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Heriberto Cerutti
University of Nebraska-Lincoln, hcerutti1@unl.edu

J. Armando Casas-Mollano
University of Nebraska-Lincoln

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Histone H3 Phosphorylation: Universal Code or Lineage Specific Dialects?

Heriberto Cerutti and J. Armando Casas-Mollano

School of Biological Sciences and Center for Plant Science Innovation, University of Nebraska–Lincoln, Lincoln, Nebraska, USA

Corresponding author – Heriberto Cerutti, School of Biological Sciences and Center for Plant Science Innovation, University of Nebraska, P.O. Box 880666, Lincoln, NE 68588 USA, email hcerutti1@unl.edu

Abstract

Post-translational modifications of histones modulate the functional landscape of chromatin and impinge on many DNA-mediated processes. Phosphorylation of histone H3 plays a role in the regulation of gene expression and in chromosome condensation/segregation. Certain evolutionarily conserved residues on histone H3—namely Thr3, Ser10, Thr11, and Ser28—are phosphorylated during interphase or mitosis in both metazoa and plants. However, many of the kinases involved in these events appear to have evolved independently in different lineages. Likewise, the mechanistic function of specific phosphorylated amino acids, although poorly understood, also seems to differ among eukaryotes. Moreover, some modifications, such as phosphorylation of histone H3 Ser10, appear to have both a positive and a negative connotation and only become meaningful in combination with other histone marks within a particular chromatin context. Thus, a detailed understanding of the influence of histone H3 phosphorylation on biological processes may require learning organismal dialects of the histone code.

Keywords: histone code, epigenetics, histone phosphorylation, transcription, gene silencing, phosphoacetylation

Introduction

In eukaryotes, nuclear DNA is associated with histones and other proteins in a complex structure referred to as chromatin. Histones are subject to multiple post-translational modifications (PTMs), including acetylation, methylation, and phosphorylation.[1,2] These histone PTMs influence chromatin organization and can regulate many DNA-templated processes, such as transcription, replication, recombination, repair, and chromosome segregation.[1,2] Certain histone PTMs may directly affect chromatin structure, for instance, by altering histone-DNA or internucleosomal interactions, whereas others may act indirectly, recruiting or preventing the binding of specific proteins to chromatin.[2,3]

The "histone code" hypothesis proposed that post-translational modifications on histone residues are recognized by effector molecules, which in turn direct unique downstream events.[4] In the strictest sense of the word, a "code" would imply that histone PTMs are predictive of specific outcomes.[2,4,5] However, emerging evidence suggests that individual histone modifications do not reliably predict a single functional output,[1,3,5] although combinations of histone PTMs appear to correlate better with a particular chromatin structure or function in a given organism.[6,7] In addition, whereas some modifications seem to have a conserved meaning in most, if not all, eukaryotes, others appear to have diverged in their biological connotation.[8,9]

Histone phosphorylation has been linked to a variety of cellular processes, such as chromosome condensation and segregation, activation of transcription, gene silencing, apoptosis, and DNA damage repair.[2,8,10] Phosphorylation of histone H3 has been specifically implicated in cell cycle progression and regulation of gene expression.[2,4,8,10] However, unlike the widely studied roles of histone methylation or acetylation, relatively little is known about the molecular mechanisms involved in translating histone phosphorylation into specific outcomes. Moreover, as discussed here, even though phosphorylation of certain histone H3 residues appears to have been conserved, their functional significance might differ among eukaryotes, in particular between metazoa and plants.

