DNA-HISTONE INTERACTIONS IN NUCLEOSOMES

K. E. Van Holde and J. R. Allen, *Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331*
K. Tatchell, *Department of Genetics, University of Washington, Seattle, Washington 98105*
W. O. Weischet, *Physiologisch-Chemisches Institut, Universitats-Krankenhaus Eppendorf, D2000 Hamburg, Germany*
Dennis Lohr, *Department of Chemistry, Arizona State University, Tempe, Arizona 85281 U.S.A.*

ABSTRACT We have utilized micrococcal nuclease digestion and thermal denaturation studies to investigate the binding of DNA to the histone core of the nucleosome. We conclude that a total of ~168 base pairs (bp) of DNA can interact with the histone core under appropriate solution conditions, even in the absence of lysine-rich histones. The interactions in this total length of DNA can be divided into three classes: (*a*) ~22 bp at the ends is bound only at moderate ionic strength. It is easily displaced, and its removal yields the 146 bp core particle. (*b*) ~46 bp near the ends of the core DNA are quite weakly bound to the core, and are displaced at quite moderate temperatures. (*c*) The remaining central 100 bp are strongly bound, and interact with all of the sites on the histones which strongly protect DNA against DNAse I digestion. A theoretical analysis of the cleavage of nucleosomal DNA by DNAse I has been used to develop evidence that the pattern of protection offered by the histone core is very similar in nuclei to that in isolated core particles.

INTRODUCTION

While the general features of the nucleosome model for chromatin structure have remained largely unchanged since their conception several years ago (1–4), certain details have been the subjects of continual debate. Among these is the question of how many base pairs (bp) of DNA can be bound to each octamer of inner histones. In the original Kornberg model (4), this was taken to be ~200 bp, and to coincide with what was then thought to be the universal chromatin repeat. It was then discovered (5–7) that the repeat length varied greatly from one cell type to another, whereas a limiting monomer DNA size of about 140 bp always seemed to be approached after moderate digestion times. This led to the "core particle plus linker" model for chromatin structure, a model which received strong support when Noll and Kornberg (8) showed that prior removal of lysine-rich histones allowed preparation of core particles with very homogeneous DNA. The size of this DNA has now been determined with considerable precision in a number of laboratories (9–11). The favored value seems to be 146 ± 2 bp. This corresponds to just 14 turns of DNA if the DNA has (as a number of experiments now indicate, 9–12) ~10.4 bp/turn.

Despite the tidiness of this model, there have been a number of lines of experimental evidence to suggest that it is an oversimplification. On the one hand, some experiments seem to show that >146 bp of DNA can be protected from micrococcal nuclease digestion even in the absence of lysine-rich histones (14). On the other hand, it is becoming clear that the way in which the DNA is associated with the histone core is by no means uniform, even over the 146 bp of the "core particle" (15–17). This paper is addressed to a fuller examination of these questions.

EXPERIMENTAL

Most of the experimental techniques used herein have been fully described in earlier publications, and need not be repeated here. Depletion of calf thymus nuclei of H-1, and subsequent micrococcal nuclease digestion is described by Weischet et al. (14). The preparation of chicken erythrocyte nucleosomes and thermal denaturation studies of these particles are as given in Weischet et al. (15) and by Tatchell (18). Finally, DNAse I digestion of both native and reconstituted core particles have been described by Tatchell and Van Holde (19, 20); similar digestions of whole nuclei are as given by Lohr et al. (13, 21).

RESULTS AND DISCUSSION

The Histone Core Can Protect as Many as 168 bp (16 Turns) of DNA from Micrococcal Nuclease

Fig. 1 *A* depicts results of the digestion of H-1 depleted calf thymus nuclei with micrococcal nuclease at moderately high ionic strengths. The limiting DNA size approached under these circumstances is ~168 bp, and there is very little degradation either to the 146 bp core particle, or to subcore fragments. These, and similar experiments, show that the retention of lysine-rich histones is not necessary for the protection of this DNA length. However, a higher salt concentration during the digestion is important; at lower salt (Fig. 1 *B*) digestion readily proceeds to the core and submonomer fragments. A possible explanation for this result is that the interaction of this extra 20-odd base pairs of DNA with the histone core is stabilized only at moderately high (~0.3 M) salt concentrations. We will return to this point in a later section.

