JB Minireview

J. Biochem. **141,** 609–614 (2007)
doi:10.1093/jb/mvm091

Role of Histone Modification in Chromatin Dynamics

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Received September 25, 2006; accepted March 25, 2007; published online March 25, 2007

The covalent modification of histone tails has emerged as a crucial step in controlling the eucaryotic genomes. Eucaryotic cells must possess mechanisms for condensing and decondensing chromatin. Moreover, chromatin condensation is particularly evident during mitosis and apoptotic cell death, whereas chromatin relaxation is necessary for replication, repair, recombination and transcription. The post-translational modifications of histone tails such as reversible acetylation, phosphorylation and methylation play a critical role in dynamic condensation/relaxation that occurs during the cell cycle. Histone phosphorylation is believed to play a direct role in mitosis, cell death, repair, replication and recombination. In this review, we discuss recent progress in studies of histone phosphorylation.

Key words: chromatin, histone code, NHK-1, nucleosome, modification, transcription.

The enormous length of the eucaryotic genome requires that it is packaged into a stable structure that can be replicated and propagated properly during mitosis and is sufficiently malleable and modifiable to enable access to genetic information. Cells protect their genetic information by organizing it as a higher-order nucleoprotein complex termed chromatin in which the basic unit is the nucleosome. Each nucleosome is composed of an octamer of core histones (two each of H2A, H2B, H3 and H4), around which two super-helical turns (80 bp) of DNA are wrapped (for review see *1–3*).

Since DNA is compacted into a highly condensed and ordered structure, considerable interest has focused on how the transcriptional machinery gains access to the genes contained within chromatin and expresses them in an organized program, as is required in the processes of cellular differentiation and development. The change of chromatin organization via its covalent modification is thought to provide access to the genes for the transcription apparatus. The location of the N-terminal tails of histone molecules outside of the relatively compact chromatin fibre makes them readily available for a variety of covalent post-translational modifications that are either thought to act as a substrate for the binding of chromatin remodelling factors or transcription factors to regulate gene expression.

The combinatorial pattern of N-terminal modifications results in a heterogeneous identity for each nucleosome that the cell interprets as an epigenetic code from the genome to the cellular machinery for various processes to occur. This concept is commonly referred to as the histone code hypothesis. One of the best understood covalent histone modification was histone acetylation, and the list has grown to include phosphorylation, methylation, ubiquitination and ADP-ribosylation. Acetylation and methylation of different lysine and arginine residues in histones H3 and H4 have been linked to either transcriptionally active or transcriptionally repressed states of gene expression, whereas phosphorylation of histone H3 was initially linked to chromosome condensation during mitosis (*4*).

In addition, evidence has accumulated that indicates the phosphorylation of histone H3 at serine 10 (Ser 10) has an important role in the transcriptional activation of eucaryotic genes in various organisms. More recently, it became evident that phosphorylation of histone H2A at Thr119 has an important role in mitosis and miosis. In this review, we focus on the importance of histone H2A and H3 phosphorylation in biological events.

HISTONE H2A PHOSPHORYLATION

In Drosophila embryos, we found that nucleosomal histone kinase-1 (NHK-1) is a histone H2A kinase (*5*). Its phosphorylation site is related to cell-cycle progression and chromosome dynamics. NHK-1 has a high affinity for chromatin and phosphorylates Thr119, at the C-terminus of H2A (Fig. 1). NHK-1 specifically phosphorylates nucleosomal H2A, but not free H2A in solution. Immunostaining of NHK-1 revealed that it travels to chromatin during mitosis and is excluded from chromatin during S phase (*5*).

