NUCLEOSOME STRUCTURE AND CONFORMATIONAL CHANGES

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ABSTRACT We have used a variety of chemical probes to measure the accessibility of DNA on the surface of the nucleosome. We review these results, and describe new experiments which show that T4 phage DNA can form complexes with the core histones, possessing the properties of normal nucleosomes. Since T4 DNA is largely occupied by glucose residues in the major groove, this suggests (as did earlier probe experiments) that the major groove is not filled with histone amino acid side chains. We also report results of recent measurements which appear to show that only a few strong charge interactions are involved in the attachment of the terminal 20 nucleotide pairs at each end of nucleosome core DNA. We speculate on the possible functional significance of the accessibility of DNA revealed by all of these experiments. We have also examined conformational changes induced in nucleosomes at high ionic strength (0.5–0.7M NaCl). The frictional coefficient is found to undergo a small increase in this region, not consistent with models in which the nucleosome is completely unfolded, but possibly reflecting the dissociation of terminal DNA from the nucleosome surface.

The chromatin subunits called nucleosomes are complexes of DNA and histones; their approximate shape and many of their physical properties have been studied, and these results are summarized in a number of recent reviews (1, 2, 3). Although it might be supposed that interactions between a negatively charged polynucleotide and the highly basic histone molecules would severely limit the reactivity of the DNA, the DNA seems in fact surprisingly exposed and reactive. In this paper we discuss evidence concerning the accessibility of DNA within the nucleosome to a variety of reagents. We report also some recent experiments concerning the conformational changes of nucleosomes that appear to provide information about histone-DNA interactions.

ACCESSIBILITY OF DNA

The subject of investigation in all the work described here is the nucleosome core particle, which contains a DNA segment ~145 base pairs in length, wrapped around an octamer of histones. In earlier studies (4, 5), we examined the accessibility of nucleosome DNA to a variety of probes, of both large and small molecular weight. A number of nucleases served as the high molecular weight probes. It is well known (6) that when pancreatic DNAse (DNAse I) is used to digest nucleosomes, the DNA is cleaved preferentially at discrete sites separated by ten nucleotide intervals along the DNA (Fig. 1 *a*). This periodic accessibility presumably arises because the backbone of the duplex is accessible to enzyme only when it faces the solvent, not when it faces the protein surface.

This result might appear to be consistent with models in which only one nucleotide in every ten is exposed, but detailed examination of the cutting sites of a series of nucleases shows that

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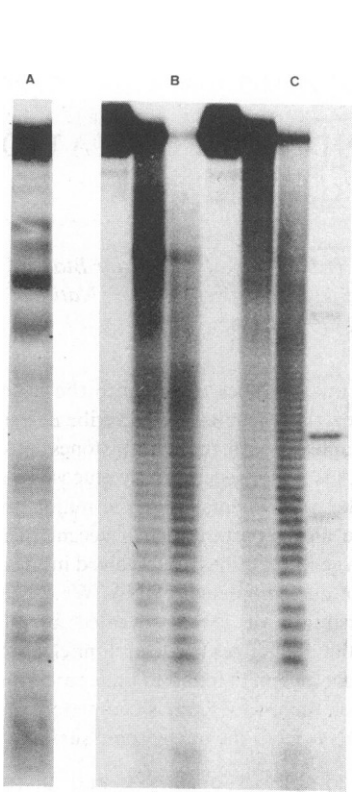

Figure 1

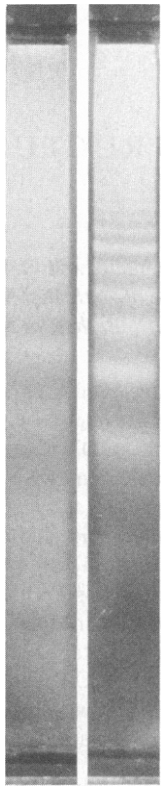

Figure 2

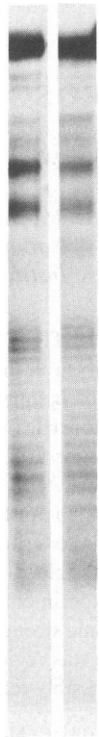

Figure 3

Figure 1 (*a*) DNase I digestion pattern of nucleosome cores. Core particles are 5′-terminally labeled with ^{32}P, digested, and the DNA electrophoresed under denaturing conditions. (*b*) Nucleosome cores, labeled as in *a*, are treated with dimethyl sulfate, the DNA isolated and subjected to chemical treatment inducing strand breakage at the guanine residues. The DNA is electrophoresed and autoradiographed as in *a*. (*c*) Same as *b*, except nucleosome DNA was prepared by deproteinization of nucleosomes and subsequently treated with dimethyl sulfate. From reference 7, which gives experimental details.

