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20 Histone modifications in plant chromosomes

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Post-translational modifications of histone proteins

In eukaryotes, genome is packed into chromosomes in a cell. Chromosome is a higher-ordered structure of DNA and proteins, and its fundamental unit is a nucleosome consisting of a histone core with 146bp DNA wrapping around it. Two heterodimers of H2A/H2B and a tetramer of H3/H4 form the core octamer and a N-terminal tail of each histone protrudes from the core [1]. The eight protruding tails are subjected to post-translational modifications at specific amino acid residues such as acetylation, phosphorylation, methylation etc. (Figure 1), which modulate various cellular functions.

The one of the major and well-documented modifications is histone acetylation occurring at N-terminal lysine residues of core histones. Throughout eukaryotes including plants, acetylations of H3 and H4 are mainly studied to date. The acetylation sites of

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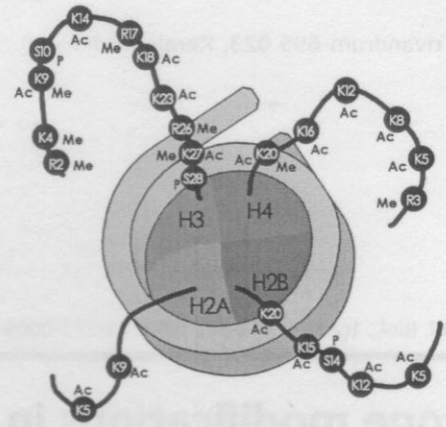

Figure 1. Modification sites of the four core histones. Ac: acetylation, Me: methylation, P: phosphorylation.

H3 are K4, K9, K14 and K18, and the sites of H4 are K5, K8, K12 and K16 [1]. In addition, plant specific acetylation was reported in H4K20 [2]. Before discovering histone acetyltransferases, histone acetylation was known to regulate transcription and to allow accessibility of a transcription factor to DNA [3]. It is also known that acetylation increases sensitivity of DNase I [4] and prevents full-condensation of chromatin fiber [5,6]. Thus it has been thought that the histone acetylation regulates transcription by relaxing interaction between DNA fibers and nucleosome core. Crystal structure of the nucleosome core revealed interaction between basic side chains of N-terminal tail of H4 (K16, R19, K20, R23) and acidic side chains of globular domain of H2A and H2B [7]. Acetylation of H4K16 and H4K20 might affect nucleosome structure itself.

A series of specific antisera that recognizes specifically acetylated lysine was constructed [8]. By using these antisera, lysine residue-specific acetylation and its functions have been revealed. For example, hyperacetylation of H4K16 was observed in a male X chromosome in *Drosophila* where up-regulation of transcription occurs [9]. Now histone acetylation is known to play important roles not only in transcription but also in chromatin assembly at mitotic S phase [10] and DNA repair [11]. Moreover, it participates chromosomal structure itself as described later.

Histone methylation occurs at lysines and arginines in N-terminal tail of H3 at K4 and K9, and of H4 at R3 and K20, etc. The methylation of H3K9 is one of the most investigated modifications because it correlates transcriptionally silenced domain [12], and methylated H3K9 is targeted to bind with HP1 protein found in heterochromatin [13,14]. In contrary, methylation of H3K4 is occurred at the euchromatic region [12,15]. Methylated H4R3 is recognized by Esa1 acetyltransferase that acetylates H4K5 for transcription [16]. As described above, histone methylation plays apparently contradictory roles for activation and suppression in transcription by residue-specific manner.

Serine residues in N-terminal tails are subjected to phosphorylation. Phosphorylation of H3S10 that is observed at pericentromeric region of mitotic condensed chromosome at mitosis is well studied [17,18], and is also involved in transcriptional activities. The

other phosphorylation sites were reported in H3S28 [19] and H2BS14 [20]. Phosphorylation of H3S10 plays roles according to different modifications in neighboring amino acids. Phosphorylation of H3S10 is antagonistic against methylation of H3K9 [21] whereas synergistic with acetylation of H3K14 [22,23].

