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ALTERATION OF NUCLEOSOME STRUCTURE AS A MECHANISM OF TRANSCRIPTIONAL REGULATION

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ABSTRACT

The nucleosome, which is the primary building block of chromatin, is not a static structure: It can adopt alternative conformations. Changes in solution conditions or changes in histone acetylation state cause nucleosomes and nucleosomal arrays to behave with altered biophysical properties. Distinct subpopulations of nucleosomes isolated from cells have chromatographic properties and nuclease sensitivity different from those of bulk nucleosomes. Recently, proteins that were initially identified as necessary for transcriptional regulation have been shown to alter nucleosomal structure. These proteins are found in three types of multiprotein complexes that can acetylate nucleosomes, deacetylate nucleosomes, or alter nucleosome structure in an ATP-dependent manner. The direct modification of nucleosome structure by these complexes is likely to play a central role in appropriate regulation of eukaryotic genes.

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INTRODUCTION

It has become increasingly apparent that modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. Chromatin structures can inhibit the binding and function of the numerous proteins that collaborate to produce appropriate levels of transcription. Recent studies have identified a large group of proteins whose primary function is to help activate transcription by altering chromatin so that its DNA sequences become more transparent to the transcriptional apparatus. Conversely, different proteins help repress transcription by making chromatin structure less transparent.

The nucleosome core is the target of many of these activities. Once thought of as a static building block of chromatin structure, the nucleosome core is now clearly understood as a dynamic structure whose stability can be regulated by posttranslational modification and by enzymatic function. In addition, regulatory proteins can bind directly to nucleosomal histones to create altered structures. Thus, the state of the nucleosome core plays a central role in determining the transcriptional competence of any region of chromatin.

The purpose of this review is to examine what is known about nucleosome core structure and how that structure might be altered during gene regulation. The nucleosome core contains about 146 bp of DNA and two copies each of the histones H2A, H2B, H3, and H4. We discuss what is known about histone-histone interactions and histone-DNA interactions within this particle, with an emphasis on how these interactions might contribute to regulation. Of particular importance are the N-terminal tails of the core histones, which are less structured and extend out of the central structured portions of the nucleosome core in a manner that frees them for interaction with DNA or with other proteins. We discuss the enzymatic acetylation and deacetylation activities that modify these tails, and we relate these modifications to changes in the structural properties of nucleosomes and to changes in gene regulation. We conclude with a discussion of ATP-dependent protein complexes that alter

nucleosome structure to increase the ability of transcription factors to interact with their DNA sequences.

STEPS IN TRANSCRIPTIONAL REGULATION

The nucleosome can inhibit several processes that must occur for a eukaryotic gene to be appropriately regulated (Figure 1). The initial event in activation is thought to be binding of sequence-specific activators to both enhancer and promoter regions. To measure the effects of nucleosomes on activator binding, investigators have assembled binding sites into mononucleosomes by salt dialysis of purified histones, or they have used either *Xenopus* or *Drosophila* chromatin assembly extracts to assemble sites into arrays of nucleosomes. These experiments demonstrate that nucleosomes inhibit the binding of most transcriptional activators; the glucocorticoid receptor is the only example of an activator that binds with similar affinity to naked and nucleosomal DNA (1). Estimation of the dissociation constants for binding indicates that the degree of inhibition for other activators varies between one and greater than four orders of magnitude and that one activator can facilitate binding of an adjacent activator (see below). Therefore the characteristics of nucleosomal structure that cause inhibition of activator binding, and the mechanisms that enhance activator binding, are critical to understanding transcriptional regulation.

Nucleosome structures also inhibit transcriptional initiation. This inhibition most likely occurs via inhibition of the binding of general transcription factors, which must associate with the template to enable specific initiation by RNA polymerase. Formation of the preinitiation complex, which includes general transcription factors and RNA polymerase, is inhibited by nucleosome formation. Prebinding the template with fractions that include TFIID, or prebinding with purified TATA-binding protein, partially relieves this inhibition (2). TBP binding is inhibited by nucleosomal formation (3), so it is possible that precisely the same mechanisms that affect access of activators to nucleosomal DNA also affect access of the preinitiation complex.

The final steps in transcription are elongation and termination. Nucleosomes create a significant topological problem for transcriptional elongation, as a megadalton RNA polymerase must proceed around 1.65 turns of DNA wrapped around the histone octamer. In vitro studies have demonstrated that purified phage RNA polymerase can elongate through a nucleosome and that eukaryotic polymerases are either significantly slowed or even stopped by a nucleosome, depending on the template and reaction conditions (4–9). Given the varied nature of the reactions that must occur, the ability of RNA polymerase to elongate through a nucleosome is likely to be affected by aspects of nucleosome structure that differ from those that affect sequence-specific DNA binding. In

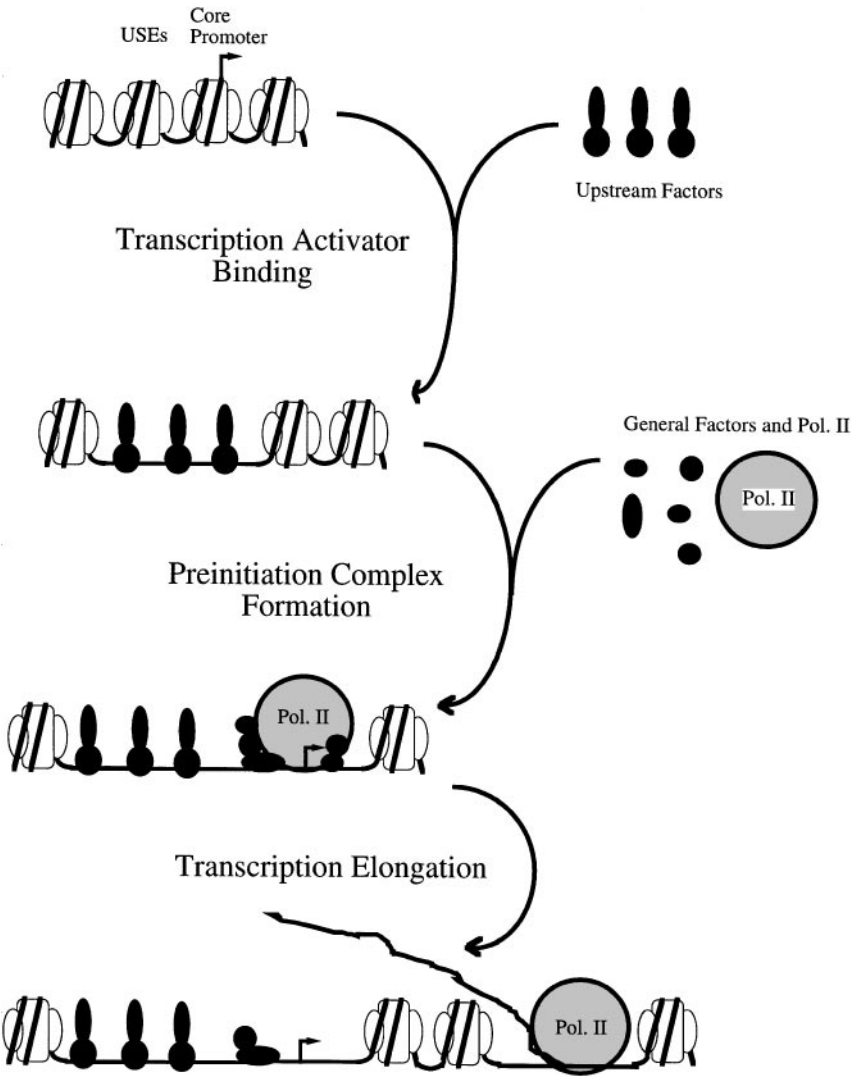


Figure 1 Nucleosomes can inhibit multiple steps required for gene transcription. The binding of upstream regulatory factors requires accessing nucleosomal DNA and may result in displacement or rearrangement of the histone octamers. Similarly the formation of preinitiation complexes at the TATA-box and transcription start site is also suppressed by the presence of nucleosomes. Finally, the elongation of RNA polymerase II is inhibited by nucleosome arrays, resulting in increasing pausing of engaged polymerases.

reviewing nucleosome structure and the activities that are believed to modify that structure, we focus on the characteristics that are likely to be important to each of the above steps in transcriptional regulation.

THE NUCLEOSOME

The discovery of the nucleosome as the repeating subunit of chromatin was a seminal event in chromatin research. The nucleosome was discovered through (*a*) its appearance in the electron microsope as the beads (nu-bodies) in the extended beads-on-a-string structure of the decondensed chromatin fiber (10–13), (*b*) by the generation of approximately 200-bp DNA ladders upon digestion of chromatin with endogenous (14) or exogenous (15) nucleases, and (*c*) by the isolation of 11.5S nucleoprotein complexes (16). These observations, histone/histone cross-linking patterns (17), and X-ray diffraction data led Kornberg to propose that the nucleosome comprised approximately 200 bp of DNA and two copies of each of the four core histones (18). The nucleosome proper is composed of (*a*) the nucleosome core (146 bp of DNA wrapped 1.65 turns around the histone octamer), (*b*) the linker histone H1 (or an H1 variant), and (*c*) the linker DNA between nucleosome cores (19). A subnucleosome particle containing the nucleosome core, histone H1, and 168 bp of DNA (i.e. the nucleosome core and 20 base pairs interacting with histone H1) has been termed a chromatosome (20). Numerous details of nucleosome structure have been revealed over the past two and a half decades. These discoveries culminated with the recent solution of a high-resolution crystal structure of the nucleosome core (21).

