CHROMATIN DYNAMICS

Histone variants — ancient wrap artists of the epigenome

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Abstract | Histones wrap DNA to form nucleosome particles that compact eukaryotic genomes. Variant histones have evolved crucial roles in chromosome segregation, transcriptional regulation, DNA repair, sperm packaging and other processes. 'Universal' histone variants emerged early in eukaryotic evolution and were later displaced for bulk packaging roles by the canonical histones (H2A, H2B, H3 and H4), the synthesis of which is coupled to DNA replication. Further specializations of histone variants have evolved in some lineages to perform additional tasks. Differences among histone variants in their stability, DNA wrapping, specialized domains that regulate access to DNA, and post-translational modifications, underlie the diverse functions that histones have acquired in evolution.

Histone chaperone
An escort protein that performs a transfer reaction on a histone, such as deposition onto DNA, eviction from DNA, transfer to another chaperone or enzyme, or storage for later use.

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Nearly all eukaryotes wrap their DNA around histones to form nucleosomes that compact the genome while still allowing access for active processes such as transcription, replication and DNA repair. Each nucleosome core particle comprises ~ 147 bp of DNA wrapped in 1.7 turns around a protein octamer of 2 molecules of each of the 4 highly conserved histones H2A, H2B, H3 and H4 (REF. 1). Genes encoding the 'canonical' histones of animals are found clustered in repeat arrays and their transcription is tightly coupled to DNA replication. Other histone genes, however, are typically found singly in the genome, are constitutively expressed and encode non-canonical histone variants that differ in primary amino acid sequence from their canonical paralogues. Unlike the canonical histones that function primarily in genome packaging and gene regulation, non-canonical variants have roles in a range of processes, including DNA repair, meiotic recombination, chromosome segregation, transcription initiation and termination, sex chromosome condensation and sperm chromatin packaging (see Supplementary information S1 (table)). This diversity of roles reflects, at least in part, structural differences from canonical histones that alter nucleosomes from within the core particle. Structural alterations in the core octamer can affect the wrap of DNA around the histones and thereby alter nucleosome dynamics[2].

The diversity of histone variants highlights their considerable innovation during their long evolutionary histories. 'Universal' variants are found in nearly all eukaryotes, reflecting ancient functions common to eukaryotic cells, whereas lineage-specific variants are specialized for the unique biology of their host organisms. In this Review, we consider the diversity and evolution of core histone variants, from archaeal ancestors to universal eukaryotic variants to lineage-specific variants and variant-specific post-translational modifications (PTMs). We summarize their diverse and dynamic interactions as 'wrap artists' of DNA and discuss recent developments in understanding their functional implications for gene regulation and epigenetic inheritance. We do not discuss histones that occupy linker DNA between nucleosomes, such as H1, or attempt to duplicate the work of many excellent reviews of the extensive literature on particular variants[3–9]. Nor do we systematically discuss PTMs[10], histone chaperones[11], or chromatin-remodelling complexes[12], except when they offer insight to the roles of variants. Our focus is on the evolutionary plasticity of core histone variants despite the extreme conservation in these protein families.

Structure and origin of nucleosomes

Each of the four core histones shares the common structure of a histone fold domain (HFD), which consists of three α-helices (α1, α2 and α3) separated by two loops (L1 and L2)[13]. All four histones also have 'tails' that extend outside the nucleosome core and are subject to diverse PTMs that are important in gene regulation and chromatin compaction. The HFDs fold together in antiparallel pairs: H3 with H4 and H2A with H2B[1]. This dimeric structure of HFDs is an ancient building block from which tetramers, hexamers and octamers can be built[2].

The HFD is found not only in eukaryotic histones but also in archaeal histones and in some eukaryotic transcription factors[13]. Histones are found in all three major

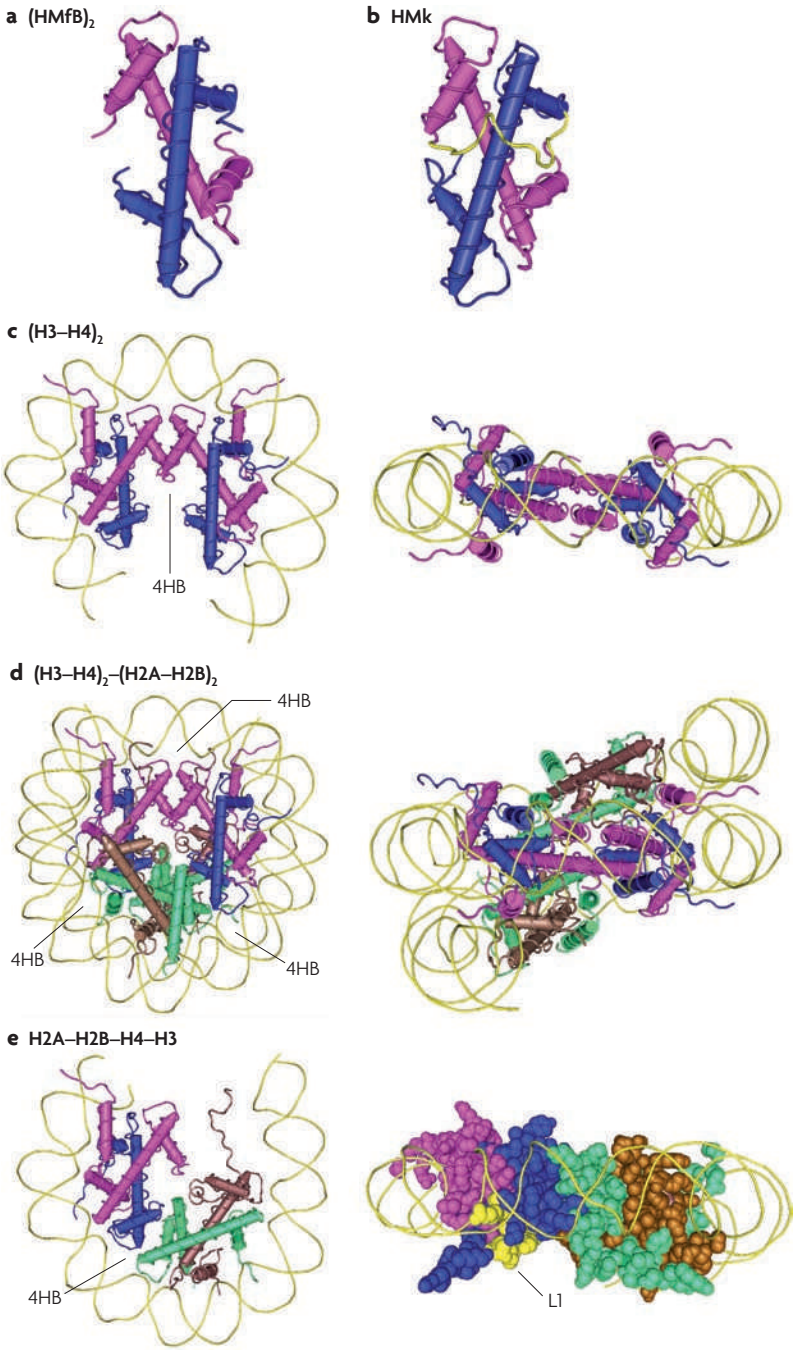


Figure 1 | **Histone dimers, tetramers and octamers. a** | The homodimer structure of archaeal histone HMfB from *Methanothermus fervidus*. The identical HMfB monomers are coloured differently for clarity. **b** | The two histone fold domains (HFDs) of histone HMk from *Methanopyrus kandleri* heterodimerize. The amino-terminal HFD is shown in pink, the carboxy-terminal HFD is shown in blue and the linking sequence between the HFDs is shown in yellow. **c** | Two views of a stable eukaryotic tetramer of H3 (pink) and H4 (blue), which can wrap DNA in either a left-handed (negative supercoil) or right-handed (positive supercoil) turn. **d** | Addition of two H2A–H2B dimers (H2A shown in brown and H2B shown in green) to an $(H3\text{-}H4)_2$ tetramer forms the canonical eukaryotic histone octamer, resulting in a left-handed wrap. **e** | The structure of a hemisome — half of an octamer. Models of centromere-specific histone H3 variant (CenH3)-containing nucleosomes have been proposed based on replacing H3 with CenH3 in the structures shown in parts **c**–**e.** Loop 1 (L1; yellow in the space-filling model) is longer in CenH3s and is proposed to stabilize the weak four-helix bundle (4HB) formed by H2B (green) and H4 (blue) in the hemisome model, and to favour a right-handed wrap.

