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Review: Chromatin Structural Features and Targets That Regulate Transcription

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The nucleosome and chromatin fiber provide the common structural framework for transcriptional control in eukaryotes. The folding of DNA within these structures can both promote and impede transcription dependent on structural context. Importantly, neither the nucleosome nor the chromatin fiber is a static structure. Histone dissociation, histone modification, nucleosome mobility, and assorted allosteric transitions contribute to transcriptional control. Chromatin remodeling is associated with gene activation and repression. Energy-dependent processes mediate the assembly of both activating and repressive proteins into the nucleosomal infrastructure. Recent progress allows the structural consequences of these processes to be visualized at the chromosomal level. DNA and RNA polymerase, SWI/SNF complexes, histone deacetylases, and acetyltransferases are targeted by gene-specific regulators to mediate these structural transitions. The mistargeting of these enzymes contributes to human developmental abnormalities and tumorigenesis. These observations illuminate the roles of chromatin and chromosomal structural biology in human disease.

INTRODUCTION

Remarkable progress in the past 10 years has brought chromatin and chromosomal structure back to center stage in transcriptional control. Although substantial contributions from structural biologists elucidated many salient features of nucleosome and chromatin fiber organization during the 1970s and early 1980s, the nucleosome and higher-order chromatin structures were viewed as essentially static entities of high intrinsic stability within which DNA was sequestered (Richmond *et al.*, 1993; Thoma *et al.*, 1979).

Unfortunately biochemists and geneticists have now demolished this simple image of a homogenous inert chromatin structure. Chromatin is more complex, but also much more interesting. Recognition that chromatin was functionally specialized and structurally heterogenous came from cell biological approaches that demonstrate the compartmentalization of particular histone variants and modifications to individual chromosomes and chromatin domains (Grossbach, 1995; Hebbes *et al.*, 1988, 1994; Turner *et al.*, 1992), from genetic analysis that indicated specialized functions for individual histone domains at particular chromosomal sites (Grunstein *et al.*, 1995), and from biochemical reconstruction of chromatin architectures that can activate (Schild *et al.*, 1993) or repress transcription (Chipev and Wolffe, 1992; Howe *et al.*, 1998; Sera and Wolffe, 1998; Tomaszewski *et al.*, 1998; Wolffe, 1989).

The dynamic quality of chromatin that provides regulatory flexibility for transcription and other processes such as replication, recombination, and repair also came more clearly into focus. Posttranslational modification of the histone proteins through acetylation of lysine residues promoted structural transitions at the mononucleosome level that facilitated access of transcription factors to DNA (Lee *et al.*, 1993; Norton *et al.*, 1989; Vettese-Dadey *et al.*, 1996). Histone acetylation further destabilizes the chromatin fiber facilitating transcription (Tse *et al.*, 1998). Events such as DNA polymerase or RNA polymerase transit through chromatin displace histones from DNA. Histone H1 and H2A/H2B deficiency promotes nucleosome mobility and transcription factor access (Chipev and Wolffe, 1992; Hayes and Wolffe, 1992; Pennings *et al.*, 1994; Tse *et al.*, 1998; Ura *et al.*, 1995). Other molecular machines of the SWI/SNF family also destabilize chromatin (Hamiche *et al.*, 1999; Langst *et al.*, 1999; Tse *et al.*, 1998). How this destabilization occurs is unknown.

Interest in chromatin structure and function relationships has been fueled by the discovery that many transcriptional activators possess histone acetyltransferase activity (Brownell and Allis, 1996; Brownell *et al.*, 1996; Wolffe and Pruss, 1996) and

that transcriptional repressors recruit histone deacetylase (Pazin and Kadonaga, 1997; Taunton *et al.*, 1996; Wolffe, 1997). Transcriptional activators can selectively associate with acetylated histones (Winston and Allis, 1999) and transcriptional repressors with deacetylated histones (Edmondson *et al.*, 1996). Activated and repressed chromatin will have intrinsically different protein compositions. The structures of activated and repressed chromatin are unknown; this remains a major gap in our knowledge. Chromatin and chromosomal structure is a rich field for future investigation. Here we briefly review established facts and indicate important problems that remain to be resolved.

STRUCTURAL FEATURES OF THE NUCLEOSOME

Histone–histone and histone–DNA interactions are now understood in considerable structural detail (Arents *et al.*, 1991). The assembly of a stable nucleosome core depends on the initial heterodimerization of H3 with H4 and the subsequent dimerization of H3 to form the $(H3, H4)_2$ tetramer (Eickbush and Moudrianakis, 1978). The $(H3, H4)_4$ tetramer can form a stable complex with more than 120 bp of DNA (Hayes *et al.*, 1991b). Histones H2A and H2B form a stable heterodimer in a manner structurally homologous to H3/H4, but do not self-assemble into stable tetramer complexes (Arents *et al.*, 1991). Rather, dimers of (H2A, H2B) bind to either side of the $(H3, H4)_2$ tetramer and extend the wrapping of DNA within the nucleosome to over 160 bp (Hayes *et al.*, 1990, 1991b). This creates a left-handed superhelical ramp of protein onto which the DNA is wrapped and that is essentially composed of the four histone dimers linked end-to-end: (H2A/H2B)–(H4/H3)–(H3/H4)–(H2B/H2A) (Arents *et al.*, 1991) (Fig. 1). The H3:H3 and H2B:H4 dimer–dimer interfaces are composed of a structurally similar four-helix bundle; however, the latter does not remain stably associated in the absence of DNA in solutions containing physiological concentrations of salt. Given the stability of the individual heterodimers (Karantza *et al.*, 1996), the H2B:H4 interface is a likely site for initial disruption of histone–histone interactions upon unfolding of the nucleosome core *in vivo*. Genetic experiments on gene regulation in yeast are consistent with this hypothesis (Santisteban *et al.*, 1997).

To follow the left-handed spiral formed by the histone fold domains, the nucleosomal DNA is severely distorted into roughly two 80-bp superhelical loops. Extended α-helical structures allow the histone fold domains within each heterodimer of the octamer structure to contact approximately three double-helical turns (~30 bp) of DNA. Each contact involves an arginine residue penetrating the minor groove; several main polypeptide chain amide interactions with two consecutive phosphates on each DNA strand, and, surprisingly, substantial hydrophobic interactions with the faces of the deoxyribose sugars in the DNA (Luger *et al.*, 1997). These precise histone–DNA interactions constrain all DNA sequences, regardless of inherent sequence-dependent structure, to adopt a relatively similar conformation in the nucleosome (Hayes *et al.*, 1991a). Because of the inherent anisotropic bending moments of most unique DNA sequences, a small number of preferred rotational orientations are found for most nucleosomal DNAs. However, at this time precise sequence-dependent translational positioning of the nucleosome has been observed for only a small number of DNA sequences (Simpson, 1991; Wolffe and Kurumizaka, 1998). This is once again an important area for future research; we need to study many more specific DNA sequences and their potential function in positioning nucleosomes. This is especially true since it has been recently established that nucleosomes have a general function in activating transcription (Wyrick *et al.*, 1999). Although still poorly understood, translational positioning probably depends on how the inherent DNA structure matches the local variations in DNA curvature and helical periodicity found in the nucleosome.

External to the histone fold domains, about 25% of the mass of the histone octamer is contained within the "tail" domains. These domains, located at the N-termini of all four core histone proteins and the C-terminus of histone H2A, were initially defined by their sensitivity to proteases (Bohm and Crane-Robinson, 1984). Proteolytic removal of the tail domains does not drastically alter the conformation or hydrodynamic properties of individual nucleosomes (Ausio *et al.*, 1989) and the tails do not play a role in nucleosome positioning or the correct assembly of nucleosomes *in vitro* (Dong *et al.*, 1990; Hayes *et al.*, 1991a). These N-termini, if fully extended, can project well beyond the superhelical turns of DNA in the nucleosome (Wolffe and Hayes, 1999). Consistent with their length, centrifugation studies with nucleosomal arrays lacking linker histones indicate that the histone tails mediate internucleosomal contacts as extended chains of nucleosomes are compacted to form the 30-nm chromatin fiber. It should be noted that the tail domains "rearrange" when chromatin fibers are digested to produce nucleosome core particules or when histone octamers are reconstituted on 200-bp vs 146-bp DNA (Lee and Hayes, 1998; Usachenko *et al.*, 1996). Similarly, the function of the tail domains in chromatin arrays is markedly different than has been observed in studies of nucleosome core particles and mononucleosomes (Hansen *et al.*, 1998).

