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Ascending the Nucleosome Face: Recognition and Function of Structured Domains in the Histone H2A-H2B Dimer

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Abstract

Research over the past decade has greatly expanded our understanding of the nucleosome's role as a dynamic hub that is specifically recognized by many regulatory proteins involved in transcription, silencing, replication, repair, and chromosome segregation. While many of these nucleosome interactions are mediated by post-translational modifications in the disordered histone tails, it is becoming increasingly apparent that structured regions of the nucleosome, including the histone fold domains, are also recognized by numerous regulatory proteins. This review will focus on the recognition of structured domains in the histone H2A-H2B dimer, including the acidic patch, the H2A docking domain, the H2B $\alpha 3$ - αC helices, and the HAR/HBR domains, and will survey the known biological functions of histone residues within these domains. Novel post-translational modifications and *trans*-histone regulatory pathways involving structured regions of the H2A-H2B dimer will be highlighted, along with the role of intrinsic disorder in the recognition of structured nucleosome regions.

Keywords

Histone H2A and H2B; acidic patch; nucleosome recognition; Sir3; post-translational modifications

1. Introduction

It has become increasingly clear that the nucleosome is not merely a static packaging mechanism for DNA, but instead serves as a dynamic hub for chromatin-regulated processes. Numerous proteins involved in these biological processes dynamically interact with specific residues or surfaces of the nucleosome [1–4]. These protein-nucleosome interactions often play key roles in regulating such cellular processes as transcription, DNA replication and repair, and chromosome segregation.

The interaction of regulatory proteins with covalently modified residues in the flexible histone tails have been extensively studied over the past decade [5–8]. Enzymes that alter the modification state of histone tail residues have also been extensively studied. These

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enzymes catalyze the acetylation, methylation, phosphorylation, poly-ADP-ribosylation, sumoylation, and ubiquitination of histone residues. Importantly, many 'reader' proteins have been identified that specifically recognize modified histone residues. Candidate reader proteins can be readily identified because they typically contain a putative modified histone interaction domain in their protein sequence. These domains include the PHD finger and chromodomains that typically recognize methylated lysine residues [5–7]; the Tudor domains that recognize methylated lysine or arginine residues [9]; and the bromodomains that recognize acetylated lysine residues [5,7,8].

A common feature of chromatin reader proteins is that their recognition domains typically bind to modified (or unmodified) histone residues located in the flexible and structurally disordered histone tails. Yet recognition of disordered protein sequences, such as the histone N-terminal tails, is often qualitatively different from the recognition of structured regions of proteins (see [10–14] for review). Hence, it is not yet clear whether the recognition paradigm derived from studies of histone tail modifications will also apply to recognition of histone residues in the structured core fold domains. In this review, we will describe our current knowledge of the functions of both modified and unmodified residues in the core fold domains of the histone H2A and H2B dimer, particularly focusing on the recognition of these residues by 'reader' proteins and the functions of these binding events.

2. Structural features of the histone H2A/H2B dimer in the Nucleosome

The nucleosome core particle is a disc-shaped structure with an interior core composed of an octamer of histone proteins, containing two copies each of the histones H2A, H2B, H3 and H4. Approximately 145 to 147 bp of nucleosomal DNA is wrapped around the outside of this protein core, forming 1.65–1.67 superhelical left-hand turns [15–18]. The N-terminal tails of the histones protrude out from the protein core and surrounding DNA, generally exiting adjacent to a minor groove in the DNA helix. In the *X. laevis* crystal structure, one of the histone H4 N-terminal tails contacts the H2A/H2B acidic patch on the adjacent nucleosome [15]; the potential significance of this interaction will be discussed in more detail in a subsequent section. The N-terminal tail regions that extend beyond the nucleosome core particle are largely disordered [10,11,19]. This intrinsic disorder presumably facilitates post-translational modification of histone tail residues, the recognition of modified (and unmodified) tail residues by 'reader' proteins, and the interaction of histone tails with adjacent nucleosomes in higher-order chromatin structures. In contrast, the central histone fold regions of the histone proteins adopt well-ordered and conserved structures that comprise the structured component of the nucleosome.

The histone fold regions of the histone H2A/H2B heterodimer have a roughly similar structure and mode of DNA binding as histone H3/H4. Both H2A/H2B and H3/H4 contact the DNA via their $\alpha 1$ - $\alpha 1$ helices and two L1–L2 loops [15,20], with histone H2A/H2B binding to superhelical locations (SHL) 3 to 6 and -3 to -6 [15]. However, histone H2A and H2B contain structured regions outside the histone fold domain that give rise to several unique features of the nucleosome. The histone H2A tail adopts a short "knuckle" α -helix as it exits adjacent to the minor groove at SHL $\pm 4 - 4.5$ [15,19], and residues in this helix contact the DNA [21]. In the nucleosome structure, histone H2A also contains a well ordered C-terminal extension, which includes the H2A 'docking domain', that buries significant surface area of the H3/H4 tetramer, potentially stabilizes the H3 α N helix, and eventually exits near the dyad axis [15,22,23] (Figure 1).

