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Review

Twenty-Five Years of the Nucleosome, Fundamental Particle of the Eukaryote Chromosome

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The proposal made 25 years ago that chromatin structure is based on a repeating unit of eight histone molecules and about 200 DNA base pairs (Kornberg, 1974) laid the basis for subsequent chromatin research. During the past few years, the X-ray structure of the repeating unit, termed the nucleosome, has been solved (Arents et al., 1991; Luger et al., 1997), and its biologic significance has emerged. The nucleosome is a focal point of transcription control, uniting previously disparate observations on gene activation and repression at the level of individual promoters, transcription units, and whole chromosomal domains (reviewed by Lewin, 1994; Grunstein, 1998; Kadonaga, 1998; Struhl, 1998; Bell and Felsenfeld, 1999). Connections with DNA replication and the cell cycle, with recombination and other DNA transactions, and with viral infection and cancer have become apparent as well. This convergence of structural and functional studies gives occasion to celebrate the silver anniversary of the nucleosome.

The Histones

The initial impact of the nucleosome idea was to dispel an aura of mystery surrounding the histones, whose existence was recognized in the last century (Kossel, 1884). As universal components of eukaryote chromosomes, histones were found at a mass level roughly equal to that of the DNA; in fact, the histones were long viewed as the genetic material itself, and later as diverse proteins likely to function as specific gene regulators. The diversity proved, however, to be an artefact of isolation. Histones were commonly extracted with salt solutions from thymus tissue, whose abundant proteases doubtless degraded them. Upon extraction in acid, preventing proteolysis, just five histone types, now designated H1, H2A, H2B, H3, and H4, were observed (Phillips and Johns, 1965). Subsequently, amino acid sequence determination of H4 disclosed a near perfect conservation across species (DeLange et al., 1969). Far from being a diverse set of molecules, the histones are among the most invariant proteins known. And yet, the possibility that histones serve as specific regulatory molecules could not be discounted, inasmuch as various combinations of the five histones might still contribute to the complexity of transcription control. The idea of combinatorial control was encouraged by apparent variation in the relative abundance of the histones, which argued against a unique repeating order of these proteins in chromatin (reviewed by Huberman, 1973); combinatorial control was finally ruled out by the recognition of just such a unique repeating order in the nucleosome.

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The mystery of the histones also derived from their bizarre biochemical behavior. Purified histones formed all manner of aggregates, both individually and in mixtures with one another (Edwards and Shooter, 1969; D'Anna and Isenberg, 1974), discouraging attempts at model building and other predictive structural analysis. The histones came to be regarded as an amorphous coating or passive polymeric counterion of the DNA. Ironically, the very breakthrough of acid extraction, which clarified the molecular composition of the histones, at the same time denatured them and impeded further studies. Once denatured, elucidation of their structure and biochemistry was doomed. With the advent of powerful protease inhibitors, histones could be extracted by milder methods (Van Der Westhuyzen and Von Holt, 1971), leading to the discovery of an $(H3)_2(H4)_2$ tetramer (Kornberg and Thomas, 1974; Roark et al., 1974), as well as an H2A-H2B dimer (Kelley, 1973). These histone oligomers could be recombined with DNA to generate the characteristic X-ray diffraction pattern of chromatin; both types of oligomer were required, whereas the H1 histone was not (Kornberg and Thomas, 1974).

The Nucleosome

The organizing principle of the nucleosome, a histone octamer, and its mode of interaction with DNA were deduced from the properties of the tetramer, as follows (Kornberg, 1974):

(1) The stoichiometry of the tetramer indicated that apparent deviations from equimolar amounts of H2A, H2B, H3, and H4 in chromatin could be disregarded, and it further implied a repeating unit that contained two each of the histones. The requirement for all four histones to form the repeating unit revealed by X-ray diffraction then led to the proposal of the histone octamer.

(2) Based on the roughly equal weights of histone and DNA in chromatin, an octamer would be associated with about 200 base pairs of DNA.

(3) The biochemical behavior of the tetramer, like that of a typical globular protein, implied a compact shape and thus the wrapping of the DNA on the outside.

(4) The occurrence in chromatin of roughly half as much H1 as each of the other histones pointed to the association of one molecule of H1 with the nucleosome. The lack of a requirement for H1 to reconstitute the X-ray diffraction pattern suggested that H1 bound on the outside of the nucleosome.