Nucleosome Structure

The histone octamer is a cylindrical wedge with an outer diameter of 6.5 nm (22, 23) that is composed of a central tetramer of two copies each of histone H3 and H4 that is flanked by two H2A/H2B heterodimers (17, 24–26). The H3/H4 tetramer is assembled by the association of two heterologous dimers of H3 and H4. Each of the core histones shares a common motif consisting of two short alpha helixes and a long central helix seperated by beta bridges. These structures constitute most of the ordered portion of the histones and have been termed the histone fold (27). The histone fold provides sites of histone DNA interactions as well as provides for the dimerization of histones via a handshake motif, in which each monomer clasps its partner in a head-to-tail arrangement (21, 27).

Homology searches have revealed that several proteins with functions related to DNA also contain putative histone fold motifs including TAFs (TBP-associated factors), which are subunits of the general transcription initiation

factor TFIID (28). The histone fold motifs in TAFs also appear to participate in dimerization of subunits (29–31). Indeed, heterodimers between TAFs and core histones can be formed in vitro (30). These observations raise the possibility that there is a histone octamer–like core structure within TFIID that might similarly wrap DNA. However, these TAFs lack arginine side chains, which are found in core histones; these side chains insert into the minor groove of the DNA helix. This difference suggests that any DNA binding by these TAFs must be by different protein-DNA interactions (21).

In addition to assisting dimerization, histone folds contribute to further oligomerization of histone proteins within the histone octamer. The central and third alpha helices of the histone folds of H3, H4, and H2A are the primary, but not only, determinants for assembly of the H3/H4 tetramer and the octamer through the formation of four helix bundles (21). An H3-H3 interaction between H3/H4 dimers drives the formation of the tetramer, and an interaction between each H4 of the tetramer with an H2B of an H2A/H2B dimer drives formation of the octamer. It is important that the H4-H2B interface is considerably more hydrophobic than the H3-H3 interfaces and thus expected to be less stable at low ionic strength (21). This characteristic may account for the early observation that whereas the H3/H4 tetramer is stable at physiological salt concentrations, the complete octamer (i.e. including binding of the H2A/H2B dimers) is stable only at high salt concentrations or when wrapped with DNA in the form of a nucleosome core (26, 32, 33). As discussed below, the lability of the H2A/H2B dimer–H3/H4 tetramer interactions may have significance for conformational changes and/or disassembly of the nucleosome core.

Within the nucleosome core, 146 bp of DNA are wrapped 1.65 times around the histone octamer in a left-handed superhelix with about 7.6 turns of the DNA helix/superhelical turn (22). The path of the central 12 turns of DNA follows a path of repeating histone-positive charges on the surface of the histone octamer (34). Each histone heterodimer within the octamer forms a cresent shape that arcs 27–28 bp of DNA along their axis in a 140° bend, leaving 4 bp linkers between them (21). The interactions of the histone folds organize the central 121 bp of DNA with additional H3 interactions, extending the DNA superhelix. Further DNA interactions are provided by the N-terminal tails of H3 and H2B, which pass through minor groove channels between the DNA helices (one tail each 20 bp) and by the binding of the H2A tail to the minor groove outside the superhelix (21). The path of the DNA helix around the histone octamer is not uniform: It is distorted by the local structure of the histone DNA–binding surface. Most notably are major bends at 10–15 and 40 bp from the dyad axis (center of the DNA superhelix), where histone interactions bulge or buckle the DNA outward (21, 22).

NUCLEOSOME DYNAMICS

Unfolding or Disassembling Nucleosome Cores

A number of physical and biochemical studies have investigated potential structural transitions in the nucleosome core when challenged with low or high ionic strengths, changes in pH, binding of intercalating agents, or denaturation caused by urea. These studies have revealed that although the nucleosome core is stable under physiological conditions, it is susceptible to unfolding and/or unwrapping of the DNA under a variety of conditions (35). The altered nucleosomal structures that form under these nonphysiological conditions are candidates for structures that might be induced in vivo by ATP-dependent remodeling complexes or by the action of acetylation or deacetylation activities. Histone-binding proteins might also be involved in stabilizing structures such as these in vivo. Only limited data address the relationship of these altered structures to structures that might be stabilized in vivo by regulatory proteins; however, it is important to consider the types of structural changes that nucleosomes can undergo when formulating models for action of regulatory proteins (Table 1).

Table 1 Proposed nucleosome conformation changes

Transition (references)	Disruption of histone-histone contacts	Disruption of histone-DNA contacts	Features
Low salt transition (36, 39–41)	+	−	Partial unfolding of the histone octamer
Loss of H2A/H2B dimer(s) (43, 44, 49, 51)	+	+	Increased factor access Decreased fiber folding
Unfolded H3-SH accessible (54, 56, 58, 60)	+	−	Enriched in transcribed sequences and acetylated histones
Transient unwrapping of nucleosome DNA (65, 67, 69, 70)	−	+	Increased factor access Facilitates cooperative factor binding
Short-range octamer movement in *cis* (74–77)	−	+	Temperature-enhanced redistribution
Long-range octamer movement in *cis* (71, 72, 80, 81)	−	+	Enhanced by high salt or ATP-dependent remodeling complexes
Histone octamer transfer (4, 7, 8, 50)	−	+	Transfers intact octamers to other DNA sites in *cis* or *trans*

The nucleosome core undergoes a structural transition when salt concentrations are dropped below 1 mM. Modeling of the low-salt nucleosome conformation has suggested an elongated partially unfolded structure (36), whereas other studies disagree with this view (37). It is interesting to note that the extent of the low-salt nucleosome transition as well as a reversible thermal transition (see below) is affected by the histone subtypes contained in the nucleosome core particle (38). The low-salt transition is thought to involve unfolding of the histone octamer in some manner because it appears to break H4-H2A contacts (suggesting weakening of the H3/H4 tetramer–H2A/H2B dimer interface) and is inhibited by histone-histone cross-linking (36, 39–42).

The interaction of the H2A/H2B dimers with the H3/H4 tetramer has been suggested to modulate many processes. For example, RNA polymerase II preferentially associates with nucleosomes deficient in one H2A/H2B dimer (43). The in vitro binding of TFIIIA adjacent to an H3/H4 tetramer reconstituted on a 5S RNA gene occurs readily but is inhibited by the association of H2A/H2B dimers that occupy its binding site (44). Acidic nucleosome assembly factors—nucleoplasmin and NAP-1, which preferentially interact with H2A/H2B(45–48)—stimulate the binding of transcription factors to nucleosome cores and can lead to the depletion of H2A/H2B dimers from factor-bound nucleosomes (49, 50). As with the low-salt nucleosome transition, stimulation of factor binding and histone removal by nucleoplasmin and NAP-1 is inhibited by cross-linking the histone octamer (50). It has also been suggested that depletion of H2A/H2B dimers can disrupt the ability of nucleosome arrays to fold into higher-order chromatin structures (51).

Genetic evidence supports an important role for association of H2A/H2B dimers within the nucleosome core. The SWI/SNF complex can remodel nucleosome core structure (see below), and depletion of one of the H2A/H2B gene pairs in yeast alters the transcription of particular genes in vivo, in a manner that supresses defects in the SWI/SNF complex (52). Moreover, single amino acid substitutions for H4 tyrosines that interact with H2A/H2B dimers cause defects in the transcription of several genes, including crucial regulators of cell cycle progression (53).

Extended unfolded nucleosomes have been described by electron spectroscopic imaging of a nucleosome subfraction isolated by Hg-affinity chromatography (54). These U-shaped particles are retained on Hg columns, in part because of accessibility of the single H3 thiol, and are enriched in acetylated histones and transcribed DNA sequences (55–58). This H3 thiol (H3-C110) is in the center of the H3-H3 four-helix bundle that binds the two H3/H4 dimers into a tetramer (21). Presumably, reactivity of this thiol to Hg would require a substantial alteration in the conformation of the tetramer, consistent with the unfolded structures described above. This conformation appears to be different

from that of the low-salt nucleosome transition. Early studies that measured energy transfer between fluorescent dyes attached to the H3-cysteins did not detect a substantial increase in the distance between the cysteins at low salt (59), suggesting that a major conformation change in the tetramer did not take place at this location. Studies in yeast have described a transcription-induced split nucleosome structure based on nuclease sensitivities that may be related to the U-shaped thiol-reactive nucleosome conformation (60). The appearance of these altered nucleosome structures is induced by transcription in vivo. These structures accumulate at the 3′ end of the gene and may result from RNA polymerase–induced positive supercoiling (60, 61). Template supercoiling may induce these structures, but in vivo nuclease digestion experiments indicate that template supercoiling does not appear to be necessary for efficient transcription in yeast (62).

