[Frontiers in Bioscience 17, 984-995, January 1, 2012]

# Histones and genome integrity

**Wes D. Williamson[1], Ines Pinto[1]**

[1]*Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701*

**TABLE OF CONTENTS**



## 1. ABSTRACT

Chromosomes undergo extensive structural rearrangements during the cell cycle, from the most open chromatin state required for DNA replication to the highest level of compaction and condensation essential for mitotic segregation of sister chromatids. It is now widely accepted that chromatin is a highly dynamic structure that participates in all DNA-related functions, including transcription, DNA replication, repair, and mitosis; hence, histones have emerged as key players in these cellular processes. We review here the studies that implicate histones in functions that affect the chromosome cycle, defined as the cellular processes involved in the maintenance, replication, and segregation of chromosomal DNA. Disruption of the chromosome cycle affects the integrity of the cellular genome, leading to aneuploidy, polyploidy or cell death. Histone stoichiometry, mutations that affect the structure of the nucleosome core particle, and mutations that affect the structure and/or modifications of the histone tails, all have a direct impact on the fidelity of chromosome transmission and the integrity of the genome.

## 2. INTRODUCTION

Eukaryotic chromosomal DNA is packaged in the cell nucleus as chromatin. The nucleosome is the fundamental repeat unit of chromatin, evolutionarily conserved and composed of histone proteins and DNA. Two molecules of histones H3 and H4 form a tetramer that is bound by two H2A-H2B dimers to form the histone octamer, to which 147 bp of DNA wrap around to form the nucleosome core particle (1-3). The high-resolution structure of the nucleosome core has provided the framework for additional studies on histone-histone and histone-DNA interactions in eukaryotes. Histones are relatively small, basic proteins that consist of globular and tail domains. The globular domain is formed by the histone fold motif (helix-loop-helix-loop-helix). The four core histones interact with each other and DNA through the histone fold domains to form the nucleosome core particle (1). The flexible N-terminal tails protrude from the nucleosome and are important for inter-nucleosome interactions, which lead to higher order chromatin structure, in combination with linker histone H1 and a

variety of non-histone proteins (2). The N-terminal tails are also subjected to various covalent post-translational modifications, including phosphorylation, methylation, acetylation, ubiquitination, ADP ribosylation, and sumoylation. These modifications have been implicated in regulating several cellular processes such as DNA replication, transcription, and chromatin condensation, among others (4-8).

Extensive research over the last two decades has led to a better understanding of chromatin function, and replaced the original notion of histones being a structural component, the mere nuclear scaffolding for DNA compaction, with histones being a dynamic and interactive participant of cellular functions (9). Although numerous *in vivo* and *in vitro* studies have demonstrated that histones affect all aspects of chromosome function, including transcription, replication, recombination and chromosome segregation, the particular roles in these processes are as yet poorly understood.

Here we provide an overview of the literature that implicates histone function in the maintenance of genome integrity. We focus on functions that affect the chromosome cycle, defined as the cellular processes involved in the maintenance, replication, and segregation of chromosomal DNA. Disruption of the chromosome cycle affects the integrity of the cellular genome, leading to aneuploidy, polyploidy, or cell death.

## 3. HISTONE GENE DOSAGE

The earliest studies that linked histones with the maintenance of genome integrity were done by investigating the effects of differing the stoichiometry of individual histones within the cell. The budding yeast *Saccharomyces cerevisiae* has proven to be an ideal model organism for these types of studies due to its relatively simple genomic organization of the histone genes. Cells subjected to overexpression of either the H2A/H2B or H3/H4 gene pairs show an increase in chromosome loss, which suggests that the ratio of H2A and H2B to H3 and H4 is important for proper chromosome segregation (10). It was later shown that underexpression of the histone H3-H4 genes could also affect mitotic chromosome transmission (11). Deletion of one of the gene pairs encoding H2A-H2B showed cell-cycle defects (12), and depletion of H2B and H4 by placing the genes under inducible promoters inhibits chromosome segregation and in turn causes cell cycle arrest (13, 14). The connection between these genetic studies and altered chromatin structure was provided by micrococcal nuclease mapping of nucleosomes on isolated nuclei. Specific genetic loci showed disrupted nucleosome arrays in yeast cells lacking one of the two H2A-H2B coding gene pair (15). One of the disrupted loci was the centromere of chromosome III. Additional chromatin mapping studies in cells repressed for expression of either H2B or H4 corroborated the sensitivity of centromeric chromatin structure to histone gene depletion (16). Recent work in fission yeast has shown that the relative levels of histone H3, H4 and the centromere-specific histone H3 variant CENP-A influence the assembly of centromeric chromatin and recruitment of kinetochore proteins, affecting the fidelity of chromosome segregation (17). In support of this finding, overexpression of H3 in budding yeast increases the rate of chromosome loss with a concomitant reduction in the levels of the centromere-specific histone H3 variant Cse4 (18). In addition, partial depletion of H4 was shown to affect chromatin assembly during DNA replication that resulted in increased levels of homologous recombination, leading to genetic instability (19). These studies clearly show that each of the four core histones must be maintained in a proper stoichiometry for normal cell cycle progression and high-fidelity chromosome segregation.

## 4. HISTONE MUTANTS

Mutational analysis of histones has allowed researchers to show that not only the balance of histones is important, but also that the histone proteins themselves can lead to phenotypes associated with defects in the chromosome cycle. Two independent mutants of H2A in *S. cerevisiae* cause increase in ploidy and increased frequency of chromosome loss. The mutations reside in evolutionarily conserved residues near the N-terminus of the structured globular domain (S19F and G29D) that make contact with DNA. These alleles show cell cycle defects, genetic interactions with kinetochore mutants, and altered centromeric chromatin structure, suggesting a role for H2A in microtubule attachment at the centromere-kinetochore (20). Work in the fission yeast *Schizosaccharomyces pombe* has led to the finding of temperature sensitive mutations in the inner region of H2B that cause defects in centromeric chromatin and chromosome segregation (21). These mutations affect DNA contact (G52D) as well as histone-histone interactions (P102L) in the core nucleosome particle, stressing the importance and stringency of the nucleosome architecture in chromatin function.

Early deletion studies in *S. cerevisiae* demonstrated that the highly conserved N-terminal tails of H3 and H4 are essential for cell cycle progression. Although H3 and H4 N-terminal tails can be individually deleted without losing cell viability, deletion of the H3 and H4 N-terminal tails in combination yields inviable cells with terminal phenotypes associated with cell division cycle defects (22). In a more detailed study of H4 N-terminal tail mutations, Megee at al. (23) reported the requirement of the four most N-terminal lysine residues (domain A, positions 5, 8, 12 and 16) for normal nuclear division. The mutant cells activate the DNA damage checkpoint and arrest at G2/M. Reintroduction of a lysine residue within domain A, without the requirement of polypeptide sequence specificity, restored cell-cycle progression, strongly supporting a role for the post-translational modifications of N-terminal lysines in cell division. In another study, a temperature-sensitive allele of H4, carrying two amino acid replacements (T82I and A89V) caused severe nuclear division and mitotic chromosome transmission defects (24). The primary mutation at position 82 is located within one of the H4 surfaces that interacts with DNA, and the T82I mutation is

lethal but rescued by the A89V mutation. Thus, similarly to the H2A mutants, H4 residues that lie in the path of DNA can have strong effects on cell cycle functions.

