What's Up and Down with Histone Deacetylation and Transcription?

Minireview

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Chromatin structure is an important component of gene expression, and recent developments have led to increased interest in the role of core histone acetylation in transcriptional regulation (for reviews, see Roth and Allis, 1996; Wade and Wolffe, 1997; Grunstein, 1997). It has been recognized for many years that there is a general correlation between core histone acetylation and gene activity, and the notion that core histone acetylation facilitates gene expression has gained further support as transcription factors such as Gcn5, CBP/p300, and TAF$_{II}$250 have been found to possess histone acetyltransferase activity.

Conversely, it has been thought that core histone deacetylation leads to transcriptional repression. This hypothesis was found to be partly consistent with the observation that a mammalian histone deacetylase, HDAC1 (also known as HD1; Taunton et al., 1996), is related to the yeast Rpd3 protein, which is required for full repression as well as full activation of gene expression (Vidal and Gaber, 1991). In addition, another mammalian protein, termed HDAC2 (also known as mRPD3), was identified as a YY1-binding protein (YY1 is a sequence-specific DNA-binding protein that can act as a repressor or activator), and a Gal4–HDAC2 fusion protein was found to repress transcription in a transient transfection assay (Yang et al., 1996).

In this context, we will summarize recent findings in the study of histone deacetylases, and then discuss a few speculative models by which protein acetylation might affect gene activity.

Sequence-Specific Transcriptional Repressors Can Recruit Deacetylases to Promoters

Seven new and interesting papers (Alland et al., 1997; Hassig et al., 1997; Heinzel et al., 1997; Kadosh and Struhl, 1997; Laherty et al., 1997; Nagy et al., 1997; Zhang et al., 1997) describe transcriptional repression by the Rpd3/HDAC1/HDAC2 deacetylases. Specifically, these papers present data that suggest a linkage between some sequence-specific DNA-binding transcriptional repressors (e.g., Mad:Max, unliganded nuclear receptors, and Ume6) and the deacetylases (Figure 1). Factors that appear to establish a protein–protein link between the DNA-bound repressors and the deacetylases are Sin3 (either yeast Sin3 protein or the related mammalian proteins, mSin3A and mSin3B) and N-CoR/SMRT, which are related proteins that were originally identified as corepressors of unliganded nuclear receptors. Other proteins that were found to be associated with Sin3 proteins include SAP18 (SAP is an abbreviation for mSin3-associated polypeptide), SAP30, RbAp46, and

RbAp48 (RbAp46 and RbAp48 are closely related proteins). Perhaps significantly, RbAp48 is associated with HDAC1 (Taunton et al., 1996) and is also a subunit of chromatin assembly factor 1 (for reviews, see Roth and Allis, 1996; Grunstein, 1997). A brief guide to the factors that were studied in each of the papers is given in Table 1.

What do these seven papers say? Do they have a common message? The major point of these papers is that transcriptional repression by a sequence-specific DNA-binding factor can be mediated by the recruitment of a deacetylase (Rpd3/HDAC1/HDAC2) to the promoter region. More specifically, each of the papers proposes a chromatin-specific mechanism for repression by a sequence-specific DNA-binding protein that involves deacetylation of core histones by a histone deacetylase that is linked to the DNA-bound repressor via Sin3 and/
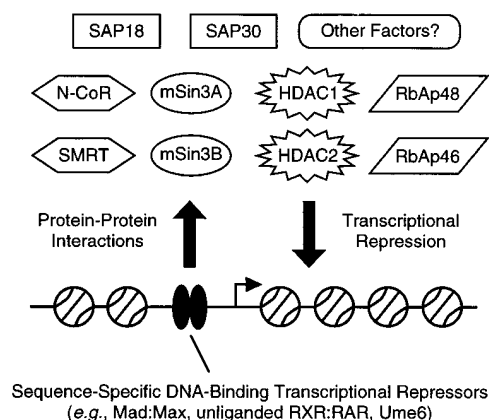

Figure 1. A Summary of Proposed Interactions That Occur between Sequence-Specific DNA-Binding Transcriptional Repressors and the Histone Deacetylases

