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Nucleosome and Chromatin Structures and Functions

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The magnitude of the problem of understanding the organization, structures, and functions of eukaryotic chromosomes can be appreciated from the fact that the human diploid genome contains more than 2 m of DNA (6×10^9bps) packaged into 46 chromosomes, each several µms in length and a half µm thick at metaphase. Despite decades of intensive research, many questions remain at the molecular level concerning the structures, nuclear organizations, and functions of eukaryotic chromosomes. Some answers will come from the completed sequences of the human genome and of the genomes of model organisms, particularly from the identification of DNA sequence motifs involved in the long range organization of chromosomes and in nuclear architecture.

In addition to the DNA, chromosomes contain an equal mass both of histones and of largely uncharacterized nonhistone proteins. Nonhistone proteins are involved in chromosomal functions, long range chromosome organization, and nuclear architecture. In an attractive model for chromosome organization the DNA is constrained into loops of average size 50 kbps by the binding of scaffold proteins to AT rich DNA sequences [Laemmli et al., 1978; Saitoh and Laemmli, 1993; see also Earnshaw and Mackay, 1994]. Thus the haploid genome would contain 60,000 average size loops, a number within the range estimated for the number of human genes of between 50,000–100,000. DNA loops are packaged by histones into chromatin domains. A chromatin domain is thought to be both a genetic unit and structural unit of eukaryotic chromosomes. The roles of centromeres and telomeres, the organization of chromosomes in the cell nucleus, and changes in that organization with cellular functions are central to an understanding of chromosome functions.

In the early view of the packaging of DNA molecules, several cms in length, into chromatids, histones were thought to bind to the outside of the linear DNA molecule and through histone:DNA and interhistone interactions coil the DNA into a very large number of sequential higher-order coilings to give the length of the metaphase chromatids. The DNA loop model provides for a much simpler process of chromosome condensation that involves nonhistone proteins, including topoisomerase II, that condense to form a protein scaffold which constrains DNA loops transverse to the axis of the chromatid. Following the long range condensation of the scaffold, the DNA loops are thought to be condensed by histones and their postsynthetic modifications into the thickness of the chromatid. This transverse mode of packaging would require only one order of chromatin coiling above the 30 nm supercoil of nucleosomes. In the early chromosome packaging model, transcriptionally active genes were thought to be devoid of histones and easily accessible to transacting factors. This view led to the long held belief that histones were no more than passive structural proteins with little involvement in DNA functions.

The major advance in understanding chromatin structures and functions came from the findings of Hewish and Burgoyne [1973] that chromatin was made up of regularly repeating subunits, later called nucleosomes. Virtually all of a eukaryotic genome is packaged into nucleosomes and they have been the focus of

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attention in chromatin research for the past 25 years.

Histones

Based on their sequences, conformational behaviors and interactions, histones fall into three groups [see van Holde, 1988]: 1) the families of very lysine rich or linker histones, $H1^A$, $H1^B$, $H1^O$, H5 etc.; 2) the H2A and H2B families; and 3) the H3 subtypes and H4. The synthesis of some members of these histone families are cell cycle dependent and synthesized in S-phase of the cell cycle, e.g., H2A1 and H2A2 and others are not, e.g., H2AX and H2AZ. All histones are highly conserved. The linker histones show the most sequence variability followed by the H2A and H2B families. Histones H3 and H4 are among the most conserved proteins in nature which implies that each and every residue is essential for their roles in chromatin structure and functions. These families of histones provide for considerable variability in the packaging of DNA for chromosome and cellular functions.

