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Cracking the Enigmatic Linker Histone Code

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Recently, the existence of a 'histone code' has been proposed to explain the link between the covalent chemical modification of histone proteins and the epigenetic regulation of gene activity. Although the role of the four 'core' histones has been extensively studied, little is known about the involvement of the linker histone, histone H1 and its variants, in this code. For many years, few sites of chemical modification had been mapped in linker histones, but this has changed recently with the use of functional proteomic techniques, principally mass spectrometry, to characterize these modifications. The functionality of many of these sites, however, remains to be determined.

Key words: epigenetic code, histone H1, histone modification, histone variant, linker histone.

Abbreviations: bp, base pairs; CHO, Chinese Hamster Ovary; ES, embryonic stem; HPLC, High Performance Liquid Chromatography; Rb, retinoblastoma protein.

For many years, almost every review of chromatin structure and function would begin with the nearly obligatory description of the four core histones: H2A, H2B, H3 and H4 and how these bind to one another to form a protein octamer, which then wraps about 200 bp of DNA around this molecular spool in order to form a nucleosome, the most basic unit of chromatin structure (for a historical perspective, see *1*). Nucleosome structure was then described as being completed upon the binding of H1, the linker histone, which bound to the outside of the nucleosome, thereby sealing the two turns of DNA and serving as a prerequisite to the higher order folding of chromatin (*1*). In the last decade, however, this canonical view of the nucleosome has undergone a radical change. First, it is now understood that chromatin is much more dynamic than previously imagined and that the histones, particularly H1, exchange on and off of nucleosomes at some frequency (*2*). Secondly, it has been shown that histone H1 is not required for viability in a number of simple eukaryotes, as will be discussed in more detail, below (*3*). This final finding places some doubt on the traditional view of histone H1 playing such a fundamental role in chromatin structure.

THE HISTONE CODE

Around the time that the *in vivo* role of histone H1 began to be questioned, a hypothesis was put forth that covalent post-translational modifications of the histone N- and C-terminal 'tails' acted sequentially or in combination to form a 'histone code', which was read by regulatory proteins to affect cell processes such as transcription, replication and chromosome condensation during mitosis (*4*). All histones, including H1, have a tripartite structure, with a central globular domain surrounded by more extended tail domains which are very basic, rich in lysines and arginines (*5*). These tails are believed to be inherently flexible and largely unstructured in solution (*5*). The N-terminal tail, especially, has many sites where covalent chemical modifications take place in the cell, namely acetylation, methylation, as well as phosphorylation (*4*). The histone code hypothesis built on a wealth of biochemical and genetic information that had been amassed over the years concerning the functionality of these, as well as other, modifications; it primarily focused on histones H3 and H4, for which most information existed, and mentioned the other three histones only in passing. A follow-up to this original paper, however, did mention two antagonistic roles that the covalent modification of histone H1 might play in the cell: the methylation of Lys^{26} (K26) in the N-terminal tail was potentially linked to gene-silencing and the assembly of heterochromatin, while the ubiquitination of histone H1 at an unknown site seemed to correlate with transcriptional stimulation (*6*). Progress on understanding the role that histone H1 plays in the histone code has been, until recently, quite slow. A recent review which discussed the link between the histone code and the developmental process mentioned this sole site-specific modification, that of K26, compared with six sites in each of the H2 histones, eight in histone H4 and 16 in histone H3 (*7*).

LINKER HISTONE VARIANTS

Covalent chemical modification is not the only means of introducing variability among linker histones in the cell, histone H1 is also found to exist in a number of variants that differ in sequence and which are often differentially

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Table 1. **A list of known mammalian linker histone subtypes comparing the three most common nomenclatures used: numeric, alphabetic, as well as a combination of the two.** List of distinctive characteristics is by no means exhaustive, and is derived from (*11*), which details many more characteristics and also contains the original references for the nomenclatures presented.

Cell Type	Histone H1 variants			Distinctive characteristics
	Numeric	Alpha.	Combo.	
Somatic	H1.0	$H1^0$		Restricted to terminally differentiated cells
	H1.1	H1a	H1a	Restricted to certain tissues[a]
	H1.2	H1c	$H1^s$-1	Highest turnover rate, not restrict. to S-phase
	H1.3	H1d	$H1^s$-2	Highest levels of expression during S-phase
	H1.4	H1e	$H1^s$-4	Turnover rate varies, depending on cell type
	H1.5	H1b	$H1^s$-3	Highest PO_4 levels at all stages
	H1.X	H1x		Identified soley in cultured cells
Testis-specfic		H1t		
		H1t2		
		HILS1		
Oocyte-specific		H1oo (H1Foo)		

[a]H1a has been found associated with thymus, testis, spleen, lymphocytic and neuronal cells.

