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Histone chaperone networks shaping chromatin function

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Abstract

The association of histones with specific chaperone complexes is important for their folding, oligomerization, post-translational modification, nuclear import, stability, assembly and genomic localization. In this way, the chaperoning of soluble histones is a key determinant of histone availability and fate, which affects all chromosomal processes, including gene expression, chromosome segregation and genome replication and repair. Here, we review the distinct structural and functional properties of the expanding network of histone chaperones. We emphasize how chaperones cooperate in the histone chaperone network and via co-chaperone complexes to match histone supply with demand, thereby promoting proper nucleosome assembly and maintaining epigenetic information by recycling modified histones evicted from chromatin.

ToC blurb

Histone chaperones safeguard the chromatin template and shield histones from promiscuous interactions to ensure their proper storage, transport, post-translational modification, nucleosome assembly and turnover.

Nucleosomes (BOX 1), which restrict DNA accessibility, must be highly dynamic in terms of their positioning and state of assembly to allow access to the base read-out of DNA. The modular nature of nucleosomes provides functional complexity through the incorporation of

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Competing interests statement

The authors declare competing financial interests: C.M.H., H.H., D.J.P. and A.G. are named inventors on a patent application covering the discoveries in Saredi et al. H4K20me0 marks post-replicative chromatin and recruits the TONSL–MMS22L DNA repair complex. *Nature* **534**, 714–718 (2016).

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histone variants and combinatorial post-translational modifications (PTMs), which in turn regulate gene expression and nuclear architecture. Beyond their nucleosomal context, histone proteins require buffering to prevent their aggregation and spurious inter actions with DNA. To meet this requirement, histones are escorted by histone chaperones[1]. These molecular chaperones guide multiple aspects of histone metabolism, including histone storage, transport, PTM, nucleosome assembly and histone turnover. Although histone chaperones share the common features of binding and shielding histones from promiscuous interactions, they are a diverse group of proteins with low or no sequence similarity and with distinct structural and functional properties.

Box 1

An overview of nucleosome architecture and assembly intermediates

Eukaryotic DNA is packaged with proteins forming a beads-on-a-string array called chromatin, the primary unit of which is the nucleosome. The nucleosome architecture consists of an octameric configuration of histone fold proteins that wrap ~146 bp of DNA. Each histone protein has a histone fold domain consisting of three helices (α1–α3) linked together by short loops (L1 and L2) that allows for heterodimerization (H2A with H2B and H3 with H4) (see the figure)[65]. Histone fold dimers can further oligomerize through four-helix bundles[65,66], with a four-helix bundle between α2–α3 helices of each copy of H3 forming the so-called H3–H4 tetramerization interface. The deposition of tetrameric H3–H4, forming the 'tetrasome', is thought to initiate nucleosome assembly as the H3–H4 tetramer occupies the central portion of DNA (the dyad position) in the nucleosome[65]. Nucleosome assembly is completed by the addition of two H2A–H2B dimers that wrap the remaining DNA at the entry and exit points of the nucleosome to form 1.7 left-handed super-helical turns of DNA[65]. Each H2A–H2B dimer associates with the H3–H4 tetramer via a four-helix bundle between H2B α2–α3 and H4 α2–α3 helices[65,66]. Structures of the H3–H4 tetramer and tetrasome are inferred from structures of the nucleosome[65] and histone octamer[66]. Beyond their histone fold domains, canonical histones include the following features: H2A has a carboxy-terminal extension that includes two short helices (the H2A docking domain) and a short amino-terminal helix (H2A αN); H2B contains a C-terminal α-helix (H2B αC); H3 contains an N-terminal helix (H3 αN); and H2A, H2B, H3 and H4 all contain N-terminal tails. N-terminal histone tails are subject to a plethora of regulatory post-translational modifications, which influence nucleosome dynamics. Other important regulatory elements include the following features: the H2A docking domain, which locks the H3 αN helix in position between the entry/exit and dyad DNA turns[65], which is otherwise structurally heterogeneous in the H3–H4 tetramer[213]; and the H2A–H2B acidic patch, which can bind the basic patch of the H4 N-terminal tail of an adjacent nucleosome[86].

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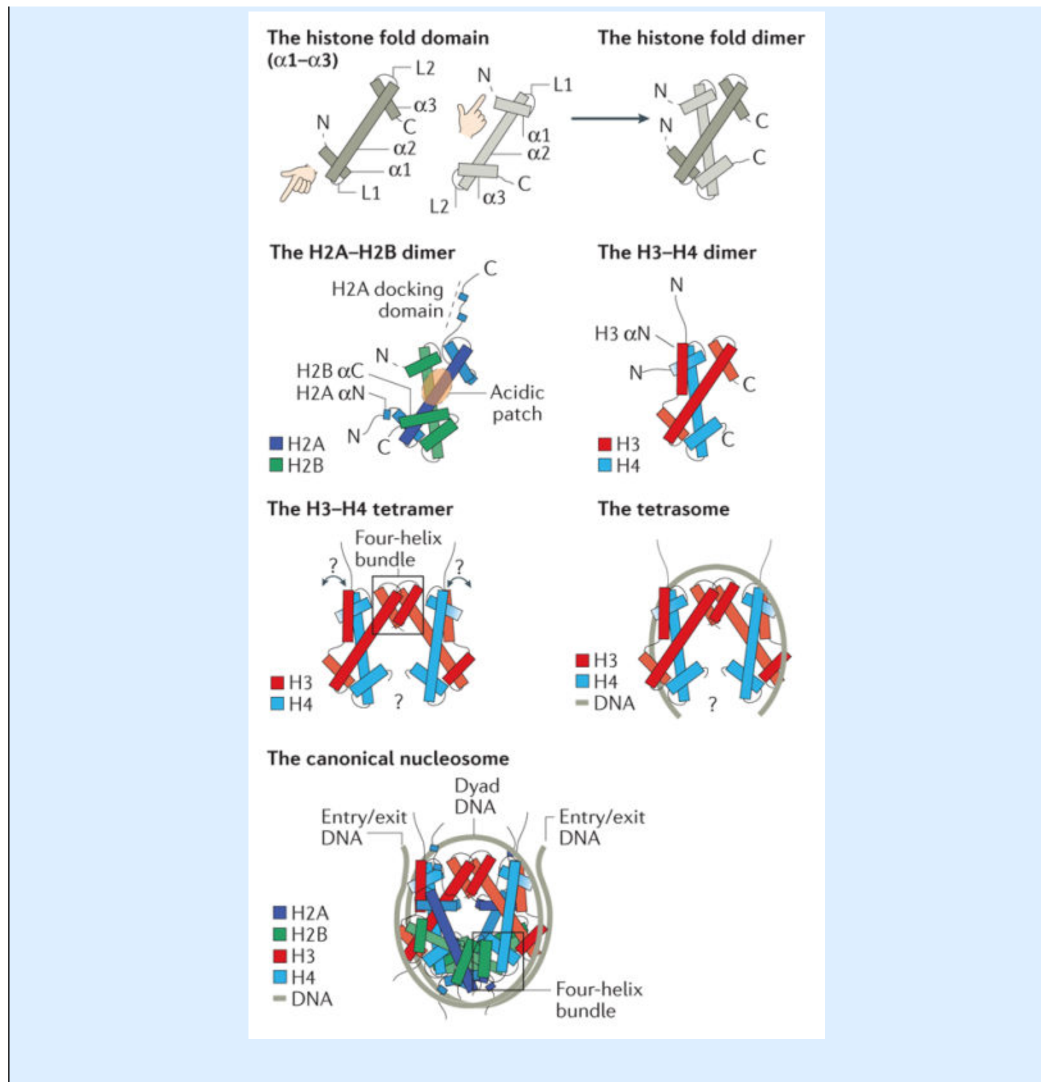

DNA replication, transcription and repair are all processes that involve chromatin disruption and restoration, requiring dynamic changes in chromatin assembly states to be coordinated with the DNA processing machinery[2–4] (FIG. 1). The challenge of the histone chaperone network is to span these diverse cellular processes[5,6], while distinguishing between canonical histones and replacement variants[7,8], to meet the demand for *de novo* histone deposition and chromatin refurbishment throughout the cell cycle and in specialized chromosome domains (FIG. 1). Failure to regulate histone supply can jeopardize functional domains such as telomeres[9] and centromeres[10–12], alter the barrier for cellular reprogramming[13,14] and challenge DNA replication[15] and genome integrity[16,17]. As such, histone chaperones function to safeguard the chromatin template and to ensure that epigenetic information is maintained.

In this Review, we first discuss the mechanistic aspects of histone chaperone function with reference to insights gained from structural studies, which have revealed the multiple properties that histone chaperones have acquired to carry out their functions. We then address how the histone chaperone network is built through histone-dependent co-chaperone

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complexes and histone-independent chaperone–chaperone interactions. Building on this molecular insight, we discuss how replication-coupled and replication-independent nucleosome assembly pathways combine to maintain chromatin integrity, which has broad implications for genome stability, epigenetic plasticity and disease. For in-depth discussions of variant-specific histone chaperones[8,18] and of transcription- and repair-coupled histone dynamics[2,4], we refer the reader to recent comprehensive reviews.

Mechanistic insights into chaperoning histones

Our understanding of histone chaperone biology has greatly benefited from structural analyses of histone chaperone complexes (FIG. 2). However, no single feature seems to demarcate a protein as a histone chaperone because both intrinsically disordered regions and structural folds are used to chaperone histones (FIG. 2; TABLE 1). Some structural folds are recognized specifically for their histone chaperone function, including the nucleosome assembly protein 1-like (NAP1-like) and nucleoplasmin (NPM) folds. Other protein folds are not restricted to chaperoning histones, including the immunoglobulin-like (Ig-like) domain, the tetratricopeptide repeat (TPR) domain, the WD40 repeat domain and the pleckstrin homology domain (FIG. 3). However, despite low levels of structural conservation among histone chaperones, some principles of histone chaperone function are beginning to emerge.

Shielding functional histone interfaces

One means of regulating nucleosome assembly is to shield the histone surfaces that associate with DNA and other histones in the nucleosome (BOX 1). Thermodynamic studies have indicated that only proper nucleosomal contacts between histones and DNA are able to compete with the interactions between histones and their chaperones[19,20]. Thus, in this way, histone chaperones can promote proper nucleosome formation by eliminating non-nucleosomal histone–DNA interactions[19]. Structural insights into the mode of interaction between histone chaperones and histones have revealed surprising aspects of histone chaperone biology.

