CHEMICAL REVIEWS

Review

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Post-Translational Modifications of Histones That Influence Nucleosome Dynamics

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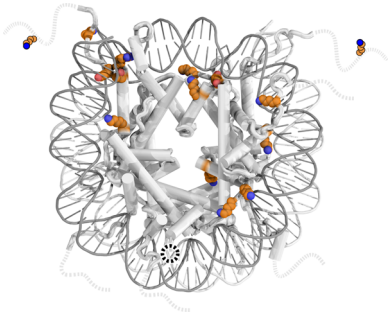

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1. INTRODUCTION

Nucleosomes are efficient DNA-packaging units. The fundamental protein unit of the nucleosome is the histone dimer, a simple α-helical domain possessing a highly basic, curved surface that closely matches the phosphate backbone of bent duplex DNA. Two copies each of histone heterodimer, H3/H4 and H2A/H2B, form a histone octamer that is wrapped with approximately 146 bp of duplex DNA in a left-handed spiral[1,2] (Figure 1). Through extensive electrostatic and hydrogen-bonding interactions, each histone dimer coordinates three consecutive minor grooves on the inner surface of the DNA spiral. The bending of DNA over the protein surface brings the phosphate backbone of the two strands closer together on the inside of the spiral, narrowing the major and minor grooves of DNA, while widening the grooves on the outside. This bent conformation of the DNA duplex, which would otherwise be energetically unfavorable, is maintained through charge neutralization from numerous arginine and lysine side chains of the histones.

A significant consequence of the intimate DNA wrapping around the histone core is that it sterically occludes other DNA-binding proteins. The inhibitory nature of this packaging is used by virtually all eukaryotic systems to regulate access to DNA. However, nucleosomes on their own are not static structures but dynamically fluctuate.[3,4] The most probable nucleosome state, captured in crystal structures, is the fully wrapped structure. However, only a small fraction of DNA–histone contacts need to be broken for the nucleosome to partially unwrap. Using restriction enzyme digestion kinetics, Polach and Widom[5] demonstrated that nucleosomes partially unwrap and rewrap spontaneously, which they termed site exposure. This behavior can be quantitatively defined as the site exposure equilibrium constant, K_{eq}, which is equal to the rate of DNA unwrapping that exposes a section of DNA for protein binding divided by the rate of rewrapping to states where the site is not accessible for binding. Values of site exposure K_{eq}

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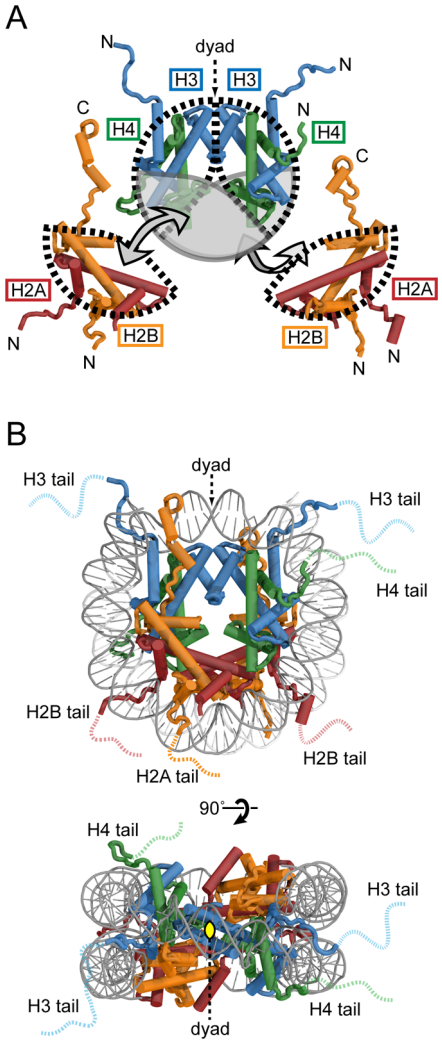

Figure 1. Overview of nucleosome architecture. (A) Illustration of H2A/H2B and H3/H4 heterodimers and how they fit together to form the histone octamer. (B) Face and top view of the nucleosome structure. For this and all subsequent molecular representations of the nucleosome, the high-resolution crystal structure (PDB code 1KX5) was used.[93]

range between 10^{-1} and 10^{-6} and correlate with the location of the DNA segment on the nucleosome, with more internal DNA segments being less accessible.[5−7] Rather than simply a binary regulator, where a DNA segment is either accessible or completely blocked, this dynamic unwrapping/rewrapping allows nucleosomes to regulate occupancy of DNA-binding proteins in a tunable, analog fashion.

In addition to DNA sequence, which influences both preferred nucleosome positioning and unwrapping characteristics,[6,8−10] two distinct mechanisms further modulate nucleosome stability and dynamics. One mechanism involves chemically altering the histones themselves, which changes the energy landscape of histone−DNA interactions and therefore greatly increases the dynamic range of DNA accessibility. These chemical changes can be in the form of post-translational modifications (PTMs) that can be dynamically added and removed enzymatically, with the best-studied modifications including acetylation, methylation, phosphorylation, ubiquitylation, and ADP-ribosylation.[11−13] These marks, plus other more recently appreciated modifications such as crotonylation, succinylation, and malonylation,[14] have the potential to alter histone−DNA and histone−histone interactions and thus provide a means for transiently targeting changes in nucleosome dynamics.[15−17]

The chemical nature of histones can also be changed at the protein sequence level, where the canonical histones used to package the majority of the genome are substituted by histone variants.[18,19] Relative to canonical histones, histone variants can have a range of sequence differences, from just four amino acid differences between the H3.3 variant and canonical H3.1 to more than 50% sequence divergence of the centromeric-specific H3 variant CENP-A.[18] Substitutions of histone variants can change multiple histone−DNA and histone−histone contacts simultaneously and are well-known for altering characteristics of single nucleosomes and chromatin fibers. Nucleosomes containing the histone H2A.Z variant, for example, compact chromatin fibers more readily than those with canonical H2A,[20] and those with the macroH2A variant wrap DNA more stably,[21] whereas nucleosomes substituted with another variant called H2A.Bbd (for Barr body deficient) do not allow chromatin to readily condense[22] and wrap DNA much more poorly.[23] Histone variants can also receive PTMs, which can further modulate the unique effects that variants have on nucleosome dynamics.[18,24]

Complementing chemical and sequence changes to histones, a second mechanism for influencing nucleosome stability and dynamics is through factors that reorganize nucleosome structure. Although many factors, such as linker histones and DNA-binding proteins, can influence characteristics such as DNA wrapping and fiber compaction, the greatest changes in nucleosome structure and stability are achieved by histone chaperones and ATP-dependent chromatin remodelers. These chromatin reorganizing factors are instrumental in catalyzing changes in nucleosome structure and dynamics that would otherwise be thermodynamically inaccessible under physiological conditions. Histone chaperones are highly acidic proteins that stabilize histones in the absence of DNA and thus play critical roles in nucleosome assembly and disassembly.[25−27] Chromatin remodelers are essential for altering the composition and position of nucleosomes by coupling ATP hydrolysis to exchange of histone variants, nucleosome sliding, and octamer assembly/disassembly.[28−30] In conjunction with histone variants, PTMs not only alter intrinsic dynamics of nucleosomes but also provide chemical signposts to help guide cellular factors to particular locations in the genome. Through recruitment of cellular factors that bind to PTM-marked histones, termed the "histone code," PTMs play an essential role in defining and maintaining functionally distinct regions of the genome.[11,13,31,32] Histone chaperones and chromatin remodelers bind to and sense PTMs as well, and in many cases the specificity of their activities can be traced to PTM-dependent interactions.[27,33−38]

This review focuses on PTMs that stimulate structural and dynamic changes in nucleosomes (Table 1). We begin by discussing PTMs that have been shown to directly affect either histone−DNA or histone−histone contacts in nucleosome core particles and modulate intrinsic nucleosome dynamics. These PTMs are located on both the tails and core of the histone octamer and affect either the unwrapping dynamics or core stability of the nucleosome. The second part of the review describes PTM-dependent changes in nucleosomes that are primarily linked with actions of chromatin remodelers. Most PTMs appear to aid in localizing particular remodeler activities

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Table 1. Sites of Post-Translational Modifications That Stimulate Dynamic Changes in Nucleosomes

modification	intrinsic effects	chromatin factors influenced
H3(K4me3)	none	recruits Chd1 remodeler; excludes NuRD and ATRX remodelers
H3(K9, K14, K18, K23ac)	entry site unwrapping	recruits SWI/SNF, RSC remodelers
H3(K36me2,3)	none	recruits ISW1b remodeler
H3(Y41ph)	entry site unwrapping[a]	
H3(R42me2a)	entry site unwrapping[a]	
H3(T45ph)	entry site unwrapping[a]	
H3(K56ac)	entry site unwrapping	alters SWR1 specificity; enhances CAF1 binding
H3(S57ph)	entry site unwrapping[a]	
H3(K64me3, K64ac)	nucleosome destabilization	
H3(K115ac)	nucleosome destabilization	
H3(T118ph)	nucleosome destabilization	stimulates disassembly by SWI/SNF
H3(K122ac)	nucleosome destabilization	
H4(K16ac)	chromatin fiber destabilization	reduces Chd1, ISWI activities
H4(S47ph)	nucleosome destabilization[a]	
H4(K77ac)	entry site unwrapping	
H4(K79ac)	entry site unwrapping	
H4(K91ac)	histone–histone destabilization	
H4(R92me)	histone–histone destabilization	
H2B(K123ub1) [yeast], H2B(K120ub1) [human]	chromatin fiber destabilization	aids FACT histone chaperone
[poly-ADP ribosylation][b]	destabilizes histone-DNA contacts	recruits Alc1 remodeler

[a]Predicted. [b]Not at a defined location.

in different biological settings. However, new evidence is emerging that some PTMs also influence the actions or specificity of remodelers.

2. DIRECT IMPACT OF HISTONE POST-TRANSLATIONAL MODIFICATIONS ON NUCLEOSOME DYNAMICS

Nucleosomes must dynamically change so that DNA binding complexes can access their binding sites. These dynamic changes, which include nucleosome unwrapping, rewrapping, sliding, assembly, and disassembly, involve the formation and/or disruption of interactions within the interfaces between the DNA, H3/H4, and H2A/H2B components of the nucleosome (Figure 1). During the past 10 years a significant number of histone PTMs have been identified that are located within DNA–histone and histone–histone interfaces.[17,39−41] These histone PTMs are poised to influence the interactions that stabilize the nucleosome structure by shifting the free energy difference between the fully wrapped nucleosome and altered nucleosome structures. This results in a change in the probability that nucleosome will spontaneously fluctuate into altered states, thereby regulating DNA accessibility and ultimately DNA processing. The free energy, ΔG, and its associated equilibrium constant, K_{eq}, are related by $K_{eq} = e^{-(\Delta G/RT)}$, so a shift in free energy of 1 kcal/mol, for example, results in a ∼5-fold change in the probability between two states.

While there are a wide range of PTM types,[11] a significant number of the PTMs within nucleosome interfaces are acetylation and phosphorylation. Lysine acetylation removes a positive charge, phosphorylation introduces negative charge, and both add steric bulk. The introduction of a single PTM can reduce the free energy of nucleosome formation by at least 2 kcal/mol.[42] This implies that a single PTM can increase the probability of altered structural states by over a factor of 25.

The PTM location impacts the type of structural fluctuation on the nucleosome.[43] We focus on four structurally and functionally distinct regions of the nucleosome: histone tails, DNA entry/exit region, dyad symmetry axis region, and interfaces between histone dimers (Figure 2). Histone tails, which refer to the disordered N-termini, extend out from the nucleosome core and help stabilize higher-order chromatin structure.[44−48] DNA entry/exit regions coordinate the outermost segments of DNA, which are the first to detach from the histones during nucleosome sliding or unwrapping (Figure 2A, red highlight). In this region the DNA is not strongly bound to the histone surface.[49,50] In contrast, the region around the dyad symmetry axis, which coordinates the most internal segment of DNA, contains the strongest DNA–histone interactions[49] (Figure 2A, blue highlight). In addition to DNA–histone interactions, nucleosome stability also depends on protein–protein interfaces between the histone dimers. We describe select PTMs between H3/H4 and H2A/H2B that appear positioned to weaken the histone–histone interface. Overall, the primary level of discussion in this review is the nucleosome core particle, as most most studies of histone PTMs in structured regions of the nucleosome to date have been investigated by use of mononucleosomes.