Histone H2B has a highly basic N-terminal extension that makes numerous contacts with both DNA gyres as it passes between them at SHL $\pm 4.5 - 5$ and SHL $\pm 2.5 - 3$. Histone H2B also has a highly ordered C-terminal α -helix (H2B α C) that is the most exposed feature of

the solvent exposed nucleosome face (Figure 1) and contains the major site of H2B ubiquitination (H2B K123 in *S. cerevisiae*, H2B K120 in human) [24,25]. A second unique feature of the nucleosome face is the H2A/H2B acidic patch, which encompasses residues from the H2A histone fold domain, H2A C-terminal extension, and the H2B α C helix (Figure 1). Finally, the H2B α C and α 3 helices form a stacked, inverted 'V' shape on the nucleosome face, which is separated from the LRS (Loss of rDNA silencing) domain [26,27] of the H3/H4 tetramer by a solvated cleft in the nucleosome face (Figure 1). Each of these structural features serve as potential recognition or binding sites for other proteins, as will be discussed below.

3. The H2A/H2B Acidic Patch regulates chromatin structure through inter-protein binding

In contrast to the largely basic nature of the core histones, the H2A/H2B acidic patch stands out due to its chemical uniqueness in the nucleosome. The acidic patch was first described by Luger et al. [15] after the 2.8 Å crystal structure was solved. Using the sequence of the *Xenopus* histones, the acidic patch consists of residues H2A E56, E61, E64, D90, E91, E92 and H2B E110. The residues in the acidic patch are all localized on the nucleosome surface and they are distributed within the central α 2 and the α C helices of H2A and the α C helix in H2B (Figure 1). Part of the acidic patch overlaps with the H2A docking domain that is important for interactions between H2A/H2B dimer and H3/H4 tetramer in the nucleosome [22].

An early clue to the function of the acidic patch came from the high resolution crystal structure of the nucleosome, where it was observed that there were trans-nucleosome interactions between the basic histone H4 N-terminal tail (residues K16 to N25) and the H2A/H2B acidic patch. This interaction is the most likely candidate to explain earlier reports that higher order chromatin structure depended on the presence of the H3 and H4 tails [28,29]. For instance, Dorigo et al. [30] showed that H4-V21C and H2A-E64C mutant residues can be reversibly cross-linked in nucleosomal arrays. Ionic interactions between H2A and H4 are critical for the stability of the trans-histone interaction surface since mutation of H4 K20 inhibited nucleosome crystallization [15]. Finally, there is evidence that these H4-H2A interactions could be regulated in cells since the reversible acetylation of H4 K16 disrupts chromatin compaction [31].

In the last six years, five chromatin-associated proteins have been shown to bind to the H2A/H2B acidic patch. The first was the N-terminus of the latency-associated nuclear antigen (LANA) from the Kaposi's sarcoma-associated herpes-virus. LANA is responsible for the association of the viral genome with mitotic chromosomes, and the N-terminal basic region is solely responsible for this activity. The crystal structure of the LANA peptide-nucleosome complex showed that there were specific interactions between two arginine residues in LANA and residues H2A E61, D90, and E92 in the acidic patch [32]. Thus, even though there is no homology between the H4 tail and the LANA peptide, both proteins bind to the same region of the nucleosome and can serve as functional mimics—for example, the LANA peptide can partially compensate for the loss of the H4 N-terminal tail in nucleosome condensation assays [33]. The latest proteins included in the H2A acidic patch binding family are IL-33 [34], RCC1 (regulator of chromatin condensation) [17], HMGN2 (high mobility group nucleosomal) [35], and the Sir3 bromo-adjacent homology domain [36]. Although the LANA peptide and IL-33 share a common H2A/H2B acidic patch binding motif (MXLRSG [34,37]), other protein regions that bind to the H2A/H2B acidic patch lack this motif. While there is limited sequence homology between the acidic patch binding domains of RCC1, HMGN2, histone H4, and LANA, their interactions with H2A/H2B are stabilized through electrostatic interactions between two arginine side chains and residues

E61, E64, and D90–E92 [35]. Looking ahead, it is almost certain that additional proteins will be identified that rely on association with the H2A-H2B acidic patch for their function.

In higher eukaryotes, there is a whole family of H2A-related proteins that regulate chromatin structure and function [22]. In most of these alternative H2A isoforms, there are significant changes in the acidic patch. For instance, in the H2A.Z nucleosome there is a slight extension of the acidic patch compared to the canonical H2A nucleosome [38], whereas in histone H2A.bbd the H2A acidic patch is nearly eliminated due to the presence of amino acid substitutions [22]. In the case of H2A.Z, the acidic patch is clearly important for the function of this isoform—for instance, residues in H2A.Z acidic patch regulate SWR1-mediated H2A.Z/H2B incorporation in yeast [39], and these same residues are critical for proper *Drosophila* development [40]. Verification that the H2A.Z/H2B acidic patch participates in protein-protein interactions comes from a recent crystal structure of this histone dimer in complex with the Chz1 histone chaperone [41]. The ability of IL-33 to bind to canonical H2A, H2A.Z, and H2A.bbd was recently tested, and indeed removal of the acidic patch in H2A.bbd eliminates association with IL-33, whereas the binding efficiency of H2A.Z was essentially identical to H2A [34]. Collectively, these studies show that the regulation of protein-protein interactions mediated by the acidic patch may be an important function of alternative H2A isoforms.