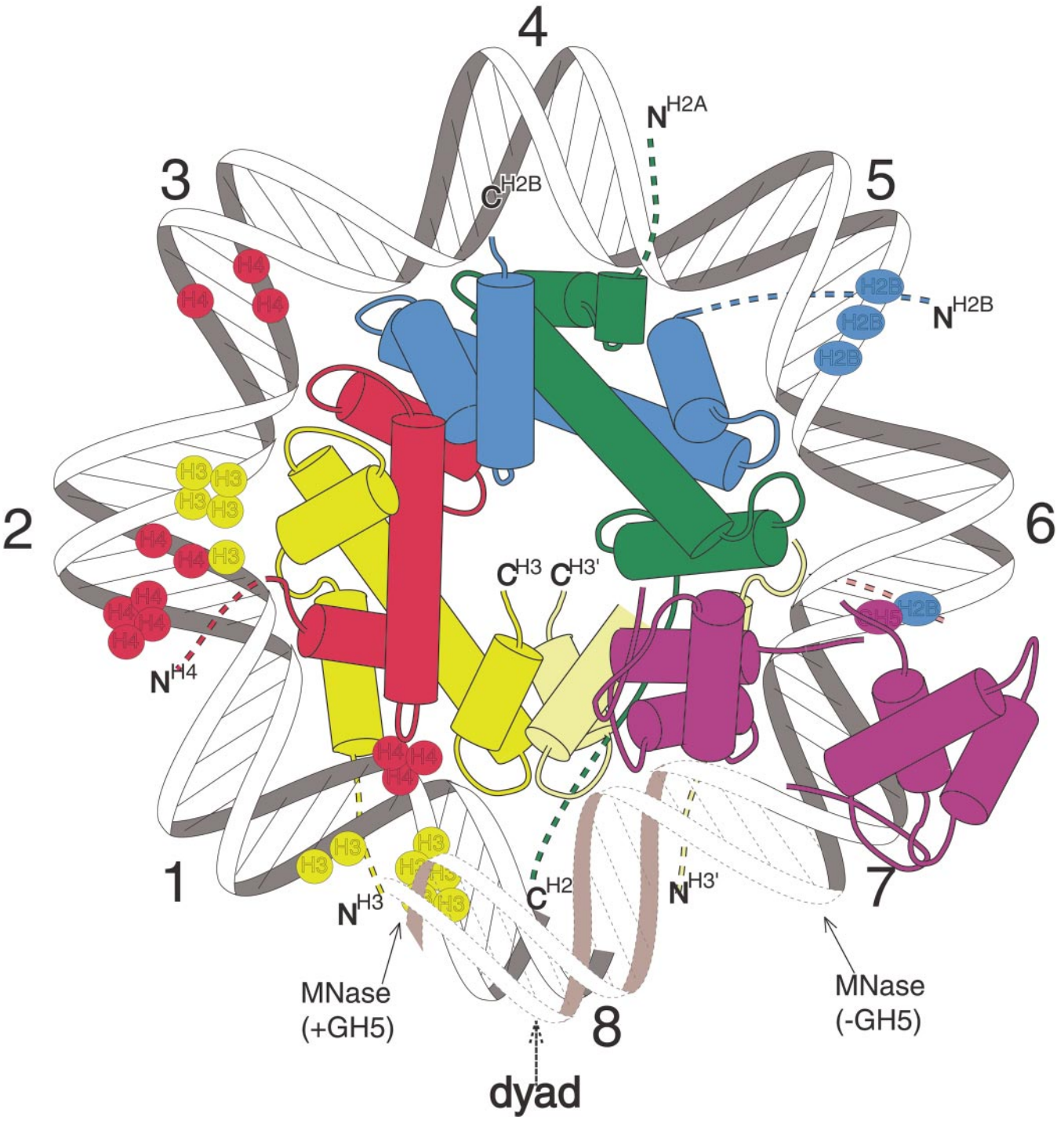


FIG. 1. The nucleosome has several possible anatomies (Hayes, 1996; Pruss *et al.*, 1995, 1996; Travers, 1999). In the view shown down the superhelical axis, the micrococcal nuclease (Mnase) digestion boundaries and sites of histone–DNA contact (revealed by crosslinking; Pruss and Wolffe (1993)) (colored circles) are shown as found on the coding sequence portion of the 5S nucleosome. For simplicity, the DNA is shown as a uniform superhelix. Core histones are color-coded in the following way: H4 (red), H3 (yellow), H2B (blue), and H2A (green). The approximate positions of the flexible histone tails are shown by broken lines. Two possible positions for the linker histone globular domain within the nucleosome are illustrated. The globular domain of histone H5 (pink) is shown in two extreme alternative positions binding to the outside or inside of the DNA superhelix. Contact with the 5S DNA is indicated by the pink circle on the double-helix.

These studies raise serious questions as to the biological relevance of many of the studies performed with native and modified nucleosome core particles, since such particles do not exist in the nucleus. This is an issue that is central to the topic of chromatin structure, and yet is almost completely ignored by most practicing members in the chromatin field. Again this is an important area for investigation. Further, the tails are critical for the self-assembly of condensed fibers into higher-order structures. Interestingly, histone tail interactions with DNA and protein change as the chromatin fiber undergoes folding or compaction (Fletcher and Hansen, 1996). Thus, certain posttranslational modifications of the tail domains may evoke specific functional and/or conformational states of the chromatin fiber by inducing a defined alteration in the array of histone tail interactions (Hansen *et al.*, 1998).

HISTONE H1 AND HIGHER-ORDER CHROMATIN STRUCTURE

Incorporation of linker histones into chromatin stabilizes nucleosomes and higher-order chromatin structures (Carruthers *et al.*, 1998). However, whereas core histones are essential for chromatin and chromosome assembly, linker histones are not required (Dasso *et al.*, 1994; Shen *et al.*, 1995). Metazoan linker histones have a three-domain structure; a central globular domain, flanked by N- and C-terminal tails. The globular domain has a winged-helix domain structure (Ramakrishnan *et al.*, 1993) and can associate with the nucleosome core in a number of distinct ways (Pruss *et al.*, 1996; Zhou *et al.*, 1998) (see Fig. 1). The N- and C-terminal tails of the linker histones bind to DNA within the nucleosome core and in the linker DNA between nucleosome cores. The preponderance of basic residues within these tail domains serves to neutralize the polyanionic backbone of DNA, thus facilitating the folding of nucleosomal arrays into higher-order structures (Clark and Kimura, 1990). Inclusion of the linker histone into the nucleosome requires the presence of an octamer of core histones and restricts the translational mobility of histone octamers with respect to DNA sequence (Pennings *et al.*, 1994; Ura *et al.*, 1995). Under physiological conditions the association of histone H1 with chromatin is much less stable than that of the core histones. Removal of histone H1 is therefore likely to represent a relatively simple means of destabilizing both local and higher-order chromatin structures and altering core histone–DNA interactions.

CHROMATIN REMODELING AND HISTONE MODIFICATION IN TRANSCRIPTION

Genetic experiments in *Saccharomyces cerevisiae* provide compelling evidence for general and specific roles for the histones in transcriptional control (Grunstein, 1990, 1997). Nucleosome depletion leads to the widespread activation of yeast promoters, and all four core histone N-termini are required for the repression of basal transcription. Acetylatable lysines in the N-termini of H3 and H4 have roles in transcriptional activation and repression. Interestingly a region in the N-terminal tail of H4 known to be critical for silencing in yeast is observed to make protein–protein contacts with the surface of a (H2A/H2B) dimer in an adjacent core in the crystal structure of a nucleosome core particle (Luger *et al.*, 1997). Certain mutations of lysine to glutamine in the N-termini of H3 and H4 relieve the requirement for histone acetyltransferase activity in transcriptional activation (Zhang *et al.*, 1998). This suggests that histone acetylation is a major function of particular coactivators. Mutation of the histone fold domains of the core histones can also lead to activation of certain yeast genes by relieving the requirement for the SWI/SNF family of molecular machines known to disrupt chromatin.

S. cerevisiae has an unusual nonessential linker histone, containing two globular domains, deletion of which has no detectable effect on gene expression (Patterton *et al.*, 1998). Deletion of *Tetrahymena* histone H1, which lacks the globular domain, does not influence transcription of the majority of genes; however, a subset of genes are either activated or repressed in H1-deficient strains (Shen *et al.*, 1995). Likewise, elimination of histone H1 containing a globular domain from *Ascobolus immersus* does not affect many normal cellular functions, general viability, and reproduction, but does lead to more subtle effects such as reduced life span (Barra *et al.*, 2000). Ablation of histone H1 during *Xenopus laevis* development leads to constitutive activation of certain oocyte-specific 5S rRNA genes and mesodermal-specific genes (Bouvet *et al.*, 1994; Steinbach *et al.*, 1997). Repression can be restored by expression of the globular domain lacking N- and C-terminal tails (Vermaak *et al.*, 1998). The molecular mechanism involved is now understood in some detail for one type of developmentally regulated gene. The globular domain of histone H1 has a precise architectural role for selective repression of the oocyte 5S rRNA genes compared to somatic 5S DNA in *X. laevis*. It binds to the 5S nucleosome asymmetrically, serving to position the histone octamer to repress certain genes while allowing continued activity of others (Chipev and Wolffe, 1992; Howe *et al.*, 1998; Sera and Wolffe, 1998; Tomaszewski *et al.*, 1998). Taken together, the histones can be seen as integral components of the transcriptional machinery with highly specific roles in gene control.

