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Control of Histone Modifications

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Abstract A role for histone modifications in transcription processes and the remodeling of chromatin structure has been established. This review highlights the recent advances made in studies on histone acetyltransferases, histone deacetylases, histone kinases, and protein phosphatases, as well as their roles in transcriptional activation and repression. Coactivators with histone acetyltransferase activity stimulate transcription, whereas corepressors with histone deacetylase activity repress transcription. Families of histone acetyltransferases and deacetylases have been identified. We have learned that their substrates are not limited to histones but also include transcription factors and architectural proteins. Studies on the composition of multiprotein complexes with histone acetyltransferase or histone deacetylase have revealed mechanisms by which these complexes are recruited to specific genomic sites that are transcriptionally active, silenced, or being repaired. A new and exciting development, presented in this review, is the role of signal transduction pathways in the phosphorylation of histone H3 and the expression of immediate-early genes. J. Cell. Biochem. Suppls. 32/33:141–148, 1999.

Key words: histone acetylation/phosphorylation; coactivators; corepressors; transcriptional activation/repression; histone acetyltransferase; histone deacetylase; histone kinase; protein phosphatase

THE NUCLEOSOME AND HISTONE TAILS

The nucleosome core particle consists of a histone octamer core around which 146 base pairs of DNA are wrapped. The core histones 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 domain, a globular domain organized by the histone fold, and a C-terminal tail (Fig. 1). The histone fold domains of the core histones mediate histone-histone and histone-DNA interactions, while their N-terminal tails, which emerge from the core particle in all directions, are involved in the genesis of a spectrum of chromatin structural states [Luger and Richmond, 1998].

Richmond and colleagues observed that the N-terminal tail of H4 (K16 to N25) binds to the H2A-H2B dimer of a neighboring nucleosome. This interaction would contribute to the folding of the chromatin fiber and may be involved in nucleosome positioning [Luger and Richmond, 1998]. Nonhistone chromosomal proteins interact with N-terminal tails of H3 or H4, or both, to form a transcriptionally competent or repressive chromatin structure. For example, HMG-14 and -17 proteins binds to the N-terminal tail of H3 and unfold the higher-order chromatin fiber, facilitating transcription [Trieschmann et al., 1998]. In another situation, the H3 and H4 N-terminal tails bind to the yeast *trans*-acting repressors, Sir3 and Sir4, leading to the formation of a transcriptionally repressed chromatin domain [Luger and Richmond, 1998]. It has been proposed that the N-terminal tails undergo an induced folding when in contact with other proteins or DNA [Hansen et al., 1998].

HISTONE MODIFICATIONS

The core histones tails are susceptible to a wide range of postsynthetic modifications, including acetylation, phosphorylation, methylation, ubiquitination, glycosylation, and ADP-ribosylation (Fig. 1). Most modifications occur on the N-terminal basic tail domain, with histone ubiquitination being the exception. The

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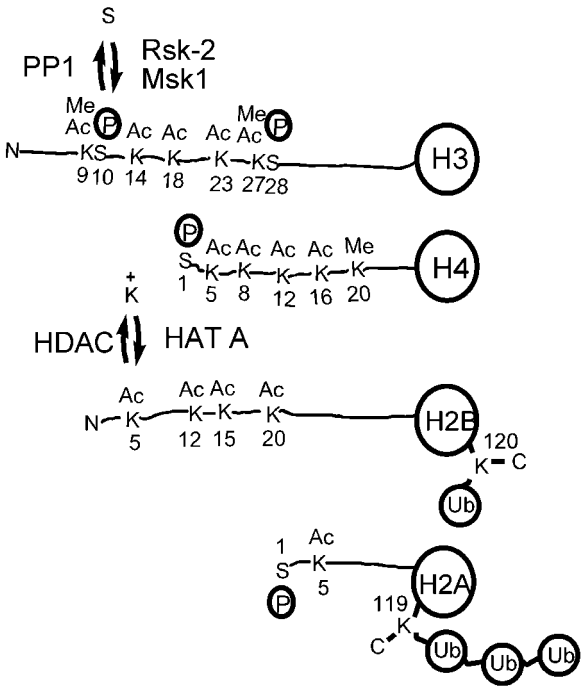

