

# Histone phosphorylation

## A chromatin modification involved in diverse nuclear events

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**Keywords:** chromatin remodeling, histone modifications, phosphorylation, mitosis, DNA repair, gene regulation

Histone post-translational modifications are key components of diverse processes that modulate chromatin structure. These marks function as signals during various chromatin-based events, and act as platforms for recruitment, assembly or retention of chromatin-associated factors. The best-known function of histone phosphorylation takes place during cellular response to DNA damage, when phosphorylated histone H2A(X) demarcates large chromatin domains around the site of DNA breakage. However, multiple studies have also shown that histone phosphorylation plays crucial roles in chromatin remodeling linked to other nuclear processes. In this review, we summarize the current knowledge of histone phosphorylation and describe the many kinases and phosphatases that regulate it. We discuss the key roles played by this histone mark in DNA repair, transcription and chromatin compaction during cell division and apoptosis. Additionally, we describe the intricate crosstalk that occurs between phosphorylation and other histone modifications and allows for sophisticated control over the chromatin remodeling processes.

### Introduction

Chromatin is a nucleoprotein structure that compacts and organizes DNA in eukaryotic cells. However, it also establishes a strong barrier for nuclear events that need to access the DNA, such as transcription, DNA repair, replication and meiotic recombination. The fundamental unit of chromatin is the nucleosome, which consists of 146 base pairs of DNA wrapped around an octamer of histones—small basic proteins that are highly evolutionarily conserved. The histone core contains two molecules of each histone H2A, H2B, H3 and H4.<sup>1</sup> Unlike the central structured domains of histone proteins that form the globular part of the nucleosome, the N-terminal domain of all four histones and C-terminal domains of H2A and H2B, called “histone tails,” are poorly structured and protrude from the nucleosome.

In order to control DNA accessibility, the chromatin structure must be dynamically modulated. Four mechanisms that modify chromatin compaction are well characterized. They are not

mutually exclusive but cooperate to regulate chromatin structure and DNA accessibility. These include (1) ATP-dependent remodeling complexes that use the energy of ATP hydrolysis to slide the nucleosomes and exchange or evict histones from the chromatin fiber; (2) histone variants that possess properties distinct from those of canonical histones and create localized specific domains within the chromatin fiber; (3) histone chaperones that control the supply of free histones and cooperate with chromatin remodelers in histone deposition and eviction and (4) post-translational modifications (PTMs) of histones, such as acetylation, methylation, phosphorylation and ubiquitination that directly or indirectly influence chromatin structure. These modifications, which occur predominately on the accessible histone tails, can directly modulate histone-DNA interactions and also form docking sites to facilitate recruitment of specific non-histone proteins to chromatin. Importantly, combinations of PTMs occurring on the same histone tail (in cis), or on another tail (in trans), have been proposed to constitute a “histone code” or signature.<sup>2-5</sup> This code is deciphered by the so-called readers, proteins that contain binding motifs specific for each modification: for example, chromodomains specifically recognize methylated residues, while bromodomains bind acetylated residues.<sup>6,7</sup> Recognition of PTMs results in the local binding of reader-containing complexes known as effectors that interpret the signal, eventually leading to chromatin remodeling.<sup>5,8</sup>

In this review, we will focus specifically on histone phosphorylation. All four nucleosomal histone tails contain acceptor sites that can be phosphorylated by a number of protein kinases and dephosphorylated by phosphatases. Histone phosphorylation can occur on serine, threonine and tyrosine residues and constitutes an essential part of the “histone code,” or combinatorial function of PTMs on chromatin. A number of proteins containing phospho-binding modules such as 14-3-3 and BRCT domains that can recognize phosphorylated histones have been identified and characterized as downstream effectors (for review, see refs. 6 and 7). While a large number of phosphorylated residues on histones have been described, identification of new phosphorylation sites and characterization of their biological functions remain areas of intensive investigation. In this review, we will summarize current knowledge concerning histone phosphorylation events, their regulation and their known functions in nuclear processes that involve modulation of chromatin structure.

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Submitted: 03/05/12; Revised: 08/20/12; Accepted: 08/26/12  
<http://dx.doi.org/10.4161/epi.21975>

