Cell, Vol. 108, 475–487, February 22, 2002, Copyright ©2002 by Cell Press

Cooperation between Complexes that Regulate Chromatin Structure and Transcription

Review

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Chromatin structure creates barriers for each step in eukaryotic transcription. Here we discuss how the activities of two major classes of chromatin-modifying complexes, ATP-dependent remodeling complexes and HAT or HDAC complexes, might be coordinated to create a DNA template that is accessible to the general transcription apparatus.

No individual factor is capable of playing a dominant role in generating the immense specificity required to regulate transcription in eukaryotes. Distinct multiprotein complexes are needed to modulate higher-order chromatin structure, to bind to promoters, to bind to enhancers, to communicate between activators and repressors and sites of transcription initiation, to modify nucleosomal structure, and to generate transcripts. Each of these complexes might be a key player in regulating a given gene. A major challenge is to determine how all of these complexes work together to ensure proper regulation.

The scope of this problem is too large to be covered in a single review. The goal here is to discuss a segment of this problem: how do ATP-dependent remodeling and histone-modifying complexes work together to modify chromatin structure and regulate function of the transcription machinery? ATP-dependent remodeling complexes use energy to modify chromatin structure in a noncovalent manner, while histone-modifying complexes add or remove covalent modifications from histone tails.

In principle, all reactions that involve DNA can be regulated by altering DNA packaging and hence DNA accessibility. Transcription requires that the DNA be accessible to sequence-specific transcription factors and RNA polymerase and requires the melting and reformation of the double helix throughout the length of the transcript. Chromatin structure, including the structure imposed by the nucleosome, impedes all steps required for transcription. Thus, in the simplest case, repression can be achieved by creating a stable, inaccessible chromatin structure, and activation can be achieved by creating an accessible chromatin structure. While regulating chromatin structure is necessary for regulating gene expression, it is not sufficient: the functions of sequence-specific activators and repressors, mediator complexes, and general transcription factors are also required to ensure proper regulation.

The conceptual problem discussed in this review, how chromatin-modifying complexes interact with each other to generate specific template structures, is fundamental to all known nuclear processes such as DNA replication, recombination, and repair. Since each of these processes functions on chromatin, regulation of the accessibility of chromatin structure can play a role in these nuclear processes much as it does in transcription.

Two major classes of complexes regulate accessibility of the template to DNA binding factors. ATP-dependent complexes can move nucleosome positions, thereby exposing or occluding DNA sequences, and can create conformations where DNA is accessible on the surface of the histone octamer. The major questions concerning this class of complexes are the relative importance, in biological systems, of "sliding" nucleosomes (as opposed to catalyzing conformational changes of the nucleosome) and the nature and stability of the remodeled state of the nucleosome.

The other class of complexes, which covalently modify nucleosomes, can add or remove many chemical moieties; acetylation, phosphorylation, and methylation of histone N termini have received the most recent attention. The chemical and enzymatic activities of many of these complexes are well understood. The major questions concern how these covalent modifications impact the structure of the template and the ability of other complexes (e.g., those in the transcription machinery) to function. For example, numerous acetyltransferase complexes have been associated with transcription activation; what is it about the action of these complexes and specific acetylation patterns of histone tails that leads to increased transcription?

We will start with a discussion of the nature and capabilities of each individual class of complexes. We will then consider how these classes of complexes can coordinate with each other and with the transcription machinery to create robust and specific regulation.

ATP-Dependent Remodeling Complexes

ATP-dependent remodeling complexes use ATP hydrolysis to increase the accessibility of nucleosomal DNA, which is a fundamental requirement for several steps in transcription. These complexes can be divided into three main classes based on the identity of their catalytic ATPase subunit (Figure 1). These ATPase subunits display homology only within the ATPase domain and contain different additional domains. Further, each ATPase subunit forms complexes with different additional proteins (Figure 1). Our knowledge of the composition of these complexes and their biochemical capabilities is constantly growing (Figures 1 and 2), but there are still some key unanswered questions that are important for understanding how these complexes function in vivo: what are the mechanisms by which these different complexes increase access to nucleosomal DNA; do the large

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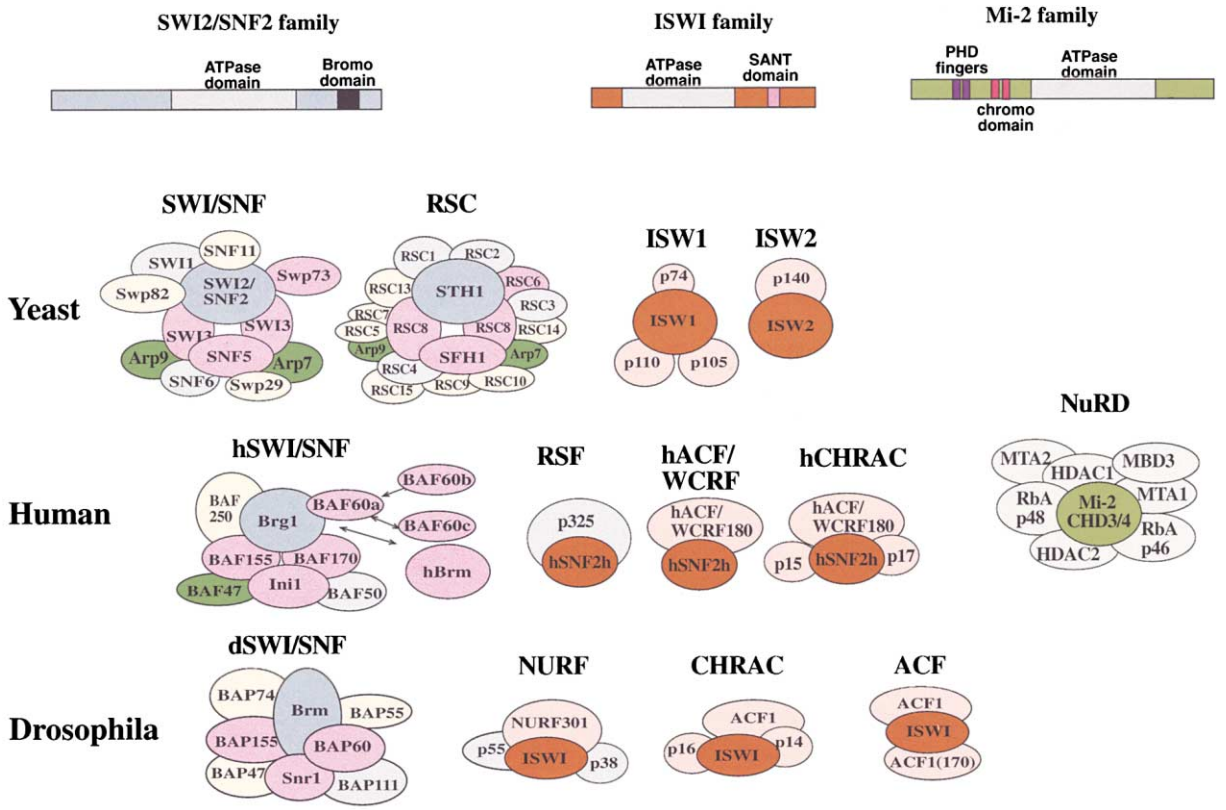

Figure 1. ATP-Dependent Remodeling Complexes

sequence differences among the three classes of ATPases result in distinct mechanisms of remodeling chromatin; and what roles do the different subunits play?

