Histone variants on the move: substrates for chromatin dynamics

Paul B. Talbert and Steven Henikoff

Abstract | Most histones are assembled into nucleosomes behind the replication fork to package newly synthesized DNA. By contrast, histone variants, which are encoded by separate genes, are typically incorporated throughout the cell cycle. Histone variants can profoundly change chromatin properties, which in turn affect DNA replication and repair, transcription, and chromosome packaging and segregation. Recent advances in the study of histone replacement have elucidated the dynamic processes by which particular histone variants become substrates of histone chaperones, ATP-dependent chromatin remodellers and histone-modifying enzymes. Here, we review histone variant dynamics and the effects of replacing DNA synthesis-coupled histones with their replication-independent variants on the chromatin landscape.

A fundamental distinction among the three domains of life — Archaea, Bacteria and Eukaryota — is in how their genomes are packaged. In all three lineages, highly basic packaging proteins have evolved to neutralize the acidic phosphates of DNA, although the resultant degree of compaction differs profoundly. Bacterial genomes are loosely packaged by HU proteins or other basic nucleoid-associated proteins[1]. By contrast, archaeal genomes organize DNA with small DNA-binding proteins or wrap DNA around a nucleosomal particle consisting of a tetramer of histone proteins[2]. Each histone contains a histone-fold domain, which is composed of three helices and serves as both a protein dimerization and DNA-binding module[3]. The emergence of eukaryotes coincided with a doubling of the number of histone subunits in a nucleosome, with DNA spiralling left-handed 1.7 times around the histone octamer core. Core histones became differentiated from as-yet unknown archaeal-like ancestors into the families H2A, H2B, H3 and H4, in which a central $(H3–H4)_2$ tetramer is flanked above and below by dimers of H2A–H2B[4]. This doubling of the DNA around the histone core relative to archaeal nucleosomes enabled the tight level of compaction of chromatin in mitotic chromosomes, which is a unique feature of eukaryotes. The H1 linker histone proteins, which differ from other histones in having a globular domain instead of a histone-fold domain, interact directly with DNA molecules entering and exiting the nucleosome to modulate its accessibility[5].

Histone genes are generally present in multiple copies, and their expression is tightly regulated at multiple levels[6,7]. In animals, so-called canonical histone genes are clustered and transcribed only in S phase. The mRNAs lack introns and the polyA tail is replaced with a special stem–loop structure that regulates their processing and stimulates translation[7]. Eukaryotic core histones have further differentiated into additional paralogues, or variants, that function in multiple processes, including transcription, chromosome segregation and DNA repair[8]. The synthesis and deposition of canonical histones in animals and plants are coupled to DNA synthesis, whereby canonical histones assemble into nucleosomes behind the replication fork and at sites of DNA repair. By contrast, the incorporation of histone variants typically occurs throughout the cell cycle and is independent of DNA synthesis. In both plants and animals, transcripts encoding histone variants typically have introns and polyA tails, and are processed like most other RNA polymerase II (Pol II) transcripts[9]. For convenience and because of their relative abundance we distinguish between DNA synthesis-coupled canonical histones and DNA synthesis-independent variant histones. However, an evolutionary analysis has suggested that variants H3.3 and H2A.X are the ancestral forms of the replication-coupled forms[8]; thus, all H3 and H2A histones can be considered variants.

The replacement of DNA synthesis-coupled histones with DNA synthesis-independent variants alters the composition and distribution of nucleosomes and DNA-binding proteins along the chromosome (the chromatin landscape). Such replacements change the properties of nucleosomes and their interactions with chromatin remodellers and modifiers. The impact of such a replacement can be profound. For example, the incorporation of the centromeric H3 variant (known as CENPA in vertebrates, Cse4 in yeast and CENH3 in plants) instead of H3 into a nucleosome forms the foundation of centromeric chromatin, which has been extensively

Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, Washington 98109–1024, USA.
ptalbert@fhcrc.org; steveh@fhcrc.org

doi:10.1038/nrm.2016.148
Published online 7 Dec 2016

reviewed elsewhere[10,11]. In other cases, the change may be subtle; for example, the replacement of human canonical H3.1 by the highly similar variant H3.3 has few apparent consequences, indicating that H3.3 has a primary role in repairing gaps in the chromatin landscape that result from nucleosome disruption[12–14]. Several H2A variants affect gene expression: H2A.Z and H2A.B are implicated in transcription initiation[15,16], whereas macroH2A in animals and H2A.W in plants seem to be associated with nucleosome immobility and transcriptional silencing[17,18].

Histones are routinely subjected to post-translational modifications, especially on the tails of H3 and H4. Histone modifications such as acetylation or phosphorylation can directly modulate chromatin structure by altering the charge on histones, thereby reducing the interaction of histone tails with the negatively charged sugar–phosphate backbone of DNA. Histone modifications can also affect histone recognition by or affinity for other proteins such as histone chaperones, chromatin remodellers and other chromatin modifiers[8,19]. Histone variants are often subjected to the same modifications as their canonical counterparts. For example, Lys4 of H3.3 is often trimethylated (H3.3K4me3) and Lys18 and Lys23 residues are often acetylated (H3.3K18ac and H3.3K23ac, respectively)[20]. A few variants, including H3.3 and H2A.X, have variant-specific modifications on residues that differ from their canonical counterparts[21,22].

The diversification of the chromatin landscape mediated by histone variants is becoming increasingly well described[23–26]. In this Review, we focus on histone variants as substrates for dynamic processes during DNA replication, transcription, and heterochromatin formation and maintenance. Owing to space constraints, we do not discuss histone variant dynamics in centromere maintenance and in DNA repair, which we and others have recently reviewed[10,11,27,28].

Chaperones mediate histone dynamics

Histones are basic proteins that neutralize the negative charge of the sugar–phosphate backbone of DNA. When histones are mixed with DNA in solution at physiological ionic strength, they precipitate unless another protein, called a chaperone, is present[29]. Histone chaperones help histones fold properly and prevent their positive charges from engaging in nonspecific interactions[19]. Histone chaperones are also essential for escorting and depositing histones into chromatin and for histone storage in oocytes[19]. Most chaperones are highly conserved across eukaryotes[19,30]. Here, we discuss only histone-specific chaperones that have been implicated in DNA replication, transcription and heterochromatin maintenance.

There are different chaperones for different histone variants and for different stages and pathways of histone assembly (Supplementary information S1 (table)). Newly synthesized histones are bound by a series of chaperones in the cytosol before entering the nucleus[31,32] (Supplementary information S2 (box)). Once soluble histone complexes enter the nucleus, chromatin assembly factor 1 (CAF1) directs histone assembly behind the replication fork, whereas the histone regulator A (HIRA) complex directs assembly into chromatin independent of DNA synthesis[33]. In many single-cell eukaryotes, such as budding yeast, both of these chaperones operate on the same H3–H4 dimers, which they may receive from another H3–H4 chaperone, anti-silencing factor (Asf1)[33–35]. In most multicellular organisms, however, separate H3 variants have evolved for DNA synthesis-coupled and DNA synthesis-independent pathways[36]. In humans, CAF1 deposits canonical H3.1 and H3.2 during replication[33,37], whereas HIRA deposits the H3.3 variant throughout the cell cycle[33]. Vertebrates have two Asf1 homologues: ASF1a and ASF1b. ASF1a interacts preferentially with HIRA, whereas ASF1b interacts preferentially with CAF1, although neither shows a preference for H3.1 or H3.3 (REF. 38). Canonical H3.1 and H3.2 differ by only four or five amino acids from the H3.3 variant, and in *Drosophila melanogaster*, substituting any of the three residues in the α2 helix of H3.2 with its counterpart from H3.3 enables DNA synthesis-independent deposition[39]. This finding suggests that CAF1 has evolved to specifically recognize the canonical form. By contrast, death domain-associated protein (DAXX), a chaperone restricted to animals, specifically recognizes the H3.3 variant[40], primarily by interacting with Gly90 in H3.3 rather than Met90 in H3.1 and H3.2. However, this specificity is not absolute because human DAXX can also deposit heterotypic nucleosomes containing H3.3 and the centromeric H3, CENPA, in ectopic locations when CENPA is overexpressed in cell lines[41,42]. Alpha thalassemia mental retardation syndrome X-linked (ATRX) is a member of the SWI/SNF DNA translocase family and works together with DAXX to deposit H3.3 at heterochromatic regions[43–45]. Before depositing newly synthesized H3.3–H4 into chromatin, DAXX escorts the dimers into nuclear bodies, where it is found with ATRX, HIRA and ASF1a; this finding suggests that nuclear bodies act as distribution centres for soluble H3.3–H4 (REF. 46).

Another important chaperone is the heterodimeric facilitates chromatin transcription (FACT) complex (BOX 1), which assists the progression of transcription and replication by traveling along with polymerases[47–49]. FACT can assemble nucleosomes onto DNA *in vitro*[48] and can bind to both H3–H4 and H2A–H2B (or H2A.X–H2B)[48,50–53]. To date, no chaperones are known to distinguish between H2A.X and canonical H2A members, nor between mammalian H3.1 and H3.2 (REF. 37).

Histone dynamics during replication

The passage of all genomic DNA through the small hole in the replicative helicase every cell cycle results in the transient release of all DNA-binding proteins, including nucleosomes. Old nucleosomes, including many with histone variants, are rapidly reassembled on the leading and lagging strands, whereas other histone variants are replaced during reassembly. Gaps between old nucleosomes are then filled with nucleosomes comprising newly synthesized canonical histones[54]. Recent evidence has helped to elucidate the mechanisms by which chaperones facilitate this dynamic process.

DNA translocase
A conserved domain of chromatin remodellers that uses ATP to move nucleosomes along DNA.

Box 1 | **How does FACT act?**

The facilitates chromatin transcription (FACT) complex comprises two subunits: suppressor of Ty16 (SPT16) and Pol I binding 3 (POB3). FACT functions in H2A variant exchange, nucleosome assembly and nucleosome eviction, and has roles in transcription and DNA replication. FACT competes with DNA for binding to H2A–H2B[151]. Human FACT can promote the eviction of a H2A–H2B dimer from the histone octamer *in vitro*, thereby forming hexasomes (sub-nucleosomal particles consisting of a $(H3\text{-}H4)_2$ tetramer and one H2A–H2B dimer). Hexasomes are proposed to facilitate transcription, which is followed by the subsequent reassembly of octameric nucleosomes (known as the dimer displacement model)[48]. However, FACT can also increase nuclease sensitivity throughout the nucleosome without displacing a dimer. This finding suggests that FACT reorganizes nucleosomes to increase DNA accessibility while tethering the eight histone components together, although H2A–H2B dimers may be more easily lost (the global accessibility model)[152]. FACT is also thought to enhance nucleosome 'breathing', or unwrapping of nucleosomal DNA, by stabilizing the unwrapped DNA and blocking its contacts with H2A–H2B (the breathing model)[51]. These models all propose that protein–DNA contacts are disrupted; they differ in the extent of disruption and in whether H2A–H2B loss is a regular or incidental occurrence. The global accessibility model perhaps best explains the various activities of FACT in replication and transcription.