To conclude this section, we note that there are hints in the literature that the acidic patch may play a role in DNA repair and replication pathways. Recent studies on comprehensive yeast libraries generated using alanine substitution at all residues in the canonical histones have shown that very few residues in yeast are essential [42]. Noteworthy is the fact that of the four essential residues in yeast H2A, two are found within the acidic patch (the yeast equivalents of E61 and D90) [42]. When challenged by hydroxyurea, a chemical that induces replication stress in yeast by depleting S-phase nucleotide pools, alanine mutants at sites equivalent to H2A E56, E64, and E92 all show lower survival compared to wild type. H2A E64 is also potentially important for proper transcriptional elongation, since its mutation to alanine makes yeast sensitive to 6-azauracil (6AU). With regards to DNA repair defects, sensitivity to methylmethane sulfonate (MMS) was observed in yeast for alanine mutants at sites equivalent to H2A E56, H2A E64, H2A E92, and H2B E110 alanine mutants (e.g., yeast H2A E57A, H2A E65A, H2A E93A, and H2B E116A) [42]. Combining these observations with the emerging paradigm that many proteins that regulate chromatin structure physically associate with the H2A/H2B acidic patch leads to the speculation that DNA replication and repair factors will be identified in the near future that rely on binding to this nucleosome region for their proper function.

4. *trans*-Regulation of histone post-translational modifications by unmodified residues in the H2A C-terminal docking domain

A number of histone modifications are regulated by the presence (or absence) of other histone modifications via so-called *trans*-histone regulatory pathways. The first and best studied example of *trans*-regulation of histone modifications is the regulation of histone H3 K4 and K79 di- and tri-methylation by histone H2B ubiquitination (reviewed in [24,25,43]). It is becoming increasingly evident that unmodified histone residues can also play important roles in regulating histone post-translational modifications. For example, histone H4 basic patch residues (17-RHR-19) are required for H3 K79 methylation because these basic residues interact with a complementarily charged acidic patch region in the Dot1 histone methyltransferase [44,45].

Recent studies in *S. cerevisiae* have identified a region of the nucleosome comprised of residues from histone H2A, H3, and H4 that is required for the Set2 histone

methyltransferase to di- and tri-methylate histone H3 K36 [46,47]. These structured residues in histones H2A, H3 and H4 appear to be unmodified *in vivo*, but have important roles in the nucleosome recognition and enzymatic activity of Set2. Of particular importance is residue K44 in histone H4 [46]. Mutation of histone H4 K44 to glutamate or glutamine (i.e., H4 K44E or H4 K44Q) disrupts Set2 di- and tri-methylation of its H3 K36 substrate *in vivo*. Moreover, introduction of an H4 K44Q mutation in recombinant nucleosomes abolishes H3 K36 methylation catalyzed by the human Set2 homolog NSD2 *in vitro* [48]. Intriguingly, the sequence adjacent to H4 K44 closely mimics the sequence surrounding the H3 K36 substrate. Indeed, the human Set2 homolog NSD2 methylates H4 K44 *in vitro* in the context of a DNA-free histone octamer, but adding DNA suppresses this reaction [48]. Although H4 K44 can be recognized and methylated by the SET domain, at least *in vitro* [48], in yeast an N-terminal acidic region in Set2 (31-DQEPDLTEE-39) likely recognizes and interacts with H4 K44 [46]. Curiously, while the H4 K44Q and H4 K44E mutants disrupted methylation of H3 K36 by Set2 and NSD2, an H4 K44A mutant has little to no effect on H3 K36 methylation *in vivo* or *in vitro* [46,48]. The origin of these distinct H4 K44 mutant phenotypes is not yet clear.

A recent study indicates that the Set2 nucleosome recognition surface also includes H2A residues L116 and L117 [47]. These residues are located in the histone H2A C-terminal docking domain (Figure 1) and are within 5 Å of H4 K44. Mutation of H2A L116 and L117 causes a significant decrease in H3 K36 tri-methylation and, to a lesser extent, di-methylation in yeast chromatin. Indeed, the H2A L116 and L117 double mutant compromises the Set2-Rpd3S pathway, leading to a decrease in Set2-catalyzed H3 K36 methylation (*in vivo* and *in vitro*), an increase in histone H4 acetylation, and up-regulation of cryptic transcripts at the *STE11* gene [47].

The authors of this study also identified additional residues in histone H2A (i.e., H2A I112, H113, and P118) and histone H3 (H3 R52, N108, I112, and to a lesser extent V117) that, when mutated, affected Set2-catalyzed tri- and di-methylation of H3 K36. These residues are relatively close to H4 K44 in the nucleosome structure [47], and could potentially comprise the nucleosome recognition surface bound by residues 31–39 in Set2. It is important to note that a histone H2A peptide containing residues 107–131 did not bind to Set2, unlike the corresponding H4 peptide containing H4 K44 [47]. This result suggests that Set2 cannot recognize the linear H2A C-terminal amino acid sequence alone; however, Set2 may recognize these histone H2A residues when they become structured in the context of the nucleosome. Alternatively, these histone H2A and H3 residues could affect Set2 activity independent of its binding to the nucleosome. Such a model has been proposed in a different study, in which it was shown that histone H3 N-terminal residues also regulate Set2 methylation, but are not required for Set2 binding to the nucleosome [49]. It will be important to further elucidate the molecular mechanisms by which Set2 recognizes its H3 K36 substrate in the context of the nucleosome.

5. Recognition of the H2B α C helix by mediators of H2B ubiquitination

The histone H2B sequence contains a unique α -helix in its C-terminal domain that is well ordered and exposed on the nucleosome face (Figure 1). This helix is the major site of histone H2B ubiquitination (H2B K123Ub in *S. cerevisiae*), which in yeast is catalyzed by the E2 ubiquitin-conjugating enzyme Rad6 and the E3 ubiquitin ligase Bre1 [50–52]. Histone H2B K123 ubiquitination levels are also regulated by the ubiquitin-specific proteases Ubp8p, which is a stable component of the SAGA (Spt-Ada-Gcn5-Acetyltransferase) transcriptional coactivator complex [53,54], and Ubp10p, which is localized to, and functions primarily within, silenced regions of the genome [55,56].

