Review

Histone variants: are they functionally heterogeneous?

David T Brown

Address: Department of Biochemistry, University of Mississippi Medical Center, Jackson, MS 39216, USA.
E-mail: dbrown@biochem.umsmed.edu

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Abstract

In most eukaryotes, histones, which are the major structural components of chromatin, are expressed as a family of sequence variants encoded by multiple genes. Because different histone variants can contribute to a distinct or unique nucleosomal architecture, this heterogeneity can be exploited to regulate a wide range of nuclear functions, and evidence is accumulating that histone variants do indeed have distinct functions.

The basic subunit of eukaryotic chromatin is the nucleosome [1,2]. Two molecules of each of the core histone proteins - H2A, H2B, H3 and H4 - form an octamer, the protein component of the nucleosome core particle, around which 147 basepairs of DNA are wrapped. One histone molecule of the linker or H1 class binds to the octamer near the point where the DNA enters and exits the nucleosome and seals two full turns (approximately 166 basepairs) of DNA around the octamer [3]. Histone H1 also associates with linker DNA between nucleosomes to stabilize higher-order structures. As nucleosomal structure is similar in all metazoans, it is not surprising that histones are among the most highly conserved proteins in terms of both structure and sequence. But in higher organisms each histone subtype, with the possible exception of histone H4, is represented by a family of genes encoding multiple non-allelic primary-sequence variants [1-5]. Why should this be the case?

There are several plausible explanations for the existence of multiple histone-encoding genes [4-6]. The first is simply gene dosage. A demand for high gene expression at specific times might require multiple active transcriptional units - for example, in the case of histones large amounts are needed during S phase when DNA is replicated and packaged into nucleosomes. In this case, heterogeneity at the protein-sequence level may be the result of genetic drift and would be of little consequence. An extension of this view might include heterogeneity at the level of regulation: multiple histone genes with distinct expression patterns during differentiation, in specific tissues, or under certain metabolic conditions might be necessary to ensure that adequate amounts of each histone are present in all cells. Evidence for this in higher organisms comes from the presence of replacement variants that, unlike most other histones, are expressed throughout the cell cycle and serve as a source of chromatin components needed during repair or recombination of DNA or to replace histones lost through turnover in quiescent cells. Protein sequence variation would be expected to be limited, but variants with greater stability might be evolutionarily selected. Finally, distinct histone variants might have evolved to confer structural heterogeneity on chromatin. Different histone variants can contribute to distinct or unique nucleosomal architectures, which could potentially be exploited to regulate nuclear functions such as transcription, gene silencing, replication or recombination. In this case, the amino-acid sequence variation among the individual variants within a subtype is presumed to be the driving force for creating and maintaining diversity. I refer to this as 'functional heterogeneity', with the reservation that the extent and mechanisms by which it achieves functional effects are far from clear. Of course, aspects of each of these driving forces may be in operation simultaneously, and experimental demonstration, especially in the case of functional heterogeneity, is difficult.

The core histones: deviant variants for deviant purposes

For the most part, the core histones have only a small number of variants and the degree of amino acid sequence

variation within a subtype is limited [1,2]. Replacement variants and partially replication-dependent variants have been described for each subtype except histone H4, and the heterogeneity is probably mostly regulatory. But there are several examples of highly divergent variants and evidence that these histones have specialized functions (Table 1).

CENP-A, a highly conserved histone H3-like variant, is specifically localized to centromeric chromatin in mammals and yeast [7]. The carboxy-terminal two thirds of the CENP-A protein is 62% identical in sequence to histone H3, contains the histone-fold domain, and is required for localizing the protein to centromeric heterochromatin. The amino-terminal 47 amino acids are not related to histone H3. CENP-A synthesis is coordinated with centromeric replication during the mid-S to early G2 phases of the cell cycle. This appears to be important, because expression of CENP-A under control of a histone H3 promoter, which is active early in G1 phase, does not result in centromeric localization of CENP-A [8]. Targeted deletion of the mouse CENP-A homolog results in early embryonic death and disruption of centromeric chromosome organization [9]. The incorporation of this variant in place of histone H3 may serve to episomally mark centromeres for kinetochore assembly, which is required for coordinated separation of sister chromosomes during mitosis.

