Core Histone Acetylation Is Regulated by Linker Histone Stoichiometry in Vivo*

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We investigated the relationship between linker histone stoichiometry and the acetylation of core histones in vivo. Exponentially growing cell lines induced to overproduce either of two H1 variants, H10 or H1c, displayed significantly reduced rates of incorporation of [3H]acetate into all four core histones. Pulse-chase experiments indicated that the rates of histone deacetylation were similar in all cell lines. These effects were also observed in nuclei isolated from these cells upon labeling with [3H]acetyl-CoA. Nuclear extracts prepared from control and H1-overexpressing cell lines displayed similar levels of histone acetylation activity on chromatin templates prepared from control cells. In contrast, extracts prepared from control cells were significantly less active on chromatin templates prepared from H1-overexpressing cells than on templates prepared from control cells. Reduced levels of acetylation in H1-overproducing cell lines do not appear to depend on higher control cell levels. Overexpression of the linker histone H10 resulted in significantly reduced levels of core histone acetylation in H1-overexpressing cell lines. These effects were also observed in nuclei isolated from these cells upon labeling with [3H]acetate. Reduced acetylation of core histones in H1-overproducing cell lines correlates with reduced H1 stoichiometry. These results suggest that alterations in chromatin structure, resulting from changes in linker histone stoichiometry may modulate the levels or rates of core histone acetylation in vivo.

Chromatin structure plays an important role in the control of gene expression by limiting the accessibility of sequence-specific binding proteins to DNA (1–3). The histone components of chromatin play an integral role in this regulation. Recently, the reversible acetylation of core histones has been recognized as a major mechanism by which chromatin-mediated gene regulation is effected (4–6).

The relationship between histone hyperacetylation and transcriptionally active chromatin was made many years ago (7, 8). Recently, it has been demonstrated that many of the histone acetyltransferases (HATs)1 that acetylate core histones are components of transcriptional activator or coactivator complexes and are specifically targeted to genes to activate transcription (9, 10). Transcriptionally silent chromatin is often hypoacetylated and histone deacetylases (HDACs) have been shown to be components of transcriptional corepressors and silencers that are also targeted to the appropriate DNA sequences (11, 12). Several recent reports provide direct evidence that the acetylation status of nucleosomal core histones has a causal relationship to gene activity (13–16).

The linker or H1 histones also modulate chromatin structure and gene expression (17–24). H1 is often perceived to function as a general repressor of transcription by stabilizing higher order structures (25, 26). Linker histones have also been demonstrated to directly occlude factor binding (27), to limit nucleosome mobility (28, 29), and to reduce the transient dynamic exposure of DNA on the nucleosome surface (30, 31), all of which would be expected to have repressive effects on transcription. Transcriptionally active chromatin has been reported to display reduced H1 stoichiometry (32–34), and thus the removal or reorganization of H1 may be a necessary aspect of gene activation (35, 36). However, recent in vivo studies indicate that the linker histones have positive and negative influences on a subset of genes and may function as specific gene regulators (17–19, 37–39).

The relationship between H1-mediated chromatin modulation and reversible core histone acetylation has received little attention. Acetylation appears to alter the interaction of linker histones with chromatin and may compromise the ability of H1 to promote the formation of condensed structures (40, 41). H1 repression of transcription factor binding to reconstituted nucleosomes was shown to be partly alleviated by increased acetylation of the core histones prior to H1 addition (27). In another study, linker histone-dependent transcriptional repression was not affected by the acetylation state of the core histones (42). However, these studies do not address the inverse relationship, that is, whether the presence of H1 in chromatin influences the acetylation status of the core histones. Binding of H1 has been shown to result in subtle but significant rearrangements of core histone interactions in the nucleosome (43, 44). Therefore, it is reasonable to suppose that histone H1 may have an effect on the properties of the amino termini tails of core histones, including their acetylation status. A recent report demonstrated that the linker histones H1 and H5 specifically inhibit the acetylation of mononucleosomes and oligonucleosomes by the histone acetyltransferase activity of p300/CBP-associated factor (PCAF) in vitro (45). Here we have addressed the influence of H1 stoichiometry on core histone acetylation in vivo.

