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SURVEY AND SUMMARY

Chromatin disruption and modification

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ABSTRACT

Chromatin disruption and modification are associated with transcriptional regulation by diverse coactivators and corepressors. Here we discuss the possible structural basis and functional consequences of the observed alterations in chromatin associated with transcriptional activation and repression. Recent advances in defining the roles of individual histones and their domains in the assembly and maintenance of regulatory architectures provide a framework for understanding how chromatin remodelling machines, histone acetyltransferases and deacetylases function.

INTRODUCTION

Chromatin appears to be an inhospitable environment for the molecular machines that use it as a substrate for transcription, replication, recombination and repair. Nucleosomes are remarkably stable to physical perturbation and under physiological conditions nucleosomal arrays fold into stable higher order structures that self-associate within the nucleus to achieve concentrations in excess of 50 mg/ml (1,2). In spite of this apparent stability and compaction, complex metabolic processes involving DNA occur very efficiently *in vivo*. This contrasting requirement between storage and functional utility is met through the use of specialized molecular machines that reversibly disrupt and modify chromatin. These dynamic properties of the chromatin template are the focus of this Survey and Summary.

We will briefly review the roles of individual histones and their domains in chromatin structure and stability. We summarize their known relevance to the control of gene expression. The structural and functional consequences of covalently modifying the histones through acetylation and phosphorylation are discussed in light of the evidence that histone tail domains are key arbiters of chromatin function. These issues have special relevance due to the increasing evidence for the control of transcription by histone acetyltransferases and deacetylases. Finally we speculate on mechanisms of chromatin disruption and reassembly in response to the action of molecular machines requiring ATP, including RNA and DNA polymerase and the SWI/SNF family of complexes.

STRUCTURAL FEATURES OF THE NUCLEOSOME CORE RELEVANT TO STABILITY AND DISRUPTION

Histone–histone and histone–DNA interactions are now understood in considerable structural detail (3–6). 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 (7,8). The $(H3/H4)_2$ tetramer can form a stable complex with >120 bp of DNA (9). 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 (3–6). Rather, dimers of (H2A/H2B) bind to either side of the $(H3/H4)_2$ tetramer (7) and extend the wrapping of DNA within the nucleosome to >160 bp (6,9,10). This creates a left-handed superhelical ramp of protein onto which the DNA is wrapped and that is essentially comprised of the four histone dimers linked end-to-end: (H2A/H2B)-(H4/H3)-(H3/H4)-(H2B/H2A) (3). The H3:H3 and H2B:H4 dimer–dimer interfaces are comprised 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 (3–6). Given the stability of the individual heterodimers (8,9), the H2B:H4 interface is a likely site for initial disruption of histone–histone interactions upon unfolding of the nucleosome core *in vivo* (7).

In order 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 (4,6,11). These precise histone–DNA interactions constrain all DNA sequences, regardless of inherent sequence-dependent structure, to adopt a relatively similar conformation in the nucleosome (9,12,13). 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, precise sequence-dependent

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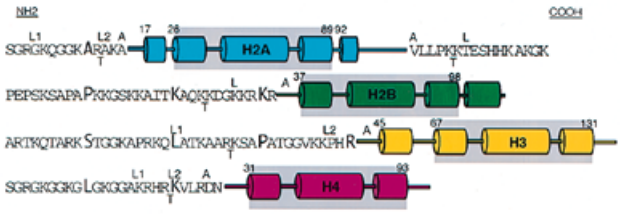

Figure 1. Core histone tail sequences and histone secondary structure. Tail sequences for the main human histone variants are shown with every tenth residue in bold type. The first (N-termini) or last (C-terminus of H2A) residues to be included in the molecular models from the crystal structures of the core histone octamer (3) or a nucleosome core (6) are indicated as A or L, respectively. L1 and L2 indicate cases where different numbers of residues for each of the two proteins present in the core structure were included in the model. α-Helical regions are indicated as columns and the three α-helices comprising the histone fold domain in each protein are indicated by the gray box. The T indicates the bond closest to the histone fold domain susceptible to trypsin proteolysis in the nucleosome.

translational positioning of the nucleosome has been observed for only a small number of DNA sequences (14,15). 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 (6,16).