STRUCTURAL AND FUNCTIONAL CONSEQUENCES OF ACETYLATION OF THE CORE HISTONES

It has been known for some time that histone acetylation is intimately connected to transcriptional regulation. However, a direct link between chromatin function and acetylation was established by the discovery that coactivator complexes required for transcriptional activation function as histone acetyltransferases (Brownell *et al.*, 1996), while corepressors containing histone deacetylases confer transcriptional repression (Taunton *et al.*, 1996). Histones are locally modified on target promoters (Kuo *et al.*, 1996) and specific lysines in particular histones are functional targets for acetyltransferases and deacetylases (Zhang *et al.*, 1998). Activator-dependent targeting of histone acetylase activity has been recapitulated *in vitro* (Utley *et al.*, 1998). With so much attention currently focused on the specific histone acetyltransferases, it is important to note that acetylation generally is a much more global process than often implied. How whole domains are targeted for acetylation (Hebbes *et al.*, 1994) remains to be resolved. Histone acetylation states are very dynamic, with the acetylated lysines of hyperacetylated histones turning over rapidly with half-lives of minutes within transcriptionally active chromatin, but much less rapidly for the hypoacetylated histones of transcriptionally silent regions (Covault and Chalkley, 1980). The dynamics of histone acetylation provide an attractive mechanistic foundation for the reversible activation and repression of transcription.

Although the exact mechanism by which acetylation affects the biophysical properties of chromatin remains somewhat undefined, it is clear that acetylation of the core histone N-termini affects the transcriptional properties of chromatin at several levels of chromatin structure. Acetylation can facilitate the binding of transcription factors to their recognition elements within isolated nucleosomes (Lee *et al.*, 1993; Vettese-Dadey *et al.*, 1996). Proteolytic removal of the N-termini of the core histones leads to comparable increases in transcription factor access to nucleosomal DNA and transcription of chromatin templates as histone acetylation (Lee *et al.*, 1993; Vettese-Dadey *et al.*, 1994), consistent with acetylation reducing the stability of interaction of the histone tails with nucleosomal DNA. It should nevertheless be noted that the N-termini of the core histones always make at least transient contacts with DNA despite acetylation (Mutskov *et al.*, 1998). Acetylated histones wrap DNA less tightly in mononucleosomes, which may result in a decrease in the amount of DNA superhelical writhe constrained by the nucleosome (Bauer *et al.*, 1994; Krajewski and Becker, 1998). These changes might be due to the fact that the acetylated N-terminal histone tails bind DNA with reduced affinity (Hong *et al.*, 1993) and are more mobile with respect to the DNA surface than unmodified tails (Cary *et al.*, 1982). Another interesting possibility is that acetylation disrupts the secondary structures that are known to exist within the H3 and H4 N-termini when they are bound to nucleosomal DNA (Baneres *et al.*, 1997). This might further destabilize interactions with DNA and the nucleosome itself.

Beyond effects on individual nucleosomes, acetylation facilitates factor access and transcription from nucleosomal arrays by decreasing the stability of the completely compacted 30-nm fiber (Tse *et al.*, 1998). It is also likely that acetylation leads to the destabilization of long-range structures through which the chromatin fiber is folded into the chromosome itself (Annunziato *et al.*, 1988). Interactions between adjacent nucleosomal arrays are reduced when they are reconstituted with acetylated histones and chromatin solubility is increased (Perry and Chalkley, 1982). *In vivo*, the region of DNase I sensitivity within the active β-globin locus also correlates with a region of increased histone acetylation (Hebbes *et al.*, 1994).

Interestingly, the level of histone modification required to facilitate the transcription process is relatively low, and a total of 12 acetylated lysines per histone octamer (of 28 potential acetylated lysines) will promote *in vitro* transcription more than 15-fold. This level of modification reduces chromatin compaction to the same extent as proteolytic removal of the N-termini (Tse *et al.*, 1998), again suggesting that the primary consequence of hyperacetylation is to reduce the interaction of the tails with the other components of chromatin including nucleosomal DNA, linker DNA, and the histones of adjacent nucleosomes. However, the level of charge neutralization necessary to facilitate the destabilization of chromatin higher-order structure is so low that other structural features must amplify the consequences of acetylation. As discussed these might include alterations to secondary structure in the tail domains and/or changes in the association of the tails with other nonhistone proteins. Acetylation of the histones probably serves to illuminate particular nucleosomes and/or segments of chromatin for interaction with other chromatin remodeling factors or components of the transcriptional machinery. The potential combination of direct chromatin structural transitions and modulation of protein–protein interactions following acetylation or deacetylation of the histone tails provides a powerful means of regulating transcription.

STRUCTURAL AND FUNCTIONAL CONSEQUENCES OF PHOSPHORYLATION AND UBIQUITINATION OF THE CORE HISTONES

In contrast to the many studies on the structural and functional consequences of histone acetylation, the impact of other posttranslational modifications of the core histones is relatively unexplored. Significant future opportunities undoubtedly lie in this research area. Histone H3 is rapidly phosphorylated on serine residues within its basic amino-terminal domain, when extracellular signals such as growth factors or phorbol esters stimulate quiescent cells to proliferate (Mahadevan *et al.*, 1991). Targeted phosphorylation of histone H3 has recently been directly connected with the RSK2 protein kinase (Sassone-Corsi *et al.*, 1999). Deficiencies in this modification pathway lead to human developmental abnormalities described as Coffin-Lowey syndrome. Global phosphorylation of serine 10 in H3 occurs in pericentromeric chromatin in late G2 phase, completely spreads throughout the chromosome just before prophase of mitosis, and is rapidly lost during anaphase (Hendzel *et al.*, 1997). This modification is spatially and temporally correlated with mitotic and meiotic chromatin condensation (Sauve *et al.*, 1999; Wei *et al.*, 1999). H3 serine 10 is located within the basic amino-terminal domain of histone H3 and, like the N-terminal domain of histone H4, may interact with the ends of DNA in the nucleosomal core particle and therefore perhaps with histone H1 (Glotov *et al.*, 1978). Indeed, based on charge effects phosphorylation of histone H3 might be expected to have structural consequences comparable to those of acetylation. A change in either nucleosomal conformation or higher-order structure concomitant with phosphorylation of H3 within the chromatin of the proto-oncogenes c-fos and c-jun occurs following their rapid induction to high levels of transcriptional activity by phorbol esters (Chen and Allfrey, 1987). DNase I sensitivity of chromatin rapidly increases and proteins with exposed sulfydryl groups accumulate on the proto-oncogene chromatin. The proteins containing exposed sulfydryl groups include both nonhistone proteins, such as RNA polymerase, and molecules of histone H3 with exposed cysteine residues. The histone H3 cysteine residues, the only ones in the nucleosome, are normally buried within the particle. Exposure of the sulfydryl groups indicates that a major disruption of nucleosome structure occurs that could involve the dissociation of an H2A/H2B dimer. Phosphorylation and acetylation of histone H3 might act in concert to cause these changes. There are likely to be increasingly important links made between cellular signal transduction pathways and chromatin targets for posttranslational modification.

Ubiquitin is a 76-amino-acid peptide that is attached to the C-terminal tail of histone H2A and perhaps H2B. Ubiquitinated H2A is incorporated into nucleosomes, without major changes in the organization of nucleosome cores (Levinger and Varshavsky, 1980). Ubiquitination of histone H2A is associated with transcriptional activity. Only 1 nucleosome in 25 contains ubiquitinated histone H2A within nontranscribed chromatin. This increases to 1 nucleosome in 2 for the transcriptionally active hsp70 genes (Levinger and Varshavsky, 1982). Enrichment in ubiquitinated H2A is especially prevalent at the 5′ end of transcriptionally active genes. Since the C-terminus of histone H2A contacts nucleosomal DNA at the dyad axis of the nucleosome (Usachenko *et al.*, 1994), ubiquitination of this tail domain might be anticipated to disrupt the interaction of linker histones with nucleosomal DNA. The bulky ubiquitin adduct might also be anticipated to disrupt higher-order chromatin structures and promote general accessibility to *trans*-acting factors by impeding internucleosomal interactions. This is an important issue for future study.

PHOSPHORYLATION OF LINKER HISTONES

Phosphorylation of histone H1 has been shown directly to weaken interaction of the basic tails of the protein to DNA. Surprisingly, these changes influence the binding of the protein to chromatin even more than to DNA and thereby potentially destabilize the chromatin fiber (Hill *et al.*, 1991). Phosphorylation of the histone H1 tails occurs predominantly at conserved (S/T P-X- K/R, serine/threonine, proline, any amino acid, lysine/arginine) motifs of which several exist along the charged tail regions. Linker histone becomes heavily phosphorylated on transcriptional activation of the micronucleus of *Tetrahymena* during the sexual cycle (Sweet and Allis, 1993). Transcriptional competence of the mouse mammary tumor virus (MMTV) promoter depends on the phosphorylation of histone H1 (Lee and Archer, 1998) and the active MMTV promoter is known to be selectively depleted in H1 (Bresnick *et al.*, 1992). In these examples it seems probable that the transcriptional machinery will target the phosphorylation of linker histones as a component of activation pathways to alleviate the repressive influence of linker histones. Recent work in *Tetrahymena* indicates that phosphorylation of linker histone H1 regulates gene expression *in vivo* by mimicking H1 removal (Dou *et al.*, 1999). This is consistent with this model.