Recent analysis of a histone H3 mutant (G44S) that causes pleiotropic phenotypes related to cell cycle progression, including benomyl and hydroxyurea sensitivity, led to the discovery of a mitotic tension-sensing function (25). Prior to anaphase, the bipolar attachment of sister chromatid kinetochores to the spindle microtubules generates tension that is monitored by the spindle assembly checkpoint. This tension-sensing checkpoint is essential to allow the cell to stall the cell cycle and correct erroneous or missing attachments that can result in aneuploidy. Yeast cells carrying the H3 G44S mutant fail to activate the spindle assembly checkpoint during tension-less situations, leading to the missegregation of chromosomes and aneuploidy. Interestingly, this impairment results from a defective interaction between H3 and Sgo1p (shugoshin), a protein required for tension sensing and present in pericentric chromatin.

Systematic histone substitution and deletion mutant collections have been created in *S. cerevisiae* to probe the contribution of each residue to chromosome function (26, 27). These collections of alleles have been screened for phenotypes associated to DNA repair and sensitivity to DNA damaging agents, providing new insights into the contribution of each residue to the DNA damage response. These valuable resources will undoubtedly provide novel information as the libraries are screened for additional phenotypes associated with the maintenance of genome integrity.

In many cases, mutational analyses of individual amino acids in each of the histones, particularly in their terminal "tails", have been carried out to study the effect of abolishing specific post-translational modifications. Those studies that link histone modifications to the chromosome cycle are discussed below.

## 5. HISTONE MODIFICATIONS

Covalent modifications of the N-terminal tails of histones have been implicated in the regulation of various cellular processes. The mechanisms by which many of these modifications carry out their effects in the cell are still largely unclear. Some may work by changing the charge of the histone, and in turn causing the DNA to associate more tightly or loosely with the nucleosome. Other modifications may serve as a "mark" to recruit chromatin remodeling complexes or other regulatory proteins. Lastly, there is an emerging "histone code" in which multiple modifications act in concert with each other and have a so-called "crosstalk" to regulate cellular functions (4, 5, 28). Importantly, covalent modifications have been found in all organisms analyzed; however, the specific amino acids that are modified, the type of modification, and the associated function can vary among species, creating an enormous challenge in the efforts to decode the histone language.

### 5.1. Methylation

Histone methylation is the result of the covalent attachment of methyl groups from S-adenosyl-L-methionine onto the epsilon-amino group of lysine, arginine, and histidine residues catalyzed by specific histone methyltransferases (29, 30). The reversible nature of this modification became clear many years later with the identification of histone demethylases (31). Three forms of methylated lysine –mono-, di- and tri-methylation- are found on histones, and each one can signal a different chromatin state. Methylation of histone H3 on Lys9 (H3K9me) has long been recognized as a determinant of silent chromatin and heterochromatin (32). Fission yeast centromeres are marked by H3K9me heterochromatin that facilitates the assembly of the essential centromere-specific H3 variant CENP-A at the central domain (33, 34). Mutations in the histone methyltransferase Clr4 distort the pericentric heterochromatin and disrupt chromosome segregation (35). Dimethylation of histone H3 Lys9 (H3K9me2) and trimethylation of histone H3 Lys9 (H3K9me3) are also present in pericentric heterochromatin in Drosophila, mouse and human cells (36). Similarly to fission yeast, loss of the suv39h histone methyltransferases disrupts mammalian heterochromatin and affects genome stability (37, 38). The regulation of pericentric heterochromatin is carried out by the chromodomain proteins Swi6/HP1, which bind H3K9me2 and are essential for mitotic progression (39-42). In mammalian cells, H3K9me3 methylation increases in late G2 phase and mitosis and rapidly decreases in G1. Loss of H3K9 methylation in G2 leads to centromere and kinetochore defects and chromosome misalignment (43, 44). Histone H4K20 trimethylation has been described in fission yeast, Drosophila and mammalian cells (36). In human cells, H4K20me3 is abundant in pericentric heterochromatin and is cell-cycle regulated. H4K20me3 decreases in S phase and increases in late G2 and mitosis (45). Interestingly, in murine cells H4K20 trimethylation is catalyzed by two histone methyltransferases, Suv4-20h1 and Suv4-20h2, which interact with HP1 and function in a suv39h dependent manner (46). This finding led to the proposal of a sequential mechanism of H3K9me3 and H4K20me3 in the formation of pericentric heterochromatin. Although these results did not provide a direct connection between these histone modifications and mitosis, recent work has shown that the loss of methylation at H3K9 and H4K20 leads to less compact pericentric heterochromatin and loss of tension at the centromere during mitosis (43). In addition, the monomethyl to dimethyl transition of histone H4K20 has been associated with chromosome behavior during mitosis and cytokinesis. Subunits of the human factor HCF-1 associate with chromatin and regulate the expression of the H4K20 methyltransferase PR-Set7. Loss of HCF-1 during mitosis leads to increased PR-Set7 expression and dimethylation of H4K20, resulting in defective chromosome alignment and segregation (47).

Methylation of H3K79 and H4K20 are the main modifications involved in DNA repair, hence, essential to the integrity of the cell's genome. Although methylated H3K79 and H4K20 are present throughout the genome, they become evident at DNA repair foci after DNA damage (48). Dimethylated H4K20 at these foci is specifically recognized and bound by the checkpoint protein

Crb2/53BP1, which triggers a G2/M arrest to allow DNA repair to take place (49, 50). Consistent with these data, depletion of the methyltransferases Suv4-20h1 and Suv4-20h2 decreases the number of DNA repair foci containing 53BP1 (51). Surprisingly, Crb2/53BP1 only recognizes H4K20me and H4K20me2, but not H4K20me3 (50, 52); therefore, it appears that different functions are associated with different proteins that recognize distinct levels of methylation at the same histone residue. Methylation of H3K79 is the main signal for DNA repair in budding yeast. Dot1 is the evolutionarily conserved methyltransferase capable of adding mono-, di-, and trimethyl groups to H3K79. Originally identified by mutations that disrupted telomeric silencing, cells lacking Dot1 are also defective in the checkpoint response to DNA damage and DNA repair pathways (53, 54). Supporting the critical function of H3K79 methylation in the chromosome cycle, mouse ES cells lacking Dot1L, the murine Dot1 homologue, show reduced levels of the heterochromatic marks H3K9me2 and H4K20me3 at centromeres and telomeres, along with the general depletion of H3K79me. These histone changes are likely the cause of the aneuploidy and telomere elongation defects observed in these cells (55).

Histone methylation can also influence cell cycle progression in a more indirect fashion. H3K4 methylation has been shown to increase in mitosis and is thought to be a mark for the activation of certain mitotic-specific genes, such as cyclin B1 (56). This finding provides a connection between the transcriptional regulation of factors driving the cell cycle and histone methyltransferases.

