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Effect of trypsinization and histone H5 addition on DNA twist and topology in reconstituted minichromosomes

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ABSTRACT

Free DNA in solution exhibits an untwisting of the double helix with increasing temperature. We have shown previously that when DNA is reconstituted with histones to form nucleosome core particles, both the core DNA and the adjacent linker DNA are constrained from thermal untwisting. The origin of this constraint is unknown. Here we examine the effect of two modifications of nucleosome structure on the constraint against thermal untwisting, and also on DNA topology. In one experiment, we removed the highly positively charged histone amino and carboxy termini by trypsinization. Alternatively, we added histone H5, a histone H1 variant from chick erythrocytes. Neither of these modifications had any major effect on DNA topology or twist in the nucleosome.

INTRODUCTION

The degree to which DNA in chromatin is constrained from undergoing motions such as twisting or sliding or transient dissociation is likely to be relevant to its accessibility *in vivo* to the molecules involved in transcription and modification. DNA which is tightly confined, as in heterochromatin, is often envisioned as being unable to participate in these dynamic processes without substantial rearrangements in higher order chromatin structure. Between such highly condensed structures and free, naked DNA, there may well exist a gradation of accessibility of DNA in chromatin, modulated both by the non-histone chromosomal proteins and by modifications to the histone proteins themselves. It has been shown, for example, that the addition of various H1 subtypes to stripped chromatin (containing only the core histones) differentially inhibits transcription *in vitro* by inhibiting binding of RNA polymerase to DNA (1). It has also been shown that native

chromatin is not as good a substrate for *in vitro* methylation as either naked DNA or trypsinized chromatin, which retains much of its complement of core histone proteins (2).

The constraint placed on DNA by chromosomal proteins will not only affect its accessibility to enzymes; it will also affect the ability of local domains of DNA to exhibit and maintain altered structures. It is impossible, for example, for torsionally strained and torsionally relaxed regions to coexist in a naked molecule of DNA. The extent to which histones and non-histone chromosomal proteins can prevent DNA from twisting or sliding will therefore define the conditions under which torsional strain can function as a regulatory mechanism (see for example references 3-7).

To investigate the constraint placed on DNA by its association with the histone proteins, we have studied the thermal untwisting of DNA incorporated into nucleosomes. Naked DNA is known to untwist with increasing temperature by 0.01° /bp/ deg. C (8, 9). We found previously that DNA incorporated into reconstituted core particles does not undergo any thermal untwisting between 4 C and 37 C, and that the length of DNA prevented from thermal untwisting corresponds to 230 $\pm$ 20 bp per core particle (10). This was a surprising result, since the length of DNA associated with the core particle is generally given as 145 bp, based on the size of the DNA fragment protected from digestion by micrococcal nuclease. We postulated that the constraint against thermal untwisting of DNA in the nucleosome beyond 145 bp was due to an interaction of the histones with the linker DNA which still left the linker DNA susceptible to micrococcal nuclease. Since the histone amino termini are positively charged at neutral pH, and are not required for the formation of nucleosome core particles (11), they seemed a likely candidate for this interaction. In this paper, we test this idea by measuring the thermal untwisting of DNA in core particles after removal of the histone amino termini by trypsinization. We also report the effect of removing the histone tails on supercoiling of DNA in the nucleosome.

As a separate test of the factors that may influence the state of the linker DNA, we asked whether adding histone H5 to

reconstituted minichromosomes would affect thermal untwisting, or the linking number, of DNA in nucleosomes. The presence of histone H5 in the nucleosome protects an additional 15-20 bp of DNA against digestion by micrococcal nuclease. Histone H5 (like histone H1) is thought to bind to the DNA at the points of entry to and exit from the core particle (12). Depending on the nature of the interaction between histone H5 and the DNA, H5 could increase or decrease the length of DNA constrained against thermal untwisting.