Histone H2B K123Ub has special historical importance because it was the first and best studied example of trans-regulation of histone modifications. In 2002, it was shown that H2B K123Ub is required for subsequent di- and tri-methylation of histone H3 K4 and K79 by the Set1/COMPASS histone methyltransferase complex and the Dot1 histone methyltransferase, respectively [57–60]. Histone H2B ubiquitination has important roles in regulating gene expression, silencing, the DNA damage response, and chromatin structure, as discussed in several recent reviews [24,25,61,62].

Of particular interest are the mechanism(s) by which H2B K123, and its adjacent residues, is recognized by multiple proteins (e.g., Rad6/Bre1, Ubp8, Ubp10, COMPASS, and Dot1). Recent studies in *S. cerevisiae* have shed light on these questions [63,64]. The Shilatifard group screened a library of single alanine mutants in each yeast core histone protein for effects on H3 K4 methylation [63]. Along with a numerous residues located in the H3 N-terminal tail (i.e., H3 R2, T3, Q5, and K14), a number of residues in histone H2A and H2B were found to be required for normal levels of H3 K4 di- and/or tri-methylation. The histone H2B residues (H2B H112, R119, K123) are all located in the H2B α C helix (Figure 2). Intriguingly the H2A residues required for H3 K4 methylation (i.e., H2A E65, L66, N69, D73; Figure 2) form a patch adjacent to, and partially overlapping with, the acidic patch (yeast H2A E65 is in common).

A subset of the histone mutants also affected H2B ubiquitination levels (H2A L66 H2B H112, and of course H2B K123), suggesting that these residues likely comprise a nucleosome surface that is recognized by Rad6/Bre1 (Figure 2), and thus affect H3 K4 methylation indirectly. In contrast, the other H2A and H2B residues (i.e., H2A E65, H2A N69, H2A D73, or H2B R119) appear to affect H3 K4 di- and/or tri-methylation independently of H2B ubiquitination (Figure 2).

A subsequent study by the Sun laboratory confirmed that the H2B R119A mutant affects H3 K4 tri-methylation independent of H2B ubiquitination [64]. Moreover, they found that H2B R119A and/or H2B T122D mutants increased H2B ubiquitination levels, while at the same time decreasing H3 K4 tri-methylation. The increase in H2B ubiquitination was due to an apparent defect in the binding and/or activity of Ubp8 and Ubp10, suggesting that these H2B residues comprise part of the Ubp8/Ubp10 recognition site. The same mutations (H2B R119A and/or H2B T122D) also caused a defect in the binding of the COMPASS subunit Spp1 (and potentially other COMPASS subunits) to chromatin [64]. Since the Spp1 subunit is specifically required for H3 K4 trimethylation by COMPASS [65,66], this could explain the concomitant decrease in H3 K4 tri-methylation in the H2B R119A and H2B T122D mutant strains.

Taken together, these results indicate that charge-altering mutations in the H2B α C helix simultaneously disrupt the binding and activity of the Ubp8 (and Ubp10) deubiquitinase and the Set1/COMPASS complex. These overlapping binding sites on the H2B α C helix may suggest that it is unlikely that Ubp8/SAGA and COMPASS bind the same nucleosome, or at least recognize ubiquitinated H2B, simultaneously. It is interesting to note that the H2B R119A and H2B T122D mutations do not affect Dot1-catalyzed H3 K79 methylation, indicating that the Dot1 binding site in the nucleosome is likely to be distinct from that of Ubp8 or Spp1/COMPASS.

Finally, a recent study has discovered that H2B S112 in human histone H2B can be post-translationally modified by the addition of β -N-acetyl-glucosamine [67]. This post-translational modification appears to regulate human H2B K120 ubiquitination in vivo [67], thus describing a potentially new regulatory mechanism involving the H2B α C helix.

6. Novel residues in the H2B α 3- α C helices regulate gene silencing and nucleosome recognition by the Sir3 Bromo-Adjacent Homology (BAH) domain

The histone H2B α 3 helix forms an inverted 'V' shape with the H2B α C helix (Figure 1). As discussed previously, this pair of H2B helices comprises a unique structural feature that is located between the H2A/H2B acidic patch and the H3/H4 LRS domain on the nucleosome face. Two recent studies have identified several functional residues in the H2B α 3 helix that regulate gene silencing and longevity in *S. cerevisiae* [68,69]. A study from our lab found that an alanine mutation in H2B R102 enhances Sir4 binding and silencing of reporter genes at yeast telomeres [68]. Furthermore, chromatin immunoprecipitation (ChIP) studies indicated that the H2B R102A mutant causes spreading of Sir4 (and presumably the other subunits of the SIR complex) up to 12–14 kb into the chromosome from telomere V-L. Enhanced SIR binding and spreading presumably is responsible for the significant decrease in expression of endogenous genes located adjacent to yeast telomeres [68]. We also observed that the H2B R102A mutant enhanced Sir4 binding to and silencing of the *HML* silent mating locus. In contrast, mutation of the H2B K111 residue, which is located in the H2B α C helix, decreased telomeric silencing and diminished Sir4 binding to telomeres and *HML* locus.