expressed (*8*). All histones, with the exception of H4, have been found to have structural variants, but the number of histone H1 variants, as well as their degree of divergence from one another, is much greater than of the core histones (*9*). Eleven different linker histone variants have been characterized in mammals to date, more than twice the number found of any core histone (*8*). These are normally classified by the cell type in which they appear, most being found in somatic cells, while three (H1t, H1t2 and HILS1) are spermatogenic variants, and one (H1oo) is oocyte-specific (*8*, Table 1). The somatic subtypes can be further subdivided into replication-dependent and replication-independent variants, with the former group containing five variants which are expressed in S-phase, and the latter group containing histone $H1^\circ$, a replacement subtype found in growth arrested cells, and histone H1x, which accumulates during G_1 phase (*8*, *10*). The five somatic replication-dependent variants have, unfortunately, been referred to in the literature using at least 12 different nomenclature systems, which has led to much confusion (*11*). The two most widely used nomenclatures include an alphabetic one, where these histones are called H1a–H1e, and a numeric one, where they are termed H1.1–H1.5. Although the authors of the review proposed yet another unifying system for nomenclature using $H1^S$-1 through $H1^S$-4, along with H1a, this system has yet to see widespread adoption in the literature (*11*). We will use the numeric system, since it seems to be gaining popularity in the literature and all of the most recent papers to be discussed in the final section of this review have adopted this system. The presence of these 11 isoforms of histone H1 must be kept in mind when envisioning a linker histone code, much more so than in the case of the core histones. The microheterogeneity represented by these subtypes provides unique sites for potential chemical modifications among the histone H1 variants, and may contribute significantly to the information contained in a histone code. The fact that this heterogeneity has been conserved across biological kingdoms suggests that these individual variants may have unique properties in the cell (*9*).

FUNCTION OF HISTONE H1

In order to 'crack' the linker histone code, it is important to elucidate testable functions for the protein *in vivo*, which may then be altered by covalent chemical modifications. Early *in vitro* studies suggested many critical roles for histone H1 and its variants. Histone H1 was seen to 'seal' the two turns of nucleosomal DNA as well as be essential for the conformational change between an extended 10 nm beads-on-a-string form of chromatin and a compacted 30 nm fibre, an important prerequisite to any higher order folding of the chromatin (*12*). Histone H1 binding was thus imagined to lead to a general repression of cellular processes such as transcription, by blocking access between the cellular machinery and chromatin-bound DNA (*12*).

Such a critical role for histone H1, however, was not supported by genetic studies which knocked out H1 and its variants, or otherwise reduced the level of these linker histones in the cell. While the precise role of H1 *in vivo* is still evolving, an analysis of these studies yields important clues to what H1 does and does not do in the cell.

DELETION IN LOWER EUKARYOTES

The first clue that histone H1 may not play an essential role *in vivo* came in 1995, when the H1 gene was knocked out in the ciliated protozoan *Tetrahymena thermophila*, without an effect on viability or growth (*13*). The mitotic chromosome structure in the mutants, however, could be seen to be less condensed in addition to the nuclei being enlarged (*13*). Two years later, the histone H1 gene in yeast, *Saccharomyces cerevisiae*, was knocked out with no detectable effects, with the exception of a change in the level of expression of one tested gene (*14*). This effect on gene expression was later investigated using microarray analysis to identify the number of genes whose activity was affected by H1 deletion (*15*). These studies found that, if affected at all, genes were moderately down-regulated in the absence of H1, not the result one would expect from removing

a general repressor of transcription from the cell. Furthermore, only a small number of genes were affected, only 27 genes showed a decrease in transcription of ~2-fold or higher, <0.5% of the total genes of this organism (*15*). More recent studies demonstrated that H1 loss in yeast increased the cell's propensity to undergo DNA repair by homologous recombination, and that an absence of the protein lead to a decrease in the life span of the yeast (*16*). The authors speculate that the H1-mediated inhibition of recombination between repeated sequences may actually lead to an increase in genome stability in *S. cerevisiae* (*16*). Arguments that both of these simple species lack a conventional H1 that contains the canonical tripartite structure, and may therefore not be representative of other species, were soon put to rest by the deletion, and silencing, respectively, of the H1 gene in fungi *Aspergillus nidulans* and *Ascobolus immersus*, which both contain H1s with this three domain structure (*17*, *18*). However, while the former manipulation had no discernable effect whatsoever, the latter did lead to an increased accessibility of the *Ascobolus* chromatin to nuclease digestion as well as a decrease in the typical lifespan of the fungus, similar to the findings using *S. cerevisiae*. The authors also noted that H1 deletion lead to hypermethylation of DNA at specific sites, namely those which already had some degree of methylation in wild-type strains (*18*).