Histone macroH2A is an extremely divergent variant consisting of an amino-terminal region that has 64% identical amino acids to full-length histone H2A, followed by a large region (57% of the total protein) that is not related to any known histone [10]. The nonhistone region contains a putative leucine-zipper domain and also has similarity to proteins involved in viral RNA replication. Immunofluorescence studies showed that macroH2A is concentrated in the inactive X chromosome of female mammals and remains associated with this chromosome through metaphase [11]. This localization may be mediated through interactions of macroH2A with *Xist*, a non-coding RNA that is tightly associated with the inactive X chromosome. MacroH2A associates with the inactive X chromosome at or near the time of its inactivation in preimplantation mouse embryos, but in differentiating mouse embryonic stem cells the association occurs well after initiation and propagation of inactivation [12]. Also, conditional deletion of part of the *Xist* locus from the inactive X chromosome leads to loss of macroH2A association but does not affect maintenance of X inactivation [13]. Thus, the precise role of macroH2A in X inactivation is unclear.

MacroH2A is found at other chromosomal locations as well as the inactive X chromosome, and it may play a more general role in gene silencing. The strong evolutionary conservation of macroH2A among species, including chickens, which do not display X-chromosome inactivation, supports the idea of a conserved function related to the regulation of gene expression [10]. Interestingly, a novel H2A variant has been recently identified and shown to have characteristics distinctly different from those of macroH2A [14]: H2A-Bbd, which is only 42% identical to histone H2A, is markedly excluded from the inactive X chromosome and may be associated with transcriptionally active regions of the genome.

H2A.Z, a minor H2A variant, is found in a wide range of organisms from yeast to mammals [6]. The sequences of H2A.Z variants of different species are more similar to one another than any single H2A.Z is to the major histone H2A in the same organism. This conservation may reflect a unique functional role, an idea that is supported by the demonstration that H2A.Z is essential for viability in both *Tetrahymena* [15] and *Drosophila* [16]. Swapping experiments, in which regions of H2A.Z were replaced with homologous regions from the major histone H2A, identified a distinct domain of H2A.Z required for the rescue of the developmental defect observed in H2A.Z-null flies [17]. This study is particularly relevant as it provides the strongest direct evidence of functional heterogeneity to date. The essential region mapped to a domain important for docking the H2A/H2B dimer to the H3/H4 tetramer to form the histone octamer, and the crystal structure of core particles containing H2A.Z revealed subtle but significant differences from that of particles containing the major H2A proteins [18]. Recent results indicate that H2A.Z can modulate the folding of nucleosomal arrays into higher-order structures and that knockout of the H2A.Z genes in mice results in embryonic death just after implantation (D. Tremethick, personal communication).

Table 1

Core histone variants with potential unique functions

Variant	Percentage of amino-acid identity to major isotype (%)	Essential for viability?	Proposed function
CENP-A	62	Yes (mouse)	Kinetochore assembly
MacroH2A	64 (in histone region)	Not known	X-chromosome inactivation; gene expression
H2A-Bbd	42	Not known	Transcriptional activation
H2A.Z	59	Yes (mouse, flies, *Tetrahymena*)	Unclear; altered higher-order chromatin structure

The linker histones: extreme diversity for subtle purposes

The linker or H1 class of histones seems the most likely to display functional heterogeneity [4]. The number of H1 variants and their degree of divergence is much greater than that

of the core histones [5,19]. This heterogeneity is limited, however, and it is conserved across biological kingdoms, suggesting that individual H1 variants might have unique properties [20] (Table 2). The H1 proteins play a direct role in stabilizing nucleosomal and higher-order chromatin structures and may function as general or specific repressors of transcription by limiting access of transcriptional activators to chromatin (Figure 1) [21]. Disruption or modification of the binding of histone H1 to the nucleosome may be a necessary step in the activation of many genes, and qualitative or quantitative differences in the modulation of chromatin structure by individual H1 variants might constitute an expression of functional heterogeneity.

