Role of H1 Linker Histones in Mammalian Development and Stem Cell Differentiation

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Abstract

H1 linker histones are key chromatin architectural proteins facilitating the formation of higher order chromatin structures. The H1 family constitutes the most heterogeneous group of histone proteins, with eleven non-allelic H1 variants in mammals. H1 variants differ in their biochemical properties and exhibit significant sequence divergence from one another, yet most of them are highly conserved during evolution from mouse to human. H1 variants are differentially regulated during development and their cellular compositions undergo dramatic changes in embryogenesis, gametogenesis, tissue maturation and cellular differentiation. As a group, H1 histones are essential for mouse development and proper stem cell differentiation. Here we summarize our current knowledge on the expression and functions of H1 variants in mammalian development and stem cell differentiation. Their diversity, sequence conservation, complex expression and distinct functions suggest that H1s mediate chromatin reprogramming and contribute to the large variations and complexity of chromatin structure and gene expression in the mammalian genome.

Key words

Linker histones, H1 variants, mammalian development, stem cell differentiation, chromatin, epigenetic gene regulation

1. Overview: Histone H1 and its variants in mammals

The DNA of all eukaryotic nuclei is packaged into chromatin by association with histone proteins. The basic repeating unit of chromatin is the nucleosome core particle, which consists of an octamer of four core histones (H2A, H2B, H3 and H4) wrapped by 147 bp of DNA [1, 2]. Linker histone H1 binds to nucleosome core particles and the linker DNA between nucleosomes to facilitate the folding of the “beads-on-a-string” extended chromatin fiber into higher order chromatin structures, the 30-nm fiber [3-6]. For gene transcription to occur, the chromatin template plays a dynamic role. Nucleosome core particles and posttranslational modifications of core histones, such as acetylation, methylation, ubiquitination, phosphorylation and sumoylation, have been shown to play critical roles in gene activation and repression. Much less is known about the role of linker histone H1 and its variants. Here we focus our discussion on recent studies about the function of mammalian H1s in development and stem cell differentiation.

The H1 histone family is the most divergent and heterogeneous group of histones among the evolutionarily conserved histone protein families. There are multiple nonallelic linker histone variants present in higher organisms which provide additional levels of regulation on chromatin structure and function [7, 8]. In mammals, 11 H1 variants have been identified, including seven somatic H1s (H1⁰, H1a, H1b, H1c, H1d, H1e and H1x) and four germ cell-specific H1s (H1t, H1T2, H1LS1 and H1oo) (Table 1). Among the multiple nomenclature systems for mammalian somatic H1 variants, the alphabetic nomenclature (H1a-e, H1⁰ and H1x) and the alternative numeric system (H1.1-1.5, H1.0 and H1.x) are most commonly used [8-12]. Table 1 summarizes their nomenclature as well as H1 variants’ distinct expression and genomic localization patterns.

The expression of H1 variants is differentially regulated during mammalian development and cellular differentiation [13-19]. H1a through H1e are the main types of H1 ubiquitously expressed in somatic cells, yet their expression is tightly regulated with distinct levels in different tissues and cell types [14, 15, 20, 21]. H1⁰ is expressed mainly in differentiated and nondividing cells [22], whereas the least characterized H1x appears to exhibit a G1 phase-dependent nucleolar accumulation in cultured human cell lines [23]. H1oo and H1t are germ cell-specific H1s with expression in oocytes and testis, respectively [24, 25]. H1T2 and H1LS1 are two H1t-related H1s specifically expressed in spermatids [26-30].

All of the mammalian H1 genes are transcribed by RNA Pol II. The genes of major somatic H1 variants (H1a, H1b, H1c, H1d and H1e) and H1t are intronless and transcribed in S phase of cell cycle in a DNA replication-dependent manner (Table 1). Their mRNAs have characteristics of DNA replication-coupled histone mRNAs, lacking a poly(A) tail but terminated by a conserved 3' stem-loop structure. This stem-loop hairpin is bound by the histone RNA hairpin-binding protein or stem-loop binding protein (SLBP) which is required for histone pre-mRNA processing and enhances translation [31-35]. The transcription of these six H1 genes involves typical cell cycle regulation of histone genes: transcription initiation, 3' end processing and mRNA stability. Interestingly, *H1c* gene also produces a polyadenylated form of mRNA besides the 3' stem-loop form mRNA, allowing for independent regulation of expression in dividing and nondividing cells [36]. These six H1 genes (*H1a-e* and *H1t*) are linked and clustered together with core histone genes on mouse chromosome 13 and the orthologous human chromosome 6 [14, 21, 37-40]. This genomic organization is conserved from mouse to human. The other five H1 genes, *H1⁰*, *H1x*, *H1oo*, *H1t2* and *H1ls1*, however, are expressed in a DNA replication-independent manner, with mRNAs polyadenylated and lacking the 3' stem-loop

structure. In addition, they are not clustered with core histones but scattered in the mammalian genomes (Table 1).

All metazoan H1s share the same tripartite domain structure that includes a short, flexible N-terminal tail domain (NTD), a central globular domain (GD) with a winged-helix motif and a long, lysine-rich C-terminal tail domain (CTD) [41-43]. The H1 globular domain is highly conserved, while the N- and C-terminal regions of H1 variants contain more sequence divergence. Comparison of the sequences of mouse and human H1 genes reveals that each mouse H1 variant gene is more similar to its human ortholog than to other mouse H1 variant genes (Fig. 1, Table 2) [44]. Table 2 summarizes the sequence comparisons among all H1 variants in mice and humans. Mouse and human H1⁰ (H1.0) are particularly conserved with 94.8% of homology, whereas they have diverged significantly from other H1 variants with homology less than 45% (Table 2) [44]. On the other hand, H1b (H1.5), H1c (H1.2), H1d (H1.3) and H1e (H1.4) are not only highly conserved from mouse to human with over 85% sequence identity, but also share most similarities with each other, with 76-86% sequence identity. Their sequence similarity suggests that H1b, H1c, H1d and H1e may be more functionally related to each other than to other H1s. Among the 7 somatic H1s, H1x is the least conserved and the most divergent, with 70.9% identity between mouse and human H1x and less than 37% similarities with other somatic H1s. H1x also contains a lower content of basic amino acid residues [45, 46]. Germ cell-specific H1 variants are generally less conserved and more divergent than somatic H1s (Table 2). Mouse H1t shares a moderate identity of 61% with human H1t and 26-55% with somatic H1s, whereas other germ cell-specific H1s (H1oo, H1T2 and H1LS1) have only 10-27% sequence identity with other H1 variants. The sequence conservation of the individual H1

variants suggests that H1 variants may have distinct roles in chromatin function and gene regulation in various cellular and developmental processes.

Consistent with the sequence heterogeneity of H1 variants, cumulative evidence suggests that H1 variants differ in their biochemical properties, affinities for chromatin, capabilities in chromatin compaction and binding partners [42, 47-62]. The globular and C-terminal domains are required for high-affinity binding of H1 to chromatin [42, 47-49]. The globular domain is suggested to bind to nucleosomes at the dyad and the linker DNA with symmetric or asymmetric models [42, 63-67], whereas the CTD is likely to bind to the linker DNA non-specifically. The globular domain of H1 variants may bind to nucleosomes in distinctive structural modes, leading to varied higher order structures [67]. Atomic force microscopy, *in vitro* biochemical assays and fluorescence recovery after photobleaching (FRAP) studies have suggested that H1b, H1d and H1e, the somatic H1s with longer C-terminal tails, display higher affinity for chromatin than H1a, H1c and H1⁰, the somatic H1s with shorter C-terminal tails [51-53]. H1b and H1d are sometimes categorized into the intermediate group of chromatin binding affinity, so is H1⁰, the most lysine-rich H1 with a short C-terminal tail [51-53]. The CTD appears to be the key determinant for the chromatin binding affinity of somatic H1s [48, 53]. The function of CTD in condensing chromatin is related to its length, the density of basic residues, the number of S/TPXK sites and its specific amino acid composition, as well as the intrinsic protein disorder in the CTD [53, 68]. The N-terminal tail appears to be dispensable for chromatin binding, nevertheless, its deletion or swapping between different H1 variants alters the binding affinity of the respective H1 variant for chromatin [47, 48, 50]. Not surprisingly, different H1 variants also display differential *in vivo* binding dynamics in oocytes and during ES cell nuclear transfer [69]. The binding of H1 to chromatin is also regulated by post-translational modifications [54, 55, 70]

and histone chaperones [71, 72]. In addition to binding to DNA and nucleosomes, H1 variants interact with a variety of cellular proteins, which contributes to their diverse functions in various cellular processes [58-62].

