Cell PRESS
Developmental Cell
Review

Histone Variants in Metazoan Development

Laura A. Banaszynski,[1,2] C. David Allis,[1] and Peter W. Lewis[1,2,*]
[1]Laboratory of Chromatin Biology and Epigenetics, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
[2]These authors contributed equally to this work
*Correspondence: plewis@rockefeller.edu
DOI 10.1016/j.devcel.2010.10.014

Embryonic development is regulated by both genetic and epigenetic mechanisms, with nearly all DNA-templated processes influenced by chromatin architecture. Sequence variations in histone proteins, core components of chromatin, provide a means to generate diversity in the chromatin structure, resulting in distinct and profound biological outcomes in the developing embryo. Emerging literature suggests that epigenetic contributions from histone variants play key roles in a number of developmental processes such as the initiation and maintenance of pericentric heterochromatin, X-inactivation, and germ cell differentiation. Here, we review the role of histone variants in the embryo with particular emphasis on early mammalian development.

Introduction

Mammalian development requires the specification of over 200 unique cell types from a single totipotent cell. This challenge is all the more impressive considering that each of these cell types carries the same genetic information and yet is able to establish and maintain the unique gene expression profile that gives rise to its cellular identity. These heritable changes in cellular phenotype or gene expression caused by mechanisms other than the underlying DNA sequence are referred to as epigenetic phenomena. Differentiated cells rely on an epigenetic memory in order to transmit and maintain gene expression patterns that distinguish the differentiated state through multiple cell divisions (Hemberger et al., 2009). The molecular basis of this epigenetic memory is currently an intense area of research.

Tissue and lineage-specific transcription factors play critical roles in regulating and defining cellular identity, but they are not its sole determinants. For instance, somatic nuclei that have undergone reprogramming by transfer to anucleated eggs exhibit molecular signs of their tissue of origin, expressing genes characteristic of their previous differentiated state (Ng and Gurdon, 2005). Similar results are seen in differentiated cells reprogrammed to earlier developmental states by the expression of a small number of transcription factors (Kim et al., 2010; Polo et al., 2010). In addition to sequence-specific transcription factors, epigenetic memory is transmitted through *trans*-acting factors such as Polycomb and Trithorax proteins, DNA methylation, noncoding RNAs, histone modifications, and histone variants (Guttman et al., 2009; Henikoff, 2008; Klose and Bird, 2006; Rando and Chang, 2009). Of these, histones, the core component of chromatin, provide a particularly attractive candidate for shaping the features of a cell's epigenetic landscape.

The highly conserved H2A, H2B, H3, and H4 histones compose the proteinaceous core of the nucleosome, the fundamental repeating unit of chromatin. In all metazoans, the majority of histones in dividing cells are transcribed and translated in a cell-cycle-dependent manner from large, multicopy, intronless clusters (Albig and Doenecke, 1997). These canonical histones are deposited into nucleosomes in a replication-coupled manner to ensure proper packaging of genomic DNA. In addition to their structural role in genome organization, histones are subject to a variety of posttranslational modifications that are associated with nearly all DNA-templated processes, with far reaching consequences for cell-fate decisions (Taverna et al., 2007). While research on the mechanism of epigenetic inheritance has focused primarily on histone modifications, an alternative means to encode and transmit information is through the incorporation of histone variant proteins into chromatin. With the exception of H4, all core histone proteins in mammals have several sequence variants. These variants can contain minor sequence differences (e.g., the canonical H3.1 and H3.2, and the variant H3.3) or significant structural dissimilarities (e.g., macroH2A, the centromere-specific protein CENP-A), and may exhibit different cell-type specific expression levels (Rogakou and Sekeri-Pataryas, 1999). Histone variants can be classified into replication dependent, replication independent, and tissue specific based on their temporal and developmental expression profiles.

Why has the cell evolved histone variants, some of which are highly conserved across the phylogenetic tree? Chromatin is, by necessity, a dynamic structure subject to factors that continuously disrupt and remodel nucleosomes in order to access the underlying DNA (Ho and Crabtree, 2010). The reestablishment of nucleosomes at these sites of activity is required to maintain genomic stability and often occurs through replication-independent pathways when newly synthesized canonical histones are unavailable. Instead, the cell has access to replication-independent histone variants, usually represented by one or two genes synthesized throughout the cell cycle, in addition to being expressed in terminally differentiated cells that no longer undergo DNA replication (Frank et al., 2003). As well as providing the cell with a continuous source of histones for nucleosome replacement outside of S phase, these histone variants allow the cell to generate biochemically unique nucleosomes for the regulation of chromatin metabolism. The major histone variants in metazoans belong to the replication-independent class of histone genes. Temporal and tissue-specific expression, coupled with exclusive deposition machinery, give histone variants a unique ability to regulate key developmental processes. Here, we will review the known functions for histone variants and their deposition machinery in mammalian development,

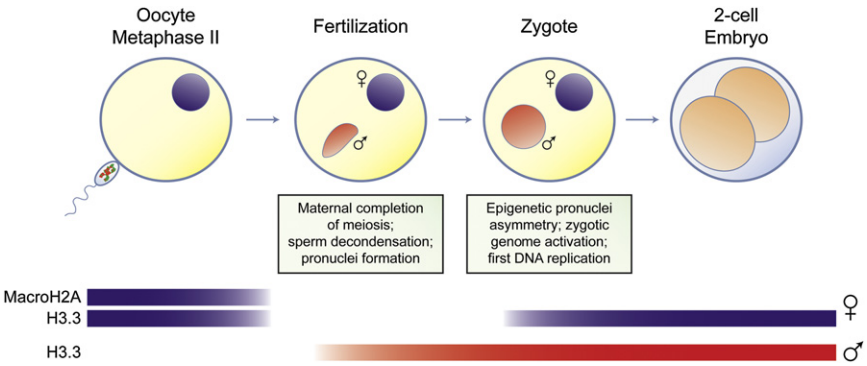

Figure 1. Chronology of Mouse Oocyte Fertilization and Associated Chromatin-Related Events

Upon fertilization, the oocyte resumes meiosis, resulting in formation of the maternal pronucleus and eviction of histones H3.3 and macroH2A from the maternal chromatin. Concurrent with sperm decondensation and formation of the paternal pronucleus, protamines are exchanged for histones resulting in nucleosomal chromatin. At this time, histone H3.3 is preferentially deposited into the male pronucleus before the onset of transcriptional activation. H3.3 incorporation in the maternal genome is not observed until nearly an hour after formation of the pronucleus. By the late pronuclear stage, after the onset of transcription and the first DNA replication, H3.3 levels in the parental pronuclei appear equivalent. MacroH2A, however, is not observed again in chromatin until later developmental stages.

with studies performed in mouse model systems unless otherwise noted, focusing specifically on the roles of histone variants in the initiation and maintenance of epigenetic memory.

H3.3 and the Developing Embryo

Upon fertilization, the genomes of two highly specialized cells, the oocyte and the sperm cell, undergo a tightly regulated sequence of reprogramming events that produce a totipotent zygote (Figure 1). Before fertilization, the oocyte is arrested in metaphase of its second meiotic division. Entry of the sperm into the oocyte cytoplasm leads to completion of maternal meiosis, resulting in formation of the polar body and the haploid maternal pronucleus. At the same time, the paternal genome of the sperm cell is stripped of sperm-specific proteins called protamines and repackaged with maternally stored histones, resulting in nucleosomal chromatin and formation of the haploid paternal pronucleus. The parental pronuclei remain physically separate in the zygote cytoplasm, undergoing one round of DNA replication before the parental genomes finally align at a common metaphase plate.

Immediately following fertilization, the maternal and paternal pronuclei exhibit asymmetric epigenetic profiles, including differential DNA methylation, posttranslational modification (PTM) of histone proteins, and incorporation of histone variants (Surani et al., 2007). The first reprogramming event of development is the establishment of nucleosomal chromatin on the paternal genome. This remodeling event occurs independently of DNA replication, with the replication-independent histone variant H3.3 preferentially incorporated into the paternal genome concomitant with formation of the paternal pronucleus (Torres-Padilla et al., 2006). The variant H3.3 differs from canonical H3.1 by just four amino acid changes in the core of the histone fold—an AAIG motif from residues 87–90 in place of the SAVM motif found in H3.1 and a C to S mutation at amino acid 96. An additional mutation of A to S is found at amino acid 31, and this residue is known to be phosphorylated in H3.3 (Hake and Allis, 2006). Unlike *h3.1*, an intronless gene repeated in multiple gene clusters whose transcript is regulated by stem-loop binding proteins (Pandey and Marzluff, 1987), H3.3 is encoded by two intron-containing genes, *h3.3A* and *h3.3B*, whose transcripts are regulated through polyadenylation and whose translation results in identical protein products (Wellman et al., 1987). Consistent with the asymmetrical incorporation of H3.3 into the paternal genome, the presence of the H3.3-specific chaperone HIRA (Tagami et al., 2004) has been reported specifically in the male pronucleus during this developmental time frame (van der Heijden et al., 2005), and both HIRA and the ATP-dependent chromatin remodeling factor CHD1 were shown to be necessary for protamine replacement by histone H3.3 in the *Drosophila melanogaster* embryo (Jayaramaiah Raja and Renkawitz-Pohl, 2005; Konev et al., 2007; Loppin et al., 2005). H3.3 is not observed in the maternal pronucleus until nearly an hour after it has been deposited into the paternal genome (Torres-Padilla et al., 2006), corresponding to the differential onset of zygotic genome activation with transcripts first observed from the paternal genome (Aoki et al., 1997). Histone H3.3 has traditionally been associated with gene activation, but several observations suggest an additional or alternative role for the variant in the early embryo. For example, while H3.3 is asymmetrically present in the male pronucleus, paternal chromatin at this stage is devoid of trimethylation of histone H3 at lysine 4 (H3K4me3), a modification associated with gene activation. Furthermore, H3.3 incorporation is observed before the onset of transcription in both the paternal and maternal pronuclei (Torres-Padilla et al., 2006). Instead, recent studies discussed below suggest that H3.3 in the paternal pronucleus is necessary for the establishment of highly specialized chromatin structures in the early embryo.