Comparison of the predicted amino acid sequence of this unique NHK-1 with known proteins revealed similarities to other known kinases, such as human Vaccinia-related kinase 1 (VRK1) (*6*), mouse VRK1, *Xenopus* VRK1 and *Caenorhabditis elegans* VRK, with conservation of 44, 43, 41 and 37% of this kinase domain, respectively. In addition to a conserved kinase domain, there is a common structure, namely an acidic amino acid region between the basic amino acid regions. This basic–acidic–basic amino acid motif (BAB motif) is conserved among species. Concerning the functions of VRK1, it has been reported that human VRK1 phosphorylates Thr18 of p53, the binding site of mdm2 (*7*). However, the biological role of human VRK1 is not well-identified (*8*). *Caenorhabditis elegans* VRK1 was characterized in a systematic analysis using

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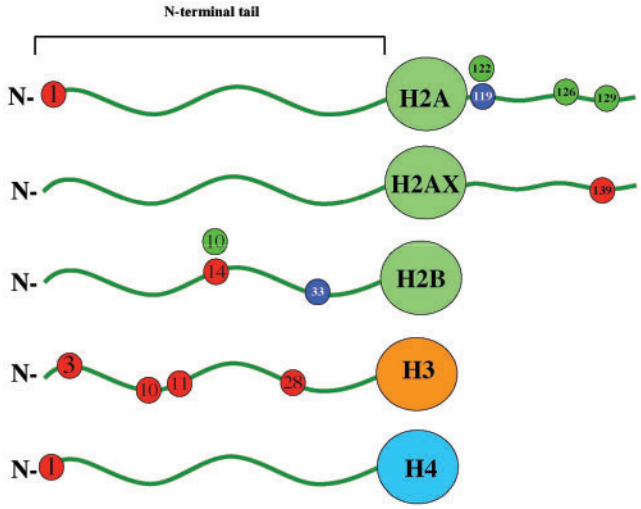

Fig. 1. **Global aspects of post-translational histone modification.** Red circle: phosphorylation detected in mammalian histones, Purple circle: phosphorylation detected in *Drosophila melanogaster* histones, Green circle: phosphorylation detected in *S. cerevisiae* histones.

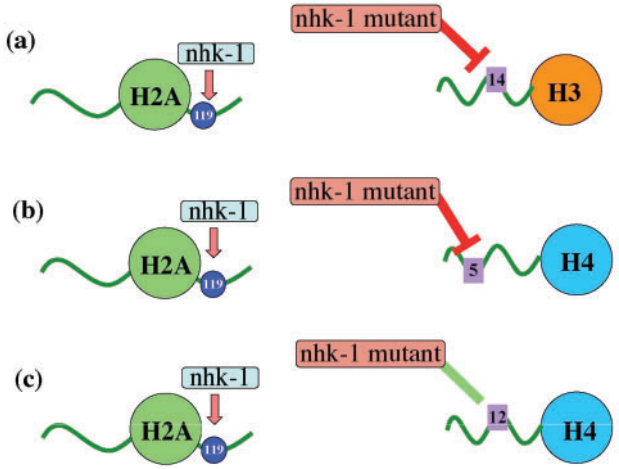

Fig. 2. **Trans-histone cross-talk between histone H2A phosphorylation on T119 and another histones methylation or acetylation in nhk-1 mutants.** (a) Histone H3 (K14) and (b) Histone H4 (K5) are not acetylated in the nhk-1 mutant. (c) Histone H4 (K12) is acetylated in the nhk-1 mutant (*10*). Phosphorylation is represented by the blue flag, and acetylation is represented by the black flag.

RNAi. Inactivation of *C. elegans* VRK1 by RNAi revealed embryonic lethality, with large cytoplasmic granules and failure to form a pronucleus (*9*). These studies suggest that VRK has important functions for viability.

In a recent report, *nhk-1* mutation leads to female sterility due to defects in the formation of the meiotic chromosomal structure, including failure to assemble a karyosome (chromosomal structure of oocyte nucleus in prophase I), a metaphase I spindle and a normal polar body (*10*). Histone H2A is phosphorylated at Thr119 in meiosis and NHK-1 is phosphorylated by itself in mitosis and meiosis (*11*). Phosphorylation of NHK-1 itself may be regulated by other mitotic kinases and therefore may play a part in coordinating mitotic or meiotic progression. Histone H3(K14) and histone H4(K5) are not acetylated in the *nhk-1* mutant, implying that histone H2A(T119) phosphorylation is a prerequisite for acetylation of these residues in meiosis. Histone H1 phosphorylation and histone H4 (K12) acetylation have no effect in this mutant (*10*) (Fig. 2). These studies demonstrate that H2A (T119) phosphorylation by NHK-1 regulates mitotic and meiotic progression.