branches of archaea, indicating that they were present in the common ancestor of eukaryotes and archaea[14]. Archaeal histones consist of HFDs without tails. Archaeal histone dimers (FIG. 1a) align well with eukaryotic histones at the structural level[14] but not at the sequence level, and combine into tetramers that protect about 60 bp of DNA[15] by forming a central 4-helix bundle (4HB) from the α2 and α3 helices of 2 of the 4 monomers. This structure is stabilized by hydrophobic interactions and a salt bridge[1]. Individual archaeal species encode up to seven HFDs and, unlike eukaryotic histones that form obligate heterodimers, archaeal histones form both homo- and heterodimers[14]. The two histones of *Methanothermus fervidus* (HMfA and HMfB) can assemble homodimers into homotetramers that differ in their affinity for DNA despite highly similar structures. The two α2 and α3 helices in the tetramer that are not engaged in the central 4HB are, in principle, available to form 4HBs with additional HFD dimers. Complexes assembled from dimers of HMfA and HMfB can wrap DNA into either positive or negative supercoils[16].

Most archaeal histones are members of the HMfB family and form homodimers, but there are others that favour heterodimerization. The histone of *Methanopyrus kandleri* (HMk) has two HFDs in the same peptide that heterodimerize with each other (FIG. 1b). HMk forms dimers (HFD tetramers) through a 4HB between two amino-terminal HFDs[17], but lacks a hydrophobic patch that would permit the carboxy-terminal HFDs to participate in 4HBs, thus limiting oligomerization[14]. MJ1647 (also known as HMvA) from *Methanocaldococcus jannaschii* has a C-terminal extension of ~ 27 amino acids that seems to destabilize the formation of a 4HB between homodimers and is therefore predicted to form heterodimers with HMfB family members *in vivo*[14,18]. In the early branching species *Nanoarchaeum equitans*, the histone NEQ288 has four additional residues at the α1–L1 junction, a feature that resembles H3 and H2B but is in contrast to H4, H2A and HMfB family members. Similar to MJ1647, NEQ288 requires another histone (NEQ348) of the HMfB family to wrap DNA, resulting in greater DNA compaction and supercoiling than with either histone alone. The occurrence of histones that structurally align with eukaryotic histone heterodimers in early branching *N. equitans* raises the possibility that the differentiation of H3-like and H4-like histones might have preceded the archaeal–eukaryotic split[19]. Thus, archaeal histones exhibit multiple strategies for diversification of nucleosome particles, suggesting flexibility in the size of those particles, their stability and the way they wrap DNA. Obligate heterodimerization in some archaeal histones offers insight into the evolution of the defined oligomerization, greater DNA compaction and tail extensions that are seen in the eukaryotic histone octamer.

Universal eukaryotic histone variants

Like tetrameric archaeal nucleosomes, assembly of the canonical eukaryotic nucleosome is thought to begin with an $(H3\text{–}H4)_2$ tetramer, held together by a strong 4HB between the two H3 molecules[1]. This tetramer

(FIG. 1c) can wrap DNA in either a right-handed helix (positive supercoils) or a left-handed helix (negative supercoils). The addition of two H2A–H2B dimers forms an octamer (FIG. 1d) with a left-handed helical ramp that locks the DNA into a negatively supercoiled configuration[20]. The H2A–H2B dimers form weak 4HBs between the α2 and α3 helices of H4 and H2B. Octameric nucleosomes occur in all eukaryotes except possibly dinoflagellates, which have lost bulk histones[21].

Other eukaryotic histone variants (centromeric histone variant H3 (CenH3; also known as CENP-A in humans, chromosome segregation protein 4 (Cse4) in *Saccharomyces cerevisiae* and centromere identifier (CID) in *Drosophila melanogaster*), H3.3, H2A.Z and H2A.X) are also of near universal occurrence, dating back to the earliest known diversifications of eukaryotic lineages[22]. As in archaeal histones, these universal variants can alter the fundamental structure of the nucleosome and its stability.

CenH3 — a right-handed wrap artist. Centromere-specific histone variants are essential for assembly of the kinetochore[23]. Centromere-specific H3s inhabit diverse forms of centromeres and are rapidly evolving[4]. They are far less conserved than canonical H3s (see Supplementary information S2 (table)), and phylogenetic analysis cannot rule out multiple origins for them in the H3 family[22,24]. The term CenH3 was therefore introduced[25] to refer functionally to all centromere-specific histones whether or not they had a common origin with the first described example, mammalian CENP-A[26]. However, a single origin for CenH3s is parsimonious, and we regard CenH3 and CENP-A as equivalent terms. CenH3s have ~ 50–60% identity with canonical H3s in the HFD and no conservation in the N-terminal tails. They have longer, non-conserved L1s[22] and usually replace Phe84 in H3 with Trp (residue numbers refer to the canonical human histones).

CenH3 tails and L1s are under positive selection in some plants and animals[4]. Oligopeptide motifs that bind the minor groove of DNA have evolved recurrently in the N-terminal tails of several CenH3s, including human CENP-A[27]. Although lacking sequence specificity, these minor groove-binding motifs prefer AT-rich DNA[28], which might be relevant to binding usually AT-rich centromeres. Despite this putative compositional preference, centromere specification is epigenetic and the mechanisms that maintain CenH3s at centromeres are not well understood[3,4].

CenH3 from the budding yeast *S. cerevisiae*, Cse4, can substitute for human CENP-A[29], which suggests that they form equivalent nucleosome structures despite considerable sequence divergence. CenH3 nucleosomes exhibit unusual properties, however, that have made interpreting their structure difficult and controversial. They have traditionally been interpreted as octamers, but unlike canonical nucleosomes that protect ~ 150 bp of DNA from nucleases, native *D. melanogaster* CenH3 (CID) nucleosomes protect much less[30]. Measurements by atomic force microscopy show that CID nucleosomes are only half the height of canonical nucleosomes[30], suggesting that they are also half the volume. In addition, like some archaeal histones, CenH3 nucleosomes in *D. melanogaster* and yeast induce positive supercoils in DNA, implying a right-handed wrap of DNA[31], in contrast to the left-handed wrap of canonical nucleosomes[1]. *D. melanogaster* CID nucleosomes contain one molecule each of CID, H4, H2B and H2A and are proposed to resemble half of an octameric nucleosome[30] (FIG. 1e) or a hemisome[2]. By contrast, the yeast Cse4 nucleosome was proposed to consist of a hexamer of two molecules each of Cse4, H4 and suppressor of chromosome missegregation 3 (Scm3), but to lack H2A and H2B[32]. Scm3 has homologues in the fission yeast *Schizosaccharomyces pombe* and in mammals that are essential for CenH3 deposition[4]. A more recent study concluded that Scm3 is not part of the Cse4 nucleosome because overexpressing Cse4 rescues an *Scm3* deletion[33]. Moreover, the detection of H2A and H2B in Cse4 particles implies that either a hemisome or octameric nucleosome occupies yeast centromeres[33,34]. Co-immunoprecipitation of two differently tagged Cse4 molecules from yeast centromeres has been cited in support of an octameric structure[33], but it is unclear whether the tags were on a single nucleosome, and a conventional octamer seems structurally incompatible with a right-handed wrap of DNA[31]. Either a hemisome or a $(CenH3–H4)_2$ tetrameric nucleosome is theoretically compatible with a right-handed wrap, although $(CenH3–H4)_2$ tetramers have not been observed[33,35]. Either tetrameric model would require a conformational change in the central 4HB. In the hemisome model, the longer L1 in CenH3 has been proposed to bring about the conformational change in the adjacent H4–H2B 4HB. The positively supercoiled tetramers are proposed to resist the mitotic condensation affecting adjacent negatively supercoiled H3-containing octamers, thereby providing an accessible asymmetric nucleosome surface on which to assemble kinetochore proteins and a possible topological basis for epigenetic inheritance of CenH3 at the centromere[31,36].