To ensure proper chromosome segregation during the first mitosis, the pronuclei of the zygote must establish chromatin structures consisting of the centromeric heterochromatin responsible for kinetochore formation and the pericentric heterochromatin that facilitates chromatid cohesion. Heterochromatin Protein 1 (HP1) is a fundamental component of pericentric heterochromatin in all metazoans. Interaction between the chromodomain of HP1 and H3K9me3, a histone modification enriched in pericentric heterochromatin, is important for recruitment of HP1 and maintenance of pericentric heterochromatin (Jacobs and Khorasanizadeh, 2002; Nakayama et al., 2001). Therefore, the establishment of H3K9me3 is a critical, early step in the formation of pericentric heterochromatin. Studies performed in *Schizosaccharomyces pombe* have uncovered a direct role for the RNAi pathway in the formation of pericentric heterochromatin that functions in part through HP1 and

Table 1. Known Functions for Histone Variants and Their Deposition Machinery in Mouse Development

Histone Variant	Chaperone	Location	Variant Phenotype	Chaperone Phenotype	References
H3.3	HIRA	euchromatin, gene bodies, promoter	N.D.	embryonic lethality (6.5–10.5 dpc)	Roberts et al., 2002
H3.3	Daxx-ATRX	telomeres, pericentric repeats	N.D.	embryonic lethality (9.5 dpc)	Michaelson et al., 1999; Garrick et al., 2006
H3T	sNASP	testes	N.D.	N.D.	Franklin and Zweidler, 1977; Witt et al., 1996
CENP-A	HJURP	centromere	embryonic lethality (3.5–8.5 dpc)	N.D.	Howman et al., 2000
H2A.Z	Chz1, SWR1	regulatory elements, promoter, pericentric repeats	embryonic lethality (4.5–7.5 dpc)	lethality (*C. elegans*)	Faast et al., 2001; Updike and Mango, 2006
H2AX	N.D.	XY Body, sites of double-strand DNA breaks	male sterility, reduced fecundity in females	N.D.	Bassing et al., 2003
MacroH2A	N.D.	inactive X, promoters	severe malformations in the brain (zebrafish)	N.D.	Costanzi et al., 2000; Buschbeck et al., 2009
H2AL	N.D.	percentric repeats	N.D.	N.D.	van Roijen et al., 1998
H2A.Bbd	N.D.	N.D.	N.D.	N.D.	Chadwick and Willard, 2001
TH2A	N.D.	testes	N.D.	N.D.	Trostle-Weige et al., 1982
TH2B	N.D.	testes	N.D.	N.D.	Brock et al., 1980

N.D., not determined

H3K9me3 (Grewal, 2010). Additionally, heterochromatic silencing in *Drosophila* is also dependent upon the RNAi machinery, suggesting involvement of this pathway is conserved in metazoan heterochromatin formation (Kavi and Birchler, 2009; Pal-Bhadra et al., 2004).

In the zygote, unique heterochromatic structures are formed consisting of pericentric and centromeric chromatin from different chromosomes arranged in rings surrounding the nucleolar-like bodies (NLBs) (Probst et al., 2007). Intriguingly, similar structures are formed in both the maternal and paternal pronuclear bodies despite their epigenetic asymmetries. While female pronuclear pericentric heterochromatin is enriched in H3K9me3 (Probst et al., 2007; Santos et al., 2005), the paternal pronuclear pericentric heterochromatin shows enrichment of H3K27me3 (Puschendorf et al., 2008) and the histone variant H3.3 (Torres-Padilla et al., 2006). Recent studies suggest that deposition of H3.3 into the paternal genome plays a key role in the establishment of pericentric heterochromatin before the first mitosis (Santenard et al., 2010). Specifically, mutation of lysine 27 to arginine on histone H3.3 (H3.3K27R), but not H3.1, resulted in decreased developmental progression from zygote to blastocyst stage. These embryos displayed defects in chromosome segregation, mislocalization of HP1β, and ineffective heterochromatinization of pericentric repeat regions at the 2-cell stage. Intriguingly, this phenotype was mimicked by deletion of HP1β's hinge region, a domain essential for pericentric enrichment of HP1β and for the interaction of HP1β with RNA (Muchardt et al., 2002). Injection of pericentric double-stranded RNA (dsRNA) into zygotes expressing H3.3K27R rescued these developmental phenotypes, allowing embryos to progress to the blastocyst stage (Santenard et al., 2010). These results suggest that the inability to posttranslationally modify H3.3K27 in the zygote leads to defects in the initiation of pericentric transcription during the first S phase, a process that may be required for the initiation of heterochromatin formation through the RNAi pathway (Grewal, 2010). However, the exact role of posttranslational modification of H3.3K27 in this process remains to be determined.

Consistent with the deposition of H3.3 into the paternal genome, the H3.3-specific chaperone HIRA is present in the paternal pronucleus (van der Heijden et al., 2005). Additionally, mutations in the *Drosophila* HIRA homolog, *sesame*, prevent male pronucleus formation (Bonnefoy et al., 2007; Loppin et al., 2005). However, recent studies demonstrate that multiple independent pathways are required for H3.3 deposition and that these pathways are highly specific for deposition at particular genomic regions (Table 1). A study in mouse embryonic stem cells demonstrated that HIRA is mainly responsible for deposition of H3.3 into protein-coding regions, specifically at the transcription start sites of genes with high CpG content promoters regardless of gene activity and in the gene body of active genes (Goldberg et al., 2010). Intriguingly, H3.3 enrichment is also highly correlated with intergenic regulatory elements that contain occupied transcription factor binding sites and at regions typically associated with heterochromatin, specifically at telomeres and other transcribed repeat regions including pericentric satellites (Figure 2). Deposition of H3.3 at these repeat regions is independent of HIRA and is mediated by specific interaction of H3.3 with Daxx, a protein previously associated with apoptosis, and ATRX, a SNF2 family chromatin remodeling protein (Drane et al., 2010; Goldberg et al., 2010; Lewis et al., 2010; Wong et al., 2010). Interestingly, ATRX is found at preicentric heterochromatin in mammalian oocytes and is important for proper chromosome alignment and segregation during meiosis (Baumann et al., 2010; De La Fuente et al., 2004). It is tempting to speculate that ATRX in the oocyte is associated with the maternal pool of H3.3 found in the germinal vesicle (this pool of H3.3 is excluded from the maternal chromatin during resumption of meiosis following fertilization) (Torres-Padilla et al., 2006). In addition, it will be important to

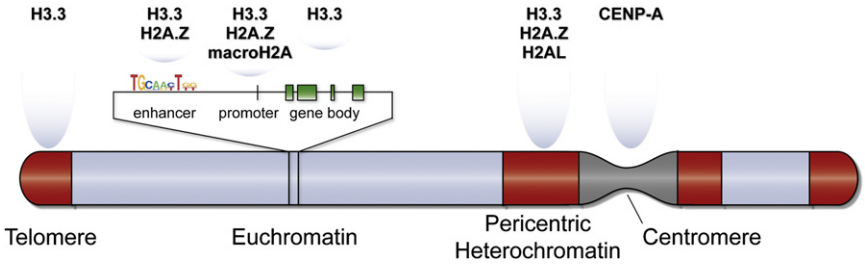

Figure 2. Known Genomic Locations of Histone Variants

Immunofluorescence and genome-wide ChIP studies have identified the chromosomal locations for some histone variants. The deposition of H3.3 at telomeres and pericentric heterochromatin occurs via the Daxx-ATRX complex, whereas the HIRA mediates H3.3 chromatin assembly in the bodies of actively transcribed and high CpG content promoter genes. An unknown deposition pathway is used to assemble H3.3 nucleosomes at enhancer elements and gene promoters. Similar to H3.3, H2A.Z is also associated with gene-specific enhancers and promoters, in addition to pericentric heterochromatin. H2AL is found in pericentric heterochromatin during the later stages of spermatogenesis. In addition to the XY body and inactive X chromosome, macroH2A is also associated with the promoter of some genes. The centromere-specific H3 variant, CENP-A, is deposited at centromeric chromatin.

analyze the maternal and paternal pronuclei for the presence of ATRX and Daxx to determine whether these pathways are instrumental for H3.3 deposition in the zygote or if they represent a deposition pathway only active in more differentiated cells. Of note, insects infected by the parasitic bacterium *Wolbachia* may provide a useful model system for discovering additional factors and pathways required for H3.3 deposition in early development, as it was recently shown that the progeny from a cross between uninfected females and bacteria-infected males exhibit early embryonic lethality, in part due to delays in H3.3 deposition following protamine removal from the male pronucleus (Landmann et al., 2009). These studies reinforce the hypothesis that H3.3 deposition plays an important role in the early developing embryo.

Multiple independent pathways are required for H3.3 deposition at specific genomic regions, but why these distinct chaperone systems have evolved and how they are targeted to specific locations remain outstanding questions. In the absence of HIRA in embryonic stem cells, a subset of transcription factor binding sites show enhanced enrichment of H3.3 with respect to wild-type cells (Goldberg et al., 2010), suggesting that these chaperones may be competing for limited pools of H3.3. Asf1 is an H3-H4 histone chaperone that is thought to bind newly synthesized histones, facilitating their genomic deposition though either replication-coupled (for H3.1) or replication-independent (for H3.3) mechanisms via chaperone-chaperone interactions with CAF-1 and HIRA respectively. Is it possible that Asf1 also facilitates the molecular hand-off of H3.3 to its multiple deposition pathways? It is likely that allocation of H3.3 to these distinct pathways is controlled by a number of factors, including intracellular concentrations and binding affinities of the various complexes for H3.3, as well as kinetics of deposition, with genomic localization possibly dictated by the chaperone systems themselves.

H2A Variants and X-Inactivation

Fertilized zygotes maintain epigenetic asymmetry until the 8-cell stage, at which time parent-specific differences in histone post-translational modification are resolved (Puschendorf et al., 2008). Until about this stage, the early blastomeres are considered to be totipotent and identical in their developmental potential. Although the exact stage at which blastomeres in the cleavage stage embryo become unequal remains controversial, it is clear that the first differentiation event in mammalian development has occurred by the time the embryo reaches the 16-cell morula stage. The morula consists of a small group of internal cells called the inner cell mass (ICM) that will give rise to the embryo, surrounded by a group of external cells that will become the trophoblast, contributing to the extraembryonic tissues that combine with the maternal endometrium to form the placenta. By the time the embryo has undergone compaction and reached the blastocyst stage, these two lineages are developmentally distinct, with cell identity reinforced through lineage-specific gene expression profiles. For a schematic of this stage of development, see Figure 3.

The histone variant H2A.Z plays an important role in the very early embryo as mice lacking this histone variant die shortly after implantation (Faast et al., 2001). Histone H2A.Z was first identified as a histone variant that was specifically enriched in the transcriptionally active chromatin of the ciliate *Tetrahymena thermophila* (Allis et al., 1980). Nucleosomes containing H2A.Z can make up 10% of mammalian genomes (Iouzalen et al., 1996), and genome-wide chromatin immunoprecipitation (ChIP) studies in *Saccharomyces cerevisiae* show that H2A.Z (Htz1) localizes primarily to promoter regions (Figure 2) (Zhang et al., 2005a). H2A.Z is deposited via the histone chaperone, Chz1, and the SWR1 chromatin-remodeling complex (Luk et al., 2007; Mizuguchi et al., 2004). Surprisingly, the role for H2A.Z in early development may be in the establishment of heterochromatin. At the blastocyst stage of development, cells found in the ICM appear to lack H2A.Z. As cells located at the outer edge of the ICM differentiate, H2A.Z accumulates at sites of pericentric heterochromatin and other nuclear sites (Figure 2) (Rangasamy et al., 2003). While H2A.Z is enriched in constitutive pericentric heterochromatin, another genomic region of constitutive heterochromatin, the inactive X chromosome, is depleted of H2A.Z (Rangasamy et al., 2003). The localization of H2A.Z nucleosomes to some, but not all, sites of constitutive heterochromatin suggests that these nucleosomes have an unknown specialized function in the establishment or maintenance of heterochromatin. In support of a specialized function in heterochromatin, cells lacking H2A.Z exhibited poor HP1α binding to heterochromatin and defects in chromosome segregation (Rangasamy et al., 2004) despite the presence of H3K9me3 (Greaves et al., 2007). In fact, in vitro binding studies found that nucleosomes containing H2A.Z bind HP1α more efficiently than those containing H2A (Fan et al., 2004). Together, these data indicate that H2A.Z plays a direct role in HP1α localization to heterochromatin.