The ability to systematically answer these questions has been enhanced by the discovery that the central ATPase subunits can alter chromatin structure in the absence of the remaining subunits (Kingston and Narlikar, 1999; Langst and Becker, 2001b; Wang and Zhang, 2001). Biochemical characterization of the activities of the central ATPase subunits together with the activities of the whole complexes has begun to provide evidence for mechanistic differences among the three classes as well as information about the role of the additional subunits.

Biochemical Comparisons of Remodeler Activities

The two best-studied families of remodeling complexes are the SWI/SNF family and the ISWI-based family of complexes. The ability of these complexes to alter the structure of chromatin has been studied using a variety of different assays (summarized in Figure 2).

These studies have indicated that the two families of complexes might act via different mechanisms, a possibility initially suggested by the discovery that they have different substrate requirements. Nucleosomes lacking the histone N-terminal tails are not detectably remodeled by NURF, a *Drosophila* ISWI-based complex, but are remodeled by yeast and human SWI/SNF (Langst and Becker, 2001b). The ATPase activities of both NURF and SWI/SNF are stimulated by substrate; however, SWI/SNF complexes can be stimulated similarly by nucleosomes and naked DNA, while NURF is stimulated significantly better by nucleosomes than by naked DNA (Tsukiyama and Wu, 1995). Subsequent work has revealed that the H4 N-terminal tail is critical for stimulation of ATPase activity (Clapier et al., 2001). Removal of the H4 N-terminal tail does not, however, diminish binding by ISWI, suggesting that this tail may play a role in coupling ATP hydrolysis to conformational changes in the nucleosome. In contrast, the rate of remodeling of a nucleosomal array by yeast SWI/SNF is not affected by the removal of the histone tails (Guyon et al., 1999; Logie et al., 1999). However, removal of the tails appears to increase the affinity of SWI/SNF for the array (Logie et al., 1999).

Further suggestions of mechanistic differences came from side-by-side comparisons of the different families using the same remodeling assays. Initial comparisons of the activities of ySWI/SNF, hSWI/SNF, dNURF, the xMi-2 complex, and dCHRAC using a restriction enzyme accessibility assay on nucleosomal arrays showed specific activities within 15-fold of each other, implying that these complexes had roughly similar efficiencies of remodeling (Boyer et al., 2000). Subsequent comparisons of the activities of BRG1, the human homolog of yeast SWI2/SNF2, and SNF2H, the human homolog of ISWI, using multiple assays suggested mechanistic differences between the two families (Aalfs et al., 2001). While both BRG1 and SNF2H could remodel nucleosomal

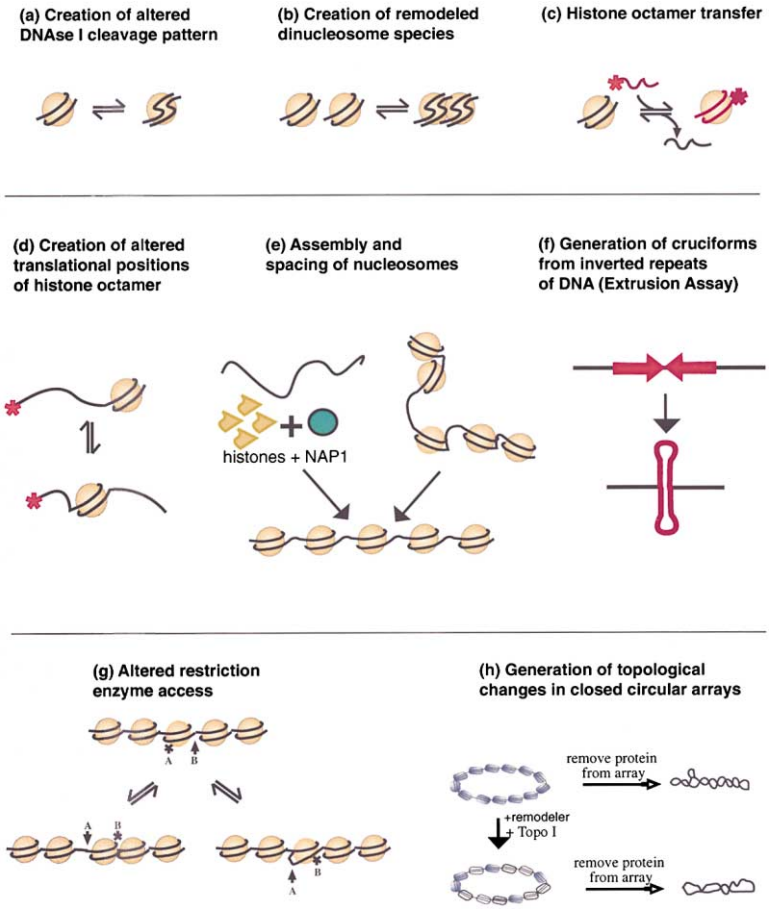

Figure 2. Biochemical Activities of ATP-Dependent Remodeling Complexes

Each panel depicts a known activity of at least one remodeling complex (see text).

(A) The 10 bp pattern generated by DNaseI on a positioned nuclesome is disrupted. Some DNA sites become hypersensitive, and some become less accessible to DnaseI.

(B) A nucleosomal species is generated that has the size of a dinucleosome and has a disrupted DNaseI cleavage pattern.

(H) Treatment of a closed circular nucleosomal array with TopoI followed by deproteinization gives one negative supercoil per nucleosome. A remodeler can reduce this number of supercoils without loss of the histone octamers.

arrays, only BRG1 could alter restriction enzyme and DNase accessibility on mononucleosomes with no significant flanking DNA. Also, only BRG1 and not SNF2H could introduce topological changes in a closed circular nucleosomal array (Figure 2H).

Another direct comparison between two classes of remodelers, performed using recombinant dMi-2 and ISWI proteins, also revealed biochemical differences (Brehm et al., 2000). Both proteins were capable of changing the translational position of a nucleosome (Figure 2D). However, dMi-2 moved the histone octamer toward central positions within a 248 bp DNA fragment, while ISWI moved the histone octamer toward the ends of the DNA. Further, unlike ISWI, Mi-2 could remodel nucleosomes that lacked the N-terminal tails of histones H4, H3, and H2A.

Differences in mechanism between remodelers were further suggested by comparisons of ySWI/SNF, BRG1, ISWI, and the x-Mi-2 complex in a DNA extrusion assay (Havas et al., 2000). This protocol detects the ability to promote the formation of cruciform DNA structures from inverted repeats of DNA (Figure 2F), an ability shared by remodelers and certain helicases. Whereas BRG1 and ySWI/SNF could extrude cruciform DNA from naked DNA and chromatin templates, ISWI and the x-Mi-2 complex could perform this function only on chromatin templates, consistent with the fact that nucleosomal substrates preferentially stimulate the ATPase activities of ISWI and recombinant Mi-2 (Langst and Becker, 2001b).