TRANSCRIPTIONAL ACTIVATORS THAT REMODEL CHROMATIN

The GCN5p containing coactivator complex was identified through a genetic screen carried out by

Guarente and colleagues to identify mutations in genes that confer resistance to the toxic chimeric transcriptional activator GAL4-VP16 (see Berger *et al.*, 1992). Genes identified by this screen might be anticipated to be involved in facilitating gene activation by the VP16 acidic activation domain. In this way two "adaptor" proteins, ADA2p and ADA3p, that were proposed to bridge interactions between activation domains and the basal transcriptional machinery were identified (Guarente, 1995). A comparable mutation in the gene *GCN5* impaired the activation of transcription by the transcription factor GCN4p (Georgakopoulos and Thireos, 1992). Subsequent genetic and biochemical experiments established that GCN5p/ADA2p/ADA3p exists as a coactivator complex in yeast (Georgakopoulos *et al.*, 1995; Georgakopoulos and Thireos, 1992; Marcus *et al.*, 1994) and that ADA2p interacts with both acidic activation domains and TBP (Barlev *et al.*, 1995). The GCN5p/ADA2p/ADA3p complex is a component of an even more elaborate coactivator known as SAGA (the *S*pt-*A*da-*G*cn5-acetyltransferase complex) (Grunstein *et al.*, 1995). The SAGA complex contains components of the basal transcriptional machinery such as the TATA-binding associated factors (TAFs). Exactly how much GCN5p is free, bound to ADA2p and ADA3p, or in the SAGA complex has not been resolved.

The GCN5p/ADA2p/ADA3p coactivator is a histone acetyltransferase (Brownell *et al.*, 1996) that selectively modifies lysine 16 in the N-terminal tail domain of histone H4 (Kuo *et al.*, 1996). This property suggested for the first time that coactivators have the capacity to directly modify the chromatin template in order to facilitate transcription. *GCN5* is not an essential gene in yeast; however, the capacity to induce gene expression by GCN4p is reduced by 60% if *GCN5* is not functional. This suggests that some of the individual histone acetyltransferases may not be essential in yeast. This might reflect the presence of numerous genes with overlapping functions and/or merely that the modification of chromatin structure is only one contributor to transcriptional regulation. However, at least one yeast histone acetyltransferase, Esa1, is essential (Smith *et al.*, 1998). The existence of multiple potentially redundant histone acetyltransferases is substantiated by recent observations in metazoans.

HISTONE ACETYLTRANSFERASES PCAF, p300, AND $TAF_{II}250$

The discovery that *S. cerevisiae* GCN5p had histone acetyltransferase activity (Brownell *et al.*, 1996) led to the recognition that comparable regulatory mechanisms exist in metazoans (Yang *et al.*, 1996b). A human homolog of GCN5p known as *p*300/*C*BP-*a*ssociated *f*actor (PCAF) acetylates histones (Yang *et al.*, 1996b), as does p300/CBP itself (Ogryzko *et al.*, 1996). p300/CBP serves as an integrator to mediate regulation by a wide variety of sequence-specific transcription factors (Kamei *et al.*, 1996) including steroid and nuclear hormone receptors, c-Jun/vJun, cMyb/v-Myb, c-Fos, and MyoD (Janknecht and Hunter, 1996). To strengthen the analogy with the GCN5p/ADA2p/ADA3p complex, p300/CBP has a domain highly similar to part of ADA2p and associates with PCAF, the homolog of GCN5p (Yang *et al.*, 1996b). A component of the DNA-binding basal transcription factor TFIID has also been shown to have histone acetyltransferase activity (Mizzen *et al.*, 1996). $TAF_{II}250$ is the architectural core of TFIID interacting with the other TAFs (TBP-associated factors) as well as with TBP. $TAF_{II}250$ is required for the activation of particular genes indicative of coactivator function and associates with components of the basal transcriptional machinery such as TFIIA, TFIIE, and TFIIF (Dikstein *et al.*, 1996). In addition, $TAF_{II}250$ functions as both a kinase and a histone acetyltransferase (Dikstein *et al.*, 1996; Mizzen *et al.*, 1996). Thus diverse proteins in metazoans (and potentially in *S. cerevisiae*) possess histone acetyltransferase activity. In an interesting link between the mammalian SWI/SNF activator complex, monoclonal antibodies against p300 immunoprecipitate a complex of p300/CBP together with at least seven other cellular proteins (Dallas *et al.*, 1998). Within this complex is TBP, $TAF_{II}250$, and hSNF2β (BRG1), suggesting that functions of histone acetyltransferases might be linked to those of other activators that contend with chromatin.

THE SWI/SNF COMPLEX

SWI (switch) and SNF (sucrose nonfermenting) genes have been found to encode proteins that together assemble a large multisubunit complex required for the regulation of a specific group of inducible genes in yeast (Cairns *et al.*, 1994; Peterson *et al.*, 1994). A major clue to the molecular mechanisms by which the SWI/SNF activator complex functions came from a genetic screen for mutations of genes that would allow transcription from the HO gene in the absence of specific SWI genes (Herskowitz *et al.*, 1992). These studies identified the SIN genes (*S*WI *in*dependent). SIN 1–4 have been found or inferred to have a direct impact on chromatin structure and function. A simple model would predict that the SWI/SNF activator complex functions by overcoming the repressive effects of the SIN gene products on transcription. Indeed *in vivo* experiments in *S. cerevisiae* establish that the SWI/SNF activator complex activates transcription by altering chromatin structure (Herskowitz *et al.*,

1992), and *in vitro* experiments using purified SWI/SNF complex indicate that stoichiometric amounts of the SWI/SNF complex can alter histone–DNA interactions in the nucleosome (Cote *et al.,* 1994). Subsequent experiments have identified several other complexes that contain proteins shared in common or highly related to those within the SWI/SNF complex including the RSC (*r*emodel *s*tructure of *c*hromatin) complex (Cairns *et al.,* 1996, 1998). These complexes also influence histone–DNA and protein–DNA interactions. Whether this is their only function remains to be resolved.

There is excellent precedent for pioneering experimental work in *S. cerevisiae* leading to the recognition of comparable regulatory mechanisms in metazoans. The identification of the SWI/SNF complex (Peterson and Herskowitz, 1992) offered insight into potential regulatory roles for related proteins in *Drosophila* (Tamkun *et al.,* 1992). It was also shown that metazoan regulatory proteins including the glucocorticoid receptor introduced into yeast could make use of the SWI/SNF complex to activate synthetic promoters containing their recognition elements (Laurent and Carlson, 1992; Yoshinaga *et al.,* 1992). Mammalian homologs of components of the SWI/SNF complex were characterized (Chiba *et al.,* 1994; Khavari *et al.,* 1993; Muchardt and Yaniv, 1993). These proteins, human brahma (hbrm) and brahma related gene product 1 (BRG1), possess amino terminal proline- and glutamine-rich regions that resemble transcriptional activation domains. Their capacity to interact with other components of the transcriptional machinery including the glucocorticoid and estrogen receptor is shown by their capacity to activate transcription in transient cotransfection assays that are largely independent of chromatin-mediated effects (Chiba *et al.,* 1994; Muchardt and Yaniv, 1993).

Evidence for the targeted disruption of chromatin by the mammalian SWI/SNF complex has taken time to emerge. A 100-fold molar excess of the 2×10^6 Da SWI/SNF complex can disrupt a synthetic nucleosome core (containing 0.1×10^6 Da of histone) *in vitro* (Imbalzano *et al.,* 1994). It has also been suggested that the RNA polymerase II holoenzyme contains SWI/SNF and might remodel chromatin (Wilson *et al.,* 1996). However, recent experiments suggest that the yeast RNA polymerase II holoenzyme might in certain circumstances disrupt chromatin independent of the presence of SWI/SNF (Gaudreau *et al.,* 1997). In a significant advance Fryer and Archer (1998) have recently obtained evidence for targeted recruitment of the BRG1/BAF complex by ligand-bound glucocorticoid receptor.

How does the SWI/SNF complex disrupt nucleosomes? So far no covalent modifications of the core histones have been shown to be conferred by SWI/SNF components. One model for disruption is that the complex tracks along DNA rather like RNA and DNA polymerase and displaces nucleosomes in a comparable way (Cairns, 1998). However, this is difficult to reconcile with the continued wrapping of DNA on the surface of the histones in SWI/SNF disrupted nucleosomes and the recovery of normal histone stoichiometries from SWI/SNF treated nucleosomes (Cote *et al.,* 1998). It is possible that the octamer may simply be transferred to a position upstream of a tracking SWI/SNF complex as has been observed for RNA polymerase under certain *in vitro* conditions (Studitsky *et al.,* 1994). An alternative idea is that histones H2A and H2B are displaced or destabilized within the nucleosome (Peterson and Tamkun, 1995). Removal of H2A and H2B facilitates access of transcription factors to nucleosomal DNA (Hayes and Wolffe, 1992; Spangenberg *et al.,* 1998; Tse *et al.,* 1998) and facilitates transcription (Hansen and Wolffe, 1994). Although complete displacement of (H2A, H2B) dimers seems unlikely, destabilization of (H2A, H2B) association would be consistent with genetic and structural data. This disruption might generate a structure prone to homologous dimerization (Schnitzler *et al.,* 1998).