## Histone Phosphorylation Involved in DNA Damage Repair

Phosphorylation of H2A(X) is an important histone modification that plays a major role in DNA damage response (for review, see refs. 9 and 10). In mammalian cells, this modification takes place on serine (S) 139 of the H2AX variant histone, and is commonly referred to as  $\gamma$ H2AX.<sup>11,12</sup> In yeast, the H2AX variant does not exist, and the corresponding phosphorylation occurs on S129 of H2A.<sup>13,14</sup> For simplicity, we will hence refer to both modifications as  $\gamma$ H2AX. This phosphorylation occurs in all phases of the cell cycle and is involved in diverse DNA-damage response (DDR) pathways including non-homologous end joining (NHEJ), homologous recombination (HR) and replication-coupled DNA repair.<sup>13-15</sup> Protein kinases  $\gamma$ Tel1 and  $\gamma$ Mec1 (ATM and ATR in mammals) carry out this phosphorylation.<sup>13,16</sup> Mutation of H2AS129 in yeast to non-phosphorylatable alanine leads to hypersensitivity to DNA-damage inducing agents such as phleomycin and methyl methane-sulphonate (MMS), highlighting a crucial role of  $\gamma$ H2AX in DNA double strand break (DSB) repair.<sup>13</sup> Phosphorylation of H2AS129 in yeast is one of the first events following DNA damage, appearing within 30 min in the immediate vicinity of the DSB.<sup>16-19</sup> This modification spreads bidirectionally over several kilobases (~50 kb) on each side of the break in yeast, while mammalian  $\gamma$ H2AX spreads over several megabases.<sup>16,20-22</sup> This wide distribution of  $\gamma$ H2AX around the break is thought to create a specific signaling platform for recruitment and/or retention of DNA damage repair and signaling factors, including the critical mediator protein MDC1 through recognition by its BRCT domain<sup>23,24</sup> (for review, see ref. 10). Studies performed in yeast suggest that  $\gamma$ H2AX also facilitates recruitment of the NuA4 acetyltransferase complex, which is thought to be the first chromatin modifier recruited to the DSB after the  $\gamma$ H2AX kinases.<sup>17,25</sup> NuA4 relocalization to the break results in H4 hyperacetylation,<sup>17,25,26</sup> which is proposed to promote local chromatin relaxation. In addition, it has been suggested that  $\gamma$ H2AX allows the recruitment to the DSB of other chromatin-modifying complexes such as the ATP-dependent remodelers INO80 and SWR1. These complexes are thought to act in concert to enhance DNA accessibility in order to facilitate the repair process.<sup>9,27,28</sup> NuA4, INO80 and SWR1 all contain a common subunit, Arp4, which was found to bind directly to  $\gamma$ H2AX in yeast, suggesting a plausible mechanism for their accumulation near the break.<sup>17</sup> Additionally, the 53BP1/Crb2-related adaptor protein Rad9 was demonstrated to interact with  $\gamma$ H2AX through its BRCT domain, while its Tudor domain binds methylated K79 of H3.<sup>29-31</sup> This specific binding promotes Rad9 recruitment to the DNA break where it is activated by the Mec1 kinase to induce activation of the DNA damage checkpoint. This mechanism plays the crucial role of delaying the cell's progression through the G<sub>1</sub>/S phase of the cell cycle, thereby permitting efficient repair of DNA damage.

Once the DNA has been repaired,  $\gamma$ H2AX must be removed from chromatin in order to stop the retention of repair proteins at the DSB, and to allow efficient recovery from the DNA damage-induced cell cycle arrest. A study performed in yeast suggested

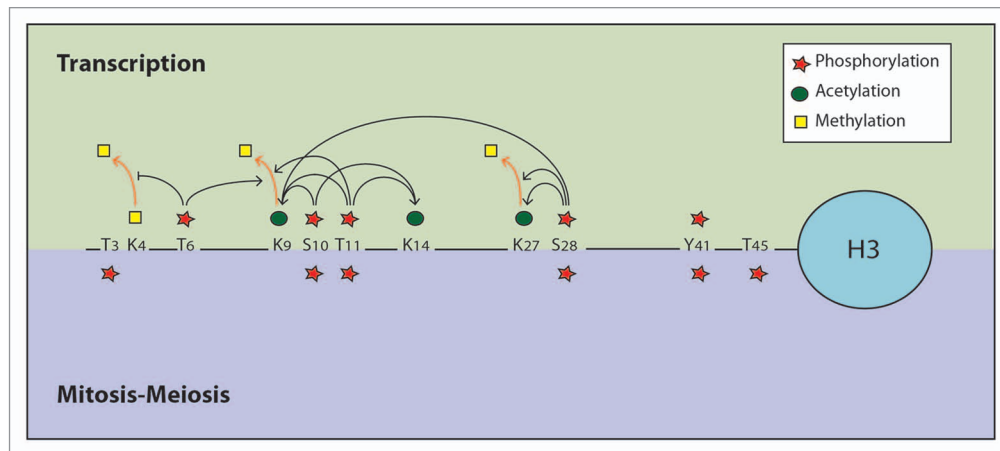
that  $\gamma$ H2AX is evicted and replaced with the Htz1 histone variant by the SWR1 remodeler.<sup>32</sup> In contrast, Van Attikum and colleagues showed that H2AZ (mammalian Htz1) and  $\gamma$ H2AX are both removed from chromatin near the DSB in an INO80-dependent manner.<sup>33</sup> Whether it is deposited or removed, the significance of H2AZ dynamics at the site of DNA damage has not been elucidated so far.

In addition to its removal by histone exchange,  $\gamma$ H2AX is also dephosphorylated. In yeast, the HTP-C phosphatase complex and its catalytic subunit Pph3 regulate H2AS129 phosphorylation status *in vivo*.<sup>34</sup> HTP-C targets H2AS129ph after its displacement from DNA, and this dephosphorylation is required for DNA damage checkpoint recovery. In parallel, mammalian  $\gamma$ H2AX phosphatases such as PP2A, Wip1, PP6 and PP4 have been described.<sup>35-39</sup> These enzymes dephosphorylate  $\gamma$ H2AX *in vivo*, and are similarly required for efficient DNA damage repair and recovery from cell cycle arrest.