Germ cell-specific H1 variants differ dramatically from somatic H1s in amino acid sequences and biochemical properties. H1t exhibits lower binding affinity for DNA and condenses chromatin to a lesser extent than somatic H1s [73-75], which may be attributed to the absence of the S/TPXK motifs, the sites for DNA binding and phosphorylation in CTD [76-78], and the single amino acid substitution of lysine observed in somatic H1s by glutamine in the H1t globular domain [79]. H1T2 is distinctive from H1t in that it is highly enriched with arginine residues and S/TPXK sites [28]. The oocyte-specific H1, H1oo, is the longest variant, with an NTD containing multiple potential phosphorylation sites and an extraordinarily long C-terminal tail rich in acidic amino acid residues [24]. Both the N-terminal and globular domains of H1oo are required for correct association with chromatin in the oocyte nucleus [69]. These intrinsic differences among H1 variants are expected to contribute to their distinct roles in chromatin compaction and gene regulation.

2. Role of histone H1 variants in mammalian development

The role of H1 in development has been interrogated in a variety of organisms. While H1 is nonessential in unicellular organisms such as yeast and *Tetrahymena* [80-84], H1 depletion in higher eukaryotes gives rise to more complex phenotypes [85-92], suggesting the involvement of H1 and its variants in regulation of diverse biological processes.

During mammalian development, the 11 nonallelic H1 variants are abundantly expressed and differentially regulated at all stages, from germ cells to embryos and adult tissues, suggesting their fundamental roles in development. The distinct properties of H1 variants as summarized above indicate that they are excellent candidates as mediators of chromatin reprogramming during mammalian development. In this section, we focus our discussions on the expression and functions of mammalian H1 variants in embryos, adult tissues and germ cells.

2.1. Embryogenesis

Mammalian development starts from a zygote, when the egg is fertilized. Fertilization induces the completion of the second meiotic division of the oocyte and the formation of two separate pronuclei within the zygote, i.e., a haploid paternal pronucleus from the sperm and a haploid maternal pronucleus from the oocyte. At this stage, the protamine-bound sperm chromatin becomes decondensed and the protamines are quickly replaced by the oocyte-specific H1, H1oo, leading to an enlarged sperm pronucleus [24, 93]. The two pronuclei subsequently fuse and cell division ensues.

Before activation of the zygotic genome at the two-cell stage, the oocyte provides the materials necessary for the first cell division. H1oo, the oocyte-specific H1 variant, remains the major H1 variant present till the late two-cell stage embryo (Fig. 2) [24, 93]. It is surmised that the incorporation of H1oo may facilitate the decondensation of paternal chromatin and nuclear reprogramming after fertilization. Evidence supporting this includes that the *Xenopus* homolog of H1oo, B4/H1M, is shown to be crucial for the reactivation of pluripotency genes in somatic nuclei transferred to *Xenopus* oocytes [94] and that ectopic expression of H1oo-GFP in mouse embryonic stem cells (ESCs) leads to increased nuclease sensitivity at specific targets of H1oo-

GFP [95]. *In vitro* experiments using *Xenopus* oocyte extracts showed that B4/H1M is necessary for the architecture and proper segregation of mitotic chromosomes even though it is not required for DNA replication and nuclear envelope assembly [96, 97]. Despite its presumed important roles, H1^{oo} is not required for early embryogenesis *in vivo* as demonstrated by the lack of observable phenotypes of H1^{oo}^{-/-} mice (Table 3) [98, 99]. This result is reminiscent of the knockouts of single somatic H1s (Table 3). While the lack of obvious phenotypes in single somatic H1 knockouts is probably due to the compensation by the remaining somatic H1 variants (as discussed below), the major somatic H1 variants, H1a-e, are unlikely to compensate upon the deletion of H1^{oo} due to the apparent lack of cross regulation of somatic H1 genes with the H1^{oo} gene.

Surprisingly, the other H1 variant protein abundantly present in the oocyte and the zygote is H1⁰, the replacement H1 variant accumulated in differentiated cells (Figs 2, 3). H1⁰ diminishes at the four-cell stage and is present at minimum levels during early embryogenesis, which is consistent with rapid DNA replication and cell divisions at this stage (Figs 2, 3) [100-102]. Similar to H1^{oo}^{-/-} mice, H1⁰ null mice are fertile and develop normally, suggesting that H1⁰ is not required for early embryogenesis either [103]. These studies suggest that, despite their unique expression patterns and properties in the oocyte and the zygote, H1^{oo} and H1⁰ are dispensable for zygotic genome activation and embryogenesis. Nevertheless, H1^{oo} and H1⁰ might help define active and inactive transcription domains in the genome and facilitate proper activation of the zygotic genome. The lack of H1^{oo} or H1⁰ may reduce the efficiency of chromatin reprogramming in zygotes, even though the surviving H1^{oo}^{-/-} or H1⁰^{-/-} embryos and mice are not affected. It is also conceivable that the protein synthesis of other H1 variants is increased in these embryos to compensate for the loss of H1^{oo} or H1⁰.

Synthesis of other somatic H1s, including H1a, H1b, H1c, H1d and H1e, from the maternal mRNA transcripts starts at late one-cell stage, reaching its maximum rate in early two-cell stage embryos (Fig. 2) [104]. The maternal mRNAs of these somatic H1s are degraded in late two-cell embryos, concomitant with the accumulation of their zygotic transcripts [101]. Resumption of somatic H1 proteins occurs during the four-cell stage of embryogenesis, accompanied by the disappearance of H1⁰ (Fig. 2) [24, 102]. The expression patterns of individual H1 variants after the four-cell stage have not been fully defined. The incorporation of somatic H1s into chromatin is likely to facilitate proper cell differentiation and germ layer specification during early embryonic development. This is suggested by studies demonstrating important roles of H1 in ESC differentiation and in loss of mesodermal competence during midblastula to neurula transition in *Xenopus* [16, 90, 105, 106]. HPLC analysis of histone extracts from *E10.5* mouse embryos at mid-gestation indicates a total H1-to-nucleosome ratio of 0.74 that is approximately 3 H1 molecules per 4 nucleosomes (Fig. 2) [13]. H1d, H1e and H1b are the most abundant H1s at this stage, accounting for ~67% of the total H1, whereas H1⁰ is present at a very low level, with an H1⁰-to-nucleosome ratio of 0.02. Given that the accumulation of H1⁰ only occurs in differentiated and non-dividing cells, this expression pattern of H1 variants is consistent with the rapid cell division in midgestation embryos [13].

The functions of somatic H1 variants in mammalian embryonic development have been extensively interrogated by targeted gene inactivation. Although somatic H1s are abundant in embryos, mice with single deletion of H1a, H1c, H1d, or H1e produce litters with normal live pups and litter size, suggesting no obvious defects in embryogenesis in these mice (Table 3) [103, 107, 108]. Even in the context of H1⁰ deficiency, further deletion of H1c, H1d or H1e generates mice that are fertile with no obvious phenotypes (Table 3) [108]. Intercrosses of H1c/H1e

double heterozygous mice produce H1c/H1e double null mice at the expected Mendelian ratio, suggesting that compound deletion of H1c and H1e does not interrupt embryogenesis [108]. However, neonatal males of H1c/H1e double knockout appear to be smaller than wild-type male littermates. Such phenotype is not observed in neonatal H1c^{-/-}H1e^{-/-} females [13]. These results suggest that the loss of H1c/H1e may not be fully compatible with the rapid growth in male embryos, resulting in moderate impairment in their growth. Due to the compensatory increase of the remaining H1 variants controlled by yet unknown mechanisms, single or double H1 deletion does not significantly alter the H1 content in mouse tissues, suggesting somatic H1 variants are, at least partially, functionally redundant during embryogenesis and in adult tissues [13, 108].

H1⁰/H1c/H1e triple homozygous knockout mice are significantly underrepresented in F2 offspring from intercrosses of H1⁰ null mice and H1c/H1e double null mice, suggesting that the loss of H1⁰/H1c/H1e impairs embryogenesis [13]. Currently it is not determined at which stage during embryogenesis some of the H1^{0/-}H1c^{-/-}H1e^{-/-} embryos are lost. Nevertheless, the surviving H1⁰/H1c/H1e triple knockout mice appear normal and fertile despite their mild growth retardation and smaller litter size [13].

