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Signaling to Chromatin through Histone Modifications

Review

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Signal Transduction and Chromatin

The ability to detect extracellular stimuli and execute the appropriate response is crucial to all cellular functions. Upon receiving external signals, be it growth factor stimulation or exposure to stress, distinct pathways are activated that culminate in the induction or repression of defined sets of genes. The transduction of signals from cell surface to the nucleus often involves phosphorylation cascades that not only allow for rapid transmission, but also serve to amplify the signal by activating multiple factors and genes. The finely tuned combination of these signal-induced nuclear effects thus leads to integrated cellular responses such as proliferation, differentiation, and apoptosis.

A central goal in the signaling field is the identification of physiologically relevant targets of transduction pathways. To that end, much attention has been focused on the signal-induced activation of transcription factors and components of the transcription machinery. However, chromatin, the physiological packaging structure of histones and genomic DNA, has largely been neglected as a relevant target of signaling pathways. Given the growing appreciation of chromatin structure as an important element regulating gene expression, we present in this review evidence that signaling pathways act directly on chromatin components to regulate different DNA-templated processes. More specifically, we propose that the N-terminal tails of histones are targeted by multiple pathways, and that reversible covalent modifications are used in combinatorial fashion to elicit appropriate downstream responses.

Core Histone Proteins and Chromatin Structure

Histone proteins, assembled with DNA to form nucleosomes, are the basic building blocks of chromatin. Not surprisingly, the four core histone molecules, H2A, H2B, H3, and H4, are among the most evolutionarily conserved proteins known (van Holde, 1988). This conservation reflects the nearly invariant way in which DNA wraps around the histone proteins in eukaryotic cells (reviewed in Kornberg and Lorch, 1999). In the classical view, first proposed by Kornberg (1974), each nucleosomal unit is formed by wrapping approximately 146 base pairs of DNA around a histone octamer core particle containing one H3-H4 tetramer and two H2A-H2B dimers. The validity of this model has been confirmed by determining the crystal structures of the histone octamer, and of the nucleosome particle (Arents et al., 1991; Richmond et al., 1993; Luger et al., 1997). As revealed by these and other studies, the C-terminal histone-fold domains of core histones have similar conformations that are critical for the assembly of nucleosomes by mediating histone–histone and histone–DNA interactions (reviewed in Wolffe, 1998). In contrast, the N-terminal tails of core histones are less structured and are not essential for maintaining the integrity of nucleosomes since removal of these tails by trypsin treatment does not diminish nucleosome stability (Whitlock and Simpson, 1977; Ausio et al., 1989). Instead, histone tails are thought to make secondary and more flexible contacts with DNA and adjacent nucleosomes (Luger et al., 1997) that allow for dynamic changes in the accessibility of the underlying genome.

How do these N-terminal histone tails participate in the modulation of chromatin architecture? One possible way is that they constitute targets for ATP-dependent chromatin remodeling factors such as Swi/Snf and NURF (Georgel et al., 1997; Lee et al., 1999; Krebs et al., 2000). Readers interested in the function of these remodeling complexes are directed to other reviews more focused on that topic (Kingston and Narlikar, 1999; Peterson and Workman, 2000). Another way is that these tails are subjected to a diverse array of posttranslational modifications, such as acetylation and phosphorylation (Figure 1), which may modulate the contacts between histones and DNA. Because these modifications are reversible, they can act as chromatin-based "on/off" switches that regulate a multitude of DNA-related processes. Moreover, since the histone tail domains are highly accessible to the nuclear environment, they constitute attractive targets for signal-activated enzymes, and may function as important links between signal transduction and gene expression. Given the prominent role that dynamic phosphorylation plays in signal transduction, in this review we have centered our discussion mainly on core histone phosphorylation. However, it is likely that the central concepts discussed here are also applicable to other covalent modifications known to influence histone structure and function.

Histone Phosphorylation and Transcriptional Activation

Perhaps the best characterized link between signal transduction and histone modification is seen in mammalian cells upon exposure to mitogen or stress. By treating mouse fibroblasts with various growth factors or protein synthesis inhibitors, Mahadevan and colleagues found that H3 and HMG14 are rapidly and transiently phosphorylated upon stimulation, an effect that they have subsequently termed nucleosomal response

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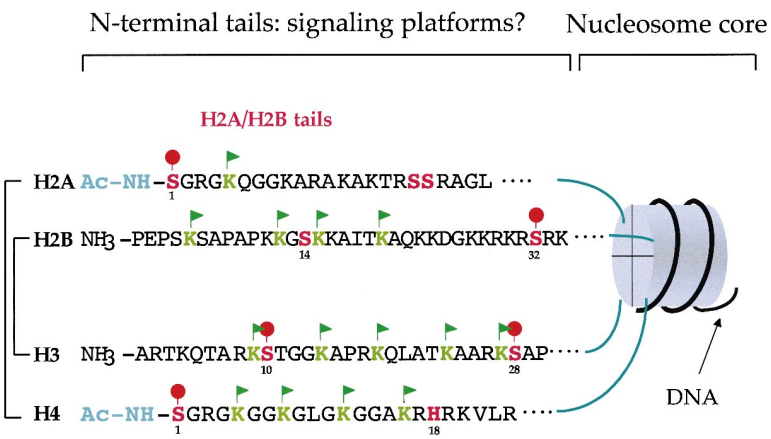

