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REVIEW

Histone variants: making structurally and functionally divergent nucleosomes and linkers in chromatin

Leilei SHI, Yuda FANG (✉)

National Key Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

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Abstract In addition to the post-translational modifications of histone proteins, emerging literature suggests that the mosaic nucleosomes formed by incorporation of various histone variants provide another mechanism for modifying chromatin structure and function. The locally defined chromatin by histone variants is involved in transcriptional regulation, DNA repair, centromere packaging, maintenance of pericentromeric heterochromatin, stress responses, temperature sensing, development, and many other biological processes. Here, we review the universal histone variants in H2A, H3 and H1 families and their roles in epigenetics.

Keywords H2AX, H2AZ, H3.3, CenH3, H1

Introduction

The fundamental repeat unit of packaging of eukaryotic genomic DNA predominantly is the nucleosome where DNA is wrapped around a histone octamer which contains two molecules of each core histones H2A, H2B, H3 and H4. Four core histones share the common histone folding domain (HFD) which is composed of three α-helices (α1,α2 and α3) separated by two loops (L1 and L2) (Luger et al., 1997). The N-terminal and C-terminal tails stretch out of the nucleosome core and are subject to diverse post-translational modifications (PTMs) (Jenuwein and Allis, 2001; Ho and Crabtree, 2010). The core histone octamer of two H2A-H2B heterodimers and an (H3-H4)2 tetramer protects ~1.7 turns, about 146 bp of DNA, giving rise to a particle called nucleosome core particle. The connecting string between adjacent nucleosome core particles consists of linker histone H1 and linker DNA.

It seems that the genetic information embedded in the one-dimensional sequence of DNA is not enough for the highly complex eukaryotes to precisely control their development and respond to the violent changes of environments. In answer to this, the “smart” organisms have evolved to obtain another branch of genetics called epigenetics. The epigenetics was first defined by Conrad Waddington in the early 1940s (Waddington, 1942) as “the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being” (Waddington, 1968), and was referred to “the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence” in recent years (Wu and Morris, 2001). The epigenetic information can be defined in different layers: the first is the modification of DNA and DNA binding proteins such as histones and high mobility group (HMG) proteins. The main modification of DNA is methylation in cytosine bases. Unlike DNA modification, histone proteins can be modulated by a variety of PTMs, including methylation, acetylation, phosphorylation, ubiquitination and poly-ADP-ribosylation (Jenuwein and Allis, 2001; Ho and Crabtree, 2010; Zhang and Wang, 2010). HMG proteins are also subject to a wide range of modifications such as lysine acetylation/methylation/formylation/SUMOylation, arginine methylation and serine/threonine phsophorylation (Zhang and Wang, 2010). These epigenetic markers are then deciphered through various modification-specific binding proteins such as methyl-CpG binding domain (MBD) proteins/DNA cytosine methylation (Fatemi and Wade, 2006); heterochromatin protein 1(HP1)/histone H3K9 methylation (Fischle et al., 2003); polycomb proteins/histone H3K27 methylation (Fischle et al., 2003), plant like-heterochromatin protein 1(LHP1)/ histone H3K27 methylation (Zhang et al., 2007), etc. The second layer is the deposition of various histone variants into divergent mosaic

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Correspondence: Yuda FANG
E-mail: yfang@sippe.ac.cn

nucleosomes to regulate the function of the histone variant-containing chromatin (Talbert and Henikoff, 2010). The third layer is mediated by some chromatin binding or remodeling proteins, for example, TATA binding protein (TBP) and some TBP-associated factors (TAFs) which bind to mitotic chromosomes of all stages including metaphase and may serve as epigenetic markers to transmit the transcriptional status of genes from mother cell to the daughter cells (Chen et al., 2002). The fourth layer is the highly-ordered three-dimensional organization of chromatin in the cell nucleus (Misteli, 2009; Misteli and Soutoglou, 2009). The non-random distribution of chromatin may correlate to the transcriptional activities of related genes. For example, in human, the gene-poor chromosome 18 is localized at the nuclear periphery, while gene-rich chromosome 19 shows the more internal localization (Cremer et al., 1996; Cremer and Cremer, 2001). The effect of subnuclear organization on gene expression was demonstrated vividly in *Drosophila* by correlating the position-effect variegation (PEV) behavior of certain mutant allele of the *Brown* (*bw*) locus to an alteration in chromatin organization that is sensitive to the developmental state (Csink and Henikoff, 1996; Dernburg et al., 1996).