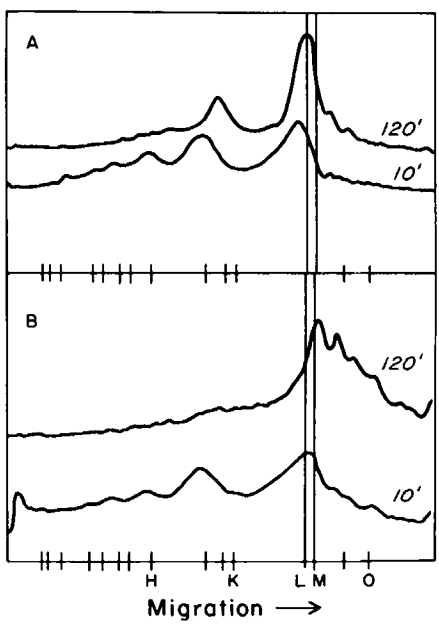

Figure 1 Digestion of H-1 depleted calf thymus nuclei at two different ionic strengths. In the lower panel (B) are shown results of digestions carried out in 0.1 mM Pipes, 1 M hexylene glycol, 1 mM $CaCl_2$, pH 7.0. In the upper panel (*A*), 0.35 M NaCl has been added to the same digestion buffer. DNA samples were extracted and electrophoresed as described in reference 14 and stained with ethidium bromide. The fluorescence of the ethidium bromide was photographed, and the photographs scanned. The marks on the abscissa correspond to positions of Hae III fragments of PM-2 DNA. Fragments designated have sizes: H = 631 bp, K = 273 bp, L = 166 bp, M = 148 bp, O = 100 bp (data from reference 14).

The band in Fig. 1 *A* is certainly not sharp enough for us to cite 168 bp as an exact number. However, we have other evidence (20) that the maximum DNA length that can associate specifically with the histone core lies between 161 and 177 bp. When ^{32}P end-labeled DNA fragments of these lengths were reconstituted with histone cores, only the former (161 bp) gave any indication of a discrete series of bands after DNAse I digestion and autoradiography of the gel. The longer (177 bp) fragment yielded only a smear of sizes, as if the ends "overlapped" the core by significant amounts.

Very recently Noll et al. (22) have also provided evidence for a value of 168 bp by reconstitution and DNAse II digestion studies. It is also important to note that while H-1 is not necessary for the stabilization of as much as 168 bp of DNA, H-1 clearly can associate with and stabilize nucleosomes containing DNA in this size range. Simpson (23) has reported the isolation of stable "chromatosomes" which he finds carry one mole of lysine-rich histone along with the core histone octamer and ~160 bp of DNA. Thus, there are at least two ways in which DNA longer than the traditional "core" length can be protected from micrococcal nuclease: moderate salt concentration or lysine-rich histones. Whether the particles produced by digestion under these two kinds of protection experiments are identical is not known.

Thermal Denaturation Shows that DNA-Histone Interaction is Nonuniform through the Nucleosome

Several years ago we analyzed the thermal denaturation of core particles by a combination of hyperchromicity, heat capacity, and circular dichroism measurements (15). The results are summarized in Fig. 2. The transition is biphasic, even up to 10 mM salt, although the first transition is rapidly shifted upward in temperature as salt concentration is increased (15). The first transition involves structural changes in the DNA alone as judged from circular

