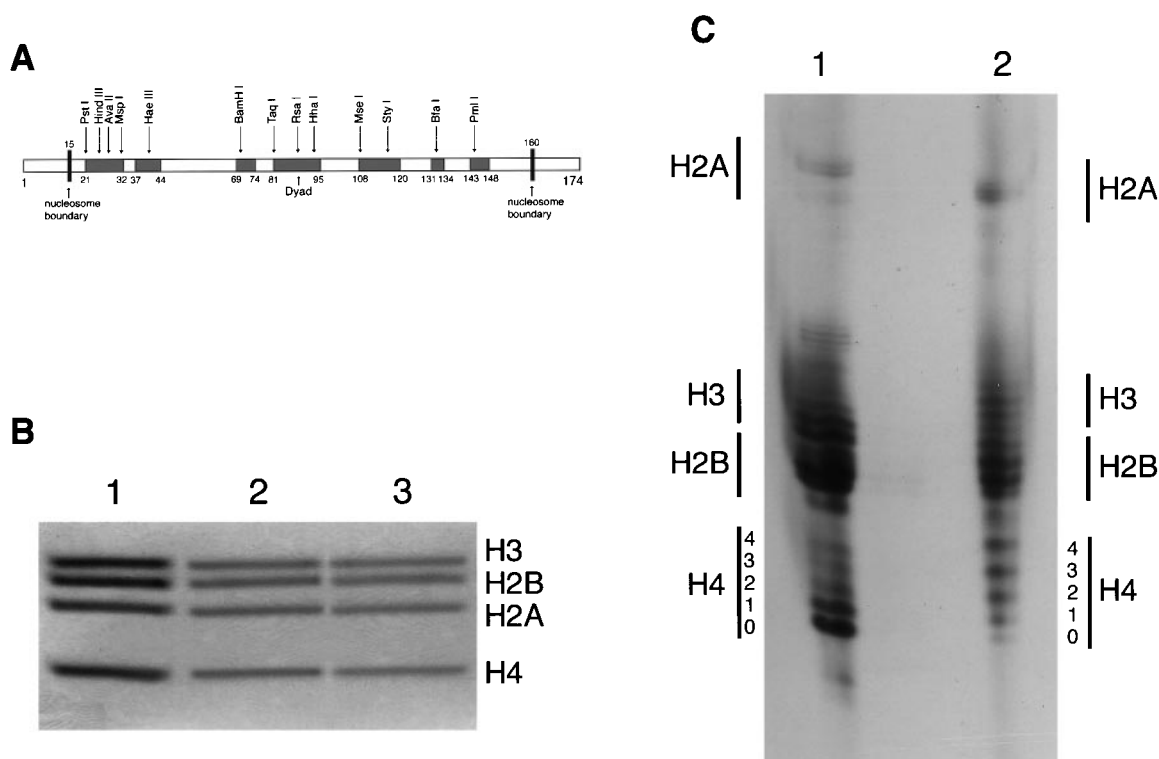


Figure 1. DNA template and histone preparations. (a) Schematic of DNA construct 601.2. The 174 bp long 601.2 parent DNA sequence is illustrated as a rectangle. The histone octamer was mapped¹⁷ to a single detectable position covering base-pairs 15 to 160, indicated by the black vertical bars. The corresponding location of the nucleosomal dyad symmetry axis is indicated by the arrow. Each nested set of restriction sites is indicated by the hatched boxes, and specific restriction sites contained therein are indicated. The numbers bordering each hatched box indicate the precise locations of those stretches of sequence within the overall 174 bp long sequence (base-pair 1 is defined as the left end of the DNA sequence). (b) Commassie-stained SDS gel of histones used in this work. Lane 1, chicken histone octamer; lane 2, control HeLa histone octamer; lane 3, hyperacetylated HeLa histone octamer. Histones H2A, H2B, H3 and H4 are labeled on the right. (c) Acid-urea gel analysis of control and hyperacetylated HeLa histones. Lane 1, control HeLa histone octamer; lane 2, hyperacetylated HeLa histone octamer. Histones H2A, H2B, H3 and H4 are labeled for each lane. The gel was run so as to optimize the resolution of the multiple acetylated H4 species. The assignment of the bands follows¹⁹ and is approximate for H2B and H3. Note the relative degree of the histone H4 acetylation for each sample. Control HeLa H4 is predominately H4⁰ and H4¹ (i.e. unacetylated and monoacetylated, respectively), whereas hyperacetylated HeLa H4 is predominately H4³ and H4⁴ (i.e. tri and tetra-acetylated, respectively).