The Mechanisms of Chromatin Remodeling

The several assays used to study ATP-dependent chromatin remodeling complexes demonstrate that these complexes can expose nucleosomal DNA. The differences in how these remodelers behave in these assays might reflect differences in how they contact the template, and might also reflect differences in their intrinsic mechanisms of catalyzing DNA exposure. The most obvious mechanism for increasing DNA exposure entails "sliding" of the DNA with respect to the histone octamer (Meersseman et al., 1992). Sliding involves identical amounts of movement of the entry and exit points of the DNA in the same direction (Figure 3A). This results in an octamer that is translationally repositioned. Thus, DNA that was originally interacting with the histones becomes nonnucleosomal.

All three families of ATP-dependent remodeling complexes can change the translational position of nucleosomes on DNA. This was first demonstrated for NURF, CHRAC, and ISWI using assays that distinguish between different translational positions of mononucleosomes assembled on ~240–350 bp of DNA (Figure 2D; Hamiche et al., 1999; Langst et al., 1999). The ability of these complexes to create regularly spaced nucleosomes from an array of randomly positioned nucleosomes (Figure 2E; Langst and Becker, 2001b) provided further evidence of their translational repositioning activity. Analysis of starting and ending nucleosome positions on defined DNA fragments demonstrated that yeast SWI/SNF could

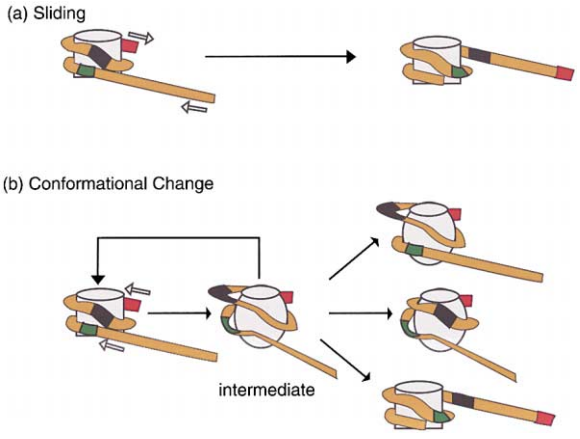

Figure 3. Two Models for the Mechanism of ATP-Dependent Nucleosome Remodeling

The structures are depicted for the intermediate, and products in (B) are hypothetical and could involve changes in the conformation of DNA, histones, or both.

also move nucleosomes in *cis* to a new position (Whitehouse et al., 1999). In separate studies performed using nucleosomal arrays, SWI/SNF action blocked certain restriction enzyme sites in linker regions, implying that SWI/SNF was able to reposition nucleosomes over these previously accessible sites (Jaskelioff et al., 2000). Further, when arrays of nucleosomes were visualized by atomic force microscopy before and after remodeling by human SWI/SNF, they showed clear changes in the distribution of nucleosome positions (Schnitzler et al., 2001).

The fact that all remodeling complexes can cause changes in the translational position of nucleosomes has led to speculation that this is achieved by a common mechanism that entails sliding. However, other mechanisms can also result in changes in translational position: a conformationally altered nucleosome could collapse to a canonical nucleosome structure that has an altered position (Figure 3B), or there could be partial or complete release of the histone octamer followed by rebinding at a new location (Lorch et al., 1999; Studitsky et al., 1994). One reason to invoke mechanisms for remodeling other than sliding is that sliding cannot explain how substantial tracts of DNA can be made accessible in regions of tightly spaced nucleosomes. Sliding mechanisms require, by definition, translational repositioning to expose DNA. Thus, sliding will not increase the amount of exposed DNA; it will simply change the location of the exposed DNA. Mechanisms that could expose DNA sequences within the boundaries of the histone octamer, therefore, without a requirement for translational repositioning (e.g., Figure 3B) would facilitate DNA exposure in regions of closely packed nucleosomes. Initial characterization of products of the remodeling reaction suggests that SWI/SNF has the ability to cause conformational changes that expose nucleosomal DNA on the surface of the histone octamer.

SWI/SNF family members can increase the DNase and restriction enzyme sensitivity of DNA sites within a mononucleosome (Kingston and Narlikar, 1999). This is achieved even though the mononucleosomes do not have flanking DNA onto which the histone octamer can slide. Restriction sites that are closer to the center of the DNA are exposed with similar or faster rates than sites that are close to the DNA ends, contrary to the result expected from sliding of the histone octamer (Narlikar et al., 2001). Further, site-specific crosslinking of the DNA to the histone octamer, which is expected to prevent sliding of the DNA, does not prevent remodeling by hSWI/SNF (Lee et al., 1999).

Another argument that sliding is not the sole mechanism for remodeling comes from studies showing that hSWI/SNF and ySWI/SNF can introduce topological changes in closed circular nucleosomal arrays (Guyon et al., 2001; Jaskelioff et al., 2000; Kwon et al., 1994). Translational repositioning of histone octamers is not expected to cause stable topological changes because nucleosomes in a standard conformation have been shown to adopt a topology that does not vary significantly. Any transient changes in the twist or writhe of linker DNA caused by the movement of nucleosomes would be expected to resolve rapidly on the unconstrained templates that have been used to study topological changes. In contrast, the topological changes introduced by hSWI/SNF are stable on a timescale of hours, suggesting that SWI/SNF alters the conformation of the nucleosome in a manner that kinetically traps the topological changes of the remodeled products (Guyon et al., 2001).

These experiments suggest that a classical sliding mechanism (Figure 3A) is not obligatory for remodeling by SWI/SNF. Yet SWI/SNF is known to be able to translationally reposition nucleosomes (Jaskelioff et al., 2000; Whitehouse et al., 1999). A simple hypothesis to resolve these observations is that translational repositioning by SWI/SNF is accomplished by a mechanism that is distinct from the classical sliding mechanism and that allows exposure of DNA within the region bound by a histone octamer. Any mechanism that involves the formation of an altered conformation of the nucleosome, accomplished by changes in the DNA path, the shape of the histone octamer, or both, is consistent with all characteristics for SWI/SNF remodeling. For example, one such model (Figure 3B) proposes that the energy of ATP hydrolysis is used to generate a high-energy intermediate that stochastically collapses to several distinct final remodeled nucleosomal states, some of which involve translational movement and some of which do not. Models of this type are also consistent with the known ability of SWI/SNF complexes to form stably remodeled dinucleosome-like structures (Figure 2B) and to promote the transfer of histone octamers (Figure 2C), both of which could occur via the high-energy intermediate.

In contrast to SWI/SNF family complexes, all of the data for ISWI-based complexes are consistent with sliding of the DNA being their main mechanism. First, a timecourse analysis of the repositioning of nucleosomes by NURF was consistent with gradual movement of the histones along the DNA (Hamiche et al., 1999). Second, all the products generated by ISWI-based complexes in the sliding, assembly, or spacing assays have so far shown characteristics of canonical nucleosomes. Third, SNF2H, the human homolog of ISWI, shows at least 100-fold lower activity on mononucleosomes without flanking DNA than on nucleosomal arrays, even though it binds mononucleo-

somes with nanomolar affinity (Aalfs et al., 2001). This has led to speculation that SNF2H requires flanking DNA onto which it can slide the histone octamer.