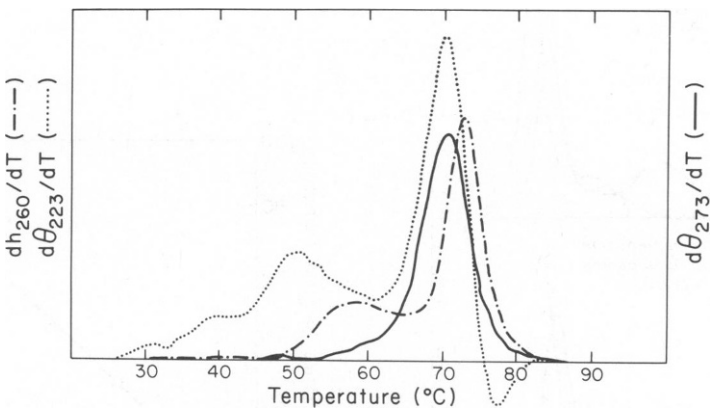

Figure 2 The thermal denaturation of nucleosomal core particles as monitored by derivative curves of hyperchromicity (dh_{260}/dT) and circular dichroism at two wave lengths ($d\theta_{223}/dT$ and $d\theta_{273}/dT$). The hyperchromicity changes reflect DNA melting. The circular dichroism at 273 nm follows conformational changes in the DNA (but not protein), whereas the circular dichroism at 223 nm is mainly sensitive to changes in histone conformation. In brief, these data (taken from reference 15) show that part of the DNA undergoes a conformational change at 40–50°C, and then melts around 60°C (the first transition). This process is not accompanied by significant changes in secondary structure of the proteins. At ~70°C, there is a highly cooperative unfolding of the protein, accompanied by a marked change in DNA conformation. The DNA becomes more like the solution form in this range. At a slightly higher temperature, the remaining DNA melts. The melting point of free DNA in the buffer used here (1 mM cacodylate, pH 7.2) is ~45°C.

TABLE I
ANALYSIS OF NUCLEOSOME THERMAL DENATURATION DATA

	*bp**	TM‡ first transition	TM main transition	Percent first transition	Bp in first transition	Bp in main transition
Core particle	144 ± 5	58	74	29	42	102
Nucleosome	172 ± 16	66	77	22	38	134
Nucleosome	184 ± 40	63	75	29	53	131
Nucleosome (−H1 and H5)	172 ± 16	54	73	41	71	101
Nucleosome (−H1 and H5)	184 ± 40	51	72	46	85	99
Reconstituted 123 bp particle	123 ± 7	—§	74	21	26	97

* ± indicates half width of DNA peak at half height in base pairs.
‡TM refers to temperature at peak of the transition in the derivative curve.
§The first transition in the case of the 123-bp reconstitution was only a shoulder of the main transition. The percent of this transition was calculated by assuming the main transition was symmetric.

dichroism studies. The amount of the DNA melting in the first stage was found to be about 40 bp for the core particle.

Recently Simpson (24) has demonstrated the same transitions in particles reconstituted with poly d(AT), and has shown that the melting of the first 40 bp is from the two ends of the DNA. One of us (18, 20) has now carried out melting studies on nucleosomes containing

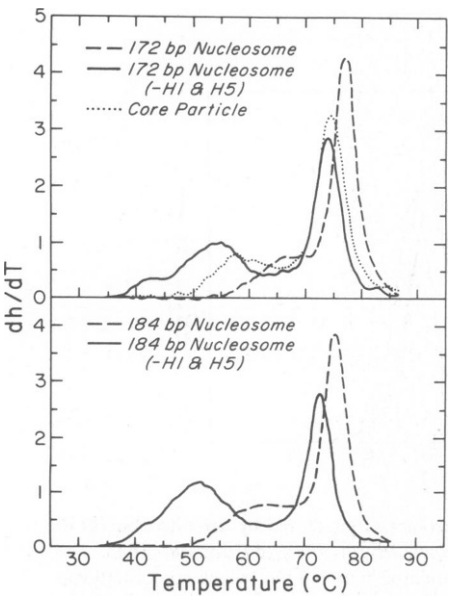

Figure 3

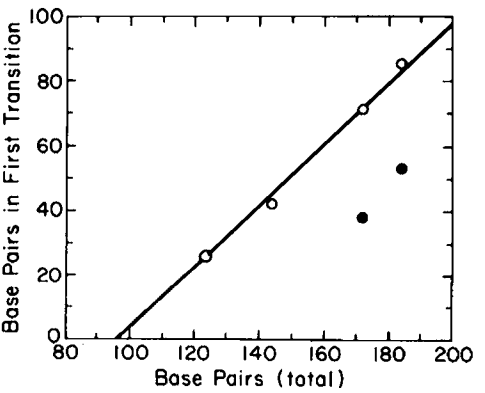

Figure 4

Figure 3 Comparison of the thermal denaturation (hyperchromicity) curves for nucleosomal particles of different types. All data were recorded in 1 mM cacodylate buffer, pH 7.2.
Figure 4 The effect of nucleosomal DNA chain length on the amount of DNA melting in the first transition for nucleosomes without (O) and with (●) lysine-rich histones. The straight line through the open circles shows that it is only that portion of DNA in excess of ~100 bp that melts in the first transition.

either longer or shorter DNA than the core particle. The results are summarized in Table I and Figs. 3 and 4.