Determining the direct influence of histone PTMs on nucleosome dynamics requires the ability to prepare histones that are homogeneously modified. This technical requirement has recently been accomplished with multiple methodologies.[51] One approach is to use a nonsense suppression strategy, where a tRNA synthetase and $tRNA_{CUA}$ are selected to incorporate nonnatural amino acids during histone expression in bacteria. This methodology is currently limited to the incorporation of acetyllysines into histone proteins.[52] Native chemical ligation (NCL)[53] and expressed protein ligation (EPL)[54] allow for a traceless connection between two peptides or a peptide and a recombinant protein. The modifications that have been incorporated into histones include acetyllysines,[55−57] phosphothreonines,[42] trimethyllysine,[58] and even ubiquitylated lysines.[59,60] One limitation of this method is that it is restricted to the N- and C-terminal regions of the histones. However, sequential NCL with multiple peptides allows for the preparation of fully synthetic histones, where multiple distinct histone PTMs can be incorporated throughout each histone.[61] Another approach is alkylation of cysteines, which enables the introduction of methyllysine[62] and acetyllysine[63] analogues throughout a histone. Although these analogue residues differ slightly from the structures of native PTMs, which may affect protein recognition, this labeling approach is significantly easier than chemical ligation and therefore a more accessible technique. Combined, these methodologies provide a diverse

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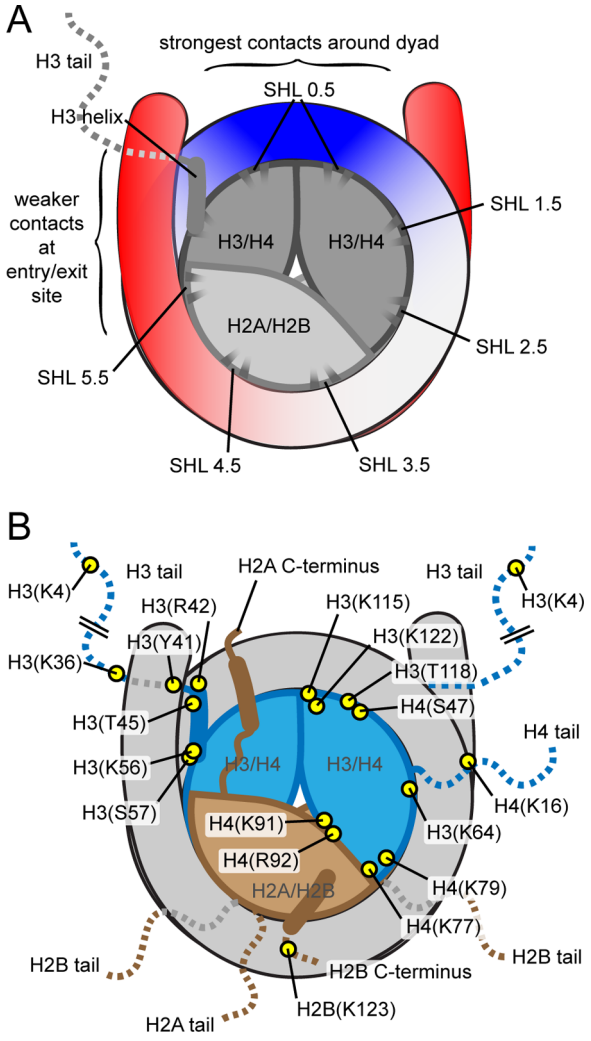

Figure 2. Schematic drawings of the nucleosome, highlighting features that contribute to nucleosome dynamics. (A) Illustration highlighting energetically important contacts within the nucleosome. The DNA entry/exit region (red) has weaker histone–DNA contacts, and the dyad region (blue) has the most energetically important contacts.[49] Superhelical locations (SHLs) indicate the histone surfaces where contact is made with the DNA minor groove.[92] The histone surface underneath the DNA at the dyad, where the major groove faces the histone octamer, is considered SHL-0, and increasing or decreasing values mark each SHL moving from the dyad to the two entry/exit regions. (B) Map of histone residues where post-translational modifications influence nucleosome dynamics.

tool set for preparing homogeneously modified histones and are necessary for identifying nucleosome characteristics influenced by specific PTMs.

2.1. Histone Tails Influence Nucleosome Unwrapping and Stability

Histone acetylation had been known to influence transcription even before the nucleosome was identified.[64,65] Long chains of nucleosomes form chromatin fibers that compact into higher-order structures.[66,67] Removal of histone tails with trypsin abolishes chromatin folding, implicating histone tails in formation of higher-order chromatin compaction.[68,69] Histone tail hyperacetylation disrupts nucleosome array folding[70,70,71] and enhances RNA transcription,[71−73] suggesting a direct link between chromatin compaction and transcription. More recent work using recombinant histones revealed that nucleosome array compaction required residues 14−19 of the histone H4 tail.[74] In agreement with these findings, a subsequent study showed that a single histone PTM in this region, acetylation of H4 at lysine 16, prevents array compaction.[56] An extensive amount of work has gone into understanding the detailed roles of histone tails and their impact on chromatin compaction, which is discussed in a number of recent reviews.[44−48]

On mononucleosomes, DNA rapidly unwraps and rewraps from the histone core, providing transient opportunities for protein binding within the nucleosome[5−7] (Figure 3A). For example, nucleosomes expose about 30 base pairs of DNA for transcription factor binding many times a second and then rewrap on the millisecond time scale.[75,76] Depending on the free energy differences between the fully wrapped nucleosome and partially unwrapped nucleosomes, these rapid fluctuations in unwrapping/rewrapping provide varying exposure and availability of binding sites.[50,77] This reduced availability effectively lowers binding affinities by decreasing protein binding rates.[75,78] Furthermore, site exposure by DNA unwrapping also occurs within compacted nucleosome arrays, suggesting that nucleosomes transiently unwrap even in compacted chromatin.[79,80] Interestingly, recent single-molecule measurements have shown that the nucleosome also dramatically increases the rate at which DNA-binding domains dissociate.[81] While the mechanism behind accelerated dissociation from the nucleosome is not known, it provides an additional pathway for regulating protein occupancy at specific DNA sites on the nucleosome.

DNA site exposure can be influenced by histone tails.[82] A number of studies have demonstrated that histone tails interact with nucleosomal DNA and influence DNA unwrapping. Cross-linking studies show that the histone tails interact with linker DNA that extends out from the core,[83,84] and removal or deletion of the histone tails enhances DNA accessibility to transcription factors and restriction enzymes,[85−88] influences nucleosome positioning,[89] and reduces nucleosome stability.[90] This is likely due to electrostatic interactions between the positive charge of the histone tails and the negatively charged phosphate backbone of the DNA. Interestingly, a recent small-angle X-ray scattering (SAXS) and fluorescence resonance energy transfer (FRET) study indicated that the H3 tail suppresses nucleosome unwrapping yet the H4 tail enhances unwrapping,[91] suggesting nucleosome unwrapping is influenced by histone tails in distinct ways.

The positively charged histone tails, which make minimal intranucleosomal interactions within crystal structures,[92,93] are thought to be largely unstructured in solution and are highly dynamic.[94−97] Perhaps due to their accessibility, the histone tails are the most post-translationally modified regions of the nucleosome,[98,99] and tail PTMs are intimately involved in many aspects of transcription, DNA repair, and DNA replication.[100−104] Acetylation neutralizes the positive charge of lysine, which reduces favorable interactions with DNA and likewise can influence the impact of the histone tails on DNA site exposure. Histone acetylation increases DNA accessibility to transcription factor binding within nucleosomes,[85] whereas restriction enzyme accessibility studies of unacetylated and hyperacetylated nucleosomes have shown that acetylation increases DNA site accessibility by up to 2-fold.[105] Single-molecule force spectroscopy studies of nucleosome arrays have revealed that histone acetylation decreases the force required to mechanically unwrap nucleosomes,[106] and likewise a separate

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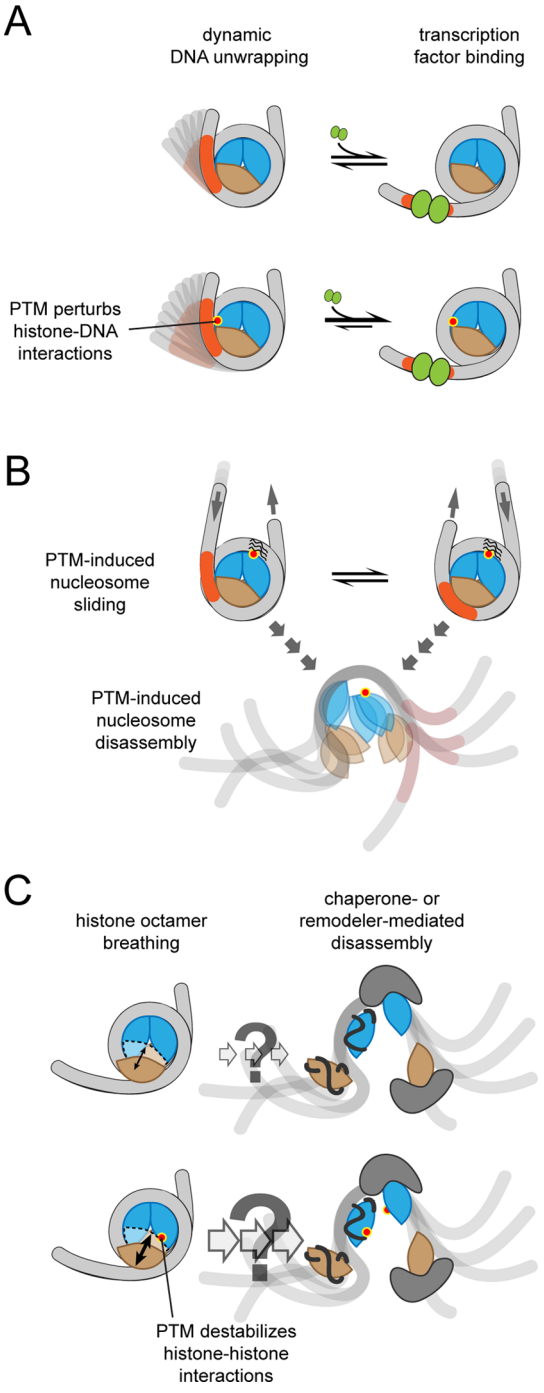

Figure 3. Types of nucleosome dynamics that can be affected by PTMs. (A) DNA unwrapping transiently exposes protein-binding sites that are buried within the fully wrapped nucleosome. (B) The DNA can slide relative to the histone octamer and nucleosomes can be disassembled to expose DNA-binding sites. With unmodified histones, these structural changes require histone chaperones and chromatin remodeling complexes. Dyad modifications can enhance both sliding and disassembly. (C) Nucleosomes can unwrap with the H2A/H2B heterodimer attached to DNA. This type of structural dynamics could be an initial step for nucleosome disassembly and H2A/H2B exchange and may be accelerated by PTMs at histone–histone interfaces.

single-molecule FRET study indicated that histone acetylation by Piccolo NuA4 increases nucleosome unwrapping.[107]

To date, however, most studies of histone tail acetylation have relied on methodologies that prepare nucleosomes that are heterogeneously acetylated, which makes it difficult to assign which lysines are modified. In addition to modifications in the tails, hyperacetylated histones can also contain acetyllysines within the folded portion of the histone core, which could increase site exposure (see below). Interestingly, a recent single-molecule study of nucleosomes with the histone tails containing 12 lysine-to-glutamine mutations that mimic lysine acetylation did not increase unwrapping.[108] However, since different histone tails appear to have opposite impacts on nucleosome unwrapping dynamics,[91] the extent to which unwrapping dynamics is influenced by tail acetylation remains unclear. Another complication is that histone tails also influence chromatin higher-order structure both within and between fibers. Histone tail interactions with DNA and the histone core are different in extended, compacted, and aggregated nucleosome arrays.[47,109−111] This suggests that single-nucleosome studies are relevant for nucleosome dynamics within extended euchromatic fibers but that the influence of histone tails and their PTMs on unwrapping dynamics within heterochromatin needs to be investigated within nucleosome arrays. To elucidate how nucleosome unwrapping and DNA site exposure are influenced by histone tail modifications, future quantitative studies will need to be performed with nucleosomes containing well-defined patterns of histone PTMs in chromatin fibers.