Histones are multidomain proteins (Fig. 1) [see reviews by Bohm and Crane-Robinson, 1984; van Holde, 1988; Bradbury, 1992]. The linker histones have a central structured domain flanked by long basic flexible domains. For the cell cycle dependent H1s from somatic cells the N-terminal domain is about 40 residues, the central structured domain is 80 residues, and the C-terminal basic domain is 100 residues. The central globular domains of the linker histones are abbreviated to GH1, GH5 etc. The solution structure of GH1 and GH5 have been solved by multidimensional NMR spectroscopy [Clore et al., 1987; Cerf et al., 1993]. The solution structure of GH5 agrees well with its crystal structure solved to 0.25 nm resolution [Ramakrishnan et al., 1993]. Histones H2A and H2B each have a structured central domain flanked by a flexible N-terminal domain of about 30 residues and C-terminal "tails" of 10–15 residues. H3 and H4 have flexible N-terminal domains of 35 and 30 residues, respectively, and a central/C-terminal structured domain. In addition H3 has a short C-terminal tail. For the linker histones and the H2A and H2B families sequence variability is confined largely to their flexible N- and C-terminal domains.

The core histones H2A, H2B, H3, and H4 form well-defined complexes (Fig. 1); the (H2A, H2B) dimer, the ($H3_2$ $H4_2$) tetramer, and the $(H2A, H2B)_2$ ($H3_2$, $H4_2$) octamer [see van Holde, 1988]. The octamer provides the structural framework of the nucleosome, particularly ($H3_2$, $H4_2$) interactions [Moss et al., 1977]. The crystal structure of the octamer has been solved to 0.31 nm resolution [Arents et al., 1991] and shows that the stability of the histone octamer involves interactions between the conserved structured domains of the core histones. Of particular interest were: 1) the identification of the histone fold motif now found in other DNA binding proteins and 2) the absence of electron density from the flexible basic N- and C-terminal domains of the core histones because of static and/or dynamic disorder in the crystal.

Histone Modifications

All five histones are subjected to reversible chemical modifications that change markedly the chemical nature of the modified residues. It is noteworthy that all of these modifications are located in the flexible basic N- and C-terminal domains of the histones (Fig. 1); acetylations of lysines in the histone octamer; phosphorylations of serines and threonines in H1, H2A, and H3 and ubiquitinations of lysines in the C-terminal tails of H2A and H2B. Acetylation and ubiquitination modify about 5% of the core histones and thus can affect only small subcomponents of chromatin. In contrast all of the H1s are hyperphosphorylated and all H3s phosphorylated at metaphase.

Phosphorylation. It has been proposed previously that the cell growth associated H1 kinase controlled the mitotic cell cycle and chromosome condensation [Bradbury et al., 1974a,b]. This H1 kinase has now been identified as the cyclin dependent kinases (cdks) particularly $p34^{cdc2}$/cyclin A and $p34^{cdc2}$/cyclin B. All in vivo sites of H1 phosphorylation can be phosphorylated in vitro by the above two cdks and $p33^{cdc2}$/cyclin A [Swank et al., 1997]. These sites however include serine 1 and threonine 3 located in the H1 N-terminal sequence Ac-SETAPA which is quite distinct from the consensus cdc2 kinase site S/TPXK [Gurley et al., 1995]. Thus the sequential phosphorylations of the linker histones through the mammalian cell cycle to reach a maximum of six to seven phosphates at metaphase come not from site specificity of the cdks but from the sequential availability of these sites.

Acetylation. Allfrey et al. [1964] proposed that histone acetylation was involved in "the