These results lead to a number of significant conclusions: (*a*) The main melting transition in particles stripped of lysine-rich histones always corresponds to ~100 bp of DNA, regardless of total DNA length. (*b*) The first transition probably corresponds to DNA at the ends of the nucleosome-wrapped coil, since its amount increases linearly with increase of the total DNA length. This is in agreement with Simpson's definitive result on poly d(AT) particles (24). (*c*) Lysine-rich histones can protect at least part of this DNA from melting in the first transition, and stabilize the rest of the DNA as well.

The latter point requires especially careful consideration. While we do not know the exact content of lysine-rich histones in these particles, gel electrophoresis indicates approximately one such molecule per nucleosome. The curves shown in Fig. 3 indicate that all of the nucleosomes are partially protected from denaturation, rather than some of the nucleosomes being strongly protected, others unprotected. In the latter case, one should observe a very extended first transition, with a very low-temperature transition remaining from those nucleosomes that are wholly unprotected. Rather, we see an upward shift of the whole curve. Analysis of the data in terms of amounts of DNA involved in the two transitions shows (Table I) that ~33 fewer bp of DNA melt in the first transition when lysine-rich histones are present. Note that a significant amount of DNA still melts in the first transition (the amount depending on the total DNA length), but that the temperature of that transition is shifted upwards by the presence of H-1 and H-5, just as is the main transition. Thus, whatever the mode of binding of lysine-rich histones may be, it is such as to (*a*) decrease the amount of DNA melting in the first transition, and (*b*) stabilize the DNA in both regions.

DNAse I Digestion Demonstrates Similarity in Structure of Isolated Nucleosomes and Nucleosomes in Chromatin

The technique of limited DNAse I digestion has been exploited by Simpson and Whitlock (25), Lutter (9) and others (11, 20) to investigate the relative accessibility of different portions of the DNA in isolated core particles. It has been known for some time that DNAse I makes single-strand cuts at intervals of ~10 bases in nucleosomal DNA. By using core particles in which the DNA has been labeled at the 5′ end with ^{32}P, it is possible to determine the relative cutting frequency at any point along the DNA (25). Such analysis leads to the idea that certain regions (notably those at about 3, 6, 8, and 11 turns from the 5′ end) are especially resistant to this enzyme, and presumably protected in some way by interaction with the histone core.

It would be of the greatest interest to extend this kind of analysis to chromatin, and to ask whether the pattern of DNA protection is the same in vivo as in isolated core particles. However, the end-labeling experiment is not possible in this case. One can digest chromatin in intact nuclei with DNAse I, and display the DNA fragments produced by staining with ethidium bromide. However, there is a considerable loss in information in these experiments (as compared to the end-labeling studies) since each fragment size can arise from a number of combinations of cutting sites.

To make at least a start on this problem, we have taken the following approach: We use an algorithm to calculate the expected fragment distribution based on a set of cutting frequencies determined from digestion of end-labeled core particles. This can first be tested by comparing the predicted pattern with that observed following ethidium bromide staining of the gel electrophorogram of a DNAse I digestion of core particles. We shall then ask how well the

relative intensities predicted for nuclear digests (using the same cutting frequencies) compare with observation of such digests. Admittedly, the route is indirect, but any gross discrepancies between the pattern of protection within the nucleus and within the isolated core particle should be detectable.

For purposes of calculation we assume a periodic structure with n equal units in each period. The period corresponds to the chromatin repeat. The term "unit" here is taken to mean that length of polymer that lies between cutting sites. In the case of DNAse I cleavage of chromatin DNA, one unit is ~10 bases. Cutting sites are assumed to be to the immediate left of each unit. For each site (numbered according to its unit) we assign a probability P_k that the site bond has not been cleaved. These probabilities are assumed to be the same in each period. The number of periods is assumed to be very large.

We wish to calculate the weight fraction of fragments which are just j units in length. Furthermore, in some cases, we shall wish to distinguish between three kinds of j-mers: class 1; those which lie entirely within one period; class 2; those which lie across one interperiod boundary; class 3; those which lie across two interperiod boundaries.

We shall neglect higher classes than these, since their frequency will be very low in any cases of interest to us.

