The right place at the right time: chaperoning core histone variants

Francesca Mattioli*†‡, Sheena D’Arcy†§ & Karolin Luger‡

Abstract

Histone proteins dynamically regulate chromatin structure and epigenetic signaling to maintain cell homeostasis. These processes require controlled spatial and temporal deposition and eviction of histones by their dedicated chaperones. With the evolution of histone variants, a network of functionally specific histone chaperones has emerged. Molecular details of the determinants of chaperone specificity for different histone variants are only slowly being resolved. A complete understanding of these processes is essential to shed light on the genuine biological roles of histone variants, their chaperones, and their impact on chromatin dynamics.

Keywords chromatin dynamics; histone chaperones; histone variants; nucleosome

Introduction

The structural organization of eukaryotic DNA into chromatin is intricately linked to the regulation of many essential cellular processes. Nucleosomes are the structural unit of chromatin and are dynamically remodeled, assembled, and disassembled to allow DNA replication, transcription, and the different types of DNA repair. In this way, nucleosomes integrate a diverse array of cell signaling events to orchestrate the spatial arrangement of, and thus timely access to, the genetic information. At the center of this integration are the proteinaceous building blocks of the nucleosome, the core histone proteins.

The core histones are small (<20 kDa), positively charged polypeptides characterized by a histone fold domain. This domain is composed of three α-helices (ξ1-3) and assembles in the presence of a binding partner, particularly another histone, to form histone fold dimers [1–3] (Fig 1A). Nucleosomes contain stable heterodimers of histones H2A and H2B, as well as histones H3 and H4. Two copies of H2A–H2B pack on either side of a (H3–H4)2 tetramer, forming a histone octamer that wraps 147 bp of DNA [4] (Fig 1B). Histones also contain less-structured tails, which protrude outward and mediate interactions between nucleosomes, as well as with nuclear signaling proteins such as transcription factors and chromatin remodelers [5]. The histone tails are heavily post-translationally modified and mediate a variety of epigenetic events. Modifications occur at virtually all amino acids and include mono-, di-, and trimethylation, acetylation, mono-ubiquitination, and phosphorylation, among others.

The bulk of the DNA is packaged by canonical histones H2A, H2B, H3 (also referred to as H3.1), and H4. Throughout evolution, however, an increase in complexity of chromatin-mediated signaling has led to a requirement for defined and localized changes in nucleosomes. A powerful mechanism to introduce such changes is the variation of histone primary sequence to produce the so-called histone variants (reviewed in [6–8]). Histone variants resemble their canonical counterparts, but alter nucleosome function, by differing in sequence composition and in some cases by carrying additional domains (Fig 2). Phylogenetic studies have shown that histone variants have evolved in eukaryotic organisms in a non-concerted manner following a “birth-and-death” process [8–10]. The most pronounced sequence divergence is found in H2A and H2B variants, with H2A also having the greatest number of variants. This suggests some tolerance for H2A–H2B diversity within chromatin. In contrast, H4 has no variant identified and most H3 variants have only minimal sequence variation. This possibly reflects the evolutionary convergence required for H3–H4 deposition onto DNA, the first critical step in nucleosome formation [11].

Further to primary sequence, canonical and variant histones also differ in their expression patterns. Canonical histones are expressed from gene clusters in a cell cycle-dependent manner, peaking during S-phase to facilitate replication-dependent packaging of DNA (reviewed in [12]). Most histone variants, however, are expressed throughout the cell cycle, meaning that their incorporation into chromatin is independent of DNA synthesis [13,14]. Histone variants can therefore fulfill specific functional niches that rely on tight temporally and spatially regulated alterations in chromatin. Such niches are required for transcriptional control of specific genes,
maintenance of epigenetic information, repair of DNA damage, chromatin transition during spermatogenesis, and the formation of a single kinetochore on each chromosome [6,15–17]. The challenge has been to determine the mechanistic basis of histone variant function and to decipher how histone variants are deposited in the right place at the right time. This review will focus on the specific crosstalk between histone variants and their chaperones, highlighting the regulation of their function and dynamics.