Does H3.3 do more than replace H3? In addition to CenH3, most eukaryotes have two similar H3 family members commonly called canonical H3 and replacement H3.3. In most animals, H3.3 differs from H3 by just four amino acid substitutions, including residue 31 in the N-terminal tail, which is an Ala in H3 and a Ser in H3.3, and substitutions at residues 87, 89, and 90 near the beginning of α2. Similarly, in other eukaryotes, differences include Ser or Thr versus Ala at residue 31 and differences at residues 87 and 89 or 90 and sometimes others — a total of 16 in the ciliate *Tetrahymena thermophila*[37]. Whereas H3 is assembled during replication in *D. melanogaster*, H3.3 can undergo either replication-coupled or replication-independent assembly. The three substitutions in α2 of *D. melanogaster* H3 exclude it from replication-independent assembly[38].

Insight into these H3.3 assembly modes first came from human HeLa cells, in which the major canonical H3 (H3.1) is assembled into chromatin by the

Supercoil
A contortion in DNA that occurs as a consequence to over- or under-twisting of the DNA helix. Supercoils can be introduced during DNA packaging and DNA–RNA synthesis. Topoisomerases sense supercoiling and can either generate or dissipate it by changing DNA topology.

Centromere
The region of a chromosome that is attached to the spindle during nuclear division.

Kinetochore
A large multiprotein complex that assembles onto the centromere of the chromosome and links it to the microtubules of the mitotic spindle. The kinetochore is also a signalling centre for many of the proteins that control the progression of mitosis.

chromatin-assembly factor 1 (CAF1) histone chaperone complex during DNA replication[39] and DNA repair[40], whereas H3.3 is assembled by the histone regulator A (HIRA) complex independently of DNA synthesis[39]. In *D. melanogaster* embryos, replication-independent assembly during sperm chromatin remodelling is mediated by HIRA and the chromatin remodeller chromodomain helicase DNA-binding protein 1 (CHD1), although the roles of these proteins in later development are unclear. Some H3.3 deposition seems to occur by replication-coupled assembly[5]. Similarly, in *T. thermophila*, canonical H3 deposition is coupled to DNA synthesis during replication and repair, whereas most H3.3 is deposited independently of replication, although inefficient replication-coupled assembly of H3.3 has also been inferred[37].

Ascomycetes, such as yeast, and some unicellular organisms, such as the algae *Cyanidioschyzon merolae* and *Chlamydomonas reinhardtii*, have only a single form of non-centromeric H3 that must undergo both replication-coupled and replication-independent assembly, corresponding functionally to H3.3. Accordingly, *S. cerevisiae* H3.3 (H3) undergoes CAF1 mediated replication-coupled assembly and Hir protein-mediated replication-independent assembly[41]. Canonical forms of H3, the synthesis of which is coupled to replication, seem to have evolved recurrently in eukaryotic evolution, presumably by divergence from H3.3-like forms[22]. Consistent with this, canonical H3s are dispensable in *T. thermophila* if H3.3s are overexpressed[37].

In both *D. melanogaster* and human cells, H3.3 is deposited into transcribed genes, promoters and gene regulatory elements[42]. It is incorporated into genes on induction of transcription and is associated with transcriptional elongation[43,44]. H3.3 is enriched relative to bulk canonical H3 in 'active' PTMs found near promoters[45], as is canonical human H3.1 when isolated from positions adjacent to H3.3 nucleosomes[46], suggesting that the deposition context is important in determining PTMs on variants. In nuclear transplantation experiments in the frog *Xenopus laevis*, H3.3 can mediate the epigenetic memory of an activated gene state that depends on Lys4 of H3.3 (H3K4)[47]. H3.3 is also assembled during genome-wide chromatin remodelling, as occurs in the decondensing male pronucleus[5] before the first round of zygotic replication, and in meiotic silencing of unsynapsed chromatin and mammalian sex chromosomes, during which H3.3 deposition is accompanied by the initial depletion and the later acquisition of PTMs[48]. This is in contrast to meiotic silencing of the X chromosome in the nematode *Caenorhabditis elegans*, which is characterized by the depletion of H3.3 and of accompanying active PTMs[49].

In *D. melanogaster*, H3.3 is turned over more rapidly than H3 (REF. 43), which might contribute to keeping chromatin accessible in transcribed genes and elsewhere. Locations of H3.3 replacement at *cis*-regulatory regions correspond to nuclease-hypersensitive sites, suggesting a dynamic process of disruption and replacement of nucleosomes at these sites[42]. Tagged H3.3 nucleosomes in chicken cells show reduced stability relative to tagged H3 controls for salt-dependent dissociation of H2A–H2B and H2A.Z–H2B dimers[50]. Because the three residues that differ in H3.3 α2 do not contact H2A or H2B, this reduced stability might result from differential disruption of H3.3 nucleosomes by cellular processes, such as transcription, chromatin remodelling and modification, rather than from inherent properties of H3.3 nucleosomes.

Surprisingly, deletions of H3.3 genes are not always lethal, although they do result in sterility in both *D. melanogaster* and *T. thermophila*[37,51,52]. *D. melanogaster* mutants display transcriptional defects but these can be rescued by overexpression of H3 (REF. 52) (although this was not seen in *T. thermophila*[37]). Therefore, *D. melanogaster* H3.3 is not required for histone replacement during somatic development. However, H3.3 mutants display meiotic defects in chromosome condensation and segregation in *D. melanogaster* spermatocytes that are dependent on residues 87, 89 and/or 90 but not on sites of PTMs[52], suggesting that H3.3 is required for germline-specific chromatin remodelling.

The conserved H3.3-specific Ser31 site becomes phosphorylated on metaphase chromosomes during mitosis and female meioisis in the urochordate *Oikopleura dioica*[53] and in metaphase I of mouse spermatocytes[48]. In pluripotent mouse and human cell lines, phosphorylated Ser31 is found in metaphase telomeric chromatin, whereas in differentiated cells it is found in metaphase pericentromeric heterochromatin[54]. Despite this association with mitotic and meiotic metaphase, Ser31 is unnecessary for fertility in *D. melanogaster* spermatogenesis[52], and its function remains unclear.

H2A.Z and transcription. Like the H3 family, the H2A family has two universal variants: H2A.Z and H2A.X. H2A.Z diverged from other H2As before the diversification of modern eukaryotes, whereas H2A.X and H2A seem to have diverged repeatedly in different lineages[22]. H2A.Z differs from H2A and H2A.X around the L1–α2 and α2–L2 junctions and in the C-terminal 'docking domain' that contacts H3 (REF. 22).

H2A.Z-containing nucleosomes are found on either side of a 'nucleosome-free region' (NFR) at transcription start sites (TSSs)[6], where they promote efficient RNA polymerase II (RNAPII) recruitment in both yeast and human cells[55,56]. In addition, H2A.Z has apparently contradictory roles in gene activation and silencing; nucleosome turnover; DNA repair; heterochromatin, boundary element and chromatin fibre formation (reviewed in REFS 6,7); suppression of antisense RNAs[57]; embryonic stem cell differentiation[58]; and antagonizing DNA methylation in plants[59].

Some of these contradictions might be explained by the effects of PTMs on H2A.Z nucleosomes (BOX 1), resistance of H2A.Z nucleosomes to binding by H1 (REF. 60), effects on nucleosome remodelling complexes[61], or variation in H2A.Z nucleosome composition. Unlike the single, non-essential H2A.Z gene in yeast (*Htz1*), vertebrates have two H2A.Z genes

Epigenetic memory
An effect on gene expression or function that is not a result of DNA sequence changes and is heritable through cell division.

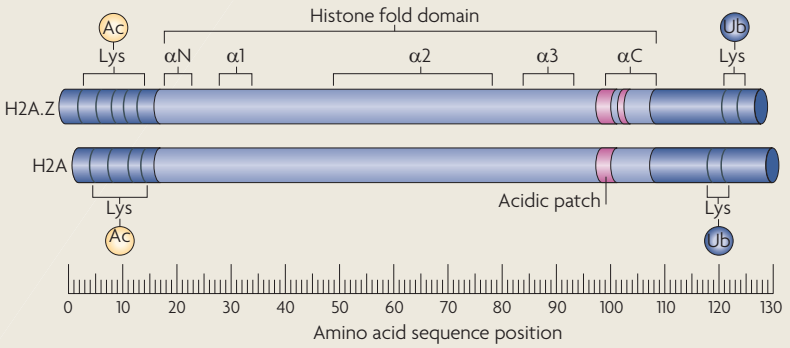
Nuclease-hypersensitive site
A chromosomal site that shows increased sensitivity to nucleases such as DNase I and that are correlated to regions of reduced nucleosome density and gene regulatory sites.

