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Extra View

Switching on Chromatin

Mechanistic Role of Histone H4-K16 Acetylation

Michael Shogren-Knaak*

Craig L. Peterson

Program in Molecular Medicine; University of Massachusetts Medical School; Worcester, Massachusetts USA

*Correspondence to: Michael Shogren-Knaak; Department of Biochemistry, Biophysics and Molecular Biology; Iowa State University; 4214 Molecular Biology Building; Ames, Iowa 50011 USA; Tel.: 515.294.9015; Fax: 515.294.0453, Email: knaak@iastate.edu

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ABSTRACT

How post-translational histone modifications regulate DNA utilization constitutes one of the central questions of chromatin biology. In studying the mechanistic role of histone H4-K16 acetylation, a mark with a functional role in maintaining transcriptionally permissive DNA domains or directly promoting gene transcription, we found that this acetylation both disrupts higher-order chromatin structure and changes the functional interaction of chromatin-associated proteins. The potential significance of this finding for in vivo chromatin structure, establishment of euchromatic domains, and promotion of gene transcription is examined.

DNA in eukaryotic organisms does not exist free in cells, but instead is present as chromatin, a complex assembly of DNA, histone proteins, and chromatin-associated proteins. Chromatin exhibits a complex hierarchy of structures, but in its simplest form it is composed of long linear arrays of nucleosomes. Nucleosomes contain 147 base pairs of DNA wrapped around a histone octamer, consisting of two copies each of histones H2A, H2B, H3 and H4, where 15-38 amino terminal residues of each histone protein extends past the DNA gyres to form histone "tails".[1] Chromatin provides a versatile regulatory platform for nearly all cellular processes that involve DNA, and improper chromatin regulation results in a wide range of diseases, including various cancers and congenital defects. One major way that chromatin regulates DNA utilization is through a wide range of post-translational modification of histones, including serine and threonine phosphorylation, lysine acetylation, methylation, ubiquitination, sumoylation and arginine methylation.[2] Histone H4 K16 acetylation is a modification that occurs on the H4 histone tail and is one of the most frequent of the known histone modifications. We have demonstrated that this mark both disrupts formation of higher-order chromatin structure and changes the functional interaction of chromatin-associated proteins.[3] Our results suggest a dual mechanism by which H4 K16 acetylation can ultimately facilitate genomic functions.

DISTRIBUTION AND ESTABLISHMENT OF H4 K16 ACETYLATION

H4 K16 acetylation is a pervasive post-translational histone modification with a functional role in maintaining transcriptionally permissive DNA domains or in directly promoting gene transcription. In the budding yeast *Saccharomyces cerevisiae* over 80% of histones are acetylated at this site,[4] and this mark is enriched in euchromatic regions of the genome.[5,6] In yeast, H4 K16 acetylation is predominantly mediated by the histone acetyl transferase (HAT) enzyme Sas2,[6] with the less specific H4 HAT, Esa1, also playing a role.[7] In higher eukaryotic organisms, H4 K16 acetylation is less ubiquitous, but can also be highly enriched in specific regions. In the fruit fly *Drosophila melanogaster*, the histones of the X chromosome of males are heavily acetylated at this site.[8] The specific H4 K16 HAT enzyme, MOF, is responsible for this enrichment and is necessary for the 2-fold enrichment of transcription of genes on the X chromosome in male flies.[9,10] While no chromosome in humans is acetylated at histone H4 K16 to a similar degree, the human homolog of this enzyme, hMOF, is responsible for most of the observed H4 K16 acetylation.[11] This acetylation occurs at less than 1/3 of all H4 histones,[12] and may also be established by the human Esa1 homolog Tip60,[13] and by the p300/CBP HAT enzymes.[14] Recent work has shown that a common feature of a number of cancers is a loss of H4 K16 acetylation.[15]