Histone code

It has been suggested that certain combinations of covalent modifications of histone tails work as epigenetic code, that is referred to as 'histone code', for regulating interaction with DNA strands, chromatin-associated proteins, or protein complexes, which in turn control chromatin functions [24,25]. There are many enzymes modifying specific amino acid residue(s) of histone tails; acetyltransferases for acetylation [26], deacetylases for deacetylation [27], kinases for phosphorylation [28] and methyltransferases for methylation [29]. Histone acetyltransferases (HATs) are found in both cytoplasm and nucleus. The cytoplasmic HATs are related to chromatin deposition of newly synthesized histones in yeast, mammals and also in maize [30,31,32]. The nuclear HATs can be classified into some families depending on their sequences. Some families such as GCN5/PCAF, $TAF_{II}250$ and CBP/p300 all function as transcriptional coactivator or transcription factor [26]. In contrast, the MYST family involves in dosage compensation of X chromosome in *Drosophila*, cell-cycle regulation and so on [26]. The acetylated histones are recognized by the bromodomain of the HATs [33,34]. In plants, two nuclear HATs were identified in maize [35,36].

Histone deacetylases (HDACs) has been categorized into RPD3, HDA1, SIR2 and HD2 families [27]. One of the RPD type histone deacetylases, mSin3A, is recruited to methylated cytosine through MeCP2 protein, and then functions for transcriptional suppression [37]. The RPD type deacetylases have been identified biochemically in maize and pea [35, 38]. The genes encoding RPD-homologues are also found in *Arabidopsis* [39]. The HD2 type deacetylase is plant specific [40,41] and forms multigene family within the plant kingdom [42,43].

Phosphorylation of H3 at S10 is regulated various types of kinases identified in mammals, yeast and insects but not in plant to date [28]. Histone methyltransferases specific for each methylation site have also been identified [29] and a H3K9 specific enzyme is found in *Arabidopsis* [44]. The methylated histones are recognized by proteins containing chromodomain, such as HP1 [45].

Histone acetylation in metaphase chromosomes

After construction of a series of antisera recognizing site-specific acetylation, acetylation pattern in a genome has been analyzed in various eukaryotes including plants. At first, metaphase chromosome has been targeted to study histone acetylation, because overall acetylation in the genome could be detected and specific chromosomal regions can be identified under a microscopy.

Histone H4 acetylation on metaphase chromosomes was detected in *Vicia faba* [46,47]. Nucleolar organizing region (NOR) showed strong acetylation at K5, K8, K12 and K16, on the other hand, heterochromatic regions were hypoacetylated at K5, K8 and K12. Similar situation was obtained in facultative heterochromatin in *Gagea lutea* (Liliaceae, 2n=72) that was characterized by a conspicuous depletion of H4 acetylation at K5, K8 and K12, but not K16 [48]. Euchromatic regions in *Silene* species displayed

strong H4 acetylation at K5, K8 and K12, whereas acetylation of H4K16 distributed evenly between euchromatin and heterochromatin [49]. Acetylation pattern is, however, different among different plant species. Acetylation of H4K16 was localized at euchromatic gene-rich region in barley [50]. Strong H4 acetylation at NORs was observed only at K5 and K12 in barley [50,51], and was not observed in *Silene* species, *Allium cepa* and *Nicotiana tabaccum* [49].

Some plants have B chromosome(s) that is supernumerary to the basic (A) chromosome set and transcriptionally inactive in most cases and acetylation of B chromosomes was also studied in *Brachycome dichromosomatica*. In the B chromosomes of *Brachycome*, acetylation at H4K5 and H4K12 were absent or reduced, whereas H4K12 and H4K16 were equally acetylated in both A and B chromosomes [52]. In mammals and *Drosophila*, sex chromosome specific hypo- or hyper-acetylation has been reported [9,53,54]. However, sex chromosomes of *Silene latiforia* showed similar pattern of H4 acetylation as in the autosomes [49].

