PROSPECTS

Histone Modifications, Chromatin Structure, and the Nuclear Matrix

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Abstract   The nuclear matrix has a role in the organization and function of nuclear DNA. A combination of stable and transient interactions between chromatin and the nuclear matrix is involved in organizing DNA within the nucleus. DNA sequences (matrix attachment regions) at the base of a loop bind to nuclear matrix proteins and arrange the nuclear DNA into chromatin loop domains. Multiple, transient interactions between the nuclear matrix and transcriptionally active chromatin are thought to be responsible for the insoluble feature of transcriptionally active chromatin. Current evidence suggests that histone acetyltransferase, histone deacetylase (enzymes that catalyze rapid histone acetylation and deacetylation), transcription factors, and the transcription machinery mediate the transient attachments between nuclear matrix and active chromatin. Highly acetylated core histones, which are associated with transcriptionally active DNA, are also ubiquitinated and phosphorylated. Recent studies show that specific H1 subtypes and their phosphorylated isoforms are localized in centers of RNA splicing in the nucleus. The implications of these findings and the impact of the histone modifications on the nuclear organization of chromatin are discussed.   © 1996 Wiley-Liss, Inc.

Key words: histone acetylation, histone phosphorylation, transcriptionally active chromatin, nuclear matrix, nuclear structure

HISTONES AND CHROMATIN ORGANIZATION

Nuclear DNA is organized in a hierarchy of structures, leading to the level of packaging needed to place 2 m of DNA into a nucleus with a diameter of 5 to 10 μm. The core histones, H2A, H2B, H3, and H4, form the protein center of the repeating structural unit in chromatin, the nucleosome. The nucleosome consists of 146 bp of DNA wrapped around a histone octamer arranged as an $(H3–H4)_2$ tetramer and two H2A–H2B dimers positioned on each face of the tetramer. The core histones have a similar structure with a NH_2-terminal unstructured domain, a globular domain organized by the histone fold, and a COOH-terminal unstructured tail (Fig. 1) [Ramakrishnan, 1994]. The globular domains of the core histones are involved in histone-histone and histone-DNA contacts that are responsible for maintaining the structural integrity of the nucleosome.

Histone H1 binds to linker DNA, the DNA that joins the nucleosomes together, and to nucleosomal histones (COOH-terminal region of H2A and, perhaps, NH_2-terminal tail of H4) [Boulikas et al., 1980; Banères et al., 1994]. H1 has a tripartite structure with a basic NH_2-terminal domain, a basic COOH-terminal tail domain, and a central globular core [Ramakrishnan, 1994] (Fig. 1). It was previously believed that chromatin in low ionic strength solutions had an extended "beads-on-a-string" conformation (the beads being the nucleosomes). However, analysis of chromatin fibers in low ionic strength solutions by tapping-mode scanning force microscopy shows that the fibers exist as three-dimensional, irregular helices [Leuba et al., 1994]. The folding is dependent upon H1. At physiological ionic strength chromatin forms a 30 nm fiber in vitro. H1 and the N-terminal tails of the core histones stabilize the 30 nm fiber.