Two conflicting structures have been proposed for how FACT binds to H2A–H2B[51,52], although both structures show that H2A–H2B interacts with the unstructured acidic carboxy-terminal domain of SPT16, which is required for FACT activity[48]. Binding sites to H2B were identified in the carboxy-terminal tails of both SPT16 and POB3, which disrupt nucleosomal DNA contacts with H2A–H2B[52]. Structurally similar binding interactions with H2B have been reported for the H2A.Z–H2B chaperones ANP32E (acidic leucine-rich nuclear phosphoprotein 32 family member E) in humans[153] and Swr1 in yeast[154]. The similarity of binding interactions suggests a common strategy for chaperoning H2A–H2B dimers, regardless of which H2A variants are involved.

The deposition of new nucleosomes behind the replication fork is mediated by the histone chaperone CAF1, which binds the replication clamp proliferating cell nuclear antigen (PCNA)[55] and travels with the replisome (FIG. 1). CAF1 is a trimeric complex responsible for assembling new canonical H3 nucleosomes behind the fork[33]. ASF1 interacts with H3–H4 by binding the homodimerization interface of H3 (REF. 56), and can only present dimers, not tetramers, to CAF1. A single CAF1 molecule can bind two H3–H4 dimers or a crosslinked $(H3\text{–}H4)_2$ tetramer; this finding suggests that CAF1 can assemble and deposit $(H3\text{–}H4)_2$ tetramers onto DNA[35,57]. One could therefore imagine that old nucleosomes might be disassembled and reassembled from their dimer components randomly behind the fork, resulting in histone octamers containing both new and old histones. This process occurs for H2A–H2B and H2A.X-H2B dimers; however, in animals, canonical H3.1 nucleosomes consist of nearly all old or all new $(H3.1\text{-}H4)_2$ tetramers[58]. This finding suggests that either old tetramers are transferred intact past the fork or they are transiently disassembled and reassembled in a way that excludes the incorporation of new dimers.

Interestingly, the amino-terminal tail of the MCM2 subunit of the minichromosome maintenance (MCM) helicase can interact with a complex containing H2A, H2B, any H3 variant, and H4 bound by FACT[59], creating a component of active replisomes that may help disassemble and reassemble nucleosomes. Crystal structures have revealed that the amino terminus of human MCM2 binds a $(H3.1\text{–}H4)_2$ tetramer or one dimer in a manner that excludes DNA and H2B[60,61]. ASF1b can bind this complex by splitting the tetramer and replacing one dimer. Thus, MCM2 might be involved in the transfer of tetramers or dimers across the fork either to CAF1 via ASF1b or directly onto DNA, perhaps with the assistance of FACT (FIG. 1). MCM2 can bind tetramers containing H3.1, H3.3 or CENPA, making MCM2 potentially capable of handling all types of old nucleosomes[61]. MCM2–(H3.1–H4)–ASF1b also exists as a soluble complex, which may act as a store of new H3.1–H4 for deposition by CAF1 (REFS 60,61).

In budding yeast, analyses of the effects of mutations in Pol I binding 3 (Pob3)[47], a subunit of FACT, revealed the involvement of FACT in replication. Furthermore, a mutation in the middle domain of the other subunit of FACT, suppressor of Ty16 (Spt16), reduced its binding to H3–H4 and compromised replication while minimally affecting transcription[53]. This finding suggests that the middle domain of Spt16 is necessary for binding H3–H4 during replication[53]. Spt16 binds to complexes of the histone chaperone Rtt106 with H4 and H3 acetylated on Lys56 (H3K56ac), thus promoting the deposition of new nucleosomes marked with H3K56ac in parallel with CAF1.

In HeLa cells, H3.3 is incorporated broadly into chromatin throughout the cell cycle, but it is not normally deposited at replication forks[12]. However, if CAF1 is depleted, H3.3 becomes localized to replication sites; this process primarily depends on HIRA-mediated deposition, with perhaps a smaller contribution from DAXX. By contrast, depletion of HIRA does not result in a significant broad deposition of H3.1 by CAF1. This result indicates that CAF1 efficiently deposits histones in gaps between old nucleosomes on nascent DNA, but lacks the ability to fill gaps uncoupled to DNA synthesis. Conversely, HIRA, in contrast to CAF1 and other tested chaperones, binds directly to DNA and deposits H3.3–H4 into gaps in the nucleosome landscape, regardless of where the gaps occur[12,14]. Although CAF1 mutants are lethal in animals[62], Caf1 mutants in yeast result only in silencing defects and CAF1 mutants in *Arabidopsis thaliana* have meristem defects[63]. This finding implies the existence of back-up systems for depositing nucleosomes during replication in these organisms. This hypothesis is supported by the abovementioned roles of FACT and Rtt106 in deposition at the replication fork in yeast[53]. Furthermore, double mutants in subunits of the CAF1 and HIRA complexes of *A. thaliana* result in more severe growth and fertility defects than mutations in either complex alone[64].

Old nucleosomes appear to be randomly distributed onto both daughter strands. In human cells, histone modifications on old nucleosomes are transmitted with them to nascent chromatin, whereas new nucleosomes show patterns of modifications similar to patterns found on soluble histones[65,66], resulting in the dilution of old modifications by half. H3.3 and H2A.X are abundant in nascent chromatin, indicating that these variants are efficiently retained. H2A.Z, however, is depleted from new chromatin and its level only recovers over several hours[65]. It is possible that H2A.Z is excluded from nascent chromatin at the replication fork by FACT, which does not assemble H2A.Z into nucleosomes[67].

Although old nucleosomes can segregate onto both daughter strands, in the asymmetric divisions of

General regulatory factors
Abundant transcription factors that are found at many promoters and augment the activity of adjacent transcription factors.

Highly positioned nucleosomes
Nucleosomes that occupy the same position on the DNA in a large majority of cells in a population.

Drosophila melanogaster male germline stem cells, old canonical H3.2 nucleosomes preferentially phosphorylated on Thr3 during mitosis are segregated to the stem cell, whereas new histones are segregated to the progenitor cell[68,69]. How this process occurs is unclear, but old nucleosomes may be recycled onto only one strand during replication, perhaps the leading strand, with new nucleosomes deposited on the lagging strand. Old nucleosomes may serve as preferential substrates for the phosphorylation of H3T3 during mitosis, which may help orient them on the spindle towards the germline stem cell[68,69]. This asymmetric pattern does not hold for H3.3 nucleosomes, which are deposited independently of replication and will therefore appear on either strand.

Transcriptional dynamics of histones

Similar to replication, transcription is disruptive to nucleosomes[70] and reshapes the chromatin landscape. These disruptions are mitigated by chromatin remodellers together with FACT and chaperones specific for the H3.3 and H2A.Z histone variants.

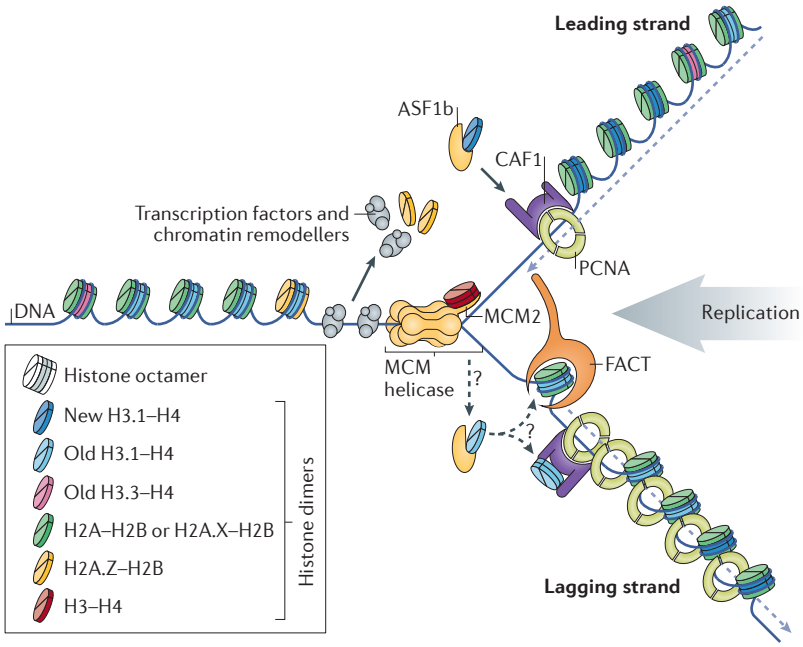


Figure 1 | **Histone variant dynamics during replication in animals.** Left to right: transcription factors, chromatin remodellers and the histone variant H2A.Z are removed during replication as the minichromosome maintenance (MCM) helicase complex progresses along the DNA. In parallel, canonical histones and variants H3.3 and H2A.X are reassembled behind the replication fork. The MCM2 subunit of the helicase can bind a H3–H4 tetramer, regardless of which H3 variant it contains (H3.1, H3.2, H3.3 or histone H3-like centromeric protein A (CENPA)) and might deposit it directly onto nascent DNA or pass it to the histone chaperone anti-silencing factor 1b (ASF1b) (dashed arrows and question marks). The chaperone complex FACT (facilitates chromatin transcription) forms a complex with all four histones and the MCM helicase complex, and may serve to disrupt old nucleosomes at the replication fork and/or reassemble them on nascent chromatin, replacing H2A.Z–H2B with H2A-H2B or H2A.X–H2B. Behind the fork, the chaperone chromatin assembly factor 1 (CAF1) binds the replication clamp proliferating cell nuclear antigen (PCNA) and assembles new tetramers from dimers supplied by ASF1b. Both old and new nucleosomes can assemble on the leading and lagging strands. Each Okazaki fragment on the lagging strand accommodates ~1 nucleosome. For clarity, polymerases and other replication proteins are not shown.

Transcriptional dynamics of yeast histone variants. Much of our mechanistic understanding of the basic enzymatic machineries that assemble, disrupt and reassemble DNA synthesis-independent nucleosomes comes from studies of yeast[71]. Yeast lack specialized canonical, DNA synthesis-dependent H2A and H3 histones. Instead, yeasts use H2A.X (called H2A in yeast) and H3, which has sequence similarities to H3.3, for both DNA synthesis-dependent and -independent nucleosome assembly. In budding yeast, nucleosome occupancy in genes, as determined by chromatin immunoprecipitation of H3 in dividing cells, correlates inversely with promoter strength. Nucleosome-depleted regions (NDRs), characterized by poly(dA:dT) tracts and/or binding sites for general regulatory factors, are adjacent to transcription start sites (TSSs)[72,73]. In non-replicating cells, high transcription levels drive high H3 turnover rates at promoters, tRNA genes and small nucleolar RNA genes[74]. Turnover rates in gene bodies are low but correlate with enrichment of Pol II, whereas turnover at promoters correlates better with sites of transcription factor binding and nucleosome remodellers. By contrast, turnover of tagged H2B is high at both active and inactive genes, although turnover of both H2B and H3 is low at telomeres[75]. This finding suggests that H2A–H2B is turned over independently of and more frequently than H3–H4, and that nucleosomes at telomeres may be incorporated primarily during replication.