Figure 2 DNase I digest of reconstituted histone T4-DNA complex. The complex was formed by mixing core histones and T4 DNA in a ratio of 1:1 and dialyzing against decreasing concentrations of salt. The complex was digested with DNase I. The DNA was extracted and electrophoresed on a 10% polyacrylamide gel under denaturing conditions. The gel was stained with ethidium bromide and photographed. (*left*) Digest of T4 DNA-histone complex. (*right*) Digest of chicken erythrocyte nuclei(control).

Figure 3 Comparison of electrophoretic patterns obtained from native (*left*) and reconstituted (*right*) nucleosome cores 5′-terminally labeled with ^{32}P, and subsequently digested with DNase I. Conditions as in Fig. 1.

although each one has a limited cutting pattern, the actual cutting sites are different for each nuclease (5). Thus, as many as four nucleotides in every ten may be accessible to one nuclease or another. The DNA is therefore much more accessible to these large probes than might have been supposed on the basis of the DNAse I digestion results alone.

We next examined the reaction of the DNA in nucleosome cores with the small chemical probes, dimethyl sulfate, and diethyl sulfate (7). DNA in nucleosome cores was labeled with ^{32}P at the 5′ terminus, then methylated. The sites of methylation are detectable because the methylated bases can be removed chemically, and strand scission induced at those points (8). Electrophoretic analysis of the cleavage products then provides a map of the frequency of methylation as a function of distance from the 5′ terminus. In this way, we were able to show

that the N7 of guanine in the large groove of DNA is as accessible to dimethyl or diethyl sulfate when it is packaged in nucleosomes as when it is free in solution. More surprisingly, there is no detectable modulation of reactivity as a function of position within the nucleosomal DNA, with the exception of a more reactive site ~62 nucleotides from the 5′ end (Fig. 1 (*b*, *c*). This is in marked contrast to the results described above for DNAse I. We have concluded from these experiments that the DNA of the nucleosome is remarkably exposed. In particular, the N7 position of guanine residues of nucleosome DNA are not in close contact with histone side chains in the large groove; with less precision, the same can be said for the N3 of adenine in the small groove. If adenine or guanine are not in special positions relative to the terminus, it seems reasonable to conclude that, to a first approximation, the grooves of the DNA are not filled with amino acid side chains.

To test this idea further, we have attempted to construct a nucleosome core particle containing T4 phage DNA. In this DNA, hydroxymethylcytosine replaces cytosine, and each such base is substituted with a glucose residue. The sugar groups occupy a considerable portion of the large groove, and should certainly interfere with large-scale interactions there. When core histones are reconstituted onto T4 DNA and the resulting complex digested with DNase I, the pattern shown in Fig. 2 is obtained. It is quite similar to the DNAse I pattern obtained with normal DNA, except for the decrease in resolution and slightly altered average mobility that arise from the contribution of the glucosylated bases.

These results suggest that T4 DNA can indeed form nucleosomes. In recent experiments (McGhee and Felsenfeld, unpublished observations) we have isolated core particles containing glucosylated DNA and possessing the hydrodynamic, thermal denaturation, and nuclease digestion properties of normal nucleosome cores, thus confirming this proposition.

These results suggest to us that the reactivity of the bases in nucleosomal DNA is an important feature of nucleosome structure. Their accessibility makes it possible, in principle, for DNA within the eukaryotic nucleus to engage in many reactions even when it is packaged in nucleosomes. (It should be noted that nucleosomes within the nucleus show the same response to nucleases as do purified nucleosomes.) If reactivity of DNA is a biologically important design feature of nucleosomes, it may place severe constraints on histone structure and amino acid sequence.

CHARGE INTERACTIONS AND CONFORMATIONAL CHANGES

The experimental results just described exclude many potential sites of histone interaction on nucleosomal DNA. The most obvious remaining candidates are the phosphodiester groups, particularly those facing toward the surface of the histone core. Again, the simplest arguments would lead us to view the complex in terms of a rather nonspecific interaction between oppositely charged polyions.

