HISTONE STRUCTURE AND
THE ORGANIZATION OF
THE NUCLEOSOME

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ABSTRACT
Chromatin structure is now believed to be dynamic and intimately related with cellular processes such as transcription. Over the past few years, high-resolution structures for the histones have become available. These structures and their implications for nucleosome organization are reviewed here.

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INTRODUCTION

Histones are associated with the DNA of eukaryotes to form chromatin. The fundamental organization of chromatin was elucidated nearly twenty years ago and is described in detail by van Holde (127). The basic subunit of chromatin is the nucleosome, which consists of 200 bp of DNA wrapped in approximately two turns around an octameric protein core containing two copies each of histones H2A, H2B, H3, and H4. A fifth histone, the linker histone H1, binds to the nucleosome and promotes the organization of nucleosomes into a higher-order structure, the 30-nm filament. Although histones as a class of proteins were discovered over a century ago, only recently have high-resolution structures of the histones become available. This review examines these structures and current ideas about how they assemble to form the nucleosome.

Since DNA is supercoiled around the octamer in the nucleosome, and nucleosomes themselves can be organized into the 30-nm filament, the association of histones with DNA results in considerable compaction and allows DNA that is over a meter long to be organized in a cell nucleus that is only microns in diameter. Initially, this packaging of the genome was considered to be the only role of the histones.

However, chromatin in most cells is far less condensed than it is, for example, in sperm nuclei, so the role of histones is not to produce the most compact form of the genome. Rather, it has long been suspected that the condensation of DNA by histones may be part of a general machinery for the repression of genes by limiting access to the transcriptional apparatus. This general repression has been considered as part of a solution to the signal-to-noise problem and becomes required as the complexity of organisms increases (9). Indeed, over the past decade, evidence has accumulated indicating that chromatin structure is dynamic and plays an important role in biological processes such as transcription (62, 63, 135). The recent discoveries of structural similarities between histones and transcription factors (discussed below) reinforces the idea that chromatin structure and transcription are intimately related. Studies on the biological role of histones have received a great impetus from genetic and biochemical experiments in yeast (see 38, 39 for review).

The discovery of nucleosome mobility (72, 85) has important biological implications, because it is important both for nucleosome positioning and disruption, depending on the biological context (63, 105). For example, nucleosomes positioned at promoters play an important role in the regulation of transcription (35, 119, 130). To allow access to the transcriptional apparatus, such nucleosomes have to be disrupted. At least in some cases, this disruption requires specific transcription factors and ATP (120–122). The Swi/Snf complex, a large multiprotein complex that is the product of genes required for induction of transcription at many promoters (24, 54, 65), is involved in remodeling of
Histone structure during transcription (58). On the other hand, because nucleosomes supercoil DNA, they can also bring two regulatory elements into close proximity. This role has been observed in the case of the heat shock elements in the hsp26 promoter in Drosophila melanogaster (67) and in the vitellogenin B1 gene (99). In both cases, a positioned nucleosome between two regulatory elements is important for transcription.

Histones are subject to posttranslational modifications such as acetylation and phosphorylation. Acetylation of histones has long been implicated in the control of transcription (11), whereas under-acetylation of histones is correlated with silencing (12). The recent discoveries that a histone acetylase purified from Tetrahymena thermophila is homologous to the yeast transcription factor GCN5 (14), whereas a mammalian histone deacetylase is related to Rpd3p, a negative regulator of transcription in yeast (113), both suggest a close relationship between acetylation and transcriptional regulation. The linker histone H1 is not subject to acetylation but becomes phosphorylated in a cell-cycle-dependent manner (11, 97). Such phosphorylation of the linker histone is associated with changes in chromatin structure.

The role of histone H1 as a general repressor of genes has been suspected for a long time (133). There are also cases where H1 may play a more specific role, such as in the repression of the Xenopus borealis oocyte 5S RNA genes in somatic cells (100). H1 depletion of chromatin in vitro allows the transcription of oocyte 5S RNA genes by initiation factors and RNA polymerase III; addition of H1 to these depleted complexes restores repression (136). The expression of the genes for the various H1 subtypes within an organism is developmentally regulated (57), which also argues for an important role for H1 in the biology of the cell. A similar phenomenon is seen in the early development of Drosophila, where HMG-D is associated with early embryonic chromatin in the absence of H1, and a switch occurs in the HMG-D/H1 ratios during development (80). A transcription factor plays the role of an anti-repressor by countering the inhibitory effects of H1 (26). An in vitro study using reconstituted chromatin showed that the presence of H1 could prevent both initiation and elongation by T7 RNA polymerase (84). Finally, linker histones reduce nucleosome mobility (86, 125); by doing so, they may interfere with disruption or repositioning of nucleosomes that are required for transcriptional activation.

Thus many recent studies point to a crucial role for the histones in the regulation of biological processes such as transcription. It has become more important than ever to have a firm structural basis for understanding the biology of histones.

**STRUCTURE OF THE CORE HISTONES**

At high ionic strengths, the octameric histone core of the nucleosome can exist as an intact entity even in the absence of DNA or cross-linking agents,
whereas at physiological ionic strengths the histone octamer dissociates into the
(H3-H4)2 tetramer and two (H2A-H2B) dimers (31). This finding made it
possible to crystallize the histone octamer (15). However, an initial structure
of the octamer at 3.3 Å resolution (16) had dimensions that conflicted with the
low-resolution structure of the octamer as determined by electron microscopy
(61) and the crystal structure of the nucleosome core particle (94), resulting in
a heated controversy (60, 77).

It turned out that the initial shape of the histone octamer determined by
crystallography (16) was incorrect owing to a subtle problem that arose because
the heavy atom positions were close to a twofold axis, so that a small error in
their location placed them on the incorrect side of the axis and resulted in a large
change in the molecular envelope after solvent flattening (132). The subsequent
structure of the histone octamer at 3.1-Å resolution (3) was a landmark in the
field of chromatin structure for two reasons: The structure was now in general
agreement with previous work on the octamer (61) and the nucleosome core
particle (94), thereby resolving the earlier controversy; and more importantly,
it revealed for the first time the tertiary structure of all the core histones. The
following sections discuss the features of the structures of core histones and
related proteins.

The Histone Fold and Tertiary Structure of the Core Histones
The four core histones share a common structural motif, termed the histone fold
(3, 5). The structures of the core histones have been used in conjunction with a
threading algorithm to identify several other proteins that are likely to contain
this fold (8). The variety of proteins found to contain this motif suggests that the
histone fold is widespread and may play a diversity of roles. The evolutionary
implications of the similarity between the histones have been discussed in a
recent review (91).