Zygotic genome activation is a hallmark of the maternal-to-zygotic transition. To equalize expression of genes encoded on the

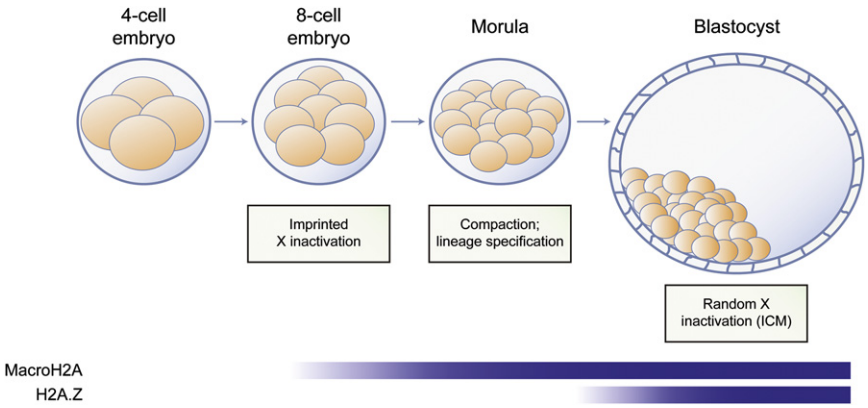

Figure 3. Developmental Transitions and Changes in Histone Variant Incorporation from the Early Embryo to the Blastocyst Stage

At the 8-cell stage, after the initiation of X-inactivation, macroH2A is deposited specifically on the silenced paternal X chromosome. MacroH2A remains present and associated with the inactive X throughout development. In the extraembryonic trophoblast, where imprinted X-inactivation is maintained, macroH2A remains associated with the paternal X chromosome. The cells of the inner cell mass (ICM) undergo transient reactivation followed by random inactivation of the X chromosome; here, macroH2A is enriched on the silenced X chromosome. H2A.Z incorporation in the early embryo is also associated with gene silencing, with enrichment observed at regions of pericentric heterochromatin as the outer cells of the ICM begin to differentiate. While present at pericentric heterochromatin, H2A.Z is absent from the inactive X chromosome.

sex-determining X chromosome, female embryos initiate dosage compensation by transcriptionally silencing one X chromosome, a process termed X-inactivation (Lyon, 1961). Imprinted inactivation of the paternal X chromosome occurs first at the 4-cell stage and is maintained in the trophectoderm and primitive endoderm of the blastocyst, whereas random inactivation of either maternal or paternal X chromosome occurs later in the epiblast. Both forms of X-inactivation are initiated by Xist, a long non-coding RNA that coats the X chromosome in *cis* (Marahrens et al., 1997; Penny et al., 1996). Xist coating is followed by a series of epigenetic events, including differential posttranslational modification of histones on the active and inactive X chromosomes (Heard et al., 2001; Mak et al., 2002; Plath et al., 2003; Silva et al., 2003) and changes in chromatin composition (Costanzi et al., 2000).

Upon inactivation, the X chromosome undergoes changes in chromatin structure that are thought to facilitate transcriptional silencing. One such change is the incorporation of an H2A variant called macroH2A. This variant represents about 5% of the total H2A protein in mammalian genomes (Timinszky et al., 2009) and is unusually large for a histone protein. Its core region shares more than 60% identity with canonical H2A, but it also contains a large (>210 amino acids) C-terminal extension composed of a nonhistone domain called the macro domain (Pehrson and Fried, 1992), which is part of a family of highly conserved ligand binding domains found in all organisms. Mammals have three different macroH2A isoforms: macroH2A.2 and splice variants macroH2A1.1 and macroH2A1.2, with the latter two subtypes found enriched on the inactive X chromosome (Chadwick et al., 2001; Costanzi and Pehrson, 1998). A transient asymmetry of macroH2A exists in the parental pronuclei of the zygote, with macroH2A enriched in the maternal pronucleus, representing a maternal store of the H2A variant likely deposited during oocyte maturation (Chang et al., 2005). Upon fertilization, macroH2A is evicted from maternal chromatin (Chang et al., 2005) and is then undetectable in the early cleavage stage embryos until after the 8-cell stage (Chang et al., 2005; Costanzi et al., 2000). Embryonic macroH2A is found in both cells of the inner cell mass and of the trophectoderm in the preimplantation embryo and is associated with the inactive X chromosome. As Xist accumulation and imprinted paternal X inactivation occur as early as the 4-cell stage, it is unlikely that macroH2A plays a role in the initiation of X inactivation. Interestingly, macroH2A appears to accumulate at times of transcriptional repression (e.g., the mature oocyte, the onset of X inactivation), leading to the hypothesis that macroH2A might be involved in this process.

Additional observations suggest a more general role for macroH2A in transcriptional repression beyond X inactivation. Indeed, macroH2A is conserved not only in mammals but also in heterogametic (ZW) female and homogametic (ZZ) male birds that likely do not achieve dosage compensation through silencing of a male Z chromosome (Mank, 2009). Moreover, in both developing embryos and more differentiated cell types, macroH2A has been shown to be associated with regions of heterochromatin (Costanzi et al., 2000; Grigoryev et al., 2004; Zhang et al., 2005b) and the inactive alleles of imprinting control regions associated with DNA methylation (Choo et al., 2006, 2007). Interestingly, the presence of the macro domain in chromatin inhibits transcription initiation in vitro, perhaps by rendering nucleosomes resistant to SWI/SNF-mediated chromatin remodeling (Angelov et al., 2003; Doyen et al., 2006; Perche et al., 2000). Further supporting a putative transcriptional repression role for macroH2A, the histone variant inactivates heat-shock-responsive genes through a repressive interaction with the poly (ADP-ribose) polymerase-1 (PARP1), with PARP1 repression relieved upon release of macroH2A from the promoter region of these genes (Ouararhni et al., 2006). Inhibition of PARP1 by macroH2A also appears to play a role in X-inactivation (Menissier de Murcia et al., 2003; Nusinow et al., 2007). Interestingly, the macro domain binds to ADP-ribose, the product of PARP1 activity, and some of its derivatives, suggesting that macroH2A responds to these enzymatic activities (Kustatscher et al., 2005; Timinszky et al., 2009). However, the consequences of metabolite binding by macroH2A remain unclear.

Two recent studies have attempted to shed light on the role of macroH2A incorporation into autosomal chromosomes (Buschbeck et al., 2009; Gamble et al., 2010). Using ChIP and genomic tiling microarrays, these studies have shown a general negative correlation between macroH2A occupancy and gene expression

levels, in agreement with the hypothesis that macroH2A plays a role in transcriptional repression. However, not all genes occupied by macroH2A are repressed (Figure 2). Indeed, about 12% of active genes profiled show macroH2A enrichment, suggesting a more complicated role for macroH2A in gene regulation (Gamble et al., 2010). MacroH2A's role in transcriptional repression is further challenged by studies showing that the presence of the variant at the inactive X chromosome is not required for the maintenance of X-inactivation in differentiated cells (Csankovszki et al., 1999). Of course, it is worth noting that multiple and redundant mechanisms likely exist to maintain X-inactivation in adult organisms. For example, while Xist is required for the initiation of X-inactivation, maintenance of the inactive X persists even in the absence of Xist (Csankovszki et al., 1999; Ng et al., 2007). While a mechanistic understanding of macroH2A's function on chromatin remains elusive, it is clearly of some importance, as macroH2A-deficient zebrafish show severe developmental defects, including deformation of the body structure and malformations in the brain (Buschbeck et al., 2009).

Recent work has extended our knowledge of the variants involved in X-inactivation beyond those of H2A. The enrichment of ATRX (a chaperone required for H3.3 deposition at DNA repeat regions) on the inactive X chromosome during random X-inactivation in differentiating stem cells suggest that H3.3 may also play a role in the process (Baumann and De La Fuente, 2009). In addition, ATRX was found to be associated with the imprinted inactive X chromosome in trophoblast stem cells and maintained its association upon differentiation (Baumann and De La Fuente, 2009). Interestingly, conditional inactivation of ATRX at the 8- to 16-cell stage of development leads to defects in formation of the extraembryonic trophoblast, resulting in male embryonic lethality at 9.5 dpc (Garrick et al., 2006). *atrx* is located on the X chromosome, so females harboring a maternal null allele should also be affected. However, some carriers of the allele are able to escape imprinted X inactivation of the wild-type *atrx* allele and are able to establish a normal placenta. These results show a clear dependence on *atrx* for maintenance of extraembryonic tissues, but it remains to be seen whether this is due to the involvement of ATRX in X-inactivation, a more general requirement of ATRX for the establishment of heterochromatin, or an as yet unknown function of ATRX. Due to the recently established role of ATRX in H3.3 deposition as discussed above, it will be interesting to see whether H3.3 is deposited specifically on the inactive X during development, similar to recent observations of H3.3 enrichment at telomeres and pericentric heterochromatin (Drane et al., 2010; Goldberg et al., 2010; Lewis et al., 2010; Wong et al., 2009).

Unlike the macroH2A, which coats the inactive X chromosome, the H2A.Bbd (H2A Barr body-deficient) histone variant is excluded from the inactive X chromosome (Chadwick and Willard, 2001). H2A.Bbd is a highly divergent H2A variant (48% identity to H2A) and is only found in vertebrates. Chromatin containing H2A.Bbd colocalizes with hyperacetylated H4, suggesting a link to active chromatin (Chadwick and Willard, 2001). GFP-tagged H2A.Bbd is exchanged in chromatin at a faster rate than GFP-H2A (Gautier et al., 2004), suggesting that H2A.Bbd nucleosomes may be inherently unstable or that H2A.Bbd is deposited at sites of active chromatin where nucleosome eviction is common. Also consistent with a role in active chromatin, reconstituted H2A.Bbd nucleosomes exhibit an altered MNase DNA protection pattern where only between 118–130 bp of DNA are protected (as compared with the ~150 bp protected fragments observed from MNase digest of chromatin containing canonical histones) (Angelov et al., 2004; Bao et al., 2004; Doyen et al., 2006). H2A.Bbd lacks a C-terminal alpha helix that makes contacts with H3 within the nucleosome and also has a smaller acidic patch than canonical H2A (Chadwick and Willard, 2001). These sequence differences are responsible for H2A.Bbd's ability to inhibit internucleosomal interactions and chromatin compaction, thereby promoting transcription in vitro. These findings suggest a direct link between a specific chromatin structure and the transcriptional state (Zhou et al., 2007).

