

H1 Family Histones in the Nucleus

CONTROL OF BINDING AND LOCALIZATION BY THE C-TERMINAL DOMAIN*

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H1 histones bind to DNA as they enter and exit the nucleosome. H1 histones have a tripartite structure consisting of a short N-terminal domain, a highly conserved central globular domain, and a lysine-and arginine-rich C-terminal domain. The C-terminal domain comprises approximately half of the total amino acid content of the protein, is essential for the formation of compact chromatin structures, and contains the majority of the amino acid variations that define the individual histone H1 family members. This region contains several cell cycle-regulated phosphorylation sites and is thought to function through a charge-neutralization process, neutralizing the DNA phosphate backbone to allow chromatin compaction. In this study, we use fluorescence microscopy and fluorescence recovery after photobleaching to define the behavior of the individual histone H1 subtypes *in vivo*. We find that there are dramatic differences in the binding affinity of the individual histone H1 subtypes *in vivo* and differences in their preference for euchromatin and heterochromatin. Further, we show that subtype-specific properties originate with the C terminus and that the differences in histone H1 binding are not consistent with the relatively small changes in the net charge of the C-terminal domains.

The H1 or “linker” histones are a family of very lysine-rich proteins that associate with the stretch of DNA that enters and exits the nucleosome. In the absence of histone H1, the nucleosome comprises ~146 bp of DNA that wraps 1.75 turns around the outer surface of the histone octamer (1–3). In the presence of histone H1, the resulting chromatosome contains two complete turns of 168-bp DNA. Histone H1, which is in an ~1:1 ratio with the number of nucleosomes in the cell, bends and alters the path of the DNA entering and exiting the nucleosome such that the nucleosome adopts a lollipop-like conformation (4, 5). This change in the trajectory of the linker DNA defines the first step in folding the polynucleosome chain into interphase chromosomes.

The discovery of the H1 histones and the identification of its seven variants in the mid 1970s suggested possible roles in development (6). An additional variant, referred to as H1oo, was

recently isolated from the oocytes of mice (7). These studies revealed associations between histone H1 subtypes in cell growth and differentiation (8–12) and in the development of higher eukaryotes (9–15). In simplistic terms, variants that have been associated with cellular differentiation are the histone H5 and histone H1.0 subtypes, and they are closer in amino acid sequences and most divergent from the other somatic histone H1 variants. Histone H5 is restricted to amphibian and reptile species, where it is found in high abundance in the nucleated but transcriptionally inert erythrocytes (16, 17). The nuclei of these mature erythrocytes are almost entirely heterochromatic, allowing them to maintain a very small total volume. In this case, histone H5 expression correlates with the cessation of the RNA polymerase II transcriptional program (18–20). It is thought that histone H5 functions to transcriptionally inactivate the genome as part of the terminal differentiation program of the erythrocyte (19, 21). Histone H1.0 is the mammalian homolog and is closer in structure to H5 than the other H1 variants (22). Early studies showed that its abundance is increased in cells that are quiescent or terminally differentiated (14). Consistent with this is a recent study showing that overexpression of histone H1.0 can slow cell cycle progression and repress gene expression (23). Logan *et al.* (24) showed that histone H1.0 and RNA polymerase II shared a common antigenic epitope and suggested that H1.0 may compete for similar regions of the chromatin with RNA polymerase II.

There are six histone H1 variants in the somatic cells in mammals, and they have a tripartite structure consisting of a central globular domain flanked by tail domains. The structure of the globular domain has been solved by X-ray crystallography (25), and this domain has routinely been used for studies on reconstituted nucleosomes and chromatin (26–29). Although there are variations in the amino acid sequences, structural studies showed that the globular domain of histone H1.0 adopts a secondary structure that is similar to those of the other variants. The major differences between the variants lie in the flanking domains. Early studies done to define the functions of these domains using reconstituted chromatin showed that the N terminus was not required for inducing higher order structures (30). In contrast, the C terminus was required, in addition to the globular domain, for H1-dependent chromatin folding but was not sufficient for the full nuclease protection afforded the nucleosome upon H1 binding (30). Several recent studies have also revealed the prominent role that the C-terminal domain (CTD)¹ plays in determining the binding properties of histone H1s *in vivo* (31–34). However, studies comparing the binding properties of the variants have been limited by the low sensitivity of the *in vitro* methods for assay-