The essentiality of histone H1 in mammalian development was demonstrated by the compound deletion of three major somatic H1 variants, H1c, H1d and H1e. Given that most of somatic H1 genes are clustered on chromosome 13 in mice (Table 1), a sequential gene targeting strategy was employed to generate *cis* triply targeted H1c^{+/-}H1d^{+/-}H1e^{+/-} ESCs for production of H1c/H1d/H1e triple knockout mice [13]. H1c/H1d/H1e triple heterozygous (H1c^{+/-}H1d^{+/-}H1e^{+/-}) mice are fertile and viable, but exhibit growth retardation. H1c/H1d/H1e triple heterozygotes are underrepresented in litters from intercrosses of H1c^{+/-}H1d^{+/-}H1e^{+/-} mice and 17% of the triple heterozygotes die during embryogenesis [13]. H1c^{-/-}H1d^{-/-}H1e^{-/-} homozygous mutants are

underrepresented at *E7.5* with increasingly fewer surviving homozygous mutant embryos at the following developmental stages. All triple knockout embryos die by *E11.5*, indicating embryonic lethality at midgestation. Recovered *E9.5* embryos display a broad spectrum of defects, ranging from mild growth restriction and modest developmental delay to severe compromise, such as neural tube aberration, failed chorioallantoic fusion, expanded pericardia, shortened tails and regions of excess tissue [13]. $H1c^{-/-}H1d^{-/-}H1e^{-/-}$ embryos have a 50% reduction in the total H1 level at *E10.5*, suggesting that compensation among somatic H1 variants is disrupted upon depletion of H1c, H1d and H1e. The embryonic lethality phenotype can be partially rescued by a single wild-type *H1d* allele, as $H1c^{-/-}H1d^{+/-}H1e^{-/-}$ (5-) mutant mice are produced from crosses of H1c/H1e double knockout mice with H1c/H1d/H1e triple heterozygotes. However, the majority of these 5(-) mice die in late embryogenesis and only one-third survive to newborns ([13]; Zhang and Fan, unpublished observation). The surviving 5(-) mutant mice are severely retarded in growth and development [13]. These findings demonstrate that significant functional redundancy exists in the H1 family and that the total H1 content, rather than any individual H1 variants, is essential for mammalian embryogenesis.

2.2. Tissue-specific functions

A plethora of studies have shown that the expression of histone H1 variants varies massively across various tissues and even at different developmental stages of the same tissue (Fig. 2) [13, 20, 108-110]. Given the presence of both replication-dependent and replication-independent H1 variant genes and the multitude levels of transcriptional and translational regulation distinct for each H1 gene, the relative abundance of mRNA levels of different H1 variants often does not correlate with the protein composition of H1 variants in specific cell types or tissues. In any

specific tissue, the protein composition of H1 variants is a result of the combinatory effects of the relative proportions of dividing and nondividing cells as well as the complex regulatory mechanisms controlling transcription, mRNA processing and the stability of mRNA and proteins for each H1 variant [14, 20, 22, 111-113].

Earlier studies using gel electrophoresis, such as two-dimensional polyacrylamide gel electrophoresis (PAGE), acid/urea PAGE and SDS PAGE, to analyze H1 histones extracted from mouse or rat tissues found that H1a through H1e are present in almost all tissues examined, consisting with the ubiquitous expression features of somatic H1s. More recent HPLC analyses of histone extracts allow the separation and quantitative measurement of different histone fractions. Using H2B as an internal control, this method provides an accurate assessment of the ratios of H1-to-nucleosome, including ratios of total H1-to-nucleosome and individual H1 variants-to-nucleosome, and standardizes the cross comparisons of the amounts of H1 variants in different cell types and tissues. Figure 2 summarizes available data on the H1 to nucleosome ratios in adult tissues.

Combining data sets from gel electrophoresis and HPLC analyses, we could note the dramatic increase of H1⁰ and H1e accompanied by the decline of H1a, H1c and H1d during tissue maturation and reduction of cell division. This trend is prominent in liver, lung, kidney and cerebral cortex during development (Fig. 2) [13, 110, 114-116]. For example, H1⁰ and H1e account for 9.5% and 19% of the total H1, respectively, in neonatal mouse liver, but their compositions reach 29% and 40% of the total H1 in adult liver (Fig. 2) [13]. Likewise, in differentiating cortical neurons, H1e reaches the highest proportion of the total H1 accompanied by an escalation of H1⁰ [110, 114-116]. During retinal maturation, however, H1c is markedly upregulated (Fig. 2) [117]. Consistent with the accumulation of H1⁰ upon terminal cell division,

H1⁰ is at minimal levels in tissues maintaining active cell proliferation, such as thymus and spleen. H1c and H1d are abundant in most tissues, including adult thymus and spleen, while the level of H1a is rather low in most adult tissues. The distantly related variant H1x is expressed in all tissues examined [45], however, it is not expressed at a comparable level as other somatic H1s that can be clearly detected by HPLC analysis of histone extracts (Fig. 2).

The distinct regulation of H1 variants expression and the specific H1 composition in each tissue during mammalian development may suggest that the abundance and relative proportion of each H1 variant are important for maintaining proper development and tissue maturation. However, mice deficient of any one of the somatic H1 variants, including those of high abundance in specific tissues, do not display obvious defects in particular tissue functions (Table 3) [13, 103, 107, 108, 117]. For example, H1c reaches 30% of the total H1 in rod photoreceptors of adult mice, yet H1c^{-/-} mice have normal rod photoreceptor packing and global heterochromatin condensation in retina [117]. Similarly, H1 proteins in adult liver are largely composed of H1e and H1⁰ which account for 42% and 27% of the total H1, respectively. The loss of either H1e or H1⁰ has no effects on the size or maturation of mouse liver [13, 103].

Functional redundancy among H1 family members and compensation of the lost H1 variants by the remaining H1s are thought to account for the lack of phenotypes in single knockouts of H1 variants. Indeed, even when two major somatic H1 variants are depleted, mice appear to be normal (Table 3). HPLC analysis of histone extracts from mouse tissues indicates that the total H1 levels in these single and double knockouts remain unchanged, suggesting compensation of the deleted H1s by upregulation of the remaining somatic H1s. For example, H1⁰ and H1e together account for 70% of the total H1 in adult liver, yet H1⁰^{-/-}H1e^{-/-} double mutant mice appear to be normal with a typical total H1-to-nucleosome ratio of 0.76 in mature

liver [108]. The compensatory effects are most prominent within the group of H1⁰, H1c, H1d and H1e, indicating potential cross regulatory mechanisms among these H1s [13, 103, 107, 108, 117]. These results suggest a large capacity of increased expression and synthesis of the remaining H1 variants that is sufficient to compensate for the loss of up to two abundant H1 variants.

Disruption of compensation by compound knockout of more than two major H1 variants leads to severe phenotypes, ranging from mild growth retardation to embryonic lethality as summarized in Table 3. In addition, H1^{0/-}H1c^{-/-}H1e^{-/-} and H1c^{-/-}H1d^{+/-}H1e^{-/-} mice have smaller thymuses than those of wild-type littermates. HPLC analysis showed a 40-50% reduction in H1 content in thymus of these mutant mice, suggesting that a low H1 content is not compatible with the rapid cell division in thymus. These studies on compound H1 knockout mice indicate that the total H1 level is critical for normal tissue development and functions [13].

Despite the compensation among H1 variants and the lack of general defects in single and double knockouts of H1 variants, emerging evidence suggests that, the loss of single somatic H1 variants can lead to specific defects. For example, H1⁰ is upregulated during cellular differentiation in all cell types, including dendritic cells [22, 118]. The deletion of H1⁰ does not lead to general defects in differentiation or cell division in most tissues examined, but specifically impairs production and function of dendritic cells in mutant mice [103, 118]. This apparent lack of compensation of H1⁰ by the remaining somatic H1s could be due to a potential failure in upregulation of the remaining H1s or may be attributed to specific cellular functions of H1⁰ in dendritic cells not directly connected to its general role in chromatin condensation, such as H1⁰-specific interactions with other proteins [119]. Similarly, H1c^{-/-} mice display increased resistance to x-ray induced apoptosis in thymocytes and intestines, which is connected to H1c

being a signal transducer in apoptosis induced by DNA double-strand breaks (DSB) [120, 121]. DSB induces the translocation of H1c from the nucleus to the cytoplasm which destabilizes mitochondrial membranes and leads to the release of cytochrome c and apoptosis [120-122].

2.3. Gametogenesis

Gametogenesis is the process of producing haploid gametes from diploid germ cells by meiotic cell division in gonads. It includes spermatogenesis and oogenesis in males and females, respectively. Mammalian gametogenesis is characterized by distinct chromatin remodeling and condensation processes where histone H1 plays a fundamental role [123].

Spermatogenesis has three phases: 1) spermatogonia renewal and proliferation, in which spermatogonial stem cells undergo multiplication to form preleptotene primary spermatocytes; 2) meiosis, in which preleptotene primary spermatocytes enter meiosis to form round spermatids; 3) spermiogenesis, in which spermatids undergo dramatic morphological changes to form elongated spermatozoa [124]. Chromatin condensation during spermatogenesis is mediated by a unique set of male germ cell specific proteins, including testis-specific linker histones, transition proteins and protamines [123]. During spermatogenesis, somatic H1 variants are progressively replaced by testis-specific H1 variants, followed by the replacement of most histones with transition proteins and protamines, resulting in dramatic chromatin condensation culminating in mature spermatozoa.

