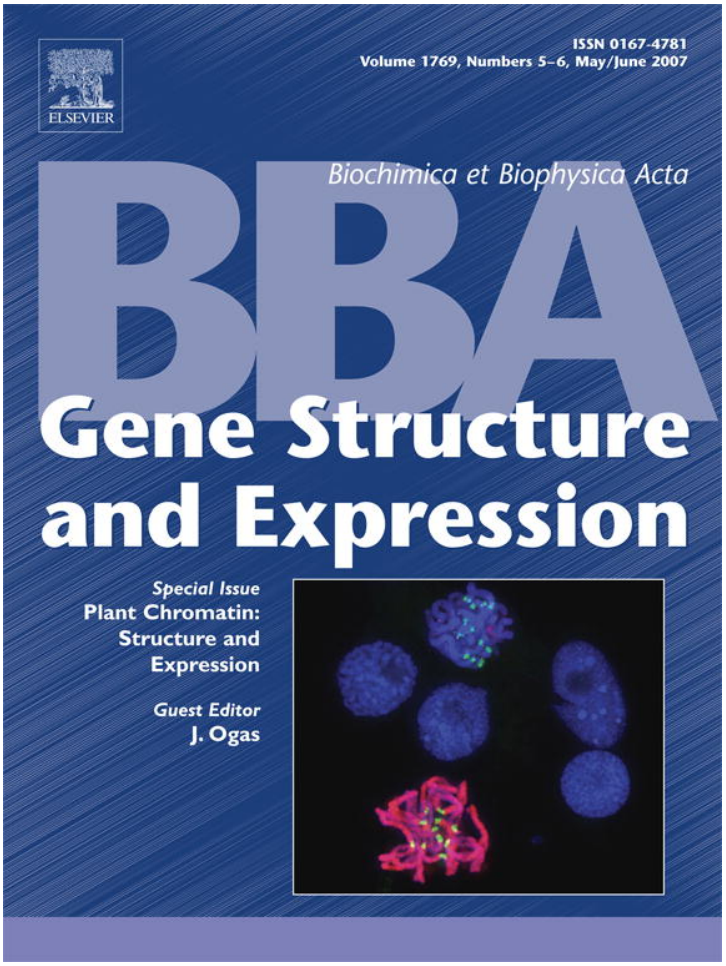

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Review

# Phosphorylation of histone H3 in plants—A dynamic affair

Andreas Houben [a,*], Dmitri Demidov [a], Ana D. Caperta [b], Raheleh Karimi [a], Francesco Agueci [a], Liudmila Vlasenko [a]

[a] Leibniz-Institute of Plant Genetics and Crop Plant Research, Chromosome Structure and Function Group, Corrensstrasse 3, D-06466 Gatersleben, Germany
[b] Centro de Botânica Aplicada à Agricultura, Secção de Genética, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017, Lisboa, Portugal

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**Abstract**

Histones are the main protein components of chromatin: they undergo extensive post-translational modifications, particularly acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation which modify the structural/functional properties of chromatin. Post-translational modifications of the N-terminal tails of the core histones within the nucleosome particle are thought to act as signals from the chromatin to the cell, for various processes. Thus, in many ways histone tails can be viewed as complex protein–protein interaction surfaces that are regulated by numerous post-translational modifications. Histone phosphorylation has been linked to chromosome condensation/segregation, activation of transcription, apoptosis and DNA damage repair. In plants, the cell cycle dependent phosphorylation of histone H3 has been described; it is hyperphosphorylated at serines 10/28 and at threonines 3/11 during both mitosis and meiosis in patterns that are specifically coordinated in both space and time. Although this post-translational modification is highly conserved, data show that the chromosomal distribution of individual modifications can differ between groups of eukaryotes. Initial results indicate that members of the plant Aurora kinase family have the capacity to control cell cycle regulated histone H3 phosphorylation, and in addition we describe other potential H3 kinases and discuss their functions.


*Keywords:* Histone phosphorylation; Chromosomes; Centromeres; Cell cycle; Aurora kinases

## 1. Cell-cycle dependent histone phosphorylation

The cell cycle dependent transition from decondensed interphase chromatin to condensed metaphase chromatin, and vice versa, is the most obvious dynamic change in chromatin structure. Histones are among numerous DNA-binding proteins that control the level of DNA condensation, and post-translational modifications of histone tails play a critical role in the dynamic condensation/decondensation process. Early observations in several eukaryotes have shown that the level of H3 phosphorylation, which is minimal in interphase, increases during mitosis [1]. With the development of an antibody specific for histone H3 phosphorylated at S10 Hendzel and colleagues [2] were able to demonstrate, *in vivo*, a precise spatial and temporal pattern of H3 phosphorylation in mammalian cells. They found that phosphorylation of H3 is initiated in late G2, and then spreads throughout the chromatin as it undergoes condensation, up to the end of mitosis. The same cell-cycle dependent histone modification was later demonstrated in plants [3]. It is noteworthy that although the process of H3 phosphorylation is highly conserved, its significance and chromosomal distribution may differ to some extent among different species groups. In mammals the cell-cycle dependent phosphorylation of H3 at serine positions 10 and 28, that originates in the pericentromere [4] and spreads throughout the chromosomes during the G2-M transition phase, is most likely to be interlinked with the initiation of chromosome condensation [5]. In yeast on the other hand, phosphorylation of S10 in H3 is not required for cell-cycle progression, where phosphorylation of histone H2B might replace the function of H3 phosphorylation [6]. Similarly, mitotic cells in *Drosophila borr* mutant embryos display only a slight undercondensation of their dividing chromosomes despite a substantial reduction of H3S10 phosphorylation [7].

* Corresponding author. Tel.: +49 39482 5486; fax: +49 39482 5137.
*E-mail address:* houben@ipk-gatersleben.de (A. Houben).

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In plants, the distribution of phosphorylated histone H3 at serine 10 [3,8,9], and serine 28 [10,11], correlates with the position of the pericentromere during mitosis and meiosis II (Figs. 1a, 2). Immunogold scanning electron microscopy supports the view that during the progression through the mitotic stages phosphorylation of histone H3 at serine 10 accumulates in the pericentromeric chromatin at metaphase [12]. The immunosignal gap observed by high resolution scanning electron microscopy represents the core centromere [12] , characterised by parallel chromatin fibres, reduced DNA and enriched protein amounts compared to the chromosome arms [13]. This agrees with the observation that histone H3 is replaced by the evolutionarily conserved centromere-specific histone H3-variant CENP-A within the core centromere [14].