The ATP-dependent RSC (remodel the structure of chromatin) complex is recruited to promoter regions by the sequence-specific general regulatory factors ARS-binding factor 1 (Abf1) and RNA polymerase I enhancer binding protein (Reb1), where it is required for the formation of NDRs[76]. The NDRs of typical divergent promoters in yeast are flanked on either side by highly positioned nucleosomes that occlude the TSSs of the divergent genes, thereby preventing Pol II loading[77,78] (FIG. 2a). SWR-C is a complex that contains the ATPase Swr1, which is a member of the SWI/SNF chromatin remodelling superfamily of proteins[79]. SWR-C binds to nucleosome-free DNA at the NDR and replaces H2A–H2B with H2A.Z–H2B in the NDR-flanking nucleosomes[76,80], which are termed the +1 and −1 nucleosomes (FIG. 2a). H2A.Z occupancy at the +1 nucleosome represents a steady state between its deposition by SWR-C and its subsequent removal by nucleosome eviction or by the INO80 complex[81]. Several studies have shown that H2A.Z nucleosome occupancy at promoters is inversely correlated with transcription[82–84]. This finding suggests that H2A.Z occupancy prevents transcription initiation and that the transcription machinery subsequently evicts H2A.Z (FIG. 2b). H2A.Z also promotes the efficient recruitment of Pol II[15]. Moreover, H2A.Z is required for the specific activation, not repression, of heat shock genes during heat shock responses[83]. Together, these findings indicate that the role of H2A.Z may be to recruit Pol II and to poise genes for activation through its subsequent eviction. This eviction of H2A.Z at the TSS of both highly and infrequently transcribed genes depends on the

TATA-box binding protein of the transcription pre-initiation complex (PIC), indicating that components associated with the PIC itself remove H2A.Z to proceed with transcription[85]. Deletion of *htz1* (which encodes H2A.Z) results in both positive and negative changes in transcription, with many genes near telomeres requiring H2A.Z to prevent their silencing by the spread of heterochromatin[86].

Nucleosomes containing H2A.Z are substrates for the RSC complex[76]. RSC promotes the formation of nucleosomes with asymmetric histone–DNA interactions[87]. Such asymmetric nucleosomes frequently flank NDRs (FIG. 2a) and are reduced in occupancy when RSC is depleted, suggesting that RSC remodels these nucleosomes. RSC protects half of the asymmetric nucleosome while disrupting the contact of H4 with DNA in the other half of the nucleosome, consistent with the *in vitro* finding that RSC unwraps a nucleosome up to the dyad axis, which represents a remodelling intermediate[88].

Following transcription, the reassembly of nucleosomes is essential for transcription repression at both the promoter and the gene body. In the absence of nucleosome reassembly by the chaperone Spt6 at the promoter, transcription activators are dispensable for transcription[89]. In gene bodies, evicted or disrupted nucleosomes are recycled by the chaperone Spt6 and by the Spt16 subunit of FACT[89–91], both of which accompany Pol II[49,92,93]. In their absence, nucleosomes are lost from gene bodies[67]. Although FACT can displace H2A–H2B dimers[48], H2B turnover continues even when Spt16 is inactivated, indicating that FACT is not solely responsible for H2B turnover[90]. Instead, H2A.Z–H2B is enriched in gene bodies in Spt16 and/or Spt6 mutants, perhaps because failure of nucleosome reassembly makes DNA available for SWR-C binding and H2A.Z–H2B deposition[67]. Incorporation of H2A.Z into gene bodies promotes aberrant transcription from cryptic promoters, which supports transcription from the 3′ ends of genes. *In vitro*, Spt6 and Spt16 can assemble H2A–H2B into nucleosomes, but they cannot assemble H2A.Z–H2B, indicating that they keep H2A.Z out of gene bodies by replacing H2A.Z–H2B dimers with H2A–H2B, as well as by recycling nucleosomes to block SWR-C binding. Similar FACT-dependent nucleosome recycling maintains very low turnover of nucleosomes with H4K20me2 and H4K20me3 in gene bodies in fission yeast[94].

Transcriptional dynamics of H3.3 in animals. Although yeasts accomplish both DNA synthesis-coupled and DNA synthesis-independent nucleosome deposition with only one form of H3, in animals, the latter process involves the variant H3.3. H3.3 is physically very similar to the canonical H3.1 and H3.2. *In vitro*-reconstituted H3.1- and H3.3-containing mononucleosomes have similar stabilities against salt-dependent dissociation, and oligonucleosomes of both types are dissociated at a similar rate under tension from magnetic tweezers[95]. In contrast to the *in vitro* similarities, endogenous H3.3-containing nucleosomes are more salt-sensitive than endogenous H3.2 nucleosomes[96].

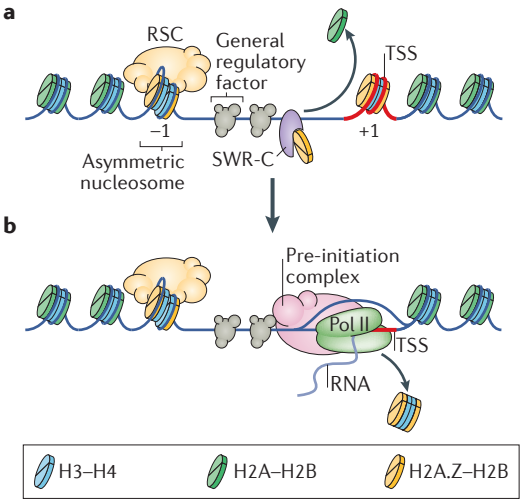


Figure 2 | **Histone variant dynamics during transcription.** **a** | In yeast, general regulatory factors and chromatin remodellers promote the formation of nucleosome-depleted regions (NDRs). The formation of many NDRs is dependent on remodel the structure of chromatin (RSC) complex, which binds to NDR-flanking nucleosomes and renders them asymmetric with respect to the contacts between H4 and DNA. SWR-C binds to DNA in the NDR and replaces H2A–H2B with H2A.Z–H2B on NDR-flanking (+1 and −1) nucleosomes. **b** | H2A.Z recruits RNA polymerase II (Pol II) and is displaced from promoters by binding the pre-initiation complex. TSS, transcription start site.

Nucleosome turnover in *D. melanogaster* is highest downstream of TSSs, gradually decreasing over gene bodies[97]. As the average Pol II density in genes increases, nucleosome occupancy decreases[98] and nucleosome turnover increases. Moreover, H3.3 levels increase in gene bodies, at promoters, at enhancers and at transcription termination sites[98–100]. Transcription activation of heat shock genes triggers the loss of canonical H3.2 and the gain of H3.3, but not at artificial promoter arrays that do not initiate transcription. This finding indicates that transcription elongation is required for H3.3 deposition throughout gene bodies[13], probably by dislodging nucleosomes and creating gaps. At enhancers, H3.3 is enriched even when adjacent genes are silent, indicating the presence of a continuous process of nucleosome disruption, probably by binding transcription factors and recruiting nucleosome remodellers. Indeed, FACT and the general regulatory factor GAGA, which work together to promote chromatin remodelling, interact with HIRA to deposit H3.3 at some GAGA binding sites[101].

In vertebrate cells, H3.3 is similarly incorporated at promoters, at enhancers, in gene bodies and at transcription termination sites, as well as in telomeres[45,95,96,102–106]. The turnover of H3.3 is lowest at telomeres, intermediate across gene bodies and highest at promoters and enhancers[105], especially at super-enhancers[106]. H3.1 has a similarly high turnover at the same sites, implying that each of these regions has a distinct nucleosome disruption rate regardless of the form of H3 that is present.

High H3.3 turnover at enhancers and TSSs also correlated with accessibility to micrococcal nuclease (MNase), indicating that histone turnover renders the DNA accessible to transcription factors[106]. In mouse embryonic stem cells, incorporation of H3.3 at bivalent promoters[107] and in gene bodies is dependent on HIRA, but HIRA is not required at telomeres, which rely on ATRX–DAXX for H3.3 deposition, nor at many transcription factor binding sites[45]. The presence of the chromatin modifications H3K4me1, H3K4me3, H3K9ac and H3K27ac, which are associated with enhancers and promoters and mark transcriptionally active chromatin, correlate with higher H3.3 turnover rates[102,105]. However, it is not known whether these modifications contribute directly to high turnover. It may be that the modifications provide docking platforms for chromatin remodellers and promote continuous turnover to enhance transcription factor binding[105].

In contrast to H3.1 nucleosomes, a fraction of H3.3 nucleosomes are 'split', consisting of one old and one new H3.3–H4 dimer[58]. Split nucleosomes are enriched at active genes and particularly at cell type-specific enhancers[108]. The processes that favour splitting of H3.3 nucleosomes in these contexts are unclear, but ASF1a is capable of splitting a $(H3.1–H4)_2$ tetramer *in vitro*[56], and seems likely to be involved in splitting $(H3.3–H4)_2$ tetramers at enhancers.

H3.3 mutants reduce the viability of *D. melanogaster*[109]. Survivors appear normal but have transcriptional defects, and males are sterile owing to chromatin-remodelling failure before meiosis. Overexpressing H3 compensates for the transcriptional defects, but not the meiotic defects. In chicken cells, incorporation of exogenous H3 into upstream regulatory elements reduces expression levels at some genes, whereas incorporation of exogenous H3.3 enhances expression[103]. These phenotypes suggest that H3.3 facilitates gene transcription and has a unique role in meiosis.

H2A.Z dynamics in animals. Vertebrates have two H2A.Z paralogues, H2A.Z.1 and H2A.Z.2, which differ by three conserved amino acid residues[110]. The genes encoding them are not redundant because H2A.Z.1 is essential for early development[111] and H2A.Z.2 is involved in DNA repair[112]. In primates, H2A.Z.2 has two splice forms, one of which, H2A.Z.2.2, has a shorter docking domain and forms highly unstable nucleosomes[113]. *In vivo*, H2A.Z.2.1 has a turnover rate similar to H2A, whereas H2A.Z.1 turns over faster[114].

H2A.Z-containing nucleosomes can be homotypic (H2A.Z|H2A.Z) or heterotypic (H2A.Z|H2A). A crystal structure of heterotypic nucleosomes revealed that the loop 1 part of H2A has the same structure as in homotypic H2A nucleosomes, whereas loop 1 of H2A.Z.1 is displaced from its location in homotypic H2A.Z.1 nucleosomes. The heterotypic H2A.Z.1 nucleosomes have greater thermal stability than the homotypic H2A.Z.1 nucleosomes[115].