Histone Variants and Gametogenesis

Once the embryo has reached the late blastocyst stage, the cells of the epiblast continue to differentiate into the somatic lineages that give rise to the adult organism. At this time, around 7.5 dpc, the specification of primordial germ cells (PGCs) occurs in a small number of cells located in the proximal epiblast (Lawson and Hage, 1994; McLaren, 2003). These cells constitute the founder population of the germ cell lineage that is the source of the next generation. By 8.5 dpc, transcriptional repression of the somatic program is established and the germ cells begin their migration to the developing gonads (Blackwell, 2004; Seki et al., 2007; Surani et al., 2004). Following PGC specification, extensive epigenetic reprogramming occurs in the germ cell lineage, including genome-wide erasure of DNA methylation, changes in histone modification status, and reactivation of the inactive X chromosome in female embryos (de Napoles et al., 2007; Hajkova et al., 2002; Sasaki and Matsui, 2008; Seki et al., 2005, 2007; Sugimoto and Abe, 2007; Surani et al., 2007). Once in the gonad, PGCs divide mitotically to produce potential gamete precursors and then undergo meiosis to form haploid gametes. For a more detailed discussion of this process, please see the accompanying review by Kota and Feil (2010) in this issue.

During mammalian spermatogeneis, global changes in chromatin structure and composition accompany other dramatic changes in cell morphology, including cytoplasm elimination and nuclear reshaping (Figure 4). As male PGCs differentiate to spermatognia and enter meiosis, the X and Y chromosomes condense together to form the XY body (Handel, 2004). This body is transcriptionally repressed through a process called meiotic sex chromosome inactivation (MSCI) and is recombinantly inactive, thus protecting the sex chromosomes from the homologous recombination event that is a hallmark of meiosis. The transcriptionally and recombinantly repressed XY body is enriched in both macroH2A (Hoyer-Fender et al., 2000) and another H2A variant called H2AX (Mahadevaiah et al., 2001). The sequence of the first 120 amino acids of H2AX is nearly identical to that of mammalian H2A (Mannironi et al., 1989). However, the C terminus of the H2AX variant shows significant homology with the form of H2A found in lower eukaryotes, including a conserved SQE motif that is a target of ATM and ATR kinase activity (Burma et al., 2001; Wang et al., 2005). Reversible phosphorylation of this conserved serine at position 139 in H2AX, known as γ-H2AX, is a hallmark of double-stranded

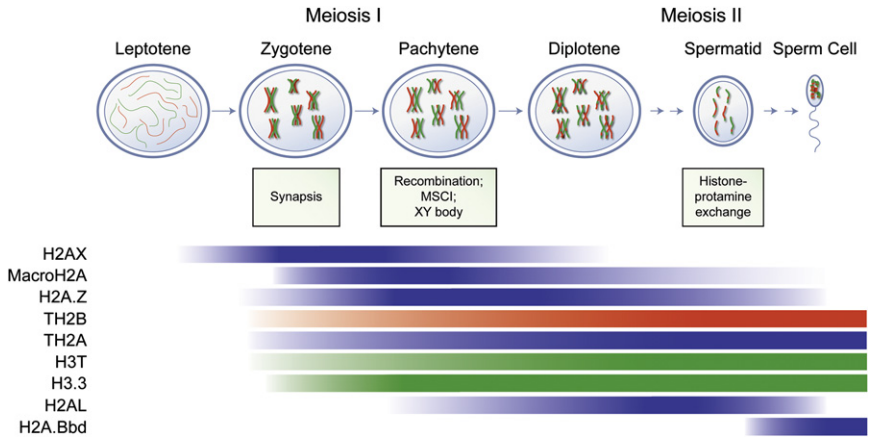

Figure 4. Global Changes in Histone Variant Deposition during Meiosis of Spermatogenesis

Germ cell development involves a number of epigenetic changes, involving differential incorporation of histone variants into chromatin. After reaching the gonad, male primordial germ cells (PGCs) divide to form spermatogonia. These cells divide to form spermatocytes, which then enter meiosis. In zygotene, homologous chromosomes synapse, leading to genetic recombination of nonsister chromatids in the pachytene stage. At this time, the X and Y chromosomes condense together to form the recombinantly inactive XY body. The sex determining chromosomes are transcriptionally inactivated at pachytene through a process called meiotic sex chromosome inactivation (MSCI). After diakinesis and completion of meiosis I, the secondary spermatocyte begins meiosis II, resulting in four haploid spermatid. At this stage, nucleosomal histones are exchanged for the sperm-specific protamines, allowing for highly condensed packaging of DNA in the mature sperm cell. The presence of histone variants at each stage is indicated, with each variant subtype represented with a different color for clarity.

DNA break and the physiologically regulated events of DNA damage repair, including homologous recombination. *h2ax*$^{-/-}$ mice are viable but are subject to pleiotropic phenotypes, one of which is the loss of male fertility, for which *h2ax* is essential. While *h2ax*$^{-/-}$ female mice are fertile, their litter size is smaller than those produced by wild-type mice (Celeste et al., 2002). Continued female fertility in the absence of H2AX (albeit reduced), along with γ-H2AX localization to the XY body in the male germ line independent of double-strand break (Mahadevaiah et al., 2001), led to the suggestion that γ-H2AX is not required for homologous recombination during meiosis. However, further studies showed that *h2ax* deficiency in the male germ line results in an inability to form the XY body and failure to undergo MSCI (Fernandez-Capetillo et al., 2003). Interestingly, in the absence of H2AX, macroH2A fails to localize to the sex chromosomes, resulting in the failure of MSCI (Fernandez-Capetillo et al., 2003). This further suggests that H2AX may play a role in transcriptional silencing. Additional studies are required to more fully understand the role of these variants in meiosis and possible sex-specific mechanisms of germ line maintenance.

Other H2A variants are also involved in the meiotic transition of spermatogonia to the more differentiated spermatozoa. The variant H2A.Z first becomes apparent in primary spermatocytes during pachytene but is excluded from the XY body. Instead, H2A.Z is localized to a facultative heterochromatic domain immediately adjacent to the large heterochromatin chromocenter that forms in postmeiotic late round spermatids (Greaves et al., 2006). Strikingly, this H2A.Z-enriched structure comprises the sex chromosome (either X or Y) of the haploid spermatid. As it has been shown that the sex chromosome silencing established at pachytene (MSCI) continues into the round spermatid stage (postmeiotic sex chromatin inactivation or PMSC) (Namekawa et al., 2006), it becomes intriguing to speculate that H2A.Z plays a role in sex chromosome inactivation, with perhaps important implications for the origins of imprinted paternal X-inactivation. Interestingly, macroH2A1.2 is associated with constitutive heterochromatin on the Y-chromosome in mouse spermatids, but not on the X chromosome (Turner et al., 2001), allowing for discrimination of the sex-determining chromosome with possible developmental consequences.

The H3.3 variant also appears to play a critical role in sexual reproduction in multiple organisms (Cui et al., 2006; Hödl and Basler, 2009; Ooi et al., 2006; Sakai et al., 2009; van der Heijden et al., 2005). H3.3 is present in germ cells prior to meiosis, and its expression and deposition increases dramatically during the first meiotic prophase when much of the nuclear H3 is replaced by H3.3 (Akhmanova et al., 1995). Patterns of *h3.3* gene expression coincide with the reported incorporation of H3.3 protein at the XY body during MSCI (Bramlage et al., 1997; van der Heijden et al., 2007). However, the function of this widespread exchange of canonical H3 for H3.3 during pachytene is not clear. The H3.3 chaperone HIRA is localized to the XY body (van der Heijden et al., 2007) and a previous study demonstrated another H3.3 chaperone Daxx localizing to the XY body in mid- to late-stage pachytene (Rogers et al., 2004). Thus, it is possible that either chaperones or both could mediate this H3.3 deposition, though it is tempting to speculate that Daxx plays a more significant role due to the recent evidence of its association with H3.3 deposition at pericentric heterochromatin and telomeres (Drané et al., 2010; Lewis et al., 2010).

After the completion of meiosis at the spermatid stage, nearly all nucleosomes in the sperm genome are exchanged for transition proteins and protamines to form the highly condensed and transcriptionally inactive sperm genome. The replacement of nucleosomes by two zinc metalloproteins, TP1 and TP2, occurs first, and these transition proteins are subsequently replaced by the highly basic, low molecular weight protamines Prm1 and Prm2 (Ammer et al., 1986; Wouters-Tyrou et al., 1998). Protamines are essential for sperm function because mice that lack either Prm1 or Prm2 produce sperm with damaged DNA and reduced chromatin compaction (Cho et al., 2001; Craig et al., 2003). Whereas older studies had estimated that between 10%–15% of histones are retained in the sperm nucleus (Gatewood et al., 1987), recent genome-wide ChIP analysis indicates that only about 4% of the haploid genome of mature human sperm is actually packaged in nucleosomes (Hammoud et al., 2009). Even more interestingly, the nucleosomes in sperm chromatin are focused at sites of developmentally regulated genes, indicating that their presence is not likely the mere consequence of inefficient histone-replacement by protamines during

spermatogenesis. For example, the *Hox* cluster, known imprinted genes, loci encoding non-coding RNAs, and other developmentally regulated genes all contain nucleosomes rather than protamines (Hammoud et al., 2009). Consistent with these nucleosomes playing a functional role, H3K4me3, a mark of gene promoters poised or activated for transcription, has been detected at the promoters of several important transcription factors and signaling proteins involved in early development. In addition, sperm chromatin were found to contain ''bivalent domains'' of histone modifications (Brykczynska et al., 2010; Hammoud et al., 2009), first identified in ESCs as unique repressed genes containing both activating H3K4me3 and repressive H3K27me3 marks (Mikkelsen et al., 2007). These bivalent domains are usually found on developmentally regulated genes and are speculated to represent genes poised for activation. The presence of these domains in sperm chromatin suggests that the origin of bivalency may trace to specific pathways in spermatogenesis. While the identity of the H3 protein present in these domains remains unclear, other studies have found H3.3 in human sperm (Gatewood et al., 1990), as well as enrichment of H3.3 at bivalent domains in ESCs (Goldberg et al., 2010). Mature sperm in *Caenorhabditis elegans* also are enriched for H3.3, suggesting that H3.3 retention on sperm chromatin may be conserved (Ooi et al., 2006). It will be important to directly determine whether H3.3 is indeed deposited in sperm chromatin at these developmentally regulated regions.

In *Drosophila*, loss of both copies of H3.3 (*h3.3A* and *h3.3B*) leads to partial but incomplete lethality (~42% viability), complete sterility, and transcriptional defects, particularly at highly expressed genes (Hödl and Basler, 2009; Sakai et al., 2009). The absolute requirement of H3.3 for fertility appears to be due to its role in chromosome segregation during spermatogenesis, as *h3.3* double null flies fail to properly condense and segregate chromosomes during male meiosis (Sakai et al., 2009). In addition, male H3.3 double null flies show an abnormal arrangement of chromatin in postmeiotic nuclei, suggesting that H3.3 is critical for the remodeling of germline chromatin. Mice harboring a hypomorphic *h3.3A* allele (produced by retroviral gene trap insertion into the histone *h3.3A* gene) exhibited partial neonatal lethality and significant defects in adult fertility (Couldrey et al., 1999). In fact, only 6% of mating events between mice homozygous for the *h3.3A* hypomorphic allele resulted in pregnancy, in comparison to 74% in wild-type mice. However, the precise function of mammalian H3.3 in reproduction is not clear, as no true knockout studies have been performed. In addition to H3.3, a testis-specific H3 protein is synthesized during pachytene and is found in developing spermatogonia (Witt et al., 1996). While this H3 protein (H3T) is most similar to canonical H3.1, it differs at several residues throughout the histone-fold domains. During spermatogenesis, H3T appears to be deposited into chromatin through its interaction with a testis-specific version of the Nuclear Autoantigenic Sperm Protein (NASP) (Osakabe et al., 2010). A somatic form of NASP is expressed during S phase in all cell types and functions as both an H1 and H3/H4 chaperone in vitro (Richardson et al., 2000; Wang et al., 2008). In vitro studies have found that nucleosomes containing H3T are less stable than conventional H3 nucleosomes (Osakabe et al., 2010), suggesting that H3T may be actively involved in gene expression or histone exchange during meiosis.

