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Regulation and Regulatory Parameters of Histone Modifications

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Abstract Histone acetylation and phosphorylation destablizes nucleosome and chromatin structure. Relaxation of the chromatin fiber facilitates transcription. Coactivator complexes with histone acetyltransferase activity are recruited by transcription factors bound to enhancers or promoters. The recruited histone acetyltransferases may acetylate histone or nonhistone chromosomal proteins, resulting in the relaxation of chromatin structure. Alternatively, repressors recruit corepressor complexes with histone deacetylase activity, leading to condensation of chromatin.This review highlights the recent advances made in our understanding of the roles of histone acetyltransferases, histone deacetylases, histone kinases, and protein phosphatases in transcriptional activation and repression. Exciting reports revealing mechanistic connections between histone modifying activities and the RNA polymerase II machinery, the coupling of histone deacetylation and DNA methylation, the possible involvement of histone deacetylases in the organization of nuclear DNA, and the role of chromatin modulators in oncogenesis are discussed. J. Cell. Biochem. Suppls. 30/31:203–213, 1998. © 1998 Wiley-Liss, Inc.

Key words: histone acetylation and phosphorylation; coactivators; corepressors; transcriptional activation and repression; histone acetyltransferase; histone deacetylase

HISTONE MODIFICATIONS

The basic repeating structural unit in chromatin is the nucleosome, which consists of an histone octamer core around which DNA is wrapped. The core histones of the octamer are arranged as a $(H3\text{-}H4)_2$ tetramer and two H2A-H2B dimers positioned on both sides of the tetramer. The core histones have a similar structure with a basic N-terminal unstructured domain, a globular domain organized by the histone fold, and a C-terminal unstructured tail (Fig. 1). Histone H1 binds to the linker DNA, which joins nucleosomes together, and to core histones, H2A and H4. H1 has a tripartite structure with a basic N-terminal domain, a basic C-terminal tail domain, and a central globular core [for review, see Davie, 1997] (Fig. 1).

The core histones are susceptible to a wide range of postsynthetic modifications, including acetylation, phosphorylation, methylation, ubiquitination, glycosylation, and ADP-ribosylation [for review, see Davie, 1998] (Fig. 1). Most modifications occur on the N-terminal basic tail domain. There has been considerable interest in histone acetylation and the enzymes catalyzing this reversible process. However, it is important to note that a dynamically acetylated histone (e.g., H3) may be phosphorylated and methylated [Barratt et al., 1994]. This review highlights recent studies on histone acetylation and phosphorylation and the enzymes that catalyze these modifications.

HISTONE ACETYLATION

With the exception of H2A, the core histones may be acetylated at four or five specific lysines located in the N-terminal tail domain of the histone (Fig. 1). Highly acetylated core histones are associated with transcribed chromatin regions [Davie, 1998; Howe et al., 1998]. The highly acetylated state of these histones is not

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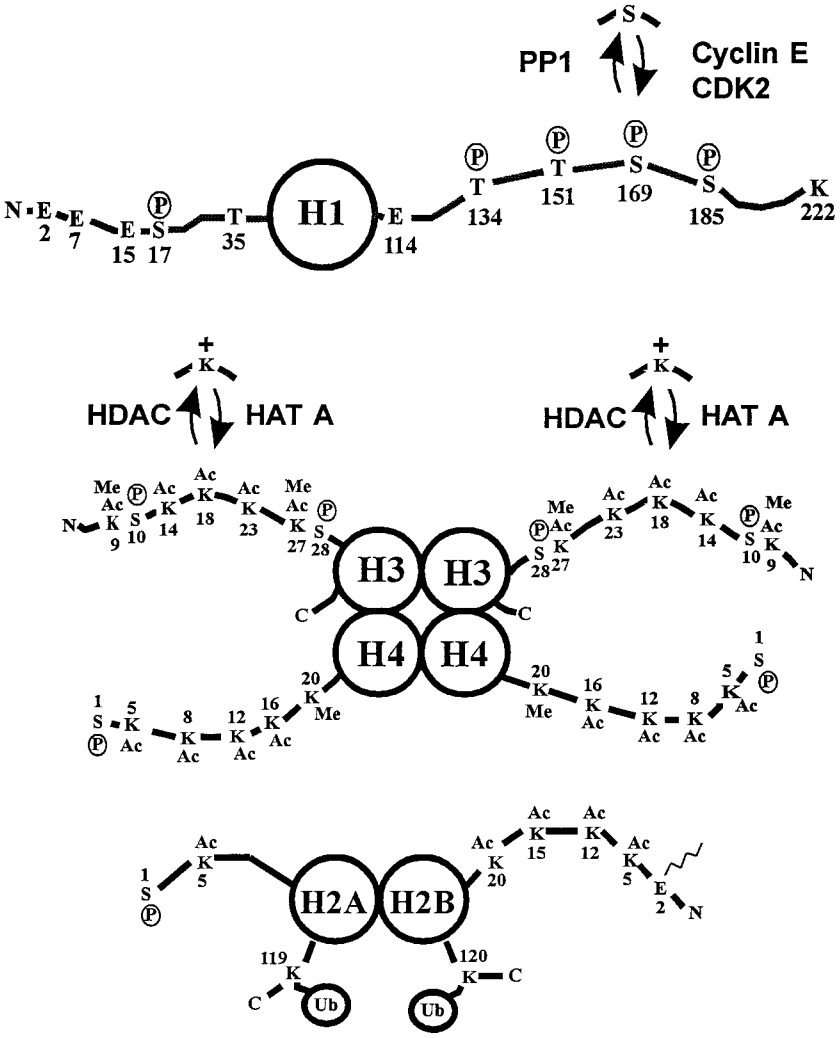