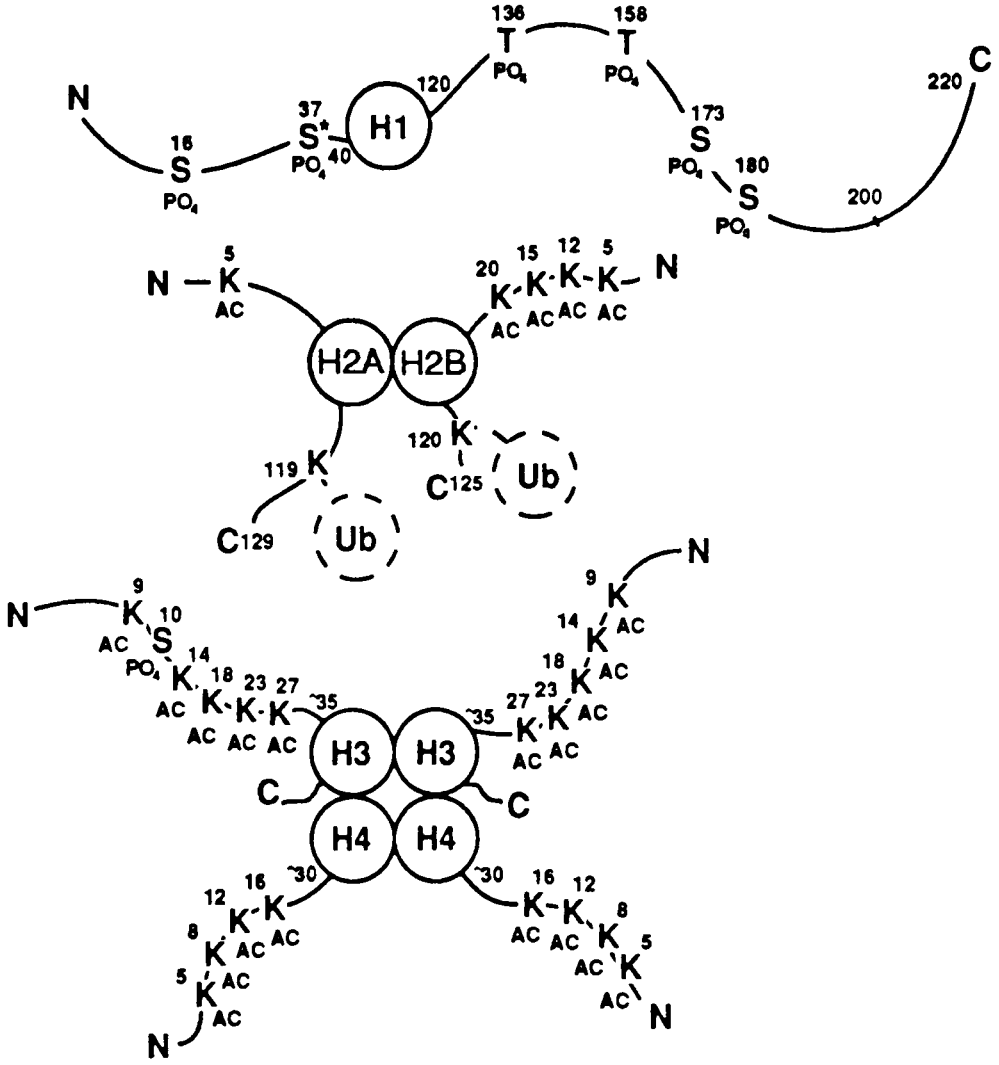

Fig. 1. Outline structures of histone H1, the (H2A, H2B) dimer and ($H3_2$, $H4_2$) tetramer showing the well-defined globular domains, the basic flexible N- and C-terminal domains and sites of reversible acetylation, phosphorylation, and ubiquitinations. From Bradbury, 1992.

regulation of RNA synthesis." Within the past few years this early proposal has been supported strongly by the identifications of families of acetyltransferases and deacetylases that are associated with the activation and inactivation of gene expression [see Struhl, 1997; Kuo and Allis, 1998; Workman and Kingston, 1998]. Previously identified protein components of the RNA polymerase II complex have now been shown to be histone acetyltransferases and thus provide a causal link between histone hyperacetylation and the requirements of gene expression. Some of the acetyltransferases and deacetylases form complexes with transacting factors to control the expression or repression of target genes. These observations clearly argue against a passive structural role for histones in chromosome functions. Histone acetylations are involved in the modulation of chromatin structure of potentially active genes for subsequent transcription. Histone acetylation/deacetylation reactions are much more complex than previously envisioned and involve large families of acetyltransferases and deacetylases. Some of these enzymes have a general activity whereas others are specific for particular histones and acetylation sites.

Ubiquitination. The third reversible modification of histone is the ubiquitinations of lysines in the C-terminal tails of H2A and H2B. The chromosomal functions of this modification are largely unknown. It has been shown that

ubiquitinated H2A is not found in metaphase chromosomes [Matsui et al., 1979] and the uH2A and a H2B are deubiquinated immediately prior to metaphase and reubiquitinated early in anaphase of the cell cycle [see Bradbury, 1992].