Recent findings suggest the following generalized model for transcriptional repression by DNA-binding repressors that can interact with Sin3 (i.e., the related mSin3A and mSin3B proteins in mammals, or the analogous Sin3 protein in yeast). First, the DNA-binding repressor binds to Sin3. Next, Sin3 binds to a deacetylase HDAC1 (also known as HD1; Taunton et al., 1996) and/or the related HDAC2 (also known as mRPD3; Yang et al., 1996), or the analogous Rpd3 protein in yeast. Then, transcription is repressed by protein deacetylation, which has been generally thought to be core histone deacetylation. In addition, the transcriptional corepressors, N-CoR and SMRT (which are related to one another), interact with unliganded nuclear receptors, as well as with mSin3 proteins, and appear to be required for transcriptional repression by unliganded receptors and by Mad/Mxi proteins. Other polypeptides (SAP18, SAP30, RbAp46, and RbAp48) were observed to copurify with mSin3A. It remains to be determined whether or not there exists a stable complex (or complexes) that contains some or all of these factors. It is important to note that this model is meant to pertain only to the subset of sequence-specific DNA-binding repressors that function with Sin3. While the magnitude of transcriptional repression of native endogenous genes by HDAC1 or HDAC2 in mammalian cells is not known, Rpd3 deletion strains in yeast exhibit approximately 2- to 5-fold defects in both transcriptional repression and transcriptional activation of endogenous genes (see, for example, Vidal and Gaber, 1991).

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Table 1. A Quick Guide to the Papers and Proteins

Paper	Mad/Mxi Proteins	Nuclear Receptors	Ume6	Sin3 Proteins	N-CoR SMRT	Rpd3 HDAC1 HDAC2	RbAp46 RbAp48	SAP18 SAP30
Laherty et al. (1997)	X			X		X		
Hassig et al. (1997)	X			X		X	X	
Zhang et al. (1997)	X			X		X	X	X
Kadosh and Struhl (1997)			X	X		X		
Nagy et al. (1997)		X		X	X	X		
Heinzel et al. (1997)	X	X		X	X	X		
Alland et al. (1997)	X			X	X	X		

or N-CoR/SMRT (Figure 1). Because HDAC1 as well as a yeast Rpd3-containing complex can deacetylate core histones (or a histone H4 peptide) in vitro (Rundlett et al., 1996; Taunton et al., 1996), it was postulated that transcriptional repression involves core histone deacetylation, but whether or not localized histone deacetylation occurs at the repressed target genes remains to be tested. In addition, a biochemical interaction (of either a direct or an indirect nature) between Sin3 and Rpd3/HDAC1/HDAC2, as shown in the new papers, is nicely in accord with the findings of genetic studies of Sin3 (= Rpd1) and Rpd3 (= Sdi2), which have suggested that the two factors function in the same regulatory pathway (Vidal and Gaber, 1991; Stillman et al., 1994).

Some, but not all, transcriptional repressors appear to function via Sin3 and deacetylases. For example, Tup1, Acr1, and ERF2 do not require Rpd3 or HDAC2 to mediate transcriptional repression (Heinzel et al., 1997; Kadosh and Struhl, 1997). The results further indicated that the histone deacetylases may not be fully responsible for repression by the corepressors, Sin3 and/or N-CoR. For instance, Laherty et al. (1997) found that deletion of the HDAC interaction domain of mSin3A did not eliminate the ability of mSin3A to mediate transcriptional repression. Thus, mSin3A also appears to be able to repress transcription by an HDAC-independent mechanism. In addition, there are specific regions of N-CoR that are important for transcriptional repression that are distinct from the sites that interact with mSin3 proteins, which suggests that N-CoR can repress transcription by Sin3-dependent and Sin3-independent mechanisms (Heinzel et al., 1997). Alland et al. (1997) have also identified a splice variant of mSin3B that interacts with N-CoR but not with HDAC1, yet is still able to repress transcription. It therefore appears that a portion of the transcriptional repression by some repressors occurs via protein deacetylation.

All of the new papers involve transcriptional repression by the Sin3 corepressor and Rpd3/HDAC1/HDAC2 deacetylases (Table 1). Examination of the Saccharomyces cerevisiae DNA sequence database suggests that yeast does not possess a protein related to N-CoR or SMRT. It is thus possible that there is a core repression mechanism conserved from yeast to humans involving Sin3 and Rpd3, and that N-CoR and SMRT are corepressors that have later evolved to allow factors, such as the unliganded nuclear receptors and Mad/Mxi proteins, to mediate transcriptional repression via the Sin3–Rpd3 pathway.

Does Core Histone Deacetylation Lead to Transcriptional Repression?

Thus far, it has been generally thought that the deacetylation of core histones by HDACs leads to transcriptional repression. This hypothesis is quite reasonable given the overall correlation between histone acetylation and transcriptional activity. Moreover, a yeast Rpd3-containing complex and recombinant HDAC1 can deacetylate core histones (or a histone H4 N-terminal peptide) in vitro (Rundlett et al., 1996; Taunton et al., 1996), and deletion of either the RPD3 gene or the HDA1 gene (which encodes a protein that is related to Rpd3) in yeast leads to increased levels of core histone acetylation in vivo (Rundlett et al., 1996). It therefore seems likely that acetylated core histones are a physiological substrate for Rpd3 and related proteins, but it remains to be determined whether deacetylation of core histones does indeed mediate transcriptional repression by these factors.