Several models have been presented for the structure of the 30 nm fiber. However, ultrastructural investigations have shown that the 30 nm fiber has considerable structural irregularity [Rattner and Hamkalo, 1978]. Woodcock
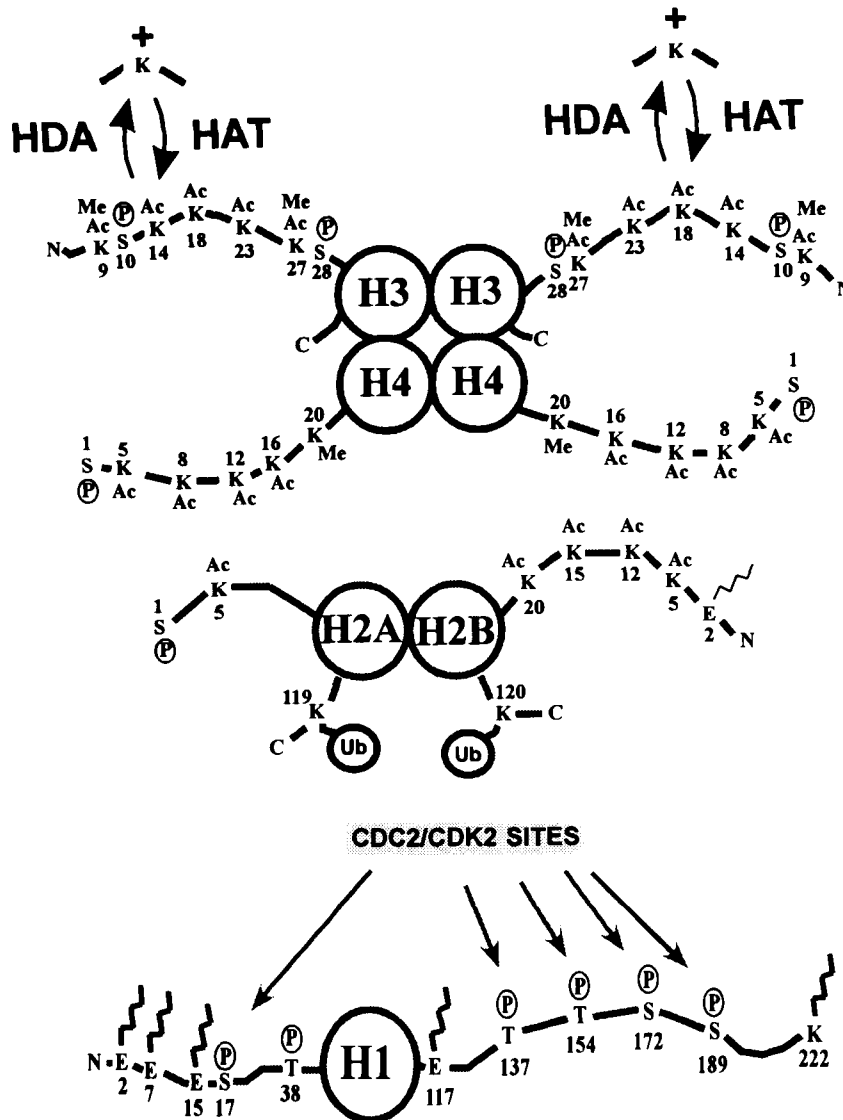
Fig. 1. Sites of modification on the histones. The structures of the H2A-H2B dimers, (H3–H4)₂ tetramers, H1, and the sites of modification are shown. The modifications shown are acetylation (Ac), phosphorylation (P), ubiquitination (Ub), poly(ADP-ribosyl)ation (the stepladder structure), and methylation (Me). All of these modifications are reversible with the possible exception of methylation. The enzymes catalyzing reversible acetylation are shown (HAT, histone acetyltransferase; HDA, histone deacetylase). CDC2 and CDK2 are cyclin dependent kinases.

and colleagues have presented a chromatin folding model that resembles actual chromatin fibers [Woodcock and Horowitz, 1995]. In this model the position of the neighboring nucleosome is determined by the length of the linker DNA and the angle at which the linker DNA enters and exits the nucleosome. H1 is thought to fix the entry-exit angle of the DNA at the nucleosome.

H1, because of its role in chromatin compaction, is considered a general repressor of transcription. However, transcriptionally active chromatin has H1. Both nucleosomes and H1 are present in the coding regions of active genes, but both are absent in the promoter regions [Belikov et al., 1993].