The Allis group has recently shown that the H2AX variant is also phosphorylated on tyrosine (Y) 142 and that this modification is regulated in a DNA-damage-dependent manner. H2AXY142 is phosphorylated by the WSTF catalytic subunit of the WICH complex and dephosphorylated by the EYA1/3 phosphatase.<sup>40</sup> Interestingly, in contrast to  $\gamma$ H2AX, H2AXY142 phosphorylation is ubiquitous in the cell and decreases in response to DNA damage. This regulation is critical for  $\gamma$ H2AX maintenance as dephosphorylation of H2AXY142ph by EYA is required to maintain  $\gamma$ H2AX-containing DNA repair foci.<sup>40</sup> A defect of H2AXY142 dephosphorylation inhibits the accumulation of repair factors at damage sites.<sup>41</sup> Full understanding of the interplay between the inverse phosphorylation of these two residues during DNA damage response requires further investigation.

Phosphorylation of serine 1 of histone H4 by Casein Kinase II (CKII) is also induced in yeast upon genotoxic stress such as exposure to UV light (UV), methyl methanesulfonate (MMS) and phleomycin.<sup>42,43</sup> ChIP experiments demonstrate that H4S1ph is restricted to a much smaller region around the DSB and appears sometime after  $\gamma$ H2AX, implicating its involvement in the later stages of the DDR. Interestingly, H4S1ph, which was initially discovered as a mark of newly synthesized histones,<sup>44</sup> is inversely correlated with H4 acetylation whose removal takes place as the repair process is nearing completion.<sup>42,43</sup> Furthermore, phosphorylation of H4S1 was found to inhibit acetylation of the H4 tail by the NuA4 HAT *in vitro*, while *in vivo* the CKII kinase associates with the Rpd3S deacetylase complex.<sup>43</sup> Thus, it seems likely that as new nucleosomes are reassembled during restoration of chromatin structure, phosphorylation of H4S1 acts to stabilize them by preventing their acetylation. However, the fact that mutation of H4S1 to a non-phosphorylatable residue does not lead to yeast's increased sensitivity to genotoxic agents<sup>43</sup> indicates that this phosphorylation event is not essential to the repair process and suggests functional redundancy with other nucleosome-stabilizing mechanisms.

In yeast, serine 122 of histone H2A is phosphorylated together with the nearby H2AS129, and their modification is similarly modulated in response to DNA damage.<sup>45-48</sup> Systematic site-directed mutagenesis of the C-terminal tail of H2A determined



**Figure 1.** Examples of crosstalk between phosphorylation and other histone post-translational modifications. The histone H3 N-terminal tail is presented as example of a platform harboring multiples PTMs showing crosstalks in *cis* with different sites of phosphorylation. Recent in vitro findings using time-resolved NMR spectroscopy suggest a slightly modified picture in which H3S10ph acts as master switch for subsequent intramolecular modifications, and inhibits T6 and T11 phosphorylation while the reverse does not apply.<sup>139</sup> This study also questioned the role of T11ph in the stimulation of K14ac.

that S122 is important for survival in the presence of DNA damage induced by various agents such as camptothecin, phleomycin, MMS, hydroxyurea (HU) or UV.<sup>47,48</sup> Phosphorylation of this residue was suggested to have a direct role in DNA repair, although it is not required for Mec1-dependent signal transduction and occurs independently of S129 phosphorylation. Since inhibition of H2AS122 phosphorylation has no effect on global chromatin structure,<sup>47</sup> it can be speculated that this modification has a role in mediating the interaction with the DDR machinery. Phosphorylation of the adjacent S122 and S129 in response to DNA damage suggests a possible interplay or combinatorial action in *cis* during recruitment, accumulation or retention of repair factors. However, further investigation is required to confirm these hypotheses and to identify any effectors that may interact with these histone modifications.

In mammals, phosphorylation of H2BS14 was also detected upon DNA damage induced by ionizing radiation and shown to colocalize with  $\gamma$ H2AX foci.<sup>49</sup> The role of this modification in DDR is unknown, while previously linked to cell apoptosis, but it is plausible that this mark plays a role in combination with  $\gamma$ H2AX and H2AXY142ph.

### Histone Phosphorylation Associated with Transcription Regulation

A substantial number of phosphorylated histone residues are associated with gene expression. Interestingly, these are often related to regulation of proliferative genes. Phosphorylation of serines 10 and 28 of H3 and serine 32 of H2B has been associated with regulation of epidermal growth factor (EGF)-responsive gene transcription. H3S10ph and H2BS32ph have been linked to the expression of proto-oncogenes such as *c-fos*, *c-jun* and *c-myc*.<sup>50-52</sup> Furthermore, targeting H3S28 phosphorylation to promoters of genes such as *c-fos* and  $\alpha$ -globin was shown to control their activation.<sup>53</sup> Notably, phosphorylation of H3S10 and H3S28 is not

exclusive to EGF stimulation, but it also increases upon UVB radiation. Phosphorylation of H3 on T11 and T6 has also been implicated in transcription regulation in response to androgen stimulation<sup>54,55</sup> and to DNA-damage in mouse cells.<sup>56</sup>