Heterochromatin
A highly condensed form of chromatin with very low transcriptional activity. It occurs at defined sites, such as around centromeres or telomeres. Typically it is composed of repetitive sequences and transposons, with few genes present.

Box 1 | **H2A.Z acetylation and ubiquitylation**

Histone variant H2A.Z is present at most promoters in yeast whether they are active promoters or not[6]. In humans, H2A.Z is found at enhancers and in heterochromatin and its presence at promoters correlates with RNA polymerase II (RNAPII) occupancy, suggesting it is only recruited to promoters when needed[56]. Apparently conflicting associations of H2A.Z with active and silenced chromatin might be partially explained by acetylation (Ac) and monoubiquitylation (Ub), which affect most H2A variants. H2A.Zs typically have multiple Lys residues in their amino-terminal tails that can be acetylated (see the figure). In *Tetrahymena thermophila*, acetylation of at least one of six Lys residues is necessary for viability, probably to regulate the charge on the tail, which might interact with DNA[122]. *Saccharomyces cerevisiae* H2A.Z (Htz1) is enriched on repressed promoters, whereas Htz1 acetylated on Lys14 is enriched on active promoters[123]. Similarly, H2A.Z in human heterochromatic regions is under-acetylated relative to euchromatic regions[56]. Acetylation of H2A.Z is necessary for its association with inducible promoters and for their activation in both yeast and *Drosophila melanogaster*[124,125]. When reconstituted with other acetylated core histones, acetylated H2A and H2A.Z are crucial for the conformational change of nucleosomes induced by global core histone acetylation, consistent with H2A and H2A.Z acetylation acting as a gene-regulation switch[65].

Monoubiquitylation on the carboxyl terminus of H2A.Z and H2A is found on the inactive X chromosome of female mammals and is mediated by the RING1B (also known as RNF2) E3 ligase, part of the Polycomb repressor complex 1 (REFS 126,127). H2A.Z is also enriched at developmental genes that are regulated by Polycomb group proteins in embryonic stem cells, and RING1B localization at these sites is reduced in H2A.Z-depleted cells[58]. RING1B is necessary for repression at these sites[128]. In *D. melanogaster*, both the RING1B homologue (RING) and H2A.Z (H2Av) are similarly necessary for repression at Polycomb-regulated genes[79,129]. Together, these results suggest that H2A.Z is likely to be a more crucial target of monoubiquitin-mediated silencing by Polycomb repressor complexes than H2A[58].



(*H2A.Z1* and *H2A.Z2*), which encode proteins that differ by three residues[62]. The genes are not redundant because mouse *H2A.Z1* mutants are lethal[63]. Although little is known about *H2A.Z2*, it is transcribed in birds and mammals and its conservation through vertebrate evolution strongly suggests that it is functional[62]. Plants can have several H2A.Zs that differ at many residues. In *Arabidopsis thaliana*, different H2A.Zs are at least partially redundant, although they differ in cell-cycle regulation[64].

In addition, H2A.Z can assemble *in vitro* into either homotypic nucleosomes or 'hybrid' nucleosomes that contain one H2A.Z and one H2A molecule. Reconstituted hybrid and homotypic H2A.Z nucleosomes protect ~ 146 bp on 5S rDNA, and hybrids display a salt-dependent stability intermediate between more stable H2A.Z homotypic nucleosomes and H2A nucleosomes[65]. Hybrid H2A.Z nucleosomes are the norm in human HeLa cells[66].

Despite the intrinsic stabilizing effect of H2A.Z in reconstituted nucleosomes, yeast Htz1 nucleosomes in chromatin have high turnover rates in promoter regions[6] and are less stable to salt washes than H2A nucleosomes[67]. Some nucleosomes containing both H3.3 and H2A.Z seem especially unstable in human cells[68], although such 'double-variant' nucleosomes are stable when reconstituted *in vitro*[60]. In chromatin prepared under very low salt conditions, these unstable nucleosomes occupy the NFR in front of genes, whereas the same region seems to be nucleosome-free in chromatin prepared in higher salt conditions[68]. This indication that NFRs are occupied at least transiently by nucleosomes but only seem to be nucleosome-free because of artefactual loss during preparation, suggests that these epigenomic features are better described as nucleosome-deficient regions (NDRs). Because nucleosomes flanking human NDRs are likely to contain H3.3 and H2A.Z but are not equally destabilized by salt[68], the instability is probably not a general property of H2A.Z–H3.3 nucleosomes in chromatin. In yeast, NDR formation for most promoters is dependent on the RSC remodelling complex, which displaces nucleosomes from the NDR and is necessary for the deposition of the flanking H2A.Z nucleosomes by the Swr1 complex[69]. The H2A.Z–H3.3 nucleosomes in human NDRs, which are not uniformly positioned[68], probably represent nucleosomes that are in the process of being disrupted.

Nucleosomes flanking the NDR are highly positioned relative to the TSS, with the +1 nucleosome being most highly positioned. In yeast, the TSS is found just inside the +1 nucleosome, whereas in *D. melanogaster* it lies in the NDR, with RNAPII frequently paused in contact with the +1 H2A.Z (H2Av) nucleosome, which might contribute to RNAPII pausing[70] and to TSS selection[71]. In human cells, positional H2A.Z nucleosomes in upstream promoter elements contribute to both positive and negative gene regulation[72,73]. The positioning of H2A.Z nucleosomes does not imply a direct sequence-dependent interaction with H2A.Z because H2A.Z is deposited into nucleosomes by multiple complexes containing Swr1 homologues, which exchange H2A.Z–H2B dimers for H2A–H2B dimers[74], and shows increased association with chromatin remodellers relative to H2A in HeLa cells[61]. The greater intrinsic stability of H2A.Z nucleosomes might contribute to their stable positioning[75].

Transcription reduces H2A.Z occupancy over promoters[6,44] and gene bodies, at which the eviction of randomly incorporated H2A.Z is followed by the preferential replacement with H2A in plants and animals[56,76]. Removal of H2A.Z from gene bodies might help prevent transcription from cryptic promoters, as has been proposed for plant genic DNA methylation, which is antagonized by H2A.Z in *A. thaliana*[59]. Methylation over gene bodies in animals[77] might serve a similar function (FIG. 2). Conversely, H2A.Z might protect promoters from methylation[56]. H2A.Z is excluded from plant heterochromatin, which is heavily methylated[59], but low or absent transcription might contribute to H2A.Z accumulation in mammalian heterochromatin[56],

