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Supercoiling energy and nucleosome formation: the role of the arginine-rich histone kernel*†

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ABSTRACT

We have formed complexes of relaxed closed circular Col E1 DNA with various combinations of histones, and examined the effects of treating the complexes with nicking-closing enzyme. Germond et al (1) have shown that when a mixture of the four core histones of the nucleosome (H2A, H2B, H3 and H4) is used in such an experiment, the subsequently isolated DNA is supercoiled. We find that the arginine-rich histone pair, H3 and H4, is sufficient to induce the supercoiling observed in this experiment. Both H3 and H4 are required, and in the absence of either, no other histones are effective. H3 and H4 are as efficient, per unit weight, as a mixture of the four histones in inducing supercoils.

We also show that there is a large difference between the DNA bending energy needed to form a nucleosome and that needed to form one turn of normal superhelical DNA. These two processes are energetically quite distinct and probably separable. We estimate the free energy of interaction between DNA-bound histone pairs, and find that one or two such interactions would generate enough energy to fold the DNA into a nucleosome.

INTRODUCTION

We have recently shown (2,3) that the arginine-rich histone pair, H3 and H4, plays a central role in the organization of the nucleosome. Reconstituted DNA-histone complexes containing the H3-H4 pair behave very much like native chromatin with respect to many chemical probes. Such complexes, when treated with staphylococcal nuclease, DNAase I, and DNAase II, give rise to DNA fragments of discrete subnucleosome size similar to those produced when chromatin is digested. The histones of these complexes are also relatively resistant to attack by trypsin and chymotrypsin. All of these effects depend upon the presence of both H3 and H4; all complexes containing both histones behave like chromatin, while all those complexes lacking either H3 or H4 do not.

The four "core" histones of the nucleosome (H2A, H2B, H3 and H4) distort the local conformation of DNA to which they are bound. Germond et al. (1) have shown that when an equimolar mixture of these histones is reconstituted with relaxed closed circular DNA, and the complex treated

with nicking-closing enzyme, the subsequently isolated DNA is supercoiled, i.e. its topological winding number has been changed. In this paper, we repeat the experiment of Germond et al. (1) using only H3 and H4, and show that the arginine-rich histone pair is capable of producing the same effect as an equal weight of the full complement of histones. All combinations of histones lacking either H3 or H4 have no detectable effect in this supercoiling assay. Our results once again support the idea that H3 and H4 are of central importance in nucleosome organization.

In the course of this work, we have also examined the possible relationship between the conformation of naturally supercoiled DNA and the conformation of DNA in the nucleosome. Although it has been assumed that native supercoiled DNA (such as that isolated from the SV40 minichromosome) is in a state suitable for the ready formation of nucleosomes when histones are added, that is not the case. Calculations suggest that the equilibrium conformation of supercoiled DNA is quite different from that required for a reasonable nucleosome model, and that the formation of nucleosomes from supercoiled DNA requires a large amount of additional free energy. The estimated interaction free energy of one or two pairs of histones within the nucleosome appears sufficient to provide the necessary driving energy for this folding of the DNA.

MATERIALS AND METHODS

Preparation of Col E1 DNA, Histones and Histone-DNA Complexes. Col E1 plasmid DNA was prepared from E. coli strain A745 met thy (Col E1) according to slight modifications of previously published procedures(4,5). Relaxed closed-circular Col E1 DNA was obtained by treating 1 mg of supercoiled DNA in 6 ml of buffer A (200 mM NaCl, 20 mM Tris·HCl (pH 8.0), 0.25 mM EDTA, 5% (v/v) glycerol) with 1000 to 1500 units of nicking closing extract (NCE, see below) at 37°C for 30 min. After the addition of 1% sodium dodecylsulfate, the DNA was purified by ethidium bromide-CsCl density equilibrium centrifugation. The relaxed closed circular DNA was collected from the gradients, the ethidium bromide removed by extraction with n-butanol, the sample was dialyzed against 50 mM NaCl, 10 mM Tris·HCl (pH 8.0) and 1 mM EDTA, and the DNA purified further by phenol-chloroform-isoamyl alcohol (48:24:1, v/v) extraction and ethanol precipitation. All DNA samples were resuspended and stored in 50 mM NaCl, 10 mM Tris·HCl (pH 8.0), and 1 mM EDTA at 4°C.

Total histones were extracted with 0.4 N H_2SO_4 from chromatin prepared from duck erythrocytes (2). Histones H2A, H2B and H4 were purified from

stock solutions of total histones according to previously published modifications (2) of the method of Van der Westhuyzen et al. (6). Purified H3 was obtained by some modifications of the procedure of Ruiz-Carrillo and Allfrey (7) to be published elsewhere (Camerini-Otero, Simon and Felsenfeld, manuscript in preparation). The four histones H2A, H2B, H3 and H4 and the pairs of slightly lysine-rich histones (H2A/H2B) and arginine-rich histones (H3/H4) were prepared by the following sequential "stripping" procedure. First, the lysine-rich histones (H1 and H5) were dissociated from chromatin by dialyzing chromatin against 0.7 M NaCl, 5 mM Tris·HCl (pH 8.0), 0.5 mM EDTA at 4°C and removing the "stripped" product by exclusion column chromatography on Bio-Gel A-5M (Bio-Rad). Part of this product was extracted with 0.4 N H_2SO_4 at 4°C to yield the four histones H2A/H2B/H3/H4. The slightly lysine-rich histones were then obtained by dissociating the remainder of the 0.7 M NaCl "stripped" product in 1.25 M NaCl, 5 mM Tris·HCl (pH 8.0), 0.5 mM EDTA at 4°C and again separating the products by exclusion column chromatography. The arginine-rich histones left on the DNA were then extracted with 0.4 N H_2SO_4 at 4°C. Further details of this purification procedure will be published elsewhere (Simon, Camerini-Otero and Felsenfeld, manuscript in preparation). The purity of all the histones was routinely monitored by electrophoresis on 15% polyacrylamide sodium dodecylsulfate stacking gels according to a method described previously (2). The individually purified histones had <1% impurities, with the exception of H2A, which had <5% contamination with nonhistone proteins. The histone pairs had <5% and <1% impurities for the (H2A/H2B) and (H3/H4) pairs respectively.

Histone-DNA complexes (reconstitutes) were prepared by mixing histones and Col E1 DNA in the presence of 50 mM NaCl, 50 mM 2-mercaptoethanol, 10 mM Tris·HCl (pH 8.0) and 1 mM EDTA; usually the DNA concentration was 50 µg/ml, and unless otherwise specified, 0.25 gm of each histone was added per gram of DNA. Histone concentrations were determined as described previously (2). These solutions were dialyzed for 2 to 12 hr at 4°C against 5 M urea, 2 M NaCl, 5 mM Tris·HCl (pH 8.0), 0.5 mM EDTA, and then subjected to step gradient dialysis. The steps were as described previously (2) except that all the dialysis steps included 5 mM Tris·HCl (pH 8.0), 0.5 mM EDTA. At the end of this reconstitution the protein to DNA ratio was exactly equal to the input ratio (8). Furthermore none of the histones was lost selectively and all of the protein was bound to the DNA. Binding of the histones was measured using an assay that detects free histone after

the histone-DNA complex has been bound to DEAE-cellulose (2).