Fig. 1. Sites of postsynthetic modifications on the histones. The structures of the H2A-H2B dimers, $(H3\text{-}H4)_2$ tetramers, H1b, and the sites of modification are shown. The modifications shown are acetylation (Ac), phosphorylation (P), ubiquitination (Ub), and poly-ADP-ribosylation (the stepladder structure). All these modifications are reversible. The enzymes catalyzing reversible acetylation and phosphorylation are shown (HAT, histone acetyltransferase; HDAC, histone deacetylase; CDK2, cyclin-dependent protein kinase 2; PP1, protein phosphatase 1).

static but dynamic, being rapidly acetylated and deacetylated [Davie, 1997]. Alterations at all levels of chromatin structure are invoked by acetylation of the core histones. Acetylation of the histone tails maintains the unfolded structure of the transcribed nucleosome, disrupts higher-order chromatin folding, and promotes the solubility of chromatin at physiological ionic strength. These combined effects of histone acetylation on the destabilization of chromatin structure facilitates transcription [Davie, 1997; Nightingale et al., 1998, Tse et al., 1998]. The enzymes catalyzing reversible histone acetylation are histone acetyltransferases (HAT) and deacetylases (HDAC). During the past several years, we have come to appreciate that proteins with HAT activity are coactivators, while proteins with HDAC activity are co-repressors.

HISTONE ACETYLTRANSFERASES AND GENE ACTIVATION

The first HAT gene to be cloned (*Tetrahymena* nuclear HAT p55) was found to be homologous to yeast GCN5, a transcriptional adaptor/coactivator with HAT activity. This pivotal discovery told us how HATs were directed to transcribed chromatin regions. A number of transcriptional coactivators with HAT activity have since been identified [for review, see Davie, 1998] (Table I). We have now come to appreciate the mechanistic connections between histone modifying activities and the RNA polymerase II machinery.

Yeast Gcn5 acetylates free H3, and when it is part of a multiprotein complex, it will acetylate histones in nucleosomes [Utley et al., 1998].

Two of the complexes, SAGA (1.8 MDa) and Ada (0.8 MDa), contain Ada3 and Ada2, which binds to yGcn5. The SAGA (Spt-Ada-Gcn5-acetyltransferase) complex contains Spt proteins (Spt20 (Ada5), -3, -7, and -8) and TAF_{II}s (TAF_{II}90, -68/61, -60, -25/23, -20/17) [Grant et al., 1998]. TAF_{II}68, which is homologous to human TAF_{II}20 and related in structure and sequence to H2B, is required for the integrity, nucleosomal acetylation, and transcriptional enhancing activities of SAGA [Grant et al., 1998]. Yeast TAF_{II}60 and -17 have sequence similarities to H3 and H4 and interact with each other as a heterotetramer through a histone fold. These observations suggest the presence of a histone octamer-like structure within the SAGA complex. Recruitment of SAGA by transcriptional activators results in localized acetylation of nucleosomal substrates in vivo and in vitro [Grant et al., 1998; Kuo et al., 1998]. Importantly, the transcriptional stimulatory activity of the recruited SAGA complex is dependent upon its HAT activity [Grant et al., 1998]. In contrast to SAGA, the ADA complex is not recruited by transcriptional coactivators such as Gal4-VP16 and Gcn4.

Tetrahymena HAT A and Gcn5 contain a bromodomain, a domain thought to be involved in protein-protein interactions. Other proteins containing this domain include TAF_{II}250, CBP/p300, PCAF, and yeast nuclear protein SNF2 (Fig. 2). The helix-turn-helix motif in the bromodomain of hGcn5 binds to the p70, the DNA-binding subunit of the Ku-DNA-dependent protein kinase complex [Barlev et al., 1998]. Phosphorylation of hGcn5 by the DNA-dependent kinase activity of this complex inactivates its HAT activity. Thus the bromodomain appears to have role in the negative regulation of HAT activity.