Phosphorylation of H3S10, T11 and S28 has been clearly associated with H3 acetylation, strongly implicating these modifications in transcription activation. Independent studies show that these phosphorylation events are mechanistically linked to Gcn5-dependent H3 acetylation (Fig. 1).<sup>57-59</sup> Indeed, in EGF-stimulated cells, phosphorylation of H3S10 is tightly coupled to H3 K9ac and K14ac, both marks of transcriptional activation.<sup>51,60,61</sup> It has been shown that phosphorylation of H3S10 promotes acetylation of H3K14 by the Gcn5 acetyltransferase in vitro and allows Gcn5-regulated gene transcription in vivo.<sup>57</sup> A similar link has been described in yeast where H3S10 phosphorylated by Snf1 acts in concert with Gcn5-dependent H3K14 acetylation to enhance transcription.<sup>62</sup> This functional link between H3S10ph and Gcn5 is likely mediated by the direct interaction of the acetyltransferase with the phosphorylated H3S10 residue, which has been observed in vitro.<sup>57,60</sup> However, analysis of the *c-jun* promoter activation showed that K14ac appears before S10ph and that inhibition of S10 phosphorylation has no effect on K14ac, suggesting that these two events can be uncoupled.<sup>61,63</sup> Interestingly, phosphorylation of the H3 tail on T11 in addition to S10 was shown to enhance its interaction with Gcn5 at promoters of Gcn5-dependent genes such as the cell-cycle regulators *cyclin B* and *cdk1*, leading to increased H3K9 and K14 acetylation and stimulation of transcription.<sup>54,56,58</sup> Likewise, phosphorylation of H3S28 was found to promote K9 acetylation.<sup>59</sup> All together, these data suggest a complex crosstalk between phosphorylation of H3S10, T11 and S28 in the control of Gcn5-dependent H3 acetylation, gene expression and cell proliferation (Fig. 1).

Importantly, H3S28ph also plays a role in combination with H3K27ac in transcription activation.<sup>53,64</sup> Mechanistically,

H3S28 phosphorylation at gene promoters is thought to displace Polycomb repressive complexes from chromatin and to induce demethylation and acetylation of the adjacent K27 residue at these loci, thereby activating transcription. Additionally, phosphorylation of H3T11 and H3T6 in response to androgen receptor-dependent gene activation has been shown to regulate transcription by controlling H3 methylation. Phosphorylation of both residues promotes removal of the repressive methyl mark on H3K9 by the Jumonji C domain-containing protein JMJD2C.<sup>54,55</sup> In addition, H3T6ph was shown to prevent LSD1-mediated removal of mono- and dimethyl-H3K4, marks of actively transcribed chromatin.<sup>55</sup> Remarkably, these studies suggest an additional interplay involving phosphorylation and methylation on the N-terminal tail of H3 in transcription regulation. Thus, phosphorylation of the H3 tail plays an important role in controlling in *cis* its acetylation and methylation in order to regulate gene expression.

H2BS32ph is ubiquitous in normally cycling mammalian cells, but is more abundant in skin cancer cells where RSK2, the kinase responsible for its phosphorylation, is also highly expressed.<sup>50,65</sup> In response to EGF treatment, H3 is phosphorylated on S10 by the specific mitogen- or stress-induced kinases RSK2 and MSK1,<sup>66,67</sup> and on S28 by MSK1, MSK2 and by the mixed lineage kinase-like mitogen-activated protein triple kinase (MLTK)- $\alpha$ .<sup>63,68</sup> Additionally, H3S10 and H3S28 are also phosphorylated in response to UVB radiation by the kinases ERK, p38 and the Src family member Fyn,<sup>69,70</sup> while H3S28 is also targeted by MLTK- $\alpha$ , MSK1, ERK1, ERK2, p38 and to a lesser degree JNK1 and JNK2.<sup>59,68,71,72</sup> Upon androgen stimulation, phosphorylation of H3T11 and T6 is catalyzed respectively by the protein kinase-C related kinase 1 (PRK1) and the protein kinase C- $\beta$  (PKC $\beta$ ), notably in prostate cancer cells.<sup>54,55</sup> Recently, it was demonstrated that H3T11 is also a specific substrate for the tumor-specific pyruvate kinase M2 (PKM2) in a transcriptional process that promotes H3 K9 acetylation and gene transcription upon EGF-signaling, leading to tumor cell proliferation.<sup>73</sup> H3T11ph can also be added by the Chk1 kinase in response to DNA damage in mammalian cells.<sup>56</sup> Upon DNA damage, this kinase rapidly dissociates from the promoters of cell-cycle regulatory genes, leading to a loss of H3T11ph, a concomitant reduction of permissive acetylation marks from the H3 tail, and a diminution of the transcription of these genes. Thus, this crosstalk between two distinct types of histone modifications appears to be responsible for transcription reduction and cell cycle arrest in response to DNA damage.

Phosphorylation of tyrosine (Y) 41 on H3 by Janus kinase 2 (JAK2) has also been described to influence transcription.<sup>74</sup> Phosphorylation of Y41 disrupts chromatin binding by HP1 $\alpha$  (heterochromatin protein 1  $\alpha$ ), which directly interacts with this region of H3 through its chromo-shadow domain. Loss of HP1 $\alpha$  from chromatin leads to transcriptional activation of JAK2-regulated genes including the oncogene *imo2*, which encodes a protein with roles in normal hematopoiesis and in leukemia. These observations suggest that, while JAK2 signaling and HP1 $\alpha$  chromatin association are tightly regulated in normally growing cells, constitutive activation of JAK2 can lead to oncogenesis by

mediating HP1 $\alpha$  displacement from chromatin through H3Y41 phosphorylation.