Regardless of the precise relationship between split nucleosomes and nucleosomes that can be bound on Hg columns, the association of these apparently altered nucleosomal structures with transcribed chromatin implies that some process connected to gene activation causes enrichment of these structures. They might be a by-product of transcriptional activation; for example, they might be induced by RNA polymerase movement through chromatin. Alternatively, they might be induced by remodeling activities prior to transcription and might therefore represent an altered nucleosomal state that is formed as a prerequisite for appropriate activation of genes. Thus, while altered structures of the nucleosome can be observed in vivo and in vitro, neither the genesis of these structures nor the role that they play in regulatory processes is clear.

Unwrapping of Nucleosomal DNA

Another reversible conformational change in the nucleosome core particle is the unwrapping of DNA from the histone octamer as revealed by thermal denaturation studies. Given that most DNA-binding proteins do not easily form a ternary complex on a nucleosome, unwrapping of nucleosomal DNA may be an important mechanism for increasing access of regulatory proteins to their target DNA sequence. With increasing temperature, denaturation of nucleosomal DNA occurs in two phases at temperatures that are higher than required for melting of naked DNA. The first phase is reversible and represents the melting of approximately 40 bp of DNA, while the second phase is irreversible and represents melting of the remaining base pairs (63). Further experiments illustrated that the first phase of nucleosomal DNA denaturation represented the melting of approximately 20 bp at each end of the nucleosome core following their release from the octamer. Moreover, this melting was not inhibited by histone-histone cross-linking, indicating that it did not require dissociation of histone protein (64). Thus, these studies demonstrated that the first 20 bp into the

nucleosome core are particularly able to reversibly dissociate from the histone octamer.

Further support for the notion that the DNA at the edge of the nucleosome core is less tightly constrained comes from analysis of transcription factor–nucleosome interactions. Numerous transcription factors interact with nucleosomal DNA albeit usually with affinities that are reduced relative to naked DNA (1). In most instances the affinity is greatest when the recognition site for the factor is located near the edge of the nucleosome core (65–67). When a single nucleosome has multiple recognition sites for the same factor, those near the edge are bound first (65). However, the binding of factors to the more accessible sites at the edge of the nucleosome core enhances the affinity of other sites deeper into the core particle (65, 68). This leads to a cooperative effect on the binding of multiple factors to a single nucleosome core that does not require direct interaction between the factors but instead appears to result from the cumulative disruption of histone-DNA interactions (67).

These data have been used to develop a model for cooperative binding of factors to nucleosomal target sites (69). The model posits that factors access their binding sites during transient unpeeling of DNA from the histone octamer (which initiates at the edge of the nucleosome core) (70). The binding of two factors may then be linked in a thermodynamic cycle governed by the equilibrium constants for exposure of each recognition site. Cooperativity could be attributable to the binding of each individual factor stabilizing the otherwise transient release of DNA sequences from the surface of the histone octamer and thereby increasing the probability of exposure of sites for additional factors.

Histone Octamer Movement

The above studies demonstrate possible mechanistic pathways for altering the nucleosome to potentially increase the ability of regulatory proteins to interact with their recognition sequences. A second type of mechanism to increase factor interaction is for the core histones to slide or jump along the DNA sequence to expose important sequences. Indeed, histone octamers are able to relocate to other positions on a DNA strand in *cis* (71, 72). This long-range movement of octamers along DNA generally requires elevated salt concentrations ($\sim$0.5 M). By contrast, short-range movement of histone octamers (i.e. tens of base pairs) can occur at physiological salt concentrations especially at 37°C. Several studies have used tandem repeats of sea urchin 5S RNA gene sequences (73) to measure nucleosome mobility at physiological ionic strength. These studies revealed that on these sequences, nucleosomes adopt a primary translational frame surrounded by others located at intervals of 10 bp (74, 75). These data illustrate a cluster of octamer positions on the 5S sequences that are in equilibrium at low ionic strength but maintain the rotational phasing

of the DNA helix (i.e. the direction of DNA bending around the octamer). Similar localized mobility of histone octamers was found to occur on other DNA sequences, suggesting that it is not solely a property of 5S RNA gene sequences (76). This localized histone octamer mobility is reduced by the binding of histone H1 (77, 78). Importantly, histone octamer sliding has been suggested to be enhanced by ATP-dependent nucleosome remodeling activities present in *Drosophila* embryo extracts (79–81).

Histone octamer transfer is another form of histone movement that appears to be mediated by the direct transfer of histones from one region or strand of DNA to another. This pathway has been illustrated by studies of phage RNA polymerase elongation through nucleosome cores. Studies by Felsenfeld and colleagues have suggested that a histone octamer can step around an elongating polymerase (4, 7, 8). The model suggests that as the elongating polymerase peels DNA off a histone octamer in front of it, the same octamer begins to reassociate with DNA behind the polymerase. That histone-histone cross-linking within the histone octamer does not interfere with elongation (82) suggested that the octamer most likely remains intact during transfer. Thus, the histone octamer appears to be directly transferred from DNA in front of the polymerase to DNA behind it. A similar pathway of histone octamer transfer might occur upon destabilization of nucleosomes by the binding of multiple factors. In vitro studies have shown that the nucleosome binding by five dimers of GAL4 derivatives can induce transhistone displacement from GAL4 sites onto competitor DNA (49, 83, 84). This transfer does not require dissociation of the histone octamer (50) and thus may also occur by direct contact between the donor and recipient DNA strands. Importantly, histone octamer transfer differs from disassembly of the histone octamer mediated by histone-binding proteins, nucleoplasmin, or NAP-1, which requires initial removal of H2A/H2B dimers (50; see above).

HISTONE ACETYLATION

The Histone Amino Terminal Tails

The N termini of core histones are central to the processes that modulate nucleosome structure. The N termini of histones H3 and H4 are the most conserved portions of these highly evolutionarily conserved proteins, and genetic studies in *Saccharomyces cerevisiae* have shown that small deletions or point mutations in the N termini lead to a remarkable breadth and severity of phenotypes (85, 86). N termini can be modified posttranslationally by acetylation and by phosphorylation. Both modifications alter the charge distribution on the N termini: Acetylation neutralizes the positive charge of N-terminal lysine residues, while phosphorylation introduces a negative charge at a conserved serine (position 10) of histone H3. Each histone can be acetylated at multiple

conserved positions; for example, histone H4 can be acetylated at four positions, and the ratio of acetylation of each position varies depending on whether H4 is newly deposited on chromatin (87, 88). This has important implications for potential regulatory mechanisms, as acetylation of multiple sites on each of the eight histone monomers in a nucleosome would dramatically alter the overall charge of the nucleosome, and hundreds of different nucleosomal charge distribution states might exist in vivo as a result of different combinations of acetylation patterns on the eight tails in each nucleosome.

Histone N termini have a disordered structure in solution and in crystals, as evidenced by nuclear magnetic resonance (NMR) structural studies (89) and by the lack of order in each increasingly resolved crystal structure (however one portion of the H4 tail is structured in the most recent crystal structure) (21; see below). These tails therefore do not appear to have a static interaction within the particle in which they are found; instead they may interact with other DNA sequences or with other proteins in a manner that contributes to changed functional properties of the nucleosome. The role of the N termini has been explored by characterizing nucleosomes with increased levels of acetylation or nucleosomes that have been treated with trypsin to remove the tails.

Treatment of nucleosome cores with limited amounts of trypsin removes the histone N termini while leaving the nucleosome core structure intact (90, 91). Initial studies revealed surprisingly few changes in the properties of mononucleosomes following trypsinization (92, 93). The extended structure that mononucleosomes form as salt increases was not significantly altered by removal of the tails; nor were there significant changes in circular dichroism (CD) spectra. Trypsinized mononucleosomes appear to be more accessible to proteins, as DNAse cleavage and binding of sequence-specific DNA-binding proteins are substantially enhanced by trypsinization (65, 93–95). Thus, the tails on purified mononucleosomes can impede access of proteins to nucleosomal DNA even though these domains are not required for the formation of nucleosomes. These studies with single nucleosome cores implicate a function of the histone tails in modulating access of factors to DNA sequences within the primary level of chromatin structure. One possibility is that removal of the histone tails enhances the transient unpeeling of DNA from the histone octamer (see above), thus facilitating factor binding. Indeed, by stimulating the binding of individual factors, removal of the core histone tails reduces the apparent cooperativity of multiple factors binding to nucleosomes (65).