Effect of histone variants on chromatin structure

One of the initial hypotheses among researchers was that histone variants would alter nucleosome structure. However, based on in vitro nucleosome reconstitution experiments and structural studies, it is clear that variants in general do not dramatically alter the overall structure or composition of nucleosomes (e.g. [18–21]). This is perhaps not surprising, as the variant residues are typically located distal to the histone–histone and histone–DNA interfaces that hold together the histone octamer and wrap the DNA. While massive reorganization of variant-containing nucleosomes is not observed, they can display subtle differences that likely contribute to their functional specialization.

A well-studied histone variant that induces a subtle conformational change is H3 variant CenH3. CenH3 is the most divergent of all H3 variants, functionally conserved from yeast (CenH3Cse4) to human (CenH3CENP-A) (Fig 2A). From a phylogenetic point of view, CenH3 does not seem to have a single origin. Similar to other variants, it has evolved multiple times and has been subject to strong positive selection, likely due to its specific function [8]. This variant plays an essential role in defining the heritable chromosomal centromere that directs kinetochore assembly [22].

The histone stoichiometry of CenH3 nucleosomes has been a matter of debate, with some groups proposing a dynamic substoichiometric composition [23–26], while others suggest it is octameric [27–29]. In yeast, this debate has been partly clarified by a study that shows the dynamic behavior of the one CenH3Cse4 nucleosome throughout the cell cycle [30]. CenH3Cse4 nucleosomes are assembled de novo during S-phase sampling sub-octameric intermediates, which eventually lead to the stable octameric form [30]. Structural studies show that in humans CenH3CENP-A can form octameric nucleosomes, at least in vitro. These studies also reveal that CenH3CENP-A nucleosomes wrap only 121 bp of DNA, compared to 147 bp wrapped in canonical nucleosomes [31] (Fig 1C). This more “loose” wrapping of DNA is consistent with biophysical studies [31,32] and may be attributed to a shorter helix, N-terminal to the histone fold (Fig 1D). This feature may contribute to the reduced stability of CenH3 nucleosomes as compared to canonical ones, when assembled on endogenous DNA templates [32].

It is not yet known whether the change in DNA wrapping is essential for CenH3-directed centromere formation. It is known, however, that CenH3 incorporation into chromatin is sufficient for kinetochore formation [33,34]. A central CENP-A targeting domain (CATD) encompassing a variant loop containing an Arg-Gly insertion and the α2 helix of the histone fold confers CenH3CENP-A function and interacts with inner kinetochore component CENP-N [35–38] (Fig 3B). CenH3CENP-A N- and C-terminal tails also directly interact with CENP-B and CENP-C kinetochore proteins [35,36,39–41]. These multiple interactions contribute to the stability of the CenH3CENP-A nucleosome, centromere identity, and the initiation of kinetochore assembly. These studies highlight how a variant-containing nucleosome can induce a specific downstream signal by exposing the histone variant surface for recognition by select nuclear proteins.

Other histone variants can also induce changes in nucleosome stability. This has been shown for H2A.B (also referred to as H2A.Bbd), which is often associated with active transcription and is involved in the histone to protamine transition in spermatogenesis [42–44]. It has also been shown for nucleosomes with both H2A.Z and H3.3, which are typically associated with transcriptional start sites [45,46]. H2A.B and H2A.Z also influence interactions between nucleosomes in a multi-nucleosome template or array. H2A.B and H2A.Z have only 35 and 51% similarity to canonical H2A, respectively, with notable sequence differences around a surface-exposed acidic patch (Fig 2B). This acidic patch mediates direct interactions with a vast number of proteins known to interact with the nucleosome, including other nucleosomes via the N-terminal tail of H4 in the neighboring particle [47]. H2A.Z has an extended acidic region [18], while H2A.B has lost its acidic residues [44]. Sedimentation studies show that these differences affect the ability of nucleosome arrays containing the variants to compact, with H2A.Z favoring compaction [48] and H2A.B reducing compaction [49]. These cases again exemplify how histone variant incorporation into chromatin can alter the nucleosome surface to facilitate the engagement of
variant-specific interactions responsible for the mediation of variant-specific functions.