A unit of chromatin structure based on a histone octamer surrounded by DNA had immediate appeal as a common denominator of many findings. Earlier work had shown that the DNA in isolated rat liver nuclei was converted by the action of an endogeneous nuclease to multiples of a unit size (Hewish and Burgoyne, 1973). A similar observation was made for the fragmentation of cellular DNA in necrotic cells, where the unit length was estimated to be about 200 base pairs (Williamson, 1970), consistent with the nucleosome hypothesis. A

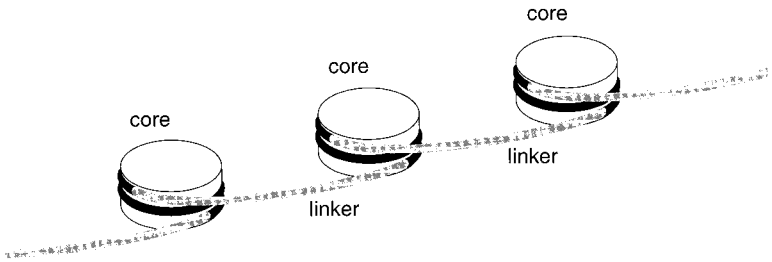

Figure 1. Schematic of Nucleosome Core Particle and Linker

The histone octamer is represented as a disk, and the DNA as a ribbon, with shading to distinguish core DNA (dark) from linker DNA (light).

shorter fragment of 110 base pairs, produced upon extended micrococcal nuclease digestion (Sahasrabuddhe and van Holde, 1974), was explained by subsequent characterization of the "core particle" of the nucleosome (see below). Finally, electron microscopists early noted a "knobby" appearance of chromatin fibers (Bram and Ris, 1971), which came into clearer focus with improved methods of specimen preparation, revealing a beaded substructure of the fibers (Olins and Olins, 1974; Woodcock et al., 1976). Soon after, electron microscopy of reconstituted histone–DNA complexes (Oudet et al., 1975) and of chromatin digested with micrococcal nuclease (Finch et al., 1975; Oudet et al., 1975) identified the beads as nucleosomes.

Subsequent solution studies provided further support for the nucleosome. Physical isolation proved the existence of the proposed histone octamer (Thomas and Kornberg, 1975). Digestion of chromatin with DNase I supported the proposed wrapping of the DNA around the octamer (Noll, 1974). Topoisomer analysis quantitated the wrapping (Germond et al., 1975). Additional studies of histone–DNA complexes completed the characterization of the nucleosome (reviewed by Pederson et al., 1986).

Structure of the Nucleosome

Micrococcal nuclease digestion of chromatin paved the way to structure determination of the nucleosome. After cleaving the DNA between nucleosomes, micrococcal nuclease digests the ends of the DNA protruding from the nucleosome to a metastable limit at 146 base pairs (van Holde et al., 1975; Noll and Kornberg, 1977). The resulting complex, containing all four histones in association with the residual DNA, has come to be known as the "core particle" of the nucleosome (Figure 1). Core particles are homogeneous enough to be crystallized. Analysis at 7 Å resolution by a combination of X-ray and electron crystallography revealed the coiling of DNA in 1 3/4 left-handed superhelical turns around the histones (Finch et al., 1977). Details of histone arrangement and structure came from crystallographic analysis of the octamer alone. The histones were seen to form a left-handed protein superhelix matching that of the DNA in the core particle (Klug et al., 1980; Arents et al., 1991). The $(H3)_2(H4)_2$ tetramer lay at the center, with H2A-H2B dimers at the ends of the DNA path. Each of the histones exhibits a similar polypeptide chain fold (the "histone fold," Figure 2), based on a long central α helix, flanked on both sides by shorter helices and loops that interact with DNA (Arents and Moudrianakis, 1995). Finally, 15–30 residues at the amino termini of all the histones are unstructured and commonly referred to as "tails."

The high-resolution structure of the core particle (Luger et al., 1997) shows details of the histone–DNA interaction, which is confined to the phosphodiester backbones of the DNA strands on the inner surface of the superhelix (Figure 2). A set of contacts is made every 10 base pairs where the minor groove of the double helix faces inward. Electrostatic interactions and hydrogen bonding with the DNA phosphates, as well as nonpolar contacts with the deoxyribose groups, are observed. The lack of contacts with DNA bases is in accord with the lack of sequence specificity of the histone–DNA interaction, evident from the capacity of the histone octamer to package virtually any DNA. Two further points that relate to nucleosome function are also noteworthy. First, the two turns of the DNA superhelix are in register, with grooves aligned, creating sufficient gaps for the amino-terminal tails of both H2B and H3 to pass through to the outside of the core particle. H2A and H4 tails pass across the superhelix on the flat faces of the particle to the outside as well. Thus, the histone tails are exposed, a feature crucial for the role of the nucleosome in transcriptional regulation. The second point of functional interest concerns the twist of the DNA double helix associated with the core particle. A stretch of about 10 base pairs of slightly altered twist, with about 1 fewer base pair per turn, is readily accommodated at various locations within the core particle. This structural plasticity may underlie the remodeling of chromatin for transcription (see below).

Structure of Chromatin Fibers

The DNA between core particles, connecting one nucleosome to the next in chromatin, is referred to as "linker" (Figure 1). Variation in the length of linker DNA (Spadafora et al., 1976) is important for the diversity of gene regulation. Despite this variation, a chain of nucleosomes can still coil or fold in a regular manner, to form a chromatin fiber. How variability is reconciled with regularity underlies a mechanism of great significance, the decondensation of chromatin for transcription, and its modulation by posttranslational modifications of the histone tails.