Fig. 1. Sites of postsynthetic modifications on the histones. The structures of the core histones H2A, H2B, H3, and H4 and the sites of modification are shown. The lengths of the N-terminal tail domains vary from 16 to 44 amino acids (H3, 44 amino acids; H4, 26 amino acids; H2B, 32 amino acids; H2A, 16 amino acids). The modifications shown are acetylation (Ac), phosphorylation (P), ubiquitination (Ub), and methylation (Me). The enzymes catalyzing reversible acetylation and phosphorylation are shown (HAT, histone acetyltransferase; HDAC, histone deacetylase; PP1, protein phosphatase).

following sections review the recent developments in histone acetylation and phosphorylation and their roles in gene expression.

HISTONE ACETYLATION

The core histones are reversibly acetylated at specific lysine residues located in the N-terminal tail domains (Fig. 1). With the exception of H2A, the core histones are acetylated at four to five sites. Thus, a nucleosome has 26 sites of acetylation. Histone acetylation can be a dynamic process; however, rates of acetylation/deacetylation vary throughout the genome. Some regions are associated with nucleosomes undergoing very rapid high levels of acetylation and very rapid deacetylation. Approximately 15% of the core histones of hepatoma tissue culture cells undergo this type of dynamic acetylation. Other chromatin regions have their histones acetylated and deacetylated at much slower rates, and some regions are associated with histones that are frozen in low or nonacetylated states [for review, see Davie and Chadee, 1998].

Histone acetylation remodels chromatin. Acetylation of the histone tails disrupts higher-order chromatin folding, promotes the solubility of chromatin at physiological ionic strength, and maintains the unfolded structure of the transcribed nucleosome [Spencer and Davie, 1999]. Recent studies show that nucleosomes do not have to be maximally acetylated to prevent higher-order chromatin folding. Acetylation to 46% of maximal site occupancy is sufficient to prevent higher-order folding and stimulation of transcription by RNA polymerase III [Tse et al., 1998]. It has been proposed that acetylation of core histone tails interferes with folding of the N-terminal tail and interactions with proteins and/or DNA, thereby destabilizing higher-order chromatin organization [Hansen et al., 1998]. These combined effects of histone acetylation on the destabilization of chromatin structure facilitate transcription. Oscillations in the expression (that is, dynamic activation and inactivation of transcription) of genes that are in a DNase I sensitive chromatin configuration has been attributed to dynamic histone acetylation [Feng et al., 1999].

Histone acetylation can affect the interaction of nonhistone chromosomal proteins with chromatin in at least two ways. First, histone acetylation facilitates the interaction of transcription factors with nucleosomal DNA [Workman and Kingston, 1998]. Partial acetylation of the core histone tails is sufficient to expose nucleosomal DNA for transcription factor binding without displacement of the N-terminal tail domains from DNA. Second, acetylation may modulate the interactions of proteins binding to the N-terminal tail domain.

The enzymes catalyzing reversible histone acetylation are histone acetyltransferases (HAT) and deacetylases (HDAC). In the past several years, we have come to appreciate that proteins with HAT activity are coactivators, while proteins with HDAC activity are corepressors.

HISTONE ACETYLTRANSFERASES AND GENE ACTIVATION

A number of transcriptional coactivators have histone acetyltransferase (HAT) activity (Table I). Characterization of these coactivators has solidified the mechanistic connections between histone acetylation and gene expression.

