PERSPECTIVE

Histone acetylation and transcriptional regulatory mechanisms

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More than 30 years ago, Vincent Allfrey proposed that histone acetylation was associated with transcriptional activity in eukaryotic cells (Allfrey et al. 1964; Pogo et al. 1966). Subsequently, acetylated core histones were shown to preferentially associate with transcriptionally active chromatin (Sealy and Chalkley 1978; Vidali et al. 1978; Hebbes et al. 1988). Acetylation occurs at lysine residues on the amino-terminal tails of the histones, thereby neutralizing the positive charge of the histone tails and decreasing their affinity for DNA (Hong et al. 1993). As a consequence, histone acetylation alters nucleosomal conformation (Norton et al. 1989), which can increase the accessibility of transcriptional regulatory proteins to chromatin templates (Lee et al. 1993; Vettese-Dadey et al. 1996). Taken together, these observations suggested how histone acetylation could result in increased transcriptional activity in vivo. However, there was essentially no information about the cause and effect relationship between histone acetylation and transcriptional activity or about the underlying molecular mechanisms.

A mechanistic and physiologically relevant connection between histone acetylation and transcriptional regulation was initially provided by two independent lines of evidence. First, yeast cells unable to acetylate the histone H4 tail because of mutations of the target lysine residues show altered patterns of transcription (Durrin et al. 1991). However, these mutations broadly affect chromatin structure in vivo, and hence are likely to influence other molecular processes involving DNA (e.g., DNA replication and repair, recombination, chromosome segregation). Second, treatment of mammalian cells with potent inhibitors of histone deacetylase activity such as trapoxin or trichostatin A resulted in increased expression of a variety of genes (Yoshida et al. 1995). However, these drugs might inhibit other cellular targets, and they affect a variety of cellular processes, including cell proliferation, apoptosis, differentiation, and DNA synthesis. Although these observations were suggestive, understanding of the relationship between chromatin structure and transcription regulation was hampered significantly by a lack of knowledge about the enzymes that acetylate and deacetylate histones.

In the past 2 years, our understanding of the causal relationship between histone acetylation and gene expression has been enhanced dramatically by the identification of proteins with intrinsic histone acetylase and deacetylase activity (Brownell et al. 1996; for recent reviews, see Grunstein 1997; Pazin and Kadonaga 1997; Wade et al. 1997). Of particular significance, some of these enzymes had been identified previously as components of the RNA polymerase II (Pol II) transcription machinery itself, proteins that associate with transcriptional regulatory factors, or proteins that positively or negatively affect transcription in vivo. These discoveries have led to a major paradigm shift. It is now clear that chromatin structure and modification can not be viewed as a process that is independent of transcriptional initiation, that is, chromatin is not simply a structure that serves to compact DNA in the nucleus and provide a relatively passive substrate for the action of transcription factors. Instead, histone acetylases and deacetylases provide a critical link between chromatin structure and transcriptional output, and this link is now accessible to experimental intervention. This review will focus on molecular mechanisms by which histone acetylation affects transcriptional activity in living cells.

A surprisingly large number of transcriptional regulatory proteins possess intrinsic histone acetylase activity

The number of histone acetylases and deacetylases in eukaryotic cells was unanticipated. Of even more importance, many of these histone acetylases and deacetylases are proteins with previously described functions in transcriptional regulation (Fig. 1). In the case of histone acetylases, these transcriptional regulatory functions are remarkably diverse. (1) The TAF130/250 histone acetylase (Mizzen et al. 1996) is a subunit of the TFIID complex, a basic component of the Pol II transcription machinery in all eukaryotic organisms; it is likely to be associated with essentially all promoters during transcriptional initiation. (2) The p300/CBP histone acetylase (Bannister and Kouzarides 1996; Ogryzko et al. 1996) was described initially as a transcriptional coactivator