The first histone chaperone complex to be crystallized was Asf1, which has an Ig-like fold[21], in complex with H3–H4 (REFS 22,23). This structure showed that Asf1 binds H3–H4 dimers through association with the H3 $\alpha2$–$\alpha3$ helices (FIG. 2Aa), which constitute the H3–H4 tetramerization interface (BOX 1). Surprisingly, the structurally related YEATS domain[24] (FIG. 3Ab) has been characterized as a reader module for the H3 tail[25,26], whereas the unrelated TPR domain of sNASP (Hif1 in yeast)[27] (FIG. 3B) also binds the H3 $\alpha3$ helix[28]. HJURP[29], Scm3 (REF. 30) and DAXX[31,32] bind dimers of H3–H4 in a variant-specific manner (reviewed in REF. 18) but their histone-binding domains (HBDs) are structurally unrelated to those of Asf1 (FIG. 2A) and sNASP. Both HJURP and the yeast homologue Scm3 form coiled-coil interactions with the $\alpha2$ helix of H3-like centromere protein A (CENP-A) (Cse4 in yeast) in a strikingly similar manner to the association of DAXX with the $\alpha2$ helix of histone H4 (REF. 31) (FIG. 2A). Thus, there seems to be some intrinsic requirement to chaperone newly synthesized H3–H4 and variants thereof as dimers, and histone chaperones achieve this goal in many ways. However, although they chaperone

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the H3–H4 tetramerization interface, these histone chaperones do not efficiently shield the DNA-binding surface of H3–H4 (FIG. 2A). Therefore, dimer-specific chaperones probably either collaborate with additional chaperones to shield the DNA-binding surface of H3–H4 (in the case of Asf1) or participate directly in H3–H4 dimer deposition (in the case of HJURP, Scm3 and DAXX).

More recently, structures of the HBDs of MCM2 and SPT2 in complex with H3–H4 tetramers have been solved (FIG. 2Ba,b). These structures show that both chaperones simultaneously shield both the DNA-binding and H2A–H2B-binding interfaces of H3–H4 (REFS 33–35). In the case of MCM2, ASF1 can split the H3–H4 tetramer to form a complex in which MCM2 and ASF1 co-chaperone a H3–H4 dimer (REF. 34) (FIG. 2 Da). In the case of SPT2, the binding to a H3–H4 tetramer seems to be more direct through recognition of the H3–H4 tetramerization interface (FIG. 2Bb). However, the majority of contacts between SPT2 and the H3–H4 tetramer are asymmetrically loaded on one dimer of H3–H4, with a relatively small helical portion of SPT2 reaching across the tetramerization interface to capture the second dimer[33] (FIG. 2Bb). Therefore, it remains possible that SPT2 also binds a H3–H4 dimer in a co- chaperone complex, analogous to that formed by MCM2 and ASF1 (REF. 34). In the absence of ASF1, H3–H4 is tetrameric and each dimer is bound by a MCM2 HBD. An intriguing possibility is therefore that one MCM2 may disengage the H3–H4 tetramer, revealing half of the DNA-binding surface that is required for deposition on DNA. The H3–H4 tetramer would thus remain tethered to MCM2 during the initial stages of DNA deposition; SPT2 might also deposit tetrameric H3–H4 via a similar mechanism.

The facilitates chromatin transcription (FACT) complex[36] (TABLE 1) binds the lateral surface of H3–H4 tetramers through the middle domain of SPT16 (SPT16-M), which forms a tandem arrangement of pleckstrin homology domains[37] (FIG. 2Bc). Interestingly, this binding mode enables SPT16-M to bind a partially disassembled nucleosome in which one H2A–H2B dimer is lost[37] and, potentially, a H3–H4 tetramer in a co-chaperone complex with MCM2 (REFS 37,38). Whether the tandem pleckstrin homology domains of Rtt106 (Rtt106-M)[39] (FIG. 3C), Pob3 (Pob3-M)[40] and the Spt16–Pob3 dimer interface (Spt16-D–Pob3-N)[41] recognize H3–H4 in a similar manner to SPT16-M is currently unclear. Rtt106-M exhibits specificity for H3–H4, and this domain recognizes histone H3 acetylated at Lys56 (H3K56ac)[42,43]. Dimerization is necessary for the function of Rtt106 in yeast[44], but this process is mediated by a novel protein fold rather than its pleckstrin homology domain[43]. Histone H3–H4-binding activity is also found in the peptidase-like fold of SPT16 (SPT16-N)[45] and SPT16 can also bind H2A–H2B[36,41,46]. Structural data on the mode of interaction between SPT16 and H2A–H2B seem to be contradictory[46], with a conserved peptide motif of SPT16 (REF. 46) (FIG. 2Cb) and SPT16-M[41] (FIG. 2Cc) both binding the same region of H2A–H2B. It is not yet clear whether these simultaneously incompatible binding modes reflect different functional states of the FACT complex. An important topic for future investigations will be to understand how the multiple histone-binding properties of the FACT complex might support the invasion and unravelling of nucleosomes and couple histone eviction with histone recycling (reviewed in REF. 47).

The NAP1-like fold is a constitutive homodimer with a headphone-like organization[48–50] (FIG. 3D). Each NAP1-like fold dimer binds a single histone dimer — as has been shown

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for binding of Vps75 or Nap1 to H3–H4 (REF. 51) and for binding of Nap1 to H2A–H2B[52] — in a manner that does not obscure the H3–H4 tetra-merization interface[51]. As such, Vps75 and Nap1 can bind H3–H4 tetramers[53] or H3–H4 dimers in complex with Asf1 (REF. 51) (FIG. 2Db). The reported cellular functions of Vps75 and Nap1 are with H3–H4 and H2A–H2B, respectively, although both chaperones are capable of binding both H2A–H2B and H3–H4 *in vitro*[54,55]. Vps75 and, to a lesser extent, Nap1 are capable of forming tetrameric ring-like assemblies[51,56] and they further oligomerize upon histone binding[51,52,57]. Tetramerization of Vps75 (FIG. 3Db) imparts a "self-chaperoning" function[51,56] as it shields key binding surfaces for H3–H4 (REF. 51) (FIG. 2Db) and the acetyltransferase Rtt109 (REFS 58–60) (FIG. 3Dc,d). Histone binding reconfigures the Vps75 tetramer[51] potentially providing a cooperative binding mechanism to accommodate a H3–H4 tetramer. Furthermore, Vps75 partially shields the DNA-binding surface of H3–H4, leaving the dyad DNA-binding surface exposed[51], which may explain its activity in promoting tetrasome assembly[53]. Similarly, Nap1 shields the DNA-binding surfaces of H2A–H2B[52] (FIG. 2Ca), possibly ensuring that proper contacts are made between H2A–H2B and the tetrasome before releasing the DNA-binding surface of H2A–H2B. NPM-fold histone chaperones also oligomerize, forming pentameric rings[61,62] (FIG. 3E) that are capable of binding multiple copies of various histone subtypes (TABLE 1) and promote tetrasome assembly[63,64].

Sequestering histones in non-nucleosomal conformations

In addition to shielding the functional interfaces of histones, histone chaperones can also trap histones in conformations that are not observed in the context of nucleosomes[65]. In doing so, these chaperones may promote the correct assembly of the histones onto nucleosomal DNA by directing the path of nucleosomal DNA towards the correct trajectory around the histones. One demonstration of this concept is the association of DAXX with H3.3–H4 (REFS 31,32). In this structure, the αN helix of H3.3 is rotated 180° compared with its location in the histone octamer[66], which probably results in a significant reorientation of the H3.3 tail[31,32] (FIG. 2Ad). As the H3 αN helix sits in a pivotal position in the nucleosome, making contacts with both dyad and entry/exit DNA (BOX 1), controlling the trajectory of this helix may be an important feature of nucleosome assembly and disassembly. The dynamics of the H2A docking domain, which supports the H3 αN helix in the nucleosome[65], may also be under the control of histone chaperones. When bound by Swr1, ANP32E and YL1, an extra helical turn is observed in the carboxy-terminal helix of H2A.Z, which could potentially affect the trajectory of the H2A.Z docking domain[67–71] (FIG. 2Cd). The interplay between the H3 αN helix and the H2A docking domain is also thought to be a key feature of FACT complex-mediated eviction of H2A–H2B from nucleosomes[37].

In addition to the already mentioned interaction of ASF1 with the tetramerization interface of H3–H4 (REFS 22,23), ASF1 makes additional contacts with the C terminus of histone H4 in what has been termed a "strand capture" mechanism[22] (FIG. 2Aa). In the nucleosome, the C-terminal β-strand of H4 associates in a parallel manner with a short β-strand of H2A[65]. When in complex with ASF1, the same region of H4 turns 90° in a hinge-like manner to associate in an anti-parallel fashion with a short β-strand at the C terminus of the ASF1

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globular domain[22,23]. This ‘strand capture’ feature of ASF1 may facilitate nucleosome disassembly in the context of larger complexes, as ASF1 alone cannot disengage H3–H4 from DNA[72]. Interestingly, DAXX anchors the H4 C terminus in another conformation distinct to that observed in complex with ASF1 (REF. 31). It is not known how sequestration of the H4 C terminus influences nucleosome assembly or disassembly processes, but flexibility in the H4 C terminus must be an important feature of histone dynamics as the H4 G94P mutation, which restricts this mobility, compromises yeast viability and chromatin structure[73].

In most of the crystal structures of histone–chaperone interactions, the histone fold is remarkably stable and similar to that observed in the nucleosome (BOX 1; FIG. 2). However, the interaction of the WD40 repeats of RBAP46 and RBAP48 with the α1 helix of H4 (REFS 74,75) (FIG. 3F) would require a significant conformational change within the histone fold domain of H3–H4. Evidence of such a conformational rearrangement has been reported and is proposed to promote the handover of H3–H4 from ASF1 to RBAP48 (REF. 76) in the chromatin assembly factor 1 (CAF1) complex. Interestingly, the WD40 repeats of RBAP46 and RBAP48 can also bind the H3 tail[77] through a different interface (FIG. 3F). However, these binding abilities are not necessarily always used. For example, the interaction of RBAP46 with H4 α1 helix directs the histone acetyltransferase activity of the HAT1 complex towards non-nucleosomal H3–H4 (REF. 78). However, this interaction is blocked in the poly-comb repressive complex 2 (PRC2) methyltransferase, in which Su(z)12 occupies the H4 α1 helix-binding surface of RBAP46 and RBAP48 and in turn directs PRC2 to a nucleosomal substrate[77]. In addition, RBAP46 may be re-directed to bind CENP-A–H4 dimers through a co-chaperone interaction with HJURP, as has been observed for orthologous yeast proteins[79,80].

Structural flexibility is not only restricted to the histones within histone–chaperone complexes. Indeed, the structures of MCM2 (REFS 34,35,81), SPT2 (REF. 33), Chz1 (REF. 82) and YL1 (REFS 70,71) (FIG. 2) imply that the chaper-one undergoes a marked transformation upon histone binding. Furthermore, the HBD of DAXX is predominantly unfolded in the absence of its histone cargo[83]; this is also expected to be the case for the minimal HBDs of SPT16, ANP32E and Swr1 (REFS 46,67–69) (FIG. 2Cb).