Serine 36 of H2B has been described as a target of AMPK (AMP-activated protein kinase) and its phosphorylation has been linked to gene expression.<sup>75</sup> ChIP experiments detect H2B S36ph within promoters and coding regions of AMPK-responsive genes, and loss of this modification leads to their reduced expression and lower rates of cell survival in response to metabolic stress. While both H3Y41ph and H2BS36ph are associated with transcription activation and cell proliferation, neither has been correlated with previously described *cis* modifications involved in expression of proliferation genes, namely T6, S10, T11 and S28 on H3 and S32 on H2B.

H2B has also been recently shown to be phosphorylated on tyrosine 37 in yeast and mammalian cells.<sup>76</sup> This mark is added by the WEE1 kinase and is important for suppression of replication-dependent core histone gene transcription. H2B Y37ph directly blocks the binding of a key transcription activator and allows recruitment of the HIRA histone chaperone.<sup>76</sup>

Studies in yeast have demonstrated that phosphorylation of H4S1 is also regulated during transcription. Indeed, levels of this modification increase upon transcription activation in a CKII-dependent manner.<sup>43</sup> Since phosphorylation of H4S1 was shown to negatively regulate H4 acetylation during DNA damage repair, it is surprising to observe the appearance of this mark upon transcription activation, a process usually associated with hyperacetylation. However, H4S1ph is enriched on the coding region compared with the promoter of the inducible *hps104* gene, and this is concomitant with a decrease of H4 acetylation at the same locus.<sup>43</sup> Thus, H4S1ph appears to play a role in transcription elongation where it regulates acetylation-dependent chromatin relaxation. As phosphorylation of H4 on S1 directly inhibits its acetylation,<sup>43</sup> one plausible hypothesis is that phosphorylation of H4 occurs on the coding region in the wake of transcribing RNA polymerase II (RNAP II), where it blocks H4 acetylation. This could be related to stabilization of nucleosomes on the chromatin fiber behind the polymerase, thereby preventing inappropriate initiation of transcription from within the coding regions of active genes, as described for histone chaperones and methylase/deacetylase.<sup>77,78</sup>

In addition, H4S1 was identified as a substrate of the sporulation specific kinase Sps1, whose deletion alters transcription of mid- and late-sporulation genes.<sup>79,80</sup> Unexpectedly, both H4S1ph and Sps1 were found to be enriched on the promoters of these genes, and to be specifically required for optimal timing of transcriptional repression.<sup>80,81</sup> While both were also detected at many other genomic loci, no correlation with transcription activity was observed. Recent ChIP-seq studies have specified more precisely H4S1ph localization during meiosis and have found that H4S1ph is enriched at the TSS (transcription start site) of a very large number of genes including sporulation genes.<sup>81</sup> Surprisingly, H4S1ph showed a local colocalization with H4ac at the TSS, in contrast to the inverted correlation that was found during DNA repair and transcription elongation. The reason for the colocalization of these two modifications has not been elucidated yet, and Govin and colleagues<sup>81</sup> suggest that it may reflect the existence

of “bivalent domains” or “bivalent promoters” presenting positive and negative marks, similar to what has been described in embryonic stem cells.<sup>82</sup> Thus, it appears that the cellular context has a determinant influence on the interplay between H4 phosphorylation and acetylation: H4S1ph can block H4 acetylation in order to stabilize nucleosomes during DNA repair or transcription elongation, or it can coexist with H4ac to tightly regulate gene expression during specific cellular events.

H4 is also phosphorylated on S47 *in vivo* and *in vitro* in a PAK2-dependent manner.<sup>83</sup> PAK2 is able to phosphorylate single H4 or the H3-H4 tetramer but not nucleosomal H4. Although H4S47ph has not been directly associated with transcription regulation, this mark is found to be preferentially enriched in H3.3-containing nucleosomes, generally deposited into chromatin in a transcription-dependent manner.<sup>83</sup> Interestingly, it was found that phosphorylation of H4S47 specifically favors H4's association with the H3.3-specific HIRA chaperone rather than with the canonical H3-specific CAF1, thereby promoting incorporation of H3.3-H4 into nucleosomes. Notably, these results demonstrate a novel mechanism by which a histone PTM can influence incorporation of histone variants into chromatin and thus, specialization of chromatin domains.

An extra-nucleosomal histone, the linker histone H1, is also known to be phosphorylated on multiple residues.<sup>84</sup> Since H1 is implicated in the stabilization of the nucleosome (as a chromatosome) and in the formation of the 30nm chromatin fiber, it has long been suggested to play a role in chromatin relaxation to allow transcription (reviewed in ref. 85).<sup>86</sup> Phosphorylation of H1 is detected at active 45S pre-rRNA promoters and at steroid hormone response elements under hormone treatment; it was also shown to assist RNAP I and RNAP II-dependent transcription.<sup>87-89</sup> These observations suggest a role of H1 phosphorylation in ribosome biogenesis and control of cell growth that certainly needs further investigation.