CHROMATIN DOMAINS AND NUCLEAR MATRIX

The chromatin fiber is organized into loop domains with an average size of 86 kb [Gerdes et al., 1994]. At the base of the loop there are sequences called MARs (matrix attachment regions) that bind to proteins associated with the nuclear matrix. This nuclear substructure consists of residual nucleoli, surrounding nuclear

pore-lamina complex, and internal nuclear matrix. Sequential extraction of nuclease-digested nuclei with intermediate and high salt reveals the core filaments of the internal nuclear matrix [Penman, 1995]. A similar structure is seen when chromatin fragments are removed from nuclease-digested nuclei by electroelution in solutions of physiological ionic strength [Jackson and Cook, 1988]. Core filaments with diameters of 10 to 13 nm appear to be the underlying structure onto which other nuclear components are bound.

The loop domains of transcriptionally active genes have a DNAase I sensitive chromatin structure, suggesting a decondensed chromatin structure. The DNAase I sensitive domains may contain one or several genes. In chicken erythrocytes, the β-globin domain has four genes, ϵ-, β^H-, β^A-, and ρ-globin genes (Fig. 2). In primitive erythrocytes of 5 day embryos, the embryonic genes, ϵ- and ρ-globin are expressed, while in definitive erythrocytes of 15 day embryos the globin gene expression switches to the adult genes, β^H- and β^A-globin. The β-globin domain is DNAase I sensitive in both the primitive and definitive erythroid cells. But at 10 kb upstream of ρ-globin and about 9 kb downstream of ϵ-globin, there is a distinct decline in DNAase I sensitivity, marking the boundaries of the 33-kb β-globin domain [Hebbes et al., 1994]. The extent of other DNAase I sensitive domains vary, for example 100 kb for chicken ovalbumin, 12 kb for chicken glyceraldehyde-3-phosphate dehydrogenase, 25 kb for chicken lysozyme, and 47.5 kb for human apolipoprotein B. The boundaries of the DNAase I sensitive domain comap with MARs [Hebbes et al., 1994].

Transcribed DNA sequences are associated with the nuclear matrix-attached chromatin fragments [Davie and Hendzel, 1994]. Adult chicken immature erythrocyte nuclear matrix-bound chromatin fragments are enriched in transcriptionally active β-globin and histone H5, but not poised ϵ-globin, DNA sequences [Davie and Hendzel, 1994]. Actively transcribed chromatin regions are thought to be immobilized on the nuclear matrix by multiple dynamic attachment sites. The transcription machinery, specific transcription factors, and enzymes (e.g., histone acetyltransferase and deacetylase) are thought to mediate the dynamic attachments between transcriptionally active chromatin and the nuclear matrix [Davie and Hendzel, 1994; Cook, 1994] (Fig. 3).