Spermatogonia express a complement of somatic H1 variants, mainly H1a and H1c [25, 125]. H1a is the predominant H1 variant in prepachytene spermatocytes, comprising approximately 70% of the total H1 [25], but its level steadily decreases in meiotic spermatocytes

and postmeiotic spermatids until it fully disappears (Fig. 3) [126]. H1t, the testis-specific H1 variant, starts to accumulate in pachytene spermatocytes and persists until round spermatids, constituting up to 55% of the total H1 (Figs 2, 3) [127-130]. Both H1a and H1t have relatively low chromatin binding affinity and are weak chromatin condensers [51-53, 73-75], which presumably contributes to the adoption of loosely compacted chromatin in early spermatocytes followed by a relatively open chromatin in pachytene spermatocytes and facilitates the genetic recombination in pachytene spermatocytes and/or the replacement of histones with transition proteins in early spermatids [131]. However, despite their unique expression patterns and abundance during these developmental stages, H1a and H1t appear to be dispensable for spermatogenesis. Mice lacking H1a or H1t are fertile and show normal spermatogenesis [107, 132-134]. HPLC analysis indicates that the total amount of H1 proteins in H1a^{-/-} and H1t^{-/-} mice remain unchanged. Interestingly, the loss of H1a is mainly compensated by increased expression of other somatic H1 variants, H1c, H1d and H1e, whereas the loss of H1t is compensated by upregulation of H1a and H1c [133, 135]. Depletion of both H1a and H1t, which together account for nearly 70% of the total H1 in male germ cells, disrupts the biochemical compensation and leads to a 25% decrease in the total H1 level. Such depletion causes specific changes in gene expression in germ cells without overt phenotypes in H1a^{-/-}H1t^{-/-} mice, which are fertile and have normal spermatogenesis [135]. No defects in homologous chromosome pairing and recombination or differences in chromatin condensation are observed in male germ cells of H1a/H1t double knockout mice, further demonstrating that H1a and H1t are dispensable for chromatin condensation and structural changes during spermatogenesis [135]. In H1a^{-/-}H1t^{-/-} male germ cells, major somatic H1 variants, H1c, H1d and H1e, are markedly upregulated,

suggesting potential roles of somatic H1s in spermatogenesis and in compensating for the loss of H1s specific for or enriched in spermatogenesis.

The other two testis-specific H1 variants, H1T2 and HILS1, are most distantly related and least conserved H1 variants (Table 2). The expression of H1T2 and HILS1 is confined to spermatids (Fig. 3) [26-29]. H1T2 is present in the nuclei of early spermatids and has a particular polarized localization in round and elongating spermatids, associated with chromatin at the apical pole of the spermatid nucleus [26]. The loss of H1T2 in mice severely impairs chromatin condensation and the morphological transformation of round spermatids [26, 28]. These results demonstrate that H1T2 is required for normal spermiogenesis and plays a critical role in chromatin condensation and in establishing cell polarity of elongating spermatids [26, 28]. H1T2 has been found to interact with protamines and its elimination leads to a decrease in protamine content, suggesting a functional role of H1T2 in histone-to-protamine transition [28]. H1T2 and the histone methyltransferase Ezh2 have been found to interact and colocalize at the apical poles of round spermatids, which may suggest a role of H1T2 in chromatin remodeling through regulating histone H3K27 methylation during spermatid elongation [136].

HILS1 is expressed in early and elongated spermatids, and its nuclear localization largely overlaps with that of transition proteins and protamines (Fig. 3) [27]. HILS1 has a higher DNA binding affinity than H1T2, which may contribute to the increased chromatin condensation in late spermatids [28]. No knockout study has been reported for HILS1. Nonetheless, decreased expression of HILS1 in sperms was found to be associated with reduced sperm mobility in human males, offering a potential link between HILS1 and male fertility [137].

Similar to spermatogenesis, the maternal germline stem cells, oogonia, go through mitotic and meiotic phases to produce fertilizable eggs. However, oogenesis does not involve a

spectacular chromatin condensation process as observed in spermatogenesis. Instead, oocytes sustain a chromatin structure much like somatic cells. Oogonia express a complement of somatic H1s, including H1a-e and H1⁰. Starting from the meiotic phase, H1a-e are gradually replaced by the oocyte-specific H1oo (Fig. 2) [24, 101]. The aforementioned unique expression pattern and properties of H1oo suggest that it may have a special role in organizing chromatin structure during oocyte maturation. Indeed, knockdown of H1oo expression by microinjection of antisense morpholino oligonucleotides against H1oo into mouse oocytes or injection of H1oo siRNA into bovine oocytes dramatically impairs first polar body extrusion and meiotic maturation of germinal vesicle stage oocytes *in vitro* [138, 139]. Such defects can be rescued by H1oo, but not somatic H1s, indicating the requirement of H1oo for proper meiotic progression during oogenesis [138, 139]. However, H1oo^{-/-} mice do not exhibit obvious phenotypes [98, 99], demonstrating that the loss of H1oo can be tolerated *in vivo* during oogenesis (Table 3). Further examination of chromatin and meiosis maturation of oocytes from H1oo^{-/-} mice will help elucidate processes and regulatory mechanisms of chromatin remodeling during mammalian oogenesis.

Although H1⁰ is retained throughout oogenesis [100-102], the role of H1⁰ in this process is not clear. H1⁰ null mice are fertile [103], suggesting that H1⁰ is dispensable. As discussed above for other developmental stages, the lack of phenotypes in single H1 knockouts is likely due to the compensation by the remaining somatic H1 variants. The mRNA transcripts of somatic H1s are present in meiotic oocytes and persist until newly fertilized embryos [100, 101], serving as a reservoir for H1 synthesis before zygotic genome activation in early embryos.

In summary, gametogenesis involves a series of chromatin and morphological reorganization steps to produce fertilizable germ cells. The replacement of somatic H1s with

germ cell-specific counterparts suggests that germ cell-specific H1s play a critical role in sperm and oocyte production. Given their distinct biochemical properties from somatic H1s, germ cell-specific H1 variants are likely to mediate or participate in the dramatic chromatin remodeling processes occurring in gametogenesis. However, their exact roles in these processes and early embryogenesis remain underexplored and warrant further investigation.

3. Role of linker histone H1 in stem cell differentiation

Stem cells have the remarkable potential to give rise to different cell types in mammalian tissues. According to their differentiation capacity, stem cells can be categorized into two major groups: pluripotent stem cells such as embryonic stem cells (ESCs) derived from the inner cell mass of a blastocyst, which have the potential to differentiate into all cell types in the body, and adult stem cells, which exist in specific tissues and are capable to generate certain cell types in the tissues. Stem cells are fundamental to embryonic development and tissue generation and can serve as great *in vitro* systems for studying the regulatory mechanisms controlling these processes. Progenitor cells, the early descendants of adult stem cells, have limited capacity in self-renewal and differentiation. Recent studies have uncovered linker histones as important regulators for differentiation of embryonic stem cells and progenitor cells.

Pluripotent stem cells have an open chromatin state with globally hyperactive transcription and undergo chromatin condensation upon differentiation, rendering the chromatin less permissive to transcription [140-142]. Differentiation of pluripotent stem cells involves dramatic molecular changes, including silencing of pluripotency genes and activation of lineage-specific genes [143], as well as global chromatin remodeling and epigenetic reprogramming [144, 145]. FRAP studies have shown that ESCs are characterized by hyperdynamic binding of

chromatin proteins, such as linker histones and heterochromatin protein 1 (HP1), and that ESC differentiation is accompanied by a reduction in the mobility of these proteins [141, 146]. The rapid exchange of H1 proteins appears to be required for ESC differentiation to proceed, as overexpression of an H1⁰ protein with a tandem duplication of CTD for restricting its mobility perturbed ESC differentiation [141]. It is surmised that hyperdynamic binding of H1 and other chromatin proteins provides a “breathing” window rendering the chromatin more accessible to remodeling factors in response to differentiation signals [141]. The high H1 mobility in ESCs may be achieved through citrullination, which neutralizes positive charges and results in a reduced binding affinity for chromatin. Peptidylarginine deiminase 4 (PADI4) catalyzes citrullination on arginine 54 within the globular domain of H1c (H1.2), H1d (H1.3) and H1e (H1.4). PADI4 expression is restricted to pluripotent stem cells and necessary to maintain pluripotency transcriptional network [147]. These findings suggest that H1 dynamics plays a fundamental role in regulating chromatin plasticity during ESC differentiation.

The role of H1 dynamics in modulating differentiation is not exclusive to pluripotent stem cells, but has also been shown in murine erythroleukemia (MEL) cells, an excellent model for studying terminal cell differentiation [148]. Concomitant with MEL differentiation is the dramatic increase in H1⁰ and H1c levels [149] as well as the increased chromatin binding affinity of all histone H1 variants [150]. H1 phosphorylation by cyclin-dependent kinase (CDK) destabilizes H1-chromatin interactions and reduces its binding to chromatin [48, 151, 152]. Overexpression of H1⁰ mutants mimicking the constitutively phosphorylated H1 sufficiently inhibits MEL differentiation, demonstrating that dephosphorylation of H1 is necessary for erythroid differentiation. It is proposed that H1 phosphorylation in MEL cells may cause an

alteration in local chromatin structure without the displacement of H1 from chromatin as observed for H1 citrullination in ESCs [150].