H4 K16 ACETYLATION DISRUPTS HIGHER-ORDER CHROMATIN STRUCTURE

We were interested in testing whether H4 K16 acetylation might exert its biological effect by directly changing higher-order chromatin structure. Chromatin undergoes compaction both through intramolecular nucleosome-nucleosome interactions that establish the 30 nm fiber and through intermolecular strand-to-strand interactions that are thought to establish the 100–400 nm interphase chromatin fiber.[16] The N-terminal histone tails have roles in mediating both of these interactions.[17,18] Among the tails, the H4 tail has special significance, and a hint of this fact came initially from the first crystal structure of a mononucleosome (Fig. 1).[1] In this structure, a crystal contact between the H4 tail of one mononucleosome and an acidic H2A/H2B patch of an adjacent mononucleosome were observed, suggesting that this interaction may directly mediate chromatin compaction. This observation has since been substantiated by solution-phase studies of chromatin model systems, nucleosomal arrays, where the H4 histone tail is the predominant mediator of both inter- and intramolecular compaction,[19] and can be cross-linked to the H2A/H2B acidic patch by the introduction of cysteine residues.[20] H4 K16 is one of the residues that mediate interaction between the H4 tail and the H2A/H2B acid patch, and we speculated that acetylation of this residue might disrupt this mechanism of compaction.

Acetylation of histones tails, especially for histone H3 and H4, has long been correlated with transcriptionally active and accessible genes.[21,22] Support for the idea that this acetylation might be causally related to increased gene accessibility and transcriptional activity came from in vitro biophysical studies of nucleosomal arrays containing hyperacetylated histone isolated from cells that were treated with butyrate.[23] The nucleosomal arrays containing the hyperacetylated histones (although not the histones from untreated cells) could not undergo intramolecular chromatin compaction suggesting that something about the highly acetylated histones was responsible for the structural change. This result was consistent with older models of the mechanistic action of acetylation, where bulk neutralization of the positive lysine residues by acetylation would prevent them from interacting with negatively charged DNA and thereby result in chromatin decondensation. However, in the highly heterogeneous system of hyperacetylated histone, whether the levels of acetylation or specific sites of acetylation were important was not clear.

To test our hypothesis that H4 K16 acetylation directly disrupts chromatin compaction, we probed the effect of this modification on the higher-order structure of a nucleosomal array model system.[3] Histone H4 uniformly acetylated at only lysine 16 was prepared by using native chemical ligation.[24,25] In this technique the desired modified histone was assembled by ligation of an acetylated H4 tail peptide (residues 1–23) and a recombinantly expressed H4 core fragment (residues 24–102) to form the full-length product connected by a canonical peptide bond. Nucleosomal arrays containing H4 K16 acetylation were prepared and compared to arrays either lacking this modification or lacking the H4 tail entirely.[19] Analysis of intramolecular chromatin compaction by analytical ultracentrifugation showed that arrays acetylated at H4 K16 could not achieve the fully compacted 30 nm fiber state adopted by the unacetylated arrays (Fig. 2).[26] Furthermore, the extent of array decondensation exhibited by the acetylated arrays matched that shown by arrays entirely lacking the H4 tail.[19] Analysis of intermolecular chromatin compaction by differential sedimentation similarly showed that H4 K16 acetylation disrupts strand-to-strand interactions to the same extent as loss of the H4 tail (Fig. 2).[18] In these array systems, the H4 tail is the only tail that significantly affects either inter- or intramolecular compaction,[19] suggesting that H4 K16 acetylation is a unique histone tail modification that can act as a reversible "switch" to disrupt two forms of higher-order chromatin structure.

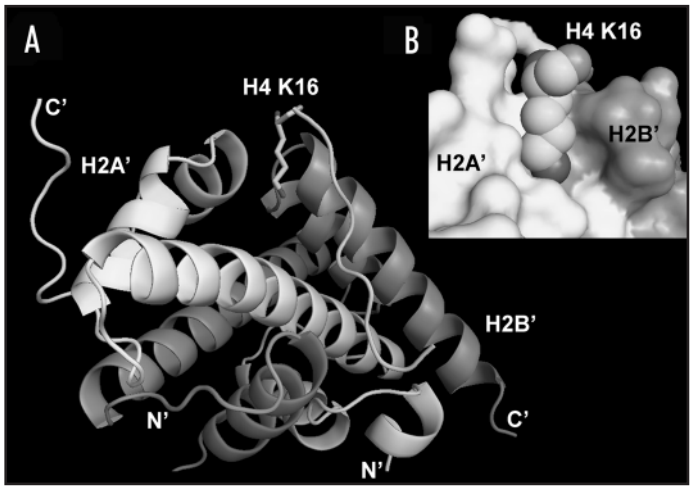

Figure 1. H4 tail interactions with a neighboring H2A/H2B interface based on the 2.8 Å mononucleosome structure solved by Luger and coworkers. (A) Histones H2A (light gray) and H2B (dark gray) are depicted as secondary structure elements. The N- and C-termini of these histones are highlighted. Residues 16–24 of histone H4 from a different mononucleosome (gray) are depicted as random coil secondary structure, with lysine 16, including the main-chain atoms, shown in stick format. The other histones and DNA in both mononucleosomes is not shown. (B) A more detailed depiction of the interaction between histone H4 K16 (sphere depictions, including main-chain atoms), and the surfaces of H2A (light gray) and H2B (dark gray) in the same orientation as (A). To more clearly visualize this interaction, the main chain and side residues of the H4 histone outside of H4 K16 are omitted. This omission removes residues that make up one of the faces of the hole into which the lysine side chain is inserted.