TABLE I. Histone Acetyltransferases and Their Substrates

HAT A (organism; proteins in complex)	Free histone of other substrate	Nucleosomal histone substrate
Gcn5 (yeast, human, *Drosophila*)	H3 > H4 (K41 of H3, K8, 16 or H4)	—
Ada (yeast; Gcn5 and Ada proteins)		H3, H2B
SAGA (yeast; Gcn5, Ada and Spt proteins, TAF_{II}s, and Tra-I, a homologue of the TRRAP)		H3 > H2B
STAGA (human; Gcn5-L, Spt3, TAF_{II}31)	H3 > H4	
TFTC (mammlian; Gcn5-L, hAda3, hSpt3, hTRAPP, TAF_{II}s	H3	H3
PCAF (human; human counterparts of yeast ADA proteins, Spt proteins, human TAF_{II}s, and PAF400, a 400-kDa protein almost identical to TRRAP)	H3 > H4, TFIIF, TFIIE	H3
Esa1 (yeast)	H4 > H3 > H2A, (K5 > K8, 12, 16 of H4; K14 of H3; K5 of H2A	
NuA4 (yeast, Esa1)		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	—
Tip60 (human)	H4 > H3 > H2A	
Elp3 (yeast, elongating RNA polymerase II holoenzyme)	H4, H3, H2A, H2B	Unknown
SRC-1 (human)	H3 > H4 (K9, K14 of H3)	H3, H4, H2A, H2B
ACTR (human)	H3, H4 > H2B	H3 > H4

Yeast Gcn5, a transcriptional coactivator with HAT activity, was among the first HATs to be characterized [for review, see Davie and Chadee, 1998; Kouzarides, 1999; Spencer and Davie, 1999]. Since the discovery that the transcriptional activator, Gcn5, had HAT activity, many other coactivators with HAT activity have been identified, including PCAF, CBP/p300, Esa1, NuA4, steroid receptor coactivators, and, most recently, Elp3 [Wittschieben et al., 1999].

The solution and crystal structures of the HAT domain of *Tetrahymena* Gcn5, yeast Gcn5 and PCAF were recently reported [Clements et al., 1999; Lin et al., 1999; Trievel et al., 1999]. Analyses of structural and functional properties of mutants showed that glutamate-173 in yeast Gcn5 and glutamate-570 in PCAF are essential residues in catalysis [Tanner et al., 1999; Trievel et al., 1999]. It is thought that the structural and functional properties of the catalytic domains of other HATs will be similar. The crystal structure of another domain commonly found in HATs, referred to as the bromodomain, has been presented [Dhalluin et al., 1999]. Interestingly, this domain in Gcn5 and PCAF interacts with the N-terminus of H3 and H4 and may be involved in targeting these coactivators to chromatin [Dhalluin et al., 1999; Ornaghi et al., 1999]. The bromodomain of human Gcn5 binds also to the DNA-dependent protein kinase. The recruited kinase phosphorylates Gcn5, inhibiting HAT activity [Berger, 1999].

The substrate specificities of the HATs differ (Table I). Further, many of the HATs are in multiprotein complexes, and the substrate specificity of the HAT will vary depending on whether the enzyme is free or in a complex [for review, see Berger, 1999; Spencer and Davie, 1999]. For example, yeast Gcn5 acetylates free H3, but inefficiently acetylates histones in nucleosomes. Yeast Gcn5 will efficiently acetylate histones in nucleosomes only when it is in high-molecular-weight multi-protein complexes, called SAGA (Spt-Ada-Gcn5-acetyltransferase) (1.8 MDa) and Ada (0.8 MDa) [Berger, 1999] (Table I). There is a longer form of Gcn5 (Gcn5-L) in mammalian cells, which is present in a SAGA-like complex, called STAGA, and in a complex called TFTC (TATA-binding protein-free TAF_{II}-containing complex) [Brand et al., 1999].

The acetyltransferase activity of several HATs is not limited to histones (Table I). PCAF acetylates the nonhistone chromosomal protein HMG-17 [Herrera et al., 1999]. Acetylation of

HMG-17 reduces the binding affinity of the protein to the nucleosome. CBP/p300 is a coactivator with HAT activity capable of acetylating the four core histones in nucleosomes and a variety of transcription factors (Table I). For example, CBP acetylates p53 and GATA-1 and potentiates the activities of these transcription factors [Berger, 1999].

