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PROMOTER TARGETING OF CHROMATIN-MODIFYING COMPLEXES

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1. ABSTRACT

The action of multi-subunit complexes that are able to overcome the repressive effects of chromatin is an important step in the regulation of eukaryotic gene expression. Identification of complexes that modify the structure of chromatin to help factors access the underlying DNA has enhanced our understanding of how some genes are controlled. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) represent one group of complexes that regulate the level of acetylation on the N-terminal tails of core histone proteins. The SWI/SNF complex is the prototype of a second group of complexes, which use the energy of ATP-hydrolysis to alter histone-DNA contacts, leading to changes in chromatin conformation. Genetic studies in yeast have revealed that some of these multi-subunit complexes interact *in vivo* to control transcription of a subset of genes. It has become apparent that some gene promoters require modifications by both types of complexes. An important question regarding these two types of complexes is how they are recruited to the promoters of genes that are dependent on their activity for their expression. This review will tie together many studies on promoter recruitment of both HATs and SWI/SNF. Emphasis will be placed on recent data that demonstrates functional interplay between these two types of chromatin-remodeling activities. In addition, this review summarizes recent data demonstrating the ability of repressors and corepressors to recruit histone deacetylase complexes. Interestingly, many subunits of chromatin-modifying complexes in humans have been implicated in the development of cancer. Thus, studying how these complexes work can help us better understand human diseases.

2. INTRODUCTION

The packaging of eukaryotic DNA into nucleosomes inhibits the access of factors to DNA and thus results in the repression of important cellular processes such as transcription, replication, and recombination. This structure, called chromatin, was linked to transcriptional regulation by genetic studies showing that mutations in histone genes affect transcription. In addition, *in vitro* transcription assays revealed that nucleosomes are able to repress basal transcription unless a preinitiation complex is assembled first. Many studies in the past few years have described conserved protein complexes whose function is to modulate the access of transcription factors to regulatory regions of genes relieving chromatin-mediated repression. They are classified into two categories based on their mode of action:

1) Histone acetyltransferase (HAT) and deacetylase (HDAC) complexes, which regulate the acetylation levels of amino-terminal tails of the core histones
2) ATP-dependent complexes, which use the energy of ATP hydrolysis to alter and/or disrupt the structure of chromatin

We will begin by introducing these two different classes of modifying complexes listed above, with an emphasis on the most widely studied complex in each group.

2.1. Histone acetyltransferase and deacetylase complexes

The neutralization of the positive charge of the histone tails by histone acetyltransferases changes the histone/DNA contacts thus affecting the higher order structure of chromatin. Ultracentrifugation studies have

shown that acetylated nucleosome arrays are less compacted than non-acetylated ones (1, 2). However, the mechanism by which histone acetylation affects the structure of chromatin is not well understood, although, this structural change of chromatin leads to transcription activation (3, 4). Consistent with this is the observation that histone hyperacetylation is linked to transcriptionally active genes (5) while histone hypoacetylation is found in transcriptionally silent genes (6). Moreover, histone acetylation has been shown to enhance transcription factor binding *in vitro* (7, 8, 9). Since the identification of the transcription coactivator Gcn5 as a nuclear histone acetyltransferase, our understanding of acetylation and its link to transcription has grown rapidly. Other proteins that had been initially described as transcriptional regulators were later shown to have histone acetyltransferase activity, such as $TAF_{II}250$ (a component of TFIID), ACTR, p300/CBP, and SRC-1 (4, 10). Recently, the identification of four major histone acetyltransferase complexes from yeast (SAGA, ADA, NuA3, and NuA4), has enabled us to show acetyl-CoA-dependent activation of *in vitro* transcription by these HATs (11, 12, 13). SAGA, ADA, and NuA3 preferentially acetylate histone H3, while NuA4 has a preference for the N-terminal tails of histone H4. The SAGA and NuA4 HAT complexes have been shown to interact directly with transcription activators and the progress in determining the functional significance for these interactions will be discussed later in this review. Histone deacetylase (HDAC) complexes and how gene-specific repressors target them will also be discussed below.