Recently, it was shown that H2A.Bbd is expressed in testes during spermatogenesis and is found in the chromatin of mature sperm (Ishibashi et al., 2010), possibly in the small nucleosome-containing compartment that exists within protamine-packaged sperm DNA. This leads to the intriguing hypothesis that the proposed role of H2A.Bbd in gene activation (Angelov et al., 2004) may facilitate early gene transcription from the male pronucleus after fertilization, in support of a significant role in early development for this histone variant. Intriguingly, the H2AL family of H2A.Bbd-like histones was recently identified in mouse spermatids, specifically marking pericentric regions (Ferguson et al., 2009; Govin et al., 2007). Like H2A.Bbd, all known members of the H2AL family have truncated C-terminal regions that may affect genome packaging. Indeed, H2AL ectopically expressed in somatic cells has been shown to interact with the testis-specific H2B variant TH2B (van Roijen et al., 1998; Zalensky et al., 2002), forming more labile nucleosomes than canonical H2A/H2B dimers (Govin et al., 2007). The presence of these histone variants in sperm chromatin leads again to the hypothesis that novel organization of sperm chromatin might have developmental consequences for fertilization. An additional H2A variant, TH2A, is also expressed during pachytene (Trostle-Weige et al., 1982), though the localization and function of this variant are unknown.

Histone Variants and Neurobiology

Thus far, we have focused on developmental pathways involved directly with reproduction. In addition, histone variants also likely play a role in a neurodevelopment, as it is known that alterations of chromatin structure play important roles in neuronal physiology and development (Borrelli et al., 2008). Both histone modifications and proteins involved in chromatin remodeling have been linked with behavioral memory and synaptic plasticity (Fagiolini et al., 2009; Levenson and Sweatt, 2005). Furthermore, multiple neurological disorders involve mutations in genes that encode chromatin-binding or chromatin-modifying enzymes (Urdinguio et al., 2009). A common theme of these disorders is that mutations in epigenetic regulators can alter chromatin structure and composition and induce a broad spectrum of neurodevelopmental defects.

The maintenance of chromatin structure in postmitotic cells relies on the deposition of replication-independent histone variants. Neurons in the mammalian central nervous system are postmitotic, and several lines of evidence suggest that macroH2A, H3.3, and H2AX are likely to play important roles in neuronal biology. *h3.3A* RNA and protein levels are observed to increase in multiple models of cell differentiation (Krimer et al., 1993; Lord et al., 1990; Wu et al., 1982; Wunsch and Lough, 1987). High levels of H3.3 protein have been found in differentiating rat neurons, and H3.3 has been shown to be the dominant H3 protein in rat cortical neurons (Bosch and Suau, 1995; Pina and Suau, 1987; Scaturro et al., 1995). In addition to embryonic lethality and reduced fertility, the previously described mice expressing the hypomorphic *h3.3A* mutant allele mice also exhibit neuromuscular deficits (Couldrey et al., 1999). This observation suggests that H3.3 may play a critical role in postmitotic cells, such as neurons, where replication-independent chromatin assembly is the only means to regulate nucleosome composition. Indeed, studies have found that H3.3

deposition in rat cortical neurons begins to increase immediately after birth, accumulating with age until this variant becomes the dominant histone H3 found in neuronal chromatin (Pina and Suau, 1987). The functional role for H3.3 in neurodevelopment remains unclear, but the ATRX component of the ATRX-Daxx deposition complex has been implicated in an X-linked mental retardation syndrome (ATR-X) (Picketts et al., 1996). We speculate that the neurodevelopmental defects observed in ATR-X patients may result in part from altered H3.3 deposition in neurons. In addition to H3.3, the histone variant H2AX also accumulates in cortical neurons (Bosch and Suau, 1995; Pina and Suau, 1987). Other histone variants, such as MacroH2A and H2A.Z, are also highly expressed in cortical neurons, though their levels do not change throughout development (Akbarian et al., 2001; Bosch and Suau, 1995). Beyond steady-state levels, little is known about the function of these histone variants in neurons. Future studies will need to address the function of histone variants in neurodevelopment.

Future Outlook

In this review, we have discussed the role of chromatin and histone variants during mammalian development, focusing on the known involvement of histone variants in fertilization, X chromosome inactivation, gametogenesis, and neurodevelopment. Histone variants are structural components of chromatin that allow for meaningful diversity in the chromatin polymer, ensuring dynamic regulation of the genome. This parsing of genetic information is essential as the embryo differentiates from the totipotent zygote into the hundreds of specialized cells that constitute the adult organism.

Decoupling histone synthesis from the cell-cycle cues provides the cell with a constant level of new histones for incorporation into chromatin. Since histone variants are expressed throughout the cell cycle, it has been postulated that some histone variants simply play a replacement role, providing structure for nucleosome remodeling events that occur independently of DNA synthesis. Indeed, it is entirely possible that histone variants evolved specifically as replacement histones. However, several observations discussed herein suggest that more specialized functions may have evolved to make use of the structural diversity that histone variants provide (much the same way that histone proteins themselves may have evolved first as scaffolds to organize our vast genetic material and second as signaling platforms to interpret cellular context). First, several studies suggest that the presence of a variant itself is related to the observed phenotype. For example, the ectopic expression of *h3.3* point mutants in oocytes results in defects in pericentric heterochromatin formation, inhibiting progression to the blastocyst stage (Santenard et al., 2010). However, the same point mutations in canonical H3 have no effect on cellular differentiation during embryonic development (Santenard et al., 2010). Studies in *Drosophila* also show that the infertility observed in H3.3 null animals can be rescued by exogenous *h3.3*, but not exogenous canonical *h3.1* (Hödl and Basler, 2009). Second, while some variants are quite similar to their canonical counterparts (H3.3, for example), others contain highly divergent sequences or structural domains. All known H2A variants fall into the latter category, as does the centromere-specific H3 variant CENP-A. Many lines of evidence suggest that these variants can alter the biochemical properties of the nucleosome, either directly be destabilizing the protein-DNA interactions (e.g., H2A.Bbd-containing nucleosomes) or indirectly through variant-specific post-translational modifications that then recruit chromatin-related proteins to bring about remodeling events (e.g., γ-H2AX). Finally, these variants are escorted to chromatin by dedicated chaperones (e.g., HIRA/Daxx/ATRX for H3.3, Chz1/SWR1 for H2A.Z), suggesting that their deposition into chromatin is highly regulated in a context-dependent manner and dictated by the availability of the variant-specific chaperones.

Intriguingly, many of the known variants appear to play a role in the establishment or maintenance of heterochromatic regions. This is an especially surprising finding for the variant H3.3, as it was first found to be a marker of gene activity associated with euchromatin. However, recent studies both in developing embryos and in vitro embryonic culture systems suggest a more complicated role for this variant, with its genomic deposition patterns likely regulated by a number of independent and highly specific chaperone systems. Another interesting aspect of histone variant biology is that several of the variants (e.g., H3.3, H2AX) appear not to be required for survival, yet are essential for fertility (*h3.3* null *Drosophila* are viable, though the corresponding studies in mouse have yet to be performed; H2AX, as discussed above, is essential for male, but not female, fertility in mice). These observations suggest that variant histones may have evolutionarily conserved roles in sexual reproduction. Interestingly, the only version of histone H3 in *S. cerevisiae* harbors the AAIG motif found in mammalian H3.3. The SQE motif found in mammalian H2AX is also conserved in the *S. cerevisiae* H2A protein. In addition to the variants discussed in this review, new histone variants no doubt remain to be discovered. Indeed, two additional primate-specific H3 variants were recently reported (Wiedemann et al., 2010). Future studies will certainly shed further mechanistic insight into the complicated use of these histone variants in a developmental setting.

ACKNOWLEDGMENTS

We express our apologies to colleagues whose work could not be included in this review. We thank Lindsey Baker and anonymous reviewers for critical reading of this manuscript and members of the Allis lab for helpful discussions. L.A.B is a Damon Runyon Cancer Research Foundation Postdoctoral Fellow. P.W.L. is supported by a National Research Service Award fellowship.

REFERENCES

Akbarian, S., Chen, R.Z., Gribnau, J., Rasmussen, T.P., Fong, H., Jaenisch, R., and Jones, E.G. (2001). Expression pattern of the Rett syndrome gene MeCP2 in primate prefrontal cortex. Neurobiol. Dis. *8*, 784–791.

Akhmanova, A.S., Bindels, P.C., Xu, J., Miedema, K., Kremer, H., and Hennig, W. (1995). Structure and expression of histone H3.3 genes in Drosophila melanogaster and Drosophila hydei. Genome *38*, 586–600.

Albig, W., and Doenecke, D. (1997). The human histone gene cluster at the D6S105 locus. Hum. Genet. *101*, 284–294.

Allis, C.D., Glover, C.V., Bowen, J.K., and Gorovsky, M.A. (1980). Histone variants specific to the transcriptionally active, amitotically dividing macronucleus of the unicellular eucaryote, Tetrahymena thermophila. Cell *20*, 609–617.

Ammer, H., Henschen, A., and Lee, C.H. (1986). Isolation and amino-acid sequence analysis of human sperm protamines P1 and P2. Occurrence of two forms of protamine P2. Biol. Chem. Hoppe Seyler *367*, 515–522.

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

Angelov, D., Verdel, A., An, W., Bondarenko, V., Hans, F., Doyen, C.-M., Studitsky, V.M., Hamiche, A., Roeder, R.G., Bouvet, P., et al. (2004). SWI/SNF remodeling and p300-dependent transcription of histone variant H2ABbd nucleosomal arrays. EMBO J. *23*, 3815–3824.

Aoki, F., Worrad, D.M., and Schultz, R.M. (1997). Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. Dev. Biol. *181*, 296–307.

Bao, Y., Konesky, K., Park, Y.-J., Rosu, S., Dyer, P.N., Rangasamy, D., Tremethick, D.J., Laybourn, P.J., and Luger, K. (2004). Nucleosomes containing the histone variant H2A.Bbd organize only 118 base pairs of DNA. EMBO J. *23*, 3314–3324.

Bassing, C.H., Suh, H., Ferguson, D.O., Chua, K.F., Manis, J., Eckersdorff, M., Gleason, M., Bronson, R., Lee, C., and Alt, F.W. (2003). Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. Cell *114*, 359–370.