In addition to the canonical histones, there exist diverse variants of core histones H2A, H3 and linker histone H1 in eukaryotic cells universally (Talbert and Henikoff, 2010). The amino acid sequences of histones H2B and H4, especially H4, are less variable. Based on the similarity between the canonical histone and its corresponding variants, the histone variants can be classified into homomorphous and heteromorphous families (West and Bonner, 1980; Ausió et al., 2001). Homomorphous variants are distinguished from canonical histones by substitution of the specific amino acid residues, i.e. histone H3 variant H3.3 (Malik and Henikoff, 2003), while heteromorphous variants have more changes in amino acid sequence, such as H2A variants H2AX, H2AZ (West and Bonner, 1980), macroH2A (mH2A) (Pehrson and Fried, 1992; Chadwick and Willard, 2001a), H2A Barr body-deficient (H2ABbd) (Chadwick and Willard, 2001b), and centromere-specific histone H3 variant (CenH3) (Talbert and Henikoff, 2010). The canonical histones in eukaryotic cells are predominantly synthesized and assembled into nucleosomes during DNA replication, while histone variants can be synthesized and deposited into nucleosomes independently of DNA replication (Grove and Zweidler, 1984; Waterborg, 1991). Incorporation of histone variants into chromatin might have great effects on structure and stability of the nucleosome, regulating function of the related chromatin.

Histone H2A variants

The H2A family has many variants including H2AX, H2AZ (West and Bonner, 1980), H2ABbd (Chadwick and Willard, 2001b) and mH2A (Pehrson and Fried, 1992; Chadwick and Willard, 2001a). The two universal variants H2AX and H2AZ can be found in a variety of species ranging from *Saccharomyces cerevisiae* to humans and plants (Redon et al., 2002). These variants play roles in regulation of transcription, heterochromatin formation, DNA damage repair and many other biological processes. Variants mH2A and H2ABbd exist only in the vertebrate cells (Henikoff and Ahmad, 2005). mH2A has a histone fold domain (HFD) following by a basic region and a C-terminal non-histone 'macrodomain' of about or more than 200 amino acid residues. mH2A is involved in the silencing of inactive X chromosome (Chadwick and Willard, 2001b; Talbert and Henikoff, 2010). In contrast to the large C terminus of mH2A, the C terminus of H2ABbd is short with a truncation of the docking domain. H2ABbd, with only 48% identical to canonical H2A, lacks the residues commonly modified in H2A (Chadwick and Willard, 2001b). Here we discuss two universal variants: H2AZ and H2AX.

Histone H2A variant H2AZ

H2AZ differs from H2AX and H2A mainly in its N- and C-terminus with little variation in its core region. Similar to the canonical H2A, the variant H2AZ is also highly conserved throughout evolution, and has been identified in a number of organisms, including chicken (H2AF) (Delahodde et al., 1986), *S. cerevisiae* (HTA3 or HTZ1) (Jackson and Gorovsky, 2000), *Schizosaccharomyces pombe* (PHT1) (Carr et al., 1994), *Arabidopsis thaliana* (partially redundant HTA8, 9, and 11) (Kumar and Wigge, 2010), *Tetrahymena thermophila* (hv1) (Liu et al., 1996), *Drosophila melanogaster* (H2AvD) (van Daal and Elgin, 1992), mice and human (H2AZ) (Faast et al., 2001).