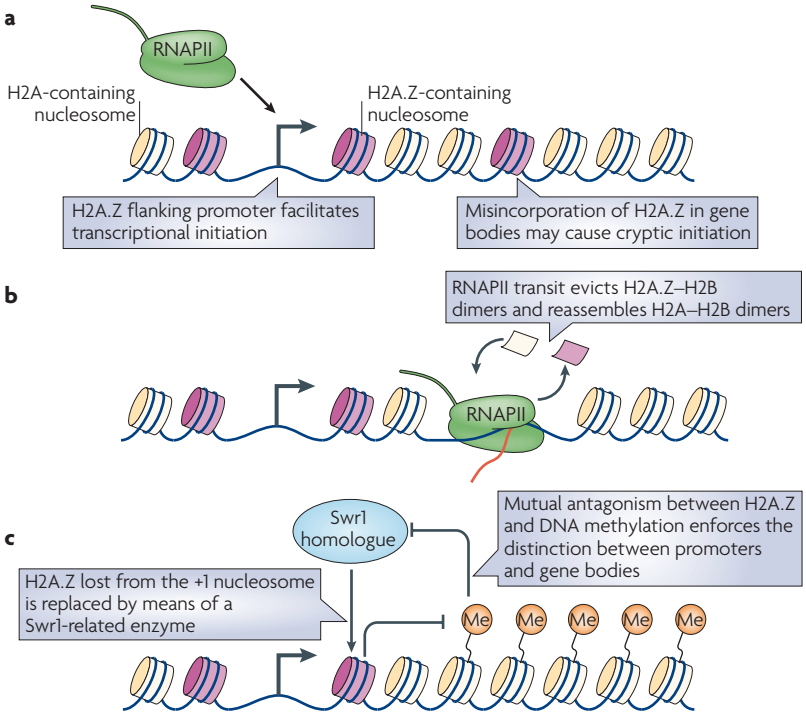


Figure 2 | **A model of H2A.Z and DNA methylation in transcription. a** | Histone variant H2A.Z facilitates RNA polymerase II (RNAPII) recruitment, but randomly incorporated H2A.Z nucleosomes (magenta) in gene bodies might contribute to aberrant transcription initiation. **b** | Transcription causes the eviction of H2A.Z–H2B dimers and the preferential reassembly of H2A–H2B dimers, which might help prevent aberrant initiation in the short term. **c** | Targeted deposition of H2A.Z at promoters by a Swr1 homologue prevents their methylation, and, conversely, DNA methylation over gene bodies inhibits the misincorporation of H2A.Z, thus enforcing the distinction between promoters (which must be kept depleted of nucleosomes) and gene bodies (which require stable nucleosomes to avoid aberrant initiation).

in which it is bound by heterochromatin protein 1α (HP1α; also known as CBX5)[78]. The binding of HP1α to H2A.Z nucleosomes is enhanced relative to H2A by the extended acidic patch in H2A.Z, a region implicated in chromatin fibre folding[78] and the stimulation of nucleosome remodelling complexes[61]. *D. melanogaster* H2Av is similarly involved in heterochromatin formation, in which the remodelling and replacement of H2A with H2Av precede methylation of H3K9 and HP1α recruitment[79,80]. A common feature of H2A.Z's different roles in gene activation, gene regulation and heterochromatin formation might be its tendency to form stably positioned nucleosomes.

H2A.X — poised for chromatin remodelling. H2A.X is similar to canonical H2A in the HFD but is distinguished by the C-terminal motif Ser-Gln-(Glu/Asp)-Φ, in which Φ represents a hydrophobic residue[22]. This motif can become rapidly phosphorylated on Ser, producing the form known as γH2A.X, by the phosphoinositide 3-kinase-like kinases, ataxia telangiectasia mutated (ATM), ataxia telangiectasia and RAD3-related (ATR) and DNA-dependent protein kinase (DNA-PK). Phosphorylation occurs in response to double-strand breaks (DSBs), at which γH2A.X helps to recruit and/or retain DNA repair proteins, histone modifying enzymes and chromatin remodelling complexes (reviewed in REFS 7,81). *X. laevis* has three H2A.X proteins, one ending in Ser-Gln-Glu-Tyr and two ending in Ser-Gln-Glu-Phe. The two ending in Ser-Gln-Glu-Phe are abundant in eggs and during early embryogenesis, where they are phosphorylated on Ser in both the presence and absence of DNA damage[82], suggesting the specialization of isoforms for distinct functions. The Ser-Gln-Glu-Tyr motif of mammalian H2A.X is phosphorylated constitutively on the terminal Tyr142, which becomes dephosphorylated on DNA damage[83]. This elaboration of the γH2A.X DNA damage response might be restricted to animals, since Tyr142 is not widely conserved in eukaryotes.

γH2A.X is also required for the remodelling and inactivation of the sex chromosomes in male mouse meiosis[84], where it precedes the chromosome-wide deposition of H3.3 (REF. 48), and more generally in meiotic silencing of unpaired chromatin[85]. γH2A.X has undefined cell-cycle regulated roles in numerous small foci that are independent of DSBs, and on the inactive X chromosome (Xi; also known as the Barr body). A common feature of these roles of γH2A.X might be to promote chromatin remodelling[8].

The multiple divergences between H2A.X and H2A in a phylogenetic tree have been attributed to multiple origins of H2A.X[22,86]. *D. melanogaster* H2Av, which is an H2A.Z-like histone that terminates in Ser-Gln-Ala-Tyr, is a clear example of such convergent acquisition of the phosphorylation motif. The topology of the H2A and H2A.X tree (FIG. 3), however, is equally consistent with multiple origins of canonical H2A from an ancestral H2A.X. Given the conserved role of γH2A.X in double-strand break repair, and the presence of H2A.X but not canonical H2A in fungi, the excavate *Giardia lamblia,* the apicomplexan *Cryptosporidium parvum* and even the dinoflagellate *Alexandrium tamarense*[21], which has lost bulk histones, we suggest that an ancestral H2A.X is a more parsimonious hypothesis than multiple acquisitions of Ser-Gln-(Glu/Asp)-Φ and its integration into the DSB repair pathway. Thus, the universal variants CenH3, H3.3, H2A.Z and H2A.X are all likely to have preceded canonical H3 and H2A. In some animals, genes exist for H2B and H4 that are transcribed independently of replication[87,88], and we suggest that these variants, rather than their canonical paralogues, recall the ancestral state.

Lineage-specific variants

Although most eukaryotes encode a single version of H4 and H2B, multiple variants of all four core histone families occur in some eukaryotic lineages. Trypanosomes have two variants of each of the four histones (BOX 2), which combine into distinct types of nucleosomes that mark transcription initiation and termination sites[89]. Ciliates have highly diversified H3s and H4s[90,91]. This diversification has been attributed to the unusual binucleate structure of the ciliate cell (BOX 3). Other notable lineage-specific novelties are described below.