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¹ The abbreviations used are: CTD, C-terminal domain; FRAP, fluorescence recovery after photobleaching; eGFP, enhanced green fluorescent protein.

ing H1 binding. We have previously shown that fluorescence recovery after photobleaching (FRAP) has the sensitivity to detect subtle differences in binding affinities of histone H1 in live cells (33), and we have used it here to examine the role of the CTD in affecting the binding affinities of individual variants. The C-terminal domain makes up half of the linker histone molecule, and the distinct features (e.g. the number of lysine residues and the (S/T)PXK phosphorylation motifs) of this domain in each variant are highly conserved between species. However, these features vary between the individual variants (35). Therefore, it is likely that the functional heterogeneity observed among the mammalian subtypes of histone H1 resides in differences in the C-terminal domain.

Relative to the histone proteins that comprise the nucleosome core particle, H1 histones have relatively low binding affinity to chromatin and have short residence times. This has been demonstrated both *in vitro* and *in vivo* (36). Using chimeric eGFP-histone H1 proteins stably expressed in human or mouse cell lines, it is possible to quantify this mobility inside the nuclei of live cells (37). Point mutations and deletion experiments using histone H1.1 showed that a single cyclin-dependent kinase phosphorylation site at specific regions within the C-terminal domain can control the association of the entire histone molecule (33). This observation is remarkably consistent with those made using partially deleted histone H1.0 on reconstituted chromatin (34), indicating that the results from these *in vitro* systems can be extrapolated to the living cell.

Further analysis of the contribution of the C terminus of histone H1.1 revealed that this domain plays a key regulatory role in controlling linker histone function, further supporting the observations made using reconstituted chromatin. This provides an explanation for the interspecies conservation of the C-terminal sequences of each variant despite intraspecies divergence of the C terminus within each species.

To further understand the mechanisms that influence both the binding and the function of H1 histones *in vivo*, we examined the binding properties of the six somatic mammalian subtypes in living cells and the influence of the C-terminal domain. We present results showing that the individual histone variants vary significantly with respect to their residency time in chromatin and that they have distinct localization patterns in the nuclei. This study, therefore, provides a mechanistic basis for the differences between individual histone H1 subtypes and sheds light on how these differences can dictate cellular functions.

MATERIALS AND METHODS

Cloning of Histone H1 Variants—PCR amplification of histone H1 variants were described by Lever *et al.* (32), using primers (synthesized by Sigma Genosys) designed from sequences available from GenBank™. The 5'-primers contained BglII restriction sequence and the 3'-primers contained BamHI restriction sequences for directional cloning into pEGFP-C1 (Clontech). The primer pairs for histone H1.0 (accession number X03473), the primer pairs were AGATCTATGACCGAGAATTCACGTC and GGATCCTCACTTCTTCTTGCCGGCCC. For histone H1.1 (accession number X57130) were AGATCTATGTCTGAAACAGTGCTCCC and GGATCCTACTTTTCTTGGGTGCCGC. For histone H1.2 (accession number X57129), the primer pairs were AGATCTATGTCCGAGACTGCTCCTGCC and GGATCCTATTTCTTCTTGGGCGCGCTT. For histone H1.3 (accession number M60747), the primer pairs were AGATCTATGTCCGAGACTGCTCCACTT and GGATCCTCACTTTTCTTCGGAGCTGC. For histone H1.4 (accession number M60748), the primer pairs were AGATCTATGTCCGAGACTGCGCCTGCC and GGATCCTACTTTTCTTGGCTGCCGC. For histone H1.5 (accession number X83509), the primer pairs were AGATCTATGTCGGAACCGCTCCTGCC and GGATCCTACTTCTTTTGGCAGCCGC. To truncate histone H1.5 by terminal 12 amino acid residues, GGATCCTAAGCTTTAGGTTTGGCTGC was used as the 3'-primer. PCR was performed with AccuTaq LA DNA polymerase (Sigma) using

genomic DNA from SK-N-SH cells as template, and the amplification products were transitionally cloned into pCR2.1 (Invitrogen), excised with BglII and BamHI, and transferred to similarly restricted pEGFP-C1.

For cloning of the C-terminal domains, the 5'-primer sequence used for histone H1.1 CTD was AAGCTTCGTCTCCGTGGAAACCAAG, and the 5'-primer for histone H1.5 was AAGCTTTTAAGGCGGCCTCCGGGGA, and the 3'-primers were those used for amplification of the individual variants. The 5'-primers contained HindIII recognition sequence for directional cloning into HindIII- and BamHI-digested pEGFP-C1. These clones were used as templates to amplify the subdomains to construct the domain-switched mutants.