Figure 1. Amino-Terminal Tails of Core Histones as Signaling Platforms

(Right) Simplified representation of a nucleosome core particle comprised of DNA wrapped around a histone octamer. (Left) Amino acid sequences of the N-terminal tails of the four human core histones. Red lollipops represent known phosphorylation sites (Pantazis et al., 1984; Hendzel et al., 1997; Goto et al., 1999; Ajiro, 2000); green flags represent known acetylation sites (Thorne et al., 1990); red serine and histidine residues represent potential phosphorylation sites. Blue Ac-NHs represent N-terminal acetylation of H2A and H4 tails. Brackets connecting the N-terminal tails of H2A and H4 versus H2B and H3 refers to potential redundancy in the function as signaling platforms for these pairs of tails (see text for further details). Due to the complexity of the nucleosome structure, this representation does not attempt to accurately portray the interactions between the histone subunits and the projections of the histone tails.

(Mahadevan et al., 1991; reviewed in Thomson et al., 1999b). Phosphorylation of H3 occurs at serine 10 (Ser10) of the N-terminal tail, and the time course of H3 phosphorylation closely corresponds to the transient expression of activated immediate-early genes, suggesting that this histone modification is linked to transcription activation. Further studies of this nucleosomal response showed that the p42/p44 mitogen-activated protein (MAP) kinase pathway (also known as ERK pathway), as well as the stress-activated p38 pathway, can each induce H3 phosphorylation (reviewed in Thomson et al., 1999b). The ERK pathway is one of the best defined signaling pathways, and many of the kinases involved in this cascade have been identified (reviewed in English et al., 1999). Simplistically, exposure of cells to extracellular growth factors induces this kinase cascade which activates the p42/p44 (ERKs 1 and 2) through Ras and Raf, and in general, induces proliferation as a response. By comparison, the related p38 pathway is not as well characterized (reviewed in Tibbles and Woodgett, 1999). It is activated by stress producing stimuli such as toxins and UV, and elicits responses including apoptosis or inflammation. Recently, it has been shown that the ERKs-activated Rsk-2 kinase is directly involved in H3 phosphorylation in vivo (Sassone-Corsi et al., 1999). Cells derived from Rsk-2-deficient Coffin-Lowry syndrome (CLS) patients, or from *Rsk-2* knockout mice, are impaired both in the transcriptional activation of c-*fos* gene and EGF-induced phosphorylation of H3 (De Cesare et al., 1998; Sassone-Corsi et al., 1999). Moreover, ectopic expression of Rsk-2 alone in CLS cells is sufficient to restore the H3 phosphorylation response to EGF. These findings together suggest that a kinase in the ERK signaling pathway can phosphorylate H3, and therefore provide a direct link between signal transduction pathways and histone phosphorylation.

Interestingly, H3 phosphorylation during mitosis occurs normally in CLS and Rsk-2 deficient mouse cells, indicating that mitotic H3 phosphorylation is mediated by a separate kinase (see later section in this review). Moreover, EGF-stimulation is not known to activate the p38 pathway, and therefore, additional kinases besides Rsk-2 must be involved in the nucleosomal response. Indeed, another study has advocated the Rsk-related Msk-1 (MAP- and Stress-activated kinase 1) as another kinase responsible for H3 phosphorylation (Thomson et al., 1999a). Msk-1 is activated by both ERK and p38 pathways, and it is possible that both these pathways can activate this kinase to phosphorylate H3. It is becoming clear that multiple enzymes can phosphorylate H3 (see later section also), and the challenge for future studies is to determine how this single histone modification, induced by different signaling pathways, can elicit diverse cellular responses. This will likely involve additional targets, and perhaps additional histone modifications that are induced by these signaling pathways

Potential Effects of H3 Phosphorylation

At present, how histone H3 phosphorylation affects gene expression is not known. One possibility is that the addition of negatively charged phosphate groups to the N-terminal H3 tails may disrupt electrostatic interactions between the basic H3 tails and the negatively charged DNA backbone, and thereby increase the accessibility of the underlying genome to nuclear factors. A similar hypothesis has been put forth to explain the facilitative effect of histone acetylation on gene expression. In fact, as depicted in Figure 2, several studies have suggested that H3 phosphorylation and acetylation are tightly coupled in response to EGF stimulation (Barratt et al., 1994; Cheung et al., 2000; Clayton et al., 2000). Nucleosomes containing phosphorylated and acetylated H3 are preferentially associated with EGF-activated genes, suggesting that both modifications may cooperate to facilitate transcription of these genes. A mechanistic link between these two enzymatic reactions is further suggested by the finding that, in vitro, several transcription-associated histone acetyltransferases (HATs) display strong preferences for H3 phosphorylated at Ser10 over the unmodified form as substrate (Cheung et al., 2000; Lo et al., 2000). These results sug-