DEPLETION IN HIGHER EUKARYOTES

Since all of the lower eukaryotes described earlier each had only a single H1 gene, the conclusions regarding the dispensability of H1 in their systems were fairly straightforward. Higher eukaryotic studies, instead of deleting all H1 homologues in a system, typically have involved the deletion of one or more H1 variants, followed by a determination of the effect a particular mutation has on growth and development. The first depletion of an H1 subtype in a higher organism was reported in the literature the same year that the *Tetrahymena* H1 was found to be dispensable. It was reported that H1° could be deleted from mice ES cells and that the resulting progeny developed normally, with no anatomical or histological defects (*19*). Subsequent studies from the same laboratory have provided a wealth of information concerning the necessity for certain histone subtypes in mice. It soon became apparent why one could delete the gene for an H1 variant in mice with no discernable effect, the chromatin from the sperm of mice in which the H1t subtype had been deleted, again with no detectable effect, was found to contain the normal ratio of H1 to nucleosomes (*20*). The authors found that other H1 subtypes were deposited in place of H1t, suggesting a mechanism for the up-regulation of different H1 variants when one type is missing from the cell (*20*). This finding contains mixed implications for the understanding of a linker histone code. The fact that different H1 subtypes can replace others, *in vivo*, with no effect suggests that the microheterogeneity between variants may not be as important as once thought. It has been proposed that the timing of histone variant expression may, at times, be more important than their primary sequence (*21*). The existence of a compensatory mechanism to ensure appropriate H1 levels are maintained in the cell, however, speaks to the importance of H1 *in vivo* and suggests that, higher organisms, unlike lower eukaryotes, cannot survive without any H1 homologue present.

Studies in mice continued with the creation of double mutants, in which double-knockouts were created which deleted two histone variants from the cell. Cells in which both H1t and H1.1 were deleted exhibited chromatin with 75% of the normal ratio of H1 to nucleosomes, but were without phenotypic effect in developing spermatocytes (*22*). Subtle effects on gene expression were detected, however, using microarray analysis, which revealed 17 genes that demonstrated at least 2-fold differences in their expression in the double mutant cells, compared with wild type ones (*22*). It was also found that mice developed normally upon deletion of H1°, as well as either H1.2, H1.3 or H1.4 (*23*). It was not until all three of these latter histones were deleted that significant results were detected (*24*). These triple null mutants exhibited about 50% of the normal ratio of H1 to nucleosomes, which led to a global shortening of the amount of linker DNA between them. A correlation between the levels of H1 and the 'nucleosome repeat length', as it is called, had been noted previously (*12*). Embryos which carried this triple mutation died by midgestation with a broad range of defects (*24*). Subsequent microarray analysis of gene expression in these triple mutants revealed 29 genes with at least a 2-fold difference in expression, a mere 0.1% of the genes in mice (*25*). The authors further noted that nearly one-third of these genes are thought to be normally regulated by DNA methylation, suggesting a role for H1 in the epigenetic regulation of gene expression (*25*). Although global changes in DNA methylation were not detected in these mutants, methylation of some of the H1-regulated genes was seen to be reduced.

While genetic studies using mice might lead one to believe that the histone variants are interchangeable, and therefore play no role in establishing a linker histone code, studies using microinjection of specific mRNAs into *Xenopus* oocytes have demonstrated otherwise. In *Xenopus* development, the oocyte-specific H1 variant, called B4, is progressively substituted by a somatic variant, namely H1A, following the midblastula transition, which results in a loss of mesodermal competence among the embryonic cells (*26*). While it is true that H1B mRNA can be injected into oocytes before H1A has appeared, resulting in the same loss of competence, injection of histone B4 mRNA does not produce this phenotype (*26*). We have gone on to show that B4 is functionally different from H1A in the way it influences chromatin structure and dynamics- B4, for instance, does not significantly restrict the accessibility of linker DNA in reconstituted chromatin, while H1.1 does so in a classical way (*27*). Our most recent work, using analytical ultracentrifugation studies of chromatin folding, has shown that H1A can either relax or condense chromatin structure in a concentration dependent fashion, something that B4 is unable to do (J.G. and K.U., unpublished observations). This would suggest that there are certain cases where histone H1 variants can replace one another

in the cell with little effect, as well as other cases where they cannot.