MATERIALS AND METHODS

Preparation and characterization of minichromosomes

Frozen chick erythrocytes were obtained from the Pocono Rabbit Farm (Canadensis, PA), and total histones isolated from purified nuclei (13) according to Stein and Bina (14) but without the final ultrafiltration. Histone concentrations were calculated using A_{230} = 4.15 at 1 mg/ml (15). In our hands, this preparation contained some contaminating histone H5. Purification of core histones by $HClO_4$ precipitation (16) led to a greatly reduced yield, and so histones containing some H5 were used in our reconstitution procedure. Histone H5 was then removed from the reconstituted core particles by elution of the complexes over Sephadex G50 in spin columns (17) at 0.6 M NaCl (see below). Histone H5 was purified from chick erythrocytes (18). About one third of the preparation consisted of histone H1 as judged by gel electrophoresis; we will refer to this preparation as "histone H5" for convenience. Analysis of histone proteins was performed on SDS-polyacrylamide gels (19) or on triton-acid-urea containing polyacrylamide gels (20).

Core histones were reconstituted onto closed circular, supercoiled pBR322 DNA by a procedure similar to that of Stein and Bina (14). Histones from a solution in 2.5 M NaCl were added to DNA in 10 mM Tris.HCl pH 8.0/ 0.2 mM EDTA plus salt at the desired weight ratio such that the final salt concentration was 1.5 M and the DNA concentration was 250 ug/ml. The solution was then dialyzed in Spectrapor dialysis tubing against 1 l of 0.8 M NaCl/ 20 mM Tris.HCl pH 7.5/ 0.2 mM EDTA for 2 hr at room temperature; then against 1 l of the same solution at 0.6 M NaCl

for one hr, after which the solution was passed over Sephadex G50 in spin columns in aliquots of 100-200 ul. Finally the eluant was dialyzed against the same solution at 0.17 M NaCl for 2 hr at room temperature, collected, and stored at 4 C. Histone H5 was reconstituted onto minichromosomes, at a ratio of 1 ug H5/3 ug core histones, according to the protocol of Stein and Bina (14). Torsionally relaxed minichromosomes were prepared by treatment with nicking-closing extract prepared from chick erythrocytes (21) and analysis of the linking number on topoisomer-resolving gels was done as described previously (10). In the analyses presented, $\overline{L}$ refers to the mean linking number of the topoisomer distribution at 37 C relative to naked pBR322 relaxed at 37 C, and $\Delta\overline{L}$ is the absolute difference in $\overline{L}$ between 4 C and 37 C for a given sample. We have also reanalyzed some densitometric scans by the method of Stein (18), in which $\overline{L}$ is determined as $\Sigma\, iI_i / \Sigma\, I_i$, where I_i is the intensity (peak area) of the ith topoisomer; this method of analysis yields the same values of $\overline{L}$ and $\Delta\overline{L}$ as the previous method, within the accuracy of the data.

Reconstituted minichromosomes were digested with micrococcal nuclease (Worthington Enzymes) by diluting the sample into 2 mM $CaCl_2$/ 20 mM Tris.HCl pH 8.5 and incubating at 37 C for 1-10 minutes with about 0.2 units of micrococcal nuclease per ug DNA. DNA was recovered and analyzed by electrophoresis on 6% polyacrylamide gels (22).

Trypsinization of minichromosomes

Material sufficient for protein analysis, micrococcal nuclease digestion, and at least three separate topoisomer analyses (usually 100-150 ug of DNA) was incubated at room temperature with 0.2-1.0 ug/ml of trypsin (Sigma) and aliquots removed at selected times. Trypsin activity was quenched in the aliquots by addition of a 10-fold weight excess of lima bean trypsin inhibitor (Aldrich). Control experiments showed that trypsin inhibitor did not interfere with the activity of nicking-closing extract. Trypsin was stored as a 1 mg/ml solution in 1 mM HCl, and trypsin inhibitor as a 5 mg/ml solution in 5 mM Tris.HCl pH 8, both at 4 C (23).