External to the histone fold domains, ~25% of the mass of the core histones 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 (17; Fig. 1). Proteolytic removal of the tail domains does not drastically alter the conformation or hydrodynamic properties of individual nucleosomes and the tails do not play a role in nucleosome positioning or the correct assembly of nucleosomes *in vitro* (9,18). These N-termini, if fully extended, can project well beyond the superhelical turns of DNA in the nucleosome (6; Fig. 2). 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 (19,20). Further, the tails are critical for the self-assembly of condensed fibers into higher order structures (21,22). Interestingly, histone tail interactions change as the chromatin fiber undergoes folding or compaction, suggesting that specific tail interactions are correlated with specific conformations of the fiber (23). Thus, certain post-translational modifications may evoke specific functional and/or conformational states of the chromatin fiber by inducing a defined alteration in the array of histone tail interactions (24; below).

It is known that *in vivo* and *in vitro* the N-termini interact with DNA and protein within chromatin (25–28). Unfortunately, the high ionic strength of the crystal environment and the lack of linker DNA preclude the observation of many of these interactions in the crystal structure of the nucleosome core (6,27). However, *in vitro* experiments under commonly used transcription conditions have allowed mapping of some contacts between the N-termini and nucleosomal DNA (29–31). For example, H2A has an extended C-terminal tail that makes contact with DNA near the dyad axis at the center of the nucleosome core (32,33). However, in nucleosome structures which contain linker DNA similar to native chromatin, significantly different interactions are found (32,33; Fig. 3). Consistent with the centrifugation studies mentioned above, mapping experiments indicate that significant rearrangements in tail–DNA interactions are found to occur as a result of the binding of linker histones and during the assembly of higher order chromatin structures (33–36). Thus these experiments provide additional evidence for a precise set of molecular interactions of the core histone tails which depends critically on the context and conformation of the chromatin fiber (24).

HISTONE H1 AND HIGHER ORDER CHROMATIN STRUCTURE

Incorporation of linker histones into chromatin stabilizes nucleosomes and facilitates the assembly of higher order chromatin structures. However, whereas core histones are essential for chromatin and chromosome assembly, linker histones are not required (37,38). 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 (39) and can associate with the nucleosome core in a number of distinct ways (40,41). 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 (42–46). 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 (47–49). Under physiological conditions the association of histone H1 with chromatin is much less stable than that of the core histones (50). As mentioned above, binding of linker histone leads to a partial rearrangement of the core histone interactions in the nucleosome (33,35,51). 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.

GENETIC EVIDENCE FOR INDIVIDUAL HISTONES AND THEIR DOMAINS IN TRANSCRIPTIONAL CONTROL

Genetic experiments in *Saccharomyces cerevisiae* provide compelling evidence for general and specific roles for the histones in transcriptional control (52,53). 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 (54). Acetylatable lysines in the N-termini of H3 and H4 have roles in transcriptional activation and repression (55,56). 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 (6). Certain mutations of lysine to glutamine in the N-termini of H3 and H4 relieve the requirement for histone acetyltransferase activity in transcriptional activation (57). 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