Early observation in *Tetrahymena* that hv1 is exclusively present in the transcriptionally active macronucleus, but not in the inactive micronucleus suggested that the histone variant hv1 (H2AZ) might play a positive role in transcription (Allis et al., 1980). In yeast, RNA polymerase II promoters normally include a nucleosome-free region about 150 bp long which is located about 200 bp upstream of the translation start codon. High-resolution chromatin immunoprecipitation (ChIP) and tilling microarray revealed that H2AZ-containing nucleosomes preferentially flank one or both sides of the nucleosome-free region in the 5′ ends of genes in euchromatin of *S. cerevisiae*. Deposition of H2AZ at the 5′ ends of genes may define a chromatin structure for regulating the gene expression through the promoters. In addition to the regulation of transcription (Adam et al., 2001; Larochelle and Gaudreau, 2003; Farris et al., 2005), studies in yeast also suggested the functions of Htz1 (H2AZ) in prevention of heterochromatin spreading to euchromatin (Meneghini et al., 2003), progression of cell cycle (Dhillon et al., 2006) and stability of genome (Carr et al., 1994).

In contrast to yeast, the biological functions of H2AZ in the higher eukaryotes are less understood as it has been shown to

be associated with both euchromatin and heterochromatin. For example, in *D. melanogaster*, H2AZ was found to be present in many euchromatic bands and heterochromatic chromocenter of polytene chromosomes (van Daal and Elgin, 1992; Leach et al., 2000). Genome-wide ChIP-sequencing or ChIP-microarray analyses of mammalian cells showed that H2AZ preferentially localizes to gene promoters (Gevry et al., 2007, 2009). However, unlike its yeast counterpart, mammalian H2AZ spread over several nucleosomes upstream and downstream of transcription start site, in addition to nucleosomes flanking the nucleosome-free region. In human cells, H2AZ is often associated with actively expressing genes, H2AZ associates with gene promoters and helps in recruiting the transcriptional machinery including RNA polymerase II (RNAPII) (Hardy et al., 2009). H2AZ also accumulates in heterochromatic domains, it seems that a battle between random H2AZ deposition and RNAPII-dependent eviction of H2AZ shapes the chromatin landscape (Ahmad and Henikoff, 2002; Hardy et al., 2009). H2AZ is also proved to be associated with chromatin compaction and heterochromatin silencing (Ahmad and Henikoff, 2002; Meneghini et al., 2003). In human cells, a fraction of mammalian H2AZ is monoubiquitylated by the RING1b E3 ligase of the human polycomb complex. The monoubiquitylated H2AZ is enriched on the inactive X chromosomes of female cells, forming a specific mark that distinguishes the H2AZ associated with euchromatin and facultative heterochromatin (Luger et al., 1997). In *A. thaliana*, H2AZ is quantitatively deficient at the heterochromatin which is transposon-rich and heavily methylated (Zilberman et al., 2008). Interestingly, a recent study in *Arabidopsis* revealed that nucleosomes containing the histone variant H2AZ are essential to perceive ambient temperature correctly (Kumar and Wigge, 2010). Moreover, the same effect was also observed in budding yeast, indicating that a chromatin thermostat mediated by histone H2AZ-containing nucleosomes is an evolutionarily conserved mechanism to sense temperature and integrate in development (Kumar and Wigge, 2010).

The deposition of the variant histone H2AZ into chromatin is mediated by the SWR1 complex which can prevent the gene to be methylated (Meneghini et al., 2003; Hardy et al., 2009). Histone H2AZ and DNA methylation are mutually antagonistic chromatin marks as DNA methylation in the gene bodies that can make the H2AZ away from them (Zilberman et al., 2008). The SWR1 complex contains up to 14 subunits, including the catalytic subunit Swr1, a DNA-dependent ATPase, which belongs to the SNF2 family of chromatin remodeling complexes. SWR1 is an ATP-dependent chromatin remodeling complex that uses the energy of ATP hydrolysis to exchange the H2A-H2B histone dimers for H2AZ-H2B dimers in nucleosomes. The evolutionally conserved SWR1 complex was described in yeast, animals and plant (March-Díaz et al., 2008; March-Díaz and Reyes, 2009).