For some experiments an alternate reconstitution procedure was used: Histones and Col E1 DNA were mixed in the same solvent and at the same concentrations as above, and then dialyzed for 2 to 12 hr at 4°C against 2 M NaCl, 5 mM Tris·HCl (pH 8.0), 0.5 mM EDTA. After 90 min the samples were then dialyzed against 5 mM Tris·HCl (pH 8.0), 0.5 mM EDTA.

Sequential addition of histones and staphylococcal nuclease digestion of reconstitutes. For these experiments histone-DNA complexes were prepared by the reconstitution procedure described above with the following exceptions: 1) Duck DNA was used (prepared as described previously (2)) and 2) the concentration of the DNA was approximately 250 µg/ml. Other histones were added to these reconstituted complexes in the following manner. At the end of the reconstitution procedure the solvent composition was 5 mM Tris·HCl (pH 8.0), 0.5 mM EDTA; to the complexes, dissolved in this solvent, an equal volume of histones in the same solvent was added dropwise at room temperature with continuous stirring. A volume of approximately 3 ml was added over a period of 3 min. In one experiment shown below (Figure 1e) histones were added dropwise to protein-free DNA. These final complexes were then digested with staphylococcal nuclease, the DNA purified, and the samples examined on 6% polyacrylamide gels as described previously (2).

Preparation of Nicking-Closing Extract. Nicking-closing extracts (NCE) were prepared from both duck erythrocyte and reticulocyte nuclei. Reticulocytes were obtained from the blood of ducks treated with 1-acetyl-2-phenylhydrazine according to a schedule described elsewhere (Zasloff and Felsenfeld, manuscript in preparation). Fresh cells were washed four times with PBS buffer by centrifugation and resuspension; they were then resuspended in 0.25 M sucrose, 10 mM Tris·HCl (pH 8.0), and 1 mM magnesium acetate and washed twice. The cells were washed five times in the same sucrose solution and 0.5% (v/v) Triton X-100. The resulting nuclei were then washed an additional three times in the absence of Triton. The nuclei were swollen by washing them in 10 mM Tris·HCl (pH 8.0), 0.5 mM EDTA. In all cases, a Dounce homogenizer (loose pestle) was used for resuspension. The nuclei, swollen to about five to ten times their original volume, were then mixed with an equal volume of 300 mM phosphate buffer (pH 7.5), homogenized with a Dounce homogenizer and centrifuged at 25,000 xg for 15 min. The supernatant is the nicking-closing extract.

The activity of the nicking-closing extracts was monitored by measuring

the conversion of supercoiled Col E1 DNA to the relaxed closed-circular form as demonstrated by agarose gel electrophoresis (see below). If a unit of relaxing activity is defined as the amount required to completely relax 1 µg of supercoiled Col E1 DNA in 30 min at 37°C in buffer A (see above), the specific activities of different extracts varied from 250 to 1200 units per mg of extract protein (8). Extracts from reticulocytes uniformly gave the higher specific activities. The extracts were all monitored for the presence of histones as demonstrated by SDS-gel electrophoresis (see above): the extracts contained less than 2 µg of each histone per mg of extract protein.

These extracts are easy to prepare, have activities comparable to those of other cells (9-13), do not require divalent ions, and are also remarkably free of proteases and nucleases as shown by the following experiments. Free histones in water, chromatin, or reconstitutes were incubated for 1 h at 37°C with 10 µg of extract protein per µg of total histone, and after the incubation the histones were examined by SDS gel electrophoresis (see above). Under these conditions there was neither any detectable diminution in the amount of protein in the histone bands nor any evidence of histone degradation products. The absence of appreciable nuclease activity was established by carrying out the unwinding reactions in the absence of EDTA and in the presence of 1 mM magnesium and/or calcium ions; under these conditions none of the supercoiled DNA was converted to either the nicked or linear forms of Col E1 DNA.

Conditions for nicking and closing DNA and agarose gel electrophoresis. Three to 5 µl of NCE containing approximately 3 to 5 µg of extract protein (3 to 5 units) were added to 200 to 400 ng of DNA (either free or complexed with histones) in 20 µl of buffer A. After 30 min. at 37°C the reaction was stopped with sodium dodecylsulfate (1%). Five µl of a mixture containing 50% sucrose, 0.02% bromphenol blue, and 1 mM EDTA (pH 7.0) were added to each sample and the samples were incubated at 45°C for 15 min; the samples were then loaded onto an agarose gel. Up to 12 samples at a time were electrophoresed in a 6 X 120 X 160 mm slab (E-C Apparatus Corp., Model 474) of 1.0% agarose (Miles Co.) with the following buffer: 40 mM Tris base, 30 mM NaH_2PO_4, 1 mM EDTA (pH 7.9). Electrophoresis was performed at 18-20°C for 12 to 14 hr at 3 V/cm. After electrophoresis the gels were stained in the dark for 4 to 6 hr in electrophoresis buffer containing 1 µg/ml of ethidium bromide, and destained for 2 to 4 hr in electrophoresis buffer or water. The slabs were photographed using two

short wave ultraviolet lamps (Type S-68, Ultraviolet Products, Inc.) and Polaroid Type 105 film, with a Wratten 23A filter over the camera lens.

RESULTS

Sequential additions of histones. We have shown that digestion within the nucleosome by staphylococcal nuclease gives rise to a large array of DNA fragments of well-defined size (2,14). Once the digestion limit is reached the double-stranded DNA fragments range in size between 158 and 38 base pairs; the fragments are separated in size by approximately (but not exactly) 10 base pairs ((2); Figure 1, slots *a* and *g*). Most of these DNA digest fragments can be generated in the absence of the lysine-rich histones, H1 and H5 (2,14). By examining the DNA of staphylococcal nuclease digests of reconstitutes involving most combinations of histones we were able to show that the arginine-rich histones are both necessary and sufficient to protect discrete DNA fragments from digestion ((2); Figure 1, slot *f*). We have reported that when arginine-rich histones, or even total histones, are added directly to DNA at low ionic strength, the DNA that is protected from digestion is polydisperse. We show here that the direct addition of histones can result in discrete fragments only when the DNA has been appropriately "primed". Figure 1 shows the results of these experiments.

The arginine-rich histones were reconstituted onto DNA by our standard reconstitution procedure (see Materials and Methods); a similar reconstitute with the slightly lysine-rich histones was also prepared. To both of these reconstitutes the complementary pair of histones was added dropwise in low ionic strength (see Materials and Methods); these complexes were digested to a limit with staphylococcal nuclease and the DNA extracted from them examined by polyacrylamide gel electrophoresis. Slots *a* and *g* of Figure 1 show the DNA of chromatin limit digest markers. When a reconstitute of H3/H4 and DNA is digested and the DNA is examined, the pattern shown in slot *f* is seen. When the H2A/H2B pair is added to this reconstitute additional discrete fragments of DNA are protected from digestion (slots *b* and *c*). On the other hand, adding the arginine-rich histone pair (H3/H4) directly to a reconstitute containing the slightly lysine-rich histones results in a complex that fails to protect any discrete DNA fragments from digestion (slot *d*). Slot *e* shows the result of adding the H3/H4 pair directly to DNA.