MECHANISM OF CHROMATIN DECONDENSATION AND IN VIVO EFFECTS

Our in vitro results show that H4 K16 acetylation disrupts both intra- and intermolecular chromatin compaction. However, how this disruption occurs is not yet certain. From the mononucleosome crystal structure,[1] interaction observed between the H4 lysine 16 side chain and a cleft in the H2A/H2B acidic patch are likely to be perturbed by the bulky acetyl group (Fig. 1B). This disruption would prevent intramolecular interactions between alternating nucleosomes, consistent with idealized models of 30 nm chromatin fibers generated from the recent low-resolution structure of a tetranucleosome.[27] How H4 K16 acetylation also disrupts strand-to-strand interactions is less clear. Previous studies have shown that intra- and intermolecular compaction can be disrupted independently of one another,[28] suggesting that disruption of the 100–400 nm fiber is not simply due to disruption of the 30 nm fiber. Work remains to be done to better establish the mechanism of chromatin condensation and decondensation. Additionally, as chromatin can adopt even more compact structures beyond the 100–400 nm fiber, the effect of H4 K16 acetylation on these compaction states remains to be determined.

In vivo, chromatin has been observed to adopt regions of 30 nm and 100–400 nm fibers, and thus, if H4 K16 acetylation can disrupt

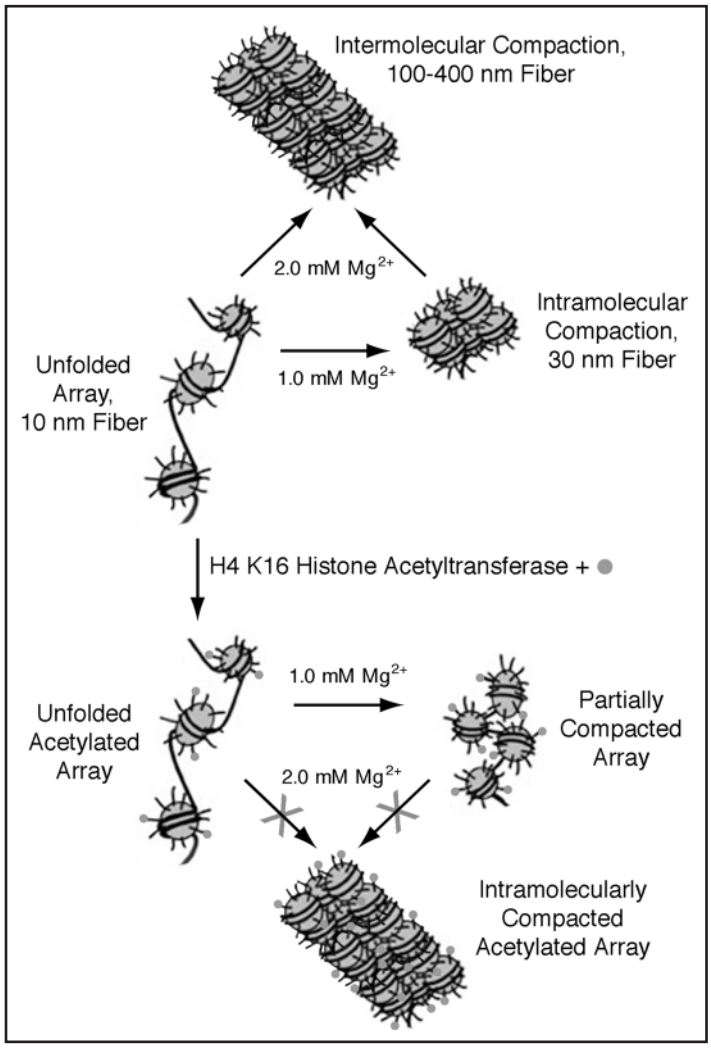

Figure 2. Schematic depiction of structural changes that chromatin undergoes when H4 K16 is either acetylated or nonacetylated. Acetyl groups are depicted as gray circles. Structural transitions and the concentration of magnesium ion used to induce these changes in chromatin model systems are shown as labeled arrows. Arrows with an 'X' through them depict conditions where no structural change is observed.