Baumann, C., and De La Fuente, R. (2009). ATRX marks the inactive X chromosome (Xi) in somatic cells and during imprinted X chromosome inactivation in trophoblast stem cells. Chromosoma *118*, 209–222.

Baumann, C., Viveiros, M.M., and De La Fuente, R. (2010). Loss of maternal ATRX results in centromere instability and aneuploidy in the mammalian oocyte and pre-implantation embryo. PLoS Genet. *6*, e1001137.

Blackwell, T.K. (2004). Germ cells: finding programs of mass repression. Curr. Biol. *14*, R229–R230.

Bonnefoy, E., Orsi, G.A., Couble, P., and Loppin, B. (2007). The essential role of Drosophila HIRA for de novo assembly of paternal chromatin at fertilization. PLoS Genet. *3*, 1991–2006.

Borrelli, E., Nestler, E.J., Allis, C.D., and Sassone-Corsi, P. (2008). Decoding the epigenetic language of neuronal plasticity. Neuron *60*, 961–974.

Bosch, A., and Suau, P. (1995). Changes in core histone variant composition in differentiating neurons: the roles of differential turnover and synthesis rates. Eur. J. Cell Biol. *68*, 220–225.

Bramlage, B., Kosciessa, U., and Doenecke, D. (1997). Differential expression of the murine histone genes H3.3A and H3.3B. Differentiation *62*, 13–20.

Brock, W.A., Trostle, P.K., and Meistrich, M.L. (1980). Meiotic synthesis of testis histones in the rat. Proc. Natl. Acad. Sci. USA *77*, 371–375.

Brykczynska, U., Hisano, M., Erkek, S., Ramos, L., Oakeley, E.J., Roloff, T.C., Beisel, C., Schubeler, D., Stadler, M.B., and Peters, A.H. (2010). Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. Nat. Struct. Mol. Biol. *17*, 679–687.

Burma, S., Chen, B.P., Murphy, M., Kurimasa, A., and Chen, D.J. (2001). ATM phosphorylates histone H2AX in response to DNA double-strand breaks. J. Biol. Chem. *276*, 42462–42467.

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

Celeste, A., Petersen, S., Romanienko, P.J., Fernandez-Capetillo, O., Chen, H.T., Sedelnikova, O.A., Reina-San-Martin, B., Coppola, V., Meffre, E., Difilippantonio, M.J., et al. (2002). Genomic instability in mice lacking histone H2AX. Science *296*, 922–927.

Chadwick, B.P., and Willard, H.F. (2001). A novel chromatin protein, distantly related to histone H2A, is largely excluded from the inactive X chromosome. J. Cell Biol. *152*, 375–384.

Chadwick, B.P., Valley, C.M., and Willard, H.F. (2001). Histone variant macroH2A contains two distinct macrochromatin domains capable of directing macroH2A to the inactive X chromosome. Nucleic Acids Res. *29*, 2699–2705.

Chang, C.-C., Ma, Y., Jacobs, S., Tian, X.C., Yang, X., and Rasmussen, T.P. (2005). A maternal store of macroH2A is removed from pronuclei prior to onset of somatic macroH2A expression in preimplantation embryos. Dev. Biol. *278*, 367–380.

Cho, C., Willis, W.D., Goulding, E.H., Jung-Ha, H., Choi, Y.C., Hecht, N.B., and Eddy, E.M. (2001). Haploinsufficiency of protamine-1 or −2 causes infertility in mice. Nat. Genet. *28*, 82–86.

Choo, J.H., Kim, J.D., Chung, J.H., Stubbs, L., and Kim, J. (2006). Allele-specific deposition of macroH2A1 in imprinting control regions. Hum. Mol. Genet. *15*, 717–724.

Choo, J.H., Kim, J.D., and Kim, J. (2007). MacroH2A1 knockdown effects on the Peg3 imprinted domain. BMC Genomics *8*, 479.

Costanzi, C., and Pehrson, J.R. (1998). Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. Nature *393*, 599–601.

Costanzi, C., Stein, P., Worrad, D.M., Schultz, R.M., and Pehrson, J.R. (2000). Histone macroH2A1 is concentrated in the inactive X chromosome of female preimplantation mouse embryos. Development *127*, 2283–2289.

Couldrey, C., Carlton, M.B., Nolan, P.M., Colledge, W.H., and Evans, M.J. (1999). A retroviral gene trap insertion into the histone 3.3A gene causes partial neonatal lethality, stunted growth, neuromuscular deficits and male subfertility in transgenic mice. Hum. Mol. Genet. *8*, 2489–2495.

Craig, J.M., Earle, E., Canham, P., Wong, L.H., Anderson, M., and Choo, K.H. (2003). Analysis of mammalian proteins involved in chromatin modification reveals new metaphase centromeric proteins and distinct chromosomal distribution patterns. Hum. Mol. Genet. *12*, 3109–3121.

Csankovszki, G., Panning, B., Bates, B., Pehrson, J.R., and Jaenisch, R. (1999). Conditional deletion of Xist disrupts histone macroH2A localization but not maintenance of X inactivation. Nat. Genet. *22*, 323–324.

Cui, B., Liu, Y., and Gorovsky, M.A. (2006). Deposition and function of histone H3 variants in Tetrahymena thermophila. Mol. Cell. Biol. *26*, 7719–7730.

De La Fuente, R., Viveiros, M.M., Wigglesworth, K., and Eppig, J.J. (2004). ATRX, a member of the SNF2 family of helicase/ATPases, is required for chromosome alignment and meiotic spindle organization in metaphase II stage mouse oocytes. Dev. Biol. *272*, 1–14.

de Napoles, M., Nesterova, T., and Brockdorff, N. (2007). Early loss of Xist RNA expression and inactive X chromosome associated chromatin modification in developing primordial germ cells. PLoS ONE *2*, e860.

Doyen, C.-M., An, W., Angelov, D., Bondarenko, V., Mietton, F., Studitsky, V.M., Hamiche, A., Roeder, R.G., Bouvet, P., and Dimitrov, S. (2006). Mechanism of polymerase II transcription repression by the histone variant macroH2A. Mol. Cell. Biol. *26*, 1156–1164.

Drane, P., Ouararhni, K., Depaux, A., Shuaib, M., and Hamiche, A. (2010). The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. Genes Dev. *24*, 1253–1265.

Faast, R., Thonglairoam, V., Schulz, T.C., Beall, J., Wells, J.R., Taylor, H., Matthaei, K., Rathjen, P.D., Tremethick, D.J., and Lyons, I. (2001). Histone variant H2A.Z is required for early mammalian development. Curr. Biol. *11*, 1183–1187.

Fagiolini, M., Jensen, C.L., and Champagne, F.A. (2009). Epigenetic influences on brain development and plasticity. Curr. Opin. Neurobiol. *19*, 207–212.

Fan, J.Y., Rangasamy, D., Luger, K., and Tremethick, D.J. (2004). H2A.Z alters the nucleosome surface to promote HP1alpha-mediated chromatin fiber folding. Mol. Cell *16*, 655–661.

Ferguson, L., Ellis, P.J.I., and Affara, N.A. (2009). Two novel mouse genes mapped to chromosome Yp are expressed specifically in spermatids. Mamm. Genome *20*, 193–206.

Fernandez-Capetillo, O., Mahadevaiah, S.K., Celeste, A., Romanienko, P.J., Camerini-Otero, R.D., Bonner, W.M., Manova, K., Burgoyne, P., and Nussenzweig, A. (2003). H2AX is required for chromatin remodeling and inactivation of sex chromosomes in male mouse meiosis. Dev. Cell *4*, 497–508.

Frank, D., Doenecke, D., and Albig, W. (2003). Differential expression of human replacement and cell cycle dependent H3 histone genes. Gene *312*, 135–143.

Franklin, S.G., and Zweidler, A. (1977). Non-allelic variants of histones 2a, 2b and 3 in mammals. Nature *266*, 273–275.

Gamble, M.J., Frizzell, K.M., Yang, C., Krishnakumar, R., and Kraus, W.L. (2010). The histone variant macroH2A1 marks repressed autosomal chromatin, but protects a subset of its target genes from silencing. Genes Dev. *24*, 21–32.

Garrick, D., Sharpe, J.A., Arkell, R., Dobbie, L., Smith, A.J.H., Wood, W.G., Higgs, D.R., and Gibbons, R.J. (2006). Loss of Atrx affects trophoblast development and the pattern of X-inactivation in extraembryonic tissues. PLoS Genet. *2*, e58.

Gatewood, J.M., Cook, G.R., Balhorn, R., Bradbury, E.M., and Schmid, C.W. (1987). Sequence-specific packaging of DNA in human sperm chromatin. Science *236*, 962–964.

Gatewood, J.M., Cook, G.R., Balhorn, R., Schmid, C.W., and Bradbury, E.M. (1990). Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. J. Biol. Chem. *265*, 20662–20666.

Gautier, T., Abbott, D.W., Molla, A., Verdel, A., Ausio, J., and Dimitrov, S. (2004). Histone variant H2ABbd confers lower stability to the nucleosome. EMBO Rep. *5*, 715–720.

Goldberg, A.D., Banaszynski, L.A., Noh, K.-M., Lewis, P.W., Elsaesser, S.J., Stadler, S., Dewell, S., Law, M., Guo, X., Li, X., et al. (2010). Distinct factors control histone variant H3.3 localization at specific genomic regions. Cell *140*, 678–691.

Govin, J., Escoffier, E., Rousseaux, S., Kuhn, L., Ferro, M., Thévenon, J., Catena, R., Davidson, I., Garin, J., Khochbin, S., et al. (2007). Pericentric heterochromatin reprogramming by new histone variants during mouse spermiogenesis. J. Cell Biol. *176*, 283–294.

Greaves, I.K., Rangasamy, D., Devoy, M., Marshall Graves, J.A., and Tremethick, D.J. (2006). The X and Y chromosomes assemble into H2A.Z-containing [corrected] facultative heterochromatin [corrected] following meiosis. Mol. Cell. Biol. *26*, 5394–5405.

Greaves, I.K., Rangasamy, D., Ridgway, P., and Tremethick, D.J. (2007). H2A.Z contributes to the unique 3D structure of the centromere. Proc. Natl. Acad. Sci. USA *104*, 525–530.

Grewal, S.I. (2010). RNAi-dependent formation of heterochromatin and its diverse functions. Curr. Opin. Genet. Dev. *20*, 134–141.

Grigoryev, S.A., Nikitina, T., Pehrson, J.R., Singh, P.B., and Woodcock, C.L. (2004). Dynamic relocation of epigenetic chromatin markers reveals an active role of constitutive heterochromatin in the transition from proliferation to quiescence. J. Cell Sci. *117*, 6153–6162.

Guttman, M., Amit, I., Garber, M., French, C., Lin, M.F., Feldser, D., Huarte, M., Zuk, O., Carey, B.W., Cassady, J.P., et al. (2009). Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature *458*, 223–227.

Hajkova, P., Erhardt, S., Lane, N., Haaf, T., El-Maarri, O., Reik, W., Walter, J., and Surani, M.A. (2002). Epigenetic reprogramming in mouse primordial germ cells. Mech. Dev. *117*, 15–23.