Co-chaperone relationships

Histone chaperones generally shield functional surfaces of histones without fully encasing the histone fold. The combination of multiple histone chaperones therefore has the potential to form a more complete shield around the histone fold as part of a co-chaperone complex. This concept is illustrated by the MCM2–H3–H4–ASF1 complex, in which MCM2 shields the DNA- and H2A–H2B-binding surfaces of H3–H4, whereas ASF1 occludes H3–H4 tetramerization[34,81] (FIG. 2Da). Despite shielding many of the interfaces required for nucleosome assembly, the MCM2–H3–H4–ASF1 complex further promotes nucleosome assembly compared with ASF1–H3–H4 and MCM2–H3–H4 alone[34]. An explanation for this observation is not yet available, but it may be that there are synergistic effects of co-chaperoning during H3–H4 deposition. Interestingly, histones bound to MCM2 and ASF1 can also engage TONSL[84], which has a dual function as a histone reader and a histone

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chaperone[84,85]. The ankyrin repeat domain (ARD) of TONSL recognizes the basic patch of the H4 tail (K16–K20) when K20 is unmethylated (H4K20me0)[84] (FIG. 2Dd). The TONSL ARD holds the H4 tail perpendicular to the DNA-binding surface of the H3–H4 tetramer and can also bind nucleosomal H4 (REF. 84). As such, TONSL ARD recognition of the H4 tail may help to guide nucleosomal DNA across the H3–H4 tetramer during deposition and potentially counteract binding of the H4 tail to the acidic patch of H2A–H2B on neighbouring nucleosomes[86] to prevent premature chromatin compaction[84].

Highlighting its central role in chaperoning H3–H4, ASF1 forms a large number of co-chaperone complexes with H3.1, H3.2, H3.3 and one or more of the chaperones Vps75, sNASP, RBAP46, RBAP48, MCM2 and the reader and chaperone TONSL[51,76,84,85,87–91] (FIG. 4). Curiously, sNASP, RBAP46 and ASF1 seem to be capable of engaging histones H3–H4 in a single co-chaperone complex[87,89–91]. However, ASF1 and sNASP may have overlapping binding sites at the H3–H4 tetramerization interface[22,23,28]. Therefore, for sNASP and ASF1 to engage histones at the same time (the interaction between ASF1 and sNASP is histone dependent[87]), additional H3–H4-binding sites may be present in sNASP to allow the partial handover of the H3–H4 tetramerization interface to ASF1. As such, the interplay among sNASP, ASF1 and H3–H4 would benefit from further biochemical and structural characterization. In addition, sNASP forms a complex with HSP90 (REF. 90), a molecular chaperone that assists in the folding of many proteins presented to HSP90 by TPR domain-containing co-chaperones[92]. Thus, sNASP may collaborate with HSP90 to assemble the H3–H4 dimer before participating in other co-chaperone complexes.

Other chaperone–chaperone interactions are direct and independent of histones and as such fall outside the definition of a co-chaperone relationship; however, these interactions are nevertheless important. Histone-independent interactions between chaperones may function to regulate the handover of histones from one chaperone to another. These interactions could also help to recruit histone chaperones with different abilities to sites of nucleosome assembly or disassembly and chromatin remodelling. The interactions of ASF1 with RBAP48 (REF. 87), and probably UBN1 (REF. 93) (FIG. 2Dc), are histone dependent. By contrast, other subunits of the CAF1 complex and the histone regulation complex (HIRA/HIR; see TABLE 1) bind to ASF1 directly via a small epitope termed the B-domain, which interacts with the β-sheet sandwich on the opposite side of H3–H4 (REFS 21,94,95) (FIG. 3Aa; TABLE 1). Other factors, including codanin 1 (REF. 96) and Rad53 (REF. 97), also bind ASF1 via B-domains, which suggests that these factors might compete with CAF1 and HIRA complexes in the histone delivery pathway. However, it remains a key open question how the interplay between histone-dependent and chaperone–chaperone interactions facilitates the presumed handover of histones from ASF1 to the CAF1 or HIRA complex.

Chaperone networks in histone supply

During chromatin assembly and histone turnover, new histones need to be synthesized, processed and delivered to specific sites in the genome. This process is orchestrated by histone chaperones, which handle histones from the time of synthesis in the cytoplasm to their delivery to chromatin.

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Matching histone supply with demand

Several layers of regulation govern the histone supply chain to meet the demand for nucleosome assembly at any given time while keeping the pool of soluble histones at a minimum. This regulation is most apparent during chromatin replication, when the histone content of the cell is doubled. The high level of new histone synthesis required to support chromatin replication is matched by high expression rates of the canonical histones genes, which are present in multiple copies in metazoans (reviewed in REF. 7). In budding yeast, an attractive negative feedback model has been proposed whereby histone chaperones monitor the level of soluble histones to dynamically control histone gene expression (reviewed in REF. 98). According to this model, the yeast histone regulation complex (Hir), Asf1 and Rtt106 assemble at a *cis*-regulatory element and, presumably in response to high levels of soluble histones, form a repressive chromatin structure that silences histone genes beyond S phase[98]. If the capacity of the histone chaperones is saturated, the Rad53 checkpoint kinase targets excess histones for ubiquitylation and proteasomal degradation[17,99] through a mechanism that may involve its ability to bind histone-free Asf1 (REF. 97). Feedback regulation remains to be explored in other organisms, but there are indications that the roles of HIRA and ASF1 in histone gene regulation may be conserved[100,101].

S phase active kinases also play a role in the spatio-temporal regulation of histone provision and nucleosome assembly. ASF1 phosphorylation by TLK1 and TLK2 promotes histone binding and interaction with downstream chaperones[102,103] to ensure efficient histone supply during DNA replication. Phosphorylation of CAF1 p150 by the CDC7–DBF4 kinase disrupts dimerization, which in turn facilitates binding to PCNA[104] and chromatin assembly. By contrast, CDK1- and CDK2-mediated phosphorylation of HJURP inhibits its recruitment to centromeres and prevents premature CENP-A incorporation[105]. These examples are probably just the tip of the iceberg, and histone chaperone PTMs are likely to emerge as an important means to regulate histone handover, deposition and eviction.

Chaperone functions in histone supply

Isolation and proteomic analyses of histone chaperone complexes have revealed that histones engage with multiple chaperones on their way to chromatin[85,87,89,90,106–108] (FIG. 4). The principles of how chaperones function together in a network to optimize histone delivery and storage are beginning to emerge[5], and a key feature seems to be that multiple chaperones can engage with a single histone dimer, as highlighted in FIG. 4. Soluble H3 and unacetylated H4 have been found together with heat shock proteins HSC70 and HSP90 (REFS 90,109), which suggests that these are early acting factors that assist histone folding before RBAP46–HAT1-mediated diacetylation of H4K5 and H4K12 (H4K5acK12ac)[110] (FIG. 4). RBAP46–HAT1 forms a conserved histone-dependent complex with sNASP[90,110], which functions in a storage capacity for soluble H3–H4 and counteracts histone degradation through HSC70- and HSP90-mediated autophagy[111]. The RBAP46–HAT1–H3–H4–sNASP co-chaperone complex is present in both the cytoplasm and the nucleus[85,87,90,112], which suggests that the ability to shuttle histones into storage and maintain the H4K5acK12ac mark is important in both cellular compartments.

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ASF1 forms a complex with H3–H4 and importin 4 (REFS 87,90,109), which suggests that ASF1 binds histones in the cytoplasm and transports them to the nucleus for handover of H3.1/H3.2–H4 to the CAF1 complex and H3.3–H4 to the HIRA complex for deposition[88,95,107,113–117]. The major function of ASF1 is presumably to prevent premature tetramerization of H3–H4 (REFS 22,23) while allowing H3–H4 dimers[107,118] in transit to engage with additional chaperones[87,90] (FIG. 4). ASF1 can bind H3–H4 within the RBAP46–HAT1–H3–H4–sNASP co-chaperone complex[91], which may provide ASF1 with access to both the cytoplasmic and nuclear stores of H3–H4. Most vertebrates have two ASF1 paralogues, ASF1A and ASF1B, which differ primarily in their amino- and carboxy-terminal regions outside the core histone-binding domain[95,119]. ASF1A is ubiquitously expressed in all tissues and throughout the cell cycle[119], whereas ASF1B is expressed in S phase in an E2F-dependent manner[120]. ASF1A preferentially interacts with the HIRA complex, whereas ASF1B binds preferentially to the CAF1 complex[95,121]. However, ASF1A and ASF1B can function redundantly in chromatin replication because the depletion of both paralogues (in contrast to single depletion) arrests DNA replication in human cell lines[88,121], similar to depletion of CAF1 (REFS 122–124). This effect phenocopies the inhibition of histone biosynthesis[15], which argues that ASF1 (ASF1A and ASF1B) and CAF1 have non-redundant functions in replication-coupled histone provision and deposition.

New H3–H4 dimers are also found in a large co-chaperone complex that includes ASF1, MCM2 and TONSL–MMS22L[84,87,89]. Binding to MCM2 can stabilize H3–H4, but it is not required for the transfer of histones to CAF1 for replication-coupled histone deposition[34]. MCM2 may therefore provide an additional storage site for new H3–H4 dimers both in solution (FIG. 4) and as part of inactive chromatin-bound MCM2–7 hexamers[34,87]. TONSL, which, together with MMS22L, promotes homologous recombination[125], specifically binds new histones by recognizing the H4 tail unmodified at K20 (H4K20me0)[84]. However, whether TONSL function is required for histone delivery or deposition remains unknown. Nevertheless, given its role as a histone reader, TONSL may utilize the new H3–H4 delivery pathway to be loaded onto replicated DNA where it can promote error-free DNA repair in case a lesion is encountered by the replication fork[84].

Similar to H3–H4, several histone chaperones are implicated in the nuclear import and delivery of H2A–H2B dimers (FIG. 4), but the chaperone network for these histones is less well understood. Notable histone chaperones include Nap1 (REF. 55), Chz1 (REF. 106), ANP32E[68,69], YL1 (REFS 70,71) and FACT[36]. Nap1 is a multifunctional chaperone that shields the DNA-binding interfaces of histones[52] and prevents unscheduled accumulation of H2A–H2B on DNA[19]. Nap1 contributes to the nuclear import of both H2A–H2B and H2A.Z–H2B, together with Kap114 (importin 9)[126], and Nap1 probably delivers H2A–H2B dimers to sites of both ongoing transcription and DNA replication. Chz1 is localized predominantly in the nucleus and specifically chaperones H2A.Z–H2B dimers in yeast[106]. Interestingly, Chz1 has overlapping functions with Nap1 in handling the H2A.Z variant[106], highlighting a degree of redundancy in the H2A–H2B delivery network. Furthermore, if Chz1 and Nap1 are absent, FACT is able to substitute these two chaperones to compensate and deliver H2A.Z–H2B to the Swr1 remodelling complex (SWR-C) for deposition[106]. In human cells, H2A.Z–H2B is handled by the variant-specific chaperones ANP32E[68,69] and YL1 (REFS 70,71); of these two Swr1-related complexes in mammals, YL1 functions in

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both SRCAP and P400–TIP60 (REF. 127), whereas ANP32E seems to be specific for p400–TIP60 (REF. 69).