Histone acetylation in interphase nuclei

An interphase nucleus has globular structure in nature. Three-dimensional microscopy is thus applied for analyzing histone acetylation patterns in barley nucleus [55]. Barley nucleus has a typical Rabl structure, and we could divide it into two hemispheres. One is a centrosphere containing a ring-like cluster of centromeres near the nuclear membrane, and the other is a telosphere having scattered telomeres and telomeric gene-rich regions. Strong acetylation of H4 at K16 was observed in the telosphere and acetylation at K5/K8/K12 was found in the centrosphere [50]. The more intact nucleus prepared without usual fixation procedures showed a ring-like cluster of maximum fourteen hyperacetylated regions (HARs) near the nuclear membrane [55]. Each HAR of H4K5 was resolved in detail by a deconvolution system and two strongly acetylated cores with surrounding less acetylated halo were detected in each HAR. Moreover, each acetylated halo was sandwiched by centromeric repetitive sequence with partial overlapping [56]. Similar ring-like formation of hyperacetylated region was observed in H4K8 and H4K12. Thus it is suggested that the hyperacetylation of H4K5/K8/K12 adjacent at the centromeric repeat sequences might involve in certain centromeric functions.

Histone acetylation on replication

Vicia faba nuclei were sorted into five fractions based on the cell cycle stages and were immunostained to reveal the degree of histone acetylation and deacetylation through the cell cycle [57]. Basically, euchromatin showed higher acetylation than that in heterochromatin, and the degree of H3 acetylation did not change throughout the cell cycle in both eu- and heterochromatins. The euchromatin was the most intensely acetylated for all acetylatable lysines of H4 during early and mid-S phase when replication occurred, whereas the heterochromatic domains became strongly acetylated during late S phase at K5, K12 and K16 of H4. Then the deacetylation in the heterochromatic domain occurred at K5 and K12 during G2, and at K16 gradually during G2 and M phases. As a result, they suggested that elevated acetylation of H4 would be correlated with replication rather than with the transcriptional activity.

Strong acetylation of H4 at K5 and K12 correlating with replication of heterochromatic domains was reported in mammals [58] and also in barley [59]. Chromatin deposition-related acetylation occurs at these two lysines in mammals and insects [10]. However, different results were reported in *Arabidopsis* where replication-linked acetylations were H4K16 and H3K18 [60]. Thus, strong acetylation of H4K5/K12 correlating with replication seems common in eukaryotes, although plant specific usage in acetylation of lysine residues may also exist.

Dynamic changes of histone acetylation during mitotic cell cycle

Chromosomes dramatically change their structure during mitotic M-phase. As nucleosome is a fundamental unit of the chromosomes, its modification should correlate large scale chromosome structure as well. Acetylation dynamics of histone H4 throughout mitotic cell cycle has been analyzed three-dimensionally and quantitatively using barley as the material [50,51]. Dynamics of specific combinations of acetylation is observed in three different chromosomal regions. Nucleolar organizing regions (NORs) are strongly acetylated at H4K5 from prometaphase until anaphase (Figure 2) and at H4K12 from prophase to anaphase. The NORs are not condensed even at metaphase forming the secondary constrictions under microscopic observation. Transcription of rRNAs starts at the telophase [61]. Thus, the pairwise acetylation of K5/K12 of H4 at the NORs should avoid usual condensation like the other chromosomal regions in preparation for the onset starting of transcription at telophase.

Centromeric regions where strongly acetylated at K5, K8 and K12 of H4 in interphase are gradually deacetylated toward metaphase, and re-acetylated again in or after telophase. Since specific inhibition of histone deacetylase using Trichostatin A compromised centromeric integrity and resulted in missegregation of chromosomes in yeast and mammals, M-phase specific deacetylation seems to be required for normal