2.2. SWI/SNF family of chromatin remodeling complexes

The second group of chromatin modifying complexes includes the yeast SWI/SNF complex, which was the first ATP-dependent chromatin remodeling complex to be identified. Genes coding for subunits of this complex were identified genetically as mutants that cause transcriptional defects of either the endonuclease gene *HO*, which is required for mating type switching (hence SWI) or the invertase gene *SUC2*, the mutants of which have been classified as sucrose non-fermentors (hence SNF). The yeast SWI/SNF complex is comprised of 11 subunits and has a molecular weight of approximately 2 MDa. These subunits are conserved among species and homologues of yeast SWI/SNF in humans and *Drosophila* have 9-10 subunits each. In *Drosophila*, the genes encoding for the SWI/SNF proteins were isolated via genetic screens looking for suppressors of the transcriptional repressor polycomb (14) and are part of a complex called Brahma (15). In humans, two SWI/SNF-like multi-subunit complexes were purified biochemically. These two different SWI/SNF homologues contain different DNA-dependent ATPase subunits, BRG1 and hBRM. Swi2/Snf2 and dBrm are the ATPase/helicase subunits in yeast and *Drosophila*, respectively. Mutations in the ATPase domain of Swi2/Snf2 have been shown to eliminate SWI/SNF function (16). All of the ATPases contain a highly conserved bromodomain in their C-terminal region. Another group of ATP-dependent remodeling complexes has been identified in flies, which contain ISWI (Imitation SWI) as their ATPase/helicase subunit. Some members of this group (NURF, CHRAC, and ACF) were purified from *Drosophila* extracts using biochemical methods based on their ability to disrupt and/or generate regularly spaced nucleosomal arrays (17, 18, 19). Complexes containing both chromatin remodeling and deacetylase activity have also been purified recently from human cells and *Xenopus* extracts, and are known as NURD (20, 21, 22, 23).

Genetic studies have suggested that SWI/SNF functions by interacting with chromatin. In one study, several mutations that suppressed SWI/SNF phenotypes were found to be in genes coding for core histones (24). A more definitive link to chromatin structure has been provided by biochemical studies demonstrating ATP-dependent disruption of nucleosome structure by purified yeast SWI/SNF complex (25, 26). This disruption increases the access of transcription factors to DNA. Thus, both classes of modifying enzymes (HATs and remodeling complexes) alter chromatin structure, leading to transcription stimulation. In this review, we will discuss the targeting of both chromatin-remodeling complexes such as SWI/SNF and histone acetyltransferase complexes such as SAGA to promoters of certain genes. Moreover, we will provide evidence of the functional interactions between these complexes. Finally, we will discuss the role that the bromodomains contained in these complexes might play in helping to stabilize them on acetylated templates.

3. ACTIVATOR TARGETING OF HISTONE ACETYLTRANSFERASE COMPLEXES

Several components of the well-characterized yeast HAT complex, SAGA, were initially identified in two genetic screens. In the first study, spt genes were isolated as suppressors of transcriptional initiation defects caused by insertion of the transposable element Ty at the promoter regions of *HIS4* and *LYS2* loci (27). In the second screen, ada genes were identified as mutations that reduce the function of the acidic activation domain from the herpes virus VP16 protein in yeast (28, 29). Further studies demonstrated that SAGA contains the proteins Gcn5, Spt3, Spt7, Spt8, and Spt20/Ada5 as well as Ada1, Ada2, and Ada3. SAGA also contains a subset of TAF proteins that were originally discovered as factors that are subunits of the TFIID basal transcription factor.

An important issue regarding the mechanism of action of chromatin-modifying complexes is how they recognize their target genes within the genome. Several ada genes were identified in genetic screens designed to identify potential transcription coactivators (28). Thus, complexes containing the Ada proteins, namely the SAGA and ADA complexes, were predicted to interact with acidic activators. The interaction of some HATs with activators has been demonstrated both in yeast and in humans (30, 31, 32). For example, the yeast SAGA and NuA4 complexes have been shown to interact directly with acidic activation domains such as those of the herpes virus VP16 and the yeast Gcn4 transcription activator, and to stimulate transcription driven by these activators. In contrast, the ADA and NuA3 HAT complexes do not interact with any of the activators tested so far, suggesting that the observed