We have previously shown by examination of the products of digestion of H3/H4 reconstitutes that this histone pair can interact with and organize

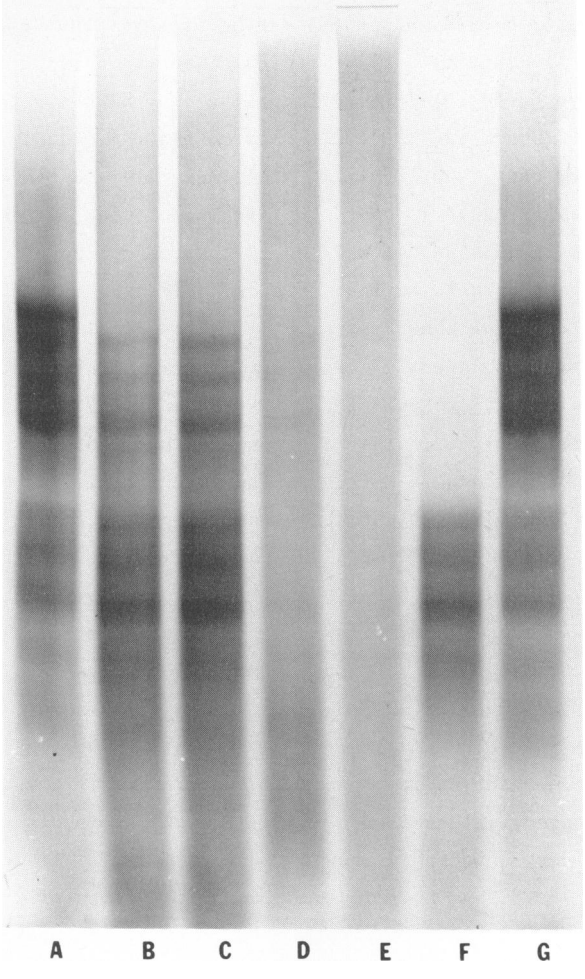

Figure 1. Sequential addition of histones. The DNA extracted from staphylococcal nuclease digests of several samples was electrophoresed on a 6% polyacrylamide slab gel. Slots *a* and *g* are markers from a limit digest of chromatin; the top band corresponds to a fragment 157 base pairs long, the lowest distinct band to a fragment 38 base pairs long. Slots *b* and *c* are two different H3/H4/DNA reconstitutes to which H2A/H2B were added; *d*, an H2A/H2B/DNA reconstitute to which H3/H4 was added; *e*, DNA to which H3/H4 was added; and *f* the H3/H4/DNA reconstitute used in *b* but without the additional H2A/H2B. All histones were at an input ratio of 0.25 g per gram of DNA.

DNA stretches at least as long as the 140 base pair nucleosome "core". We proposed that this "arginine-rich histone kernel" was the substrate on which the slightly-lysine rich histones H2A/H2B complete the nucleosome. This proposal is supported by the above results.

Conversion of supercoiled DNA to relaxed covalently closed circular DNA. Our endonuclease-free and protease-free nicking-closing extract (NCE) is similar in activity to extracts that have been isolated previously from other eukaryotes (9-13). The effect of the nicking-closing extract on supercoiled DNA is shown in Figure 2. Supercoiled DNA has a high electrophoretic mobility, while contaminating nicked circular DNA forms a slower moving band ((1,15); Figure 2, slot *a*). Nicking-closing extract converts the fast moving band of superhelical DNA to a series of bands of electrophoretic mobility comparable to that of nicked circular DNA ((1,15); Figure 2, slot *b*). These bands represent a Boltzmann distribution, about the relaxed state, of covalently closed circular DNA molecules with different topological winding, or linking, numbers (see below) (16,17). Partially supercoiled (or relaxed) molecules have intermediate mobilities and are also seen as a series of bands each corresponding in principle to a different value of the linking number (Figure 2, slots *h* and *j*), though the resolution of the bands varies with linking number and with conditions of electrophoresis.

Treatment of reconstitutes with nicking-closing extract. Germond et al. (1) have shown that the four histones H2A, H2B, H3 and H4, when reconstituted onto closed circular DNA, deform the DNA in a manner topologically equivalent to the induction of slightly more than one superhelical turn for each nucleosome observed in the electron microscope. However, under the reconstitution conditions used by these workers, this efficiency of induction could only be achieved when two grams of the four histones were added for each gram of DNA; this is twice the ratio ordinarily found in the nucleosome.

In this study we confirm these results; however, using our reconstitution methods, it is possible to induce about one superhelical turn for each added nucleosome-equivalent of the four histones (1 g of histones per gram of DNA). We have also used these methods to examine the effect of individual histones and combinations of histones on supercoiling. Briefly, the experimental scheme is as follows: Histones are reconstituted onto covalently closed circular DNA that has previously been relaxed by treatment with nicking-closing enzyme. After reconstitution the complex is then treated with nicking-closing enzyme, deproteinized, and the DNA electrophoresed in a gel system that separates covalently closed circular species according to linking number, as described above.

When a reconstitute of 1 g of the four histones (isolated as a

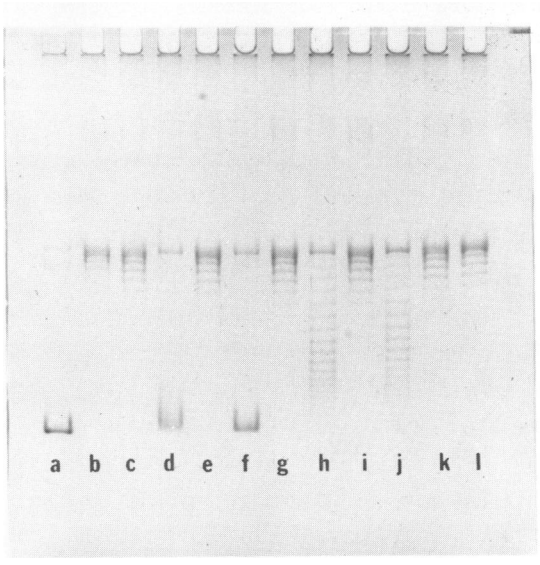

Figure 2. Gel electrophoresis of histone-Col E1 DNA complexes untreated and treated to the limit with nicking-closing extracts. Slots *a* and *b* contain supercoiled Col E1 DNA before and after treatment with nicking-closing extract. Slots *c* through *l* are paired samples, before and after treatment, of reconstitutes onto relaxed DNA. Slots *c* and *d*, all four histones, purified as a group of four; *e* and *f*, the four histones, purified as pairs; *g* and *h*, histones H3 and H4, purified as a pair; *i* and *j*, histones H3 and H4, purified as single histones; and *k* and *l*, histones H2A and H2B, purified as a pair. All histones were reconstituted at an input ratio of 0.25 g per gram of DNA. In this and the following figures, the samples in slots *a* and *b* are a control demonstrating the presence of enzyme activity. The 'relaxed' DNA in slot *b* is not the same sample used in the reconstitution experiments; small differences between the patterns in slot *b* and slot *c* (for example) arise from variation between 'relaxed' DNA samples, and not from the effects of histones.