2.2. Histone Post-Translational Modifications within the Nucleosome DNA Entry/Exit Region Directly Influence Unwrapping Dynamics

There are a number of histone PTMs that are near the DNA entry/exit region of the nucleosome (Figure 2B) and positioned to potentially interrupt the DNA–histone interface, including H3(Y41ph), H3(R42me2a), H3(T45ph), H3(K56ac), and H3(S57ph). These histone PTMs are involved in a range of biological processes including transcription,[112−116] DNA repair,[117] replication,[118,119] and apoptosis.[120] Each of these PTMs are under the nucleosomal DNA, so distortion of the nucleosome structure is required for protein binding to any of these PTMs within the nucleosome (Figures 4 and 5). This positions them to directly impact nucleosome structure or dynamics. H3(K56ac) significantly increases nucleosome unwrapping,[52] while the impact of the other PTMs has yet to be reported. In addition, H3(K56ac) influences nucleosome assembly by altering the interaction of H3/H4 with histone chaperones. The studies of H3(K56ac) suggest that entry/exit PTMs both increase site exposure to enhance DNA accessibility and alter H3/H4 histone chaperone binding to regulate nucleosome assembly.

H3(K56ac) is the most studied of the entry/exit PTMs. This PTM has been incorporated into histone H3 by exploiting the pyrrolysyl-tRNA synthetase/$tRNA_{CUA}$ pairs that were evolved to incorporate acetyllysines.[52] This study found that H3(K56ac) does not alter chromatin compaction and only slightly impacts (by 20%) chromatin remodeling by SWI/SNF and RSC. However, they determined with single-molecule FRET distribution measurements that H3(K56ac) increases by 7-fold the amount of DNA that is unwrapped at the nucleosome entry/exit region.[52] Interestingly, this study found that there was no measurable increase in unwrapping further into the nucleosome at the 27th base pair. In contrast, a separate study, which prepared H3(K56ac) by sequential NCL, found that this PTM increased transcription factor occupancy at a site extending 27 base pairs into the nucleosome by 3-fold due to

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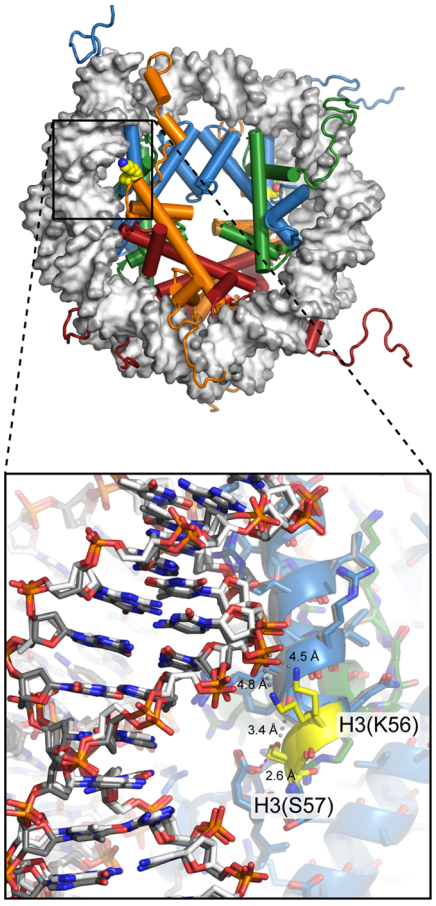

Figure 4. View of the nucleosome (1KX5), with H3(K56) and H3(S57) highlighted in yellow. Located under the DNA near the nucleosome entry/exit region, H3(K56ac) increases site exposure by increasing the DNA unwrapping rate[52,61] and influences histone chaperone binding,[126] while H3(S57A) substitution interferes with octamer formation and increases H2A/H2B dimer exchange.[90] Close-up view (bottom) shows the two sides of the nucleosome superimposed, with one copy of each histone in color and one copy in gray. In the crystal structure, H3(S57) hydrogen bonds with neighboring H3(E59) (magenta dotted line) and makes van der Waals contacts with carbonyl oxygens of the αN-helix of histone H3 (gray spheres) but is too far to make direct interactions with DNA. Although the neighboring H3(K56) makes a closer approach to DNA, the lysine side chain is too distant to directly hydrogen-bond to the phosphate backbone. The two positions observed for the H3(K56) side chain suggest some mobility, and the small gray spheres highlight the shortest path from the lysine to the closest DNA phosphates.

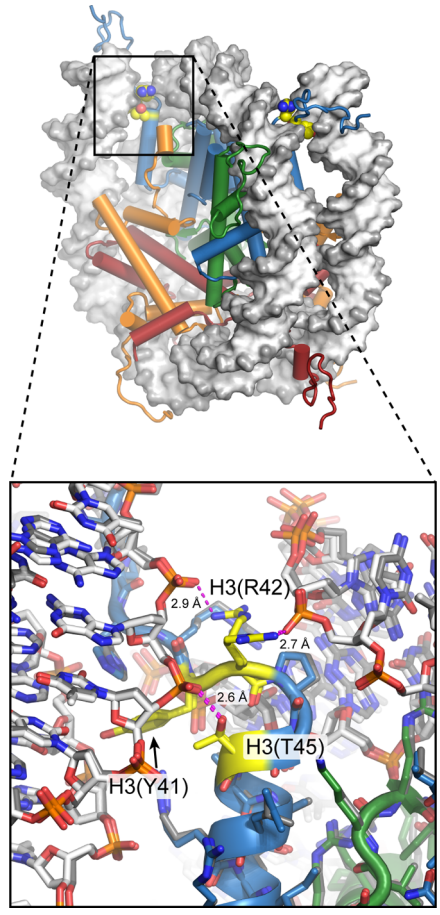

Figure 5. View of the nucleosome (1KX5), with H3(Y41), H3(R42), and H3(T45) highlighted in yellow. These residues are located right where the DNA enters and exits the nucleosome. Close-up view (bottom) with the two sides of the nucleosome superimposed shows the same rotamers for H3(Y41) and H3(T45) and two different conformations for H3(R42). In this structure, both H3(R42) and H3(T45) make direct hydrogen bonds to the DNA phosphate backbone (magenta dotted lines). Phosphorylation of Y41 or T45 would be expected to cause steric clashes and electrostatic repulsion with the DNA. The direct impact of PTMs at these positions on nucleosome dynamics has yet to be reported, but they are predicted to increase DNA unwrapping on the basis of studies of H3(K56ac).

enhanced unwrapping.[61] The results of these two studies can be understood by the observation that nucleosomes iteratively unwrap from the DNA entry/exit region, and therefore a reduction in unwrapping free energy at the edge of the nucleosome also enhances DNA site exposure further into the nucleosome.[50] This property of the nucleosome also results in cooperative binding of transcription factors to adjacent binding sites within the nucleosome.[121,122] In addition, H3(K56Q), which is often used to mimic lysine acetylation, produced a similar increase in transcription factor occupancy,[61] indicating that this mimic effectively captures the impact of the acetylation on nucleosome unwrapping. Subsequent studies, which used rapid association kinetics of transcription factor binding to indirectly measure unwrapping rates, found that the shift in equilibrium toward partially unwrapped nucleosomes is due to an increase in the unwrapping rate.[78] Somewhat counter to expectations, the X-ray crystal structure of a nucleosome containing the H3(K56ac) mimic did not reveal distortions at the histone−DNA interface.[123] Thus, although H3(K56ac) does not prevent formation of a fully wrapped nucleosome structure, this modification shifts the site exposure equilibrium toward partially unwrapped states, enhancing nucleosomal DNA accessibility.

H3(K56ac) is involved in nucleosome assembly during DNA replication[118] and repair[117] and disassembly during transcription.[112−114] The location of H3(K56), both adjacent to H2A in the nucleosome and underneath DNA at the entry/exit site, is positioned to impact the interactions that could influence nucleosome formation (Figure 4). An indirect effect of H3(K56ac) on nucleosome formation is evident from altered interactions of H3 with histone chaperones, which bind H3/H4 tetramers/dimers and H2A/H2B dimers. A pulldown study found that H3(K56ac) increased the affinity of the H3/H4 tetramer to the human histone chaperone CAF-1,[118] while a

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study of the steps of nucleosome assembly by the histone chaperone Nap1 found that H3(K56ac) reduced the affinity of the tetrasome to DNA relative to tetrasome−Nap1 binding.[124] A magnetic tweezers study of unmodified and H3(K56ac)-containing nucleosome arrays detected no difference in DNA−histone binding following mechanical disruption of nucleosomes.[43] NMR and isothermal titration calorimetry (ITC) studies of H3/H4 binding to the yeast histone chaperone RTT106 found that H3(K56ac) significantly enhanced binding to a double pleckstrin-homology (PH) domain,[125] while fluorescence studies found that H3(K56ac) increases H3/H4 binding to the yeast histone chaperone CAF-1.[126] These studies suggest that H3(K56ac) influences assembly/disassembly through interactions between H3/H4 tetramers and histone chaperones. Following nucleosome assembly, H3(K56ac) enhances DNA site exposure in the entry/exit region without significantly increasing the propensity for dissociation of the H2A/H2B dimer.[61]

Other histone PTMs in the DNA entry/exit region—H3(Y41ph), H3(R42me2a), H3(T45ph), and H3(S57ph)—have not been extensively studied. H3(Y41), H3(R42), and H3(T45) are located at the N-terminal end of the αN-helix of H3, which extends between the two gyres of DNA, while H3(S57) is at the C-terminal end of the same helix, adjacent to H3(K56) (Figures 4 and 5). As for H3(K56ac), these PTMs could affect assembly/disassembly by altering interactions with histone chaperones and have the potential of impacting nucleosome site exposure by disrupting histone−DNA interactions and increasing unwrapping. H3(Y41) is phosphorylated by JAK2, implicated in transcriptional regulation[127] and anticorrelated with the occupancy of HP1α.[115] Phosphorylation of H3(T45) in human cells is carried out by PKCδ[120] and DYRK1A,[116] is enriched in apoptotic cells,[120] and regulates HP1 binding.[116] In budding yeast, H3(T45) is phosphorylated by the S-phase kinase Cdc7-Ddf4.[119] The level of this modification peaks during DNA replication, while loss of H3(T45ph) causes replicative defects. Interestingly, the double mutant H3(T45A,K56R) caused a significantly slower growth phenotype type than either of the single mutations.[119] Since phosphorylation at these positions would introduce additional negative charge near the phosphate backbone, these PTMs could significantly enhance unwrapping; however, this remains to be directly tested.

Also on the αN-helix of H3, H3(R42) was recently reported by Allis and co-workers[128] to be asymmetrically dimethylated in human cells by CARM1 and PRMT6. They used expressed protein ligation (EPL) to prepare H3(R42me2a) and found that this modification increased in vitro transcription from a chromatinized template by 2.5 times. They hypothesize that this increase is due to disruption of DNA−histone interactions. However, it is not known whether this effect directly results from increased unwrapping or if this PTM acts via nucleosome-interacting factors.

At the C-terminal end of the H3 αN-helix, H3(S57) has been shown to be phosphorylated during mitosis.[116] This phosphorylation, which appears to be carried out by the DYRK1A kinase, antagonizes binding of some HP1 isoforms in human cells and correlates with transcriptional activation.[116] Although this residue is too distant to directly contact nucleosomal DNA, H3(S57) hydrogen bonds with H3(E59) and packs against carbonyl oxygens of the H3 N-terminal helix, which would likely be destabilized upon phosphorylation (Figure 4). An H3(S57A) substitution was found to interfere with octamer formation and increase exchange of H2A/H2B dimers in the nucleosome,[90] consistent with an important role of this side chain in maintaining the canonical structure of the nucleosome.