A role for histone chaperones

An important part of variant and canonical histone biology is the dynamics of their incorporation into and eviction from chromatin. It is important to highlight that within the nucleosome, H2A–H2B dynamics have less impact on overall nucleosome stability than H3–H4 dynamics. In this light, incorporation or removal of H3 variants will require a more dramatic restructuring of chromatin as compared to H2A or H2B variants. H2A and H2B variants can be more easily exchanged without dismantling the central (H3–H4)2 tetramer–DNA complex. These dynamics are influenced by histone chaperones and ATP-dependent chromatin remodeling complexes. The latter primarily organize chromatin by sliding or ejecting assembled nucleosomes from chromatin. In this process, they can influence nucleosome composition by allowing the exchange of the more labile histone H2A–H2B dimers [50]. Histone chaperones instead

Figure 1. Canonical and variant nucleosomes. (A) Elements of the histone fold and structures of Xenopus laevis H2A–H2B, H3–H4 and (H3–H4)2 (PDB ID: 1KX5). (B) Structure of the canonical Xenopus laevis nucleosome (PDB ID: 1KX5). Other nucleosome structures, such as the human nucleosome, are structurally similar. (C) Structure of the CenH3CENP-A-containing nucleosome (PDB ID: 3AN2). (D) Zoomed view of the αN helix of CenH3CENP-A (left) and H3 (right) involved in stabilizing the DNA ends. Histone H3 is blue, CenH3CENP-A is cyan, H4 is green, H2A is yellow, H2B is red, and DNA is white.
are a class of proteins that bind histones and facilitate their interactions to form the nucleosome [51].

Chaperones are structurally diverse and are involved in histone trafficking between the nucleus and cytoplasm, as well as histone deposition into and eviction from chromatin [17,52,53]. With very few exceptions, it is important to realize that interactions between chaperones and histones are not always exclusive or binary. In fact, a particular histone can be bound by different chaperones. H2A-H2B, for example, is handled by numerous chaperones, including FACT and NAP1 [54,55]. Variant H3.3-H4 can also bind chaperones ASF1, HIRA, and DAXX [56]. On the flipside, a chaperone can also often bind more than one histone heterodimer. NAP1, for example, interacts with both H2A-H2B and H3-H4 as either a dimer or tetramer [57]. ASF1 can also bind both H3.1-H4 and H3.3-H4 [58,59]. This creates a redundancy that hinders the ability to fully understand the specific contributions of each chaperone in vivo.

The structural underpinnings of chaperone redundancy stem from the fact that chaperones can bind histones in various manners. The most promiscuous chaperones, such as NAP1, interact primarily with the histone backbone, explaining their inability to discriminate between H2A-H2B and H3-H4, as well as many histone variants [54]. Some more selective chaperones interact with histone surfaces that are conserved between canonical histones and their variant counterpart. ASF1, for example, binds the tetramerization H3 interface within a H3-H4 dimer [60,61] (Fig 2A). This region is conserved among most H3 variants such that ASF1 can handle both H3.1-H4 and H3.3-H4 [58,59]. Similarly, FACT mainly interfaces with H2B, explaining how it can recognize H2A-H2B containing different H2A variants [55] (Fig 3A). There are nonetheless chaperones specialized in handling specific histone variants (Table 1). These chaperones discriminate their substrate by recognizing specific side chains, unique to the variant.

It is important to note that while specificity in chaperone recognition can be critical in determining the timely and localized dynamics of histone variants, it is by far not the only controlling factor. Regulation of histone chaperones themselves, in particular their expression pattern, modification, sub-cellular and sub-nuclear localization, and interacting partners, can greatly impact these dynamics. Understanding how the cell integrates these different layers of regulation

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Figure 2. Schematic of select H3 and H2A variants.
Schematic representation of select human H3 (A) and H2A (B) variants and the yeast homologs. The histone fold is shown in solid blue shades for H3 isoforms and in orange shades for H2A isoforms. The tails are shown as lines. Specific amino acid substitutions are reported. Different shades of color are used to indicate the degree of sequence divergence. Residues involved in forming the acidic patch on H2A isoforms are shown in red (where the acidic residues are present) and white (for H2A.B which lacks these residues). Sequence identity to the human canonical histone is shown in gray.
to control histone variants has proven more complex than initially predicted.