We choose a unit at random. The probability that this is the i-th unit of some period is $1/n$. The probability that this unit exists in a j-mer is:

$$P_{ij} = \frac{1}{n}[(1 - P_i)P_{i+1}P_{i+2} \cdots P_{i+j-1}(1 - P_{i+j}) + (1 - P_{i-1})P_iP_{i+1} \cdots P_{i+j-2}(1 - P_{i+j-1}) + \cdots + (1 - P_{i-j+1})P_{i-j+2}P_{i-j+3} \cdots P_i(1 - P_{i+1})]. \quad (1)$$

The various terms in P_{ij} correspond to the different ways a j-mer can include unit i. Now the weight fraction of j-mers equals the probability that any unit picked at random will lie in a j-mer. That is:

$$W_j = \sum_{i=1}^{n} P_{ij}. \quad (2)$$

As a check, we note that if all P_k are the same ($=P$), we get

$$W_j = j(1-P)^2P^{j-1}, \quad (3)$$

which is the well-known result for random cleavage.

It should be noted that it is easy with this analysis to sort out the terms corresponding to the three classes of j-mers described above, since we have the criteria: class 1: $1 - j + 1 > 0$, and $i + j \leq n$, class 2: $i - j + 1 \leq 0$, or $i + j > n$, but not both, class 3: $i - j + 1 \leq 0$, and $i + j > n$.

A simple computer program has been written to compute either the weight fractions of j-mers in each class, or the total weight fraction of j-mers with any assigned set of cutting probabilities.

For preliminary tests of the program, we have first calculated the fragment distribution when all P_k are equal. The result agrees exactly with Eq. 3. Next, we calculated distributions expected for cleavage of core particles using the cutting probabilities experimentally determined by Lutter (9). The cutting of core particle DNA is simulated by setting $n = 14$ and $P_1 = 0$; that is, inserting an obligatory cut at the beginning of each period.

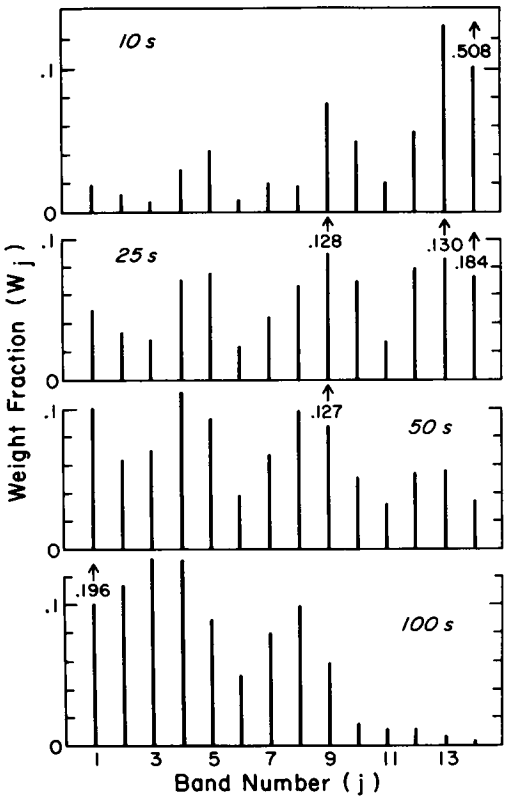

Figure 5 Predicted distributions of single strand fragments at different times of digestion, using the rate constants for cleavage at the various sites as determined by Lutter (16). Note that band 8 becomes the most prominent only after considerable digestion has occurred.

Fig. 5 depicts the distributions predicted at various digestion times under Lutter's conditions, and Fig. 6 compares observed and computed distributions. It is evident that for core particles, at any rate, the fragment distribution can be quite nicely predicted as should be expected. Fig. 5, in addition, shows how the pattern should evolve with continued digestion.

The main question, of course, is whether the distribution of fragment sizes obtained by digesting chromatin can be equally well predicted. A test is more difficult than would appear at first glance. To compare with data on something like chicken erythrocyte or calf thymus chromatin would be very awkward, for in computing the pattern we would have no idea of what cutting probabilities to assign to sites in the rather long spacer regions. Indeed, we do not even know how DNAse I cutting sites are arranged in the spacer regions. Any such calculation would, at this point, involve far too many arbitrary quantities.