The importance that H1 plays in development was also seen in *Caenorhabditis elegans*, where RNA interference studies of H1.1 expression affected germline proliferation and differentiation, and produced such phenotypes as severely disordered gonad structures as well as infertility (*28*). In addition, transgenic tobacco plants in which levels of the two major histone H1 variants were decreased showed aberrations in flower development, which correlated with changes in the temporal pattern of specific genes, coupled with male sterility (*29*). Despite this treatment, a compensatory response by the plant was able to restore overall linker histone levels to nearly normal levels, as was seen in studies using mice (*29*). This same laboratory later achieved a >90% reduction in the levels of H1 in *Arabidopsis thaliana*, which led to a number of developmental aberrations in the plant, including the formation of irregular leaves, as well as changes in the timing and morphology of flower production (*30*). Since a number of these phenotypes resembled those normally observed in *Arabidopsis* mutants displaying DNA hypomethylation, the methylation patterns of a number of DNA regions were investigated in mutant plants and found to be altered in the absence of H1 (*30*).

SUMMARY OF *IN VIVO* STUDIES

From the various studies described earlier, it is clear that the *in vivo* roles of histone H1 include: (i) compaction of chromatin and establishment of the spacing between nucleosomes, (ii) maintenance of the level of DNA methylation in certain regions of the genome, (iii) both positive and negative regulation of the expression of a subset of cellular genes, perhaps due to changes in chromatin compaction and/or DNA methylation and (iv) a degree of developmental control, possibly linked to the regulation of specific genes. With these roles in mind, we will turn to what is currently known about covalent modifications of H1 and its subtypes, with the hopes of understanding how these contribute to the function of the linker histones.

STUDIES OF COVALENT MODIFICATIONS: USING TRADITIONAL METHODS

The first covalent modification to be studied extensively in histone H1 was phosphorylation, since it had long been recognized that H1 was phosphorylated at different serine and threonine residues in response to different stimuli (*31*). Of particular interest was this modification's link with transcriptional regulation as well as chromosome condensation during mitosis (*4*). More recently, H1 phosphorylation has been linked with DNA repair, apoptosis and ATP-dependent chromatin remodeling (*32–34*). An early review lists no less than seven regions of the protein which had been seen to undergo phosphorylation *in vivo*: S15 or 16; T17, 19 or 20; S37 or 38; S145 or T136; T153; S161 or 173 and S180 or 182 (*31*). The positions given are variable, since the studies in question were performed using a number of different sources of H1, as well as a variety of subtypes. One of the best studied organisms with regard to the positioning and timing of *in vivo* H1 phosphorylation has been *Tetrahymena thermophila*, but since this organism lacks the canonical tripartite structure of a linker histone, we will limit our discussion to modification sites on metazoan histones; the reader is referred to a representative *Tetrahymena* mapping paper (*35*). A more recently reported mapping of phosphorylation in histone H1 was the identification of sites associated with mitosis in CHO cells (*36*). Here, tryptic digested extracts from synchronized cells were subjected to HPLC to purify phosphopeptides, and the peptide which was purified from mitotic cells was then sequenced. The peptide, which was identified as belonging to histone H1.2 group (based on sequence comparison with the human placental H1 subtypes), was found to be phosphorylated at both S1 and T3 (*36*).

Another covalent modification associated with histone H1 is methylation, which has been correlated with transcriptional repression (*6*). While K26 has long been assumed to be the site of modification, this was only recently demonstrated *in vivo* using antibodies raised against a peptide from H1.4 which had been methylated at K26 (*37*). Subsequent studies revealed that chromatin condensation at Rb-regulated genes was dependent on the binding of a specific protein to histone H1.4 which had been mono- or di-, but not tri-methylated, at K26 (*38*).

STUDIES OF COVALENT MODIFICATIONS: USING MASS SPECTROMETRY

The advent of functional proteomics has lead to an increase in the use of mass spectrometry to monitor changes in post-translational modifications of specific proteins (*39*). Since 2004, these newer methods have been applied to investigating covalent chemical modifications of histone H1. The first reported use of mass spectrometry to study H1 modification coupled traditional chromatographic techniques with electrospray ionization (ESI) tandem mass spectrometry (MS/MS) to investigate global changes in H1 phosphorylation in mouse cell culture, in response to hormone treatment (*39*). Here, basic proteins such as H1 were extracted from the cells in question using an acid treatment, followed by a purification step using HPLC, the obtained fractions were then analyzed using the mass spectrometer (*39*). Although the authors did not use these techniques to map the specific sites of H1 modification, studies soon followed which coupled enzymatic digestion of the histone proteins with this same basic procedure to do just that, this time using human HeLa cell culture as a source of linker histone (*40*). Again, while mass spectrometry has recently been applied to map the sites of phosphorylation within histone H1 in *Tetrahymena*, this review will continue to focus on metazoan H1 (*41*). The studies using Hela cells as a source of histones identified all five major somatic isoforms of H1, as well as a lesser studied isoform, H1.X, and confirmed almost all of the phosphorylation sites detailed in an early review, as well as those found later using CHO cells (*31*, *36*, *40*, Fig. 1A). One site not found using mass

