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H3Ser10 Histone Phosphorylation in Plant Cell Division

D. B. Loginova* and O. G. Silkova

Federal Research Center Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia

**e-mail: loginova@bionet.nsc.ru*

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Abstract—Histones, the main protein components of the chromatin, are exposed to posttranslational modifications that influence on peculiarities of structural and functional organization of the chromosomes. Phosphorylation, methylation, acetylation, and ubiquitination are the most spread posttranslational modifications. Phosphorylation of histones mainly happens on N-terminal domains of serines (Ser) and threonines (Thr) and is involved in regulation of different processes in mitotic and meiotic divisions. To date, it was demonstrated that this type of modification is required for the activation of transcription, repair of DNA breaks, and recombination, as well as for the condensation and divergence of chromosomes. Among the four main histones, the presence of a larger number of modification sites is typical for the H3 histone. In plants, H3 histone phosphorylation at serine in positions 10 and 28 and at threonine in positions 3, 11, 32, and 133 are the most well studied. The data known to date on the spatiotemporal distribution of H3 phosphorylation at serine in position 10 (phH3Ser10) in the mitosis and meiosis of different plant species are collected in the review. For most species, phosphorylation of only pericentromeric regions in mitosis and the second division of meiosis and along the entire length of the chromosomes in the first meiotic division is typical. However, there are exceptions in the phH3Ser10 distribution in mosses and in the *Cestrum* genus, as well as in species with holocentric chromosomes. Controversial data on the phH3Ser10 distribution in mitosis and meiosis in the same species are found. The functional significance of phH3Ser10 in the cellular division in plants is associated with the activity of the centromere, the cohesion of the centromeres and sister chromatids, and chromosome segregation. The involvement of candidates of kinases and phosphatases known to date in the dynamics of H3Ser10 phosphorylation is discussed. The review provides an overview of the role of phH3Ser10 modifications in the chromosome division and segregation in mitosis and meiosis.

Keywords: higher plants, phosphorylation, H3 histone, serine, mitosis, meiosis

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INTRODUCTION

The eukaryotic chromatin is organized in main units (nucleosomes) consisting of approximately 147 DNA base pairs (bp) wrapped around the histone octamer, which includes two molecules of H2A, H2B, H3, and H4 histones. Histone proteins consist of the core domain, which carries out the interaction between different histones within the octamer, and N-terminal fragment, whose posttranslational modifications (PTMs) influence the chromatin structure and functions (Wang and Higgins, 2013). At least eight different types of histone modifications exist, among which acetylation, methylation, and phosphorylation are the most studied (Fuchs et al., 2006; Ito, 2007; Wang and Higgins, 2013; Zhang et al., 2014). The largest number of modification sites from the four main histones is described for the H3 histone, and these modifications are involved in the regulation of genes and chromatin assembly (Ito, 2007).

The phosphorylation of H2A and H3 histones by the threonine and serine residues was studied in plants: H2A histones, by threonine in positions 120 and 133 (phH2AThr120 and phH2AThr133); and H3 histone, by threonine in positions 3, 11, and 32 (phH3Thr3, phH3Thr11, and phH3Thr32) and serine in positions 10 and 28 (phH3Ser10 and phH3Ser28) (Gernand et al., 2003; Houben et al., 2005; Caperta et al., 2008; Dong and Han, 2012; Demidov et al., 2014; Zhang et al., 2014). All these modifications are dynamical and are directly related to the chromosome divergence (started at the stage of prophase and finishing in the late anaphase or telophase). The H2AThr133 and H2AThr120 phosphorylation is observed in the centromeric and pericentromeric regions both in mitosis and meiosis and is associated with the centromere activity (according to the authors) (Dong and Han, 2012; Demidov et al., 2014). The H3Thr3, H3Thr11, and H3Thr32 phosphorylation occurs along the entire chromosome in mitosis and the first meiotic division; however, in the second meiotic division, phH3Thr3 is only limited by the centromeric region, while phH3Thr11 and phH3Thr32 are distributed

along the chromosomes (Houben et al., 2005, 2007; Caperta et al., 2008). It is assumed that these modifications are involved in the chromosome compaction.

The H3Ser10 phosphorylation is known for all higher plants; however, the character of this PTM localization varies depending on the type of cell division (mitosis and meiosis) and plant species (Houben et al., 1999; Kaszas and Cande, 2000; Manzanero et al., 2000; Pedrosa et al., 2001; Fernandes et al., 2008; Marcon-Tavares et al., 2014). The H3Ser10 phosphorylation in the pericentromeric chromosome region is associated with the centromere activity (Han et al., 2006, 2009; Gao et al., 2011). According to other data, H3Ser10 phosphorylation plays an important role in activating the transcription (Ito, 2007). The dynamics of H3 histone phosphorylation at Ser28 and Ser10 residues in mitosis and meiosis barely differ; however, phH3Ser28 is mainly localized in the core part of the centromere (directly at the primary constriction), while phH3Ser10 is localized in the pericentromeric region (Gernand et al., 2003). Based on the different pattern of phH3Ser10 and phH3Ser28 localization on the chromosomes in mitosis and meiosis in plants and animals, it is assumed that both modifications in plants are required to attain the cohesion of the sister chromatid (Kaszas and Cande, 2000; Manzanero et al., 2000; Gernand et al., 2003; Houben et al., 2007), while in animals, it is required for the condensation of chromatin (Hendzel et al., 1997).