CenH3 and HJURP: a paradigm for chaperone specificity

As introduced above, CenH3 is a striking example of a functionally specific histone variant. Such specificity requires a CenH3-specific chaperone to orchestrate the timely and accurate deposition of CenH3 into chromatin [22]. CenH3-specific chaperones have been identified in a number of organisms. In humans, HJURP handles CenH3CENP-A [62–65], in yeast, Scm3 binds CenH3Cse4 [66–68], and in fly, CAL1 is responsible for deposition of CenH3CID [69] (Table 1). Although these chaperones have low sequence similarity and largely diverge in domain composition, they fulfill the same functional niche with regards to CenH3.

Structures of the N-terminal region of yeast Scm3 and human HJURP bound to their cognate CenH3-H4 orthologues reveal a conserved mode of binding [70–72] (Fig 3B). The CATD domain of CenH3 is orange. (C) Structure of H3.3–H4 and of the complex with the histone-binding domain of DAXX (PDB ID: 4H9N). Shown in orange are the H3.3-specific residues, with G90 shown as spheres. (D) Zoomed view of the DAXX Glu225 and H3.3 Gly90 interface, the key determinant for specificity in vivo. (E) Structure of H3.3–H4 with the histone-binding peptide of UBN1 and ASF1 (PDB ID: 4ZBJ). Shown in orange are the H3.3-specific residues, with G90 shown as spheres. In all panels, H3 isoforms are blue, CenH3 is cyan, H4 is green, and ASF1 is gold. Specific chaperones HJURP, Scm3, UBN1, and DAXX are shown in maroon.

Figure 3. Structures of H3 variants and their chaperones.

(A) Structure of the H3-H4 (PDB ID: 1KXS) and of the complex with the histone-binding domain of ASF1 (PDB ID: 2HUE). (B) Structure of CenH3CENP-A–H4 and of the complex with the histone-binding domain of HJURP (PDB ID: 3R45) and Scm3 (PDB: 2YLV). The CATD domain of CenH3 is orange. (C) Structure of H3.3–H4 and of the complex with the histone-binding domain of DAXX (PDB ID: 4H9N). Shown in orange are the H3.3-specific residues, with G90 shown as spheres. (D) Zoomed view of the DAXX Glu225 and H3.3 Gly90 interface, the key determinant for specificity in vivo. (E) Structure of H3.3–H4 with the histone-binding peptide of UBN1 and ASF1 (PDB ID: 4ZBJ). Shown in orange are the H3.3-specific residues, with G90 shown as spheres. In all panels, H3 isoforms are blue, CenH3 is cyan, H4 is green, and ASF1 is gold. Specific chaperones HJURP, Scm3, UBN1, and DAXX are shown in maroon.
HJURP binding [72]. It seems that Ser68 and the CATD work in concert to ensure accurate and faithful deposition of CenH3CENP-A by HJURP. The CATD was initially identified as it was sufficient to cause an H3 swap mutant to be incorporated into centromeric chromatin [38,62]. More recent studies highlight the complexity of this deposition in human cells, where phosphorylation at Ser68 and ubiquitination at Lys124 also coordinate CenH3CENP-A binding and deposition [73,74].

The complexity of CenH3 deposition is enhanced by the regulation of the chaperone itself. The necessity of HJURP for CenH3CENP-A deposition is obvious from the dramatic reduction of CenH3CENP-A incorporation in HJURP knockdown cells [65]. Artificial tethering of HJURP to non-centromeric loci is also sufficient to induce kinetochore assembly [64]. In yeast, Scm3 remains associated with the centromeric nucleosome, likely ensuring stability of the particle [30]. HJURP function at centromeres relies on its dimerization [75] and DNA-binding capacity [76]. It is also modulated by post-translational modifications, in particular phosphorylation that coordinates its action in a cell cycle-dependent manner [76]. More recently, an additional role for HJURP in assembling de novo centromere was described and shown to be dependent on a direct interaction with CENP-C, a member of the kinetochore complex [34].