Interestingly, during meiosis in monocentric plants the distribution of S10 and S28 phosphorylation varies between the two meiotic divisions [8–10] (Fig. 2). During the first division the chromosomes are highly phosphorylated throughout their entire length, while in the second division the H3 phosphorylation is restricted to the pericentromeric regions, as in mitotic chromosomes (Fig. 2a, c) . Surprisingly, at the same time, single chromatids resulting from equational division of univalents at anaphase I show no H3 phosphorylation (Fig. 2c). Irrespective of their low level of H3 phosphorylation, however, prematurely separated chromatids show normal condensation and their kinetochores interact with the microtubules. These observations led to the hypothesis that in plants pericentromeric H3 phosphorylation at both serine positions is required for cohesion of sister chromatids during metaphase I, and for sister chromatid pericentromeres during mitosis and metaphase II, respectively [9,10] (Fig. 3). This hypothesis was further supported by the observation that in a maize mutant (*afdI*) defective in sister chromatid cohesion the univalents at metaphase I showed strong phosphorylation only at the pericentromeric regions [8], and that a dicentric chromosome revealed hyperphosphorylated H3 only at the functional centromere [3,15]. In contrast the polycentric chromosomes of *Rhynchospora tenuis* [16] and of the genus *Luzula* [10,17] (Fig. 1c), were labelled along the entire length of chromosomes during mitosis. The fact that histone H3, at the positions described, becomes dephosphorylated at interkinesis and phosphorylated again during prophase II indicates that this post-translational modification is reversible, and it can occur independent of the DNA replication process.

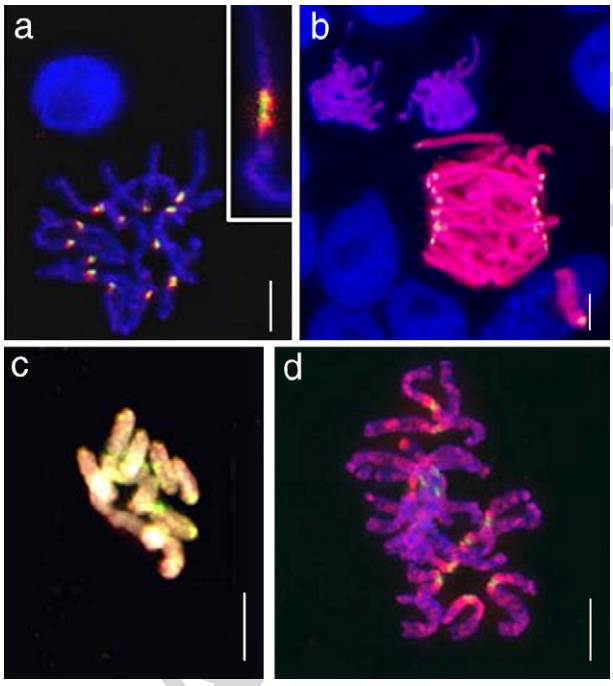


Fig. 1. Mitotic cell cycle regulated histone H3 phosphorylation in plants (a: *Hordeum vulgare,* b: *Vicia faba*, c: *Luzula luzuloides*, d: *Secale cereale*). (a) On monocentric chromosomes the pericentromeric regions show H3S10ph (red), whereas H3S28ph (yellow) is confined to the central part of the pericentromeric region (inset). DAPI-stained interphase nuclei (blue) display no detectable H3 phosphorylation. (b) H3T11ph (red) correlates with condensation of metaphase chromosomes [18]. (c) The polycentric chromosomes of *L. luzuloides* are entirely labelled with H3 phosphorylated at both serine positions (red/yellow) [10]. (d) H3T3ph (red) correlates with condensation of metaphase chromosomes. H3S28ph (yellow) is confined to the pericentromeric region [10]. Bars represent 5 μm.

Compared to serine phosphorylation there are fewer reports for histone threonine phosphorylation. Phosphorylation at T3 (Fig. 1d) and T11 [18] (Fig. 1b) in plants occurs along entire chromosome arms, and correlates with the condensation of mitotic and meiotic chromosomes (Fig. 3). In mammals, in contrast, where phosphorylation of T3 and T11 is most abundant in the centromere [19–21], it may instead serve as a recognition code for centromere assembly. The reverse may be true for S10/28 modifications, which may provide a label for the pericentromere region in plants (Fig. 1a, d), but has a function in chromosome condensation in mammals.

The coincidence of H3 phosphorylation (at S10/28) with chromosome condensation in animal cells had led to the proposal of a causal relationship [2,4]. However, since this correlation does not exist in plants and other organisms this proposal has been modified to the “ready production hypothesis” [22,23], meaning that H3 phosphorylation during mitosis simply serves as a ‘memory’ that chromosomes are ready for separation. With the finding that in plant cells phosphorylation at T3/11, rather than S10/28, correlates with chromosome condensation the original proposal by Hendzel et al. [2] may well be valid, due to species-specific differences in the biological significance of the histone code.

The dynamics of histone H3 phosphorylation has been artificially altered by the application of phosphatase and kinase inhibitors. The protein phosphatase inhibitor cantharidin increases histone H3S10/28 phosphorylation along chromosome arms from prophase to telophase in plants [10,24]. Unlike the situation in mammals, where phosphatase inhibitors induced premature chromosome condensation and stimulate H3 phosphorylation in interphase nuclei [25], no such severe effect of interphase histone H3 phosphorylation could be found in plants. The H3S10 phosphorylation pattern after *in vitro* treatment with cantharidin resembles that of the chromosomes at first meiotic division in plants. It could be that the phosphorylation of the pericentric chromatin, and of chromosome arms, is controlled by different kinases. Alternatively, there might only be one

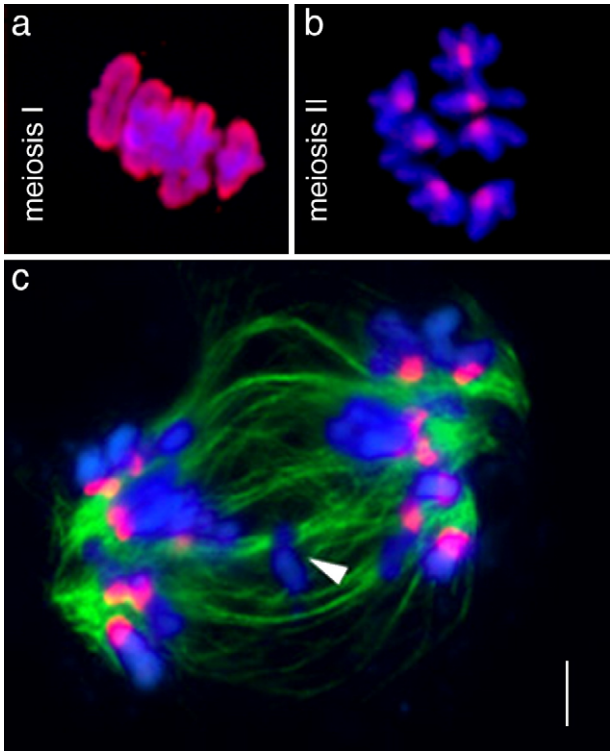


Fig. 2. Distribution of phosphorylated H3S10 differs between the first and second meiotic division [9]. At the first division (a) entire *Secale cereale* chromosomes are labelled with H3S10ph-specific signals (red), whereas at second division (b) H3S10ph is confined to the pericentromeric regions. (c) Histone H3 of chromatids that are condensed prematurely and of separated chromatids (arrowed) are not S10-phosphorylated at the pericentromeric regin. Note: irrespective of their low level of H3 phosphorylation prematurely separated chromatids are normally condensed and their kinetochores interact with microtubules (green). Bar represents 5 μm.