We developed a system to overproduce H1 histone variants in homologous mouse cells (17–20). Cell lines stably transfected with plasmids containing the coding regions of either of two H1

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1 The abbreviations used are: HAT, histone acetyltransferase; HDAC, histone deacetylase; PCAF, p300/CBP-associated factor; HPLC, high-performance liquid chromatography; TAU, Trichosantin-urea; PMSF, phenylmethylsulfonyl fluoride; CHX, cycloheximide; TSA, trichostatin A.
Regulation of Core Histone Acetylation by H1

Table I

| Cell line | H1α | H1b | H1d/e | H1c | Total |
|-----------|-----|-----|-------|-----|-------|
| MTA       | 0.16 ± 0.02 | 0.16 ± 0.03 | 0.23 ± 0.01 | 0.19 ± 0.01 | 0.81 ± 0.03 |
| MTH10     | 0.74 ± 0.12 | 0.12 ± 0.03 | 0.23 ± 0.01 | 0.11 ± 0.01 | 1.15 ± 0.10 |
| MTH1c     | 0.12 ± 0.02 | 0.11 ± 0.01 | 0.23 ± 0.01 | 0.19 ± 0.01 | 0.70 ± 0.03 |

In Vivo Assays for HAT Activity in Nuclear Extracts—Nuclear HAT activity in cultured mammalian cells is very tightly bound within the nuclei and requires 2 mM salt for extraction (49). A strategy for preparing nuclear extracts containing HAT activity was devised based on the observation that a mild digestion with nuclease renders the nuclear HAT activity susceptible to extraction with less than 0.4 mM salt (50). All steps were carried out on ice unless indicated otherwise. Nuclei were resuspended in DNase I digestion buffer (1× wash buffer for nuclei, 1 mM MgCl₂, 0.5 mM CaCl₂) containing 200 units/ml of RNase-free DNase I (Sigma), incubated at 37 °C for 20 min, and then pelleted by centrifugation at 16,000 × g for 2 min at 4 °C. DNase I treatment renders some HATs soluble; therefore, the supernatant was concentrated 5-fold in a Microcon 10 concentrator by centrifugation at 16,000 × g for 45 min at 4 °C. The concentrated supernatants were pooled together and used in HAT assays.

Soluble chromatin for substrate in HAT assays was prepared as follows. Nuclei from exponentially growing cells were resuspended in 900 μl of DNase I digestion buffer containing 200 units/ml of RNase-free DNase I (Sigma). The nuclei were digested with DNase I at 37 °C for 10 min, and the nuclear suspension was mixed by gentle tapping every 5 min. Nuclei were pelleted by centrifugation at 16,000 × g at 4 °C for 2 min, resuspended in nuclei lysis solution containing 2 mM EDTA and 1 mM EGTA, and incubated on ice for 15 min. Nuclei were pelleted by centrifugation at 16,000 × g at 4 °C for 10 min. The supernatant was collected and concentrated 5-fold in a Microcon 30 concentrator and adjusted to A₂₆₀ = 10.

Assays for HAT activity in nuclear extracts were carried out in 1× wash buffer for nuclei supplemented with 0.5 mM PMSF, 1 mM DTT, 1% thiodiglycol, 20 mM butyrate, 15 μM sodium fluoride, 0.1 mM sodium vanadate, 0.5 mM sodium selenite, 0.5 mM sodium fluoride, 0.5 mM sodium selenite, and 0.5 mM sodium fluoride. Reactions were incubated for 2 h at 37 °C. Reactions were stopped by the addition of H₀S₀, and NH₄OH to a final concentration of 0.2 mM and 0.75 mM respectively, and precipitated by the addition of 3 volumes of ice-cold 100% ethanol. The pellet was washed twice with 70% ethanol, resuspended in 100 μl of water, and counted by liquid scintillation. For analysis of the acetylated proteins, the assay was scaled up and the products separated on TAU gels.