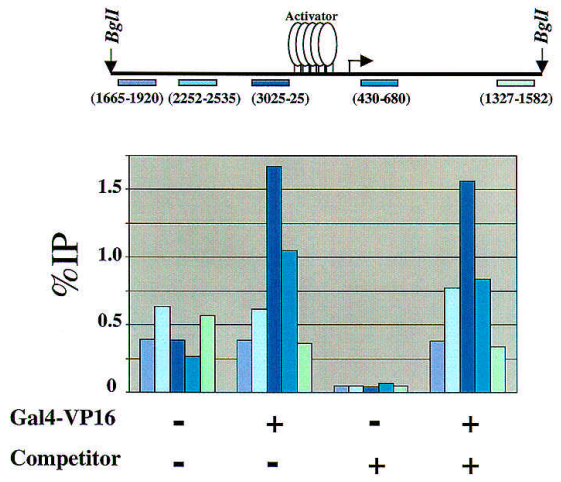

Figure 1. Targeted acetylation of the SAGA complex to promoter-proximal nucleosomes by the activator Gal4-VP16. The diagram on top shows the position of the different probes along the template. The pG5E4T plasmid, which contains 5 Gal4-binding sites upstream of the adenovirus 2 E4 minimal promoter, was digested with BglI, reconstituted, and used for this experiment. The template was incubated with SAGA and acetyl-CoA in the presence or absence of Gal4-VP16 and competitor chromatin as indicated. Next, the chromatin was digested with MNase and immunoprecipitated with anti-acetylated H3 antibody. DNA was then extracted from bound and unbound fractions, slot-blotted, and the membranes were successively hybridized with the probes shown in the diagram. The graph shows that Gal4-VP16 directs the HAT activity of the yeast SAGA complex to promoter-proximal nucleosomes.

interactions of SAGA and NuA4 with activators are specific.

Targeted promoter specific histone acetylation has been shown both *in vivo* and *in vitro*. Chromatin immunoprecipitation (ChIP) experiments revealed enrichment in histone H3 acetylation over approximately 600 bp of the promoter region of the HIS3 gene (33). A similar pattern of localized acetylation was observed for the human interferon-beta (IFN-beta) promoter, where H3 and H4 acetylation was restricted to about 600 bp around the transcription start site (34). Localized histone acetylation is also observed on the *HO* gene, where H3 and H4 acetylation was found in the upstream regulatory sequences during G1phase of the cell cycle (35). Using *in vitro* assays, Vignali *et al*. have shown that targeting of the SAGA complex by acidic activation domains results in a sharp localized region of acetylation near the activator binding sites (figure 1, and (36)). In contrast, targeted acetylation by the NuA4 complex is more widely spread over a nucleosome array (36). These results taken together argue for the transcription activator-targeting of some HATs to promoters leading to increased acetylation of promoter nucleosomes, which in turn results in transcription activation. The recruitment of other chromatin modifying complexes such as SWI/SNF or the Rpd3/Sin3 histone deacetylase complex to promoters through interactions with sequence specific activators and repressors has also been shown and will be discussed below.

4. ACTIVATOR TARGETING OF SWI/SNF COMPLEXES

SWI/SNF is required for the transcriptional regulation of a subset of highly inducible yeast genes, such as *INO1*, *PHO8*, *GAL1-10*, and *SUC2* (37). Genome-wide DNA microarray expression analysis has found that approximately 5% of yeast genes are dependent on the ATPase activity of the yeast SWI/SNF complex (38, 39). Since SWI/SNF exists at a relatively low copy number in the cell (approximately 100 copies per cell, (40)), it is imperative to understand how it is specifically recruited to its target genes. The complex has the ability to bind DNA and nucleosomes, but it does so without displaying DNA-sequence specificity (25, 41). Two primary models for recruitment of SWI/SNF have been suggested. The first model proposes that SWI/SNF is recruited to promoters via a direct association with RNA polymerase II holoenzyme, based on the fact that both yeast and human SWI/SNF components have been found in RNA polymerase II "holoenzyme" preparations (42, 43, 44). The second model, called the 'Activator model', is supported by numerous recent studies, and proposes that the SWI/SNF complex is recruited to target genes through direct interactions with sequence-specific activators. Several laboratories have demonstrated that yeast SWI/SNF can interact directly with acidic activation domains, including those of Gal4-VP16, Gcn4, Hap4, and Swi5 (45, 46, 47). These activators were shown to contact both highly purified SWI/SNF as well as SWI/SNF present in yeast whole-cell extracts (figure 2A). This interaction was abolished when activators that harbored mutations in the activation domain that affect their ability to activate genes, were used. Additionally, yeast SWI/SNF did not interact with glutamine- or proline-rich activators (45, 46, 47). Moreover, an early report suggested that yeast SWI/SNF could interact with the glucocorticoid receptor (GR) in whole cell extracts. A more recent study found that the major transactivation domain of the GR, tau1, which is highly acidic, interacts directly with the complex (48, 49). *In vivo* chromatin immunoprecipitation studies at the yeast *HO* promoter further suggested that SWI/SNF is recruited to the promoter via an interaction with the Swi5 transcription activator (35, 51). The interaction of SWI/SNF with activators is able to target the chromatin remodeling activity of SWI/SNF to different chromatin substrates, such as mononucleosomes and nucleosome arrays (figure 2B, and (47, 50)). This targeting can also result in the stimulation of *in vitro* transcription from nucleosome arrays (45).