H3(K36) is at the boundary between the N-terminal tail and the entry/exit region of the nucleosome (Figure 6). Since it is adjacent to the DNA as it exits the nucleosome, a PTM at this site or a protein bound to this site could directly influence nucleosome unwrapping dynamics. This residue can be either methylated[129] or acetylated[130] in vivo. H3(K36me3) is located within the transcribed regions of active genes,[100] recruits a number of histone PTM binding domains (readers),[131] and is involved in DNA repair, alternative splicing, and transcription[132] (see below). Acetylation of this H3 residue is located within promoter regions of RNA polymerase II genes.[130] At present, it is not known whether H3(K36ac) directly influences nucleosome dynamics. A recent study did investigate nucleosomes containing the trimethyllysine analogue at H3(K36) and showed that methylation alone does not enhance nucleosome unwrapping.[133] However, H3(K36me3) could be specifically bound by the Tudor domain of Phf1, and in the context of the nucleosome, this interaction enhanced

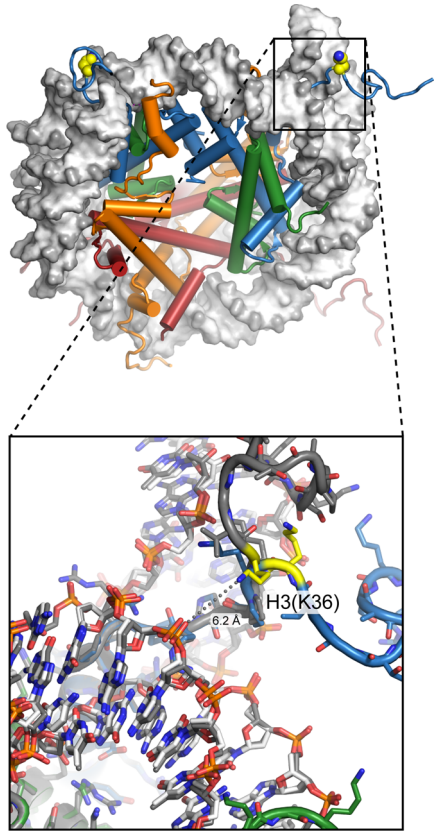

Figure 6. View of the nucleosome (1KX5), with H3(K36) highlighted in yellow. This residue is located on the H3 tail just outside where the tail enters between the two gyres of DNA at the entry/exit region. Close-up view (bottom) with the two sides of the nucleosome superimposed shows that even the backbone position of H3(K36) differs between the two copies in this structure. Neither copy of H3(K36) has the lysine side chain within direct hydrogen-bonding distance of the DNA backbone. While modification of this residue does not influence DNA unwrapping,[43] the binding of the Phf1 Tudor domain enhances DNA unwrapping and accessibility.[35] This suggests that other histone PTM readers that bind in the entry/exit region may also alter nucleosome unwrapping/rewrapping dynamics.

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transcription factor binding through increased site exposure. These experiments suggested that tethering a protein domain at the entry/exit site sterically interferes with nucleosome wrapping and thus provides greater accessibility to nucleosomal DNA.[133] Given these results, it is expected that other proteins that bind modified H3(K36) would also increase site exposure and potentially other forms of nucleosome dynamics. Likewise, although it is not currently known if PTMs on entry/exit residues H3(Y41), H3(R42), or H3(T45) are specifically bound by other factors, the ability of H3(K36me3) to increase its own accessibility through the Phf1 Tudor domain suggests that other binding domains targeted to this region would also have a significant impact on nucleosome unwrapping. Future studies looking for and characterizing these types of histone readers may have the added technical challenge of requiring the full nucleosome for binding studies, but they will be important for identifying new factors that take advantage of these entry/exit PTMs to regulate site accessibility on the nucleosome.

Increased DNA unwrapping from the nucleosome has also been observed with acetylation of two residues, H4(K77) and K4(K79),[43] which are located approximately 50 bp from the entry site, near superhelical location (SHL) ± 2.5 (Figure 7). These residues have been found to be acetylated in metazoans[39] and map to a region originally identified with yeast genetic screens that cause a loss of rDNA silencing (LRS), which include the point substitutions H4(R78G), H4(K79M), and H4(T80A).[134] By use of in vivo chromatin immunoprecipitation (ChIP), *lrs* mutants have been shown to have reduced binding of the yeast silencing information regulatory (SIR) proteins at loci normally targeted for silencing.[135−137] The Sir2−3−4 complex binds to nucleosomes via Sir3, and the molecular interactions required for nucleosome recognition were revealed in a crystal structure of the nucleosome bound to the Sir3 BAH domain.[138] Interestingly, however, some positions giving LRS phenotypes, such as H4(K79) and H3(R83), do not directly contact the Sir3 BAH domain but instead are located at histone−DNA interfaces. Thus, one interpretation is that although these substitutions may not directly alter a histone surface recognized by silencing factors, the increased unwrapping may indirectly interfere with nucleosome binding or other aspects required for silencing. This connection between loss of silencing and increased DNA unwrapping was also supported by the observation that H3(K56Q) substitution leads to loss of silencing in yeast.[139] Although it has not been reported whether acetylation of H4(K77) and H4(K79) occurs in yeast as in metazoans, the acetyl mimic H4(K79Q) but not H4(K77Q) disrupted telomeric silencing in *Saccharomyces cerevisiae*.[140] Recently, a number of additional histone PTMs have been identified within the DNA−histone interface along the first 40 base pairs of DNA,[41] and it will be interesting to investigate both the biophysical and in vivo consequences of these modifications.

2.3. Histone Post-Translational Modifications near the Nucleosome Dyad Symmetry Axis Destabilize the Nucleosome

The dyad region around SHL ± 0.5 contains the strongest histone−DNA interactions[49] (Figure 2A), and PTMs in this region significantly destabilize the nucleosome.[40] Histone−DNA contacts between the H3/H4 tetramer and DNA at the nucleosomal dyad are presumably the first contacts made upon assembly and the last contacts broken during nucleosome disassembly. There are four histone PTMs near the dyad

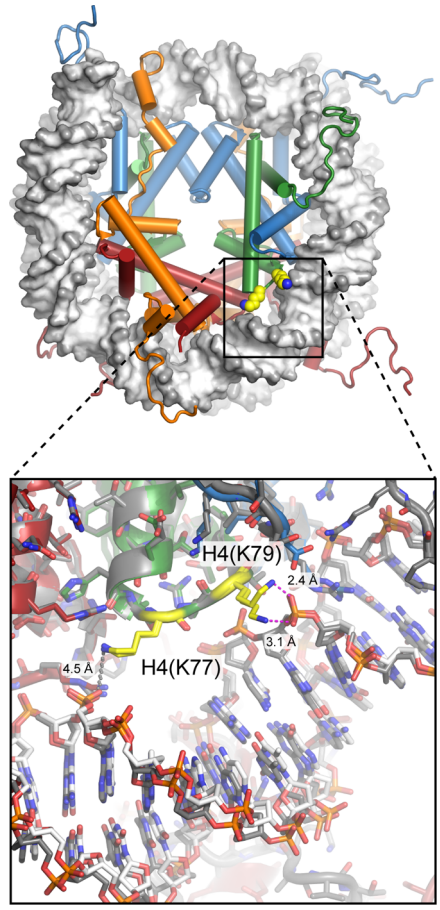

Figure 7. View of the nucleosome (1KX5), with H4(K77) and H4(K79) highlighted in yellow. These residues are located around SHL ± 3, where histone mutations result in the loss of rDNA silencing (LRS).[134] Close-up view (bottom) with the two sides of the nucleosome superimposed shows that H4(K79) can occupy two different conformations yet still directly hydrogen-bond to the DNA phosphate backbone and that H4(K77) is too distant to directly hydrogen-bond in this structure. Despite their location relatively far from the edge of the nucleosome, acetylation of these two residues increases DNA unwrapping at the entry/exit site,[43] which may underlie their connection to disrupting transcriptional silencing.

symmetry axis: H3(K115ac), H3(T118ph), H3(K122ac), and H4(S47ph)[39,41] (Figure 8). These PTMs do not influence nucleosome unwrapping, suggesting that PTMs near the dyad function distinctly from PTMs in the entry/exit region.[43]

This region was first demonstrated to be important for nucleosome dynamics through genetic screens in *S. cerevisiae* that identified five SIN (SWI/SNF-independent) histone mutations.[141] These five separate histone point mutations in budding yeast partially relieved the reduced transcription of the HO gene in a SWI/SNF chromatin remodeler mutant.[141] Three of these mutations—H4(R45H), H3(R116H), and H3(T118I)—reside in the DNA−histone interface near the dyad symmetry axis. Amino acid substitutions at these positions significantly increased thermal mobility of nucleosomes,[142,143] and H4(R45H) reduced higher-order chromatin structure.[144] Consistent with weakening histone−DNA contacts, substitutions at SIN positions H4(R45) and H3(T118) were found to reduce the nucleosomal barrier to transcription by RNA polymerase II.[108,145] However, akin to H3(K56Q), SIN

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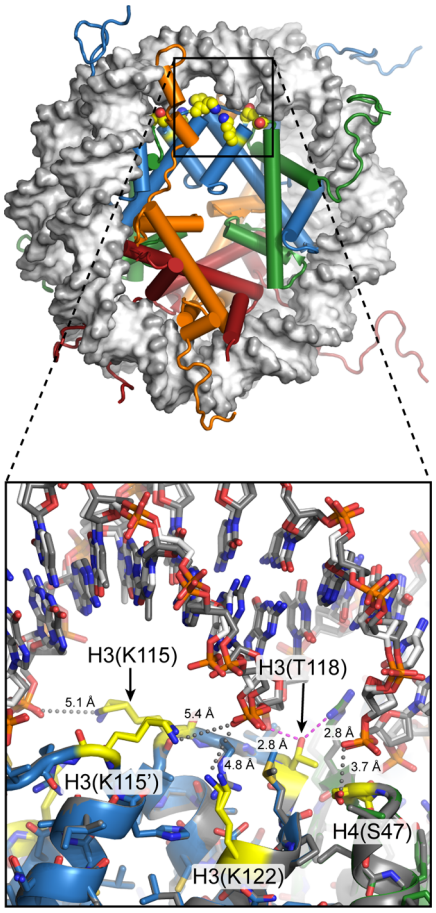

Figure 8. View of the nucleosome (1KX5), with H3(K115), H3(T118), H3(K122), and H4(S47) highlighted in yellow. Located around SHL ± 0.5, these residues are positioned within the most energetically important histone–DNA contacts. Close-up view (bottom) with the two sides of the nucleosome superimposed shows very similar conformations for these residues. H3(T118) generates a SIN phenotype when mutated and directly hydrogen-bonds to both the DNA phosphate backbone and another SIN residue, H4(R45) (magenta dotted lines). Phosphorylation of H3(T118), which would likely disrupt these energetically important interactions, has been shown to destabilize the nucleosome, similar to SIN mutations.[142,143,146]

substitutions did not significantly disrupt the wrapped organization of the nucleosome in crystal structures.[142]

Amino acid substitutions and phosphorylation of H3(T118) significantly impact nucleosome stability and dynamics. Interestingly, mutation at this position had the largest impact on HO expression among the SIN mutations.[141] The phosphorylation mimic H3(T118E) is lethal in budding yeast, while low-level expression leads to a loss of rDNA and telomeric silencing.[140] These results suggest that H3(T118) is an essential histone H3 residue. While little is known about its role in vivo, the impact of this PTM on nucleosome structure and dynamics has been investigated in vitro.[42,146] By use of nucleosomes containing H3(T118ph) prepared by EPL, these studies found that this modification reduced the free energy of nucleosome formation by approximately 2 kcal/mol and increased mobility at a temperature of 53 °C by 30-fold relative to unmodified nucleosomes. As measured by restriction enzyme and DNase I digestion, H3(T118ph) did not increase DNA site accessibility in the entry/exit region of the nucleosome but did increase DNA accessibility near the nucleosome dyad. This modification was also found to induce dramatic changes in the canonical histone–DNA organization that allowed formation of nucleosome monomers, dimers, and alternative intermediately sized structures called "altosomes"[146] (Figure 3B). Altosomes have previously been observed as remodeling products of SWI/SNF[147−150] and are believed to represent disassembly intermediates[150,151] (see below). Accordingly, the disassembly activity of SWI/SNF is dramatically increased on nucleosomes possessing H3(T118ph).[146] Although further work is needed to elucidate when and where H3(T118) phosphorylation occurs in vivo, this PTM is likely short-lived on nucleosomes and coupled to processes that require nucleosome-free stretches of DNA.