A simple model in which core histone acetylation leads to gene activation predicts that global hyperacetylation of chromatin would lead to widespread transcriptional activation. In yeast rpd3 deletion strains, however, a defect in both transcriptional repression as well as transcriptional activation is observed (see, for example, Vidal and Gaber, 1991; Rundlett et al., 1996). Specifically, there is a 2- to 5-fold derepression of uninduced genes and a 2- to 5-fold reduction in the levels of expression of activated genes (Vidal and Gaber, 1991). In addition, Van Lint et al. (1996) performed a differential display analysis of cells treated with the histone deacetylase inhibitor, trichostatin A. In that study, the deacetylase inhibitor induced a change (i.e., either an increase or a decrease) in the transcriptional activity of only 8 out of the approximately 340 genes that were examined, even though the inhibitor did increase the overall level of core histone acetylation. (Van Lint et al. [1996] also observed induction of HIV-1 transcription and repression of c-myc transcription upon treatment with either of the deacetylase inhibitors, trichostatin A or trapoxin. The c-myc repression was rapid, as transcripts were no longer detectable 2 hr after treatment, and hence, it is difficult to ascribe this repression to the transcriptional induction of a gene that encodes a c-myc repressor.) Therefore, the available data indicate that a global increase in core histone acetylation does not induce widespread transcription.

Studies of position–effect variegation and telomeric silencing, which are forms of localized transcriptional repression that occur in the vicinity of heterochromatin,

**Acetylation Induces a Conformational
Change in the Core Histones**

EXAMPLE

Lysine-rich tails bind tightly to
DNA and repress transcription
by blocking access of factors
to the DNA template.

REPRESSED

Acetylation of lysine residues
alters chromatin structure
and allows binding of
transcription factors.

ACTIVE/COMPETENT

**Acetylation Is a Transducing
Signal**

$\overset{+}{N}H_3$

Protein

EXAMPLE

Acetylation of a histone at
a specific site might
create (or eliminate) a
recognition site for the
binding of another factor.

NHAc

Protein

**Acetylation Affects the Activity
of a Nonhistone Protein**

$\overset{+}{N}H_3$

Protein

EXAMPLE

Acetylation of a nonhistone
factor, such as an HMG
protein or a transcription
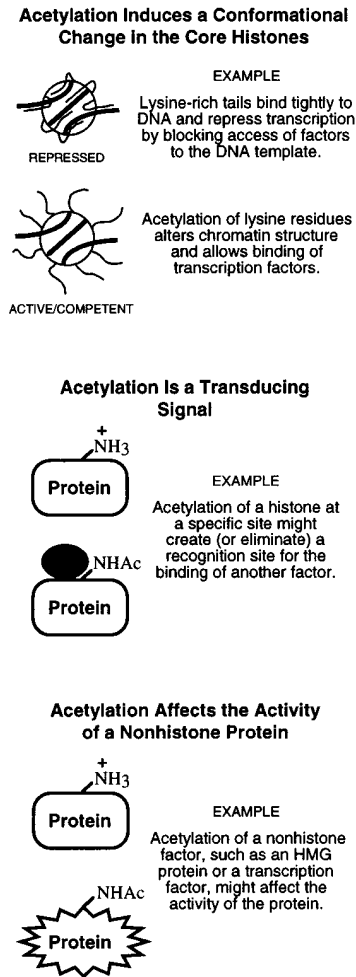factor, might affect the
activity of the protein.

NHAc

Protein

Figure 2. Some Hypothetical Mechanisms by which Protein Acetylation Could Affect Transcriptional Activity

These models are not mutually exclusive and are not necessarily nonoverlapping.

have also led to unexpected findings with regard to core histone acetylation and gene expression. In particular, *rpd3* deletion strains in yeast (which possess increased levels of histone acetylation) exhibit decreased transcriptional activity near telomeres relative to their wild-type counterparts (De Rubertis et al., 1996; Rundlett et al., 1996). In addition, enhancement of position–effect variegation (i.e., a decrease in transcriptional activity near heterochromatin) was seen in Drosophila *rpd3* mutant strains (De Rubertis et al., 1996). Thus, in these instances, an increase in histone acetylation correlated with a decrease in transcriptional activity. These and other findings collectively suggest that the relation between Rpd3/HDAC function and transcriptional activity may be more complex than a matter of histone deacetylation and consequent transcriptional repression.

How Might Protein Acetylation
Regulate Transcription?

A few hypothetical models for the regulation of transcriptional activity by protein acetylation are depicted in Figure 2. These models are not mutually exclusive and may partially overlap.