A study by the Boeke laboratory also found that the H2B R102A mutant enhanced silencing and Sir2 binding to yeast telomeres [69]. Intriguingly, they found that the H2B R102A mutant completely abolished silencing at the yeast rDNA locus [69]. They also found that the H2B R102A mutant caused a significant increase in recombination rates at the rDNA locus, to the level of rDNA recombination observed upon deletion of *sir2*. Increased rDNA recombination in the H2B R102A mutant led to an accumulation of extrachromosomal rDNA circles (ERCs) and a subsequent decrease in replicative lifespan. In contrast, mutation of H2A R95, which like H2B R102 is located in the H2B α 3 helix (Figure 3A), abolished telomeric silencing and decreased Sir2 binding to telomeres, but enhanced silencing at the rDNA locus. Hence, like H2B R102 and K111, the adjacent H2B R95 and R102 residues have opposing effects on silencing in yeast (Figure 3A). Recent studies have found that some mutants (e.g., dot1) can alter the readout of silencing reporter genes without significantly affecting endogenous telomeric silencing [70,71]. However, this is unlikely to be an issue with the H2B R95 and R102 mutants, as microarray data indicate that both mutants affect the silencing of endogenous genes adjacent to yeast telomeres [68,69].

Multiple genetic and biochemical studies [27,72–76] have indicated that Sir3 recognizes nucleosomes in part through its Bromo-Adjacent Homology (BAH) domain (Sir3 residues 1–214), which was thought to interact in large part with the H3/H4 LRS domain residues. A docking model of the Sir3 BAH domain bound to the nucleosome [72] suggested that H2B R95, R102, and K111 may potentially interact with the Sir3 BAH domain upon its binding to the nucleosome, although it was not completely clear why H2B R95 and K111 mutants decreased silencing and SIR binding to telomeres, while the H2B R102 mutant enhanced silencing and SIR binding to telomeres.

Recently, the crystal structure of the yeast Sir3-D205N BAH domain bound to the *Xenopus* nucleosome has been published [36]. In the structure, the Sir3 BAH domain binds to an extensive nucleosome surface that includes the structured domains of multiple histones, including residues in the H2B α 3- α C helices, the H3/H4 LRS domain, and, surprisingly, the H2A/H2B acidic patch. The Sir3 BAH domain also makes extensive contacts with the H4 N-terminal tail, including the H4 K16 residue that is critical for silencing.

Many of the histone residues that interact with the Sir3 BAH domain in the crystal structure have been previously shown to regulate silencing in yeast, including residues in the H4 N-terminal tail, the H3/H4 LRS domain, and the H2B α 3- α C helices [36]. Indeed, the *Xenopus* histone residues H2B R96 and K105 (H2B R102 and K111 in yeast) make contacts with Sir3 BAH domain [36], while H2B R89 (H2B R95 in yeast) appears to hydrogen bond with residue N80 in Sir3. Other H2B α C residues also make polar contacts with the Sir3 BAH domain (e.g., *Xenopus* H2B Q92, H2B S109, and H2B T112), although it is not known whether these histone H2B residues regulate silencing in yeast. However, it remains unclear how the yeast H2B R102A mutant would necessarily enhance telomeric gene silencing and SIR complex binding. Future studies are needed to resolve this issue, as well as the role of this and other residues in the H2B α 3 helix residues in regulating rDNA silencing.

One of the surprises from the structure is that a “poorly ordered” region of the Sir3 BAH domain encompassing loop 1 and helix A0 (Sir3 residues 17–37) apparently binds to the H2A/H2B acidic patch [36]. Much of this region is missing from the crystal structures of the Sir3 BAH domain alone [77,78], indicating that it is likely to be intrinsically disordered. This hypothesis is further supported by bioinformatics analysis (Figure 3B) using the PONDR-FIT disorder predictor [79]. One possible hypothesis is that the intrinsic disorder of this region of the Sir3 BAH domain may facilitate its docking to the nucleosome face.

Moreover, intrinsically disordered proteins are thought to bind with lower affinity due to the entropic cost of the disorder-to-order transition upon binding [12–14]. A recent review suggested a ‘Goldilocks’ model of silencing by the Sir3 protein, in which Sir3 binding affinity to the nucleosome could be neither “too tight” or “too loose” for proper metastable silencing of yeast telomeres [27]. This review suggested that the “too tight” binding affinity was avoided because of unfavorable electrostatic interactions between the Sir3 BAH domain and the nucleosome (e.g., between H3 D77 and Sir3 D205 [36]). However, it is also possible that the potential entropic cost of nucleosome binding by intrinsically disordered regions of the Sir3 BAH domain may also prevent the “too tight” binding scenario, and thus ensure metastable silencing of yeast telomeres. A potential caveat to this model is that it is unclear whether the Sir3 loop1-A0 region becomes fully ordered upon nucleosome binding, since this region of the Sir3 BAH domain yielded weak and discontinuous electron density in the crystal structure of the Sir3 BAH domain-nucleosome complex [36]. Hopefully, future experiments will determine the potential role of intrinsically disordered regions in Sir3 in nucleosome binding and silencing.

7. The histone H2A repression (HAR) domain affects histone H2B ubiquitination and condensin binding

Previous studies identified a sequence cassette comprised of residues 16–20 in yeast histone H2A that played a role in gene repression and the response to UV damage [80,81]. For these reasons this cassette was named the histone H2A repression (HAR) domain. Alanine-scanning mutagenesis of this domain identified the HAR residues H2A S17 and R18 as being required for repression of the *BNA2* reporter gene, and an H2A S17A,R18A double mutant recapitulated the de-repression of the *BNA2* reporter [80]. Residues in the HAR cassette contact the DNA minor groove at superhelical location $\pm 4 - 4.5$ in the nucleosome structure [15,20]. A high resolution *Xenopus* nucleosome structure [21] revealed that H2A R17 (homologous to H2A R18 in yeast) hydrogen bonds with the DNA phosphodiester backbone. These structural features and other data suggest that the HAR domain may repress transcription by enhancing nucleosome occupancy in yeast promoter regions [81].