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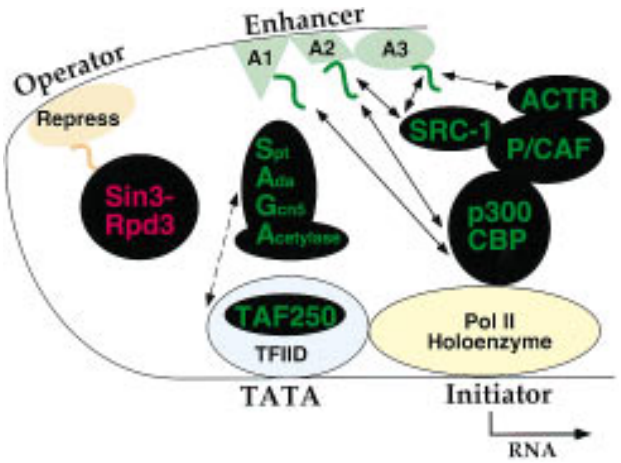

Figure 1. Histone acetylases and deacetylases are associated with the Pol II transcription machinery. Relationship of histone acetylases (black ovals with green text) and deacetylases (black oval with red text) with components of the basic machinery (TFIID and Pol II holoenzyme, blue and yellow ovals, respectively), gene-specific activators (A1, A2, A3 with activation domains indicated by wavy lines), and gene-specific repressors (peach oval with repression domain indicated by wavy line). TAF250 is an intrinsic subunit of TFIID; Gcn5 is a subunit of the SAGA complex that contains Spt and Ada proteins; Rpd3 is a subunit of multiprotein complex containing Sin3; it is unknown whether the other histone acetylases are found as isolated proteins are as part of protein complexes. Biochemically defined interactions are indicated by direct contact of the relevant ovals or by solid arrows. The allele-specific genetic interaction between Spt3 and the TBP subunit of TFIID is indicated by a broken arrow.

that functions by interacting with a wide variety of enhancer-binding proteins (Janknecht and Hunter 1996). However, p300/CBP is tightly associated with the Pol II holoenzyme (Nakajima et al. 1997; D. Reinberg, pers. comm.), suggesting the possibility that this histone acetylase could be viewed as a more general component of the transcription machinery. p300/CBP is found in a variety of multicellular organisms from worms to humans, but it does not exist in yeast. (3) Histone acetylase activity is also an intrinsic function of ACTR (Chen et al. 1997) and SRC-1 (Spencer et al. 1997), two transcriptional coactivators that associate with a variety of nuclear receptors in a hormone-dependent manner. As is the case with p300/CBP, these histone acetylases appear to restricted to multicellular organisms. (4) The P/CAF histone acetylase (Yang et al. 1996) is structurally similar to the Gcn5 enzymes from *Tetralhymena* and yeast (Brownell et al. 1996), although it is a protein distinct from the probable human Gcn5 homolog (Candau et al. 1996). Interestingly, P/CAF associates both with p300/CBP (Yang et al. 1996b) and with the nuclear receptor coactivators ACTR and SRC-1 (Chen et al. 1997; Spencer et al. 1997), thereby forming protein complexes with multiple histone acetylases. (5) Gcn5, the first nuclear histone acetylase to be identified (Brownell et al. 1996), is likely to present in all eukaryotes. In yeast, Gcn5 is not essential for cell growth, but it is important for the expression of a subset of genes (Georgakopoulos and Thireos 1992). Yeast Gcn5 is found in at least two distinct multiprotein complexes, Ada and SAGA, neither of which is tightly associated with TFIID or the Pol II holoenzyme (Grant et al. 1997). Both Gcn5 complexes contain Ada proteins, but the SAGA complex also contains Spt proteins, including Spt3 which interacts with the TATA-binding protein (Eisenmann et al. 1992).