Nucleosomes

For most somatic cells the nucleosome contains 195 ± 10 bp DNA, the histone octamer and a linker histone. For unknown reasons the nucleosomes from some specialized cells contain different DNA repeats. Nucleosomes are isolated following micrococcal (m) nuclease digestion of chromatin or nuclei. Further m. nuclease digestion trims the DNA ends of the nucleosome to give two well-defined subnucleosomal particles; the chromatosome with 168 bp DNA and the full complement of histones and the core particle with a precise 146 ± 1 bp DNA and the histone octamer.

The Nucleosome Core Particle

The core particle has been subjected to intense structural studies. Early neutron scatter studies of core particles in solution proved that DNA was coiled on the outside of the histone octamer and the model that fitted best the scatter data was a disc 11.0 nm diameter, 5.5 to 6.0 nm thick with 1.7 ± 0.2 turns of DNA with a mean radius 4.5 nm coiled with a pitch of 3.0 nm [see Bradbury, 1992; van Holde, 1988]. Low resolution X-ray and neutron diffraction of crystals of core particles gave a model of a wedge-shaped disc 11.0 nm in diameter and 5.7 nm thick with 1.8 turns of DNA of mean radius 4.4 nm coiled with a pitch of 2.8 nm around the histone octamer [Finch et al., 1977; Bentley et al., 1981]. Thus at low resolutions, the solution and crystal models appear to be very similar. The resolution of the core particle crystal structure has been extended to 0.7 nm [Richmond et al., 1984] and most recently to 0.28 nm [Lugar et al., 1997]. In this highest resolution core particle structure, the structure of the histone octamer is very similar to the structure of the isolated octamer [Arents et al., 1991] the 146 bp DNA is coiled in 1.65 turns of a left-handed supercoil of mean radius 4.18 nm and pitch 2.39 nm which is lower than the previously reported pitch of 2.8 nm [Finch et al., 1977; Bentley et al., 1981] and of 3.0 to 2.5 nm [Richmond et al., 1984]. The DNA is not bent uniformly around the histone octamer but follows a more irregular path of bends and straighter segments. As found for the 0.7 nm crystal structure much of the electron density expected for the flexible core histone N- and C-terminal domains is not observed in the 0.28 nm structure. At this higher resolution, however, portions of the N terminal domains adjacent to the core histone structured regions have been identified. The N-terminal domains of H2B and H3 are observed to extend between the DNA gyres to the outside of the core particle. The electron density for the portion of the N-terminal outside the core particle is very weak and uninterpretable. The regions that extend outside of the core particle have the potential to interact with adjacent nucleosomes in stabilizing higher order chromatin structure [Lugar et al., 1997]. One of the H4 N-terminal portions is found bound to an acidic regions of the H2A/H2B dimer of the adjacent particle. Whether this is a physiological binding sites remains to be seen.

The Chromatome and Nucleosome

Models for the 168 bp chromatosome and the 195 ± 10 bp nucleosome are based on the crystal structure of the core particle [Lugar et al., 1997], the accessibility of DNA in chromatin to nuclease digestion, DNA footprinting, and zero length histone-DNA crosslinking. In the 0.28 nm resolution core particle crystal structure the 146 bp DNA is constrained in 1.65 turns of DNA superhelix. Based on the parameter of this superhelix the 168 bp of chromatosome DNA would be constrained in 1.9 turns of DNA, i.e., approximately two full turns of DNA. In an early model (Fig. 2) GH1 protects 168 bp DNA against m. nuclease digestion by binding at the pseudyad axis with DNA entering and leaving the nucleosome [see Bradbury, 1992; Zlatanova and van Holde, 1996; Crane-Robinson, 1997]. In this model globular H1 interacts with three DNA segments whereas the crystal structure of the globular domain of H5 contains two putative DNA binding sites [Ramakrishnan et al., 1993]. If the globular domain of H1 is moved out from the nucleosome along the dyad axis it could interact with the DNA entering and leaving the nucleosome and induce a DNA cross over as found for the binding GH1 to supercoiled DNA [see Zlatamova and van Holde, 1996]. The location of the very lysine rich histones on the chromatosome/nucleosome is currently under intense investigation. In detailed studies of the interaction of GH1 and GH5 with a nucleosome assembled on the *Xenopus* 5S