The length of linker DNA varies in many cases almost at random, but the locations of nucleosomes are sometimes constrained by barriers, such as sequence-specific DNA-binding proteins on the DNA, as well as by more subtle effects, such as sequence dependence of the energy of bending DNA. As a consequence, nucleosomes often appear at preferred positions in the vicinity

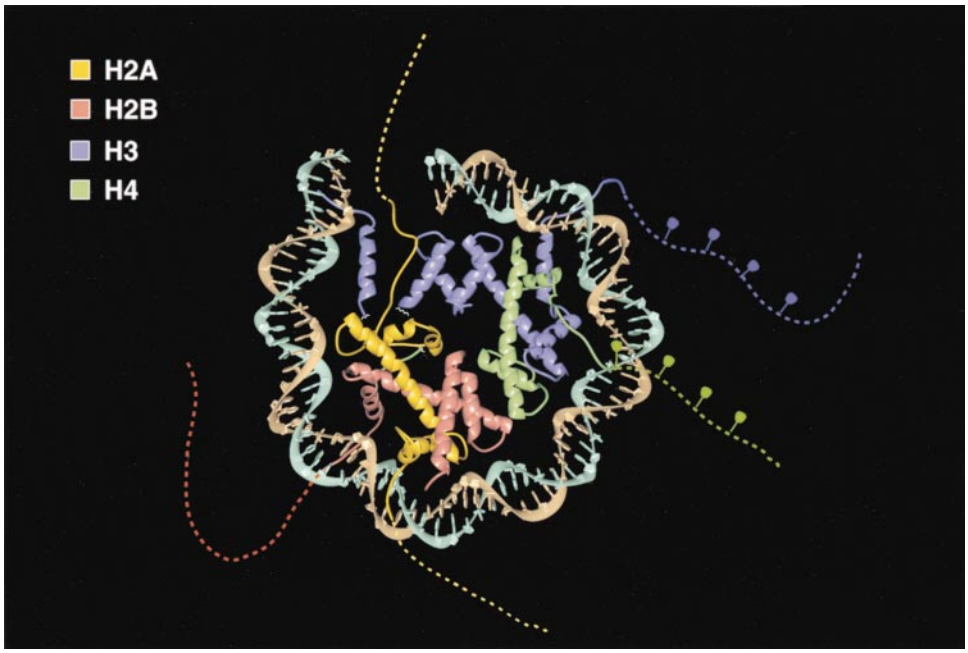

Figure 2. X-Ray Structure of Nucleosome Core Particle

The disk in Figure 1 is split between the two turns of DNA and viewed from above. Half the core particle, with four histone molecules and 73 DNA base pairs, is shown. Three helices from the H3 molecule in the other half of the core particle are included in this view at the upper left. The unstructured histone tails, absent from the X-ray structure, are drawn as dashed lines of appropriate length, with lollipops to indicate acetylation sites. H2A tails are both N terminal (bottom) and C terminal (top), while tails shown for the other histones are all N terminal. (Adapted from Luger et al., 1997, courtesy of Luger and Richmond).

of promoters, regulatory elements, and other special sites in DNA (reviewed by Simpson, 1991; Thoma, 1992). Positioning may serve to prevent specific protein binding to sites within the nucleosome or to potentiate binding to sites exposed in linkers (see, for example, Straka and Horz, 1991). Positioning may also facilitate the binding of proteins evolved to recognize DNA sequences located on the surface of the nucleosome or juxtaposed by coiling around the nucleosome (see, for example, Lu et al., 1995).

A degree of sequence preference is found also for interaction of the so-called "linker histones," H1 and its avian counterpart H5. The main mass of the protein binds near one end of the core DNA, which frequently contains the sequence AGGA (Travers and Muyldermans, 1996). Neutron diffraction places H1, and thus the linker DNA, on the inside of the chromatin fiber (reviewed by Ramakrishnan, 1997), in keeping with evidence that linker histones promote coiling or folding of chromatin in the fiber. Histone tails protruding from nucleosomes also appear to promote fiber formation, perhaps by contacting adjacent nucleosomes or by influencing the configuration of linker DNA (Schwarz et al., 1996). An interaction, seen in core particle crystals (Luger et al., 1997), of an H4 tail from one nucleosome with a region of the H2A-H2B dimer on the flat face of an adjacent nucleosome, could play such a role. Modifications of the histone tails may promote or disrupt these contacts, controlling fiber formation, and thereby modulating the accessibility of chromatin for transcription (Tse et al., 1998).

Nucleosomes Repress Transcription

Packaging promoters in nucleosomes prevents the initiation of transcription by bacterial and eukaryotic RNA polymerases in vitro (Knezetic and Luse, 1986; Lorch et al., 1987). Nucleosomes exert a similar inhibitory effect upon transcription in vivo: turning off histone synthesis by genetic means in yeast, and consequent nucleosome loss, turns on transcription of all previously inactive genes tested (Han and Grunstein, 1988). These and other observations have led to the conclusion that histones serve as general gene repressors.