kinase whose activity along the chromosome is regulated differently in mitosis and meiosis. Following cantharidin treatment the observed meta/anaphase cells appeared normal, with the exception of very rare lagging chromatids at anaphase, and a delayed transition from metaphase to anaphase (Manzanero, A. and Houben, A., unpublished data). Inhibition of histone deacetylation by trichostatin in *Nicotiana sylvestris* protoplasts during mitosis induces the accumulation of metaphase cells and reduces H3S10ph at anaphase and telophase [26], which suggests a direct or indirect interaction between H3 phosphorylation and acetylation.

For threonine 11 of histone H3 a different response to cantharidin has been reported [18]. T11 became phosphorylated during interphase, but this phosphorylation was restricted to pericentromeric regions. The simplest explanation for this finding is that phosphorylation takes place in interphase as well, but remains undetected because the rate of dephosphorylation exceeds that of phosphorylation. Alternatively, the kinase phosphorylating T11 may be inactivated by dephosphorylation during interphase. Thus, inhibition of phosphatase activity could result in activation of this kinase. Since cantharidin affects a number of different PP2A and PP1 phosphatases [27] the specific enzyme involved cannot be deduced. It could be however, that the artificially induced H3T11 phosphorylation of the pericentric interphase chromatin and the phosphorylation of the chromosome arms is controlled by different kinases/phosphatases.

A reduced level of H3S10/28 phosphorylation and an aberrant segregation of mitotic chromosomes have been revealed by varying the Aurora kinase activity of cultured tobacco cells with the inhibitor Hesperadin [28]. Hesperadin, which has been developed as potential anticancer drug [29], is a small molecule that inhibits Aurora B activity. Because the Hesperadin binding sites of Aurora B are conserved between human and *Arabidopsis*, Hesperadin potentially inhibits plant Aurora kinases in the same manner as ATP competitive inhibitors in animals [28]. Since chromatids were normally condensed without H3S10/S28 phosphorylation, and a high frequency of lagging anaphase chromosomes in Hesperadin-treated tobacco cells were found, it is likely that H3S10/S28 phosphorylation is required for the dissociation of mitotic sister chromatid cohesion in plants. Further experiments will be necessary to clarify the interrelationship between a reduction in H3S10/S28ph staining and defects in chromatid segregation.

## 2. Proposed functions of histone H3 phosphorylation during cell cycle

Given the varied and often conflicting data on histone H3 phosphorylation its function remains controversial. The primary function of the cell cycle dependent histone phosphorylation may be to identify different domains of the chromosomes, and to mark their progress through the cell cycle [23]. Older models proposed that histone modifications may directly influence either the structure or the folding dynamics of nucleosomal arrays, but there is little evidence for such models [30]. Analysing the folding patterns of synthetic *in vitro* assembled nucleosomal arrays has demonstrated that arrays assembled with homogeneously phosphorylated H3S10 do not behave any differently from unmodified H3S10, indicating that H3S10 phosphorylation has no direct effect on inter-nucleosomal tail interactions which are important for higher-order folding [32]. It seems to be more likely that histone modifications control the binding of non-histone proteins to the chromatin fibre. For example, some chromodomains bind to methylated lysines, whereas bromodomains specify binds to acetylated lysines (reviewed in [30–32]. Also phosphorylation of H3S10 in *S. cerevisiae*, facilitates the sequential acetylation of lysine 14 by directly enhancing the binding of the GCN5 acetyltransferase [33,34].

The reversible cycle of histone phosphorylation/dephosphorylation may govern the capacity of chromatin-binding proteins to bind methylated lysines, and to re-release these binding factors at the appropriate stage of the cell cycle. To date, however, no protein has yet been described that could ‘read’ and interact with the phosphorylated histone. Rather, evidence exists that in mammals phosphorylation of H3S10 is responsible for the dissociation of the methyl H3K9-binding protein HP1 during mitosis [35,36]. Mass spectrometry data provided some *in vivo* support for the ‘methyl/phos’ binary switch hypothesis [35,37], since phosphopeptides containing lysine

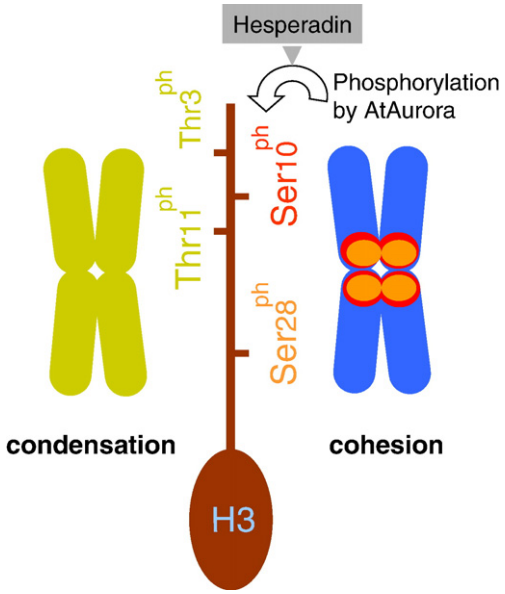


Fig. 3. Model: In plants the cell cycle dependent phosphorylation at H3T11/3 may serve as a signal for other proteins involved in chromosome condensation, while H3S10/28ph is involved sister chromatid cohesion at the pericentromeres of metaphase chromosomes during mitosis and meiosis II. Aurora-like kinase phosphorylates H3 at S10/28, and Hesperadin inhibits phosphorylation activity of Aurora.

methylation were often found. This suggests that these binary switches could also possibly extended to K27/S28 and T3/K4 [38]. This finding is in contrast to earlier reports suggesting that the coexistence of H3K9 methylation and S10 phosphorylation was unlikely [39]. A reduction in the levels of the JIL-1 histone H3S10 kinase resulted in the spreading of the major heterochromatin markers H3K9me2 and HP1 to ectopic locations on the chromosome arms, with the most pronounced increase on the X chromosomes [40]. Whether a similar 'methyl/phos binary switch' exists in plants remains to be studied. However, the finding that the subnuclear localisation of HP1-like protein was unaffected in Arabidopsis mutants displaying a significant reduction in H3K9 methylation raises some doubt about the role of H3-K9 methylation in HP1 recruitment to chromocentres in plants [41]. In the same way, Arabidopsis DNA methylation mutants (DDM1 and MET1) did not reveal any altered H3S10 phosphorylation sites at the microscopical level (Houben, A., unpublished data).