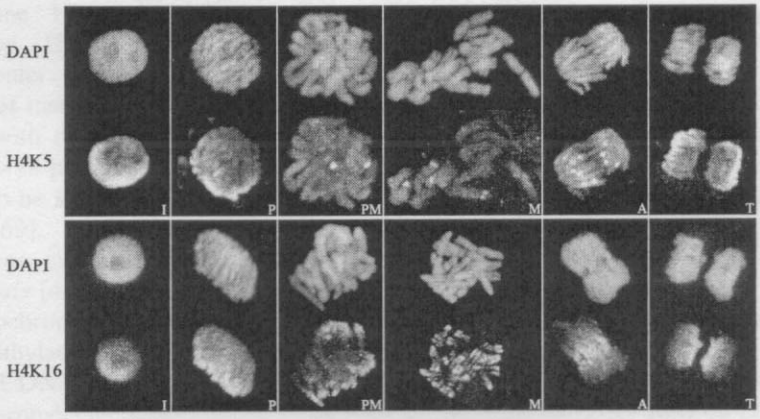

Figure 2. Stage-specific acetylation in barley chromosomes at H4K5 (upper panels) and H4K16 (lower panels). DAPI indicates counterstaining of chromosomes. H4K5 and H4K16 indicate immunostained signals of acetylated histone H4. I: interphase, P: prophase, PM: prometaphase, M: metaphase, A: anaphase, T: telophase. (Wako et al., 2002)

mitosis [62,63]. Specific deacetylation of centromeric region in barley could also play an essential role for the normal mitosis by maintaining the normal status or structure of centromeric regions. It is also worth noticing that the highly acetylated regions are not the regions of centromeric repeats [56].

Telomeric regions of barley chromosome show specific acetylation at H4K8 and H4K16 [50,51]. Acetylation of H4K16 at the telomeric regions is constantly higher than the other chromosomal regions (Figure 2), and the level of acetylation becomes minimum at metaphase when chromosomes condense the most. Strong acetylation of H4K8 occurs only at metaphase and anaphase. Because the telomeric regions of barley correspond to gene-rich regions [64], constant and high acetylation of H4K16 may involve in transcriptional activity. Stage-specific acetylation of H4K8 when the acetylation of H4K16 is the minimum, may compensate the decrease of K16 acetylation [51].

Dynamics of H4 acetylation during mitotic cycle is summarized in Figure 3. Three chromosomal regions, NORs, telomeric and centromeric regions show specific combinations of acetylated lysines. A pairwise combination H4K5/K12 involves in NORs and centromeric region, and an additional acetylation of H4K8 occurs at the centromeric region. Another pairwise acetylation of H4K8/K16 occurs at telomeric

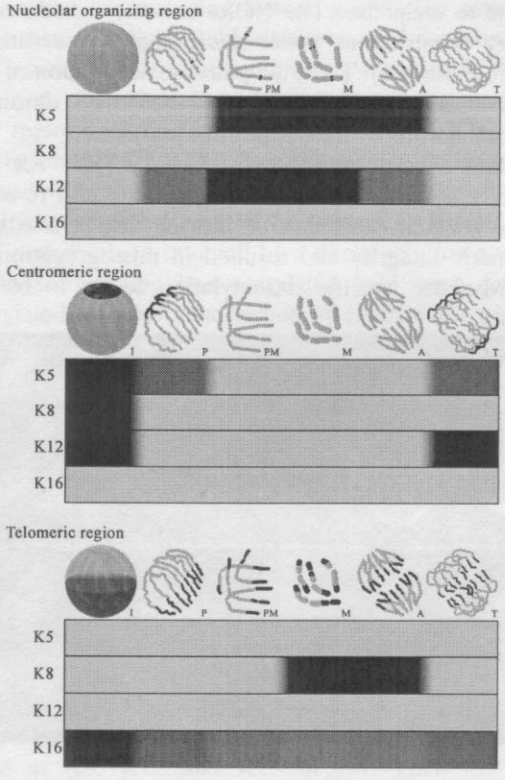

Figure 3. Dynamics of histone modification during mitotic cycle. Black and light gray periods indicate high level and basal level of acetylation, respectively. I: interphase, P: prophase, PM: prometaphase, M: metaphase, A: anaphase, T: telophase.

region. These pairwise combinations of H4 acetylation should have specific roles for the chromosome structure by stage- and region-specific manner.

Histone H3 phosphorylation during mitosis and meiosis

Phosphorylation of histone H3 at S10 is known to correlate with chromosome condensation in many eukaryotes. The phosphorylation in plants has been studied in rye, barley and *V. faba* [65]. The plant mitotic cells immunolabeled with an antibody recognizing phosphorylated histone H3 at S10 show strong signals at pericentromeric heterochromatic regions, whereas interphase nuclei show no phosphorylated signals. Correlation between H3 phosphorylation and centromere integrity is also examined in this study. A barley translocation line with semi-dicentric chromosomes bearing a partial and inactive additional centromere is used. Only the active centromere is immunolabeled, and the additional inactive centromere is not phosphorylated [65].

