Chemical probes of DNA structure in chromatin

Understanding the way genes work requires detailed knowledge of the organization of DNA in the chromatin complex. The difficulties associated with the study of this large macromolecular assembly present an interesting challenge to both biologists and chemists.

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The genetic instructions for construction, maintenance and propagation of our human form are encoded within the primary structure of approximately two dozen nucleic acid biopolymers, the chromosomes. The amount of information is huge, resulting in the requirement that the DNA molecules in which it is stored be extremely long, with about three meters total of DNA per diploid cell. These large macromolecules must then be confined within the tiny space of the cell's nucleus, roughly 10 μm in diameter. The fact that severe organizational and topological problems will result is obvious.

Eukaryotic cells solve the problem of how to make their DNA fit into their nucleus by complexing it with histones and other chromosomal proteins into a composite known as chromatin [1]. The amazing characteristic of chromatin is not so much the overall compaction of the DNA that it makes possible, but rather that this compaction occurs in such a precisely-ordered manner as to allow efficient retrieval of the genetic information within the DNA despite the compression of its structure. The interesting challenge for those studying this complex is to understand how these two seemingly mutually-incompatible requirements for chromatin structure have been satisfied.

Importance of understanding DNA structure in chromatin

The recent realization that chromatin structure has been functionally integrated into nuclear processes has rekindled interest in how DNA is organized within this complex [2]. For example, recent experiments have demonstrated that the transcriptional control mechanisms associated with the promoter of a yeast gene that is induced by monophosphate anion do not function properly in the absence of chromatin proteins (see [3] for review). A precise chromatin organization in this promoter strategically blocks the binding of an important transcription factor to its site within the promoter region. The presence of monophosphate anion was found to cause a cascade of events which resulted in disruption of the chromatin organization in the promoter region, presumably allowing binding of the transcription factor and, ultimately, transcription of the gene. Further confirmation of the essential role of chromatin structure came from studies on yeast mutants that lacked crucial chromatin proteins. In these yeast mutants, the chromatin structure of the gene's promoter was inherently disrupted. Although the cells that carry this mutation are sick, the monophosphate-inducible gene was found to be fully active even in the absence of the inducer, presumably because the transcription factor was no longer blocked from binding to its recognition site [4].

In other cases transcription is thought to be assisted by the organization of DNA into chromatin, as elements that may be distant from each other in the DNA sequence can be brought into close proximity by the fact that the intervening DNA is wrapped into a nucleosome core (see below) [4–6]. This juxtaposition of DNA sites is thought to allow cooperative interactions which assist binding of the transcription factors and ultimately enhance transcription of the gene. Thus the ordered packaging of DNA into chromatin has been incorporated into transcriptional regulatory mechanisms by providing an architectural scaffold for building the tertiary structure of an active promoter complex [7]. These observations provide clear evidence that a precise understanding of the way in which DNA is organized within the chromatin complex is a prerequisite for a complete understanding of all nuclear processes that involve DNA.

Traditional enzymatic and crystallographic approaches

Like the structure of DNA, the structure of chromatin is repetitive. At its most basic level chromatin comprises repeating units known as nucleosomes. Each nucleosome unit encompasses ~200 base pairs (bp) of DNA, ~150 bp of which is wrapped nearly twice around a spool comprised of 'core' histone proteins (Fig. 1). A 'linker' histone binds to the core and to the other ~50 bp of DNA within each nucleosome and promotes the formation of a coil of nucleosomes into a fiber 30 nm in diameter, but of unknown construction (Fig. 1). This structure, the predominant form of chromatin in the interphase cell nucleus, is compacted further, via obscure intermediates, to attain the recognizable highly-condensed metaphase chromosome.

Careful enzymatic cleavage of the DNA between nucleosomes in native chromatin releases nucleosome core particles for physical study (see Figs 1 and 2). These preparations yield collections of nucleoprotein complexes containing DNAs of somewhat heterogeneous length, reflecting the diverse sequences found in the genome. The heterogeneity of these complexes, together with the large size of a single nucleosome complex (250 kDa) has limited the amount of structural detail that can be