nucleosomes prepared from construct 601.2 DNA and control HeLa histones, *versus* the same DNA reconstituted with chicken erythrocyte histones. We obtained values of K_{eq}^{conf} for the two samples that are identical within experimental error. In a previous study we measured the position-dependent values of K_{eq}^{conf} for nucleosomes containing 601.2 DNA and chicken erythrocyte histones.¹⁷ Taken together, these observations allow us to relate the present relative measurements of K_{eq}^{conf} for hyperacetylated *versus* control HeLa nucleosomes back to the *absolute* measurements of the position-dependent values of K_{eq}^{conf} for 601.2 measured in our earlier studies on chicken erythrocyte histones. The resulting values for K_{eq}^{conf} for control and hyperacetylated HeLa nucleosomes are summarized in Figure 4.

K_{eq}^{conf} values are strongly dependent on position in the nucleosome

The approximately 1.1 to 1.8-fold relative increases in equilibrium accessibility (K_{eq}^{conf}) detected here are superimposed on a strong dependence of K_{eq}^{conf} with position within the nucleosome: K_{eq}^{conf} decreases progressively by two to three orders of magnitude with distance from either nucleosome end in toward the middle (dyad axis) of the nucleosomal DNA.^{13,17} Thus the small effects on K_{eq}^{conf} arising from histone hyperacetylation are a positive demonstration of small changes, not a negative finding that might have resulted were the assay unable to detect large changes in protection when these in fact exist.

The strong dependence of K_{eq}^{conf} on position inside the nucleosome, together with the substan-

Table 1. Effects of histone hyperacetylation on site exposure in nucleosomes

Restriction enzyme	Region probed (approximate number of base-pairs from the nucleosome end)	K_{eq}^{conf} (hyperacetylated)/ K_{eq}^{conf} (control)
<i>Pst</i> I	6-11	1.51 ± 0.18
<i>Hind</i> III	12-17	1.08 ± 0.14
<i>Ava</i> II	18-22	1.10 ± 0.16
<i>Msp</i> I	22-25	1.27 ± 0.37
<i>Hae</i> III	26-29	1.48 ± 0.11
<i>Bam</i> HI	54-59	1.75 ± 0.73
<i>Taq</i> I	66-69	1.53 ± 0.80
<i>Rsa</i> I	72-75	1.16 ± 0.10
<i>Hha</i> I	76-79	1.39 ± 0.13
<i>Mse</i> I	93-96	1.09 ± 0.05
<i>Sty</i> I	100-105	1.42 ± 0.42
<i>Bfa</i> I	116-119	1.60 ± 0.62
<i>Pml</i> I	128-133	1.11 ± 0.13
		Average: 1.35 ± 0.06

Fold-enhancement of DNA site accessibility (average ± one standard deviation) from multiple independent experiments. Fold-enhancement for each independent experiment are obtained from ratios of best-fit rate constants for digestions of hyperacetylated nucleosomes *versus* control nucleosomes, scaled for the enzyme concentrations used (see Materials and Methods). The fold-enhancement averaged over all sites throughout the nucleosome is reported as average ± standard error.

tial protection afforded to even the most accessible regions at the ends of the nucleosomal DNA, provide additional evidence that mispositioning of nucleosomes is not contributing significantly to the results obtained here. Given these known protection factors, any non-nucleosomal DNA protruding beyond an end of a mispositioned nucleosome would be digested to completion within the first timepoint, and therefore would not contribute to the present analysis, since we fit the disappearance of full-length reactant subsequent to the first timepoint. (We do this to prevent the assays from being sensitive to small amounts of contaminating naked DNA created when the nucleosomes are adjusted to digestion conditions; see Materials and Methods.) Actually, however, we find that the fraction of total template DNA digested within the first time-point is small and moreover is closely similar to the small fraction of naked DNA present in mock-digested samples as assessed by native gel electrophoresis and sucrose gradient ultracentrifugation (data not shown). Thus we conclude that any mispositioned nucleosomes are present in at most small amounts, not detectable in the assays used here (and therefore also not contributing to the results obtained), consistent with the presence of single bands in native gel electrophoresis.