It is therefore possible that SWI/SNF and ISWI-based complexes expose nucleosomal DNA by different mechanisms. A mechanism based on classical sliding will lead to identical amounts of movement of the entry and exit points of the DNA in the same direction (Figure 3A). One way to generate such movement involves twisting of the double helix "into" the nucleosome at the entry point, with the twist propagating through the nucleosome to the exit point ("twist diffusion;" Luger et al., 1997). An alternative mechanism, consistent with the characteristics of SWI/SNF function, results in minor movements of the DNA at the entry and exit points toward each other (Figure 3B) with either both or only one DNA end involved in the movement. This might be coupled to changes in histone-DNA or histone-histone contacts that result in stable exposure of a segment of DNA, possibly in the form of a DNA loop (Figure 3B).

The ability of SWI/SNF and ISWI to extrude DNA from inverted repeats (Figure 2F) suggested that both these complexes can introduce changes in superhelicity by twisting the DNA (Havas et al., 2000). It has been proposed that the ability to cause these changes in superhelicity contributes directly to disruption of histone-DNA contacts (Gavin et al., 2001). Recent work with ISWI shows, however, that introduction of single-strand nicks into nucleosomal DNA, which is predicted to dissipate any superhelicity, does not inhibit remodeling (Langst and Becker, 2001a). In fact, one specifically positioned nick caused a small increase in the ability of ISWI to slide the histone octamer. Thus, generation of superhelicity might not be part of the mechanism of ISWI action, but might be a consequence of a related event (e.g., transient unpairing of basepairs) that leads to disruption of histone-DNA contacts.

While there are clues about the ways in which ATP-dependent remodeling complexes alter chromatin structure, we are still in the early stages of understanding the molecular details of remodeling mechanisms. Further work, including detailed kinetic and thermodynamic analyses of these enzymes coupled with structural information on the products, will be required to understand the molecular strategies used by this class of molecular motors and to elucidate differences and similarities in function.

Roles of the Remaining Subunits

The discovery that the central ATPase subunit alone has remodeling activity raised obvious questions about the roles of the remaining subunits of remodeling complexes. In principle, the remaining subunits can perform two distinct types of functions. They can modulate the remodeling activity of the ATPase subunit, or they can be involved in targeting of the remodeling complex to specific promoters directly or via interactions with transcriptional activators. Very little is currently known about the roles of these subunits in targeting; more is known concerning the mechanistic effects, particularly with ISWI-based complexes.

In the ISWI-based complexes, the remaining subunits affect both the efficiency and outcome of remodeling as well as substrate specificity. The Acf1 subunit in the ACF complex increases the ability of ISWI to assemble chromatin from histones and DNA (Figure 2E) by ~30-fold (Ito et al., 1999). Consistent with this result, ACF and CHRAC, both of which contain Acf1, are 10-fold better than ISWI alone at translational repositioning of the histone octamer (Figure 2D) (Langst and Becker, 2001b). Acf1 also alters the predominant product: ISWI alone moves the histone octamer to the ends of the fragments of DNA that are used in the positioning analysis, but it moves the octamer predominantly to the center of the DNA when Acf1 is present. In contrast, NURF moves the histone octamer to positions that are intermediate between the center and the ends of the DNA (Hamiche et al., 1999). This effect appears to require only the NURF301 subunit in addition to ISWI (Xiao et al., 2001). The extra subunits in CHRAC confer a different substrate specificity; unlike ISWI alone, CHRAC can translationally reposition nucleosomes that lack H3 and H2A N-terminal tails (Clapier et al., 2001).

One explanation for the different outcomes in these sliding assays is based on the hypothesis that ISWI acting alone can generate rapid movement of the histone octamer. In the absence of the remaining subunits, the octamer predominantly adopts the most thermodynamically stable position, which is often at the ends of the DNA. The additional subunits may exert their effect by binding to the DNA ends and forcing the histone octamer to adopt intermediate positions. Whether these observations are relevant to function in vivo, where there are very few if any ends of double-stranded DNA, is unclear. Alternatively, additional subunits might cause changes in position because they directly alter fundamental properties of ISWI mechanism.

Less is known about the role of the additional subunits in the SWI/SNF and Mi-2 based complexes. Initial work suggests that Mi-2 activity can be enhanced by up to two orders of magnitude in the NuRD complex (Wang and Zhang, 2001), and that SWI/SNF components can increase BRG1 function under conditions of limiting enzyme and substrate concentrations (Kingston and Narlikar, 1999). These effects could be caused by changes in substrate binding and/or enzymatic activity.

Implications for In Vivo Functions of ATP-Dependent Chromatin Remodelers

At the simplest level, all ATP-dependent remodeling complexes help regulate transcription by regulating the access of nucleosomal DNA to various transcription factors. However, the challenges of exposing DNA differ according to local nucleosome organization: some promoter regions contain regularly spaced nucleosomes, while others have gaps in nucleosome organization. At promoters where multiple closely spaced nucleosomes occlude different factor binding sites, the DNA has to be made accessible at several sites without significantly altering the translational position of the histone octamers. This is different from the task of creating DNA access in regions of low nucleosome density, where there is sufficient space for moving the nucleosomes and DNA can be exposed by creating nucleosome-free regions. Exposing DNA from densely packed nucleosomes also differs from the essential task, often the

responsibility of ATP-dependent remodelers, of creating appropriately spaced nucleosomes throughout the genome following replication. As described above, ATP-dependent remodeling can in theory be accomplished by sliding mechanisms, or instead by mechanisms that do not require translational movement of the histone octamer. It appears that nature has evolved different classes of ATP-dependent remodelers that use different mechanisms, each suited to a specific biological task.

The proposed ability of SWI/SNF-based complexes to expose nucleosomal DNA without requiring sliding of the histone octamer would allow it to function at promoters, such as the mouse mammary tumor virus promoter (Deroo and Archer, 2001), which have closely spaced nucleosomes. On the other hand, the ability to translationally reposition nucleosomes would allow ISWI-based complexes to establish specifically spaced nucleosomal structures at various promoters. Indeed, ISWI-based complexes have been implicated in establishing X chromosome structure in male *Drosophila* and nucleosome positions at the hsp26, hsp70, and yeast early meiotic gene promoters (Deuring et al., 2000). An important goal over the coming years will be to develop techniques to determine whether or not a nucleosome has been remodeled, and how it has been remodeled, on a given segment of DNA in vivo.

Covalent Modifications

Hyperacetylation of lysines in the N-terminal tails of the core histones was proposed to be involved in activation of transcription almost 40 years ago and has subsequently been strongly correlated with active genes (Allfrey et al., 1964). The relatively recent discovery and characterization of complexes that can add and remove acetyl groups and the development of stringent antibodies to specifically acetylated histone tails has helped provide a more comprehensive picture of these initial correlations.

Studies of bulk acetylation levels have shown that up to 13 of the 30 tail lysine residues in a histone octamer are acetylated (Roth et al., 2001). Genetic studies in combination with ChIP studies suggest that this steady-state level of acetylation is maintained by the opposing actions of histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes (Reid et al., 2000; Vogelauer et al., 2000). It is believed that targeting of HAT and HDAC complexes to promoter regions then creates specific patterns of hyper- and hypoacetylation in a background of global acetylation that correlate with transcription activation and repression, respectively. Indeed, ChIP studies using antibodies to specifically acetylated histone tails have shown that increased acetylation in promoter proximal regions of specific genes correlates with recruitment of HAT complexes and increased gene expression (Kuo et al., 2000). Hypoacetylation at specific promoters has analogously been correlated with recruitment of HDAC complexes to repressed genes (Khochbin et al., 2001). The discovery that certain HAT complexes contained general transcription factors and that certain HDAC complexes contained known transcriptional repressors further strengthened the correlations between hyperacetylation and activation and between hypoacetylation and repression.