Mutation of the core histone fold domains can generate yeast strains that are *S*WI/SNF *in*dependent (SIN). These SIN mutations lie either in regions of the core histones that mediate interaction between the $(H3, H4)_2$ tetramer and the (H2A, H2B) dimers (Santisteban *et al.,* 1997) or at sites that destabilize histone–DNA interactions (Kruger *et al.,* 1995). The boundaries of the nucleosome core are known to be mainly defined by the (H2A, H2B) dimers. Destabilization of (H2A, H2B) interactions in the nucleosome alone are insufficient to explain all of the features of SWI/SNF-mediated nucleosomal disruption, because removal of (H2A, H2B) dimers will not eliminate rotational constraint of DNA in the nucleosome cores. Such loss of rotational constraint as assayed by DNase I cleavage is seen in the presence of SWI/SNF (Cote *et al.,* 1994) and in nucleosome cores containing SIN2 mutant histones (Kurumizaka and Wolffe, 1997). The $(H3, H4)_2$ tetramer rotationally constrains DNA as efficiently as the histone octamer; thus the interaction of the (H3, H4) tetramer with DNA must also be destabilized during SWI/SNF-mediated nucleosome disruption. It is possible that the binding of the SWI/SNF complex to the nucleosome destabilizes both (H2A, H2B) dimer and (H3, H4) tetramer interactions with DNA and that this is accomplished by protein–protein interactions with the SWI/SNF complex on the face of the nucleosome. This interaction may require contact with the core histone tails (Georgel *et al.,* 1997) and may

resemble the interaction of other nucleosome core-binding proteins, such as the globular domain of linker histones, HNF3 and NF1 (Alevizopoulos *et al.*, 1995; Cirillo *et al.*, 1998; Pruss *et al.*, 1996). Binding of SWI/SNF to the face of the nucleosome would allow contact with all four core histones and might be predicted to alter the contacts with DNA as has been observed following binding of linker histones (Guschin *et al.*, 1998; Usachenko *et al.*, 1996).

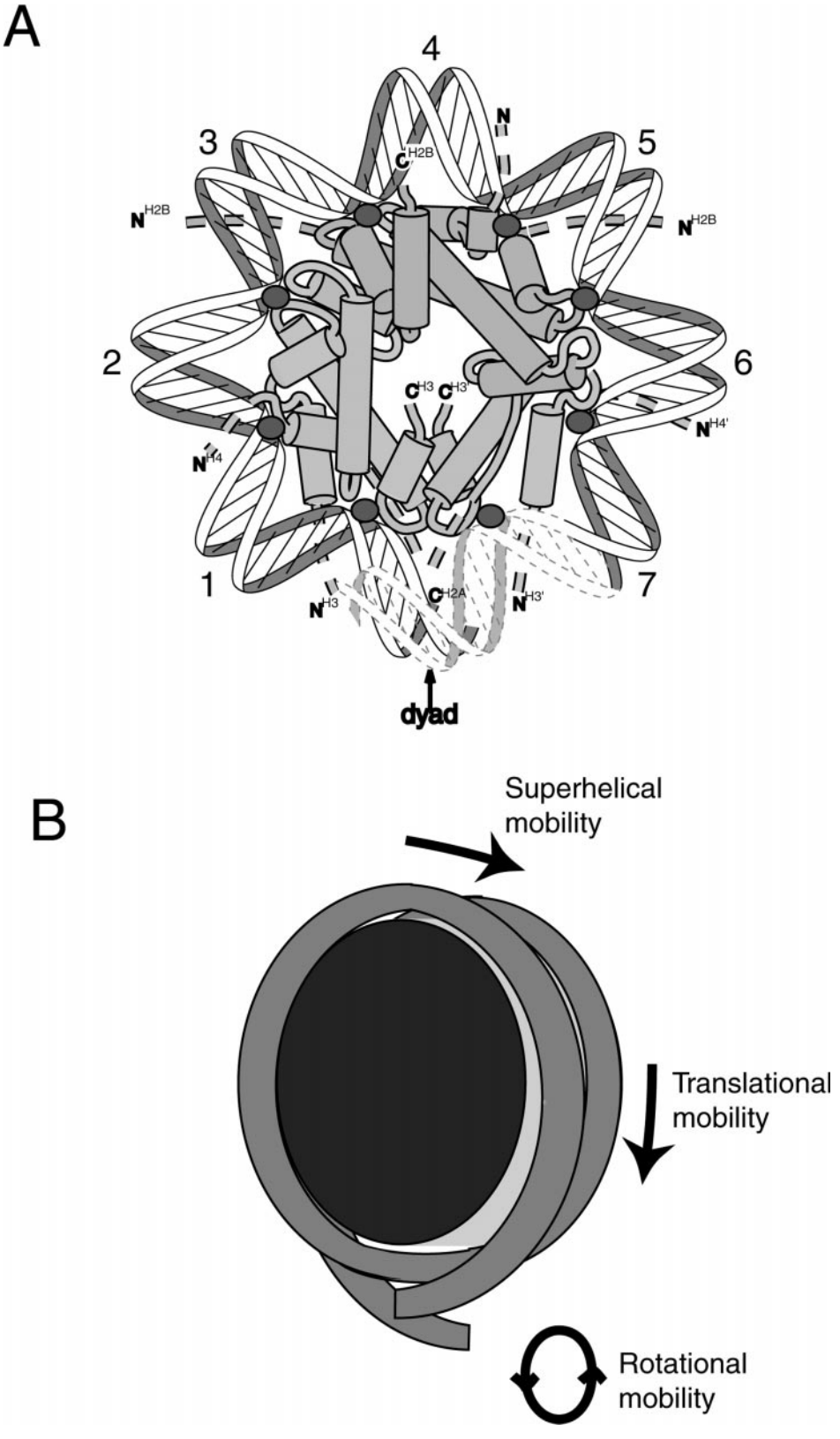
THE ISWI COMPLEXES PROMOTE NUCLEOSOME MOBILITY

Additional insights into the molecular mechanisms used by enzyme complexes similar to SWI/SNF come from research in metazoan systems. The *n*ucleosome *r*emodeling *f*actor (NURF) and *ch*romatin *a*ccessibility *c*omplex (CHRAC) have been purified from *Drosophila* embryo extracts. Both complexes are relatively small (~0.5 MDa), containing fewer than five components including ISWI, a member of the SWI/SWNF2 superfamily of ATPase found in the SWI/SNF complexes. NURF also contains a 215-kDa subunit yet to be defined, a 55-kDa WD repeat protein that can bind histones with considerable specificity, and a 38-kDa inorganic pyrophosphatase (Hamiche *et al.*, 1999). It has been suggested that inorganic pyrophosphatase might facilitate NURF activity through the elimination of pyrophosphate during the rapid replication cycles of early *Drosophila* development. The only other component of CHRAC to have been identified aside from ISWI is a dimer of topoisomerase II. This is an enzyme competent to relax superhelical DNA in an ATP-dependent process, to resolve catananes, and to facilitate chromosome assembly. Three other smaller components of CHRAC are as yet uncharacterized (Langst *et al.*, 1999). The subunit compositions of NURF and CHRAC are consistent with roles in histone and chromosome metabolism; however, the exact *in vivo* biological roles of these molecular machines have not been defined nor has their existence yet been described in cells or organisms other than those of *Drosophila*. Nevertheless experiments with NURF and CHRAC, and more recently with the purified ISWI ATPase, which is specific for nucleosomal DNA, have proven remarkably informative with respect to the mechanisms of chromatin disruption as defined using *in vitro* model systems.

Both NURF and CHRAC facilitate chromatin disruption as assayed by access of transcription and replication factors. Overall cleavage of DNA within chromatin by enzymes such as micrococcal nuclease or DNase I does not appear to increase markedly in the presence of NURF and CHRAC. In addition, NURF disrupts the regular positioning of histone octamers relative to one another within a previously assembled nucleosomal array, while CHRAC promotes the assembly of a spaced nucleosomal array. Experiments with the purified ISWI nucleosomal ATPase demonstrated that this component alone would facilitate the spacing of nucleosomes, so the contradictory behavior of NURF relative to CHRAC is dependent on other specialized subunits interacting with chromatin. The unifying theme for the NURF and CHRAC chromatin remodeling machines is that histone–DNA interactions change to facilitate the access of regulatory proteins to specific recognition elements, while retaining the efficient packaging of DNA in chromatin. How might this be accomplished? NURF, CHRAC, and the ISWI nucleosomal ATPase have now been discovered to share the capacity to actively promote nucleosome mobility.