HISTONE ACETYLATION AND TRANSCRIPTIONALLY ACTIVE CHROMATIN DOMAINS

The core histones are reversibly modified by acetylation of lysines located in the basic NH_2-terminal part of these molecules (Fig. 1). Histone acetylation and deacetylation are catalyzed by histone acetyltransferases and deacetylases, with the level of acetylation being governed by the net activities of these two enzymes. Histone acetylation is not limited to transcriptionally active chromatin, but also has a role in DNA replication, DNA repair, and in spermatogenesis where histones are replaced by protamines. These latter processes are not occurring in adult chicken mature and immature erythrocytes which are arrested in G_0 phase of the cell cycle. Thus, in studying the relationship between histone acetylation and gene expression, the chicken erythrocyte has been an informative system. Further, incubation of mature and immature erythrocytes with sodium butyrate or trichostatin A, inhibitors of histone deacetylase, can be used to alter the level of the acetylated histone isoforms. The effects of acetylation on the properties of specific chromatin fragments can then be studied. This is a further advantage of the chicken erythrocyte system. In cycling cells such studies are complicated by butyrate and trichostatin A arresting cells in G_1 and/or G_2 phase of the cell cycle, and in the induction of histone $H1^0$ expression [Yoshida et al., 1995]. When cycling cells are treated with these drugs,
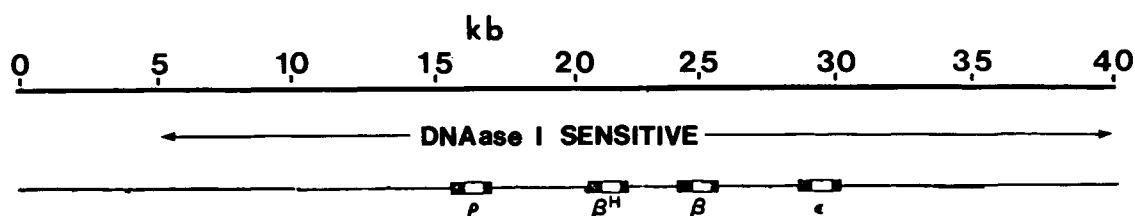
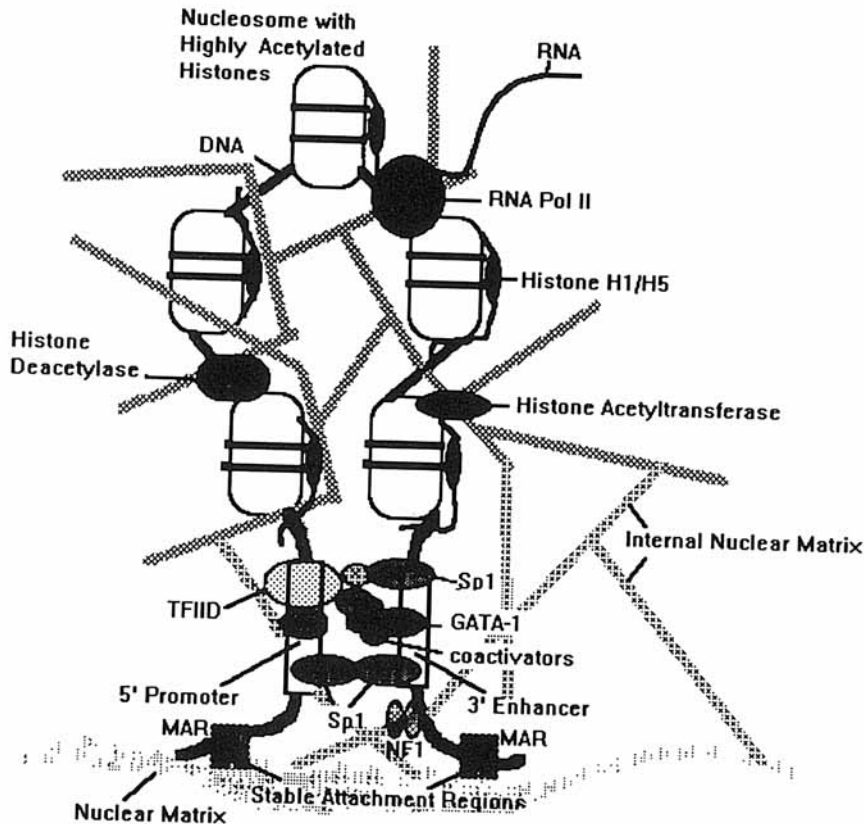**Fig. 2.** Chicken erythrocyte DNAase I sensitive β-globin domain.

Fig. 3. A model for transcriptionally active chromatin. At the base of the loop are nuclear matrix association regions (MARs). Multiple dynamic attachment sites between the transcriptionally active domain and the internal nuclear matrix are presented. Histone acetyltransferase, histone deacetylase, transcription machinery, transcription factor NF1, and RNA processing machinery are shown associated with the internal nuclear matrix. Histone acetyltransferase and deacetylase are depicted as continually contacting nucleosomes, catalyzing rapid reversible acetylation.

caution must be used in deciding whether the effect on the expression of a particular gene is due to changes in histone acetylation or a blockage in cell cycle.