Circumstantial evidence in favor of this hypothesis comes from several types of observation. The somatic variants differ in their expression patterns during development and differentiation [22], in their turnover rates [4], and in the extent and schedule of phosphorylation during the cell cycle [23]. An extensive collection of *in vitro* data, starting from the work of R.D. Cole, demonstrates that individual variants differ in their ability to condense a variety of chromatin and DNA substrates [4,24]. Finally, if H1 variants have distinct functions one would expect them to be non-randomly distributed within the nucleus and with respect to active versus inactive genes, and evidence for this has recently been reported [25]. As persuasive as these observations are collectively, the case for functional heterogeneity would be strengthened if specific physiologically significant effects associated with perturbing the stoichiometry of individual H1 variants could be demonstrated *in vivo*.

A clear example of the importance of H1 variant heterogeneity in gene expression is the developmental regulation of 5S rRNA synthesis in frogs. In *Xenopus laevis* there are two clusters of 5S genes, the somatic and the oocyte clusters, which share the same transcription factors, such as TFIIIA.

Table 2

Linker histone variants with potential unique functions

Variant	Organism	Essential for viability?	Proposed function
H1A	*Xenopus*	Not known	Specific gene repression during development
H1t	Mouse	No	Promotion of open chromatin structure; recombination during spermatogenesis
$H1^0$	Mouse	No	Repression of gene expression; stabilization of chromatin structure during differentiation
H1c	Mouse	No	Activation of gene expression
$H1^S$-2, $H1^S$-4	Human	No	Activation of gene expression; selective depletion from active genes

The somatic 5S genes are expressed throughout embryogenesis and in somatic cells. In contrast, the oocyte 5S genes are expressed in oocytes and during early embryogenesis but are repressed near the mid-blastula transition. At the same approximate time during embryogenesis, adult histone H1 genes are expressed and begin to replace the cleavage-stage variant H1M(B4), which is incorporated into nucleosomes during embryogenesis. Manipulation of the levels of adult H1 protein by injection of cDNA or ribozymes demonstrated that the switch in H1 variant type was causal for the specific repression of oocyte 5S gene expression [26,27]. Subsequent studies suggested that differential nucleosome positioning underlies this selective repression, such that the oocyte 5S genes bind adult histone H1 more avidly than they bind the transcription factor TFIIIA, whereas the opposite holds for the somatic 5S genes [28,29]. Of note for this discussion, although nucleosome positioning is important, the replacement of embryonic H1M by adult histone H1 is essential to reinforcing the selective repression of the oocyte 5S genes.

In the mouse there are at least eight H1 variants, including six somatic variants found in most cells and two germline-specific isotypes [5,19]. The testis-specific H1t variant is found in substantial amounts only in pachytene spermatocytes and early spermatids. *In vitro* reconstitution studies suggest that, relative to somatic variants, histone H1t imparts a more open chromatin structure [24]. *In vivo* this property could facilitate recombination or the subsequent chromosomal protein transitions during sperm maturation when histones are replaced by sperm-specific proteins. Surprisingly, three groups have independently demonstrated that mice lacking H1t are fertile and undergo normal spermatogenesis [30-32]. In these H1t-null mice other H1 variants are deposited on chromatin in place of H1t to maintain normal, or near normal, amounts of total H1 histone. Presumably, at least some of these variants are able to compensate for the function of H1t. It may be significant that even in the H1t-deficient mice the chromatin of the germ cells contains, relative to somatic cells, higher levels of histones H1a and H1c and much less H1d and H1e. The function, if any, of the recently described oocyte-specific H100 variant is unclear, but its expression pattern and its sequence similarity to the *Xenopus* embryonic variant H1M suggest a potential role in the regulation of gene expression during oogenesis and early embryogenesis [33].