rDNA positioning sequence with the core histones it was observed that the DNA extensions to the core particle to give the chromatosome were not each 10 bp but 5 bp and 15 bp [Hayes and Wolfe, 1993]. From DNA footprinting, histone-DNA crosslinking and UV induced crosslinking of GH5 to a nucleosome formed with a 331 bp DNA containing arylazide groups at known locations the major UV crosslinking site was in the major groove 60–68 bp from the dyad axis [see Crane-Robinson, 1997]. This places the binding site for GH5 within the nucleosome core particle DNA and because of the pseudodyad axis a second binding site would be expected at the diametrically opposite site in the core particle. The accepted stoichiometry is one H1 per nucleosome and the only unique H1 binding site is at the pseudodyad axis where the DNA enters and leaves the nucleosome (Fig. 1 or Fig. 1 with GH1 located further out along the pseudodyad axis) [Zlatanova and van Holde, 1996]. A prediction of the off axis model for GH5 binding is that two GH5s should bind to the core particle as well as to the chromatosome. This off axis binding site of GH5 to the nucleosomes assembled on Xenopus rDNA may be unique to this constitutively expressed gene. After more than 2 decades of intensive research on the locations and modes of interactions of the three linker histone domains it is sobering to realize that binding site of the globular domain remains controversial! To understand chromatin structure above the level of the nucleosome it is essential to know the in vivo bindings of linker histones to the nucleosome, the paths of linker DNAs between nucleosomes and changes in these interactions and paths with chromosome functions.

Histone N and C Terminal Domains

Figure 1 strikingly shows the importance of the flexible N-terminal domains and C-terminal tails in understanding chromatin functions. We need to know the binding modes of these N- and C-terminal flexible, basic domains in the nucleosome and in chromatin; the effects of the reversible chemical modifications on these interactions and whether there are any subsequent interactions of the chemically modified terminal domains in chromatin with other proteins or nucleic acids. The crystal structure of the core particle at 0.28 nm resolution does not give the modes of interactions of the N- and C-terminal domains. Most of the electron densities from these domains are not observed. A major problem in understanding the structures