Histone modifications during supply

Newly synthesized histones can be modified by various PTMs, which subsequently can affect chaperone binding, histone deposition and final chromatin state. The H4K5acK12ac mark is both highly conserved[128] and abundant (being present on ~80% of total soluble H4 (REF. 129) and ~98% of ASF1-bound H4 (REF. 87) in HeLa cells). Paradoxically, the exact function of this mark is unclear, although it has been implicated in nuclear import, replication-coupled nucleosome assembly, chromatin maturation and replication fork repair (reviewed in REFS 5,128). In yeast, H3K56ac is a highly abundant mark on newly synthesized histones[130]. H3K56ac is imposed by Rtt109 on Asf1-bound histones (reviewed in REF. 54) and it promotes association with the downstream chaperones Rtt106 and CAF1 and thus facilitates nucleosome assembly[42,43,131]. This function does not seem to be conserved in mammalian cells, as H3K56ac is only present on ~1% of newly synthesized H3 before[87] and after deposition[132,133]. In yeast, H3 ubiquitylation by Rtt101–Mms1 can also promote histone handover from Asf1 to downstream chaperones[134] by reducing the affinity of H3 for Asf1. Similarly, the ubiquitylation of CENP-A by CUL4–RBX1–COPS8 promotes its interaction with HJURP and facilitates the deposition of CENP-A at centromeres[135].

A key consideration regarding the PTM of soluble histones is whether these modifications are universally imposed during delivery or are restricted to histones assembled into particular chromatin domains. In metazoans, H3 can be monomethylated at Lys9 (REF. 129) during translation[136] and replication[137], and only a fraction of newly synthesized histones carry this modification (5–30%)[87,129,132]. By functioning as a precursor for H3K9me3, monomethylation may contribute to heterochromatin establishment (reviewed in REF. 3); however, it is unknown whether monomethylation has a function in histone delivery or how it is targeted to heterochromatin sites.

Chaperones in histone deposition and recycling

The activity of histone chaperones is measured by their ability to mediate the deposition of histones onto DNA *in vitro*. However, *in vivo*, some chaperones are restricted to handling histones at earlier stages in the delivery pathway and only a few chaperones are currently recognized as actual deposition factors. It remains unclear what functional properties are required to assemble nucleosomes, but shared features of the chaperones involved in this process include specificity for histone variants (reviewed in REF. 18) and recruitment to chromatin through a mechanism that is linked to their mode of action.

De novo histone deposition

Nucleosome assembly factors are recruited to their sites of action through highly distinct mechanisms that can be coupled to a process (such as DNA replication, repair or transcription) or linked to chromatin features (such as histone marks, transcription factors or free DNA). For example, the CAF1 complex mediates replication-coupled nucleosome assembly of H3.1/H3.2–H4 by associating with the replication machinery through its largest

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subunit (p150), which binds the homotrimeric sliding-clamp PCNA[138] (FIG. 5a). In this way, newly synthesized DNA is almost immediately assembled into nucleosomes in a manner that makes the processes of DNA replication and nucleosome assembly inter-dependent[15,139]. The interaction with PCNA can also recruit CAF1 to assemble nucleosomes at sites of DNA repair[140,141] (FIG. 5b), although CAF1 has been observed to function independently of PCNA and DNA synthesis during double-stranded break repair[142,143]. In yeast, Rtt106 can also mediate replication-coupled H3–H4 deposition[144] (FIG. 5a), but the recruitment mechanism for Rtt106 is less well defined and may involve concomitant interactions with DNA[39], Cac1 (CAF1 p150)[144] and FACT[145].

Replication-independent incorporation of newly synthesized H3.3 may occur through a histone exchange reaction or via a gap-filling mechanism. Analysis of H3.3 occupancy on DNA by chromatin immunoprecipitation followed by sequencing (ChIP-seq) shows that this histone variant is enriched at *cis*-regulatory elements, gene bodies and telomeres, with turnover rates being highest at active promoters and enhancers (reviewed in REFS 2,146). The underlying mechanism of H3.3 turnover depends on the genomic location and is governed by the HIRA complex in transcribed regions and promoters and by DAXX–ATRX in heterochromatin at pericentromeres and telomeres[108,147–152] (FIG. 5c,d). The HIRA complex has multiple modes of recruitment through interactions with RNA polymerase II[150,151] and transcriptional regulators[150,152,153] (FIG. 5c). In addition, the HIRA complex performs a gap-filling function that may involve interactions with naked DNA[151]. Although further experiments are required to resolve the role of DNA binding in the functions of the HIRA complex, this mode of recruitment could explain HIRA-mediated deposition of H3.3–H4 at DNA repair sites[154] (FIG. 5b). The histone chaperone DAXX mediates H3.3–H4 deposition in heterochromatic regions via interactions with the SWI/SNF-like chromatin remodeller ATRX[108,147,149]. ATRX specifically recognizes H3K9me3 through its ADD (ATRX-DNMT3-DNMT3L) domain[155–157], providing a means to direct DAXX–ATRX-dependent H3.3–H4 deposition to heterochromatic loci (FIG. 5d). How histones H3.3–H4 are delivered to ATRX is currently unclear, but the presence of DAXX in the cytosolic fraction and its interaction with RBAP46 or RBAP48 (REF. 108) suggest that it could chaperone H3.3–H4 during nuclear import.

Directed deposition of CENP-A–H4 is required for centromere function and for faithful chromosome segregation (reviewed in REF. 10). In budding yeast, a single Cse4 (CENP-A)-containing nucleosome is found at a sequence-defined centromere. During DNA replication, the old Cse4-containing nucleosome is removed and a new one is installed by the Scm3 chaper-one[158–163]. In fission yeast and metazoans, the presence of CENP-A-containing chromatin, rather than the DNA sequence, defines the centromere and, in this case, the old CENP-A is recycled during DNA replication[164,165]. New CENP-A is not incorporated in a replication-dependent manner; rather, it is deposited by HJURP through an exchange reaction before the next round of DNA replication (to counteract the dilution with H3–H4 taking place during replication)[165–168]. Scm3 is recruited to DNA in a sequence-dependent manner and stays with the CENP-A-containing nucleosome throughout the cell cycle[158,163], whereas HJURP-dependent deposition of CENP-A is cell cycle regulated[105,166,167] and requires several constitutive centromere components as well the MIS18 complex (FIG. 5e) and RBAP46 or RBAP48 (reviewed in REF. 10). Given that nucleosomal CENP-A itself

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directs the recruitment of centromeric core proteins and MIS18 (REFS 169,170), this process represents a self-sustaining epigenetic mechanism for centromere maintenance.

Although the principles of histone variant specificity and recruitment of chaperones are emerging, understanding the actual histone deposition process remains a major challenge. However, H2A.Z–H2B deposition by the SWR-C remodelling complex is particularly well understood, as this process has been extensively characterized by biochemical investigations[67,171–174]. SWR-C catalyses a two-step, ATP-dependent histone exchange reaction triggered by the presence of H2A-containing nucleosomes and non-nucleosomal H2A.Z[174]. This process involves two distinct chaperone activities of SWR-C, provided by the catalytic subunit Swr1 (REFS 67,172) and the accessory subunit Swc2 (REF. 171) (YL1 (REFS 70,71)). Interestingly SWR-C function is directed to nucleosomes flanking the transcription start site due to the preference of SWR-C for nucleosomes with long linker DNA[175,176], which is exposed in the nucleosome-free region (FIG. 5c). The coupling of histone chaperone activity and chromatin remodelling activity is also observed for the DAXX–ATRX complex. However, the exchange of nucleosomal H3–H4 for soluble H3.3–H4 would require extensive remodelling of the nucleosome to access the central H3–H4 tetramer. It will therefore be important to investigate whether H3.3–H4 is deposited by DAXX–ATRX through an exchange reaction or a gap-filling mechanism.

Deposition of H3–H4 to form the tetrasome could foreseeably proceed in two ways: the deposition of a pre-assembled H3–H4 tetramer on DNA or by the sequential deposition of individual dimers on DNA. As H3–H4 is mainly dimeric at physiological-like salt concentrations[72], stabilization of the H3–H4 tetramer is a necessary requirement for H3–H4 to be deposited as a tetramer. Several chaperones have the ability to bind H3–H4 tetramers, including CAF1 (REFS 177,178), MCM2 (REFS 34,35), SPT2 (REF. 33), Rtt106 (REFS 43,44), Vps75 (REFS 51,53), Nap1 (REF. 53) and FACT[37]. By contrast, Scm3 mediates assembly of a Cse4–H4 tetrasome through a mechanism involving the consecutive deposition of Cse4–H4 dimers[179]. HJURP also handles CENP-A–H4 dimers, but through dimerization has the capacity to chaperone two CENP-A–H4 dimers, which may facilitate CENP-A–H4 tetramer formation during deposition[180].

Once histone chaperones are recruited to their site of action, their histone variant specificity directs the genomic localization of these histone subtypes[8,18]. Mutation and domain-swapping experiments have helped to identify the key features of histone variants that specify their assembly pathway and genomic localization[167,181,182]. Although some chaperones have a natural preference for one histone variant, an imbalance in the chaperone network may skew the system. For example, in the absence of DAXX, CAF1 is able to bind H3.3–H4 (REF. 108), and upon CENP-A overexpression, DAXX can bind CENP-A–H4 and mediates its incorporation at ectopic sites through an exchange reaction generating heterotypic H3.3- and CENP-A-containing nucleosomes[12].

Histone recycling

The recycling of modified histones that are evicted during replication, transcription and repair could be key to maintaining the epigenetic state of the locus, and histone chaperones work in an integrated manner with DNA processing machineries to ensure this process.

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Here, we focus on histone recycling during DNA replication and transcription, as these processes are the best characterized.

The kinetics of histone turnover highlight enhancers and promoters as major sites of histone replacement, whereas gene bodies have slower rates of histone turnover (reviewed in REF. 2). Asf1 can aid histone eviction at promoters and in coding regions[183–185]. In moderately transcribed genes, a single H2A–H2B dimer may be displaced per nucleosome, whereas highly transcribed genes are characterized by more pronounced nucleosome disruption (reviewed in REF. 2). Displacement of a H2A–H2B dimer can be facilitated by FACT[36], and the resultant hexasome can be maintained by FACT[36] or Nap1 (REFS 52,186). The loss of either chaperone leads to the depletion of histones in transcribed regions[187–189]. The re-establishment of chromatin structure after the passage of RNA polymerase II prevents the initiation of transcription from cryptic promoters[190,191], and a substantial number of histone chaperones, including SPT2, SPT6, Rtt106, FACT, Vps75, Asf1 and HIRA[2,185,192], are implicated in this process (reviewed in REF. 2) (FIG. 5c). SPT6 is a H3–H4 chaperone[193] that is coupled to the transcription machinery through interaction with the phosphorylated form of the RNA polymerase II C-terminal domain (reviewed in REF. 2). SPT2 chaperones H3–H4 tetramers[33,194] and has overlapping functions with SPT6 (REF. 195), which suggests that H3 and H4 can be recycled as tetramers during transcription. In addition, Nap1-like chaperones (such as Vps75) show genetic interactions with transcription elongation factors[192,196] and could also contribute to the recycling of H3–H4 tetramers[51,53,56]. The FACT complex also has the capacity to handle H3–H4 tetramers[37] and is an additional candidate for mediating H3–H4 recycling during transcription. Understanding the division of labour among these many chaperones, whether they act together or in distinct settings, is an important challenge for future research.