Domain Switch between Histone H1 Variants—For domain switching between histone H1 variants, the N-terminal half containing the N-terminal and globular domains were amplified using the 5'-primers specific for each of the variants described above, and the antisense primer (GCCTTCTTGTGAGTTTAAAGG) was complementary to the 3'-end of the highly conserved globular domain. The 5'-primer used to amplify the C-terminal domain has the sequence CTGGCTCCTTTAACTCAACAAG and contains a stretch of nucleotides that is complementary to the antisense primer use for the globular domain. The 3'-primers used to amplify the CTD were the specific primers used to amplify the histone variants. The N-terminal and C-terminal halves were amplified from the individual clones and gel-purified and were mixed for reamplification using the 5'- and 3'-primers for the individual histone variants that were to be switched. The amplification products were gel-purified and cloned into pEGFP-C1 as described above. PCR products were sequenced by the Paleo-DNA Laboratory (Lakehead University) to verify the mutations.

Fluorescence Recovery after Photobleaching—Cells were transfected with individual histone H1 constructs using Effectene (Qiagen), and stably expressing cells were generated by selection in G418 (Sigma). Following selection in G418, cells were plated onto number 1.5 glass coverslips, mounted in a small volume of tissue culture medium by creating a "well" with vacuum grease (Dow-Corning) on a glass slide. A 1.5-micrometer diameter region passing across the width of the cell nucleus was then photobleached, and recovery was monitored and quantified according to detailed procedures published elsewhere (37).

RESULTS

Sequence Conservation between Human Histone H1 Subtypes—We generated eGFP fusion proteins of histone H1 subtypes 1.1–1.5 as well as H10, a subtype that is up-regulated during entry into G₀ as cells enter contact inhibition, senescence, or differentiation. Fig. 1 shows a diagrammatic representation of the C-terminal domain illustrating the sequence differences and subdomains where structure or function have been mapped. To define the exact roles played by the CTDs in each of the variants, we generated eGFP fusion proteins of histone H1 subtypes 1.1–1.5 as well as H10. The eGFP was attached to the N terminus of each histone H1 variant because of our previous finding that fusion to the C terminus greatly influences binding of histone H1 to chromatin, as the CTD contains the variations between the individual subtypes. Histone H1.1 and H1.2 have the shortest C-terminal regions, whereas histones H1.4 and H1.5 have the longest C-terminal domains. Regions of the C-terminal domain where functions have been defined are indicated with bars in Fig. 1. Although these domains are very lysine-rich, each variant contains a different amount of lysines and arginines that confer different overall net positive charges on the C termini. The 103-amino-acid long CTD of histone H1.1 has the fewest with 38 lysines and 2 arginines, whereas histones H1.4 (110 amino acids containing 43 lysines and 1 arginine) and H1.5 (114 amino acids containing 46 lysines) have the largest number of positively charged amino acids. Not only is histone H1.1 the shortest subtype, it also differs from the remaining subtypes in having only two of the conserved cdk-dependent S/TPXK phosphorylation sites. Interestingly, histone H1.0 is the shortest of the linker histones, with a CTD of 97 amino acids but contains the highest density of basic amino acids with 1 arginine and 41 lysines. It also has one more S/TPXK motif than histone H1.1.