Histone H2A variant H2AX

The variant H2AX has a similar HFD domain to the core histones, but with a C-terminal Ser-Gln-(Glu/Asp)-(Ile/Leu/Phe/Tyr) motif (Malik and Henikoff, 2003). The Ser in this motif can be phosphorylated massively and rapidly to produce the phosphorylated H2AX, or called γH2AX, in response to DNA double-strand breaks (DSBs) environmentally or metabolically induced, and also to programmed DNA DSBs generated by non-homologous end joining (NHEJ), homologous recombination, and caspase-activated DNase (Sedelnikova et al., 2003). Initially a physical signal such as a DNA break end or a chromatin deformation near the site of a DSB, presents a high-affinity for a phosphoinositide 3-kinase-like kinase (PI3 kinase) such as atxia telangiectasia mutated (ATM), atxia telangiectasia and RAD3-related (ATR) or DNA-dependent protein kinase (DNA-PK). The recruited PI3 kinase then phosphorylates serine in the C-terminal tail of H2AX to generate γH2AX, starting near the break and progressing away from it. As H2AX is a histone anchored into the chromatin, it marks the break site to form γH2AX foci *in vivo* (Paull et al., 2000). Mobile factors such as DNA repair proteins, histone modifying enzymes (van Attikum and Gasser, 2009) and chromatin remodeling complexes(Altaf et al., 2009) are then recruited to the foci by either affinity for the C-terminal tail of γH2AX or an altered chromatin conformation induced by the tail. ATM molecules accumulate and form visible foci as soon as the γH2AX foci appeared. In addition, cohesins are recruited to the DSBs to keep DNA ends in close proximity during repair, preventing the loss of large DNA fragments in a γH2AX-dependent manner (Ström et al., 2004; Ünal et al., 2004). When the DSB is repaired, PI3-like kinase molecules can no longer be recruited, and the γ-phosphates in γH2AX are then removed by phosphatases (Redon et al., 2002).

In addition to DNA repair, the phosphorylation of H2AX is involved in the initiation of facultative heterochromatinization of the XY body and the male meiotic sex chromosome inactivation (MSCI) in mice. The phosphorylated H2AX accumulates in the XY body in a manner independent of meiotic recombination-associated double-strand breaks (Mahadevaiah et al., 2001; Fernandez-Capetillo et al., 2003). The male mice deficient in H2AX are infertile and have increased levels of X-Y asynapsis (Celeste et al., 2002).

Histone H3 variants

The number of histone H3 and its variants among different species are different, for example, in addition to CenH3, yeast and some unicellular organisms such as the alge *Cyanidioschyzon merolae* and *Chlamydomonas reinhardtii* have only one type of histone H3, which is a H3.3-like protein. In *Neurospora elegans*, *D. melanogaster*, *Xenopus laevis* and *A. thaliana*, there are two variants including CenH3 and H3.3

which deposit into chromatin in a replication-independent manner. In mammals, however, H3 family comprises five main members, including centromere-specific centromere protein (CENP)-A, H3.1, H3.2, H3.3 which is identified as a replication-independent histone expressed throughout the whole cell cycle, and H3t identified in testis (Govin et al., 2005). Recently, H3.X and H3.Y are identified as two novel primate-specific H3 variants (Nagaki et al., 2005).

Histone H3 variant H3.3

In most animals, histone variant H3.3 differs in four amino acid residues from H3.1, which are residue 31 in the N-terminal tail with Ser in H3.3 instead of Ala in H3.1 and residues 87, 89 and 90 in the HFD near the beginning of α2. In other species, the numbers can be different, such as a total of 16 in *T. thermophila* (Cui et al., 2006). The residues 87, 89 and 90 in the core domain of histone H3.3 define its replication-independent assembly into nucleosomes (Ahmad and Henikoff, 2002). The residue Ser31 in N-terminal tail of H3.3 becomes phosphorylated during mitosis in human cells (Hake et al., 2005) and meiosis in the urochordate *Oikopleura dioica*. At diakinesis and meiosis II, the phosphorylation of Ser31 is found in all chromosomes, but to a much greater degree in sex chromosomes (van der Heijden et al., 2007). Despite this association with mitotic and meiotic metaphase, Ser31 is unnecessary for fertility in *D. melanogaster* spermatogenesis (Sakai et al., 2009), and its function remains unclear.