The C-terminal domain of human PCAF, a nuclear HAT, is similar to yeast Gcn5. The N-terminal domain of PCAF and metazoan Gcn5 proteins is not found in fungal, plant, and protozoan Gcn5 [Smith et al., 1998a]. The N-terminal domain of PCAF associates with other coactivators, CBP/p300 and ACTR, both of which are HATs. Alternate splicing of the human *GCN5* gene can generate Gcn5 proteins with and without the N-terminal domain [Smith et al., 1998a]. Presumably, the short form of hGcn5 is not recruited by CBP or ACTR. Similar to yeast SAGA, human PCAF and Gcn5 are in large multiprotein complexes consisting of human counterparts of yeast ADA2, ADA3, Spt3, and human TAF_{II}31 and -20/15, which have the histone fold structure found in histones H3 and H2B, respectively. The PCAF complex contains two TAF-like proteins, PAF65α and PAF65β. PAF65α has similarity to $hTAF_{II}$80 and has an H4-like region, while PAFβ has similarity to the WD40 repeat containing TAF_{II}100 [Ogryzko et al., 1998]. Thus, the PCAF and Gcn5 complexes are considered as the human counterparts of yeast SAGA. Several of the TAF_{II}s found in the PCAF and Gcn5 complexes are also present in TFIID. TAF_{II}250 subunit of TFIID has HAT activity [for review, see Struhl and Moqtaderi, 1998]. Independent of CBP, the PCAF and/or Gcn5 complex can be directly recruited by several factors, including NF-Y (CCAAT-binding factor), nuclear hormone receptors (RXR-RAR heterodimers, ERα, AR, and GR), and the viral oncoprotein E1A [Blanco et al., 1998; Jin and Scotto, 1998; Reid et al., 1998]. NF-Y recruitment of PCAF HAT activity has a key role in the transcriptional activity of the human *MDR1* promoter [Jin and Scotto, 1998].

CBP/p300 is a coactivator with a HAT activity capable of acetylating the four core histones. It is a limiting cofactor within the cell; many transcription factors compete in the recruitment of this coactivator (Fig. 2). In humans, the loss of one allele of CBP is the underlying defect in Ruberstein-Tabyi syndrome. Patients with this syndrome are more susceptible to cancer, consistent with the suggestion that CBP/p300 may function as a tumor suppressor. Further somatic translocations involving the *CBP* gene are found in various types of hematological malignancies [Giles et al., 1998].

CBP is an integrator of many signaling pathways (Fig. 2). Many transcription factors, including hormone receptors, CREB, and fos-jun, loaded onto promoters or enhancers bind either directly or indirectly to CBP/p300, recruiting a coactivator with HAT activity. Further, it is a component of the RNA polymerase II holoenzyme [Neish et al., 1998]. As with Gcn5, the HAT activity of CBP is directly involved in stimulating transcription [Martinez-Balbas et al., 1998]. Recent studies show the recruitment of CBP by Smad2/3 proteins in the TGF-β-signaling pathway [Feng et al., 1998; Janknecht et al., 1998]. There is evidence that mutation of