There is a presence of phH3Ser10 in a large number of taxa—ciliates (*Tetrahymena thermophile*) (Wei et al., 1998), yeasts (*Saccharomyces cerevisiae*), mammals (Hendzel et al., 1997), as well as fungi (*Aspergillus nidulans*), and insects (*Drosophila melanogaster*) (Wei et al., 1998)—which indicates a high level of conservatism of this modification among eukaryotes and its important role in cell division. In spite of the large number of works associated with the study of H3Ser10 phosphorylation, the function of this modification in cell division has not yet been completely determined. In this review, known to date data on spatiotemporal pattern of H3Ser10 phosphorylation on the plant chromosomes in mitosis and meiosis are presented, the role of this modification in the plant cell cycle is discussed, and the candidates of kinases involved in the dynamics of phH3Ser10 are considered.

H3 HISTONE PHOSPHORYLATION IN MITOSIS

H3 Histone Phosphorylation in Species with Monocentric Chromosomes

For most of the studied vascular plants with monocentric chromosomes, it was demonstrated that H3 histone phosphorylation at serine in position 10 in mitosis is observed in the pericentromeric region starting from the prophase and disappears in the telophase (Figs. 1a–1c). Most of the studies are conducted on monocots (cereals): rye (*Secale cereale*) (Houben et al., 1999; Manzanero et al., 2000; Manzanero et al., 2002; Gernand et al., 2003; Oliver et al., 2013); barley (*Hordeum vulgare*) (Houben et al., 1999; Manzanero et al., 2002; Gernand et al., 2003); maize (*Zea mays*) (Kaszas and Cande, 2000); and wheat (*Triticum aestivum*) (Gernand et al., 2003); as well as on dicotyledons: beans (*Vicia faba*) (Houben et al., 1999; Manzanero et al., 2002) and arabidopsis (*Arabidopsis thaliana*) (Gernand et al., 2003; Oliver et al., 2013).

The first work on the analysis of the phH3Ser10 distribution in mitosis of rye (*S. cereale*), barley (*H. vulgare*), and beans (*V. faba*) was made in 1999 by the method of indirect immunofluorescence (immunofluorescence assay, IFA) using antibodies that recognize H3 histone phosphorylated at serine in position 10 (Houben et al., 1999). The immune localization signal was mainly detected in the pericentromeric region of the chromosome. The same type of results were obtained in maize (*Z. mays*) (Kaszas and Cande, 2000). The H3Ser10 phosphorylation was initiated in the prophase in the pericentromeric region of some chromosomes; a bright signal was observed in the pericentromeric region in the metaphase and anaphase in all chromosomes, which disappeared in the telophase (Kaszas and Cande, 2000).

The centromeric localization of phH3Ser10 immune fluorescence signals was also observed in wheat (*T. aestivum*), arabidopsis (*A. thaliana*), and barley (*H. vulgare*) (Gernand et al., 2003). However, according to Oliver et al. (2013), the H3Ser10 phosphorylation in the *A. thaliana* in the metaphase–anaphase occurred along the entire chromosomes (Oliver et al., 2013). The observed differences could be associated with the complexity of interpreting the data on the small chromosomes. However, the distribution of phosphorylation along the entire chromosomes in the mitosis anaphase was also detected in dividing BY-2 cells (Kawabe et al., 2005; Kurihara et al., 2006) and the protoplasts isolated from the tobacco leaves (*Nicotiana tabacum*) (Li et al., 2005). As in other cases, phosphorylation started in the prophase, and it was observed at the stages of metaphase and early anaphase only in the centromere region; however, it was distributed along the entire chromosomes in the late anaphase and gradually disappeared in the telophase (Kawabe et al., 2005; Li et al., 2005; Kurihara et al., 2006). The reproducibility of the IFA results in some works on tobacco excludes the method's error. It is possible that such a phosphorylation pattern is species-specific; in addition, all three works were conducted not on the cells of the root apical meristem (as in most other works) but on protoplasts or certain cellular cultures (which also could be the reason behind such differences).