### Roles of Histone Phosphorylation in Chromatin Compaction

**Histone phosphorylation and chromatin compaction associated with mitosis and meiosis.** Histone H3 phosphorylation is highly conserved among eukaryotes from yeast to human, and has been extensively studied for many years. While H3 phosphorylation is involved in chromatin relaxation and regulation of gene expression, this modification was originally identified to be associated with chromosome compaction during mitosis and meiosis. In total, four phosphorylated residues within the N-terminal tail of H3 were discovered to be associated with chromosome condensation and segregation: T3, S10, T11 and S28. However, it is still unclear whether these modifications are functionally related to chromatin condensation and inter-related between each other. Phosphorylation of H3 on S10 is likely the best-documented mark related to chromatin condensation associated with mitosis and meiosis in many eukaryotic organisms, and is commonly used as a reference mark of these events.<sup>90-93</sup> Interestingly, the patterns of H3S10 and H3S28 phosphorylation in early mitosis are quite similar, commencing at the onset of

chromosome condensation during prophase.<sup>94</sup> Phosphorylation of both residues occurs along the same chromosome during mitosis, but it is important to note that there is no evidence that these two marks are present together on the same histone tail. In fact, immunofluorescence and sequential immunoprecipitation analyses performed in quiescent cells have demonstrated that H3S10ph and H3S28ph are found on distinct populations of H3.<sup>95,96</sup> Nevertheless, whether their apparent separation applies to mitotic cells has not been described yet. Since both residues present the same consensus sequence, ARKS, it is not entirely surprising that they are phosphorylated by the same kinase Aurora B (yeast Ipl1) and dephosphorylated by protein phosphatase 1 (PP1; Glc7 in yeast).<sup>93,97,98</sup> The balance between Aurora B/ Ipl1 and PP1/yGlc7 was shown to be critical for the control of H3 phosphorylation and for proper chromosome segregation.<sup>98</sup> Supporting the notion that these enzymes play an important role in chromosome stability in mammalian cells, Aurora B was reported to be overexpressed in a variety of human cancers.<sup>99-101</sup> Interestingly, the H3 K9 residue adjacent to H3S10 was shown to be methylated in silenced regions associated with heterochromatin, and this methylation defines a docking site for the binding of HP1. Moreover, HP1 was shown to dissociate from chromatin during the M phase<sup>102</sup> and, importantly, phosphorylation of H3S10 during mitosis was demonstrated to be sufficient to eject the HP1 $\alpha$ , HP1 $\beta$  and HP1 $\gamma$  bound to methylated H3K9.<sup>103</sup> This result suggests a role for H3S10ph in the regulation of protein binding to chromatin during mitosis. Full understanding of the functional link between H3S10ph and H3S28ph and of the exact molecular mechanisms by which these PTMs influence chromosome condensation and segregation still needs further examination.

During mitosis, histone H3 is also phosphorylated on threonine 3. Distribution of this PTM is similar to that of H3S10ph in early prophase, but diverges when cells enter prometaphase.<sup>104</sup> H3 T3ph becomes highly enriched at inner centromeric regions on prometaphase and metaphase chromosomes, but its levels decline during anaphase and disappear entirely from decondensed chromosomes.<sup>105</sup> H3T3 is phosphorylated by Haspin, a mitotic chromatin-associated kinase.<sup>105-109</sup> Haspin is known to play a role in sister chromatid cohesion during mitosis, and its function appears to be mediated, at least in part, by phosphorylated H3T3. Recent studies in yeast and human cells have demonstrated that Haspin-dependent phosphorylation of H3T3 is required for recruitment of the Aurora B-containing chromosomal passenger complex (CPC) to the centromere, and this relocalization is mediated by a direct interaction between H3T3ph and Survivin, another subunit of CPC.<sup>110-113</sup> Furthermore, Aurora B can also phosphorylate Haspin, promoting further H3T3 phosphorylation at the centromere, and thereby establishing a positive-feedback loop.<sup>114</sup> Haspin was also shown to cooperate with the Bub1 kinase in targeting CPC to the centromere.<sup>110,114</sup> Bub1 localizes to kinetochores where it phosphorylates H2A on T120 in mammalian cells (and on the corresponding S121 in fission yeast) and regulates H3T3ph distribution.<sup>110</sup> H2AT120ph facilitates nucleosome binding by shugoshin, the centromeric CPC adaptor,<sup>110,115</sup> and was previously known to be targeted by

NHK-1 in *Drosophila* where it is required for meiotic and mitotic chromosomal architecture.<sup>116,117</sup>

Depletion of Haspin and reduction of H3T3ph levels by RNA interference affects sister chromatin cohesion, suggesting that H3T3 phosphorylation plays a role in the regulation of chromosome separation rather than in chromosome condensation.<sup>106,109</sup> Unexpectedly, it was found that H3T3ph always occurs in *cis* with tri-methylated K4 and di-methylated R8.<sup>118</sup> These marks appear at multiple sites at the periphery of the prophase nucleus, then cluster at the centromeres of metaphase chromosomes, and finally spread to the distal areas of segregating chromatids. The joint role of these histone PTMs has not been fully investigated, and the presence of H3K4me3, a mark of active transcription, together with the mitotic H3T3ph is very surprising.