In fly and human cells, histone H3.3 is incorporated into specific regions including transcribed genes, promoters and gene regulatory elements (Henikoff, 2008). It is deposited into genes upon induction of transcription and is associated with transcription elongation. In *D. melanogaster*, H3.3 can be displaced dynamically by H3.1 and reloaded at the site of transcription (Schwartz and Ahmad, 2005). In fungi and insects, H3.3 is exchanged in a transcriptional manner (Schwartz and Ahmad, 2005; Thiriet and Hayes, 2005). The nucleosome turnover might have functional significances in epigenome maintenance, gene regulation and DNA replication (Deal et al., 2010). Since various evidences showed that H3.3 is involved in active transcription, one may think H3.3 is essential for the viability or development of the H3.3-containing organisms. However, when H3.3 gene is knockout in *D. melanogaster*, they show the sterility but not lethality, and the transcriptional defects can be rescued by over-expression of H3.1 (Hodl and Basler, 2009; Sakai et al., 2009), which demonstrates that H3.3 is not necessary for the somatic development. However, H3.3 mutants display meiotic defects in the chromosome condensation in fly spermatocytes depending on residues 87, 89 and/or 90 (Kapros et al., 1995), suggesting a germline-specific role of H3.3 in chromatin remodeling. In the mouse embryo, H3.3 localizes to paternal pericentromeric chromatin during S phase, and has an important role in the transcription of pericentromeric repeats. Mutation of H3.3K27 results in aberrant accumulation of pericentromeric transcripts, HP1 mislocalization, dysfunctional chromosome segregation and developmental arrest, and this phenotype is rescued by pericentromeric repeats-derived double-strand RNAs, suggesting an important role of H3.3K27 in the dsRNA-dependent formation of pericentromeric heterochromatin (Santenard et al., 2010). H3.3 incorporation into the promoter can mediate the epigenetic memory of an activated gene state that depends on H3.3K4, and this memory can persist through 24 cell divisions without transcription, as revealed in the nuclear transplantation in frog *Xenopus* (Ng and Gurdon, 2008).

Assembly of H3.1 into nucleosomes is mainly mediated by chromatin-assembly factor 1(CAF1) interaction with proteins at replication foci such as proliferating cell nuclear antigen (PCNA), while H3.3 is assembled in a replication-independent pattern by histone chaperones including the histone regulator A (HIRA), death-associated protein DAXX, ATRX or DEK (Loyola, 2004; Tagami et al., 2004; Goldberg et al., 2010; Sawatsubashi et al., 2010). In *D. melanogaster*, HIRA and chromatin remodeller chromodomain helicase DNA binding protein 1(CHD1) are critical for the assembly of paternal chromatin at fertilization and chromatin remodeling during egg fertilization and embryo development (Bonnefoy et al., 2007; Konev et al., 2007). In *S. cerevisiae*, there is only one form of non-centromeric H3, and H3.3-like, which is assembled in two ways: replication-dependent assembly mediated by CAF1 and replication-independent assembly mediated by HIRA protein (Mousson et al., 2007). Similar to yeast, the same situation occurs to the assembly of histone H3.3-like variant in some unicellular organisms like the algae. Interestingly, no defects during sexual reproduction were detected in an *Arabidopsis* null mutant *At-hira* (Ingouff et al., 2010), the plant homolog of HIRA. In addition, a survey of plant homologs of animal H3 chaperones suggests that a new mechanism might be involved in the removal of parental H3 from the zygote nucleus (Ingouff et al., 2010).