During ESC differentiation, not only is the mobility of H1 reduced, but also the level of total H1 is progressively increased. ESCs have a total H1-to-nucleosome ratio of around 0.46, compared with that above 0.7 in differentiated cells [13, 15, 153]. During embryoid body (EB) differentiation of ESCs, the H1-to-nucleosome ratio increases to 0.62 (Table 3) [16]. The increase in the total H1 level during ESC differentiation is due to the elevation in protein levels of H1c, H1d, H1e and H1⁰ [16], which likely contributes to chromatin condensation. The dynamic changes in H1 expression suggest histone H1 and its variants as important modulators of cellular differentiation. However, deletion of H1c, H1d, H1e, or H1⁰, despite a slight decrease in the total H1 level in mouse ESCs, does not impair ESC differentiation (Table 3) ([154] and Pan, Zhang, Fan unpublished observations). This is consistent with the findings from single H1 knockout studies that individual H1 variants are not required for mammalian development (see Section 2.1). Nevertheless, H1⁰ (H1.0) knockdown in human ESCs impairs the induction of *HNF4*, *Sox17* and *FoxA2*, genes associated with differentiation [106], suggesting a potential role of H1⁰ in regulating specific genes during human ESC differentiation.

The important role of H1 in ESC differentiation has been demonstrated in H1c/H1d/H1e triple null ESCs. These ESCs, derived from H1c/H1d/H1e triple knockout (H1 TKO) blastocysts, have 50% reduction in the total H1 level, offering a useful system to test the necessity of H1 in ESC differentiation [16, 105, 153]. The loss of H1c, H1d and H1e does not appear to affect self-renewal or expression of pluripotency factors in undifferentiated ESCs, and H1 TKO ESCs display growth rate, cell morphology and overall transcriptome characteristic of WT ESCs [16, 153]. However, H1 TKO ESCs are more resistant to spontaneous differentiation, and H1 TKO

EBs are impaired in producing cells and morphologies of three primitive germ layers (Table 3) [16]. When induced for neural differentiation, putative H1 TKO EBs display severe defects in neurite outgrowth and the induction of neural lineage specific genes. A common theme in the various differentiation schemes is the failure in effective repression of pluripotency genes such as *Oct4* and *Nanog* in H1 TKOs, which also occurs *in vivo* in H1 TKO embryos [16]. H1 TKO ESCs are dysregulated in specification of mesendoderm and neuroectoderm [105], further suggesting specific effects of H1 in these lineages. The fact that H1 occupancy at the promoter region of the pluripotency gene *Oct4* increases during ESC differentiation and that the loss of H1 impairs DNA methylation and changes of histone marks at this region necessary for *Oct4* silencing suggests H1 as a critical regulator for epigenetic silencing of *Oct4* [16, 106]. Thus, the effects of H1 on the silencing of pluripotency genes are likely to be direct.

Other evidence establishes specific H1 variants as important regulators for differentiation of progenitor cells. During terminal differentiation of retinal cells in mice, the expression of H1c, along with H1⁰ and H1e, is dramatically increased, contributing to the highly compact heterochromatin and nuclear architecture in mouse rod cells [117]. Although the loss of H1c does not lead to abnormalities in rod cell differentiation, H1c/H1e double knockouts and H1⁰/H1c/H1e triple knockouts displayed defects in rod development and rod cell packing, presumably due to the impairment in the terminal differentiation of rod photoreceptors.

While aforementioned studies suggest the necessity of a sufficient total H1 level for proper differentiation, H1b has been found to participate in the inhibition of myogenesis during myoblast differentiation [90, 155]. H1b is specifically recruited by the homeoprotein Msx1 to the core enhancer region of the *MyoD* gene, which establishes a repressive chromatin state at the *MyoD* gene locus and inhibits the expression of *MyoD* and myogenic differentiation of myoblast

C2C12 cells [155]. Knockdown of H1b by shRNA abolishes the Msx1-mediated repression of myogenic differentiation, and coexpression of Msx1 and H1b in *Xenopus* embryos synergistically inhibits *Xenopus MyoD* [155]. These results are reminiscent of the previous findings in *Xenopus* embryos where inhibition of the accumulation of *Xenopus* H1c (xH1c), the ortholog of mouse H1b, by ribozymes, leads to the failure in loss of mesodermal competence and the lack of *MyoD* silencing [90]. The effects appear to be specific to H1b, as injection of H1e into *Xenopus* embryos does not inhibit *MyoD* expression [155].

4. Potential mechanisms of histone H1 in the regulation of mammalian development and stem cell differentiation

The broad defects in mammalian development and stem cell differentiation uncovered through H1 manipulation as discussed above suggest that H1 plays crucial and versatile roles in these processes. While the exact mechanisms underlying the regulatory roles of H1 in these processes remain to be explored, a few potential mechanisms can be gleaned from the studies of functions of H1 in chromatin structure and gene expression.

Chromatin structure undergoes dramatic changes during development and cellular differentiation. As a key architectural chromatin protein, H1 modulates the bulk chromatin structure in various aspects, such as chromatin condensation, nucleosome repeat length (NRL), histone modifications and chromatin dynamicity. The H1 stoichiometry is positively correlated with nucleosome repeat length (NRL) (reviewed in ref. [15]), both of which are key determinants of the degree of chromatin compaction [156]. Higher H1 content leads to a longer NRL and more compact chromatin fibers, while H1 depletion causes reduction in NRL and chromatin decondensation [13, 109, 117, 153, 157]. The H1 content progressively increases during stem

cell differentiation or terminal cell differentiation, as shown in the total H1-to-nucleosome ratio changing from 0.45 in ESCs to 0.62 in differentiating EBs, or from 1.02 in new born retina to 1.25 in adult retinal cells (Fig. 2, Table 3) [16, 117]. H1 stoichiometry regulates histone modifications in bulk chromatin as shown for specific histone methylations and histone acetylation [153, 158, 159]. The H1 level is also pivotal for promoting heterochromatin formation, such as the establishment and proper function of pericentric heterochromatin and chromocenter clustering [86, 109, 159-162].

The amount and modifications of H1 histones can regulate chromatin dynamicity. Chromatin in pluripotent stem cells is found to be hyperdynamic [141]. The proper level of chromatin flexibility and dynamicity seems to be necessary for chromatin remodeling during development and cellular differentiation, as both the increase of H1 binding affinity in ESCs (by overexpression of the H1⁰ mutant with two C-terminal domains) and the failure to increase H1 binding by dephosphorylation during MEL differentiation (by overexpression of a H1⁰ mutant mimicking the constitutively phosphorylated H1⁰) inhibit cellular differentiation in respective mouse ESCs and erythroleukemia cells [141, 146, 150]. In addition, H1 depletion reduces chromatin flexibility in gene-rich topologically associating domains (TADs) [163].

It is not determined if any or all of the aspects of bulk chromatin structure per se are responsible for the defects in development and cell differentiation displayed in compound H1 knockouts. The lack of overall phenotype *in vivo* in single knockouts, combined with increasingly more severe phenotypes caused by more dramatic depletion of H1 in compound knockouts, establishes a compensatory effect among the H1 family and a common role of H1 variants in development and cellular differentiation. These results, together with the studies showing the necessity of H1 in chromatin architecture and assembly of mitotic chromosomes as

well as the roles of H1 in pericentromeric heterochromatin function and proper chromocenter clustering [86, 97, 109, 164], indicate that H1's regulatory roles in bulk chromatin structure could contribute to general cell functionality required for robust development and proper cell differentiation.

In contrast to its global effects on bulk chromatin, H1 plays rather specific roles in gene regulation *in vivo*. Even though early studies with *in vitro* constituted chromatin indicate a general repressive role of H1 for transcription by all three types of RNA polymerases (reviewed in [165, 166]), depletion of H1 in a variety of organisms and cells shows limited gene expression changes *in vivo* [81, 84, 135, 153, 167]. rRNA genes, transgenes, major satellites and genes at pericentromeric regions, imprinted genes, sex chromosome genes and pluripotency genes, as well as developmental genes important for embryogenesis and gametogenesis, have been characterized as target genes of H1 in various systems [16, 85, 88, 90, 91, 109, 153-155, 168-171]. Interestingly, these genes are often subjected to epigenetic regulation.