Both histone fold–DNA interactions in the core particle and histone tail interactions in the chromatin fiber are thought to contribute to repression, and each is counteracted by specific mechanisms. Repression due to interactions in the core particle is opposed by "chromatin-remodeling" complexes, while that due to condensation in the chromatin fiber may be relieved by histone acetyltransferase (HAT) and reestablished by histone deacetylase (HDAC) complexes. A number of observations suggest that derepression at the level of the chromatin fiber precedes that at the level of the individual nucleosome. Transcriptionally active genes in vertebrates reside in chromosomal domains, as large as 100 kb, characterized by an elevated rate of digestion by DNase I, increased acetylation of histone tails, and well-defined boundaries (Hebbes et al., 1994 and references therein). DNase I sensitivity and histone acetylation are more or less uniform across a domain, including nontranscribed, intergenic regions. Thus, these features are not simply a consequence of transcription, but rather relate to a

more general property, such as decondensation of the chromatin fiber encompassing the domain. The DNA sequences governing this decondensation have not been identified with certainty, but they may include "locus control regions," which can regulate the activity of several genes within a single domain (reviewed by Grosveld, 1999), and "insulators," which define the boundaries of a domain (reviewed by Bell and Felsenfeld, 1999).

The pattern of micrococcal nuclease digestion within a domain, reflecting chromosome structure at the level of the nucleosome, is not much affected by transcription, except at sequences such as enhancers and promoters, which become "hypersensitive" to digestion, due to the loss of nucleosomes (Varshavsky et al., 1979; Wu, 1980; Almer and Horz, 1986). Hypersensitive sites are formed independently of and subsequent to the appearance of general DNase I sensitivity of a domain. They are typically a few hundred base pairs in length and appear to consist of naked DNA with specifically bound transcription factors. Some of these factors display HAT activity, directed toward neighboring nucleosomes.

Histone Acetylation

The recent discovery of the HAT activity of transcription factors provided key evidence for a regulatory role of the nucleosome (reviewed by Grunstein, 1997; Struhl, 1998; Bjorklund et al., 1999). The connection between histone acetylation and transcription had long been suspected (Allfrey, 1977). Acetylation of multiple sites in the core histone tails was known to be associated with transcriptional activity in isolated nuclei and partially purified chromatin preparations. Furthermore, constitutively inactive heterochromatin shows a lack of acetylation at sites in the histone tails important for transcription. Finally, substitution of the lysine residues in the H4 tail with arginine, which cannot be acetylated, virtually abolishes transcription of inducible genes in yeast.

The connection between acetylation and transcription still remained uncertain until the demonstration that yeast Gcn5 protein, a positive transcriptional regulator of many genes, has HAT activity (Brownell et al., 1996), and stimulation of transcription by Gcn5 requires the HAT activity (Kuo et al., 1998). Chromatin in the vicinity of a Gcn5-dependent promoter showed an increase in the level of H3 acetylation upon induction. Mutation of the catalytic domain of Gcn5 eliminated the effects on both transcription and acetylation (Kuo et al., 1998). Gcn5 may be classified as a type A HAT, nuclear in origin, active on chromosomal histones, with a role in transcription. Cytoplasmic type B HATs, by contrast, are mainly involved in the acetylation of newly synthesized histones prior to their assembly with newly replicated DNA in chromatin.

The implications of Gcn5 HAT activity were soon reinforced by similar results on mammalian transcription factors (reviewed by Grunstein, 1997; Struhl, 1998). The human coactivator, p300/CBP, was identified as a HAT, whose activity is closely correlated with its effect on transcription. Interaction of p300/CBP with numerous DNA-binding regulatory proteins integrates and transduces signals for control of the cell cycle, differentiation, DNA repair, and apoptosis. Two further coactivators that interact with nuclear hormone receptors are also HATs, as is the general transcription factor subunit $TAF_{II}250$. The targets of these multiple HATs can include not only histones but also other components of the transcription machinery. For example, p300/CBP can acetylate the p53 transcriptional activator protein, as well as TFIIE and TFIIF, and $TAF_{II}250$ can also acetylate TFIIE and TFIIF.

Recombinant Gcn5 protein acetylates histones in the free state but not in nucleosomes. This lack of activity on the natural substrate prompted investigation of the natural form of Gcn5, which resulted in the discovery of the SAGA complex (Table 1), so-called for its content of *S*pt and *A*da proteins, *G*cn5, and *a*cetyltransferase (Grant et al., 1997); *SPT* and *ADA* genes had been identified previously by screens for mutations affecting transcriptional activation and promoter function. A human counterpart of the SAGA complex, containing the acetylase PCAF (related to Gcn5), as well as Spt and Ada homologs, has been described (Ogryzko et al., 1998).