### 5.2. Acetylation

Histones are reversibly acetylated on lysine residues primarily in the N-terminal tails. The transfer of the acetyl group from acetyl-coenzyme A is catalyzed by histone acetyltransferases (HATs). Histone acetylation has been mainly implicated in transcriptional regulation, with histones in transcriptionally active regions being acetylated. The reversal of acetylation has been associated with transcriptional repression and chromatin compaction (57, 58). Underacetylated histones H3 and H4 are abundant in centromeric heterochromatin of metaphase chromosomes (59), and an overall reduction of histone H3 and H4 acetylation occurs in the transition from interphase to mitosis (60). However, histones H3 and H4 remain acetylated in loci that are still transcriptionally active during mitosis or need to be reactivated quickly following mitosis (56). Deletion of the H3 acetyltransferases *SAS3* and *GCN5* in *S. cerevisiae* leads to G2/M mitotic arrest (61), perhaps as a result of transcriptional defects.

Histone deacetylase activity is essential for mitotic progression. Inhibition of deacetylation has been associated with delayed G2/M transition (62) and mitotic arrest (63). Treatment of cells with histone deacetylase inhibitors affects the formation of pericentric heterochromatin, resulting in kinetochore assembly defects (64), chromosomal instability and defective checkpoint activation (65). Depletion of the mammalian histone deacetylase HDAC3 also affects chromosome condensation, sister chromatid cohesion, and kinetochore-microtubule attachment, leading to defective chromosome segregation (66-68). Depletion of the human histone deacetylase HDAC3 causes premature dissociation of sister chromatids and acetylation of centromeric H3K4, which correlates with the loss of dimethylation at the same position, illustrating the complexity of the "histone code" in the regulation of mitotic events (67). It has also been suggested that deacetylation of H4K16 by the SirT2 deacetylase during the G2/M transition is required for chromatin condensation (69).

Additionally, deletion of a histone deacetylase complex (Hda1) in *S. cerevisiae* suppresses a histone H2A mutant that causes increase in ploidy and increased frequency of chromosome loss, providing a genetic link between histone deacetylation and mitotic function (70). These histone H2A mutants alter the nucleosome architecture and pericentric chromatin structure in a significant way, leading to the hypothesis that pericentric chromatin contributes to kinetochore formation and microtubule attachment in budding yeast (20). The increased acetylation observed in the suppressors may compensate directly for a defective histone post-translational modification in the H2A mutant-containing nucleosomes, restoring an epigenetic mark specific for pericentric chromatin. Alternatively, indirect suppression may occur by bypassing the chromatin structural defect, creating an epigenetic environment favorable for the formation of a functional centromere-kinetochore complex and microtubule attachment. Further studies will be necessary to decipher the factors that interact with pericentric chromatin and contribute to the bipolar kinetochore-microtubule attachment and proper chromosome segregation in *S. cerevisiae*.

Acetylation of H3K56 deviates from the well-characterized modifications of the histone tails, but this modification has been shown to play an important role in DNA replication and repair. Lysine 56 resides in the H3 core and is acetylated in yeast cells by the Rtt109 acetyltransferase as a mark of newly synthesized chromatin during S phase. Although this modification was originally described in yeast, it has recently been identified in mammalian cells (71). In the absence of DNA damage H3K56 acetylation is removed during the G2/M phase of the cell cycle. In contrast, cells with DNA lesions maintain high levels of acetylated H3K56, modification that is crucial for the DNA damage response (72, 73). Consistently, *rtt109* mutants display hypersensitivity to DNA damaging agents and elevated levels of spontaneous chromosome breaks (74, 75). Moreover, H3K56R mutants are also sensitive to DNA-damaging agents and unable to reassemble chromatin after DNA repair (76, 77).

### 5.3. Phosphorylation

Phosphorylation of histones, mainly at serine residues, has long been recognized as an important modification involved in chromosome dynamics during mitosis and DNA repair processes. Phosphorylation of histone H3 at serine10 (H3S10pho) has been found in all organisms analyzed so far, and shown to be required for

chromatin compaction and condensation in mammals and most eukaryotes (78, 79). During mitosis, levels of H3S10pho are high through the activity of the evolutionarily conserved Aurora B kinase (80). Mutants of H3S10 in *Tetrahymena* that are unable to be phosphorylated display problems in chromosome segregation caused by lack of chromosome condensation (81). In a converse study, increased mitotic levels of H3S10pho induced by overexpression of the mammalian AIM-1 (Aurora B) kinase led to lagging chromosomes and aneuploidy (82). Thus, regulated levels of H3S10pho are required for proper mitotic progression. One of the proposed mechanisms by which H3S10pho may function is a binary switch responsible for the association /dissociation of the chromodomain protein HP1 from mitotic chromosomes. Phosphorylation of H3S10 in mitosis induces the dissociation of HP1 bound to H3K9me, the latter required for heterochromatin maintenance, while the levels of H3K9me remain unchanged (83, 84). Another study has suggested that phospho-acetylation of H3 (SP10-K14Ac) is required for eviction of HP1 from chromatin (85). Recent work has shown that H3S10pho also regulates the binding of two human SR protein splicing factors, SRp20 and ASF/SF2, with chromatin. These SR proteins associate with interphase and late post-mitotic chromatin, but are dissociated from mitotic chromatin following H3S10 phosphorylation. They also interact with HP1, which fails to dissociate from chromatin when the SR proteins are absent (86). Much like H3S10, H3S28 is also phosphorylated and is closely correlated with chromatin condensation (87). An additional phosphorylation event at threonine 3 (H3T3), catalyzed by the Haspin kinase, has been shown to be required for metaphase chromosome alignment (88). The functional connection between the H3T3 and H3S10 phosphorylation events remains unknown. Phosphorylation of H3 at Thr 45 has been recently reported in budding yeast and linked to DNA replication (89). Although the specific functions of H3 phosphorylation remain to be elucidated, the emerging information points to a dynamic interaction between H3 kinases, phosphatases, and chromatin associated factors required for the formation of the proper chromatin conformation of the mitotic chromosome.

Two other phosphorylations, H2A-S1 and H4S1, are also associated with mitotic chromatin condensation (90). While most histone modifications are at the N-terminal tails, they can be modified elsewhere as well. Phosphorylation of H2A-T119 takes place at the C-terminus and happens specifically during mitosis (91), where it is enriched at centromere regions in Drosophila (92). Recent work has provided a functional link to this modification. In fission yeast, H2A-S121 (equivalent to Drosophila H2A-T119) is phosphorylated by the mitotic kinase Bub1 and recruits shugoshin/Sgo1 to centromeres, which secures proper chromosome partitioning (93). These data establish an essential function for H2A phosphorylation in maintaining mitotic chromosome stability.

### 5.4. Ubiquitination

Ubiquitination is the covalent conjugation of ubiquitin to lysine residues. Histones are usually momoubiquitinated, a modification that does not lead to protein degradation. Monoubiquitination of H2BK123 in *S. cerevisiae* is mediated by the Rad6/Ubc2 ubiquitin conjugating enzyme and the Bre1 ubiquitin ligase. Mutants that are unable to be ubiquitinated at H2BK123 show mitotic and meiotic defects (94). Recently, it has also been shown that ubiquitination of H2BK123 is required for trimethylation of H3K4 and H3K79 (95), a cross talk that has been mainly implicated in the regulation of gene expression. Histone ubiquitination has also been linked to DNA repair. DNA lesions caused by UV-irradiation induce monoubiquitination of histone H2A by the Ring2 ubiquitin ligase (96), as well as ubiquitination of H3 and H4 by the CUL4-DDB-Roc1 ubiquitin ligase complex (97). It is likely that these modifications alter the chromatin structure and facilitate the recruitment of repair proteins to the damage loci.