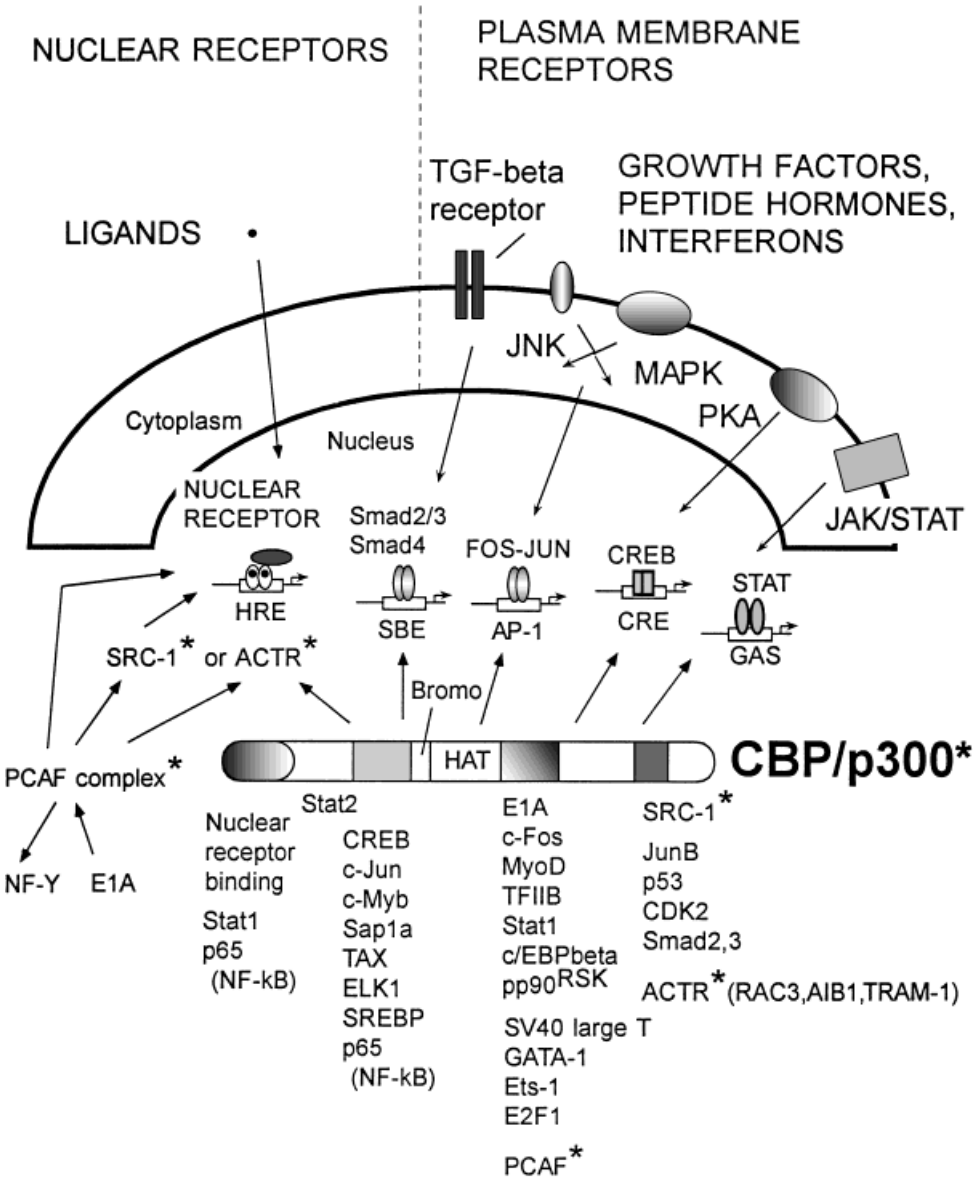

Fig. 2. The histone acetyltransferase/coactivator, CPB/p300, co-integrates diverse signaling pathways. A variety of sequence-specific transcription factors and coactivators bind to different regions of the CBP (CREB-binding protein)/p300 protein [for review, see Davie, 1998; Giles et al., 1998; Torchia et al., 1998]. Kinases such as JAK (Janus kinase), PKA (protein kinase A), MAPK (mitogen-activated protein kinase), and JNK (Jun N-terminal kinase), once activated, will phosphorylate a variety of transcription factors. The signal-dependent transcription factor, once bound to its DNA binding site (HRE, hormone-responsive element; CRE, CREB-responsive element; GAS, interferon-stimulated gene response element; and SBE, Smad binding element), will recruit CBP/p300 (*arrows*, from CBP/p300). CBP/p300 and steroid receptor coactivators have histone acetyltransferase (HAT) activity (*asterisk*).

the players in this pathway (e.g., Smad2, Smad4, p300) leads to the development of cancer (e.g., colorectal carcinomas) [Janknecht et al., 1998; Zhou et al., 1998].

A phosphorylation event is often the key step for a transcription factor to recruit CBP/p300. For example, phosphorylation of NF-κB p65 by PKA induces a conformational change within the protein, resulting in the exposure of two sites that associate with different regions on CBP [Zhong et al., 1998]. In the competition to recruit the limiting CBP/p300, an enhancer with an array of transcription factors in a specific arrangement that provides multiple interactions with CBP/p300 will succeed. The arrangement of factors and architectural proteins in

the IFNβ enhanceosome, for example, results in the effective recruitment of CBP [Merika et al., 1998].

The steroid receptor coactivators SRC-1 and ACTR, which bind to a variety of nuclear receptors in a ligand-dependent manner, associate with CBP/p300 and PCAF. Both SRC-1 and ACTR (and related proteins RAC3, AIB1, and TRAM-1) have HAT activity. AIB1 (amplified in breast cancer-1) is of particular interest as it is expressed at high levels in breast cancer, but not in normal breast tissue. Thus, a ligand-activated nuclear receptor could recruit multiple coactivators with HAT activity (e.g., SRC-1, CBP, and PCAF) [for review, see Davie, 1998; Torchia et al., 1998].

BRCA1 and *BRCA2* are tumor suppressor genes involved in familial breast cancers. BRCA1 and BRCA2 are thought to have roles in transcription, cell cycle control, and DNA repair. BRCA1 associates with CBP, whereas BRCA2 has HAT activity [Cui et al., 1998; Siddique et al., 1998]. Thus, HAT activity may play a role in the function of these tumor suppressor proteins.

Essential SAS2-related acetyltransferase (Esa1) (the *ESA1* gene is essential for yeast growth) and Tat interacting protein 60 (Tip60) are members of the MYST family of proteins (named after the founding members, MOZ, YBF2/SAS3, SAS2, and Tip60). Esa1 and Tip60 are HATs with similar substrate specificities [Smith et al., 1998b]. Neither protein can acetylate chromatin substrates. However, there is evidence that Esa1 is the catalytic unit in a protein complex (possibly NuA4) that acetylates nucleosomal H2A and H4 [Smith et al., 1998b] (Table I). Yeast NuA4 complex can be recruited by Gcn4 and the VP16 activation domain [Utley et al., 1998]. Other members of the MYST family of proteins, including *Drosophila* MOF, a protein involved in regulation of dosage compensation, may exhibit HAT activity [Smith et al., 1998b].

It has been proposed that the recruited coactivator(s) with HAT activity acetylates surrounding histones in nucleosomes, leading to the destabilization of higher-order chromatin structure. However, histones are not the only substrates for these enzymes [Davie, 1998]. The high mobility group proteins (HMG) 1, 2, 14, 17, and I are acetylated in vivo. Further several transcription factors may be acetylated, including general transcription factors TFIIF and TFIIE, p53 and the erythroid Krüppel-like factor (EKLF) (Table I) [Zhang and Bieker, 1998].