The histone fold consists of a long central helix flanked on either side by a
loop and a short helix (Figure 1A). The fold has an approximate twofold axis of
symmetry, where the axis is normal to the plane of the drawing in Figure 1A and
bisects the central helix. This observation suggests that the fold itself may have
arisen from a gene duplication event, in which a module that consists of a helix-
strand-helix (HSH) is duplicated. In this scenario, the central long helix of the
histone fold would consist of a fusion of two shorter helices, the C-terminal
helix of the first HSH motif and the N-terminal helix of the second. The central
helices of H2A and H4 appear to be slightly bent (3, 5). Apart from the central
histone-fold region, histones H2A and H3 contain additional helices at the N-
terminus, whereas histone H2B contains an additional helix at the C-terminus.

The high-resolution structures of two classes of proteins containing the hi-
stone fold were determined recently. The structure of the histone-like protein
**Figure 1.** 

A. The histone fold, showing the helix-strand-helix (HSH) motif (5) at each end. The two HSH motifs that make up the fold are related by an approximate twofold axis as shown. The protein shown here is dTAFII 42 (137). B. Alignment of proteins whose high-resolution structures have revealed that they contain the histone fold. These proteins include the four core histones (3, 5), dTAFII 42, dTAF II 62 (137), and HMfB (107). These proteins dimerize, and a subset of residues involved in dimerization (137) is indicated with d’s. The exact residues vary for a particular dimer. Residues involved in tetramerization are indicated with t’s (137). These include residues from H3, which associates with another H3 molecule in the (H3-H4)_2 tetramer; dTAFII 42, which likewise associates in the dTAF II 42/62 tetramer; and H2B and H4, which similarly associate in the formation of the histone octamer from the tetramer.

HMfB from the hyperthermophilic archaeabacterium *Methanothermus fervidus* has been determined by NMR (107). The crystal structures of two subunits of *Drosophila* TFIID, dTAFII 42 and dTAFII 62, have also been determined (137), with important implications for the formation of nucleosome-like structures as part of the transcription complex. One of these subunits, dTAFII 42, also has a bent central helix. Thus structures are now known for a total of seven proteins with the histone fold. The sequence alignment based on the structures is shown in Figure 1B.

The C-α root mean square (rms) deviation between the histone-fold elements for various pairs of core histones ranges from 1.46 Å (H2B-H3) to 2.56 Å.
(H2A-H2B) (5). Interestingly, the C-α rms deviation of the two subunits of the HMfB dimer when compared to H4 are 1.56 and 1.66 Å, respectively (107), whereas the C-α rms deviation between the histone-fold regions of dTAFII42 and dTAFII62, dTAFII42 and H3, and dTAFII62 and H4 are all 1.6 Å (137). Thus it appears that these proteins are at least as related structurally to the core histones as the core histones are to one another.

In addition to the structured regions that include the histone fold, the core histones have N-terminal tails that have a basic region. These tails are not visible in the octamer structure (3), suggesting that they are disordered in the absence of DNA. However, they could take on some definite conformation when bound to DNA. Mutational studies in yeast H4 suggested that the basic region together with an adjacent relatively uncharged region formed a DNA-induced amphipathic α-helix (55).

The Dimer

Each of the core histones exists as part of a dimer, in which the two monomers are intimately associated in a head-to-tail manner in a so-called handshake motif (3) (Figure 2). The histone-fold portion of each monomer is related to its partner in the dimer by an approximate twofold axis. Thermodynamic studies on H2A-H2B show that the histones are stable structured entities only when they are complexed as part of a dimer (56). In the case of the dTAFII42/62 dimer, the association results in the burial of about 3390 Å² of solvent-accessible surface area, of which 56% is hydrophobic (137). There are two histone-like proteins in the archaebacterium *M. fervidus*, HMfA and HMfB. Each of these two proteins can self-associate, but when mixed together, they can also form heterodimers (37). The recent structure of HMfB (107) shows that this archaebacterial protein self-associates to form a symmetric homodimer (Figure 2), lending support to the view that the ancestral dimer may have been a homodimer with a true twofold axis (5).

In each case, the dimer is stabilized largely through hydrophobic interactions that span the entire length of the histone-fold regions of each monomer, with the long central helices associated in an antiparallel manner. The residues involved in dimerization are indicated in Figure 1b. The complementarity of the interface dictates the formation of specific heterodimers in the case of the core histones and dTAFII42/62, and homodimers in the case of HMfB.

In the dimer, the N- and C-termini of each histone are close together (Figure 2). The strand in the HSH motif in the N-terminal half of a monomer pairs with the strand in the HSH motif of the C-terminal half of its partner to form a short two-stranded β-sheet or β-bridge (Figure 2). A similar sheet is found at the other end in the symmetric position. The solvent-exposed surfaces of these sheets consist of conserved basic residues that are likely to be involved in binding DNA (see below).
The histone dimer. The homodimer of HMβ (107) is used as an example. This dimer has a perfect twofold axis of symmetry, which is only approximate in the heterodimers. The strand from the N-terminal HSH motif of one member pairs with the C-terminal strand of its partner to form a β-bridge (4), and the ends of the N-terminal helices are also close together to form another paired element (4).

The Tetramer
The (H3-H4) dimer further associates to form a tetramer (31), which is the stable entity at physiological ionic strength. The tetramer is important because it is involved in the initial step in nucleosome assembly and in determining nucleosome positioning (28, 46). Similarly, it has been shown that the dTAFII42/62 dimer associates to form a stable tetramer (137). In both the (H3-H4)₂ and (dTAFII42/62)₂ tetramers, the two halves of the tetramer, which consist of the heterodimers, are related by a crystallographic twofold axis. The shape of the tetramer is relatively flat, resembling a twisted open horseshoe (3, 137).
Figure 3  The histone tetramer. The dTAF_{II42/62} tetramer (137) is used to illustrate how two histone dimers come together to form a tetramer. Tetramerization occurs via interactions between the C-terminal halves of two dTAF_{II42} molecules and results in a twofold axis of symmetry for the tetramer as shown. The histone (H3-H4)$_2$ tetramer is similar; the analogous interactions occur between the C-terminal domains of H3. In the histone octamer (not shown), similar interactions occur between the C-terminal halves of H2B and H4, one on each side of the tetramer. A schematic path of the DNA illustrates how the repeated paired elements of $\beta$-bridges and N-terminal ends form a superhelical ramp that interacts with minor grooves of the DNA (4).

The basis of tetramer formation in dTAF_{II42/62} is the association between the C-terminal HSH motif of dTAF_{II42} from each dimer (Figure 3). The formation of the tetramer buries an additional 670 Å$^2$ of surface area, of which about half is hydrophobic. The dissociation constant is 1 $\mu$M (137). In the case of the (H3-H4)$_2$ tetramer, the interaction is between the C-terminal HSH motif of H3, which is the counterpart of dTAF_{II42}. Seven residues in dTAF_{II42} that are
involved in tetramer formation in dTAF_{II}42/62 have been identified. Three of the corresponding residues are identical in H3, whereas two others are conservative substitutions. Tetramerization occurs largely via hydrophobic interactions.