RESULTS

Role of the histone tails

To determine whether the histone tails were involved in constraining core particle DNA against thermal untwisting, reconstituted minichromosomes, comprising closed circular pBR322 and nucleosomes derived from chick erythrocyte core histones, were treated with trypsin to remove the histone termini. This reduces the histones to a discrete set of limit proteolysis fragments, as seen in the gel shown in Figure 1. Quantitation of the proteolysis products by densitometry indicates that the total

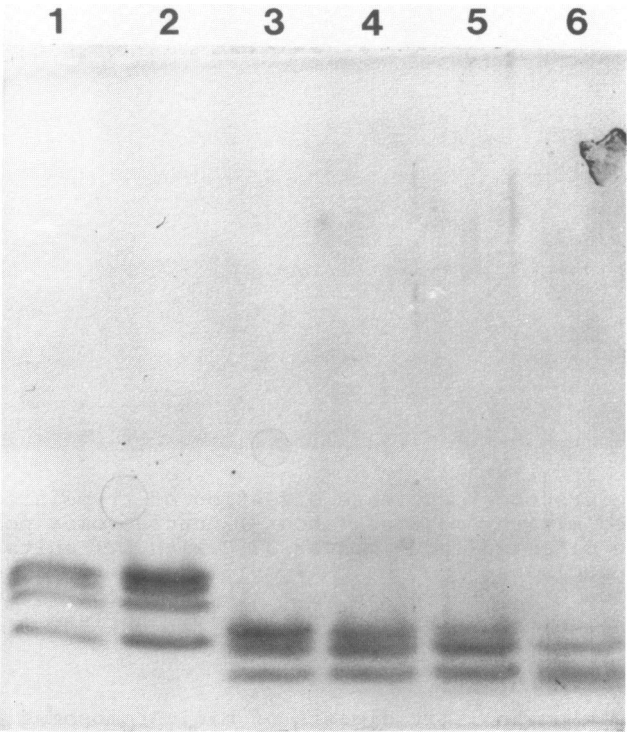

Figure 1. Trypsin digestion of reconstituted minichromosomes. Reconstituted minichromosomes were digested with 1 ug/ml trypsin and the digestion stopped by the addition of lima bean trypsin inhibitor. Samples were incubated with 1% SDS for 15 min at room temperature before electrophoresing on an 18% polyacrylamide-SDS gel at 50 V for 16 hrs. Lane 1, core histones from chick erythrocytes; lane 2, untrypsinized minichromosomes; lane 3, 30 min trypsinization; lane 4, 1 hr trypsinization; lane 5, 2 hr trypsinization; lane 6, 6 hr trypsinization.