The other phosphorylation site, H4(S47), is adjacent to both H3(T118) and the SIN residue H4(R45) (Figure 8). In budding yeast, the mutation H4(S47E) causes a slow growth phenotype and an increase in rDNA and telomeric silencing.[140] In human cells, PAK2 kinase phosphorylates H4(S47) and appears to promote CAF-1-mediated nucleosome assembly.[152] There are no reported studies of the impact of this histone PTM on nucleosome dynamics. Given its proximity to H3(T118), however, phosphorylation of H3(S47) could function similarly to H3(T118ph) and significantly impact nucleosome stability.

H3(K64) has been reported to be both trimethylated[153] and acetylated.[154] This site is located near SHL ± 1.5, and although the side chain does not make direct contact with DNA in crystal structures, it lies just underneath the DNA duplex and thus does not appear easily accessible in the fully wrapped state (Figure 9). Trimethylation of H3(K64) is localized within heterochromatic regions and repetitive sequences and is significantly reduced during cell differentiation.[153] Acetylation of this residue is found in transcription start sites, with other histone PTMs that activate transcription and with RNA polymerase II occupancy.[154] P300/CBP can acetylate H3(K64) in vitro, and knockdowns reduced the levels of H3(K64ac) in vivo. In vitro, nucleosomes containing H3-(K64ac) were less stable and enhanced histone eviction within a transcription assay.[154] Combined, it appears that the acetylation of H3(K64) may function similarly to H3(K122ac) to facilitate transcription, while methylation of H3(K64) may either stabilize nucleosomes or simply regulate the acetylation of this residue. However, more direct measurements of the methylation and acetylation of this site are required to determine their impacts on nucleosome dynamics and stability.

2.4. Histone Post-Translational Modifications at Histone–Histone Interfaces Are Poised to Regulate Nucleosome Stability

In addition to PTMs at the DNA–histone interface of the nucleosome, a number of histone PTMs are located within interfaces between H3/H4 tetramers and/or H2A/H2B dimers.[39] The most extensively studied histone PTM located within a histone–histone interface is the acetylation of H4(K91) (Figure 10), which was first identified by mass spectrometry of histones from calf thymus nuclei[39] and later in budding yeast.[155] In budding yeast, H4(K91ac) is associated with transcriptionally active chromatin and is reduced at telomeres. In one study, the mutation H4(K91A) caused a reduction in telomeric silencing,[155] while a separate study reported that H4(K91Q) induced a subtle reduction in rDNA silencing but not in telomeric silencing.[140] This modification is

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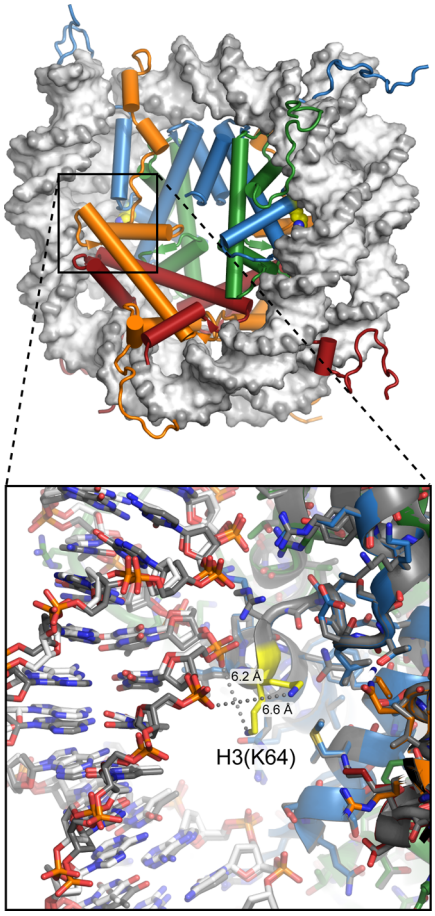

Figure 9. View of the nucleosome (1KX5), with H3(K64) shown in yellow. Located at SHL ± 2, this position lies underneath the major groove of DNA and would not be easily accessible to a histone-modifying enzyme in a fully wrapped nucleosome. Close-up view (bottom) with the two sides of the nucleosome superimposed shows very different positions of the lysine side chain, neither within hydrogen-bonding distance of the DNA phosphate backbone (gray spheres). From the crystal structure, the manner in which modification of H3(K64) would directly impact nucleosome dynamics is not clear.

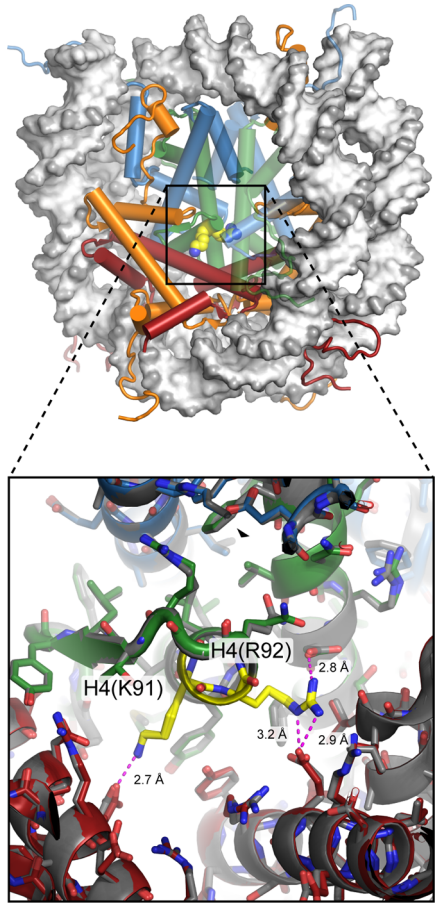

Figure 10. View of the nucleosome (1KX5), with H4(K91) and H4(R92) highlighted in yellow. These residues are located near the center of the histone octamer, at the interface between H2A/H2B dimers and H3/H4 tetramer, and are not readily accessible from the exterior of the nucleosome in the crystal structure. In the far view (top), the backbone of H3/H4 on the right side is semitransparent so that H4(R92) can be seen. Close-up view (bottom) with the two sides of the nucleosome superimposed shows that both copies are in very similar conformations, with each making direct hydrogen bonds to residues on H2B (magenta dotted lines). Modification of either H4(K91) or H4(R92) would be expected to interfere with these hydrogen bonds. H4(K91ac) is involved in nucleosome assembly and may increase fluctuations or dissociation of H2A/H2B on the nucleosome.

associated with the histone acetyltransferase and chaperone complex Hat1p−Hat2p−Hif1p, suggesting that it is acetylated before deposition and involved in regulating nucleosome assembly. Nucleosomes containing H4(K91A) have enhanced sensitivity to micrococcal nuclease digestion and a predisposition to disassemble at lower NaCl concentrations.[155] In addition, human HAT4 acetylates H4(K91) before H3/H4 is assembled onto DNA.[156] These findings are consistent with the hypothesis that H4(K91ac) regulates nucleosome assembly, but more work is needed to clarify the extent that these effects arise from chaperone-mediated contacts versus direct histone−histone destabilization.

Additional sites of histone PTMs within histone−histone interfaces that have been investigated include H4(R92), which can be methylated, and H3(C110), which can be glutathionylated. In budding yeast, mutations H4(R92A) and H4(R92K) eliminate telomeric silencing, suggesting that this modification is important in regulating transcription.[140] Glutathionylation of H3(C110) was recently reported to occur within proliferating cells and destabilize nucleosomes.[157] Furthermore, there are over 10 additional histone PTMs that reside near or within histone−histone interfaces that have been detected by mass spectrometry but remain to be studied.

On the basis of studies of H4(K91ac), histone PTMs within histone−histone interfaces appear to regulate nucleosome assembly and disassembly. Assembly/disassembly could be regulated by two nonexclusive mechanisms where PTMs (i) directly disrupt nucleosome stability and/or (ii) alter histone chaperone binding. The report that lower concentrations of NaCl are required to disassemble nucleosomes with H4(K91ac) suggests that this PTM directly disrupts nucleosome stability. Interestingly, two of the five SIN mutations identified in genetic screens, H3(E105 K) and H4(V43I), are also located near histone−histone interfaces.[141] In vitro, H4(V43I) only slightly altered nucleosome thermal mobility,[142,143] suggesting that changes at histone−histone interfaces do not always directly impact nucleosome stability. In addition, unmodified nucleosomes have been reported to transiently breathe where the H2A/H2B dimer transiently dissociates from the H3/H4

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tetramer.[158] Although such breathing may change the extent to which nucleosomal DNA is wrapped, this mode of structural fluctuation is distinct from site exposure. Given this dynamic nature of histones in nucleosomes, the degree of structural fluctuations may be impacted by PTMs at histone−histone interfaces (Figure 3C). With multiple PTMs at histone−histone interfaces,[41] several PTMs may work together or in parallel to influence chromatin remodeling and/or nucleosome assembly/disassembly. Future work in this area will be essential to understand the impact of these histone PTMs on nucleosome stability, dynamics, and assembly/disassembly.

2.5. Nucleosome Destabilization by Poly(ADP-ribosylation)

A PTM that has long been associated with chromatin and changes in chromatin structure is poly(ADP-ribosylation).[159−162] Carried out by poly(ADP-ribose) polymerases (PARPs), ADP-ribosylation shares some commonalities with, but also has important differences from, other PTMs. As for other PTMs, single or multiple ADP-ribose units, polymerized into so-called PAR chains, are recognized specifically by reader domains, called macrodomains, and are removed by eraser enzymes called poly(ADP-ribose) glycohydrolases (PARGs).[163,164] However, what distinguishes PARylation from other modifications is its dominant electrostatic character. With two phosphates for every ADP-ribose unit, and often many dozens of such units strung together in branched and unbranched chains, PARylation produces strong anionic polyelectrolytes that can compete with DNA and RNA binding.[159−162] Although the predominant substrate for PARP-1 is itself,[165] it also modifies histone H1 and the core histones, particularly H2B, in vivo.[166] In vitro, both direct PARylation of histones and noncovalent associations with PAR chains disrupt histone−DNA interactions,[167−169] correlating with early observations of PARP-dependent decondensation of purified chromatin fibers.[170] In vivo, PARP activity in *Drosophila* embryos was responsible for normal chromatin decondensation, visualized as chromatin puffs, and robust gene expression upon heat shock.[171] Similarly, in *Drosophila* S2 cells, the rapid spread of PARP-1 and PAR chains in the activated HSP70 locus was shown to be necessary for histone eviction, preceding transcription by RNA polymerase II.[172,173]

The mechanisms of nucleosome destabilization by PARylation have been difficult to discern due to technical challenges and complexity of the system. PARP-1 is a large, multifunctional enzyme regulated through DNA and protein interactions and post-translational modifications.[174,175] PARylation activity is greatly stimulated by binding to double-stranded DNA breaks, histones, and polynucleosomes.[175−180] The mechanisms of PARP-1 activation are not currently clear, but transcription-coupled activity has been shown to require C-terminal phosphorylation of the histone variant H2A.Z[181] and N-terminal acetylation of histone H2A.[173] Subsequent to PARP activation, the recruitment and stimulation of PARG enzymes add further complexity to the system, as the lengths and distributions of PAR chains change dynamically.[182] At sites of DNA damage, PARylation is required for recruiting the macrodomain-containing chromatin remodeler Alc1 (amplified in liver cancer 1), which likely plays an active role in destabilizing and evicting nucleosomes[183−185] (see below). For PARylation, an intriguing idea is that PAR chains may be important for stabilizing histones that have been displaced from DNA.[169,171−173] In this model, a dense mesh of PAR chains, synthesized in situations where rapid and large-scale nucleosome eviction is occurring (e.g., activated heat shock loci or sites of DNA damage), could provide a scaffolding to locally maintain a reservoir of displaced histones. The subsequent breakdown of the PAR chains by PARGs would then make histones available for redeposition, a process referred to as histone shuttling.[169] To further complicate matters, however, PARylation is not only involved in stripping histones from DNA but also has been shown to be needed to maintain chromatin structure.[180,186] PARylation has been found to be important for maintenance of heterochromatin and rDNA silencing,[186,187] and in mammals, the inactive X chromosome is enriched in a macrodomain-containing H2A variant called macroH2A.[188] Thus, although great strides have been made, more work will be needed to elucidate the nature and mechanisms by which PARylation alters chromatin structure. As this system will likely continue to be challenging to interrogate in vitro, progress will be greatly aided by application of high-resolution and real-time cell imaging.[189−191]