The relative balance between histone turnover (eviction and incorporation of newly synthesized histones) and histone recycling is also influenced by histone modifications. H3K56ac can promote histone turnover[197], whereas co-transcriptional methylation of H3K36 reduces histone exchange and favours the retention of old histones[198]. Furthermore, transcription-coupled mono-ubiquitylation of H2BK120 (K123 in yeast) cooperates with FACT in promoting transcription and ensuring the re- assembly of nucleosomes in the wake of the polymerase[199,200]. Interestingly, H2B monoubiquitylation is also present at replication origins in yeast, where it may aid progression of the replication fork and chromatin assembly[201].

During DNA replication, nucleosomes are disrupted ahead of the replicative helicase and H2A–H2B dimers and H3–H4 tetramers are recycled to the daughter strands (reviewed in REFS 3,202) (FIG. 6). The force of the progressing replicative helicase, composed of CDC45, MCM2–7 and GINS (the CMG complex), may be sufficient to trigger nucleosome disruption. Meanwhile, the FACT complex associates with the CMG helicase and is thus in a good position to aid nucleosome disruption[38,203–205] (FIG. 6). In yeast, approximately one FACT complex is present for every five nucleosomes, which could facilitate efficient nucleosome disassembly and recycling[206]. A quantitative analysis of new and old histones in nascent chromatin has shown that histone recycling is highly efficient, resulting in a 1:1 ratio of new and old H3–H4 and H2A–H2B histones on replicated DNA[132]. The histone variants

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H3.3 and H2A.X are recycled with similar efficiency as their canonical counterparts[132], whereas H2A.Z is lost at least partially upon replication fork passage[132,207]. The segregation of new and old histones on nascent DNA has been intensely studied[5,202], and current data support the segregation of new and old H3.1–H4 dimers into separate nucleosomes post-replication[208]. This finding suggests that H3.1–H4 tetramers are preserved during recycling. By contrast, the transfer of the two nucleosomal H2A–H2B dimers seems to be uncoupled, with nucleosomes in post-replicative chromatin containing a mixture of new and old H2A–H2B dimers associated with new or old H3.1–H4 tetramers[208].

Efficient re-incorporation of old histones behind the replication fork probably requires integration of histone recycling with DNA replication, as best illustrated by the chaperone activity of MCM2 (REFS 34,35,89), which is part of the CMG helicase. The flexible N-terminal tail of MCM2 contains a highly conserved HBD[34,35,38,209], which can chaperone[210] histones in the context of soluble and chromatin-bound MCM2, including inactive MCM2–7 double hexamers and active CMG helicases[34,38,87,89] (FIG. 6). MCM2 chaperones a H3–H4 tetramer *in vitro*[34,35] and *in vivo*[34] by mimicking nucleosomal DNA. This mode of interaction renders MCM2 able to chaperone all H3 variants, including CENP-A[34], which allows the CMG complex to handle old evicted H3–H4 tetramers genome-wide. The CMG complex could thus function as a platform for retaining evicted histones at the replication fork[34]; however, it remains less clear how these histones are transferred to newly synthesized DNA. The MCM2 tail may directly facilitate the re-deposition of histones; yet, this process might create a bias towards segregation onto the lagging DNA strand, as structural studies suggest that the N-terminal region of MCM2 is located close to polymerase-α[211]. Thus, other histone-binding platforms may exist within the DNA replication machinery or soluble histone chaperones may mediate histone transfer. ASF1 interacts with MCM2 within the CMG complex at both active and stalled replication forks in a histone-dependent manner[34,87,89], which may facilitate histone transfer in a reaction involving splitting of the MCM2-bound H3–H4 tetramer[34,89] (FIG. 6). However, given that old and new H3.1–H4 dimers generally do not mix during DNA replication[208], old H3.1–H4 dimers would have to be maintained as tetramers or channelled as dimers into a deposition pathway specific to old histones. Alternatively, the role of ASF1 could be to ensure that evicted histones are chaperoned when the replication fork stalls and recycling is interrupted[87,208]. The FACT complex is recruited to active replication forks[38,40,203–205,212] through an apparent multitude of interactions that include polymerase-α[212], replication protein A (RPA)[40], MCM4 (REF. 205) as well as histone-dependent inter actions with MCM2 (REF. 38) (FIG. 6). Alignment of MCM2–H3–H4 (REFS 34,35) and SPT16-M–H3–H4 (REF. 37) structures support that FACT and MCM2 may collaborate in a co-chaperone complex[37,38] during parental histone transfer. It is possible that one old H2A–H2B dimer is evicted and the resultant hexasome can be recycled. In this instance, if MCM2 and FACT collaborate during histone transfer, additional H2A–H2B binding surfaces such as those present in the FACT complex[36,41,47] would need to be used. Old histones maintain their PTMs during recycling[132] and these PTMs may, via recruitment of their cognate enzymes, function as a blueprint for the modification of neighbouring new histones (reviewed in REF. 3) to maintain epigenetic states. Thus, understanding histone recycling and whether the

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process can be challenged by developmental cues and/or cellular stresses therefore represents one key to understand epigenetic cellular memory.

Concluding remarks

The chromatin landscape must remain flexible to enable the regulation of gene expression and programmed changes in cell identity to occur while also protecting DNA from deleterious events. The functions of histone chaperones are crucial for allowing dynamic accessibility to genomic loci, which underscores the importance of unveiling the modes of action and biological functions of this large and diverse group of proteins. As discussed in this Review, key questions regarding nucleosome dynamics and histone chaperone function remain unresolved. For example, what is the biological significance of co-chaperone relationships? Is it important to shield the entire histone fold dimer? How do histone chaperones mediate nucleosome assembly and disassembly *in vivo*? How is chaperone function integrated with chromatin remodelling and the larger machineries that are involved in DNA transcription, replication and repair? Further structural analyses of histone chaperones, in the context of larger protein assemblies and of the deposition and disassembly machineries, by crystallography and cryoelectron microscopy should provide an exciting new entry point to answer these long-standing questions. Furthermore, genome editing holds great promise for translating basic molecular understanding into a broader biological context, which should reveal the importance of the individual chaperones in histone logistics.

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Glossary

Histone chaperones

Defined here as proteins that handle non-nucleosomal histones *in vivo* and mediate the assembly of nucleosomes from isolated histones and DNA *in vitro*.

Histone storage

The sequestration of histones in the soluble fraction of the cell that prevents their degradation.

Histone turnover

The eviction of nucleosomal histones, followed by deposition of new histones at the same genomic loci.

Histone chaperone network

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The integration of histone chaperone functions to support histone dynamics across various cellular processes.

Canonical histones

Core histone subtypes (H3.1, H3.2, H4, H2A and H2B) that are expressed in S phase of the cell cycle and mainly incorporated into nucleosomes in a DNA replication-dependent manner.

Replacement variants

Histone subtypes (such as H3.3, CENP-A, and H2A.Z) incorporated into nucleosomes via DNA replication-independent pathways and for which expression is not restricted to S phase.

***De novo* histone deposition**

Incorporation of newly synthesized histones into chromatin.

Co-chaperone

Here defined as a complex containing two or more histone chaperones brought together in a histone-dependent manner.

Epigenetic plasticity

Heritable information other than DNA sequence that maintains cellular traits while also being subject to change without said changes being permanent.

H3K56ac

A mark of newly synthesized H3–H4 in yeast, catalysed by Rtt109 in an Asf1-dependent manner, that promotes replication-dependent histone deposition.

Histone recycling

Re-deposition of histones evicted from chromatin by cellular processes that require access to the DNA template

Dyad DNA

The dyad position locates the pseudo axis of symmetry, which coincides with the central base pair (or pairs) of nucleosomal DNA and the H3–H4 tetramerization interface, around which the nucleosome can be rotated 180° and map back onto itself

Tetrasome

Thought to be the first assembly intermediate during nucleosome assembly, the tetrasome is the product of the deposition of a H3–H4 tetramer on DNA

RBAP46 and RBAP48

Histone chaperone homologues that are almost identical and seem to be interchangeable in most of their chromatin-modifying complexes, apart from HAT1 (RBAP46) and CAF1 (RBAP48).

Histone reader

A protein that binds to histones in a post-translational modification-dependent manner.

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H4K20me0

Histone H4 unmethylated at lysine 20 (H4K20me0); a signature of newly synthesized histones that marks post-replicative chromatin until G2/M phase of the cell cycle, when H4K20 methylation is established on those new histones.

Soluble histones

Non-nucleosomal histones.

H4K5acK12ac

Highly conserved diacetylation mark, catalysed by RBAP46–HAT1, that marks newly synthesized histone H4 before deposition.

Histone exchange

The replacement of nucleosomal histones with the corresponding canonical histones (H2A–H2B, H3–H4) or replacement variants (H2A.Z–H2B, H3.3–H4).

Hexasome

A nucleosome intermediate generated by either the loss of one H2A–H2B dimer from the nucleosome or the addition of one H2A–H2B dimer to the H3–H4 tetrasome.

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Biographies

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Key points

- Chromatin integrity and functionality is governed by the controlled assembly and disassembly of nucleosomes.
- An elaborate histone chaperone network governs histone provision, chromatin assembly, histone recycling and histone turnover.
- Histone chaperone networks operate through histone-dependent co-chaperone interactions and direct chaperone–chaperone contacts.
- The mode of action of histone chaperones is interpreted from structural and biochemical studies of histone–chaperone complexes.
- Key molecular functions of histone chaperones include the shielding of functional histone interfaces and trapping histones in non-nucleosomal conformations.
- The integration of histone chaperone function across DNA metabolic processes acts to maintain genome and epigenome integrity.