In adult chicken mature and immature erythrocytes, the bulk of histones is frozen at low acetylation states. A small population of core histones is rapidly acetylated to the high acetylation states (e.g., tetra-acetylated H2B) and then rapidly deacetylated. This rapidly modified population of core histones is associated with transcriptionally active DNA [Davie and Hendzel, 1994]. In immunoprecipitation studies with an antibody to ϵ-acetyl lysine, Crane-Robinson and colleagues showed directly that acetylated histones are associated with transcriptionally active genes in human and chicken erythroid cells [Hebbes et al., 1994]. They have recently extended these studies to show that the entire β-globin DNAase I sensitive domain of 15-day chicken embryo erythrocytes is associated with highly acetylated histones [Hebbes et al., 1994].

Further, in 5 day chick embryo erythrocytes, the transcriptionally active ρ- and inactive (poised) β^A-globin DNA sequences were in acetylated nucleosomes. It remains to be decided whether the core histones associated with the active and poised regions of the domain are engaged in rapidly reversible, high level acetylation. However, current evidence suggests that dynamically acetylated-deacetylated histones are bound primarily to the active DNA [Davie and Hendzel, 1994].

Most of the chicken erythrocyte chromatin fragments, which are associated with low levels of acetylated histones, are insoluble in 150 mM NaCl. H1 is responsible for this insolubility, for removal of H1 results in salt solubility of the chromatin fragments. However, transcriptionally active chromatin fragments with highly acetylated core histones and H1 are salt soluble. Active chromatin fragments isolated from butyrate-treated cells and reconstituted with H1 and H5 remain soluble in 150 mM NaCl, while active

chromatin fragments isolated from cells incubated without butyrate are salt insoluble following reconstitution with H1 [Davie and Hendzel, 1994]. These observations suggest that histone acetylation alters the capacity of H1 to condense the chromatin fragment. Possibly, histone acetylation alters nucleosome structure such that acetylated nucleosomes no longer bind H1. Current evidence shows that H1 binds to nucleosomes reconstituted with highly acetylated histones [Ura et al., 1994]. However, it is doubtful that nucleosomes associated with transcriptionally active DNA have the same structure as nucleosomes reconstituted with highly acetylated histones. Transcriptionally active DNA-enriched chromatin fragments isolated from chicken immature erythrocytes had atypical nucleosomes, described as U-shaped nucleosomes [Locklear et al., 1990]. Further, transcriptionally active nucleosomes isolated from human cancer cell lines, rat liver, and mouse fibroblasts have a reactive thiol group in H3 that is inaccessible in canonical nucleosomes, and these active nucleosomes have an atypical U-shaped structure [Chen-Cleland et al., 1993; Czarnota et al., 1995]. Nucleosomes reconstructed with highly acetylated histones do not have reactive H3 thiol groups [Marvin et al., 1990]. Thus, events occurring in situ, for example, torsional stress, may be required to generate the atypical nucleosome structure [Ausio, 1992]. Future studies will decide whether H1 binds to these atypical nucleosomes of active chromatin.

NUCLEAR MATRIX ASSOCIATION OF HISTONE ACETYLTRANSFERASE AND DEACETYLASE

Vertebrate histone acetyltransferase and deacetylase are associated with the nuclear matrix [Davie and Hendzel, 1994; Hendzel et al., 1994]. Chicken immature erythrocyte nuclear skeletons isolated from micrococcal nuclease-digested nuclei retain less than 10% of the nuclear DNA, 36% of the nuclear protein, and 50 to 75% of the transcriptionally active DNA. These nuclear skeletons have 80% of the nuclear histone acetyltransferase and deacetylase activities. The enzymes bound to the nuclear skeletons catalyze reversible acetylation using as substrate the chromatin fragments associated with this structure [Hendzel et al., 1994]. These studies suggest that histone acetyltransferase and deacetylase are colocalized to specific sites on the nuclear matrix. Based on these observa-

tions, we proposed a model in which nuclear matrix-bound histone acetyltransferase and deacetylase mediate dynamic interactions between the nuclear matrix and transcriptionally active chromatin [Davie and Hendzel, 1994; Hendzel et al., 1994] (Fig. 3).