In the first (upper) model, modification of charged lysine residues in the histone tails by acetylation increases the access of transcription factors to the DNA template. While there is not a perfect correlation between acetylation and transcriptional activity, it is possible that the changes in core histone structure that occur upon acetylation may facilitate transcription but not be sufficient to induce transcription of an inactive gene. In fact, core histone acetylation could be involved at the level of transcriptional competence (i.e., the facility by which a gene can be transcribed) rather than transcriptional induction. For example, analysis of both coding and noncoding regions of the chicken β-globin locus revealed a 33 kbp region of core histone hyperacetylation, which comprises transcriptionally active and inactive β-globin genes, that corresponds closely with a DNase I general sensitivity domain (Hebbes et al., 1994). In such instances, core histone acetylation may contribute to the establishment and/or maintenance of a transcriptionally competent state in which the chromatin is in an altered ("open") conformation.

In the second (middle) model, the specific acetylation of a lysine residue (of a histone or nonhistone protein) creates (or eliminates) a signal that is recognized by another factor. This model is suggested, for example, by the distinct acetylation patterns of conserved lysine residues in histone H4 of Drosophila (Turner et al., 1992). In Drosophila polytene chromosomes, H4 acetylated at positions 5 or 8 is distributed throughout euchromatin, H4 acetylated at position 12 is preferentially associated with β-heterochromatin, and H4 acetylated at position 16 is preferentially associated with the hyperactive male X chromosome. This specificity in the localization of differently acetylated forms of H4 suggests that acetylated H4 is acting as a signal. (In addition, the localization of H4 acetylated at lysine 12 in Drosophila β-heterochromatin indicates, once again, that histone acetylation does not necessarily correlate with gene activity.) It would be interesting to investigate the consequences of acetylation at specific sites in histones. For instance, downstream effector molecules, such as coactivator or corepressor complexes, might bind to H4 that is acetylated at specific positions (see, for instance, Edmondson et al., 1996).

The third (bottom) model postulates that acetylation of nonhistone proteins is important for transcriptional regulation. For example, high mobility group (HMG) proteins are acetylated at lysine residues, and the deacetylation of HMG proteins is inhibited by sodium butyrate (a reagent that is commonly used to inhibit histone deacetylation). Therefore, HMG protein deacetylation may contribute to the transcriptional effects that are seen with Rpd3 and related proteins. In addition, other proteins, such as transcription factors and components of the basal transcription machinery, could possibly be modified by acetylation.

The specificity of protein acetylation is reminiscent of that of protein phosphorylation. Some phosphorylation events induce a conformational changes in proteins that alter their functions. Other phosphorylation sites serve as docking sites for effector molecules. Further studies will reveal the extent of such parallels between protein acetylation and protein phosphorylation.

Are the Deacetylases Involved
in Transcriptional Activation?

While there has been considerable effort devoted to the study of deacetylases as transcriptional repressors, is it possible that they also function as transcriptional activators? For example, the yeast Rpd3 protein is required for full activation as well as for full repression of gene expression (Vidal and Gaber, 1991; Rundlett et al., 1996). In addition, a transcriptional activation function of Rpd3 is consistent with the reduced transcriptional activity that is observed upon the loss of Rpd3 function in Drosophila or yeast (De Rubertis et al., 1996; Rundlett et al., 1996). Alternatively, these data could be interpreted to indicate that loss of Rpd3 leads to repression of a transcriptional activator (and hence, a reduction in gene activity), and such an indirect effect for activation by Sin3 has been proposed.

The recent studies of the effects of histone deacetylases upon transcription have generally characterized the deacetylases as Gal4 or LexA fusions with synthetic reporter genes containing multiple (typically, four or five) Gal4- or LexA-binding sites upstream of the core promoter. It is therefore possible that these assays did not detect an activation function for the deacetylases. If histone deacetylases were components of large protein complexes that regulate transcription, it seems plausible that such complexes could possess the capacity to activate or to repress transcription. In this regard, it is interesting to note that many of the sequence-specific DNA-binding factors that function via the Sin3 and Rpd3/HDAC repression pathway (such as Max, TR, RAR, Ume6, and YY1) can also function as activators in other contexts.

Conclusions and Prospects

There is little doubt regarding the biological significance of protein acetylation. Several new and important papers have shown that deacetylases can function in transcriptional repression. These studies additionally suggest many new lines of experimentation. For instance, are core histones and/or HMG proteins the critical downstream targets of the deacetylases? What are the functional consequences of protein acetylation? Why does Rpd3 affect both transcriptional repression and activation? To address some of these questions, it will be important to analyze the expression of endogenous or stably integrated genes rather than transiently transfected templates that are not efficiently packaged into chromatin (note, however, that many of the studies of Sin3 and Rpd3 in yeast have used native endogenous genes). It will also be interesting to investigate whether or not there is a large protein complex containing some or all of the factors shown in Figure 1. There are many other questions and issues remaining, as we are in the early stages of understanding the various functions of protein acetylation. In the near future, we can look forward to many more interesting and important discoveries in this area.

Selected Reading

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