Histone hyperacetylation increases the equilibrium accessibility of nucleosomal DNA

We find that nucleosomes prepared with hyperacetylated histones yield a 1.4-fold increase in K_{eq}^{conf} averaged over the full set of measurable locations

in the nucleosomes (with individual values ranging from 1.1 to 1.8-fold increases). This 1.4-fold averaged increase, while small, is nevertheless significantly different from 1, as the standard error is 0.1. We cannot state with confidence whether the individual position-dependent fluctuations are real or not, as each of them is based on fewer measurements with a correspondingly greater standard error. We emphasize that this finding of a small but significant averaged increase in K_{eq}^{conf} is a positive demonstration of a small increase, not a result of an inability of the assay to detect larger differences when those exist.

It is illustrative to compare the present results with results from earlier studies on nucleosomes from which the tail domains have been fully removed by proteolysis. We showed previously using both restriction enzyme digestion kinetics and equilibrium binding assays that removing the tail domains altogether leads to 1.5 to 14-fold increases in K_{eq}^{conf} .¹² Those findings are consistent with results of studies on tailless nucleosomes¹⁰ when we analyze those experiments as described by the site exposure model (see Introduction and ref. 12).

We have similarly analyzed more recent equilibrium binding experiments by on hyperacetylated nucleosomes to extract values for K_{eq}^{conf} ¹¹ and, from these, the fold-changes in K_{eq}^{conf} resulting from histone hyperacetylation. Their data show that the proteins USF and GAL4-AH bind to single sites on hyperacetylated nucleosomes (butyrate-isolated HeLa histones) with ~2.6-fold and ~1.7-fold greater values for K_{eq}^{conf} , respectively, than for binding to control nucleosomes. Thus the previous studies, which utilized quite different approaches, are consistent with the present conclusion that histone hyperacetylation leads to small but significant increases in the equilibrium accessibility of nucleosomal DNA target sites.

While histone hyperacetylation and complete removal of the tail domains both lead to significant increases in equilibrium accessibilities of nucleosomal DNA target sites, complete removal of the tail domains appears to allow for a larger magnitude of effect. We observed analogous greater effects from complete removal of the tail domains *versus* their hyperacetylation in recent studies on the rate of transcriptional elongation through nucleosomes.¹⁵

Thus while the experiments on fully tailless nucleosomes may represent one extreme model system for the possible effects of histone acetylation, the use of hyperacetylated histones may represent an opposite situation, i.e. giving a lower bound, for the possible effects of the highest acetylation state, since the fraction of tetra-acetylated H4, which may show the strongest effects, is substantially less than one. The results of an experiment that is sensitive to the most highly acetylated states of histone H4 (tri and tetra-acetylated H4)¹¹ are consistent with this view. When we analyze those data as described by the site