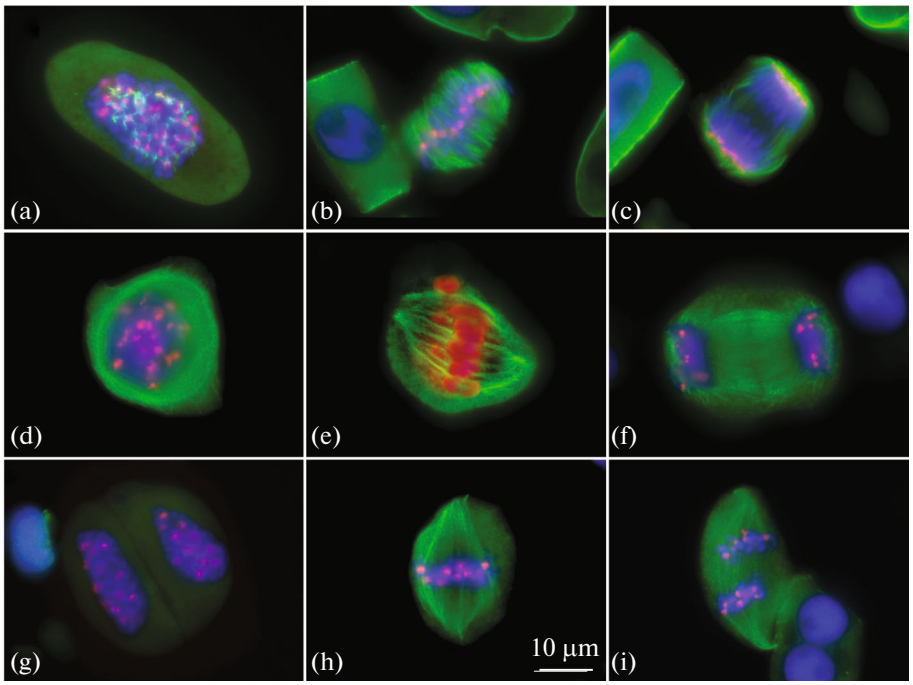

Fig. 1. H3Ser10 phosphorylation in mitosis (a–c) and meiosis (d–i) in *Triticum aestivum* (author's photo). (a) Prophase; (b) metaphase; (c) late anaphase; (d) prophase I; (e) metaphase I; (f) telophase I; (g) prophase II; (h) metaphase II; (i) anaphase II; the chromosomes are stained with DAPI (blue); immunofluorescence staining of microtubule on α-tubulin, green; immunofluorescence staining of H3Ser10, red; scale interval, 10 μm.

In species with large chromosomes, many authors registered the presence of the signal of immune localization of antibodies to phosphorylated H3Ser10 histone in the pericentromeric region and its absence directly in the place of primary constriction in mitosis (Houben et al., 1999; Fernandes et al., 2008; Marcon-Tavares et al., 2014).

The absence of phH3Ser10 in the core centromere region was clearly illustrated on the barley chromosomes by means of immunostaining with gold nanoparticles and scanning electron microscopy (Schroeder et al., 2003). This peculiarity can be associated with H3 histone substitution in the centromere region on its centromere-specific variant (CENH3), while the absence of such a gap in the localization of antibodies to phH3Ser10 in species with smaller chromosomes, may be due to the smaller size of the centromeric region (Houben et al., 1999; Marcon-Tavares et al., 2014).

Among vascular plants, the pericentromeric phH3Ser10 pattern was also found in other members of monocots (Pedrosa et al., 2001; Manzanero et al., 2002; Feitoza and Guerra, 2011; Marcon-Tavares et al., 2014) and dicotyledons (Fernandes et al., 2008; Marcon-Tavares et al., 2014), as well as in fern-like plants, lycopsids, and gymnosperms (Marcon-Tavares et al., 2014). We note that the correlation of phH3Ser10 with the presence of centromeric tandem repeat Sat1 (present both in the centromere and in the intercalary heterochromatin region (in smaller amounts) was demonstrated in the plants of ornithogalum (*Ornithogalum longibracteatum*) from the *Asparagaceae* family (Pedrosa et al., 2001).

Phosphorylation in Species with Holocentric Chromosomes and in Mosses

Among higher plants, the members of the species—*Chionographis japonica* Maxim. from the Liliaceae family and nutmeg (*Myristica fragnans* Houtt.) from the Myristicaceae family; one of the *Cuscuta* subgenus from the Convolvulaceae family; and the *Drosera* genus (but not other Droseraceae); as well as the members of two families (Juncaceae and Cyperaceae)—have holocentric chromosomes (Guerra et al., 2006), whose behavior and structure in mitosis significantly differs from the monocentric ones. The presence of primary constriction in prometaphase and metaphase, attachment of the division spindle microtubules along the entire chromosomes, and parallel divergence of the sister chromatids in anaphase are the main peculiarities of holocentric chromosomes (Germand et al., 2003; Guerra et al., 2006).

Differences in the structure of the mitotic chromosomes resulted in the difference in the H3Ser10 phosphorylation pattern (Germand et al., 2003; Guerra et al., 2006). In the *Luzula luzuloides* (the Juncaceae family), the phH3Ser10 immunofluorescence signals

became visible with the beginning of the chromosome condensation at the early prophase stages. A stable signal was observed along the entire chromosome in the meta- and anaphase. The fluorescence disappeared with the chromosome decondensation in the telophase (Germand et al., 2003). Similar results were obtained for the *Rhynchospora tenuis* (the Cyperaceae family) plants (Guerra et al., 2006). Marcon-Tavares et al. (2014) also observed phosphorylation of mitotic chromosomes along the entire length in other members of the Cyperaceae family, namely, in another *Rhynchospora pubera* species, *Eleocharis geniculata*, and *Fimbristylis miliacea*.

Recent studies in this area demonstrated that phosphorylation along the entire length of the chromosome in mitosis among the higher plants is also observed in mosses (bryophytes) (in addition to the plants with holocentric chromosomes) (Marcon-Tavares et al., 2014). In the *Notothylas vitalii* plants (the Notothylaceae family) and *Riccia plano-biconvexa* (the Ricciineae family) plants, the fluorescent signal became visible in the prophase diffusely along the entire chromosome, reached the maximum at metaphase, and disappeared in the early telophase. In the *N. vitalii* and *R. plano-biconvexa*, no primary constrictions were observed; however, the V- and I-shape chromosomes in the anaphase allowed us to assume that they are typical monocentrics. The authors assumed that the character of chromosome phosphorylation in mitosis in bryophytes is closer to phosphorylation of the animal chromosomes than the phylogenetically isolated holocentric plant chromosomes. It was also noted in the work that the acquisition of compensatory modifications due to the need to adapt to the environment was a possible reason for the absence of phH3Ser10 on the chromosome arms in vascular plants with monocentric chromosomes (Marcon-Tavares et al., 2014).