mixture of the four histones, see Materials and Methods) per gram of relaxed Col E1 DNA is deproteinized and the DNA is examined by electrophoresis the pattern shown in Figure 2, slot *c* is obtained. Figure 2, slot *d* shows the pattern of the DNA from the same reconstitute if the sample is treated with nicking-closing enzyme before being deproteinized. The DNA has an electrophoretic mobility similar to that of native, fully supercoiled Col E1 DNA; as Germond et al. (1) reported, the presence of the histones induces a deformation topologically equivalent to supercoiling (see Discussion for an explanation of this "induction"). In our experiment this deformation has been accomplished with one nucleosome-equivalent of histones. Figure 2, slots *e* and *f* show a similar experiment in which the DNA from a

reconstitute of a mixture of the four histones, isolated as the pairs H2A/H2B and H3/H4 (see Materials and Methods), is examined before and after treatment with nicking-closing extract. The results are the same.

We next examined the effect of various other combinations of histones on supercoiling. The H3/H4 histone pair (isolated as a pair) was reconstituted with relaxed Col E1 DNA at 0.5 g protein/g DNA, and treated with NCE. The resulting DNA (before and after NCE treatment) is shown in Figure 2, slots *g* and *h*. It is clear that at this protein to DNA ratio, approximately that found in native nucleosomes for this pair of histones, a significant amount of supercoiling has been induced, but not as much as that achieved with a full complement of histones (cf. Figure 2, slots *d* and *f*). In Figure 2, slots *i* and *j*, are shown the results of a similar experiment in which H3 and H4 were purified separately, rather than as a pair. The small difference between the amount of supercoiling in slots *h* and *j* is neither reproducible nor significant. In contrast, when the slightly lysine-rich histone pair H2A/H2B, purified as a pair, was used in the reconstitutes, no supercoiling was induced (Figure 2, slots *k* and *l*).

Other combinations that did not include the H3/H4 pair also failed to induce supercoiling. Some of these experiments are shown in Figure 3. The triplets of purified histones H2A/H2B/H3 (Figure 3, slots *e* and *f*) and H2A/H2B/H4 (Figure 3, slots *g* and *h*) and the single arginine-rich histones H3 (Figure 3, slots *i* - *l*) and H4 (not shown) did not alter the final topological conformation of relaxed closed circular DNA. In the case of reconstitutes with the single purified histones H3 or H4, experiments were carried out at protein to DNA weight ratios of 0.25, 0.5 and 1.0; the results were all the same as in Figure 3, slots *j* and *l*. As a control, a mixture of the four individually purified histones was reconstituted onto DNA; full supercoiling was induced (Figure 3, slots *c* and *d*).

In order to estimate more accurately the amount of supercoiling that can be induced by a given amount of histone, reconstitutions were carried out at varying protein to DNA ratios, and the complexes examined as above. With equimolar mixtures of the four histones, full supercoiling occurred at between 0.8 g and 1.0 g of total added histones per gram of DNA. "Full supercoiling" is used here to denote degrees of supercoiling that are electrophoretically indistinguishable from native supercoiled DNA. Since the resolution of this gel system is less than optimal for highly supercoiled molecules (18), it may be that the actual supercoiling at the end point is slightly less than that found in native Col E1 DNA. It

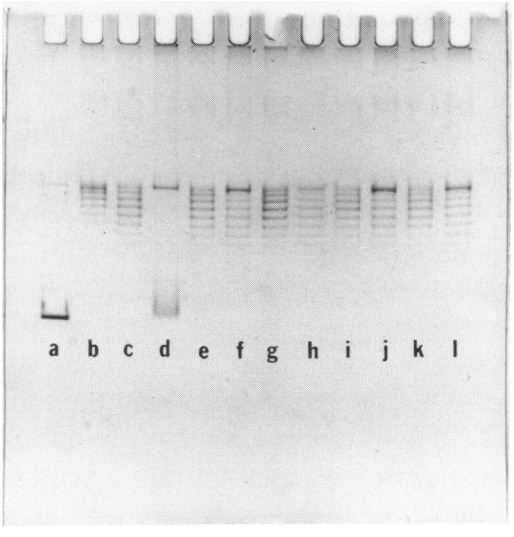

Figure 3. Gel electrophoresis pattern of histone-Col E1 DNA complexes. Slots *a* and *b*, supercoiled DNA before and after NCE treatment. Slots *c* through *l* are paired samples, before and after treatment with NCE, of reconstitutes onto relaxed DNA. Slots reconstituted with: *c* and *d* all four histones, all purified as individual histones; *e* and *f*, histones H2A, H2B and H3, purified as single histones; *g* and *h*, histones H2A, H2B and H4, purified as single histones; *i* and *j*, histone H3; and *k* and *l* histone H3 at an input ratio of 0.5 g per gram of DNA. All histones, except where noted, were at an input ratio of 0.25 g per gram of DNA.

is possible in principle to analyze the electrophoretic band pattern at intermediate points in a titration of DNA with histones, and to deduce the stoichiometry more precisely in that way. Such precision is probably not justifiable until it can be shown that every histone molecule is correctly recombined with DNA.

A similar titration with the histone pair H3/H4 is shown in Figure 4. Here again full supercoiling occurred at somewhere between 0.8 g and 1.0 g of added histones per gram of relaxed Col E1 DNA. It is highly unlikely that any of the supercoiling is due to the gratuitous addition of histones present in the nicking-closing extract: we estimate that the addition of NCE contributes to each reaction at most 0.02 g of each histone per gram of DNA.

Experiments similar to those presented above can be carried out using reconstitutes of histones with unrelaxed, supercoiled, DNA. When such reconstitutes, carrying a mixture of the four histones at 1 g total histones per gram of DNA, are treated with NCE, the deproteinized DNA has the same

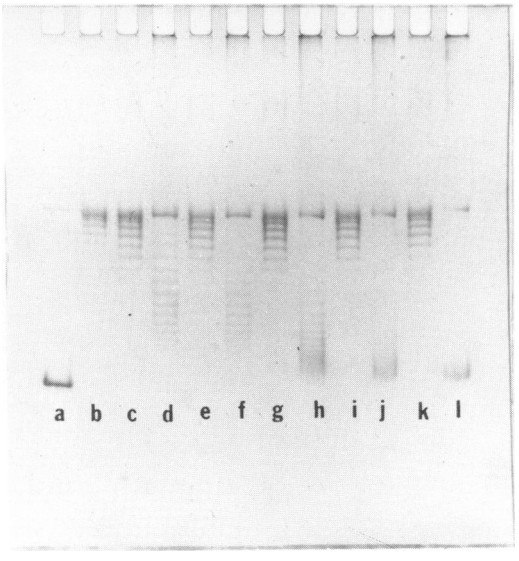

Figure 4. The effect of varying histones H3 and H4 on the induction of supercoils. Slot *a* and *b*, supercoiled DNA before and after NCE treatment. Slots *c* through *l* are paired samples, before and after treatment with NCE, of reconstitutes with varying amounts of H3 and H4, purified as the pair, onto relaxed Col E1 DNA. For the pair, the input amount per gram of DNA was: *c* and *d*, 0.4 g; *e* and *f*, 0.5 g; *g* and *h*, 0.6 g; *i* and *j*, 0.8 g; and *k* and *l*, 1.0 g.