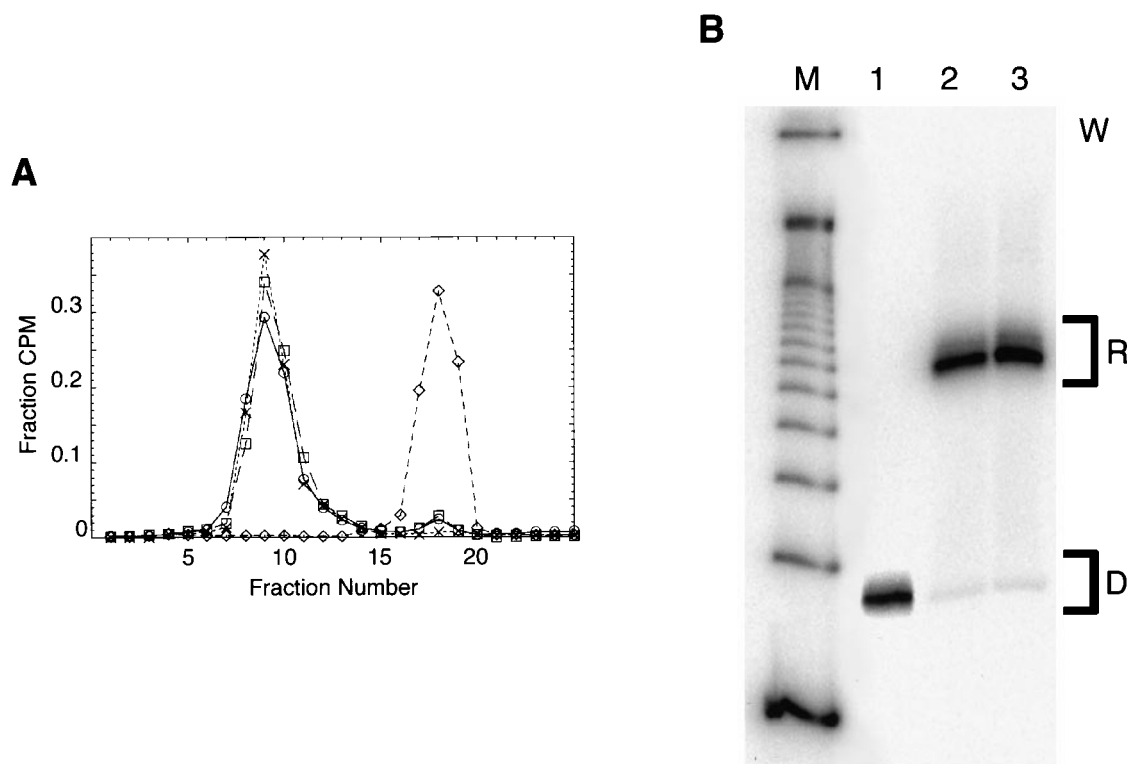


Figure 2. Sucrose gradient purification, and re-analysis by sucrose gradient and native gel electrophoresis. Nucleosomes are reconstituted by gradual salt dialysis and separated from naked DNA on 5-30% (w/v) sucrose gradients (a). Purified nucleosomes are further analysed on a (a) second sucrose gradient and by (b) native gel electrophoresis. (◇) naked 601.2 DNA; (○) preparative run of reconstituted control HeLa nucleosomes; (□) Preparative run of reconstituted hyperacetylated HeLa nucleosomes; (×) re-analysis of gradient purified hyperacetylated HeLa nucleosomes. (c) Native gel analysis: W indicates the location of the loading wells; R indicates the mobility of the reconstituted nucleosomes; D indicates the mobility of naked DNA. Lane M, 100 bp DNA marker; lane 1, naked 601.2 DNA; lane 2, purified control HeLa nucleosomes; lane 3, purified hyperacetylated HeLa nucleosomes. Phosphorimager analysis of the gel reveals contamination by free DNA to be $\leq 0.5\%$.

exposure model, they reveal that nucleosomes containing tri and tetra-acetylated H4 yield an additional ~ 3.7 -fold increase in affinity for GAL4-AH protein above the ~ 1.7 -fold increase obtained with (butyrate-isolated) hyperacetylated histones, for an overall ~ 6.3 -fold increase in affinity.

In summary, we conclude that: (i) histone hyperacetylation leads to significant increases in the equilibrium accessibility of nucleosomal DNA target sites. And, (ii) studies on completely tailless nucleosomes and on bulk hyperacetylation nucleosomes likely place upper and lower bounds, respectively, on the effects of the highest acetylation states, as regards the equilibrium accessibility of DNA target sites in nucleosomes.

Biological significance

Even very modest changes in equilibrium accessibility of nucleosomal DNA target sites (K_{eq}^{conf}) can have important biological consequences. Our earlier work shows that increases in K_{eq}^{conf} lead directly to increased occupancy by gene activator proteins *in vitro*.¹² We presume this also likely to be the case *in vivo*. One expects that increased occupancy

by gene activator proteins leads to increased frequency of transcription initiation. Moreover, studies on a model system for nucleosome transcription show that the increased K_{eq}^{conf} caused by histone hyperacetylation or complete removal of the tail domains is also accompanied by an increased rate of transcriptional elongation through a nucleosome.¹⁵

Multiple relatively small effects may also combine to yield a large dynamic range of gene regulation. One clear demonstration of the biological significance of even modest quantitative effects attributable to histone acetylation is the phenomenon of dosage compensation in *Drosophila*.²² In this case, histone acetylation acts in combination with other factors to yield an overall twofold increase in X chromosome transcription in males. Histone acetylation presumably contributes only a fraction of the overall twofold increase; and even that modest contribution may arise from the combined effects of multiple independent mechanisms, each likely yielding even-smaller separate effects on transcription initiation or elongation.¹⁵