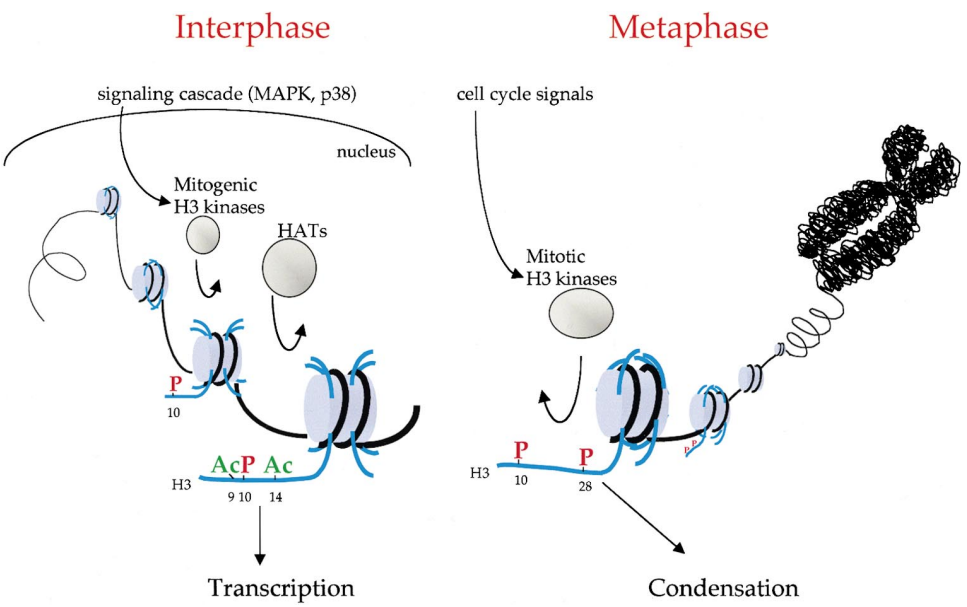

Figure 2. Duality of Histone H3 Phosphorylation in Interphase and Metaphase Cells

(Left) Schematic representation of the sequential H3 phosphorylation and acetylation events seen upon mitogen (EGF) stimulation. Activation of MAP kinase or p38 pathways results in the activation of mitogenic H3 kinases (Rsk-2 or Msk1) and leads to rapid and transient phosphorylation (red P) of H3 at serine 10. This H3 phosphorylation is coupled to acetylation of nearby residues (green Ac's, acetylation of Lys9 and Lys14 are highlighted, but additional sites may also be involved). Together, these events can facilitate transcription of the immediate-early genes. The blue protrusions represent the N-terminal tails of the core histones. (Right) Schematic drawing of a mitotic chromosome with representative nucleosomes from the chromosome arm region. During mitosis, not yet well defined cell cycle signals activate mitotic H3 kinases (Ipl1 in yeast or NIMA in *A. nidulans*) which in turn phosphorylate the N-terminal tails of H3 (red P's on extended blue tails) at Ser10. Ser28 is also phosphorylated during mitosis; however, the identity of the kinase responsible for this has not been determined. These phosphorylation events likely contribute to the chromosome condensation process.

gest dynamic interplay between different modifications that occur on the same histone tail, a phenomenon that adds yet another layer of complexity to the regulation of gene expression through histone modifications.

Another model of how phosphorylated—and/or acetylated—H3 may promote gene activation is that these modifications may serve as recognition sites for recruitment of transcription factors or regulatory complexes. An informative paradigm for phospho-dependent protein binding is exemplified by SH2 domains found in many elements of receptor tyrosine kinase–mediated signal transduction pathways (reviewed in Hunter, 2000). The discovery of SH2 and other phospho-tyrosine binding motifs was an important breakthrough that provided a mechanistic basis for the ability of phosphorylation to recruit and target enzymes involved in propagating signaling cascades. At present, much less is known about factors that may have phosphoserine binding motifs, and as yet, no nuclear factors have been identified that specifically bind to phosphorylated H3 molecules. However, phosphoserine-specific binding proteins are known. For example, the transcription coactivator CBP (CREB binding protein) was first identified as a nuclear factor that specifically binds to CREB phosphorylated at Ser133, and this phosphate-dependent interaction with CBP is critical for the stimulus-induced activation property of CREB (Chrivia et al., 1993; reviewed in De Cesare et al., 1999; and Shaywitz and Greenberg, 1999). Given that CBP itself has HAT activity, one intriguing possibility that merits further investigation is whether CBP or other HATs can directly interact with phosphorylated H3. In that respect, it is noteworthy that CBP was found to interact with Rsk-2 (Nakajima et al., 1996), and protein complexes with multiple histone modifying-enzymes may be targeted to histones at the same time.