Concomitant with H3T3ph, mitotic H3T11ph enrichment is restricted from prophase to early anaphase, and is also found to preferentially associate with the centromere.<sup>119</sup> Phosphorylation of H3 on T11 was first identified in rat and in the human breast carcinoma cell line MCF7 and shown to be restricted to mitotic cells. Dlk was suggested to be the kinase responsible for T11 phosphorylation since the GFP-Dlk fusion associated with the centromere within the same time frame.<sup>119</sup> This modification has also been reported in plants where it temporally correlates with chromatin condensation during mitosis and meiosis.<sup>120</sup> In yeast, H3T11 is also phosphorylated during meiosis and this modification is catalyzed by the meiotic Mek1 kinase.<sup>120,121</sup> In contrast to the H3S10A substitution in yeast that strikingly had no negative effect on sporulation, the T11A mutant showed a reduction in sporulation efficiency, arguing that phosphorylation of T11 is a key modification during meiosis.<sup>121</sup>

Phosphorylation of H4S1 and H2BS10 was also demonstrated to occur during meiosis in yeast. Additionally, H4S1ph has been observed during fruit fly and mouse spermatogenesis.<sup>80</sup> Examination of H4S1ph timing relative to H3S10 phosphorylation shows that these two marks have a distinct pattern during sporulation.<sup>80</sup> Unlike the transient H3S10ph that coincides with early sporulation, H4S1ph appears as a stable mark after meiotic I and II divisions.<sup>80,121</sup> In fact, S10 residues of both H2B and H3 are phosphorylated during meiotic chromosome condensation and disappear during meiotic divisions,<sup>122</sup> whereas H4S1 phosphorylation appears later in meiosis and increases in post-meiotic cells.<sup>80,81</sup> H4S1ph is stable and persists in post-meiotic cells, including mature yeast spores and mature spermatids. This meiotic phosphorylation is dependent on Sps1, a mid-sporulation-specific kinase member of the Ste20 family.<sup>80</sup> There is no *in vitro* evidence that Sps1 phosphorylates H4S1 directly, but deletion of this kinase results in a loss of H4S1ph signal. Supporting the role of H4S1ph in chromatin compaction during later stages of meiosis, deletion of Sps1 as well as mutation of H4S1 to alanine in yeast results in sporulation deficiency and increased DNA and nuclear volumes.<sup>80,121</sup>

Finally, H2BS10 phosphorylation has been detected during the prophase of meiosis, although the kinase responsible for this modification in meiosis remains unknown.<sup>122</sup> While Ipl1 can phosphorylate both H3 and H2B *in vitro*, the possibility that it phosphorylates H2B on S10 *in vivo* during meiosis remains

to be addressed. Intriguingly, this histone mark was found to colocalize with Zip1, a component of the synaptonemal complex characteristic of meiotic pachytene chromosomes. Overall, current evidence indicates that H2BS10ph plays a major role in chromatin condensation during various cellular processes such as meiosis but the molecular mechanism by which this modification influences chromatin structure still needs to be elucidated.

Phosphorylation of linker histone H1 is also regulated in a cell cycle-dependent manner (reviewed in ref. 123). It significantly increases during mitosis and S phase, being more specifically detected through metaphase. Multiple phosphorylated sites have been identified<sup>84,124</sup> and the state of H1 phosphorylation is thought to be dependent on the balance of protein phosphatase I and CDC2/CDK2 kinases.<sup>125</sup> H1 phosphorylation appears to be associated with chromatin decondensation rather than chromatin condensation.<sup>123</sup> While H1 was shown to bind HP1 and to stabilize the compacted chromatin structure, its phosphorylation by CDK2 is proposed to disrupt this binding, resulting in chromatin destabilization and efficient cell-cycle progression.<sup>126</sup> Whereas N- and C-terminal tails of H1 contain consensus sequences with potential phosphorylatable sites by CDC2/CDK2 kinases, it is suggested that it is the number of phosphorylated sites that is functionally important, not the specific residues per se.<sup>127</sup>

**Histone phosphorylation and chromatin compaction associated with apoptosis.** Several intriguing connections exist between histone phosphorylation and apoptosis. Early work demonstrated that phosphorylation of the N-terminal tail of histone H2B was essential for apoptosis-induced chromatin condensation.<sup>128,129</sup> Subsequently, serine 14 was identified as the phosphorylated residue in apoptotic mammalian cells.<sup>130</sup> The corresponding phosphorylation on S10 of yeast H2B was confirmed to be essential for hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced chromatin condensation and apoptosis.<sup>122,131</sup> Phosphorylation of H2B S10 in yeast is mediated by the sterile 20 kinase (Ste20), while the homologous Mst1 modifies H2BS14 in mammals.<sup>130,131</sup> Deletion of Ste20 abrogates H2BS10ph, increases cell survival under H<sub>2</sub>O<sub>2</sub> treatment, as does mutation of serine 10 to non-phosphorylatable alanine. In contrast, mutation of S10 to glutamate, which mimics phosphorylation, promotes cell death and induces constitutive chromatin compaction.<sup>132</sup> Thus, H2BS10ph appears to play a key role in apoptotic chromatin condensation. While further investigation is necessary to understand the mechanisms by which this mark can induce such drastic chromatin structure changes, a number of studies have addressed the issue of its regulation. It has been demonstrated that the K11, immediately adjacent to H2BS10, can be acetylated in a Gcn5-dependent manner and is enriched in exponentially growing cells.<sup>133</sup> More recently, it was shown that H2BS10 phosphorylation is negatively regulated by the acetylation state of H2BK11, pointing to a mutually exclusive existence of these marks in the tail of H2B.<sup>132</sup> Deacetylation of H2BK11 by the Hos3 HDAC is a prerequisite for H2BS10 phosphorylation by Ste20 and for subsequent induction of apoptotic chromatin compaction.