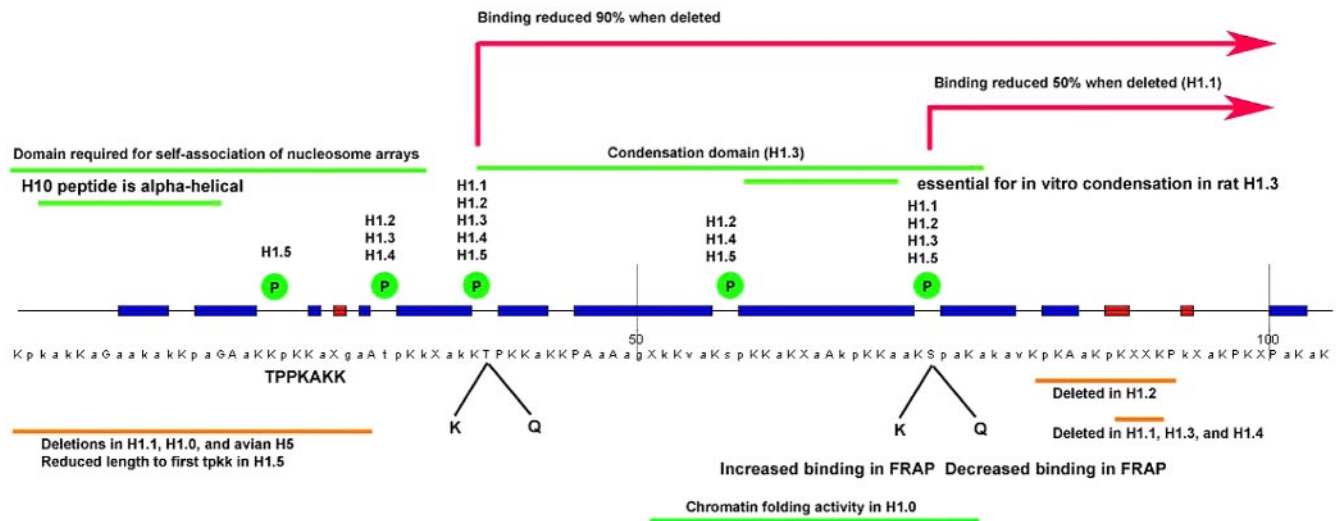


FIG. 1. **Diagrammatic representation of subdomain structure within the C terminus of histone H1.** The amino acid sequence represents a consensus sequence derived from variants H1.1–H1.5. The blue bars above the sequence represent regions that have α -helix-forming potential, whereas the red bars indicate potential β -turns. The remaining annotations indicate regions where structural or functional information has been published on specific subregions of the histone H1 C terminus. The secondary structure prediction was performed using Accelrys DS Gene 1.5 using the Robson and Garnier algorithm.

Binding of Histone H1 Variants in Living SK-N-SH Cells—FRAP has proven to be a powerful tool for studying the chromatin binding properties of histone H1 and allows for measurements to be made within living cells. We have previously used this technique to reveal an unexpected destabilization of histone H1.1 binding to chromatin through a single amino acid substitution at position 152. This result, consistent with a recent *in vitro* study on the contribution of the C-terminal tail of histone H1.0 and histone H5, is inconsistent with the prevailing model of histone H1–DNA interaction that is based solely on electrostatic effects. Based on this result, we reasoned that the small but significant differences (or variations) in primary sequence among the six human somatic cell H1 histones will result in quite distinct binding properties *in vivo*. Fig. 2 shows examples of FRAP experiments comparing the recovery of fluorescence for each of the six major somatic H1 subtypes. The H1 histones, in fact, show remarkably different binding properties *in vivo*. Histones H1.1 and H1.2, which are distinguished by having the shortest C-terminal tails, rapidly undergo binding and dissociation events, requiring only 1–2 min to reach equilibrium. As mentioned previously, histone H1.0 has diverged in amino acid sequence, and the highly divergent C-terminal domain is the shortest among all of the H1 histones. Unlike H1.1 and H1.2, however, histone H1.0 takes longer to recover, requiring several minutes to equilibrate in the FRAP experiment. It is more closely aligned with the remaining H1 histones that have longer C-terminal domains, which exchange much more slowly. These histones, H1.3, H1.4, and H1.5, all require as much as 15 min to equilibrate after the initial photobleaching event.

When we analyzed the recovery curves of ~30 cells and compared the individual histone H1 subtypes for quantitative differences in binding, it can be seen that the different histone H1 subtypes spanned a broad range of recovery times (Fig. 3). The results suggest that the histone H1 variants bound to chromatin with different affinities *in vivo*. Fig. 3 shows the recovery profiles for histone H1 variants in human SK-N-SH neuroblastoma cells. Both histone H1.1 and histone H1.2 bound to chromatin with the lowest affinity, and this affinity increased sequentially with increasing CTD lengths of histones H1.3, H1.4, and H1.5. We tested the statistical significance of the differences in relative recovery required for 50% recovery,