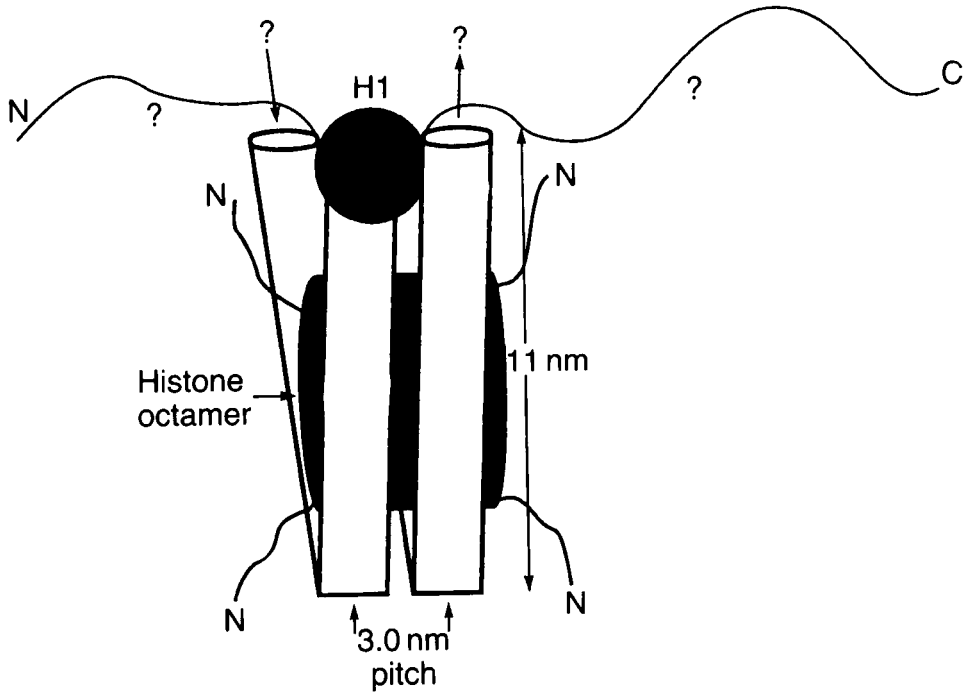

Fig. 2. Model for the chromatosome based on the known structure of the nucleosome core particle and the possible binding site of histone H1. From Bradbury, 1992.

and interactions of the N- and C-terminal domains is that the 146 bp core particle lacks both the linker DNAs and H1 of the 195 bp nucleosome. A major question is whether removal of approximately 25 bp DNA from each end of the nucleosome to give the core particle removes native DNA binding sites for the basic N- and C-terminal domains of the core histones resulting in the rearrangements of these domains in the core particles and core particle crystals. At the molecular level it will be necessary to solve the crystal structures of the 195 bp nucleosome, dinucleosomes, and higher oligosomes with nonmodified and fully modified histones. It is essential to know the in vivo binding sites of the histone N- and C-terminal domains in chromosomes and whether there are rearrangements of these domains with chromosomal functions through the cell cycle. Answers to questions concerning the in vivo locations of flexible N- and C-terminal domains will come from zero length covalent histone-DNA crosslinking [Levina et al., 1981]. Using this method, it has been shown that the H2A C-terminal tail binds at the pseudodyad axis of the 146 bp core particle [Usachenko et al., 1994]. More recently it has been found that the H2A C-terminal domain makes a very strong contact with the DNA at the pseudodyad axis in nucleosomes from native hypoacetylated nucleosome whereas in the native hyperacetylated domain this contact was absent [Usachenko and Bradbury, unpublished]. Because the C-terminal and structured regions of H2A are not acetylated, the loss of this contact in the hyperacetylated chromatin domain probably results from a structural rearrangement of the nucleosome. It has been shown that the full acetylation of H3 and H4 cause a reduction in the nucleosome linking number [see Bradbury, 1992]. It will be necessary to identify the binding sites of histone N- and C-terminal domains in nuclei at different stages of the cell cycle to determine whether functionally related rearrangements of these domains occur in vivo. In addition to the effects of acetylation on nucleosome structure and stability, it has been shown that the constraints of nucleosomes to transcription can be relieved by protein complexes in an ATP dependent manner [see Workman and Kinston, 1998].

Nucleosome Core Positioning and Mobility

Nucleosomes lacking H1 are called nucleosome cores. H1 depleted chromatin is a linear array of nucleosome cores. One of the strongest nucleosome positioning sequences is the 5S ribosomal gene DNA from the sea urchin *Lytechinus* [Simpson and Stafford, 1983; Simpson et al., 1985]. Head to tail tandem repeats of 18 × 207 bp and 45 × 172 bp DNAs have been constructed by Simpson's group for studies of chromatin structure. The 172 bp DNA contains seven unique restriction sites and the 207 bp contains eight such sites. These unique restriction sites were used to map the position(s) of the histone octamer on nucleosome core particles obtained by m. nuclease digestion of the assembled arrays of nucleosome cores [Meersseman et al., 1991; see Pennings and Bradbury, 1997]. This analysis showed that many but not all histone octamers, assembled on one position from nucleotide 6 to 153 bp in both the 207_{18} and 172_{45} head to tail dimer assembled chromatin. In addition to this dominant position, minor positions were identified at 10 and 50 bp upstream and 10, 20 and 40 bp downstream of the dominant position. The 172_{45} gave the same octamer positions except for the absence of the most distant upstream and downstream positions. It is to be noted that all of the minor positions are multiples of the B form DNA repeat of 10 bp from the dominant position. The above behavior most probably results from the dynamic nature of the mainly electrostatic interaction between the lysines and arginines of the basic histones and the DNA superhelix around the octamer. The addition of linker histones caused a redistribution of the populations of the bands without affecting their positions. The above behavior of nucleosome core positioning suggested that nucleosome cores have the ability to move between major and minor positions. The effect of temperature on nucleosome cores assembled onto 207_{18} showed that at 37°C, but not at 4°C, the nucleosome cores redistributed themselves between the major and minor positions on the 207 bp DNA [Pennings et al., 1991]. A similar behavior was found for native chromatin depleted of H1 showing that nucleosome core mobility is a general property of H1 depleted chromatin. This has important functional significance because actively transcribing chromatin is largely depleted of linker histones resulting most probably from the hyperacetylations of the core histone. Thus through nucleosome core mobility, cis acting DNA elements packaged into nucleosome cores would become available for the binding of trans