Transcription factors may recruit one or more HATs. Transcriptional activators with an acidic activation domain (e.g., VP16) or helix-loop-helix proteins with the LDFS motif (e.g., yeast transcription factor Rtg3) recruit SAGA [Ikeda et al., 1999; Massari et al., 1999]. VP16, however, appears to recruit several HATs in situ, including Gcn5, PCAF, and CBP/p300 [Tumbar et al., 1999]. CBP/p300 is an integrator of multiple signaling pathways [for review, see Davie and Chadee, 1998]. Transcription factors, including hormone receptors, CREB, and fos-jun, loaded onto promoters or enhancers bind directly or indirectly to CBP/p300. Further, CBP is a component of the RNA polymerase II holoenzyme. The steroid receptor coactivators SRC-1 and ACTR (and related proteins RAC3, AIB1, and TRAM-1) bind to a variety of nuclear receptors in a ligand-dependent manner. These coactivators associate with CBP/p300 and PCAF. Thus, a ligand-activated nuclear receptor could recruit multiple coactivators with HAT activity (e.g., Tip60, SRC-1, CBP and PCAF) [for review, see Brady et al., 1999; Spencer and Davie, 1999] (Fig. 2).

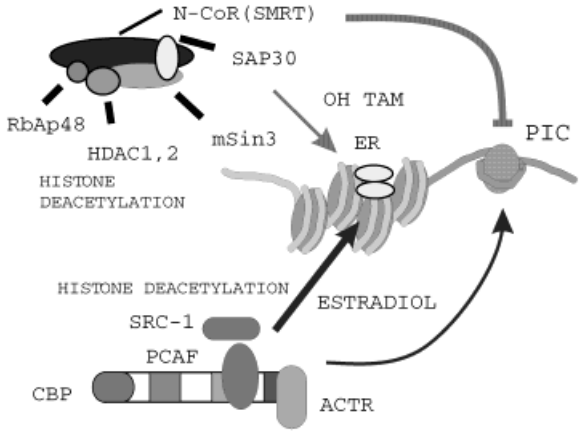

Fig. 2. Ligand-dependent recruitment of coactivators or corepressors by estrogen receptor. The model shows estrogen receptor (ER) bound to a nucleosome. When ER is associated with estradiol, ER recruits several coactivators (CBP, PCAF, ACTR) that have histone acetyltransferase activity. The recruited coactivator will acetylate nucleosomal histones and/or transcription factors and will aid in the formation of the preinitiation complex. The net result is stimulation of transcription. However, when the ER is associated with hydroxytamoxifen (OH TAM), the ER recruits the Sin3A histone deacetylase (HDAC) complex. The corepressor complex will deacetylate histones and transcription factors and prevent the formation of a preinitiation complex. The net result is repression of transcription.

ROLE OF HAT IN TRANSCRIPTION

Coactivator multiprotein complexes with HAT activity can stimulate transcription at several levels, including stimulating the formation of the preinitiation complex and by remodeling chromatin [Berger, 1999; Ikeda et al., 1999; Liu et al., 1999; Madisen et al., 1998](Fig. 2). Several lines of evidence support the view that recruited coactivators with HAT activity acetylate surrounding histones in nucleosomes, leading to the destabilization of higher-order chromatin structure and stimulation of transcription [Ikeda et al., 1999; Kouzarides, 1999]. However, it has been questioned whether the histones are the bona fide in vivo substrates of some HATs. Some HATs may affect transcription by acetylating transcription factors.

Acetylation of chromatin components can activate or repress transcription. The activity of the interferon-β (IFN-β) enhanceosome is regulated, in part, by acetylation. The enhanceosome consists of NF-κB, IRF1, ATF2/c-Jun and HMGI(Y), an essential architectural protein involved in the stereospecific assembly of this complex. Once assembled, the complex effectively recruits CBP, which then acetylates H3 and H4 in neighboring nucleosomes. This, in turn, results in the remodeling of chromatin and the recruitment of the RNA polymerase II holoenzyme [Parekh and Maniatis, 1999]. The net result is the turning on of IFN-β gene expression. However, CBP can also acetylate HMG-I(Y) at a site important in DNA binding. The result of HMG-I(Y) acetylation is disruption of the enhanceosome and the turning off of IFN-β gene expression [Munshi et al., 1998].