HISTONE DEACETYLASE AND GENE REPRESSION

In 1996, the cloning of mammalian histone deacetylase (HDAC1) demonstrated that is was related to yeast transcription regulator RPD3, providing a link between transcription regulation and histone deacetylation. Several HDACs have since been reported, including HDAC2 (the mammalian homologue of RPD3) and mammalian HDAC3 [reviewed in Davie, 1998]. HDACs, bacterial acetoin utilization proteins, and acetylpolyamine amidohydrolases appear to be members of an ancient protein superfam-

TABLE I. Histone Acetyltransferases and Their Substrates*

HAT A (organism)	Free histone or other substrate	Nucleosomal histone substrate
ACTR (human)	H3, H4 > H2B	H3 > H4
SRC-1 (human)	H3 > H4 (K9, K14 of H3)	H3, H4, H2A, H2B
PCAF (human)	H3 > H4, TFIIF, TFIIE	H3
GCN5 (yeast, human, *Drosophila*)	H3 > H4 (K14 of H3, K8, 16 of H4)	—
Ada (yeast)		H3, H2B
SAGA (yeast)		H3 > H2B
NuA4 (yeast)		H4, H2A
NuA3 (yeast)		H3
CBP/p300 (human)	H3, H4 > H2A, H2B, (K5, 8, 12, 16 of H4) TFIIF, TFIIE, p53, EKLF	H3, H4, H2A, H2B
$TAF_{II}250$ (human, *Drosophila*, yeast)	H3 > H4, (K14 of H3), TFIIE	—
Esa1 (yeast)	H4 > H3 > H2A, (K5 > K8, 12, 16 of H4; K14 of H3; K5 of H2A	
Tip60 (human)	H4 > H3 > H2A	

*For references, see Histone Acetyltransferase page at http://mdanderson.org/~genedev/Bone/hathome.html

ily. These proteins share nine blocks of sequence similarity, with 20 amino acids being invariant in these alignments [Leipe and Landsman, 1997]. Some of these conserved amino acids could be involved in binding a metal atom, e.g., zinc; there is evidence that HDAC1 is a metalloenzyme [Hassig et al., 1998].

Mammalian HDAC1 and HDAC2, but not HDAC3, are in large multiprotein complexes containing mSin3, N-CoR, or SMRT (co-repressors), SAP18, SAP30, RbAp48, and RbAp46 [for review, see Davie, 1998]. Similarly, yeast Sin3 and RPD3 are in a large 2-MDa protein complex. Several signal transduction pathways are regulated by the HDAC co-repressor complex. The Sin3A-N-CoR/SMRT-HDAC1,2 complex is recruited by unliganded nuclear receptors and the Mad family of bHLH-Zip proteins (Fig. 3). The Notch signal transduction pathway is mediated by interaction between SMRT-HDAC complex and CBF1 [Kao et al., 1998]. SAP30, which binds to mSin3 and N-CoR, is required for N-CoR/mSin3-mediated repression of hydroxytamoxifen-bound ER and homeodomain protein Rpx, but not unliganded RAR and TR [Laherty et al., 1998; Zhang et al., 1998]. Interestingly, microinjection of anti-N-CoR or anti-SMRT IgG into cells can convert hydroxytamoxifen-bound ER and RU486-bound PR from repressors to activators [Lavinsky et al., 1998]. There is intriguing evidence that decreasing the levels of N-CoR can lead to tamoxifen resistance in breast cancer [Lavinsky et al., 1998].

An exciting development is the realization that methyl-CpG-binding protein 2 (MeCP2) binds to Sin3, recruiting the HDAC1/2 complex [Jones et al., 1998; Nan et al., 1998]. These reports suggest that DNA methylation and his-