In vitro, H2A.Z-containing nucleosomes, whether they contain H3.1 or H3.3, are more stable to salt than H2A-containing nucleosomes[95]. However, H2A.Z|H3.3 double-variant nucleosomes are unstable in chicken 6C2 cells[96]. Similarly, in HeLa cells, H2A.Z|H3.3 nucleosomes are enriched at DNase I-hypersensitive sites, at NDRs and at CTCF binding sites, from which they are lost during chromatin preparation by standard methods[104]. This observation is somewhat reminiscent of fragile nucleosomes in yeast[116]. The Swr1 homologue p400, which can exchange H2A–H2B with H2A.Z–H2B, is found at promoters, at enhancers and in coding regions in U2OS cells and is also required for maintaining H3.3 levels[117]. *In vitro*, p400 can introduce H3.3 as well as H2A.Z into chromatin, indicating that p400 may have a role in depositing H2A.Z–H3.3 nucleosomes.

In *D. melanogaster*, homotypic H2A.Z|H2A.Z nucleosomes, but not heterotypic H2A.Z|H2A nucleosomes, are soluble in low-salt conditions and are enriched at the +1 nucleosome of transcribed genes, with little enrichment at inactive genes[118]. In contrast to yeast, in which H2A.Z enrichment is confined almost entirely to the +1 nucleosome, homotypic H2A.Z nucleosomes are also present over the subsequent +2 to +4 nucleosomes in diminishing occupancy and at 5′ exon boundaries. In addition, sub-nucleosomal-sized H2A.Z particles of ~55 bp are enriched in the NDR ~20 bp upstream of the TSS. These smaller fragments may represent disrupted nucleosomes that have been internally cleaved by MNase. The +1 nucleosome in yeast typically encompasses the TSS, whereas it is downstream of the TSS in *D. melanogaster* and other animals. In nascent Pol II transcripts, the +1 nucleosome represents a major barrier to Pol II progression, and H2A.Z reduces the barrier relative to H2A, with knockdown of H2A.Z resulting in increased Pol II stalling[119]. H2A.Z occupancy inversely correlates with H3–H4 occupancy, suggesting that H2A.Z dimers may be more easily lost, whereas $(H3–H4)_2$ tetramers are retained to allow transcription to proceed. The +1 nucleosome may be a larger barrier than nucleosome barriers downstream because little DNA has been unwound when Pol II reaches it.

In vertebrates, H2A.Z is enriched around both promoters and enhancers (BOX 2), but varies over the cell cycle. In mouse trophoblast stem cells, H2A.Z nucleosome levels are reduced from S phase to mitosis (inclusive), with the loss of heterotypic H2A.Z at the TSS, and the gain of homotypic H2A.Z on centromeres[120]. This finding suggests that H2A.Z has a cell cycle-regulated role in centromere function and that H2A.Z distribution depends on the cell cycle; however, the functional relevance of these changes is currently unclear.

In mouse testes, TSSs of X chromosome genes that are active in round spermatids and encode proteins involved in transcription, RNA metabolism and spermatogenesis are occupied by nucleosomes containing the H2A.B variant[16]. H2A.B has a shorter docking domain and forms nucleosomes that wrap only ~120 bp of DNA[121], which might facilitate the transit of Pol II through them. Heterotypic H2A.Z nucleosomes are also found at promoters in round spermatids, but in different genes with a broader range of functions than those occupied by H2A.B nucleosomes. This result suggests that different variants may be used to activate different sets of genes in mammalian tissues.

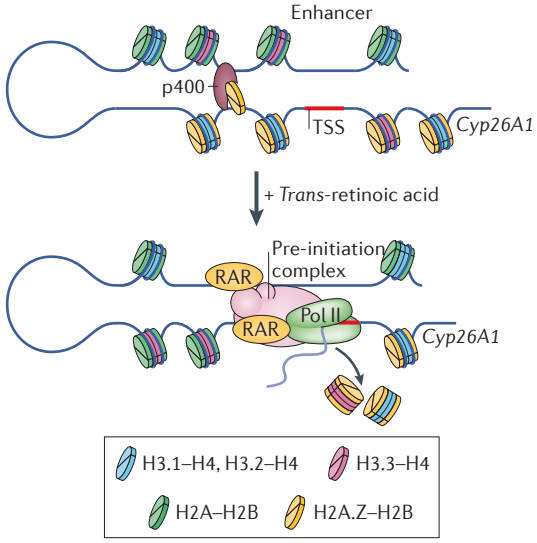
Bivalent promoters
Transcription start sites flanked by nucleosomes that are enriched for trimethylation of both the Lys 4 and Lys 27 residues of histone H3, thereby comprising a mark of 'poised' activation.

Docking domain
The carboxy-terminal region of H2A variants, which interacts with H3 and H4.

CTCF
A protein that binds the motif CCCTC and regulates the formation of long-range chromatin interactions and of topologically associated domains.

Fragile nucleosomes
A nucleosome with increased sensitivity to micrococcal nuclease, which forms at yeast promoters that have a nucleosome-depleted region > 150 bp.

Box 2 | **Roles of histone variants at enhancers**



H2A.Z appears to have seemingly contrasting roles in vertebrate enhancers, assuming both positive and negative roles in transcription. In enhancers of oestrogen receptor-α (ERα) target genes, H2A.Z is present in a cell type-specific manner at a subset of active enhancers that have greater DNase I accessibility and higher levels of ERα binding[155]. At these sites H2A.Z is required for the recruitment of RNA polymerase II (Pol II) and cohesin and for oestradiol induction of enhancer RNA transcription. By contrast, ERα enhancers without H2A.Z recruit Pol II and cohesin independently of H2A.Z and transcribe a basal level of enhancer RNA. This finding suggests that by recruiting Pol II, H2A.Z may promote enhancer RNA transcription, ERα and cohesin binding and the stabilization of enhancer–promoter associations. Similarly, H2A.Z is present with 48 kDa TATA box-binding protein-interacting protein (TIP48; also known as RuvB-like 2), a H2A.Z-specific chaperone, at both the promoter and 3′ enhancer of the cyclin D1 gene (*CCND1*), which together form a repressive chromatin loop[156]. Following oestradiol binding to ERα, TIP48 promotes the acetylation and exchange of H2A.Z by the histone acetyltransferase TIP60 (also known as KAT5). This process disrupts the loop and enables ERα binding, which stimulates transcription.

At the mouse retinoic acid-inducible gene *Cyp26A1* (which encodes cytochrome P450 26A1), H3.3 is predominantly found at the enhancer before induction (see the figure, top), and is necessary for the recruitment of the retinoic acid receptor (RAR) upon its induction by *trans*-retinoic acid[95]. At the transcription start site (TSS) H2A.Z is rapidly depleted upon induction, whereas the occupancy levels of H3.3, Pol II and RAR increase (see the figure, bottom). A model was proposed whereby H3.3-nucleosome turnover at the enhancer maintains an open chromatin structure that is permissive for RAR binding and assists in recruiting chaperones that can deposit H2A.Z at the promoter to repress transcription. Upon induction, RAR replaces H3.3 at the enhancer and H2A.Z is evicted and replaced by H2A–H3.3 nucleosomes. These examples suggest that promoter–enhancer interactions, chaperones and acetylation can all affect the role of H2A.Z in transcription.

Transcriptional dynamics of histone variants in plants. H3.1 and H3.3 in plants evolved independently from but in parallel to those in animals[36], probably because the chaperones that direct their assembly are largely conserved[19,30]. Functional characterizations of plant chaperones point to both similarities with and differences from their roles in other organisms[63,64,122], but patterns of H3.1 and H3.3 incorporation are similar to their animal counterparts. In *A. thaliana*, H3.1 nucleosomes are enriched with the H3K9me2 and H3K27me3 modifications and are found in heterochromatin and transposons[123,124], thus correlating with gene silencing. H3.3 nucleosomes are enriched in chromatin marked by H3.3K4me3 and are found across gene bodies and transcription termination sites. At gene bodies, H3.3 enrichment correlates with Pol II occupancy levels, gene body DNA methylation and gene expression[123,124].

H2A.Z is found primarily at promoters, and H2A.Z levels inversely correlate with DNA methylation and H3K9 dimethylation at gene bodies[125,126]. H2A.Z is necessary for gene expression but its occupancy is inversely correlated with gene expression levels, indicating that it is evicted during transcription[122]. H2A.Z mediates the plant thermosensory response and is lost from promoters at elevated temperatures, although this effect is not correlated with a transcriptional response[127]. More generally, H2A.Z in plants has been proposed to act as a sensor for environmental changes[128].

G-quadruplexes
A four-stranded helical structure formed in DNA and RNA that is composed of four runs of three or more guanines, separated by up to seven other nucleotides.

Heterochromatin maintenance

A large fraction of the genome of most eukaryotes comprises transposons and other repetitive elements that must be kept transcriptionally silent; this state is often achieved through DNA and histone methylation. Replication-coupled processes aid in this task in multiple ways, primarily by methylating new canonical H3 nucleosomes (BOX 3). However, replication-independent variants also have crucial roles in heterochromatin maintenance.

H3.3 dynamics in heterochromatin maintenance. Histone variants have shown unexpected roles in maintaining heterochromatin independently of replication. Genome-wide studies have found that ATRX and associated proteins are enriched at heterochromatin regions. Associated proteins include DAXX, H3.3, H3K9me3, H4K20me3 and the transcription co-repressor complex that includes the histone methyltransferase SETDB1 and KRAB-associated protein 1 (KAP1; also known as TRIM28). Specifically, heterochromatin is found in telomeres, pericentric regions, short tandem repeats, the 3′ exons of zinc finger genes, endogenous retroviruses and retrotransposons, and silenced alleles of imprinted genes[129–133]. ATRX targets heterochromatin by recognizing and binding multiple heterochromatin components. Such components include H3K9me3 (REFS 134,135), heterochromatin protein 1 (HP1), methyl-CpG-binding protein 2 (REFS 136,137), and, *in vitro*, G-quadruplexes[138],

which are predicted to form at telomeres. H3.3 enrichment at heterochromatin was generally found to be dependent on ATRX and DAXX[43–45,129,130].

Knockdown of H3.3 reduces H3K9me3 at heterochromatin regions and leads to the increased transposition of endogenous retroviruses, including intracisternal A-particle retrotransposons (IAPs)[130,139]. H3.3 knockdown also results in the de-repression of transcription at retrovirus-adjacent genes and increased telomere damage and sister chromatid exchange at telomeres[130,139]. Depletion of SETDB1 strongly reduces H3.3K9me1 and H3.3K9me3 at telomeres, whereas depletion of the histone methyltransferases SUV39H1 and SUV39H2 mostly reduces H3.3K9me3 levels[139]. *In vitro* studies have indicated that SETBD1 is capable of mono- and dimethylating H3K9 efficiently, but is inefficient at trimethylation[140]. Together, these results suggest a model in which ATRX recognizes existing H3K9me3 in a complex (or complexes) with DAXX, H3.3–H4, KAP1, SETDB1 and SUV39H1 (FIG. 3). SETDB1 may monomethylate H3.3 before its chromatin deposition, after which SUV39H1 and SUV39H2 are primarily responsible for trimethylating H3.3K9 to maintain heterochromatin structure[139]. In contrast to this model, another study using an IAP transgene construct in the β-globin locus found that silencing of the transgene depended on ATRX, DAXX, KAP1 and SETDB1, but was not affected by knockdown of H3.3 (REF. 132). This discrepancy might arise because IAP elements appear to retain H3.3 better than other sequences following H3.3 knockdown[130].