Unlike the case of the heterodimers above, the (H2A-H2B) heterodimer does not show a marked tendency to associate further into a tetramer. Although some of the residues involved in tetramerization are also found in H2A and H2B, presumably not enough of the interactions that are found in the (H3-H4)\textsubscript{2} tetramer interface can be formed by either H2A or H2B to form a stable tetramer. In the case of the HMf dimer, tetramer formation appears to require association with DNA (78) but may be mediated by interactions analogous to those in the (H3-H4)\textsubscript{2} or the dTAF\textsubscript{II}42/62 tetramers.

**The Octamer**

An (H2A-H2B) dimer associates on each side of the (H3-H4)\textsubscript{2} tetramer to form the histone octamer, both in vivo as part of the nucleosome and in vitro at high ionic strength. This association occurs primarily via an interaction between the C-terminal halves of H2B and H4 that mimics the interaction between two H3 molecules in the tetramer. Although the association of (H2A-H2B) to the tetramer is weaker than the association of the two halves of the tetramer both in the nucleosome and for free histones, the interface between each H2A-H2B dimer and the (H3-H4)\textsubscript{2} tetramer is larger than that between the two halves of the tetramer (3). Despite its more extensive nature, the interface between (H2A-H2B) dimers and the tetramer is more open and accessible to solvent (3). This observation is consistent with what is known about the equilibria between the dimers and the tetramer.

The binding of H2A-H2B dimers to opposite sides of the tetramer results in a tripartite structure for the octamer. Depending on the point of view, the octamer looks like a disk or can resemble the wedge shape previously seen in electron micrographs (61). Electron microscopy had also detected the presence of a left-handed superhelical ramp on the surface of the octamer, which presumably was the path of interaction with DNA (61). The high-resolution structure of the octamer confirms the presence of this ramp but also suggests the detailed nature of the interactions with DNA (discussed below).

An excellent illustration of the assembly of histones into the dimer, tetramer, octamer, and the nucleosome can be seen on the World Wide Web at the URL maintained by D. Pruss (http://www.online.ru/people/pruss/nucleosome.html).

**THE NUCLEOSOME CORE PARTICLE**

The nucleosome core particle consists of about 146 bp of DNA wrapped in 1-3/4 turns around the histone octamer. The particle was originally produced
as the limit of digestion of nucleosomes by micrococcal nuclease. The crystal structure of the core particle at 7 Å resolution is still the highest resolution structure of this molecule to date (94); it has been reviewed extensively (127) and remains a milestone in chromatin structure. At this resolution, major and minor grooves of the DNA are visible, as are rod-like regions of density that correspond to α-helices of the histones. A striking feature of the structure is that the DNA is not wrapped uniformly around the octamer but instead varies in curvature, especially at positions ±1 and ±4 turns of DNA from the dyad (94). The width of the major and minor grooves also varies considerably throughout the nucleosome core.

The technique of hydroxyl-radical cleavage (123) has proved to be extremely useful for high-resolution footprinting experiments on the nucleosome in solution (45, 48). These experiments showed variation in the helical periodicity of DNA on the nucleosome, and in particular showed that the periodicity at the dyad differed significantly from that on the rest of the nucleosome. Other experiments used the fact that the ability of HIV integrase to direct integration of DNA into a target sequence depends among other things on the DNA curvature, which results in nonrandom integration in nucleosomal DNA. These integration sites were also correlated with regions of higher curvature on the nucleosome (89).

The high-resolution structure of the histone octamer has clarified many of the features of the nucleosome core particle. The β-bridges formed by the various histone dimers occur repeatedly on the surface of the octamer. In addition, the amino-terminal ends of the histone-fold region of each dimer partner also point toward the surface (3, 4). These repeating elements include a number of conserved positive charges that are candidates for interaction with DNA (4, 76). They also form a superhelical ramp that is consistent with the known path of DNA in the 7-Å structure of the nucleosome core particle (94). The molecular dyad lies between β-bridges formed by (H3-H4) dimers. Since the nucleosomal dyad has a DNA minor groove at the exposed surface (94), the position of the repeating β-bridges and N-terminal helices is such that they are likely to interact with minor grooves (Figure 3). This observation is in general agreement with the idea that nonspecific DNA-binding proteins often interact with the minor groove (18).

In the model of DNA binding to repeating elements, the histones are found along the superhelical path in the following order, proceeding from the outermost point toward the twofold axis at the dyad: H2A1, H32, H2A3, H2B3, H2A1, H2B1, H44, H31, H41, with H31-H32 overlapping at the dyad. This order is consistent with cross-linking data on the primary organization of histones along core-particle DNA (102). The order is different from that deduced from an analysis of the nucleosome core particle structure (94), where the identification
of regions of density with particular histones used cross-linking data with the assumption that the histones were globular and distinct. However, the discovery that the histones are both elongated as well as intimately associated results in the possibility of each histone chain making noncontiguous contacts along the DNA.

Recently, the electron density for the core-particle at 7-Å resolution was re-analyzed based on the structure of the histone octamer (95), and it was found that the rod-like densities that correspond to the various α-helices of the individual histones can all be assigned. The result is a picture consistent with the model for wrapping around the histone octamer (4, 76).

Direct evidence of the interaction of a β-bridge with DNA comes from site-specific cleavage experiments (32) on a core particle with a defined DNA sequence. In these experiments, Ser-47 of histone H4, which is part of one strand of a β-bridge, was mutated to a cysteine and used as a point of attachment for Fe(II), which was used to generate hydroxyl-radicals via the Fenton reaction. The presence of cleavage of nucleosomal DNA confirmed the proximity of Ser-47 to DNA. The pattern of cleavage was consistent with the model of histones binding to DNA. An interesting result from the cleavage experiments was that the dyad axis was right over a base pair, so that the canonical core particle that is symmetric about the dyad must consist of an odd number of base pairs, i.e. 145 or 147. The site-specific cleavage from H4 used in these studies is also an excellent way to define the nucleosomal dyad, since by definition, the cuts from the two H4 molecules must be located symmetrically about the dyad. The dyad position in general is difficult to define by other methods such as footprinting of the nucleosome.

NUCLEOSOME PHASING AND MOBILITY

By their nature, histones are designed to bind to a variety of DNA sequences. However, it was discovered that in a defined sequence of DNA from the sea urchin *Lytechinus variegatus* that included the gene for 5S RNA, the histone octamer preferentially bound to one sequence within the fragment (106). This property, which directs the octamer to bind preferentially to a particular sequence rather than others including overlapping flanking sequences, is called nucleosome phasing. Other sequences also have the ability to phase nucleosomes. These include a sequence that contains the 5S RNA gene from *Xenopus borealis* (48, 93) and a viral promoter (35).