Histone H3 Phosphorylation during Mitosis

Phosphorylation of histone H3 seems to be important for the proper condensation and segregation of chromosomes.[4,8,10] In mammalian cells, histone H3 is phosphorylated at several sites during mitosis, including serines 10 and 28 (H3S10ph and H3S28ph) and threonines 3 and 11 (H3T3ph and H3T11ph).[8,11–13] The mitotic phosphorylation of at least some of these residues is conserved in a variety of metazoa, fungi, plants and protozoa.[8–11] Moreover, several kinases implicated in phosphorylating histone H3 during mitosis in mammalian cells, Aurora B for H3S10 and H3S28 and Haspin for H3T3,[2,8,14] appear to have orthologs in a wide spectrum of eukaryotes (Fig. 1). However, mitotic histone H3 phosphorylation also seems to be carried out by lineage-specific kinases. In metazoa, H3T11 is likely phosphorylated by the Dlk/ZIP kinase,[15] a member of the death-associated protein kinase family that lacks true orthologs in plants.[16] H3T3 and H3S10 also seem to be phosphorylated by Vaccinia-Related Kinase 1 (VRK1),[17] another metazoa-specific protein (Fig. 1).

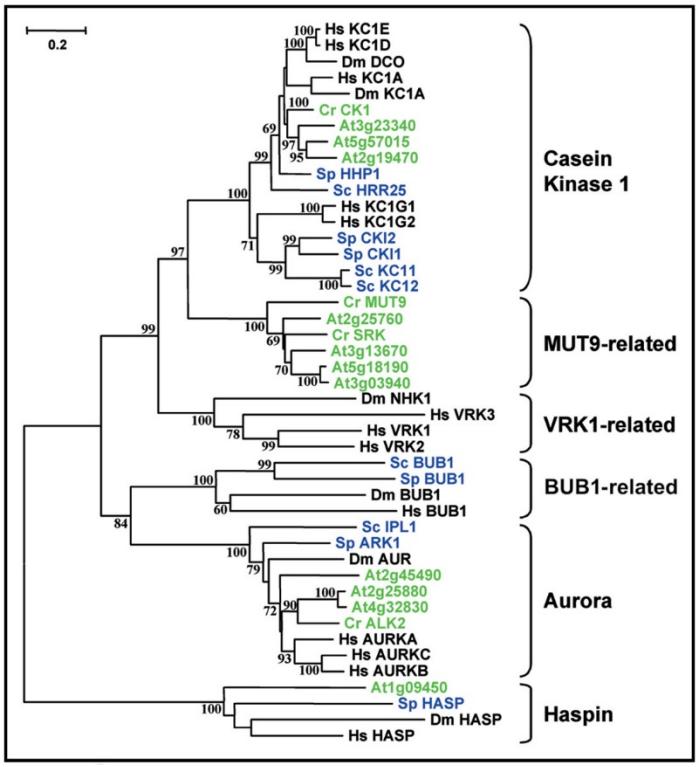

Figure 1. Phylogenetic analysis of several histone H3 kinases and other closely related eukaryotic kinases. Ser/Thr kinase domains in the examined proteins were identified using the SMART database. Sequences were then aligned with the ClustalX program, and the tree was drawn with the MEGA3.1 program using the Neighbor-Joining method. Numbers on the branches indicate bootstrap values higher than 60%, based on 1,000 pseudoreplicates. Kinase subclasses are indicated to the right of the tree: MUT9, Mutant 9 kinase; VRK1, Vaccinia Related Kinase 1; BUB1, Budding Uninhibited by Benzimidazole 1 kinase. Species are designated by a two-letter abbreviation preceding the name of each protein: At, *Arabidopsis thaliana* (green); Cr, *Chlamydomonas reinhardtii* (green); Dm, *Drosophila melanogaster* (black); Hs, *Homo sapiens* (black); Sc, *Saccharomyces cerevisiae* (blue); Sp, *Schizosaccharomyces pombe* (blue).