H3 HISTONE PHOSPHORYLATION IN MEIOSIS

H3 Histone Phosphorylation in Normal Plants

Phosphorylation of H3Ser10 histone in meiosis in plants significantly differs from those in animal meiosis or in plant mitosis (in case of first division) (Manzanero et al., 2000). In rye (*S. cereale*) and wheat (*T. aestivum*), the first diffuse phH3Ser10 signals were observed during the transfer from leptotene to zygotene. In diakinesis, the immune localization of antibodies to phH3Ser10 was observed along the entire chromosomes; however, a more intensive signal was registered in the centromere region. The peak of phosphorylation along the chromosome accounted for metaphase I; and phosphorylation gradually disappeared in telophase I. The chromosome condensation at the beginning of prophase II coincided with the new stage of H3Ser10 phosphorylation beginning in some areas. Bright signals of immune localization were observed in the centromeric region at the end of prophase II and in metaphase II, while the chromatid arms were very poorly labeled. Phosphorylation in the centromere region was maintained after the separation of sister chromatids in anaphase II, and it gradually disappeared in telophase II, coinciding with the beginning of the decondensation of the chromosome (Manzanero et al., 2000). Similar data were obtained on maize (*Z. mays*) (Kaszas and Cande, 2000). Differences from rye (*S. cereale*) and wheat (*T. aestivum*) consisted in the fact that two signals on nucleolus-forming regions were present in maize in prophase I at the leptotene and diakinesis stages, while only one signal was present in pachytene (when the chromosomes were completely paired). The signal distribution along the entire length of the chromosomes in prometaphase coincided with the destruction of the nuclear envelope. Phosphorylation in the second division in maize completely disappeared by the beginning of telophase II (as opposed to rye and wheat).

H3 Histone Phosphorylation in Plants with Univalent Chromosomes

The character of h3Ser10 phosphorylation on univalent chromosomes was studied on the example of the rye meiosis (*S. cereale*), whose genome contains additional B-chromosomes, and haploid wheat (*T. aestivum*), as well as the monosomic supplemented wheat line, to which genome long arm of the rye 5RL chromosome was added (Manzanero et al., 2000). The total picture of the phH3Ser10 signal distribution in the first meiotic division corresponded to H3 histone phosphorylation in the rye plants without B-chromosomes (Manzanero et al., 2000). The rye B-chromosomes and wheat univalents were phosphorylated along the entire length of the chromosomes on a par with bivalents in metaphase I and anaphase I. Differences in the phosphorylation pattern became obvious in the metaphase–anaphase of the second division. Single chromatids located separately from other chromosomes in metaphase II or lingering in the equational plane in anaphase II had no fluorescent phH3Ser10 signals in the pericentromeric region, but were attached to the division spindle microtubules.

Peculiarities of H3 Histone Phosphorylation in Cestrum strigilatum Carrying the Single B-Chromosome

To date, pericentromeric (as opposed to phosphorylation along the entire length of the chromosomes in other species) phH3Ser10 localization in the first meiosis division was demonstrated for the single plant species (*Cestrum strigilatum*). The *C. strigilatum* is a dicotyledon relating to the Solanaceae family and is characterized by the presence of B-chromosomes in

the genome (Fernandes et al., 2008). In the studied plants, the presence of the univalent B-chromosome (always located separately from A chromosomes) was observed in 23% of the meiocytes. The B-chromosome delayed on the equator in the first division and divided on the chromatids in the second meiotic division. Both in the first and second meiotic divisions, phosphorylation of the A-chromosomes occurred only in the pericentromeric region and was detected starting from the pachytene up to the late anaphase I (completely disappearing in telophase I). Then the signal detection was reproduced starting from prophase II up to the early telophase II. The temporal distribution of phosphorylation on B-chromosomes in the first division did not differ from those in the A-chromosomes; however, it occurred along the entire length except for the terminal region of the long arm. No fluorescent signal on the diverged B-chromatids was observed in the second division. The authors explained the difference of the meiotic phosphorylation of the *C. strigilatum* H3Ser10 from the other species described in the literature by their specific peculiarities and assumed that it may have been associated with the motility of the chromosomes but not with condensation or cohesion (Fernandes et al., 2008). Although the authors made conclusions about the difference of the phosphorylation pattern in the A- and B-chromosomes, the size of the antibody localization sites was almost the same. It is possible that the phosphorylated pericentromeric B-chromosome region occupies almost all its length, especially since the size of the blocks of the immune localization of the antibodies to phH3Ser10 in vascular plants is relatively stable between the chromosomes within the karyotype even in species with a significantly different morphology of the chromosomes, for example, telocentric and metacentric in the *Nothoscordum pulchellum* or with a large difference in the chromosome size, as in the *Cipura paludosa* (Marcon-Tavares et al., 2014). In the *Eleutherine bulbosa*, the largest chromosome is five times larger than the small chromosomes; however, they all demonstrated pericentromeric fluorescently stained phH3Ser10 blocks that were similar in size (Feitoza and Guerra, 2011).