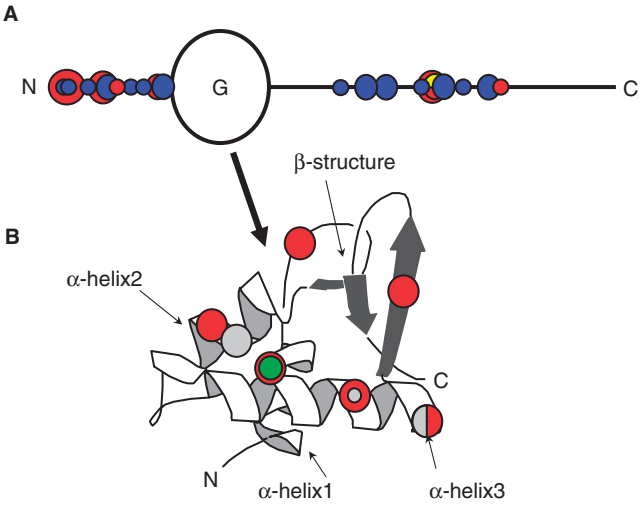

Fig. 1. **Covalent modifications mapped to linker histones.** A representation of many of the covalent modifications that have been mapped to specific regions of the somatic H1 subtypes. (A) Modification of amino (N) and carboxyl (C) terminal tails. Globular domain (G) modifications are detailed in the following section. Diagram is based on the alignment of H1.1-H1.5 shown in (*44*), and has adopted the color scheme shown there. Data concerning specific sites of modification was obtained from this paper, as well as (*40*) and (*47*). The area of the circles are meant to represent how many of the five main somatic subtypes have been reported to contain a particular modification, the largest (at the N-terminus) representing all five and the smallest (for example, the modification farthest toward the C-terminus) representing only one. Colour scheme is as follows: red, acetylation; yellow, methylation; blue, phosphorylation; green, ubiquitination. Circles are divided in half when two modifications occur at the same site in equal numbers of variants. (B) Modification of globular domain. Structure is based on data found in (*49*). Placement of modifications is approximate. Colour scheme is as above, with the addition of gray for formylation. Concentric circles are used if more than one type of modification occurs at the same site, but in different numbers of subtypes.

spectrometry was modification of S15 or 16, while two previously unknown sites were revealed: S27 of H1.4 and T31 of H1.2 (*40*). In retrospect, it is not surprising that these two sites had not been previously characterized, since neither of these amino acids were conserved within the other somatic subtypes in humans. Of these, S27 was of particular interest, due to its proximity to K26 and the possibility that it participated in a 'methyl/phos switch', a binary switch that has been hypothesized to play a role in the core histone code (*42*). Indeed, it was soon discovered that an essential component of heterochromatin, HP1, binds specifically to H1.4 which is methylated at K26, while phosphorylation at S27 blocks its binding (*43*). In addition to phosphorylation sites, the mass spectrometric study in question confirmed the long held view that the H1 isoforms were acetylated at their N-termini, and also reported on one site of acetylation which was internal to the protein (*36*, *40*, Fig. 1).

While the study discussed earlier provided a baseline of typical posttranslational modifications found in asynchronously growing HeLa cells, an even more ambitious study of this, plus one other tissue culture cell type, along with nine different mouse tissues was undertaken which revealed an even greater array of mapped covalent groups (*44*). As in the previous study, six somatic subtypes (including H1.X) were found associated with HeLa cells, however, this new study also detected low levels of H1° as well (*44*). This new analysis not only confirmed most of sites of serine and threonine phosphorylation detailed above (including S16, which had yet to be confirmed), it also found a number of modified lysines which had not been previously characterized (Fig. 1A). Since we have been dealing mostly with histone H1.4 in this review, we will discuss the results from this variant only, as well as use a numbering scheme based on its sequence. Acetylation was detected on the following lysines at various times: 17, 34, 46, 52, 64, 85, 90, 97, 168 and 169, but three of these sites were also found to have alternate covalent modifications as well. Lysines 46, 90 and 169 were also found to be ubiquitinated, formylated, and methylated, respectively, in certain cells, while K63 was found to be another site where formylation occurred (*44*, Fig. 1).

Ubiquitination of histones had been previously linked with transcriptional activation. It had been found that the co-activator $TAF_{II}250$ mediated the monoubiquitination of histone H1, but the site of modification had not been characterized (*45*). It is interesting that this modification (along with more than half of the sites of acetylation, as well as all three sites of formylation) is found in the globular region of histone H1, as it has been speculated that covalent modifications in this region may affect H1 binding to DNA (*44*). While the addition of a formyl group to lysines in histone proteins had not been reported previous to this study, it was recently reported that histone H1 is formylated in response to DNA damage in the nucleosomal linker regions (*46*). The authors speculated that this modification may interfere with the signaling functions normally associated with acetylation (*46*). Indeed, all sites of formylation are either adjacent to, or overlap with, sites to which aceytlation was mapped (*44*, Fig. 1B).