Concerning another phosphorylation site of histone H2A, Barber *et al*. (*12*) report that histone H2A is highly phosphorylated at Serine 1 residues during mitosis in the worm, fly and mammalian cells. This phosphorylation by MSK1 negatively regulated transcription on chromatin templates (*13*). However, it remains unclear what is the biological function of other histone H2A posphorylation sites and how they impact the many other histone modifications.

HISTONE H2AX PHOSPHORYLATION

Histone H2AX, one of several variants of H2A is phosphorylated on Ser 139 (*14*) (Fig. 1). The Serine phosphorylated form of H2AX, termed g-H2AX, occurs in response to DNA double-strand breaks for recruitment of DNA-damage-response proteins, including DNA damage checkpoint proteins (*15*), cohesin complex (*16*, *17*) and chromatin remodelling protein (*18*, *19*) to regions of damaged chromatin. The phosphatidylinositol-3-OH kinase-related kinase (PIKK) family includes ataxia-telangiectasia mutated (ATM), ATM and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK) (*20*, *21*).

These kinases are important for DNA-damage signalling (*22*), however there is functional redundancy among the pathways such that g-H2AX is detectable in individual kinase dead mutants (*23*). In mice lacking functional H2AX, the efficiency of DSB repair is impeded, the DNA damage-induced checkpoint fails and numerous morphological abnormalities are observed (*24*, *25*).

Recent studies have demonstrated that mammalian MDC1 directly binds to g-H2AX and this direct interaction plays a central role in the mammalian response to DNA damage (*26*). Bassing *et al*. (2003) reported that H2AX had been shown to function as a dosage-dependent suppressor of oncogenic translocations and tumours in mice, whereas in humans, H2AX maps to a cytogenetic region frequently altered in human cancers (*27*). These observations are consistent with a role for altered or mutated H2AX expression in the onset and progression of at least some human tumours. Current evidence shows that H2AX is also phosphorylated during apoptotic DNA fragmentation (*28*).

In *Saccharomyces cerevisiae*, histone H2A is more similar to mammalian H2AX than to mammalian H2A. The counterparts of ATM and ATR, Tel1 and Mec1, similarly phosphorylate histone H2A (S129) in response to DNA damage (*29*). Furthermore, recent work has shown that the phosphorylated histone H2A (S129) interacts with the NuA4 histone acetylferase and INO80 ATP-dependent chromatin remodelling complexes (*18*).

We have discussed recent studies about phosphorylation of histone H2AX in mammals or H2A in yeast, and it is one of the key events in the response to DNA damage. This phosphorylation recruits cohesin complex, chromatin remodelling complex and histone acetyltranseferase to DSBs, and promotes the accumulation of checkpoint and repair proteins at these sites. However, some groups report that another complex, for example SWR1 complexes including H2AZ (another histone H2A variant), also recruit to double-strand break regions. There still remain some questions about how other histone modification or other histone variants respond to DNA damage.

HISTONE H3 PHOSPHORYLATION

One of the prominent histone modifications of H3 is phosphorylation of histone H3 at Ser 10. This modification has been observed in different processes, including the activation of transcription and chromosome condensation during mitosis (*30*, *31*) and meiosis (*32*) (Fig. 1). Mutation of this phosphorylation site impairs chromosome condensation and segregation *in vivo* (*33*). Histone H3 at S10 is phosphorylated by multiple kinases, such as mitogen and stress-activated protein kinases 1 and 2 (MSK1 and MSK2), cAMP-dependent protein kinase A (PKA), NIMA kinase, Aurora B kinase, ribosomal S6 kinase 2 (RSK2) and IkB kinase a (IKKa) (*34*). MSK1/2 are downstream targets of the Ras mitogen-activated protein kinase (MAPK) signal transduction pathway.