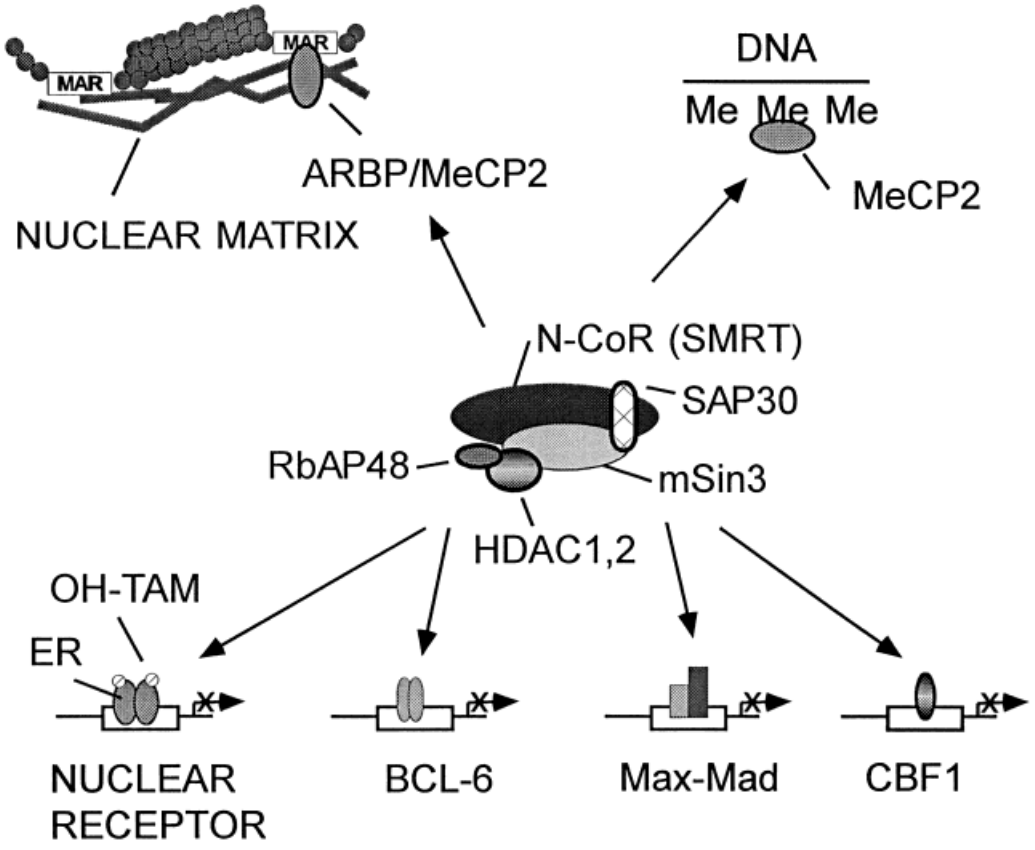

Fig. 3. The N-CoR (SMRT)-Sin3-HDAC1,2 complex is involved in several signaling pathways. The mammalian HDAC complex consists of Sin3A, N-CoR (nuclear receptor co-repressor) or silencing mediator of retinoid X receptor and thyroid hormone receptor (SMRT), mSin3-associated protein (SAP30), RbAP48, and several other peptides [Davie, 1998; Laherty et al., 1998]. This large multiprotein complex may be recruited to specific sites by nonliganded hormone receptors or hydroxytamoxifen (OH-TAM)-bound estrogen receptor (ER) to a hormone-responsive element (HRE), by Mad-Max to E-box-related sequences, by LAZ3/BCL6 to the interferon-stimulated gene response element (GAS) motif, and by CBF1, a factor involved in the Notch signal transduction pathway. ARBP/MeCP2 may recruit the HDAC complex to methylated DNA or to matrix-associated region (MAR) DNA.

tone deacetylation are coupled events in the formation of repressive chromatin structures and gene silencing.

PML-RARα, PLZF-RARα, and AML-1-ETO, oncoproteins in acute promyelocytic or myeloid leukemia generated by chromosomal translocations, recruit SMRT-mSin3A-HDAC1 and N-CoR-mSin3A-HDAC1,2 complexes [Grignani et al., 1998; Lin et al., 1998; Lutterbach et al., 1998]. The SMRT-mSin3A-HDAC1 complex is recruited by the BTB/POZ domain found in the oncoprotein LAZ3/BCL6 [for review, see Davie, 1998]. The recruitment of HDAC1 is crucial to the transforming potential of these oncoproteins. Inhibiting the HDAC activity with new-generation HDAC inhibitors appears to be a promising approach to the treatment of these cancers [Lin et al., 1998; Nakajima et al., 1998; Richon et al., 1998].

Several transcription factors can recruit HDAC directly without the assistance of the mSin3, N-CoR, and SMRT. HDAC 1, 2, and 3 bind to YY1 [reviewed in Davie, 1998]. Rb and E2F form a complex with HDAC1 [DePinho, 1998; Luo et al., 1998]. The IXCXE motif located in the C-terminal region of HDAC1 and HDAC2 associates with the Rb "pocket" domain of hypophosphorylated Rb. The recruitment of the E2F-Rb-HDAC1 complex is partly responsible for the repression of the cyclin E promoter in G1 phase of the cell cycle. Phosphorylation of Rb by CDK4 and CDK6 results in the dissociation of Rb from E2F. The promoter-associated E2F may now associate with CBP/p300, recruiting a HAT activity that stimulates transcription of the cyclin E gene. The human papilloma virus E7 oncoprotein and presumably E1A and SV40 large T antigen disrupt the interaction between Rb and HDAC1. The recruitment of Rb-HDAC1 to a promoter of a transiently expressed construct resulted in deacetylation of the H3 associated with the promoter [Luo et al., 1998].

HDAC has a principal role in transcription repression. Targeting HDAC1 or 2 to a promoter by fusing HDAC to a DNA-binding domain (e.g., Ga14 DNA-binding domain) results in transcriptional repression of a Ga14-dependent reporter in transient transfection assays [for review, see Davie, 1998]. HDAC inhibitors, trapoxin or trichostatin A, nullify this repressive activity. The popular model shows the recruited HDAC deacetylating histones in nucleosomes, leading to condensation of chromatin. However, acetylated HMG proteins and transcription factors may also be targets of the HDAC activity. Further, N-CoR of the HDAC complex can interact with $TAF_{II}32$, $TAF_{II}70$, and TFIIB, and SMRT and mSin3A can bind to TFIIB [Muscat et al., 1998]. Thus, the HDAC complex may interfere with the generation of a functional initiation complex.