Given that histones are not sequence-specific proteins, why do they show any sequence preference? The reason has to do with the fact that the structure and bendability of DNA is sequence dependent. If DNA with a particular sequence had an intrinsic ability to bend in the direction required to form a nucleosome,
this would reduce the free energy of binding, because energy would not have to be expended to bend it. In circular DNA, runs of AT tended to be positioned with their minor grooves facing inward (30) and GC minor grooves facing outward. This is because AT steps can accommodate a narrower minor groove. Analysis of 177 core particle DNA sequences confirmed that in the nucleosome core particle, minor grooves at AT steps predominantly faced inward, whereas those at GC steps faced outward (98). Thus a major determinant of nucleosome positioning comes from its so-called rotational setting, which is related to the ability of the particular DNA sequence to curve around the histone octamer. This idea was tested by the synthesis of DNA that had repeats of the form (A/T)\textsubscript{3} NN(G/C)\textsubscript{3}, so that tracts of AT or GC were repeated with a roughly 10-bp period (103). Given the helical periodicity of DNA, this would mean that (A/T) minor grooves would always be on the inside. Such DNA sequences had a 100-fold greater affinity for the histone octamer compared to bulk DNA, confirming that the rotational signal was indeed a major component of nucleosome positioning.

However, a position that is approximately 10 bp away from a phasing sequence has, except for end effects, the same rotational setting, because the same parts of the DNA will face inward as they would in the original position. As a result, when histone octamers are reconstituted on a fragment of DNA containing a nucleosome phasing sequence, they do not occupy just the major site, but they also occupy minor sites at roughly 10-bp intervals on either side (27). The translational signal determines why the histone octamer should bind to a particular sequence rather than one with the same rotational setting roughly 10 bp away. Apart from the obvious end effects, the features of DNA that confer a preferred translational setting to the octamer are not well understood. However, the analysis of several nucleosome positioning sequences has led to some empirical rules for translational positioning in terms of the probability of occurrence of specific dinucleotides at various locations (124).

Experiments to determine the histone determinants of phasing showed that the (H3-H4)\textsubscript{2} tetramer by itself is enough to confer nucleosome positioning on a defined DNA sequence (28, 46). This observation supports the idea that in nucleosome assembly, the tetramer assembles first, followed by the H2A-H2B dimers. Not only are octamers distributed among the major binding site and other sites with the same rotational setting, but they are also mobile and can redistribute themselves among the various sites (72, 73, 85). These studies show that the redistribution is reduced at 4°C. Recently, it was found that incubation at 37°C of reconstituted nucleosomes containing sequences derived from the L. variegatus 5S DNA was able to drive the population into the major site (32). This observation suggests that reconstitution at low temperature can kinetically trap nucleosomes at less favorable sites, such as ones with the same rotational
setting as the major site, but incubation at higher temperature can increase mobility and thereby allow re-equilibration to the major site. Studies also show that binding of the linker histone H1/H5 reduces nucleosome mobility (73, 125).

There is little doubt that nucleosome mobility, with its implications for nucleosome disruption and repositioning plays a major role in chromatin remodeling during transcription. However, at least in principle, transcriptional elongation does not require the prior disruption of a nucleosome in its path. Experiments show that the SP6 polymerase can transcribe through a nucleosome. In the process, the nucleosome “steps around” the polymerase, so that it ends up behind the polymerase after transcription without actually leaving the DNA (20, 109, 110). Thus nucleosome disruption may have more to do with the formation of a transcriptional complex for initiation of transcription than with elongation of transcripts.

Recent experiments with important implications for the interaction between transcription factors and nucleosomes showed that in nucleosomes with target sequences for restriction enzymes, the sites within nucleosomal DNA can be exposed transiently to enzymes (88). This observation implies that transcription factors could invade nucleosomes once they gain transient access.

The histone-like protein HMf associates into tetramers on binding DNA but forms positive supercoils (78), unlike the normal left-handed (H3-H4)₂ tetramer that results in negatively supercoiled DNA in the nucleosome. Also, the (H3-H4)₂ tetramer can form both positive and negative supercoils on closed circular DNA, showing that it may switch between left- and right-handed forms (40). This result suggests that quite apart from nucleosome mobility, which involves association and dissociation from DNA, there is also much greater intrinsic conformational variability in the tetramer than had been suspected previously.

These discoveries indicate that chromatin is a dynamic structure, with important implications for its role in biological processes.

HISTONE ACETYLATION AND NUCLEOSOME STRUCTURE

The N-terminal tails of the core histones are subject to a variety of posttranslational modifications, including acetylation. As pointed out earlier, histone acetylation is correlated in striking ways with transcriptional activation, whereas deacetylation is correlated with inactivation or silencing. It has also been found that during replication, newly deposited histones are acetylated, and the recently discovered cytoplasmic acetylase from yeast (59) may play a role in this process. This acetylase, hat1, has a specificity for Lys-12 of H4, raising the possibility
that the cell has other acetylases with varying specificities or cofactors that modify hat1 to alter its specificity.

An early study found that the transcription factor TFIIIA could bind to its recognition site on the *X. borealis* 5S gene even though it was part of a nucleosome (93). However, later studies found that TFIIIA bound preferentially to nucleosomes that either lacked H2A and H2B (49) or contained acetylated core histones (66). The interesting question is whether the acetylation of core histones changes nucleosome structure, or whether the acetylation simply prevents or promotes interaction with various other proteins. Core histones that had their N-terminal tails trimmed by trypsin also facilitate binding of TFIIIA (66), a result suggesting that the tails have a major role in restricting transcription factor access to the nucleosome, and that release of the tails by acetylation facilitates transcription factor binding.

Acetylation of the histone tails could also result in a structural change in the nucleosomal DNA. Neutron scattering experiments on nucleosome core particles containing acetylated histones showed no significant change in the shape of the octamer or the nucleosome, so any structural changes on acetylation, if they occur, must be too subtle to detect by scattering techniques (53). In an in vitro system, acetylation of histones leads to a change in the linking number of DNA-containing nucleosomes (82, 83). However, studies in vivo on SV40 chromatin show no change in linking number with histone acetylation (68). Hydroxyl-radical footprinting shows no effect of acetylation on the apparent helical periodicity of DNA or the extent of protein-DNA contacts in the nucleosome (7). These data were interpreted to suggest that the shape of the octamer changes on acetylation, with a resultant wrapping of DNA that accounts for the previously observed change in linking number (83) but that at the same time leaves unaltered the apparent surface helical periodicity observed in hydroxyl-radical footprinting. However, as pointed out above, the linking number change is controversial, so it is still an open question whether there is any structural change on acetylation. The facilitation of transcriptional factor access to the nucleosome could simply be the direct result of a weakening of the interactions of histone tails with DNA (66).