Activator targeting of chromatin remodeling complexes is not a mechanism unique to yeast. A human SWI/SNF complex, also named E-RC1, was found to be functionally important for the efficient transcription regulation of the beta-globin gene by EKLF (erythroid kruppel-like factor). Indeed, hSWI/SNF was later found to

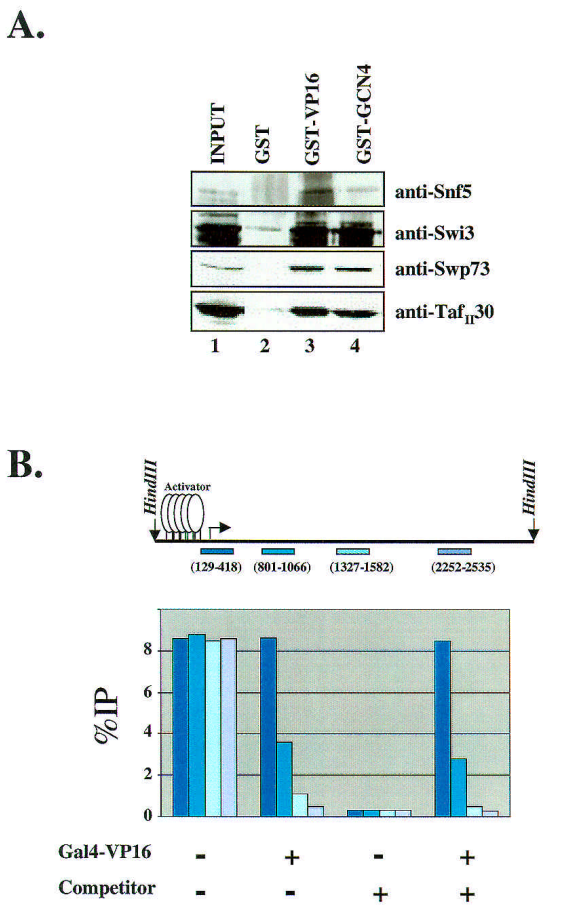

Figure 2. Activator targeting localizes the SWI/SNF complex to promoter-proximal nucleosomes. A) Activator interaction with the SWI/SNF complex in yeast whole cell extracts. The proteins associated with GST-VP16, GST-GCN4, and GST alone bound to glutathione sepharose beads were immunoprecipitated and detected with antisera to SWI/SNF subunits. B) Recruitment of SWI/SNF to nucleosomes proximal to the activator binding sites. The diagram on top shows the position of the different probes along the template plasmid. The pG5E4T plasmid was digested with HindIII, reconstituted, and used for this experiment. The template was incubated with SWI/SNF in the presence or absence of Gal4-VP16 and competitor chromatin as indicated. The substrates were then digested with MNase and immunoprecipitated with anti-HA antibody (the Swi2/Snf2 subunit of the complex is HA-tagged). DNA was then extracted from bound and unbound fractions, slot-blotted, and the membranes were successively hybridized with the probes shown above. The results show that Gal4-VP16 recruits the SWI/SNF complex to promoter-proximal nucleosomes.

interact directly with the zinc finger DNA-binding domain of EKLF (52, 53, 54). C/EBPbeta was also found to interact with hSWI/SNF, leading to activation of myeloid-specific genes (55). Furthermore, hSWI/SNF has been reported to interact with c-Myc (56), MyoD (57), EBNA2 (58), and nuclear hormone receptors, including GR, estrogen receptor, and retinoic acid receptor (59, 60, 61, 62).

Studies with human SWI/SNF have investigated which subunits of the complex are directly contacting transcription activators. The initial studies suggest that different subunits facilitate interactions with different activators, which may explain gene-specific recruitment of this chromatin-remodeling activity. For example, BAF155, BAF170, and the BRG1 ATPase subunit directly contact the zinc finger DNA-binding domain of EKLF (53, 54). On the other hand, the alternative ATPase subunit that is found in some human SWI/SNF complexes, hBRM, facilitates the interaction of hSWI/SNF with C/EBPbeta (55). The hSNF5 subunit, also named INI1 due to its ability to interact with HIV-1 integrase (63), has been shown to interact with c-Myc and the viral transcription activator, EBNA2 (56, 64). In the case of c-Myc and EBNA2, hSNF5 interacts with the DNA-binding domain region of the activator, which seems to be a more common mechanism for recruitment of hSWI/SNF. Another report showed that BAF250 directly contacts the glucocorticoid receptor (62). Importantly, both BRG1 and hSNF5/INI1 are often mutated in many types of human cancers (65, 66, 67, 68, 69, 70). Additionally, heterozygous SNF5 knockout mice develop nervous system and soft tissue sarcomas in early embryogenesis (71, 72). This suggests that there may be a link between recruitment of hSWI/SNF chromatin-remodeling activity and the control of cell growth.