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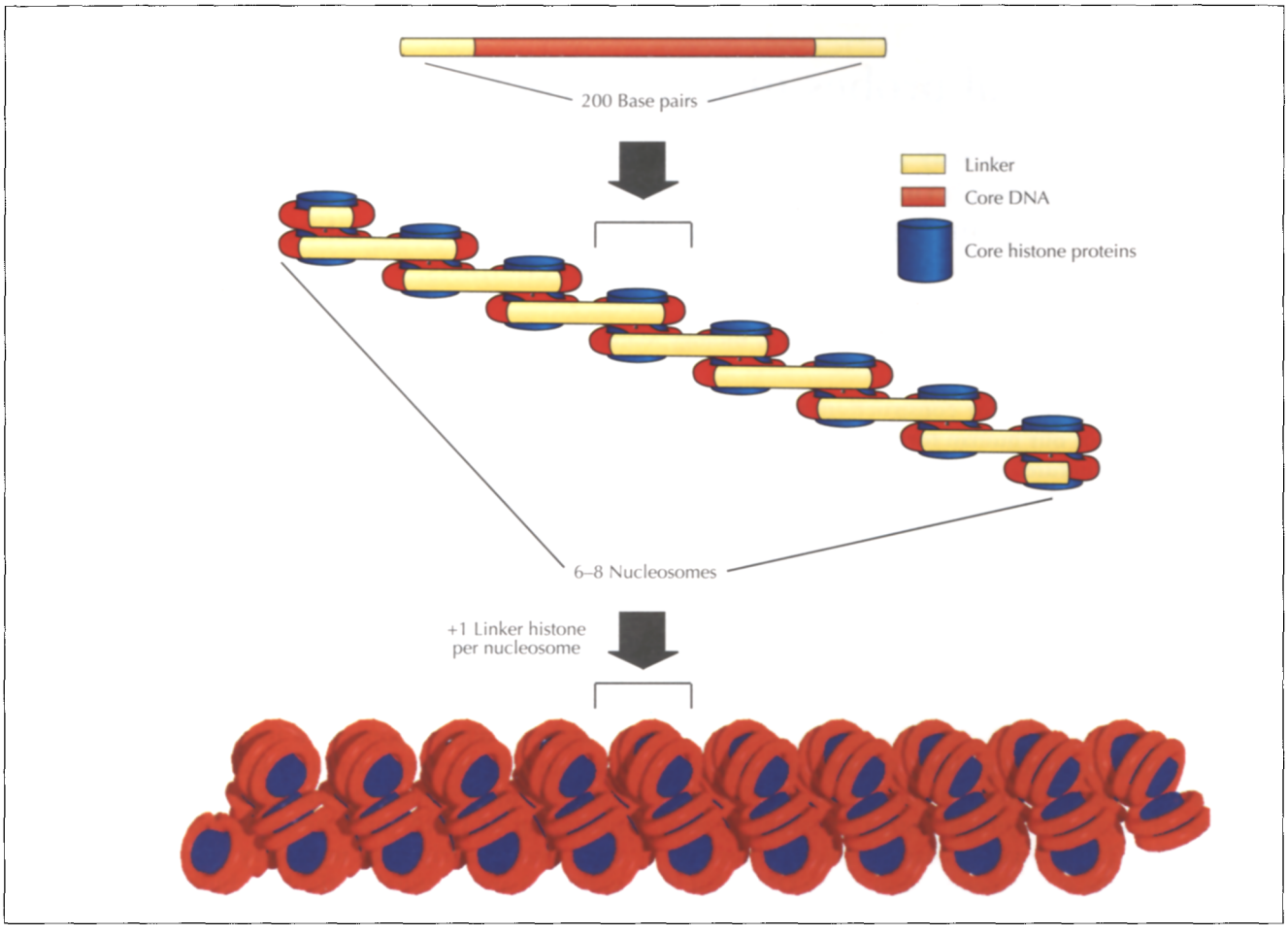


Fig. 1. The first steps of DNA packaging into chromatin. Portions of genomic DNA ~200 bp in length (top) are wrapped about twice around a spool composed of the core histone proteins (blue), forming an extended repeating structure (center). Each repeating unit, upon binding a single molecule of an additional histone protein (linker histone) is formally known as a nucleosome. About 145 base pairs of the DNA (red) in the nucleosomal repeat are in tight contact with core histone proteins and relatively protected from micrococcal nuclease digestion, while the linker DNA (yellow), which connects the nucleosomes, is digested away. The complex remaining after digestion contains the nucleosome core DNA (red) and the core histone proteins (see also Fig. 2) and is known as the nucleosome core particle. The binding of the linker histones promotes further compaction into a structure known as the 30-nm fiber (bottom). The conformation of the linker DNA in this structure is unknown. The approximate degree of relative compaction of the different structures is indicated.

obtained via traditional crystallographic techniques. Still, a crystallographic analysis of random-sequence nucleosome cores (~7 Å resolution) has revealed the basic dimensions of the structure (Fig. 2) and confirmed that the DNA is severely bent into roughly two 80-bp superhelical turns around the outside of a protein core [8–10]. The crystal structure also showed that the bending and rise of the superhelix is not uniform (Fig. 2). The resolution of the data was not sufficient to reveal any additional details of the DNA structure, however. A recent crystallographic analysis of just the histone protein core of the structure, crystallized in the absence of DNA, has revealed many interesting details of the structure of the protein component of this complex [11].