3. EPIGENETIC GUIDANCE OF CHROMATIN REMODELERS AS A MEANS OF DETERMINING NUCLEOSOME STABILITY AND DYNAMICS

In addition to influencing nucleosome dynamics directly, PTMs also play important roles in reshaping the chromatin landscape by directing actions of chromatin remodelers. Chromatin remodelers appear to be involved in most processes that reorganize nucleosomal architecture, such as nucleosome assembly, disassembly, histone variant exchange, and histone octamer repositioning.[28−30] These distinct nucleosomal rearrangements are all driven by a conserved helicase-like ATPase motor, named Snf2 for the founding SWI/SNF remodeler, that can translocate along DNA.[192−194] For SWI/SNF and ISWI remodelers, the Snf2 motor has been shown to engage with nucleosomal DNA at an internal location, approximately 20 bp from the nucleosomal dyad.[195−199] At a simple level, shifting DNA past the histone octamer is presumably achieved by translocation of the Snf2 motor on nucleosomal DNA while maintaining contact with some portion of the histone octamer. However, recent single-molecule studies with ISW2 unexpectedly revealed that DNA appears to exit the nucleosome before more DNA is pulled onto the histone core.[200] Although the details remain to be worked out, these results suggest that the canonical structure of the nucleosome is likely distorted during the remodeling process.

Whereas the ATPase motor plays a central role in powering the structural reorganization of nucleosomes, the outcome of the remodeling reaction appears to be guided by auxiliary domains outside the Snf2 motor.[194] The number and types of auxiliary domains and subunits vary extensively among different remodeler families.[201−203] Many domains are recognizable for a direct connection to reading and modifying the histone code. Associated reader domains that can recognize unmodified, acetylated, and methylated lysines include bromodomains, chromodomains, PHD (plant homeodomain) fingers, BAH (bromodomain-associated homology) domains, ADD (ATRX−DNMT3−DNMT3L) domains, and PWWP (Pro-Trp-Trp-Pro) domains.[32,131,204,205] One or more of these lysine-recognition domains are commonly found in chromatin remodeling complexes, either covalently attached to the Snf2 motor or as part of non-covalently-associated subunits.[30,203] Another type of reader domain is the macrodomain, which recognizes ADP-ribosylation and appears unique to the Alc1 remodeler.[183,184]

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These reader domains can help localize remodeling activities to chromatin containing particular PTMs. The SWR1 remodeler, for example, is believed to be preferentially recruited to nucleosomes with acetylated H2A and H4 tails through its bromodomain-containing subunit Bdf1.[206] While individual reader−PTM interactions are generally weak (micromolar K_D), many remodelers possess multiple reader domains, suggestive of multivalent interactions that would increase both affinity and specificity.[207,208] One example of a multivalent reader is the BPTF subunit of NURF, an ISWI-type remodeler, which preferentially binds nucleosomes possessing both H3(K4me3) and H4(K16ac) marks through a PHD−bromodomain module.[209] Reader domains can also increase specificity through preferences for unmodified residues at specific positions, alone or in combinations with other PTMs. Methylation of H3(K4) interferes with recruitment of the multisubunit NuRD remodeler,[210] and similarly, the DPF3b subunit of the BAF remodeler[211] and ADD domain of the ATRX remodeler,[212] which recognize H3(K14ac) and H3(K9me3), respectively, require unmodified H3(K4) for binding to the H3 tail.

The sections below give examples where PTMs help direct actions of chromatin remodelers. Each example describes the known activities of particular remodelers and how these activities fit into the biological contexts in which the PTMs are found. As an efficient packaging medium, chromatin is relatively stable on its own and therefore requires active intervention by chromatin remodelers to rapidly alter nucleosome position, occupancy, and composition. In many cases, PTMs stimulate changes in chromatin through binding and recruitment of remodelers, with the specificity of the remodeling reaction dictated by the type of remodeler that is recruited. In addition to recruitment, PTMs can influence remodeler specificity by changing the chemical nature of histones, which can both affect intrinsic nucleosome dynamics and how remodelers engage with their nucleosome substrates.

3.1. Nucleosome Disruption through Recruitment of SWI/SNF Remodelers

One of the earliest connections between PTMs and chromatin remodeling was made with the discoveries that the SWI/SNF remodeler and acetylation accompany transcriptional activation. From work in budding yeast focused on genes required for mating-type switching (SWI) and growth on sucrose (SNF, for sucrose nonfermenting), subunits of the SWI/SNF remodeler were first identified as transcriptional activators[213−216] that participate in altering chromatin structure.[217] The discovery that SWI/SNF-related remodelers stimulated transcription by disrupting chromatin structure[218−222] spawned several exciting areas of exploration that continue to this day: what are the mechanisms underlying chromatin remodeling, in what cellular processes are remodelers involved, where do they act, and how are remodelers specifically localized? Just on the heels of finding SWI/SNF to be a chromatin remodeler was the discovery that the GCN5 transcriptional activator is an acetyltransferase that modifies nucleosomes to regulate transcription,[223,224] which provided the foundation for modern epigenetics.

Histone acetylation has since been shown to stimulate chromatin remodeling activities by SWI/SNF remodelers.[225−228] This stimulation stems from acetylation-dependent targeting to nucleosome substrates via bromodomains, which primarily read out the acetylation state of histone H3.[227−231] Although directly recognizing epigenetic modifications, SWI/SNF remodelers have also long been known to be recruited to chromatin through sequence-specific DNA-binding factors.[232−236] These distinct mechanisms for remodeler recruitment not only provide an environmentally sensitive means for focusing SWI/SNF action but also likely underlie the dynamic cycles of SWI/SNF-stimulated nucleosome disruption followed by re-establishment of the chromatin barrier.[237−239]

SWI/SNF remodelers are perhaps best known for their roles in disrupting chromatin structure, an important step for transcriptional activation.[240−242] By reorganizing histone−DNA contacts, SWI/SNF remodelers promote binding of sequence-specific factors that would otherwise be occluded by nucleosomes.[218,219,222,243] This increased access to DNA arises from the ability to reposition or slide nucleosomes along DNA. One defining characteristic of SWI/SNF remodelers is that they are insensitive to whether DNA flanking the nucleosome is available to accept new positions of the histone core.[244,245] On mononucleosome substrates, this insensitivity allows movement of nucleosomes up to 50 bp "off the ends" of DNA, which corresponds to translocation of DNA ends up to the internal location of the Snf2 motor on the nucleosome, approximately 20 bp from the dyad.[196,197,244,245] This insensitivity has two consequences. First, nucleosomes can be shifted greater distances, thus exposing larger stretches of DNA that were previously occluded by histones (Figure 11A). Second, this insensitivity allows nucleosomes to be shifted into each other. Nucleosomes become unstable when positioned too closely together, and the active collision of neighboring nucleosomes has been proposed to underlie the disruptive ability of SWI/SNF.[150,151]

Consistent with the ability to shift nucleosomes into each other, SWI/SNF has been shown to generate altosomes, a noncanonical organization that resembles a dinucleosome on a short stretch of DNA[147−149] (Figure 11A). Although difficult to study in vitro, these alternative structures are less stable than canonical nucleosomes and therefore provide an attractive starting point for nucleosome disassembly.[148] Despite their active involvement in increasing nucleosome dynamics, however, SWI/SNF remodelers likely do not evict nucleosomes single-handedly. Histone chaperones are essential for maintaining soluble pools of histone dimers in the absence of DNA and take part whenever histones are deposited or removed from DNA.[25−27] Remodelers such as SWI/SNF and histone chaperones are therefore ideally suited to work together: remodelers can disrupt the histone architecture, either directly or through collisions with neighboring nucleosomes, and chaperones may both increase the ease of ATP-dependent disruption and provide efficient acceptors for displaced histones. Indeed, although the RSC remodeler can displace histone dimers and weakly transfer octamers its own in vitro,[246,247] nucleosome eviction is greatly aided in the presence of the NAP1 histone chaperone.[248] How exactly these two systems work together, though, and how the remodeling reactions are resolved, is far from clear. On its own, RSC promotes loss of the H2A/H2B dimer during transcriptional elongation by RNA polymerase II; however, inclusion of NAP1 under identical conditions actually prevented dimer loss and effectively stabilized the nucleosome.[249] Thus, whereas SWI/SNF remodelers possess an intrinsic ability to destabilize nucleosomes and increase nucleosome dynamics through repositioning, the outcome of the remodeling reaction is dictated by the presence of other chromatin-associated

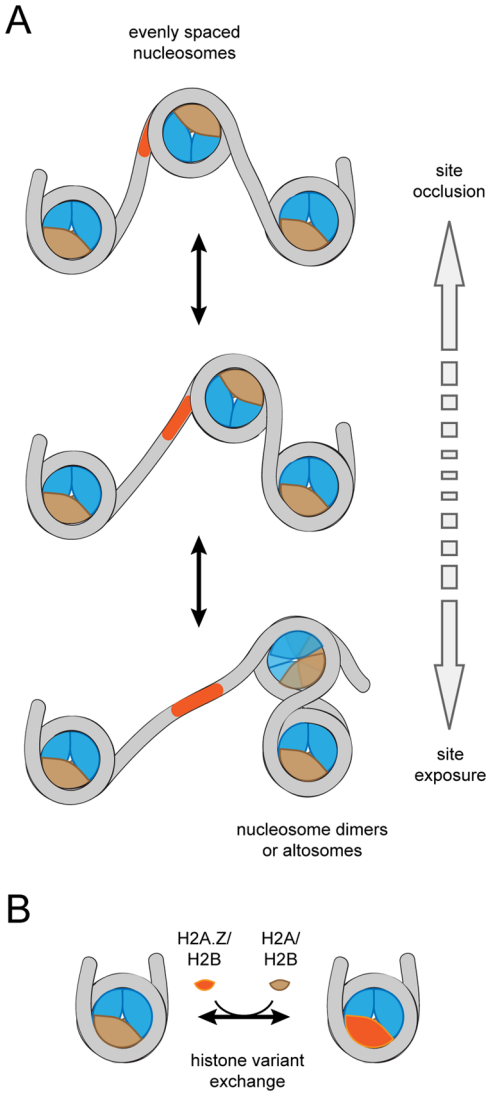

Figure 11. Changes in nucleosome organization carried out by chromatin remodelers. (A) Most chromatin remodelers can reposition or "slide" nucleosomes along DNA. Depending on the direction of sliding, this repositioning can bury or expose DNA binding sites. Remodelers like Chd1 and many ISWI-type remodelers are sensitive to DNA flanking the nucleosome and generate evenly spaced nucleosome arrays (top). In contrast, other remodelers such as SWI/SNF and RSC can shift nucleosomes into their neighbors, generating dimeric or altosome structures, which are believed to be intermediates for nucleosome disassembly (bottom). The altosome organization depicted here was adapted from a model of Ulyanova and Schnitzler.[356] (B) Some remodelers specialize in histone variant exchange. Exchange of canonical and variant H2A/H2B dimers, highlighted here, is carried out by SWR1 and INO80.

factors,[250] which ultimately rely on DNA sequence and epigenetic signatures.