While the studies described earlier have provided us with a wealth of information on potential sites of covalent modification within H1, and are useful as baseline studies as well as enabling comparison between cell types, they do not really speak to the functionality of such sites. A true 'cracking' of the linker histone code will occur only when modification at specific sites is linked with a given function. Along these lines, mass spectrometric studies have been performed using human cell lines synchronized in different stages of growth (*47*). Human lymphoblastic T-cells, which had been enriched in either logarithmically growing, or mitotic cells were subjected to mass spectrometry to map the sites of phosphorylation on different H1 subtypes (*47*). During interphase, both H1.2 and H1.3 were seen to be monophosphorylated, at S172 and S188, respectively. H1.4, however, was found to be diphosphorylated at both of these corresponding sites, while H1.5 was triphosphorylated, still at these sites, with the addition of S17 (*47*). This specific pattern changed in mitosis, with H1.5 taking a pentaphosphorylated form. This form resulted from the phosphorylation of T10, a previously uncharacterized site that is exclusive to H1.5, as well as

either, but not both, T137 and T154 (*47*, Fig. 1A). Thus, although H1.5 has five phosphorylation motifs with a consensus site of (S/T)P(K/A)K, these five are never all phosphorylated simultaneously, with the pentaphosphorylated form using the non-consensus site at T10 (*47*). Of course, further studies are required to determine the significance, if any, of this ordered phosphorylation process. In fact, recent studies where these five consensus sites have been sequentially mutated observe that total phosphorylation levels are most important for the disruption of H1.5 binding to HP1, not the specific sites of modification (*48*). These authors argue in favour of a simple, redundant histone code (*48*).

Regardless of whether the linker histones end up encoding rather simple, or exceedingly complex, epigenetic instructions, great strides have been made in cracking their enigmatic code within recent years. Although over 20 years had gone by without adding significantly to the known sites of covalent modification in histone H1, the last few years has seen this number triple, mostly due to functional proteomics approaches that use mass spectrometry for the identification of purified peptides (*31*, *40*, *44*, *47*). Although the functionality of most of these sites remains to be determined, and the *in vivo* role of histone H1 and its variants requires further study, it would appear that we are finally closing in on an understanding of the linker histone code.