CASES OF CONTRADICTORY DATA ON H3 HISTONE PHOSPHORYLATION IN MITOSIS AND MEIOSIS

For plants with a small genome, the immunofluorescence analysis is complicated, since it is difficult to obtain a clear pattern of the signal distribution with a small size of the chromosomes (Gernand et al., 2003; Oliver et al., 2013; Paula et al., 2013). As a result, the data on the phosphorylation pattern are different for the same objects (Gernand et al., 2003; Oliver et al., 2013) or the data of illustrations and material text support are difficult to compare (Paula et al., 2013). Thus, the first distinguishable signals of the localization of antibodies to phH3Ser10 in the arabidopsis (*A. thaliana*) mitosis appeared with the beginning of the chromosome condensation in the early prophase. In the metaphase and anaphase, bright signals were clearly distinguishable in the pericentromeric region of the chromosomes and disappeared with the beginning of their decondensation in the telophase (Gernand et al., 2003). However, according to Oliver et al. (2013), phosphorylation started not with the beginning of the chromosome condensation but in the middle–end of the prophase. By metaphase, it spread on the chromosome arms and disappeared at the end of the telophase. In addition, differences from the already known data on other plants were observed in the *A. thaliana* meiosis (Manzanero et al., 2010). The H3ser10 phosphorylation started in diplotene in the pericentromeric region (but not during the transfer from leptotene to zygotene as in maize, Manzanero et al., 2000). The phH3Ser10 distribution on the chromosome arms in the second meiotic division was the next surprise; i.e., the phosphorylation pattern was similar to those observed in the first meiotic division (Oliver et al., 2013).

Another phosphorylation pattern was described in the meiosis of Brachiaria (*Brachiaria ruziziensis*, *B. decumbens*, and *B. brizantha*) (the Gramineae family) (Paula et al., 2013). In all the studied plants, the H3 phosphorylation during the first division was distributed on the whole chromosomes and disappeared in telophase I. In spite of the fact that the authors themselves described the phosphorylation pattern in the second meiotic division as pericentromeric, the fluorescent signal distribution on the illustrations to the article coincides with the contours of the DAPI-stained chromosomes (that is, it goes along the entire length of the chromosomes) (Paula et al., 2013). The interpretation error is possible due to the small size of the compacted meiotic chromosomes in the second division.

Inconsistencies in the phosphorylation pattern can also be noted in the works with plants with large chromosomes (for example, with beans) (*V. faba*) (Houben et al., 1999; Demidov et al., 2005). Thus, a weak staining of the chromosome arms and a bright signal in the pericentromeric region in the mitosis metaphase–anaphase were registered (Houben et al., 1999). A strict pericentromeric localization of fluorescent signal on the acrocentric chromosome and the absence of such on the chromosome arms were demonstrated in a later work conducted on the same object (Demidov et al., 2005). The observed difference could be associated with peculiarities of the used method. The polyclonal antibodies were used for indirect IFA in both cases, while the fluorescent signal was detected by epifluorescence microscopy (EfM). Both these factors have an error associated with the signal specificity and optical resolution. The polyclonal antibodies have a number of peculiarities, promoting the

appearance of a background (nonspecific) signal (following from the production method). The polyclonal antibody serum consists of the products of the secretion of several B-lymphocyte clones that have a different specificity (antibodies to different antigen binding sites and even to different antigens), affinity to the bound antigens, etc. (Sveshnikov et al., 2006). In addition, the optical resolution of the chromosomes analyzed by means of the usual EfM microscopy is limited due to the large volume of cellular preparations.

Differences in the H3Ser10 phosphorylation pattern were registered during the analysis of the mitotic preparations of the chromosomes of rye (*S. cereale*) (Houben et al., 1999). The authors associated the differences in the fluorescent signal distribution with the use of different methods of data fixation, namely, the usual epifluorescence microscopy (EfM) and computational deconvolution microscopy (CDM). In the case of using EfM, a weak signal on the chromosome arms and brighter signal in the pericentromeric region were observed. However, when using CDM, bright signals were observed in the pericentromeric region with single point signals in the region of the chromosome arms. However, the authors noted that deconvolution microscopy tends to increase the bright signals and decrease or even remove the weak signals (Houben et al., 1999; Wallace et al., 2012).

FUNCTIONAL SIGNIFICANCE OF PHOSPHORYLATION IN PLANT CELL DIVISION

H3Ser10 Histone Phosphorylation Is Correlated to the Cohesion of the Sister Chromatids

Although the H3Ser10 phosphorylation is correlated to chromosome condensation in mitosis in animals (Hendzel et al., 1997), as well as in mitosis and meiosis in lower eukaryotes (for example, infusorians) (Wei et al., 1999), the causal relationship between them is still unclear. It was assumed that the phosphorylated H3 histone in animals attracts topoisomerase 2 to the chromosome (Hendzel et al., 1997). However, it was not so for meiosis. In the meiosis of mice, the localization of topoisomerase 2 on the chromosomes and their condensation started in the early prophase, while phosphorylation was observed in the late prophase I (Cobb et al., 1999).