Genome-wide expression analysis using DNA microarrays has indicated that the genes affected by inactivation of SWI/SNF and HATs overlaps to some extent. Genetic and biochemical data have shown that these two types of complexes interact with a similar set of gene-specific transcription activators. In the next section, we will discuss recent studies aimed at understanding how these complexes work together to activate the genes that depend on them both for their full expression.

5. COMMUNICATION BETWEEN HATS AND SWI/SNF AT PROMOTER NUCLEOSOMES

Information regarding the mechanism by which different chromatin modifying complexes such as SWI/SNF and HATs work together to regulate transcription has only begun to emerge. In this section we will discuss the functional link between SWI/SNF and the yeast histone acetyltransferase complexes. Both SWI/SNF and the SAGA HAT complex can be recruited to the same promoters by transcriptional activators. Thus, these complexes may act together or in series. Genetic interactions between chromatin components and both of these modifying complexes suggest functional connections (73, 74). Roberts and Winston found that the Swi2/Snf2 subunit of the SWI/SNF complex is synthetically lethal with Spt20 (a subunit of SAGA). Furthermore, double mutants of spt20 and snf5 or swi1 as well as double mutants of ada2 and swi2 were found to be inviable (73, 75). These observations suggest that the two types of chromatin-modifying complexes may perform overlapping functions. Consistent with this possibility, some SWI/SNF regulated genes (e.g. *HO*, *SUC2*, *ADH2*, *INO1*, and Ty insertions) are also regulated by SAGA. Similar to deletions of components of SWI/SNF, deletion of Ada2,

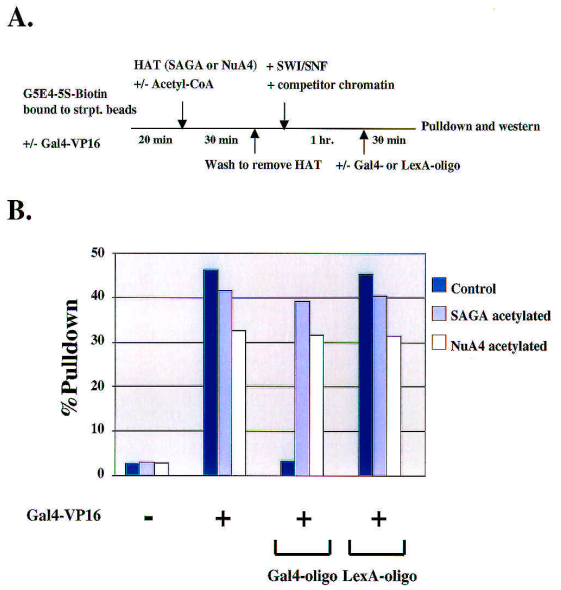

Figure 3. Acetylation of the nucleosomal array template by SAGA or NuA4 HAT complexes stabilizes the subsequent SWI/SNF binding to the array. A) Diagram of the experimental strategy. The G5E4-5S template, which contains 5 Gal4-binding sites upstream of the adenovirus 2 E4 minimal promoter flanked on both sides by five 5S rDNA nucleosome positioning sequences, was biotinylated, reconstituted, and bound to streptavidin beads. Immobilized G5E4-5S nucleosome arrays were bound by Gal4-VP16, followed by the addition of SAGA or NuA4 and acetyl-CoA. After a 30 minute incubation and several washes to remove the HATs, SWI/SNF and competitor chromatin were added, followed by competition with an oligonucleotide containing binding sites for Gal4. The template beads were pulled-down with a magnet, and the presence of SWI/SNF was analyzed by Western blotting. B) The percent of SWI/SNF pulled-down in the presence or absence of Gal4-VP16 and the percent of SWI/SNF retained after the removal of the activator with oligo were determined. The results show that acetylation enhances SWI/SNF binding to a nucleosomal array template.

Ada3, or Gcn5, subunits of SAGA, leads to reduced expression of these genes (73, 76).