These studies left many questions concerning the structure of DNA in chromatin unanswered. How accessible is the DNA within the nucleosome core and how are contacts to DNA made by histone proteins? How does the conformation of DNA within the core differ from that of classic B-form DNA? In other words, how is the severe bending that must be required of the DNA accommodated within the helix? Is the conformation of DNA in a nucleosome dependent upon the sequence of the DNA, like the conformation of pure, uncomplexed DNA? How much DNA is contacted by core histone proteins? And finally, how is the DNA structure further changed in higher-order chromatin complexes such as the 30-nm fiber? This review focuses on some of the chemical and photochemical-based approaches which have proved instrumental in answering many of the questions concerning the structure of DNA in chromatin. Protein–DNA and protein–protein crosslinking strategies and biophysical analyses of higher-order chromatin structures are not presented here; for excellent reviews on these topics, see [10,12–15].

DNA alkylation measures DNA accessibility

One of the most widely-used chemical reagents for probing protein–DNA interactions *in vivo* and *in vitro* has

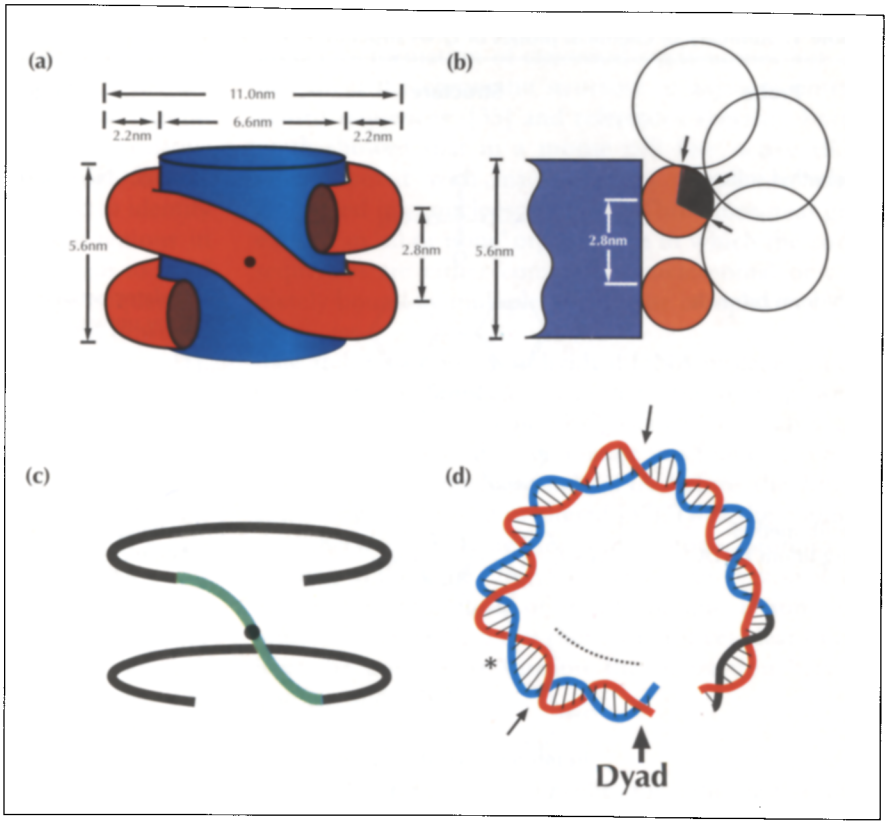


Fig. 2. Details of DNA structure within the nucleosome core particle. **(a)** Schematic of the nucleosome core particle showing relative size of the core DNA and the core histone proteins and overall dimensions of the structure (from [9,11]). Dot in center indicates the position of the dyad symmetry axis, nearly perpendicular to the plane of the page. **(b)** Cut-away view of the nucleosome core particle showing how the histone proteins and the adjacent turns of DNA restrict access to a DNA-binding factor (modeled as an sphere of 30 kDa with density of ~1 g cm^{-3}). **(c)** Ribbon depicting the superhelical path of the DNA in the core particle. The green portion (center) and the exterior portions (black) are found to have different helical periodicities, of about 10.7 and 10.0 base pairs/turn of DNA, respectively (see text and [46]). **(d)** Top view showing only top turn of DNA within the nucleosome core particle. This view shows the degree of curvature of the DNA and the positions of the most severe bends (indicated by arrows). The position of reactivity to singlet oxygen is indicated by the asterisk and the region of altered helical periodicity in the center of the structure is indicated by the dotted line.