## 3. Candidate kinases involved in cell cycle-dependent histone phosphorylation

Genetic and biochemical data indicate that members of the Aurora kinase family, in particular Ipl1p of *Saccharomyces cerevisiae* and the B-type Aurora of *Caenorhabditis elegans, Drosophila* and mammals, can control cell cycle regulated histone H3 phosphorylation at serine 10 and 28, as opposed to a type 1 phosphatase (Glc7p in *S. cerevisiae*) [6,42–44]. At the same time several other kinases, have also been reported to be involved in the phosphorylation of H3 after mitogenic stimulation or stress (listed in [45]). Thus many kinases, which appear to be involved in histone H3 phosphorylation, may be stimulus- and cell type-dependent. The first plant type Aurora kinases have recently been characterised [46,47]. Consistent with the mitotic function of Aurora kinases described in other systems, transcripts and proteins of Arabidopsis Auroras are most abundant in tissues containing actively dividing cells. Localisation of GFP-tagged AtAuroras revealed that AtAuroras primarily associate with mitotic structures such as microtubule spindles, centromeres and with the emerging cell plate of dividing BY-2 culture cells of tobacco. All three members of the Arabidopsis *Aurora*-like gene family (*At4g32830, At2g25880, At2g45490*) phosphorylate histone H3 at serine 10 *in vitro*. At Aurora-specific immunosignals at the pericentromeres that are coincident with histone H3 that is phosphorylated at serine position S10 further supports histone H3 substrate specificity. But in contrast to the mammalian B-type Aurora [44] recombinant AtAurora1 of Arabidopsis reveals no *in vitro* kinase activity towards H3 at position serine 28. Overexpression data of [47] indicate that AtAurora3 also acts on gamma-tubulin disposition or recruitment to promote spindle formation. The generation of knockdown mutants will help to further define the mitotic and meiotic function of plant Aurora kinases.

Evolutionary analysis shows that Aurora kinases, while having a consistent sequence theme throughout eukaryotes, have undergone lineage-specific expansions and functional specialization in metazoans. The occurrence of two subclades in plants raises the interesting possibility that plants possess two different isoforms of Aurora, called plant Aurora α and Aurora β [46].

In animals, Aurora B is an enzymatic core of chromosomal passenger complex, which also includes the inner centromere protein INCENP, survivin, and borealin/DasraB. The complex localises to the centromere during prometaphase and metaphase [48]. Members of the Aurora B complex in fission yeast and budding yeast have been characterised and shown to have similar functions to their animal-cell orthologues (reviewed in [49]). However, homologues of INCENP, survivin, and borealin/DasraB could not be found in the Arabidopsis genome and other plant proteins using NCBI conserved domain database and blastp [47]. Whether the plant kinetochore-localised histone H3 variant CENH3 (CENPA) undergoes phosphorylation by an Aurora kinase, as reported for other organisms, remains to be answered. In human S7 of CENH3 is phosphorylated in a temporal pattern that is similar to H3S10 [50]. In plants it is noteworthy that CENH3-S50 and H3S28 are phosphorylated with virtually identical kinetics [11]. Based on the temporal coordination of CENH3 and H3S28 phosphorylation Zhang et al. [11] suggest that one signal for centromere-mediated cohesion accumulation is a histone kinase, which binds first at CENH3 and then diffuses outwards over histone H3 to define the boundaries of the pericentromeric domains. Identification of additional plant Aurora kinase substrates will be very fruitful in determining details of their molecular function.

NIMA kinase (never in mitosis, [51]) activity is required for both phosphorylation of histone H3 Serine 10 and chromo-

some condensation in *Aspergillus nidulans* [52]. The fission yeast NIMA homologue, Fin1p, can induce profound chromosome condensation in the absence of condensin and topoisomerase II, indicating that Fin1p-induced condensation differs from mitotic condensation [53]. Yet, despite similarity to NIMA of *Aspergillus*, no evidence for histone H3-specific kinase activity has been found for Fin1p [51]; although *in vitro* evidence exists that the human NIMA-like kinase Nek6 has potential histone H3 substrate specificity [54]. While yeast and filamentous fungi have only a single NIMA-like kinase, *Caenorhabditis elegans* encodes four [55], humans encode at least 11 [56] and Arabidopsis encodes a *NIMA*-related gene family with 7 members (*At1g54510*, *At3g04810, At5g28290*, *At3g63280*, *At3g20860*, *At3g44200*, *At3g12200*) [56]. The first plant NIMA-like kinases were described in *Antirrhinum majus* [57], tomato [58] and poplar [56]; but whether histone H3 is the substrate for any of these plant NIMA-like proteins remains to be analysed.

The human haspin kinase (haploid germ cell-specific nuclear protein [59]) phosphorylates histone H3 on T3 *in vitro*, and probably also *in vivo* in human cultured cells. Haspin RNA interference in mammals causes misalignment of metaphase chromosomes, and overexpression delays progression through early mitosis [60]. Genes encoding *haspin* homologues are present in all major eukaryotic phyla, but not in any of the sequenced prokaryotic or archaea genomes [61]. In mammals haspin localises to the nucleus, to condensed chromosomes throughout mitosis, to the centrosomes and spindle microtubules during metaphase and to the midbody in telophase [19]. Despite its crucial role in mitosis, a cell cycle dependent kinase activity could not be detected for haspin [19]. Similarly, based on publicly available microarray datasets [62] the expression profile of the *haspin*-like gene of Arabidopsis (*At1g09450*) does not change during mitosis. *In vitro* kinase assays testing histone substrate specificity of the *Saccharomyces cerevisiae* haspin-related proteins Alk1 and Alk2 failed to identify histones as putative substrates [63].

The potential kinase(s) involved in phosphorylation of H3T11 in plants remain to be identified. In mammals, Dlk/ZIP kinase seems to be a likely candidate since it phosphorylates H3 at T11 *in vitro* and its association with centromeres parallels precisely the appearance of T11 phosphorylation [21]. A BLAST search of the Arabidopsis databank for homologues of DAP kinase family members [64] only revealed protein kinases with low similarity.

The identification of different kinases with H3 substrate specificity suggests that various kinases could function as mitotic H3 kinases in different organisms. It is also conceivable that within any single organism many kinases can phosphorylate histones during cell division.

## 4. Histone phosphorylation in the activation of transcription, apoptosis and DNA damage repair

Histone phosphorylation, like other post-translational histone modifications, has also been linked to the activation of transcription, apoptosis, DNA damage repair and even sex chromosome dosage compensation (reviewed in [23,65,66]); although our knowledge of its involvement in these processes in plants is limited.