Regulation of CenH3CENP-A by HJURP is of crucial importance for fidelity in chromosome segregation and cell division. Aberrant functions of the chaperone, as well as the histone variant, have been associated with chromosomal instability and ultimately cancer. Overexpression of HJURP and CenH3CENP-A has been observed in tumors, where their elevated mRNA levels are associated with lower patient survival [22,77–81]. Cells overexpressing CenH3CENP-A fail to arrest in the presence of DNA-damaging agents, suggesting that CenH3CENP-A overexpression may contribute to resistance against current chemotherapeutic drugs [82]. Remarkably, when overexpressed, CenH3CENP-A will localize at sites of high histone turnover, forming heterotypic nucleosomes with the H3.3 variant [82]. This ectopic localization of CenH3CENP-A is not dependent on HJURP, but rather on DAXX, a histone chaperone known to primarily bind H3.3–H4 [82]. These data illustrate the redundancy among chaperones and the malleability of histone binding. Nonetheless, the tight functional connection between CenH3CENP-A and HJURP highlights a unique specific link between this histone variant and chaperone. All together, these observations suggest that alongside the co-evolution of specific histone variant–chaperone pairs, flexibility can be accommodated in the system, particularly in disease conditions.

### H3.3 and DAXX: water-mediated specificity

Compared to CenH3, the histone variant H3.3 is fairly widespread throughout the genome, marking euchromatin, pericentromeres, and telomeres [56,83]. It differs from canonical H3.1 and H3.2 by modifications of Lys27 and Lys36, key residues targeted by the histone tail, Lys27 to Met and Gly34 to Val or Arg, have been found in humans, H3.3 is encoded by two genes with identical sequence and is expressed in a replication-independent manner [13]. Knockout experiments result in phenotypes ranging from lethality in mice to infertility in flies [84,85]. Interestingly, replacing the endogenous H3.3 gene with H3.2 in flies can rescue the infertility phenotype, suggesting that histone supply outside of S-phase may be more relevant than sequence specificity in this context [86]. Nonetheless, engineering single-point mutations on H3.1 to make it resemble H3.3 leads to a replication-independent incorporation of H3.1 in fly cells [13]. This highlights that the variant residues are important for histone dynamics, which are likely mediated by histone chaperones.

H3.3 was the first histone variant to be shown to be directly involved in carcinogenesis. Two-point mutations in the N-terminal tail, Lys27 to Met and Gly34 to Val or Arg, have been found in patient samples [87,88]. These mutations affect post-translational modifications of Lys27 and Lys36, key residues targeted by the mammalian epigenetic program. H3.3 chaperones are also mutated in a wide range of cancers [89,90], and their mutation can correlate with H3.3 mutations in the same tumors [88]. To date, two distinct H3.3 chaperones have been identified with separate functional niches. The first is HIRA, which controls H3.3 deposition at

**Table 1.** Function of specific histone variant–chaperone pairs.

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transcriptionally active loci, while the second is DAXX, which functions at pericentromeric and telomeric regions [56] (Table 1).

DAXX was discovered as H3.3 incorporation into chromatin that was not completely abolished in HIRA knockout cells [56,91]. DAXX directly interacts with H3.3, although its chromatin-related functions are linked to ATRX, a member of the SNF2 ATP-dependent chromatin remodeling family [56,92]. Structures of the central domain of DAXX bound to H3.3–H4 have shed light on the mode of histone binding and H3.3 specificity [93,94] (Fig 3C). DAXX envelops H3.3–H4 and makes extensive contacts with both H3.3 and H4. DAXX forms a coiled coil with α2 helix of H4, somewhat similar to the one formed by HJURP or Scm3 with CenH3, suggesting a conserved mode of interaction. Like many other chaperones, DAXX interferes with H3.3–H4 tetrameric formation and DNA binding as required for nucleosome formation [93,94].

Alongside structural studies, pull-down experiments using whole cell extracts found that a single DAXX residue, Glu225, was key in determining H3.3 specificity [93,94]. In cells, a Glu225Ala DAXX mutation is sufficient to induce a gain-of-binding effect for H3.2 in cells. As seen in the structure, Glu225 engages in a network of water-mediated hydrogen bonds to the histone pair [93]. This network also involves H3.3 residue Gly90, which is a Met in H3.1 and H3.2 (Fig 3D). Pull-down experiments using single swap mutants between H3.1/2 and H3.3 residues show that Gly90 is the primary determinant for DAXX binding. A Gly90Met H3.3 mutant can no longer bind DAXX, while a Met90Gly H3.1 mutant has gained the ability to bind DAXX [93,94]. Despite these pull-down results, the Gly90Met H3.3 mutant can be crystallized in complex with DAXX, and the DAXX Glu225Ala mutant can be crystallized in complex with H3.3–H4 [93]. These structures have a different number of water molecules at the interface, suggesting a role for water-mediated interactions in DAXX recognition of H3–H4 variants. DAXX once again highlights the lack of tight structural stringency in chaperone recognition of histone variants, reinforcing the need for combined in vivo and in vitro studies.