In addition to the effects of the histone tails observed with mononucleosomes, studies with arrays of nucleosomes highlight additional functions of the core histone tails in chromatin structure. Arrays of nucleosomes form a compacted structure, as measured by sedimentation rate, as the NaCl concentration increases from less than 10 mM to 150 mM or as Mg concentration increases to

2 mM (96–98). Increased compaction under these conditions can also be seen by electron microscopy and by nondenaturing gel electrophoresis. Trypsinized cores do not form this compacted structure (99, 100). Each nucleosome in a trypsinized array is more senstive than normal nucleosomes to micrococcal nuclease cleavage at low salt, creating a 106-bp protected fragment instead of a 146-bp fragment. This sensitivity decreases as salt is increased, but the fully compacted structure is not formed. This sensitivity to micrococcal nuclease is similar to the increased sensitivity of acetylated mononucleosomes to DNase (101) and might reflect the same alteration. Although the effect of the histone tails on the condensation of nucleosome arrays may be indirect (e.g. contributing to necessary charge shielding of the DNA backbone), these experiments raise the intriguing possibility of important interactions between the tails and linker DNA and/or adjacent core particles that participate in formation of a higher-order structure. Thus, interactions involving the histone tails may not be constrained to their own nucleosome, and histone tails may be available for interaction with other proteins (see below).

Similar conclusions can be drawn from comparisons of nucleosomes with high and low levels of acetylation. There are no dramatic changes in biophysical properties of mononucleosomes upon acetylation. However, there are changes in access of DNA-binding proteins to the mononucleosome: DNase cleavage is enhanced at a position 60 bp from one end (73, 102, 103), and transcription factor access can be increased by acetylation (94, 104). Acetylation does have a pronounced effect on supercoiling of arrays of nucleosomes (105, 106). In a normal nucleosomal array, each nucleosome introduces approximately one negative supercoil into a closed circular plasmid. In arrays formed with hyperacetylated nucleosomes, each nucleosome introduces approximately 0.8 negative supercoils, and this 20% decrease appears to be due largely to acetylation on histones H3 and H4 (105). Similar effects can be seen on a subpopulation of plasmids in intact cells following butyrate treatment to increase intracellular acetylation levels (107); however, no changes in linking number were seen in simian virus 40 (SV40) minichromosomes as a result of increased acetylation (108). Thus, in a purified in vitro system, or in vivo at very high template concentration, acetylation induces a change in topology. This change is not seen in vivo at lower template concentration. One possible interpretation is that tails can alter nucleosome structure to affect long-range interactions (as above), and acetylation can affect that process. However, in vivo other proteins might interact with tails to alter these changes.

Histone tails might interact with DNA, with other proteins, or with both. Nonacetylated tails have a strong positive charge, suggesting an ability to bind tightly to DNA. The ability of tails to interact with DNA has been examined by chemical strategies designed to map regions of histones that contact DNA and

by thermal denaturation experiments. A peptide representing the N-terminal tail of histone H4 is able to interact with DNA, as shown by an increase in the denaturation temperature of DNA when the peptide is present (109). However, this effect is seen only at low salt concentrations and is not seen at physiological levels of salt. Acetylation of tails reduces this interaction, a result that is consistent with the decreased positive charge of acetylated tails.

An analysis of the interaction of H2A N terminus with nucleosomal DNA was obtained by placing a cross-linking agent at specific residues on the H2A tail and assembling the modified histone into mononucleosomes (110). This site-specific cross-linking agent interacts with DNA at two specific sites on the nucleosomal DNA that are symmetrically disposed about 40 nucleotides from the dyad. This is consistent with a specific interaction between each of the two H2A tails in a nucleosome and two specific, symmetric sites. The specificity of the observed interaction is most simply interpreted as showing a specific localization of the H2A tail in these mononucleosomes. The histone H4 N terminus can be cross-linked to regions near the dyad (111). Chemical modification studies have been used to examine how the sites where H2B and H3 contacts DNA change in sea urchin sperm chromatin when linker DNA is present. The modified H2B that is found in this organism was shown to contact linker DNA (112). These data show that histone tails can interact with DNA. However, all of these studies have been performed using either purified mononucleosomes or purified arrays of nucleosomes. It is unclear how many of these contacts would occur in a nuclear environment where many other proteins might interact with the tails.

Whereas histone tails are not ordered, the high-resolution crystal structure of the nucleosome shows that the tails of histones H3 and H2B emerge from the core by tracking through a tunnel made by the minor grooves of adjacent DNA helices; thus they have significant DNA contact (21). These contact points are not near the points of acetylation, however, and there is no observed contact between DNA and the acetylated regions of the tails that are not visible in the crystals. The nucleosome preparation used in the crystal structure study has no linker DNA, so it is possible that an ordered interaction between tails and DNA would be seen in the presence of linker DNA. It seems likely, however, from the biochemical data discussed above and from genetic studies, that the tails are not bound tightly to DNA but rather might have transient interactions with DNA that make them available for interactions with nuclear proteins.

One such interaction is seen in the crystal structure (21). Amino acids 16–24 of histone H4 are bound to a negatively charged face of histones H2A and H2B on an adjacent nucleosome. There might be an artifactual contribution of crystal packing to this interaction. The interaction is particularly interesting because this region of histone H4 has been shown by genetic and biochemical studies to

interact with the gene-silencing protein SIR3 of *S. cerevisiae* (113–117). The SIR proteins are believed to form a stable structure that coats nucleosomal arrays, with SIR3 functioning via a direct contact with the histone H4 tail. SIR3, therefore, might alter long-range nucleosomal stability by removing one interaction between nucleosomes and replacing it with a presumably more stable structure. This interaction site is directly adjacent to where acetylation occurs, and it indeed contains lysine 16, one of the sites of acetylation. Acetylation might be expected to weaken the interaction between the H4 N-terminal tail and this negatively charged region of H2A and H2B by decreasing the positive charge on H4. This structural information provides a possible explanation for a paradoxical observation: Mutation of a yeast deacetylase complex increases silencing at telomeres, the opposite of the expected effect (deacetylation most normally being associated with repression, thus mutating a deacetylase complex should decrease silencing) (115). Silencing at telomeres requires SIR3 interaction, so it is possible that the increased acetylation of H4 in mutant deacetylase strains increases the access of SIR3 to the H4 tail by decreasing H4 interactions with the adjacent nucleosome or with DNA.

The basic hypothesis that underlies the above example can be stated as follows: Histone tails are able to form transient interactions with various elements of nucleosome structure in a manner that leaves them sufficiently weakly bound (with a sufficiently high off-rate) that they are available for interactions with other proteins, including repressing proteins such as the SIR complex, or enzymatic activities such as the acetylase and deacetylase activities discussed in the following section. This hypothesis is consistent with the biochemical and genetic data and implies that the tails form critical handles that can be used to alter nucleosome structure in a manner that facilitates appropriate regulation. A central role for tails, particularly H3 and H4 tails, in modulating nucleosome structure might be the underlying reason that the sequence of the tails has been so strictly conserved in evolution.

Histone Acetyltransferases

Since the initial observation that core histones are posttranslationally modified by acetylation (118), strong correlations between histone acetylation and gene transcription (119–121) and histone deposition during chromatin assembly (122–126) have been developed. This generated a long-standing interest in the enzymes that catalyzed this modification. For example, numerous studies have used fractionated cell extracts to identify and characterize histone acetyltransferases (HATS) from organisms as diverse as rat (127–130), calf (131, 132), *Drosophila* (133), yeast (134–137), *Tetrahymena* (121), corn (138, 139), and *Physarum* (140). These studies made the important observation that there are multiple forms of cytoplasmic and nuclear histone acetyltransferases

and that they often differed with regard to the histones they acetylated (127, 129, 135, 138, 140). Thus, HATs are grouped into two broad categories. These are the nuclear type A HATs, which acetylate chromosomal histones (131, 141–144), and cytoplasmic type B HATs, which acetylate free cytoplasmic histones prior to chromatin assembly (133, 141, 144). The recent purification and cloning of type A and type B HATs have revealed remarkable insights regarding their functions.

CYTOPLASMIC, TYPE B, HATS Newly synthesized histones H3 and H4 are modified by acetylation (123) and are subsequently deacetylated after their assembly into chromatin (124). The newly sythesized and acetylated H3 and H4 are assembled into a chromatin assembly complex (CAC), which also contains the chromatin assembly factor CAF-1 (145). CAF-1 comprises three subunits (p160, p60, and p48) and promotes nucleosome assembly during in vitro DNA replication and DNA repair (146–149). Analysis of CAC with H4 acetyl-lysine–specific antibodies suggests that it contains H4 proteins acetylated at lysines 5, 8, and 12 (145). H4, which is acetylated at lysines 5 and 12, is also found in newly assembled chromatin (150), partially consistent with the premise that it is deposited by CAC. The small subunit of CAF-1, p48, is identical to RbAp48 (retinoblastoma-associated protein 48) (145), previously identified through interactions with retinoblastoma protein in vitro (151, 152). *Drosophila* CAF-1 also contains an RbAp48 homologue (153). p48 appears to play multiple roles in histone metabolism. It is also tightly associated with HD1, a human histone deacetylase (154, also see below). Its *S. cerevisiae* homologue, Hat2p, is a subunit of a yeast type B HAT (155). Moreover, that p48 and Hat2p bind directly to histone H4 (145, 155) suggests that p48 may funtionally link histone H4 acetylation with assembly into chromatin and maturation/deacetylation (156).