Residues in the HAR domain are also located in close proximity to the H2B C-terminal helix, which is the site of H2B ubiquitination. Intriguingly, a recent paper has shown that the HAR cassette, particularly residues S17 and R18, is required for histone H2B ubiquitination in *S. cerevisiae* [82]. Histone H2A N-terminal deletions that eliminated the HAR domain (i.e., H2A Δ 4–20, H2A Δ 12–20, and H2A Δ 16–20) or mutated residues in this domain (i.e., H2A S17A R18A) significantly decreased H2B ubiquitination. A similar decrease was observed in H3K4me3 in these HAR mutant strains. Furthermore, there was a significant association between the gene expression changes in the H2A N-terminal deletion mutant (H2A Δ 4–20) and the expression changes in mutants that obviate histone H2B ubiquitination (H2B K123R, rad6 Δ , or bre1 Δ) or H3 K4me3 (e.g., set1 Δ) (unpublished studies and [82]).

Importantly, the defect in H3K4me3 levels could be rescued by deleting the histone H2B deubiquitinases ubp8 and ubp10, which restore high H2B ubiquitination levels in the histone H2A mutant strains [82]. This result indicates that the defect in H3K4me3 in the H2A mutant strains was likely a downstream consequence of decreased H2B ubiquitination. Yet, paradoxically the H2A N-terminal deletion mutants did not disrupt recruitment of Rad6 E2 ubiquitin conjugase to the *GAL1* reporter gene. The authors suggested that HAR domain may not be required for the chromatin association of Rad6, which is likely mediated by interactions with transcriptional activator proteins and with elongating RNA polymerase II complex [83–85]. Instead, the HAR domain may be required for proper binding of Rad6 and Bre1 to the nucleosome and recognition of the H2B K123 substrate following recruitment of Rad6 and Bre1 to chromatin [82]. Perhaps the HAR domain and the H2B α C helix represent a bipartite nucleosome interaction surface for Rad6 and/or Bre1, similar to the multipartite nucleosome surface that is required for Set2 activity. Alternatively, the HAR domain may be required for Rad6 or Bre1 enzymatic activity, or it may regulate the binding the Ubp8 and Ubp10 deubiquitinases (e.g., HAR mutants could enhance the binding of Ubp8 and/or Ubp10).

A recent study has indicated that residues in the HAR domain may also serve as a binding or recognition site for the condensin complex [86]. The condensin complex plays important roles in the condensation and proper segregation of chromosomes during cell division [87,88], yet the mechanism by which condensin is recruited to chromosomes has yet to be fully elucidated. The study by Watanabe and colleagues identified the N-terminal tails of histone H2A and H2A.Z as likely “chromatin receptors” of the condensin complex [86]. The condensin I kleisin subunit (Cnd2 in *S. pombe*; CAP-H in humans) specifically recognized histone H2A and H2A.Z in vitro, and depletion or mutation of these histones impaired condensin localization. Note that this interaction was specific to the condensin I complex (i.e., CAP-H2 in condensin II did not interact with histone H2A) and required phosphorylation of the kleisin subunit by Aurora kinase [86]. Mutational studies of histone H2A in *S. pombe* indicated that the mutation of three residues (H2A K13, R18, and K21) to alanine abolished the Cnd2-histone H2A interaction. Of these, the HAR domain residue H2A R18 appeared to be most important for Cnd2 binding [86].

A key question arising from each of these studies is whether histone H2A residues within, or immediately adjacent, to the HAR domain, directly interact with Rad6, Bre1, or Cnd2/CAP-H. Examination of the nucleosome structure suggests that H2A R18 residue is likely to be somewhat inaccessible for binding, at least in the canonical nucleosome structure. A possible resolution of this conundrum is that this region of histone H2A may dynamically alter its conformation to become transiently more solvent exposed and thus accessible for binding. A similar model has been put forward as a potential explanation of how the H2A R18 residue in *S. cerevisiae* regulates H2B ubiquitination [82]. In this case, it was suggested that RNA polymerase II passage could trigger transient solvent exposure of H2A R18 [82].

If a similar mechanism is operating to facilitate condensin binding to histone H2A, what triggers the increased solvent exposure of H2A R18 (and potentially H2A K21) to facilitate condensin binding? It will also be important to determine whether condensin binds to similar histone H2A and H2A.Z residues in other species. The study from the Watanabe laboratory suggests that the human condensin subunit CAP-H interacts with a similar region of human histone H2A [86]. This suggests that the role of this histone H2A region in condensin recruitment is likely to be conserved; however, future studies will be required to rigorously test this hypothesis.

8. Post-translational modifications in the histone H2B repression domain

The histone H2B repression (HBR) domain was initially identified in the same genetic studies as the HAR domain [81,89], and is located in close proximity to the HAR domain in the nucleosome structure. Deletion of the HBR domain (H2B Δ 30–37 in yeast) causes a striking de-repression of a large fraction of the yeast genome [90,91], and renders cells more sensitive to UV irradiation [90,91]. This repression function may be due to the strategic location of the HBR domain in the nucleosome structure. The HBR domain is located between the DNA gyres, and in the *Xenopus* nucleosome structure (in which the entire HBR domain is visible [15]) multiple residues in the HBR domain make contacts with the DNA. Moreover, H2B R36 in yeast (H2B R30 in *Xenopus*) is one of the few arginine residues that insert into the minor groove of the DNA. These observations suggest that the HBR domain may repress transcription by affecting nucleosome positioning and stability. A study by Smerdon and colleagues has indicated that the H2B Δ 30–37 mutant enhances DNA accessibility at a variety of loci (*HML*, *GAL1–10*, *RPB2*) in the yeast genome [91], in accordance with this hypothesis.