HIRA is a heterotrimer composed of HIRA, UBN1, and CABIN1. It is functionally connected to H3.3 dynamics as its depletion in cell culture affects H3.3 incorporation at promoters and gene bodies [56]. Chromatin immunoprecipitation followed by high-throughput DNA sequencing shows that HIRA co-localizes with H3.3, primarily at transcriptional start sites of highly expressed genes [95]. This link between HIRA and active transcription is further supported by its co-localization with both initiating and elongating RNA polymerase [83]. Moreover, HIRA functions at sites of UV damage, where it is required for transcriptional restart [96]. A short peptide of the UBN1 subunit has been identified as the primary interface of the HIRA complex to H3.3–H4 dimers [97]. This peptide is specific for the H3.3 variant as it interacts with Gly90 [97]. The crystal structure of this peptide with H3.3–H4 bound by ASF1 confirms that these chaperones interact with structurally distinct regions on the H3.3–H4 dimer (Fig 3E). Little is known about how HIRA and DAXX functions are regulated, what directs them to separate genomic loci, and how H3.3 is understood between the two chaperones. Understanding these pathways will shed light on the mechanisms of regulating H3.3 dynamics.

Another interesting feature of H3.3 is that phylogenetic studies suggest that it is the ancestral H3 isoform [8,9]. In simpler eukaryotes, such as fungi, H3.3 is the only non-centromeric H3 variant, and H3.1 and H3.2 are not present [8]. Despite only having a single, non-centromeric H3 variant, however, yeast contains two distinct H3 chaperones. The first is a homolog of HIRA (Hir) that performs replication-independent functions [59], while the second is CAF-1 [58]. In higher eukaryotes, CAF-1 is typically H3.1-specific and performs replication-dependent functions [98–102]. This raises a few interesting points about histone chaperone specificity. On the one side, it seems feasible that CAF-1 specificity for H3.1 evolved after yeast, and that yeast CAF-1 in fact chaperones H3.3-like histones. On the other side, it highlights that in these organisms, although a single H3 isoform is present, a separation of function at

Figure 4. Structures of H2A variants and their chaperones. (A) Structure of H2A–H2B (PDB ID: 1KXS) and of the complex with the histone-binding domain of the chaperone FACT (PDB ID: 4KHA). (B) Structure of H2A.Z–H2B and of the complex with the histone-binding region of Chz1 (PDB ID: 2J5S). (C) Structure of the complex between the Swr1 histone-binding region and the H2A.Z–H2B dimer (PDB ID: 4M68). (D) Superposition of the H2A–H2B and H2A.Z–H2B dimers, close up view on the αC helix which is extended in H2A.Z due to a single amino acid deletion. (E) Structure of the ANP32E histone-binding region–H2A.Z–H2B complex (PDB ID: 4CAY). Shown in dark green is the region of the αC helix important for H2A.Z specificity. H2A is yellow, H2A.Z is gold, H4 is red, and FACT is light blue. Chaperones Chz1, Swr1, and ANP32E are purple.
the level of histone chaperones has already evolved. This provides a unique situation where specificity in histone dynamics may primarily reside in chaperone function and regulation. Chaperone function may thus play an equal and, in some cases, more important role than the actual sequence of the histone.

**H2A.Z and Swr1 or ANP32E: the importance of a single-residue insertion**

Another functionally important and clinically relevant histone variant is H2A.Z. Like the H3 variants, H2A.Z is overexpressed in a number of malignancies and is deposited independent of DNA replication [7,103–106]. H2A.Z has roles in diverse processes such as telomere stabilization and DNA damage repair, although it is best characterized as a transcriptional regulator. H2A.Z is enriched at promoters and gene regulatory regions such as enhancers and insulators [107–112]. It is generally associated with active transcription and often co-localizes with H3.3, even in the same nucleosome [46]. As mentioned previously, these composite nucleosomes are unstable and possibly facilitate nucleosome disassembly [46]. H2A.Z, however, can also associate with repressed genes [113], convoluting our understanding of its specific roles.