Recent transcriptome analysis in yeast demonstrated that H3S10 phosphorylation is not a general requirement for transcription at all promoters, but it may play a role tailored to specific promoters [67]. In addition diverse stimuli, including salt stress [68] or treatment of cells with growth factors [69], elicit rapid and transient phosphorylation of different serine/threonine positions on histones. Probably H3 phosphorylation also has a role in the transcriptional activation of plant genes [70]. Its involvement in stress response of plants is indirectly supported by the observation that cold, salt or hormone stress enhances the activity of the Arabidopsis MSK homologues ATPK6/19 kinases [71]. MSK kinases are enzymes involved in the process of histone phosphorylation during stress conditions [72]. In the same way, the treatment of cultured BY-2 cells of tobacco with different concentrations of sucrose, or of NaCl, can lead to an increase in phosphorylation of histone H3 at S10 and T3, particularly at intermediate concentrations, independently of any increase in the rate of cell division (Fig. 4, Demidov, D., unpublished data). Similar changes in the modifications of histone H3 after salt stress were reported for a tobacco cell culture [73]. We assume that the treatment with sucrose and NaCl might induce in a time and concentration dependent manner the chromatin environment of genes involved in the processes of energy and carbohydrates metabolism and osmotic-salt stress responsive pathway, respectively.

The correlation of H3S10 phosphorylation with gene transcription received further support after increased phospho H3S10-specific immunolabelling was observed in actively transcribing shock puffs in *Drosophila* polytene chromosomes following heat shock [74]. Cold treatment of plant root meristems has also been found to result in additional chromosomal sites (45S rDNA loci and heterochromatic sites) of H3S10 phosphorylation, besides that in the centromeres [24]; although

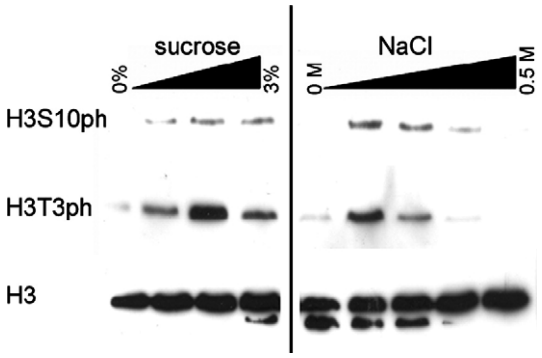


Fig. 4. Western blot analysis of histone H3 phosphorylation on total protein extracts from tobacco BY-2 cells after treatment with different concentrations of sucrose (0, 0.1, 1 and 3%) and NaCl (0, 0.1, 0.1, 0.25 and 0.5 M). Cell suspension was cultivated in a basic medium [87] and the treatment was performed for 1 h. Total protein isolation and immunodetection by Western blot was performed according [47]. Left: BY-2 suspension after treatment with 0–3% sucrose and subjected for Western blot analysis with antibodies against H3S10ph, H3T3ph and unmodified histone H3, respectively. Right: The same antibodies as above were used for the Western blot on protein extracts isolated from BY-2 cells treated with 0–0.5 M NaCl.

it is not clear whether chromosomal cold-sensitive regions are indirectly correlated with cold-stress activated genes.

Histone phosphorylation has also been observed in association with the apoptosis-induced chromatin condensation process (reviewed in [75]). Besides histone H3, there is also evidence for a correlation between histone H2A.X phosphorylation [76] and H2B phosphorylated at serine 14 [77] with the onset of apoptotic chromatin condensation and DNA fragmentation in human cells. In contrast with S10/28 phosphorylation of H3, the H2BS14 phosphorylation mark is not detected in mitotic chromosomes. This has led to the hypothesis of a non-overlapping set of phosphorylation marks discriminating mitotic from apoptotic chromatin, a 'life versus death histone code' [77]. Chromatin apoptotic-like features partly similar to mammalian cells have been observed in plants (reviewed in [78]) but a relationship between H3 phosphorylation and apoptosis is not yet established for plants.

In yeast and in metazoa the histone variant H2A.X becomes phosphorylated (known as gamma-H2A.X) at its C-terminal S139 on either side of a DNA double-strand break, induced by ionising radiation, meiosis, replication and recombination, at a distance of 1 to 50 kb by specific kinases (ATM, ATR). Phosphorylated H2A.X reversibly triggers the accumulation of components involved in DNA recombination repair, and in cell cycle checkpoint activation including histone acetyltransferase and cohesin (for review see [79]). In plants, phosphorylation of H2A.X is induced by gamma-irradiation at only one third the rate observed in yeast and mammals, suggesting that plants are more resistant than mammals to the induction of DNA lesions [80]. It is possible that in plants, fewer DNA lesions are formed in response to ionising radiation, or plants possess a greater capacity for DNA repair and/or damage tolerance than mammals do [80].

## 5. Outlook

Except for centromeric histone CENPA of maize [11], plant histone H3 investigations have not yet looked specifically at individual H3 isoform phosphorylation. It is known however, that Arabidopsis encodes 15 histone H3 genes, including five H3.1, three *H3.3* and five *H3.3* like genes [81]. Amongst them are H3 variants with highly similar protein sequences, but different function, suggesting that phospho-specific antibodies are insufficient to distinguish between the phosphorylation status of different H3 isoforms. As mentioned by [39], future work on histone H3 phosphorylation cannot overlook the fact that modification differences in H3 variants may exist and should be taken into account. Complementary approaches, combining mass spectrometry with immunological assays, will be necessary to decipher the meaning of the diverse combinations of histone H3 modifications. Besides further research on H3 and isoforms, the functional analysis of phosphorylation of the other core histones including linker histones will improve our knowledge on the complex interplay of post-translational histone modifications in plants. Recently, chromatin condensation has been linked to phosphorylation of histone H4 [82,83], H2A [82], H2B [84] and H1 [76] in non-plant species.

Although the components of the chromatin code are fairly well conserved throughout eukaryotes, evidence is growing that the interpretation of the code has diverged in different organisms, particular in plants versus animals [85,86]. We look forward to further exciting insights that are certain to arise as researchers continue to investigate the function and regulation of histone modifications in diverse model systems.