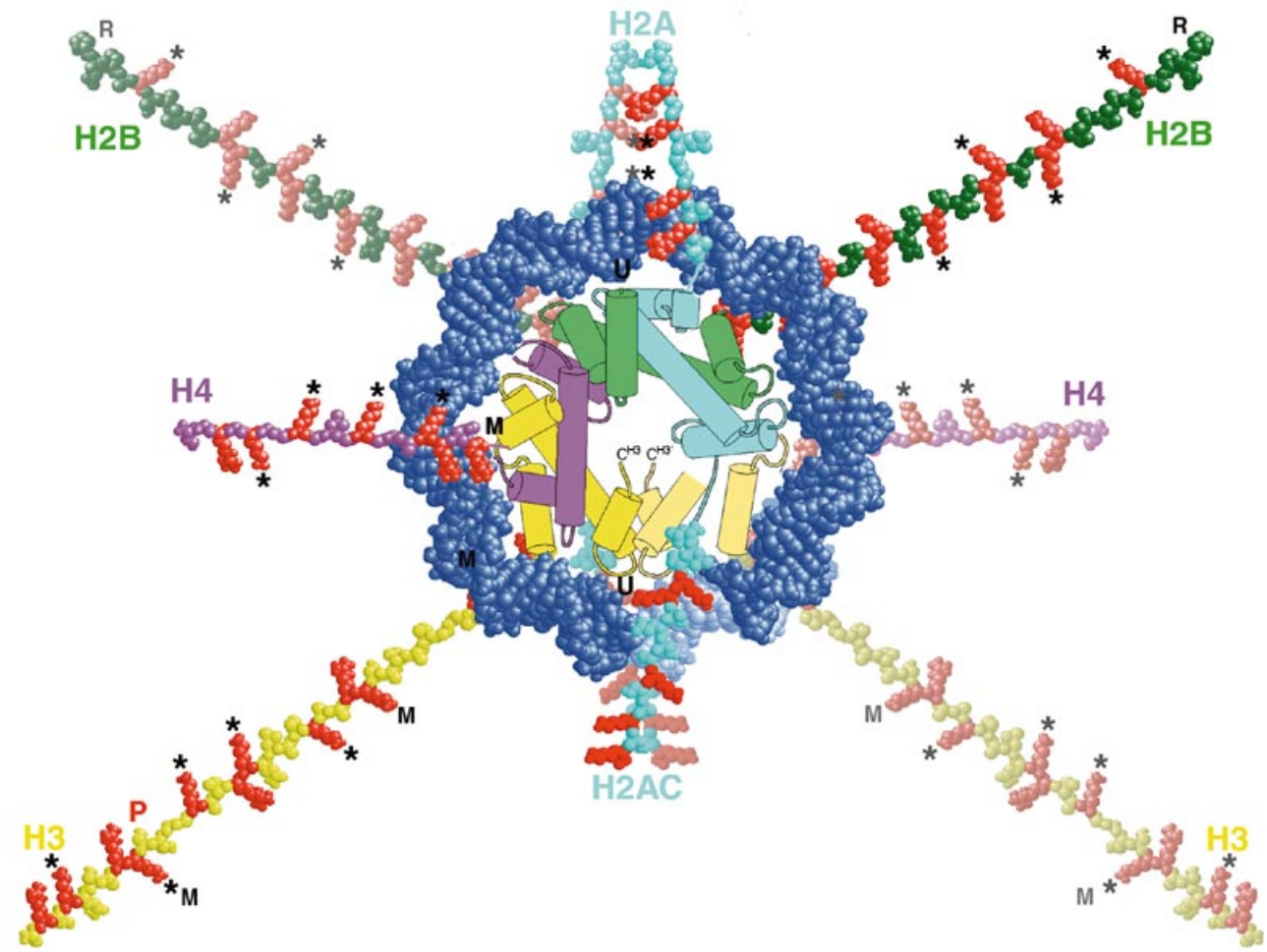

Figure 2. Sites of post-translational modifications within the histone tail domains. The histone tail domains and the nucleosome core proper are viewed along the superhelical DNA axis. The tail domains are modeled as fully extended polypeptide chains to show the approximate length of these domains with respect to the largely α-helical histone fold domains (columns). Tail sequences are positioned according to the X-ray crystal structure of a nucleosome core (6). The top and bottom superhelical turns of core DNA are colored blue and light blue, respectively. H2A, H2B, H3 and H4 are colored cyan, green, yellow and magenta, respectively, while arginine and lysine residues in the tails are colored red. The H2A C-terminal tail is indicated as H2AC. Note that only the top four polypeptides are shown in their entirety; a portion of H3 from the bottom half of the nucleosome is shown (light yellow). Likewise, tails from histones in the bottom half of the nucleosome are shaded lighter than those from the top half. Well-characterized sites of acetylation on lysines are indicated by an asterisk (1). Sites of methylation (M), the site of phosphorylation (P) in the H3 tail (Ser10), and sites of ribosylation (R) and ubiquitination (U) in H2A and H2B are also indicated (1). Note that other sites of modifications such as phosphorylation of the N-terminal serines of H2A and H2B are not represented here.