In mammalian cells, deubiquitination of H2A is required for normal mitosis and cell cycle progression. It is also apparent that deubiquitination of H2A is required for H3S10 phosphorylation (98). Thus, histone ubiquitination has emerged as an important signal for various cellular processes. Further research is needed to determine the specific involvement of this modification in cell cycle progression.

## 6. HISTONE VARIANTS

Histone variants are specialized histones that replace core histones in a DNA-replication independent manner, generating an altered chromatin structure with distinct cellular functions (99).

### 6.1. CenH3

All eukaryotes, from yeast to humans, have a histone H3 variant (called CenH3, in general) that takes the place of the canonical H3 in centromeric nucleosomes. CenH3 is called Cse4 in *S. cerevisiae,* Cnp1 in *S. pombe*, CID in *Drosophila,* and CENP-A in mammals. CenH3 is only 50% identical to the canonical H3, compared with most other histone variants that are more conserved with respect to the canonical histone. CenH3 in *Saccharomyces cerevisiae*, called Cse4, occurs only in one nucleosome per chromosome directly at the centromere because the budding yeast centromeres are only 125bp long (100). On the other hand, higher eukaryotes have regional centromeres that can be up to 1 megabase long; blocks of CenH3-containing nucleosomes are interspersed with blocks of H3-containing nucleosomes (101).

The composition of the centromeric nucleosome in *S. cerevisiae* has been a topic of recent debate. It was reported that a nonhistone protein, Scm3, could assemble with Cse4 and histone H4 to form a centromeric nucleosome hexamer that lacked H2A-H2B (102). A later study showed that Cse4 forms an octameric nucleosome with H2A, H2B, and H4 (103). The latter study suggests that Scm3 is perhaps intimately associated with Cse4-H4 tetramers as an intermediate complex before nucleosome formation, but is not included in the resultant histone octamer.

CenH3-containing nucleosomes are assembled into centromeric chromatin, which becomes the scaffolding

on which the kinetochore is formed during mitosis. CenH3 is essential for the formation of a stable kinetochore. Mutational analysis in *S. cerevisiae* has demonstrated that loss of CenH3 leads to mitotic arrest and missegregation of chromosomes (104). Lastly, CenH3 is an important epigenetic mark in organisms with regional centromeres, as the highly variable centromeric DNA of higher eukaryotes is not sufficient for kinetochore formation. Specification of kinetochore location is directed by the epigenetic mark of CenH3 dilution to daughter DNA strands following S phase, allowing the centromeric chromatin to be heritable (105).

### 6.2. H3.3 and H3.1

In addition to CenH3, there are two other histone H3 variants in higher eukaryotes called H3.1 and H3.3. While not as well characterized as the other histone variants with respect to genome integrity, they do appear to play significant roles in the chromosome cycle. In metazoans, H3.3 is a replication-independent H3 variant that has mainly been implicated as an epigenetic mark for active chromatin (106). Interestingly, *Drosophila* mutants which are deficient for H3.3 display widespread transcriptional defects, sterility, and semi-lethality (107). H3.1 and H3.3 have nearly identical sequences to the canonical H3, with only a stretch of 4 amino acids contributing to the difference in function and selective deposition at specific genetic loci (108). H3.1 is a replication-dependent H3 variant found in mammals, the function of which remains unknown.

### 6.3. H2A.Z

Another histone variant that has been implicated in genome integrity is the H2A variant H2A.Z. Studies in *D. melanogaster* have shown that the loss of H2A.Z leads to depletion of HP1alpha from chromosome arms, thus affecting the integrity of heterochromatin. This defect in forming higher order chromatin structures is likely the cause of the chromosome segregation errors (109). H2A.Z has also been shown to affect chromosome segregation and centromere silencing in the fission yeast *S. pombe.* It was determined that H2A.Z is required for the expression of Cnp3, the *S. pombe* homolog of CENP-C, which is a centromere protein that is essential for maintenance of centromere silencing (110). H2A.Z is not an essential protein in the budding yeast *S. cerevisiae*, but phenotypic and genetic studies have implicated it in genome stability (111). Unlike CenH3, which has a direct effect on chromosome segregation at centromeric regions, H2A.Z has a more indirect effect on genome integrity by affecting heterochromatin at chromosome arms as well as transcription of certain centromeric proteins.

### 6.4. H2AX

Histone modifications provide a critical signal during the DNA damage response, by marking the sites of DNA lesions and making them accessible to the repair machinery (48). In mammalian cells, the histone variant H2AX becomes rapidly phosphorylated in response to double-strand breaks (DSB) (112). The phosphatidylinositol-3-OH kinase-like family of protein kinases, which include ataxia telangiectasia mutated (ATM), ataxia telangiectasia-related (Rad-3 related or ATR) and DNA-dependent protein kinase (DNA-PK), catalyzes the phosphorylation of Ser 139 in the highly conserved carboxy terminal Ser-Gln-Glu (SQE) motif, generating gamma-H2AX (113, 114). In *S. cerevisiae* and *D. melanogaster*, which lack H2AX, a conserved SQ motif is found at the C-terminus of the canonical H2A and the H2Av variant, respectively. Phosphorylation of Ser 129 of H2A in yeast signals DSB repair via non-homologous end-joining (115). The presence of gamma-H2AX on the chromatin surrounding the DNA lesion triggers a signal cascade for the recruitment and retention of the DNA repair proteins to the damaged site, along with chromatin remodeling complexes and mitotic checkpoint factors (116). Recent work has provided evidence that additional post-translational modifications, including acetylation and ubiquitination of gamma-H2AX and other chromatin components, are necessary for the repair process, either through the non-homologous end-joining or homologous recombination pathways (116, 117).

### 6.5. MacroH2A

This is the most atypical histone variant. MacroH2A (mH2A) is a vertebrate specific variant, consisting of an N-terminal domain homologous to the canonical H2A and a large C-terminal region referred to as the macro domain, connected by a basic hinge region (118). This non-histone like region accounts for two thirds of the molecular mass of mH2A. There are two closely related variants, macroH2A1 and macroH2A2, which preferentially associate with the inactive X chromosome (Xi), suggesting a role in transcriptionally repressed chromatin. However, they are also found in autosomes, where they appear to exert a function in gene repression and heterochromatization (119). *In vitro* studies have shown that nucleosomes containing mH2A1 can interfere with chromatin remodeling and transcription initiation (120, 121). Interestingly, the macro domain can bind and maintain in an inactive form poly(ADP-ribose) polymerase 1 (PARP-1), contributing to X chromosome inactivation and gene silencing. Release of mH2A from promoters activates PARP-1, which in turn activates transcription through ADP ribosylation (122). Recently, extensive analysis of hundreds of mH2A targets reveled that they are enriched in genes controlling developmental processes and cell fate decisions (123).

Like other histones, mH2A variants are also subjected to post-translational modifications (124). Phosphorylation of S137 in mH2A1, which resides in the hinge region of mH2A, was shown to be present in male and female cells during mitosis, but excluded from the X chromosome (125). This finding suggests a role for mH2A in chromatin function throughout the cell cycle, outside Xi, and regulated by its own post-translational modifications.