The HDAC/Rpd3 family of histone deacetylases is associated with transcriptional regulatory proteins

The best described histone deacetylases are members of a common family that includes the founding member from human, HDAC1 (Taunton et al. 1996), and yeast Rpd3 (Rundlett et al. 1996). HDAC/Rpd3 homologs have been found in a wide variety of eukaryotes, and there are typically multiple family members in each organism (e.g., there are five in yeast). These family members are presumed to have histone deacetylase activity, but this has been directly demonstrated only in a limited number of cases. HDAC1 and Rpd3 are found in large multiprotein complexes that include the Sin3 corepressor and other proteins (for review, see Pazin and Kadonaga 1997). More importantly, these HDAC/Rpd3 complexes associate with DNA-binding repressors such as Mad (Hassig et al. 1997; Laherty et al. 1997), Ume6 (Kadosh and Struhl 1997), YY1 (Yang et al. 1996a), or with transcriptional corepressors for nuclear receptors such as SMRT (Nagy et al. 1997) and NCoR (Alland et al. 1997; Heinzel et al. 1997). Aside from the HDAC/Rpd3 family, a structurally distinct histone deacetylase has been identified from maize nucleoli (Lusser et al. 1997).

Distinct biochemical specificities of histone acetylases and deacetylases

For many years, it was assumed that chromatin existed either in a transcriptionally active state in which histones were acetylated or in a repressed state in which histones were not acetylated. This view became complicated by the observations that histone H4 isoforms acetylated at specific lysines are selectively localized on *Drosophila* chromosomes (Turner et al. 1992), and that lysine 12 of histone H4 is preferentially acetylated in yeast heterochromatin (Braunstein et al. 1996). Biochemical characterization of the various enzymes described above has revealed a much greater level of complexity in histone acetylation patterns.

When assayed on a variety of substrates including histone tail peptides, isolated histones, or nucleosomes, the individual histone acetylases and deacetylases display distinct specificities. Some enzymes are relatively promiscuous in their action, whereas others are quite specific in terms of the individual lysine residues and particular histones they affect. For example, Gcn5 preferentially acetylates lysine 14 of histone H3 and lysines 8 and 16 of histone H4 (Kuo et al. 1996), and HDAC/Rpd3

preferentially deacetylates lysines 5 and 12 of histone H4 (Rundlett et al. 1996; Taunton et al. 1996). In addition, the histone acetylases differ in their ability to act on nucleosomal or free histones, and in this regard, the recombinant form of the enzyme can differ dramatically from the histone acetylase complex that exists in cells. For example, recombinant Gcn5 can only acetylate free histones, whereas the Ada and SAGA complexes that contain Gcn5 can acetylate nucleosomes (Grant et al. 1997). This suggests that the Ada (and perhaps Spt) proteins are required for Gcn5 to utilize nucleosomal substrates. At present, the physiological roles of these distinct acetylation patterns are poorly understood, although it seems likely this enzymatic specificity will be reflected in biological selectivity.

Are histone acetylase and deacetylase activities physiologically relevant?

The fact that the various histone acetylases and deacetylases can catalyze enzymatic reactions on histone substrates in vitro does not necessarily mean that histones are physiologically relevant substrates in vivo. This issue is particularly important because some of the enzymes can acetylate nonhistone proteins that are part of the transcription machinery. P/CAF, TAF250, and p300 can efficiently acetylate the β subunit of TFIIE, and both subunits of TFIIF can be acetylated by P/CAF and p300 (Imhof et al. 1997). In addition, p300 can acetylate the p53 transcriptional activator protein, and this acetylation results in a striking increase in specific DNA-binding activity (Gu and Roeder 1997). In the case of these histone acetylases (and also ACTR and SRC-1), it has yet to be demonstrated whether histones are physiological substrates.

A related and equally critical question is whether the histone acetylase or deacetylase activities per se are relevant for the transcriptional effects in vivo. The fact that *gcn5*- or *rpd3*-deletion strains alter transcriptional activation or repression of selected genes does not necessarily mean that the histone acetylase or deacetylase activities are involved directly in transcriptional control. These proteins could have some other transcriptional function, particularly since they are found in large multiprotein complexes. Similarly, although potent histone deacetylase inhibitors significantly reduce repression mediated by Mad and SMRT in mammalian cells (Hassig et al. 1997; Laherty et al. 1997; Nagy et al. 1997), the specificity of these inhibitors for the various cellular deacetylases is poorly understood. Moreover, the possibility of indirect effects on transcription can not be ignored especially given the fact that these drugs affect a variety of complex cellular processes.