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REFERENCES

1. Kornberg, R.D. and Lorch, Y. (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98**, 285–294
2. Bustin, M., Catez, F., and Lim, J-H. (2005) The dynamics of histone H1 function in chromatin. *Mol. Cell* **17**, 617–620
3. Ausio, J. (2000) Are linker histones (histone H1) dispensable for survival? *BioEssays* **22**, 873–877
4. Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature* **403**, 41–45
5. Ramakrishnan, V. (1997) Histone structure and the organization of the nucleosome. *Annu. Rev. Biophys. Biomol. Struct.* **26**, 83–112
6. Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. *Science* **293**, 1074–1080
7. Margueron, R., Trojer, P., and Reinberg, D. (2005) The key to development: interpreting the histone code? *Curr. Opin. Genet. Dev.* **15**, 163–176
8. Ausio, J. (2006) Histone variants- the structure behind the function. *Brief. Funct. Genet. Prot.* **5**, 228–243
9. Brown, D.T. (2001) Histone variants: are they functionally heterogeneous?. *Genome Biol.* **2**, reviews 0006.1–0006.6
10. Stoldt, S., Wenzel, D., Schulze, E., Doenecke, D., and Happel, N. (2007) G_1 phase-dependent nucleolar accumulation of human histone H1x. *Biol. Cell* **99**, 541–552
11. Parseghian, M.H. and Hamkalo, B.A. (2001) A compendium of the histone H1 family of somatic subtypes: an elusive cast of characters and their characteristics. *Biochem. Cell Biol.* **79**, 289–304
12. Woodcock, C.L., Skoultchi, A.I., and Fan, Y. (2006) Role of linker histone in chromatin structure and function: H1 stoichiometry and nucleosome repeat length. *Chromosome Res.* **14**, 17–25
13. Shen, X., Yu, L., Weir, J.W., and Gorovsky, M.A. (1995) Linker histones are not essential and affect chromatin condensation *in vivo*. *Cell* **82**, 47–56
14. Ushinshy, S.C., Bussey, H., Ahmed, A.A., Wang, Y., Friesen, J., Williams, B.A., and Storms, R.K. (1997) Histone H1 in *Saccharomyces cerevisiae*. *Yeast* **13**, 151–161
15. Hellauer, K., Sirard, E., and Turcotte, B. (2001) Decreased expression of specific genes in yeast cells lacking histone H1. *J. Biol. Chem.* **276**, 13587–13592
16. Downs, J.A., Kosmidou, E., Morgan, A., and Jackson, S.P. (2003) Suppression of homologous recombination by the *Saccharomyces cerevisiae* linker histone. *Mol. Cell* **11**, 1685–1692
17. Ramon, A., Muro-Pastor, M.I., Sazzocchio, C., and Gonzalez, R. (2000) Deletion of the unique gene encoding a typical histone H1 has no apparent phenotype in *Aspergillus nidulans*. *Mol. Microbiol.* **35**, 223–233
18. Barra, J.L., Rhounim, L., Rossignol, J-L., and Faugeron, G. (2000) Histone H1 is dispensable for methylation-associated gene silencing in *Ascobolus immersus* and essential for long life span. *Mol. Cell Biol.* **20**, 61–69
19. Sirotkin, A.M., Edelmann, W., Cheng, G., Klein-Szanto, A., Kucherlapati, R., and Skoultchi, A.I. (1995) Mice develop normally without the $H1^0$ linker histone. *Proc. Natl Acad. Sci. USA* **92**, 6434–6438
20. Lin, Q., Sirotkin, A., and Skoultchi, A.I. (2000) Normal spermatogenesis in mice lacking the testis-specific linker histone H1t. *Mol. Cell Biol.* **20**, 2122–2128
21. Khochbin, S. (2001) Histone H1 diversity: bridging regulatory signals to linker histone function. *Gene* **271**, 1–12
22. Lin, Q., Inselman, A., Han, X., Xu, H., Zhang, W., Handel, M.A., and Shoultchi, A.I. (2004) Reduction in linker histone levels are tolerated in developing spermatocytes but cause changes in specific gene expression. *J. Biol. Chem.* **279**, 23525–23535
23. Fan, Y., Sirotkin, A., Russell, R.G., Ayala, J., and Skoultchi, A.I. (2001) Individual somatic H1 subtypes are dispensable for mouse development even in mice lacking the $H1^0$ replacement subtype. *Mol. Cell Biol.* **21**, 7933–7943
24. Fan, Y., Nikitina, T., Morin-Kensicki, E.M., Zhao, J., Magnuson, T.R., Woodcock, C.L., and Skoultchi, A.I. (2003) H1 linker histones are essential for mouse development and affect nucleosome spacing *in vivo*. *Mol. Cell Biol.* **23**, 4559–4572
25. Fan, Y., Nikitina, T., Zhao, J., Fleury, T.J., Bhattacharyya, R., Bouhassira, E.E., Stein, A., Woodcock, C.L., and Skoultchi, A.I. (2005) Histone H1 depletion in mammals alters global chromatin structure but causes specific changes in gene regulation. *Cell* **123**, 1199–1212
26. Steinbach, O.C., Wolffe, A.P., and Rupp, R.A.W. (1997) Somatic linker histones cause loss of mesodermal competence in *Xenopus*. *Nature* **389**, 395–399
27. Saeki, H., Ohsumi, K., Aihara, H., Ito, T., Hirose, S., Ura, K., and Kaneda, Y. (2005) Linker histone variants control chromatin dynamics during early embryogenesis. *Proc. Natl Acad. Sci. USA* **102**, 5697–5702
28. Jedrusik, M.A. and Schulze, E. (2001) A single histone isoform (H1.1) is essential for chromatin silencing and germline development in *Caenorhabditis elegans*. *Development* **128**, 1069–1080
29. Prymakkowska-Bosak, M., Przewloka, M.R., Slusarczyk, J., Kuras, M., Lichota, J., Kilianczyk, B., and Jerzmanowski, A. (1999) Linker histones play a role in male meiosis and the development of pollen grains in tobacco. *Plant Cell* **11**, 2317–2329
30. Wierzbicki, A.T. and Jerzmanowski, A. (2005) Suppression of histone H1 genes in *Arabidopsis* results in heritable