Box 3 | **Assembly of heterochromatin during replication**

In mammals, DNA and histone methylation are coordinated to maintain heterochromatin. During replication, DNA methylation is maintained by DNA (cytosine-5)-methyltransferase 1 (DNMT1), which interacts with proliferating cell nuclear antigen (PCNA) at the replication fork, recognizes hemi-methylated DNA and methylates the newly synthesized strand[157]. Methyl-binding protein 1 (MBD1) binds the methylated DNA and forms a complex with the histone methyltransferase SETDB1 and the large subunit of the chromatin assembly factor 1 (CAF1) complex during S phase; SETDB1 monomethylates H3.1 on Lys9 (H3K9me1) before CAF1 deposits it into chromatin[158]. H3K9me1 is a substrate for the histone methyltransferases SUV39H1 and SUV39H2, which trimethylate it. CAF1 and SETDB1 are also in a complex with heterochromatin protein 1 (HP1), which binds H3K9me3 during replication to re-establish the heterochromatin structure[159]. Upon binding H3K9me3, HP1 forms protein bridges across nucleosomes and recruits SUV39H1, SUV39H2 and the H4 methyltransferase SUV420H, which catalyses H4K20 trimethylation[160,161]. These enzymes then methylate adjacent nucleosomes, thereby spreading and reinforcing a local heterochromatic environment. In contrast to other histone modifications, the heterochromatic modifications H3K9me3 and H3K27me3 remain underrepresented on new nucleosomes after one cell cycle. However, their overall levels are maintained by the methylation of both old and new nucleosomes[65,66]. This finding suggests that the re-establishment of silencing modifications following replication is not processive or precise at the nucleosome level, but that silencing depends on maintaining a certain overall level of repressive modifications.

As in animals, replication-coupled processes help maintain heterochromatin in plants. H3K27me1 is a mark of condensed heterochromatin in *Arabidopsis thaliana*[162]. The histone methyltransferases *Arabidopsis* Trithorax-related protein 5 (ATXR5) and ATXR6 bind to PCNA[163] and recognize the Ala31 residue of H3.1, but not the Thr31 residue of H3.3 (REF. 164). Consequently, ATXR5 and ATXR6 monomethylate Lys27 only of H3.1, leaving H3.3-enriched genic regions unsilenced[164]. This finding suggests that ATXR5 and ATXR6 maintain silencing by specifically recognizing and methylating the replication-coupled form of H3.

In addition, SETDB1, SUV39H1 and SUV39H2 recruited by ATRX–DAXX can modify both H3.1 and H3.3 (REF. 20). Consequently, if H3.1 can be more easily incorporated at the β-globin locus than in heterochromatin, whether through histone recycling or another mechanism, it might be able to substitute for H3.3 in ATRX–DAXX-directed silencing, at least at this locus.

Nucleosome-free gaps in the chromatin are required for ATRX–DAXX to deposit H3.3. In *D. melanogaster*, ATRX (XNP) and HIRA independently bind to nucleosome-depleted chromatin and remain there until displaced by H3.3 nucleosomes[14]. One plausible cause for the occurrence of nucleosome gaps in heterochromatin is transcription, which can dislodge nucleosomes and result in turnover[13]. As depletion of ATRX in mouse embryonic stem cells results in de-repression of transcription from endogenous retroviruses[130], from telomeres[139] and from imprinted alleles[129,130], it is likely that transcription from these elements triggers the histone turnover that leads to their silencing. Other potential sources of nucleosome gaps include DNA damage, replication stress, spontaneous nucleosome unwrapping, remodelling, and nucleosome-inhibitory sequences such as the G-quadruplexes in telomeric repeats.

macroH2A: silence or stability? ATRX also interacts with macroH2A.1, but not together with DAXX and not with H3.3, which is largely absent from macroH2A.1.2-containing nucleosomes[17]. Instead, knockdown of ATRX increases macroH2A.1 deposition at telomeres and throughout the subtelomeric α-globin locus. Thus, the increased deposition of macroH2A.1 at this locus might underlie the silencing of α-globin in the alpha thalassemia phenotype of human *ATRX* mutations. MacroH2A has generally been regarded as a repressive variant that inhibits chromatin remodelling[141], but its role may be more nuanced. In the Burkitt lymphoma Namalwa cell line, single macroH2A nucleosomes occupy the promoters of both expressed and non-expressed genes in a cell type-specific manner[142]. Moreover, gene activity depends on whether these macroH2A nucleosomes occlude an activating or repressing transcription factor binding site[142]. A subset of macroH2A nucleosomes is bound by the transcription factor nuclear respiratory factor 1, which stabilizes the nucleosomes against perturbation by constitutive or viral transcription factors, thereby reducing transcriptional noise. This finding indicates that macroH2A can help enforce existing expression patterns in fluctuating conditions.

Roles of H2A variants in heterochromatin formation and maintenance. H2A.Z has a role in the formation of heterochromatin in animals. In *D. melanogaster*, H2A.Z is present in pericentric heterochromatin, and H2A.Z mutants have reduced amounts of H3K9 methylation and HP1; this finding suggests that H2A.Z is required for efficient heterochromatin assembly[143]. H2A.Z mutants suppress position-effect variegation and also have mitotic segregation defects, both of which are similar to phenotypes of HP1 mutants. In early mouse

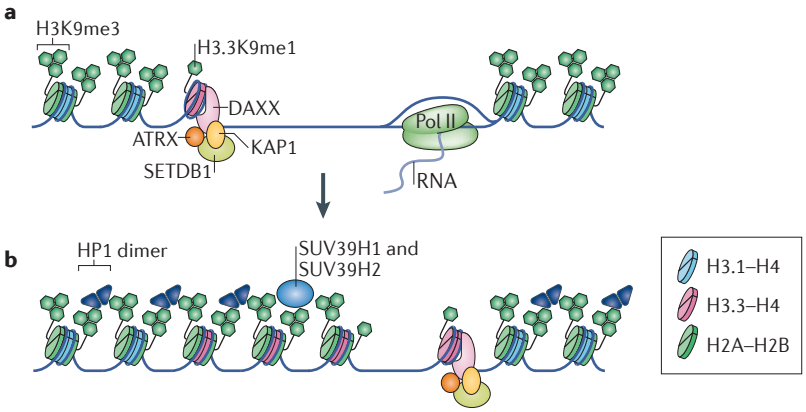


Figure 3 | **H3.3 in heterochromatin maintenance. a** | Nucleosome gaps appear in heterochromatin, for example, as a result of nucleosome eviction during transcription or other processes. Alpha thalassemia mental retardation syndrome X-linked (ATRX) in a complex with death domain-associated protein (DAXX), H3.3–H4 dimers, the transcriptional co-repressor KRAB-associated protein 1 (KAP1) and the histone methyltransferase SETDB1, can recognize the heterochromatin modification of trimethylation of histone H3 on Lys9 (H3K9me3) and direct DAXX to deposit H3.3K9me1–H4 dimers onto DNA. **b** | H3.3K9me1 can be converted to H3.3K9me3 by the histone methyltransferases SUV39H1 and SUV39H2. Heterochromatin protein 1 (HP1) binds to trimethylated Lys9 and forms protein bridges across nucleosomes to stabilize them, thereby providing a platform for heterochromatin spreading and maintenance (not shown).

embryos, H2A.Z is absent from the undifferentiated inner cell mass, but is deposited into heterochromatin upon differentiation and interacts *in vitro* with the pericentric inner centromere protein (INCENP)[144]. Knockdown of H2A.Z in monkey COS-7 cells results in mitotic defects and partial loss of HP1α localization, even though H2A.Z is not detectably enriched in the pericentromere in these cells[145]. In human HEK293 and mouse L929 cells, H2A.Z and H3K4me2 are present on chromatin fibres in domains that are interspersed with centromeric CENPA domains and embedded within regions of flanking heterochromatin[146]. The linearly interspersed CENPA domains self-associate in 3D to form the kinetochore that faces the pole at anaphase, whereas the domains of H2A and H3K9me3 self-associate and mediate sister chromatid cohesion.

In 3T3 cells and mouse embryonic fibroblasts, new H2A.Z is deposited in pericentromeric heterochromatin primarily in G1 phase, whereas H2A is deposited during S phase, although both appear to be deposited in euchromatin in all phases. An intact heterochromatin structure appears to limit the timing of H2A.Z deposition to G1 (REF. 147). A possible interpretation of these results is that low transcription levels in heterochromatin limits the deposition of H2A by FACT or other chaperones except during replication. By contrast, H2A.Z can be deposited following nucleosome losses during mitosis, in damaged heterochromatin or in developmental chromatin remodelling processes. In this scenario, mutations or knockdown of H2A.Z might leave damaged heterochromatin unrepaired and contribute to the loss of methylated H3K9 and HP1, and to mitotic defects resembling the loss of HP1.

H2A.Z is absent from heterochromatin in plants[122], in which heterochromatin formation is dependent on a special H2A variant, H2A.W. This variant has an extended carboxy-terminal tail with an SPKK motif that wraps an additional 16 bp of DNA[148], and may protect nucleosomes from unwrapping. H2A.W colocalizes with H3K9me2 but does not depend on H3K9me2 or DNA methylation for its localization or ability to condense chromatin *in vivo*[149]. The extended tail of H2A.W promotes long-range chromatin interactions *in vitro*, which may be relevant to promoting chromatin condensation.

Conclusion and future perspective

For decades, progress in the study of histone variants was mostly limited to bulk biochemical characterization. Since then, the use of revolutionary genomic technologies has provided high-resolution maps of histone variants and modified nucleosomes. These maps have revealed where and to what extent different variants are enriched on a genome-wide scale so that the processes involved in their deposition and mobilization can be understood *in vivo*. Over the past few years we have also seen remarkable progress in our understanding of the action of the complexes that use histones and nucleosomes as their substrates, including chromatin remodellers, chaperones and other chromatin regulators that participate in nucleosome dynamics. We are also seeing progress in resolving the rapid dynamics of the replication fork, and the dynamics of DNA synthesis-independent histone replacement during transcription. In the near future, we anticipate that advances in super-resolution microscopy and live cell imaging will bring us closer to visualizing these processes by tracking individual particles during replication, transcription and DNA damage repair[150].