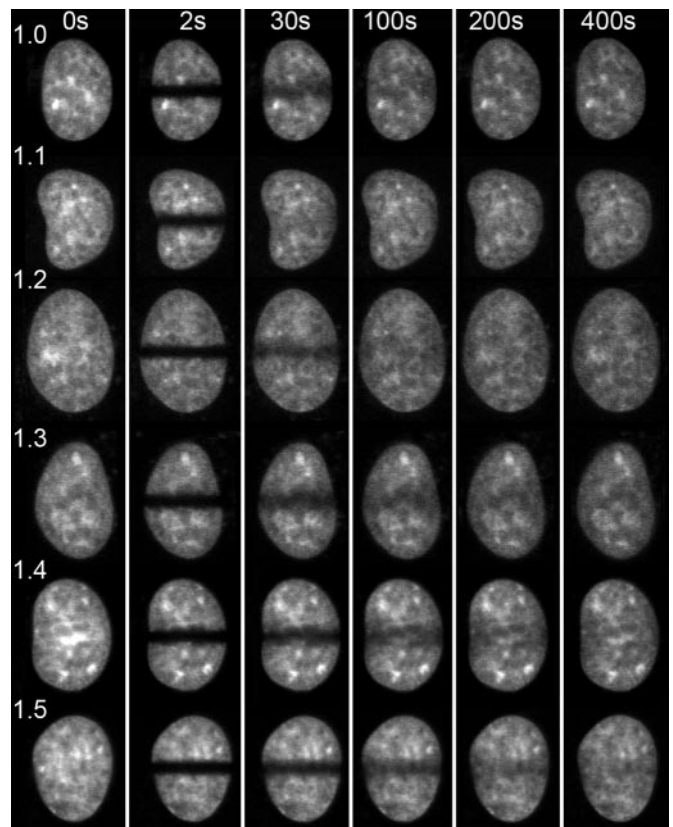


FIG. 2. **FRAP experiments showing the properties of each of the major somatic histone H1 subtypes.** SK-N-SH neuroblastoma cells stably expressing eGFP-histone H1 proteins were examined by fluorescence recovery after photobleaching. The subtypes are shown in sequence according to the Doenecke nomenclature H1.0–H1.5 top to bottom. The elapsed time is shown at the top of each column.

and this analysis segregated the H1 variants into three major binding groups. Histones H1.1 and H1.2 each had binding properties distinct from all other histone H1 variants. Histone H1.0 and histone H1.3 showed only modest differences that were not statistically significant from each other but were significantly different from the remaining histone H1 variants.

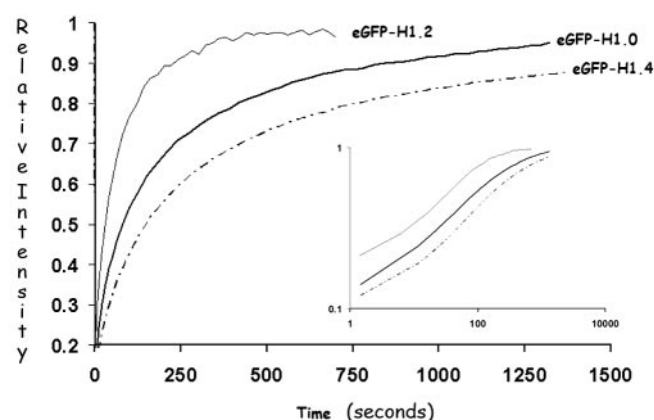


FIG. 3. **FRAP recovery curves for histone H1 subtypes H1.2, H1.0, and H1.5.** The plots show the relative intensity *versus* time profiles for three histone H1 subtypes representing low affinity (H1.2), moderate affinity (H1.0), and high affinity (H1.5) subtypes. The inset replots the recovery profile as the log of relative intensity *versus* the log of time. Each plot represents the mean of 30 separate FRAP experiments.

Similarly, H1.4 and H1.5 had recoveries that were similar to each other but differed significantly from H1.0 and H1.3. This result suggests that the lengths of the CTD of histone H1.1–H1.5 have a major influence on the binding to chromatin. Although histone H1.0 has the shortest CTD, the binding studies suggest that other variations in the rest of the molecule may contribute to its increased binding affinity, such as the higher density of lysine content, and also the density and distribution of the DNA-binding S/TPXK motifs.