## 7. CONCLUSIONS

In order to maintain the integrity of the genome the cell requires precise temporal and spatial chromatin dynamics during the chromosome cycle. Ample evidence indicates that the proper balance of histones is essential for

maintaining nucleosome assembly and chromatin structure. The centromeric and pericentric regions of the chromosome are particularly sensitive to histone balance, since the incorporation of the cenH3 variant to centromeric regions and the structure of pericentric chromatin are perturbed when histone stoichiometry is altered. Aneuploidy is a common consequence of altered histone balance, likely the result of defective centromere-kinetochore structures, although not necessarily the only cause of it. More studies are needed to understand genomic instabilities associated with defective chromatin assembly during DNA replication as a consequence of histone imbalance.

Histone mutations can be generally divided into two groups, those that affect the globular domain of histones in the core nucleosome particle, and those that affect the flexible histone tails. The first group of mutations usually leads to distortions in the nucleosome architecture, which correlate with phenotypes that can be associated with specific cellular functions. It is becoming apparent that there are domains within the nucleosome particle that may be recognized by specific proteins devoted to distinct cellular functions. This possibility raises questions of specificity, recognition, and targeting that will require extensive research to be elucidated. Most mutations within the histone tails have been induced to study the effect of their post-translational modifications. It is clear that all four histones undergo modifications that are crucial for the chromosome cycle; what is not so clear yet is the specific role of each modification, although some correlations are evident. Examples of such associations are the methylation of H3 lysines and heterochromatin formation, histone deacetylation and mitotic progression, and H3 phosphorylation with chromosome condensation. Most of what we have learned so far comes from studies that abolish individual modifications, either by mutations in the modified amino acid, or by mutations in the modifier enzyme (methyltransferase, acetyltransferase, etc.). The use of modification-specific antibodies has provided major advances in connecting specific histone modifications to cellular functions. Undoubtedly, histones provide key signals in the dynamic behavior of chromatin throughout the chromosome cycle. The difficult task ahead lies in the identification of the proteins that recognize and bind nucleosomal histones in their specific modified state, and to link them to their respective cellular pathway.

## 8. ACKNOWLEDGEMENTS

We would like to thank Dr. David McNabb for critical reading of the manuscript. We would also like to acknowledge support from the National Science Foundation and the Arkansas Biosciences Institute.

## 9. REFERENCES

1. K Luger, AW Mader, RK Richmond, DF Sargent, TJ Richmond: Crystal structure of the nucleosome core particle at 2.8 A resolution, *Nature* 389, 251-260 (1997)

2. CL White, RK Suto, K Luger: Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosome interactions, *EMBO J* 20, 5207-5218 (2001)

3. K van Holde: Chromatin. *Springer-Verlag*, NY (1988)

4. D Xu, J Bai, Q Duan, M Costa,W Dai: Covalent modifications of histones during mitosis and meiosis, *Cell Cycle* 8, 3688-3694 (2009)

5. T Kouzarides: Chromatin Modifications and Their Function, *Cell* 128, 693-705 (2007)

6. BD Strahl, CD Allis: The language of covalent histone modifications, *Nature* 403, 41-45 (2000)

7. K Luger, TJ Richmond: The histone tails of the nucleosome, *Curr Opin Genet Dev* 8, 140-146 (1998)

8. M Grunstein: Yeast heterochromatin: regulation of its assembly and inheritance by histones, *Cell* 93, 325-328 (1998)

9. S Chakravarthy, YJ Park, J Chodaparambil, RS Edayathumangalam, K. Luger: Structure and dynamic properties of nucleosome core particles, *FEBS Lett* 579, 895-898 (2005)

10. D Meeks-Wagner, LH Hartwell: Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission, *Cell* 44, 43-52 (1986)

11. M Smith, V Stirling: Histone H3 and H4 gene deletions in Saccharomyces cerevisiae, *J Cell Biol* 106, 557-566 (1988)

12. D Norris, MA Osley: The two gene pairs encoding H2A and H2B play different roles in the Saccharomyces cerevisiae life cycle, *Mol Cell Biol* 7, 3473-3481 (1987)

13. M Han, M Chang, UJ Kim, M Grunstein: Histone H2B repression causes cell-cycle-specific arrest in yeast: effects on chromosomal segregation, replication, and transcription, *Cell* 48, 589-597 (1987)

14. UJ Kim, M Han, P Kayne, M Grunstein: Effects of histone H4 depletion on the cell cycle and transcription of Saccharomyces cerevisiae, *EMBO J* 7, 2211-2219 (1988)

15. D Norris, B Dunn, MA Osley: The Effect of Histone Gene Deletions on Chromatin Structure in Saccharomyces cerevisiae, *Science* 242, 759-761 (1988)

16. MJ Saunders, E Yeh, M Grunstein, K Bloom: Nucleosome depletion alters the chromatin structure of Saccharomyces cerevisiae centromeres, *Mol Cell Biol* 10, 5721-5727 (1990)

17. AG Castillo, BG Mellone, JF Partridge, W Richardson, GL Hamilton, RC Allshire, AL Pidoux: Plasticity of fission yeast CENP-A chromatin driven by relative levels of histone H3 and H4, *PLoS Genet* 3, e121 (2007)

18. WC Au, MJ Crisp, SZ DeLuca, OJ Rando, MA Basrai: Altered dosage and mislocalization of histone H3 and

Cse4p lead to chromosome loss in Saccharomyces cerevisiae, *Genetics* 179, 263-275 (2008)

19. F Prado, A Aguilera: Partial depletion of histone H4 increases homologous recombination-mediated genetic instability, *Mol Cell Biol* 25, 1526-1536 (2005).

20. I Pinto, F Winston: Histone H2A is required for normal centromere function in Saccharomyces cerevisiae, *EMBO J* 19, 1598-1612 (2000).

21. T Maruyama, T Nakamura, T Hayashi, M Yanagida: Histone H2B mutations in inner region affect ubiquitination, centromere function, silencing and chromosome segregation, *EMBO J* 25, 2420-2431 (2006).

22. BA Morgan, BA Mittman, MM Smith: The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. *Mol Cell Biol* 11, 4111-4120 (1991)

23. PC Megee, BA Morgan, MM Smith: Histone H4 and the maintenance of genome integrity, *Genes Dev* 9, 1716-1727 (1995)

24. MM Smith, P Yang, MS Santisteban, PW Boone, AT Goldstein, PC Megee: A novel histone H4 mutant defective in nuclear division and mitotic chromosome transmission, *Mol Cell Biol* 16, 1017-1026 (1996)

25. J Luo, X Xu, H Hall, EM Hyland, JD Boeke, T Hazbun, MH Kuo: Histone h3 exerts a key function in mitotic checkpoint control, *Mol Cell Biol* 30, 537-549 (2010)

26. J Dai, EM Hyland, DS Yuan, H Huang, JS Bader, JD Boeke: Probing nucleosome function: a highly versatile library of synthetic histone H3 and H4 mutants, *Cell* 134, 1066-1078 (2008)