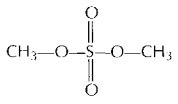
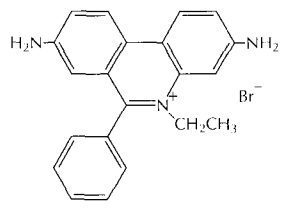
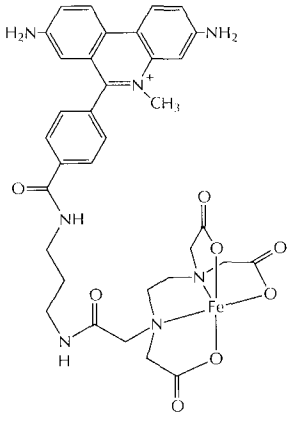
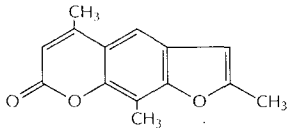
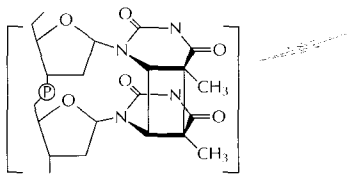
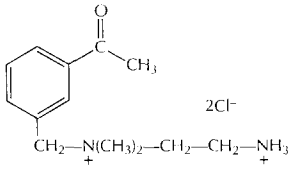
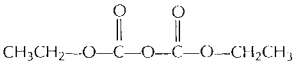
been dimethylsulfate [16] (see Table 1). This reagent efficiently alkylates the N7 position of guanine bases in double-stranded DNA and to a lesser extent the N3 position of adenine bases. This specificity, coupled with the propensity of such modified bases to undergo base-catalyzed elimination, forms the basis of the Maxam–Gilbert sequencing reaction for guanine residues [17]. McGhee and Felsenfeld [18] used dimethylsulfate to show that histone core proteins contact the bases of the DNA in a way which leaves the major or minor grooves very accessible to bulk solvent. They further demonstrated the accessibility of the major groove of DNA in the nucleosome by showing that even glucosylated DNA, which contains a glucose monomer attached to cytosine bases in the major groove, could be accommodated within the structure [19]. This work demonstrated that histone proteins do not significantly contact DNA in either the major or the minor groove, and that the contacts between DNA and histone proteins leave the grooves of DNA in chromatin remarkably accessible to solvent. This conclusion is consistent with other data showing that histone proteins make only about one phosphate contact per helical turn of DNA (per strand), at least in the more peripheral regions of the particle [20].

Intercalating agents probe the conformational flexibility of DNA

Ethidium (see Table 1) is a planar aromatic dye which readily intercalates between the base pairs of DNA [21]. Because of the distortion in DNA caused by intercalation and the restricted motility of DNA in the nucleosome core [22] (and see discussion in [1]), intercalation preferentially occurs in the more-deformable linker regions of chromatin [23]. This has been exploited by Widom and colleagues [24] to demonstrate that there are severe constraints on the amount of 'twist' permitted in the linker between nucleosomes. Low levels of ethidium intercalation were found to cause decondensation of compacted dinucleosome substrates due to a very small change in twist imparted into the linker DNA as a result of the binding. This change amounted to ~1/4 of a helical turn in about five turns of linker DNA or ~5% change. Thus, only a very limited range of twist values may be tolerated in the linker DNA of compacted structures, supporting the notion that the twist of the linker in folded nucleosome arrays must be quantized, that is, constrained to values that differ by integral numbers of turns of the DNA helix [24]. A corollary to this proposal is that chromatin-containing DNA sequences with different inherent twists will require slightly different numbers of base pairs between nucleosomes — perhaps accounting for the observed heterogeneity in nucleosome repeat length [1].

Methylpropidium EDTA (MPE; see Table 1) is an effective non-enzymatic chemical reagent developed by Dervan and colleagues [25] for probing drug–DNA and protein–DNA complexes. One advantage of this reagent over enzymatic probes is that it binds DNA in a relatively non-sequence-specific manner via a methidium moiety which intercalates into the DNA helix. An EDTA–Fe(II) center is attached by a propyl tether and DNA cleavage is accomplished by production of hydroxyl radicals via Fenton chemistry. Subsequent

Table 1. Summary of chemical probes of DNA structure in chromatin.

Probe	Structure	Target/activity	References
Dimethyl sulfate		Methylates N7 of guanine and N3 of adenine.	18
Ethidium bromide		Intercalates between DNA bases; distorts DNA by untwisting and extension of the helix.	1,23,24
Fe (II) methyl-propidium EDTA		Intercalates in flexible 'linker' regions; causes oxidative cleavage of DNA backbone.	25–29
4, 5', 8 Trimethyl-psoralen		Intercalates into DNA; when activated by light, forms interstrand cross-links, predominantly between thymidine bases. Detects linker DNA in chromatin fibers.	31–37
Hydroxyl radical	•OH	Probes bulk solvent accessibility to DNA backbone; minor groove-centered abstraction of a hydrogen atom from the C4' position causes a DNA-strand break.	38,46
UV light		Causes dimerization of contiguous pyrimidines, primarily thymines.	59–63
Acetophenone derivative (N-(*m*-acetyl-benzyl)-N,N-dimethylethylene diammonium dichloride)		Sensitizes DNA to the effects of UV light	63
Singlet oxygen	$^1O_2$	Oxidizes unstacked or partially-unstacked guanines in double-helical DNA.	66–67
Diethyl-pyrocarbonate		Carbethoxylates N7 of guanine and N3 of adenine.	68–70

oxidative degradation of a nearby deoxyribose results in a one-base gap in the DNA backbone (see below).