However, the HBR domain may also regulate gene expression through an alternative mechanism mediated by post-translational modifications of residues in, or immediately adjacent to, the HBR domain (Figure 4). For example, it was recently discovered that human H2B K34 (equivalent to yeast H2B K37) is ubiquitinated in human cells by the RING finger domain-containing protein MSL2 [92]. MSL2, which is a subunit of the conserved MOF-MSL histone acetyltransferase complex, can function as an E3 ubiquitin ligase to primarily ubiquitinate H2B K34 in vitro and in vivo [92]. Moreover, MSL2-mediated H2B K34 ubiquitination stimulates the methylation activity of MLL (of H3 K4) and DOT1L (of H3 K79) on recombinant nucleosomes in vitro, and depletion of MSL2 from HeLa cells results in a concomitant decrease in the cellular levels of H3 K4me3 and K79me2 [92]. MSL2 depletion also causes a decrease in the H2B K120 ubiquitination in human cells, suggesting that there is likely crosstalk between these two ubiquitination sites. However, rescue of H2B K120 ubiquitination levels (by RNF20/RNF40 overexpression) does not completely rescue H3 K4me3/H3 K79me2 levels, suggesting that MSL2 ubiquitination of H2B K34 may directly regulate methylation of these histone H3 residues.

Intriguingly, *Drosophila* dMSL2, which is a subunit of the MOF-MSL dosage compensation complex, can also ubiquitinate histone H2B in vitro, suggesting this modification may play role in X chromosome dosage compensation [92]. Histone H2B K34 ubiquitination, as well as other novel H2B ubiquitination sites, had been previously identified in mouse brain tissue [93]. However, this modification pathway does not appear to be conserved in *S. cerevisiae*, as yeast H2B K37 ubiquitination has not been detected, and mutation of the corresponding H2B residue (H2B K37A) does not affect H3 K4 or H3 K79 methylation levels in yeast [63,94]. Histone H2B K37 is di-methylated in *S. cerevisiae*, although both the function of this modification and the enzyme responsible for catalyzing this methylation has yet to be elucidated [94]. Other studies have detected formylation and crotonylation of human H2B

K34 [95], indicating that this histone residue can be targeted by multiple post-translational modifications.

Like the rest of the HBR domain, human H2B K34 is located in a relatively inaccessible location in the nucleosome structure (Figure 1). Ubiquitination, methylation, or other post-translational modifications of this residue in chromatin would presumably require significant remodeling of the nucleosome structure, including potential displacement of the adjacent nucleosomal DNA. Ubiquitination of this residue, in particular, would likely result in a significant alteration in the nucleosome structure to accommodate the ubiquitin moiety at this location. It was noted that MSL2-catalyzed H2B ubiquitination was significantly diminished for nucleosome substrates relative to free histone H2B [92], which may be due to the relative inaccessibility of the H2B K34 residue in nucleosomes. It will be important to determine the prevalence of these HBR modifications in chromatin relative to free histones, and to elucidate the potential mechanism(s) by which this histone residue could be made accessible to histone modifying enzymes.

Intriguingly, a serine residue immediately adjacent to the HBR domain (mouse and human H2B S36) is also post-translationally modified (Figure 4). A recent study discovered that adenosine monophosphate activated kinase (AMPK) phosphorylates H2B S36 in mammalian cells [96]. AMPK is a key regulator of energy homeostasis in eukaryotic cells by sensing levels of AMP relative to ATP in the cell [97]. The study by Berger, Jones, and colleagues found that AMPK is recruited to chromatin by transcription factors such as p53, and there phosphorylates H2B S36 [96]. Introduction of a histone H2B S36A mutant in mouse embryonic fibroblasts decreased expression of p53 target genes, as well as cell viability in response to external stressors [96]. Taken together, these results highlight the biologically important role of histone H2B S36 as an AMPK substrate. The histone H2B S36 residue is located in a relatively inaccessible location in the nucleosome structure [98]. While AMPK can phosphorylate free histone H2B or H2B peptides [96], it is not yet clear whether AMPK can phosphorylate histone H2B in the context of the nucleosome in vitro, or if accessory factors are required for AMPK to recognize the H2B S36 substrate.

One possible model is that perturbation of the nucleosomal DNA during RNA polymerase II transcription (or pausing) could render the H2B S36 substrate more accessible to AMPK phosphorylation. This model could explain the high levels of H2B S36 phosphorylation detected at transcribed regions of p53 activated target genes [96]. Alternatively, AMPK could phosphorylate free histone H2B [98], which would then be dynamically exchanged into chromatin to stimulate (or accompany) active transcription of the AMPK target genes. Future studies will be needed to discern whether either of these hypotheses can explain how AMPK phosphorylates histone H2B in vivo. Since H2B S36 can also be modified by the addition of β -N-acetyl-glucosamine [99], it will be important to characterize the potential interplay between these two distinct post-translational marks, as well as their abundance in chromatin.