Indeed, recent studies have established H1 as an important epigenetic factor for gene regulation. Genes affected by triple deletion of H1c/H1d/H1e in ESCs are enriched in genes normally regulated by DNA methylation, and H1 depletion leads to hypomethylation at the regulatory regions of these H1-regulated genes, such as the imprinting control regions (ICR) of *H19/Igf2* and *Gtl2/Dlk* loci in ESCs [153]. These results reveal a novel link between H1 and DNA methylation, which was also strengthened by studies on the immunoglobulin heavy chain locus [172], the *Rhox* gene cluster [170] and the pluripotency gene *Oct4* locus [154]. The identification of DNA methyltransferases DNMT1 and DNMT3B as H1 interacting partners further provides a mechanism for H1 regulating DNA methylation through direct recruitment of DNMTs [173, 174]. Likewise, H1 has been shown in various studies to regulate histone

modifications by interaction with histone modifying enzymes, including PRC2-Ezh2 polycomb complex, which catalyzes H3K27 di- and tri- methylation [175-178], SirT1, a histone deacetylase preferentially targeting to H3K9Ac and H4K16Ac [179, 180], and Cul4A, an E3 ubiquitin ligase responsible for H4K31 ubiquitination [62]. H1 occupancy can also prevent the binding of certain histone modifying enzymes to specific regulatory loci [174]. Nevertheless, given the general non-DNA-sequence-dependent nature for the binding of H1, DNMTs, and histone modifying enzymes to DNA, how the specificity is achieved at these target gene loci remains elusive. It is noteworthy that many of these H1-regulated genes, such as imprinted genes, are particularly sensitive to local changes in chromatin structure and epigenetic modifications, with a lower threshold for eliciting changes in gene expression and epigenetic states.

H1 variants may also participate in specific gene regulation in a variant-specific manner. As mentioned earlier, H1 variants have distinct expression patterns during development and cell differentiation. They differ from each other in amino acid sequence, biochemical property and binding affinity for chromatin, which may confer specificity in chromatin compaction, protein interactions and gene regulation (See Section 1). While genome-wide mapping studies of H1 variants categorize H1 binding as a repressive epigenetic mark by its positive correlation with repressive histone marks, such as H3K9me3, and negative correlation with active histone marks, such as H3K4me3, different H1 variants do have distinct enriched regions despite their overall co-localization and common binding patterns at the genome-wide scale [109, 181, 182]. Indeed, it has been shown that *in vivo* occupancy of H1a (H1.1) and H1d (H1.3) differ dramatically at the regulatory region of *H19* gene [171], suggesting that distinct binding by different H1 variants

could contribute to the establishment and maintenance of epigenetic patterns and gene regulation at specific loci.

H1 variants may interact with transcription factors, resulting in specific gene regulation of the target genes of those regulatory proteins. The best examples come from studies on H1c (H1.2) and H1b (H1.5). H1c was found to repress p53 target genes in HeLa cells through its interaction with p53, a group of cofactors, and ribosomal proteins [61]. During early embryogenesis, H1c forms a complex with CHD8 to suppress p53-mediated transcription and apoptosis [169]. H1b interacts with the homeoprotein Msx1 to repress *myoD* expression, thus inhibiting myogenic differentiation in myoblast C2C12 cells and mesodermal competence in *Xenopus* animal caps [90, 155]. On the other hand, H1b was found to bind forkhead box transcription factor FoxP3 in human regulatory T (Treg) cells to regulate the expression of FoxP3 target genes, such as *IL2* and *CTL4*, thus modulating Treg function [183]. In the human promonocytic THP-1 cells, H1b binds to NF- κ B factor RelB and forms a repressor complex at the promoters of *TNF- α* and *IL-1 β* , silencing these proinflammatory genes [184]. These results suggest that H1 variants regulate specific gene expression in a context dependent manner.

A variety of other specific roles of H1 variants in cellular processes in mammalian cells have been identified. For instance, H1⁰ (H1.0) was found to preferentially interact with a group of proteins in the nucleolus, indicating a specific role of H1⁰ in rRNA biogenesis and mRNA metabolism [119]. Other than the aforementioned role of H1 in apoptosis through regulating p53 and CHD8 target genes, H1c was shown to translocate from the nucleus to the cytoplasm and stimulate cytochrome c release from mitochondria upon DSB damage, thus acting as a signal transducer in apoptosis induced by DSB [120]. More recently, H1c and H1x have been found to be ubiquitinated at K63 and that H1 is a key target for E3 ubiquitin ligase RNF8 and E2

ubiquitin-conjugating enzyme UBC13 in DSB signaling [185]. In addition, H1 may stimulate non-homologous end-joining and homologous recombination mediated repair after DNA damage [186, 187].

Most of the mechanistic studies mentioned in this section were carried out in cell lines, which are more amiable for genetic manipulation and environmental stimuli. It is not yet clear whether any or all of the specific roles of H1 variants discussed here are responsible for the phenotypes observed in mammalian development and stem cell differentiation in compound H1 knockouts. Future studies in *in vivo* setting to identify the key regulatory pathways that are responsible for the defects in compound H1 knockouts should provide new insights.

5. Concluding remarks and future directions

During the past decades, we have seen an enormous amount of advances in the understanding of chromatin structure and function, especially in connection with chromatin modifying activities and chromatin remodeling complexes. Compared with core histones, linker histones have not drawn adequate attention and thus remain underexplored [57]. Investigating the role of H1 variants in mammalian development and stem cell differentiation *in vivo* as well as the underlying mechanisms has been challenging partly due to the complexity/heterogeneity and the compensatory effects of mammalian H1 variants and the lack of antibodies of high affinity and specificity for H1 variants and their post-translational modifications. However, a plethora of recent studies as summarized in this review have shed significant new insights on the role of H1 in these processes.

We have focused our discussion on the pivotal roles of H1 in mammalian development, including embryogenesis, tissue maturation and gametogenesis, and also reviewed its role in stem cell differentiation with connection to development. These studies establish that the total H1 level, rather than individual H1s, is critical for viability and robust development and required for proper ESC differentiation. There is significant redundancy and compensation among somatic H1 variants during development. While the regulatory circuit governing the compensation among somatic H1s remains poorly understood, such redundancy suggest commonality in their structural and functional roles at the genome-wide scale, which is also supported by their largely overlapping genomic localization in multiple cell types [109, 181, 182]. On the other hand, the high sequence conservation and the exquisite differential expression patterns of H1 variants are indicative of an amazing evolutionary adaption. The presence of multiple H1 variants may also provide a powerful cushion for potential perturbations in chromatin milieu.

Disruption of the compensation among somatic H1s results in global effects on chromatin structure but specific gene expression changes [13, 16, 103, 107, 108, 132-135, 154]. The mechanisms underlying the global vs. specific effects regulated by H1 are important avenues to pursue. New links between H1 and other epigenetic regulatory mechanisms are likely to emerge from future studies. In addition, identification of key H1 target genes and the signaling pathways involved in physiological and pathological contexts should lead to new insights into the role of H1.

Given their differences in biochemical properties, interaction partners, chromatin binding affinity and spatial temporal expression patterns, H1 variants are bound to have distinct functions, probably in a specific and context-dependent manner, in development and cellular differentiation.

These differential properties of H1 variants offer a means to ‘fine-tune’ the regulation of chromatin structures and gene expression, culminated in optimal physiological performance and response to environmental stress. Indeed, such variant-specific functions may be especially important in diseases, such as cancers and immunological diseases, as nicely illustrated by the prominent effects arising from manipulation of H1 variants in cancer cells and the identification of H1 variant mutations as driver mutations in tumors [167, 171, 188-191]. Future research aimed at H1 variant-specific functions in the context of various diseases should provide novel insights into the role of H1 variants in mammalian development and cellular differentiation.

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Figure Legends

Fig. 1. Multiple alignment of mouse and human somatic H1 variants. Genbank accession numbers of the protein sequences are listed in Table 1. The black line on top of amino acid sequences marks the globular domain. The conserved and the similar residues are highlighted in black and grey colors, respectively.

Fig. 2. The expression patterns of histone H1 variants during mouse development.

Currently available data of indicated developmental stages in embryogenesis and postnatal tissues are shown. From zygotes to four-cell stage embryos, “++”, “+” and “-” represent relative “high expression”, “low expression” and “no expression detected”, respectively, of the temporal expression change of each H1 variant, not intended for comparisons among different H1 variants. N/D: expression not determined. Data derived from refs. [24, 93, 100-102, 104]. RNA levels were measured by semi-quantitative RT-PCR, and protein levels were measured by Western blotting or immunofluorescence microscopy. The oocyte-specific H1^{oo} is also enriched in zygotes but gradually decreased until complete disappearance at the four-cell stage [24, 93]. The maternal H1^o mRNA and protein are readily detected at the zygotic stage but are degraded and replaced by zygotic H1^o counterparts at the late two-cell stage [100-102]. Synthesis of H1a-e from the maternal mRNA transcripts starts at the late one-cell stage, resulting in appearance of H1a-e proteins in two-cell stage embryos [104]. The maternal mRNAs of H1a-e are degraded in late two-cell embryos concomitant with the accumulation of their zygotic transcripts [101]. Individual H1 variant- and the total H1-to-nucleosome ratios of midgestation embryos and postnatal tissues were adapted from refs. [13, 109, 117, 135]. No expression data in mice is available for H1x. As H1d and H1e are co-eluted in HPLC analysis, they were quantified together in embryos and certain tissue samples.