In yeast, H2A.Z (Htz1) interacts with chaperones Chz1, Nap1, and FACT [114–117]. Of these three chaperones, Chz1 is purported to prefer H2A.Z–H2B over canonical H2A–H2B [118] (Table 1). The basis for such specificity, however, is unclear. The NMR structure of the Chz1 histone-binding region shows how Chz1 recognizes H2A.Z–H2B, but does not clearly reveal how it discriminates H2A.Z from H2A [116] (Fig 4B). It is tempting to speculate that either additional regions of Chz1 or additional regulatory proteins or modifications determine the H2A.Z preference of Chz1.

H2A.Z–H2B is also incorporated into chromatin by the ATP-dependent remodeling complex Swr1 [119–121] (Table 1). Swr1 associates preferentially with H2A.Z–H2B compared to canonical H2A–H2B and is involved in the acetylation-dependent exchange of canonical H2A–H2B with variant H2A.Z–H2B at precise chromatin regions [122]. A short region of Swr1 directly binds H2A.Z–H2B, and the complex has been structurally characterized [123] (Fig 4C). Swr1 contacts H2A.Z on a C-terminal helix, which harbors the single-residue deletion in H2A.Z compared to canonical H2A. This deletion extends the C-terminal helix, ultimately changing the exposed residues (Fig 4D). Intriguingly, this helix of H2A.Z is critical for survival in the fly [124]. In humans, the Swr1-related complexes are SRCAP and p400/Tip60. These have been implicated in exchanging H2A.Z–H2B at transcriptionally active loci [125,126].

ANP32E is a novel component of the human p400/Tip60 complex that is able to chaperone H2A.Z–H2B in vitro [127,128] and with roles in DNA damage response [129,130] (Table 1). Structural analysis reveals that a C-terminal region of ANP32E binds H2A.Z–H2B in a remarkably similar fashion to Swr1 (Fig 4E). Structural conservation, however, is not mimicked by functional conservation, as in vitro ANP32E removes H2A.Z–H2B from chromatin, rather than depositing or exchanging it [127–129]. In fact, loss of ANP32E in cells induces a 20% increase in H2A.Z at promoters, as well as de novo localization of H2A.Z at enhancers and insulators [127,128]. Overexpression of ANP32E also induces loss of H2A.Z [127], reinforcing an H2A.Z–H2B removal function. This function of ANP32E plays a key role in the DNA damage response, where it affects H2A.Z dynamics and DNA repair pathway choice [129,130]. The chaperoning of H2A.Z is thus a complicated process where binding modes have been maintained but functional dynamics seem to have diverged through evolution, possibly mediated by functions of chaperone domains outside the histone-binding region.

**Histone variants H2AX and macroH2A: elusive chaperones**

Despite the many connections between histone variants and chaperones, there remain several histone variants that are yet to be linked to a specific chaperone. For these variants, little is known about the underlying mechanisms of their chaperone-mediated deposition into and eviction out of chromatin. Two key examples of these variants are H2A.X and macroH2A. These and the many other variants perform functions that critically depend on their temporal and spatial deposition into chromatin. As such, it seems likely that future studies will uncover many pathways and players involved.

The H2A.X variant is involved in DNA damage signaling. Although stochastically distributed throughout the genome, its function at DNA damage sites becomes relevant in response to a specific post-translational modification. Compared to canonical H2A, this variant contains a short C-terminal extension that contains an important serine residue, Ser139 (Fig 2B). This residue is phosphorylated in response to DNA damage, creating γH2A.X, and initiating a repair cascade [131,132]. γH2A.X also plays a role in meiosis, where it is required for deposition of H3.3 [133]. In yeast, H2A.X is absent, but canonical yeast H2A contains a similar C-terminal extension with a serine residue (Ser129) [134,135] (Fig 2B). In mice, H2A.X is not essential, but knockout does cause chromosomal instability, sensitivity to radiation, growth retardation, immunodeficiency, and male infertility [136]. Specific chaperones for H2A.X and/or γH2A.X have not been identified. FACT can exchange γH2A.X, as well as other H2A isoforms [137]. Biochemical and structural details of a potentially specific interaction with this variant are outstanding.