1. Macvanin, M. & Adhya, S. Architectural organization in *E. coli* nucleoid. *Biochim. Biophys. Acta* **1819**, 830–835 (2012).
2. Peeters, E., Driessen, R. P., Werner, F. & Dame, R. T. The interplay between nucleoid organization and transcription in archaeal genomes. *Nat. Rev. Microbiol.* **13**, 333–341 (2015).
3. Arents, G. & Moudrianakis, E. N. The histone fold: a ubiquitous architectural motif utilized in DNA compaction and protein dimerization. *Proc. Natl Acad. Sci. USA* **92**, 11170–11174 (1995).
4. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260 (1997).
5. Song, F. *et al.* Cryo-EM study of the chromatin fiber reveals a double helix twisted by tetranucleosomal units. *Science* **344**, 376–380 (2014).
6. Kurat, C. F. *et al.* Regulation of histone gene transcription in yeast. *Cell. Mol. Life Sci.* **71**, 599–613 (2014).
7. Marzluff, W. F. Metazoan replication-dependent histone mRNAs: a distinct set of RNA polymerase II transcripts. *Curr. Opin. Cell Biol.* **17**, 274–280 (2005).
8. Talbert, P. B. & Henikoff, S. Histone variants — ancient wrap artists of the epigenome. *Nat. Rev. Mol. Cell Biol.* **11**, 264–275 (2010).
9. Waterborg, J. H. & Robertson, A. J. Common features of analogous replacement histone H3 genes in animals and plants. *J. Mol. Evol.* **43**, 194–206 (1996).
10. Steiner, F. A. & Henikoff, S. Diversity in the organization of centromeric chromatin. *Curr. Opin. Genet. Dev.* **31**, 28–35 (2015).
11. McKinley, K. L. & Cheeseman, I. M. The molecular basis for centromere identity and function. *Nat. Rev. Mol. Cell Biol.* **17**, 16–29 (2016).
12. Ray-Gallet, D. *et al.* Dynamics of histone H3 deposition *in vivo* reveal a nucleosome gap-filling mechanism for H3.3 to maintain chromatin integrity. *Mol. Cell* **44**, 928–941 (2011).
13. Schwartz, B. E. & Ahmad, K. Transcriptional activation triggers deposition and removal of the histone variant H3.3. *Genes Dev.* **19**, 804–814 (2005).

14. Schneiderman, J. I., Orsi, G. A., Hughes, K. T., Loppin, B. & Ahmad, K. Nucleosome-depleted chromatin gaps recruit assembly factors for the H3.3 histone variant. *Proc. Natl Acad. Sci. USA* **109**, 19721–19726 (2012).
15. Adam, M., Robert, F., Larochelle, M. & Gaudreau, L. H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. *Mol. Cell. Biol.* **21**, 6270–6279 (2001).
16. Soboleva, T. A. *et al.* A unique H2A histone variant occupies the transcriptional start site of active genes. *Nat. Struct. Mol. Biol.* **19**, 25–30 (2011).
17. Ratnakumar, K. *et al.* ATRX-mediated chromatin association of histone variant macroH2A1 regulates α-globin expression. *Genes Dev.* **26**, 433–438 (2012).
18. Yelagandula, R. *et al.* The histone variant H2A.W defines heterochromatin and promotes chromatin condensation in *Arabidopsis*. *Cell* **158**, 98–109 (2014).
19. Gurard-Levin, Z. A., Quivy, J. P. & Almouzni, G. Histone chaperones: assisting histone traffic and nucleosome dynamics. *Annu. Rev. Biochem.* **83**, 487–517 (2014).
20. McKittrick, E., Gafken, P. R., Ahmad, K. & Henikoff, S. Histone H3.3 is enriched in covalent modifications associated with active chromatin. *Proc. Natl Acad. Sci. USA* **101**, 1525–1530 (2004).
21. Chang, F. T. *et al.* CHK1-driven histone H3.3 serine 31 phosphorylation is important for chromatin maintenance and cell survival in human ALT cancer cells. *Nucleic Acids Res.* **43**, 2603–2614 (2015).
22. Ismail, I. H. & Hendzel, M. J. The γ-H2A.X: is it just a surrogate marker of double-strand breaks or much more? *Environ. Mol. Mutagen.* **49**, 73–82 (2008).
23. Filipescu, D., Szenker, E. & Almouzni, G. Developmental roles of histone H3 variants and their chaperones. *Trends Genet.* **29**, 630–640 (2013).
24. Skene, P. J. & Henikoff, S. Histone variants in pluripotency and disease. *Development* **140**, 2513–2524 (2013).
25. Turinetto, V. & Giachino, C. Histone variants as emerging regulators of embryonic stem cell identity. *Epigenetics* **10**, 563–573 (2015).
26. Gaume, X. & Torres-Padilla, M. E. Regulation of reprogramming and cellular plasticity through histone exchange and histone variant incorporation. *Cold Spring Harb. Symp. Quant. Biol.* **80**, 165–175 (2015).
27. Gursoy-Yuzugullu, O., House, N. & Price, B. D. Patching broken DNA: nucleosome dynamics and the repair of DNA breaks. *J. Mol. Biol.* **428**, 1846–1860 (2015).
28. Talbert, P. B. & Henikoff, S. Environmental responses mediated by histone variants. *Trends Cell Biol.* **24**, 642–650 (2014).
29. Laskey, R. A., Honda, B. M., Mills, A. D. & Finch, J. T. Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA. *Nature* **275**, 416–420 (1978).
30. Tripathi, A. K., Singh, K., Pareek, A. & Singla-Pareek, S. L. Histone chaperones in *Arabidopsis* and rice: genome-wide identification, phylogeny, architecture and transcriptional regulation. *BMC Plant Biol.* **15**, 42 (2015).
31. Alvarez, F. *et al.* Sequential establishment of marks on soluble histones H3 and H4. *J. Biol. Chem.* **286**, 17714–17721 (2011).
32. Campos, E. I. *et al.* The program for processing newly synthesized histones H3.1 and H4. *Nat. Struct. Mol. Biol.* **17**, 1343–1351 (2010).
33. Tagami, H., Ray-Gallet, D., Almouzni, G. & Nakatani, Y. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell* **116**, 51–61 (2004).
34. Green, E. M. *et al.* Replication-independent histone deposition by the HIR complex and Asf1. *Curr. Biol.* **15**, 2044–2049 (2005).
35. Liu, W. H., Roemer, S. C., Port, A. M. & Churchill, M. E. CAF-1-induced oligomerization of histones H3/H4 and mutually exclusive interactions with Asf1 guide H3/H4 transitions among histone chaperones and DNA. *Nucleic Acids Res.* **40**, 11229–11239 (2012).
36. Waterborg, J. H. Evolution of histone H3: emergence of variants and conservation of post-translational modification sites. *Biochem. Cell Biol.* **90**, 79–95 (2012).
37. Latreille, D., Bluy, L., Benkirane, M. & Kiernan, R. E. Identification of histone 3 variant 2 interacting factors. *Nucleic Acids Res.* **42**, 3542–3550 (2014).
38. Abascal, F. *et al.* Subfunctionalization via adaptive evolution influenced by genomic context: the case of histone chaperones ASF1a and ASF1b. *Mol. Biol. Evol.* **30**, 1853–1866 (2013).
39. Ahmad, K. & Henikoff, S. The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell* **9**, 1191–1200 (2002).
40. Elsasser, S. J. *et al.* DAXX envelops a histone H3.3–H4 dimer for H3.3-specific recognition. *Nature* **491**, 560–565 (2012).
Recognition of Gly90 found in H3.3 but not H3.1 or H3.2 by the histone chaperone DAXX distinguishes the otherwise almost identical variants.
41. Lacoste, N. *et al.* Mislocalization of the centromeric histone variant CenH3/CENP-A in human cells depends on the chaperone DAXX. *Mol. Cell* **53**, 631–644 (2014).
42. Athwal, R. K. *et al.* CENP-A nucleosomes localize to transcription factor hotspots and subtelomeric sites in human cancer cells. *Epigenetics Chromatin* **8**, 2 (2015).
43. Drane, P., Ouararhni, K., Depaux, A., Shuaib, M. & Hamiche, A. The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. *Genes Dev.* **24**, 1253–1265 (2010).
44. Wong, L. H. *et al.* ATRX interacts with H3.3 in maintaining telomere structural integrity in pluripotent embryonic stem cells. *Genome Res.* **20**, 351–360 (2010).
45. Goldberg, A. D. *et al.* Distinct factors control histone variant H3.3 localization at specific genomic regions. *Cell* **140**, 678–691 (2010).
46. Delbarre, E., Ivanauskiene, K., Kuntziger, T. & Collas, P. DAXX-dependent supply of soluble (H3.3–H4) dimers to PML bodies pending deposition into chromatin. *Genome Res.* **23**, 440–451 (2013).
47. Schlesinger, M. B. & Formosa, T. POB3 is required for both transcription and replication in the yeast *Saccharomyces cerevisiae*. *Genetics* **155**, 1593–1606 (2000).
48. Belotserkovskaya, R. *et al.* FACT facilitates transcription-dependent nucleosome alteration. *Science* **301**, 1090–1093 (2003).
49. Mason, P. B. & Struhl, K. The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation *in vivo*. *Mol. Cell. Biol.* **23**, 8323–8333 (2003).
50. Heo, K. *et al.* FACT-mediated exchange of histone variant H2AX regulated by phosphorylation of H2AX and ADP-ribosylation of Spt16. *Mol. Cell* **30**, 86–97 (2008).
51. Hondele, M. *et al.* Structural basis of histone H2A–H2B recognition by the essential chaperone FACT. *Nature* **499**, 111–114 (2013).
52. Kemble, D. J., McCullough, L. L., Whitby, F. G., Formosa, T. & Hill, C. P. FACT disrupts nucleosome structure by binding H2A–H2B with conserved peptide motifs. *Mol. Cell* **60**, 294–306 (2015).
53. Yang, J. *et al.* The histone chaperone FACT contributes to DNA replication-coupled nucleosome assembly. *Cell Rep.* **14**, 1128–1141 (2016).
54. Ramachandran, S. & Henikoff, S. Replicating nucleosomes. *Sci. Adv.* **1**, e1500587 (2015).
55. Shibahara, K. & Stillman, B. Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell* **96**, 575–585 (1999).
56. Natsume, R. *et al.* Structure and function of the histone chaperone CIA/ASF1 complexed with histones H3 and H4. *Nature* **446**, 338–341 (2007).
57. Winkler, D. D., Zhou, H., Dar, M. A., Zhang, Z. & Luger, K. Yeast CAF-1 assembles histone $(H3–H4)_2$ tetramers prior to DNA deposition. *Nucleic Acids Res.* **40**, 10139–10149 (2012).
58. Xu, M. *et al.* Partitioning of histone H3–H4 tetramers during DNA replication-dependent chromatin assembly. *Science* **328**, 94–98 (2010).
59. Foltman, M. *et al.* Eukaryotic replisome components cooperate to process histones during chromosome replication. *Cell Rep.* **3**, 892–904 (2013).
60. Richet, N. *et al.* Structural insight into how the human helicase subunit MCM2 may act as a histone chaperone together with ASF1 at the replication fork. *Nucleic Acids Res.* **43**, 1905–1917 (2015).
61. Huang, H. *et al.* A unique binding mode enables MCM2 to chaperone histones H3–H4 at replication forks. *Nat. Struct. Mol. Biol.* **22**, 618–626 (2015).
The MCM2 subunit of the replicative helicase co-chaperones with ASF1 H3–H4 dimers regardless of variant composition.
62. Houlard, M. *et al.* CAF-1 is essential for heterochromatin organization in pluripotent embryonic cells. *PLoS Genet.* **2**, e181 (2006).
63. Kaya, H. *et al. FASCIATA* genes for chromatin assembly factor-1 in *Arabidopsis* maintain the cellular organization of apical meristems. *Cell* **104**, 131–142 (2001).
64. Duc, C. *et al.* The histone chaperone complex HIR maintains nucleosome occupancy and counterbalances impaired histone deposition in CAF-1 complex mutants. *Plant J.* **81**, 707–722 (2015).
65. Alabert, C. *et al.* Two distinct modes for propagation of histone PTMs across the cell cycle. *Genes Dev.* **29**, 585–590 (2015).
Most histone modifications are restored rapidly after replication, but H2A.Z levels are only slowly restored over the course of multiple cell generations.
66. Xu, M., Wang, W., Chen, S. & Zhu, B. A model for mitotic inheritance of histone lysine methylation. *EMBO Rep.* **13**, 60–67 (2012).
67. Jeronimo, C., Watanabe, S., Kaplan, C. D., Peterson, C. L. & Robert, F. The histone chaperones FACT and Spt6 restrict H2A.Z from intragenic locations. *Mol. Cell* **58**, 1113–1123 (2015).
68. Tran, V., Lim, C., Xie, J. & Chen, X. Asymmetric division of *Drosophila* male germline stem cell shows asymmetric histone distribution. *Science* **338**, 679–682 (2012).
Asymmetric division of germline stem cells in *D. melanogaster* testes results in newly deposited H3.1, but not H3.3, segregating to the progenitor cell with retention of old H3.1 in the self-renewing stem cell.
69. Xie, J. *et al.* Histone H3 threonine phosphorylation regulates asymmetric histone inheritance in the *Drosophila* male germline. *Cell* **163**, 920–933 (2015).
70. Studitsky, V. M., Clark, D. J. & Felsenfeld, G. A histone octamer can step around a transcribing polymerase without leaving the template. *Cell* **76**, 371–382 (1994).
71. Kornberg, R. D. & Lorch, Y. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98**, 285–294 (1999).
72. Bernstein, B. E., Liu, C. L., Humphrey, E. L., Perlstein, E. O. & Schreiber, S. L. Global nucleosome occupancy in yeast. *Genome Biol.* **5**, R62 (2004).
73. Lee, C. K., Shibata, Y., Rao, B., Strahl, B. D. & Lieb, J. D. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat. Genet.* **36**, 900–905 (2004).
74. Dion, M. F. *et al.* Dynamics of replication-independent histone turnover in budding yeast. *Science* **315**, 1405–1408 (2007).
75. Jamai, A., Imoberdorf, R. M. & Strubin, M. Continuous histone H2B and transcription-dependent histone H3 exchange in yeast cells outside of replication. *Mol. Cell* **25**, 345–355 (2007).
76. Hartley, P. D. & Madhani, H. D. Mechanisms that specify promoter nucleosome location and identity. *Cell* **137**, 445–458 (2009).
77. Raisner, R. M. *et al.* Histone variant H2A.Z marks the 5′ ends of both active and inactive genes in euchromatin. *Cell* **123**, 233–248 (2005).
78. Rhee, H. S. & Pugh, B. F. Genome-wide structure and organization of eukaryotic pre-initiation complexes. *Nature* **483**, 295–301 (2012).
79. Mizuguchi, G. *et al.* ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* **303**, 343–348 (2004).
80. Ranjan, A. *et al.* Nucleosome-free region dominates histone acetylation in targeting SWR1 to promoters for H2A.Z replacement. *Cell* **154**, 1232–1245 (2013).
81. Papamichos-Chronakis, M., Watanabe, S., Rando, O. J. & Peterson, C. L. Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity. *Cell* **144**, 200–213 (2011).
82. Guillemette, B. *et al.* Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. *PLoS Biol.* **3**, e384 (2005).
83. Zhang, H., Roberts, D. N. & Cairns, B. R. Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell* **123**, 219–231 (2005).
84. Zanton, S. J. & Pugh, B. F. Full and partial genome-wide assembly and disassembly of the yeast transcription machinery in response to heat shock. *Genes Dev.* **20**, 2250–2265 (2006).