9. Conclusions

It is clear that a diverse set of functionally important proteins interact with structured regions of the nucleosome, including unique structural features of the histone H2A and H2B dimer (Figure 5). Further, it is likely that the examples discussed in this review encompass only a small portion of the proteins that recognize the structured domains of the nucleosome in vivo. However, based on these initial examples, one can begin to draw conclusions about common mechanisms of nucleosome recognition. First, unlike other histone reader proteins that often share common binding domains (e.g., chromodomains or bromodomains), it appears that proteins that recognize the nucleosome face do so via largely distinct protein

sequences. For example, the LANA peptide, the histone H4 N-terminal tail, the RCC1 switchback loop, and the Sir3 BAH domain loop 1-A0 region all bind to the H2A/H2B acidic patch (Figure 5), although they do not seem to share any significant sequence similarity.

A second emerging theme is that nucleosome recognition surfaces are often multipartite, and often include regions from multiple histones as well as the nucleosomal DNA. For example, the Sir3 BAH domain interacts with residues in histone H2A, H2B, H3, and H4, while Set2 likely interacts with residues in histone H2A, H3, and H4. These multipartite recognition motifs could provide a mechanism to specifically recognize nucleosomes as opposed to free histone dimers or tetramers. In contrast, many reader proteins that bind to modified histone tail residues interact with amino acids from a single histone protein that are immediately adjacent to the histone modification site.

Some nucleosome recognition motifs may even require alterations in the canonical nucleosome structure. For example, the recognition of HAR or HBR domain residues may occur in structurally altered nucleosomes, which could occur, for example, during the process of RNA polymerase II transcription.

Finally, there are hints that proteins may use intrinsically disordered sequences to bind to the nucleosome face. For instance, many of the proteins that bind to the H2A/H2B acidic patch (e.g., RCC1, Sir3 BAH, histone H4 N-terminal tail) appear to do so using protein regions that are intrinsically disordered or “conformationally diverse” in the absence of binding [10,17,36,100]. Such intrinsic disordered regions could provide the flexibility needed to dock with the structured nucleosome face. Or, as discussed above for the Sir3 BAH domain, intrinsically disordered regions may be used to limit the binding affinity to the nucleosome, which in this particular example may be important for proper regulation of gene silencing. Since a recent study indicates that a significantly high fraction of chromatin remodeling proteins contain regions of intrinsic disorder [101], perhaps this mode of nucleosome recognition may be more common than has been previously appreciated.

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Highlights

- Reviews protein recognition of structural features in the histone H2A-H2B dimer.
- Describes novel post-translational modifications and trans-histone regulatory pathways involving the histone H2A-H2B dimer.
- Discusses the role of intrinsically disordered regions in recognizing structured histone regions.

Fig. 1.

Location of key structural features of the Histone H2A/H2B dimer in the nucleosome structure. The *Xenopus* nucleosome structure (1aoi [15]) was drawn using POLYVIEW-3D [102]. Histone H2A is colored magenta; histone H2B is colored cyan. The approximate locations of various structural features are indicated with shaded circles.

Fig. 2.

Location of yeast (*Saccharomyces cerevisiae*) histone H2A and H2B residues that regulate histone H3 K4 methylation and H2B K123 ubiquitination. The yeast nucleosome structure (1id3 [103]) was drawn using POLYVIEW-3D [102]. Histone H2A is colored magenta; histone H2B is colored cyan. The histone H2A and H2B residues are color-coded based on the effects of mutants in these residues on histone H2B K123 ubiquitination (H2B-Ub) and H3 K4 tri-methylation (H3 K4me3). Data from references [63,64].

Fig. 3.

(**A**) Structural position of histone residues in the H2B α 3 and α C helices that regulate silencing and SIR complex binding in *Saccharomyces cerevisiae*. The yeast nucleosome structure (1id3 [103]) was drawn using POLYVIEW-3D [102]. (**B**) Predicted intrinsic disorder in the yeast Sir3 BAH domain (residues 1–213). Disorder predictions and output graph were generated using PONDR-FIT [79]. The Loop1-A0 region (residues 17–37) of the Sir3 BAH domain [36] is indicated in magenta.

		<i>Ub</i> <i>GlcNAc</i>
<i>H.sapiens</i>	...AQKKGKKRKRSRKESYSVYVY...	
		<i>Ub</i> <i>Ph</i>
<i>M.musculus</i>	...AQKKGKKRKRKRSRKESYSVYVY...	
		<i>Me</i>
<i>S.cerevisiae</i>	...STSTDGKKRSKARKETYSYIY...	
	<u>HBR domain</u>	

Fig. 4.

Alignment of histone H2B repression (HBR) domain and adjacent sequences from human, mouse, and yeast (*S. cerevisiae*) H2B. Previously identified post-translational modifications in this region of H2B are indicated (references are indicated in text). Abbreviations are as follows: *Ub* = ubiquitination; *GlcNAc* = β -N-acetyl-glucosamine; *Ph* = phosphorylation; and *Me* = methylation.

Fig. 5.

Summary of proteins that interact with different structural features of the histone H2A/H2B dimer. The *Xenopus* nucleosome structure (1aoi [15]) was drawn using POLYVIEW-3D [102]. Histone H2A is colored magenta; histone H2B is colored cyan. The approximate locations of various structural features are indicated with shaded circles, and the proteins that interact with these structural features are indicated. An asterisk (e.g., RCC1*) indicates the protein (or protein complex) is known to interact with other histones (H3 and H4) or the nucleosomal DNA, in addition to histone H2A and H2B.