The assays for the movement of the histone core with respect to DNA sequence rely on nondenaturing gel electrophoresis sensitive to nucleoprotein conformation and nuclease mapping of the boundaries of strong histone–DNA interactions (Pennings *et al.*, 1991; Ura *et al.*, 1995). In experiments using NURF, CHRAC, and ISWI, DNA fragments of 250 to 359 bp in length are used to provide ample opportunity for the histone octamer to redistribute to alternate positions. The results clearly show an increase in the rate of nucleosome movement dependent on the two remodeling machines and their ISWI nucleosomal ATPase activity. The stoichiometry of histones to DNA is retained and the integrity of the nucleosomal templates resist competition *in trans* of more than 3 000-fold excess (50 μg/ml). Interestingly CHRAC and ISWI move nucleosomes in different directions with respect to the particular DNA sequence used, and NURF fails to move nucleosomes at all on one DNA sequence encoding a 5S rRNA gene. Thus the mobility of nucleosomes will be sensitive to the particular components in association with ISWI and on the preexisting stability or conformation of the histone–DNA complex. Although these determinants remain to be understood, the important conclusion is that NURF and CHRAC stimulate nucleosome mobil-

FIG. 2. Possible mobilities of DNA relative to the histone octamer. (A) One face of the core histone octamer is shown with DNA coiled around it. Histones H3/H4 and H2A/H2B are shaded. The dyad axis and the positions where the amino- and carboxyl-terminal tails of the histones exit the turns of DNA are indicated. The positions where the core histones contact the DNA minor groove are marked by circles. (B) Possible directions of nucleosome mobility. Superhelical mobility: the histone octamer (dark shaded ellipsoid) with DNA (gray tube) wrapped around it might move along the axis of the DNA superhelix. Translational mobility: the histone octamer might move along the axis of the DNA double-helix. Rotational mobility: the DNA double-helix might rotate with respect to the surface of the histone octamer.


A
4
3
5
2
6
1
7
N
C H2B
N H2B
N H2B
C H3
C H3'
N H4'
N H4
N H3
C H2A
N H3'
dyad
B
Superhelical mobility
Translational mobility
Rotational mobility

ity. This movement of the histone octamer with respect to DNA sequence will offer the potential for at least transient access of all recognition elements within a DNA fragment to regulatory factors while retaining the overall wrapping of DNA within chromatin.

Nucleosome movement by CHRAC, NURF, and ISWI can be accomplished with neither disruption of the histone octamer nor displacement of the entire octamer from association with DNA. Only the exact DNA sequence bound by the octamer changes. Nucleosome mobility could occur most simply through three distinct mechanisms (Fig. 2). The translational mobility of a nucleosome relative to DNA sequence could be promoted by local looping of DNA in contact with the histones; small loops of 20–30 bp perhaps originating from linker DNA could break one or two contacts at any time allowing movement, while retaining histone DNA association. In this model the rotational orientation of the DNA molecule itself relative to the histone surface remains constant. The detailed analysis of spontaneous nucleosome mobility shows that this translational movement of the octamer relative to DNA sequence occurs in integral helical steps. Whether this happens in the mobility driven by CHRAC and NURF has not yet been determined. An alternate means of mobilizing DNA with respect to the histone octamer is to rotate the double-helix with respect to the histone surface. This rotational mobility appears unlikely for CHRAC-mediated disruption because DNase I cleavage retains a 10- to 11-bp periodicity typical of rotationally positioned DNA sequences. Any nonintegral rotation of DNA with respect to the histone surface would be detected in this analysis. Rotation of integral turns of DNA would not be detected in this assay and could contribute to facilitating translational movement in steps of 10–11 bp. A third means of promoting the mobility of the histone octamer relative to DNA sequence is to make use of the alignment of major and minor grooves of DNA on the surface of the octamer. Movement of the histone octamer in integral superhelical turns of DNA each containing 80 bp would require the transient disruption of all contacts of DNA with the histone fold domains. There is no evidence for such a large mobilization of histone–DNA contacts in the *in vitro* experiments using ISWI alone or within the NURF or CHRAC complexes.

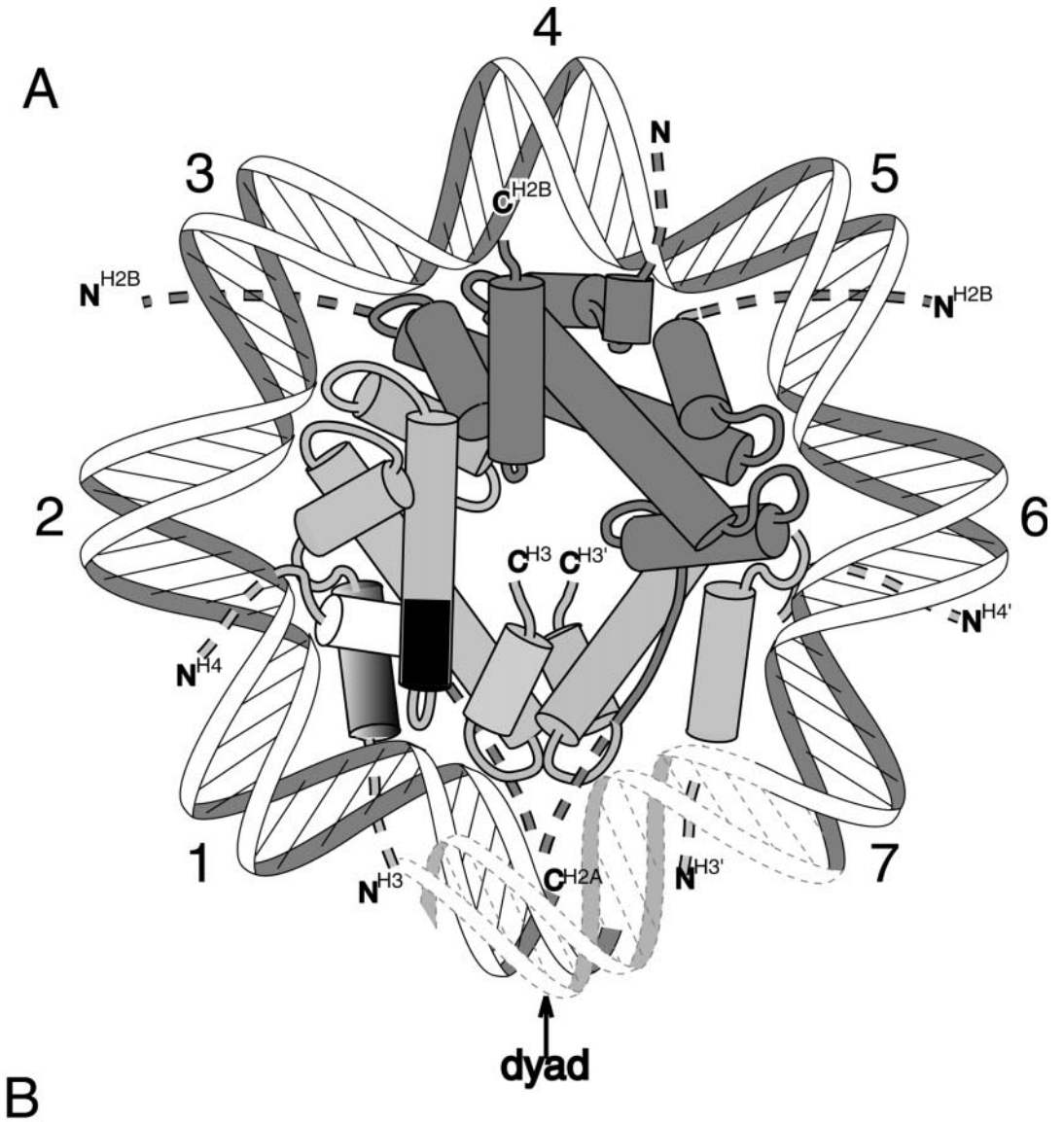
How might NURF, CHRAC, and ISWI promote nucleosome mobility? One possibility is that the local disruption of histone–DNA contacts at the periphery of the nucleosome core might have a large influence on the integrity of the whole particle (Fig. 3). The N-terminal α-helix of histone H3 makes contact with the DNA at the edge of the nucleosome core, providing additional interactions beyond those imparted by contacts with the minor groove, and may contribute to determining the boundaries of the nucleosome core as detected by micrococcal nuclease. Interestingly, mutant forms of histone H3 exist that relieve the requirement for the yeast SWI/SNF proteins to destabilize histone–DNA contacts at the boundary of the nucleosome core (Kurumizaka and Wolffe, 1997).

TRANSCRIPTIONAL REPRESSORS THAT REMODEL CHROMATIN

The purification of the mammalian histone deacetylase and the recognition of the similarities to *S. cerevisiae* RPD3p (Taunton *et al.*, 1996) have provided considerable insight into transcriptional repression in metazoans. The first direct evidence for mammalian homologs of RPD3p being involved in transcriptional repression came from two-hybrid screens indicating that the transcriptional regulatory YY1 interacted with mouse and human RPD3p (Yang *et al.*, 1996a). The fusion of mammalian RPD3p to a targeted DNA-binding domain directed transcriptional repression by more than 10-fold. Mutations in a glycine-rich domain of YY1 that directs binding to RPD3p could abolish transcriptional repression by YY1, suggesting that YY1 negatively regulates transcription by tethering RPD3. YY1 is a mammalian zinc-finger transcription factor (Shi *et al.*, 1991) that is proposed to regulate cell growth and differentiation (Shrivastava and Calame, 1994). Interestingly, a *Drosophila* homolog of YY1 is *Pleiohomeotic* (PHO), which is a member of the Polycomb group complex of proteins that control gene expression by altering chromosomal structure (Taunton *et al.*, 1996).