Yeast chromatin, with its much shorter spacer, presents a much more tractable model. There is, however, a special complication here, which requires a bit of explanation. It has been recently shown (13) that many of the yeast spacers must be of lengths (10 m ± 5) bp where m is an integer. Thus, the DNAse I digestion pattern of yeast chromatin consists of two overlapping series of bands displaced by 5 bp; one set coming from intranucleosomal cuts, the other from cuts in adjacent nucleosomes.

The computer program described above can sort out these different classes of fragments, and predict the overall pattern, if values for the probabilities P_k are given. As a first trial, we

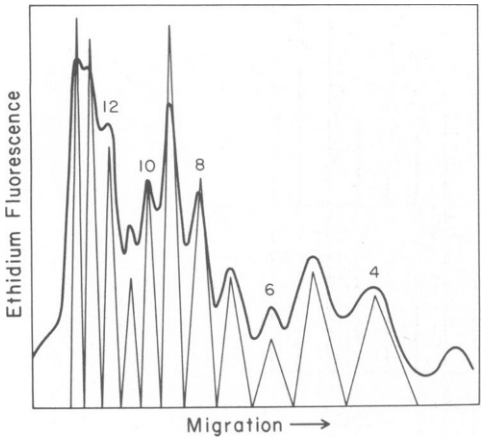

Figure 6

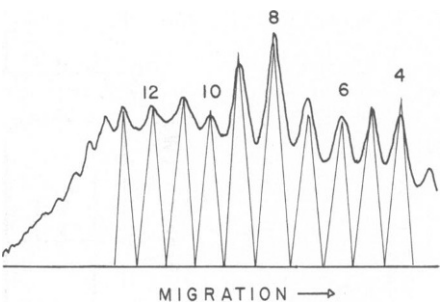

Figure 7

Figure 6 A "best fit" to the ethidium bromide scan of a DNAse I digestion pattern (solid curve) to the relative intensities predicted by Lutter's (16) cutting frequencies (triangles). The widths of the triangles have been set by the observed band spacing; their relative areas are calculated from Eqs. 1 and 2. It should be pointed out that observed fluorescence (as measured by film darkening) may not be exactly proportional to DNA concentration. Band numbers are shown above several peaks. Bands smaller than 4 were not compared, for we find inefficiency in ethidium staining and losses in DNA isolation with small fragments.

Figure 7 Comparison of a scan of an ethidium bromide stained gel of fragments obtained by DNAse I digestion of yeast nuclei (solid line) with intensities predicted by Lutter's (16) cutting frequencies for core particles. The number of residues in the repeat was taken to be 16, corresponding to a repeat length of 168 bp. The cutting rate at site 8 was taken to be $6.6 \times 10^{-3}\ s^{-1}$ (as compared to Lutter's value of $2.3 \times 10^{-3}\ s^{-1}$) and two cuts in linker were assumed, each at a rate of $9.6 \times 10^{-3}\ s^{-1}$. Only the "core" region of the pattern is shown. As in Fig. 6, the smallest bands have not been included.

assign the Lutter frequencies for all internal sites. It is necessary to then decide on a number of linker sites and values for the cutting probabilities at these sites. We have tried numbers of linker sites from 1 to 3, and various values of the cutting probability at linker sites. The best result from this trial and error calculation is shown in Fig. 7. In initial calculations, using the Lutter frequencies, the intensity of band 6 was far too low. Again, trial and error shows that this can be improved by decreasing P_8; that is, increasing the cutting probability at the nucleosome midpoint (see legend to Fig. 7). It may be significant that Altenberger et al. (26) have reported that DNAse II appears to cut more frequently at the nucleosome midpoint in chromatin than in isolated nucleosomes. Furthermore, both these authors and Lohr and Van Holde (13) have observed that DNAse I yields a half-nucleosome repeat pattern with chromatin, but not with core particles. However, the major conclusion is that the cutting probabilities determined by Lutter for rat liver core particles do rather well in prediction of the DNAse I cleavage pattern for yeast nuclei. The important inference from this is that the pattern of protection afforded by the histones is probably quite similar in vivo and in isolated nucleosomes. In turn, this can be taken as evidence for the existence of an in vivo structure rather like that found in the purified particles.