developmental defects and stochastic changes in DNA methylation. *Genetics* **169**, 997–1008
31. Hohmann, P. (1983) Phosphorylation of H1 histones. *Mol. Cell Biochem.* **57**, 81–92
32. Guo, C.Y., Mizzen, C., Wang, Y., and Larner, J.M. (2000) Histone H1 and H3 dephosphorylation are differentially regulated by radiation-induced signal transduction pathways. *Cancer Res.* **60**, 5667–5672
33. Talasz, H., Helliger, W., Sarg, B., Debbage, P.L., Puschendorf, B., and Linder, H. (2002) Hyperphosphorylation of histone H2A.X and dephosphorylation of histone H1 subtypes in the course of apoptosis. *Cell Death Differ.* **9**, 27–39
34. Horn, P.J., Carruthers, L.M., Logie, C., Hill, D.A., Solomon, M.J., Wade, P.A., Imbalzano, A.N., Hansen, J.C., and Peterson, C.L. (2002) Phosphorylation of linker histones regulates ATP-dependent chromatin remodeling enzymes. *Nature Struct. Biol.* **9**, 263–267
35. Roth, S.Y., Schulman, I.G., Richman, R., Cook, R.G., and Allis, C.D. (1988) Characterization of phosphorylation sites in histone H1 in the amitotic macronucleus of *Tetrahymena* during different physiological states. *J. Cell Biol.* **107**, 2473–2482
36. Gurley, L.R., Valdez, J.G., and Buchanan, J.S. (1995) Characterization of the mitotic specific phosphorylation site of histone H1. *J. Biol. Chem.* **270**, 27653–27660
37. Kuzmichev, A., Jenuwein, T., Tempst, P., and Reinburg, D. (2004) Different Ezh2-containing complexes target methylation of histone H1 or nucleosomal histone H3. *Mol. Cell* **14**, 183–193
38. Trojer, P., Li, G., Simms, R.J., III, Vaquero, A., Kalakonda, N., Boccuni, P., Lee, D., Erdjument-Bromage, H., Tempst, P., Nimer, S.D., Wang, Y-H., and Reinburg, D. (2007) L3MBTL1, a histone-methylation-dependent chromatin lock. *Cell* **129**, 915–928
39. Deterding, L.J., Banks, G.C., Tomer, K.B., and Archer, T.K. (2004) Understanding global changes in histone H1 phosphorylation using mass spectrometry. *Methods* **33**, 53–58
40. Garcia, B.A., Busby, S.A., Barber, C.M., Shabanowitz, J., Allis, C.D., and Hunt, D.F. (2004) Characterization of phosphorylation sites on histone H1 isoforms by tandem mass spectrometry. *J. Proteome Res.* **3**, 1219–1227
41. Garcia, B.A., Joshi, S., Thomas, C.E., Chitta, R.K., Diaz, R.L., Busby, S.A., Andrews, P.C., Ogorzalek Loo, R.R., Shabanowitz, J., Kelleher, N.L., Mizzen, C.A., Allis, C.D., and Hunt, D.F. (2006) Comprehensive phosphorylation analysis of linker histone H1 from *Tetrahymena thermophila*. *Mol. Cell Proteomics* **5**, 1593–1609
42. Fischle, W., Wang, Y., and Allis, C.D. (2003) Binary switches and modification cassettes in histone biology and beyond. *Nature* **425**, 475–479
43. Daujat, S., Zeissler, U., Waldmann, T., Happel, N., and Schneider, R. (2005) HP1 binds specifically to Lys^{26}-methylated histone H1.4, whereas simultaneous Ser^{27} phosphorylation blocks HP1 binding. *J. Biol. Chem.* **280**, 38090–38095
44. Wisniewski, J.R., Zougman, A., Kruger, S., and Mann, M. (2007) Mass spectrometric mapping of linker histone H1 variants reveals multiple acetylations, methylations, and phosphorylation as well as differences between cell culture and tissue. *Mol. Cell Proteomics* **6**, 72–87
45. Phram, A-D. and Sauer, F. (2000) Ubiquitin-activating/conjugating activity of $TAF_{II}250$, a mediator of activation of gene expression in Drosophila. *Science* **289**, 2357–2360
46. Jiang, T., Zhou, K., Taghizadeh, K., Dong, M., and Dedon, P.C. (2007) N-formylation of lysine in histone proteins as a secondary modification arising from oxidative DNA damage. *Proc. Natl Acad. Sci. USA* **104**, 60–65
47. Sarg, B., Helliger, W., Talasz, H., Forg, B., and Linder, H.H. (2006) Histone H1 phosphorylation occurs site-specifically during interphase and mitosis. *J. Biol. Chem.* **281**, 6573–6580
48. Hale, T.K., Contreras, A., Morrison, A.J., and Herrera, R.E. (2006) Phosphorylation of the linker histone H1 by CDK regulates its binding to HP1α. *Mol. Cell* **22**, 693–699
49. Cerf, C., Lippens, G., Ramakrishnan, V., Muyldermans, S., Segers, A., Wyns, L., Wodak, S.J., and Hallenga, K. (1994) Homo- and heteronuclear two-dimensional NMR studies of the globular domain of histone H1: full assignment, tertiary structure, and comparison with the globular domain of histone H5. *Biochemistry* **33**, 11079–11086