Histone acetyltransferases are generally divided into two groups, nuclear A-type enzymes and cytoplasmic B-type enzymes. Nuclear A-type enzymes can acetylate free core histones and nucleosomal histones, while B-type enzymes acetylate H4 and H3 [Sobel et al., 1995]. Multiple forms of histone deacetylases, which are nuclear enzymes with different substrate specificities, have been found in plants, animals, yeast, and Physarum [Brosch et al., 1992]. Recently, Sanchez del Pino et al. [1994] reported that yeast histone deacetylase was isolated as a high-molecular-mass complex (500 kDa) in low ionic strength solutions. Elevating the ionic strength with 500 mM NH_4Cl or NaCl resulted in the dissociation of the histone deacetylase complex to a low-molecular-mass form of 150 kDa. We have obtained similar results in our analysis of the chicken erythrocyte histone deacetylase [Li and Davie, 1995]. The enzyme extracted from the adult chicken immature erythrocyte nuclear matrix has a molecular mass greater than 400 kDa. Importantly, we found that chromatography of the high-molecular-mass histone deacetylase form on an ion exchange column (Q Sepharose) resulted in its dissociation to a 55 kDa form, and this dissociation was accompanied with a change in the enzyme's substrate specificity. These observations suggest that the multiple histone deacetylase forms may have originated from a high-molecular-mass histone deacetylase complex.

Analysis of histone deacetylation rates in chicken immature erythrocytes displayed a multiplicity of deacetylation rates for the core histones [Zhang and Nelson, 1988]. H2B is more rapidly deacetylated than H3 and H4. The nuclear-skeleton-associated histone deacetylase prefers nucleosomal H2B [Hendzel et al., 1994]. Similarly, the nuclear matrix histone deacetylase prefers H2B. The similarity in deacetylation rates of the core histones in situ with the histone specificity properties of the nuclear matrix-bound histone deacetylase suggests that the high-molecular-mass, nuclear matrix-bound enzyme is an active histone deacetylase form in vivo. To achieve the nucleosomal histone specificity observed in situ, our results suggest that

histone deacetylase is in a complex with proteins that are components of the nuclear matrix.

MULTIPLE MODIFICATIONS OF CORE HISTONES

Besides acetylation the core histones are modified by methylation, poly (ADP-ribosyl)ation, ubiquitination and phosphorylation (Fig. 1). In adult chicken immature erythrocytes, dynamically acetylated histones H3 and H4 are selectively undergoing methylation, and dynamically acetylated H2A and H2B are ubiquitinated [Hendzel and Davie, 1989; Li et al., 1993]. In *Physarum polycephalum* and mouse myeloma cells ADP-ribosylation occurs preferentially on the highly acetylated core histone isoforms [Golderer and Grobner, 1991]. Addition of growth factors, phorbol esters, or okadaic acid to G_0-phase-arrested mouse fibroblasts leads to the rapid transcriptional activation of immediate early genes (e.g., c-*fos*, c-*jun*), and these events are accompanied with a rapid phosphorylation of H3. The small population of H3 that participates in dynamic acetylation is engaged in the mitogen-stimulated phosphorylation [Barratt et al., 1994]. The simultaneous multiple modification of the core histones is likely to have an impact on nucleosome and higher order chromatin structure, particularly if several histones in the nucleosome are coordinately highly modified. Crosslinking studies have shown that ubiquitinated H2A is paired to ubiquitinated H2B in nucleosomes [Li et al., 1993]. The presence of these ubiquitinated dimers appears to destabilize the interaction of the H2A-H2B dimer with the $(H3-H4)_2$ tetramer. Besides modified core histones, there are core histone variants and their modified isoforms. Interestingly, a dimer containing H2A.Z, a histone variant thought to be associated with transcriptionally active chromatin, stabilizes the association of the H2A-H2B dimer in nucleosomes [Li et al., 1993]. Thus, histone modifications and variants may generate asymmetry in nucleosomes with respect to the binding and dissociation of H2A-H2B dimers.