MPE has been useful in the identification of the regions that are positioned between nucleosome cores in native chromatin. The common enzymatic probe used for this purpose is micrococcal nuclease, which is used to identify the location of the nuclease-sensitive linker regions in chromatin (see Fig. 1). This approach often fails to yield interpretable patterns when applied to unique sequences, especially those containing repeated structures, however, due to the sequence selectivity of the enzyme [1]. Cartwright, Elgin and colleagues [26,27] have used MPE to map the organization of chromatin in several unique sequence loci which had yielded uninterpretable cleavage patterns when probed with micrococcal nuclease. The patterns clearly indicate the presence or lack of a phase nucleosomal repeat on these sequences *in vivo*. MPE also has been effectively used to detect the changes that occur in chromatin structure upon gene activation in heat-shock loci in *Drosophila* and in the mouse β-major globin gene [28,29]. Because of the structural distortion imparted by the binding of this reagent, however, one must be careful about the interpretation of very fine details from the cleavage pattern [30].

Photocrosslinking intercalators probe nucleosome position

Trimethylpsoralen (see Table 1) has been an effective agent for mapping nucleic acid structure because of its ability to crosslink the strands of a double helix when activated by light [31]. The three-ring structure of this natural product intercalates into the DNA helix, perfectly positioning two photoreactive moieties to face opposite strands of the DNA. If the sequence into which the drug is intercalated contains adjacent pyrimidine bases on the two strands, cyclobutane addition products form, crosslinking the DNA. Crosslinking sites for trimethylpsoralen have been used to identify regions of helical structure in folded RNAs and in non-B-form DNAs [31,32].

As expected, the distortion in the DNA that is required to accommodate the intercalation of trimethylpsoralen is disfavored within the nucleosome-core DNA; crosslinking therefore occurs mainly in the linker regions of chromatin. Thus, trimethylpsoralen crosslinking has been incorporated into a clever assay for long-range nucleosome organization on individual DNA molecules [33–35]. Crosslinked sites are detected by partial denaturation of the DNA and direct observation by electron microscopy. Crosslinked linker regions remain double-stranded while intranucleosome core regions appear as single-stranded bubbles.

The disposition of nucleosomes in the face of biological activities such as transcription and replication has been investigated by trimethylpsoralen crosslinking methods. Ribosomal DNA in *Dictyostelium* cells was found to be organized into nucleosomes except for the portion of the DNA containing the ribosomal coding sequence. Thus, the binding of the large numbers of polymerase molecules known to occur on these genes efficiently precludes formation of canonical nucleosomes but does not affect the chromatin structure of adjacent untranscribed sequences ([35] and references therein). Further work showed that in a mouse cell line, active rRNA genes coexist with inactive genes which are wholly assembled into nucleosomes [36]. Thus ribosomal genes exhibit an 'all or none' organization in which the coding sequences are either completely nucleosomal or completely bound by multiple polymerase complexes.

The ability to observe individual DNA molecules being transcribed or replicated is perhaps the most powerful application of the trimethylpsoralen technique. For example, an elongating polymerase was found to directly encroach upon nucleosomal DNA before the histone octamer was displaced or altered [37]. Thus, the competition between polymerases and histone proteins for binding to the same DNA can occur over very small distances. The resolution of this technique is somewhat limited, however, and it is therefore not yet clear exactly how the polymerase disrupts the interaction between histones and DNA.

Radical probes detect fine detail

One of the best chemical probes for defining the structure of DNA in macromolecular assemblies is the hydroxyl radical [38]. Because of the small size and lack of sequence specificity of this diffusible reactive species, very fine details of the conformation of DNA and of protein–DNA contacts can be resolved. These features are reflected in the rates of DNA cleavage at individual nucleotide positions. Cleavage is (probably) effected by the abstraction of a hydrogen atom by a hydroxyl radical from a deoxyribose carbon center in the minor groove of the DNA. This leaves a carbon-centered radical which rapidly disintegrates to leave 5' and 3' monophosphoester termini on either side of a one nucleoside gap. Hydroxyl radicals can be efficiently produced in solution in virtually any salt conditions and in the presence of any soluble protein–DNA assembly via the one-electron reduction of hydrogen peroxide by an Fe(II)EDTA complex to produce •OH and OH^-:

$$[Fe(II)EDTA]^{2-} + H_2O_2 \leftrightarrow \bullet OH + OH^- + [Fe(III)EDTA]^-$$

The oxidation of Fe(II) is readily reversible by mild reducing agents such as sodium ascorbate, thus effectively recycling the iron complex until the peroxide is depleted. Other attributes of the hydroxyl radical, such as its lack of sequence specificity, have been reviewed in [38].