3.2. Poly(ADP-ribosylation) Recruits and Activates the Alc1 Remodeler

In metazoans, PARP-1 is activated in response to certain stresses, such as DNA damage and heat shock, and rapidly poly(ADP-ribosyl)ates (PARylates) itself, histones, and other factors as an early step in chromatin reorganization and signaling.[159−162] In addition to intrinsically destabilizing nucleosomes by generating PAR chains (see above), PARP-1 further promotes chromatin reorganization by recruiting and activating the Alc1 remodeler. Alc1, related to Chd-type remodelers and also known as Chd1L for Chd1-like, is a monomeric and relatively small remodeler, with a C-terminal macrodomain being the only identifiable domain outside of the Snf2 motor.[183,184] DNA- and nucleosome-dependent ATPase activities and nucleosome sliding ability of Alc1 are stimulated in the presence of PARP-1 and NAD^+, the substrate for generating PAR, but not PARP-1 alone. This PARylation-dependent stimulation requires the macrodomain, suggesting that the macrodomain plays a crucial role in localization by binding to PAR chains.[183,184] Accordingly, Alc1 rapidly colocalizes with PARylation at sites of DNA damage in cells, and this localization relies on the PAR-binding ability of the macrodomain.[183,184]

It has not yet been demonstrated what remodeling products are preferentially generated by Alc1, though nucleosome eviction, observed to accompany PARylation, would be consistent with promoting disassembly. The related Chd1 remodeler assembles but does not remove nucleosomes from DNA.[251] However, yeast Chd1 gained the potential to disrupt nucleosomes when the native DNA-binding domain was replaced with monomeric streptavidin.[252] This Chd1−streptavidin fusion remodeler shifted nucleosomes off the ends of DNA fragments and generated SWI/SNF-like nucleosome products when the histones were biotinylated. In contrast, when DNA was biotinylated, the remodeler was not disruptive and instead repositioned nucleosomes on top of the biotinylated DNA sites, which reduced nucleosome binding affinity. These results suggested that the Chd1 remodeler could be transformed into a more disruptive remodeler simply by utilizing recruitment sites that are unaffected by nucleosome sliding.[252] For Alc1, recruitment via PAR chains, attached to either histones or PARP-1, may similarly enable the remodeler to disrupt nucleosomes in a SWI/SNF-like fashion.

3.3. Recruitment of Chd1 to the +1 Nucleosome Helps Early Elongation of RNA Polymerase II

Promoters of activated genes typically maintain nucleosome-free or nucleosome-depleted regions (NFRs/NDRs) flanked by well-positioned nucleosomes, with a nucleosome that overlaps or is adjacent to the transcription start site (TSS), called the +1 nucleosome.[253] Two important epigenetic marks at the promoter/gene body boundary of active genes are the H2A.Z variant histone and H3(K4me3).[12,13,254−256] In vitro, the H3(K4me3) mark increases the association of mammalian Chd1 with nucleosomes and appears to enhance chromatin remodeling toward modified nucleosomes.[257,258] Interestingly, although the H3(K4me3) mark is present in budding yeast, the yeast Chd1 chromodomains do not maintain the aromatic cage necessary for binding to this modification,[259,260] and accordingly deletion of Chd1 has little effect on +1 nucleosomes in yeast.[261,262] As with recognition of most PTMs, the interaction of the mammalian Chd1 chromodomains with H3(K4me3) is relatively weak.[259,263] Though the presence of H3(K4me3) did stimulate transcriptional elongation in a Chd1- and ATP-dependent manner, the H3(K4me3) mark alone was not sufficient for robust recruitment of Chd1 in vitro.[258] Instead, recognition of this mark is one of several interactions that localizes Chd1 to the transcriptional machinery; protein−protein interactions have been found between Chd1 and the mediator subunits med1 (*S. cerevisiae*)[258] or med15 (*Schizosaccharomyces pombe*),[264] the splicing factor SF3a subcomplex of the spliceosome,[265] and components of the Paf1, FACT

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(Spt16−Pob3), and DSIF (Spt4−Spt5) elongation factors.[266−269]

It is presently unclear how localization of Chd1 to +1 nucleosomes may stimulate transcription in mammalian cells. Decreasing the levels of active Chd1 in mammalian cells correlated with a small but significant reduction in histone occupancy and turnover at the promoter, and also an increased fraction of RNA polymerases that were paused at promoter-proximal nucleosomes.[270] Nucleosomes are natural barriers for RNA polymerase II, with distinct pause sites corresponding to stable histone−DNA interactions on the nucleosome.[49,271−273] Whether the increased pausing from lack of Chd1 activity is due to a direct or indirect effect is not presently known. Chd1 can assemble and reposition nucleosomes,[251,274−276] and therefore it could potentially assist RNA polymerase II directly by disrupting histone−DNA interactions. It has been demonstrated that several different chromatin remodelers introduce torsional strain into DNA during the remodeling reaction.[277] Interestingly, nucleosomes were recently found to be sensitive to torsional strain in single-molecule experiments.[278] By use of a specialized optical trap that allowed nucleosomal DNA to be both stretched and twisted, the H2A/H2B dimer was found to be selectively lost at high torque (∼38 pN·nm). In addition to chromatin remodelers, DNA torque accompanies all enzymes that translocate along duplex DNA, and significant torque is generated from transcribing RNA polymerases.[278,279] Although nucleosomes pose a significant barrier for RNA polymerases, increasing ionic strength to facilitate transcription through the nucleosome results in loss of H2A/H2B dimers[280] or entire octamers.[273] A challenge for factors assisting RNA polymerase is to therefore coordinate with the mechanics of transcription to reduce the nucleosomal barrier while maintaining or reestablishing histones and their marks for subsequent polymerases.

3.4. Post-Translational Modifications in Gene Bodies Reduce Histone Turnover and Coordinate Chromatin Dynamics with Passage of RNA Polymerase II

Although RNA polymerase II can transcribe through nucleosomes and leave the histone octamer intact, it often stimulates loss of H2A/H2B and sometimes the entire octamer from DNA.[273,280−285] RNA polymerase II is accompanied by several factors, many of which are elongation factors, that play important roles in maintaining the chromatin barrier during transcription. Failure to effectively maintain the chromatin barrier can allow improper transcriptional initiation at cryptic sites within coding regions, resulting in truncated gene products and antisense transcripts.[286−288] Also associated with the elongating polymerase are PTMs, which appear to help establish and maintain the transcriptionally refractory environment of chromatin yet also aid polymerase transiently as it transcribes through nucleosomes. Two marks that play important roles in these processes are monoubiquitylation of the H2B C-terminus (K123 in *S. cerevisiae* and K120 in humans) and methylation of H3(K36).

Di- and trimethylation of H3(K36) is established in gene bodies by Set2, which interacts with the phosphorylated C-terminus of elongating RNA polymerase II.[289−292] This methyl mark appears to serve as a basic recruitment signal for several factors. The Rpd3 histone deacetylase complex recognizes methylated H3(K36) through a chromodomain-containing subunit, Eaf3, and its localization to gene bodies reduces acetyl marks to maintain chromatin in a less transcriptionally accessible state.[293−295] Additional factors associated with the H3(K36me3) mark were identified by MudPIT (multidimensional protein identification technology) mass spectrometry, which, in addition to Rpd3 subunits, included several chromatin remodelers (ISW1a, ISW1b, ISW2, and Chd1) and histone chaperones (Spt16, Pob3, Spt6, Rtt106, Hir1, Hir2, and Hir3).[296] The ISW1b remodeler, consisting of the Isw1 ATPase subunit and two auxiliary subunits, Ioc2 and Ioc4,[297] appeared to be the most likely candidate for a direct interaction with H3(K36me3) due to PHD and PWWP domains on its Ioc2 and Ioc4 subunits, respectively. Although these subunits failed to directly bind methylated H3(K36)-containing peptides in vitro and the PWWP domain only modestly improved interactions with methylated nucleosomes, the PWWP domain was shown to be required for localization of Ioc4 to gene bodies in a Set2-dependent manner.[296] Also consistent with H3(K36me3) recognition, deletion of the ISW1 gene produced a similar phenotype as *set2*Δ, with increased cryptic transcription and histone turnover.[296,298] Both cryptic transcription and increased histone turnover were more pronounced in an *isw1*Δ*chd1*Δ background, consistent with colocalization to H3(K36me3)-containing chromatin and functional redundancy for these two remodelers.[262,296] Although Chd1 failed to specifically recognize methylated H3(K36) peptides or nucleosomes,[296] the interactions between Chd1 and elongation factors (FACT,[266−268] DSIF,[268] and Paf1 complex[267−269]) may account for its colocalization to the H3(K36me3) mark.

In the coding region, Chd1 and ISWI-type remodelers may help maintain the chromatin barrier by several mechanisms. One common characteristic shared by Chd1 and many but not all ISWI-type remodelers is the ability to generate evenly spaced arrays both in vitro[251,299−301] and in vivo[262,302] (Figure 11A). Close packing of nucleosomes is expected to be refractory to transcription initiation, as it would limit the availability of DNA. Unlike SWI/SNF remodelers, which can shift nucleosomes into their neighbors to create altosomes and nucleosome-free regions,[147,150,151] Chd1 and ISWI-type remodelers maintain nucleosomes at a minimum distance from each other. This distinct remodeling characteristic arises from a preference to shift nucleosomes toward longer stretches of flanking DNA.[274,275,303,304]

Another potential mechanism for reducing cryptic transcription and reestablishing the chromatin barrier is through nucleosome assembly. In vitro, both Chd1 and Iswi-type remodelers have been shown to catalyze the formation of nucleosomes with histones that have been deposited on DNA but not properly wrapped into nucleosomes.[251,276,299−301] For highly expressed genes where polymerase density is high, most nucleosomes are evicted, and reassembly by remodelers and histone chaperones is essential for resetting the chromatin barrier once transcription levels are reduced.[286,305−307]

Another connection between Chd1 and maintenance of the chromatin barrier in gene bodies is the ubiquitylation of H2B(K123) [yeast numbering, corresponding to H2B(K120) in humans]. Although no direct link has yet been made, Chd1 was found to be necessary for high levels of the H2B monoubiquitin mark.[308] Ubiquitylation of H2B is a dynamic mark associated with elongating RNA polymerase, which both regulates histone methylation pathways and participates in reorganization of chromatin structure.[309] In *S. cerevisiae*, and similarly in metazoans, ubiquitin is added to H2B(K123) by Rad6 (a ubiquitin-conjugating E2 enzyme) and Bre1 (an E3 ubiquitin ligase), a reaction that depends on the Paf1 complex

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and transcriptional elongation.[310−315] The monoubiquitylation mark on H2B is required for subsequent di- and trimethylation of H3(K4) and H3(K79) by Set1/COMPASS and Dot1 methyltransferases[316,317] and also reduces methylation of H3(K36) by Set2.[318] The presence of ubiquitin on the H2B C-terminus appears to directly influence nucleosome−nucleosome packing, as this mark interferes with chromatin fiber compaction.[319] The ubiquitin mark is only present transiently, however, as deubiquitylation enzymes, such as Ubp8 in *S. cerevisiae*, also travel along with the elongating polymerase complex and dynamically remove ubiquitin from H2B.[320] Preventing either removal of ubiquitin (*ubp8*Δ) or its deposition [H2B(K123A) substitution] reduces gene expression, highlighting the importance of the dynamic ubiquitylation/deubiquitylation cycle for proper transcriptional elongation and gene expression.[320−322]

Intriguingly, beyond just influencing other PTMs, the ubiquitin mark on H2B(K123) has been found to affect chromatin dynamics and passage of RNA polymerase. With a reconstituted chromatin transcription system, robust transcription was found to require both ubiquitylation of H2B and the presence of the FACT elongation factor.[315] Although ubiquitylated H2B does not alter transcription through chromatin on its own, the increased stimulation of transcriptional elongation in the presence of FACT suggests that the ubiquitin modification either intrinsically destabilizes the nucleosome or assists destabilization through action of FACT.[315] In vivo, simultaneous disruption of FACT and prevention of H2B ubiquitylation (K123A substitution) produced a transcription-dependent lowering of histone occupancy.[322] Blocking ubiquitylation of H2B with K123A also disrupted chromatin structure and increased cryptic transcription.[322]

Since disruption of Chd1 and ISWI-type remodelers, FACT, and H2B-ubiquitin and H3(K36me3) marks increase histone turnover and decrease histone occupancy, a generally accepted view is that these factors are important for histone octamer reassembly after passage of RNA polymerase II. However, the increased histone turnover and decreased occupancy may instead result indirectly from increased polymerase pausing. After forward motion of polymerase unwraps some DNA from the nucleosome, the rewrapping of DNA can trap polymerases in a backtracked state that prevents nucleotides from being added to the 3′ end of the growing RNA strand.[273,284,323] On moderately transcribed genes, increased pausing would allow some polymerases to catch up to others. When traveling in pairs, polymerases can assist each other in transcribing through nucleosomes, as the trailing polymerase limits the backtracking of the leading polymerase, and the leading polymerase helps maintain an unwrapped state for the trailing polymerase.[323] In contrast to single polymerases transcribing through nucleosomes, which can leave the nucleosome intact, multiple polymerases are much more destructive, presumably because they diminish opportunities for nucleosomes to re-form histone−DNA contacts.[283,324] A major remaining challenge is to elucidate the mechanisms by which transcription of RNA polymerase through nucleosomes can be assisted by chromatin remodelers and histone chaperones, which help to leave the nucleosome barrier intact.