Similar to the animals, the possible involvement of phosphorylation in the condensation of the chromosome was speculated in the first works on plants (Houben et al., 1999), since the beginning of phosphorylation coincided with the beginning of condensation and disappeared with the decondensation of the chromosome. However, it was demonstrated in the later works that phosphorylation is rather associated with the cohesion of the sister chromatids in mitosis and meiosis, but not with the degree of their condensation (Kaszas and Cande, 2000; Manzanero et al., 2000; Manzanero et al., 2002; Brasileiro-Vidal et al., 2005; Feitoza and Guerra, 2011). A number of data indicate in favor of this assumption: (1) there are no signals of the immune localization of antibodies to phH3Ser10 on normally condensed delayed chromatids and chromosome fragments in the mitosis of hybrids with an unstable genome (Brasileiro-Vidal et al., 2005), as well as on single chromatids in the second meiotic division of haploid wheat plants and rye B-chromatids (Manzanero, 2000); (2) for the *E. bulbosa* plants (for which a clearly determined early condensation of the first chromosome pair in the prophase is known), neither premature nor more intensive phosphorylation of these chromosomes was observed (Feitoza and Guerra, 2011); and (3) the beginning of the chromosomes' phosphorylation does not coincide with the beginning of their condensation in the first meiotic division in maize. The clear phH3Ser10 signals at all chromosomes were only observed during the transition from diakinesis to prometaphase, when the chromosomes were already completely condensed (Kaszas and Cande, 2000).

The absence of the association of phH3Ser10 with the chromosome condensation was established in barley and rye (Manzanero et al., 2002). The use of phosphatase inhibitors (cantharidin and okadaic acid) did not have a strong effect on the H3 histone phosphorylation and did not result in premature chromosome condensation in the interphase (Manzanero et al., 2002). The decrease in the length of the condensed chromosomes hyper-phosphorylated along the entire length (observed in mitosis) was comparable with the shortening of the chromosomes caused by the cold treatment, during which additional phosphorylation sites appeared only in some arm regions but not along the entire length of the chromosomes (Manzanero et al., 2002). These data differ from the results obtained on animals (Guo et al., 1995). The phosphorylation of the H3 histone and the complete chromosome condensation were already observed at the G2 stage during the treatment of cancer cells from a mouse culture by okadaic acid and fostriecin (inhibitors of PP1 and PP2A phosphatases) (Guo et al., 1995).

In addition, the H3Ser10 phosphorylation, which started in the pericentromeric regions and spread to the chromosome arms in metaphase I, and was observed only in the pericentromeric regions in metaphase II of the meiocytes, is consistent with the changes in the cohesion of the sister chromatids but not with the condensation of the chromosome (Kaszas and Cande, 2000; Manzanero et al., 2000). However, the phosphorylation of the H3 histone cannot be completely responsible for the cohesion of the chromatid, since the interconnection between them is not complete: both processes do not start and finish simultaneously. For example, the sister chromatids are separated in the mitosis anaphase, while phosphorylation

disappears only in the telophase. The cohesion of the sister chromatids is also observed in the leptotene of the first meiotic division; however, it can change in a zygotene and is almost completely absent by the beginning of anaphase I (Cai et al., 2003). For the same reason, phosphorylation cannot be responsible for the initiation of the cohesion (Kaszas and Cande, 2000). According to Kaszas and Cande (2000), phosphorylation can be involved in regulating or maintaining cohesion in the centromeric regions. During the study of the *afd1* (*absence of first division 1*, the gene encoding the meiosis-specific Rec8 cohesin), in the first meiotic division of which 20 univalents are generated, the authors registered the separation of the sister chromatids in anaphase I (as well as during the mitotic division). When the chromosomes generate a metaphase plate, phosphorylation is observed only in the pericentromeric regions, while the signal of the immune localization of antibodies to the phosphorylated H3 histone is absent from the individual sister chromatids in the second meiotic division (Kaszas and Cande, 2000). The involvement of phosphorylation in the cohesion of the centromeric regions is also confirmed by the work on *C. strigilatum*, for which the authors demonstrated the pericentromeric localization of the immunofluorescence signal of the antibodies to phH3Ser10 both in mitosis and in the two meiotic divisions (Fernandes et al., 2008).

phH3Ser10, Epigenetic Marker of an Active Centromere

The association of H3Ser10 phosphorylation with the activity of centromeres was registered in several works on the study of semidicentric (Houben et al., 1999) and dicentric chromosomes (Han et al., 2006; 2009; Gao et al., 2011; Zhang et al., 2013). Only a single active centromere is present in the studied semidicentric and dicentric chromosomes, in spite of the presence of traditional sequences of centromeric DNA in both centromeres (Ty/gypsy barley retro-elements or 156 bp satellite tandem repeats and retrotransposons of CRM maize). Semidicentric 5^3 chromosomes of T3-5u line barley were generated during reciprocal translocation with the breaking point in the region of the chromosome 3 centromere and chromosome 5 arm. The absence of violations in mitosis indicated the activity of only one centromere. Only the chromosome 5 centromere, which generates a clearer primary constriction rather than a partial constriction (with a low number of copies of centromeric sequences) than the chromosome 3 centromere, had a clear localization signal of the antibodies to the phosphorylated H3 histone. In addition, only 14 signals corresponding to the number of active centromeres in the cell were in the mitotic prophase (Houben et al., 1999).