THE LINKER HISTONE H1/H5

The linker histone H1 binds to the nucleosome and promotes the organization of nucleosomes into a higher-order structure (33, 114). In avian erythrocytes, the predominant linker histone is H5, a variant of H1 in which many lysines have been replaced by arginines. Digestion of chromatin by micrococcal nuclease results in a metastable intermediate, the chromatosome, which consists of about 165 bp of DNA wrapped around the histone octamer, and histone H1 (81, 104,
Further digestion with micrococcal nuclease results in the dissociation of H1 and the digestion of an additional 20 bp of DNA to produce the nucleosome core particle (see above) that consists of about 146 bp of DNA. In the absence of H1, the chromatosome is not observed as an intermediate, whereas restoring H1 to H1-depleted chromatin restores the pause at the chromatosome. Thus histone H1, in binding to the nucleosome, protects an additional 20 bp of nucleosomal DNA (Figure 4).

The Globular Domain

Nearly all members of the linker histone family consist of a trypsin-resistant globular domain that is flanked by N- and C-terminal tails that are relatively unstructured in the free histones in solution (6, 43), although the tails are likely to adopt a definite structure upon binding DNA. The tails are highly basic, and in particular, over half the residues in the C-terminal tail consist of lysines and arginines. The globular domain by itself is capable of protecting an additional 20 bp of nucleosomal DNA just like full-length H1/H5. Experiments that probed the protection against chemical modification of lysines in H5 bound to chromatin show that the most strongly protected lysines are in the globular domain, even though most of the lysines are in the tails (118). This result suggests that the globular domain binds chromatin in a fundamentally different way from the tails. Thus the globular domain of H1/H5 can be thought of as an independent module that is the nucleosome-binding domain, whereas the C-terminal tail is likely to bind to the linker DNA between nucleosomes. In constructs of H5 in which only the globular domain and the C-terminal tail are present, the chromatosome pause is not seen in nuclease digestion (2), showing that the highly charged C-terminal tail can misposition the histone in the absence of the N-terminal tail.
The structure of GH5, the globular domain of histone H5, has been determined by X-ray crystallography (92). The protein consists of a three-helix bundle, with a \( \beta \)-hairpin or “wing” at its C-terminus. The \( \beta \)-hairpin, along with a short strand that connects helices I and II, forms a three-stranded sheet at the base of the three-helix bundle. The structure of the \( \beta \)-hairpin is very different in the two molecules in the asymmetric unit of the crystal, suggesting that it is flexible in the free protein in solution.

A similarity between GH5 and the DNA-binding domain of the catabolite gene activator protein CAP (101) had been suspected on the basis of the fold of GH5 determined by NMR (23). This similarity was confirmed by a comparison of the crystal structures of the two molecules, and subsequently, a model for the binding of GH5 to DNA was proposed (92). In this model, helix III of GH5 is the recognition helix, which would bind in the major groove of DNA. The \( \beta \)-hairpin would also be involved in making contacts with DNA and would assume a definite conformation upon binding DNA.

However, GH5 is not a true helix-turn-helix (HTH) protein like CAP, but an HTH variant in which the canonical four-residue turn between helices II and III is replaced by a seven-residue loop. The subsequent structure of the DNA-binding domain of the transcription factor HNF-3 complexed with DNA (22) had interesting implications for histone H5, because the two proteins are very similar structurally: despite only 10% sequence identity, they have a C-\( \alpha \) rms deviation of only 1.3 Å (Figure 5A). Like GH5, HNF-3 is also an HTH variant, with a three helix bundle and a loop between helices II and III. It contains two wings that bind DNA, the first of which corresponds to the \( \beta \)-hairpin of GH5, whereas the second is not present in the GH5 structure. The structural similarity has led to the classification of HNF-3, GH5, and other proteins as belonging to the winged-helix family of proteins (13).

The binding of HNF-3 to DNA is similar to the model proposed for GH5 (Figure 5B). Given the striking similarity between the two proteins, the model of GH5 binding to DNA is likely to be correct at least in broad outline. The model also explains a number of biochemical features, such as the chemical protection of specific lysines (118), as well as the cross-linking of two histidines, His-25 and His-62, to DNA in chromatin (74).

The structure of GH1, the globular domain of histone H1, was determined by NMR (17) and is similar to that of GH5. The \( \beta \)-hairpin in GH1 has a variable conformation in solution, whereas in the crystal structure of GH5, the two conformations of the \( \beta \)-hairpin are determined by crystal packing. A significant difference between GH5 and GH1 is in the conformation of the loop between helices II and III. In GH5, this loop contains His-62, which has been cross-linked to DNA in chromatin (74). However, in GH1, not only is this histidine absent, but the loop contains negative charged residues that would be
Figure 5  A. The crystal structures of GH5, the globular domain of histone H5 (92) and the transcription factor HNF-3 (22). Despite only a 10% sequence identity, the two structures are remarkably similar and belong to the winged-helix class of DNA-binding proteins. B. A model of GH5 binding to DNA, based on its similarity to DNA-binding proteins (92). The seven highly conserved lysines/arginines and the two histidines that have been cross-linked to DNA in chromatin (75) are also shown. Reproduced with permission from Reference 90.
expected to repel DNA. Also, the loop is bent away from the putative DNA relative to the loop in GH5. This conformational difference could account for the lower affinity of GH1 for the nucleosome compared to GH5 (115).

**DNA and Nucleosome Binding Sites on the Globular Domain of H1/H5**

The structural similarity between GH5 and DNA-binding proteins such as HNF-3 suggests one clear primary DNA-binding site. This site includes helix III, β-hairpin, and the loop between helices II and III that contains His-62 (Figure 5B). However, linker histones bind preferentially to nucleosomes compared to free DNA (50). What is the chemical basis for this preference?

Evidence that GH5 has more than one DNA-binding site comes from several experiments. It has long been known that linker histones bind preferentially to crossovers in supercoiled DNA (64, 131). Its binding to free DNA is cooperative (29, 117) and results in tramlines in which two DNA duplexes are linked by GH5 molecules. Linker histones bind preferentially to four-way junctions (128), and the isolated globular domains also exhibit this preference (129). The implication of these results is that GH5 has more than one DNA-binding site, and the preference of linker histones for four-way junctions is related to their preference for nucleosomes. This preference for the nucleosome could be the result of more than one DNA-binding site on GH5. The supercoiling of DNA in the nucleosome results in two duplexes of DNA coming together in spatial proximity. If GH5 were able to bind to the crossover on the nucleosome where the entry and exit duplexes of nucleosomal DNA come together (1), this would explain its preference for the nucleosome. A similar interaction would occur in its binding to four-way junctions.