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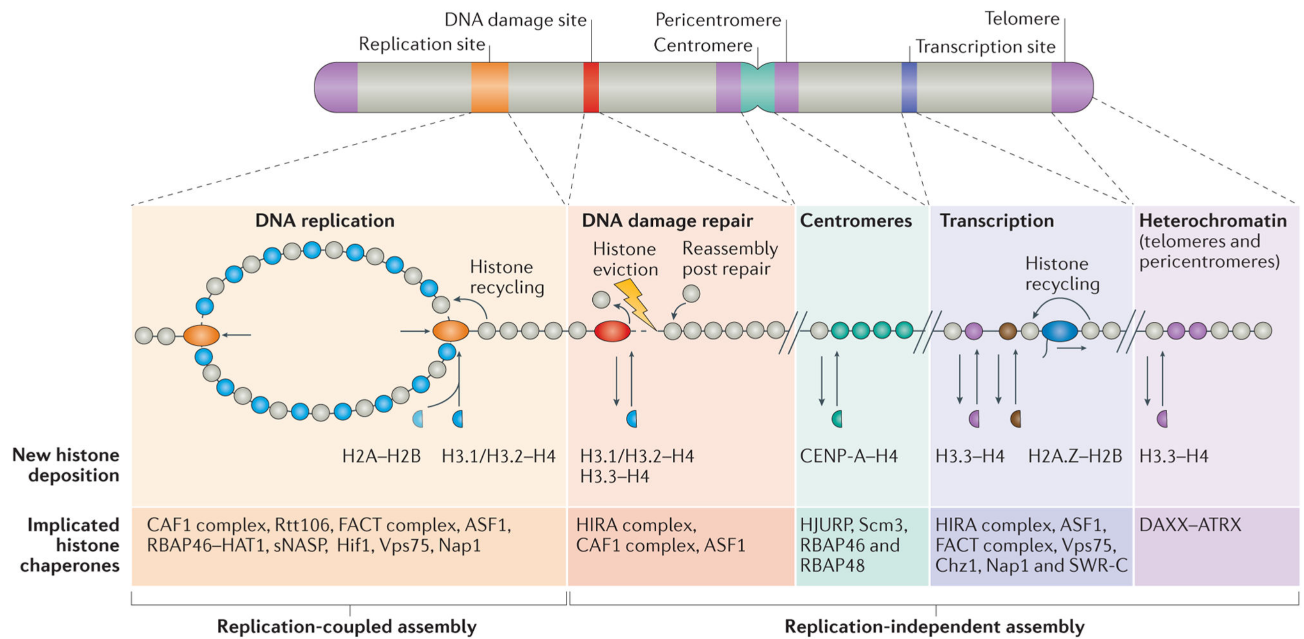

Figure 1. Overview of histone deposition mechanisms

Newly synthesized histones are incorporated into chromatin via globally and locally acting mechanisms. A network of specialized histone chaperones controls histone delivery and deposition. The figure provides an overview of replication-coupled and replication-independent pathways that require the incorporation of newly synthesized canonical histones and replacement variants, together with parental histone recycling. The histone chaperones that are implicated in each process are listed; for definitions of histone chaperone abbreviations see TABLE 1.

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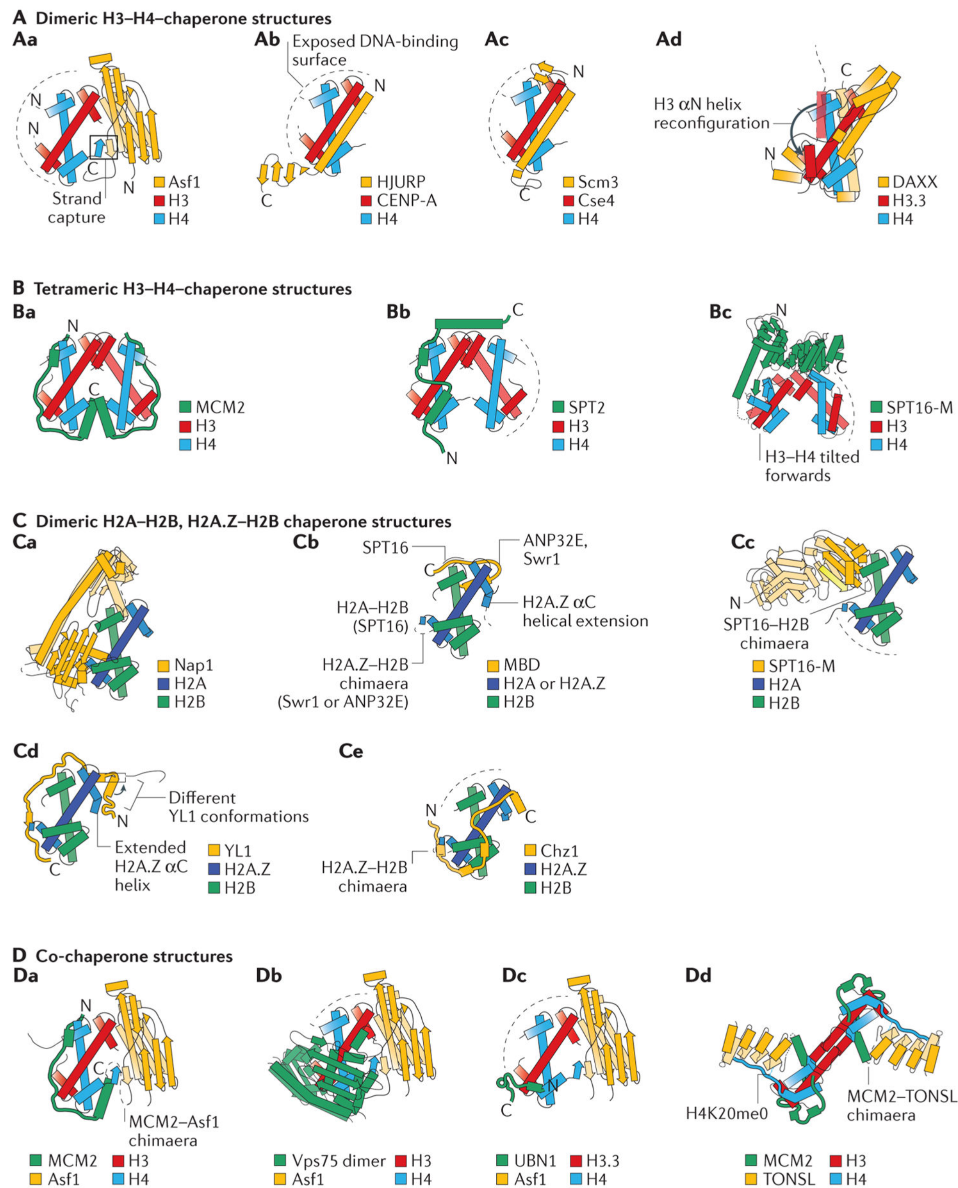

Figure 2. Structural features of histone–chaperone complexes

Two-dimensional depictions of 3D structures in the RCSB Protein Data Bank (PDB). Protein secondary structures are represented by arrows (β-strand**s**), rectangles (α-helices) and black lines (loops). In some cases, loops are represented as a thick line and free DNA-interaction surfaces are indicated by a broken line. For definitions of histone chaperone abbreviations see TABLE 1. **A.** Histone chaperones binding dimeric H3–H4: (part **Aa**) Asf1–H3–H4 (PDB identifier: 2HUE)[22]; (part **Ab**) HJURP–CENP-A–H4 (PDB ID: 3R45)[29]; (part **Ac**) Scm3–Cse4–H4 (PDB ID: 2YFV)[30]; and (part **Ad**) DAXX–H3.3–H4 (PDB ID: 4H9N)[31], the location of the nucleosomal H3 αN helix (PDB ID: 1AOI)[65] is indicated by broken lines. **B.** Histone chaperones binding tetrameric H3–H4: (part **Ba**) MCM2–H3–H4 (PDB ID: 5BNV)[34] — note that the structure includes two MCM2 HBDs; (part **Bb**) SPT2–H3–H4 (PDB ID: 5BSA)[33]; and (part **Bc**) SPT16 middle domain (SPT16-

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M)–H3–H4 (PDB ID: 4Z2M)[37]. **C.** Histones chaperones binding dimeric H2A– or H2A.Z–H2B: (part **Ca**) Nap1–H2A–H2B (PDB ID: 5G2E)[52]; (part **Cb**) minimal binding domain (MBD) of ANP32E (PDB ID: 4CAY, 4NFT)[68,69] and Swr1 (PDB ID: 4M6B)[67] with a H2A.Z–H2B chimaera (H2A.Z αC helix extension indicated), and SPT16 with H2A–H2B (PDB ID: 4WNN)[46]; (part **Cc**) SPT16-M–H2B chimaera with H2A (PDB ID: 4KHA)[41]; (part **Cd**) YL1 in complex with H2A.Z–H2B (PDB ID: 5FUG, 5CHL)[71,70]; and (part **Ce**) Chz1–H2A.Z–H2B (PDB ID: 2JSS)[82]. **D.** Co-chaperone complexes: (part **Da**) MCM2–Asf1 chimaera bound to H3–H4 (PDB ID: 5BO0)[34]; (part **Db**) the Vps75–Asf1–H3–H4 co-chaperone complex[51]; (part **Dc**) UBN1–H3.3–H4–Asf1 (PDB ID: 4ZBJ)[93]; and (part **Dd**) MCM2-TONSL bound to a H3–H4 tetramer (PDB ID: 5JA4)[84].

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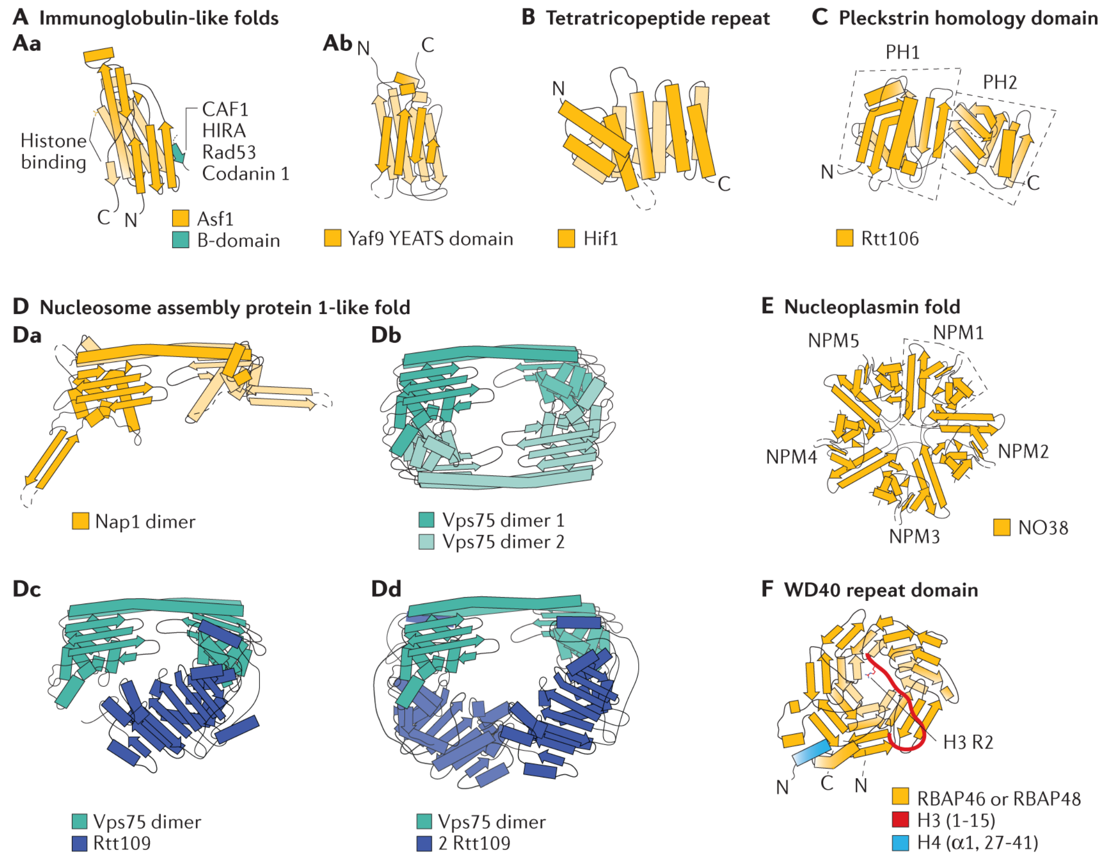