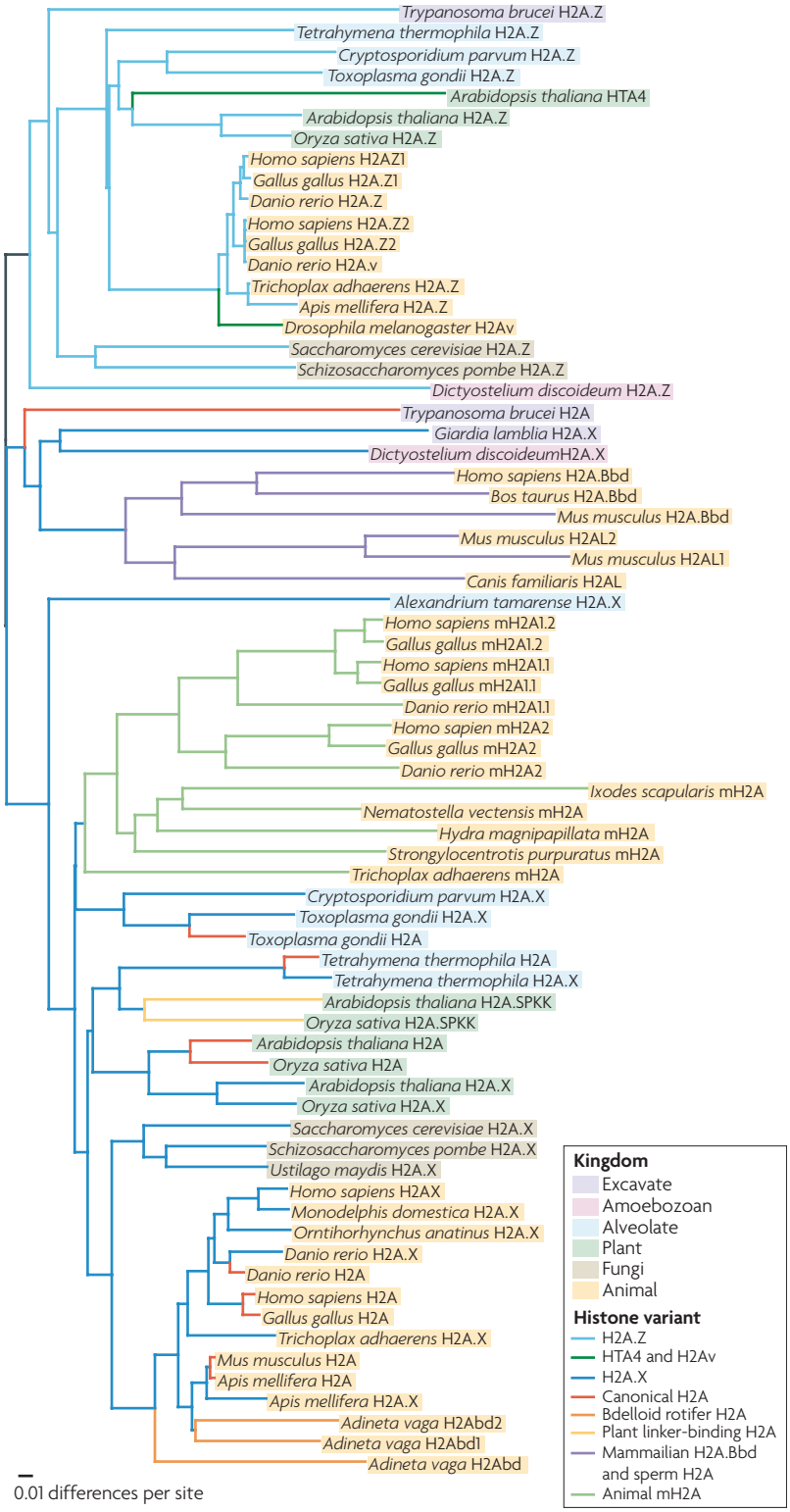


Figure 3 | **Phylogeny of H2A variants.** A neighbour-joining protein tree rooted between histone variant H2A.Z and other H2A variants. H2A.Z diverged from other H2As before the diversification of eukaryotes. *Drosophila melanogaster* H2Av and *Arabidopsis thaliana* HTA4 are H2A.Zs with features resembling H2A.X and H2A Barr body-deficient (H2A.Bbd), respectively. H2A.Xs have probably repeatedly given rise to 'canonical' H2As and to bdelloid rotifer H2As and plant H2As with minor-groove-binding motifs. Mammalian H2A.Bbds and sperm H2As are rapidly evolving proteins with shortened carboxyl termini, and are anomalously placed near the base of the tree. Animal macroH2As (mH2As) have a large C-terminal macrodomain. Tree drawn using Dendroscope[148].

Rotifer H2As — DNA damage protection? Bdelloid rotifers are asexual invertebrates that lack both males and meiosis. They are extraordinarily resistant to radiation and undergo frequent desiccation that causes severe DNA damage[92]. Surprisingly, they have no H2A.X, H2A.Z, or canonical H2A, but they encode three forms of H2A with C-terminal tails that extend 30–50 residues past the docking domain. These are highly conserved between two bdelloid families but are absent from the sexual monogont rotifers. The function of the large C termini is unknown, but it is speculated to aid in survival of desiccation and its accompanying DNA damage[86]. If so, these might be the only histone variants that have evolved to adapt to special environmental conditions.

MacroH2As and polyADP-ribosylation. MacroH2As (mH2As) are highly conserved H2As that are characterized by a HFD domain followed by a much larger > 200 residue C-terminal non-histone domain that includes a basic region and a 'macrodomain'. A mH2A gene is probably ancestral in animals since one can be found in placozoans, cnidarians, chelicerates, and echinoderms, as well as in vertebrates, which have two genes: *mH2A1* and *mH2A2* (FIG. 3). The variant mH2A1 (also known as H2AFY) encodes two differentially expressed forms in the same transcript using alternatively spliced exons, yielding either mH2A1.1 or mH2A1.2, both of which are conserved between mammals and birds[93]. mH2A2 (also known as H2AFY2) shares 80% identity with mH2A1. Both mH2A1 and mH2A2 are enriched on the Xi chromosome of female mammals, suggesting a role in transcriptional silencing[94,95].

Native chicken mH2A1-containing nucleosomes have greater salt-dependent stability than canonical H2A nucleosomes, are modified with polyADP-ribose and seem to exclude H1, perhaps by steric hindrance from the large macrodomain[96]. In reconstituted mH2A1.2 nucleosomes, the macrodomain reduces transcription factor access and represses transcriptional activation mediated by the histone acetyltransferase p300 (REF. 97). Nucleosomes reconstituted using the mH2A1 HFD preferentially assemble hybrid nucleosomes with one copy each of H2A and mH2A[98], suggesting a hybrid mH2A nucleosome *in vivo*.

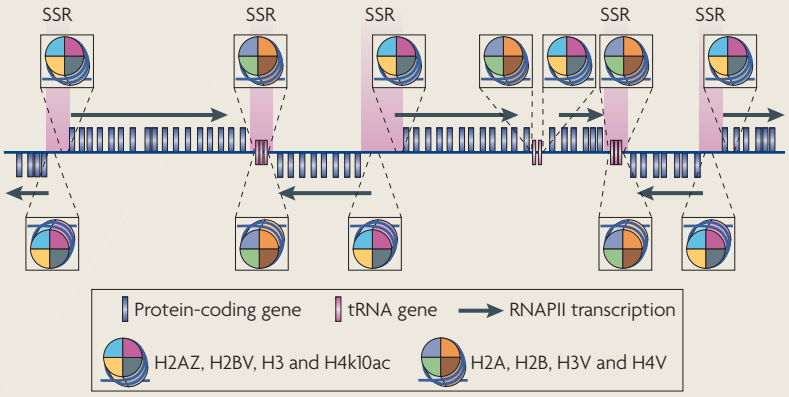
The enzyme polyADP-ribose polymerase 1 (PARP1) is necessary to maintain Xi chromosome silencing, and the H2A1.2 macrodomain was shown to bind PARP1 catalytic and zinc-finger domains. The macrodomains of mH2A1.1, mH2A1.2 and mH2A2 were all reported to inhibit PARP1 activity *in vitro*[99]. In apparent conflict with this, binding to full-length PARP1 was reported to be specific to the mH2A1.1 macrodomain, which binds polyADP-ribose on the autoADP-ribosylated PARP1[100]. At inducible heat-shock genes, human mH2A1.1 binds and inhibits PARP1 activity, and its release when heat shocked allows PARP1 to ADP-ribosylate promoter-associated histones, perhaps thereby facilitating transcription[101]. Consistent with a role in conditional silencing of genes, mH2A1 and mH2A2 nucleosomes in a human pluripotent cell line were enriched at the promoter regions of developmental genes, including homeobox genes,

Box 2 | **Trypanosome histone variants punctuate transcription**

Trypanosomes — the single-cell blood parasites that cause African sleeping sickness, Chaga's disease and other mammalian afflictions — are often considered to be among the most primitive eukaryotes[130] and are unusual in having polycistronic transcription units that are separated into mRNAs post-transcriptionally[131]. Neighbouring convergent or divergent transcription units are separated by strand-switch regions (SSRs). RNA polymerase II (RNAPII) transcription initiates at divergent SSRs and terminates at convergent SSRs[132], but recognizable RNAPII promoter elements are lacking[131].

Trypanosomes have two forms of each of the four histones (H2A and H2A.Z, H2B and H2BV, H3 and H3V, and H4 and H4V), all of which are considerably diverged from the canonical histones of animals. In *Trypanosoma brucei*, nucleosomes at presumed transcription start sites of divergent SSRs are enriched for H2A.Z, H2BV and H4 acetylated on Lys10 (H4K10ac). Nucleosomes containing H2A, H2B, H3V and H4V are found at putative transcription termination sites of convergent SSRs (see the figure). Thus, trypanosome histone variants appear to punctuate transcription with distinct nucleosomes[89]. Acetylation of histone tails decreases nucleosome stability[65] and tagged H2A.Z or H2BV nucleosomes become more destabilized with increasing salt levels than tagged H2A or H2B nucleosomes[89], suggesting that unstable nucleosomes at transcription start sites facilitate transcription initiation. The use of histone variants to mark initiation and termination sites suggests an ancestral mode of transcriptional regulation based on histone variants, perhaps before transcription factors took over. Interestingly, other early branching eukaryotes with putatively primitive promoter structures[133] also have highly divergent H3 variants[24].

Neither the *T. brucei* H3 nor H3V corresponds to centromere-specific histone H3 variant (CenH3), which, surprisingly, is missing from trypanosome genomes[134]. How trypanosomes form kinetochores without a CenH3 remains a mystery. Image is modified, with permission, from REF. 119. © (2009) Cold Spring Harbor Laboratory Press.



significantly overlapping with sites of H3 Lys27 trimethylation (H3K27me3) by the Polycomb repressive complex 2, with greatest enrichment in the least transcribed genes. Supporting a role in development, a zebrafish mH2A2 knockdown results in developmental defects[102].