Solution of the linking number paradox

One of the most interesting and sought-after structural parameters describing DNA in the nucleosome was its helical periodicity — the number of base pairs per helical turn (bp/turn) of DNA within the nucleosome. The reason for the intense interest in this parameter was a problem known as the linking number paradox. As first stated by Germond *et al.* [39], the paradox lies in the

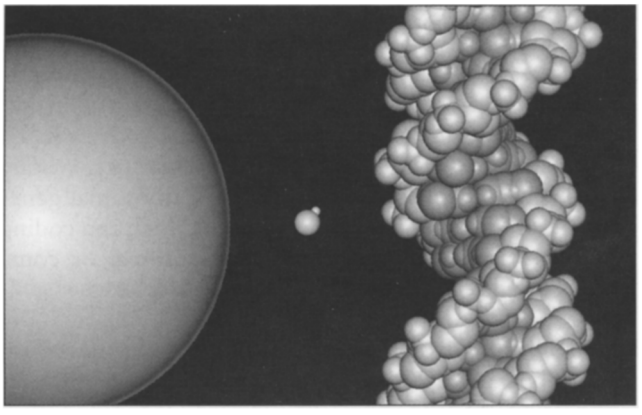


Fig. 3. DNase I is sterically hindered in its approach to DNA in the nucleosome core. Left: DNase I is shown as a sphere approximately to the same scale as DNA (right). When the DNA is complexed with histones, much of its surface becomes inaccessible to DNase I (see Fig. 2b). For comparison, the size of hydroxyl radical is also shown (center). Most of the DNA in a nucleosome core is accessible to hydroxyl radical, with the exception of those sections that are actually in contact with histone proteins.

difference between the observed number of coils of DNA in the nucleosome (two) and the number of topological supercoils of DNA actually taken up by each nucleosome, which should be about the same as the number of coils in the nucleosome but is in fact only one [40]. This paradox could be resolved if the helical twist (related to helical periodicity) of the DNA is changed upon nucleosome formation from its native state by about 0.5 bp/turn (see [41,42]). If this indeed is the explanation for the paradox, the helical periodicity of DNA in the nucleosome should be different from that in solution.

Initial structure-probing experiments with nucleases revealed, as expected, that the helical backbone of the DNA was oriented toward the solvent and accessible to cleavage by nucleases approximately every 10 base pairs, a value clearly dependent upon the helical period [8]. Unfortunately, precise measurements of DNase I cleavage showed that the periodicity of these cleavage sites [43,44] was related to but did not accurately reflect the true helical period of nucleosomal DNA [45]. This was due to the steric hindrance encountered by the relatively-large enzymatic probe in approaching the DNA in the nucleosome core ([45]; Fig. 3).

The hydroxyl radical, being about the size of a water molecule, is not expected to be subject to the steric impediments encountered by the much larger (31 kDa) DNase I (Fig. 3). Cleavage of either unique-sequence DNA reconstituted into a nucleosome core or random-sequence nucleosome cores revealed that on average all nucleosomal DNA has a helical periodicity of 10.19 ± 0.05 bp/turn [46,47]. Hydroxyl-radical analysis can also be used to determine the helical periodicity of DNA in a linear state, while adsorbed onto a calcium phosphate crystal surface [38]. This approach showed that the same DNA used in the above experiments, when not assembled into a nucleosome, has a helical periodicity of ~10.5 bp/turn [46]. Thus this new approach resulted in a direct demonstration that a change in helical periodicity does in fact occur upon nucleosome formation [46].

Detailed structure of the nucleosome core

In addition to the determination of the average helical repeat, hydroxyl-radical analysis clearly revealed that local regions of structure exist within the nucleosome core. Two symmetrically-related exterior regions are found which have a helical periodicity of ~10.0; the central region has a higher helical periodicity (see Fig. 2). This helical discontinuity correlates well with details of the path of the DNA as seen in the crystal structure which shows that the winding of DNA around the histone octamer is not uniform. The rise of the DNA superhelix is relatively flat at either end and makes a very steep 'key ring'-like jog in the center (Fig. 2). These results show that the DNA within the nucleosome core is organized into three parts, like the organization of histones in the nucleosome [48]; as noted above, a linker histone binds to either side of the core histone proteins.

Nuclease-digestion studies indicate that about 145 bp of DNA is in tight association with the histone core proteins ([1], see Fig. 1). The actual range of histone–DNA interactions, as revealed by hydroxyl-radical analysis, is much more extensive than previously detected, however, due to core histone–DNA contacts outside the nucleosome core DNA region, which appear to be weaker than contacts within the core [46]. When these weaker contacts are included, almost the entire nucleosomal repeat (~180 bp of ~200) is associated with histone protein. These weaker peripheral interactions are not biologically irrelevant; they can, for example, block the binding of a sequence-specific transcription factor [49]. A significant fraction of the linker DNA is involved in such contacts. These weaker interactions, together with interactions with linker histones, may be involved in directing the condensation of the nucleosomal array into the 30 nm fiber [50].