Histone acetylation by promoter-associated transcription factors is localized. For example, increased acetylation of H3 and H4, attributed to p300/CBP, was found upon viral infection in two to three nucleosomes surrounding the interferon-β promoter (Parekh and Maniatis, 1999). Whether this increase is in addition to the domain-wide acetylation found for chromosomal genes (see above), or whether it occurs instead of such acetylation at certain promoters, remains unclear.

Histone Deacetylation

The connection between acetylation and transcription is further shown by the fact that deacetylation can cause repression (reviewed by Grunstein, 1997; Struhl, 1998). As in the discovery that many coactivators are HATs, proteins originally identified as corepressors have now been shown to possess deacetylase activity. Genetic analysis in yeast, as well as inhibitor studies in higher cells, have established the physiologic relevance of the activity.

The deacetylation–repression connection was most clearly demonstrated by the isolation of a human histone deacetylase, HDAC1, whose sequence was highly similar to that of a yeast negative regulatory protein Rpd3. Many additional deacetylases have been identified in yeast and human cells (Grozinger et al., 1999; reviews by Grunstein, 1997; Struhl, 1998). All of the known deacetylases occur in multiprotein complexes (Table 2), with important functional consequences. First, the complexes are able to deacetylate histones in nucleosomes, whereas the isolated deacetylase subunits cannot; second, the complexes contain other proteins previously implicated in transcriptional repression and chromosome transactions; and finally, the deacetylase complexes interact with DNA-binding proteins, bringing the deacetylases to promoters (reviewed by Grunstein, 1997; Struhl, 1998).

Other members of deacetylase complexes include chromodomain proteins, retinoblastoma protein–associated proteins, and Sin3. The chromodomain occurs in protein components of heterochromatin (Paro and

Table 1. Some Transcription-Related Histone Acetyltransferase Complexes

Complex	Organism	HAT	Other Subunits
SAGA/ADA	*S. cerevisiae*	Gcn5	Ada2, Ada3, Spt3, Spt7, Spt8, Spt20, TAF_{II}s, Tra1
NuA4	*S. cerevisiae*		
Elongator	*S. cerevisiae*	Elp3	Iki3
PCAF	*H. sapiens*	PCAF	TAF_{II}s
p300/CBP	*H. sapiens*	p300/CBP	None
TFIID	*H. sapiens*	TAF_{II}250	TBP, TAF_sS
TFTC	*H. sapiens*	hGcn5	TRRAP, hAda3, hSpt3, hPAF65β, TAF_ss

Many additional HATs, such as Esa1, Sas3, and Tip60, are not included because of uncertain relevance to transcription. For references, see Workman and Kingston, 1998; Bjorklund et al., 1999; Brand et al., 1999; Wittschieben et al., 1999.

Hogness, 1991), in which a low level of histone acetylation is associated with a virtual absence of gene expression. Sin3 interacts with Mad to bring deacetylases to the Myc promoter and repress expression. Sin3 also interacts with two corepressors, which, in turn, interact with nuclear hormone receptors to bring deacetylases to hormone-responsive promoters and repress their expression (reviewed by Grunstein, 1997; Struhl, 1998). Finally, Sin3 in a complex with methyl CpG-binding protein recruits deacetylases to methylated DNA regions, thereby repressing their transcription (reviewed by Ng and Bird, 1999).

As an alternative to the recruitment of deacetylases by corepressors that interact with DNA-binding proteins, deacetylases can be fused directly to DNA-binding proteins (reviewed by Struhl, 1998). Tethering yeast and mammalian Rpd3 to promoters in this way significantly represses transcription. Therefore, action of the deacetylase itself, and not merely that of an associated protein in a deacetylase complex, can be responsible for repression.

Chromatin Remodeling

Although acetylation of histone tails may counteract the condensation of nucleosomes in chromatin fibers, it is unlikely to disrupt the structure of the core particle for transcription. The reason is that the sites of acetylation in the tails lie outside the core particle and make little, if any, contribution to its structure. A requirement for nucleosome disruption is apparent from inhibition of RNA polymerase binding, of activator protein binding, and of other interactions with nucleosomal DNA (reviewed by Workman and Kingston, 1998). This inhibitory effect doubtless explains why enhancers and promoters of transcriptionally active genes are found in apparently naked ("nuclease-hypersensitive") DNA regions. How are nucleosomes displaced from these regions in preparation for gene expression? An appealing answer has come with the discovery of chromatin-remodeling complexes (Table 3).

Genetic studies in yeast revealed a set of genes, termed SWI and SNF, whose products oppose the inhibition of transcription by histones in vivo (reviewed by Winston and Carlson, 1992; Bjorklund et al., 1999). A multiprotein SWI/SNF complex was isolated and shown to alter the structure of nucleosomes in an ATP-dependent manner (Cairns et al., 1994; Cote et al., 1994 and references therein; Imbalzano et al., 1994). A second multiprotein complex, termed RSC, isolated from yeast on the basis of homology with SWI/SNF (Cairns et al., 1996), exerts a similar effect on nucleosome structure. RSC is far more abundant than SWI/SNF and is encoded by essential genes, whereas none of the genes for SWI/SNF proteins is required for cell viability. Human cells appear to contain counterparts of these yeast complexes (Imbalzano et al., 1994).