HISTONE DEACETYLASE (HDAC) AND GENE REPRESSION

In contrast to HATs, recruitment of HDACs can lead to repression. It is important to note, however, that chromatin regions engaged in transcription are associated with dynamically acetylated histones [for review, see Davie and Chadee, 1998]. Thus, both HATs and HDACs are recruited to these regions. When the balance of activity of these two enzymes favors

deacetylation, the chromatin region will take on a repressive higher-order structure.

The HDACs have been categorized into two classes. The first class consists of yeast histone deacetylases Rpd3, Hos,1 and Hos2 and mammalian HDACs, HDAC1, HDAC2 (the mammalian homologue of yeast RPD3) and HDAC3 [for review, see Davie and Chadee, 1998]. Class 2 consists of yeast Hda1 and mammalian HDAC4, HDAC5, and HDAC6 [Grozinger et al., 1999]. Mammalian HDAC1 and HDAC2 are in large multiprotein complexes (e.g., mSin3A and NuRD). The mSin3A complex contains mSin3, N-CoR or SMRT (corepressors), SAP18, Sap30, RbAp48, RbAp46, and c-Ski [Nomura et al., 1999]. Another complex called NuRD (nucleosome remodeling histone deacetylase complex) consists of N-CoR, MTA2 (highly related to metastasis-associated protein MTA1), Mi2, RbAP46/48, and MBD2 (methyl-CpG-binding domain-containing protein), and has both ATP-dependent chromatin remodeling and HDAC activities [Zhang et al., 1999]. HDAC3 and the class II HDAC are not found in the mSin3A and NuRD complexes [Grozinger et al., 1999].

Class I and II HDAC can deacetylate the four core histones. However, substrate preference is regulated by components of the multiprotein complexes. For example, free avian HDAC1 preferentially deacetylates H3, but not nucleosomal H3. HDAC1 in a multiprotein complex associated with the nuclear matrix preferentially deacetylates free H2B and will deacetylate histones in nucleosomes [Sun et al., 1999].

HDAC or HDAC complexes are recruited to specific genomic sites by transcription factors (repressors). HDAC1, 2, and 3 bind to YY1, while Rb and E2F form a complex with HDAC1 [for review, see Davie and Chadee, 1998; Kouzarides, 1999]. The methyl-CpG-binding protein 2 (MeCP2) recruits the mSin3A complex, and the methyl-CpG-binding domain-containing protein (MBD2) binds to the NURD complex, providing mechanisms for coupling DNA methylation and histone deacetylation in gene silencing [Zhang et al., 1999]. Several signal transduction pathways regulate the recruitment of the HDAC corepressor complex to specific loci. The Sin3A-N-CoR-HDAC1, 2 complex, for example, is recruited by unliganded nuclear receptors and the Mad family of basic helix-loop-helix-zipper proteins [Davie and Chadee, 1998; Kouzarides, 1999].

Recently BRCA1 was shown to bind to RbAp46, RbAp48, HDAC1 and HDAC2, suggesting that BRCA1 may be a component of one or more of the HDAC1/2 multiprotein complexes. BRCA1 functions as a transcriptional coactivator that associates with the RNA polymerase II holoenzyme, and is also involved in transcription-coupled DNA repair [Abbott et al., 1999]. Thus, BRCA1 may recruit HDAC complexes to sites of transcription and repair.

ROLE OF HDAC IN TRANSCRIPTION

HDAC has a principal role in transcription repression [for review, see Davie and Chadee, 1998]. Once recruited to a specific promoter, HDAC deacetylates histones in nucleosomes, leading to the condensation of chromatin [Kouzarides, 1999]. However, acetylated HMG proteins and transcription factors may also be targets of the HDAC activity.

The HDAC corepressor complex can also repress transcription by mechanisms that do not require deacetylation. N-CoR and mSin3A of the HDAC complex interacts with components of the preinitiation complex. Thus, the HDAC complex may interfere with the generation of a functional initiation complex (Fig. 2).