The identification of complexes containing either histone acetyltransferase or histone deacetylases activities together with ATP-dependent remodeling activity has provided additional evidence for the combined role of remodeling complexes and HATs in living cells (20, 23, 75). It is likely that these complexes can work together on a promoter for the normal regulation of RNA polymerase II transcription. But, how do these complexes affect each other? And, which of the two acts first? A better understanding of the possible ordered recruitment of chromatin modifying complexes to specific promoters would have a significant impact on the field.

Two recent papers have addressed the ordered recruitment of chromatin modifying complexes *in vivo* by analyzing the cross-linking of proteins to the *HO* promoter in yeast. Cosma *et al*. have shown that the binding of the Swi5 transcription activator to its UAS site is required for SWI/SNF recruitment to the regulatory region of the *HO* locus. In turn, SWI/SNF is required for the recruitment of a Gcn5-containing histone acetyltransferase complex, SAGA. Both complexes are required for the maintenance of an active chromatin state, the binding of a second activator SBF and full transcriptional stimulation. Interestingly, the SWI/SNF complex remains bound to the promoter even after the activator Swi5 is dissociated (51). The mechanism by which SAGA is recruited to this promoter is not yet understood. In an independent study, Krebs *et al*. demonstrated that the recruitment of SAGA to the regulatory region of the *HO* gene results in cell-cycle regulated acetylation of approximately one kb of upstream regulatory sequences including factor binding sites and the TATA box (35). This step precedes transcription and possibly SBF binding. The data together support an interesting model, in which SWI/SNF is recruited by transcriptional activators to the regulatory regions of specific genes, and in turn allows the binding of the SAGA histone acetyltransferase complex. Then, the acetylation of the amino terminal tails of the core histones by SAGA would facilitate the binding of other activators resulting in enhanced transcription from those promoters.

Other experiments suggest that acetylation actually enhances the interaction of SWI/SNF with promoters. For example, using immobilized template assays, we have recently been able to show that acetylation of nucleosomal array templates by either the SAGA or NuA4 yeast HAT complexes stabilizes SWI/SNF binding to promoter nucleosomes even after the dissociation of the activator (figure 3, and (50)). The enhanced retention of SWI/SNF on acetylated nucleosomes might be due to an interaction of the bromodomain in the Swi2/Snf2 subunit with acetylated histones. Indeed, it has been shown that bromodomains can interact specifically with acetylated histones (77, 78); and Gcn5, the catalytic subunit of SAGA, has been shown to participate in the stabilization of SWI/SNF binding to promoters *in vivo* (79). Additional studies are needed to determine the importance of bromodomains in the stabilization of these chromatin-modifying complexes on acetylated promoters. In addition, two recent papers have also argued that the function of the HAT complexes on particular promoters could precede that of SWI/SNF. Agalioti *et al*. show that histone acetylation by Gcn5 is followed by SWI/SNF recruitment during activation of the interferon-beta (INF-beta) promoter *in vitro* (80). In another report, transactivation by RAR/RXR is shown to require histone acetylation prior to SWI/SNF action (60). Collectively, these data demonstrate that at certain genes acetylation precedes SWI/SNF action (figure 4).

6. REPRESSOR TARGETING OF HISTONE DEACETYLASE COMPLEXES

It has been long recognized that transcriptional activators and repressors share a common architecture and analogous, but opposite, mechanisms of action. In other

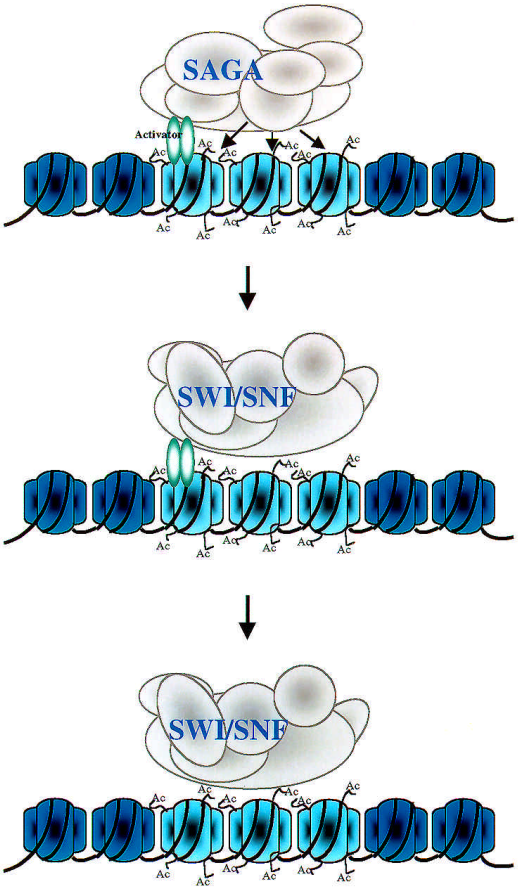

Figure 4. Model depicting how targeted nucleosomal acetylation by SAGA retains the SWI/SNF complex at promoters. Activator recruitment of the SAGA complex leads to acetylation of promoter-proximal nucleosomes (A). Then, the SWI/SNF complex is also recruited to promoter-proximal nucleosomes by the same activator (B). Finally, once the activator had dissociated, acetylation by the SAGA complex helps retain SWI/SNF at the promoter via interactions of bromodomain-containing subunits of the complex with acetylated lysines.