A number of experiments suggest that this is not a correct view. We have examined (McGhee and Felsenfeld, manuscript in preparation) the ways in which cloned fragments of DNA of unique, defined sequence bind to the core histones to form nucleosome-like structures. We find that one such fragment, 140 base pairs in length, has a preferred binding position in which the 5′ terminus is displaced inward from the usual terminal position by ~17 nucleotides. A slightly longer fragment does not behave this way. One possible explanation of this behavior is that there is a strong binding site at positions 0 and 145, but not in the region between positions 0 and 17. Tatchell and Van Holde (9) have reported that chicken DNA of nucleosome core size can be reconstituted onto the core histones to form a particle in which the DNA is in perfect "register". We confirm this result. As shown in Fig. 3, when

nucleosomes with 5'-terminally labeled DNA are dissociated and recombined, the DNAse I cutting pattern of the product is almost indistinguishable from that of the native complex, which shows the characteristic position-dependent modulations in cutting intensities. This suggests at once that, in the absence of any sequence-dependent effect, DNA binding sites must be highly localized on the histone octamer surface, since otherwise some "sliding degeneracy" should occur, i.e., there should be a number of structures of roughly equal energy, but different relative positions of DNA and histones. From an examination of reconstituted complexes containing DNA fragments shorter and longer than 145 base pairs in length, Tatchell and Van Holde (9) concluded that there are particularly strong binding sites at positions 0 and 145 which fix the position of the DNA terminus, and therefore of the fragment.

Further information about the histone-DNA interaction can be obtained from a study of conformational changes in the nucleosome. Such changes can be induced by altering, for example, the temperature or ionic strength of the solvent. We consider first the effects of temperature, which are usually monitored by measuring DNA hyperchromism. The thermal denaturation curve of nucleosome DNA is biphasic; the first phase is reversible, does not measurably perturb the histone structure, and involves ~30% of the DNA. A very careful study by Weischet et al. (10) has led to the conclusion that the first step in this process is the release from the nucleosome surface of ~20 base pairs at the end of the core DNA. Thus, the combination of denaturation and reconstitution data suggest that these regions of nucleosome DNA may be bound to histone by only a small number of interactions, located near the ends.

The existence of a reversible dissociation and denaturation of terminal DNA makes it possible for us to ask another question: how many charge interactions are involved in holding down these regions? To obtain an estimate of this number, we have studied (McGhee and Felsenfeld, manuscript in preparation) the ionic strength dependence of the early thermal transition, and compared it to the ionic strength dependence of the denaturation of naked DNA. Given certain simplifying assumptions, the thermodynamic analysis of the data provides an estimate of the number of sodium ions absorbed by the terminal DNA when it is released from the nucleosome surface. We find that a maximum of perhaps three ions are absorbed for each ten base pairs freed. Given further reasonable assumptions about the nature of the DNA counterion atmosphere, we conclude that this is also the approximate number of strong charge interactions holding histone and terminal DNA together. This corresponds to only 15% of all the phosphodiester groups present, and to many fewer interactions than might have been expected. We conclude that in this region at least, there are only a small number of short range electrostatic interactions involved in holding the terminal DNA in place. It may be that this is true of the central part of the nucleosome core as well; it is easy to show that even a limited number of ion pairs provide sufficient free energy to stabilize the folded nucleosome. Our results lead us to speculate that the DNA need be held to the histones by very few interactions of any kind.

NUCLEOSOME CORES AT "HIGH" SALT CONCENTRATIONS

We now turn to hydrodynamic studies of nucleosome conformational changes at high ionic strength. For reasons discussed above, we expect that with increasing monovalent salt concentration oppositely charged macromolecules should complex less strongly, and this is indeed observed in nucleosome cores. Their conformational properties at ionic strengths at and above physiological conditions disclose interesting information with respect to chromatin dynamic behavior. We well know that nonhistone proteins and H1 and H5 histones as well as

the H2A,H2B and H3,H4 complexes can be sequentially removed by raising the ionic strength; selected components can be reconstituted by the reverse process. We now consider that the four core histones presumably form a rather unstable octamer at 2 M NaCl (11, 12); moreover, at reconstitution salt concentrations the core histones most probably present themselves as the separate H2A,H2B dimer and the H3,H4 tetramer. It then becomes clear that the function of the DNA in the reconstitution process is not solely to wrap itself around a preformed histone core, but also to organize the two histone complexes referred to above, into the proper octamer.