Phosphorylation of H3 during meiosis was also studied in meiotic chromosomes of wheat and rye [66]. Phosphorylated histone H3 was detectable during the transition from leptotene to zygotene. At metaphase I, entire chromosomes were strongly phosphorylated not only in bivalents of rye and wheat, but also rye B chromosomes and wheat univalents. Then the phosphorylation of H3 gradually disappeared at telophase I. During the second meiotic division, phosphorylation of H3 was restricted to pericentromeric regions as in mitosis.

It has been reported that H3 phosphorylation is not required for the chromosome condensation in mammals and *Tetrahymena* [18, 67]. It was also suggested that H3 phosphorylation alone was not sufficient for maintaining chromosome condensation in plants, because the phosphorylation of H3S10 disappeared at interkinesis in plants when the degree of chromatin condensation was higher than that at mitotic interphase [66].

Histone H3 methylation in plants

Histone H3 methylation in *Arabidopsis* showed alternative distribution of methylated H3K4 in euchromatin and methylated H3K9 in heterochromatic chromocenter where methylated DNA was also located [60, 68]. The distribution patterns of methylated H3 were the same as in other eukaryotes [12]. However, plant species with the larger genome had methylated H3K9 even in euchromatic region [69]. It was proposed that large genome had high amounts of repetitive sequence needed to be silent and thus methylation of H3K9 was required also in euchromatic regions [69].

Histone methyltransferase, KRYPTONITE, specific for H3K9 was identified in *Arabidopsis* [44]. A mutant (*kyp*) of KRYPTONITE with reduced methylated H3K9 in the heterochromatic chromocenters had no change in size and shape of chromocenters or DNA methylation pattern from those in the wild-type [60]. On the contrary, in the decreased DNA methylation mutants of *Arabidopsis*, *ddm1* and *met1* showed smaller size of chromocenters and reduced methylation of DNA and H3K9 [68]. In *Drosophila* and mouse, null mutants of H3K9 specific methylase had heterochromatins with appearance of wild-type, although methylated H3K9 was much reduced [70,71]. Thus, it is suggested that high level of H3K9 methylation is dispensable for the formation of constitutive heterochromatin also in *Arabidopsis* nuclei [60].

Conclusion

Because of the highly conserved structure of histone H3 and H4 in eukaryotes, specific modification in plants has been studied by using specific antibodies recognizing the post-translational modifications in human histones. Many evidences clearly indicate the usages of the modifications are similar and have similar roles in plants like as in the other eukaryotes. However, species specific combination of modified histones is also revealed in the NOR acetylation and in the replication-related acetylation. Some modification sites are remained to study whether they would have specific roles in histone code usages. Microscopic studies have revealed structural relationship and dynamics of the modification in the entire chromosomes and nucleus. Combination of indirect immunofluorescent and FISH methods provide clear data on the modification status on the target sequence and its physical locations on the chromosomes. In the near future, electron and scanning probe microscopy are expected to be utilized for the investigation of the modifications with the higher resolution such as a single nucleosome level.

Relationships between histone modifications and cellular functional processes that require accessibility of certain factors to DNA such as transcription, replication and repairing mechanisms have been studied especially in relation to the modifications of H3 and H4. Moreover, correlations between the histone modifications and structural changes of the chromosomes would also be an interesting target to be elucidated. For the detailed analysis, new molecules and genes that modulating and/or recognizing modifications in plants must be identified and characterized. Recently, the other modification sites are found even in globular domain of core histones such as H3K79 in yeast [72]. More than 20 modification sites in mammal have also been reported [73]. Next challenges may be studying these sites located in nucleosomal globular domain and the elucidation of their function in plants. New antisera and/or the advanced detection methods should be necessary for these studies.

The nucleosome is packing not only DNA but serving as regulating codes for various cellular functions and also relating chromosome structure. Unpacking the roles of histone codes will give us a new insight for global understanding of chromosomal structure and function.

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