CHIP AND MAPPING OF ACETYLATED HISTONES

The mapping of histone acetylation to particular regions of the genome by the chromosomal histone immunoprecipitation (CHIP) assay has become a valuable experimental approach for understanding the role of this histone modification in transcription. The CHIP assay involves the isolation of DNA regions associated with modified histones by immunoprecipitation with antibodies recognizing specifically modified histone isoforms [reviewed in Spencer and Davie, 1999]. Using this approach, researchers have discovered that transcriptionally active DNA regions are associated with greater levels of highly acetylated histones compared to inactive DNA regions [reviewed in Crane-Robinson et al., 1999; Spencer and Davie, 1999]. Thus, histone acetylation appears to play an important role in the transcriptional process.

More recently, CHIP studies fine-mapping highly acetylated H3 and H4 isoforms to specific regions within a gene show that the promoter region of a transcriptionally active gene is associated with more highly acetylated H3 and/or H4, than coding regions and regions

upstream of the promoter [Krebs et al., 1999; Parekh and Maniatis, 1999]. Furthermore, this hyperacetylation appears to take place over a limited range of DNA only within the promoter region. However, in contrast to these observations, we showed that histone hyperacetylation is required to maintain the transcriptionally active nucleosome in an open conformation for transcriptional elongation [Walia et al., 1998]. In addition, CHIP studies using an antibody recognizing all acetylated histones isoforms show that highly acetylated histones are not restricted to the promoter region of transcriptionally active genes [Crane-Robinson et al., 1999; Madisen et al., 1998]. This provides evidence for an uneven distribution of acetylated histone isoforms within a gene [Madisen et al., 1998], and, therefore, suggests that the promoter-targeted histone acetylation observed in the former studies may be a result of the H3 and H4 specificity of the antibodies used. Such antibodies would not have been able to detect other acetylated core histones such as H2B [Spencer and Davie, 1999].

HISTONE PHOSPHORYLATION

The core histones and histone H1 undergo phosphorylation on specific serine and threonine residues. Phosphorylation of H1 and H3 is cell cycle dependent, with the highest level of phosphorylation of these histones occurring in M-phase. H1 is phosphorylated on Ser/Thr residues on the N-terminal and C-terminal domains of the molecule, while H3 is phosphorylated on Ser/Thr residues on its N-terminal domain (Fig. 1). The latest developments in H3 phosphorylation will be discussed.

Phosphorylation of H3 has been implicated in the establishment of transcriptional competence of immediate-early response genes. H3 is rapidly phosphorylated when the Ras-mitogen-activated protein kinase (MAPK) pathway of serum starved cells is stimulated with growth factors and phorbol esters. H3 phosphorylation is concurrent with the transcriptional activation of the early response genes c-*fos* and c-*jun* [Chadee et al., 1999]. Recently, we demonstrated that the newly phosphorylated H3 is located in numerous small foci scattered throughout the interphase nuclei; the foci were found outside condensed chromatin regions [Chadee et al., 1999]. Highly acetylated H3 is also observed in similarly positioned numerous small foci, which agrees with the observation that H3 phosphorylation is restricted to a small fraction of H3 histones that are dynamically highly acetylated [Hendzel et al., 1998]. Using CHIP, we provided direct evidence that the newly phosphorylated H3 is associated with induced c-*fos* and c-*myc* genes [Chadee et al., 1999] (Fig. 3).

The c-*fos* gene is transcribed in quiescent cells; however, elongation of the gene is blocked approximately 100 nucleotides from the site of initiation. Stimulation of the Ras-MAPK pathway results in the release of this block in elongation. It is possible that phosphorylation and likely, acetylation of H3 associated with the c-*fos* gene allows the chromatin fiber to be less compact, favoring elongation. Consistent with this hypothesis, the c-*fos* chromatin becomes more DNase I sensitive following activation of the Ras-MAPK pathway [Chadee et al., 1999]. As the H3 tail contributes to the folding and