Whereas little is known about cytoplasmic HATs that modify H3, type B HATs that acetylate H4 have been purified from yeast and corn (155, 157). Both enzymes are specific to free histones (i.e. not associated with DNA) and contain two subunits of approximately 45 and 50 kDa. Sequencing of the large subunit of the yeast enzyme, Hat1p, led to its identification as the catalytic subunit and product of the HAT1 gene (155). The HAT1 gene was also identified by enzymatic screens of mutants defective in H4 acetylation (158). Yeast Hat1p is the predominant cytoplasmic HAT and acetylates H4 primarily on lysine 12 (155, 158). Moreover, the activity of the corn enzymes generates diacetylated H4, suggesting that it also acetylates a second lysine, presumably lysine 5 (157). Thus, the higher eukaryotic enzymes may have expanded specificity, generating H4 products more closely resembling those found in human CAC (145) or newly assembled chromatin (150). As mentioned above, the small subunit of the yeast

HATB, Hat2p, was identified as the histone-binding subunit and the homologue of RbAp48, leading to the proposal that the Hat2p/RbAp48 family of proteins serve as escorts of histone metabolism enzymes (155).

NUCLEAR, TYPE A, HATS A major breakthrough in the study of type A HATs came with the initial purification and cloning of a catalytic subunit. Brownell & Allis used an in gel activity assay to identify the polypeptide, p55, corresponding to the catalytic subunit of a type A HAT from *Tetrahymena* (159). Purification and cloning of p55 led to its identification as a homologue of the yeast transcriptional adaptor protein Gcn5 (160). Gcn5 was originally identified through genetic screens in yeast as a protein that functionally interacts with the transcriptional activator Gcn4 (161) and with the activation domain of the herpes simplex virus protein VP16 (162). Thus, the discovery of Brownell and colleagues that Gcn5 is a type A HAT provided a direct link between pathways of transcription activation and histone acetylation (160). Indeed, recombinant yeast Gcn5 was shown to acetylate lysines in histones H3 (lysine 14) and H4 (lysines 8 and 16) that are thought to be sites of transcription-linked acetylation in vivo (163). Moreover, the function of Gcn5 in vivo requires its identified HAT domain indicating that this catalytic activity is crucial to the function of the Gcn5 transcriptional adaptor (164, 165).

Additional genetic screens in yeast for mutants that relieved GAL4-VP16–mediated toxicity in vivo revealed additional genes whose products were thought to be functionally related to that of Gcn5. These include ADA2 (166), ADA3 (167), ADA5/SPT20 (168, 169), and ADA1 (170). Ada2, Ada3, and Gcn5 were shown to physically interact with each other (162, 171, 172). High molecular weight complexes of 170–200 kDa, 800–900 kDa, and 1.8–2.0 MDa containing Ada and Gcn5 proteins have been identified in yeast (170, 173–175). Moreover, analysis of mutant strains has shown that Gcn5 is the primary if not only catalytic HAT subunit of each of these HATA complexes (174, 175).

Mutants in ADA1 and ADA5 show more severe phenotypic defects than mutants in the other ADA genes and also display *spt*-phenotypes (168–170). In fact, ADA5 was simultaneously discovered in a screen for SPT mutants and named SPT20 (169). SPT genes have been isolated in genetic screens for suppressors of Ty and delta promoter insertion mutations (176). Genetic and biochemical studies illustrated interactions between Spt3, Spt7, Spt8, Spt20, and TBP (177–179), suggesting that these proteins might constitute a multiprotein complex. These observations prompted testing of the 0.8-MDa and 1.8-MDa ADA/GCN5 HAT complexes for the presence of Spt proteins. Both complexes contain Ada2, Ada3, and Gcn5, and the larger complex was found to also contain Spt3, Spt7, Spt8, and Spt20/Ada5 (174). Although both complexes contain several yet unidentified proteins, neither complex appears to contain

the TATA-binding protein, TBP, which is encoded by SPT15. Thus, the 0.8-MDa complex has been termed the ADA complex while the 1.8-MDa complex has been termed the SAGA complex (Spt-Ada-Gcn5-Acetyltransferase) (174).

The SAGA complex provides a link between histone acetylation by Gcn5p and the function of the Spt and Ada proteins. This complex contains proteins that interact with acidic activation domains and TBP (179–182) and appears to be a primary transcriptional regulator in yeast. Further genetic analysis indicates that SAGA carries out multiple functions, a subset of which are dependent on Gcn5 and thus presumably histone acetylation (179). Moreover, mutations in components of the SWI/SNF chromatin remodeling complex (see below) and SRB/Mediator coactivator complexes cause lethality in *spt20/Ada5* deletion mutants. Thus, the SAGA, SWI/SNF, and SRB/Mediator complexes appear to represent a trio of partially redundant activities that provide a mutual backup system for appropriate regulation of RNA polymerase II transcription (179).

In addition to the Gcn5-containing ADA and SAGA adaptor HAT complexes, there are other HAT complexes in yeast that neither contain the Spt or Ada proteins described above nor use Gcn5 as a catalytic subunit (174). However, GCN5 belongs to a diverse superfamily of genes (GNATs) that share four regions of sequence homology, including domains implicated in enyzmatic activity (183). In addition, screens for genes enhancing mating defects in *Saccharomyces* have revealed a separate family of tentative nuclear histone acetyltransferases (the MYST family). Of these, the SAS2 and SAS3 (SAS, something about silencing) genes contain regions of homology to GCN5, HAT1, and other acetyltransferases as well as extensive homology to each other throughout the rest of their sequence (184). SAS2 and SAS3 are also closely homologous to the HIV Tat-interacting protein, Tip60 (185), and the MOZ gene product, which is a translocation partner in the MOZ-CBP chimaeric oncogene (186). Thus, the GNAT and MYST gene families represent potential catalytic subunits of yet uncharacterized nuclear HAT complexes.

The discovery of nuclear proteins that function as type A HATs from mammals has strengthened the link between histone acetylation and transcription activation and provided a possible link to cellular transformation. Both p300 and p/CAF can function as HATs. p300 and its closely related functional homologue, CBP, are transcriptional adaptors that interact with numerous transcription factors including CREB, Jun, Myb, Fos, MyoD, and nuclear hormone receptors (187–196). Moreover, p300/CBP interacts with the adenovirus E1A transforming protein (197, 198). P/CAF binds to p300 and CBP (199) and is also closely homologous to yeast GCN5 and the human homologue hGCN5 (200). Recombinant p/CAF acetylates primarily H3 while recombinant p300/CBP acetylates all four core histones, suggesting that the two catalytic subunits might function within a single adaptor complex to efficiently acetylate nucleosomal histones.

In addition, recombinant forms of p/CAF and p300/CBP effectively acetylate histones within nucleosomes (199, 201). By contrast, recombinant yeast Gcn5 does not modify nucleosomal histones (199), but Gcn5 does so within native HAT complexes (174).

While these data suggest that histone acetylation might be one mechanism through which these proteins regulate gene expression, other nuclear proteins may be crucial acetylation targets. For example, in addition to histones, p300 will acetylate the tumor suppressor protein, p53, enhancing its in vitro DNA-binding activity (202). In this instance it is not clear whether histones, p53, and/or other nuclear proteins are the critical targets for p300 action in vivo, an issue that also pertains to other proteins that have been identified as histone acetyltransferases.

Acetylation might be involved in other regulatory events in mammals. p300/CBP and pCAF are recruited to ligand-bound nuclear hormone receptors by an additional coactivator, ACTR, which also has HAT activity on both free histones and nucleosomes (203). Thus, during transcriptional activation by ligand-bound nuclear hormone receptors it appears that a multiprotein complex (204) containing three distinct HAT activities (ACTR, p300/CBP, and p/CAF) is assembled by the receptor to modify nucleosomal histones. Importantly, the recruitment of these HATs by the receptors, and presumably the acetylation of nearby nucleosomes, is ligand dependent (203). The importance of histone acetylation during activation by the receptors is further illustrated by the fact that the unliganded receptors instead recruit corepressor complexes that carry histone deacetylase activity (see below). Steroid receptor–responsive promoters are sufficiently defined in terms of nucleosomal structure that it should be possible to examine in detail how the acetylation state of these promoters is changed as various receptor agonists and antagonists are introduced.

In addition to the yeast ADA and SAGA complexes and mammalian p/CAF-p300/CBP, further coactivator complexes might carry out histone acetylation as part of their functions. For example, human $TAF_{II}250$ and its yeast homologue also contain HAT activity (205). $TAF_{II}250$ is part of the TFIID complex that also contains TBP, the TATA-binding protein. The presence of a HAT activity in TFIID suggests that histone acetylation may be particularly important at the core promoter (i.e. TATA-box) to mediate transcription factor interactions with nucleosomal DNA. In addition to HAT activity, $TAF_{II}250$ preparations are able to phosphorylate the basal transcription factor RAP74 (206). $TAF_{II}250$ is therefore a multifunctional enzyme that catalyzes at least two distinct reactions as well as serving as a core subunit of TFIID. Thus, $TAF_{II}250$ provides an example of a protein that can function as a HAT but that has other activities relevant to gene regulation. This is likely to be true of other HATs and HAT complexes as well (e.g. see 179).