27. S Nakanishi, BW Sanderson, KM Delventhal, WD Bradford, K Staehling-Hampton, A Shilatifard: A comprehensive library of histone mutants identifies nucleosomal residues required for H3K4 methylation, *Nat Struct Mol Biol* 15, 881-888 (2008)

28. JA Latham, SY Dent: Cross-regulation of histone modifications, *Nat Struct Mol Biol* 14, 1017-1024 (2007)

29. WK Paik and S Kim: Enzymatic methylation of histones, *Arch Biochem Biophys* 134, 632-637 (1969)

30. SS Ng, WW Yue, U Oppermann, RJ Klose: Dynamic protein methylation in chromatin biology, *Cell Mol Life Sci* 66, 407-422 (2009)

31. AJ Bannister, T Kouzarides: Reversing histone methylation, *Nature* 436, 1103-1106 (2005)

32. C Martin, Y Zhang: The diverse functions of histone lysine methylation, *Nat Rev Mol Cell Biol* 6, 838-849 (2005)

33. HD Folco, AL Pidoux, T Urano, RC Allshire: Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres, *Science* 319, 94-97 (2008)

34. E Dunleavy, A Pidoux, R Allshire: Centromeric chromatin makes its mark, *Trends Biochem Sci* 30, 172-175 (2005)

35. RC Allshire, ER Nimmo, K Ekwall, JP Javerzat, G Cranston: Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation, *Genes Dev* 9, 218-233 (1995)

36. K Ekwall: Epigenetic control of centromere behavior, *Annu Rev Genet* 41, 63-81 (2007)

37. AH Peters, D O'Carroll, H Scherthan, K Mechtler, S Sauer, C Schofer, K Weipoltshammer, M Pagani, M Lachner, A Kohlmaier, S Opravil, M Doyle, M Sibilia, T Jenuwein: Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability, *Cell* 107, 323-337 (2001)

38. J Nakayama, JC Rice, BD Strahl, CD Allis, SI Grewal: Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly, *Science* 292, 110-113 (2001)

39. A Serrano, M Rodriguez-Corsino, A Losada: Heterochromatin protein 1 (HP1) proteins do not drive pericentromeric cohesin enrichment in human cells, *PLoS One* 4, e5118 (2009)

40. C Maison, G Almouzni: HP1 and the dynamics of heterochromatin maintenance, *Nat Rev Mol Cell Biol* 5, 296-304 (2004)

41. AJ Bannister, P Zegerman, JF Partridge, EA Miska, JO Thomas, RC Allshire, T Kouzarides: Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain, *Nature* 410, 120-124 (2001)

42. M Lachner, D O'Carroll, S Rea, K Mechtler, T Jenuwein: Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins, *Nature* 410, 116-120 (2001)

43. R Heit, JB Rattner, GK Chan, MJ Hendzel: G2 histone methylation is required for the proper segregation of chromosomes, *J Cell Sci* 122, 2957-2968 (2009)

44. KJ McManus, VL Biron, R Heit, DA Underhill, MJ Hendzel: Dynamic Changes in Histone H3 Lysine 9 Methylations, *J Biol Chem* 281, 8888-8897 (2006)

45. JC Rice, K Nishioka, K Sarma, R Steward, D Reinberg, CD Allis: Mitotic-specific methylation of histone H4 Lys 20 follows increased PR-Set7 expression and its localization to mitotic chromosomes, *Genes Dev* 16, 2225-2230 (2002)

46. G Schotta, M Lachner, K Sarma, A Ebert, R Sengupta, G Reuter, D Reinberg, T Jenuwein: A silencing pathway to

induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin, *Genes Dev* 18, 1251-1262 (2004)

47. E Julien, W Herr: A Switch in Mitotic Histone H4 Lysine 20 Methylation Status Is Linked to M Phase Defects upon Loss of HCF-1, *Mol Cell* 14, 713-725 (2004)

48. D Huertas, R Sendra, P Munoz: Chromatin dynamics coupled to DNA repair, *Epigenetics* 4, 31-42 (2009)

49. SL Sanders, M Portoso, J Mata, J Bahler, RC Allshire, T Kouzarides: Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage, *Cell* 119, 603-614 (2004)

50. MV Botuyan, J Lee, IM Ward, JE Kim, JR Thompson, J Chen, G Mer: Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair, *Cell* 127, 1361-1373 (2006)

51. H Yang, JJ Pesavento, TW Starnes, DE Cryderman, LL Wallrath, NL Kelleher, CA Mizzen: Preferential dimethylation of histone H4 lysine 20 by Suv4-20, *J Biol Chem* 283, 12085-12092 (2008)

52. NT Greeson, R Sengupta, AR Arida, T Jenuwein, SL Sanders: Di-methyl H4 lysine 20 targets the checkpoint protein Crb2 to sites of DNA damage, *J Biol Chem* 283, 33168-33174 (2008)

53. F van Leeuwen, PR Gafken, DE Gottschling: Dot1p modulates silencing in yeast by methylation of the nucleosome core, *Cell* 109, 745-756 (2002)

54. R Wysocki, A Javaheri, S Allard, F Sha, J Cote, SJ Kron: Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9, *Mol Cell Biol* 25, 8430-8443 (2005)

55. B Jones, H Su, A Bhat, H Lei, J Bajko, S Hevi, GA Baltus, S Kadam, H Zhai, R Valdez, S Gonzalo, Y Zhang, E Li, T Chen: The histone H3K79 methyltransferase Dot1L is essential for mammalian development and heterochromatin structure, *PLoS Genet* 4, e1000190 (2008)

56. E Valls, S Sanchez-Molina, MA Martinez-Balbas: Role of histone modifications in marking and activating genes through mitosis, *J Biol Chem* 280, 42592-42600 (2005)

57. WL Cheung, SD Briggs, CD Allis: Acetylation and chromosomal functions, *Curr Opin Cell Biol* 12, 326-333 (2000)

58. M Grunstein: Histone acetylation in chromatin structure and transcription, *Nature* 389, 349-352 (1997)

59. P Jeppesen, A Mitchell, B Turner, P Perry: Antibodies to defined histone epitopes reveal variations in chromatin conformation and underacetylation of centric heterochromatin in human metaphase chromosomes, *Chromosoma* 101, 322-332 (1992)

60. MJ Kruhlak, MJ Hendzel, W Fischle, NR Bertos, S Hameed, XJ Yang, E Verdin, DP Bazett-Jones: Regulation of global acetylation in mitosis through loss of histone acetyltransferases and deacetylases from chromatin, *J Biol Chem* 276, 38307-38319 (2001)

61. L Howe, D Auston, P Grant, S John, RG Cook, JL Workman, L Pillus: Histone H3 specific acetyltransferases are essential for cell cycle progression, *Genes Dev* 15, 3144-3154 (2001)

62. A Mikhailov, M Shinohara, CL Rieder: Topoisomerase II and histone deacetylase inhibitors delay the G2/M transition by triggering the p38 MAPK checkpoint pathway, *J Cell Biol* 166, 517-526 (2004)

63. V Sandor, AR Robbins, R Robey, T Myers, E Sausville, SE Bates, DL Sackett: FR901228 causes mitotic arrest but does not alter microtubule polymerization, *Anticancer Drugs* 11, 445-454 (2000)