Fig. 3. The expression patterns of histone H1 variants during gametogenesis. During spermatogenesis, male germ cells undergo unique chromatin remodeling. Somatic H1s were detected in spermatogonia, predominated by H1a and H1c, whose levels steadily decrease in meiotic spermatocytes until their disappearance in round spermatids [126, 133]. H1t is expressed in pachytene spermatocytes and persists until round spermatids [127-130]. The expression of H1T2 and HILS1 is confined to spermatids, with H1T2 in the nuclei of early spermatids and HILS1 in both early and elongated spermatids [26-29]. Similar to spermatogonia, oogonia express a complement of somatic H1s. During oogenesis, somatic H1s are gradually replaced by the oocyte-specific H1oo [24, 101]. However, H1⁰ is retained throughout oogenesis [100-102].

Table 1. Mammalian histone H1 variants^a

Histone H1 variants (Alt. symbols)	Mouse histone H1 genes			Human histone H1 genes			Gene Intronic ^b	Expression Cell types ^c	Expression Dependence on DNA replication	mRNA 3'UTR
	Gene name	Accession no.	Chr location	Gene name	Accession no.	Chr location				
H1a (H1.1)	<i>Hist1h1a</i>	NM_030609	13	<i>HIST1H1A</i>	NM_005325	6	No	Somatic	Yes	Stem-loop
H1b (H1.5)	<i>Hist1h1b</i>	NM_020034	13	<i>HIST1H1B</i>	NM_005322	6	No	Somatic	Yes	Stem-loop
H1c (H1.2)	<i>Hist1h1c</i>	NM_015786	13	<i>HIST1H1C</i>	NM_005319	6	No	Somatic	Both ^g	Both ^g
H1d (H1.3)	<i>Hist1h1d</i>	NM_145713	13	<i>HIST1H1D</i>	NM_005320	6	No	Somatic	Yes	Stem-loop
H1e (H1.4)	<i>Hist1h1e</i>	NM_015787	13	<i>HIST1H1E</i>	NM_005321	6	No	Somatic	Yes	Stem-loop
H1 ⁰ (H1.0, H1f0)	<i>H1f0</i>	NM_008197	15	<i>H1F0</i>	NM_005318	22	No	Somatic ^f , oocyte	No	Poly-A
H1x (H1.x, H1fx)	<i>H1fx</i>	NM_198622	6	<i>H1FX</i>	NM_006026	3	Y/N ^c	Somatic	No	Poly-A
H1oo (H1foo)	<i>H1foo</i>	NM_138311	6	<i>H1FOO</i>	NM_153833	3	Yes	Oocytes, zygote and 2-cell embryo	No	Poly-A
H1t	<i>Hist1h1t</i>	NM_010377	13	<i>HIST1H1T</i>	NM_005323	6	No	Spermatocytes spermatids	Yes	Stem-loop
H1T2	<i>H1fnt</i>	NM_027304	15	<i>H1FNT</i>	NM_181788	12	No	Spermatids	No	Poly-A
HILS1	<i>Hils1</i>	NM_018792	11	<i>HILS1</i>	AY286318	17	Y/N ^d	Spermatids	No	Poly-A

^a Gene names and accession numbers are adapted from [192];^b Data derived from GenBank accessions, AceView [193] and refs. [26-29, 32, 55, 194];^c Human H1FX gene encodes multiple transcript variants with or without introns (see refs. [55, 193]);^d Human *HILS1* gene consists of two exons and a small 106-bp intron and mouse *Hils1* gene is intronless (ref. [27]);^e Expression data derived from refs. [14, 22, 24-29, 39, 45, 46, 93, 194-197];^f H1⁰ is enriched in differentiated cells (see ref. [22]);^g The *H1c* gene encodes two versions of mRNAs, polyadenylated and stem-loop forms, allowing for both replication-dependent and replication-independent expression (see ref. [36]).

Table 2. Sequence similarity among H1 variants in mouse and human^a

	mH1a	mH1b	mH1c	mH1d	mH1e	mH1 ⁰	mH1x	mH1t	mH1T2	mHILS1	mH1oo	
hH1a	78.3	66.2	64.3	67.7	66.2	41.0	30.4	51.8	13.0	21.6	17.9	mH1a
hH1b	66.4	90.7	74.0	81.7	79.2	41.8	31.2	52.8	15.0	21.2	22.3	mH1b
hH1c	67.7	76.5	88.3	81.0	82.3	39.3	32.2	50.9	14.5	20.5	20.2	mH1c
hH1d	67.7	77.8	81.1	87.8	86.4	40.8	32.5	54.7	15.1	19.5	23.1	mH1d
hH1e	70.7	83.8	84.5	86.0	94.1	41.9	32.1	51.8	15.0	21.1	23.1	mH1e
hH1.0	42.6	43.2	43.4	38.0	41.9	94.8	27.2	33.8	10.3	15.4	21.4	mH1 ⁰
hH1.x	36.4	32.0	33.8	32.3	34.1	29.7	70.9	26.2	10.0	16.4	16.5	mH1x
hH1t	52.1	49.6	52.2	48.2	52.0	29.7	24.2	61.0	13.4	24.7	21.5	mH1t
hH1T2	26.8	22.3	25.9	23.5	20.7	16.7	24.7	20.8	31.7	10.7	17.9	mH1T2
hHILS1	17.1	16.2	15.6	16.2	14.7	8.7	12.1	18.0	14.9	28.5	14.1	mHILS1
hH1oo	20.1	26.4	21.9	23.7	22.4	19.2	20.1	18.6	22.1	10.9	43.1	mH1oo
	hH1a	hH1b	hH1c	hH1d	hH1e	hH1.0	hH1.x	hH1t	hH1T2	hHILS1	hH1oo	

^a The numbers indicate the percentage of identity between two aligned H1 variants. Sequence comparisons between mouse H1 variants are shown in the upper half of the table (non-shaded) and those between human H1 variants are shown in the lower half (shaded). The bold numbers at the diagonal represent the sequence similarity between mouse H1 variants and their human counterparts.

Table 3. Summary of properties of mice and ESCs with altered H1 expression

H1 manipulation ^a		H1/nucleosome ratio ^b	Phenotype	References
In mice				
H1 OE	H1 ⁰	N/D	Normal (10-20 fold increase in H1 ⁰ mRNA, N/C in H1 ⁰ protein)	[198]
Single H1 KO	H1 ^{0/-}	N/C (adult liver: 0.68)	Normal Decreased dendritic cell production; reduced response to vaccination with antigens	[103] [118]
	H1a ^{-/-}	N/C (male germ cells: 0.73)	Normal	[107, 135]
	H1c ^{-/-}	N/C	Normal Increased resistance to x-ray induced apoptosis in thymocytes and intestine	[108] [120]
		RD (PN56 retina: WT 1.25/KO 0.99)	Normal retina	[117]
	H1d ^{-/-}	N/C	Normal	[108]
	H1e ^{-/-}	N/C	Normal	[108]
	H1oo ^{-/-}	N/D	Normal Or homeostasis	[98] [99]
	H1t ^{-/-}	N/C (male germ cells: 0.68)	Normal	[132-134]
	H1T2 ^{-/-}	N/D	Abnormal sperms and male infertility	[28]
Double H1 KO	H1 ^{0/-} H1c ^{-/-}	N/C (adult liver: 0.71)	Normal	[108]
	H1 ^{0/-} H1d ^{-/-}	N/C (adult liver: 0.71)	Normal	[108]
	H1 ^{0/-} H1e ^{-/-}	N/C (adult liver: 0.76)	Normal	[108]
	H1c ^{-/-} H1e ^{-/-}	N/C (adult liver: 0.72) RD (retina)	Mild growth retardation Abnormal rod photoreceptor packing	[13] [117]
	H1a ^{-/-} H1t ^{-/-}	RD (male germ cells: WT 0.78/KO 0.59)	Normal	[135]
Triple H1 KO	H1c ^{-/-} H1e ^{-/-} H1 ^{0/-}	RD (adult liver: WT 0.79/KO 0.64) RD (retina)	Growth retardation; lower birth rate; small thymus Abnormal rod photoreceptor packing	[13] [117]
	H1c ^{-/-} H1d ^{-/-} H1e ^{-/-}	RD (midgestation embryo: WT 0.74/KO 0.40)	Embryonic lethality by E11.5	[13]
Other compound H1 KO with multiple H1 null alleles	H1c ^{-/-} H1d ^{+/-} H1e ^{-/-}	RD (thymus: WT 0.83/KO 0.41)	Low birth rate; severe growth retardation	[13]
	H1c ^{-/-} H1d ^{+/-} H1e ^{-/-} H1 ^{0/-}	N/D	Low birth rate; neonatal lethality	[13]
	H1c ^{-/-} H1d ^{-/-} H1e ^{-/-} H1 ^{0/-}	N/D	Embryonic lethality	[13]
In ESCs				
Single H1 KO (mouse)	H1 ^{0/-}	N/D	Normal ESC differentiation	#
	H1c ^{-/-}	RD (WT 0.45/KO 0.38)	Normal cell morphology and proliferation; normal ESC differentiation and teratoma formation; specific expression changes of Hox genes	[154]
	H1d ^{-/-}	RD (WT 0.45/KO 0.35)		[154]
	H1e ^{-/-}	RD (WT 0.45/KO 0.35)		[154]
Triple H1 KO (mouse)	H1c ^{-/-} H1d ^{-/-} H1e ^{-/-}	RD (ESCs: WT 0.46/KO 0.25)	Reduced chromatin compaction; specific changes in gene expression and DNA methylation Chromocenter clustering and de-repression of major satellite repeats Enhanced DNA damage response Decreased chromatin flexibility in gene rich TAD ESCs more resistant to spontaneous differentiation; impaired EB and neural differentiation Dysregulated in specification of mesendoderm and neuroectoderm	[153] [109] [199] [163] [16] [16] [105]
		RD (EBs: WT 0.62/KO 0.36)		
H1 KD (human)	H1 ⁰	N/D	Lack of induction of a few differentiation associated genes	[106]