In addition to its extensively studied role in DDR, phosphorylation of H2AXS139 in mammals was recently shown

**Table 1.** Sites of histone phosphorylation, their known kinases and functions

Histone	Phosphorylated residue	Kinases	Role
H2A	S1	?	Mitosis
	S16	RSK2	EGF signaling
	S122*(Sc)/T120(Hs)	Bub1, NHK-1 (Dm)	DNA repair/mitosis/meiosis
	S129*(Sc)/S139(Hs,H2AX)	Mec1, Tel1 (Sc)/ATM, ATR, DNA-PK (Hs)	DNA repair
	Y142 (H2AX)	Mst1 WSTF	Apoptosis DNA repair
H2B	S10 (Sc)/S14 (Hs)	Ste20 (Sc)/Mst1 (Hs) (Ipl1?)	Apoptosis Meiosis
	S32	RSK2	EGF signaling
	S36	AMPK	Transcription
	Y40 (Sc)/Y37 (Hs)	Swe1 (Sc) / WEE1 (Hs)	Transcription
H3	T3	Haspin	Mitosis
	T6	PKC $\beta$	Transcription
	S10	Ipl1 (Sc)/AuroraB (Hs), RSK2, MSK1, ERK1, p38, Fyn, Chk1, PRK1	Transcription, chromatin condensation, UVB response
	T11	Mek1 (Sc)/Dlk (Hs, ?) PRK1, PKM2 Chk1	Meiosis (Sc), Mitosis (Hs) Transcription DNA damage response
	S28	AuroraB, ERK1/2, p38 MLTK- $\alpha$ , JNK1/2, MSK1	Meiosis Mitosis, transcription
	Y41	JAK2	Transcription
	T45	PK-C $\delta$	Apoptosis
H4	S1	CKII Sps1	DNA repair, transcription Meiosis, transcription
	S47	PAK2	(H3.3-H4) deposition
H1	S/T	CDK2	Mitosis Transcription

This table summarizes the histone residues currently known to be phosphorylated *in vivo*; identified responsible kinases are indicated as well as the cellular processes implicated. Sc, *Saccharomyces cerevisiae*; Hs, *Homo sapiens*; Dm, *Drosophila melanogaster*; ?, kinase speculated without being formally tested. \*These coordinates count methionine as the first amino acid, unlike the other histone coordinates.

to function in apoptosis.<sup>93</sup> Notably, H2AX phosphorylation increases upon DNA fragmentation and apoptosis.<sup>134,135</sup> Mst1 has been identified as the kinase responsible for apoptotic H2AX phosphorylation.<sup>136</sup> Overexpression of Mst1 induces phosphorylation of H2AXS139 in HeLa cells, accompanied by DNA fragmentation. In line with this, a study by the Pommier group found that H2BS14 phosphorylation coincides with  $\gamma$ H2AX staining at the nuclear periphery following induction of apoptosis by death receptor agonists such as TRAIL or Fas-Ligand, or by treatment with staurosporine.<sup>137</sup> Furthermore, it was demonstrated that a defect of H2AXY142 dephosphorylation resulted in recruitment of the pro-apoptotic factor JNK1, rather than the repair apparatus, to  $\gamma$ H2AX.<sup>41</sup> Based on such evidence, Solier et al. proposed the existence of an H2AX-H2B phosphorylation code during apoptosis in mammalian cells. They suggested that the interplay between phosphorylation of H2BS14, H2AXS139 and H2AXY142 determines the cell fate decision between repair and apoptosis, with H2BS14ph being a hallmark of the latter.<sup>137</sup>

H3T45 phosphorylation was also observed to occur during apoptosis. While this site has been identified as the target

of the PKC $\delta$  kinase *in vitro* and *in vivo* in normally cycling human cells,<sup>138</sup> its phosphorylation increases in apoptotic cells. This occurs concomitantly with or shortly after DNA nicking, and the kinetics of its appearance closely resembles those of caspase-3 activation. The role of H3T45ph during apoptosis is still unknown and further investigation is needed to understand whether this mark has an influence on nucleosome structure and a function in chromatin condensation during apoptosis.

### Concluding Remarks

Identification and characterization of histone phosphorylation events and of the kinases that carry them out have shown that histone phosphorylation is prominently involved in various essential cellular processes associated with chromatin remodeling and gene expression (Table 1). It is interesting to observe that the same phosphorylation events can be implicated in multiple cellular processes involving chromatin modulation. For instance, H4S1ph in yeast is involved in chromatin compaction or nucleosome stabilization during such independent processes

as DNA repair, transcription and sporulation. Even more remarkable is the fact that the same phosphorylated residue can have significantly distinct effects on chromatin structure depending on the context in which it occurs. Phosphorylation of H3S10 and H3S28 is a good example of this duality: both phosphorylated residues are involved in chromatin condensation associated with mitosis and meiosis, as well as in chromatin relaxation linked to transcription activation (Fig. 1). Such divergent roles played by these phosphorylation events make it clear that, like most other histone modifications, phosphorylation of individual residues cannot be considered in isolation. The cellular context, the crosstalk with other modifications in *cis* or in *trans*, and the local histone “code” or signature formed by diverse PTMs determine the

identities of the effector proteins that are recruited to particular chromatin domains, thereby dictating the ultimate outcomes of the phosphorylation events (Fig. 1).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Acknowledgments

We apologize to our colleagues for work that could not be cited owing to space limitations. Work in our laboratory is supported by operating grants from the Canadian Institutes of Health Research (CIHR, MOP-14308/64289). N.A. was a CIHR-Institute of Aging Fellow and J. Côté holds a Canada Research Chair.

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