85. Tramantano, M. *et al.* Constitutive turnover of histone H2A.Z at yeast promoters requires the preinitiation complex. *eLife* **5**, e14243 (2016).
Components of the transcription pre-initiation complex cause H2A.Z turnover in budding yeast.
86. Meneghini, M. D., Wu, M. & Madhani, H. D. Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell* **112**, 725–736 (2003).
87. Ramachandran, S., Zentner, G. E. & Henikoff, S. Asymmetric nucleosomes flank promoters in the budding yeast genome. *Genome Res.* **25**, 381–390 (2015).
Half-unwrapping of RSC-bound nucleosomes flanking promoters *in vivo* indicates that RSC partially unwraps a nucleosome before performing ATP-dependent DNA translocation.
88. Lorch, Y., Maier-Davis, B. & Kornberg, R. D. Mechanism of chromatin remodeling. *Proc. Natl Acad. Sci. USA* **107**, 3458–3462 (2010).
89. Adkins, M. W. & Tyler, J. K. Transcriptional activators are dispensable for transcription in the absence of Spt6-mediated chromatin reassembly of promoter regions. *Mol. Cell* **21**, 405–416 (2006).
90. Jamai, A., Puglisi, A. & Strubin, M. Histone chaperone Spt16 promotes redeposition of the original H3–H4 histones evicted by elongating RNA polymerase. *Mol. Cell* **35**, 377–383 (2009).
91. Kato, H. *et al.* Spt6 prevents transcription-coupled loss of posttranslationally modified histone H3. *Sci. Rep.* **3**, 2186 (2013).
92. Kaplan, C. D., Morris, J. R., Wu, C. & Winston, F. Spt5 and Spt6 are associated with active transcription and have characteristics of general elongation factors in *D. melanogaster*. *Genes Dev.* **14**, 2623–2634 (2000).
93. Hartzog, G. A., Wada, T., Handa, H. & Winston, F. Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in *Saccharomyces cerevisiae*. *Genes Dev.* **12**, 357–369 (1998).
94. Svensson, J. P. *et al.* A nucleosome turnover map reveals that the stability of histone H4 Lys20 methylation depends on histone recycling in transcribed chromatin. *Genome Res.* **25**, 872–883 (2015).
95. Chen, P. *et al.* H3.3 actively marks enhancers and primes gene transcription via opening higher-ordered chromatin. *Genes Dev.* **27**, 2109–2124 (2013).
96. Jin, C. & Felsenfeld, G. Nucleosome stability mediated by histone variants H3.3 and H2A.Z. *Genes Dev.* **21**, 1519–1529 (2007).
97. Deal, R. B., Henikoff, J. G. & Henikoff, S. Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. *Science* **328**, 1161–1164 (2010).
98. Mito, Y., Henikoff, J. G. & Henikoff, S. Genome-scale profiling of histone H3.3 replacement patterns. *Nat. Genet.* **37**, 1090–1097 (2005).
99. Mito, Y., Henikoff, J. G. & Henikoff, S. Histone replacement marks the boundaries of *cis*-regulatory domains. *Science* **315**, 1408–1411 (2007).
100. Wirbelauer, C., Bell, O. & Schubeler, D. Variant histone H3.3 is deposited at sites of nucleosomal displacement throughout transcribed genes while active histone modifications show a promoter-proximal bias. *Genes Dev.* **19**, 1761–1766 (2005).
101. Nakayama, T., Nishioka, K., Dong, Y. X., Shimojima, T. & Hirose, S. *Drosophila* GAGA factor directs histone H3.3 replacement that prevents the heterochromatin spreading. *Genes Dev.* **21**, 552–561 (2007).
102. Chow, C. M. *et al.* Variant histone H3.3 marks promoters of transcriptionally active genes during mammalian cell division. *EMBO Rep.* **6**, 354–360 (2005).
103. Jin, C. & Felsenfeld, G. Distribution of histone H3.3 in hematopoietic cell lineages. *Proc. Natl Acad. Sci. USA* **103**, 574–579 (2006).
104. Jin, C. *et al.* H3.3/H2A.Z double variant-containing nucleosomes mark 'nucleosome-free regions' of active promoters and other regulatory regions. *Nat. Genet.* **41**, 941–945 (2009).
105. Kraushaar, D. C. *et al.* Genome-wide incorporation dynamics reveal distinct categories of turnover for the histone variant H3.3. *Genome Biol.* **14**, R121 (2013).
106. Deaton, A. M. *et al.* Enhancer regions show high histone H3.3 turnover that changes during differentiation. *eLife* **5**, e15316 (2016).
Active enhancers are characterized by high turnover of H3.3 during differentiation.
107. Banaszynski, L. A. *et al.* Hira-dependent histone H3.3 deposition facilitates PRC2 recruitment at developmental loci in ES cells. *Cell* **155**, 107–120 (2013).
108. Huang, C. *et al.* H3.3–H4 tetramer splitting events feature cell-type specific enhancers. *PLoS Genet.* **9**, e1003558 (2013).
Although H3.1-containing nucleosomes are reassembled with an intact four-helix bundle behind the replication fork, H3.3 nucleosomes at enhancers tend to split.
109. Sakai, A., Schwartz, B. E., Goldstein, S. & Ahmad, K. Transcriptional and developmental functions of the H3.3 histone variant in *Drosophila*. *Curr. Biol.* **19**, 1816–1820 (2009).
110. Eirin-Lopez, J. M., Gonzalez-Romero, R., Dryhurst, D., Ishibashi, T. & Ausio, J. The evolutionary differentiation of two histone H2A.Z variants in chordates (H2A.Z-1 and H2A. Z-2) is mediated by a stepwise mutation process that affects three amino acid residues. *BMC Evol. Biol.* **9**, 31 (2009).
111. Faast, R. *et al.* Histone variant H2A.Z is required for early mammalian development. *Curr. Biol.* **11**, 1183–1187 (2001).
112. Nishibuchi, I. *et al.* Reorganization of damaged chromatin by the exchange of histone variant H2A.Z-2. *Int. J. Radiat. Oncol. Biol. Phys.* **89**, 736–744 (2014).
113. Bonisch, C. *et al.* H2A.Z.2.2 is an alternatively spliced histone H2A.Z variant that causes severe nucleosome destabilization. *Nucleic Acids Res.* **40**, 5951–5964 (2012).
114. Horikoshi, N. *et al.* Structural polymorphism in the L1 loop regions of human H2A.Z.1 and H2A.Z.2. *Acta Crystallogr. D Biol. Crystallogr.* **69**, 2431–2439 (2013).
115. Horikoshi, N., Arimura, Y., Taguchi, H. & Kurumizaka, H. Crystal structures of heterotypic nucleosomes containing histones H2A.Z and H2A. *Open Biol.* **6**, 160127 (2016).
116. Kubik, S. *et al.* Nucleosome stability distinguishes two different promoter types at all protein-coding genes in yeast. *Mol. Cell* **60**, 422–434 (2015).
In budding yeast, fragile nucleosomes form in nucleosome-depleted regions that are widened by the binding of general regulatory factors.
117. Pradhan, S. K. *et al.* EP400 deposits H3.3 into promoters and enhancers during gene activation. *Mol. Cell* **61**, 27–38 (2016).
118. Weber, C. M., Henikoff, J. G. & Henikoff, S. H2A.Z nucleosomes enriched over active genes are homotypic. *Nat. Struct. Mol. Biol.* **17**, 1500–1507 (2010).
119. Weber, C. M., Ramachandran, S. & Henikoff, S. Nucleosomes are context-specific, H2A.Z-modulated barriers to RNA polymerase. *Mol. Cell* **53**, 819–830 (2014).
Nucleosome barriers to Pol II are lowered by H2A.Z *in vivo*.
120. Nekrasov, M. *et al.* Histone H2A.Z inheritance during the cell cycle and its impact on promoter organization and dynamics. *Nat. Struct. Mol. Biol.* **19**, 1076–1083 (2012).
121. Bao, Y. *et al.* Nucleosomes containing the histone variant H2A.Bbd organize only 118 base pairs of DNA. *EMBO J.* **23**, 3314–3324 (2004).
122. Deal, R. B., Topp, C. N., McKinney, E. C. & Meagher, R. B. Repression of flowering in *Arabidopsis* requires activation of *FLOWERING LOCUS C* expression by the histone variant H2A.Z. *Plant Cell* **19**, 74–83 (2007).
123. Stroud, H. *et al.* Genome-wide analysis of histone H3.1 and H3.3 variants in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **109**, 5370–5375 (2012).
124. Wollmann, H. *et al.* Dynamic deposition of histone variant H3.3 accompanies developmental remodeling of the *Arabidopsis* transcriptome. *PLoS Genet.* **8**, e1002658 (2012).
125. Coleman-Derr, D. & Zilberman, D. Deposition of histone variant H2A.Z within gene bodies regulates responsive genes. *PLoS Genet.* **8**, e1002988 (2012).
H2A.Z in plants acts as a general sensor of environmental conditions.
126. Bewick, A. J. *et al.* On the origin and evolutionary consequences of gene body DNA methylation. *Proc. Natl Acad. Sci. USA* **113**, 9111–9116 (2016).
127. Kumar, S. V. & Wigge, P. A. H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. *Cell* **140**, 136–147 (2010).
128. Coleman-Derr, D. & Zilberman, D. DNA methylation, H2A.Z, and the regulation of constitutive expression. *Cold Spring Harb. Symp. Quant. Biol.* **77**, 147–154 (2012).
129. Voon, H. P. *et al.* ATRX plays a key role in maintaining silencing at interstitial heterochromatic loci and imprinted genes. *Cell Rep.* **11**, 405–418 (2015).
130. Elsasser, S. J., Noh, K. M., Diaz, N., Allis, C. D. & Banaszynski, L. A. Histone H3.3 is required for endogenous retroviral element silencing in embryonic stem cells. *Nature* **522**, 240–244 (2015).
131. He, Q. *et al.* The Daxx/Atrx complex protects tandem repetitive elements during DNA hypomethylation by promoting H3K9 trimethylation. *Cell Stem Cell* **17**, 273–286 (2015).
132. Sadic, D. *et al.* Atrx promotes heterochromatin formation at retrotransposons. *EMBO Rep.* **16**, 836–850 (2015).
133. Valle-Garcia, D. *et al.* ATRX binds to atypical chromatin domains at the 3' exons of zinc finger genes to preserve H3K9me3 enrichment. *Epigenetics* **11**, 398–414 (2016).
134. Dhayalan, A. *et al.* The ATRX-ADD domain binds to H3 tail peptides and reads the combined methylation state of K4 and K9. *Hum. Mol. Genet.* **20**, 2195–2203 (2011).
135. Eustermann, S. *et al.* Combinatorial readout of histone H3 modifications specifies localization of ATRX to heterochromatin. *Nat. Struct. Mol. Biol.* **18**, 777–782 (2011).
136. McDowell, T. L. *et al.* Localization of a putative transcriptional regulator (ATRX) at pericentromeric heterochromatin and the short arms of acrocentric chromosomes. *Proc. Natl Acad. Sci. USA* **96**, 13983–13988 (1999).
137. Nan, X. *et al.* Interaction between chromatin proteins MECP2 and ATRX is disrupted by mutations that cause inherited mental retardation. *Proc. Natl Acad. Sci. USA* **104**, 2709–2714 (2007).
138. Law, M. J. *et al.* ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size-dependent manner. *Cell* **143**, 367–378 (2010).
139. Udugama, M. *et al.* Histone variant H3.3 provides the heterochromatic H3 lysine 9 tri-methylation mark at telomeres. *Nucleic Acids Res.* **43**, 10227–10237 (2015).
H3.3 deposited by ATRX at mammalian telomeres is the preferred substrate for H3K9 trimethylation and heterochromatin formation.
140. Basavapathruni, A. *et al.* Characterization of the enzymatic activity of SETDB1 and its 1:1 complex with ATF7IP. *Biochemistry* **55**, 1645–1651 (2016).
141. Doyen, C. M. *et al.* Mechanism of polymerase II transcription repression by the histone variant macroH2A. *Mol. Cell. Biol.* **26**, 1156–1164 (2006).
142. Lavigne, M. D. *et al.* Composite macroH2A/NRF-1 nucleosomes suppress noise and generate robustness in gene expression. *Cell Rep.* **11**, 1090–1101 (2015).
143. Swaminathan, J., Baxter, E. M. & Corces, V. G. The role of histone H2Av variant replacement and histone H4 acetylation in the establishment of *Drosophila* heterochromatin. *Genes Dev.* **19**, 65–76 (2005).
144. Rangasamy, D., Berven, L., Ridgway, P. & Tremethick, D. J. Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. *EMBO J.* **22**, 1599–1607 (2003).
145. Rangasamy, D., Greaves, I. & Tremethick, D. J. RNA interference demonstrates a novel role for H2A.Z in chromosome segregation. *Nat. Struct. Mol. Biol.* **11**, 650–655 (2004).
146. Greaves, I. K., Rangasamy, D., Ridgway, P. & Tremethick, D. J. H2A.Z contributes to the unique 3D structure of the centromere. *Proc. Natl Acad. Sci. USA* **104**, 525–530 (2007).
147. Boyarchuk, E., Filipescu, D., Vassias, I., Cantaloube, S. & Almouzni, G. The histone variant composition of centromeres is controlled by the pericentric heterochromatin state during the cell cycle. *J. Cell Sci.* **127**, 3347–3359 (2014).
148. Lindsey, G. G., Orgeig, S., Thompson, P., Davies, N. & Maeder, D. L. Extended C-terminal tail of wheat histone H2A interacts with DNA of the "linker" region. *J. Mol. Biol.* **218**, 805–813 (1991).
149. Yuan, W. *et al.* Dense chromatin activates Polycomb repressive complex 2 to regulate H3 lysine 27 methylation. *Science* **337**, 971–975 (2012).
150. Yi, J., Stypula-Cyrus, Y., Blaha, C. S., Roy, H. K. & Backman, V. Fractal characterization of chromatin decompaction in live cells. *Biophys. J.* **109**, 2218–2226 (2015).