electrophoretic mobility as the starting DNA. These results are similar to those reported by Germond et al. (1), except that the efficiency of protection (per gram of histone) is about twice as great in our experiments. When such reconstitutions are carried out with other combinations of histones, we find again that the H3/H4 pair is required to "protect" supercoils. In the absence of other histones, the DNA is fully protected against electrophoretic mobility changes at a protein to DNA weight ratio of approximately 0.8 to 1.0. These results are summarized in Table 1. Also shown in Table 1 are the results of experiments carried out with samples prepared with a different reconstitution procedure that did not include urea, and involved only one gradient dialysis step (see Matérials and Methods). The results obtained with this method of reconstitution are similar to those obtained by our usual methods. Both forms of reconstitutes "protect" supercoils with the same efficiency. However, reconstitutes formed without urea required about 25% more protein per unit relaxed DNA to achieve a given effect in the supercoil induction experiments.

Table 1

Results of treating reconstitutes with nicking-closing extract

Histones in Reconstitute[a]	Method of Reconstitution A(urea, NaCl)[b] Type of DNA I	Ir	B(NaCl)[b] Type of DNA I	Ir
H2A/H2B/H3/H4(S)	++*	++*		
H2A/H2B/H3/H4(P)	++*	++*	++*	++*
H2A/H2B/H3/H4(SP)		++		
H3/H4(S)		+		
H3/H4(P)	+*	+*	+*	+*
H2A/H2B(S)		-		
H2A/H2B(P)	-	-	-*	
H2A/H2B/H3(P)		-		
H2A/H2B/H4(P)		-		
H3(P)		-[c]	-	
H4(P)		-[c]	-	

++ = complete protection of supercoils and complete induction of supercoils for reconstitutes onto supercoiled (I) and relaxed (Ir) DNA respectively.

+ = roughly one half protection and induction of supercoils (cf. Fig. 2)

* = reconstitutes done at concentrations of DNA of both 50 and 200 µg/ml, otherwise reconstitutes were only done at DNA concentrations of 50 µg/ml.

(S) Purified by "stripping" of histones.

(P) Individually purified histones.

(SP) Four histone mixture of the two purified "stripped" pairs.

[a] In the experiments represented here all the histones were at an input of 0.25 g of each histone per gram of DNA. For reconstitutes involving different amounts of H3 and H4 see Figure 2.

[b] For different methods of reconstitution see Materials and Methods.

[c] Experiments were performed with 0.25 g, 0.5 g and 1.0 g of each histone per gram of DNA.

DISCUSSION

The "induction" and "protection" of supercoils. A covalently closed double-stranded DNA molecule is characterized by its topological winding number, α (19) also termed the linking number, Lk (20,21). This quantity is

an integer and is invariant with respect to all changes that keep the covalent bonds of the backbone intact.

Fuller (21) has shown how to relate Lk to the geometry of superhelices: The linking number can be expressed as the sum of two other quantities, the writhing number, W, and the total twist number, Tw:

$$\alpha \equiv Lk = W + Tw \qquad (1)$$

W is determined (21,22) by the space curve formed by the axis of the double-stranded helix. For purposes of this discussion we consider only the deviations, ΔLk and ΔTw, of these parameters from their values in relaxed DNA. Since $W \sim 0$ for relaxed covalently closed circular DNA, $\Delta Lk = W + \Delta Tw$. The exact conformation assumed by the relaxed circular DNA double helix which we take as our reference point varies with salt and temperature. The discrete bands of the gel electrophoretic patterns correspond to the integral values of Lk (15-17). However, the actual mobility of each band is determined by the average shape of the molecular species with that linking number. Fully relaxed circular DNA, with $W \sim 0$, has the smallest mobility.

Our experimental results (Figs. 2, 3 and 4) can be explained in terms of these parameters. When histones bind to a relaxed covalently closed DNA circle, the deformations they induce in DNA shape and twist will contribute both to changes in W and Tw. Unless these fortuitously balance, the regions of DNA that are histone-free must experience compensatory deformations that keep the linking number constant. As Germond et al. (1) first pointed out, treatment of the complex with nicking-closing enzyme removes these energetically unfavorable deformations in the regions of DNA not constrained by histones, resulting in a molecule with ΔLk equal to the sum of the changes in W and Tw induced by the histones. It is not possible, using this kind of experiment, to separate the contributions to ΔLk made by twisting of the double helix from those made by bending of the helix axis (1). Any decrease in the value of Lk from its value for relaxed circular DNA is seen as an "induction" of supercoils. The results shown in Figures 2, 3 and 4 are consistent with the series of events postulated above. It is easy to apply a similar line of reasoning to explain the "protection" of supercoils.

Supercoiling and folding in the nucleosome. Crick (22) has recently pointed out some of the pitfalls in attempting to calculate the linking number of a known structure. Conversely, one must be cautious in inferring the details of DNA folding from measurements of the linking number. It might seem attractive to suppose, for example, that a closed supercoiled SV40 DNA molecule, as isolated from the cell, has a conformation similar

to the one it assumes in the nucleosome. As we will show, however, this is not the case; the two DNA structures must differ markedly in conformation and energy.

Models of DNA folding in the nucleosome (23,24) all assume that the DNA is wrapped around the histone core. For purposes of our discussion, we will assume that 140 base pairs of DNA are tightly bound to the core, as suggested by previous investigations (25-27). We take the diameter of this DNA coil as 100 to 110 Å (28-30). We assume that the DNA is uniformly wound around a cylinder of length equal to the 110 Å diameter, so that 140 base pairs make about 1.4 turns around the cylinder. This gives a coil with a pitch of 82 Å and a pitch angle of about 12°. Such a structure has a packing ratio of 4.3. The conclusions we will reach below do not depend strongly upon the numerical details of our model. In fact, our model has the minimum uniform DNA curvature possible, consistent with nucleosome dimensions.

What energy is required to bend DNA into this conformation? We assume a free energy of bending of the form (31)

$$\Delta G_B = (1/2)\ B\ k^2\ L, \qquad (2)$$

where B is a bending force constant, k is the curvature (the inverse of the radius of curvature) of the bend, and L is the total length of DNA being considered (here, 476 Å). The curvature of the superhelix formed by the DNA in this structure is about (1/55) $Å^{-1}$. The value of the constant B is equal to RT times the persistence length of DNA in Angstrom units (31-33). If we take the persistence length as 600 Å (34,35), B has the value 360 Kcal-Å/ rad^2-mole at T = 298° K. Alternatively, B can be determined (32) from the temperature dependence of the persistence length, which gives a value of about 260 Kcal-Å/rad^2-mole. Using these values of B, we estimate the bending energy of DNA per mole of nucleosome as 20 to 28 Kcal. Note that we have not considered possible contributions from DNA twisting, which could make the total free energy of deformation even larger.