Recent work on yeast Gcn5 and Rpd3 strongly suggests that histones are physiologically relevant substrates for these enzyme complexes, and that the histone acetylase and deacetylase activities are critical for transcriptional regulation. Two papers in this issue describe detailed mutational analyses of Gcn5, leading to the conclusion that there is a strict correlation between histone acetylase activity in vitro and transcriptional activity in vivo (Kuo et al. 1998; Wang et al. 1998). In one of these studies (Wang et al. 1998), histone acetylation by the various Gcn5 derivatives was performed in the context of the Ada and SAGA complexes on nucleosomal substrates. In the other study, additional evidence for physiological relevance was obtained by analyzing directly the acetylation state of chromatin in yeast cells (Kuo et al. 1998). Overexpression of Gcn5 leads to increased acetylation of core histones. More interestingly, Gcn5 increases histone acetylation at promoter regions in a manner that is correlated with Gcn5-dependent transcriptional activation and histone acetylase activity in vitro.

Histones are also physiological substrates for yeast Rpd3 and Hda1 histone deacetylases (Rundlett et al. 1996). In accord with the enzymatic specificities of these deacetylases, yeast strains lacking either Rpd3 and Hda1 show increased acetylation at lysines 5 and 12 of histone H4. Furthermore, mutant derivatives of Rpd3 that abolish histone deacetylase activity but do not affect Sin3–Rpd3 complex formation are defective for transcriptional repression in vivo (Kadosh and Struhl 1998). These experiments are consistent with and extend the observations in mammalian cells that HDAC-dependent repression in vivo is sensitive to histone deacetylase inhibitors. Nevertheless, the key issue of whether Rpd3-dependent repression is associated with increased acetylation at the level of specific target genes has yet to be demonstrated.

Models for how histone acetylases and deacetylases selectively affect gene expression

Although histones are associated with virtually all eukaryotic genomic DNA sequences, histone acetylases and deacetylases do not universally affect the transcription of all genes. In yeast cells, Gcn5 and Rpd3 are important for the transcription of a small subset of genes; expression of most genes is unaffected in *gcn5*- or *rpd3*-deletion strains. Conversely, the five Rpd3-like histone deacetylases in yeast do not have equivalent functions, because the selective transcriptional effects in *rpd3*-deletion strains occur even though the other four deacetylases are present. These observations are in accord with the fact that histone acetylation patterns are not uniform in eukaryotic chromosomes.

There are three basic models to account for how histone modifying activities can affect the transcriptional activity in a gene-specific manner (Fig. 2). These models are distinguished by whether the histone acetylases and deacetylases are untargeted, generally targeted, or specifically targeted to promoters. Individual histone acetylases and deacetylases will differ with respect to which of these models applies.

In the untargeted model (model 1), the histone modifying activities operate on a genome-wide basis with minimal specificity for particular chromosomal regions. In this case, selective gene expression would arise from promoter-specific responses to a generally altered chromatin structure. For example, promoters whose DNA

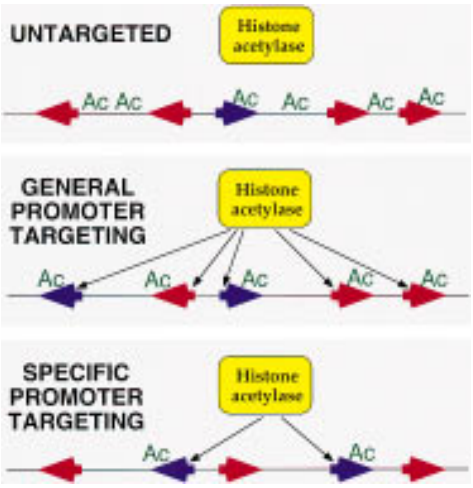

Figure 2. Models for how histone acetylases selectively affect transcriptional activity. For each model, sites of acetylation (green Ac) are indicated with respect to five promoters (arrows) that either are (blue) or are not (red) affected by acetylation. In model 1, histone acetylases are not targeted, and histone acetylation occurs at promoter and nonpromoter regions. In model 2, histone acetylates are generally targeted to promoters (arrows), because of their association with a general component of the Pol II transcription machinery. For both models 1 and 2, selective effects on transcription are attributable to inherent differences in the promoters with respect to the state of histone acetylation (see Fig. 3). In model 3, histone acetylases are targeted to specific promoters by gene-specific activator proteins, leading to selective effects on transcription.