RESULTS AND DISCUSSION

Overexpression of Histone H1α or H1c in Exponentially Growing Cells Results in Decreased Incorporation of Acetate into Core Histones—Exponentially growing cultures of H1-overexpressing cell lines (MTH10, MTH1c) and a control line (MTA) were treated for 4 days with the inducer ZnCl₂. In H1-overexpressing lines this protocol results in major perturbations of the normal ratio of individual H1 variants and in the total amount of linker histone per nucleosome relative to that of control cells. Variant-specific differences in gene expression associated with linker histone overexpression were detected in these cell lines (17–19). In this study we utilized this system to demonstrate that increased H1 stoichiometry inhibits core histone acetylation in vitro.

EXPERIMENTAL PROCEDURES

Cell Culture—The H1α- and H1-overexpressing cell lines, MTH10 and MTH1c, and the control line MTA (transfected with the expression vector lacking H1 sequences) were described previously (17, 20). All experiments were initiated from stocks of stable cell lines stored in liquid nitrogen and were maintained as described previously (17). For the overexpression of H1 histone variants during exponential growth conditions, cells were seeded at a low density; typically less than 10% of the total surface area of the flask was covered. 12 h after the initial seeding, cells were treated with 50 μM ZnCl₂ for 12 h. This was followed by another 4 h of induction with 100 μM ZnCl₂. The media was replaced every 24 h. Cells were harvested prior to confluence; i.e. no more than 75% of the surface area of the flask was covered by cells at the time of harvesting. Total chromatin-bound histones were extracted with 0.2 M H₃O₂ and separated by high performance liquid chromatography (HPLC) as described previously (17, 18) or separated on Triton-acylurea (TAU) gels as described below.

TAU Polyacrylamide Gel Electrophoresis—TAU polyacrylamide gel electrophoresis was employed to separate post-translationally modified forms of histone proteins, as described by Zweidler (47). These gels contained 0.37% Triton X-100, 5% acetic acid, 8 M urea, 12% acrylamide, and 0.08% bisacrylamide. Gels were fixed and stained for 1 h at room temperature in fixing and staining solution (0.1% Coomassie Brilliant Blue R-250 in 50% methanol and 7% acetic acid) and processed for fluorography using Entensify (PerkinElmer Life Sciences) fluor solution according to the manufacturer’s instructions. Dried gels were exposed to Kodak X-OMAT AR autoradiography film with intensifying screen at −80 °C for 2 weeks to 6 months.

Isolation and Treatment of Nuclei—Nuclei were prepared as described previously (48). Isolated nuclei were resuspended in an appropriate volume of 1× wash buffer for nuclei (10 mM Tris-HCl, pH 7.4, 15 mM NaCl, 60 mM KCl, 0.15 mM spermine, 0.5 mM spermidine) and processed further as described in the figure legends and text. For experiments involving acetylation of core histones following micrococal nuclease treatment (Fig. 6), nuclei isolated as described above were resuspended in 450 μl of wash buffer for nuclei supplemented with 200 μM CaCl₂ and 3 units/ml micrococal nuclease (Sigma) and incubated at 37 °C for 15 min. The digestion was stopped by pelleting the nuclei by centrifugation at 16,000 × g for 2 min at 4 °C. The supernatant was discarded, and the nuclei were processed further as described in the figure legends and text. For the analysis of nuclease-digested DNA, parallel aliquots were stopped by adding 50 μl of 10× stop buffer (20 mM EDTA, 10 mM EGTA, 5% SDS) and proteinsase K to a final concentration of 200 μg/ml. After overnight digestion at 50 °C, the DNA was extracted with phenol:chloroform and ethanol-precipitated. Samples were resuspended and electrophoresed on a 1.8% Metaphor-agarose gel.