The macroH2A variant is localized primarily on the inactive X chromosome in mammals [138]. It plays roles in both autosomal gene activation and silencing, and acts as a tumor suppressor [7,139–142]. MacroH2A has a divergent histone fold region and contains a C-terminal macrodomain, which causes the macroH2A–H2B molecular weight to be double that of H2A–H2B (Fig 2B). Nucleosomes containing the histone fold of macroH2A resemble canonical nucleosomes, while the macrodomain is thought to protrude outward from the nucleosome, potentially hindering binding of some ligands [21]. The linker region between the histone fold and macrodomains also stabilizes the wrapping of DNA, promoting chromatin condensation [143].

It is important to note that the macrodomain is not exclusive to macroH2A and is found in a number of other, non-histone proteins. It often confers binding to monomers or polymers of ADP ribose [144]. ADP-ribose chains are the product of ART (also known as PARP) enzymes, and recently, auto-modified versions of PARP1 were shown to have histone chaperone activity [145]. While intriguing, a direct link between PARP1 chaperone activity and macroH2A has not yet been shown. Another candidate for macroH2A chaperone is APLF.
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APLF binds to macroH2A–H2B in vitro and in vivo, and its depletion reduces macroH2A levels at sites of DNA damage [146]. It is intuitive to predict that the presence of the macrodomain would facilitate specific interactions of this variant with chaperones and other factors, both within and outside of the nucleosome.

Outlook: regulation of histone variant dynamics by histone chaperones

Understanding how histone chaperones regulate histone variant dynamics has been challenging. Variant chaperone specificity has been mostly demonstrated by functional overlap using knockout/knockdown phenotype analysis in vivo, pull-down experiments with amino acid swap mutants and, in some cases, by the interconnected behavior in disease conditions. Nonetheless, in most cases, it still lacks a comprehensive description of the structural determinants of such specificities (Table 1). Recapitulating the in vivo observations in an in vitro setup has proven arduous.

Structural studies have been limited by the complexity of the histone chaperone proteins and they have often only focused on minimal histone-binding regions rather than full-length proteins, hindering the full understanding of chaperone structure and functions. Biochemical studies have shown that binding affinity differences between canonical histones and their variant counterpart are often small, rather than a clear cut, all-or-nothing effect. It seems plausible that on one side, such small affinity differences may be magnified in the cellular context, where relative concentrations of histone isoforms and mass action effects may play important roles. On the other end, this may also suggest a prime role for additional layers of regulation of histone chaperones themselves in vivo. Post-translational modifications, expression pattern, sub-cellular localization, and interactions with other nuclear proteins have all been observed as modulators of histone chaperone function, and these are all likely candidates for further regulating histone variant dynamics. In this light, the regulatory step in the deposition/eviction pathway may not be specificity in histone binding, but rather the context that drives the chaperone to act at a specific genomic site. This is exemplified by the fact that H3.3 is handled by both HIRA and DAXX, but at distinct genomic loci [56]. It is also interesting to consider that the lack of stringency in variant chaperone binding affinity may have evolved to sustain the redundancy that has been observed for chaperones in vivo. This redundancy may well have important functional roles in protecting cell homeostasis in disease conditions, where the specific histone–chaperone pairs are deregulated.

It is clear, however, that histone variants diversify chromatin signals in a temporally and spatially regulated manner. Variants are involved in a plethora of cell processes and are important agents in a cells epigenetic memory. Tight regulation of histone variant functions by histone chaperones is crucial for cell homeostasis and the prevention of tumorigenesis. The most compelling model is that a combination of factors determines a chaperone’s ability to influence the dynamics of a histone variant. Subtle structural and biochemical aspects are likely reinforced by the favorable timing of expression and localization for both the chaperone and histone variant. Integration of structural, biochemical, and in vivo studies will be instrumental in understanding the complex interplay between histone variants and their chaperones.

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Conflict of interest

The authors declare that they have no conflict of interest.

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