The Effect of C-terminal Domain Swapping on Subtype Binding *in Vivo*—To further investigate the extent that the C-terminal domain defines the *in vivo* binding properties of the histone H1 subtypes, we performed domain switches between histone H1.1, the histone H1 subtype with the lowest binding affinity, and histones H1.4 and H1.5, the subtypes with the highest binding affinity. Fig. 4 shows that the H1.1-H1.4 hybrid, consisting of the N terminus and globular domains of histone H1.1 fused to the CTD of histone H1.4, requires considerably longer to equilibrate than the H1.4-H1.1 hybrid, which consists of the N terminus and globular domains of histone H1.4 fused to the CTD of histone H1.1. The replacement of the C-terminal domain of either H1.4 or H1.5 with that of H1.1 significantly reduced the duration of time required for the protein to equilibrate following photobleaching, down to that typical for histone H1.1. Conversely, when the C-terminal domain of histone H1.1 was replaced with either the C-terminal domain of histone H1.4 or histone H1.5, the duration of time required for the protein to equilibrate following photobleaching was significantly increased. For comparison, the wild type proteins are also shown.

Role of the Extended C Terminus in Histone H1.5 Binding—One of the more obvious differences between the tight binding histone H1 variants, H1.4 and H1.5 *versus* the weaker binding histone H1.1 and H1.2 is the length of the C terminus. This contributes to a slight difference in the overall net charge but, when comparing H1.5 with H1.2 for example, there is no change in the number of T/SPXK sites previously implicated as DNA binding domains. Therefore, we tested whether this extension of the C terminus made a significant difference in the binding affinity of the H1.5 protein. Fig. 5 shows the recovery profiles of full-length and truncated histone H1.5. The C-terminal truncation of histone H1.5 results in a significant reduction in the time required for the H1.5 to equilibrate in the photobleached region.

Localization of Histone H1 Variants within the Nucleus—Using variant-specific antibodies, histone H1 subtypes have

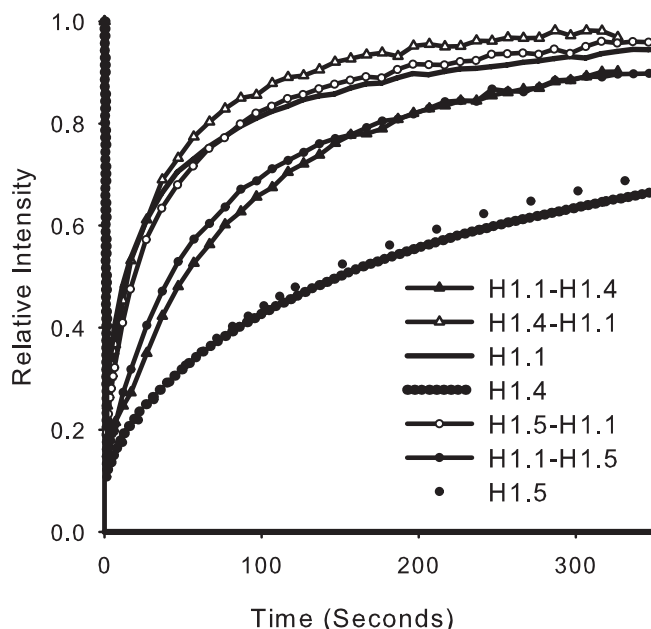


FIG. 4. **FRAP recovery curves for synthetic histone H1 proteins where the C-terminal domains were switched between the individual subtypes.** The plot shows the relative intensity *versus* time profiles for the recovery of synthetic histone H1 subtypes where the C terminus was replaced with the C-terminal domain from a separate histone H1 subtype. For comparison, the wild type proteins are also shown. Each plot represents the mean of 30 separate FRAP experiments.

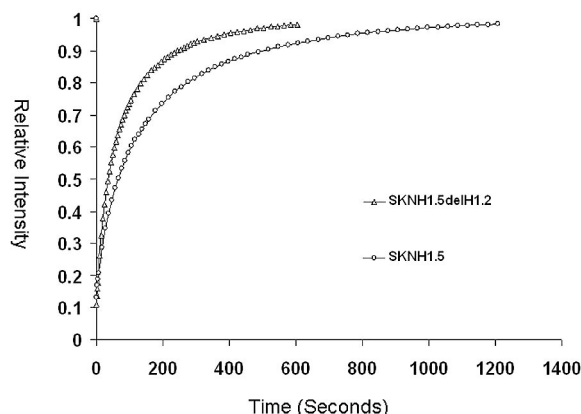


FIG. 5. **FRAP recovery curves for histone H1.5 and truncated histone H1.5.** The plot shows the relative intensity *versus* time profiles for the recovery of histone H1.5 and truncated histone H1.5. Histone H1.2 is also shown for comparison. Each plot represents a mean of 30 separate experiments.