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## References

[1] L.R. Gurtley, R.A. Walters, R.A. Tobey, Sequential phosphorylation of histone subfractions in the Chinese hamster cell cycle, J. Biol. Chem. 250 (1975) 3936–3944.
[2] M.J. Hendzel, Y. Wei, M.A. Mancini, A. Van Hooser, T. Ranalli, B.R. Brinkley, D.P. Bazett-Jones, C.D. Allis, Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation, Chromosoma 106 (1997) 348–360.
[3] A. Houben, T. Wako, R. Furushima-Shimogawara, G. Presting, G. Kunzel, I. Schubert, K. Fukui, The cell cycle dependent phosphorylation of histone H3 is correlated with the condensation of plant mitotic chromosomes, Plant J. 18 (1999) 675–679.
[4] H. Goto, Y. Tomono, K. Ajiro, H. Kosako, M. Fujita, M. Sakurai, K. Okawa, A. Iwamatsu, T. Okigaki, T. Takahashi, M. Inagaki, Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation, J. Biol. Chem. 274 (1999) 25543–25549.
[5] A. Van Hooser, D.W. Goodrich, C.D. Allis, B.R. Brinkley, M.A. Mancini, Histone H3 phosphorylation is required for the initiation, but not maintenance, of mammalian chromosome condensation, J. Cell Sci. 111 (1998) 3497–3506.
[6] J.Y. Hsu, Z.W. Sun, X. Li, M. Reuben, K. Tatchell, D.K. Bishop, J.M. Grushcow, C.J. Brame, J.A. Caldwell, D.F. Hunt, R. Lin, M.M. Smith, C. D. Allis, Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes, Cell 102 (2000) 279–291.
[7] K.K. Hanson, A.C. Kelley, M. Bienz, Loss of *Drosophila borealin* causes polyploidy, delayed apoptosis and abnormal tissue development, Development 132 (2005) 4777–4787.
[8] E. Kaszas, W.Z. Cande, Phosphorylation of histone H3 is correlated with changes in the maintenance of sister chromatid cohesion during meiosis in maize, rather than the condensation of the chromatin, J. Cell. Sci. 113 (2000) 3217–3226.
[9] S. Manzanero, P. Arana, M.J. Puertas, A. Houben, The chromosomal distribution of phosphorylated histone H3 differs between plants and animals at meiosis, Chromosoma 109 (2000) 308–317.
[10] D. Gernand, D. Demidov, A. Houben, The temporal and spatial pattern of histone H3 phosphorylation at serine 28 and serine 10 is similar in plants but differs between mono- and polycentric chromosomes, Cytogenet. Genome Res. 101 (2003) 172–176.
[11] X.L. Zhang, X.X. Li, J.B. Marshall, C.X. Zhong, R.K. Dawe, Phosphoserines on maize CENTROMERIC HISTONE H3 and histone H3 demarcate the centromere and pericentromere during chromosome segregation, Plant Cell 17 (2005) 572–583.
[12] E. Schroeder-Reiter, A. Houben, G. Wanner, Immunogold labeling of chromosomes for scanning electron microscopy: a closer look at phosphorylated histone H3 in mitotic metaphase chromosomes of *Hordeum vulgare*, Chromosome Res. 11 (2003) 585–596.