The role of histone modification in mediating protein–protein interaction is better illustrated by a structure known as bromodomain that binds acetyl-lysines. Bromodomains are found in a number of nuclear histone acetyltransferases and transcription regulators (reviewed in Jeanmougin et al., 1997; Winston and Allis, 1999). A direct interaction between bromodomains and acetyl-lysines was initially suggested by NMR structural studies of PCAF's bromodomain and confirmed by in vitro peptide binding and mutagenesis experiments (Dhalluin et al., 1999). More recent crystallographic analyses of the double bromodomain of human $TAF_{II}250$ showed that these modules have the capacity to bind histones that are multiply acetylated (Jacobson et al., 2000; Figure 5A). Interestingly, the distance between the two binding pockets ideally suits interaction with acetylated lysines that are seven amino acids apart, a configuration that is seen on H4 in vivo (K5, K8, K12, and K16 of the N-terminal tail are known to be acetylated [Thorne et al., 1990]). Furthermore, in vitro peptide binding assays showed that multiply acetylated H4 peptides bind to the double bromodomain with much greater affinity than unacetylated peptides. Given that $TAF_{II}250$ is a component of TFIID, these findings immediately suggest a model whereby acetylation of histones, partic-

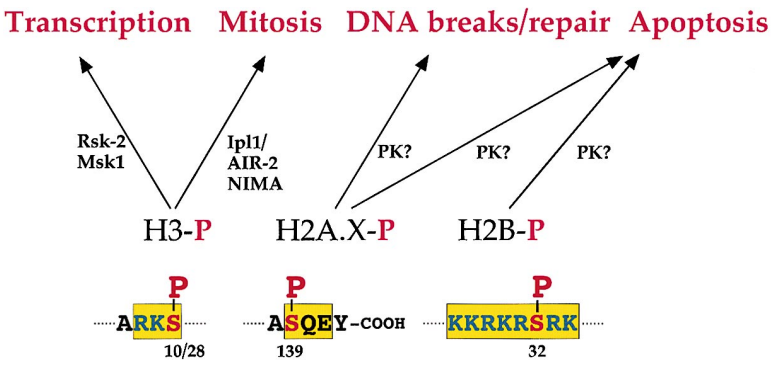

Figure 3. Multiple Cellular Processes Are Associated with Histone Phosphorylation

Phosphorylation of H3, H2A.X, H2B has respectively been associated with mitogen stimulation (Transcription), chromosome condensation (Mitosis), DNA damage, and apoptosis. The putative enzymes responsible for these phosphorylation events and the sites of phosphorylation, where known, are as indicated, and PK? refers to unknown protein kinases. The amino acid sequence surrounding those phosphorylation sites are indicated at the bottom, and the yellow boxes typically indicate stretches of basic amino acid residues flanking the phosphorylated serines.

ularly at H4, may serve to recruit TFIID to relevant promoters and facilitate the assembly of preinitiation complexes. Moreover, the intrinsic HAT and kinase activities of $TAF_{II}250$ (Mizzen et al., 1996; Rossignol et al., 1999) may further modify other histones or factors present at the initiation complex. In that case, acetylation-dependent binding of $TAF_{II}250$ may serve as yet another step linking signal-induced histone modifications to transcription activation.

In mammalian cells, transcription-associated H3 phosphorylation appears to be unique to a small set of genes (the immediate-early genes) that are rapidly turned on and off in response to extracellular signals. This is particularly well illustrated by immunofluorescence studies which showed speckled nuclear staining for phosphorylated H3 in EGF-stimulated cells (Chadee et al., 1999; Sassone-Corsi et al., 1999). At present, our knowledge of the identity of the genes regulated by this response is limited, as is our understanding of the targeting mechanisms that direct the H3 kinases to the selected loci. Therefore, future research into these two areas is needed in order to further our understanding of the link between H3 phosphorylation and transcription.

Histone Phosphorylation in Response to DNA Damage and in Association with Apoptosis

As illustrated in Figure 3, in addition to transcription regulation, histone phosphorylation is also linked to other cellular processes. For example, several reports have shown that an H2A variant in mammalian cells, H2A.X, is rapidly phosphorylated at its C-terminal tail (at Ser139) upon exposure to ionizing radiation (Rogakou et al., 1998, 1999). Phosphorylation occurs at the H2A.X-specific C-terminal sequence motif [KATQAS*QEY-COOH] (Figure 3) and this event has been termed γ-phosphorylation. Upon exposure to ionizing radiation, phosphorylation at this site peaks within 10 min, suggesting that it is mediated by a presently unknown signaling pathway that has rapid kinetics. Further evidence suggests that γ-phosphorylation is triggered by radiation-induced DNA double-strand breaks, and analysis of DNA damage induced by laser microbeam showed that the H2A.X phosphorylation is localized to mega-base regions of DNA that flank sites of double-strand breaks. Incidentally, the ASQE motif is conserved in the C-terminal tail of the major H2A species in yeast, and phosphorylation of the corresponding Ser129 of H2A in *S. cerevisiae* has been found in association with DNA damage (Rogakou et al., 1999). Therefore, C-terminal phosphorylation of H2A and H2A.X is a conserved mechanism which may function to facilitate access of the damaged areas to repair mechanisms.