The conformation of B-form DNA is clearly dependent upon its sequence content. For example, the A/T-rich sequences tend to adopt a conformation with a narrow minor groove, while in G/C-rich DNA this groove is typically much wider [51]. Further, helical periodicity, base-stacking parameters, and deoxyribose backbone conformation all vary as a function of sequence [21]. Given that nucleosome organization appears to require the same precisely-organized structure regardless of DNA sequence, how are these sequence-dependent structural variations affected by nucleosome assembly? It is possible that intrinsic DNA structure is lost upon incorporation into the nucleosome. It is also possible that the core complex accommodates only some of these intrinsic DNA structures. Indeed, early reports suggested that such intrinsic properties caused certain DNA sequences to be completely refractory to nucleosome formation [2,52].

Recent work has, however, shown that all sequences can in fact be assembled into nucleosomes [53–55]. Further, hydroxyl-radical analysis of assembled complexes has shown that, regardless of the original structure, all DNA sequences are constrained to adopt the same conformation upon nucleosome assembly [56]. Indeed, the amount of change in conformation undergone by a particular DNA sequence, as determined by hydroxyl-radical analysis, correlates with the relative change in free energy ($\Delta\Delta G$) upon histone protein binding [56–58]. These results reinforce 'histone-dominant' models of the nucleosome in which the DNA helix is contorted to make contacts with precisely-positioned DNA-contacting residues on the surface of the histone core [56].

UV light-induced pyrimidine dimer formation

Contiguous pyrimidine bases within DNA can undergo UV light-activated dimerization to form primarily cyclobutane pyrimidine dimers (see Table 1). The yield of dimerized product is affected by the relative position of the two reacting bases, and the appearance of the cyclobutane dimer product can thus be used as a measure of the local conformation of the DNA helix. This has been exploited to probe the structure of nucleosomal DNA both in isolation and within the native structure of the nucleus. Pyrimidine dimer formation within UV-irradiated nucleosome cores is modulated with approximately the periodicity of the DNA [59]. This effect is due to the influence of bent DNA conformation on the quantum yield of photoproducts, rather than direct suppression of dimer formation by histone–DNA contacts through restricted motility or energy-transfer effects [60]. The 10-bp modulation is not uniform, with the highest peaks of pyrimidine dimer formation corresponding to the observed locations of sharpest curvature within the DNA of the nucleosome core crystal structure [9,61].

When nucleosome cores were assembled using DNA containing pre-formed pyrimidine dimers, the dimers were found in a similar, but not identical, pattern of distribution, again tending to be positioned near the locations of sharpest curvature [62]. The probability of DNA containing a pyrimidine dimer being found in different regions of the nucleosome varies in an interesting way. The central three turns of DNA in the nucleosome core are less likely to contain a dimer than are the outer regions. This correlates well with the twist of the DNA; the central three turns are overwound (10.7 bp/turn), and the remaining DNA is slightly underwound (10.0 bp/turn) [46]. These data suggest that pyrimidine dimer formation varies with twist as well as with bending of the DNA helix [60].

Interesting linker-histone-dependent changes in the pyrimidine dimer pattern are observed when the DNA in native chromatin is photosensitized to pyrimidine dimer formation by irradiation in the presence of an acetophenone derivative ([63]; see Table 1) . In the presence of linker histones, the small peak of dimer production due to bending of the DNA across the dyad axis in the center of the nucleosome core is suppressed and two smaller peaks appear just to either side. This result has been interpreted to indicate that the binding of linker histones to the exterior of the nucleosome core changes the structure of the DNA in some way. Previous pyrimidine-dimer experiments [64] and hydroxyl-radical analyses do not detect any changes in DNA structure upon linker–histone association, however. An alternative interpretation is that the binding of linker histones to the nucleosome core somehow suppresses dimer formation for only the centermost bases in the core, so that the peaks that remain are the 'shoulders' of the parent peak. The suppression of dimer production in the center of the nucleosome might occur via direct contact with the linker histone or by allosteric changes in the core proteins upon binding [64].

Higher-order chromatin structures

The pyrimidine-dimer approach has also been used to probe the conformation of DNA in higher-order chromatin structures. Irradiation of intact nuclei was used to show that the structure of the DNA within isolated nucleosome cores is not detectably changed when higher-order native chromatin structures are formed (61). Moreover, this method has provided perhaps the only direct information about the structure of the linker DNA in native chromatin to date — information that is crucially important for deducing the structure of the 30-nm fiber ([63]; Fig. 1). These experiments indicate that the linker DNA in native chromatin is organized differently from nucleosome-core DNA. Indeed, the reactivity of linker DNA in these experiments is suggestive of a non-curved conformation, supporting models which incorporate this feature [15,63]. Overall dimer formation appears to be somewhat suppressed in this region, however, perhaps due to protein–DNA contacts, which may hinder investigation of the structural characteristics of the linker. Further work is needed to resolve this issue.