In mammalian cells, increased phosphorylation of H3S10 and H3S28 starts in late G2 in pericentromeric regions and then spreads throughout the chromosome arms as mitosis proceeds.[8,18–20] H3S10ph becomes later more prominent at the periphery of the metaphase plate (i.e., at the ends of chromosomes) and persists until late anaphase.[14,18,19] This pattern of phosphorylation and other analyses suggested that H3S10ph and H3S28ph might play a role in chromosome condensation.[8,17,20] Phosphorylation of H3T3 initially overlaps with that of H3S10ph but, at metaphase, H3T3ph is more intensely concentrated in the central region of the metaphase plate.[14,19] In metazoa, H3T3ph has been implicated in metaphase chromosome alignment and centromeric cohesion,[8,14] although given the similarities with the H3S10ph distribution, it might also function in chromosome condensation.[17] Finally,

H3T11ph appears to be limited to the centromeric regions of metaphase chromosomes and has been proposed to serve as a recognition code for kinetochore assembly.[13,15] The histone variant H3.3 also undergoes phosphorylation at serine 31 in mitotic mammalian cells, predominantly in regions bordering centromeres during metaphase.[11,21]

In higher plants with monocentric chromosomes, like *Hordeum vulgare* and *Secale cereale*, phosphorylation of H3S10 and H3S28 during mitosis remains limited to pericentromeric regions.[8,9,22,23] In contrast, H3T3ph and H3T11ph start near centromeres but then disperse along entire chromosome arms.[8,24] H3T32 is also phosphorylated during mitosis of plants cells, with a distribution similar to that of H3T11ph.[24] Based on these patterns of phosphorylation, unlike those observed in metazoa, H3S10ph and H3S28ph have been postulated to play a role in sister centromere cohesion whereas H3T3ph and H3T11ph (and perhaps H3T32ph) appear to correlate with chromosome condensation.[8,9,23,25] Members of the aurora family of kinases have been implicated in the phosphorylation of H3S10 in *Arabidopsis thaliana*,[8] as in mammals, but other mitotic kinases remain uncharacterized in plants.

Given the varied and often conflicting data on mitotic histone H3 phosphorylation in different organisms, its function remains controversial. Mass spectrometry analyses of human histone H3 during mitosis revealed that phosphorylation and methylation can be found on adjacent residues of the same H3 molecule such as Lys9/Ser10 and Lys27/Ser28,[11,12] providing support for the "methylation/phosphorylation" binary switch hypothesis.[26] This hypothesis proposed that post-translational modifications on adjacent amino acids, such as methylation of Lys9 and phosphorylation of Ser10 on histone H3, could modulate the binding of effector molecules to histones.[26] In mammalian cells, constitutive heterochromatin is characterized, among other modifications, by enrichment in trimethylated H3K9 (H3K9me3).[2,9,27] Heterochromatin Protein 1 (HP1) is recruited to chromatin by the binding of its chromo domain to di- or trimethylated H3K9 and mediates heterochromatin organization and gene silencing.[26,28,29] As cells enter mitosis, phosphorylation of H3S10 (and perhaps also the acetylation of H3K14) has been shown to promote dissociation of HP1β from chromosomes without an alteration in H3K9me3 levels.[28,30] This HP1 displacement provides a molecular mechanism for the action of H3S10 phosphorylation during mitosis. In metazoa, H3S10ph could conceivably mediate the release of tightly associated chromatin-binding proteins, such as HP1, and allow the dynamic rearrangements of chromatin higher-order structure required for mitotic chromosome condensation. In addition, avoiding erasure of the H3K9 methylation mark would allow for epigenetic "memory maintenance" during mitotic progression.

However, the role of H3S10ph in the mitotic release of HP1 does not seem to have been conserved in the plant lineage. In several plant species, in contrast to metazoa, mitotic H3S10ph does not extend to heterochromatic domains in chromosomal arms.[8,9,22] Moreover, heterochromatin in angiosperms is characterized by mono- or dimethylation of H3K9,[9,23,27] rather than H3K9me3, and the only homolog of HP1 encoded in the *A. thaliana* genome, Like Heterochromatin Protein 1 (LHP1), does not seem to be a component of heterochromatin.[31,32] Arabidopsis LHP1 binds instead to trimethylated H3K27 and mediates the silencing of euchromatic genes.[32,33] Like metazoan HP1, LHP1 does dissociate from chromosomes during mitosis31 but the mechanism involved in this process is presently unknown. It might be tempting to argue that a Lys27/Ser28 methylation/phosphorylation switch might