Finally, we emphasize that additional distinct possibilities for roles of histone acetylation remain,

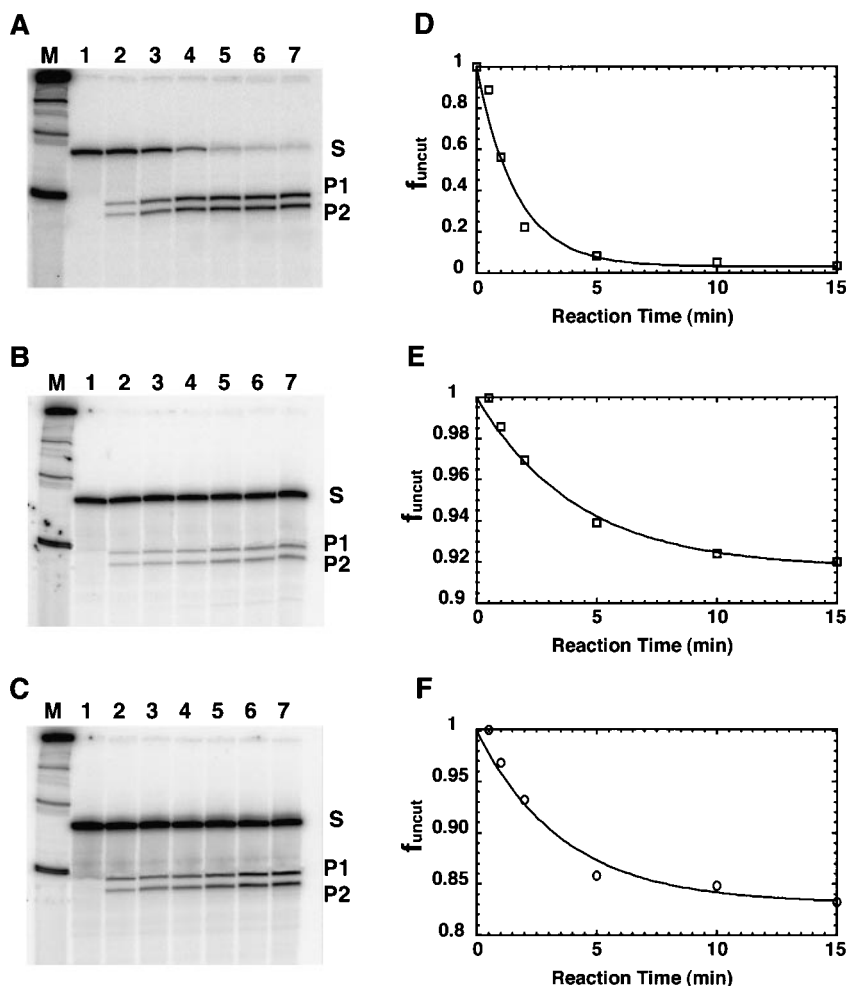


Figure 3. Representative kinetic analyses, probing site-exposure at a site spanning 76-79 bp from the 5' end of the predominant core particle position. (a)-(c) Denaturing polyacrylamide gel analysis of the time-course of digestion using the enzyme *HhaI*. Lanes 1 through 7 in all digestion gels are samples removed at 0, 0.5, 1, 2, 5, 10 and 15 minutes, respectively, from reaction initiation. In each case, the substrate (S; 174 nt) is converted over time to two products (93 nt, P1, and 83 nt, P2, for 601.2). The sizes of S, P1, and P2 expected from the DNA sequence are confirmed against the 100 bp DNA markers in lane M. (a) Naked 601.2 DNA, digested with *HhaI* at 0.1 units ml⁻¹. (b) Control HeLa nucleosomes, digested with *HhaI* at 2000 units ml⁻¹. (c) Hyperacetylated HeLa nucleosomes, digested with *HhaI* at 2000 units ml⁻¹. (d)-(f) Quantitative analysis of the time-course of digestion from the data in (a)-(c), respectively. The fraction of DNA remaining uncut is plotted versus time. The superimposed lines represent the results of fits to the following equation: fraction uncut = $(m2 + (1 - m2) \times \exp(-m1 \times \text{time}))$; see Materials and Methods for details. As described in Materials and Methods and in ref. 12, alternative fits based on double exponentials do not change

the interpretation. Note that the digestion on naked DNA utilized a 20,000-fold lower enzyme concentration.

and that these are non-exclusive with those outlined above. Among these additional possibilities are: (i) acetylation may act at higher levels of chromatin folding,^{23,24} destabilizing the condensed chromatin fiber and thereby decreasing the free energy penalty that otherwise must be paid to allow regulatory protein binding. (ii) A chief role of acetylation may be to regulate the binding of other (non-histone) proteins to the histones in chromatin through direct protein-protein contacts between these other proteins and specifically acetylated (or unacetylated) histone N-terminal domains.