How does this value compare to the free energy of superhelix formation in histone-free DNA? The free energy ΔG_s required to convert a covalently closed, relaxed circular DNA to a molecule with ΔLk topological turns, has the form

$$\Delta G_s = A\ (\Delta Lk)^2 \qquad (3)$$

where A is estimated to have the value 0.120 Kcal/mole for SV40 DNA (16,17,58). Continuing the use of SV40 DNA for illustrative purposes, we calculate the

free energy of supercoiling for the DNA isolated from minichromosomes, which has been shown (18) to have $\Delta Lk = -26$ in its "native" supercoiled state. We find $\Delta G_S = 81$ Kcal per mole of DNA. This energy must be compared to the bending energy of 400 to 560 Kcal required to form 20 nucleosomes, the approximate number seen in SV40 minichromosomes in vivo (1,36,37).

The validity of this comparison of course depends upon the correctness of equation (2) for ΔG_B. Since this equation was originally derived for small deformations (those with radius of curvature of the order of a persistence length) it is possible that the value of 21-28 Kcal per mole of nucleosome or 400-560 Kcal per mole of SV40 minichromosome is an overestimate of ΔG_B. Indeed, if minimization of bending energy were an important consideration in the formation of nucleosomes, it might be more advantageous to bend the DNA by forming about a dozen kinks (38,39), at an estimated cost of 1-2 Kcal per kink in lost energy of base stacking.** As we will show, the energy of folding derived from histone-histone interactions is probably large enough to overbalance even the largest energies of bending discussed here, so neither mechanism of bending can be eliminated for energetic reasons. Whatever the bending mechanism, it is likely that a considerable amount of energy must be introduced to fold supercoiled DNA into nucleosomes: supercoiled DNA is energetically only slightly more favorable than relaxed DNA for nucleosome formation.

Supercoiled DNA and nucleosome DNA differ not only in their conformational energy, but also in their shape. Although the protein-free SV40 DNA with $\Delta Lk = -26$ could in principle assume a left-handed toroidal conformation, most evidence suggests that the structure actually observed in solution is the topologically equivalent, but conformationally distinct, right-handed, interwound form (40,41; Camerini-Otero & Felsenfeld, manuscript in preparation). The dimensions of this interwound superhelix can be estimated from hydrodynamic measurements and electron microscopy: the structure is rodlike (40,41) with a large pitch and therefore little curvature (see below). As we will now show, this low curvature accounts for the low free energy of supercoiling relative to that required to form the highly bent DNA of the nucleosome.

The free energy of supercoiling can be decomposed into a contribution from bending, and another from twisting (21,58)

$$\Delta G_S = \Delta G_B + \Delta G_{Tw}, \qquad (4)$$

where ΔG_B is given by equation (2). In a regular interwound superhelix of

total DNA contour length L with N superhelical turns (L/2 length and N/2 turns in each direction along the superhelix), the twist relative to the relaxed form is given by $\Delta Tw = \Delta Lk + N^2p/L$, (21,22), where ΔLk is -26 for SV40 and p is the pitch of the superhelix. ΔG_B can also be expressed in terms of N and p. The curvature of a helical curve of pitch p and radius r is given by (21) $k = 4\pi^2 r/(4\pi^2 r^2 + p^2)$. Since $L^2 = N^2(4\pi^2 r^2 + p^2)$, equation (2) can be written as

$$\Delta G_B = 2\pi^2 BN^2 (L^2 - N^2p^2)/L^3. \quad (5)$$

The calculation of the equilibrium dimensions of such a uniform interwound superhelix is not in general straightforward since the equilibrium, as Fuller (21) has pointed out, is determined by the balance between bending and twisting forces operating under additional (and unknown) steric constraints. At equilibrium the number of turns, N, will not in general equal the change in linking number, ΔLk, and in most cases the total energy of supercoiling (ΔG_s) will contain both bending and torsional contributions. For the purposes of our argument, we are only interested in estimating the configuration in which bending makes the maximum possible contribution to the free energy, given a known value of ΔG_s. This structure has the maximum possible uniform curvature of DNA, and corresponds to the requirement that $\Delta Tw = 0$, so that all the supercoiling energy is derived from bending. [If some of the measured supercoil free energy derives from torsion, the contribution from bending must be smaller, and the total curvature of the DNA is also smaller. We neglect contributions from the 'ends' of the rodlike structure.] When $\Delta Tw = 0$, ΔG_{Tw} will vanish, and $N^2p/L = 26$. From equation (3), we find $\Delta G_s = 81$, and since L = 18270 Å for SV40 DNA (calculated from a molecular weight of 3.6×10^6 daltons (42)), substitution in equation (5) gives $N \sim 30$, $p \sim 500$ Å, and $r \sim 50$ Å. Thus the dimensions of this regular superhelix are very different from the ones required for nucleosome formation. Even if the left-handed toroidal form of SV40 DNA were more stable than the right-handed interwound form, it is evident from similar arguments that the DNA could not be highly curved. The arguments about shape difference are simply another way of emphasizing the differences in energy of curvature between DNA in SV40 supercoils and DNA in nucleosomes.

Finally, the analysis given above should alert us to the possibility that while certain histones might well be able to induce a gentle fold in the DNA (such as is present in an SV40 DNA superhelical turn) they might not be able to completelely fold the DNA into a nucleosome. These two

processes are energetically distinct: the first involves relatively little energy while the latter requires considerably more energy. Also, since it is most likely that the final folding of the DNA into a nucleosome is accompanied by a further induction of supercoils, histones that do not carry out this final compaction might not induce supercoils as efficiently as those that do.

Histone-Histone interactions and nucleosome structure. Are lower energy kinks preferable to higher energy continuous deformations of DNA? Is it necessary that nucleosome models invoke the largest possible number of histone-histone interactions? In this section we suggest that in fact many different models of nucleosome structure can be accommodated because of the large energies available from interactions between histones.

It is now generally accepted (23, 43-45) that each histone molecule consists of two kinds of domains: a highly basic portion that interacts primarily with the DNA, and a hydrophobic region which is the site of histone-histone interactions. We now assume that ΔG°_{T}, the total free energy of folding, can be decomposed into two parts, that due to the principally ionic histone-DNA interactions, ΔG°_{I}, and that due to principally hydrophobic histone-histone interactions, ΔG°_{H}, so that $\Delta G^{\circ}_{T} = \Delta G^{\circ}_{I} + \Delta G^{\circ}_{H}$. We further assume that at physiologic ionic strengths ΔG°_{I} (incorporating the free energy of approximately 20 salt linkages per histone) is much larger than ΔG°_{H} (46,47).

We next imagine that we start with a folded nucleosome, and unfold the particle while still keeping the histones bound to their initial sites on the DNA. The histone-histone interaction free energy lost during this unfolding is the free energy we wish to estimate. Though we do not know the nature of the contacts, we will suppose that the forces holding the nucleosome in its folded form are derived from the histone-histone hydrophobic interactions measured in solution. In the discussion that follows we estimate the free energy for a DNA-bound histone complex undergoing an internal rearrangement: (unfolded nucleohistone complex) → (folded nucleosome). In the unfolded complex, some histone dimer interactions may already exist. Since we assume that the DNA of the unfolded structure is completely unbent, neither these interactions nor the ionic interactions will contribute to the free energy of folding.