both forms of higher-order chromatin structure, regions containing high levels of this mark should show some decondensation. Indeed, it has long been observed that the yeast genome, which contains over 80% of histones acetylated at H4 K16,[4] exists in a decondensed state relative to chromatin of higher eukaryotes.[29] Similarly, the X chromosome of male fruit flies, which is highly enriched for histone H4 K16 acetylation,[8] exists in a decondensed state relative to other fly chromosomes.[30] Thus, bulk in vivo chromatin structural data corroborates our in vitro observations on the effect of H4 K16 acetylation.

H4 K16 ACETYLATION CHANGES THE BINDING OF CHROMATIN-ASSOCIATED PROTEINS

While histone H4 K16 acetylation directly perturbs higher-order chromatin structure, it also has the potential to change the binding of chromatin-associated proteins. For example, histone lysine acetylation has been shown to stabilize chromatin association of both the SAGA HAT complex and the SWI/SNF ATP-dependant remodeling complex through binding to subunits harboring bromodomains, thereby promoting transcription.[31] In contrast, H4 K16 acetylation has been proposed to destabilize associations between the H4 histone tail and chromatin-associated proteins. In fruit flies the ATP-dependent ISWI remodeling enzyme binds to the H4 histone tail, with specific recognition of residues surrounding H4 K16.[32] It was proposed that H4 K16 acetylation might disrupt this interaction, and indeed, competition experiments with H4 tail peptides showed that peptides unacetylated at K16 were more effective than those acetylated at K16 as competitors for ISWI activity on nucleosomes.[33] To test whether such effects were observed with substrates that themselves contained H4 K16 acetylation, mononucleosomes containing ligated H4 K16 acetylated histone were generated and compared to nucleosomes containing unacetylated histone.[3] Octamer mobilization by an ISWI-containing complex was assayed, and we found that H4 K16 acetylation reduced the rate of octamer mobilization relative to unacetylated substrates. As these mononucleosome substrates cannot adopt higher-order chromatin structures, these studies suggest that H4-K16 acetylation directly modulates ISWI binding, providing a complementary mechanism for the action of this histone mark.

HOW DOES H4 K16 ACETYLATION ESTABLISH EUCHROMATIC DOMAINS?

Budding yeast provides one of the best-understood models for how euchromatic domains are established and maintained. In this system, loss of H4 K16 acetylation results in the spreading of heterochromatin protein Sir3 into euchromatic regions.[5,6] How Sir3 binding is antagonized by H4 K16 acetylation is not absolutely clear, but it is likely to involve binding to the H4 tail as well as higher-order chromatin structure. Sir3 interacts with the H4 tail, with key contacts involving H4 K16, but not other sites of H4 acetylation (H4 K5, K8 and K12).[34] In fact, studies of Sir3 binding to acetylated H4 tail peptides have shown that H4 K16 acetylation does decrease binding affinity for the H4 tail.[35] Yet, this loss in binding affinity is roughly equivalent to that seen with acetylation of other H4 acetylation sites, leading the authors to suggest that additionally the "in vivo context is important." Structural changes that occur during chromatin decondensation may provide such a context. Sir3 and other associated silencing proteins may make simultaneous binding contacts with both the H4 tail and histone residues in the vicinity of the H2A/H2B acidic patch or other histone tails and require that the nucleosomes be close to one another to stabilize their binding. The unfolding of nucleosomal arrays by H4-K16 acetylation would be expected to disrupt these Sir protein interactions, blocking the spread of heterochromatic structures and maintaining euchromatic domains.

In higher eukaryotes, silencing is established using different proteins than in yeast, and these proteins (e.g., HP1 and polycomb) tend to interact with methylated lysines within the histone H3 tail.[36,37] Therefore, it is unlikely that H4 K16 acetylation directly changes the binding of these proteins. However, because it is speculated that these silencing proteins can associate and spread to form heterochromatic regions, decondensation of chromatin may also destabilize heterochromatin protein binding and spreading.

HOW DOES H4 K16 ACETYLATION AFFECT GENE TRANSCRIPTION?