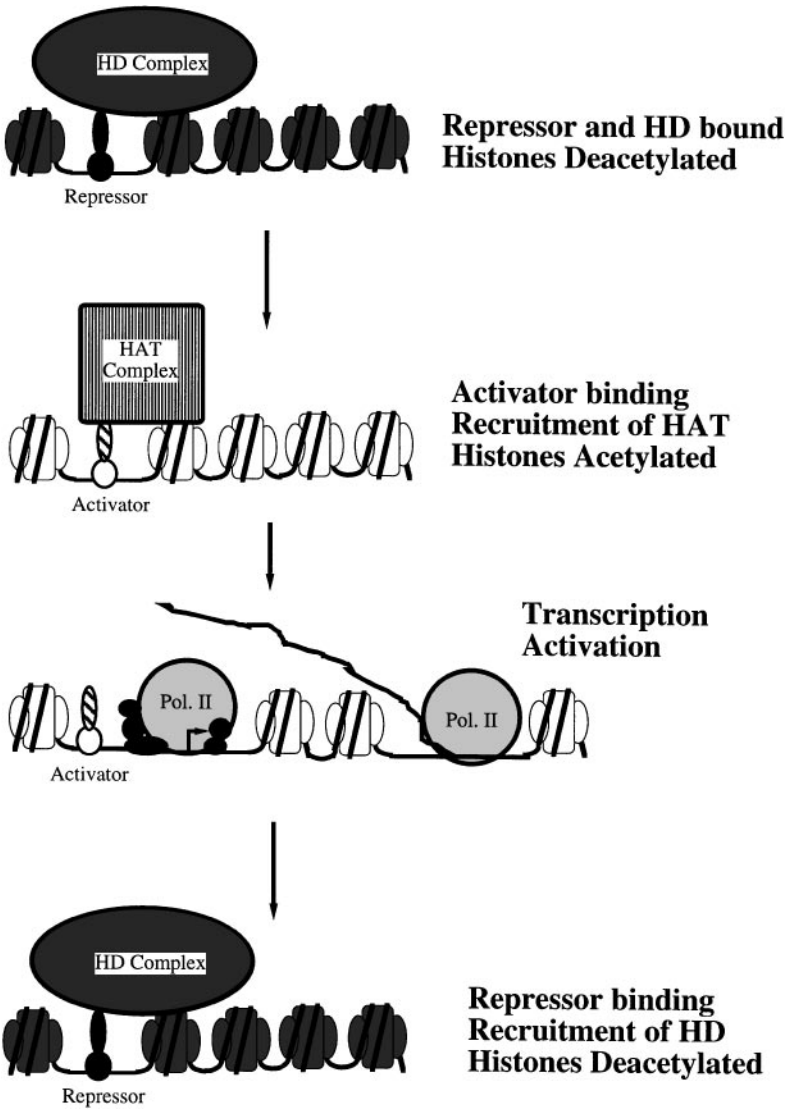
Histone Deacetylases

For histone acetylation to play an important role in signaling pathways controlling transcription, efficient reversal of the modification (i.e. histone deacetylation) is crucial (Figure 2). The importance of histone deacetylase enzymes (HDs) in the turnover of the acetate modification became clear from early studies that illustrated that inhibition of HD activity led to an accumulation of acetylated histones in vivo (207). Multiple forms of HDs have been characterized in several organisms (139, 140, 208–210), and like HATs, they appear to be found in high molecular weight complexes (211–214). As discussed below, some histone deacetylases have been linked to RNA polymerase II transcription; however, there are likely to be additional HDs that perform other functions in chromatin. For example, purification of a Maize histone deacetylase, HD2, has led to its identification as an acidic nucleolar phosphoprotein that may regulate ribosomal gene chromatin structure and function (215).

A direct link between histone deacetylases and transcription resulted from the purification and cloning of a mammalian HD, called HDAC1, which was found to be homologous to the yeast Rpd3 protein (154). A related protein, HDAC2, was found in screens for corepressors that interact with the YY1 transcription repressor/activator (216). Rpd3 was found to be the catalytic subunit of a 600-kDa yeast HD complex, HDB (213, 217). A second yeast HD complex, HDA, is distinct from HDB (350 kDa) and contains the Hda1 protein, which is similar to Rpd3 (213, 217). Moreover, the presence of three additional ORFs in yeast (HOS1, HOS2, HOS3) that are similar to RPD3 and HDA1 suggest that there may be additional HD complexes (217).

Rpd3 and Hda1 may act as both negative and positive regulators of transcription. Deletions of Rpd3 or Hda1 increase repression at telomeric loci in yeast, and mutations in a *Drosophila* RPD3 homologue increase position effect variegation (115, 217). These observations suggest that in these contexts, Rpd3 and Hda1 counteract repression mechanisms. However, it is not yet known in this instance whether Rpd3 and Hda1 act directly or indirectly in relief of repression.

Figure 2 Tentative model of the action of HD and HAT complexes. Sequence-specific DNA-binding repressor proteins recruit HD complexes to the promoter region of a gene, maintaining nucleosomal histones in the deacetylated state (black nucleosomes), which is refractory to transcription. The binding of sequence-specific activator proteins recruits HAT complexes, which leads to acetylation of the nucleosomal histones (white nucleosomes). This acetylation in turn leads to conformational changes within the nucleosomal array. The acetylated nucleosomes permit the recruitment of general transcription factors and RNA polymerase II, which bind the promoter and initiate transcription. The rebinding of a sequence-specific repressor recruits HD complexes, which deacetylates the nucleosomal histones and thus leads to the cessation of transcription.


HD Complex
Repressor
Repressor and HD bound
Histones Deacetylated
HAT
Complex
Activator
Activator binding
Recruitment of HAT
Histones Acetylated
Pol. II
Pol. II
Activator
Transcription
Activation
HD Complex
Repressor
Repressor binding
Recruitment of HD
Histones Deacetylated

For example, Rpd3 might counteract silencing by acting as a repressor, reducing expression of genes encoding other repressor proteins.

The possibility that Rpd3 functions directly as a repressor protein is supported by numerous biochemical and genetic studies. Rpd3 acts as a negative regulator of genes that are also negatively regulated by the Sin3 protein (218–222). Sin3 is physically associated with Rpd3; both are contained in a very large, 2-MDa, complex (214, 223). The relationship between this 2-MDa complex and the 600-kDa HDB complex described above is not clear (other than that they both contain Rpd3). One possibility is that the 600-kDa complex is a subcomplex that associates with additional proteins to form the larger complex. Alternatively, Rpd3 may function in two distinct complexes. Neither Sin3 nor Rpd3 binds DNA directly; however, LexA fusion proteins containing either Sin3 or Rpd3 repress transcription from reporter genes bearing lexA sites, suggesting that they act as corepressors (223, 224).

The Sin3/Rpd3 corepressor complex interacts with sequence-specific DNA-binding proteins, which suggests that this interaction is the mechanism of its targeting to specific promoters. Sin3 binds directly to Ume6, a sequence-specific DNA-binding protein that recognizes URS1, an upstream repression sequence found in several yeast promoters (223). Thus, the picture emerging from the studies in yeast is that a specific DNA-binding protein that binds repressor elements recruits the Sin3/Rpd3 corepressor complex, which mediates transcriptional repression and histone deacetylation. It will be interesting to see whether HDA is similarly targeted to yeast promoters. It is not yet clear whether histone deacetylation is the primary mechanism of transcriptional repression mediated by the Sin3/Rpd3 complex. It is likely that the complex performs multiple functions, some of which may play a more prominent role in transcription repression. However, the catalytic deacetylase subunit, Rpd3, is clearly required for repression by Sin3 (223). Generation of mutants in Rpd3 that reduce its deacetylase activity but leave the Sin3/Rpd3 corepressor complex intact will help address the importance of histone deacetylation in its repression function.

Simultaneous studies of corepressor complexes in mammals have strengthened the connection between transcriptional repression and histone deacetylases. Mad/Max and Mxi/Max heterodimers and unliganded retinoid and thyroid hormone receptors function as sequence-specific transcriptional repressors (225–228). Mad represses transcription via interactions with mSin3A or mSin3B, mammalian homologues of yeast Sin3 protein (229, 230). Repression of the unliganded thyroid and retinoid receptors is mediated via SMRT and N-CoR corepressors (227, 228). N-CoR and SMRT interact directly with mSin3A/B (231–233). mSin3A/B interacts with the Rpd3 homologues HDAC1 and/or HDAC2 (231–234) to form high molecular weight corepressor complexes

that also contain RbAP 46/48 and several novel proteins (235, 236). Transcriptional repression by the mSin3/HDAC complexes is blocked by histone deacetylase inhibitors (Tricostatin A, trapoxin), implicating deacetylase activity in the transcriptional repression mechanism. As mentioned above, RbAp48 is also the histone-binding subunit (HAT2) of the yeast cytoplasmic type B HAT (155), suggesting that it might similarly connect the corepressor/deacetylase complex to histone substrates.