Other modifications, such as methylation, ubiquitination, and phosphorylation have also been discovered and shown to be crucial in regulation of transcription (Berger, 2001). Here, we briefly summarize the vast literature on functional consequences of acetylation and deacetylation and the nature of the complexes that perform these modifications. We then discuss the regulatory interactions between these complexes and the ATP-dependent remodeling complexes and transcription machinery.

Functional Consequences of Modifications

There are multiple mechanisms by which acetylation of the histone tails might facilitate transcription. In vitro, histone acetylation has been shown to enhance the accessibility of DNA to restriction enzymes and transcription factors (Lee et al., 1993; Anderson et al., 2001; Sewack et al., 2001). This might be caused in part by the lowered positive charge on acetylated N termini and a consequent lowered stability of interaction with DNA. Histone acetylation can also decrease compaction of nucleosomal arrays by disrupting internucleosomal interactions made via the histone tails (Tse et al., 1998). A third possibility is that acetylated residues in different combinations can be involved in recruiting additional transcription factors. When other tail modifications are taken into account, the combinatorial possibilities expand rapidly. This has led to the hypothesis that certain combinations of modifications in one or more tails act sequentially or concomitantly to form a histone code (Strahl and Allis, 2000). Recognition of this code via binding of specific regulatory proteins is proposed to lead to additional downstream events.

Histone Acetyltransferase Complexes

There are two main classes of histone acetyltransferases (HATs), the type A nuclear HATs and the type B cytoplasmic HATs. Here we focus on nuclear regulatory complexes, which mainly contain type A HATs. Three families of Type A HATs have been identified (Figure 4). All of them share a highly conserved motif containing an Acetyl-CoA binding site, and several of these HATs have been shown to have activity on histones in vitro. However, when in vivo they always seem to act as part of large complexes.

The different complexes have different subunit compositions and different histone specificities (Table 1). Correspondingly, these complexes appear to be involved in distinct biological functions (Roth et al., 2001). For example, genetic and biochemical studies implicate the Spt3, 7, and 8 proteins, which are unique to the SAGA complex, in stabilizing TBP binding to the TATA box, suggesting that SAGA might function as a coactivator at the site of initiation in addition to its acetylation activity. In contrast, Spt16, a subunit of NuA3, or its mammalian homologs have been implicated in transcriptional elongation and replication, events that might require more large-scale acetylation over several kb. A distinct role for NuA4 is suggested based on its HAT, Esal, which, unlike GCN5, is essential in yeast and is homologous to MOF, the HAT implicated in 2-fold upregulation of the *Drosophila* male X chromosome. Mice that are homozygous for deletion of the HAT proteins p300, CBP, PCAF, or GCN5 exhibit distinct developmental de-

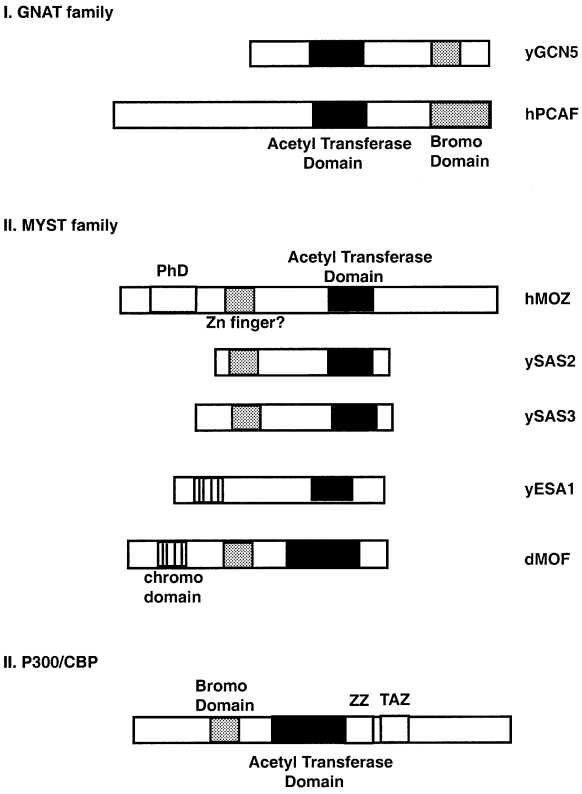

Figure 4. Representative Members of the Three Different HAT Families

fects, further suggesting differences in function of these highly related HAT subunits.

The different functions of HAT complexes are likely caused by differences in the histone residues that are acetylated and differences in targeting. Each complex contains a specific set of non-HAT subunits, which might interact with different sequence-specific activators that target the complexes to distinct genes. Indeed, whereas SAGA and NuA4 can be functionally recruited by the glucocorticoid receptor and acidic activators such as Gal4-VP16 (Brown et al., 2000; Roth et al., 2001), the distinct HAT complexes ADA and NuA3 do not appear to interact with these activators, leading to the speculation that these might be involved in more global, nontargeted acetylation events. The subunits may also differentially modulate HAT activity; GCN5-containing complexes have different substrate specificities than isolated GCN5 (Brownell et al., 1996; Grant et al., 1999). Finally, there are many examples where sequence-specific transcription factors directly affect HAT activity (Roth et al., 2001) and where adjacent histone modifications such as phosphorylation and methylation regulate HAT activity (Berger, 2001; Zhang and Reinberg, 2001).

Table 1. Some Representative HAT Complexes

yADA	ySAGA	hPCAF	yNuA3	yNuA4
GCN5	GCN5	hPCAF	Sas3	Esa1
	Ada1			
Ada2	Ada2	hAda2		
Ada3	Ada3	hAda3		
	Ada5/Spt20			
	Spt3	hSpt3	Spt16	
	Spt7			
	Spt8			
	Tra1	PAF400		Tra1
	TAFII90	PAF65β	TAFII30	
	TAFII61/68	TAF15/20		
	TAFII60	PAF65α		
	TAFII23/25	TAF30		
	TAFII17/20	TAF31		
	Sin4			
Ahc1				
				Act3/ARP
				Act1
				Epl1
				Eaf3

Adapted from Roth et al., 2001.

Table 2. Subunits in the Sin3 and NuRD Complexes

Sin3 Complex	NuRD Complex
HDAC1	HDAC1
HDAC2	HDAC2
RbAp46	RbAp46
RbAp48	RbAp48
	Mi-2α, Mi-2β
	MTA1
	MTA2
	MBD3
	p66
Sin3	
SAP18	
SAP30	

Adapted from Ahringer, 2000.

Histone Deacetylase Complexes

HDAC proteins identified to date fall in three main classes (reviewed in Khochbin et al., 2001). The Sin3 complex and NuRD complex contain members of the class I HDAC family, HDAC1 and HDAC2. Complexes containing members of class II HDACs have yet to be purified. Two complexes containing a member of class III HDACs, Sir2, have been recently identified and show distinct deacetylase activities. Sir2 is involved in heterochromatin silencing at silent mating loci, telomeres, and ribosomal DNA (reviewed in Moazed, 2001).