Additional studies showed that phosphorylation of mammalian H2A.X at Ser139 is also triggered upon apoptosis-induced signals (Rogakou et al., 2000). The timing of phosphorylation coincides with the initiation of DNA fragmentation seen at early stages of apoptosis, and may be linked to double-stranded DNA breakage induced by the apoptotic program. Histone H2B has been reported to be phosphorylated in apoptotic mammalian cells as well (Ajiro, 2000), and this phosphorylation event also initiates around the time of nucleosomal DNA fragmentation. Phosphorylation of both H2A.X and H2B is dependent on activation of caspases, and therefore may be linked to caspase-induced signaling pathways. The identification of kinases that phosphorylate H2A.X or H2B will help clarify any potential links between apoptotic pathways and histone modifications.

Multiple Personalities of H3 Phosphorylation

Phosphorylation of histone H3 at Ser10 has long been correlated with mitosis and chromosome condensation (reviewed in Bradbury, 1992; Koshland and Strunnikov, 1996), and more recently, H3 Ser28 was found to be phosphorylated during mitosis as well (Goto et al., 1999). It has always been paradoxical that H3 phosphorylation at Ser10 is associated with seemingly contrasting functions of transcription activation and chromosome condensation. One possible, but yet to be proven, hypothesis is that H3 phosphorylation during mitosis actually opens up the chromatin fiber for nuclear factors that promote condensation to access the genome. Phosphorylation at Ser10 starts at G2/M of the cell cycle, and is initially localized to pericentromeric heterochromatin of each chromosome (Hendzel et al., 1997). By metaphase, Ser10 phosphorylation spreads throughout all chromosomes. The tight correlation in timing of H3 phosphorylation with chromosome condensation suggests a functional link between the two events. Although a direct causal connection between the two events has not been fully established, the finding that mutation of H3 Ser10 to alanine (S10A) in *Tetrahymena* leads to abnormal patterns of chromosome segregation and extensive chromosome loss strongly suggests that H3 phosphorylation plays an important role in mitosis and meiosis (Wei et al., 1999).

With a potential functional role of H3 phosphorylation in chromosome condensation, great efforts have been invested in identifying the kinase(s) responsible for this phosphorylation during mitosis. Two recent papers have identified the Ipl1/AIR-2 kinases in yeast and nematodes, and the NIMA kinase in *Aspergillus nidulans* as mitotic H3 kinases (Hsu et al., 2000; De Souza et al., 2000). Although Ipl1/AIR-2 and NIMA are evolutionarily divergent kinases, they share a number of similar features that led to the conclusions that they function as the mitotic H3 kinases in their respective organisms. In yeast, expression of Ipl1 protein peaks at M phase and strains bearing temperature-sensitive mutations of gene encoding Ipl1 display defects in chromosome segregation during mitosis (Francisco et al., 1994; Biggins et al., 1999). Similarly, NIMA mRNA and protein levels peak at G2/M, and ectopic expression NIMA induces in S phase cells premature chromatin condensation (Osmani et al., 1987, 1988). More strikingly, mutations in Ipl1 or NIMA in their respective organisms result in the complete loss of H3 phosphorylation during mitosis (Hsu et al., 2000; De Souza et al., 2000). Also, both kinases can phosphorylate H3 at Ser10 in vitro, and Ipl1 can do so in the context of nucleosomes (this ability has not been tested for NIMA). It is possible that different organisms may employ different kinases to perform a critical function. In addition, it has been reported that the NIMA-related kinase 2 (Nek2) in mammalian cells is complexed with protein phosphatase 1 (PP1) (Helps et al., 2000), and the yeast PP1 homolog, Glc7, has been suggested to mediate dephosphorylation of H3 postmitotic chromosome condensation (Hsu et al., 2000). Therefore, regulated activities of kinases and phosphatases can affect the overall H3 phosphorylation levels during mitosis.

Both NIMA and Ipl1 are phosphoproteins and their activities are regulated by upstream kinase pathways. Phosphorylation of NIMA requires $NIMX^{cdc2}$ (Ye et al., 1995), whereas the kinase(s) that phosphorylates Ipl1 is still unknown. Interestingly, recent data demonstrated that a member of the Survivin/BIR family has an indirect regulatory role in H3 phosphorylation in *C. elegans*. This family of proteins all contain BIR domains and were originally identified as proteins that have inhibitory effects on apoptosis (reviewed in Deveraux and Reed, 1999). Using RNA-mediated interference (RNAi) to suppress expression of BIR-1 in *C. elegans*, it was found that these mutant embryos lack H3 phosphorylation, and exhibited problems with chromosome condensation and segregation of chromosomes (Speliotes et al., 2000). These phenotypes are virtually identical to those of *air-2* mutants, and this observation is explained by the finding that BIR-1 functions to target AIR-2 to chromosomes. Insofar as Survivin and Aurora (the human homologs of BIR-1 and AIR-2) are often overexpressed in human cancer cells (reviewed in Reed and Bischoff, 2000), these proteins likely participate in a regulatory pathway that maintains proper histone phosphorylation, chromosome condensation and segregation.