sequences inherently cause nucleosomes to be tightly packed or preferentially positioned over binding sites for transcription factors might be particularly sensitive to acetylation status (Fig. 3). Alternatively, gene-specific activators and repressors differ dramatically in their ability to bind to nucleosomal DNA, and such differences might underlie promoter-specific responses to acetylation status. Although the nontargeted model is typically disfavored (or ignored), it is a plausible alternative that needs to be addressed experimentally.

In model 2, the histone modifying activity is selectively targeted to promoter regions in a manner that is relatively nonspecific for individual genes (Fig. 2). If the TAF130/250 histone acetylase activity is physiologically relevant, the general targeting model will almost certainly be correct because the TFIID complex is presumed to present at all functional promoters. Although TAF130 is not required for transcription of most yeast genes (Moqtaderi et al. 1996; Walker et al. 1996), it is tightly associated with TBP in yeast and mammalian cells, and TBP is generally required for transcription in vivo (Cormack and Struhl 1992). The general targeting model is compatible with gene-specific gene expression because individual promoters vary considerably with respect to intrinsic chromatin structure, protein-binding sites, and rate-limiting steps for transcriptional initiation (Fig. 3); hence, promoters are likely to differ considerably in their response to and requirement for histone acetylation.

In principle, the general targeting model will apply to any histone-modifying activity that is a basic component of the Pol II machinery or that strongly associates with the basic machinery. Furthermore, transcriptional activators that function by increasing recruitment of the Pol II machinery to promoters (Struhl 1996; Ptashne and Gann 1997) should increase acetylation concomitantly in the promoter region; the activator does not have to contact the histone-modifying activity directly for this to occur. In this regard, activation of the HIV-1 enhancer in vitro is accompanied by increased acetylation of histone H4 (Sheridan et al. 1997). Thus, histone acetylases that function by general promoter targeting might be part of the mechanism that accounts for the long-standing and general correlation between an active chromatin structure and gene transcription.

The gene-specific targeting model (model 3) involves recruitment of a histone modifying activity to particular promoters by sequence-specific, DNA-binding proteins (Fig. 2). The best case for such gene-specific targeting is transcriptional repression by the Sin3–HDAC/Rpd3 histone deacetylase complexes. This repression mechanism appears to be conserved from yeast to mammals, and the supporting arguments for this mechanism derive and benefit from complementary experimental approaches and observations (Yang et al. 1996a; Alland et al. 1997; Hassig et al. 1997; Heinzel et al. 1997; Kadosh and Struhl 1997; Laherty et al. 1997; Nagy et al. 1997). First, the yeast and mammalian histone deacetylase complexes specifically interact with DNA-binding repressor proteins (e.g., Mad, YY1, Ume6) or their associated corepressors (NCor, SMRT). Second, mutational analyses indicate that the ability of these proteins to interact physically with the histone deacetylase complexes is strictly correlated with the ability to repress transcription of appropriate target genes in vivo. Third, artificial recruitment of histone deacetylase complexes to promoters via heterologous DNA-binding domains is sufficient to mediate transcriptional repression; in mammalian cells, this repression is sensitive to histone deacetylase inhibitors. Fourth, yeast Rpd3 deacetylates histones in vivo (Rundlett et al. 1996), and histone deacetylase activity per se is important for repression (Kadosh and Struhl 1998). Fifth, yeast Rpd3 is specifically required for Ume6- and Sin3-dependent repression but not for other transcriptional repressors assayed under comparable experimental conditions (Kadosh and Struhl 1997). Taken together, these observations strongly suggest that transcriptional repression occurs by recruitment of the HDAC/Rpd3 complexes to specific promoters and targeted histone deacetylation leading to local changes in chromatin structure (Fig. 4). However, conclusive proof of this mechanism will require direct analysis of chromatin structure of the transcriptionally repressed genes.