been reported to have specific distributions in the nucleus. Because these differences were not observed when C-terminal GFP-tagged histones were used in a previous study of mouse H1 subtype binding, we reevaluated these results with our N-terminally tagged H1 histone proteins. Mouse fibroblast cell lines have visually distinct heterochromatic and euchromatic regions in their nuclei and were used to evaluate whether or not these H1 subtypes differed in how they distribute throughout the genome. The association of each H1 histone protein with pericentromeric heterochromatin is particularly easy to identify within mouse cells because they are visible as large condensed spheres of chromatin. Although all subtypes were at least occasionally present in mouse pericentromeric heterochromatin, we observed distinct differences in the overall relative distributions of the individual H1 subtypes. The most interesting example is histone H1.0, which was found to in-

crease in quiescent and differentiated cells and has the greatest number of sequence changes when compared with the other somatic subtypes examined (see Fig. 1). Fig. 6 shows a three-dimensional deconvolution image of a mouse fibroblast where GFP-histone H1.0 is visualized simultaneously with Hoechst 33258 in a living cell nucleus. The DNA stained by Hoechst 33258 is false-colored red so that regions of overlap with the green fluorescent histones will appear as yellow. The green fluorescent histone H1.0 tended to localize in the euchromatin regions of the nucleus. Interestingly, a single heterochromatin domain is enriched in histone H1.0, whereas the pericentromeric heterochromatin tended to be depleted of histone H1.0. The other subtypes of linker histones also showed different distinct sublocalization patterns through the nucleus. The green fluorescence staining patterns showed that histones H1.1–H1.3 are found more commonly in euchromatin regions, whereas histones H1.4 and H1.5 are preferentially located in heterochromatin regions of the genome. Table I summarizes the relative distributions observed for ~50 cells examined for each H1 histone variant.

DISCUSSION

The sequence conservation between human and mouse within individual variants is higher than the conservation between individual variants within each species (16, 17). It was, therefore, surprising, when a previous study comparing the *in vivo* binding of histone H1 subtypes H10 and H1c, the murine homolog of histone H1.2, failed to observe a significant differ-

ence between the two (38). This has been well documented *in vitro*, where differences between histone H1 subtypes has been shown for both binding and the capacity to aggregate polynucleosomes into condensed structures (39). This implies functional differences between the different histone H1 subtypes, at least in the context of development of a complex organism. Genetic evidence for functional differences between histone H1 variants is beginning to emerge, and the earlier conclusions that specific subtypes are not critical for developmental regulation may turn out to be an oversimplification based on the ability of embryos to come to term and survive. For example, although histone H1.0 was originally defined as having no phenotype, it has recently been determined that there is a specific defect in dendritic cell differentiation.

In this study, we have examined the *in vivo* binding properties of human histone H1 variants to determine whether or not these variants differ in their binding in living cells. One previous study examined this question using mouse histone H1 subtypes (38). A complication of this study was the use of C-terminal eGFP fusion proteins. We have recently demonstrated that C-terminal fusion of eGFP to histone H1 destabilizes the binding of this protein *in vivo* (33). This is now supported by the clear differences between histone H1 variants that are observed in these studies, which make use of N-terminal eGFP fusion proteins. We found that fusion to the N terminus has minimal effects on chromatin binding, most likely because physical studies showed that the distal subregion of the N-terminal domain is unstructured and do not bind chromatin (40). Furthermore, when we switched the CTDs between histones H1.1 and those of H1.4 and H1.5, this domain exerted a dominant influence over the binding of the hybrid histone, adopting the recovery profile of the native histone that donated the CTD. This shows clearly that the N-terminal domain does not exert much influence on the binding to chromatin.

Initial examination of the recovery profiles of the histone H1 variants suggested a direct correlation between the length of the protein molecule and binding affinity. Because the globular domains are virtually identical, this correlation is with the C-terminal domain. However, this correlation was not seen with the shortest variant histone H1.0. When we compared the content of the lysine/arginine residues in the CTD, histone H1.0 has the highest density (42 basic residues in 97 amino acids), more than the stronger binding histones H1.4 (44 basic residues in 110 amino acids) and H1.5 (46 basic residues in 114 amino acids). The presence of more of the S/TPXK phosphorylation sites in histone H1.4 and H1.5 would suggest that these DNA-binding motifs have greater influence on the binding affinities. The relative contributions to histone H1 binding of these basic amino acids and the S/TPXK motifs are currently being examined. Notably, the truncation of the C terminus of histone H1.5 to the length of histone H1.2 results in a significant reduction in the binding of the H1.5 protein. These results