3.5. H3(K56) Acetylation Modulates the Specificity of Histone H2A.Z Variant Exchange by the SWR1 Remodeler

Histone modifications not only provide platforms for recruitment but also influence how chromatin remodelers act on their nucleosome substrates. Recently, the H3(K56ac) mark was shown to dramatically affect histone exchange activity of the SWR1 remodeler.[325] SWR1, together with INO80, constitute a unique remodeler family with the defining characteristics of a large (>250 residue) insertion in the Snf2 motor and tight association with AAA+ RuvB helicase-like ATPases.[326−330] These remodelers are involved in many aspects of DNA processing and maintenance, including DNA damage signaling and repair, DNA replication, telomere maintenance, stability of centromeres, and transcriptional regulation.[329,330] The unique and defining characteristic of these remodelers is an ability to exchange H2A variants into and out of nucleosomes. Multiple variants of histone H2A have appeared throughout evolution, and SWR1 and INO80 target the only two variants common to all eukaryotes, H2A.X and H2A.Z[18] (Figure 11B).

Deposition of the histone H2A.Z variant is tightly integrated into transcriptional activation. For both transcriptionally active genes and those poised for activation, H2A.Z histones are specifically deposited into nucleosomes flanking the promoter NFR.[256,331−334] This deposition relies on the ATPase remodeling action of the SWR1 complex.[327,328] The SWR1 complex is specifically stimulated by nucleosomes possessing the canonical H2A/H2B dimers and, with the help of the NAP1 or Chz1 chaperones, replaces these canonical dimers with H2A.Z/H2B in a unidirectional reaction.[327,335,336] The reverse reaction, where nucleosomes containing H2A.Z variants are replaced with canonical H2A/H2B dimers, has been shown to be specifically catalyzed by INO80.[337] Thus, these two remodelers complement one another, with removal by INO80 important for sharpening the distribution of H2A.Z deposited by SWR1.[337]

The +1 nucleosomes of active promoters are rapidly turned over, which means that they possess the H3(K56ac) mark indicative of a newly deposited H3/H4 tetramer.[305−307,338] The high correlation between the H3(K56ac) mark and the presence of H2A.Z on +1 nucleosomes led Peterson and coworkers[325] to investigate the influence of this mark on SWR1 activity. Remarkably, it was found that the H3(K56Q) acetylation mimic disrupts the ability of the remodeler to distinguish H2A from H2A.Z nucleosomes. Unlike unmodified substrates, where SWR1 did not exchange H2A.Z/H2B dimers into nucleosomes already containing two copies of H2A.Z,[336] the presence of the H3(K56Q) acetylation mimic allowed SWR1 to replace existing H2A.Z/H2B on the nucleosome with H2A.Z/H2B dimers in solution. Therefore, some element in SWR1 senses H3(K56) and, when unmodified, effectively blocks ATPase stimulation and dimer exchange when the nucleosome already possesses the H2A.Z variant.[325] Two different H2A.Z-interacting elements in SWR1 have been proposed to link recognition H2A.Z on the nucleosome with the acetylation status of H3(K56). One is the Swc2 subunit,[325] which is required for interacting with H2A.Z/H2B dimers.[339] Loss of Swc2 reduced overall ATPase activity of SWR1 but, importantly, also prevented preferential ATPase stimulation by H2A-containing nucleosomes over those with H2A.Z.[325] However, SWR1 complexes lacking Swc2 are severely impaired for H2A.Z exchange[339] and also show some instability of other SWR1 subunits,[339,340] complicating interpretation of these experiments. Another candidate sensor element is the Swr1-Z

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domain, which is N-terminal to the Snf2 motor on the Swr1 subunit.[341,342] This element forms an extended chain that wraps over one end of the H2A.Z/H2B dimer.[342] Although not yet tested biochemically, the Swr1-Z domain contacts the H2A.Z/H2B dimer in a location that would be in close proximity to H3(K56) in the context of the nucleosome, making it attractive as a potential sensor of H3(K56) acetylation status.[342]

What might be the purpose in coupling H3(K56ac) with loss of the ability of SWR1 to discriminate H2A.Z- from H2A-containing nucleosomes? Like other histones, H2A.Z can acquire PTMs after deposition into nucleosomes, and the exchange of H2A.Z in nucleosomes with soluble pools of H2A.Z may be one mechanism of removing those marks. In *S. cerevisiae*, H2A.Z can be acetylated by NuA4 and SAGA at positions K5, K8, K10, and K14,[343−345] and in mammals it can be monoubiquitylated at its C-terminus.[346] These PTMs alter the cellular response to H2A.Z in chromatin. Acetylation of the H2A.Z tail is important for DNA damaging sensing. In *S. cerevisiae*, widespread H2A.Z deposition, which occurs in the absence of functional INO80, renders cells highly sensitive to DNA replication inhibitors, DNA damaging agents, and double-stranded DNA breaks.[326,337,347,348] These sensitivities can be suppressed when the four lysines that become acetylated are simultaneously mutated to glutamine, serving as acetyl mimics.[337] Therefore, the DNA damaging checkpoints that fail to resolve upon mislocalization and overincorporation of H2A.Z in an *ino80*Δ background arise from recognition of nonacetylated H2A.Z.[337] Another mark that would be expected to be disrupted by exchanging nucleosome-associated H2A.Z with the soluble pool is monoubiquitylation, which has been observed in mammalian cells.[346] Monoubiquitylated H2A.Z is highly enriched on the inactive X chromosome and, similarly to H2A, deubiquitination has been found to be required for gene activation.[346,349,350] The ability for SWR1 to replace these modified forms of H2A.Z therefore has the potential to have a dramatic impact on H2A.Z-sensitive signaling pathways. Importantly, however, the H3(K56ac) mark is required for this behavioral change in SWR1, which limits the SWR1-dependent refreshing of H2A.Z nucleosomes to those where H3/H4 has been newly deposited, perhaps as a mechanism for keeping H2A.Z in a more naïve state.

4. CONCLUDING REMARKS

Nucleosome dynamics plays an important role in relieving the intrinsically inhibitory nature of chromatin and granting access to DNA. Post-translational modifications (PTMs) can modulate dynamics by directly altering the energy landscape of the nucleosome and by influencing binding of histone chaperones and chromatin remodelers. PTMs near histone−DNA interfaces can promote DNA unwrapping, which increases access for DNA-binding proteins such as transcription factors (Figure 3A), or can destabilize the entire nucleosome by perturbing energetically important contacts near the dyad (Figure 3B). Likewise, PTMs at histone−histone interfaces can increase fluctuations of histone dimers in the octameric core to aid assembly/disassembly (Figure 3C). PTMs that enhance the specificity of chromatin remodelers may not only promote nucleosome sliding, disassembly, and histone exchange (Figure 11) but also direct remodelers to maintain the integrity of the chromatin barrier in the presence of disruptive machinery such as RNA polymerase II.

An area in which very little is currently known is how chromatin remodelers and core PTMs that affect nucleosome dynamics may work together or in opposition to alter histone−histone and histone−DNA interactions. One reported example of functional synergy is between phosphorylation of the SIN residue H3(T118) and the SWI/SNF remodeler: each destabilizes the nucleosome on its own, but together they are much more disruptive.[146] Another PTM that potentially acts synergistically with remodelers is H3(K56ac), which increases unwrapping at the entry/exit region[52,61] but also alters specificity of H2A.Z exchange for the SWR1 remodeler.[325] Although this effect on SWR1 specificity may be due to direct recognition of the acetylation status of H3(K56), the increased DNA unwrapping due to this or other PTMs may bias exchange of H2A/H2B dimers. In addition to acting synergistically, increased DNA unwrapping dynamics due to PTMs could potentially have negative consequences if, for example, unwrapping were to interfere with actions of Chd1 and ISWI-type remodelers that are sensitive to DNA around the entry/exit region.

Compared to modifications on the flexible histone tails, PTMs on the histone core are generally less accessible. Core histone PTMs can be introduced onto histones before assembly into nucleosomes, such as with H3(K56ac) and H4(K91ac). In the context of the nucleosome, however, enzymes to add or remove such marks likely require assistance to access target residues. Some access may be gained through the increased nucleosome dynamics provided by the core PTMs themselves, which would be expected to also increase the likelihood that modifications are added or removed at neighboring residues. The dramatic structural changes catalyzed by chromatin remodelers would also offer greater opportunities for adding or removing core PTMs. Indeed, the NuRD remodeler is named for having a nucleosome remodeling Snf2 motor associated with histone deacetylase subunits HDAC1 and HDAC2[202] and has also been reported to associate with the lysine demethylase LSD1.[351] Other types of modifying enzymes have been shown to associate with remodelers, such as the kinase-containing WSTF protein that joins ISWI-type remodelers[352,353] and the TIP60 acetytransferase complex that associates with the SWR1 remodeler.[354] A relatively uncharted area for future discovery will be to identify what structural and dynamic changes in the nucleosome are required to write and erase core PTMs, and how remodelers and other factors directly contribute to these processes.

In addition to perturbing nucleosome dynamics, PTMs on the histone core may also be utilized to recruit other factors. The binding of reader domains to core histone PTMs would likely stabilize nucleosomes in altered conformations, further enhancing nucleosome dynamics. While the face of the histone octamer interacts with protein domains, such as RCC1[355] and Sir3 BAH domain,[138] there are currently no known factors that bind histone PTMs in the DNA−histone or histone−histone interfaces. The apparent lack of such readers could reflect the reduced accessibility of core PTMs but may also stem from poor reagents to detect such readers. A continuing technical challenge in studying PTMs on the histone core is raising good antibodies for chromatin immunoprecipitation. Although this has been achieved for some modifications, such as H3(K56ac) and H3(K122ac), the distinct structure and environment of PTMs in the context of the histone core is not maintained when these PTMs are presented on peptides. The loss of structural context for core PTMs, as well as potential steric interference that would reduce binding, make it difficult to

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isolate antibodies that robustly recognize these marks in the context of native nucleosomes.

Since their identification of histone PTMs within the nucleosome core in the early 2000s, we have begun to learn how these modifications can influence nucleosome dynamics. Given the large and growing number of histone PTMs that have since been identified and the limited number of core histone PTMs that have been studied, what we have learned so far may be only the tip of the iceberg.

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The authors declare no competing financial interest.

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Michael G. Poirier received his Ph.D. (2002) in physics from the University of Illinois, Chicago, for his work on mitotic chromosome structure and function with John F. Marko. He moved to the Université Louis Pasteur (2002−2004) for postdoctoral training with Dr. Didier Chatenay. He then moved to Northwestern University (2004−2006) as a postdoctoral fellow and worked on chromatin structure and dynamics with Dr. Jonathan Widom. He has been at The Ohio State University since 2006 and is currently an associate professor in the Department of Physics. His lab has been investigating the direct impact of histone post-translational modifications on nucleosome and chromatin structural dynamics.

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