H2A.Bbd — a short wrapper. In contrast to the large C-terminal macrodomain of mH2As, the mammal-specific H2A Barr body-deficient (H2A.Bbd) has a short C terminus with a truncation of the docking domain. It is only 48% identical to canonical H2A and lacks the residues that are modified in other H2As[103]. H2A.Bbd seems to be associated with active chromatin, being deficient on Xi chromosomes in fibroblasts and coinciding cytologically with acetylated H4 (REF. 104).

In A431 human cell lines, H2A.Bbd tagged with green fluorescent protein (H2A.Bdb–GFP) is exchanged more quickly in the nucleosome than H2A–GFP[105]. *In vitro*, nucleosome assembly protein 1-like 1 (NAP1L1) preferentially exchanges H2A.Bbd–H2B for H2A–H2B dimers in reconstituted nucleosomes, and this is more efficient in H3.3 nucleosomes than H3 nucleosomes[106]. Reconstituted H2A.Bbd nucleosomes are unstable without DNA, protect only 110–130 bp of DNA, have more divergently oriented DNA ends (~ 180°) than canonical nucleosomes and do not undergo remodelling by SWI/SNF — properties that are largely attributable to the H2A.Bbd docking domain[9]. H2A.Bbd has a smaller acidic patch than H2A, which inhibits the folding of reconstituted H2A.Bbd nucleosome arrays into 30-nm fibres and reduces the ability of these arrays to inhibit transcription[107]. Despite the evidence that H2A.Bbd forms accessible chromatin, no specific function has yet been identified for this interesting variant.

H2A.Bbd is evolving faster than other H2As, resulting in a probably misleading placement in phylogenetic trees (FIG. 3) near the base of the H2A family[103]. It is most closely related to mouse sperm-specific H2As that also have a truncated docking domain (FIG. 3; BOX 4). In an unusual case of convergence between mammals and plants, the most divergent of the four H2A.Z variants in *A. thaliana* (HTA4; also known as H2A.8)[108] has a similar C-terminal truncation. HTA4 has no known function[108], but its structural similarity to H2A.Bbd suggests independent selection for nucleosomes that wrap less DNA.

Canonical histone variants. Plants and animals encode several genes for each canonical histone. In humans, all H4 genes encode the same protein, but the H3 genes encode two proteins (H3.1 and H3.2) that differ at a single residue. The ancestral Ala96 residue of H3.2 is replaced by Cys96 in H3.1. Intriguingly, H3.1 and H3.2 differ in the abundance of key PTMs[45], which suggests that even minor differences between variants can have a major impact on chromatin metabolism. Human canonical H2A proteins vary at ten or more positions, as do H2B proteins, with frequent variation in the final six residues of H2A. Similar levels of heterogeneity of H2A and H2B proteins occur in mouse[87], sea urchin[88] and *A. thaliana*[108,109]. Ten of the eleven *A. thaliana* H2B proteins differ mostly in the Lys-rich N-terminal tail and in tissue-specific expression, but there is as yet no evidence for their functional specialization[109]. *A. thaliana* has three H2As with minor-groove-binding motifs in the C terminus[108]. Similar H2As in wheat protect an additional 16 bp of linker DNA from nuclease treatment compared with canonical H2A nucleosomes[110]. These oligopeptides might help stabilize quiescent chromatin in seeds but they become rapidly phosphorylated during germination, perhaps destabilizing the linker DNA interaction[111].

Sea urchins have two sets of canonical histones, one expressed from the oocyte to gastrulation and the other expressed throughout the life cycle. Uniquely, the late genes include a gene encoding H3.3, which is also constitutively expressed from a separate gene, as in other animals. Sea urchins also have a unique set of 'cleavage stage' histones, with minor differences from the canonical histones. These are expressed constitutively in the oocyte and during the first few cleavages, and replace the sperm histones during pronuclear decondensation[88].

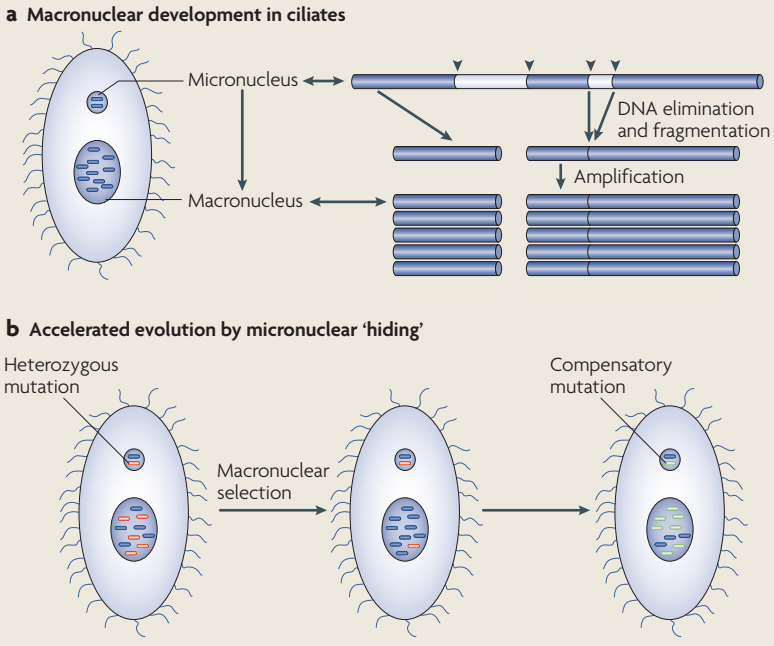
Homeobox gene
One of a family of genes that encode homeodomain-containing transcription factors, which are involved in the patterning of the body during development.

Box 3 | Ciliate histones and nuclear dualism

Ciliates are single-cell alveolates that have unusually diversified histone H3s and H4s. Despite diversification, patterns of conserved residues suggest that ciliate H4s have similar functional constraints to other H4s[90,91]. This diversification, which also affects other conserved proteins, has been attributed to the unusual binucleate structure of the ciliate cell. Ciliates have two different nuclei: a transcriptionally active macronucleus and a micronucleus that is transcriptionally inactive in vegetative cells. Following sexual reproduction, the diploid 'germline' micronucleus gives rise to a polyploid 'somatic' macronucleus through a process of DNA elimination, fragmentation and amplification (see the figure, part **a**). Differential loss of partially recessive deleterious alleles in the amitotic macronucleus can shield deleterious alleles in the micronucleus from selection, allowing time for compensatory mutations to arise and thus accelerating diversification[91] (see the figure, part **b**).

The ciliate life cycle also presents opportunities for specialized adaptation of histones. For example, *Euplotes crassus* has an H3 variant (H3(P)) that is expressed exclusively in the developing macronucleus, coincident in timing with the expression of another variant (H2A(P)) and with the DNA elimination and fragmentation process, suggesting that H3(P) may have a role in these processes[135].

In *Tetrahymena thermophila*, the centromere-specific histone H3 variant (CenH3; known as Cna1) also has a role in DNA elimination. In addition to its presence at centromeres in the micronucleus, Cna1 is observed in some developing macronuclei and is required for formation of DNA elimination structures[136]. Recently, mammalian CenH3 (CENP-A) was found to be recruited to DNA double-strand breaks and was proposed to have a role in DNA repair[137]. If Cna1 is also involved in double-strand break repair, this could explain why it is required for proper DNA elimination, and why it is present throughout the chromosomes as well as at centromeres during meiotic prophase I at the time of recombination[136,138]. In *Arabidopsis thaliana*, CenH3 (known as HTR12) has also been observed on both chromosome arms and at centromeres during prophase I[25].



Sperm histones. Animal sperm DNA is tightly packaged with basic proteins such as variant histones, protamines, and protamine-like proteins. Protamine and protamine-like classes evolved from H1 linker histones and replace histones to varying degrees in different lineages, often following a wave of post-meiotic histone acetylation and initial replacement by transition proteins[112]. Testis- or sperm-specific histone variants, including both core and linker histones, are common. Whereas some linker histone variants seem to help condense chromatin, the role of core histone variants in spermatogenesis is not well understood (BOX 4). Nucleosomes from human sperm that contain the testis-specific variant TH2B (also known as TSH2B) are enriched in genes for spermatogenesis[113], suggesting that TH2B might promote their transcription or prevent their packaging in protamines.