Centromere-specific histone H3 variant CenH3

In centromeres, canonical histone H3.1 is replaced by a centromere-specific histone H3 variant CenH3 which was given different names in different organisms, such as CENP-A in humans (Earnshaw and Rothfield, 1985), chromosome segregation protein 4 (Cse4) in *S. cerevisiae* (Stoler et al., 1995), HCP-1 in *Caenorhabditis elegans* (Moore et al., 1999), centromere identifier (CID) in *D. melanogaster* (Malik and Henikoff, 2001), HTR12 in *A. thaliana* (Fang and Spector, 2005; Tablbert et al., 2002), and Cna1 in *T. thermophila* (Cui and Gorovsky, 2006). Surprisingly, in contrast to the octamer of bulk nucleosome which contain two copies of each canonical histone H2A, H2B, H3 and H4, the

centromeric nucleosome mainly contains only one molecule of each canonical histone H2A, H2B, H4 and variant CenH3 in *Drosophila* (Dalal et al., 2007) and human (Dimitriadis et al., 2010), forming a tetrameric “hemisome.” In yeast, almost all the centromeric nucleosomes adopt a hexameric state (Mizuguchi et al., 2007), although a part of octameric nucleosome exits in budding yeast (Loppin et al., 2005). The phylogenetic analysis shows that there maybe lie multiple origins of rapidly evolving CenH3 families (Malik and Henikoff, 2003; Dawson et al., 2007). Currently, many centromere-specific histones functionally have been classified as CenH3s which have a low identity with the canonical H3.1 in the HFD domain (Talbert and Henikoff, 2010), and no conservation in their longer N-tails and L1s (Smith, 2002). Mislocalized histone CENP-A which are incorporated into non-centromeric regions is degraded by ubiquitin-mediated proteolysis rapidly (Collins et al., 2004). In yeast, an E3 ubiquitin ligase psh1 prevent ectopic localization of Cse4, the budding yeast CenH3 protein, through centromere targeting domain of Cse4 (Hewawasam et al., 2010). In fission yeast, the CHD (chromo-helicase/ATPase DNA binding) remodeling factor Hrp1 contributes to stimulating CENP-A loading to centromeres and plays a direct role in chromatin assembly during DNA replication (Walfridsson et al., 2005). Recently, it was shown that the assembly of CENP-A into centromeric nucleosomes is mediated by the deposition factor HJURP via a highly conserved N-terminal domain (Shuaib et al., 2010). However, we still have a long way to go to unravel the mystery of the loading factors and function of CenH3.

Histone H1 variants

In contrast to the relative conservation in structure and sequence of the core histones, the linker histone H1 and variants are more variable. Histone H1 contains a highly conserved central globular domain and less conserved relatively short N-terminal and long C-terminal domains. Fluorescence recovery after photobleaching (FRAP) analyses of mammalian cells expressing H1- green fluorescent protein (GFP) fusions have revealed that H1 variants bind to chromatin dynamically *in vivo* and that both the globular and C-terminal domains contribute to chromatin binding (Lever et al., 2000; Misteli et al., 2000).

Despite the presumed role of linker histones in chromatin organization, studies in *Tetrahymena*, *S. cerevisiae*, *Aspergillus nidulans*, and *Ascobolus immerses* suggested that the linker histone H1 is not essential in these unicellular eukaryotes (Shen et al., 1995; Ushinsky et al., 1997; Patterton et al., 1998; Barra et al., 2000; Ramón et al., 2000; Oishi et al., 2007). In yeast, linker histone Hho1p has a limited role in transcriptional regulation, although the yeast cells without histone H1 have a shortened life span (Ushinsky et al., 1997; Schäfer et al., 2008). It was also shown that histone H1 represses the recombination at the rDNA by a mechanism which is independent of the recombination pathways regulated by Sir2 (Li et al., 2008).