The histone specificities of the class I HDAC family complexes are just beginning to be characterized. Recent in vivo studies using highly specific antibodies suggest that the yeast homolog of HDAC1, Rpd3, deacetylates all sites except lysine 16 on histone 4, a site that is strongly linked to heterochromatic silencing and a site whose acetylation by dMOF is linked to activation of the *Drosophila* male X chromosome (Roth et al., 2001; Suka et al., 2001). This is consistent with Rdp3 having distinct functions from the Sir 2 deacetylases. Further, this suggests that Rpd3 does not act antagonistically to Esal, the yeast homolog of dMOF (Figure 4).

Analogous to the HAT complexes, the different subunit compositions of Sin3 and NuRD suggest that they participate in distinct biological functions (Table 2). The biochemical activities of the Sin3 and NuRD complexes have not yet been compared side by side, but it is possible that quantitative comparison of their deacetylase

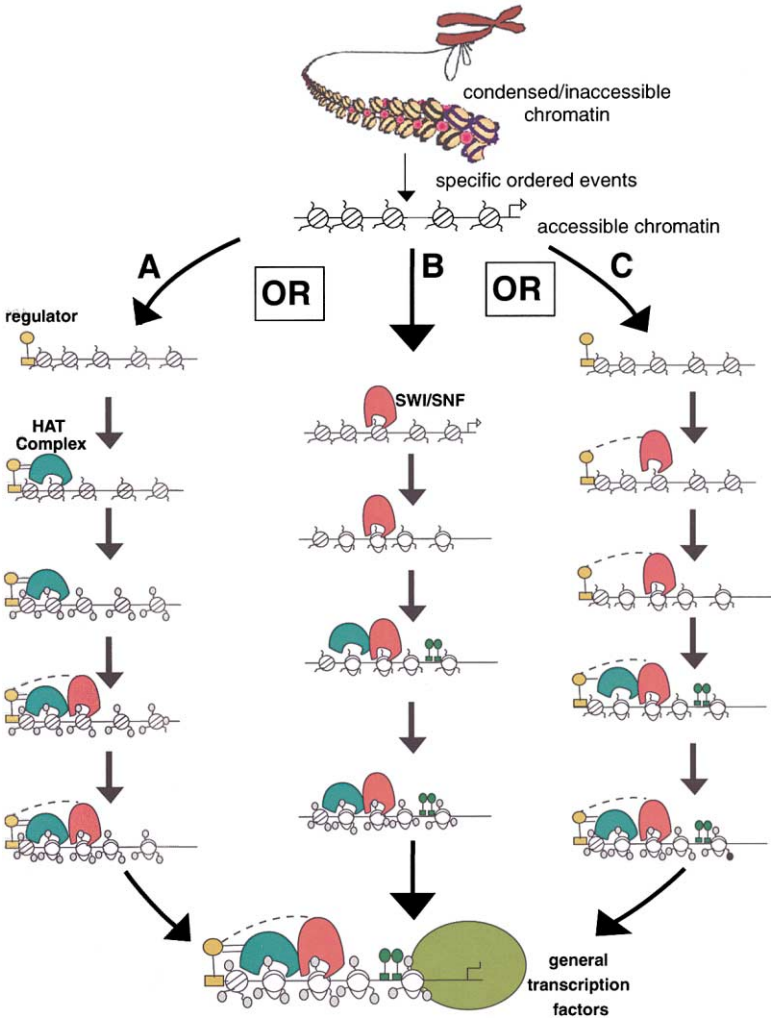

Figure 5. Models Depicting Different Orders of Action by Regulators and Chromatin-Remodeling Complexes

Regulators, HAT complexes, and ATP-dependent remodeling complexes can act in different orders (pathway A, B, or C) and still give the same end result: a template competent for transcription. Although not shown, it is also possible that binding by the general transcription factors precedes the action and recruitment of HAT complexes and ATP-dependent remodelers.

activities will reveal differences in substrate specificity and efficiencies of deacetylation. The Mi-2 protein that is unique to NuRD is an ATP-dependent chromatin remodeling factor (Tong et al., 1998; Wade et al., 1998; Zhang et al., 1998). Correspondingly, the deacetylase activity of NuRD on nucleosomal substrates is enhanced by ATP, suggesting that remodeling by Mi-2 increases the accessibility of the histone tails for HDAC1 and 2 (Tong et al., 1998). The inclusion of both ATP-dependent remodeling activity and covalent modification activity within the NuRD complex provides a telling example of cooperation between these two classes of activity. This cooperation appears to be common and to occur between remodelers and covalent modifiers that reside in physically distinct complexes.

Interactions between Complexes

To understand how a cell establishes its unique repertoire of expressed genes, it is essential to understand how the chromatin-modifying complexes described above interact with other components of the transcription machinery in a spatially and temporally coordinated manner. Genes that start in a highly condensed state are expected to require chromatin decondensation early in the process of regulation (Figure 5). The complexes and processes that regulate transitions between highly condensed chromatin structures and decondensed chromatin are not well understood; the discussion that follows pertains to regulation of target genes once they are in a decondensed and accessible chromatin state.

When transcription of a gene is altered, a specific event, frequently the binding of a gene-specific activator or repressor to accessible chromatin, triggers a cascade of reactions. These reactions result in a chromatin template, appropriately remodeled, which is bound by regulatory factors and the general transcription machinery. There is no a priori requirement that ATP-dependent remodeling, covalent modification of histones, or binding by regulatory factors or the transcription machinery occur in any specific order; the sole requirement is that the appropriate end stage, e.g., a properly structured template with a functional preinitiation complex poised for transcription, be attained in a timely manner (Figure 5). While the functional interactions between the numerous complexes that regulate transcription require further intense study, the emerging story suggests that these complexes are able to act in many different orders and can assist each other's function. Thus, multiple pathways are available for regulation, allowing multiple options to be considered during the evolution of a specific pathway for regulation of an individual gene.

Regulation of any biological reaction can be accomplished kinetically, by regulating the rate of a rate-determining step, or thermodynamically, by regulating the affinity of an enzyme for its substrate. For example, prokaryotic transcription initiation can be regulated, among other mechanisms, by changing the rate of promoter DNA melting and by modulating the stability of RNA polymerase binding. While eukaryotic transcription can be regulated at many more steps, similar concepts apply. Several previous reviews have addressed how ATP-dependent remodeling complexes and histone modifiers can individually contribute to transcription by increasing transcription factor access to DNA. Here we explore an additional level of regulation, in which ATP-dependent remodelers, histone modifiers, and transcription factors can kinetically and/or thermodynamically modulate each other's activities in the context of activation or repression on different genes.

How Are Chromatin-Modifying Complexes Recruited to Specific DNA Loci?

While both ATP-dependent remodeling and histone modifying complexes can bind nonspecifically to DNA, a wide variety of data implies that the activity of these complexes is regulated both spatially and temporally. For example, genome-wide expression studies performed with mutants of ySwi/Snf subunits have revealed that this complex is involved in the expression of only 6% of all yeast genes (Holstege et al., 1998; Sudarsanam et al., 2000). Similarly, Gcn5p-containing HAT complexes seem to be required for the expression of only 5% of yeast genes (Holstege et al., 1998). How is this specificity achieved? Accumulating evidence indicates that at least some sequence-specific transcription factors bind directly to both ATP-dependent chromatin-remodeling and histone acetyltransferase complexes to "target" these activities to specific locations.