One major function of both sperm-specific histones and protamines might be to erase paternal epigenetic states. Nucleosomes that survive chromatin remodelling in the sperm and pronucleus have the potential to maintain epigenetic information at particular loci, with CENPA being the most notable example[114]. H3.3 persists in sperm in *C. elegans*[49], and might potentiate gene expression, as was seen in *X. laevis* nuclear transplantation[47].

The nucleosome-bound DNA content of human sperm is estimated at 4–15%, and sperm contains all four canonical histones plus H2A.X, H2A.Z, H3.3 and CenH3 (REFS 115,116). Genomic profiling of sperm nucleosomal DNA reveals that H2A.Z nucleosomal DNA is enriched in pericentric heterochromatin[113]. Dimethylation of H3K4 (or H3.3K4) is enriched at developmental promoters, whereas both H3K4me3 and H3K27me3 are enriched in a subset of developmental genes including at the bivalent (H3K4me3–H3K27me3) promoters found in embryonic stem cells. H3.1 and/or H3.2 in sperm survive pronuclear remodelling[116], offering a potential vehicle for H3K4 and H3K27 methylation to survive into the zygote and influence zygotic transcription.

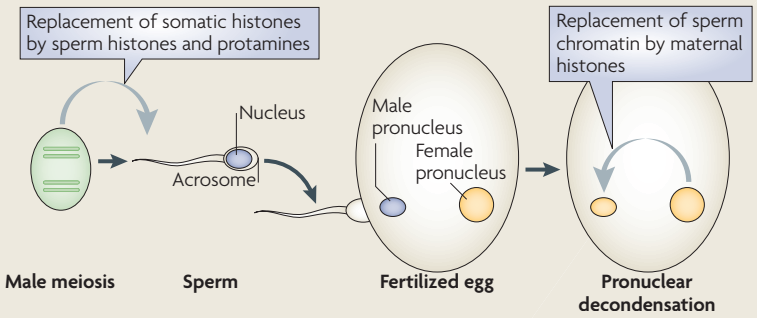
Plants also have sperm- and pollen-specific histone variants that might participate in chromatin remodelling. Divergent H2A, H2B and H3 paralogues occur in male gamete cells of *Lilium longiflorum*[117]. In *A. thaliana*, HTR12 (CenH3) and a male gamete-specific H3.3-like variant, HTR10, are both present in the sperm nuclei, which carry out double fertilization of the zygote and endosperm. HTR10 tagged with red fluorescent protein (HTR10–RFP) is removed from the paternal zygotic chromosomes before first mitosis, in contrast to HTR12–GFP. In the endosperm, however, HTR10–RFP persists at diminishing intensity through three mitoses[118] and could potentially influence endosperm development.

Perspectives

From archaeal tetramers to hybrid octamers to sperm-specific subnucleosomal particles, histone variants exhibit an astonishing diversity in basic structure, intrinsic stability, the length of DNA that they wrap and even the direction of wrapping. The diversity of H2A variants probably reflects the position of H2A–H2B dimers on the 'edges' of the canonical octamer, where they are more easily mobilized and can better regulate access to the bound DNA. In contrast to their partner H2B, which forms a 4HB with H4, H2A variants have fewer structural constraints, as demonstrated by the diversity of their C-terminal tails, which exit the nucleosome at a favourable position to promote or preclude interactions with the linker DNA and/or linker histones. They also vary in their acidic patches, which have been

Protamine
A small, highly basic protein that tightly packages sperm DNA, replacing histones completely or to varying degrees in many animals.

Box 4 | **Sperm histones — for packaging or mobilization?**



Sperm histones have poorly understood properties. Sea urchins package sperm DNA in nucleosomes using histone H2B and H1 variants with reversibly phosphorylated minor-groove-binding motifs, rather than using protamines[111]. These histones are proteolysed during pronuclear decondensation[139] and replaced with maternal cleavage-stage histones[88] (see the figure). Animals that package sperm DNA using protamines also have sperm-specific histones. The human H3 variant H3t and its mouse counterpart are transcribed in primary spermatocytes[140]. Although H3t differs from H3.1 in only four residues, the histone chaperone nucleosome assembly protein 1-like 1 (NAP1L1) assembles H3t into nucleosomes less efficiently than other H3 variants[141]. Two human testis-specific H2B variants have been described: TH2B (also known as TSH2B), which is present in spermatogonia and spermatids and in only a fraction of mature sperm[142], and H2BFWT, which might be part of a sperm-specific telomere complex[143]. In reconstituted nucleosomes, TH2B forms an unstable octamer with canonical histones in the absence of DNA, and H2BFWT octamers are unable to recruit chromosome condensing factors[142,144]. A mouse sperm variant, H2BL1, is similar to bovine SUBH2BV[140], which is a non-nucleosomal sperm histone present in the subacrosomal perinuclear theca that is suggested to have a role in pronuclear chromatin remodelling[145]. Mice also have two similar H2A variants, H2AL1 and H2AL2 (REF. 140), which resemble H2A Barr body-deficient (H2A.Bbd) in having an Arg-rich amino terminus and a shorter docking domain[146]. An antibody recognizing H2AL1 and/or H2AL2 reveals preferential localization in pericentric heterochromatin in early condensing spermatids. H2AL1 and/or H2AL2 co-fractionate with TH2B and an ~ 60 bp DNA fragment in a micrococcal nuclease-resistant subnucleosomal structure that lacks H3 and H4. When H2AL2 and TH2B are co-expressed in somatic cells they form octamers that are less stable than canonical octamers[140]. Reconstituted H2AL2–H2B-containing octamers protect only 130 bp of DNA and have linker DNA ends that exit at a large angle, similar to H2A.Bbd nucleosomes[146]. A common theme is that testes- and sperm-specific histones form less stable nucleosomes, which might facilitate their mobilization when they are eventually replaced[147].

implicated in interactions between nucleosomes[75,107] and in the stimulation of nucleosome remodellers[61]. Variants of H2B, H3 and H4 are more limited in number and in structural diversity, with the notable exception of CenH3, which seems to be unique in having a right-handed wrap that is consistent with a tetrameric nucleosome structure. Histone variants also provide opportunities for the use of PTMs, DNA-binding motifs, and even macrodomains for specialized functions in chromatin. These diverse properties support the long-held notion that nucleosomes regulate accessibility to DNA. Evidence from trypanosomes suggests that deployment of variants in regulating transcription might have preceded sophisticated gene regulation by transcription factors[89,119]. Although most attention has focused on accessibility for transcription, chromatin accessibility is also important for replication[120,121] and for repair and recombination[81].

Loss of function for most non-canonical histone variants produces severe phenotypes, which are often attributable to interference with specific functions such as kinetochore formation, DNA repair, sex chromosome inactivation and developmental regulation. In other cases, such as H3.3 in *D. melanogaster* soma or Htz1 in yeast, phenotypes are surprisingly mild, suggesting a significant overlap in function with canonical histones. Among the chromatin components that are increasingly seen to be crucial to the deployment of histone variants are chromatin remodellers and their attendant acetyltransferases, ubiquitin ligases and other modifiers. Recent genome-wide localization studies have provided a detailed view of the specific contexts in which particular histone variants occur, but our knowledge of what put them there lags behind. Further insights into the biology of histone variants and dynamic chromatin processes[42] is likely to require a better understanding of the functions, contexts and substrates of the remodellers[7,12] and chaperones[11] that position them and of the enzymes[10] that modify them.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
Htz1
UniProtKB: http://www.uniprot.org
ATM | ATR | CENP-A | CHD1 | CID | Cse4 | H2Av | H2A.X | H2A.Z | H3 | H3.1 | H3.3 | HMfA | HMfB | HMk | HP1α | HTA4 | HTR12 | mH2A1 | mH2A2 | MJ1647 | NAP1L1 | NEQ288 | NEQ348 | p300 | PARP1 | Scm3 | TH2B

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