Distinguishing between general and gene-specific targeting models requires experimental work, and the distinctions can be semantic. For example, the p300/CBP histone acetylase might be recruited to a subset of pro-

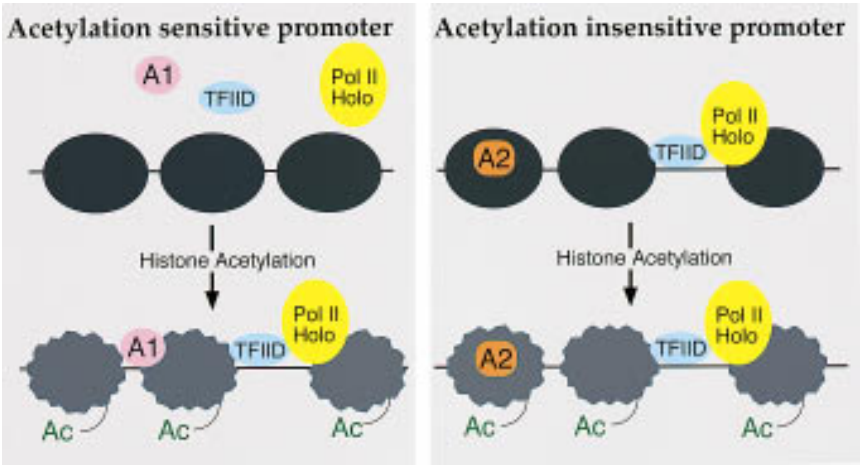

Figure 3. Potential differences between promoters that could affect the response to histone acetylation. The chromatin structural change due to histone acetylation (vertical arrows) is depicted by the conversion of nonacetylated nucleosomes (black ovals) to acetylated nucleosomes (wavy gray ovals with green Ac); increased accessibility to transcription factors (colored ovals) is shown by increased space between nucleosomes. Two putative, and nonmutually exclusive differences between acetylation-sensitive and acetylation-insensitive promoters are indicated. First, because of their inherent DNA sequence preferences, nucleosomes on the acetylation-sensitive promoter are more tightly packed and/or positioned to preclude the accessibility to the Pol II machinery. In contrast, nucleosomes on acetylation-insensitive promoters are less tightly packed and/or positioned to facilitate accessibility of the Pol II machinery to the promoter. Second, the acetylation-sensitive promoter responds to an activator protein (A1) whose binding is strongly inhibited by nucleosomes, whereas the acetylation-insensitive promoter responds to an activator (A2) that is largely unaffected by nucleosomes. The diagrams and description above are intended to illustrate general ideas, not explicit molecular mechanisms.

moters because it interacts specifically with a variety of DNA-binding proteins (Janknecht and Hunter 1996). However, the promoter specificity of p300/CBP function remains to be clarified, because p300/CBP appears to be associated with the Pol II holoenzyme (D. Reinberg, pers. comm.). If p300/CBP is an integral part of the Pol II holoenzyme, it would be present at essentially all promoters and hence function by model 2; in this view, the interactions with enhancer-binding proteins would be analogous to interactions between other components of the Pol II machinery and transcriptional activation domains. Alternatively, if p300/CBP associates with, but is not an integral part of, the Pol II holoenzyme, it could affect gene expression selectively by virtue of being selectively recruited to promoters. These issues also apply to P/CAF, which interacts with p300/CBP, and to ACTR and SRC-1, which interact with P/CAF. It seems likely that some or all of these histone acetylases will be recruited to specific promoters and then locally affect chromatin structure, but the physiological roles of these proteins and their histone acetylase activities remains to be clarified.