A second well-defined protein complex that influences cell growth and differentiation in mammalian cells is the Mad–Max heterodimer (Chen *et al.*, 1995; Hurlin *et al.*, 1995; Lahoz *et al.*, 1994). Max is a widely expressed sequence-specific transcriptional regulator of the basic region-helix-loop-helix-leucine zipper family (bHLH-ZIP). Max heterodimerizes with

FIG. 3. Regions of the core histones that may be particularly important for the action of SWI/SNF nucleosome-destabilizing complexes. Views of the nucleosome are shown (a) from the top and (b) from the side. Histones H3/H4 are shaded light and histones H2A/H2B dark. The amino-terminal α-helix of the histone fold domain of H3 is in a graded gray tone; this is proposed to stabilize histone octamer–DNA interactions at the edge of the nucleosome core. The amino-terminal α-helix of the histone fold domain of H4 is unshaded; this is the key histone-recognition domain for NURF 55. The part of the long central α-helix of the H4 histone fold domain shaded black is the site of mutations that relieve the requirement for the SWI/SNF complex is *S. cerevisiae*. These histone domains are close to each other in the nucleosome, and their interaction with a protein, for example, a component of a nucleosome-destabilizing complex, might alter their DNA-binding surfaces, such as those labeled "β-bridges" or "paired ends of α-helices."


A
4
3
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7
N
C H2B
N H2B
N H2B
C H3
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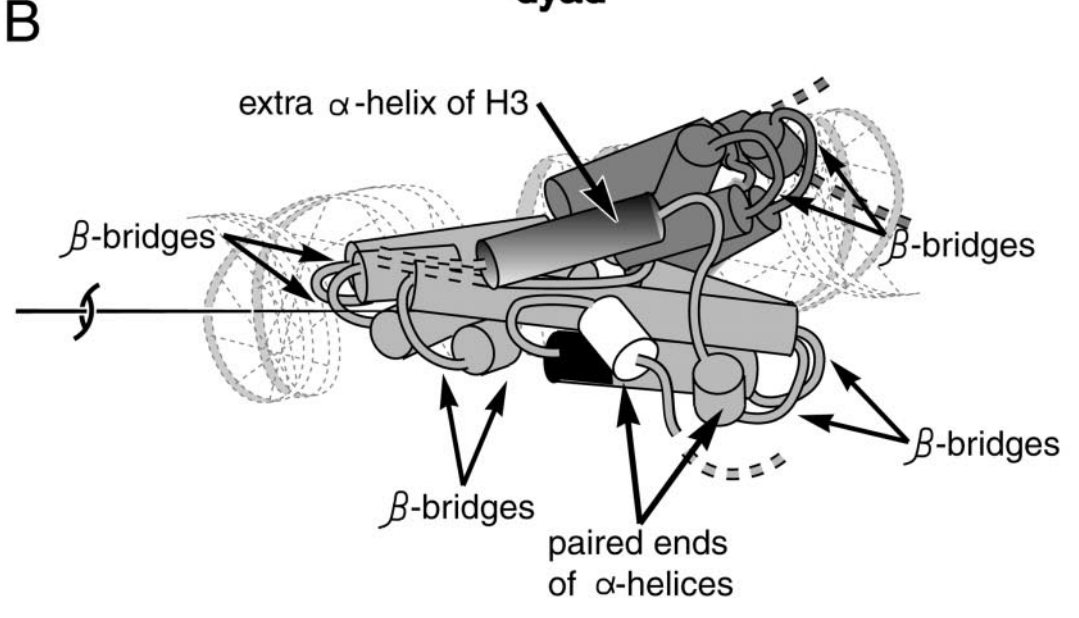
N H4'
N H4
N H3
C H2A
N H3'
dyad


B
extra α-helix of H3
β-bridges
β-bridges
β-bridges
β-bridges
paired ends
of α-helices

the Myc family of bHLH-ZIP proteins including Myc, Mad, and Mxi-1 (Ayer *et al.*, 1993; Zervos *et al.*, 1993). While the Myc–Max complex activates transcription and transformation, the Mad–Max complex represses these events. Eisenman and colleagues identified two mammalian proteins, mSin3A and mSin3B, that interact with Mad and that have striking homology to *S. cerevisiae* Sin3p, including the four paired amphipathic helix (PAH) domains (Ayer *et al.*, 1995). The association between Mad–Max and mSin3A and B requires the second PAH domain. Mutations in this domain eliminate the interaction with mSin3A and prevent the Mad–Max complex from repressing transcription (Ayer *et al.*, 1995). The next step was to establish that the mSIN3 proteins interact with the mammalian histone deacetylases. Mad, mSIN3, and the mammalian histone deacetylases coimmunoprecipitate (Alland *et al.*, 1997; Laherty *et al.*, 1997). The third PAH domain of mSIN3 interacts with the mammalian RPD3p homologs and can confer transcriptional repression when attached to a DNA-binding domain. More subtle mutational analysis suggests that the cell transformation and transcriptional repression suppressed by the Mad–Max complex depend on distinct domains of the mSIN3 proteins (Alland *et al.*, 1997). However, an active role for histone deacetylation in transcriptional control is demonstrated by the use of deacetylase inhibitors such as Trichostatin A (Yoshida *et al.*, 1990) that abolish Mad's ability to repress transcription. The existence of a conserved transcriptional repression mechanism that utilizes SIN3p and histone deacetylase emphasizes the significance of the chromatin environment for transcriptional control. Histone deacetylation directs the assembly of a stable repressive chromatin structure.

NUCLEAR HORMONE RECEPTORS AND HISTONE DEACETYLASE

A role for chromatin had already been established in the control of transcription by the thyroid hormone receptor (Wong *et al.*, 1995, 1997a). These studies provide a useful example of how the histones can contribute to gene regulation. The assembly of minichromosomes within the *Xenopus* oocyte nucleus has been used to examine the role of chromatin in both transcriptional silencing and activation of the *Xenopus* TRβA promoter. Transcription from this promoter is under the control of thyroid hormone and the thyroid hormone receptor (Ranjan *et al.*, 1994), which exists as a heterodimer of TR and RXR. Microinjection of either single-stranded or double-stranded DNA templates into the *Xenopus* oocyte nucleus offers the opportunity for examination of the influence on gene regulation of chromatin assembly pathways that are either coupled or uncoupled to DNA synthesis (Almouzni and Wolffe, 1993). The staged injection of mRNA encoding transcriptional regulatory proteins and of template DNA offers the potential for examining the mechanisms of transcription factor-mediated transcriptional activation of promoters within a chromatin environment. In particular, it is possible to discriminate between preemptive mechanisms in which transcription factors bind during chromatin assembly to activate transcription and postreplicative mechanisms in which transcription factors gain access to their recognition elements after they have been assembled into mature chromatin structures. TR/RXR heterodimers bind constitutively within the minichromosome, independent of whether the receptor is synthesized before or after chromatin assembly. Rotational positioning of the TRE on the surface of the histone octamer allows the specific association of the TR/RXR heterodimer *in vitro*. The coupling of chromatin assembly to the replication process augments transcriptional repression by unliganded TR/RXR without influencing the final level of transcriptional activity in the presence of thyroid hormone.

The molecular mechanisms by which the unliganded thyroid hormone receptor makes use of chromatin in order to augment transcriptional repression also involve mSin3 and histone deacetylase (Alland *et al.*, 1997; Heinzel *et al.*, 1997). The unliganded thyroid hormone receptor and retinoic acid receptor bind a corepressor, NcoR (Horlein *et al.*, 1995). NCoR interacts with Sin3 and recruits the histone deacetylase (Alland *et al.*, 1997; Heinzel *et al.*, 1997). All of the transcriptional repression conferred by the unliganded thyroid hormone receptor in *Xenopus* oocytes (Wong *et al.*, 1995, 1997a) can be alleviated by the inhibition of histone deacetylase using Trichostatin A (Wong *et al.*, 1998), indicative of an essential role for deacetylation in establishing transcriptional repression in a chromatin environment.

The addition of thyroid hormone to the chromatin-bound receptor leads to the disruption of chromatin structure (Wong *et al.*, 1995, 1997b). Chromatin disruption is not restricted to the receptor-binding site and involves the reorganization of chromatin structure before targeted histone acetylation by the PCAF and p300/CBP activators can have a contributory role (Li *et al.*, 1999; Ogryzko *et al.*, 1996; Yang *et al.*, 1996a). It is possible to separate chromatin disruption from productive recruitment of the basal transcription machinery *in vivo* by deletion of regulatory elements essential for transcription initiation at the start site and by the use of transcriptional inhibitors (Wong *et al.*, 1995, 1997a). Therefore

chromatin disruption is an independent hormone-regulated function targeted by DNA-bound thyroid hormone receptor. It is remarkable just how effectively the various functions of the thyroid hormone receptor are mediated through the recruitment of enzyme complexes that modify chromatin. These results provide compelling evidence for the productive utilization of structural transitions in chromatin as a regulatory principle in gene control.