words, activators recruit histone modifying- and chromatin-remodeling activities that positively affect transcription through their activation domains as discussed above; whereas, transcriptional repressors recruit histone modifying and nucleosome positioning activities that negatively affect transcription. Here we discuss the role of transcriptional repressors in recruiting chromatin-regulating activities and parallel their mechanisms to that of transcriptional activators.

A number of studies have documented that repressors function, at least in part, by recruiting histone deacetylases to their target promoters. This in turn results in the localized deacetylation of histones (For review see 32, 81, 82, 83, 84, 85, 86). Two mechanisms of targeted histone deacetylation have been described in yeast recently. The first mechanism involves the direct recruitment of HDACs by the gene-specific repressor itself, such as that observed for Ume6 (87, 88, 89). The second involves the indirect recruitment of HDACs to promoters through the Ssn6/Tup1 corepressor complex (90, 91, 92). Whichever mechanism is utilized, the recruitment of HDACs results in the localized deacetylation of the nucleosomes adjacent to the URS region (87, 89, 91, 92). There are a number of views on the mechanism of Ssn6/Tup1-mediated repression (91, 92, 93, 94, 95, 96). It has been documented that Ssn6/Tup1 is required for positioning nucleosomes on a wide variety of genes (95, 97, 98, 99, 100), and Li and Reese, submitted). However, the role of Ssn6/Tup1 in nucleosome positioning is unclear, but it may be dependent upon the ability of Tup1 to interact with under-acetylated histone H3 and H4 tails (93). It is also not known if the binding of Ssn6/Tup1 complexes to nucleosomes promotes their positioning, or acts exclusively as stabilizers of arrays set up by other activities.

Gene expression requires the recruitment of both histone acetylation and nucleosome remodeling complexes by activator proteins *in vivo* (45, 46, 51), indicating a collaboration between these two types of activities in chromatin remodeling as described above. By analogy, it is expected that repressors recruit both HDACs and nucleosome positioning activities, but this had not been documented until a recent study. The protein encoded for by *UME6* was initially identified as a repressor of meiotic genes throughout the mitotic cell cycle, but it has since been verified to regulate additional classes of genes (For review see (101)). Ume6 represses transcription by recruiting the Rpd3-Sin3 histone deacetylase complex to its target promoters, resulting in the deacetylation of nucleosomes near the URS (87, 89). The recent work of Tsukiyama and colleagues (88) reveals that Ume6 recruits the Rpd3-Sin3 HDAC complex and the Isw2 complex, an ATP dependent nucleosome positioning activity, to the promoters of early meiotic genes. They demonstrated that the Isw2 and Rpd3-Sin3 complexes function in parallel pathways to repress transcription synergistically. Their analysis suggests that at least the Ume6 repressor functions analogously to activators in transcription, in that it can recruit both a histone-modifying activity and an ATP-dependent chromatin-remodeling complex. This begs the question of whether or not it is unique among all transcriptional repressors or if it reveals a paradigm for repressor function. Insights into this question are building from our recent analyses of the repression mechanism of the *RNR3* gene.

DNA damage responsive genes, such as *RNR3*, are repressed by the binding of Crt1 to the upstream repression sequences (URS), the damage responsive elements (DREs) or x-boxes (102). Crt1 represses the transcription of *RNR3* by recruiting the Ssn6-Tup1 corepressor complex to the promoter (102, 103). Chromatin mapping studies indicates that nucleosome positioning plays a crucial, if not predominant, role in transcriptional repression of *RNR3*. In the absence of DNA damage-generated signals, an array of positioned nucleosomes cover the promoter region of *RNR3*, which undergo extensive remodeling upon gene activation (Li and Reese,