Direct interactions between activators and these complexes can have three functional consequences that may affect the overall rate of chromatin remodeling. These interactions can specifically increase the affinity of the remodeling complex for a given DNA region due to the contacts between the activator and the complex. This would increase overall activity by increasing the local concentration of the remodeling complex. Targeting can also regulate remodeling by increasing the rate at which the remodeling complex binds to the chromatin template, if the binding event is rate limiting. Finally, interactions with the specific DNA binding factors may directly affect the activity of the remodeling complex. Many studies have provided examples of the first role of targeting with recent data providing initial examples of the third role.

Transcription Activators Target SWI/SNF and HATs

A widely accepted model has SWI/SNF targeted to specific promoters by direct interactions with sequence-specific transcription activators. Examples of targeting exist both in vivo and in vitro, from yeast to man. As an illustration, human SWI/SNF participates in nuclear receptor-mediated transcriptional regulation, and the glucocorticoid receptor (GR) and the estrogen receptor have been shown to recruit SWI/SNF to responsive promoters (Deroo and Archer, 2001; Hassan et al., 2001a) In addition to nuclear receptors, transcription activators as divergent as erythroid Kruppel-like factor, C/EBPβ, c-Myc, MyoD, HSF1, and EBNA2 have also been found to recruit SWI/SNF to specific promoters and, in some cases, to activate gene expression (Hassan et al., 2001a; Peterson and Workman, 2000; Sullivan et al., 2001).

Although the molecular details of the association between SWI/SNF and transcription activators remain largely unknown, there is evidence that points to the importance of specific features of transcription activation domains. A study of the chimeric Gal4-VP16 acidic transcription activator has shown that targeting is reduced or eliminated by mutations that disrupt its acidic residues (Hassan et al., 2001a; Peterson and Workman, 2000). Additionally, hydrophobic residues within the transcription activation domain of the human heat shock factor HSF1 have been shown to be important for mediating SWI/SNF recruitment (Sullivan et al., 2001).

Nuclear HAT complexes and transcription coactivators with intrinsic HAT activities have also been found to interact with transcription activators (Roth et al., 2001). Similar to SWI/SNF targeting, the direct contact between an activator and a HAT complex will contribute to an increased affinity for a particular DNA region, thus facilitating recruitment to specific DNA loci. For example, the transactivation domain of GR has been shown to interact with CBP, a protein with intrinsic HAT activity (Deroo and Archer, 2001). As described above for SWI/SNF, it has been demonstrated that transcription activators containing acidic domains can directly interact with SAGA and NuA4 and target these HAT complexes to specific promoters (Hassan et al., 2001a; Peterson and Workman, 2000). Recently, Tra1p, a common subunit of SAGA and NuA4, has been shown to mediate the interaction of these complexes with acidic transcription activators (Brown et al., 2001).

Repressors Can Target ATP-Dependent Remodelers and HDACs

In contrast to ySWI/SNF, which can be targeted by acidic activators, Isw2p has been shown to be targeted to promoters of early mitotic genes by the transcriptional repressor UME6 (Goldmark et al., 2000). In further contrast to the examples discussed above, the recruitment of Isw2p leads to the formation of inaccessible chromatin structure proximal to the Ume6p binding site and, consequently, represses gene expression (Goldmark et al., 2000). The Sin3-Rpd3 complex, a member of the yeast HDAC family, is one of the most extensively studied histone deacetylase complexes. This complex has been shown to be targeted to specific promoters by the same transcription repressor Ume6p, resulting in local histone deacetylation and transcription repression (Fazzio et al., 2001).

Another example demonstrating the generality of transcription repressor-mediated recruitment is the targeting of the NuRD complex, which contains an ATP-dependent chromatin remodeler, Mi-2, and a histone deacetylase (Khochbin et al., 2001; Kingston and Narlikar, 1999). Upon T cell activation, the DNA binding protein Ikaros recruits NuRD to regions of heterochromatin (Kim et al., 1999). It has been proposed that this recruitment either maintains an inactive chromatin state or

converts an accessible chromatin conformation to an inaccessible structure. The transcription repressor, Kap-1, can also target NuRD to specific promoters to repress gene expression (Schultz et al., 2001). Another candidate for this type of recruitment is the hunchback protein of *Drosophila*, which associates with the *Drosophila* Mi-2 homolog (Kehle et al., 1998).

DNA microarray analyses, biochemical studies, and genetic studies indicate that yeast and human SWI/SNF are also involved in gene repression. The transcription corepressors Hir1p and Hir2p of yeast can recruit ySwi/Snf to a responsive promoter and repress gene expression (Dimova et al., 1999), and in *Drosophila* and mammals SWI/SNF has been found to participate in Rb-E2F repression pathways (Zhang and Dean, 2001). Evidence connecting SWI/SNF and deacetylation came from biochemical studies which have shown that components of the Sin3-HDAC complex copurify with a BRG1-containing SWI/SNF complex, and mSin3 interacts with components of SWI/SNF in vitro (Sif et al., 2001).

It is therefore clear that sequence-specific DNA binding regulatory factors can directly target ATP-dependent remodeling complexes, HAT complexes, and HDAC complexes to specific locations. In certain instances, there is concerted recruitment of a remodeler and HDAC activity. In many cases, targeting has mainly a thermodynamic effect, with the direct contacts between the gene-specific regulatory protein and the chromatin-modifying complex contributing to increased binding energy. DNA binding factors can also have a kinetic effect by directly affecting the activity of a remodeling complex. Factors such as Twist and E1a have been shown to directly alter acetyltransferase activity of p300 (Roth et al., 2001). To date, there is no example of an activator or repressor directly altering the rate of chromatin remodeling, although it is early days and such kinetic effects are likely to be uncovered. These targeting mechanisms likely serve to initiate a cascade of events at a given promoter that results in local alteration of chromatin structure to facilitate formation of an active or repressed state.

How Do Chromatin Remodelers and HATs and the Transcription Machinery Function Together?

There is strong evidence to support the common-sense notion that ATP-dependent remodelers and covalent modifiers work together to regulate gene expression. A functional link between ATP-dependent remodeling and HAT complexes was first suggested by genetic studies in yeast (Pollard and Peterson, 1997; Roberts and Winston, 1997). Mutations in subunits of the SAGA complex (excluding GCN5) were lethal in combination with mutations in the ySwi/Snf complex, though none of the single mutants showed any severe growth defects, indicating a synergistic interaction between components of these two complexes. In mammalian cells, ChIP experiments have found that both BRG1 and p300/CBP are present on Estrogen receptor-responsive promoters following estrogen treatment of MCF7 cells, consistent with cooperative interactions (DiRenzo et al., 2000; Shang et al., 2000). Increasing levels of histone acetylation, by treating cells with an HDAC inhibitor while simultaneously overexpressing BRG1, synergistically enhances ER-responsive gene expression. This suggests that BRG1 and p300/CBP function interdependently; the interdependence between ATP-dependent remodelers and histone-modifying enzymes could be a general feature.