Compared to unicellular eukaryotes, higher eukaryotes express multiple variants of histone H1, for example, chickens have six H1 variants; mice possess at least eight H1 subtypes; and *A. thaliana* contains three linker histone H1 proteins differentially localized in the nuclei (Ascenzi and Gantt, 1999; Gantt and Lenvik, 1991). H1-1 and H1-2 are two major forms in most *Arabidopsis* tissues while the H1-3 type, which is much more divergent, is induced by drought stress (Ascenzi and Gantt, 1997). The linker histones participate in the epigenetic regulation of gene expression. Simultaneous knockdown of three *Arabidopsis* H1 genes by RNAi results in developmental defects and DNA hypo or hypermethylation (Wierzbicki and Jerzmanowski, 2005). The histone H1 null chicken cells show decreased global nucleosome spacing, expanded nuclear volumes, and increased chromosome aberration rates, but maintain proper mitotic chromatin structure, suggesting roles of linker histone H1 in nucleosome spacing, interphase chromatin compaction and global regulation of transcription (Hashimoto et al., 2010). In mice, embryos without the three H1 subtypes (H1c, H1d, and H1e) and with 50% of the normal ratio of H1 to nucleosomes die by mid-gestation, suggesting that the total amount of H1 is crucial for proper embryonic development (Fan et al., 2003). Downregulation of linker histone H1 of *D. melanogaster* by RNAi to 20% of the level in wildtype larvae causes lethality in the late larval. In addition, histone H1 was shown to play roles in heterochromatin structural integrity, the deposition or maintenance of major pericentric heterochromatin-associated histone marks such as H3K9me2 and H4K20me2, the alignment of endoreplicated sister chromatids and the organization of pericentric regions of all polytene chromosomes into a single chromocenter, demonstrating a fundamental role of H1 in the architecture and activity of chromosomes *in vivo* (Lu et al., 2009).

In addition to chromatin in nucleoplasm, histone H1 variants also play roles in controlling the structure and expression of nucleolar chromatin, or rDNA arrays. It was shown that the polymerase I transcription factor UBF (upstream binding factor) inhibits the assembly of inactive rDNA structures by displacing or dissociating histone H1 from nucleolar nucleosomes (Kermekchiev et al., 1997). Another player in this process is the nucleolin, a major nucleolar protein involved in the transcription and processing of 45S pre-rRNA (Ginisty et al., 1999; Mongelard and Bouvet, 2007). The nucleolin induces chromatin decondensation by interacting with histone H1 (Erard et al., 1988). In *Arabidopis*, the expression of the two nucleolin genes (*AtNUC-L1* and *AtNUC-L2*) is regulated in response to stress and developmental conditions, and disruption of the constitutively expressed *AtNUC-L1* gene causes the specific decondensation of 45S rDNA chromatin (Kojima et al., 2007; Pontvianne et al., 2007).

The timing and pattern of expression of H1 variants is tightly

regulated. Moreover, the post-transcriptional modification state of the histone H1 variants is also regulated in a cell-cycle-dependent way. Histone H1 phosphorylation affects chromatin condensation and function, but little is known about how specific phosphorylations affect the function of H1 variants. In human cells, the specific sites in H1.2 and H1.4 are phosphorylated only during mitosis or during both mitosis and interphase, distributed throughout nuclei and enriched in nucleoli. Moreover, interphase phosphorylated H1.4 is enriched at active 45S pre-rRNA gene promoters and is rapidly induced at steroid hormone response elements by hormone treatment, suggesting that site-specific interphase H1 phosphorylation facilitates the transcription by RNA polymerases I and II and has an unanticipated function in ribosome biogenesis and control of cell growth. Differences in the numbers, structure, and locations of phosphorylation sites in histone H1 variants may further contribute to their functional diversity (Zheng et al., 2010).

Perspectives

We are now realizing the importance of incorporation of various histone variants into octameric, tetrameric or hexameric nucleosomes in defining chromatin structure and functions. However, we still have a long way toward understanding the evolution of various histone variants, dynamics of histone variants in nucleosomes of various cell types and developmental stages, histone charporones and signals for assembly and disassembly of histone variant-containing nucleosomes, crosstalk between histone variants and DNA or histone modifications, the enzymes to modify them and functions of histone variants in various biological processes including regulation of gene, genome stability and replication, epigenetic memory and programming or reprogramming in different organisms. Application of new techniques such as long-term live-cell imaging (Fang and Spector, 2010; Ingouff et al., 2010) to investigating the distributions of histone variants in real time has begun to shed light on the dynamic loading or the depletion of histone proteins *in vivo*, essential for giving insights into the functions of dynamic histone variants.

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