In our initial studies we have examined (Fig. 4) the stability of the chicken erythrocyte nucleosome core particle in the range between 0.1 and 0.7 M NaCl. The weight average molecular weight, determined by equilibrium sedimentation, is essentially unchanged over the whole range of salt concentrations. The average of all measurements, $(2.11 \pm 0.05) \times 10^5$, corresponds well to the value 2.05×10^5 calculated for 145 base pairs of DNA and one histone octamer. For the buoyancy parameter ϕ' (13) required for the calculation of the molecular

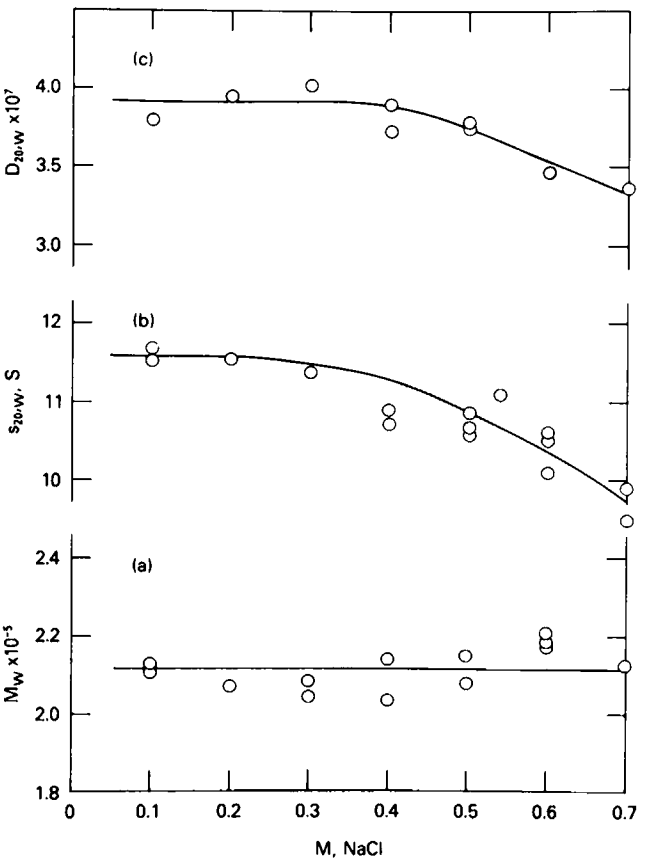

Figure 4 Properties of chicken erythrocyte nucleosome core particles at room temperature, at concentrations of NaCl between 0.1 and 0.7 M NaCl; buffer also contains 1 mM Tris, pH 8.1, 0.1 mM EDTA. (*a*) Equilibrium sedimentation; initial DNA concentration in nucleosome cores, $A_{260} = 0.35$, solution column, 1.5 mm; angular velocity 10,000 rpm; base line determined by boundary depletion at twice the angular velocity. (*b*) sedimentation velocity, 30,000 and 40,000 rpm; $A_{260} = 0.7$. (*c*) Translational diffusion coefficient calculated by combination of velocity and equilibrium sedimentation at identical solvent compositions. For the value of the buoyancy parameter ϕ' we have used 0.646, 0.651, 0.654, 0.656, 0.657, 0.659, 0.660 ml/g between 0.1 and 0.7 M NaCl (in steps of 0.1) calculated as described in the text.

weights, we have used a series of values (see legend to Fig. 4) between 0.1 and 0.7 M NaCl, arrived at by assuming additivity of the values reported for DNA (14) as a function of ionic strength, and a 0.75 ml/g value for the histone octamer (15) independent of ionic strength.

The sedimentation coefficients $s_{20,w}$ are close to 11.6S between 0.1 and 0.3 M NaCl and then decrease smoothly to 9.7S at 0.7 M NaCl. Whereas this clearly indicates a conformational change,it does not resemble the abrupt transitions reported in the low ionic strength regions. With increasing ionic strength we observe an additional minor slow component in the sedimentation pattern ($s_{20,w}$= 5.0–5.2S) above 0.5 M NaCl.Its concentration increases from 5.4% at 0.5 to 6.7% at 0.6, and to 13.5% at 0.7 M NaCl. These observations are consistent with the recent work of Stein (15), who found that at 0.6 M NaCl the nucleosome core, though essentially composed of the canonical 145 base pairs of DNA and an octamer of histones, is in equilibrium with a small amount of free 145-base pair DNA ($s_{20,w} \simeq 5$) and an equivalent amount of nucleosome cores carrying two complements of histone octamers. The dynamic nature of the nucleosome core, as expressed in its ability to complex additional histone octamers without undergoing a major change of conformation, has previously been reported by Voordouw and Eisenberg (16). The internal rearrangement of components as manifested in the appearance of additional boundaries in the sedimentation pattern is not easily discernible in the appearance of the equilibrium sedimentation patterns.