Analogous to the effects of sequence-specific activators, these two types of complexes could help each other in multiple ways. Direct physical interactions between the ATP-dependent remodelers and chromatin modifiers could increase their affinity for the chromatin template. Such direct interactions could also affect the activities of each complex. Finally, alteration of the chromatin template by one complex could make it a better substrate for the other complex. For example, remodeling of the nucleosomes by ATP-dependent remodelers may increase the accessibility of the histone N termini for acetylation or deacetylation. Alternatively, ATP-dependent remodelers might bind more strongly to, or dissociate more slowly from, nucleosomes having N termini acetylated at specific positions. Acetylation states of histones may directly affect the kinetics of ATP-dependent remodelers' activities. Thus far, few biochemical analyses have afforded the temporal resolution necessary to resolve these issues, and detailed quantitative studies will be required in the future to dissect out the different ways in which HAT complexes and ATP-dependent remodeling complexes can affect each other's activities.

Two studies raise the possibility that acetyltrasferase complexes might stabilize the interaction of yeast SWI/SNF complexes with the template. Work performed using an altered PHO5 promoter in yeast suggested that the Gcn5p-containing SAGA complex might serve two functions (Syntichaki et al., 2000). The catalytic Gcn5p subunit first acetylates a promoter region, and then the bromodomain of Gcn5p is proposed to stabilize the binding of Swi/Snf to the newly hyperacetylated nucleosomes. Biochemical analysis provides a somewhat different story that supports the basic notion that acetyltransferases can stabilize binding of yeast SWI/SNF. It was shown using an in vitro system that SWI/SNF is preferentially bound on an acetylated template, implying that acetylation stabilizes SWI/SNF association (Hassan et al., 2001b).

ISWI family ATP-dependent remodeling complexes can also interact with covalent modifying complexes. Although Isw2p and Sin3p-Rpd3p have unique biochemical activities, genetic studies and genome-wide microarray analyses indicate that *ISW2* and *SIN3-RPD3* function synergistically to regulate gene expression (Fazzio et al., 2001; Goldmark et al., 2000). In an in vitro transcription system, p300 and P/CAF enhanced NURF-mediated transcriptional activation from a chromatin template (Mizuguchi et al., 2001). And yet, acetylated histones were not found to increase the amount of NURF-remodeled template, as judged by the steady-state accessibility of restriction enzymes or micrococcal nuclease (Mizuguchi et al., 2001). It remains possible, however, that acetylated chromatin might have changed the rate of remodeling catalyzed by NURF.

The importance of changing the rate of remodeling is demonstrated by a study of the PHO5 promoter (Barbaric et al., 2001). It had been accepted that Gcn5p has little effect on transcription activation of the yeast *PHO5* gene; however, Barbaric et al. showed that whereas the steady-state level of the PHO5 message is not altered in *gcn5* mutants, the rate of nucleosome remodeling at

the *PHO5* locus is decreased (Barbaric et al., 2001). Thus, HATs can increase the rate of gene induction, without affecting steady-state expression levels, apparently by stimulating the rate of remodeling.

Is There a General Order of Recruitment?

It appears that there is no obligate order for function of ATP-dependent remodelers and covalent modifiers that is general for all promoters. Rather, it seems that each individual promoter will work using a set order of action by these complexes that differs from promoter to promoter. On the yeast HO promoter, the recruitment of ATP-dependent chromatin remodelers precedes that of HAT complexes (Cosma et al., 1999; Krebs et al., 1999). The reverse order of complex recruitment has been observed on the IFN-β promoter and for retinoic acid-induced transcription (Agalioti et al., 2000; Dilworth et al., 2000). The precise order seems to depend upon the nature of the promoter, the complement of transcription factors present, and the chromatin structure in which the promoter resides.

Indeed, for a particular gene, the requirement for specific chromatin remodelers may vary. This is best revealed by the cell-cycle dependence of ATP-dependent remodeling and HAT activities in yeast. Activation of the inducible *GAL1* promoter during interphase requires Gcn5p but not ySwi/Snf. Induction of this gene in late mitosis, however, requires both ySwi/Snf and the Gcn5p complex. This indicates that ATP-dependent remodeling complexes are needed to assist HAT-dependent gene expression when chromatin is highly condensed (Krebs et al., 2000).

Many Paths Can Lead to the Same Place; Coordination with the Transcription Machinery

The analysis described above implies that ATP-dependent remodelers and chromatin-modifying complexes can work in any order, and that these two types of complexes can directly influence each other's activity. For example, acetylation can enhance ATP-dependent remodeling, and remodeling can increase the rate of covalent changes such as deacetylation (DiRenzo et al., 2000; Guschin et al., 2000). While space constraints preclude a detailed discussion of interactions involving the general transcription machinery, we describe below a few examples to make the point that the components of the general transcription machinery can assist in chromatin remodeling, and that chromatin-remodeling complexes can increase function of the transcription machinery. Thus, the notion that there is no obligate order of action for ATP-dependent remodeling and covalent modification can be extended to state that there is no obligate order of action for chromatin-modifying complexes and complexes in the general transcription machinery.

Transcription factors and regulatory complexes can bind chromatin prior to recruitment of chromatin-modifying complexes, and are capable of altering chromatin structure. Biochemical studies have shown that binding of activators such as GAL4 can displace nucleosomes in *cis* and in *trans* to create nucleosome-free regions (Workman and Kingston, 1992). Elongation by RNA polymerase can also cause changes in the chromatin structure, including changes in nucleosome position (Lee and Garrard, 1991).

Chromatin-modifying/remodeling complexes can facilitate specific steps in transcription. ATP-dependent remodeling complexes can increase binding by gene-specific activators and by components, such as TBP, of the preinitiation complex (Burns and Peterson, 1997; Cote et al., 1994; Imbalzano et al., 1994). Acetylation of the template can also increase binding of transcription factors (Sewack et al., 2001). Both HAT complexes and ATP-dependent remodeling complexes can significantly increase the rate of overall transcription from nucleosomal templates in defined in vitro transcription systems (Ikeda et al., 1999; Neely et al., 1999).

It appears that each gene has "chosen" a specific pathway to achieve appropriate regulation from a menu of different possible pathways. This is consistent with recent biochemical studies that emphasize the requirement for specific chromatin-modifying complexes in an individual system. For example, activation of the β-globin locus via action of the transcription factor EKLF requires a specific SWI/SNF-family remodeling complex (Hassan et al., 2001a). Transcriptional activation in vitro of a specific promoter by hormone receptors in a defined in vitro system remarkably requires one particular SWI/SNF family complex, while a different SWI/SNF family complex will not function (Lemon et al., 2001). It appears that these very specific requirements for a given complex on a given promoter reflect the particular pathway that has evolved to work on that promoter, not a requirement that is general for the activator involved or for other promoters.

This small subset of examples serves to make the point that there is no obligate order for function of the transcription machinery with respect to the chromatin-modifying machinery. The sole requirement is that the end point, which is the structure of the template and association of appropriate components of the general transcription machinery, be reached in a timely manner (Figure 5). Each component is capable of functioning in the absence of the other components. What is apparent from the studies described above, and is the key to allowing different pathways to occur on different genes, is that each step is able to facilitate another step. Remodeling can facilitate transcription factor binding, and factor binding can facilitate remodeling. Thus, each player can help the other, and a pathway can be chosen that offers the most parsimonious solution to the problem of achieving specific and robust transcriptional regulation.

Acknowledgments

We apologize to our colleagues whose primary papers could not be cited due to space constraints. We thank I. King, J. Dennis, R. Lake, and D. Reinberg for comments.

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