Variant genes, H1α or H1c, under control of a mouse metallothionein gene promoter were created. Treatment of these lines with the inducer ZnCl₂ results in perturbations of the normal ratio of individual H1 variants. This treatment also results in an increase in the total amount of linker histone per nucleosome relative to that of control cells. Variant-specific differences in cell-cycle progression and both variant-specific and variant-independent differences in gene expression associated with linker histone overexpression were detected in these cell lines (17–19). In this study we utilized this system to demonstrate that increased H1 stoichiometry inhibits core histone acetylation in vivo.

According to the nomenclature proposed by Parseghian et al. (46), H1c is a member of the H1α-1 class of somatic H1 variants. Some relative to that of control cells. Variant-specific differences in cell-cycle progression and both variant-specific and variant-independent differences in gene expression associated with linker histone overexpression were detected in these cell lines (17–19). In this study we utilized this system to demonstrate that increased H1 stoichiometry inhibits core histone acetylation in vivo.
Exponentially growing MTA, MTH10, and MTH1c cells were treated with [3H]acetate into the core histones in exponentially growing cells. Sodium [3H]acetate (4.1 Ci/mmol; 10 mCi/ml; PerkinElmer Life Sciences) was added to the culture medium to a final concentration of 20 μCi/ml. Where indicated cycloheximide (CHX) and trichostatin A (TSA) were added to final concentrations of 50 and 100 ng/ml, respectively. Cultures were labeled for 6 h at 37 °C. Total histones were acid-extracted as described previously (17, 18) except that 10 mM sodium butyrate was added to all buffers to inhibit endogenous HDAC activity. Aliquots from equal numbers of cells were resolved on TAU gels and subjected to fluorography.

only partially compensated such that the total amount of H1 per cell is increased. Because we do not have extinction coefficients at 210 nm for each of the variants, the absolute amount of H1 per cell is an estimate. However, in the overexpressing lines the levels of accumulation are 1.2 to 1.3 times that of control cells and are within the physiological range displayed by mouse tissues. We observed no effect on cell viability or cell cycle progression in these or previous studies (19). Importantly, under these conditions we found no evidence of aberrant chromatin structures, although subtle but significant changes in nucleosome repeat length and nucleosome conformation were previously documented (20).

Following this induction protocol cultures were labeled by the addition of [3H]acetate to the culture medium. Parallel cultures were treated during the labeling period with cycloheximide (CHX) to prevent incorporation of label into proteins as acetate-derived amino acids and with trichostatin A (TSA) to inhibit HDAC activity. Total chromatin-bound acid-soluble proteins were isolated and separated by TAU gels (Fig. 1). The fluorograph shows that, relative to control cells, the H1-overexpressing cell lines displayed significantly lower levels of incorporation of label into the core histones. This was observed both in the presence and absence of TSA-mediated hyperacetylation, although the effect is better visualized after TSA treatment due to incorporation of more label. Treatment with CHX alone does not affect acetylation of the core histones (19) (Fig. 1). This indicates that the incorporation of label most likely represents the action of nuclear Type A HATs acting on histones in a chromatin context. In a separate experiment, cultures of these cell lines were treated as above but were not induced with ZnCl2 and therefore displayed similar H1 stoichiometries to one another. No differences were observed among the cell lines in the incorporation of label into core histones (data not shown). We detected no labeling of any of the linker histone variants in this protocol.