From a combination of sedimentation equilibrium and velocity it is possible to obtain a translational diffusion coefficient, and therefore a frictional coefficient, which is independent of any assumption concerning the value of ϕ' (cf. Fig. 4). $D_{20,w}$ closely parallels the behavior of the sedimentation coefficient $s_{20,w}$.

The complex interaction of nucleosome cores with additional histones will be the subject of a separate contribution (Eisenberg and Felsenfeld, manuscript in preparation). The central issue of interest here is an understanding of the nature of the conformational transition manifested in a 14–15% increase in the frictional coefficient, as monitored by both sedimentation and diffusion, when the ionic strength is increased from 0.1 to 0.7. Dieterich et al. (17) have suggested that at 0.6 M NaCl the single sulfhydryl groups on the two H3 histones in chicken erythrocyte nucleosome cores are separated by a distance of 7 nm. Such large separations might imply dissociation into half nucleosomes, involving heterotypic tetramers connected by a stretch of freely coiling DNA (18). However, our data do not appear to be consistent with this suggestion, since such unfolding would be accompanied by much larger changes in frictional coefficient than the 10% increase we observe in 0.6 M NaCl (see Fig. 4). A major rearrangement of the structure, with an accidental equivalence of frictional properties, also seems unlikely, since we find that DNAse I digestion patterns are not greatly changed at these salt concentrations from those seen in low salt. It seems possible that the fluorescent substituent might affect high salt behavior of nucleosomes, but hydrodynamic studies of such modified structures have yet to be carried out.

Additional support for the idea that dissociation into half nucleosomes does not occur at 0.6 M NaCl comes from a recent result (Gould and Eisenberg, unpublished observations) that nucleosomes crosslinked *in situ* at the closely neighboring H3 sulfhydryl sites (19) exhibit sedimentation behavior at both 0.1 and 0.7 M NaCl identical to the nontreated particles. It is therefore more reasonable to assume that the change in translational frictional coefficients reflects a more subtle change in histone DNA interactions. On the basis of results described earlier in this paper, we suggest as one reasonable possibility that the conformational effect of salt involves an unwrapping of a limited number of base pairs of double helical DNA at the

extremities of the chain, with subsequent increase in frictional resistance, somewhat akin to the premelting transition observed at low ionic strength.

It is our hope that these studies will tell us something both about the nature of the interactions between DNA and histones, and about the conformational changes which nucleosomes can undergo in the course of their biological activity.

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DISCUSSION

Session Chairman: Victor Bloomfield *Scribe*: Nanibhushan Dattagupta

BLOOMFIELD: We'll start with a written question from Don Crothers.

CROTHERS: "In your paper you state that only ~3 charge interactions per 10 bp stabilize the terminal ~20 bp on the core particle. Consider, however, that the DNA in the terminal regions overlaps the DNA in the single complete

turn around the core. Hence one can expect both electrostatic repulsion and electrostatic attraction to affect the terminal regions. At low enough salt concentration, the repulsion term is presumably responsible for the observed low-salt expansion of the particle. The number 3 is, I assume, the net difference in ion binding per 10 terminal bp by the intact nucleosome and the putative structure in which the terminal sections have become disengaged. (No data are given to support this interpretation.) The ion binding of the folded form should be enhanced by having two duplexes adjacent to each other, whereas the ion binding of the unfolded form should be increased by the loss of basic amino acid interactions. I don't see how one can interpret this process simply in terms of breaking electrostatic interactions between histones and DNA."

MCGHEE: Relating the number of sodium ions released in the melting to the number of histone-DNA interactions disrupted, one gets an estimate of only 1.5 such interactions per 10 bp of terminal DNA. As Dr. Crothers points out, on the core particle surface the terminal 27 bp must be closely juxtaposed to the DNA duplex on the next turn of the core particle DNA. This would lead to an increased local charge density and hence to an increased Na^+ binding solely because of the DNA conformation. This would further complicate the relation between the number of Na^+ ions released and the number of interactions disrupted.