As noted above in discussing acetylase activities, a caveat in interpreting these data is that there may be further protein substrates for these deacetylase complexes. The finding that tumor suppressor protein, p53, is acetylated by p300 makes this possibility more intruiging (202). However, the model of recruitment of corepressor/deacetylase complexes by sequence-specific DNA-binding repressor proteins fits nicely with the recruitment of coactivator/HAT complexes by sequence-specific DNA-binding activators. This is illustrated most clearly by the switch for nuclear hormone receptors, which in their unliganded form recruit mSin3-HDAC repressor complexes (231–233) and when bound by hormone recruit the p300/pCAF coactivator/HAT complex (195, 196). Moreover, the recruitment of histone-modifying enzymes (HATs, HDs) for both activation and active repression strongly implicate nucleosome functions in the control of transcription.

ATP-DEPENDENT REMODELING COMPLEXES

Genetic and biochemical studies have identified complexes that are able to alter nucleosome structure in an ATP-dependent manner. These studies imply that at least some, if not all, of these complexes play important roles in regulating the activation of transcription by increasing access of the transcription machinery (237). Five related complexes of this type have been identified (the SWI/SNF, NURF, RSC, CHRAC, and ACF complexes), and each of these complexes can increase either transcription factor binding or restriction enzyme access to nucleosomal DNA (238–246). Although the mechanism(s) by which these complexes function has not yet been elucidated, it appears that they increase accessibility to the DNA of the nucleosome without removing histones. This suggests that they must alter histone-DNA contacts either by remaining in contact with the nucleosome to actively disrupt these contacts or by catalyzing the formation of a remodeled configuration of histones and DNA that has weakened histone-DNA contacts. Four important questions regarding function of these complexes are as follows: (*a*) Does each of the ATP-dependent remodeling complexes function by the same mechanism? (*b*) What is the configuration of histones and DNA in a remodeled nucleosome? (*c*) Will a remodeled nucleosome remain remodeled following the removal of the remodeling complex

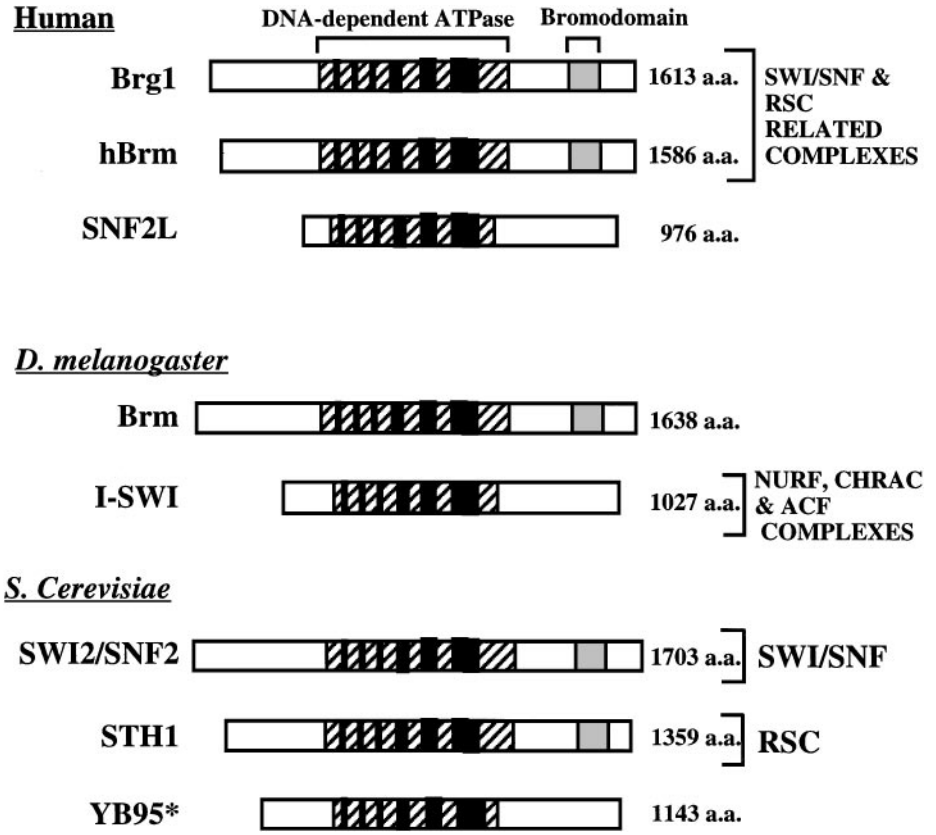


Figure 3 The ATPase subunits of known ATP-dependent chromatin remodeling complexes. SWI/SNF2 and STH1 are homologous to BRG1 and hBRM in humans and Brm in *Drosophila*. ISWI is homologous to YB95 (and several other potential homologues) in yeast and hSNF2L in humans. Complexes are indicated where they have been identified and shown to alter some aspect of chromatin structure in an ATP-dependent manner.

(i.e. how stable is the remodeled state?) (*d*) Are these complexes targeted to specific regions of chromatin, and if so, what does the targeting?

There are likely to be significant differences in the mechanisms used by these complexes to remodel nucleosomes. The five complexes can be divided into two families based on protein composition (Figure 3). The SWI/SNF and RSC complexes each contain numerous subunits: SWI/SNF has 11 subunits in yeast (240, 245, 247), and the putative homologue has 8 subunits in humans (238, 248, 249), while the RSC complex has approximately 15 subunits (246).

The DNA-dependent ATPase subunits of SWI/SNF and RSC are similar to each other (see Figure 3), as are at least three other subunits (246). This similarity suggests that SWI/SNF and RSC might alter nucleosome structure using similar mechanisms, and it further suggests that these complexes are evolutionarily related. In humans, the hBRM and BRG1 genes (250–252) appear to be homologues of the SWI2/SNF2 and STH1 genes of yeast, so it is possible that the hBRM and BRG1 proteins organize two separate complexes that correspond to SWI/SNF and RSC. Both BRG1 and hBRM proteins have been associated with complexes that can alter nucleosome structure in an ATP-dependent manner, and immunoprecipitation experiments imply that complexes exist that contain only hBRM or only BRG1 (248, 249, 253).

Genetic studies suggest that there are differences between the roles that SWI/SNF and RSC play in vivo. Deletion of genes that encode components of the SWI/SNF complex is not lethal to growth, whereas deletion of RSC components is lethal (239, 254, 255). Temperature-sensitive mutations in subunits of RSC affect cell cycle progression, whereas there are no known effects of SWI/SNF mutations on cell cycle (256). In human cells, the BRG1 gene cannot be deleted unless BRG1 is expressed from an ectopic source, suggesting that the BRG1-based complex might be essential to cell viability, similar to RSC (257). Deletion of the hBrm gene is not lethal. These considerations, in addition to the fact that BRG1 is slightly more similar to STH1 than to BRG1, raise the possibility that the BRG1-based complex and RSC have similar functions in yeast and humans. These differences between SWI/SNF and RSC function in vivo might reflect differences in their mechanism of action. Alternatively, these phenotypic differences might reflect differential requirements for SWI/SNF and RSC in cellular processes and different abilities of these complexes to receive signals and be targeted to specific regions of chromatin.

The NURF (241, 242, 258), CHRAC (243), and ACF (244) remodeling complexes have been isolated from *Drosophila* extracts. Each contains the ISWI protein, a DNA-dependent ATPase that has homology to SWI2/SNF2 only in the ATPase domain. In addition to ISWI, these complexes contain either three or four additional subunits, so these complexes are smaller than the SWI/SNF family of complexes. Another difference is that the SWI/SNF ATPase is stimulated by bare DNA or by nucleosomal DNA whereas the NURF ATPase is stimulated only by nucleosomal DNA (242). These data suggest that there might be differences in the function between the ISWI-based complexes and the SWI/SNF family of complexes.

The potential for differences in function between ATP-dependent remodeling complexes is further substantiated by the finding that the different ISWI family complexes have different characteristics in vitro. CHRAC will remodel nucleosome arrays to increase restriction enzyme access in a manner that is

not seen with NURF when the two activities are compared side by side (243). In addition, CHRAC contains Topoisomerase II as a subunit, whereas NURF does not. Although it is not clear what activity is contributed to CHRAC by Topoisomerase II, because inhibitors of Topoisomerase II function do not alter the basic remodeling activity of CHRAC, it seems likely that Topoisomerase II plays an important role in the mechanism of action of CHRAC. Whereas ACF and CHRAC share similar abilities in promoting appropriate spacing of nucleosomes on plasmid templates, ACF does not contain Topoisomerase II and thus is likely to have some mechanistic differences when compared to CHRAC (244).