Materials and Methods

Preparation of DNA and histones

Construct 601.2 (174 bp) was prepared by PCR as described¹⁷ and purified by ion exchange HPLC on an anion-exchange column (Mono-Q HR5/5) using a linear gradient of 0.65 M NaCl in TE (pH 8.0, room temperature) to 0.8 M NaCl in TE over 90 minutes at a flow rate

of 0.25 ml min⁻¹. The purified products were concentrated on Centricon 30 filters (Millipore) and resuspended in 0.1 × TE. Typical yields from 10 ml of PCR synthesis were 150 μg of DNA after HPLC purification.

Control (i.e. non-hyperacetylated) HeLa core histones and hyperacetylated HeLa core histones were prepared from HeLa cells and butyrate-treated HeLa cells produced at the National Cell Culture Center (Minneapolis, MN) as described^{18,25} with modifications. For isolation of hyperacetylated HeLa histones, 10 mM sodium butyrate was included throughout the preparation; butyrate was omitted for preparation of control HeLa histones. Protease inhibitors PMSF (0.1 mM) and benzamidine (1 mM) were included throughout both preparations. Cells were washed and nuclei prepared and digested as described,¹⁸ except replacing the Pipes and Mes buffers with Tris (pH 7.5). For control samples, soluble chromatin was isolated as in our standard protocols.²⁶ Digested nuclei were collected by low speed sedimentation, lysed by resuspension in TE buffer, and residual nuclear debris removed by centrifugation. For hyperacetylated samples, the most highly acetylated chromatin fragments were enriched and collected as described.¹⁸ A fraction of the hyperacetylated nucleosomes diffuse out of the nuclei in the digestion buffer; these are separated from the