The presence of nucleosomes can have a repressive effect on transcriptional activation by blocking the DNA binding sites of proteins involved in initiation of transcription.[38] Similarly, in vitro studies suggest that formation of higher-order chromatin structure can also repress gene transcription.[39] We presume that chromatin decondensation resulting from H4 K16 acetylation will increase accessibility of gene promoters to transcription factors, facilitating gene transcription.

In budding yeast, the effect of H4 K16 acetylation on transcription has appeared to be relatively weak, but is difficult to analyze due to the near ubiquitous levels of H4 K16 acetylation in euchromatic regions. Genome-wide expression studies of strains depleted in histone H4 (50% levels) have been performed, and this reduction has been shown to largely affect genes localized to regions near silenced telomeres.[40] It is not surprising that genes already in decondensed euchromatic regions are not as strongly affected as those in heterochromatic regions that are structurally disrupted, as histone H4 depletion reduces the number of H4 tails, decondensing chromatin and preventing Sir3 binding. Attempts to perform similar genome-wide analysis with a more targeted mutation, H4-K16R, have shown similar, overall mild effects, where many of the affected genes are the same as those perturbed by *SIR* gene mutations.[41] H4-K16R is often used as a model for a nonacetylatable lysine because it maintains the positive charge of lysine. However, based on the fact that small structural changes to H4 K16 (i.e., acetylation) have dramatic effect on the specific binding interaction between H4 K16 and the H2A/H2B acidic patch, it seems likely that an arginine residue at position 16 cannot mediate this interaction and may resemble persistently acetylated H4-K16. In such a context, this substitution would only be expected to affect genomic regions that do not already have H4 K16 acetylation, e.g., heterochromatin. Thus, because only heterochromatic regions would be disrupted, this substitution would be expected to affect gene transcription in a similar manner as deletions of the heterochromatic SIR proteins.

As opposed to studies that genetically perturb yeast histones, genome-wide studies that simply correlate transcription and histone acetylation have also been performed.[42] Surprisingly in these studies a negative correlation was found between transcription level and H4-K16 acetylation. However, in this study, loss of acetylation due to histone loss was not accounted for. Loss of histones is emerging as a feature of active promoters,[43] and this may explain the negative correlation. Despite the difficulty of assessing the role of H4 K16 acetylation on yeast gene activation, the fact remains that this mark is prevalent in euchromatic regions, including, potentially, genes that are not transcriptionally active. Thus it seems that for yeast, H4 K16 acetylation, and therefore decondensation, is not sufficient to stimulate transcription. However, its presence may be a necessary condition for many euchromatic genes, and poise them toward rapid activation.

In fruit flies, the connection between H4 K16 acetylation and transcription appears clearer. Targeting of the MOF complex to genes either in vitro or in vivo results in increased transcription.[10] In addition to direct decondensation of chromatin by H4 K16 acetylation, our work and previous work suggest that binding of ATP-dependent remodeling complexes containing ISWI is reduced in the presence of such acetylation.[3,33] It has been proposed that one function of ISWI is to more uniformly space nucleosomes and thereby facilitate formation of compacted chromatin.[33] In both mechanisms, H4 K16 acetylation would ultimately promote the decondensed state, facilitating transcription factor access and gene transcription.

In humans, H4 K16 acetylation and transcription also appear to be linked. It has long been appreciated that regions of active transcription are correlated with chromatin decondensation.[22] Moreover, transcription itself does not appear to be the cause of the decondensation, as targeted recruitment of modified acidic gene activators can promote chromatin decondensation without resulting in transcription.[44] Our collaborators in the Davie lab showed that decondensed regions of chromatin are correlated with higher H4 K16 acetylation,[3] and thus this modification may be the cause of the decondensed state and subsequent increase in transcriptional activity. Further support that H4 K16 acetylation has a direct link to transcription comes from recent studies that show hMOF, the main source of human H4 K16 acetylation, is associated with the MLL complex and directed to gene promoter regions.[45] The MLL complex mediates H3 K4 methylation, which has been well established as a mark associated with actively transcribed genes.

The fact that loss of H4 K16 acetylation is a common hallmark of a number of cancers implies that maintenance of properly regulated chromatin structure plays a key role in cell growth and division and that loss of acetylation may cause alterations in gene expression or chromosome structure that predisposes cells to cancer. Future work will help to elucidate what role H4 K16 acetylation-mediated changes in both higher-order chromatin structure and chromatin-associated protein binding play in the establishment and maintenance of abnormal gene expression and disease.

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