Although the primary DNA-binding site on GH5 is reasonably clear, any other DNA-binding sites are less well defined. A possible second site was identified from the crystal structure as consisting of a cluster of highly conserved, basic residues on the opposite side of the molecule. When highly conserved basic residues in either the primary or putative secondary binding sites were mutated to alanines or glutamic acids, the resulting GH5 mutants showed negligible or greatly reduced cooperative binding to DNA, would not bind preferentially to four-way junctions, and would not protect the additional 20 bp of nucleosomal DNA that is characteristic of wild-type GH5 (36). The experiments show that these residues are important for correct binding of GH5 on the nucleosome. Because these residues occur on opposing faces of the molecule, they are unlikely to be part of a single contiguous DNA-binding site. Therefore, the mutagenesis studies suggest that the second cluster of residues must be part of at least one other DNA-binding site. The extent and nature of this site and the mode of its interaction with DNA remain to be clarified. It has also been proposed that
GH5 could bind three DNA duplexes, namely the central duplex at or near the dyad, and the entry and exit points of nucleosomal DNA (1, 25). Although the structure suggests at least two DNA-binding sites, it is unclear whether a third site exists, and if so, what residues make up the third site. The underlying assumption in the analysis of the mutagenesis experiments is that the cooperative formation of tramlines of two DNA-duplexes and the preferential binding of GH5 to nucleosomes and four-way junctions are all related to GH5 having more than one DNA-binding site. As is discussed below in the section on the chromatosome, experiments done with a nucleosome reconstituted on the X. borealis 5S gene appear to give very different results.

**Structure of the Tails of H1/H5**

The tails of the linker histones comprise over half the molecule. In particular, the very basic C-terminal tail is known to be required for condensation of nucleosomes into the 30-nm filament (115) and also contains the sites of phosphorylation of linker histones. Such phosphorylation occurs in a cell-cycle-dependent manner and is correlated with changes in chromatin structure (see 11 and 97 for reviews). Little is known about the structure of the tails of H1. Given its highly basic nature and its role in condensation, the C-terminal tail is believed to interact with DNA, in particular with linker DNA. The details of this interaction are not clear; however, some models exist for the interaction of the tails with DNA.

Although the C-terminal tail appears to be unstructured in solution, it assumes an \( \alpha \)-helical conformation in buffers such as perchlorate that may mimic the negatively charged phosphate groups of DNA (21). From this observation, it was suggested that upon binding chromatin, the C-terminal tail assumes a segmented \( \alpha \)-helical conformation that could track one of the grooves on DNA. The \( \alpha \)-helical segments would be broken by the prolines that occur throughout the sequence of the tail. Some evidence of this process can be seen in an electron microscopic study of histone H5 reconstituted onto defined sequence nucleosomes (41). The binding of H5 to the nucleosome brings together the two ends of nucleosomal DNA to form a stem that presumably consists of two duplexes of DNA. The length of this stem is correlated with the length of the C-terminal tail of H5, suggesting that the stem is defined by the region of interaction with the tail of H5. Analysis of the length of the stem suggests that the C-terminal tail of H5 could not be fully extended, consistent with the idea that the tail is made up of \( \alpha \)-helical segments.

The tails of linker histones are rich in lysines or arginines, prolines, and alanine. Analysis of the sequence shows that the prolines are often preceded by serines or threonines, which are the sites of phosphorylation, and followed by basic residues. This observation has led to the suggestion that the sequence SPKK, or the variants (S/T)PXX where X is a lysine or arginine, form a motif
involved in DNA binding (87, 111) that consists of a $\beta$-turn, with a hydrogen-bond between the hydroxyl of the serine or threonine and the amide of the third lysine, resulting in additional stabilization of the turn. Hydroxyl-radical footprinting studies have shown that a peptide containing this motif will preferentially bind in the minor groove of AT-rich DNA (19), and a model for the binding of this structural motif to the minor groove of DNA has been proposed (112).

However, only weak evidence for a $\beta$-turn in this motif was provided by an NMR study on octapeptides (112) containing two repeats of the motif. The study was done in over 90% dimethylsulfoxide, a solvent known to favor the formation of hydrogen bonds. Even in this solvent, the peptide was in exchange with a more extended structure. Further, in the context of the structure of the transcription factor GAL4 bound to DNA (69), the (S/T)PXX sequence occurs not as part of a turn, but in an extended structure with the basic residues making contacts with the phosphate backbone of the DNA. Thus it is still unclear whether the sequence SPKK or its variants form a structural motif at all, let alone interact with DNA in the specific manner proposed in this model (111, 112).

Even less is known about the structure and interactions of the N-terminal tails of linker histones, which consist of both a basic region and a region rich in prolines and alanines. Clearly, detailed structural studies on the interaction of the tails of the linker histone with DNA are needed. Given the unstructured nature of these tails in isolated histones, such studies will have to involve complexes with DNA or chromatin, in the appropriate context.

THE CHROMATOSOME

The chromatosome consists of about 166 bp of DNA, wrapped around the histone octamer, and histone H1/H5 (104). DNAse I footprinting experiments suggested that 10 bp of DNA have been added to each end of the ~146-bp core particle DNA to make up the chromatosome.

The simplest explanation of the protection by H1/H5 of an additional 20 bp of nucleosomal DNA is that it is the result of direct interaction between the globular domain of H1/H5 with the entry and exit points of nucleosomal DNA (1, 25). This crossover of the entry and exit points of DNA would also be close to the nucleosomal dyad if one assumes symmetrical 10-bp extensions at each end of the core particle DNA, leading to a model for the binding of the globular domain that would be at or over the dyad, where it would be in a position to contact both ends of nucleosomal DNA directly. In support of this hypothesis, footprinting studies using DNase I showed a strong protection by H1 and H5 at the nucleosomal dyad (108) and moderate protection at the
ends of nucleosomal DNA (Figure 6A). Mapping of the H5 binding sites by immuno-electron microscopy (34) as well as recent cryo-electron microscopy studies using various domains of H5 (41) are consistent with the notion that GH5 binds to the entry and exit points of DNA and the C-terminal tail binds to linker DNA.

Direct binding of GH5 to the dyad raises some problems. The nucleosomal dyad has an exposed minor groove (94). If the primary binding of GH5 is similar to that of the HTH proteins (as discussed above), then binding at the dyad would involve an alpha helix in a minor groove, which would be surprising for a member of this family. Thus binding at the dyad, if it occurs, must involve a different mode of interaction.