[13] G. Wanner, H. Formanek, A new chromosome model, J. Struct. Biol. 132 (2000) 147–161.
[14] A.A. Van Hooser, I.I. Ouspenski, H.C. Gregson, D.A. Starr, T.J. Yen, M.L. Goldberg, K. Yokomori, W.C. Earnshaw, K.F. Sullivan, B.R. Brinkley, Specification of kinetochore-forming chromatin by the histone H3 variant CENP-A, J. Cell. Sci. 114 (2001) 3529–3542.
[15] F. Han, J.C. Lamb, J.A. Birchler, High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 3238–3243.
[16] M. Guerra, A.C. Brasileiro-Vidal, P. Arana, M.J. Puertas, Mitotic micortubule development and histone H3 phosphorylation in the holocentric chromosomes of *Rhynchospora tenuis* (Cyperaceae), Genetica 126 (2006) 33–41.
[17] K. Nagaki, K. Kashihara, M. Murata, Visualization of diffuse centromeres with centromere-specific histone H3 in the holocentric plant *Luzula nivea*, Plant Cell 17 (2005) 1886–1893.
[18] A. Houben, D. Demidov, T. Rutten, K.H. Scheidtmann, Novel phosphorylation of histone H3 at threonine 11 that temporally correlates with condensation of mitotic and meiotic chromosomes in plant cells, Cytogenet. Genome Res. 109 (2005) 148–155.
[19] J. Dai, J.M.G. Higgins, Haspin—A mitotic histone kinase required for metaphase chromosome alignment, Cell Cycle 4 (2005) 665–668.
[20] H. Polioudaki, Y. Markaki, N. Kourmouli, G. Dialynas, P.A. Theodoropoulos, P.B. Singh, S.D. Georgatos, Mitotic phosphorylation of histone H3 at threonine 3, FEBS Lett. 560 (2004) 39–44.
[21] U. Preuss, G. Landsberg, K.H. Scheidtmann, Novel mitosis-specific phosphorylation of histone H3 at Thr11 mediated by Dlk/ZIP kinase, Nucleic Acids Res. 31 (2003) 878–885.
[22] F. Hans, S. Dimitrov, Histone H3 phosphorylation and cell division, Oncogene 20 (24) (2001) 3021–3027.
[23] C. Prigent, S. Dimitrov, Phosphorylation of serine 10 in histone H3, what for? J. Cell Sci. 116 (2003) 3677–3685.
[24] S. Manzanero, T. Rutten, V. Kotseruba, A. Houben, Alterations in the distribution of histone H3 phosphorylation in mitotic plant chromosomes in response to cold treatment and the protein phosphatase inhibitor cantharidin, Chromosome Res. 10 (2002) 467–476.
[25] K. Ajiro, K. Yoda, K. Utsumi, Y. Nishikawa, Alteration of cell cycle-dependent histone phosphorylations by okadaic acid. Induction of mitosis-specific H3 phosphorylation and chromatin condensation in mammalian interphase cells, J. Biol. Chem. 271 (1996) 13197–13201.
[26] Y. Li, Y. Butenko, G. Grafi, Histone deacetylation is required for progression through mitosis in tobacco cells, Plant J. 41 (2005) 346–352.
[27] C. MacKintosh, R.M. MacKintosh, Inhibitors of protein kinases and phosphatases, Trends Biochem. Sci. 19 (1994) 444–448.
[28] D. Kurihara, S. Matsunaga, A. Kawabe, S. Fujimoto, M. Noda, S. Uchiyama, K. Fukui, Aurora kinase is required for chromosome segregation in tobacco BY-2 cells, Plant J. 48 (2006) 572–580.
[29] S. Hauf, R.W. Cole, S. LaTerra, C. Zimmer, G. Schnapp, R. Walter, A. Heckel, J. van Meel, C.L. Rieder, J.M. Peters, The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint, J. Cell Biol. 161 (2003) 281–294.
[30] C.L. Peterson, M.A. Laniel, Histones and histone modifications, Curr. Biol. 14 (2004) R546–R551.
[31] C.J. Fry, M.A. Shogren-Knaak, C.L. Peterson, Histone H3 amino-terminal tail phosphorylation and acetylation: synergistic or independent transcriptional regulatory marks? Cold Spring Harbor Symposia on Quantitative Biology 69 (2004) 219–226.
[32] W. Fischle, Y. Wang, C.D. Allis, Histone and chromatin cross-talk, Curr. Opin. Cell Biol. 15 (2003) 172–183.
[33] A. Clements, A.N. Poux, W.S. Lo, L. Pillus, S.L. Berger, R. Marmorstein, Structural basis for histone and phosphohistone binding by the GCN5 histone acetyltransferase, Mol. Cell 12 (2003) 461–473.
[34] W.S. Lo, R.C. Trievel, J.R. Rojas, L. Duggan, J.Y. Hsu, C.D. Allis, R. Marmorstein, S.L. Berger, Phosphorylation of serine 10 in histone H3 is functionally linked in vitro and in vivo to Gcn5-mediated acetylation at lysine 14, Mol. Cell 5 (2000) 826–917.
[35] W. Fischle, B.S. Tseng, H.L. Dormann, B.M. Ueberheide, B.A. Garcia, J. Shabanowitz, D.F. Hunt, H. Funabiki, C.D. Allis, Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation, Nature 438 (2005) 1116–1122.
[36] T. Hirota, J.J. Lipp, B.H. Toh, J.M. Peters, Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin, Nature 438 (2005) 1176–1180.
[37] W. Fischle, Y. Wang, C.D. Allis, Binary switches and modification cassettes in histone biology and beyond, Nature 425 (2003) 475–479.
[38] B.A. Garcia, C.M. Barber, S.B. Hake, C. Ptak, F.B. Turner, S.A. Busby, J. Shabanowitz, R.G. Moran, C.D. Allis, D.F. Hunt, Modifications of human histone H3 variants during mitosis, Biochemistry 44 (2005) 13202–13213.
[39] S. Rea, F. Eisenhaber, D. O'Carroll, B.D. Strahl, Z.W. Sun, M. Schmid, S. Opravil, K. Mechtler, C.P. Ponting, C.D. Allis, T. Jenuwein, Regulation of chromatin structure by site-specific histone H3 methyltransferases, Nature 406 (2000) 593–599.
[40] W. Zhang, H. Deng, X. Bao, S. Lerach, J. Girton, J. Johansen, K.M. Johansen, The JIL-1 histone H3S10 kinase regulates dimethyl H3K9 modifications and heterochromatic spreading in *Drosophila*, Development 133 (2006) 229–235.
[41] A. Zemach, Y. Li, H. Ben-Meir, M. Oliva, A. Mosquna, V. Kiss, Y. Avivi, N. Ohad, G. Grafi, Different domains control the localization and mobility of LIKE HETEROCHROMATIN PROTEIN1 in Arabidopsis nuclei, Plant Cell 18 (2006) 133–145.
[42] C. Crosio, G.M. Fimia, R. Loury, M. Kimura, Y. Okano, H. Zhou, S. Sen, C.D. Allis, P. Sassone-Corsi, Mitotic phosphorylation of histone H3: spatio-temporal regulation by mammalian Aurora kinases, Mol. Cell. Biol. 22 (2002) 874–885.
[43] R. Giet, D.M. Glover, *Drosophila* aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis, J. Cell Biol. 152 (2001) 669–682.
[44] H. Goto, Y. Yasui, E.A. Nigg, M. Inagaki, Aurora-B phosphorylates histone H3 at serine28 with regard to the mitotic chromosome condensation, Genes Cells 7 (2002) 11–17.
[45] A.M. Bode, Z. Dong, Inducible covalent posttranslational modification of histone H3, Sci. STKE 2005 (281) (2005) re4.
[46] D. Demidov, D. Van Damme, D. Geelen, F.R. Blattner, A. Houben, Identification and dynamics of two classes of aurora-like kinases in Arabidopsis and other plants, Plant Cell 17 (2005) 836–848.
[47] A. Kawabe, S. Matsunaga, K. Nakagawa, D. Kurihara, A. Yoneda, S. Hasezawa, S. Uchiyama, K. Fukui, Characterization of plant Aurora kinases during mitosis, Plant Mol. Biol. 58 (2005) 1–13.
[48] P. Vagnarelli, W.C. Earnshaw, Chromosomal passengers: the four-dimensional regulation of mitotic events, Chromosoma 113 (2004) 211–222.
[49] P.D. Andrews, E. Knatko, W.J. Moore, J.R. Swedlow, Mitotic mechanics: the auroras come into view, Curr. Opin. Cell Biol. 15 (2003) 672–683.
[50] S.G. Zeitlin, R.D. Shelby, K.F. Sullivan, CENP-A is phosphorylated by Aurora B kinase and plays an unexpected role in completion of cytokinesis, J. Cell Biol. 155 (2001) 1147–1157.
[51] A.H. Osmani, K. O'Donnell, R.T. Pu, S.A. Osmani, Activation of the nimA protein kinase plays a unique role during mitosis that cannot be bypassed by absence of the bimE checkpoint, EMBO J. 10 (1991) 2669–2679.
[52] C.P. De Souza, A.H. Osmani, L.P. Wu, J.L. Spotts, S.A. Osmani, Mitotic histone H3 phosphorylation by the NIMA kinase in *Aspergillus nidulans*, Cell 102 (2000) 293–302.
[53] M.J. Krien, R.R. West, U.P. John, K. Koniaras, J.R. McIntosh, M.J. O'Connell, The fission yeast NIMA kinase Fin1p is required for spindle function and nuclear envelope integrity, EMBO J. 21 (2002) 1713–1722.
[54] Y. Hashimoto, H. Akita, M. Hibino, K. Kohri, M. Nakanishi, Identification and characterization of Nek6 protein kinase, a potential human homolog of NIMA histone H3 kinase, Biochem. Biophys. Res. Commun. 293 (2002) 753–758.
[55] M.J. O'Connell, M.J. Krien, T. Hunter, Never say never. The NIMA-related protein kinases in mitotic control, Trends Cell Biol. 13 (2003) 221–228.