Singlet oxygen as a probe of base stacking

The hydrophobic interactions that result from flat stacking of the aromatic bases are a major driving force in the self-assembly and maintenance of DNA structure [21]. The stacking also protects the aromatic bases from suprafacial attack (attack at right angles to the plane of the base) by common oxidizing agents such as OsO_4, $KMnO_4$ and singlet oxygen. Such reagents are therefore useful to detect DNA conformations in which the base stacking is opened up, or deformations within the DNA which disrupt this stacking [16]. Some models of DNA in the nucleosome predict that the severe bending is accomplished via stretches of straight DNA interrupted by kinks where base unstacking is expected to occur [65]. However, the crystal structure of the nucleosome core suggests that the bending of the DNA is relatively uniform compared to kinked models [9]. Unfortunately, the resolution of this structure (see above) does not allow a definitive statement to be made concerning the details of base stacking.

Singlet oxygen causes specific, if inefficient, oxidation of guanine residues within B-form DNA [16]. Hogan *et al.*

[66] have used this reagent to address the question of base-stacking in nucleosomal DNA. Previous work by these authors [67] suggested that the base-stacking angle between adjacent bases (about 5° in B-form DNA) is proportional to the accessibility of diffusible reactive species to the base. It turns out that singlet oxygen reacts primarily with only one site within the core, about 1.5 turns of DNA to either side of the dyad symmetry axis. This results strongly suggests that most of the DNA in the core is relatively smoothly deformed, not kinked at 5 or 10 base-pair intervals as has been suggested with the exception of this one site.

Although smoothly deformed, the arc of DNA bending is found to be slightly sharper at about 1 and 4 helical turns away from the dyad axis in the crystal structure [9]. The site of singlet oxygen reactivity, 1.5 turns to either side of the dyad, is near to but does not coincide with the more severe bending site [66]. Interestingly, the singlet oxygen site exactly corresponds to the junction between the regions of DNA that are overwound and those that are underwound (these regions therefore have different helical periodicity) as identified by hydroxyl-radical analysis at about 1.5 turns to either side of the dyad [46,47]. Thus, this single site of unique reactivity indicative of base-pair unstacking may be due to a combination of classical kinking and accessibility resulting from a junction between two regions of different structure.

Diethylpyrocarbonate detects major alterations in DNA conformation

Like dimethylsulfate, diethylpyrocarbonate (DEPC) specifically modifies (carbethoxylates) the N7 position of guanine and the N3 position of adenine. Similarly, the glycosidic bond of the modified base is sensitive to alkaline hydrolysis, leading ultimately to strand breakage at the reactive position. The rate of modification of these major and minor groove positions by DEPC in normal B-form DNA is much less than that found for dimethylsulfate, however [16], probably because of the size of the reagent. DEPC has thus been a useful probe for identifying altered (non-B form) DNA conformations in which these sites are much more exposed to solvent, such as the single-stranded loops found at the end of extruded cruciforms or the left-handed Z-form of DNA [68,69].

DEPC has recently been successfully used to probe DNA structure in nucleosome cores by Fox and colleagues [55,70]. Alkylation of nucleosomal DNA is modulated with a periodicity related to the helical period of the DNA. Interestingly, this reagent detects bases located in the major groove oriented directly away from the histone octamer in the nucleosome core. Thus DEPC yields information complementary to DNase I, which cleaves in the minor groove, and gives a pattern of cleavage which is 'out of phase' with the enzymatically-produced pattern by 180°. This reagent may be useful for probing the interactions of major-groove-binding proteins which associate with the nucleosome core such as linker histones, since the binding of these proteins has been difficult to detect with minor-groove-reactive probes such as DNase I and hydroxyl radical [64].

Future directions

The chemical tools for probing DNA are many and varied, as summarized in Table 1, and the use of these tools has contributed significantly to our understanding of chromatin structure. Many interesting questions still remain to be answered, however. It is still not clear how the severe DNA bending in the core affects the conformation of the individual nucleotides, nor is it yet possible to resolve the many models of how arrays of nucleosomes are folded into the 30-nm fiber. Additional information about the structure of linker-DNA structure in folded chromatin will be necessary for this purpose. The effect of linker histones on the structure of the DNA in chromatin is also still an open question. Finally, it is not yet known how the many known post-translational modifications of histone proteins influence DNA structure; without information on this point, we will not be able to understand how these modifications influence various nuclear processes. Clearly the development of new chemical approaches that can focus on specific regions of DNA within a large structure will be crucial to the resolution of these and other questions of how DNA is organized in chromatin.

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