To calculate the maximum effect which this complication might be expected to produce, we considered all $4 \times 27 - 108$ negative charges to be uniformly arranged on a chord joining the ends of the 27 bp terminal region, and calculated the resulting increase in condensed Na^+ ions. The final result, which should be an overestimate, is an estimated ~4 interaction per 10 bp. The number given in the text is the average, ~3 interactions per 10 bp.

YAGIL: We have been studying the interaction of intact chromatin, as well as of nucleosomes, with a second alkyating reagent, nitrogen mustard $ClCH_2CH_2(CH_3)CH_2CH_2Cl$, which is a well known bifunctional reagent for DNA (A. Yerushalmi and G. Yagil, 1980, *Europ. J. Biochem.* **45**:211–221). We have followed quantitatively the course of the mono-functional substitution of both proteins and DNA parts of chromatin (substitution is primarily on N7 as with dimethylsulfate). Unexpectedly, the extent of DNA substitution when in intact chromatin was found to be several-fold higher than of DNA free in solution. The substitution of DNA in chromatin leveled off when nearly 5% of the base pairs (2% in nucleosomes) were substituted. This raises the possibility that the bases of DNA are in a different, more reactive conformational state when DNA is wrapped around the histone core. The fraction of the nucleotide, which we situated in a reactive position towards the mustard reagent, is limited to approximately one per turn of the double helix. We don't know whether this is because the mustard is a larger reagent than dimethylsulfate or because of a difference in conditions of reaction and analysis. Could you tell whether you have reached with DMS the level of reaction at which substitution with nitrogen mustard levels off?

MCGHEE: The extent of reaction with dimethylsulfate ranged from less than one per nucleosome up to ~10/nucleosome. We saw no evidence for protection, under conditions where we should have detected protection, of 1 bp out of 10. We do not find what you see with nitrogen mustard.

KUNTZ: I am a little confused about this picture of half empty particles, and I would like to hear something more about it.

H. EISENBERG: We are not surprised that nucleosomes, which are functional particles, have empty spaces, filled with solvent or maybe with other biological components in the functional state. I was recently told that a similar situation may apply in the case of ribosomes. From dimensions given by neutron scattering and x-ray diffraction data in the literature we calculate 524 nm^3 for the total volume of the particle. The frictional coefficient determined by us agrees rather well with the size and shape of the particle as determined by Klug and his colleagues from x-ray diffraction. From neutron scattering or straightforward calculation the "dry" volume of the particle is only ~220 nm^3. Bradbury and his colleagues find ~60 nm^3 occupied by water of hydration, determined by contrast matching with glycerol in neutron scattering. We have confirmed this by measurements with sucrose in the ultracentrifuge . A fairly large amount of space has therefore still to be accounted for. We hope that experiments now in progress in our laboratory will provide a clue to the resolution of this problem.

BUTLER: The nucleosome x-ray map is still at low resolution. But there is space inside.

SUBIRANA: An empty space in the nucleosome can be seen by x-ray diffraction. Upon dehydration of the sample you preserve the structure up to a stage where the volume of the nucleosome would be ~450 nm^3. When you dehydrate more, there is a reversible x-ray pattern change. The shape of the particle changes considerably at this point so there is certainly void space.

REVZIN: I would like to direct a question to Jim McGhee. There is strong binding sites at 0 and 145 bp and I was wondering whether an end effect of DNA plays any role.

MCGHEE: I don't know whether this should be addressed to me or to Ken Van Holde. Our ion release measurement is an average property so we can get so many ions released per 27 bp of denatured DNA. We have no way *a priori* to decide whether all those are bound as one big bundle or whether every phosphate is 15% neutralized.

If you take a specific restriction fragment and try to reconstitute nucleosomes, depending on the length of the fragment and its exact property, you can get specific reconstitution. This would argue, in the absence of sequence dependent effect, that the binding sites are really quite localized. You assemble in such a way to maximize these very strong interactions—exactly the way Ken Van Holde described for his reconstitution.

MARTIN: Does this void space observed in the particle occur in the cell or it is an artifact of preparation?

MCGHEE: We don't know whether within the cell there is something sticking in the hole.