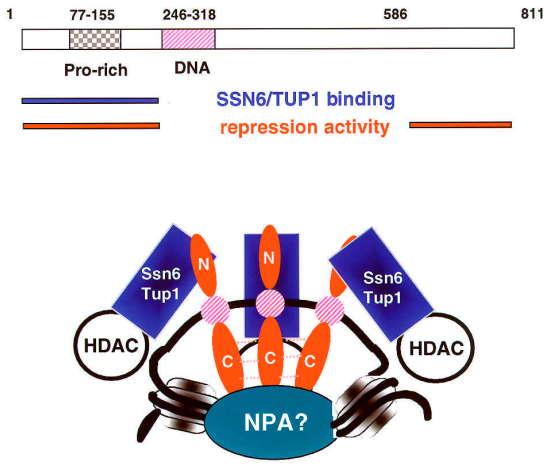

Figure 5. Model for co-repressor recruitment by Crt1. The diagram shows the two repression domains revealed by domain mapping studies of Crt1 ((104), and Zhang and Reese, unpublished). The N-terminal repression region spans amino acids 1-240 and is the Ssn6/Tup1- and HDAC-dependent repression domain, whereas repression by amino acids 586-811 is independent of these two complexes. An elaborate protein-nucleosome structure is predicted from chromatin mapping studies performed on the URS region of *RNR3* (Li and Reese, submitted for publication). Red indicates Crt1 bound to each of the three DRE elements. NPA, green, is an unidentified nucleosome positioning activity that is predicted to regulate chromatin structure at the *RNR3* promoter.

submitted). Nucleosome positioning is strictly dependent upon Crt1 and the Ssn6-Tup1 complex, as deletion of these genes individually results in fully remodeled chromatin. We have mapped the regions of Crt1 required for transcriptional repression. The N-terminus of Crt1 is responsible for recruiting the Ssn6/Tup1 complex; thus, fusing this region to the DNA binding domain represses a transgene containing lexA operators (104). Surprisingly, additional mapping studies showed that a LexA DNA binding domain fusion containing the last 226 amino acids of Crt1 represses a reporter gene to a significant degree (Zhang and Reese, unpublished). This suggests that it either does so by forming a second Ssn6/Tup1 interaction surface or by recruiting additional corepressors. The later scenario was confirmed by showing that this second repression domain is both Ssn6/Tup1- and HDAC-independent (Zhang and Reese, unpublished data); thereby suggesting that it recruits a second repressor complex (figure 5). Therefore, repression by Crt1 is likely to involve both a Ssn6/Tup1- and HDAC-dependent mechanism, and a HDAC-independent mechanism. The HDAC-independent mechanism likely requires an ATP-dependent nucleosome positioning activity, as nucleosome positioning plays a dominant role in *RNR3* repression. Studies are currently underway to identify this activity.

Thus, it appears that repressors, like activators, can recruit multiple types of chromatin modifying and remodeling activities. What remains to be seen is if there exists interdependence of the nucleosome positioning activities (such as Isw2 for meiotic genes) on the chromatin modifying activities (HDACs), as seen for transcriptional activators. The continued reinforcement of the architectural and functional similarities between activators and repressors suggest that such as dependence does exist.

7. PERSPECTIVES

New insights into the regulation of eukaryotic gene regulation have recently emerged from *in vivo* and *in vitro* studies. It appears that transcription from chromatin templates often requires more than one type of modifying complex. Furthermore, the data suggest that these complexes need to function in an ordered fashion to exert their combined effects on a particular gene. It is likely that bromodomains within some subunits of these complexes contribute significantly to binding and stabilization of these complexes on promoters and hence affect transcriptional regulation of the target genes. It is possible that, in addition to stabilizing SWI/SNF, acetylation could stabilize other bromodomain containing complexes. However, many questions remain unanswered. Are bromodomain-containing HATs also stabilized on acetylated nucleosomes? Are there other domains within SWI/SNF or HATs that are important for this enhanced binding? What are the effects of histone acetylation or other histone modifications such as phosphorylation or methylation on binding of SWI/SNF, HATs, or other chromatin modifying complexes? Do HDACs function in large part by eliminating the stable interactions of bromodomain-containing proteins with promoters? Undoubtedly, experiments in the future will increase our knowledge of how these different chromatin-modifying complexes communicate with each other to affect the regulation of specific genes.

In conclusion, it is clear that promoter recruitment of chromatin modifying complexes is a central mechanism of gene regulation exploited by both activators and repressors. Indeed, the parallels between the functions of sequence specific activators and repressors as well as the recruited co-activator and co-repressor complexes are striking. Thus, it seems that transcription activation and repression are both “active” rather than “passive” processes that exploit changes in chromatin structure. Hence, the importance of promoter recruitment of chromatin-modifying complexes.

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