The SWI/SNF family of chromatin-remodeling complexes contains several related components, most notably an ATPase subunit, termed Swi2/Snf2 in SWI/SNF and Sth1 in RSC. A second family of chromatin-remodeling complexes contains a more distantly related ATPase termed ISWI, also conserved from yeast to humans. The founding member of this second family, the *Drosophila* NURF complex, was revealed by assaying a *Drosophila* embryo extract for the ability to generate a nuclease-hypersensitive site within an array of nucleosomes (Tsukiyama et al., 1994). NURF activity appears to require the histone tails (Georgel et al., 1997). Additional members of the ISWI family, CHRAC and ACF, were isolated from *Drosophila* embryo extract on the basis of assays for increased exposure to nuclease digestion and improved regularity in the spacing of nucleosomes on DNA (Ito et al., 1997; Varga-Weisz et al., 1997).

The difference between the modes of action of SWI/SNF-related and ISWI complexes appears to be fundamental. SWI/SNF-related complexes destabilize the

Table 2. Histone Deacetylase Complexes

Complex	Organism	HDAC	Other Subunits
HDA	*S. cerevisiae*	Hda1	
HDB	*S. cerevisiae*	Rpd3	
Mi-2	*X. laevis*	Rpd3	RbAp46/48
NRD	*H. sapiens*	HDAC1, HDAC2	CHD3, CHD4, RbAp46/48
mSin3	*H. sapiens*	HDAC1, HDAC2	mSin3, RbAp46/48, SAP18, SAP30
MeCP2	*H. sapiens*	HDAC1, HDAC2	mSin3
Hda1-like	*H. sapiens*	HDAC3, HDAC4, HDAC5	

For references, see Zhang et al., 1997; Wade et al., 1998; Xue et al., 1998; Grozinger et al., 1999; Ng and Bird, 1999.

Table 3. Chromatin-Remodeling Complexes

Complex	Organism	ATPase	Mass (MDa)	No. of Subunits
SWI/SNF family				
SWI/SNF	*S. cerevisiae*	Swi2/Snf2	2	11
RSC	*S. cerevisiae*	Sth1	1	15
Brahma	*D. melanogaster*	brahma	2	ND
h SWI/SNF	*H. sapiens*	hBRM	2	~10
h SWI/SNF	*H. sapiens*	BRG1	2	~10
NRD	*H. sapiens*	CHD4	1.5	18
ISWI family				
I SWI1	*S. cerevisiae*	ISWI1	0.4	4
I SWI2	*S. cerevisiae*	ISWI2	0.3	2
NURF	*D. melanogaster*	ISWI	0.5	4
CHRAC	*D. melanogaster*	ISWI	0.7	5
ACF	*D. melanogaster*	ISWI	0.2	4
RSF	*H. sapiens*	hISWI	0.5	2

For references, see LeRoy et al., 1998; Varga-Weisz and Becker, 1998; Xue et al., 1998; Bjorklund et al., 1999; Kornberg and Lorch, 1999; Tsukiyama et al., 1999.

nucleosome, disrupting DNA–histone contacts, as shown by a change in accessibility to nuclease digestion (Cote et al., 1994; Imbalzano et al., 1994; Lorch et al., 1998): the nucleosomal DNA becomes almost uniformly accessible to digestion, whereas it is ordinarily protected by the histones from cutting on one side. One member of the SWI/SNF family, RSC, has been found capable of disrupting the nucleosome completely and transferring the histone octamer to another molecule of DNA (Lorch et al., 1999). By contrast, ISWI complexes cause no such perturbation of the nucleosome, leaving the pattern of nuclease digestion unaltered. ISWI complexes are nonetheless able to create nucleosome-free regions, apparently enabling movement ("sliding") of histone octamers to adjacent positions on the same DNA (Hamiche et al., 1999; Langst et al., 1999).

Mechanisms of octamer transfer and sliding may be inferred from the structure of the nucleosome. Such movements had long seemed unlikely, in view of the high affinity of the histone octamer for DNA. The dissociation of a central segment of DNA from the nucleosome is well nigh impossible, because the DNA flanking it is bound so tightly on both sides. The "Achilles heel" of the nucleosome may be at the periphery, where DNA enters and leaves it. A terminal DNA segment may dissociate by breaking only one of the 14 sets of contacts between the phosphodiester backbone and the octamer. In fact, DNA does spontaneously "unpeel" from the ends as far back as the center of the nucleosome (Polach and Widom, 1996). Nucleases and polymerases evidently can exploit this weakness by invading a nucleosome from the ends, advancing as the DNA dissociates and blocking reassociation (Prunell and Kornberg, 1978; Lorch et al., 1987; Studitsky et al., 1994). SWI/SNF-related complexes may function in an analogous manner, binding to a terminal segment of DNA and advancing by ATP-driven translocation toward the center of the nucleosome. A bulge of DNA associated with the remodeling complex might be accessible to nuclease attack, while the exposed histone octamer surface could provide a point of entry for binding and transfer to another DNA molecule.