[56] M. Cloutier, F. Vigneault, D. Lachance, A. Seguin, Characterization of a poplar NIMA-related kinase PNek 1 and its potential role in meristematic activity, FEBS Lett. 579 (2005) 4659–4665.
[57] H. Zhang, G. Scofield, P. Fobert, J.H. Doonan, A nimA-like protein kinase transcript is highly expressed in meristems of *Antirrhinum majus*, J. Microsc. (Oxford) 181 (1996) 186–194.
[58] L. Pnueli, T. Gutfinger, D. Hareven, O. Ben-Naim, N. Ron, N. Adir, E. Lifschitz, Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering, Plant Cell 13 (12) (2001) 2687–2702.
[59] H. Tanaka, Y. Yoshimura, M. Nozaki, K. Yomogida, J. Tsuchida, Y. Tosaka, T. Habu, T. Nakanishi, M. Okada, H. Nojima, Y. Nishimune, Identification and characterization of a haploid germ cell-specific nuclear protein kinase (Haspin) in spermatid nuclei and its effects on somatic cells, J. Biol. Chem. 274 (1999) 17049–17057.
[60] J. Dai, S. Sultan, S.S. Taylor, J.M. Higgins, The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment, Genes Dev. 19 (2005) 472–488.
[61] J.M. Higgins, Structure, function and evolution of haspin and haspin-related proteins, a distinctive group of eukaryotic protein kinases, Cell Mol. Life Sci. 60 (2003) 446–462.
[62] M. Menges, L. Hennig, W. Gruissem, J.A.H. Murray, Cell cycle-regulated gene expression in Arabidopsis, J. Biol. Chem. 277 (2002) 41987–42002.
[63] A. Nespoli, R. Vercillo, L. di Nola, L. Diani, M. Giannattasio, P. Plevani, M. Muzi-Falconi, Alk1 and Alk2 are two new cell cycle-regulated haspin-like proteins in budding yeast, Cell Cycle 5 (2006) 1464–1471.
[64] D. Kogel, J.H. Prehn, K.H. Scheidtmann, The DAP kinase family of pro-apoptotic proteins: novel players in the apoptotic game, BioEssays 23 (2001) 352–358.
[65] K.M. Johansen, J. Johansen, Regulation of chromatin structure by histone H3S10 phosphorylation, Chromosome Res. 14 (2006) 393–404.
[66] R. Loury, P. Sassone-Corsi, Analysis of histone phosphorylation: coupling intracellular signaling to chromatin remodelling, Methods Enzymol. 377 (2004) 197–212.
[67] W.S. Lo, E.R. Gamache, K.W. Henry, D. Yang, L. Pillus, S.L. Berger, Histone H3 phosphorylation can promote TBP recruitment through distinct promoter-specific mechanisms, EMBO J. 24 (2005) 997–1008.
[68] T. Reitsema, D. Klokov, J.P. Banath, P.L. Olive, DNA-PK is responsible for enhanced phosphorylation of histone H2AX under hypertonic conditions, DNA Rep. 4 (2005) 1172–1181.
[69] I.S. Strelkov, J.R. Davie, Ser-10 phosphorylation of histone H3 and immediate early gene expression in oncogene-transformed mouse fibroblasts, Cancer Res. 62 (1) (2002) 75–78.
[70] G.F. Li, K.J. Bishop, T.C. Hall, De novo activation of the beta-phaseolin promoter by phosphatase or protein synthesis inhibitors, J. Biol. Chem. 276 (2001) 2062–2068.
[71] T. Mizoguchi, N. Hayashida, K. Yamaguchishinozaki, H. Kamada, K. Shinozaki, 2 genes that encode ribosomal-protein-S6 kinase homologs are induced by Cold or salinity stress in *Arabidopsis thaliana*, FEBS Lett. 358 (1995) 199–204.
[72] A.L. Clayton, L.C. Mahadevan, MAP kinase-mediated phosphoacetylation of histone H-3 and inducible gene regulation, FEBS Lett. 546 (2003) 51–58.
[73] A. Sokol, M. Prymakowska-Bosak, A. Jerzmanowski, Changes in the modifications of core histone H3 after salt stress in the BY-2 tobacco cell line and *Arabidopsis thaliana* cells, Abstract book of the 15th International Conference on Arabidopsis Research Published by: Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg1 14476 Potsdam, T12-020, 2004.
[74] S.J. Nowak, V.G. Corces, Phosphorylation of histone H3 correlates with transcriptionally active loci, Genes Dev. 14 (2000) 3003–3013.
[75] J.P. Th'ng, Histone modifications and apoptosis: cause or consequence? Biochem. Cell. Biol. 79 (2001) 305–311.
[76] B. Sarg, W. Helliger, H. Talasz, B. Forg, H.H. Lindner, Histone H1 phosphorylation occurs site-specifically during interphase and mitosis: identification of a novel phosphorylation site on histone H1, J. Biol. Chem. 281 (2006) 6573–6580.
[77] W.L. Cheung, K. Ajiro, K. Samejima, M. Kloc, P. Cheung, C.A. Mizzen, A. Beeser, L.D. Etkin, B. Chernoff, B. Earnshaw, C.D. Allis, Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase, Cell 113 (2003) 507–517.
[78] E. Lam, Controlled cell death, plant survival and development, Nat. Rev., Mol. Cell Biol. 5 (2004) 305–315.
[79] C. Thiriet, J.J. Hayes, Chromatin in need of a fix: phosphorylation of H2A.X connects chromatin to DNA repair, Mol. Cell 18 (2005) 617–622.
[80] J.D. Friesner, B. Liu, K. Culligan, A.B. Britt, Ionizing radiation-dependent gamma-H2AX focus formation requires ataxia telangiectasia mutated and ataxia telangiectasia mutated and Rad3-related, Mol. Biol. Cell 16 (2005) 2566–2576.
[81] T. Okada, M. Endo, M. Singh, P.L. Balla, Analysis of the histone H3 gene family in Arabidopsis and identification of the male-gamete-specific variant AtMGH3, Plant J. 44 (2005) 557–568.
[82] C.M. Barber, F.B. Turner, Y.M. Wang, K. Hagstrom, S.S. Taverna, S. Mollah, B. Ueberheide, B.J. Meyer, D.F. Hunt, P. Cheung, C.D. Allis, The enhancement of histone H4 and H2A serine 1 phosphorylation during mitosis and S-phase is evolutionarily conserved, Chromosoma 112 (2004) 360–371.
[83] T. Krishnamoorthy, X. Chen, J. Govin, W.L. Cheung, J. Dorsey, K. Schindler, E. Winter, C.D. Allis, V. Guacci, S. Khochbin, M.T. Fuller, S.L. Berger, Phosphorylation of histone H4 Ser1 regulates sporulation in yeast and is conserved in fly and mouse spermatogenesis, Genes Dev. 20 (2006) 2580–2592.
[84] S.H. Ahn, K.A. Henderson, S. Keeney, C.D. Allis, H2B (Ser10) phosphorylation is induced during apoptosis and meiosis in *S. cerevisiae*, Cell Cycle 4 (2005) 780–783.
[85] P. Loidl, A plant dialect of the histone language, Trends Plant Sci. 9 (2004) 84–90.
[86] J. Fuchs, D. Demidov, A. Houben, I. Schubert, Chromosomal histone modification patterns—From conservation to diversity, Trends Plant Sci. 11 (2006) 199–208.
[87] K. Czempinski, J.M. Frachisse, C. Maurel, H. Barbier-Brygoo, B. Mueller-Roeber, Vacuolar membrane localization of the Arabidopsis 'two-pore' K+ channel KCO1, Plant J. 29 (2002) 809–820.