Hake, S.B., and Allis, C.D. (2006). Histone H3 variants and their potential role in indexing Mamm. Genomes: the "H3 barcode hypothesis". Proc. Natl. Acad. Sci. USA *103*, 6428–6435.

Hammoud, S., Nix, D., Zhang, H., Purwar, J., Carrell, D., and Cairns, B. (2009). Distinctive chromatin in human sperm packages genes for embryo development. Nature *460*, 473–478.

Handel, M.A. (2004). The XY body: a specialized meiotic chromatin domain. Exp. Cell Res. *296*, 57–63.

Heard, E., Rougeulle, C., Arnaud, D., Avner, P., Allis, C.D., and Spector, D.L. (2001). Methylation of histone H3 at Lys-9 is an early mark on the X chromosome during X inactivation. Cell *107*, 727–738.

Hemberger, M., Dean, W., and Reik, W. (2009). Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. Nat. Rev. Mol. Cell Biol. *10*, 526–537.

Henikoff, S. (2008). Nucleosome destabilization in the epigenetic regulation of gene expression. Nat. Rev. Genet. *9*, 15–26.

Ho, L., and Crabtree, G.R. (2010). Chromatin remodelling during development. Nature *463*, 474–484.

Hödl, M., and Basler, K. (2009). Transcription in the Absence of Histone H3.3. Curr. Biol. *19*, 1221–1226.

Howman, E.V., Fowler, K.J., Newson, A.J., Redward, S., MacDonald, A.C., Kalitsis, P., and Choo, K.H. (2000). Early disruption of centromeric chromatin organization in centromere protein A (Cenpa) null mice. Proc. Natl. Acad. Sci. USA *97*, 1148–1153.

Hoyer-Fender, S., Costanzi, C., and Pehrson, J.R. (2000). Histone macroH2A1.2 is concentrated in the XY-body by the early pachytene stage of spermatogenesis. Exp. Cell Res. *258*, 254–260.

Iouzalen, N., Moreau, J., and Mechali, M. (1996). H2A.ZI, a new variant histone expressed during Xenopus early development exhibits several distinct features from the core histone H2A. Nucleic Acids Res. *24*, 3947–3952.

Ishibashi, T., Li, A., Eirín-López, J.M., Zhao, M., Missiaen, K., Abbott, D.W., Meistrich, M., Hendzel, M.J., and Ausió, J. (2010). H2A.Bbd: an X-chromosome-encoded histone involved in mammalian spermiogenesis. Nucleic Acids Res. *38*, 1780–1789.

Jacobs, S.A., and Khorasanizadeh, S. (2002). Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. Science *295*, 2080–2083.

Jayaramaiah Raja, S., and Renkawitz-Pohl, R. (2005). Replacement by Drosophila melanogaster protamines and Mst77F of histones during chromatin condensation in late spermatids and role of sesame in the removal of these proteins from the male pronucleus. Mol. Cell. Biol. *25*, 6165–6177.

Kavi, H.H., and Birchler, J.A. (2009). Interaction of RNA polymerase II and the small RNA machinery affects heterochromatic silencing in Drosophila. Epigenetics Chromatin *2*, 15.

Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I.R., et al. (2010). Epigenetic memory in induced pluripotent stem cells. Nature *467*, 285–290.

Klose, R.J., and Bird, A.P. (2006). Genomic DNA methylation: the mark and its mediators. Trends Biochem. Sci. *31*, 89–97.

Konev, A.Y., Tribus, M., Park, S.Y., Podhraski, V., Lim, C.Y., Emelyanov, A.V., Vershilova, E., Pirrotta, V., Kadonaga, J.T., Lusser, A., et al. (2007). CHD1 motor protein is required for deposition of histone variant H3.3 into chromatin in vivo. Science *317*, 1087–1090.

Kota, S.K., and Feil, R. (2010). Epigenetic transitions in germ cell development and meiosis. Dev. Cell *19*, this issue, 675–686.

Krimer, D.B., Cheng, G., and Skoultchi, A.I. (1993). Induction of H3.3 replacement histone mRNAs during the precommitment period of murine erythroleukemia cell differentiation. Nucleic Acids Res. *21*, 2873–2879.

Kustatscher, G., Hothorn, M., Pugieux, C., Scheffzek, K., and Ladurner, A.G. (2005). Splicing regulates NAD metabolite binding to histone macroH2A. Nat. Struct. Mol. Biol. *12*, 624–625.

Landmann, F., Orsi, G.A., Loppin, B., and Sullivan, W. (2009). Wolbachia-mediated cytoplasmic incompatibility is associated with impaired histone deposition in the male pronucleus. PLoS Pathog. *5*, e1000343.

Lawson, K.A., and Hage, W.J. (1994). Clonal analysis of the origin of primordial germ cells in the mouse. Ciba Found. Symp. *182*, 68–84, discussion 84–91.

Levenson, J.M., and Sweatt, J.D. (2005). Epigenetic mechanisms in memory formation. Nat. Rev. Neurosci. *6*, 108–118.

Lewis, P.W., Elsaesser, S.J., Noh, K.-M., Stadler, S.C., and Allis, C.D. (2010). Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. Proc. Natl. Acad. Sci. USA *107*, 14075–14080.

Loppin, B., Bonnefoy, E., Anselme, C., Laurençon, A., Karr, T.L., and Couble, P. (2005). The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus. Nature *437*, 1386–1390.

Lord, K.A., Abdollahi, A., Hoffman-Liebermann, B., and Liebermann, D.A. (1990). Dissection of the immediate early response of myeloid leukemia cells to terminal differentiation and growth inhibitory stimuli. Cell Growth Differ. *1*, 637–645.

Luk, E., Vu, N.-D., Patteson, K., Mizuguchi, G., Wu, W.-H., Ranjan, A., Backus, J., Sen, S., Lewis, M., Bai, Y., et al. (2007). Chz1, a nuclear chaperone for histone H2AZ. Mol. Cell *25*, 357–368.

Lyon, M.F. (1961). Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature *190*, 372–373.

Mahadevaiah, S.K., Turner, J.M., Baudat, F., Rogakou, E.P., de Boer, P., Blanco-Rodríguez, J., Jasin, M., Keeney, S., Bonner, W.M., and Burgoyne, P.S. (2001). Recombinational DNA double-strand breaks in mice precede synapsis. Nat. Genet. *27*, 271–276.

Mak, W., Baxter, J., Silva, J., Newall, A.E., Otte, A.P., and Brockdorff, N. (2002). Mitotically stable association of polycomb group proteins eed and enx1 with the inactive x chromosome in trophoblast stem cells. Curr. Biol. *12*, 1016–1020.

Mank, J.E. (2009). The W, X, Y and Z of sex-chromosome dosage compensation. Trends Genet. *25*, 226–233.

Mannironi, C., Bonner, W.M., and Hatch, C.L. (1989). H2A.X. a histone isoprotein with a conserved C-terminal sequence, is encoded by a novel mRNA with both DNA replication type and polyA 3′ processing signals. Nucleic Acids Res. *17*, 9113–9126.

Marahrens, Y., Panning, B., Dausman, J., Strauss, W., and Jaenisch, R. (1997). Xist-deficient mice are defective in dosage compensation but not spermatogenesis. Genes Dev. *11*, 156–166.

McLaren, A. (2003). Primordial germ cells in the mouse. Dev. Biol. *262*, 1–15.

Menissier de Murcia, J., Ricoul, M., Tartier, L., Niedergang, C., Huber, A., Dantzer, F., Schreiber, V., Ame, J.C., Dierich, A., LeMeur, M., et al. (2003). Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. EMBO J. *22*, 2255–2263.

Michaelson, J.S., Bader, D., Kuo, F., Kozak, C., and Leder, P. (1999). Loss of Daxx, a promiscuously interacting protein, results in extensive apoptosis in early mouse development. Genes Dev. *13*, 1918–1923.

Mikkelsen, T.S., Ku, M., Jaffe, D.B., Issac, B., Lieberman, E., Giannoukos, G., Alvarez, P., Brockman, W., Kim, T.K., Koche, R.P., et al. (2007). Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature *448*, 553–560.

Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S., and Wu, C. (2004). ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science *303*, 343–348.

Muchardt, C., Guilleme, M., Seeler, J.S., Trouche, D., Dejean, A., and Yaniv, M. (2002). Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha. EMBO Rep. *3*, 975–981.

Nakayama, J., Rice, J.C., Strahl, B.D., Allis, C.D., and Grewal, S.I. (2001). Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science *292*, 110–113.

Namekawa, S.H., Park, P.J., Zhang, L.F., Shima, J.E., McCarrey, J.R., Griswold, M.D., and Lee, J.T. (2006). Postmeiotic sex chromatin in the male germline of mice. Curr. Biol. *16*, 660–667.

Ng, R.K., and Gurdon, J.B. (2005). Epigenetic memory of active gene transcription is inherited through somatic cell nuclear transfer. Proc. Natl. Acad. Sci. USA *102*, 1957–1962.

Ng, K., Pullirsch, D., Leeb, M., and Wutz, A. (2007). Xist and the order of silencing. EMBO Rep. *8*, 34–39.

Nusinow, D.A., Hernández-Muñoz, I., Fazzio, T.G., Shah, G.M., Kraus, W.L., and Panning, B. (2007). 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.

Ooi, S.L., Priess, J.R., and Henikoff, S. (2006). Histone H3.3 variant dynamics in the germline of Caenorhabditis elegans. PLoS Genet. *2*, e97.

Osakabe, A., Tachiwana, H., Matsunaga, T., Shiga, T., Nozawa, R.-S., Obuse, C., and Kurumizaka, H. (2010). Nucleosome formation activity of human somatic nuclear autoantigenic sperm protein (sNASP). J. Biol. Chem. *285*, 11913–11921.

Ouararhni, K., Hadj-Slimane, R., Ait-Si-Ali, S., Robin, P., Mietton, F., Harel-Bellan, A., Dimitrov, S., and Hamiche, A. (2006). The histone variant mH2A1.1 interferes with transcription by down-regulating PARP-1 enzymatic activity. Genes Dev. *20*, 3324–3336.

Pal-Bhadra, M., Leibovitch, B.A., Gandhi, S.G., Rao, M., Bhadra, U., Birchler, J.A., and Elgin, S.C.R. (2004). Heterochromatic silencing and HP1 localization in Drosophila are dependent on the RNAi machinery. Science *303*, 669–672.

Pandey, N.B., and Marzluff, W.F. (1987). The stem-loop structure at the 3′ end of histone mRNA is necessary and sufficient for regulation of histone mRNA stability. Mol. Cell. Biol. *7*, 4557–4559.

Pehrson, J.R., and Fried, V.A. (1992). MacroH2A, a core histone containing a large nonhistone region. Science *257*, 1398–1400.

Penny, G.D., Kay, G.F., Sheardown, S.A., Rastan, S., and Brockdorff, N. (1996). Requirement for Xist in X chromosome inactivation. Nature *379*, 131–137.