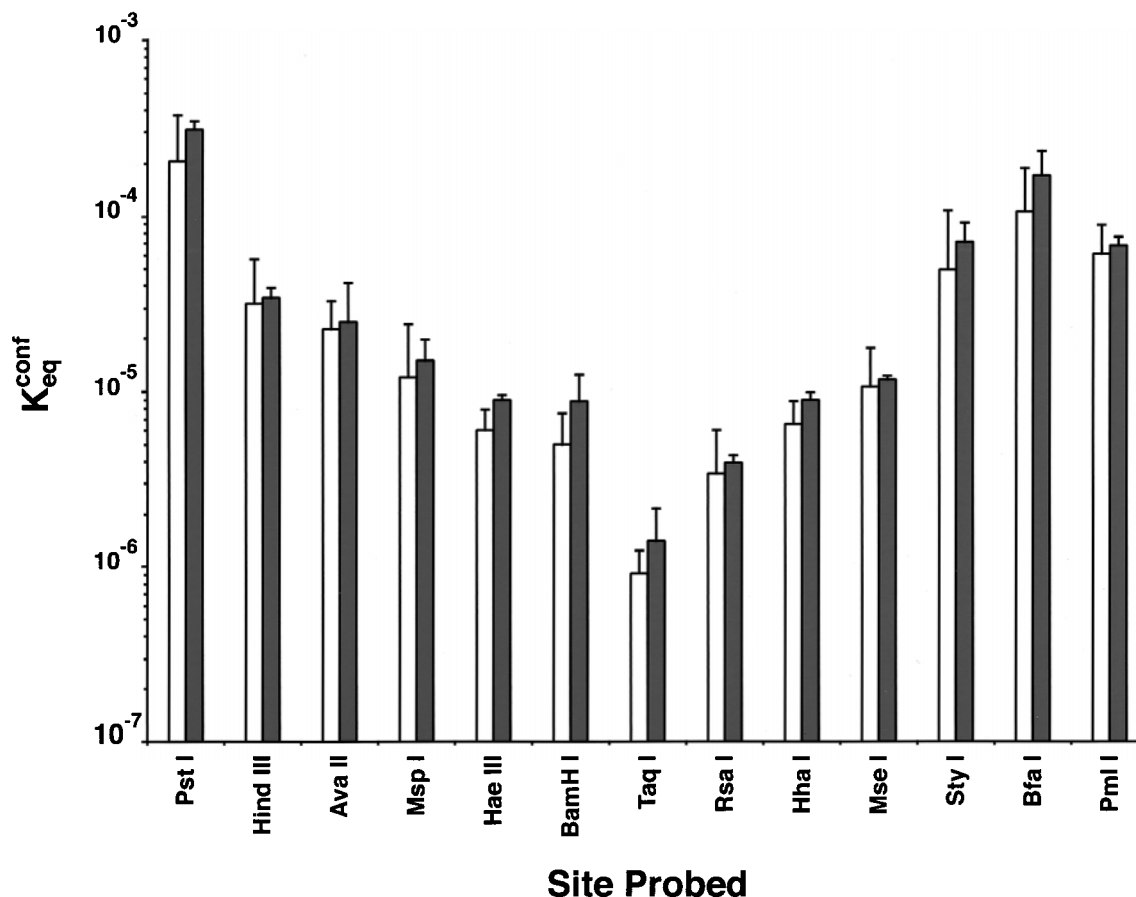


Figure 4. Position-dependent values of K_{eq}^{conf} for hyperacetylated and control nucleosomes. See Figure 1 for the nucleosomal locations of the different restriction sites, and the text for details. Note the log scale for K_{eq}^{conf} . Filled bars, hyperacetylated nucleosomes; open bars, control nucleosomes.

residual nuclei by low speed centrifugation. The collected nuclei are then resuspended in buffer containing 150 mM NaCl, which allows additional hyperacetylated nucleosomes to diffuse out. These are again separated from the residual nuclei, and the two samples of hyperacetylated nucleosomes (which are comparably enriched in the highest histone acetylation states¹⁸) are pooled. Control and hyperacetylated histone octamer were purified from soluble chromatin by chromatography on hydroxylapatite as described,²⁵ and further purified by size exclusion chromatography on sephacryl S200 in 2 M NaCl. The extent of hyperacetylation (particularly monitoring histone H4) was assessed by electrophoresis on acid urea gels as described.¹⁹ Chicken erythrocyte histones were prepared as described.²⁷

Reconstitution and purification of nucleosome core particles

Prior to reconstitution, construct DNAs were labeled with [γ -³²P]ATP using phage T4 polynucleotide kinase (NEB). Reconstitution reactions contained 200 ng of labeled construct DNA, 19.2 μ g of chicken erythrocyte core particle DNA, 15.5 μ g of HeLa core histone octamer (hyperacetylated or control) or chicken erythrocyte octamer, in a 50 μ l volume of 2.0 M NaCl, in 1 mM Tris (pH 8.0), 0.1 mM EDTA (0.1 \times TE), 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.1 mM benzami-

dine (BZA). The reconstitutions were performed by a gradual stepwise salt dialysis, beginning at 2.0 M NaCl and the stepping successively to 1.5 M NaCl, 1.0 M NaCl, 0.5 M NaCl, and 5 mM NaCl, each for a minimum of two hours and each supplemented with 0.5 \times TE, 0.1 mM BZA and 0.5 mM PMSF. A final overnight dialysis step into 0.5 \times TE was performed before further processing. All dialyses were performed at 4 $^{\circ}$ C.

Core particle samples were run on 5% to 30% (w/v) sucrose gradients (in 0.5 \times TE) at 41,000 rpm in a Beckman SW41 rotor for 24 hours at 4 $^{\circ}$ C. Gradients were fractionated into 0.5 ml fractions and quantified by Cerènkov counting. Fractions containing nucleosome core particles were pooled and exchanged into 0.5 \times TE on Centricon-30 concentrators and analyzed by native polyacrylamide gel electrophoresis.