D'Anna and Isenberg (48) and others (49,50) have studied the pairwise interactions of the four histones in solution. They found that four pairs of histones interact strongly and in the following order of strength

(48): H3/H4 > H2B/H4 ∿ H2A/H2B >H3/H2A. Using the data of D'Anna and Isenberg (48) and Roark et al. (50) one can estimate that the unitary free energy (51) for pair formation ranges from -16 Kcal to -9 Kcal, or on the average about -12 Kcal per mole of dimer. Since two monomers bound to DNA will have a more favorable free energy of dimerization than when free in solution, this energy represents the minimum energy available for pair formation.

Ignoring nonideality, and assuming a true equilibrium, there are at least two factors which will increase the favorable free energy of interaction of the bound histone pair relative to that for histones in solution. First, the electrostatic repulsion energy of highly positively charged histone molecules interacting in solution is reduced when the histones are bound to DNA. We estimate that this contributes at least -5 Kcal/mole to ΔG_H (52,53). Most importantly, we must consider those contributions to ΔG_H arising because the histones that remain bound to DNA upon dissociation do not regain their full translational and rotational entropies as they do when they dissociate in solution (54). This also increases the available free energy of association. The change in entropy for the formation of protein dimers has been calculated for other purposes by Doty and Myers (52) and Chothia and Janin (55). If, when bound to the DNA, the hydrophobic portions of the histones are as immobile in the monomers as in the dimer, this contribution could be as large as -25 Kcal/mole of dimer. Allowing for some increased mobility in the monomer, and for additional internal vibrational and rotational modes in the dimer, would decrease the magnitude of this number. It is not unreasonable to suppose, however, that after taking account of these two effects, the effective ΔG_H for dimer formation while the histones remain DNA-bound could be at least -20 Kcal/mole of dimer.

We do not know how many histone-histone contacts are formed during the folding reaction we have considered. If several of the D'Anna and Isenberg strong interactions are involved in the folding reaction it is clear that the energy derived from these interactions (whether corrected (-20 Kcal/per mole of dimer) or not (-12 Kcal/per mole of dimer)) can supply more than enough energy to deform the DNA (20 to 28 Kcal). More interestingly, however, our calculations show that even if most of these strong interactions were involved in forming a structure such as a cyclic heterotypic tetramer (45) which did not fold DNA, one or two histone pair interactions would be sufficient to stabilize the folded nucleosome structure relative to the

unfolded form. In one of the simplest versions of such a model this folding could be imagined to arise from the dimerization of these two unfolded half-nucleosomes. With the above mentioned corrections, the relevant histone interactions might be the relatively weak reaction $2(H3/H4) \rightarrow (H3/H4)_2$ (-4 Kcal/mole of product in free solution (50)). Our estimates suggest that structures stabilized by only one or two histone pair interactions are energetically reasonable, and that they might involve a delicate balance between the opposing forces of pair formation and DNA bending. Such a situation might be advantageous for a biological system in which nucleosome unfolding (during replication, for example) might occur.

Reconstitutes of the arginine-rich histones. The observation that a strongly interacting tetramer of the arginine-rich histones, H3 and H4, forms in solution (48,49) led Kornberg (24) to suggest that such a tetramer has a central role in nucleosome structure. Only recently, however, has direct evidence for the structural importance of the arginine-rich histones become available. We have shown that the arginine-rich histones, when reconstituted onto DNA, are unique in their ability to create structures with many of the features of native chromatin or nucleosomes. In particular, complexes of DNA with H3 and H4, when digested with a variety of nucleases or proteases, yield discrete DNA and protein fragments similar to those obtained by digestion of chromatin. The presence of H3 and H4 is necessary and sufficient to obtain such results (2,3).

We also examined the kinetics of digestion of complexes of DNA with H3 and H4, and found that at early times of digestion with either staphylococcal nuclease or DNAase I, fragments as large as 130 nucleotide pairs and 180 nucleotides, respectively, are produced as transient intermediates. On the basis of these findings we concluded that most of the DNA of the nucleosome is in fact interacting with these histones (2,3). We have proposed that the arginine-rich histone-DNA complex forms a kernel on which the slightly lysine-rich histones (H2A and H2B) can complete the nucleosome. This evidence is supported by the results of Boseley et al. (56), who have shown that certain H3/H4/DNA complexes have many of the X-ray diffraction properties of whole chromatin. The data in Figure 1 are clearly consistent with the important role we have suggested for the arginine-rich histones.

The results in Figures 2-4 show that only those reconstitutes containing both H3 and H4 deform relaxed circular DNA in a way which leads to a change in linking number upon treatment with nicking-closing enzyme. We cannot, of course, show that other combinations of histones do not form

significant structures that distort DNA locally, but if they do, the distortion must involve equal and opposite changes in W and Tw which do not affect Lk.

We also cannot rule out the possibility that other reconstitution methods would reveal presently undetected contributions to structure from histone combinations lacking H3 and H4. On the other hand, the urea-salt gradient method of reconstitution used here works well when a nucleosome-equivalent of the four histones (H2A/H2B/H3/H4) is combined with a DNA fragment 140 base pairs long. Particles formed in this way are identical to native nucleosomes in their nuclease digestion patterns and have identical sedimentation properties (Simon, Camerini-Otero & Felsenfeld, manuscript in preparation). Furthermore, this method has been used with partial complements of histones to produce complexes which are indistinguishable, by a variety of probes, from those obtained by stripping selected histones from whole chromatin (2,3). It should also be pointed out that use of a quite different reconstitution method has no effect upon the results of supercoiling experiments (Table 1).

The results shown in Figure 4 suggest that H3 and H4 are as effective for supercoil induction, on a weight basis, as a mixture of the four histones (57). A nucleosome-equivalent of H3 and H4 (0.5 g per gram of DNA) induces about half as much supercoiling as a nucleosome equivalent of the four histones (1 g per gram of DNA). If we take into account the possibility that not every histone molecule may recombine perfectly, this result must be interpreted as a lower limit to the efficiency of the H3/H4 pair in induction of supercoils.

It is quite possible, however, that even under the best of circumstances a nucleosome-equivalent of the arginine-rich histones (0.5 g per gram of DNA) cannot induce full supercoiling, and that only when the nucleosome is completed by the slightly lysine-rich histones will the DNA be completely supercoiled. In any case, the data on the induction and protection of supercoils by the arginine-rich histones is consistent with the results obtained with other techniques. The data taken as a whole strongly support the concept that these histones play the central role in nucleosome structure.

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REFERENCES

* Dedicated to the memory of Jerome Vinograd, a good friend and teacher.

† A preliminary report of some of these results was presented at a Symposium on Chromatin Structure and Function at the First International Congress on Cell Biology, Boston, Massachusetts, September 6, 1976. Felsenfeld, G., Camerini-Otero, R. D. and Sollner-Webb, B. (1977) in "International Cell Biology 1976-1977". Rockefeller University Press, B. Brinkley and K. Porter (eds.), in press.