Histone H3 phosphorylation at S10 by MSK1/2 has been linked to transcriptional activation of mitogen-stimulated immediate-early response genes, such as c-fos and c-jun (*35*), and, more recently, has been reported in conjunction with many other inducible genes and oncogenes (*36*). Recent studies have demonstrated that there exists a cross-talk between phosphorylation of histone H3 Ser 10 and acetylation or methylation of histone H3. Histone H3 phoshorylation at S10 can enhance acetylation of histone H3 at K14 (*37*, *38*), abolish acetylation of histone H3 at K9 (*39*) and inhibit methylation of histone H3 at K9 (*40*). Furthermore, methylation of histone H3 at K9 interferes with phosphorylation of histone H3 at S10 (*40*) (Fig. 3).

Further evidence about phosphorylation of histone H3 Ser 10 is that overexpression of Aurora kinase, which has been observed in many cancer cell lines, causes increased phosphorylation (*41*). Increased phosphorylation was found during the heat shock response (*42*) and in oncogene-transformed mouse fibroblasts (*43*). Phosphorylation of histone H3 at Ser 10 by AuroraB disrupts the HP1 (Heterochromatin protein 1) and methylation of histone H3 (K9) interaction, and release of HP1 from chromosomes during mitosis (*44*, *45*). These data suggest that switching of methylation and phosphorylation roles permit the progression of mitosis.

Recent studies have demonstrated other phosphorylation sites of histone H3 at Threonine 3 (*46*) and Threonine 11 (*47*) (Fig. 1). Histone H3 (T11) phosphorylation occurs during mitosis by Dlk/ZIP kinase (Dlk: death-associated protein (DAP)-like kinase, ZIP: Zipper interacting protein kinase) (*47*).

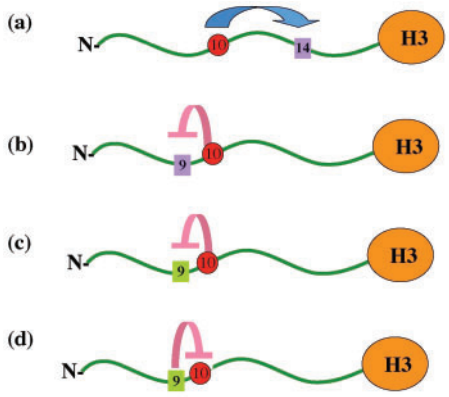

Fig. 3. **Trans-histone cross-talk between phosphorylation of histone H3 (S10) and acetylation of histone H3 (K14) or methylation of histone H3 (K9).** (a) Histone H3 phoshorylation at S10 can enhance acetylation of histone H3 at K14 (*37*, *38*). (b) Histone H3 phoshorylation at S10 abolish acetylation of histone H3 at K9 (*39*). (c) Histone H3 phoshorylation at S10 inhibit methylation of histone H3 at K9 (*40*). (d) Methylation of histone H3 at K9 interferes with phosphorylation of histone H3 at S10. Phosphorylation is represented by red circle, acetylation is represented by purple square and methylation represented by green square.

Histone H3 at Serine 28 is phosphorylated by Aurora B kinase at mitosis and this phosphorylation coincides with chromosome condensation (*48*, *49*) (Fig. 1). Histone H3 (S28) phosphorylation initiates at prophase, whereas histone H3 (S10) phosphorylation initiates during the late G2 phase (*50*).

Centromere protein A (CENP-A), one of several variants of histone H3, is phosphorylated on Ser 7 by Aurora B kinase which is equivalent to Ser 10 of histone H3 (*51*). Recent studies demonstrate that Aurora A kinase also phosphorylates CENP-A (S7) (*52*). The presence of CENP-A in centromeric nucleosomes is required for kinetochore organization and function (*53*). Loss of CENP-A phosphorylation function at Ser 7 caused a mislocalization of Aurora B, a putative partner phosphatase (PP1g1) and inner centromere protein (INCENP). H3.3, another variant of histone H3 is phosphorylated on Ser 31 *in vivo*. H3.3 (S31) is a mitosis-specific modification that is present only in late prometaphase and metaphase. Furthermore, H3.3 (S31) is excluded from centromeres. However it is enriched in distinct chromosomal areas immediately adjacent to centromeres (*54*).