HATs, HDACs, AND THE NUCLEAR MATRIX

Both HATs and HDACs are associated with the nuclear matrix [reviewed in Davie, 1997]. We proposed that nuclear matrix-associated HAT and HDACs mediate a transient attachment of transcriptionally active chromatin to the nuclear matrix. Many of the transcription factors that associate with the HATs or HDACs are nuclear matrix associated (e.g., YY1, hypophosphoryated Rb, GATA-1, ER) [reviewed in Davie et al., 1998]. HATs (e.g., $TAF_{II}250$, CBP), HDACs, and dynamically acetylated histones are found gathered around interchromatin granule clusters [Hendzel et al., 1998]. The nuclear matrix may have a role in establishing this organization. Importantly, these interactions between HATs, HDACs, transcription factors, and active chromatin should be viewed as dynamic [e.g., see Bex et al., 1998].

Recently, we reported that HDAC1 is associated with matrix-associated regions (MAR) DNA in human breast cancer cells [Davie et al., 1998; Samuel et al., 1998]. These results suggest that HDAC1 may have a role in the organization of nuclear DNA. It is interesting to note that attachment region binding protein (ARBP), a nuclear matrix protein that binds to MARs, is homologous to MeCP2 [reviewed in Holth et al., 1998]. Thus, N-CoR-Sin3A-HDAC1 complex could be recruited to the nuclear matrix and to MAR-DNA by MeCP2/ARBP (Fig. 3).

HISTONE PHOSPHORYLATION

All core histones and histone H1 can undergo phosphorylation on specific serine and threonine residues. H1 can be phosphorylated on Ser/Thr residues on the N-terminal and C-terminals domains of the molecule, and H3 can be phosphorylated on Ser/Thr residues on its N-terminal domain [Van Holde, 1988] (Fig. 1). The phosphorylation of both H1 and H3 is cell cycle dependent with the highest level of phosphorylation of both histones occurring in M phase. In G1 phase of the cell cycle, the lowest number of H1 sites is phosphorylated,

and there is a gradual increase in the number of sites phosphorylated throughout S and G2 phases. In M phase, when chromatin is highly condensed, the maximum number of sites is phosphorylated. The strong correlation between highly phosphorylated H1 and chromatin condensation at mitosis lead to the assumption that H1 phosphorylation drives mitotic chromatin condensation; however, chromatin condensation can occur in the absence of H1 phosphorylation [Ohsumi et al., 1993]. H1 phosphorylation destabilizes chomatin structure and weakens its binding to DNA. Therefore, H1 phosphorylation may lead to decondensation of chromatin and access of the DNA to factors involved in transcription and replication in G1 and S and to condensing factors present in mitosis [Chadee et al., 1995, and references cited therein].

Recent studies show an involvement of H1 phosphorylation in gene transcription. H1b phosphorylation is dependent upon ongoing transcription and replication processes; the inhibition of these processes may alter accessibility of H1b to the H1b kinase, which would result in decreased levels of phosphorylated H1b [Chadee et al., 1997]. Further, inactivation of the MMTV promoter is associated with dephosphorylation of H1, and reactivation of the promoter is associated with rephosphorylation of histone H1 [Lee and Archer, 1998].

Though most studies on H3 phosphorylation have focussed on the phosphorylation that occurs in mitosis, H3 phosphorylation also occurs in G1. Phosphorylation of H3 has been implicated in the establishment of transcriptional competence of early-response genes. H3 is rapidly phosphorylated when serum starved cells are treated with growth factors and phorbol esters, and this phosphorylation is concurrent with the transcriptional activation of the early-response genes c-*fos* and c-*jun*. Furthermore this H3 phosphorylation is restricted to a small fraction of H3 histones that are hyperacetylated [Barratt et al., 1994].

Studies on H3 phosphorylation during mitosis have revealed that Ser-10 phosphorylation of H3 is correlated with both mitotic and meiotic divisions in *Tetrahymena* micronuclei; this phosphorylation closely coincides with chromosome condensation [Wei et al., 1998]. In mammalian cells, Hendzel et al. [1997] showed that mitosis-specific phosphorylation of H3 on Ser-10 initiates primarily within percentromeric heterochromatin during late G2 and spreads in an ordered fashion throughout the condensing chromatin and is complete just before the formation of the prophase chromosomes. These investigators suggest that phosphorylation of H3 at Ser-10 may promote the binding of factors that drive chromatin condensation as cells enter mitosis. H3 phosphorylation may also be involved in coordinating chromatin decondensation associated with M phase.