promote the mitotic release of LHP1 in plants, but the patterns of H3K27me3 and H3S28ph hardly overlap.[8,9] For instance, in *H. vulgare*, H3K27me3 is strongly enriched in the gene-rich termini of mitotic chromosomes[9] whereas H3S28ph is restricted to pericentromeric regions.[8,22] Thus, the phosphorylation of H3T3, H3S10, H3T11, and H3S28 during mitosis is apparently conserved between metazoa and plants. However, the chromosomal localization, the timing and the putative functional significance of these phosphorylation events, with the possible exception of H3T3ph, seem to have diverged substantially. Yet, an important caveat in this interpretation is that the precise mechanistic role of mitotically phosphorylated histone H3 residues is still unclear in nearly all organisms.

Histone H3 Phosphorylation during Interphase

Phosphorylation of histone H3 has also been linked to transcriptional regulation during interphase. In mammalian cells, H3S10ph has been implicated in the activation of various genes responding to stress or mitogen-stimulated signaling pathways, such as *c-fos* and *c-jun*, and is tightly associated with acetylation of H3K9 and H3K14.[2,34,35] In *Drosophila melanogaster*, phosphorylation of H3S10 has been linked to dosage compensation on the male X chromosome and transcription induced by the heat shock response.[10,36] In the latter case, H3S10ph has been proposed to serve as a platform for the recruitment of the Positive Transcription Elongation Factor b (P-TEFb) that, in turn, induces the release of RNA polymerase II from promoter-proximal pausing.[36,37] However, these results have been recently challenged, and H3S10ph has been suggested, instead, to cause structural alterations of chromatin, counteracting heterochromatization and gene silencing at certain loci.[38] Indeed, in metazoa, as appears to be the case in *Saccharomyces cerevisiae*,[39] histone H3S10 phosphorylation may not be universally required for transcription but may play a distinct role tailored to specific promoters.[10,34,35,38] Intriguingly, H3S10ph, as part of a double modification with H3K9me3, has also been found in association with facultative heterochromatin in differentiated postmitotic mammalian cells.[29] This phosphorylation of H3S10 leads to displacement of HP1β from chromatin,[29] perhaps allowing the binding of an unidentified (heterochromatic) protein(s) that recognizes the double modification (Fig. 2A).

A number of protein kinases have been implicated in H3S10 phosphorylation in interphase cells of metazoa, such as Ribosomal Protein S6 Kinase (RPS6K) family members (MSK1, MSK2, and JIL-1), IκB Kinase α, PDZ binding kinase, the proto-oncogenic PIM1, and Transglutaminase 2.[2,10,35,37,40] With the exception of RPS6K family members, most of the metazoan kinases phosphorylating H3S10 do not appear to be conserved in the plant lineage.[16] Interestingly, environmental stresses enhance the activity of two *A. thaliana* RPS6K homologs[41] and, in both tobacco and *A. thaliana* cell lines, H3S10 phosphorylation has been observed to increase in response to high salinity or cold treatment.[8,42] In interphase nuclei of *A. thaliana* plants grown under normal conditions, H3S10ph also appears to be associated with heterochromatin.[27,43] However, the role of H3S10ph in transcriptional activation or repression remains to be examined in plant species.