The processes of histone methylation, phosphorylation and dynamic acetylation are not directly coupled; neither modification predisposes H3 to the other [Barratt et al., 1994]. However, these observations suggest that histone acetyltransferases, deacetylases, H3 kinases, histone methyltransferases, and poly-

(ADP-ribose) polymerase are targeted to specific locations in the nucleus. In addition to histone acetyltransferase and deacetylase, poly(ADP-ribose) polymerase is associated with the nuclear matrix [Golderer and Grobner, 1991]. Chicken erythrocyte H3 methyltransferase is associated with salt soluble chromatin and with the residual nuclear material (the nuclear skeleton) [Hendzel and Davie, 1989]. It is not known whether the H3 methyltransferase present in the latter fraction is associated with the nuclear matrix.

HISTONE H1 SUBTYPES AND PHOSPHORYLATION

The H1 histones are a heterogeneous group of several subtypes that differ in amino acid sequence. The expression of the subtypes is differentially regulated throughout development, through the cell cycle, and during differentiation. Since H1 subtypes differ in their abilities to condense DNA and chromatin fragments, it has been proposed that the differential distribution of the H1s with chromatin domains may generate chromatin regions with different degrees of compaction. Indirect immunofluorescence studies with H1 subtype-specific antibodies have shown that the nuclear location of specific H1 subtypes is non-uniform. Human H1-3 is found primarily in the nuclear periphery, and human H1-1 is distributed in parallel to the DNA concentration [Parseghian et al., 1994]. Antibodies to human H1-2 and H1-4 generated punctate staining patterns, reminiscent of the speckled staining patterns described when the nuclear sites of splicing factors, small nuclear RNAs and RNA synthesis were localized [Parseghian et al., 1994; Blencowe et al., 1994].

H1 is phosphorylated at serine and threonine residues located in the N- and C-terminal domains of the protein (Fig. 1). Several of these phosphorylation sites contain the phosphorylation consensus sequence -Ser/Thr-Pro-X-Lys/Arg which corresponds to the p34[cdc2] kinase consensus motif. Histone H1 is also phosphorylated by cAMP- or cGMP-dependent kinases, for example at serine residue 37 in rat liver or rabbit thymus H1. Phosphorylation of H1 subtypes is likely to influence their interaction with DNA and, in turn, modulate chromatin structure. Several studies support the idea that H1 phosphorylation leads to the decondensation of chromatin [for review see Roth and Allis, 1992].

PHOSPHORYLATED H1 SUBTYPES ARE LOCALIZED TO CENTERS FOR RNA SPLICING

Ras- and c-*myc*-transformed mouse fibroblasts have a less condensed chromatin structure than the parental cells [Chadee et al., 1995]. These oncogene-transformed cells and those cells transformed with *raf-, fes-, mos-,* and constitutively active mitogen-activated protein kinase kinase have elevated levels of phosphorylated H1s. This increase in H1 phosphorylation may lead to chromatin decondensation in oncogene-transformed cells.

Allis and colleagues [Lu et al., 1994] isolated an antibody that is selective for the highly phosphorylated isoform of *Tetrahymena* H1. This antibody also detects highly phosphorylated HeLa H1 and mouse fibroblast H1b [Lu et al., 1994; Chadee et al., 1995]. In indirect immunofluorescence experiments with the antibody, a punctate pattern of nuclear staining was observed for HeLa cells that were in G_1 phase of the cell cycle. In parental and ras-transformed mouse fibroblasts, a similar punctate/speckled pattern of nuclear staining was also observed with this antibody. This pattern of fibroblast nuclear staining closely matched the pattern found with the B1C8 monoclonal antibody. B1C8, a nuclear matrix protein, colocalizes with other RNA splicing components [Blencowe et al., 1994]. Several transcribed genes (e.g., c-*fos*) are positioned near sites containing RNA splicing components [Durfee et al., 1994]. These observations suggest that phosphorylated H1b is associated with transcriptionally active chromatin. These findings are consistent with the current view that RNA processing occurs co-transcriptionally, reflecting nuclear organization that is probably governed by the underlying nuclear substructure, the nuclear matrix.