Figure 3. Other histone chaperone structures

Two-dimensional depictions of 3D structures in the RCSB Protein Data Bank (PDB). Histone chaperones are shown in yellow. For definitions of histone chaperone abbreviations see TABLE 1. **A** | Immunoglobulin-like folds: (part **Aa**) Asf1 (PDB identifier: 1ROC)[21], showing the location of B-domain interactions with Rad53 (PDB ID:2YGV)[97], Cac2/CAF1 p60 (PDB ID: 2Z3F)[94], Hip1/Hir1/HIRA (PDB ID: 2Z34, 2I32)[94,95] and codanin 1 (REF. 96); and (part **Ab**) the YEATS domain of Yaf9 (PDB ID: 3FK3)[24]. **B** | Tetratricopeptide repeat of Hif1 (PDB ID: 4NQ0)[27]. **C** | Tandem pleckstrin homology domains (PH1 and PH2) of Rtt106 (PDB ID: 3GYP)[39]. **D** | Nucleosome assembly protein 1 (Nap1)-like folds of (part **Da**) the Nap1 dimer (PDB ID: 2AYU)[49]; (part **Db**) Vps75 tetramer (PDB ID: 5AGC)[51]; and Vps75–Rtt109 complexes with (part **Dc**) 2:1 (PDB ID: 3Q66)[60] and (part **Dd**) 2:2 (PDB ID: 3Q35)[58] stoichiometries. **E** | Nucleoplasmin (NPM) fold of *Xenopus laevis* NO38 (PDB ID: 1XB9)[62]. **F** | WD40 repeat domains of RBAP46 and RBAP48 proteins with the H4 α1 (PDB ID: 3C9C, 3CFV)[74,75] and the H3 tail (PDB ID: 2YBA)[77].

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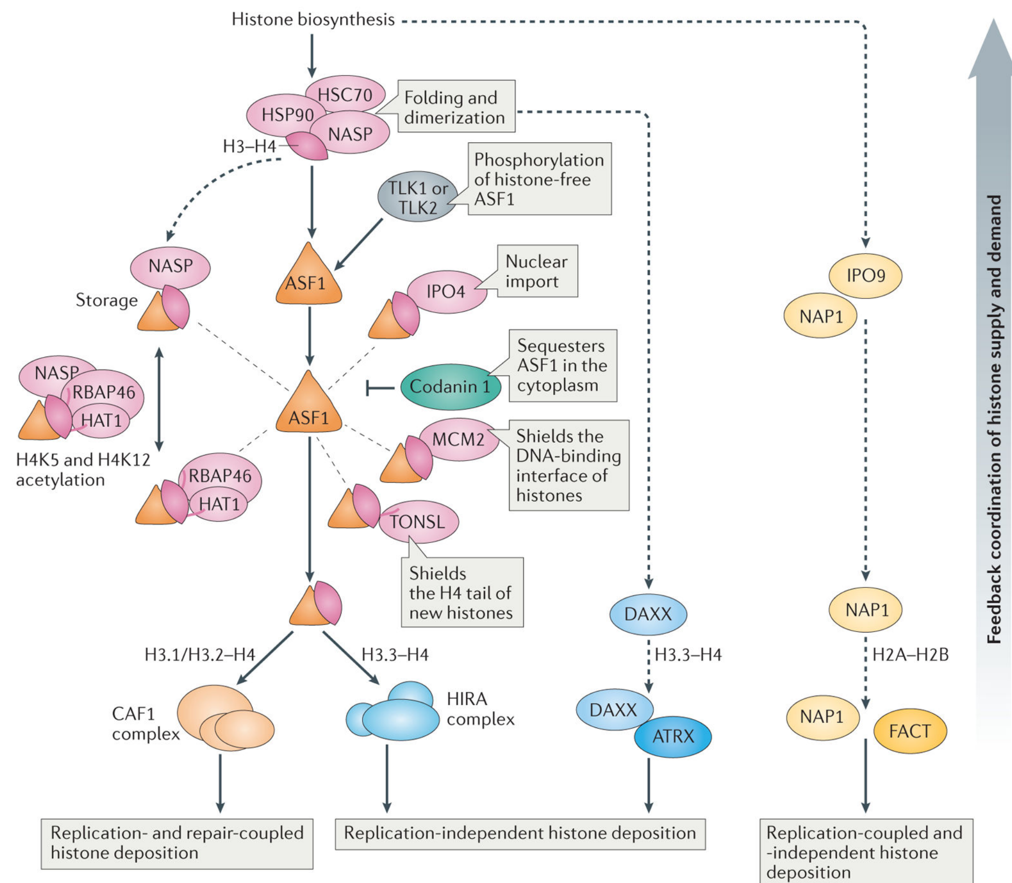

Figure 4. The histone supply network

After synthesis, histones H3–H4 and H2A–H2B engage with multiple different chaperones and enzymes on their way to chromatin. ASF1 is a central chaperone in the delivery of newly synthesized H3.1–, H3.2– or H3.3–H4 dimers. Histone-free ASF1 is phosphorylated by tousled-like kinases (TLKs), promoting histone binding. Histones bound by ASF1 are engaged in multiple different co-chaperone complexes, which further shields their functional interfaces and facilitates their acetylation, nuclear import and storage. Co-chaperoning may be a general paradigm, supporting branching of the pathway and the modification of histones 'on the go'. ASF1 shuttles H3.1/H3.2–H4 and H3.3–H4 dimers to the CAF1 and HIRA complexes for replication-coupled and replication-independent deposition, respectively. H3.3–H4 dimers are also deposited by DAXX–ATRX, whereas the H2A–H2B supply is handled by NAP1 and FACT. Histone chaperones also have an important role in the feedback regulation of histone supply. Although several histone chaperones are implicated in H3–H4 delivery with ASF1, it is still unclear whether other chaperones collaborate with DAXX and NAP1 in the delivery of H3.3–H4 and H2A–H2B dimers, respectively. See TABLE 1 for definitions of histone chaperone abbreviations.

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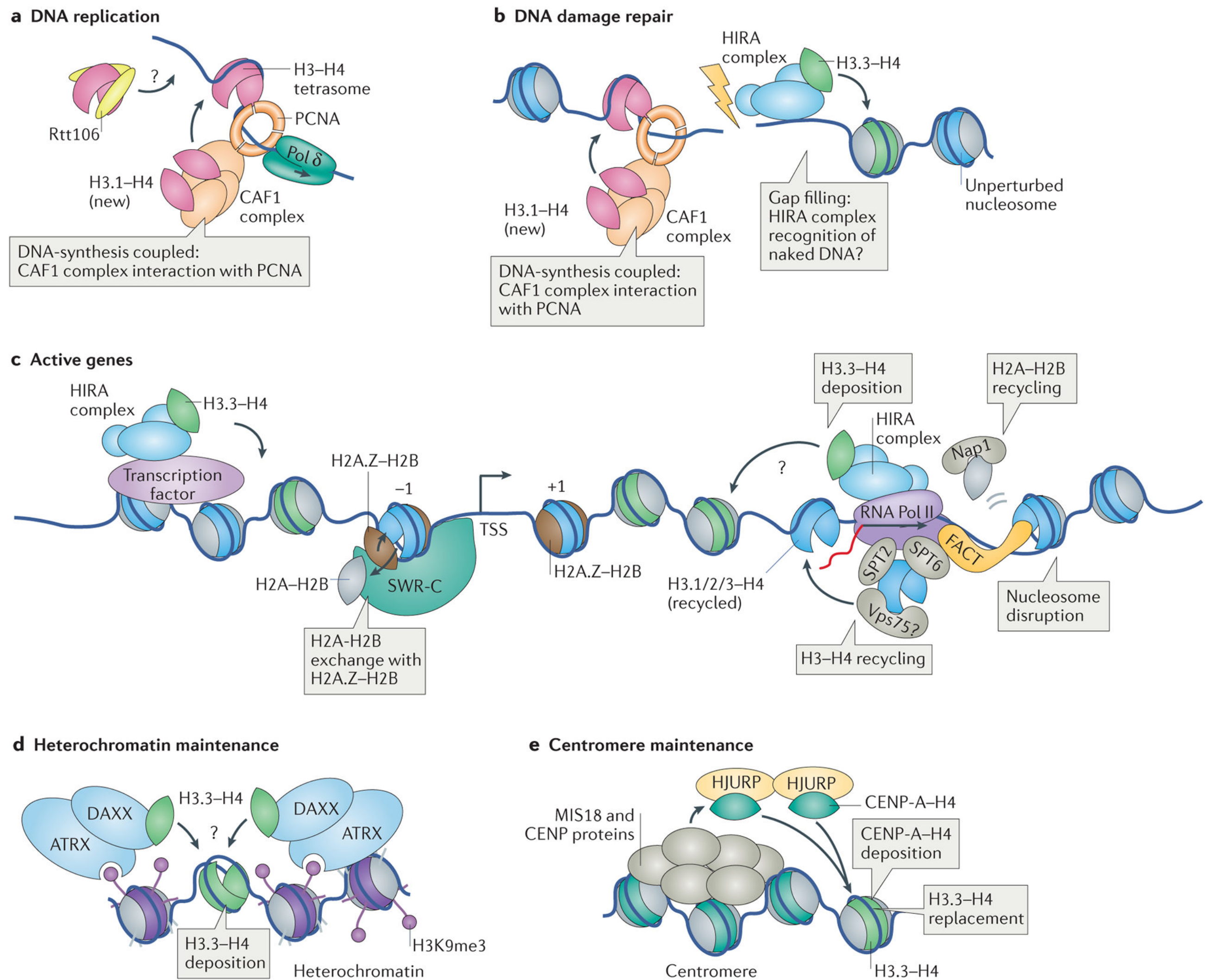

Figure 5. Recruitment of histone chaperones to chromatin

Histone chaperones recognize specific factors or features of chromatin to target canonical and variant histones to designated genomic loci. The CAF1 complex binds to PCNA to promote deposition during DNA replication (part **a**) and DNA repair (part **b**), whereas the HIRA complex may be recruited by naked DNA (part **b**). At transcription start sites (TSSs), Swr1 as part of the SWR-C complex recognizes the nucleosome-depleted region and exchanges H2A–H2B for H2A.Z–H2B at the −1 and +1 nucleosome (part **c**). Furthermore, the HIRA complex interacts with RNA polymerase II (Pol II) and transcription factors, which could facilitate H3.3–H4 deposition in gene bodies and at promoters, respectively. SPT2, SPT6 and potentially Vps75 mediate recycling of H3–H4 during ongoing transcription and SPT6 can be recruited by binding the phosphorylated carboxy-terminal repeat domain of Pol II. NAP1 and FACT are also recruited to transcription sites and can facilitate histone H2A–H2B eviction. In heterochromatin (telomeres, pericentromeres and repetitive elements), DAXX–ATRX is recruited through ATRX-mediated binding to H3K9me3 (part **d**). At centromeres, HJURP recruitment requires priming by the MIS18

complex together with core centromeric components (part **e**). For definitions of histone chaperone abbreviations, see TABLE 1.