For histone acetylases, our knowledge is most advanced for yeast Gcn5. Studies from David Allis' laboratory reported in this issue indicate that Gcn5 acetylates histones in vivo with a strong preference for promoter regions (Kuo et al. 1998). Preferential histone acetylation at the promoter is correlated with Gcn5-dependent transcriptional activation and hence is physiologically relevant. These observations demonstrate that Gcn5 histone acetylase is targeted to promoter regions, but they do not distinguish between general and specific targeting. In this regard, it would be of particular interest to examine Gcn5-dependent histone acetylation in the promoter regions of genes whose transcriptional activity is not influenced by Gcn5.

How does the Gcn5 histone acetylase complex selectively affect gene expression in yeast? It has been suggested that Gcn5 might be selectively recruited to promoters because activation domains can interact with the Ada2 component of the Gcn5 histone acetylase complex (Silverman et al. 1994) and because *gcn5* and *ada* mutations can selectively affect the function of activation domains (Berger et al. 1992). On the other hand, analysis of mutant strains indicates that the subset of genes sensitive to Gcn5 histone acetylase are remarkably similar to those affected by the Swi/Snf nucleosome remodeling complex (Pollard and Peterson 1997). As there is no obvious relationship among the genes affected by both the Gcn5 and Swi/Snf complexes, it seems unlikely that the similar biological functions of these very different chromatin-modifying activities reflect activator-specific recruitment to a common subset of promoters. Instead, it is more likely that the chromatin structure of Gcn5- and Swi-Snf-dependent promoters is inherently ''restrictive'' because of nucleosome positioning and/or density. In such promoters, wild-type levels of transcription will require chromatin to be ''loosened'' by either of these nucleosome-modifying activities. In this view, Gcn5 (and perhaps Swi/Snf; Wilson et al. 1996) will be generally targeted to promoters, but will only affect transcription of genes that are inherently sensitive to the state of chromatin.

How is transcriptional activity affected by targeted histone acetylation and deacetylation?

Histone acetylation weakens the association of histones with DNA, thereby altering nucleosomal conformation and stability. It seems likely that the basic biochemical properties of acetylated and deacetylated chromatin are relevant for the general correlation between histone acetylation and transcriptional activity. In accordance with this general correlation, the histone acetylases de-

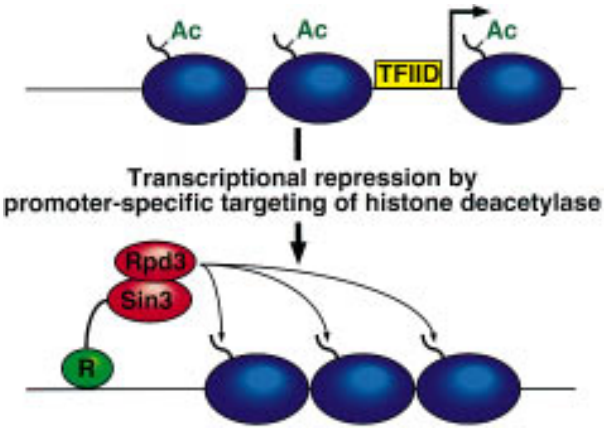

Figure 4. Transcriptional repression by promoter-specific targeting of the Sin3-Rpd3 histone deacetylase. A DNA-binding repressor (R) recruits a complex containing Sin3 and Rpd3 histone deacetylase to the promoter. As a consequence, nucleosomes (dark blue ovals) in the vicinity of the promoter have deacetylated histone tails, which leads to an inactive chromatin structure (depicted as nucleosomes close together and loss of TFIID binding) and inhibition of transcription (lack of arrow). Aside from the acetylation state of the histone tails, the model does not specify the molecular nature of inactive and active chromatin structures or the step at which transcriptional repression occurs. This model is strongly supported, but direct evidence for locally perturbed chromatin at the level of histone acetylation in vivo is lacking at present.

scribed above are typically associated with transcriptional activation whereas the Rpd3/HDAC histone deacetylases are typically associated with transcriptional repression. Furthermore, histone acetylation increases HIV-1 enhancer activity in vitro by facilitating transcriptional reinitiation (Sheridan et al. 1997).