Two types of models explain how any of these remodeling complexes might use the energy of ATP hydrolysis. This energy might be used in a power-stroke mechanism that catalyzes an isomerization of the nucleosome structure into a different structure with the same components, but with altered histone-DNA contacts (Figure 4*b*,*c*), or ATP hydrolysis might be used to promote continual movement of the complex around the nucleosomal DNA (259), with the complex physically prying the DNA away from the histone core as it moves (Figure 4*a*). These two models currently cannot be rigorously evaluated for either the SWI/SNF or ISWI families of complexes. The latter model is argued against in the case of SWI/SNF function by the observation that ATP is not required to maintain a disrupted state of a nucleosome (260), although it is conceivable that removal of ATP freezes the nucleosome in a disrupted state with SWI/SNF still present. The central distinguishing feature of these models is that the first model posits the existence of an altered nucleosome structure that might be stable in the absence of the remodeling complex.

Functional analysis of the NURF complex implies that NURF contacts the histone N termini to alter nucleosomal structure. Truncation of the N termini from nucleosomes blocks remodeling by NURF and also inhibits the ability of these nucleosomes to stimulate ATPase activity (261). This inhibition appears to be caused by a direct interaction between the N termini and NURF, because isolated tails of histones can act as inhibitors of NURF ATPase activity. These data are most consistent with a model in which NURF alters structure of the nucleosomes and uses the tails as part of a handle to accomplish this alteration. These data are less consistent with a model in which NURF continually translocates around the nucleosome, because it is not clear why histone tail contact would be required for this translocation.

The ability of chromatin remodeling complexes to catalyze formation of an altered nucleosomal state might be the first step of a mechanistic pathway that leads to a stable alteration in chromatin structure. For example, the combined action of SWI/SNF and the binding of multiple GAL4 derivatives can mediate displacement of histone octamers from nucleosome cores and generate

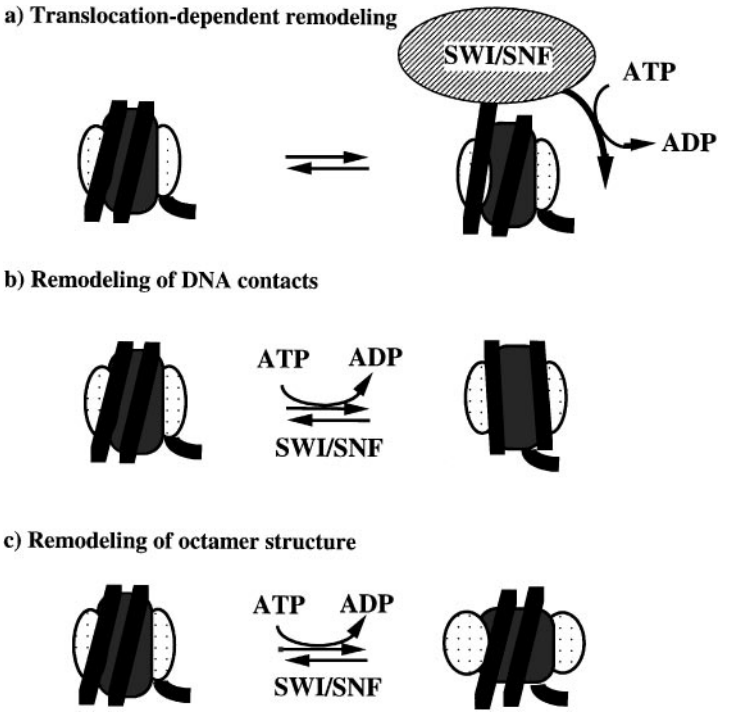


Figure 4 Three different mechanisms that might be used to alter nucleosomal structure by ATP-dependent remodeling complexes (using SWI/SNF as an example): (*a*) Continual ATP hydrolysis might be required to separate DNA from histones (259); (*b*) histone-DNA contacts might be altered by a mechanism that alters the path of DNA around an unaltered histone octamer; and (*c*) histone-DNA contacts might be altered by a mechanism that changes the conformation of the histone octamer in a manner that weakens bonds to DNA. An ATP-dependent movement such as that shown in (*a*) could lead to more stable structural changes such as those shown in (*b*) or (*c*). It is also possible that both the path of the DNA and the histone octamer might be altered—that is, a combination of (*b*) and (*c*).

DNAse 1 hypersensitive sites that persist after removal of the SWI/SNF complex (262). It is not clear whether this persistent chromatin remodeling requires the SWI/SNF complex and the binding of the transcription factors to happen in concert or whether SWI/SNF first catalyzes a remodeled state, and GAL4 binds only after the large SWI/SNF complex has dissociated from the remodeled nucleosome. The conformation of nucleosomes that have been remodeled by these ATP-dependent remodeling activities may resemble one of the altered nucleosome states that has been identified by varying solution conditions (discussed

above). These biophysical data emphasize that nucleosome structure can be dynamic in solution, providing precedence for the possibility that these complexes use the energy of ATP hydrolysis to drive the nucleosome into an altered structure.

The SWI/SNF complex was initially identified via genetic studies in *Saccharomyces cerevisiae*, and mutations in histones have been isolated that suppress mutations in the SWI/SNF genes (263, 264). The location of these mutations on the crystal structure of the nucleosome informs possible models for SWI/SNF action (21). Two of the six isolated point mutations of histone H4 mutate an Arginine residue (R45) that inserts into the minor groove of DNA and is one of the key contact points between histones and DNA. Two other point mutations affect amino acids that contribute to the shape of the loop that contains R45, or directly contact R45 and DNA, indicating that weakening a normal histone-DNA contact confers a phenotype that alleviates the lack of SWI/SNF function. This further validates the belief that the primary role of this remodeling complex is to weaken histone-DNA contacts. Perhaps more intriguing are the locations of the other two point mutations. One (H3 R116) is buried in the structure and thus might contribute to altering the overall structure to favor that of a remodeled nucleosome. Another mutant (H3 E105) mutates a surface negative charge to a positive charge in a region that does not contact DNA in the solved structure. It is intriguing to speculate that this region of the surface might somehow be involved in redirecting histone-DNA contacts in a remodeled nucleosome structure (the increased positive charge stabilizing the remodeled interactions). Another possibility, however, is that altered contacts between this region and adjacent nucleosomes cause the phenotype.

Regardless of how these complexes are able to remodel nucleosomes, a critical question concerning function of the complexes in vivo concerns targeting of these activities. Several different remodeling complexes are found in cells; thus each is anticipated to play a different role in regulating nuclear processes. It is therefore important to understand the mechanism(s) that target each complex to a specific region of chromatin and those that regulate activity at specific stages of cellular development. Very little concrete information is available on this issue.

The clearest example of targeting comes from immunoprecipitation studies that show that the glucocorticoid receptor can interact with the SWI/SNF complex (265). An analysis of the possible function of this interaction demonstrated that binding the glucocorticoid receptor to a nucleosome increased the ability of SWI/SNF to disrupt the nucleosome, a result that is most simply interpreted as arguing that SWI/SNF was targeted to the nucleosome by the receptor (266). Although it is not clear from these experiments whether SWI/SNF directly contacts the glucocorticoid receptor, or whether intermediary proteins are involved,

these experiments suggest that targeting of a remodeling complex might be an important aspect of activation by the glucocorticoid receptor. Taken together with the observations that many steroid receptors bind nucleosomal DNA (267–269) and that many have also been linked to acetylation and deacetylation activities (see above), these results all point toward the possibility that regulation of chromatin structure is a general mechanism used by receptors to affect gene expression. A different mechanism of targeting is suggested by the association of SWI/SNF with yeast RNA polymerase holoenzyme (270). This might allow targeting of SWI/SNF via the targeting of RNA polymerase, although the potency of this interaction has been questioned (246). It will be informative to determine whether associations exist between SWI/SNF and RNA polymerase in mammalian systems and whether this association results in the targeting of remodeling activities when polymerase is bound to a template.

SUMMARY

Descriptive studies of chromatin structure in vitro and in vivo have led to the unequivocal conclusion that altered states of the nucleosome exist. More recent functional analysis of the role that chromatin structure plays in gene regulation has led to the possibility that these altered nucleosomal states play an important role in establishing the proper chromatin structure on a gene. Generation of a chromatin structure that is more accessible to regulatory proteins is believed to be an important mechanism in gene activation. Conversely, a more tightly ordered chromatin structure is believed to be important in repression. Stable alterations in nucleosome structure might be an important means of creating an altered chromatin state, or alterations in nucleosome structure might be transient but be an essential step in a pathway that creates a stably altered chromatin state.

The discovery of complexes of proteins that acetylate nucleosomes, that deacetylate nucleosomes, and that remodel nucleosome structure in an ATP-dependent manner provides an essential advance in understanding the role that chromatin structure plays in regulating nuclear events. The purification and characterization of these complexes will allow several questions to be addressed: How do these complexes alter nucleosome structure? What are the functional consequences of these alterations for regulation of nuclear processes? How are these activities spatially and temporally targeted? The answers to these questions are vital to an understanding of regulatory mechanisms in the eukaryotic nucleus.

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