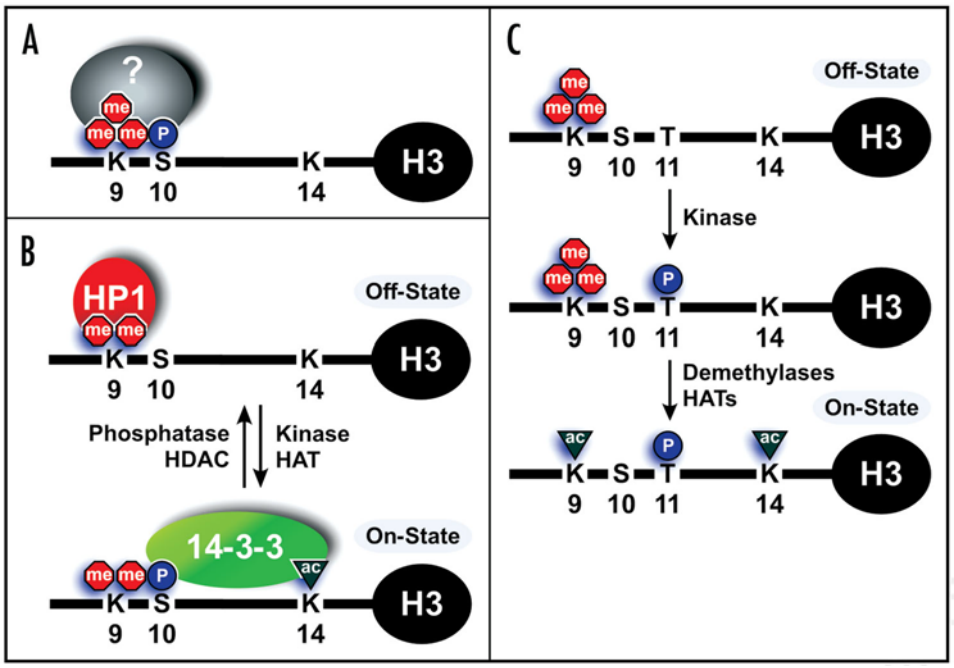

Figure 2. Models for the role of histone H3 phosphorylation in transcriptional regulation during interphase (see text for details). (A) The double H3K9me3/H3S10ph modification may generate a novel binding platform for a protein(s) involved in facultative heterochromatin organization and gene silencing.[29] (B) Phosphorylation of H3S10 and acetylation of H3K14 may trigger the displacement of repressive HP1, without removal of H3K9me2, the binding of 14-3-3 proteins and promoter activation.[35] HAT, histone acetyltransferase; HDAC, histone deacetylase. (C) Phosphorylation of H3T11 may enhance demethylation of H3K9 and histone H3 acetylation, resulting in chromatin changes conducive to transcriptional induction.[47] HATs, histone acetyltransferases.

The paradoxical correlation of H3S10ph with both (heterochromatic) gene silencing and gene activation in interphase could be explained if it modulates, in combination with other histone modifications, the interaction with H3 of distinct effectors that influence specific outcomes (Fig. 2A and B). In mammalian systems, 14-3-3 proteins have recently been shown to bind histone H3 phosphorylated on Ser10 (or on Ser28) and this association is more stable if Lys9 or Lys14 (but not both) is acetylated.[35,44–46] Moreover, 14-3-3 proteins are recruited to nucleosomes at the *c-fos* and *c-jun* promoters following gene activation and seem to be required for transcriptional induction.[35,44,46] Thus, 14-3-3 proteins appear to act as phosphoacetylation recognition modules (Fig. 2B) and counterparts of the repressive HP1 molecule.[35,46] The precise role of 14-3-3 proteins in transcriptional activation is not known but they seem to detect phosphorylated H3 residues specifically during interphase, since they are excluded from mitotic chromosomes.[35,44] For some genes like *hdac1*, the recognition of H3S10ph and H3K14ac by 14-3-3 proteins may allow overriding the repressive influence of H3K9 methylation, without erasure of this mark (Fig. 2B), and the rapid switching between expression/repression states.[35,46]