^a OE, overexpression; KO, knockout; KD, knockdown.^b N/C, no change; RD, reduced; N/D, not determined; WT, wildtype.

Pan, Zhang, Fan unpublished observation.

Figure 1

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mH1a MS----ETAPVAQAASTATEKPAAAKTKKPAKAAAP-RKKPAGP-SVSELIVQAVSSSKERSGVSLA-ALKKSLAAAGYDV 75
hH1a MS----ETVPPAPAASAAPEKPLAGKKAKKPAKAAAASKKKPAGP-SVSELIVQAASSSKERGGVSLA-ALKKALAAAGYDV 76
mH1b MS----ETAPAETAAPAPVEKSPAKKKTTKK---AGAAKRKATGP-PVSELITKAVSASKERGGVSLP-ALKKALAAAGYDV 73
hH1b MS----ETAPAETAAPAPVEKSPAKKKATKKAAGAGAAKRKATGP-PVSELITKAVAASKERNGLSLA-ALKKALAAAGYDV 76
mH1c MS----EAPAAPAAAPPAEKAPAKKKAAKK---PAGVPRKASGP-PVSELITKAVAASKERSGVSLA-ALKKALAAAGYDV 73
hH1c MS----ETAPAAPAAAPPAEKAPVKKKAANK---AGGTPRKASGP-PVSELITKAVAASKERSGVSLA-ALKKALAAAGYDV 73
mH1d MS----ETAPAAPAAAPPAEKTTPVKKKAANKT---GAAAGKRKASGP-PVSELITKAVAASKERSGVSLA-ALKKALAAAGYDV 74
hH1d MS----ETAPLAPTIPAPAECTPVKKKAANKA---GATAGKRKASGP-PVSELITKAVAASKERSGVSLA-ALKKALAAAGYDV 74
mH1e MS----ETAPAAPAAAPPAEKTTPVKKKAANKA---AGGAKRKTSGP-PVSELITKAVAASKERSGVSLA-ALKKALAAAGYDV 73
hH1e MS----ETAPAAPAAAPPAEKTTPVKKKAANKS---AGAAKRKASGP-PVSELITKAVAASKERSGVSLA-ALKKALAAAGYDV 73
mH10 MT-----ENSTSAPAA-----KPKR-----AKASKSTDHP-KYSDMIVAATQAEKNRAGSSRO-SIQKYIKSHYKVG 61
hH1.0 MT-----ENSTSAPAA-----KPKR-----AKASKSTDHP-KYSDMIVAATQAEKNRAGSSRO-SIQKYIKSHYKVG 61
mH1x MSVELEEALPPTSADGTARKTAKAGGSAAPTQPKRRKN-RKKNQPGKYSQLVVETIRKLGERGGSSSLARIYAEARKVAFWDQ 81
hH1.x MSVELEEALPVTTAEGMAKKVTKAGGSAALSPSKRRKNSKKKNQPGKYSQLVVETIRRLGERNGSSSLAKIYTEAKKVPWFDQ 82

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mH1a EKNNSRIKLGKLSLVNKGTLVQTKGTGAAGSFKLNKKAEESKAITTT---KVSV-KA-KASGAACKPKKTAGAAA-KKTV-KTP 150
hH1a EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKASSVETKPGASKVAT-KT-KATGASKKLKKATG-AS-KKSV-KTP 153
mH1b EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKAAASGEAKPKAKKTGAACKKPKAGAT--PKKPKKTAGAKKTVPKKT 153
hH1b EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKAAASGEAKPKAKKAGAAKAKKPKAGAT--PKKAKKAGAKKAVKKT 156
mH1c EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKAAASGEAKPKAKKAGAAKAKKPKAGAT--PKKAKKAGAKKAVKKT 155
hH1c EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKAAASGEAKPKVKKAGGTPPKPVGAACKPKKAAGGATPKKSAKKT 155
mH1d EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKAAASGEAKPKAKKAGAAKAKKPKAGAAKPKKAAGATPKKTAKKT 156
hH1d EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKAAASGECKPKAKKAGAAKPKPKAGAAKPKKVAGATPKKSLKKT 156
mH1e EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKAAASGEAKPKAKKAGAAKAKKPKAGAAKPKKAAGTATAKKSTKKT 155
hH1e EKNNSRIKLGKLSLVSKGLTVQTKGTGASGSFKLNKKAAASGEAKPKAKKAGAAKAKKPKAGAAKPKKAAGTATPKKSACKT 155
mH10 ENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKGDEPKRSVAFKTKKEVKKVATPKKAAKPKKAASKAPSKPKATPV 143
hH1.0 ENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKGDEPKRSVAFKTKKEIKKVATPKKASKPKKAASKAPTCKPKATPV 143
mH1x QNGRTYIKYSIRALVQNDTLLQVKGTDANGSFKLNKKLEGGGAERRGASAASSPAPK-----ARTAAD--R-TP 148
hH1.x QNGRTYIKYSIKALVQNDTLLQVKGTDANGSFKLNKKLEGGGERGAPAAATAPAPTAKHAKKAAPGAAGSRRAD--K-KP 161

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mH1a KKPKKPAV---SKKTSKSPKKPKVV-KAKKVAKSPAKAKAVKPKASKAKVTTPKPTPAKPKKAAPKKK----- 213
hH1a KKAKKPAA---TRKSSKNPKPKPTV-KPKKVAKSPAKAKAVKPKAAKARVTTPKT-AKPKKAAPKKK----- 215
mH1b KKAKKPAAAGV-KKVAKSPKKAKAAAKPKKAASPAKPKAVKSKASKPKVTTPKT-AKPKAAKAKKAVSKKK 223
hH1b KKAKKPAAAGV-KKVAKSPKKAKAAAKPKKATKSPAKPKAVKPKAAKPKAAKPKA-AKPKAAKAKKAAAKKK 226
mH1c KKAKKPAAAVTKKVAKSPKKAKVT-KPKKV-KSASKA-----VKPKAAKPKV-AKAKKVAACKK----- 212
hH1c KKAKKPAAATVTKKVAKSPKKAKVA-KPKKAASAKA-----VKPKAAKPKV-VKPKKAAPKKK----- 213
mH1d KKAKKPAAAAGAKKVSKSPKKVKAA-KPKKAASPAKAKAPKAKASKPKASKPKA-TAKKKAAPRKK----- 221
hH1d KKVKKPAATAAGTKKVAKSAKKVKT-OPKKAASPAKAKAPKPKAAKPKSGKPKV-TAKKKAAPKKK----- 221
mH1e KKAKKPAAAAGAKK-AKSPKKAKAT-KAKKAPKSPAKAKTVKPKAAKPKTSKPKA-AKPKKTAACKK----- 219
hH1e KKAKKPAAAAGAKK-AKSPKKAKAA-KPKKAPKSPAKAKAVKPKAAKPKTAKPKA-AKPKKAAAKKK----- 219
mH10 KKAKK--KPAATPKKAKPKVVVV--KPVKASK-PKKAKTVKPKAKSSAKRASKKK----- 194
hH1.0 KKAKK--KLAATPKKAKPKVTVA--KPVKASK-PKKAKFVKPKAKSSAKRAGKK----- 194
mH1x --ARPQ-PERRAHKS-----KKAAAAASAKKVKAA-KPSVPKVPKGRK----- 188
hH1.x --ARGQKEPQRSHKKGAGAKKDKGGKAKKTAAGGKKVKKAA-KPSVPKVPKGRK----- 213

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Figure 2

Figure 3

Spermatogenesis

Oogenesis