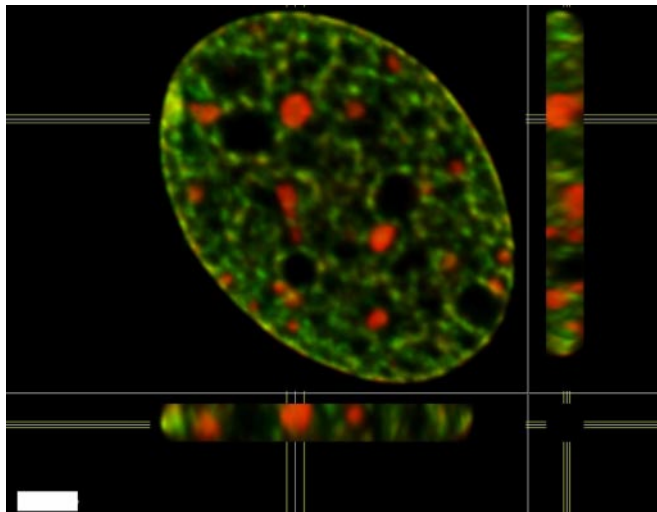


FIG. 6. Three-dimensional reconstruction of histone H1.0 distribution in mouse 10T1/2 cell chromatin. The image shows a two-dimensional projection of a three-dimensional maximum point reconstruction of histone H1.1 distribution (green) relative to total DNA (red). XY (middle) XZ (bottom) and YZ (right) projections were generated from a deconvolved optically sectioned mouse fibroblast nucleus. The scale bar represents 3 μ m.

TABLE I

The percentage of cells showing enrichment in fluorescence in morphologically distinct chromatin types

Five types of chromatin are distinguished, the dense chromatin surrounding the nucleolus (nucleolar HC), the pericentromeric heterochromatin (pericentromeric HC), regions of dense 4',6-diamidino-2-phenylindole (DAPI) staining outside of heterochromatic regions (Eu-high), regions where DAPI staining is evident as discrete chromatin fibers or domains (Eu-intermediate), and regions where DAPI is present but distinct chromatin structures are difficult to resolve (Eu-low).

H1 subtype	Nucleolar HC	Pericentromeric HC	Eu-high	Eu-intermediate	Eu-low
1.0	33	18	22	57	22
1.1	46	50	54	23	23
1.2	28	36	2	21	77
1.3	7	39	2	32	66
1.4	58	70	44	44	12
1.5	33	57	39	45	16

demonstrate that the S/TPXK motifs are not the sole determinants of the affinity of histone H1 binding.

Histone H1.2 is the most abundant nuclear histone in most cell types and represents one of the more weakly bound subtypes, along with histone H1.1. This property may make histone H1.2 a very sensitive probe for double-strand breaks in the DNA. Histone H1.2 has recently been defined as a cytoplasmic cytochrome *c*-releasing factor that is genetically required for the induction of apoptosis following the introduction of double-strand breaks (41).

Histone H1.0 has been the most actively studied single histone H1 variant from mammalian species. Its sequence divergence and accumulation in terminally differentiated and aging tissues has led to the speculation that it is important in the terminal differentiation process. It was recently demonstrated that histone H1.0 is genetically required in the terminal differentiation of dendritic cells (42). H1.0 knock-out mice are specifically deficient in this immune cell type. We find that histone H1.0 binds to chromatin with an intermediate affinity. More interesting, perhaps, is its preference for euchromatic regions of the genome and its depletion in heterochromatin. Enrichment in euchromatic regions of the nucleus imply that it is preferentially involved in the regulation of expressed and expression-competent regions of the genome. Its association with nondividing cells suggests that it may function to repress expression of genes that are no longer required, such as those associated with cell cycle progression.

In summary, the results of our study show that the C-terminal domains, which distinguish the linker histone H1 variants from each other, also control binding to the chromatin. This domain was shown to be important for chromatin organization in early studies using reconstituted systems (30), and we have confirmed this using live cells (33). The importance of these regions is further supported by the conservation between the mouse and human sequences in the distribution of the lysine residues and the cdk-dependent phosphorylation sites. These differences in the C terminus most likely determine the specific functions of the variants in mammalian cells and determine their distinct roles in the development of mammals.

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