ORGANIZATION OF DNA IN THE NUCLEUS

Usually the 30 nm chromatin fibers are not seen inside nuclei [Woodcock and Horowitz, 1995]. Rather, the chromatin is observed as matted patches. It has been proposed that the 30 nm fibers interdigitate [Woodcock and Horowitz, 1995]. The basic NH_2-terminal tails of the core histones may stabilize the interaction between fibers. How is active chromatin organized in the nucleus? We often visualize active chromatin as a decondensed, extended chromatin fiber. In support of this view, we observed that chicken erythrocyte salt-soluble polynucleosomes en-

riched in active DNA and highly modified core histones had an extended "beads-on-a-string" structure. However, reevaluation of the structure of the salt-soluble polynucleosomes by tapping-mode scanning force microscopy has revised our thinking. In 80 mM NaCl the salt-soluble polynucleosomes compacted, with parts of a few fibers showing an extended structure [Leuba et al., 1995]. Clearly, these fibers do not exist as "beads-on-a-string." Although the salt soluble polynucleosomes have a compacted structure, these fibers do not precipitate in 150 mM NaCl. At 100 mM NaCl the bulk of chromatin fragments aggregate and then precipitate. The aggregates appear to consist of one or more 30 nm filaments packed tightly together in an end-to-end and side-to-side arrangement. It is possible that salt soluble polynucleosomes do not partake in the fiber-fiber packaging events, and thus escape precipitation. An extension of this idea is that in situ transcriptionally active domains have a compact structure but interdigitation of neighboring fibers is disfavoured. Acetylation and phosphorylation of the basic NH_2-terminal tails of the core histones, ubiquitination of the COOH-terminal regions of H2A and H2B, and phosphorylation of H1 could cooperate to prevent fiber-fiber interactions. The reversibility of core histone modifications and torsional stress in the chromatin domain may result in dynamic changes in nucleosome structure. These structural changes will likely be accompanied with changes in the angle at which linker DNA enters and exists the nucleosome (especially when H1 is absent) and therefore changes in chromatin folding. These oscillations in chromatin folding of active chromatin regions associated with dynamically modified histones would disfavour interdigitation of neighboring fibers. The individual chromatin fibers would then be exposed to transcription factors, the transcription machinery and nucleases.

FUTURE DIRECTIONS

Recent studies show that transcription factors and nuclear enzymes are recruited from one nuclear site to another [Bregman et al., 1995; Larsson et al., 1995]. How do specific proteins get targeted to specific nuclear regions will be an important question to address. These events also highlight the dynamics of factors/enzymes associating with the nuclear matrix and/or nuclear matrix bound components. Purification of the histone acetyltransferases and deacety-

lases, and the cloning of the genes encoding the catalytic subunit of these enzymes are current projects. Once completed, the tools (antibodies, fusion proteins) needed to reveal their nuclear locations and to understand nuclear targeting of these enzymes will be available. These reagents will also be used to capture the nuclear matrix proteins associated with these enzymes. The observations that specific phosphorylated H1 subtypes are localized in specific nuclear regions suggest that the H1 subtype and/or kinases are located in specific nuclear regions (e.g., at or near sites engaged in RNA splicing). Immunolocalization studies with antibodies to nuclear enzymes (cyclin dependent kinases, CDK2, CDC2) and specific H1 subtypes will help provide answers. Immunoprecipitation of chromatin fragments or nucleosomes (± crosslinking) with antibodies to specific modified histone isoforms and subtypes will decide whether the histone subtypes and their modified forms (e.g., phosphorylated H1b, phosphorylated/acetylated H3) individually or in combination are associated with transcriptionally active genes. Finding that phosphorylated H1b in mouse fibroblasts is associated with transcriptionally active DNA would strengthen the idea that RNA processing occurs cotranscriptionally.

ACKNOWLEDGMENTS

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