Perche, P.Y., Vourc'h, C., Konecny, L., Souchier, C., Robert-Nicoud, M., Dimitrov, S., and Khochbin, S. (2000). Higher concentrations of histone macroH2A in the Barr body are correlated with higher nucleosome density. Curr. Biol. *10*, 1531–1534.

Picketts, D.J., Higgs, D.R., Bachoo, S., Blake, D.J., Quarrell, O.W., and Gibbons, R.J. (1996). ATRX encodes a novel member of the SNF2 family of proteins: mutations point to a common mechanism underlying the ATR-X syndrome. Hum. Mol. Genet. *5*, 1899–1907.

Pina, B., and Suau, P. (1987). Changes in histones H2A and H3 variant composition in differentiating and mature rat brain cortical neurons. Dev. Biol. *123*, 51–58.

Plath, K., Fang, J., Mlynarczyk-Evans, S.K., Cao, R., Worringer, K.A., Wang, H., de la Cruz, C.C., Otte, A.P., Panning, B., and Zhang, Y. (2003). Role of histone H3 lysine 27 methylation in X inactivation. Science *300*, 131–135.

Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T., et al. (2010). Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat. Biotechnol. *28*, 848–855.

Probst, A.V., Santos, F., Reik, W., Almouzni, G., and Dean, W. (2007). Structural differences in centromeric heterochromatin are spatially reconciled on fertilisation in the mouse zygote. Chromosoma *116*, 403–415.

Puschendorf, M., Terranova, R., Boutsma, E., Mao, X., Isono, K., Brykczynska, U., Kolb, C., Otte, A.P., Koseki, H., Orkin, S.H., et al. (2008). PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. Nat. Genet. *40*, 411–420.

Rando, O.J., and Chang, H.Y. (2009). Genome-wide views of chromatin structure. Annu. Rev. Biochem. *78*, 245–271.

Rangasamy, D., Berven, L., Ridgway, P., and Tremethick, D.J. (2003). Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. EMBO J. *22*, 1599–1607.

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

Richardson, R.T., Batova, I.N., Widgren, E.E., Zheng, L.X., Whitfield, M., Marzluff, W.F., and O'Rand, M.G. (2000). Characterization of the histone H1-binding protein, NASP, as a cell cycle-regulated somatic protein. J. Biol. Chem. *275*, 30378–30386.

Roberts, C., Sutherland, H.F., Farmer, H., Kimber, W., Halford, S., Carey, A., Brickman, J.M., Wynshaw-Boris, A., and Scambler, P.J. (2002). Targeted mutagenesis of the Hira gene results in gastrulation defects and patterning abnormalities of mesoendodermal derivatives prior to early embryonic lethality. Mol. Cell. Biol. *22*, 2318–2328.

Rogakou, E.P., and Sekeri-Pataryas, K.E. (1999). Histone variants of H2A and H3 families are regulated during in vitro aging in the same manner as during differentiation. Exp. Gerontol. *34*, 741–754.

Rogers, R.S., Inselman, A., Handel, M.A., and Matunis, M.J. (2004). SUMO modified proteins localize to the XY body of pachytene spermatocytes. Chromosoma *113*, 233–243.

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

Santenard, A., Ziegler-Birling, C., Koch, M., Tora, L., Bannister, A.J., and Torres-Padilla, M.-E. (2010). Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3. Nat. Cell Bio. *12*, 853–862.

Santos, F., Peters, A.H., Otte, A.P., Reik, W., and Dean, W. (2005). Dynamic chromatin modifications characterise the first cell cycle in mouse embryos. Dev. Biol. *280*, 225–236.

Sasaki, H., and Matsui, Y. (2008). Epigenetic events in mammalian germ-cell development: reprogramming and beyond. Nat. Rev. Genet. *9*, 129–140.

Scaturro, M., Cestelli, A., Castiglia, D., Nastasi, T., and Di Liegro, I. (1995). Posttranscriptional regulation of H1 zero and H3.3B histone genes in differentiating rat cortical neurons. Neurochem. Res. *20*, 969–976.

Seki, Y., Hayashi, K., Itoh, K., Mizugaki, M., Saitou, M., and Matsui, Y. (2005). Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev. Biol. *278*, 440–458.

Seki, Y., Yamaji, M., Yabuta, Y., Sano, M., Shigeta, M., Matsui, Y., Saga, Y., Tachibana, M., Shinkai, Y., and Saitou, M. (2007). Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. Development *134*, 2627–2638.

Silva, J., Mak, W., Zvetkova, I., Appanah, R., Nesterova, T.B., Webster, Z., Peters, A.H.F.M., Jenuwein, T., Otte, A.P., and Brockdorff, N. (2003). Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed-Enx1 polycomb group complexes. Dev. Cell *4*, 481–495.

Sugimoto, M., and Abe, K. (2007). X chromosome reactivation initiates in nascent primordial germ cells in mice. PLoS Genet. *3*, e116.

Surani, M.A., Ancelin, K., Hajkova, P., Lange, U.C., Payer, B., Western, P., and Saitou, M. (2004). Mechanism of mouse germ cell specification: a genetic program regulating epigenetic reprogramming. Cold Spring Harb. Symp. Quant. Biol. *69*, 1–9.

Surani, M.A., Hayashi, K., and Hajkova, P. (2007). Genetic and epigenetic regulators of pluripotency. Cell *128*, 747–762.

Tagami, H., Ray-Gallet, D., Almouzni, G., and Nakatani, Y. (2004). Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell *116*, 51–61.

Taverna, S.D., Li, H., Ruthenburg, A.J., Allis, C.D., and Patel, D.J. (2007). How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat. Struct. Mol. Biol. *14*, 1025–1040.

Timinszky, G., Till, S., Hassa, P.O., Hothorn, M., Kustatscher, G., Nijmeijer, B., Colombelli, J., Altmeyer, M., Stelzer, E.H.K., Scheffzek, K., et al. (2009). A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. Nat. Struct. Mol. Biol. *16*, 923–929.

Torres-Padilla, M.-E., Bannister, A.J., Hurd, P.J., Kouzarides, T., and Zernicka-Goetz, M. (2006). Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos. Int. J. Dev. Biol. *50*, 455–461.

Trostle-Weige, P.K., Meistrich, M.L., Brock, W.A., Nishioka, K., and Bremer, J.W. (1982). Isolation and characterization of TH2A, a germ cell-specific variant of histone 2A in rat testis. J. Biol. Chem. *257*, 5560–5567.

Turner, J.M., Burgoyne, P.S., and Singh, P.B. (2001). M31 and macroH2A1.2 colocalise at the pseudoautosomal region during mouse meiosis. J. Cell Sci. *114*, 3367–3375.

Updike, D.L., and Mango, S.E. (2006). Temporal regulation of foregut development by HTZ-1/H2A.Z and PHA-4/FoxA. PLoS Genet. *2*, e161.

Urdinguio, R.G., Sanchez-Mut, J.V., and Esteller, M. (2009). Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. Lancet Neurol. *8*, 1056–1072.

van der Heijden, G.W., Dieker, J.W., Derijck, A.A.H.A., Muller, S., Berden, J.H.M., Braat, D.D.M., van der Vlag, J., and de Boer, P. (2005). Asymmetry in histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote. Mech. Dev. *122*, 1008–1022.

van der Heijden, G.W., Derijck, A.A.H.A., Pósfai, E., Giele, M., Pelczar, P., Ramos, L., Wansink, D.G., van der Vlag, J., Peters, A.H.F.M., and de Boer, P. (2007). Chromosome-wide nucleosome replacement and H3.3 incorporation during mammalian meiotic sex chromosome inactivation. Nat. Genet. *39*, 251–258.

van Roijen, H.J., Ooms, M.P., Spaargaren, M.C., Baarends, W.M., Weber, R.F., Grootegoed, J.A., and Vreeburg, J.T. (1998). Immunoexpression of testis-specific histone 2B in human spermatozoa and testis tissue. Hum. Reprod. *13*, 1559–1566.

Wang, H., Wang, M., Wang, H., Böcker, W., and Iliakis, G. (2005). Complex H2AX phosphorylation patterns by multiple kinases including ATM and DNA-PK in human cells exposed to ionizing radiation and treated with kinase inhibitors. J. Cell. Physiol. *202*, 492–502.

Wang, H., Walsh, S.T., and Parthun, M.R. (2008). Expanded binding specificity of the human histone chaperone NASP. Nucleic Acids Res. *36*, 5763–5772.

Wellman, S.E., Casano, P.J., Pilch, D.R., Marzluff, W.F., and Sittman, D.B. (1987). Characterization of mouse H3.3-like histone genes. Gene *59*, 29–39.

Wiedemann, S.M., Mildner, S.N., Bonisch, C., Israel, L., Maiser, A., Matheisl, S., Straub, T., Merkl, R., Leonhardt, H., Kremmer, E., et al. (2010). Identification and characterization of two novel primate-specific histone H3 variants, H3.X and H3.Y. J. Cell Biol. *190*, 777–791.

Witt, O., Albig, W., and Doenecke, D. (1996). Testis-specific expression of a novel human H3 histone gene. Exp. Cell Res. *229*, 301–306.

Wong, L.H., Ren, H., Williams, E., McGhie, J., Ahn, S., Sim, M., Tam, A., Earle, E., Anderson, M.A., Mann, J., et al. (2009). Histone H3.3 incorporation provides a unique and functionally essential telomeric chromatin in embryonic stem cells. Genome Res. *19*, 404–414.

Wong, L.H., McGhie, J.D., Sim, M., Anderson, M.A., Ahn, S., Hannan, R.D., George, A.J., Morgan, K.A., Mann, J.R., and Choo, K.H. (2010). ATRX interacts with H3.3 in maintaining telomere structural integrity in pluripotent embryonic stem cells. Genome Res. *20*, 351–360.

Wouters-Tyrou, D., Martinage, A., Chevaillier, P., and Sautiere, P. (1998). Nuclear basic proteins in spermiogenesis. Biochimie *80*, 117–128.

Wu, R.S., Tsai, S., and Bonner, W.M. (1982). Patterns of histone variant synthesis can distinguish G0 from G1 cells. Cell *31*, 367–374.

Wunsch, A.M., and Lough, J. (1987). Modulation of histone H3 variant synthesis during the myoblast-myotube transition of chicken myogenesis. Dev. Biol. *119*, 94–99.

Zalensky, A.O., Siino, J.S., Gineitis, A.A., Zalenskaya, I.A., Tomilin, N.V., Yau, P., and Bradbury, E.M. (2002). Human testis/sperm-specific histone H2B (hTSH2B). Molecular cloning and characterization. J. Biol. Chem. *277*, 43474–43480.

Zhang, H., Roberts, D.N., and Cairns, B.R. (2005a). Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell *123*, 219–231.

Zhang, R., Poustovoitov, M.V., Ye, X., Santos, H.A., Chen, W., Daganzo, S.M., Erzberger, J.P., Serebriiskii, I.G., Canutescu, A.A., Dunbrack, R.L., et al. (2005b). Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev. Cell *8*, 19–30.

Zhou, J., Fan, J.Y., Rangasamy, D., and Tremethick, D.J. (2007). The nucleosome surface regulates chromatin compaction and couples it with transcriptional repression. Nat. Struct. Mol. Biol. *14*, 1070–1076.