H1 AND H3 KINASES

The different kinases, which have been shown to phosphorylate H1 in vitro, are cAMP and cGMP dependent kinases and the growth associated H1 kinases (cyclin-dependent kinases [CDKs]) [Van Holde, 1988]. The CDKs are believed to be involved in the cell cycle dependent phosphorylation of H1. Four distinct cyclin-dependent kinases can phosphorylate H1 at all of the growth related phosphorylation sites in vitro [Swank et al., 1997]. As there is no site selectivity among these kinases, it is thought that the in vivo cell cycle-dependent H1 phosphorylations must involve differential accessibility of H1 sites at different stages of the cell cycle [Swank et al., 1997].

Phosphorylation of H1 is cell cycle regulated, and when there is deregulation of cell cycle control such as in cancer, an accompanying change in histone phosphorylation and possibly chromatin structure may also occur. There is evidence that oncogene-transformed cells have an altered chromatin structure [for review, see Holth et al., 1998]. Such a change in chromatin structure could be a result of alterations of levels of histone subtypes or in the post-translational modification of the histones. Increased levels of phosphorylated histone H1b in mouse fibroblasts transformed with oncogenes or constitutively active mitogen-activated protein (MAP) kinase have been reported [Chadee et al., 1995]. Elevated cyclin E-associated H1 kinase activity resulting from persistent activation of the MAP kinase pathway in the transformed cells may lead to the increased level of phosphorylated H1b. Interestingly, fibroblasts lacking the tumour suppressor Rb also exhibit an increased level of phosphorylated H1 and relaxed chromatin structure; it was suggested that deregulation of CDK2 may be directly involved [Herrera et al., 1996]. As discussed earlier in this review, Rb is in a complex with HDAC1 and the transcriptional activator E2F

[DePinho, 1998]. When Rb is phosphorylated, E2F is released allowing transcription of E2F regulated genes (e.g., cyclin E). Elevated activity of cyclin E-CDK2 would result in H1 phosphorylation and chromatin relaxation in transformed cells. Possibly, an altered chromatin structure acquired by deregulation of processes, which control histone modifications such as phosphorylation of H1 and H3, would facilitate aberrant gene expression in the process of cellular transformation.

The identity of the H3 kinase remains unknown, however, phosphorylation of H3 has been correlated with protein kinase A (PKA) activity. Treatment of thymocytes with gliotoxin, which induces apoptosis, is accompanied by phosphorylation of H3 and DNA fragmentation. When apoptosis is inhibited by genistein (which also inhibits PKA), H3 phosphorylation is also inhibited [Waring et al., 1997]. These results provide evidence for involvement of PKA in H3 phosphorylation. During mitosis, H3 phosphorylation correlates with CDK1 activity in syncytial divisions of Drosophila embryos [Su et al., 1998]. In vitro studies identify H3 as a substrate for a number of other different kinases such as $pp90^{rsk}$ [Chen et al., 1992]. The identification the in vivo kinase(s), however, which phosphorylates H3 at mitosis and in G1 remains to be determined.

The state of phosphorylation of a protein is dependent upon a balance of phosphatase and kinase activities in the cell. Protein phosphatase 1 appears to be the H1 phosphatase [Paulson et al., 1996]. Inhibition of H1 phosphatase activity in metaphase arrested HeLa cells was achieved with okadaic acid and microcystin LR at concentrations that strongly suggest that PP1 (protein phosphatase 1) is the H1 phosphatase. Interestingly, PP1 is also subject to phosphorylation by CDK2, which results in a decrease in its activity. Changes in PP1 activity occur during the cell cycle with a high level of activity in G0, a progressive decrease in the activity in G1 and S phases, and in M phase there is a sharp increase in the activity again [Dohadwala et al., 1994]. Possibly, the phosphorylation of both PP1 and H1 by CDKs may control the level of H1 phosphorylation throughout the cell cycle.

FUTURE DIRECTIONS

The recruitment and nuclear localization of coactivators and corepressors with HAT, HDAC, and kinase activity are dynamic processes. The mechanisms underlying these processes are now being revealed. It will be important to find the extent of histone and transcription factor acetylation and deacetylation resulting from the recruitment of coactivators and co-repressors with HAT and HDAC activity, respectively. For example, when a coactivator/HAT is recruited to a promoter, does the HAT activity only acetylate nucleosomes located at and near the promoter? Modified histones are found along the coding region of genes. If coactivator/co-repressor-mediated histone modification is local, which HATs, HDACs, and kinases are responsible for the acetylation and phosphorylation of histones associated with transcribed chromatin domains? Perhaps HATs, HDACs, and histone kinases recruited to the nuclear matrix are responsible [Davie, 1998]. Future studies will need to determine the role of nuclear structures such as interchromatin granule clusters and the nuclear matrix in the organization of sites of transcribed chromatin and dynamic histone acetylation/phosphorylation. Our current thinking of transcribed chromatin as an extended 10-nm fiber will have to be revised. A recent study shows that transcribed acetylated chromatin is more condensed that we envisaged [Hendzel et al., 1998]. Much still needs to be learned about the role of histone acetylation/phosphorylation in chromatin structure. Further, an issue not explored in this review and needs to be addressed, is the association of nonhistone chromosomal proteins with the histone tails, the interactions of which are altered by histone modifications [Davie, 1997].

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