The role of somatic histone H1 heterogeneity in mammals was explored by inducibly overexpressing individual variants in homologous 3T3 fibroblasts [34]. Overexpression of the mouse $H1^0$ variant led to a transient delay in cell-cycle progression and to reduced steady-state levels of all the polymerase II transcripts that were studied. Overexpression of another variant, H1c, had no effect on cell-cycle progression, however, and led to either no change or a dramatic increase in steady-state transcript levels of all the genes tested. It was subsequently shown that the differential effects of overexpression

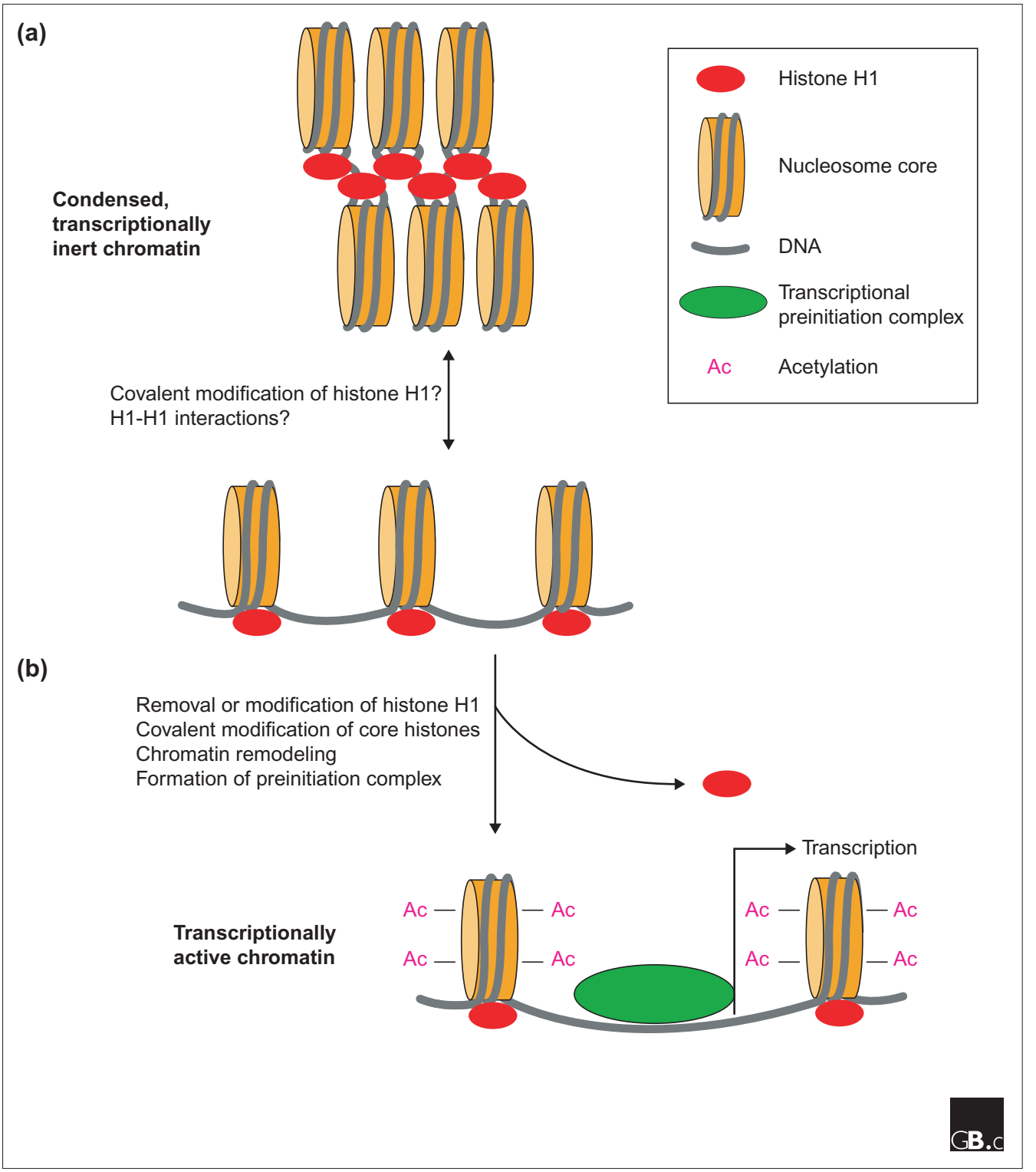

Figure 1
Potential mechanisms of H1-mediated regulation of transcription. **(a)** Linker histones stabilize and/or promote the formation of transcriptionally inert higher-order chromatin structures. **(b)** The removal or modification of the binding of histone H1 to relaxed chromatin structures may be a prerequisite for initiating a series of events resulting in transcriptional activation. These events may include covalent modification (for example acetylation) of core histones, chromatin remodeling by sliding or removal of nucleosome cores, and the establishment of a transcription preinitiation complex. Differences among H1 variants in their ability to promote or antagonize any of these transitions could contribute to transcriptional regulation.

of these two variants are due to differences in their central globular domains [35]. Structural studies indicate that the globular domains of these two variants are very similar, suggesting that subtle differences in the structures of these regions and perhaps in the way they interact with the nucleosome have important consequences for chromatin function. It should be noted that chronic overexpression of either variant for several days results in compensatory responses and the development of cell populations with cell-cycle parameters and gene-expression profiles identical to those of unperturbed cells (D. Sittman, personal communication).