Two distinct kinetic classes of histone acetylation have been described previously (51). One class, representing ~10% of the histones, displays rapid labeling and turnover. The second, which includes the bulk of core histones, is labeled more slowly and is more stable. The quantitative differences observed among the cell lines upon labeling with [3H]acetate could reflect a specific effect on a minor rapidly labeling subset of substrates. To investigate this possibility, total chromatin-bound histones from the TSA-treated samples displayed in Fig. 1 were fractionated by HPLC. Individual fractions containing all the acetylated species of histone H4 were collected and run on TAU gels. Equal amounts of material, based on absorbance at 210 nm, were loaded from each cell line (Fig. 2). Coomassie Blue staining of the gel showed significant differences in the relative amounts of individual acetylated isoforms of these variants. This suggests that the H1-overexpressing cells display reduced bulk levels of acetylated histones under these conditions. However, these differences appear even more dramatic in the resulting fluorographs. This may reflect, in part, differences in the sensitivity of Coomassie Blue staining versus fluorography. Alternatively, and perhaps more interestingly, this may indicate that certain kinetic classes of histone acetylation are differentially affected in H1-overexpressing cells.

As histone acetylation/deacetylation is a dynamic process, the observed differences in label incorporation could conceivably be due to alterations in HAT activity, HDAC activity, or both. Furthermore, at this point we cannot differentiate between effects due to alterations in the chromatin substrate or to changes in the amounts or activities of the enzyme complexes. In the previously described experiments we measured incorporation after a 6-h labeling period. Using a similar protocol we measured the incorporation of label during multiple, shorter labeling periods. Fig. 3A shows quantitation of label incorporation into the tetra-acetylated form of H4. Incorporation was fairly linear for the first 3 h, and the rate of incorporation was approximately 3-fold greater in control cells relative to those overexpressing H1. Analysis of Coomassie Blue-stained gels from the same experiment, although not as sensitive, revealed a similar 3-fold difference between the rate of appearance of tetra-acetylated H4 in control cells relative to those overexpressing H1. Furthermore, the levels of tetra-acetylated H4 prior to TSA treatment allowed us to determine that the specific activity of the label was roughly the same in all cell lines and remained constant throughout the experiment. This indicates that we are approaching initial rate conditions in this assay and, as expected due to the inclusion of TSA, no significant turnover occurred. Qualitatively similar results were obtained for the labeling of each of the core histone variants in this assay.
We next measured the rates of deacetylation of labeled core histones in these cells. Cultures were labeled for 6 h in the presence of CHX and TSA, washed, and then incubated in fresh medium lacking the inhibitors and label (Fig. 3B). Although we see some evidence of a biphasic loss of label, no differences between the cell lines were observed. We conclude that the rates of HDAC activity on the labeled histones are not significantly different among the cell lines in this assay. These kinetic results suggest that the reduced levels of labeled histones reflects decreased HAT activity on the chromatin of H1-overexpressing cell lines.

Core Histone Acetylation in Isolated Nuclei—Differences in the incorporation of label into core histones among the cell lines might be due in part to different rates of entry of acetate into the cell or conversion of acetate to acetyl-CoA, the substrate for HATs. Measurements of the rate of disappearance of the label from the culture media and its appearance in the cytoplasmic and nuclear fractions showed no significant differences among the three cell lines (data not shown). This implies that the rate of isotope entry is likely to be the same in the three cell lines. As an alternative assay, we measured acetylation in nuclei isolated from exponentially growing cultures that were treated with ZnCl2 as described above. These nuclei were briefly labeled with 3Hacetyl-CoA followed by separation of total chromatin-bound acid-soluble proteins on TAU gels (Fig. 4). Due to the short treatment and labeling time, no differences among the cell lines were observed in the Coomassie Blue-stained pattern of the core histones (lanes 1–3). However, there was a clearly less label incorporated into the core histones of nuclei isolated from MTH1c cells relative to the control (compare lanes 8 and 9 to lane 7). We conclude that the reduction in incorporation of tritium label into core histones either in whole cells labeled with tritiated acetyl-CoA, reflects reduced core histone acetylation upon histone H1 overexpression.