Restriction enzyme assays

Nucleosome samples were digested with the following enzymes, all at 37 $^{\circ}$ C: *Pst*I, *Hind*III, *Ava*II, *Msp*I, *Hae*III, *Bam*HI, *Rsa*I, *Hha*I, *Mse*I, *Sty*I, *Bfa*I, and *Pml*I. *Taq*I digestions were carried out at 65 $^{\circ}$ C. All enzymes were obtained in their most concentrated commercially available form from New England Biolabs (NEB), and all digestions were carried out with the buffer supplied by NEB, supplemented with 100 μ g/ml bovine serum albumin. Glycerol was added to all naked DNA digests to

the same final concentration present in the corresponding core particle digestion, and never exceeded 5% (v/v) in any reaction. All reactions (both hyperacetylated and control) were supplemented with 10 mM sodium butyrate, to limit histone deacetylase activity in the hyperacetylated samples and to eliminate any butyrate-dependent effects in comparisons between the two samples. The specific buffers used for each enzyme are as follows: NEB buffer #1 (10 mM Bis tris propane-HCl, 10 mM MgCl₂, 1 mM DTT, pH 7.0) for *RsaI* and *PmlI*; NEB buffer #2 (10 mM Tris-HCl, 10 mM MgCl₂, 50 mM NaCl, 1 mM DTT, pH 7.9) for *HindIII*, *MspI*, *HaeIII*, and *MseI*; NEB buffer #3 (50 mM Tris-HCl, 10 mM MgCl₂, 100 mM NaCl, 1 mM DTT, pH 7.9) for *PstI* and *StyI*; NEB buffer #4 (20 mM Tris-OAc, 10 mM MgOAc, 1 mM DTT, pH 7.9) for *AvaII*, *HhaI* and *Bfal*; NEB *TaqI* buffer (10 mM Tris-HCl, 10 mM MgCl₂, 100 mM NaCl, pH 8.4) for *TaqI*; NEB buffer *BamHI* (10 mM Tris-HCl, 10 mM MgCl₂, 150 mM NaCl, 1 mM DTT, pH 7.9) for *BamHI*.

At various time-points during the reactions, 10 μ l aliquots were removed and quenched with 40 mM EDTA. Samples were analyzed on denaturing polyacrylamide gels and quantified on the phosphorimager. Background values were obtained from regions between bands on each gel and subtracted from the integrals measured for each band of interest. The substrate DNA (S) and the two products (P1 and P2) are simultaneously resolved, allowing the fraction of uncut DNA was calculated as follows: (counts in S)/(counts in S + P1 + P2), all after background subtraction. This definition is insensitive to variations in gel loading. A thorough explanation of the kinetic analysis of restriction enzyme digestions is described elsewhere.^{13,14}

The data analysis was complicated by two issues, described in more detail in our earlier work.¹² (i) The initial timepoint in the nucleosomal restriction digestions exhibits an anomalously large extent of digestion. This was most pronounced for nucleosomes prepared with the hyperacetylated HeLa histones, less pronounced with the control HeLa histones, and even less pronounced with the chicken erythrocyte histones. Native gels of parallel mock digestions exhibit quantitatively similar behavior. We conclude that a small fraction of the nucleosomes is dissociating upon exposure to digestion conditions, thus allowing a burst of digestion on the newly generated naked DNA. Due to the high concentrations of restriction enzyme utilized in the nucleosome digestions, the naked DNA is digested nearly instantaneously. To address this issue, we omit the initial timepoint, define the amplitude of the second timepoint as 1.0, and rescale the amplitude of the remaining timepoints accordingly. (ii) A fraction of the nucleosomes is never digested. We traced this behavior to a fraction of nucleosomes being insoluble in the digestion buffer, as measured by an ultracentrifuge-based sedimentation assay (J.D.A. & J.W., unpublished results). This behavior has been demonstrated before in nucleosome solubility experiments.²⁸ We address this issue by allowing the baseline to float in the non-linear-least squares fit of the data. We fit the data from each digestion using the following equation: fraction uncut = $(m2 + (1 - m2) \times \exp(-m1 \times \text{time}))$. The ratio of ($m1$) values for control nucleosomes versus experimental hyperacetylated nucleosomes (scaled for their enzyme concentrations, which are generally identical between pairs of nucleosomal samples) yields the fold-enhancement of site exposure due to the hyperacetylated histones.

In order to assess the appropriateness of this analysis, several of the data sets were fit to a double exponential

(results not shown). Examining the resulting pairs of rate constants separately or averaging them to obtain amplitude-weighted mean values leads to results that are quantitatively similar and qualitatively equivalent to those obtained in the single exponential analysis. We therefore focus the analysis on the single exponential fits as these have fewer adjustable parameters.

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