SUMMARY

By combining the results from a number of techniques (thermal denaturation, digestion by various nucleases), it is possible to arrive at a more detailed view of nucleosome structure than

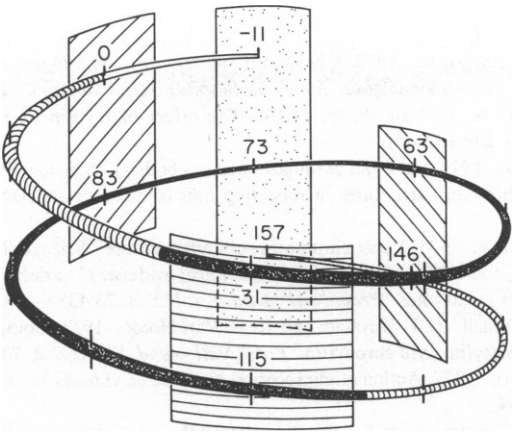

Figure 8 A schematic model of the 168 bp nucleosome. Residue numbering is according to the core particle (0–146) with 11 bp additional DNA shown at each end. The areas shaded by diagonal and horizontal lines indicate regions of strong binding to the protein core, and the weak binding region is denoted by the lightly stippled area. The darker part of the coil corresponds to the 100 bp of DNA deduced to be most strongly bound to the histones. The partially shaded part of the coil is that which is released from the core particle in the first transition in thermal denaturation.

we have had available hitherto. The salient points are depicted in a schematic view in Fig. 8, and can be stated as follows: (*a*) The central 100 bp are, as judged by thermal denaturation, most strongly bound to the histone core. It should be noted that this region includes all of the sites strongly protected against DNAse I digestion. (*b*) The additional 46 bp of DNA incorporated in the core particle are bound to the histone core at room temperature, but relatively easily released in thermal denaturation. From a number of criteria, we judge this DNA to be at the ends of the core DNA. It should be noted that Mirzabekov et al. (17) present data to indicate that about 20 bp of DNA at each 5′ end are not histone bound; the binding in this region seemingly is only to the 3′ end. This could account for the thermal denaturation effects we see. (*c*) Beyond the core DNA, there lie ~22 bp of additional DNA which can, under some circumstances, be associated with the histone core. Ruiz-Carrillo et al. (27) and Noll et al. (22) have recently come to the same conclusion from chromatin reconstitution studies. From our micrococcal nuclease digestion studies, it would appear that this association is most stable at moderate (0.3–0.4 M) salt concentrations. (*d*) As well as can be judged from DNAse I digestion studies, the pattern of protection afforded the DNA by histone association is much the same in whole chromatin as in isolated nucleosomes. There may, however, be a significant decrease in accessibility of the central point in the core DNA when nucleosomes are isolated.

The results described above, together with recent demonstrations of relative "phasing" of nucleosomes (13, 21) suggest that the overall chromatin structure is more finely arranged than many of us first thought. How such arrangement relates to higher order structure is, however, yet to be revealed.

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DISCUSSION

Session Chairman: Victor Bloomfield *Scribe*: Andrew W. Fulmer

BLOOMFIELD: Let us again begin with a question from Don Crothers: "The short time (10 min) digestion data in Fig. 1 *a* indicate a particle with DNA size appreciably larger than 166 bp. Does this mean that a nucleosome can offer at least partial protection to a DNA length greater than 166 bp? What is the upper limit on the size of DNA that can associate with a nucleosome and be partially protected by that association?"

VAN HOLDE: One always observes DNA bands which are larger than 168 base pairs, especially in the early stages of digestion. The point I want to make is that in 0.35 M NaCl, even with very long (~120 min) digestion times, digestion comes to a stop at ~168 bp in a way that it doesn't in the absence of H-1 under low salt conditions. Digestions at lower ionic strength, for the same amount of time, result in extensive degradation of the DNA. Reconstitution experiments (Tatchell and Van Holde, 1979) also support the protection of ~168 bp of DNA by the core histones. Different lengths of DNA were reconstituted with core histones. The DNA ends were then labeled before digestion with DNAse I. A discrete banding pattern of the DNAse I-digested DNA on gels was used as a criterion for a fixed and regular binding position between DNA and core histones. The results indicated that reconstitution with 125, 146 or 161 bp DNA satisfied the above test for specific binding, whereas the 177 bp fragment resulted in a smear of the digested DNA on the gel. This latter fragment was considered to be longer than the length of DNA which can be specifically organized by the core histones. Thus, various possibilities for DNA end location could exist which would tend to smear the DNA gel pattern. This would place a limit of 161–177 bp of DNA which could be specifically organized by the core histones.