We also utilized isolated nuclei to investigate the effect of H1 depletion on core histone acetylation. Parallel samples from the experiment described above were briefly extracted with 0.6 M KCl. This treatment releases H1 histones (and other chromosomal proteins) but not core histones from the nucleus (Fig. 4, compare lanes 4–6 to lanes 1–3). The extracted nuclei were then labeled with 3Hacetyl-CoA. The resulting fluorograph indicates that removal of H1 results in a slight increase in labeling of control nuclei but, most importantly, a very significant increase in labeling of the core histones of MTH1c nuclei (compare lanes 11 and 12 to lanes 8 and 9).

These results are consistent with the notion that H1, in particular H1 at stoichiometries greater than one per nucleosome, is antagonistic to core histone acetylation.

It is obvious from the fluorograph that extraction with 0.6 M KCl alters the pattern of core histone acetylation in all cell lines. Notably, there is a complete loss of detectable labeling of H2B. The Coomassie Blue-stained gel indicates that these proteins are present in the gel. We attribute the lack of labeling to a loss or lability of the major H2B HAT activity during the salt extraction. Results presented in a later section support this contention.

Regulation of Core Histone Acetylation by H1 Stoichiometry Is Mediated through Chromatin Structure—An important issue in the interpretation of these results is whether the observed effects of H1 overexpression on acetylation are due to alterations in the chromatin substrate or to changes in the levels or activities of nuclear HATs and/or HDACs. Previously, we showed that overexpression of H1 variants leads to changes in the expression of a number of genes (17–19). If overexpression of H1 histones results in either a down-regulation of HAT expression or an up-regulation of HDAC expression, this could contribute to the inhibition of core histone acetylation. To measure the nuclear HAT activities in these cells, crude nu-

![Fig. 3. Time course of acetylation and deacetylation of core histones.](image)

![Fig. 4. Effect of H1 variant overexpression on the incorporation of acetyl-CoA into core histones of isolated nuclei.](image)
clear extracts were prepared from control and H1-overexpressing cells as described in “Experimental Procedures.” The substrate was a soluble mixture of oligonucleosomes and mononucleosomes isolated from control (MTA) nuclei following a mild DNase I digestion. HAT activity in the extracts was measured as [3H]acetyl-CoA incorporation into total acid-soluble proteins and was nearly identical in control and H1-overexpressing cells (Fig. 5A), suggesting that H1 overexpression does not affect bulk nuclear HAT levels. We also assayed the extract from control cells on chromatin substrates derived from the H1-overexpressing cell lines. The control extract was found to be approximately 3-fold less active on chromatin templates derived from cell lines overexpressing H10 or H1c, relative to the control templates (Fig. 5A). The values shown in Fig. 5A are the total amount of label incorporated into chromatin-bound acid-soluble material. Nuclear extract and chromatin substrates prepared were scaled up to allow analysis of the labeled products on TAU gels (Fig. 5B). The fluorograph shows that histones H2A, H3, and H4 are clearly labeled in the extracts but H2B is not. The pattern is very similar to that obtained with salt-washed intact nuclei (Fig. 4) and is consistent with loss or lability of HAT activity toward H2B.

Total nuclear HDAC activity in the same extracts was assayed on free hyperacetylated core histones, and no significant differences among the extracts was detected (data not shown). Collectively, these data suggest that the inhibition of core histone acetylation observed upon H1 variant overexpression in vivo is due to differences in the structure of the chromatin rather than a change in the level of nuclear HAT or HDAC activities.

Overexpression of Histone H1 Variants Appears to Inhibit Core Histone Acetylation at the Level of the Nucleosome—Histone H1 could limit accessibility of the core histones to the HATs due to the formation of higher order structures. H1 might also affect nucleosomal structure through interactions with DNA or proteins at the nucleosomal level. Isolated nuclei were prepared, and aliquots were digested with micrococcal nuclease. Analysis of the DNA from these samples by agarose gel electrophoresis revealed that most of the chromatin was digested to mono- or dinucleosomes (Fig. 6A). Parallel aliquots of nuclease-treated nuclei were washed and then labeled with [3H]acetyl-CoA (Fig. 6B). The nuclei prepared from cells overexpressing H10 or H1c incorporated significantly less label than nuclei from control cells. It is therefore likely that the histone H1-mediated inhibition of core histone acetylation occurs primarily at the level of the nucleosome, possibly as a consequence of H1 binding to the nucleosome. As in the salt-washed nuclei and the extract experiments, labeling of H2B was not observed.