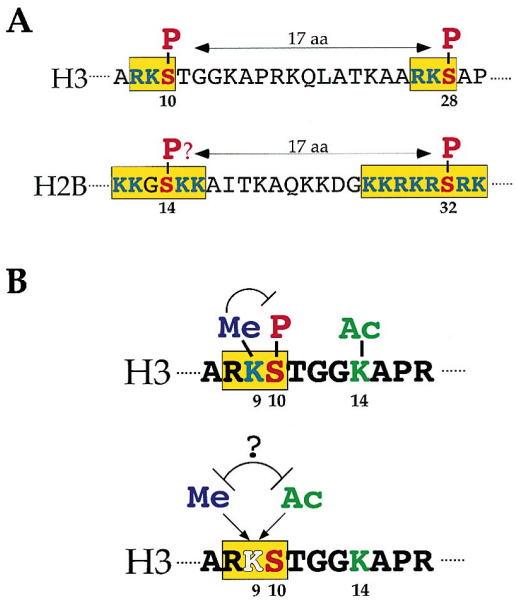

Figure 4. Symmetry of N-Terminal Tails of H3 and H2B, and Potential Interplay between Nearby Histone Modifications

(A) Partial sequence alignment of N-terminal tails of H3 and H2B highlighting symmetry between these two histone tails. Red P's represent known phosphorylation sites whereas red P's with question marks represent potential sites. Yellow boxes indicate highly basic amino acid residues that flank these phosphorylation sites. Ser10 and 28 are known to be phosphorylated during mitosis; however, the existence of H2B tails bearing one or both phosphorylation marks at Ser14 and 32 is yet to be demonstrated. (B) Potential interplay between histone modifications. Evidence from *Suv39h1* knockout murine cells suggests that methylation of H3 at Lys9 by this histone methyltransferase can inhibit phosphorylation of the adjacent Ser10 residue. Also, since H3 Lys9 can be methylated or acetylated, these two mutually exclusive processes may be antagonistic to each other. If correct, histone deacetylation may be required to permit subsequent methylation of the same residue.

Functional Redundancy of Histone Tails

One of the more puzzling observations with respect to the role of H3 phosphorylation in mitosis is that mutation at H3 Ser10 in *Tetrahymena* resulted in defects in chromosome segregation, whereas the corresponding mutation in yeast H3 Ser10 did not show any defects (Wei et al., 1998; Hsu et al., 2000). It is possible that the yeast mitotic H3 kinase, failing to locate the preferred Ser10 site, may phosphorylate histones at alternative sites which could compensate for the function of H3 phosphorylation. Examination of the specificity of yeast Ipl1 kinase on nucleosomal substrates showed that in addition to H3, H2B is also phosphorylated (Hsu et al., 2000). Could H2B harbor a consensus Ipl1 phosphorylation site that is utilized in the yeast S10A mutant? Given that most of the kinases that can phosphorylate H3, such as Ipl1, PKA, Rsk-2, and Msk1, are basic residue-directed kinases, i.e., they tend to target Ser/Thr sites that are surrounded by basic residues, it is intriguing that the human H2B N terminus contains two serines (Ser14 and Ser32) that are both flanked by series of basic residues (Figure 4). More thought-provoking still is that these serines are not found in H2B of *Tetrahymena*. Therefore, we speculate that H2B may be phosphorylated during mitosis in the absence of H3 Ser10 in yeast.

Striking pair-wise similarities between the N-terminal tails of H2A–H4, and H2B–H3 (see Figure 1) invite additional speculations regarding the redundancy or

complementarity of modifications in these histones. For example, the N-terminal tails of H2A and H4 are considerably shorter than those of H3 and H2B. Crystallographic studies suggested that the portions of H2B and H3 immediately adjacent to the histone fold domains have more extensive interactions with DNA as they exit the nucleosome core particle compared to that of the H2A and H4 tails (Luger et al., 1997). In addition, a number of HATs have pair-wise preferences for histone as substrates: H3 and H2B are targets for the prototypical HAT Gcn5p in the yeast SAGA complex (Grant et al., 1997), whereas H4 and H2A are the preferred substrates for Esa1p, the catalytic component of the NuA4 complex (Allard et al., 1999). Upon closer inspection of the histone tails, we note that both H4 and H2A in fact have an identical 5 amino acid stretch at their N termini (Figure 1). While H3 and H2B tails do not share identical sequences, they both have highly conserved serine residues that are adjacent to or flanked by basic amino acids. These include the well-known sites of phosphorylation at serines 10 and 28 of H3, and the serines at positions 14 and 32 of H2B (Figure 4). Curiously, these conserved serines are seventeen amino acids apart in both histone tails, a feature that further illustrates the symmetry of these two histones. Finally, although assembly of H2A–H2B and H3–H4 heterodimers are well known, the characteristics listed above compel us to hypothesize that the N-terminal tails of H2A and H4, and those of H2B and H3, may act in concert as "signaling platforms", and that modifications on these pairs of tails may share some functional redundancy.