Dicentric T1-5 maize (*Z. mays*) chromosomes were obtained as a result of the translocation between A-chromosomes 1 and 5 (with the capture of the centromere between the breaking points) (Gao et al., 2011). The authors demonstrated that the presence of the CENP-C and phH3Ser10 signals was observed only in the place of the primary constriction corresponding to the active centromere. The second cluster of centromeric sequences had no localization signals of the antibodies to CENP-C and phH3Ser10 (Gao et al., 2011). The maize Dic-15 (Han et al., 2006, 2009) and sDic-15 (Zhang et al., 2013) chromosomes contain two centromeres of B-chromosomes, and only one of them is active and is phosphorylated at the H3Ser10 histone. The activity of this centromere was confirmed by the presence of the localization signal of the antibodies to CENH3, as well as other modifications peculiar to the active centromere, such as phH2AThr133 and phH3Thr3 (Zhang et al., 2013). Regardless of whether the inactive centromeres originated from the A- or B-chromosomes, the histone phosphorylation occurred only at the active centromeres.

However, Manzanero et al. (2000) observed the presence of delayed single chromatids without the immunofluorescence phH3Ser10 signal in the centromere region (associated with the spindle microtubules) in the anaphase of the second meiotic division in the haploid and 5RL monosomic supplemented wheat (*T. aestivum*) lines. Similar results were also obtained for the delayed B-chromatids in the second meiotic division in rye plants (Manzanero et al., 2000). According to the author's conclusions, phosphorylation did not determine the ability of the kinetochores to bind to the spindle micrortubules.

Both the cohesion and functional activity of the centromeric regions are directed to segragate the chromosome in the first meiotic division and sister chromatids in mitosis and meiosis II. The association of phosphorylation with the segregation of the centromeres in mitosis was demonstrated during the analysis of intergenic wheat–wheatgrass hybrids (Brasileiro-Vidal et al., 2005). Brasileiro-Vidal et al. (2005) observed a deviation in H3Ser10 phosphorylation in the cells with different anomalies in descendants of the 5th and 7th generations from the self-pollination of twice backcrossed wheat–wheatgrass hybrids (*T. aestivum* × *Thinopyrum ponticum*). The H3 histone phosphorylation in the mitosis of the *T. aestivum* × *Th. ponticum* hybrids started in the prophase; in the metaphase, the signal was seen at all chromosomes and centric chromosome fragments in the region of the pericentromeric heterochromatin. The pericentromeric regions were still strongly phosphorylated at the H3 histone in the anaphase; however, the signal was not observed in some cells on the chromatids delayed in the equational plane. Phosphorylation completely disappeared in the telophase. Based on the data obtained, the authors assumed that the absence of the H3 histone phosphorylation is associated with the functional violations of the centromeric regions

(reflected on the segregation of the chromatids in the anaphase) (Brasileiro-Vidal et al., 2005).

ROLE OF KINASES IN REGULATING H3 HISTONE PHOSPHORYLATION

In contrast to the detailed analysis of the candidates of kinases and other proteins associated with the H3 histone modifications in animals, there are only a few reports concerning plants. To date, only three conservative kinases, Haspin, Aurora (including AtAurora 1, 2, and 3), and BRK1, were cloned in plants (Wang et al., 2012; Zhang et al., 2014). However, the association with phH3Ser10 was only demonstrated for the Aurora and BRK1 kinases. The arabidopsis (*A. thaliana*) Haspin-kinase phosphorylates of the H3 histone at Thr3 and Thr11 (Kurihara et al., 2011).

For the *A. thaliana*, three genes of Ser/Thr-protein kinases from the Aurora family (*AtAurora1*, *AtAurora2*, and *AtAurora3*) are described in the literature; they are actively transcribed in tissues containing many dividing cells (Demidov et al., 2005; Kawabe et al., 2005). A specific localization of recombinant AtAurora proteins labeled by GFP fluorochrome was observed on the dynamical mitotic structures, spindle microtubules, and centromeres, as well as on the developed cellular plate in the dividing tobacco (*N. tabacum*) BY-2 cells (Demidov et al., 2005). By means of IFA, the coincidence of the localization signals of the antibodies to AtAurora 1 with phH3Ser10 was established in the centromeres of the metaphase chromosomes in the *V. faba* plants (with large chromosomes). The signal was located in the pericentromeric regions between the kinetochores of the homologous chromosomes; however, phosphorylation captured a small region of the chromosome arms, where the Aurora 1 kinase was not detected (Demidov et al., 2005). Centromeric localization was demonstrated for the AtAUR3 starting from the mitosis prophase up to the early anaphase in the BY-2 tobacco cells. The localization signal of the antibodies to AtAUR3 in the late anaphase was observed along the entire length of the chromosomes, which corresponded to the mitotic pattern of the distribution of the H3Ser10 phosphorylation (Kawabe et al., 2005).