The H1 histones are architectural components of chromatin capable of modulating gene expression in general and specific ways (21–23). Here we demonstrate that perturbation of H1 variant stoichiometry in vivo affects the rate of core histone acetylation observed upon H1 variant overexpression.
acetylation and that this is likely mediated by alterations in nucleosomal structure. These results confirm and extend in vitro observations (45) and suggest a possible mechanism by which linker histones could directly influence transcription.

The simplest mechanistic explanation for H1-mediated inhibition of core histone acetylation is that H1 is promoting the formation of higher order structures in which the core histone tails are inaccessible to the HAT enzymes. However, the results from the nuclease digestion experiments suggest that the effect is mediated at least in part at the nucleosomal level. A similar conclusion was reached from an in vitro study of H1-mediated inhibition of PCAF HAT activity (45). In seems unlikely that H1 physically occludes all the core histone acetylation sites at the nucleosomal level. However, a number of studies indicate that H1 binding to nucleosomes results in significant rearrangements of core histone tails and in their interactions with DNA (43, 44).

Although we cannot derive absolute values, the H1/nucleosome ratio exerts unity in the overexpressing cell lines implying that some nucleosomes contain two H1 molecules. In vitro and in vitro evidence for a second H1-binding site has been presented (52–54), and some mammalian tissues naturally contain high H1 stoichiometries (1). The observation that removal of all H1 by high salt extraction preferentially increased acetylation in the overexpressing cell lines (Fig. 4) suggests that the H1 in excess of one per nucleosome is critical for inhibition. Herrera et al. (45) also noted that acetylation of H3 by a multiprotein complex containing PCAF was only inhibited at relatively high H1 stoichiometries. The possibility that some chromatin subdomains have higher H1 stoichiometries is of interest but has not been directly demonstrated. Based on our results, these regions should be relatively hypoacetylated. High H1 stoichiometry and core histone hypoacetylation could conceivably work in concert to silence chromatin subdomains. The observation that HDAC activity is not affected by H1 stoichiometry is also interesting. Acetylated histones are generally believed to be associated with chromatin in a more active or open conformation. Thus, even in the H1-overexpressing lines, the substrates for the HDACs might be in accessible chromatin subdomains.

Biochemical studies have demonstrated that active or potentially active genes are partially depleted in H1 (32–34) or that the manner of H1 binding is altered relative to bulk chromatin (55). Recruitment of HAT activity is a likely early step in gene activation and may require removal or remodeling of H1 (56). The observation that removal of all H1 by high salt extraction preferentially increased acetylation in the overexpressing cell lines (Fig. 4) suggests that the H1 in excess of one per nucleosome is critical for inhibition. Herrera et al. (45) also noted that acetylation of H3 by a multiprotein complex containing PCAF was only inhibited at relatively high H1 stoichiometries. The possibility that some chromatin subdomains have higher H1 stoichiometries is of interest but has not been directly demonstrated. Based on our results, these regions should be relatively hypoacetylated. High H1 stoichiometry and core histone hypoacetylation could conceivably work in concert to silence chromatin subdomains. The observation that HDAC activity is not affected by H1 stoichiometry is also interesting. Acetylated histones are generally believed to be associated with chromatin in a more active or open conformation. Thus, even in the H1-overexpressing lines, the substrates for the HDACs might be in accessible chromatin subdomains.

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The differential temporal and spatial pattern of expression of individual H1 variants or the number of H1 molecules bound to the nucleosome could contribute to specific gene expression in vivo. The results presented here suggest that one mechanism for this regulation may be through modulation of core histone acetylation.

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