Skoultchi and colleagues have developed a systematic series of histone H1 knockouts in mice ([36] and A. Skoultchi and Y. Fan, personal communication). Homozygous inactivation of any one of the somatic H1 variants does not affect viability or development. In each of the single-gene knockout mice, upregulation of the remaining histone H1 genes resulted in a normal, or near normal, stoichiometry of total H1 histone to nucleosomes. Skoultchi and co-workers went on to create compound knockouts, in which several H1 genes were simultaneously disrupted. Triple-knockout mice lacking H1c, H1d, and H1e die during embryogenesis at embryonic day 9.5-10.5. Analysis of the chromatin in these embryos revealed a reduction in the ratio of H1 histone to nucleosomes of approximately 50%. One clear conclusion from these results is that a major reduction in total histone H1 stoichiometry, which would be expected to have a significant impact on chromatin higher-order structure, is indeed detrimental to mammalian development. But the embryonic lethality in mice lacking multiple H1 variants might actually make it difficult to demonstrate a functional significance for specific variants. What is required are rescue experiments, to create animals in which the total amount of H1 histone is near normal but the repertoire of expressed variants is reduced.

The available evidence seems to indicate that for the extremely modified core histones a strong case can be made for functional heterogeneity. The widespread observation of compensatory responses and the lack of any demonstration of an essential function for an H1 variant suggest a level of functional redundancy. Does this mean that the presence of multiple H1 variants in mammals represents primarily a dosage-compensation or 'regulatory' heterogeneity? I would argue that this is not necessarily the case. The evolutionary and circumstantial evidence cited above cannot be dismissed and certainly suggests that functional heterogeneity among H1 variants is possible. The loss of one variant might be compensated for by another variant that has similar biological properties but perhaps not by any H1 variant. Finally, it should be stressed that the range of assayable phenotypes is limited both in knockout studies with whole organisms and in overexpression studies with cultured cells. The development of new techniques, such as the direct measurement of histone dynamics in living cells [37,38], should provide additional insights into the properties and potential functions of specific histone variants *in vivo*.

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