BINA: Can you speculate on where the excess octamer might be bound to the nucleosome?

H. EISENBERG: No, but we will know eventually.

KALLENBACH: In your melting experiment you determine the slope of Tm for the lower transition. What does that measurement say about the screening that is going on in the remainder of the core?

MCGHEE: The screening would cancel out, if I understood your question.

KALLENBACH: You are saying that the ion release you measure must be less than or equal to the number that you would need to affect the interior of the particle; that is, the ionic interaction must be stronger.

MCGHEE: We are only looking at 35% of the DNA at the end of the core particle. We would suggest that the same density of interactions could potentially be representative of the entire core particle. We can calculate the actual free energy provided by such a limited number of interactions, and it would seem to be ample to stabilize the overall core particle.

KALLENBACH: What about the slope for the Tm of the second transition, the major transition as a function of ionic strength? What does that correspond to?

MCGHEE: The reason we did not look at the upper melting phase is that it is irreversible; the sample starts to aggregate.

KALLENBACH: The whole thing seems to hinge on whether this really is a valid bound, and what the electrostatic interactions are in the interior.

MCGHEE: I quite agree. Again our point throughout is simply to put an upper limit on the number of interactions.

HARRINGTON: My poster corroborates many of the points raised in the talk. We have condensed T4 phage DNA with polylysine into a highly condensed structure. This surprised me, and I was prepared to argue on quite rational grounds about the very existence of this sort of thing. I was gratified to see that other people have seen the corresponding phenomenon.

We also looked at the nucleosome at high salt concentrations using intrinsic viscosity and streaming birefringence measurements based on rotational dynamics. The intrinsic viscosity data are roughly comparable to the 15% decrease in sedimentation coefficient that Henryk Eisenberg reported. If you assume something like 15% increase in Scheraga Mandelkern β going from 0.1 M salt to 0.6 M salt. I'd like to ask Dr. Eisenberg whether the change of f/f_0 can be interpreted as being due to some kind of structural or conformational change.

H. EISENBERG: At 0.6 M NaCl we find only a ~10% decrease in $s_{20,w}$ (as compared to 0.1 M NaCl) which corresponds to at most 30–40% increase in intrinsic viscosity. There thus appears to be some discrepancy with the data of Rodney Harrington which may be due to the preparation of the samples. We calculated all kinds of possibilities and don't believe there is considerable unfolding. This is supported by the crosslinking experiments in collaboration with Hanna Gould, referred to in the text. There occurs no doubt a conformational change. We hope to be able to define it better by small angle x-ray scattering experiments now in progress. It appears though that up to 0.7 M NaCl no considerable unfolding occurs.

The work on the flexibility of DNA referred to was undertaken at the Weizmann Institute by Nia Borochov and Tsvi Kam. DNA flexibility is an old problem in the literature which can now be settled with the availability of homogeneous DNA samples and laser light scattering, allowing precise measurements to low angles of scattering. We

have determined the radii of gyration, *Rg* and the thermodynamic nonideality (second virial coefficients, A_2) for $ColE_1$ DNA ($M = 4.35 \times 10^6$) over the range 4 M down to 5 mM NaCl. Application of standard excluded volume theory to our light scattering data yields a flexibility of DNA that is higher than previously believed, fairly constant at high salt, decreasing rather mildly at low salt concentrations.

These findings may be relevant to the folding of DNA in compact biological structures.

LIPPARD: We studied the binding of another small reagent with nucleosome core particle, *viz* the antitumor drug *cis*-dichlorodiammineplatinum II. Indeed, as with dimethylsulfate, the reaction with the nucleosome core particle was more or less the same as that with free core particle DNA. You can ultimately load up to 30–40 platinum atoms per core particle after a long-time incubation.

I am curious about the case of dimethylsulfate or any such reaction which involves small molecules binding to nucleosomal DNA. How do you know at the end of the experiment that the core particle has a structure that resembles what it had at the beginning? It is also known that DNA can slip relative to the octamer. How do you know you are not unravelling the structure at the end, for example?

MCGHEE: After reaction, we isolate the 11S particle on a sucrose gradient. This gives at least a gross measure of conformational integrity. What I have done at the same time is to add DNAse I into the reaction mixture containing dimethylsulfate. We still observed the 10 bp repeat. Every way we looked at it, the core particle seemed to be perfectly happy with 5–10 methyl groups on the DNA.