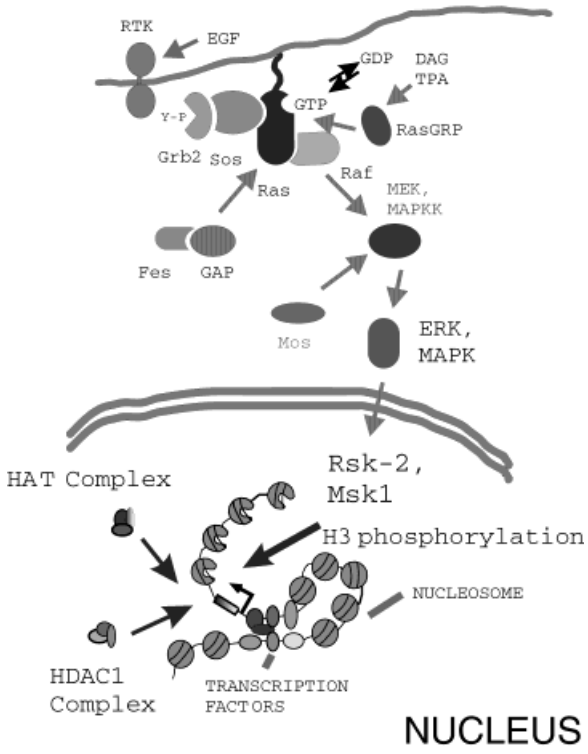

Fig. 3. The Ras/MAPK signaling pathway and the phosphorylation of H3. Stimulation of the Ras/MAPK pathway by EGF or TPA results in the activation of ERK and then Rsk-2 and Msk1. In this model, Rsk2 and/or Msk1 phosphorylate H3 associated with immediate-early response genes like c-*fos*. As the H3 that is being phosphorylated is engaged in dynamic acetylation, complexes with histone acetyltransferases (HAT) or histone deacetylases (HDAC) would be recruited to these regions. To learn more about the Ras/MAPK pathway, see http://kinase.oci.utoronto.ca/signalingmap.html.

inter-association of chromatin fibers, modification of the H3 tail by acetylation and phosphorylation may destabilize higher-order compaction of the chromatin fiber and contribute to maintaining the unfolded structure of the transcribing nucleosome (Fig. 3).

The steady-state level of H3 phosphorylation is dependent on a balance of phosphatase and kinase activities in the cell. Protein phosphatase 1 appears to be the H3 phosphatase [Chadee et al., 1999]. Allis and colleagues have presented evidence that the activity of Rsk2, a member of the $pp90^{rsk}$ kinases, is required for the mitogen-stimulated phosphorylation of H3 [Sassone-Corsi et al., 1999]. Coffin-Lowry patients have a mutation in the Rsk-2 gene. Fibroblasts from these patients do not exhibit EGF- or TPA-stimulated phosphorylation of H3 and, interestingly, growth factor-induced expression of the c-*fos* gene is severely impaired. However, Mahadevan and colleagues recently reported that MSK1 may be the H3 kinase [Thomson et al., 1999]. Both Rsk2 (MAPKAP kinase-1β) and MSK1 are members of a subfamily of MAPK-activated protein kinases, which have two distinct protein kinase domains [Deak et al., 1998].

Persistent activation of the Ras-MAPK signaling pathway results in elevated levels of phosphorylated H3 in oncogene-transformed mouse fibroblasts. The remodeling of chomatin structures resulting from increased H3 phosphorylation may contribute to aberrant gene expression [Chadee et al., 1999].

FUTURE DIRECTIONS

During the past few years, genetic and biochemical approaches have shown the mechanistic connections between histone acetylation and the transcription process. However, it is also evident that reversible acetylation of nonhistone chromosomal proteins has a role in transcriptional activation and repression of genes. To clarify the contributions of HAT and HDAC in gene expression, the bona fide substrates of HATs and HDACs will have to be determined. Also, to appreciate the role of acetylated histone isoforms in remodeling chromatin, we need to understand the position and role of the core histone tails in chromatin and to identify proteins binding to the tails. We are beginning to appreciate the role of histone modifications other than histone acetylation. More needs to be known about the enzymes catalyzing modifications other than acetylation. Histones associated with transcribed chromatin are modified by several types of modifications (e.g., H3 is phosphorylated and acetylated). How the enzymes catalyzing these modifications are jointly recruited to specific sites in three dimensional nuclear space needs to be determined.

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