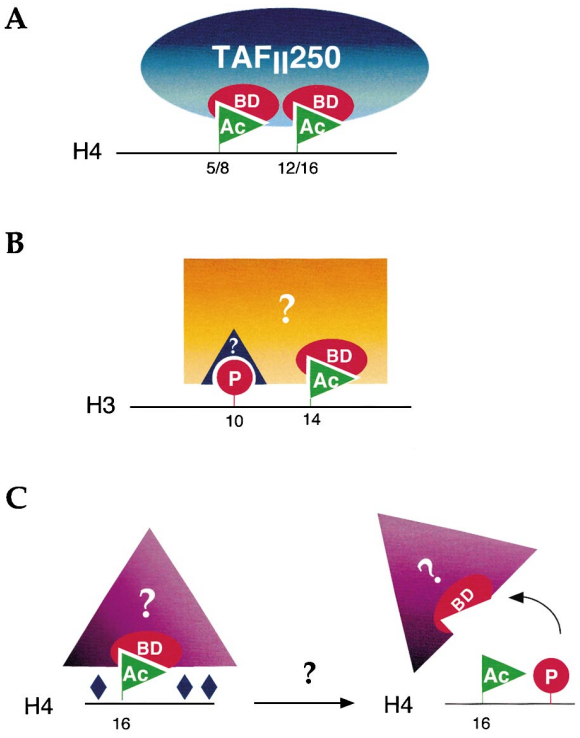

Figure 5. Possible Functional Roles of Combinations of Histone Modifications

(A) The pair of acetyl-groups (Ac flags) on multiply-acetylated H4 function as interaction modules and bind to the double-bromodomains (BD) of $TAF_{II}250$ (Jacobson et al., 2000). (B) By analogy, double modifications such as Phos Ser10 and Ac Lys14 on H3 may also act as unique epitopes that specifically interact with nuclear factors (question mark). (C) Interaction of hypothetical bromodomain containing protein (question mark) with acetylated H4 could be stabilized by specific contact points on H4 flanking the acetylated site (blue diamonds). Additional nearby modifications, such as phosphorylation (red lollipop), may disrupt these stabilizing contacts and thereby prevent binding of the nuclear protein to acetylated H4.

Unzipping Polar Zippers via Histone Phosphorylation?

One of the effects of phosphorylation is the alteration of the electrostatic charge of this surrounding region. We are struck by the frequent presence of short stretches of basic amino acids flanking potential phosphorylation sites within the histone tails. These "basic patches" may engage acidic residues on other histones or on chromatin-associated factors, and that reversible phosphorylation may be utilized to disrupt these electrostatic interactions and thereby unfold the chromatin fiber. Indeed, recent evidence suggests that modulation of "charge patches" in linker histone H1 via reversible phosphorylation can regulate transcription, presumably through altering higher-order chromatin structure (Dou and Gorovsky, 2000).

In addition to basic patches flanking serines 14 and 32 of H2B, another critical and well-studied basic region is present adjacent to the histone fold domain of H4. This [KRHRK] sequence, representing amino acids 16–20 on the H4 N-terminal tail, is within a well-studied domain that is critical for silencing in yeast (Kayne et al., 1988; Smith, 1991; Johnson et al., 1992). This stretch of basic residues was observed to contact acidic residues of H2A/H2B molecules in an adjacent nucleosome in crystallized core particles (Luger et al., 1997), and may form an internucleosomal "bridge" in higher-order chromatin. We note that the histidine (His18 in most H4s) found in this basic patch has the potential to be phosphorylated, and therefore, may have a role in disrupting these potential internucleosomal interactions. While Ser/Thr phosphorylation predominates in histones, acid-labile, P-N linkages to histidine, lysine, and arginine residues of histones have been reported (reviewed in van Holde, 1988). We speculate that short basic stretches within histones may represent "spot-welding" modules used to stabilize higher-order chromatin structure. Moreover, phosphorylatable (or acetylatable) amino acid residues located within these basic patches may be targets for signal-activated kinases/acetyltransferases, and may regulate dynamic changes in chromatin structure at selected loci of the genome.