Histone acetylases and deacetylases that are generally or specifically targeted to promoters almost certainly cause localized perturbations of chromatin structure. At present, there is virtually no information on the localization of modified chromatin in vivo. Do these enzymatic activities modify a single nucleosome, or is chromatin structure perturbed over a larger distance? Where is the location of the modified chromatin structure with respect to binding sites for DNA-binding activators and repressors or for components of the basic transcription machinery such as TFIID or the Pol II holoenzyme? In addition, the histone acetylases and deacetylases differ with respect to the individual lysine residues and specific histones that are affected, and there is limited information on how such differences affect chromatin structure and protein accessibility in vivo. These questions should be addressed in the near future, and it is likely that the answers will differ depending on the histone-modifying activity and the promoter.

Local perturbations of chromatin structure should specifically affect the accessibility and/or function of transcriptional regulatory proteins that bind DNA sequences in the region where histone acetylation or deacetylation occurs. However, accessibility to the promoter is also influenced strongly by (1) the inherent ability of a given DNA-binding protein to bind nucleosomal templates, (2) the inherent positioning of nucleosomes on particular promoter DNA sequences, (3) the intracellular levels of the DNA-binding proteins, (4) the inherent quality of the binding site, and (5) competition between binding sites in promoter regions and those located throughout the genome. Furthermore, local perturbations of chromatin structure could affect the communication between enhancer-bound proteins and the general Pol II transcription machinery. Clearly, there is a complicated and poorly understood interplay between these additional parameters and the local state of histone acetylation. Thus, to understand the molecular mechanism by which histone acetylation affects transcription of particular genes, it will be essential to experimentally determine the occupancy of the relevant promoter DNA sequences by activators, repressors, TFIID, and Pol II holoenzyme in vivo.

Although the effects of histone acetylation and deacetylation are typically viewed in terms of promoter accessibility, it is also possible that acetylated or deacetylated histones could serve as signals for interaction with proteins. For example, the transcriptional repression domain of the Tup1 corepressor interacts with underacetylated forms of histones H3 and H4 (Edmondson et al. 1996). In cases where histone acetylation or deacetylation is targeted, recognition of such signals by relatively general chromatin-associated proteins could lead to local chromatin structures that differ considerably from that of bulk chromatin. For example, lysine 12 of histone H4 is preferentially acetylated in transcriptionally silent heterochromatin (Braunstein et al. 1996), and Rpd3 histone deacetylase (which affects this lysine residue) counteracts heterochromatic silencing in yeast and flies (DeRubertis et al. 1996; Vannier et al. 1996). These observations are surprising because, in striking contrast to the usual correlation, histone acetylation is associated with deceased transcriptional activity. One explanation for this paradoxical situation is that the acetylated lysine 12 of histone H4 is recognized by proteins that lead to the formation of heterochromatin.

Conclusion

The molecular description of histone acetylases and deacetylases has revealed two fundamental principles. First, histone acetylases can be basic components of, or closely associated with, the Pol II machinery. Thus, recruitment of the Pol II machinery to promoters is concomitant with recruitment of histone acetylases, thereby providing a simple mechanism to account for the general correlation between histone acetylation and transcriptional activity. Second, some histone acetylases and deacetylases interact with specific DNA-binding activator and repressor proteins, strongly suggesting that they modulate transcriptional activity of specific promoters by locally perturbing chromatin structure. Furthermore, specific targeting of chromatin modifying activities could occur independently of recruitment of the

Pol II machinery, thereby providing an explanation for situations in the development of multicellular organisms, in which changes in chromatin structure precede changes in transcriptional activity. More speculatively, targeting of histone modifying activities to specific genomic regions could underlie long-range chromatin structures, such as occur in heterochromatin, locus control regions, and chromosome inactivation. Given the recent excitement in this area and the powerful experimental tools now available, it should not be too long to wait for long-standing correlations to metamorphose into detailed molecular mechanisms.

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