64. AR Robbins, SA Jablonski, TJ Yen, K Yoda, R Robey, SE Bates, DL Sackett: Inhibitors of histone deacetylases alter kinetochore assembly by disrupting pericentromeric heterochromatin, *Cell Cycle* 4, 717-726 (2005)

65. HJ Shin, KH Baek, AH Jeon, SJ Kim, KL Jang, YC Sung, CM Kim, CW Lee: Inhibition of histone deacetylase activity increases chromosomal instability by the aberrant regulation of mitotic checkpoint activation, *Oncogene* 22, 3853-3858 (2003)

66. D Cimini, M Mattiuzzo, L Torosantucci, F Degrassi: Histone hyperacetylation in mitosis prevents sister chromatid separation and produces chromosome segregation defects, *Mol Biol Cell* 14, 3821-3833 (2003)

67. G Eot-Houllier, G Fulcrand, Y Watanabe, L Magnaghi-Jaulin, C Jaulin: Histone deacetylase 3 is required for centromeric H3K4 deacetylation and sister chromatid cohesion, *Genes Dev* 22, 2639-2644 (2008)

68. S Ishii, Y Kurasawa, J Wong, LY Yu-Lee: Histone deacetylase 3 localizes to the mitotic spindle and is required for kinetochore-microtubule attachment, *Proc Natl Acad Sci U S A* 105, 4179-4184 (2008)

69. A Vaquero, MB Scher, DH Lee, A Sutton, HL Cheng, FW Alt, L Serrano, R Sternglanz, D Reinberg: SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis, *Genes Dev* 20, 1256-1261 (2006)

70. H Kanta, L Laprade, A Almutairi, I Pinto: Suppressor analysis of a histone defect identifies a new function for the hda1 complex in chromosome segregation, *Genetics* 173, 435-450 (2006)

71. J Yuan, M Pu, Z Zhang, Z Lou: Histone H3-K56 acetylation is important for genomic stability in mammals, *Cell Cycle* 8, 1747-1753 (2009)

72. NL Maas, KM Miller, LG DeFazio, DP Toczyski: Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4, *Mol Cell* 23, 109-119 (2006)

73. I Celic, H Masumoto, WP Griffith, P Meluh, RJ Cotter, JD Boeke, A Verreault: The sirtuins hst3 and Hst4p preserve genome integrity by controlling histone h3 lysine 56 deacetylation, *Curr Biol* 16, 1280-1289 (2006)

74. R Driscoll, A Hudson, SP Jackson: Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56, *Science* 315, 649-652 (2007)

75. J Han, H Zhou, B Horazdovsky, K Zhang, RM Xu, Z Zhang: Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication, *Science* 315, 653-655 (2007)

76. CC Chen, JJ Carson, J Feser, B Tamburini, S Zabaronick, J Linger, JK Tyler: Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair, *Cell* 134, 231-243 (2008)

77. A Ozdemir, S Spicuglia, E Lasonder, M Vermeulen, C Campsteijn, HG Stunnenberg, C Logie: Characterization of lysine 56 of histone H3 as an acetylation site in Saccharomyces cerevisiae, *J Biol Chem* 280, 25949-25952 (2005)

78. SJ Nowak and VG Corces: Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation, *Trends Genet* 20, 214-220 (2004)

79. KM Johansen, J Johansen: Regulation of chromatin structure by histone H3S10 phosphorylation, *Chromosome Res* 14, 393-404 (2006)

80. JY Hsu, ZW Sun, X Li, M Reuben, K Tatchell, DK Bishop, JM Grushcow, CJ Brame, JA Caldwell, DF Hunt, R Lin, MM Smith, CD Allis: Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes, *Cell* 102, 279-291 (2000)

81. Y Wei, L Yu, J Bowen, MA Gorovsky, CD Allis: Phosphorylation of Histone H3 Is Required for Proper Chromosome Condensation and Segregation, *Cell* 97, 99-109 (1999)

82. T Ota, S Suto, H Katayama, ZB Han, F Suzuki, M Maeda, M Tanino, Y Terada, M Tatsuka: Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability, *Cancer Res* 62, 5168-5177 (2002)

83. W Fischle, BS Tseng, HL Dormann, BM Ueberheide, BA Garcia, J Shabanowitz, DF Hunt, H Funabiki, CD Allis: Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation, *Nature* 438, 1116-1122 (2005)

84. T Hirota, JJ Lipp, BH Toh, JM Peters: Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin, *Nature* 438, 1176-1180 (2005)

85. B Mateescu, P England, F Halgand, M Yaniv C Muchardt: Tethering of HP1 proteins to chromatin is relieved by phosphoacetylation of histone H3, *EMBO Rep* 5, 490-496 (2004)

86. RJ Loomis, Y Naoe, JB Parker, V Savic, MR Bozovsky, T Macfarlan, JL Manley, D Chakravarti: Chromatin binding of SRp20 and ASF/SF2 and dissociation from mitotic chromosomes is modulated by histone H3 serine 10 phosphorylation, *Mol Cell* 33, 450-461 (2009)

87. H Goto, Y Tomono, K Ajiro, H Kosako, M Fujita, M Sakurai, K Okawa, A Iwamatsu, T Okigaki, T Takahashi, M Inagaki: Identification of a Novel Phosphorylation Site on Histone H3 Coupled with Mitotic Chromosome Condensation, *Journal of Biological Chemistry* 274, 25543-25549 (1999)

88. J Dai, S Sultan, SS Taylor, JM Higgins: The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment, *Genes Dev* 19, 472-488 (2005)

89. SP Baker, J Phillips, S Anderson, Q Qiu, J Shabanowitz, MM Smith, JR Yates 3rd, DF Hunt, PA Grant: Histone H3 Thr 45 phosphorylation is a replication-associated post-translational modification in S. cerevisiae, *Nat Cell Biol* 12, 294-298 (2010)

90. CM Barber, FB Turner, Y Wang, K Hagstrom, SD Taverna, S Mollah, B Ueberheide, BJ Meyer, DF Hunt, P Cheung, CD Allis: The enhancement of histone H4 and H2A serine 1 phosphorylation during mitosis and S-phase is evolutionarily conserved, *Chromosoma* 112, 360-371 (2004)

91. H Aihara, T Nakagawa, K Yasui, T Ohta, S Hirose, N Dhomae, K Takio, M Kaneko, Y Takeshima, M Muramatsu and T Ito: Nucleosomal histone kinase-1 phosphorylates H2A Thr 119 during mitosis in the early Drosophila embryo, *Genes Dev* 18, 877-888 (2004)

92. AL Brittle, Y Nanba, T Ito, H Ohkura: Concerted action of Aurora B, Polo and NHK-1 kinases in centromere-specific histone 2A phosphorylation, *Exp Cell Res* 313, 2780-2785 (2007)

93. SA Kawashima, Y Yamagishi, T Honda, K Ishiguro, Y Watanabe: Phosphorylation of H2A by Bub1 prevents chromosomal instability through localizing shugoshin, *Science* 327, 172-177 (2010)

94. K Robzyk, J Recht, MA Osley: Rad6-Dependent Ubiquitination of Histone H2B in Yeast, *Science* 287, 501-504 (2000)

95. S Nakanishi, JS Lee, KE Gardner, JM Gardner, YH Takahashi, MB Chandrasekharan, ZW Sun, MA Osley, BD Strahl, SL Jaspersen, A Shilatifard: Histone H2BK123 monoubiquitination is the critical determinant for H3K4 and H3K79 trimethylation by COMPASS and Dot1, *J Cell Biol* 186, 371-377 (2009)