Histone Phosphorylation, Acetylation, and More

Interest in chromatin structure and histone modifications has grown dramatically over the last few years. Not only is the link between histone acetylation and transcription activation now firmly established, there is growing evidence that histone phosphorylation, methylation, ADP-ribosylation, and even ubiquitination are associated with a wide variety of biological processes (Bradbury, 1992; Strahl et al., 1999; Tanny et al., 1999; Thomson et al., 1999b; Rea et al., 2000; Robzyk et al., 2000). In addition to the well-documented link between

transcription-associated H3 phosphorylation and MAP kinase pathways, indirect evidence suggest that histone methylation may also be linked to the JAK/STAT signaling pathway. By yeast two-hybrid assay, Pollack et al. (1999) identified a novel JAK2 kinase interacting protein, Jak binding protein (JBP1), which has methyltransferase activity that can methylate histones H2A and H4 in vitro. In addition, Hsl7, the yeast homolog of JBP1, was initially identified through a screen for histone H3 synthetic lethal mutants (Ma et al., 1996), suggesting that it plays a functional role in conjunction with H3. Hsl7 participates in the yeast MAP kinase pathway and has the ability to methylate histones H2A and H4 as well (Lee et al., 2000). At present, the validity of histones being in vivo substrates of Hsl7 remains to be ascertained; nevertheless, it would not be surprising if other signaling pathways can induce additional histone modifications.

Recently, the heterochromatin-associated murine SUV39H protein has been found to possess a Lys9-specific histone H3 methyltransferase (HMTase) activity (Rea et al., 2000). As genes within heterochromatin are silenced, the SUV39H-mediated histone methylation may have functions in transcription repression. Interestingly, mouse cells carrying a targeted mutation of the gene encoding SUV39H display increased levels of adjacent Ser10 phosphorylation and mitotic abnormalities, suggesting a potential inhibitory role of H3 Lys9 methylation on H3 Ser10 phosphorylation. In support of this finding, Ipl1/aurora kinase phosphorylates unmodified or Lys9-acetylated H3 peptides equally well in in vitro assays, but fails to phosphorylate the corresponding H3 peptide methylated at Lys9. These data again highlight the dynamic interactions between nearby histone modifications.

With the possibility of multiple histone modifications present at the same time on the N-terminal histone tails (for example see Figure 1), what kinds of interplay between them can occur? Could these modifications functionally regulate each other or could they be used in combination to affect the function of nucleosomes and chromatin? For example, as mentioned already, there is now evidence that one modification can affect, be it cooperative or antagonistic, the establishment of another modification (Figure 4B). In other instances, such as Lys9 of H3 which can be acetylated or methylated, the mutual exclusive nature of modifying the same amino acid residue could develop into a reciprocal-regulatory mechanism where the effects on chromatin alternate depending on the modification present (see Figure 4B). Also, as suggested by the in vitro binding of $TAF_{II}250$ to multiply acetylated H4 tails (Jacobson et al., 2000, Figure 5A), pairs of acetylated residues with defined spacing could act as binding sites for protein factors. Analogously, different combinations of modifications, such as the phosphorylated Ser10 and acetylated Lys14 of H3, could potentially be presented as regulatable binding platforms for nuclear factors (Figure 5B). In vitro binding studies also suggested that, besides binding to acetyl-lysines, the bromodomain of Gcn5p can also interact with nearby residues on the backbone of H4 (Ornaghi et al., 1999). Therefore, the addition of a modification at these contact residues could present steric hindrance and potentially regulate the binding of bromodomain-containing factors to the acetyl-lysine residue (Figure 5C). All in all, the many possible combinations of different histone modifications are staggering, and the challenge, and excitement, for future research is to systematically dissect the functional relationship between these histone modifications.

Conclusions and Perspectives

As first pointed out more than 25 years ago, the symmetry displayed in the nucleosome particle of chromatin is both simple and elegant (Kornberg, 1974). Today, this fundamental packaging unit of eukaryotic chromatin is yielding unsuspected links to DNA-templated process with far-reaching implications to human biology and disease (Kornberg and Lorch, 1999). In this article, we have proposed that the histone tails function to integrate upstream signals that, in turn, impart regulatable dynamic changes to the chromatin structure of localized regions of the genome. While H2A–H2B and H3–H4 pairings make up the physical construction of histone octamers, we propose that the N-terminal tails of H2B–H3 and H2A–H4 act as pairs that function as signaling platforms (see Figure 1). We also speculate that histone tails are punctuated by highly basic patches which interact with acidic regions in yet to be identified partners to lead to the formation of polar zippers that can be "unzipped" by covalent modifications such as phosphorylation or acetylation. The extent to which these "zippers" stabilize or maintain higher-order chromatin polymers remains unclear, but is already hinted at from views of the crystal structure of nucleosome core particles (Luger et al., 1997).

Signaling to chromatin promises to be an exciting area for future investigation. The combinatorial and sequential modification of histone tails offers numerous regulatory opportunities at the level of upstream cross talk and converging cascades. With the widespread usage of chromatin immunoprecipitation techniques and advances in microarray technology, future research combining these technologies can globally identify sets of genes that are associated with histones bearing specific modification marks. Similar approaches in defining genes regulated by other chromatin remodeling factors or MAP kinase pathways (Roberts et al., 2000; Sudarsanam et al., 2000) have already revealed complex circuitries of gene regulation which histone modifications may also affect. Based upon the concepts presented here, we suspect that there will be many more "tales" to tell.

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