A mechanism of octamer sliding is suggested by the twist variation of the DNA double helix in the crystal structure of the nucleosome core particle (van Holde and Yager, 1985; Hamiche et al., 1999). A deficit of a base pair in one turn of the helix, thought to result from packing constraints in the crystal, might be introduced in a nucleosome in solution by the action of an ISWI complex. Propagation of the defect around the nucleosome would bring about octamer sliding. This mechanism is more attractive than movement of the core DNA molecule as a unit across the octamer surface. Were there such movement, all histone–DNA contacts would have to be broken simultaneously, whereas sliding by defect propagation requires the alteration of only one histone–DNA contact at a time.

Silencing

Negative regulation of transcription can also occur by "silencing." This mechanism is entirely different from that of repression by gene-specific DNA-binding proteins, yet it also involves the nucleosome, as well as the histone tails. Silencing results from the formation of heterochromatin, a condensed structure that spreads from defined sites such as silencer DNA elements and chromosome ends (telomeres), inactivating the genes it encompasses and persisting throughout the cell cycle. The extent of spreading varies from one cell to the next and may be inherited by the daughters of a cell division, resulting in position-effect variegation and epigenetic regulation of gene expression.

Genetic studies in yeast have defined a set of proteins required for silencing, which include H3, H4, SIR3, and SIR4 (reviewed by Grunstein, 1998). H3 tail residues 4–20 are important, as are H4 tail residues 16–29, which overlap the part of the H4 tail (residues 4–23) needed for activation. SIR3 and SIR4 interact with these tail regions, and SIR3 binding extends along the whole length of the chromosomal region subject to silencing. An important site of acetylation in transcribed chromatin, lysine 16, lies within the required H4 tail domain and may prevent SIR3 binding, while lack of acetylation at this site allows the interaction in heterochromatin.

Nucleosomes and Transcription Elongation

In contrast with a lack of initiation by RNA polymerases at promoters in nucleosomes, the polymerases can transcribe through nucleosomes. The mechanism doubtless

entails unpeeling of nucleosomal DNA in the course of transcription (see above), and it results in the complete displacement of the histone octamer from the DNA (Lorch et al., 1987). If the DNA is of sufficient size, it can recapture the octamer to form a new nucleosome shifted slightly upstream from the original location (Studitsky et al., 1994). In addition to this capacity for readthrough, polymerases can also receive help from other factors to diminish pausing or stalling and speed up transcription of nucleosomal DNA (Brown and Kingston, 1997; Hartzog et al., 1998; Orphanides et al., 1998).

Multiple Mechanisms of Activation and Repression

At least two modes of transcriptional regulation can be discerned, one based on repression by the nucleosome and the other involving RNA polymerase itself. Activators (and repressors) impinge on a multiprotein Mediator complex, which interacts with the C-terminal domain of RNA polymerase II and modulates the initiation of transcription (Kim et al., 1994; Ito et al., 1999). Following initiation, Mediator is replaced on the C-terminal domain by Elongator complex, as well as components of the RNA processing machinery (Wittschieben et al., 1999 and references therein; reviewed by Neugebauer and Roth, 1997; Bjorklund et al., 1999). One of the subunits of Elongator has been identified as a highly conserved HAT, which may play a role in regulation involving nucleosomes as well (Wittschieben et al., 1999).

The multiple mechanisms of regulation discussed here are exemplified by results on nuclear hormone receptors reviewed above. These receptors bind enhancers in the vicinity of hormone-responsive promoters. In the absence of ligand, hormone receptors interact with corepressors, which attract the Sin3–HDAC complex to repress transcription. In the presence of ligand, receptors recruit instead p300/CBP with its HAT activity to potentiate transcription, and they also interact with Mediator (Ito et al., 1999), which delivers the final signal for RNA polymerase II to initiate transcription.

Viral Infection and Cancer

Chromatin transactions play a central role in the etiology of viral infection and cancer (reviewed by Archer and Hodin, 1999; Jacobson and Pillus, 1999). Both altered histone acetylation/deacetylation and aberrant forms of chromatin-remodeling complexes are involved. For example, adenoviral oncoprotein E1A interferes with the growth-suppressive action of p300/CBP by inhibiting its HAT activity and also by displacing the PCAF HAT complex. Tumor suppression by Mad depends on its interaction with Sin3 in an HDAC complex.