For plants, the association of Aurora kinases with H3Ser10 phosphorylation was demonstrated both in vitro (Demidov et al., 2005; Kawabe et al., 2005) and in vivo (Kurihara et al., 2006). During the analysis of the AtAurora 1 kinase activity in vitro, Demidov et al. (2005) observed the preferential phosphorylation of the H3 histone at Ser10 but not at Ser28 or Thr3, 11, or 32. In addition to AtAUR1, AtAUR2 and AtAUR3 also showed kinase activity relative to the H3Ser10 phosphorylation in vitro in the arabidopsis plants (Kawabe et al., 2005). During the treatment of the BY-2 tobacco cells with hesperadin (which inhibits *A. thaliana* Aurora-kinase 3 (AtAUR3)), a decrease in the level of phH3Ser10 and phH3Ser28, as well as the anomalous segregation of the mitotic chromosomes were found (Kurihara et al., 2006). In the rice plants (*Oryza sativa*), the Bub1-related kinase 1 (BRK1) is presumably required for the normal localization of Aurora kinase before the beginning of metaphase I (Wang et al., 2012). The authors made this conclusion based on two characteristics of meiosis in the *brk-1* rice mutants: phH3Ser10 is absent in the centromere region during diakinesis, while the bipolar tension between the kinetochores of homologous chromosomes significantly decreases in metaphase I. Since the normal functioning of Aurora kinase is required both in the first and in the second cases, the inactivation of the BRK1 kinase probably induces the anomalous localization of Aurora kinase before the beginning of metaphase I.

As well as phosphorylation, dephosphorylation plays a large role in the cell cycle. Dephosphorylation of the H3 histone in plants is associated with analogs of the PP2A phosphatase (dephosphorylates cohesin combined in the SGO–PP2A complex) and the PP1 phosphatase in animals. In the barley (*H. vulgare*), bean (*V. faba*), and rye (*S. cereale*) plants, a high level of phH3Ser10 along the chromosome arms was detected in mitosis after the plants' treatment with cantharidin, which is a specific inhibitor of phosphatases such as PP2A and PP1 (Manzanero et al., 2002). The distribution of the phH3Ser10 signal during the treatment with cantharidin was similar to the chromosome phosphorylation pattern in the first meiotic division in plants; however, the absence of dephosphorylation did not result in serious violations of the cell cycle, since the cells with normal anaphases and telophases were observed among the analyzed cells (Manzanero et al., 2002).

The existence of the association of PP2A with phH3Ser10 dephosphorylation was proven for the *A. thaliana*. In addition, it was established for the plants from this species that NAP-related proteins (NRPs) are potential regulators of phH3Ser10 dephosphorylation (Biro et al., 2012). NRPs are plant orthologs of the SET/I_2^{PP2A} protein, which is an inhibitor of the PP2A-mediated phH3Ser10 dephosphorylation in animal cells. It was demonstrated that the arabidopsis NRP1 inhibits the activity of immunoprecipitated PP2A in vitro and interacts with the catalytic PP2A subunit in vivo (Biro et al., 2012). Based on these data, it is possible to assume that the dynamics of H3Ser10 phosphorylation in the plants' mitosis is associated with the BRK1-mediated activity of Aurora kinase and the NRP-controlled activity of PP2A phosphatase.

The H3Ser10 histone phosphorylation in plants is directly associated with the process of cell division, since it is not observed in the interphase, but starts in the prophase, reaches a maximum in metaphase, and disappears both in mitosis and in both meiotic divi-

sions. Small differences in the time when phosphorylation begins (early or late prophase), as well as in the activity of dephosphorylation (anaphase–telophase), can be observed. The phosphorylation of the pericentromeric regions is typical for mitosis and the second meiotic division, and it is typical along the entire length of the chromosomes for the first meiotic division. However, there are several exceptions to this regularity. Thus, the distribution of H3Ser10 phosphorylation over the entire length of the chromosomes was registered in the plants with holocentric chromosomes and mosses, as well as in some works on arabidopsis and tobacco. In addition, phH3Ser10 in the *C. strigilatum* was only observed in the pericentromeric region both in the first and second meiotic divisions.

In spite of the significant differences in the pattern and insignificant differences in the dynamics of the phosphorylation of the chromosome, it is possible to assume that H3Ser10 phosphorylation for most plants is tightly associated with the cohesion and functional activity of the centromere that, together, are required for the correct chromosome segregation in mitosis and meiosis. The data accumulated to date allow us to assume that the H3Ser10 phosphorylation is associated with the segregation of the centromeres in homologs in the first meiotic division and the sister centromeres in mitosis and the second meiotic division. The absence of phH3Ser10 signals in the second meiotic division on delayed rye B-chromatids and single chromatids of haploid wheat confirms this assumption, since the chromosome separated in meiosis I. In addition, the association of the segregation of the sister centromeres in mitosis with Aurora-dependent H3Ser10 phosphorylation was established for plants.

The presence of differing data relative to the H3Ser10 phosphorylation on the chromosome arms allows us to suggest that the function of this modification can differ in different plant species. Phosphorylation of the chromosome arms in meiosis is most probably associated with the cohesion of the sister chromatids required for the correct separation of the homologous chromosomes. The distribution of phH3Ser10 on the chromosome arms (observed in the species with holocentric chromosomes) is associated with their morphological peculiarities and refers to the phosphorylation of the pericentromeric regions. It is possible that the H3Ser10 phosphorylation in mosses and some species of vascular plants preserved the association with the chromosome condensation both in animals and infusorians. However, further studies in this area are required in order to confirm this assumption. In addition, in spite of the large number of works associated with the study of H3Ser10 phosphorylation, very little is known to date about the proteins responsible for the regulation of its dynamics in the plants (which also requires further studies).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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