151. Hsieh, F. K. *et al.* Histone chaperone FACT action during transcription through chromatin by RNA polymerase II. *Proc. Natl Acad. Sci. USA* **110**, 7654–7659 (2013).
152. Xin, H. *et al.* yFACT induces global accessibility of nucleosomal DNA without H2A–H2B displacement. *Mol. Cell* **35**, 365–376 (2009).
153. Obri, A. *et al.* ANP32E is a histone chaperone that removes H2A.Z from chromatin. *Nature* **505**, 648–653 (2014).
154. Hong, J. *et al.* The catalytic subunit of the SWR1 remodeler is a histone chaperone for the H2A.Z–H2B dimer. *Mol. Cell* **53**, 498–505 (2014).
155. Brunelle, M. *et al.* The histone variant H2A.Z is an important regulator of enhancer activity. *Nucleic Acids Res.* **43**, 9742–9756 (2015).
156. Dalvai, M., Fleury, L., Bellucci, L., Kocanova, S. & Bystricky, K. TIP48/reptin and H2A.Z requirement for initiating chromatin remodeling in estrogen-activated transcription. *PLoS Genet.* **9**, e1003387 (2013).
157. Chuang, L. S. *et al.* Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for $p21^{WAF1}$. *Science* **277**, 1996–2000 (1997).
158. Sarraf, S. A. & Stancheva, I. Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. *Mol. Cell* **15**, 595–605 (2004).
159. Loyola, A. *et al.* The HP1α-CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. *EMBO Rep.* **10**, 769–775 (2009).
160. Souza, P. P. *et al.* The histone methyltransferase SUV420H2 and heterochromatin proteins HP1 interact but show different dynamic behaviours. *BMC Cell Biol.* **10**, 41 (2009).
161. Krouwels, I. M. *et al.* A glue for heterochromatin maintenance: stable SUV39H1 binding to heterochromatin is reinforced by the SET domain. *J. Cell Biol.* **170**, 537–549 (2005).
162. Fischer, A., Hofmann, I., Naumann, K. & Reuter, G. Heterochromatin proteins and the control of heterochromatic gene silencing in *Arabidopsis*. *J. Plant Physiol.* **163**, 358–368 (2006).
163. Raynaud, C. *et al.* Two cell-cycle regulated SET-domain proteins interact with proliferating cell nuclear antigen (PCNA) in *Arabidopsis*. *Plant J.* **47**, 395–407 (2006).
164. Jacob, Y. *et al.* Selective methylation of histone H3 variant H3.1 regulates heterochromatin replication. *Science* **343**, 1249–1253 (2014).

Acknowledgements
The authors thank K. Ahmad, S. Ramachandran, S. Kasinathan and anonymous reviewers for their helpful comments on the manuscript.

Competing interests statement
The authors declare no competing interests.

SUPPLEMENTARY INFORMATION
See online article: S1 (table) | S2 (box)
ALL LINKS ARE ACTIVE IN THE ONLINE PDF