We have discussed phosphorylation of histone H3, which has been studied in many organisms. Phosphorylation of histone H3 (S10) has two opposite main functions. One is necessary to initiate chromosome condensation during mitosis and meiosis, while the other is transcriptional activation. Current evidence shows that a combination of phosphorylation of H3 (S10) and methylation of H3 (K9) or acetylation H3 (K9, K14) play important roles in these phenomena including cell cycle related chromosome dynamics and transcriptional activation. These results suggest that a combination of different histone modifications execute different biological outcomes (Fig. 3).

HISTONE H2B AND H4 PHOSPHORYLATION

In addition to histone H2A and H3 phosphorylation, histone H2B and H4 phosphorylation plays an important role for chromatin dynamics. For example histone H2B amino-terminal tail is essential for chromatin condensation (*55*). In Xenopus, chicken and human cells phosphorylation of H2B at Serine 14 by Mst1 (Mammalian Sterile Twenty) kinase has been linked to chromatin compaction during apoptosis (*56*, *57*) and DNA double-strand breaks (*58*). Recent studies demonstrate that histone H2B phosphorylation is also a key role in response to DNA double-strand breaks, apoptosis, meiosis and transcription activation events. However, this detail mechanism is poorly understood.

Furthermore, phosphorylation of histone H4 (S1) increase during cell-cycle, same as histone H2A (S1) (*12*, *59*). To compare directly the timing of histone H3 (S10 or S28) and H2A/H4 (S1) phosphorylation during the different stages of mitosis with individual antibodies, Barber *et al*. (*12*) found that the overall timing of these modifications was similar. They also detected a lower level of histone H2A/H4 phosphorylation in early S-phase cells, possibly on newly deposited histones on replicating DNA. The phosphorylation of H2A/H4 (S1) is an evolutionarily conserved modification that may have separate roles during mitosis and S-phase (*12*).

Recent studies have demonstrated that Histone H4 (S1) phosphorylation is also a key role in response to DNA double-strand breaks, cell-cycle progression and gene expression. Specially, this modification may have important roles during mitosis and S-phase-associated events in cell-cycle and its phosphorylation found on newly synthesized histones during S-phase. However this phosphorylation residue is novel histone modification site, and this detail mechanism will be evident by the future experiments.

CONCLUSIONS AND PERSPECTIVES

Here we discuss recent progress in understanding what occur as a consequence of histone phosphorylation. Given the number of different histone tails and the number of different histone modifications, the potential complexity of regulation is immense. Strahl and Allis (*4*) have proposed the histone code hypothesis, according to which each combination of post-translational modifications on a histone tail has a specific function (*60*). For example, mitotic chromatin condensation is associated with histone H3 (S10) phosphorylation, but this modification has not been observed during apoptotic-induced chromatin condensation. Both mitosis and apoptosis have chromatin condensation phenomena, however histone marks that regulate these phenomena are different. There is insufficient knowledge to address how these different histone modifications regulate chromatin condensation in the different contexts.

Histone phosphorylation function is still poorly understood and there are many questions. For example, the role of histone H3 variants during transcriptional activation needs to be explored further in addition to histone modification. Recent results (*61*) suggest that transcription of rDNA arrays in Drosophila correlates with the replacement of histone H3 for the H3.3 variant. Perhaps the de novo phosphorylation that is observed at sites of active transcription might affect histone H3.3 rather than histone H3, whereas the Ser 10 phosphorylation leading to chromosome condensation is specific for histone H3. This could account for the multiple observations of histone H3 acetylation rather than phosphorylation as a mechanism for transcriptional activation of yeast genes (*37*, *38*). Because the histone H3.3 variant is the only form of histone H3 that is found in yeast (*61*) an exchange between histone H3 and H3.3 (and therefore histone H3 phosphorylation) might not be required for the activation of transcription in this organism. Future experimentation will provide answers for many of these questions.

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