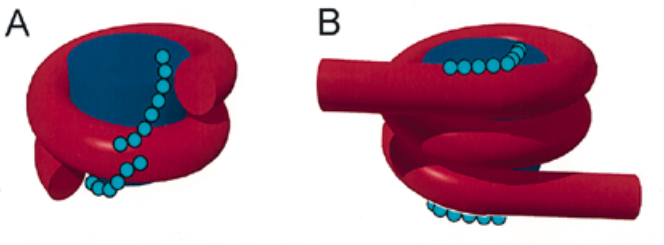

Figure 3. Location of the C-terminal tail of H2A in nucleosome core particles (**A**) and in nucleosomes containing linker DNA (**B**). The sites of DNA contact by the C-terminal tail as mapped by general and specific crosslinking experiments are shown (31,32,51). The DNA is shown as a red tube, the core histones as a blue column and residues comprising the C-terminal tail of H2A as light blue spheres.

relieving the requirement for the SWI/SNF family of molecular machines known to disrupt chromatin (below; 58,59).

Saccharomyces cerevisiae has an unusual non-essential linker histone, containing two globular domains, deletion of which has no detectable effects on gene expression (60,61). 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 (62). Ablation of histone H1 during *Xenopus laevis* development leads to constitutive activation of certain oocyte-specific 5S rRNA genes and mesodermal-specific genes (63–65). Repression can be restored by expression of the globular domain lacking N- and C-terminal tails (66). 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 with somatic 5S DNA in

Xenopus laevis. It binds to the 5S nucleosome asymmetrically serving to position the histone octamer to repress certain genes while allowing continued activity of others (67–71). Comparable mechanisms may operate on the highly divergent *Xenopus borealis* oocyte and somatic 5S rRNA gene repeats (72), however, functional studies remain to be carried out. 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 (53,73–75). 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 (76–78), while corepressors containing histone deacetylases confer transcriptional repression (79–84). Histones are locally modified on target promoters (78,85) and specific lysines in particular histones are functional targets for acetyltransferases and deacetylases (57,85). Activator-dependent targeting of histone acetylase activity has recently been recapitulated *in vitro* (86). Histone acetylation states are 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 (87,88). The dynamics of histone acetylation provides an attractive mechanistic foundation for the reversible activation and repression of transcription (89,90).

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 (91–94). 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 (91,94–98), consistent with acetylation reducing the stability of interaction of the histone tails with nucleosomal DNA (99). It should nevertheless be noted that the N-termini of the core histones always make contact with DNA even when they are acetylated (100). 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 (101–103; but see ref. 104). These changes might be due to the fact that the acetylated N-terminal histone tails bind DNA with reduced affinity (99,105) and are more mobile with respect to the DNA surface than unmodified tails (29). 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 (106). 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 (20,22,95,96; but see ref. 107). 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 (108). Interactions between adjacent nucleosomal arrays are reduced when they are reconstituted with acetylated histones (22) and chromatin solubility is increased (22,109). *In vivo*, the region of DNase I sensitivity within the active β-globin locus also correlates with a region of increased histone acetylation (110). Acetylation of core histones does not have major consequences for the association of histone H1 with mono- or dinucleosomes (95,111) or for the recovery of H1 from nuclear chromatin (112). However, maintenance of diacetylated histone H4 in the nascent chromatin assembled on newly replicated DNA reduces the incorporation of histone H1 (113). Thus, in some circumstances, acetylation of the core histones, which causes the destabilization of the chromatin fiber (20,22), might lead to increased dissociation of histone H1 (113). Within the 75 mg/ml nucleoprotein environment of the eukaryotic nucleus all of these structural transitions might contribute to facilitating transcription.