Studies of the tumor suppressor retinoblastoma protein (RB), mutated in many cancer cells, have revealed a dual mechanism of transcriptional repression (reviewed by Archer and Hodin, 1999; Jacobson and Pillus, 1999). RB antagonizes the E2F family of transcription factors, which activate expression of genes for entry into S phase of the cell cycle. Binding of RB to the activation domains of E2F proteins prevents their contact with the transcription machinery. RB bound to E2F also interacts with HDACs, which deacetylate nucleosomal histones in the vicinity of the promoter, thereby repressing transcription. The importance of both mechanisms is indicated by the consequences of RB mutations in cancer cells. The mutant RB proteins bind neither E2F nor HDACs. Moreover, several viral oncoproteins appear to function by blocking the RB–HDAC interaction.

Chromosomal translocations associated with leukemias create fusions of various cellular genes to those for HATs and for subunits of remodeling complexes. The *MOZ* (monocytic leukemia zinc finger) and *MLL* (mixed lineage leukemia) genes are frequently fused to the gene encoding the coactivator HAT CBP. AF9, whose fusion to MLL can cause leukemia, shows homology to a component of the SWI/SNF complex. Mutations of the *hSNF5* gene, which encodes a member of the human SWI/SNF complex, are also frequent in malignancy.

General Principles

The nucleosome is fundamental to DNA coiling and gene regulation. It serves as a basis for chromatin condensation, whose modulation controls transcription. The primary event in gene activation may be the modification of histones and the resulting decondensation of large chromosomal domains. Further histone modification by acetylation or its reversal then determines the state of transcriptional activity of promoters within a domain. Only after the completion of chromatin transactions, including the displacement of nucleosomes from promoter DNA, does the transcription machinery come into play.

The structure of the nucleosome and its role in transcription are based on the following principles:

(1) A nucleosome may be defined as a histone octamer, made up of two each of H2A, H2B, H3, and H4, with DNA wound on the outside.

(2) Each histone is organized into two domains: a central fold, which lies within and constrains the DNA superhelix, contributing to the compact core of the nucleosome; and an unstructured amino-terminal tail, which extends outside the core and provides a basis for interaction and regulation.

(3) A chain of nucleosomes is coiled in a chromatin fiber through interactions of the histone tails with adjacent nucleosomes and additional proteins; these interactions may be modulated by acetylation of the tails.

(4) Chromatin-remodeling complexes clear nucleosomes from enhancers, promoters, and other specific protein-binding sites in chromatin.

(5) Many DNA-binding regulatory proteins stimulate or repress transcription by recruiting histone acetyltransferases or deacetylases, which act on nearby nucleosomes.

(6) Stable repression of transcription by the formation of heterochromatin is based on the nucleosome. Interaction of histone tails with silencing proteins starts at special sites and spreads along the chromosome to form a repressive structure that may persist through many cell generations.

Perspectives

The chromatin field needs much more information about structure beyond the nucleosome. Even the trajectory

of the DNA entering and exiting the nucleosome, immediately beyond the core particle, is unclear (reviewed by Prunell, 1998). The pattern of coiling a chain of nucleosomes in a thicker fiber remains uncertain (reviewed by Ramakrishnan, 1997). Whereas additional coiling in a succession of higher helices would be a most plausible mechanism of further condensation, alternative hypotheses have been advanced. Many of the difficulties of analyzing chromatin problems stem from the variability inherent in higher order chromatin structures. Existing methods of structure determination require averaging and thus are of limited use in the face of variability.

Solution of the higher order structure problem is crucial for understanding chromatin function. Histone tail modifications and interactions with other proteins, important for regulation, seem likely to influence higher order structure more than core particle structure. There is, however, insufficient evidence that acetylation actually causes chromatin unfolding, and only a suggestion of the interplay between acetylation and the chromatin remodeling events that affect core particle structure (Cosma et al., 1999; Sudarsanam et al., 1999). Histone phosphorylation, long correlated with chromosome condensation, has recently been linked to transcriptional activity (reviewed by Bjorklund et al., 1999). There is no information as to the structural or functional consequences of other modifications, such as ubiquitination and glycosylation. Histone H1 is thought to promote condensation, but an understanding of its structural role and the connection with gene activity is lacking. Elucidation of gene silencing by heterochromatin similarly depends on determination of its structure.

Finally, functional analysis in cell-free systems must be extended beyond the nucleosome to the chromosomal context. Histone–DNA complexes assembled in vitro reveal effects of HAT and chromatin-remodeling complexes on transcription (Mizuguchi et al., 1997; LeRoy et al., 1998; Utley et al., 1998), but in vivo, promoters are associated with additional, nonhistone proteins, which influence the locations of nucleosomes and undoubtedly the higher order configuration of chromatin as well. These associations extend, in the broadest sense, to such DNA elements as locus control regions, which regulate the structure and activity of entire chromosomal domains. Only when transcription, replication, recombination, and other DNA transactions have been reconstituted in vitro with naturally assembled chromatin templates will a full understanding of the nucleosome be achieved.

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