acting protein factors prior to gene activation. It is to be expected that attempts by the factors to bind to their DNA site in the nucleosome core would influence the position of the nucleosome core making the site available for binding. This behavior has been observed for the binding of TFIIIA to the Xenopus 5sr DNA assembled into nucleosome cores [Ura et al., 1995].

Higher Order Chromatin Structures

Early electron microscopy studies showed that a "30 nm fibre" was the major component of isolated chromatin [Ris and Kubai, 1970]. A major problem with determining its molecular structure is the paucity of high-resolution structural data. X-ray diffraction patterns of orientated fibers of chromatin are very diffuse with few reflections: a broad meridional reflections at 10.5–11.0 nm; broad equatorial reflections at 6.0 nm and 2.7–3.0 nm; and diffuse rings at 5.5 and 3.5 nm [see van Holde, 1988]. Such a diffuse X-rays fibre pattern bespokes a great deal of disorder in higher orders chromatin structures. No more than a handful of structural repeats are required to give this type of diffuse pattern. The discovery of the nucleosome led to renewed studies of the "30 nm" chromatin fiber. From electron micrographs Finch and Klug [1976] proposed a solenoid model of a supercoil of nucleosomes 30 nm in diameter with pitch of about 11 nm. From neutron fiber diffraction studies of H1 depleted chromatin fibers [Carpenter et al., 1976] the 11.0 nm reflection was found to be semi-meridional and it was proposed that at the concentration of chromatin in fibers the supercoil formed in the absence of H1. Thus H1 is probably involved in stabilizing the 30 nm supercoil. Transverse neutron scatter studies of chromatin solutions gave a mass/unit length corresponding to six to seven nucleosome per turn of the fibre [Suau et al., 1979]. The diameter of the hydrated fibre was 34 nm compared to 30 nm for the dehydrated fibre in electron microscographs. In the model for the "30 nm" fibre the nucleosome discs are arranged close to radial leaving a hole of 10 nm diameter down the axis of the supercoil. From neutron scatter studies [Graziano et al., 1994] of chromatin fibres assembled with deuterated H1 it was found that the average distance of H1 from the fiber axis was 6–6.5 nm suggesting that H1 is located largely inside the hole down the axis of the "30 nm" supercoil. It seems very unlikely that structural studies of native chromatin will give sufficiently high resolution data that will lead to a detailed model for the "30 nm" supercoil. It will be necessary to investigate fully defined chromatins assembled on head to tail tandem repeats of nucleosome position sequences.

Summary

From the above discussion it can be appreciated that the major unknowns to advancing our understanding of nucleosome and chromatin structure and functions are: 1) the binding sites of the histone N- and C-terminal domains in native nucleosomes and in chromatin; 2) the effects of histone reversible chemical modifications of phosphorylations, acetylations and ubiquitinations on the binding of the histone N- and C-terminal domains in nucleosome and chromatin structures; 3) the path(s) of the linker DNA between nucleosome in the different orders of chromatin structure; 4) the position of the globular, N- and C-terminal domains of linker histones in the nucleosome and chromatin structures; and 5) the lack of a detailed structure for the 30 nm chromatin fiber. The above unknowns provide formidable challenges for future researchers.

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