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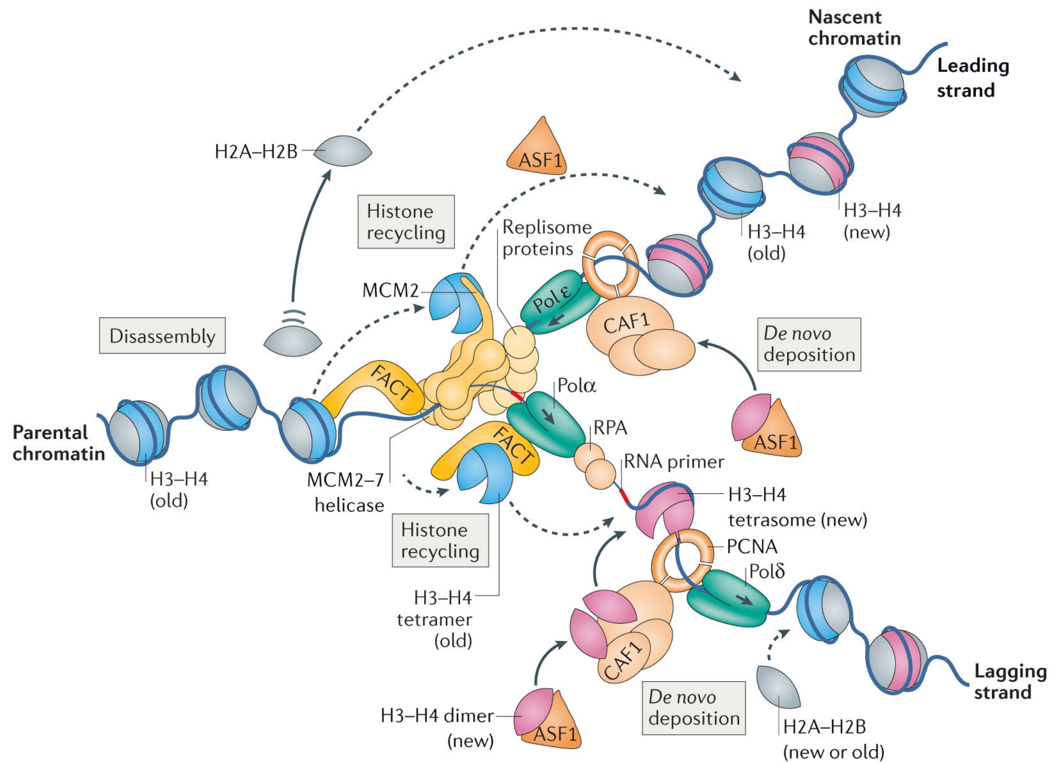

Figure 6. Parental histone recycling during DNA replication

Evicted parental histones are randomly segregated to daughter DNA strands. The FACT complex may contribute to nucleosome disruption and histone recycling through interaction with the CMG complex (which includes CDC45–MCM2–7–GINS), polymearase α(polα) and RPA. MCM2, which is part of the CMG helicase complex, provides a binding platform for evicted H3–H4 tetramers and may facilitate their recycling directly or in collaboration with FACT or as dimers with ASF1, which splits H3–H4 tetramers and forms a co-chaperone complex with MCM2. It remains unknown whether other components of the DNA replication machinery have histone chaperone activity and whether deposition of old and new histones occurs by separate pathways. For definitions of histone chaperone abbbreviations, see TABLE 1.

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Table 1

Details of histone chap erones

Histone chaperone	*Saccharomyces cerevisiae* homologue (or homologues)*	Histone preference	Complex (or complexes)	Domains
Anti-silencing function 1A/B (ASF1A/B)	Anti-silencing function 1(Asf1)	H3.1–, H3.2–, H3.3–H4	Multiple	ASF1, Ig-like, B-domain
Minichromosome maintenance protein 2 (MCM2)	Minichromosome maintenance protein 2 (Mcm2)	CENP-A–, H3.1–, H3.2–, H3.3–H4	MCM2–7 complex	AAA+ ATPase, HBD
Tonsuku Like (TONSL)	ND	H3–H4	MMS22–TONSL complex	ARD, TPR, LRR
Retinoblastoma associated protein 46 (RBAP46)	Histone acetyltransferase 2 (Hat2)	H3–H4	HAT, HDAC, NuRF, NuRD, PRC2	WD40
Heat shock protein 90A/B (HSP90A and HSP90B)	Heat shock cognate (Hsc82), Heat shock protein 82 (Hsp82)	H1, H2A, H2B, H3, H4	Heat shock family	HSP90, ATPase-like, TPR binding motif
Heat shock cognate 70 (HSC70)	Stress-seventy subfamily A (Ssa1, Ssa2, Ssa3, Ssa4)	H1, H2A, H2B, H3, H4	Heat shock family	HSC70, ATPase domain, peptide binding domain
Somatic nuclear autoantigenic sperm protein (sNASP)	Hat1 interacting factor (Hif1)	H3.1–, H3.2–, H3.3–H4, H1	H AT	TPR
Importin 4 (IPO4)	ND	H3.1–, H3.2–, H3.3–H4	Nuclear import receptor	HEAT domain, Armadillo-type-fold
Suppressor of Ty 2 (SPT2)	Suppressor of Ty 2 (Spt2)	H3–H4	ND	SPT2, HMG box
Suppressor of Ty 6 (SPT6)	Suppressor of Ty (Spt6)	H3–H4	ND	SPT6 core domain, SH2
ND	Regulator of Ty 1 transposition 106 (Rtt106)	H3–H4	ND	Dimerization, pleckstrin homology
ND	Swi2/snf2-related 1 (Swr1)	H2A.Z–H2B	SWR-C	Swi2/Snf2-related helicase, Swr1-Z domain
Acidic-leucine-rich nuclear phosphoprotein 32E (ANP32E)	ND	H2A.Z–H2B	P400–TIP60	LRR, ZID domain
ND	Chaperone for Htz1/H2A–H2B dimer 1 (Chz1)	H2A.Z–H2B	Delivery to SWR-C	H2A.Z-H2B binding
Protein YL1 (YL1), vacuolar protein sorting 72 homologue (VPS72)	SWr complex 2 (Swc2)	H2A.Z-H2B	SRCAP/SWR-C, P400–TIP60	ZID domain
Holliday junction recognition protein (HJURP)	Suppressor of chromosome missegregation 3 (Scm3)	CENP-A-H4 (Cse4-H4)	Centromere assembly	Scm3/HJURP
Patient SE translocation (SET)	Vacuolar protein sorting 75 (Vps75)	H3–H4	INHAT, Vps75–Rtt109	NAP1-like
Nucleosome assembly protein 1-like (NAP1L1-6)	Nucleosome assembly protein (Nap1)	H2A–, H2A.Z–H2B, H3–H4, H1	Nuclear import importin 9, Kap114	NAP1-like
Nucleophosmin (NPM1)	ND	H3–H4, CENP-A–H4, H1	SWAP	Nucleoplasmin
Nucleoplasmin 2 (NPM2)	ND	H2A–H2B	ND	Nucleoplasmin
Nucleoplasmin 3 (NPM3)	ND	ND	NPM1	Nucleoplasmin, NPM1 binding
Nucleolin (NCL)	ND	H2A–H2B, H1	SWAP	RNA binding, DNA helicase/ ATPase

Histone chaperone	*Saccharomyces cerevisiae* homologue (or homologues)*	Histone preference	Complex (or complexes)	Domains
Chromatin assembly factor 1, subunit A (CHAF1A or p150)	Chromatin assembly complex 1 (Cac1)	H3.1–H4	CAF1	PIP-box, p60 binding, HP1-binding, WH domain
Chromatin assembly factor 1, subunit B (CHAF1B or p60)	Chromatin assembly complex 2 (Cac2)	H3.1–H4	CAF1	WD40, B-domain
Retinoblastoma associated protein 48 (RBAP48)	Multi-copy suppressor of IRA1 (Msi1)	H3.1–, H3.2–, H3.3–H4	CAF1, HDAC, NuRF, NuRD, and PRC2	WD40
Histone regulation A (HIRA)	Histone regulation (Hir1, Hir2)	ND	HIRA/HIR	WD40, B-domain
Ubinuclein 1 (UBN1)	Histone periodic control (Hpc2)	H3.3–H4	HIRA/HIR	UBN1, HRD
Calcineurin-binding protein cabin-1 (CABIN1)	Histone regulation (Hir3)	ND	HIRA/HIR	TPR
Suppressor of Ty 16 (SPT16)	Suppressor of Ty 16 (Spt16)	H2A–H2B, H3–H4	FACT	Peptidase-like, pleckstrin homology
Structure-specific recognition protein 1 (SSRP1)	POl1 binding 3 (Pob3)–non-histone protein 6 (Nhp6A/B)	H2A–H2B, H3–H4	FACT	Pleckstrin homology, HMG box
Death domain-associated protein 6 (DAXX)	ND	H3.3–H4	DAXX–ATRX	HBD, four-helix bundle, SIM, PML targeting
Alpha-thalassaemia/ mental retardation syndrome X-linked (ATRX)	ND	ND	DAXX–ATRX	Swi/Snf2-related helicase, ADD, HP1 binding, MeCP2 binding

AAA+, ATPases associated with diverse cellular activities; ADD, ATRX-DNMT3-DNMT3L domain; ARD, ankyrin repeat domain; B-domain, Asf1 binding domain; CAF1, chromatin assembly factor 1 complex; FACT, facilitates chromatin transcription; HAT, histone acetyltransferase; HBD, histone-binding domain; HDAC, histone deacetylase; HEAT, domain also present in Huntington, Ef3, protein phosphatase 2A and Tor1; HIR, histone regulation complex (yeast); HIRA, histone regulation A complex (human); HMG-box, high mobility group box; HRD, Hpc2-related domain; HP1, heterochromatin protein 1; Ig-like, immunoglobulin-like; INHAT, inhibitor of acetyltransferase; Kap114, karyopherin 114; LRR, leucine-rich repeat; MCM2–7, the complex of minichromosome maintenance proteins 2, 3, 4, 5, 6 and 7; MeCP2, methyl-CpG-binding protein 2; MMS22L, methyl methanesulfonate sensitivity 22-like; NAP1-like, nucleosome assembly protein 1-like; ND, not defined; NuRD, nucleosome remodelling and histone deacetylase; NuRF, nucleosome remodelling factor; PIP-box, PCNA-interacting protein box; PML, promyelocytic leukaemia nuclear body; PRC2, polycomb repressive complex 2; Rtt109, regulator of Ty 1 transposition 109; SIM, SUMO-interacting motif; SH2, Src homology 2 domain; SRCAP, Snf-2-related CREB-binding protein activator protein; SWAP, switch-associated protein complex; SWR-C, Swr1 complex; Swi2/Snf2, switch/sucrose nonfermenting 2; TIP60, 60 kDa Tat-interactive protein; TPR, tetratricopeptide repeat; WD40, 40 amino acid repeat terminating in tryptophan-aspartic acid; WH, winged helix; ZID, H2A.Z-interacting domain.

*Yeast and human homologue (or homologues) were identified using http://yeastmine.yeastgenome.org and functional evidence in the literature.

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