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 (out of 28 potential acetylated lysines) will promote *in vitro* transcription >15-fold (22). This level of modification reduces chromatin compaction to the same extent as proteolytic removal of the N-termini (21,22), 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 (99) and the histones of adjacent nucleosomes (24). 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 non-histone proteins (114). 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 (115–117). 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, UBIQUITINATION, ADP-RIBOSYLATION AND METHYLATION OF THE CORE HISTONES

In contrast to the many studies on the structural and functional consequences of histone acetylation, the impact of other post-translational 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 N-terminal domain, when extracellular signals such as growth factors or phorbol esters stimulate quiescent cells to proliferate (118). Global phosphorylation of Ser10 in H3 occurs in pericentromeric chromatin in late G_2 phase, completely spreads throughout the chromosome just before prophase of mitosis and is rapidly lost during anaphase (119). This modification is spatially and temporally correlated with mitotic and meiotic chromatin condensation (120). H3 Ser10 is located within the basic N-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 (121). Interestingly, the low level of phosphorylation of H3 that is detectable in interphase cells is likely to be related to preparing chromatin for transcription. Indeed, based on charge effects phosphorylation of histone H3 might be expected to have structural consequences comparable with acetylation. Several studies have suggested 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* following their rapid induction to high levels of transcriptional activity by phorbol esters (118,122,123). 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 non-histone 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 implies that a major disruption of nucleosome structure occurs which could involve the dissociation of a H2A/H2B dimer. Phosphorylation and acetylation of histone H3 might act in concert to cause these changes. There is likely to be an important link between cellular signal transduction pathways and chromatin targets for post-translational 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 (124,125). Ubiquitination of histone H2A is associated with transcriptional activity. Only one nucleosome in 25 contains ubiquitinated histone H2A within non-transcribed chromatin. This increases to one nucleosome in two for the transcriptionally active *hsp70* genes (126). Enrichment in ubiquitinated H2A is especially prevalent at the 5′-end of transcriptionally active genes (127). Since the C-terminus of histone H2A contacts nucleosomal DNA at the dyad axis of the nucleosome (32), ubiquitination of this tail domain might be anticipated to disrupt higher order chromatin structures.

ADP-ribosylation of core histones may also lead to localized unfolding of the chromatin fiber. ADP-ribosylation may play a particularly important role in DNA repair. Here the disruption of chromatin structure cannot always rely on the processive enzyme complexes involved in DNA replication or transcription or the specific recruitment of acetyltransferases or SWI/SNF complexes. The synthesis of long negatively charged chains of ADP-ribose may well facilitate a partial disruption of nucleosomes, presumably by exchange of histones to this competitor polyanion (1,2).

Core histones are methylated on their lysine residues without clearly defined functional consequences. Most methylation in vertebrates occurs on histone H3 at Lys9 and Lys27 and histone H4 at Lys20. These are not known sites of acetylation (1,2). Methylation of H3 seems to be correlated with acetylated regions of chromatin while methylation of H4 seems to have the opposite correlation (128). However, the exact role(s) of this modification has not been elucidated.