More recently, experiments on a phased nucleosome reconstituted on the oocyte 5S gene from X. borealis have resulted in a different picture of linker histone binding to the nucleosome. Hydroxyl-radical footprinting experiments failed to see any protection by linker histone, at the dyad or elsewhere, whereas the protection of the ends of nucleosomal DNA by micrococcal nuclease was shown to be asymmetric: 15 bp is protected at one end and 5 bp is protected at
the other (50). Because micrococcal nuclease strongly prefers to cut after AT base pairs (70), the protection seen in these digestion experiments could be the result of sequence preferences of micrococcal nuclease rather than protection by linker histone. Indeed, over 95% of bulk chromatosomal sequences that were cloned and sequenced had AT base pairs at their ends (79). Protection experiments on bulk chromatin are less susceptible to potential problems arising from the sequence preferences of the nuclease.

However, more direct evidence for an asymmetric location for GH5 on the 5S nucleosome came from protein-DNA cross-linking studies, which showed that GH5 contacted DNA near the end of nucleosomal DNA and roughly a quarter-turn away from the dyad spatially (47) (Figure 6B). The cross-linking data suggested two possible locations for GH5. One of these was an external location, in which GH5 would still be in a position to contact two DNA double helices, from adjacent gyres on the nucleosome. However, the authors favored the possibility of a location inside the DNA superhelix, in which GH5 would bind at a “cavity” between the DNA superhelix and the histone octamer. An interior location would explain why GH5 fails to protect nucleosomal DNA in hydroxyl-radical footprinting and would also explain various other biochemical data such as its interaction with core histones, especially H2A (10). The interior location would also have the DNA bend toward the GH5 molecule, which is similar to the bend of the DNA in the HNF-3/DNA complex (22). In this model of GH5 binding, protection of the ends of nucleosomal DNA from nuclease digestion would be indirect, especially since GH5 would be unable to protect even the proximal end of nucleosomal DNA directly. The protection would come as a result of indirect allosteric effects that would involve changes in core histone interactions with DNA, and indeed such changes have been observed as a consequence of linker histone binding (126). More recently, site-specific cleavage experiments with Fe(II) tethered to H1° and reconstituted on the 5S nucleosome (44) show cleavage patterns that are consistent with the interior location suggested by the cross-linking experiments discussed above.

The location of the globular domain at an asymmetric location on the 5S nucleosome is somewhat surprising, because the nucleosome without linker histone has an approximate dyad axis of symmetry. This observation suggests that in the 5S nucleosome, the globular domain strongly prefers one of the two roughly equivalent sites on either side of the dyad, implying a strong DNA sequence preference on the part of GH5, which could be a direct sequence preference or an indirect one as a result of the sequence modulating the structure of the binding pocket.

It has been argued that the protection seen at the dyad on bulk nucleosomes by DNase I (108) is the result of cleavage done on dinucleosomes, so that protection could result from steric hindrance that comes from adjacent nucleosomes
rather than directly as a result of H1/H5 binding (44). However, this hypothesis has not been proved, for example, by footprinting of phased dinucleosomes containing the 5S gene.

Therefore, there appears to be a conflict between data obtained on the 5S nucleosome and other experiments. One possibility for reconciling the two lines of evidence is if linker histones were to bind in a fundamentally different way to the 5S nucleosome than to bulk nucleosomes, as has been suggested previously (36, 138). The sequence preference of H1/H5 on the 5S nucleosome is strong enough to make it select one of the two potential binding sites almost exclusively, and this strong preference may also preclude its binding at a possibly more usual site. Interestingly, in a study of bulk chromatosomal sequences, an AGGA sequence was observed near one of the termini of chromatosomal DNA but not the other (79), raising the possibility that this sequence may be involved in binding the globular domain of H1/H5. This sequence does not occur at the same place on the 5S nucleosome.

Given that H1 is involved in specific repression of the oocyte 5S gene in somatic cells (136), its location on the 5S nucleosome may be related to its regulatory role, whereas in the case of bulk chromatin, its role in condensing nucleosomes may require binding at an alternative site. Despite many years of effort in various laboratories, a successful 30-nm filament from tandem repeats of the 5S gene has yet to be demonstrated, suggesting that H1 fails to perform its packaging function with the 5S nucleosome, perhaps because it binds to a special site related to its regulatory role.

However, in these speculations about a dual role and binding site for H1, one should bear in mind that such dual binding would require H1 to bind to two very different binding sites on the nucleosome. The site favored by studies on bulk nucleosomes is an external DNA pocket close to the dyad, involving more than one duplex of DNA. The site favored by studies on the 5S nucleosome is an asymmetric interior location in which one face of GH5 would bind nucleosomal DNA while other parts would interact directly with core histones. Such completely different modes of binding of the same molecule to the same entity, the nucleosome, would be extremely unusual. The high-resolution mapping techniques that have been used so successfully with the 5S nucleosome need to be applied to other nucleosomes including bulk nucleosomes in order to settle this question unambiguously.

HISTONES AND TRANSCRIPTION FACTORS

The similarity between the globular domain of H5 and the transcription factor HNF-3 suggested that there was a relationship between linker histones and transcription factors. The observation that HNF-3 is associated with a positioned
nucleosome array in a cell-specific manner (71) is consistent with the idea that linker histones are involved in the repression of transcription. For example, the role of certain transcription factors is to counter the repressive effects of H1 (26), and deposition of H1 onto nucleosomal arrays prevents both initiation and elongation of transcripts by T7 RNA polymerase (84).

A striking discovery was made recently that two subunits of the transcription complex TFIIID, dTAFII42 and dTAFII62, have the histone fold and form a tetramer similar to the (H3-H4)2 tetramer (137). Biochemical studies show that a tetramer of the human equivalents hTAFII31 and hTAFII80 combine with two dimers of hTAFII20 to form an octameric structure (52). This observation suggests that the transcription complex is likely to contain a histone octamer-like structure. The conserved basic residues that are proposed to be involved in DNA binding in the histone octamer (4) are also present in these proteins, supporting the idea that a nucleosome-like structure could be present in the transcription complex.

Together, these findings blur the distinction between histones and transcription factors, again reinforcing the idea that histones and the transcription machinery of eukaryotes may have co-evolved as a solution to the signal-to-noise problem that became an issue as the gene number and complexity of organisms increased (9).

CONCLUSIONS

In the past few years, high-resolution structures of the histones have become available for the first time and have greatly extended our understanding of chromatin structure. Serious gaps remain in our knowledge, however.

The histone octamer structure does not contain any information about the important N-terminal tails of the core histones, presumably because they are disordered in the absence of DNA. These tails are the sites of specific modifications such as acetylation and are also the sites of interaction with specific proteins such as SIR3 and SIR4, which are involved in silencing of transcription (51). Therefore, it is important to obtain information about the basic structural features of these tails to understand how they may become modified during various cellular processes.