** It is of course also possible that the kinking mechanism is involved in DNA bending in solution, and that the constant B of equation (2) itself reflects the kinking process. Presently available data do not permit us to distinguish kinking from continuous bending.

1 Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 1843-1847.
2 Camerini-Otero, R. D., Sollner-Webb, B. and Felsenfeld, G. (1976) Cell 8, 333-347.
3 Sollner-Webb, B., Camerini-Otero, R. D. and Felsenfeld, G. (1976) Cell 9, 179-193.
4 Clewell, D. B. and Helinski, D. R. (1969) Proc. Nat. Acad. Sci. U.S.A. 62, 1159-1166.
5 Sakakibara, Y. and Tomizawa, J. (1974) Proc. Nat. Acad. Sci. U.S.A. 71, 802-806.
6 Van der Westhuyzen, D. R., Böhm, E. L. and Von Holt, C. (1974) Biochim Biophys. Acta 359, 341-345.
7 Ruiz-Carrillo, A. and Allfrey, V. G. (1973) Arch. Biochem. Biophys. 154, 185-191.
8 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
9 Champoux, J. J. and Dulbeceo, R. (1972) Proc. Nat. Acad. Sci. U.S.A. 69, 143-146.
10 Baase, W. A. and Wang, J. C. (1974) Biochemistry 13, 4299-4303.
11 Keller, W. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 2550-2554.
12 Pulleyblank, D. E. and Morgan, A. R. (1974) Biochemistry 14, 5205-5209.
13 Vosberg, H. P., Grossman, L. I. and Vinograd, J. (1975) Eur. J. Biochem. 55, 79-93.
14 Axel, R., Melchior, W., Sollner-Webb, B. and Felsenfeld, G. (1974) Proc. Nat. Acad. Sci. U.S.A. 71, 4101-4105.
15 Keller, W. and Wendel, I. (1974) Cold Spring Harbor Symp. Quant. Biol. 39, 199-208.
16 Depew, R. E. and Wang, J. C. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 4275-4279.
17 Pulleyblank, D. E., Shure, M., Tang, D., Vinograd, J. and Vosberg, H. P. (1975) Proc. Nat. Acad. Sci. U.S.A. 72 4280-4284.
18 Shure, M. and Vinograd, J. (1976) Cell 8, 215-226.
19 Vinograd, J. and Lebowitz, J. (1966) J. Gen. Physiol. 49, 103-125.
20 Glaubiger, D. and Hearst, J. E. (1967) Biopolymers 5, 691-696.
21 Fuller, F. B. (1971) Proc. Nat. Acad. Sci. U.S.A. 68, 815-819.
22 Crick, F. H. C. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 2639-2643.
23 Van Holde, K. E., Sahasrabuddhe, C. G. and Shaw, B. R. (1974) Nucl. Acids Res. 1, 1579-1586.
24 Kornberg, R. (1974) Science 184, 868-871.

25 Sollner-Webb, B. and Felsenfeld, G. (1975) Biochemistry 14, 2915-2920.
26 Axel, R. (1975) Biochemistry 14, 2921-2925.
27 Shaw, B. R., Herman, T. M., Kovacik, R. T., Beaudreau, G. S. and Van Holde, K. E. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 505-509.
28 Baldwin, J. P., Boseley, P. G., Bradbury, E. M. and Ibel, K. (1975) Nature 253, 245-249.
29 Pardon, J. F., Worcester, D. L., Wooley, J. C., Tatchell, K., Van Holde, K. E. and Richards, B. M. (1975) Nucl. Acids Res. 2, 2163-2176.
30 Bradbury, E. M., Hjelm, R. P., Carpenter, B. G., Baldwin, J. P. and Hancock, R. (1977) in Molecular Biology of the Mammalian Genetic Apparatus, in press.
31 Landau, L. D. and Lifshitz, E. M. (1958) Statistical Physics pp. 478-482, Addison-Wesley, Reading, Mass.
32 Gray, H. B. and Hearst, J. E. (1968) J. Mol. Biol. 35, 111-129.
33 Schellman, J. A. (1974) Biopolymers 13, 217-226.
34 Jolly, D. and Eisenberg, H. (1976) Biopolymers 15, 61-95.
35 Godfrey, J. E. and Eisenberg, H. (1976) Biophys. Chem. 5, 301-318.
36 Griffith, J. (1975) Science 187, 1202-1203.
37 Cremisi, C., Pignatti, P. F., Croissant, O. and Yaniv, M. (1976) J. Virol. 17, 204-211.
38 Crick, F. H. C. and Klug, A. (1975) Nature 255, 530-533.
39 Sobell, H. M., Tsai, C. C., Gilbert, S. G., Jain, S. C. and Sakore, T. D. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 3068-3072.
40 Gray, H. B., Jr. (1967) Biopolymers 5, 1009-1019.
41 Upholt, W. B., Gray, H. B. and Vinograd, J. (1971) J. Mol. Biol. 61, 21-38.
42 Tai, H. T., Smith, C. A., Sharp, P. A. and Vinograd, J. (1972) J. Virol. 9, 317-325.
43 Bradbury, E. M. and Rattle, H. W. E. (1972) Eur. J. Biochem. 27, 270-281.
44 De Lange, R. J. and Smith, E. L. (1975) in Ciba Symposium on Structure and Function of Chromatin 28, 59-70.
45 Weintraub, H., Worcel, A. and Alberts, B. (1976) Cell 9, 409-417.
46 Bradbury, E. M., Cary, P. D., Crane-Robinson, C., Rattle, H. W. E., Boublik, M. and Sautière, P. (1975) Biochemistry 14, 1876-1885.
47 Record, M. T., Lohman, T. M., de Haseth, P. (1976) J. Mol. Biol. 107, 145-158.
48 D'Anna, J. A. and Isenberg, I. (1974) Biochemistry 13, 4992-4997.
49 Kornberg, R. D. and Thomas, J. O. (1974) Science 184, 865-868.
50 Roark, D. E., Geoghegan, T. E. and Keller, G. H. (1974) Biochem. Biophys. Res. Commun. 59, 542-547.
51 Kauzmann, W. (1959) in Advances in Protein Chemistry 14, p. 35, Academic Press, New York.
52 Doty, P. and Myers, G. E. (1952) Discussions Faraday Soc. 13, 51-58.
53 Tanford, C. (1961) Physical Chemistry of Macromolecules. pp. 519-521 John Wiley and Sons, New York.
54 Page, M. I. and Jencks, W. P. (1971) Proc. Nat. Acad. Sci. U.S.A. 68, 1678-1683.
55 Chothia, C. and Janin, J. (1975) Nature 256, 705-708.
56 Boseley, P. G., Bradbury, E. M., Butler-Browne, G. S., Carpenter, B. G. and Stephens, R. M. (1976) Eur. J. Biochem. 62, 21-31.
57 That H3 and H4 can induce supercoils in DNA has been recently confirmed by M. Bina-Stein and R. Simpson (personal communication).
58 Bauer, W. and Vinograd, J. (1970) J. Mol. Biol. 47, 419-435.