PHOSPHORYLATION OF LINKER HISTONES

Phosphorylation of histone H1 has been shown directly to weaken interaction of the basic tails of the protein with DNA. Surprisingly, these changes influence the binding of the protein to chromatin even more than to DNA and thereby potentially destabilize the chromatin fiber (129). 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 (130). Linker histone becomes heavily phosphorylated on transcriptional activation of the micronucleus of *Tetrahymena* during the sexual cycle (131). Transcriptional competence of the mouse mammary tumor virus (MMTV) promoter depends on the phosphorylation of histone H1 (132) and the active MMTV promoter is known to be selectively depleted in H1 (133). 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. In some cases, phosphorylation may also inhibit transcription. For example, phosphorylation of *Tetrahymena* H1 appears to inhibit activation of the *Cyp* gene *in vivo*, an effect similar to that observed in H1 knockouts (Y.Dou and M.A.Gorovsky, personal communication; 62). A possible explanation of these seemingly contradictory effects of H1 phosphorylation *in vivo* is that cell cycle or developmentally regulated phosphorylation weakens the association of H1 with chromatin *in vivo*, allowing access by transcriptional repressors whose cognate sequences are blocked by H1 or, alternatively, preventing H1 from functioning as a coactivator (M.A.Gorovsky and Y.Dou, personal communication).

CHROMATIN DISRUPTION BY DNA POLYMERASE, RNA POLYMERASE AND SWI/SNF COMPLEXES

In addition to the consequences of covalent modification, chromatin can also be disrupted by molecular machines driven by ATP hydrolysis, including DNA and RNA polymerases and SWI/SNF-type complexes, such as NURF, RSC, CHRAC and ACF (134). Nucleosomes are disrupted by DNA polymerase with the pre-existing histone $(H3/H4)_2$ tetramers being distributed between both daughter DNA duplexes and reassociating with pre-existing and newly synthesized histone (H2A/H2B) dimers (1,2,135,136). Nucleosomes appear to fall apart once the replication complex has penetrated into the structure (135). Half of the newly assembled nucleosomes on nascent DNA contain newly synthesized diacetylated H4 and consequently will be more accessible to the transcriptional machinery (95). Felsenfeld and colleagues have shown that RNA polymerase needs to disrupt histone–DNA contacts in half of the nucleosome approximately up to the dyad axis in order to effect cooperative displacement of the remaining histone–DNA interactions (137–139). Prokaryotic DNA and RNA polymerases have remarkable success in traversing chromatin templates (136–138), however, eukaryotic RNA polymerases II and III have some difficulty progressing along nucleosomal arrays (140–144).

Eukaryotic polymerases make use of additional factors to promote elongation through chromatin (145,146). These include proteins of the SWI/SNF class (146). Components of the yeast SWI/SNF complex and their metazoan homologs were originally identified from genetic analysis (147,148). Molecular insights into how these molecular machines are targeted to particular promoters are beginning to emerge (149), however, the majority of studies have concerned the untargeted disruption of chromatin (150). Mononucleosomal substrates lose the rotational constraint of DNA on the histone surface in the presence of yeast or mammalian SWI/SNF complexes (151–153). This loss requires ATP hydrolysis and facilitates the access of DNA-binding proteins to DNA in the nucleosome. Interestingly DNA remains

wrapped on the core histones over at least 80 bp under these conditions (154). The continued wrapping of DNA but the increase in conformational flexibility is similar to the consequences of histone acetylation (102,103). Experiments using nucleosomal arrays lacking H1 establish that the yeast SWI/SNF complex facilitates the association of transcription factors, thereby generating a DNase I hypersensitive site that persists after removal of the SWI/SNF complex. Nucleosomes within the array lose defined boundaries and their DNA becomes more accessible to restriction endonucleases (155,156). This latter endonuclease accessibility assay has been used to unequivocally demonstrate a catalytic activity for the yeast SWI/SNF complex in disrupting nucleosomes within arrays (156). The metazoan SWI/SNF complexes show a comparable disruption of nucleosomal arrays dependent on ATP hydrolysis (157,158).

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 polymerases and displaces nucleosomes in a comparable way (159). 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 (150,154). An alternative idea is that histones H2A and H2B are displaced or destabilized within the nucleosome (160). Removal of H2A and H2B facilitates access of transcription factors to nucleosomal DNA (161,162) and facilitates transcription (141). Although complete displacement of (H2A/H2B) dimers seems unlikely (151), destabilization of (H2A/H2B) association would be consistent with genetic and structural data. This disruption might generate a structure prone to homologous dimerization (163).