96. S Bergink, FA Salomons, D Hoogstraten, TA Groothuis, H de Waard, J Wu, L Yuan, E Citterio, AB Houtsmuller, J Neefjes, JH Hoeijmakers, W Vermeulen and NP Dantuma: DNA damage triggers nucleotide excision repair-dependent monoubiquitylation of histone H2A, *Genes Dev* 20, 1343-1352 (2006)

97. H Wang, L Zhai, J Xu, HY Joo, S Jackson, H Erdjument-Bromage, P Tempst, Y Xiong, Y Zhang: Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage, *Mol Cell* 22, 383-394 (2006)

98. HY Joo, L Zhai, C Yang, S Nie, H Erdjument-Bromage, P Tempst, C Chang, H Wang: Regulation of cell cycle progression and gene expression by H2A deubiquitination, *Nature* 449, 1068-1072 (2007)

99. HS Malik, S Henikoff: Phylogenomics of the nucleosome, *Nat Struct Biol* 10, 882-891 (2003)

100. S Furuyama, S Biggins: Centromere identity is specified by a single centromeric nucleosome in budding yeast, *Proc Natl Acad Sci U S A* 104, 14706-14711 (2007)

101. DW Cleveland, Y Mao, KF Sullivan: Centromeres and Kinetochores: From Epigenetics to Mitotic Checkpoint Signaling, *Cell* 112, 407-421 (2003)

102. G Mizuguchi, H Xiao, J Wisniewski, MM Smith, C Wu: Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere-specific nucleosomes, *Cell* 129, 1153-1164 (2007)

103. R Camahort, M Shivaraju, M Mattingly, B Li, S Nakanishi, D Zhu, A Shilatifard, JL Workman, JL Gerton: Cse4 is part of an octameric nucleosome in budding yeast, *Mol Cell* 35, 794-805 (2009)

104. S Stoler, KC Keith, KE Curnick, M Fitzgerald-Hayes: A mutation in CSE4, an essential gene encoding a novel chromatin-associated protein in yeast, causes chromosome nondisjunction and cell cycle arrest at mitosis. *Genes Dev* 9, 573-586 (1995)

105. RS Gieni, GK Chan, MJ Hendzel: Epigenetics regulate centromere formation and kinetochore function, *J Cell Biochem* 104, 2027-2039 (2008)

106. K Ahmad, S Henikoff: The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly, *Mol Cell* 9, 1191-1200 (2002)

107. A Sakai, BE Schwartz, S Goldstein, K Ahmad: Transcriptional and developmental functions of the H3.3 histone variant in Drosophila, *Curr Biol* 19, 1816-1820 (2009)

108. SJ Elsaesser, AD Goldberg, CD Allis: New functions for an old variant: no substitute for histone H3.3, *Curr Opin Genet Dev* 20, 110-117 (2010)

109. D Rangasamy, I Greaves, DJ Tremethick: RNA interference demonstrates a novel role for H2A.Z in chromosome segregation, *Nat Struct Mol Biol* 11, 650-655 (2004)

110. H Hou, Y Wang, SP Kallgren, J Thompson, JR Yates 3rd, S Jia: Histone variant H2A.Z regulates centromere silencing and chromosome segregation in fission yeast, *J Biol Chem* 285, 1909-1918 (2010)

111. NJ Krogan, K Baetz, M Keogh, N Datta, C Sawa, TCY Kwok, NJ Thompson, MG Davey, J Pootoolal, TR Hughes, A Emili, S Buratowski, P Hieter, JF Greenblatt: Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4, *Proc Natl Acad Sci U S A* 101, 13513-13518 (2004)

112. J Fillingham, MC Keogh, NJ Krogan: GammaH2AX and its role in DNA double-strand break repair, *Biochem Cell Biol* 84, 568-577 (2006)

113. EP Rogakou, DR Pilch, AH Orr, VS Ivanova, WM Bonner: DNA Double-stranded Breaks Induce Histone H2AX Phosphorylation on Serine 139, *J Biol Chem* 273, 5858-5868 (1998)

114. Y Shiloh: ATM and related protein kinases: safeguarding genome integrity, *Nat Rev Cancer* 3, 155-168 (2003)

115. JA Downs, NF Lowndes, SP Jackson: A role for Saccharomyces cerevisiae histone H2A in DNA repair, *Nature* 408, 1001-1004 (2000)

116. H van Attikum, SM Gasser: Crosstalk between histone modifications during the DNA damage response, *Trends Cell Biol* 19, 207-217 (2009)

117. AE Escargueil, DG Soares, M Salvador, AK Larsen, JAP Henriques: What histone code for DNA repair? *Mutat Res* 658, 259-270 (2008)

118. JR Pehrson, V Fried: MacroH2A, a core histone containing a large nonhistone region, *Science* 257, 1398-1400 (1992)

119. C Costanzi, JR Pehrson: Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals, *Nature* 393, 599-601 (1998)

120. D Angelov, A Molla, PY Perche, F Hans, J Cote, S Khochbin, P Bouvet, S Dimitrov: The histone variant macroH2A interferes with transcription factor binding and SWI/SNF nucleosome remodeling, *Mol Cell* 11, 1033-1041 (2003)

121. CM Doyen, W An, D Angelov, V Bondarenko, F Mietton, VM Studitsky, A Hamiche, RG Roeder, P Bouvet, S Dimitrov: Mechanism of polymerase II transcription repression by the histone variant macroH2A, *Mol Cell Biol* 26, 1156-1164 (2006)

122. DA Nusinow, I Hernández-Muñoz, TG Fazzio, GM Shah, WL Kraus, B Panning: Poly(ADP-ribose) Polymerase 1 Is Inhibited by a Histone H2A Variant, MacroH2A, and Contributes to Silencing of the Inactive X Chromosome, *J Biol Chem* 282, 12851-12859 (2007)

123. M Buschbeck, I Uribesalgo, I Wibowo, P Rue, D Martin, A Gutierrez, L Morey, R Guigo, H Lopez-Schier, L Di Croce: The histone variant macroH2A is an epigenetic regulator of key developmental genes, *Nat Struct Mol Biol* 16, 1074-1079 (2009)

124. AA Thambirajah, A Li, T Ishibashi, J Ausio: New developments in post-translational modifications and functions of histone H2A variants, *Biochem Cell Biol* 87, 7-17 (2009)

125. E Bernstein, TL Muratore-Schroeder, RL Diaz, JC Chow, LN Changolkar, J Shabanowitz, E Heard, JR Pehrson, DF Hunt, CD Allis: A phosphorylated subpopulation of the histone variant macroH2A1 is excluded from the inactive X chromosome and enriched during mitosis, *Proc Natl Acad Sci U S A* 105, 1533-1538 (2008)

**Key Words:** Histones, Histone mutations, Histone modifications, Chromosome Cycle, Genome Integrity, Chromosome Stability, Review

**Send correspondence to:** Ines Pinto, Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701Tel: 479-575-6339, Fax: 479-575-4010, E-mail: ipinto@uark.edu

http://www.bioscience.org/current/vol17.htm