BUTLER: How precise is that limit? One should be able to count single bases up to ~160–170 bp on good gels. The fit of your data (Fig. 6) taking the cutting frequencies is not as good as the agreement Lutter and I found using the bandwidths from DNAse I digests. Do you think that you could fit your data better by using the bandwidths of the cutting frequencies? There is a bit of slop at all of these cutting sites. If one uses precise gel techniques, one can accurately determine that width distribution.

VAN HOLDE: Our gel techniques are not that precise; we have not resolved individual bases on the gel.

BINA: Have you tried to digest your chromatin at salt concentrations above 0.40 M to see if the major digestion product returns to the 145 bp core particle?

VAN HOLDE: We have found that digestions around 0.45 M NaCl are the same as those at 0.35 M NaCl. Higher salt concentrations appear to induce sliding of core histones along the DNA.

KALLENBACH: Do these nucleosomes undergo an overall expansion at very low ionic strengths? Is there a conformational change which results in an overall unwinding of the nucleosome? If so, then what do you believe to be the actual unit which is undergoing the thermal transitions in your denaturation studies?

VAN HOLDE: Several people have observed a conformational transition over a range of very low ionic strengths. Reports of the midpoint of this transition have been somewhat variable and range from 1.3 mM (Crothers, et al.) to 0.3 mM (Small et al.). The latter study examined very homogeneous core particles and would put our studies in a comfortable range. I believe that many of these studies have dealt with inhomogeneous preparations of core particles. Extra DNA on the ends of the nucleosomes would generate additional electrostatic repulsion between the turns of DNA superhelix around the core histones. This could lead to conformational transitions at higher ionic strengths or lower temperatures. We feel that preparations of core particles which are truly homogeneous in DNA length undergo this transition below 1 mM ionic strength.

BINA: We (J. M. Sturtevant and A. Stein) have recently crosslinked the histone octamer in the core particle and repeated the calorimetric measurements which Van Holde and coworkers have reported previously. This crosslinking should eliminate the possibility of observing heats of unfolding which correspond to this low salt conformational transition. The total heats of unfolding were the same for crosslinked and uncrosslinked core particles.

VAN HOLDE: Thank you.

DATTAGUPTA: We have measured this salt induced unfolding transition for nucleosomes containing various lengths of DNA. Depending on temperature the midpoint of transition for nucleosome containing 175 bp DNA is ~5 mM, while those containing 145 bp of DNA is at ~1.3 mM.

VAN HOLDE: So you still find the transition at ~1.3 mM?

DATTAGUPTA: Yes.

DEARBORN: What are the effects of polyamines (spermine and spermidine) on the structure of the nucleosome? Do these ligands differ significantly from the metal ions?

VAN HOLDE: We have not looked into this. In general we have preferred to avoid the use of polyamines.

SANDERS: There appear to be at least two major models in the literature for the protection of DNA against cleavage by nucleases: special sites on the surface of the histone core which protect particular sites on the DNA against cuts, and simple geometrical orientational protection with a beating effect due to the non-integral number of base pairs per turn of the double helix. You calculate the distribution of DNA fragment sizes resulting from digestion by assuming certain probabilities of cutting at different cutting sites. Do you see an experimental way of distinguishing between these two models?

VAN HOLDE: I don't see a clear way of distinguishing between these two models now. Certainly the calculations that we did do not contribute to making such a distinction. They are simply based on Lutter's experimentally determined cutting frequencies. Perhaps the kind of experiments that Jim McGhee has been doing will help answer your question.

MCGHEE: We certainly cannot distinguish between these two models, but we can rule out both models in their most strict sense. Our data on the general accessibility of nucleosome DNA to small chemical probes indicates that the DNA is rather largely exposed at the surface of the nucleosome. This would suggest that the DNA is not covered up with large regions of histone. The other model, which was proposed by Trifonov, predicts that if the DNA has a nonintegral number of base pairs per turn then you would observe an interference pattern on the surface of the nucleosome. If this is the case, then the cutting frequency should be a pure function of the DNA and not of the nuclease used to make the cut. Experimentally the periodicity of the cutting pattern varies with the type of nuclease used. Obviously, both models appear to explain part of the data, but neither is strictly true.