Mutation of the core histone fold domains can generate yeast strains that are SWI/SNF independent (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 (59) or at sites that destabilize histone–DNA interactions (58,164–166). The boundaries of the nucleosome core are known to be mainly defined by the (H2A/H2B) dimers (4,6). 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 (154) and in nucleosome cores containing SIN2 mutant histones (164). The $(H3/H4)_2$ tetramer rotationally constrains DNA as efficiently as the histone octamer (9), thus the interaction of the $(H3/H4)_2$ tetramer with DNA must also be destabilized during SWI/SNF-mediated nucleosome disruption. This essential role for the $(H3/H4)_2$ tetramer lends a note of caution to experiments in which histone H4 is mutated at an amino acid (Ser47) in the vicinity of key tetramer–DNA contacts in order to reconstruct nucleosomes that can be used for positioning studies (72,167,168). Mutation at Arg45 of histone H4 within the nucleosome leads to a SIN2 phenotype (58,166). Interference with the association of key arginine residues in the $(H3/H4)_2$ tetramer with DNA might suffice to reduce rotational constraint of the double helix. We speculate that the binding of the SWI/SNF complex to the nucleosome destabilizes both (H2A/H2B) dimer and $(H3/H4)_2$ 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 (169) and may resemble the interaction of other nucleosome core-binding proteins, such as the globular domain of linker histones, HNF3 and NF1 (67,170,171). 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 (33,35,51). Replacement of histone H1 might also facilitate nucleosome mobility as reflected in the loss of clearly defined spacing (155,157,158) and destabilize higher order chromatin structure. Protein compositional analysis within nucleosome arrays containing H1 and SWI/SNF crosslinking to nucleosomal substrates will be necessary to test this hypothesis.

FUTURE PROSPECTS

Structural work on chromatin has lagged behind the impressive advances made in documenting the existence of coactivators and corepressors that target the covalent modification of histones and the genetic definition of the role of individual histone domains in transcriptional control. There is a compelling need to determine the exact structural and functional consequences of modifying the histones in the exact manner inferred to be of major significance by genetic analysis. These modifications are likely to elicit concerted rearrangments of histone–DNA and histone–protein interactions, especially those involving the histone tail domains. Assays are now available with sufficient sensitivity to assess the effect of particular histone tails and modest levels of histone acetylation on higher order structure (21,22). As histones are now established as *bona fide* regulators of gene expression, this type of analysis will be of increasing importance until eventually the transcriptional machinery itself ought to be incorporated into an integrated structural model. We still do not know at which point(s) histone acetylation exerts influence in the regulation of transcription. It could be during pre-initiation complex assembly, recruitment of RNA polymerase, escape of RNA polymerase into the transcription unit or transcriptional elongation. Our lack of knowledge concerning the structural and functional consequences of other histone modifications is even more extreme and offers considerable opportunity for analysis.

Chromatin is conformationally dynamic with DNA polymerase gaining access to the entire genome once every cell cycle and RNA polymerase to the active transcription units several times per hour for an active gene (172). Histone modifications and nucleosome disruption will follow as a consequence of these events as chromatin is reassembled after the polymerase. Although the replication fork will provide an opportunity to alter a state of gene activity, it is unlikely to be used in the control of gene expression in the absence of cell division. The SWI/SNF complex provides an ATP-driven motor that can disrupt chromatin independent of cell cycle progression. The assays for chromatin disruption by SWI/SNF complexes currently use low resolution techniques such as nucleases and topological assays. Here protein–DNA crosslinking and sedimentation analysis could provide much useful information. A challenge so far unexplored for SWI/SNF is not only how to take a nucleosome apart but how to reconstitute chromatin as a component of repression pathways. Here the recent discovery of an SWI/SNF family ATPase as a component of a histone deacetylase complex suggests that SW1/SNF might have an important role in the covalent modification

of the histones coupled to repression (173,174). The future offers the exciting prospect of integration of both covalent modification of histones and the ATP-driven molecular machine into the regulation of transcription through the targeted reversible disruption of chromatin.

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