DNA METHYLATION AND TRANSCRIPTIONAL CONTROL

The covalent modification of DNA provides a direct and powerful mechanism to regulate gene expression (Kass *et al.*, 1997b). Considerable experimental evidence supports the existence of such a mechanism in the majority of plants and animals (Bird, 1986, 1995; Szyf, 1996; Yoder *et al.*, 1997). The genome of an adult vertebrate cell has 60–90% of the cytosines in CpG dinucleotides methylated by DNA methyltransferase (Riggs and Porter, 1996). This modification can alter the recognition of the double-helix by the transcriptional machinery and the structural proteins that assemble chromatin (Kass *et al.*, 1997a; Nan *et al.*, 1997).

DNA methylation could control gene activity either at a local level through effects at a single promoter and enhancer or through global mechanisms that influence many genes within an entire chromosome or genome (Tate and Bird, 1993). An attractive suggestion is that DNA methylation evolved as a host-defense mechanism in metazoans to protect the genome against genomic parasites such as transposable elements (Yoder *et al.*, 1997). An increase in methyl-CpG correlates with transcriptional silencing for whole chromosomes, transgenes, particular developmentally regulated genes, and human disease genes (Li *et al.*, 1993; Szyf, 1996). All of these systems exhibit epigenetic effects on transcriptional regulation in which identical DNA sequences are differentially utilized within the same cell nucleus. These patterns of differential gene activity are clonally inherited through cell division. Because specific methyl-CpG dinucleotides are maintained through DNA replication, DNA methylation states also provide an attractive mechanism (epigenetic mark) to maintain a particular state of gene activity through cell division and, thus, to contribute to the maintenance of the differentiated state (Holliday, 1987).

Bird and colleagues identified two repressors, MeCP1 and MeCP2, that bind to methyl-CpG without apparent sequence specificity (Meehan *et al.*, 1989, 1992; Ng *et al.*, 1999). Like DNA methylation itself, MeCP2 is dispensable for the viability of embryonic stem cells; however, it is essential for normal embryonic development. Recent results show that MeCP2 function is essential for human development, since individuals afflicted by Rett syndrome, the leading cause of female mental retardation, have point mutations in the gene encoding MeCP2 (Amir *et al.*, 1999). Consistent with the capacity of methylation-dependent repressors to operate in chromatin, MeCP2 is a chromosomal protein with the capacity to displace histone H1 from the nucleosome (Chandler *et al.*, 1999; Nan *et al.*, 1996). Moreover, MeCP2 contains a methyl-CpG DNA-binding domain, which might alter chromatin structure directly, and a repressor domain, which might function indirectly to confer long-range repression *in vivo* (Jones *et al.*, 1998; Nan *et al.*, 1993, 1997). The capacity for MeCP2 to function in chromatin explains several phenomena connected with unique aspects of chromatin assembled on methylated DNA.

A role for specialized chromatin structures in mediating transcriptional silencing by methylated DNA has been suggested by several investigators. High levels of methyl-CpG correlate with transcriptional inactivity and nuclease resistance in endogenous chromosomes (Antequera *et al.*, 1989, 1990). Methylated DNA transfected into mammalian cells is also assembled into a nuclease-resistant structure containing unusual nucleosomal particles (Keshet *et al.*, 1986). These unusual nucleosomes migrate as large nucleoprotein complexes on agarose gels. These complexes are held together by higher-order protein–DNA interactions despite the presence of abundant micrococcal nuclease cleavage points within the DNA. Individual nucleosomes assembled on methylated DNA appear to interact together more stably than on unmethylated templates (Keshet *et al.*, 1986). The replacement of histone H1 with MeCP2 is a possible explanation for the assembly of a distinct chromatin structure on methylated DNA (Nan *et al.*, 1997).

Early experiments using the microinjection of templates into the nuclei of mammalian cells suggested that the prior assembly of methylated, but not unmethylated, DNA into chromatin represses transcription (Buschhausen *et al.*, 1987). The importance of a nucleosomal infrastructure for transcriptional repression dependent on DNA methylation was reinforced by the observation that immediately after injection into *Xenopus* oocyte nuclei, methylated and unmethylated templates both have equivalent activity (Kass *et al.*, 1997a). However, as chromatin is assembled, the methylated DNA is repressed with the loss of DNase I hypersensitivity and the loss of engaged RNA polymerase. The requirement for nucleosomes to exert efficient repression can be explained in several ways. The repression domain of MeCP2 recruits a corepressor complex containing SIN3 and histone deacetylase that directs the modi-

fication of the chromatin template into a more stable and transcriptionally inert state (Jones *et al.*, 1998; Nan *et al.*, 1998). In addition, MeCP2 might bind more efficiently to nucleosomal rather than to naked DNA (Chandler *et al.*, 1999). Any cooperative interactions between molecules could propagate the association of MeCP2 along the nucleosomal array even into unmethylated DNA segments. This latter mechanism is analogous to the nucleation of heterochromatin assembly at the yeast telomeres by the DNA-binding protein RAP1, which then recruits the repressors SIR3p and SIR4p that organize chromatin into a repressive structure (Grunstein *et al.*, 1995; Hecht *et al.*, 1996). All of these potential mechanisms could individually or together contribute to the assembly of a repressive chromatin domain. Two other proteins, MBD2 and MBD3, also interact selectively with methylated DNA (Hendrich and Bird, 1998; Wade *et al.*, 1999). The properties of these proteins establish a general link between recognition of methylated CpGs and chromatin modification. MBD2 is a component of MeCP1 (Ng *et al.*, 1999). MBD3 is a component of the Mi2/NURD deacetylase complex (Wade *et al.*, 1999). This complex is the most abundant macromolecular form of histone deacetylase found in *Xenopus* eggs and embryos (Wade *et al.*, 1998) and in cultured mammalian cells (Zhang *et al.*, 1998). In *Xenopus*, this complex consists of six polypeptides: MBD3, the histone-binding protein RbAp48, histone deacetylase, a 66-kDa GATA zinc finger protein, a DNA-binding protein Mta 1-like, and the Mi-2 nucleosomal ATPase (Wade *et al.*, 1998). These diverse polypeptides have an interesting set of properties, which, taken together as a complex, provide significant insight into how DNA methylation can be associated with histone deacetylation.

The Mi-2 protein is a member of the SWI2/SNF2 superfamily of ATPases that use energy to disrupt histone–DNA interactions. In this context, Mi-2 must disrupt the nucleosome to allow access of RbAp48 to the histone fold domain of histone H4. This domain normally lies sequestered inside the coils of nucleosomal DNA. RbAp48 interacts with histone deacetylase directly and enhances enzymatic activity presumably by tethering the deacetylase next to the target site for deacetylation at the N-terminal tail of histone H4. The Mi2/NURD deacetylase complex resolves a paradox: the histone deacetylase catalytic subunit will modify free histone with low efficiency, but is without effect on nucleosomal histone. In the presence of ATP, the Mi-2 nucleosomal ATPase facilitates deacetylation of nucleosomal histone (Tong *et al.*, 1998). Presumably the presence of the Mta1-like and MBD3 proteins will stabilize the interaction of the Mi-2 deacetylase complex with methylated nucleosomal DNA.

If methylated DNA directs the assembly of a specialized repressive chromatin structure, it might be anticipated that the transcriptional machinery will have less access to such a structure than the orthodox chromatin assembled on unmethylated promoters and genes. Activators such as Gal4-VP16 can normally penetrate a preassembled chromatin template to activate transcription, even in the presence of histone H1 (Laybourn and Kadonaga, 1992). However, once chromatin has been assembled on methylated DNA, Gal4-VP16 can no longer gain access to its binding sites and activate transcription (Kass *et al.*, 1997a). This suggests that the specialized features of chromatin assembly on methylated DNA provide a molecular lock to silence the transcription process permanently (Siegfied and Cedar, 1997). This capacity of DNA methylation to strengthen transcriptional silencing in a chromatin context could be an important contributor to the separation of the genome into active and inactive compartments in a differentiated cell. This hypothesis presents an important area for future investigation with particular emphasis on developmental roles that may become misdirected in afflictions such as Rett syndrome.

CONCLUSION

Chromatin and chromosomes represent the true environment for transcriptional control. Experiments have now established that chromatin and chromosomal architecture have essential functions in transcriptional control. Genetic and biochemical approaches have defined numerous chromatin remodeling machines that control nucleosome structure and transcriptional activity or repression. An immediate challenge for the future is to understand how changes in nucleosome and chromatin fiber structure are targeted, how these changes are achieved by the molecular machines in mechanistic terms, and how they contribute to the compartmentalization of gene activity within the nucleus itself. In the longer term it will be important to connect these events in normal individuals with developmental decision-making and to understand how they might go wrong, leading to abnormal developmental programs.

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