In other systems, such as androgen receptor-dependent gene expression, active removal of H3K9 methylation from promoters appears to be part of the activation process. Phosphorylation of H3T11, mediated by Protein kinase C Related Kinase 1 (PRK1), has been implicated in this type of transcriptional regulation.[40,47] H3T11ph appears to enhance the activity of JMJD2C, a protein containing a Jumonji C catalytic domain that cooperates with Lysine-Specific Demethylase 1 (LSD1) to remove tri-, di-, and monomethyl groups from H3K9.[47,48] In addition, H3T11ph may facilitate the recruitment of histone acetyltransferases, such as the transcriptional coactivator p300, that add acetyl groups to H3K9 and H3K14.[47] These combined modifications (Fig. 2C) likely lead to HP1 release and chromatin structure changes that contribute, at least partly, to transcriptional induction. Phosphorylation of H3T11 by Checkpoint kinase 1 (Chk1), possibly enhancing the recruitment to chromatin of the GCN5 histone acetyltransferase, also appears to be involved in the activation of the *cyclin B1* and *cdk1* promoters.[40,49] Intriguingly, demethylation of H3K9me3 by JMJD2A, a close homolog of JMJD2C, is blocked by phosphorylation of H3S10.[50] Thus, in metazoa, histone H3 phosphorylation can result in transcriptional activation by different mechanisms, depending on the particular modified residue (Fig. 2B and C).

To our knowledge, a potential role of H3T11ph in transcription regulation has not been examined in plant species. However, H3T3ph has recently been implicated in gene repression in the green alga *Chlamydomonas reinhardtii*.[51] Based on chromatin immunoprecipitation assays, both H3T3ph and H3K4me1 associate with silent chromatin and their distribution appears to be mutually exclusive with that of the activating di- or trimethyl forms of H3K4.[51,52] It is tempting to speculate, by analogy to the proposed function of H3T11ph, that H3T3ph may promote the recruitment and/or the activity of a demethylase that erases the H3K4me3 and H3K4me2 states. MUT9, the kinase responsible for H3T3 phosphorylation in *C. reinhardtii*, seems to have evolved in the plant lineage and lacks metazoa orthologs (Fig. 1).[51] Yet, H3T3ph does appear to occur in mammalian interphase cells, although its function has not been characterized.[17,19]

As for mitotic cells, certain histone H3 residues are phosphorylated in interphase cells of both metazoa and plants. However, many of the kinases involved in these events seem to have evolved independently in different lineages and target specific subsets of genes. The mechanisms by which histone H3 phosphorylation is translated into transcriptional regulation also appear to differ depending on the particular modified residue and the system under study. Nonetheless, assessing the significance of these differences will require further research since the machinery decoding histone H3 phosphorylation is still poorly understood in almost all organisms and the functional outcomes, which seem to depend on complex combinatorial PTMs, are only beginning to be deciphered.

Perspective

One significant challenge in the chromatin field is to define the molecular events that link histone post-translational modifications with specific biological outcomes, such as transcriptional activation or repression. A loosely defined "histone code" that describes a specific set of histone PTMs for a given output (regardless of whether they are causative or consequential) would be very useful to begin decoding genomic information into function.

However, chromatin marks cannot be easily interpreted, some seem to have both a positive and a negative connotation and only become meaningful in combination with other modifications within a genomic and regulatory context.[1,2,5] An added level of complexity is that the language of covalent histone modifications may not be universal.[8,9,27] In the case of histone H3, the phosphorylation of several residues appears to be conserved in widely divergent eukaryotes, both during mitosis and in interphase cells. However, upon closer inspection of the data, the functional significance of some of these modifications seems to have diverged. Several phosphorylation events appear to be carried out by lineage specific kinases that evolved after the divergence of metazoa and plants and conceivably integrate differentially with specific DNA-mediated processes. The readers of these modifications are poorly characterized in nearly all eukaryotes. Yet, it is becoming apparent that there are organismal differences in the mechanistic connotation of phosphorylated H3 residues. For instance, the effect of H3S10ph on the binding affinity of HP1 is unlikely to be recapitulated in plant species where the HP1 homolog associates with H3K27me3. Thus, some histone PTMs may have an almost invariant meaning across the eukaryotic spectrum, even if difficult to comprehend. Others, including the phosphorylation of certain histone H3 residues, may require learning organismal dialects to fully understand their influence on biological processes. Elucidating these dialects will involve detailed characterization of the mechanisms that interpret histone post-translational modifications in evolutionarily divergent eukaryotes.

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