A high-resolution structure of the nucleosome core particle would not only shed light on the details of histone-DNA interactions in the nucleosome, but also has the potential to reveal structural features of the histone tails and their interaction with DNA, because these tails may adopt a definite structure on binding DNA. The use of defined sequences of DNA that are known to phase nucleosomes (96) as well as the use of a palindromic sequence to produce symmetric nucleosome core particles (42) have resulted in the ability to produce
crystals that now diffract to better than 3-Å resolution. A high-resolution structure of the core-particle appears to be imminent, with exciting consequences for our understanding of histone-DNA interactions.

In the case of linker histones, although the structure of the globular domain has suggested a mode of interaction with DNA, the details of its interaction with the nucleosome or even DNA are not well understood. As in the case of core histones, the tails of the linker histones are the sites of important modifications, and their structure and the way in which they interact with chromatin remain unknown. Future work in these areas will be extremely useful.

The high-resolution structures of the histones and the anticipated high-resolution structure of the nucleosome core particle will provide a firm basis to interpret the avalanche of results emerging on the role of chromatin structure in the biology of the cell.

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Literature Cited

21. Clark DJ, Hill CS, Martin SR, Thomas JO.

22. Clark KL, Halay DE, Lai E, Burley SK.


108 RAMAKRISHNAN
66. Lee DY, Hayes JJ, Pruss D, Wolfe AP. 1993. A positive role for histone acetylation...
tion in transcription factor access to nu-
cleosomal DNA. Cell 72:73–84
The role of a positioned nucleosome at 
the Drosophila melanogaster hsp26 pro-
moter. EMBO J. 14:4738–46
Effects of histone acetylation on chro-
12:5004–14
69. Marmorstein R, Carey M, Ptashne M, 
Harrison SC. 1992. DNA recognition by 
GAL4: structure of a protein-DNA com-
70. McGhee JD, Felsenfeld G. 1983. Another 
potential artifact in the study of nucle-
osome phasing by chromatin digestion 
with micrococcal nuclease. Cell 32:1205– 
15
71. McPherson CE, Shim E-Y, Friedman DS, 
Zaret KS. 1993. An active tissue-specific 
enhancer and bound transcription factors 
existing in a precisely positioned nucleo-
72. Meersseman G, Pennings S, Bradbury 
EM. 1992. Mobile nucleosomes—a gen-
eral behavior. EMBO J. 11:2951–59
73. Meersseman G, Pennings S, Bradbury 
EM. 1991. Chromatosome positioning on 
assembled long chromatin. Linker his-
tones affect nucleosome placement on 5 
S rDNA. J. Mol. Biol. 220:89–100
74. Mirzabekov A, Pruss DV, Ebralidse 
KK. 1989. Chromatin superstructure-
dependent crosslinking with DNA of the 
histone H5 residues Thr1, His25 and 
75. Mirzabekov AD, Pruss DV, Ebralidse 
KK. 1990. Chromatin superstructure-
dependent crosslinking with DNA of the 
histone H5 residues Thr1, His25 and 
76. Moudrianakis EN, Arents G. 1993. Struc-
ture of the histone octamer core of the 
nucleosome and its potential interactions 
with DNA. Cold Spring Harbor Symp. 
Quant. Biol. 58:273–79
77. Moudrianakis EN, Love WE, Wang BC, 
Xuong NG, Burlingame RW. 1985. Sci-
entific correspondence. Science 229:1110– 
12
78. Musgrave DR, Sandman KM, Reeve JN. 
1991. DNA binding by the archaeal his-
tone HMf results in positive supercoiling. 
79. Muyldermans S, Travers AA. 1994. DNA 
sequence organisation in chromatosomes. 
J. Mol. Biol. 235:855–70
80. Ner SS, Travers AA. 1994. HMG-D, the 
Drosophila melanogaster homologue of 
HMG 1 protein, is associated with early 
embryonic chromatin in the absence of 
histone H1. EMBO J. 13:1817–22
81. Noll M, Kornberg RD. 1977. Action of 
micrococcal nuclease on chromatin and 
the location of histone H1. J. Mol. Biol. 
109:393–404
82. Norton VG, Imai BS, Yau P, Bradbury 
EM. 1989. Histone acetylation reduces 
nucleosome core particle linking number 
change. Cell 57:449–57
83. Norton VG, Marvin KW, Yau P, Bradbury 
EM. 1990. Nucleosome linking num-
ber change controlled by acetylation of 
histones H3 and H4. J. Biol. Chem. 
265:19848–52
84. O’Neil T, Meersseman G, Pennings S, 
Bradbury EM. 1995. Deposition of his-
tone H1 onto reconstituted nucleosome 
arrays inhibits both initiation and elon-
gation of transcripts by T7 RNA poly-
merase. Nucleic Acids Res. 23:1075–82
85. Pennings S, Meersseman S, Bradbury 
EM. 1991. Mobility of positioned nu-
cleosomes on SS rDNA. J. Mol. Biol. 
220:101–10
86. Pennings S, Meersseman S, Bradbury 
EM. 1994. Linker histones H1 and H5 
prevent the mobility of positioned nu-
cleosomes, Proc. Natl. Acad. Sci. USA 
91:10275–79
87. Poccia D. 1987. Regulation of chromatin 
condensation and decondensation in sea 
urchin pronuclei. In Regulation of Chro-
matin Condensation and Decondensation 
in Sea Urchin Pronuclei, ed. RA Schlegel, 
MS Halleck, PN Rao, pp. 149–77. New 
York: Academic
of protein access to specific DNA se-
quences in chromatin: a dynamic equi-
librium model for gene regulation. J. Mol. 
Biol. 254:130–49
89. Pruss D, Reeves R, Bushman FD, Wolfe 
AP. 1994. The influence of DNA and nu-
cleosome structure on integration events 
directed by HIV integrase. J. Biol. Chem. 
269:25031–41
91. Ramakrishnan V. 1995. The histone fold: 
Sci. USA 92:11328–30
92. Ramakrishnan V, Finch JT, Graziano V, 
Lee PL, Sweet RM. 1993. Crystal struc-
ture of globular domain of histone H5 and 
its implications for nucleosome binding. 
Nature 362:219–23
93. Rhodes D. 1985. Structural analysis of 
a triple complex between the histone 
octamer, a Xenopus gene for SS RNA
and transcription factor IIIA. EMBO J. 4:3473–82
111. Suzuki M. 1989. SPKK, a new nucleic acid-binding unit of protein found in histone. EMBO J. 8:797–804
120. Tsukiyama T, Becker PB, Wu C. 1995. ISWI, a member of the SWI/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell 83:1021–26