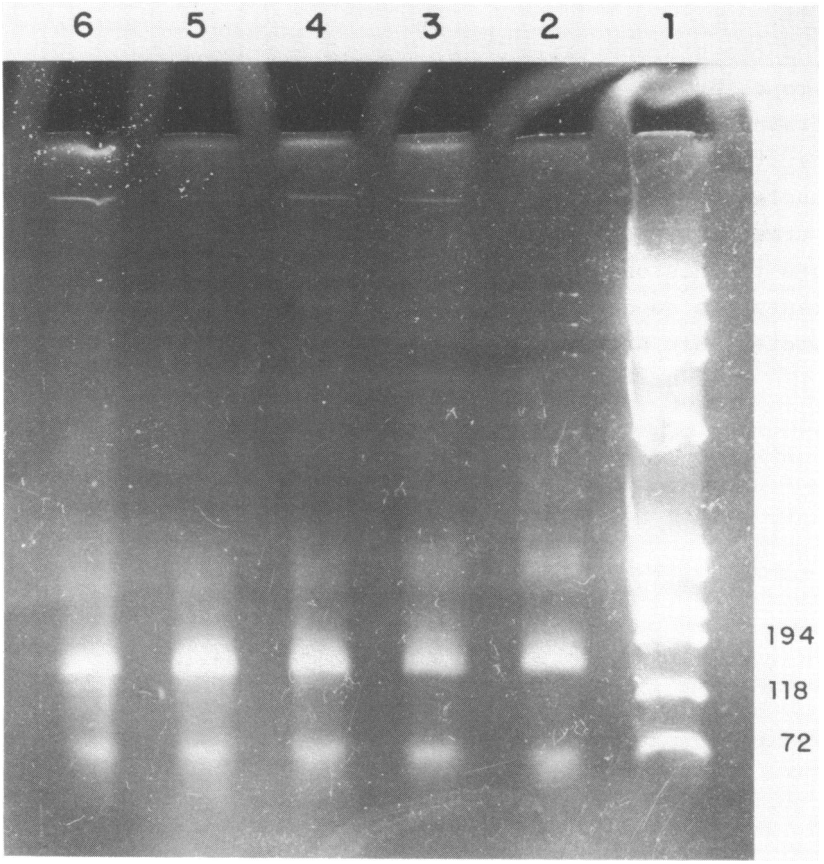

Figure 2. Micrococcal nuclease digestion of trypsinized reconstituted minichromosomes (about 12 nucleosomes per plasmid). Samples were digested for 2 min at 37 C with 0.1 units micrococcal nuclease, and the DNA purified and run on a 6% polyacrylamide gel. Lanes 2-6 were treated with 1 ug/ml trypsin for 0, 0.5, 1, 2, and 4 hrs, respectively, and lane 1 is ϕx174 rfDNA cut with Hae III.

protein remaining in limit digests of minichromosomes is 55-72% of the original, in good agreement with the determination by Diaz and Walker that 64% of the original histone mass is resistant to trypsinization in nucleosomes (24). Extended trypsinization eventually leads to reduction of the histone mass to less than 50% of the starting material, probably at least in part due to loss of entire nucleosomes (see below). We have also electrophoresed the trypsinization products on triton-acid-urea

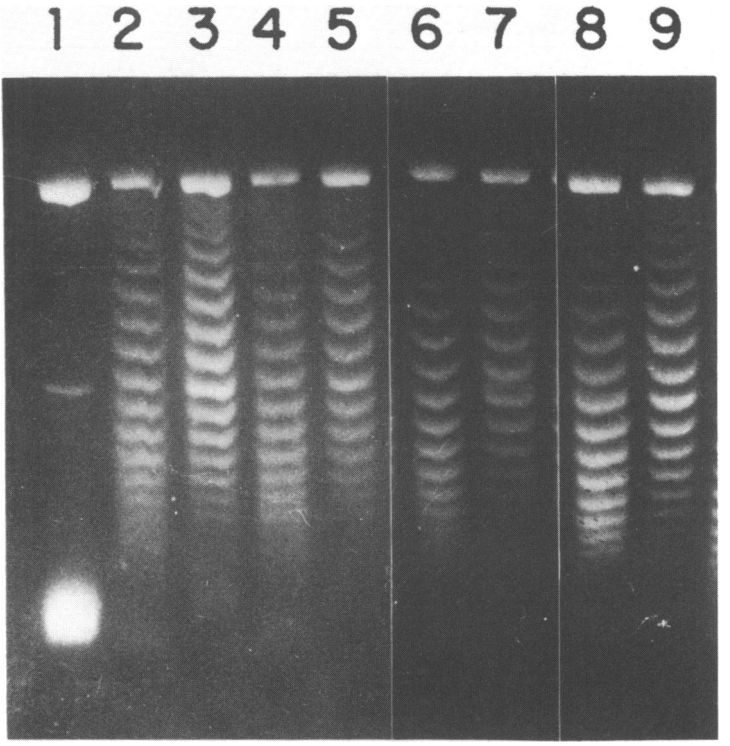

Figure 3. Relaxation of intact and trypsinized minichromosomes at 4 C and 37 C. Reconstituted minichromosomes (about 13 nucleosomes per plasmid) were digested with 0.2 ug/ml trypsin and digestion stopped by addition of lima bean trypsin inhibitor. Samples relaxed with nicking-closing extract were deproteinized and electrophoresed on a 1.5% agarose gel containing 7 ug/ml chloroquine at 50 V for 30 hrs. The topoisomers are running as positively supercoiled molecules, and the bright staining band near the top of the gel is nicked circular DNA. Lane 1, naked pBR322; lanes 2, 4, 6, and 8, samples treated with trypsin for 0, 0.5, 2, and 6 hr, and relaxed at 4 C; lanes 3, 5, 7, and 9, the same samples relaxed at 37 C.

containing polyacrylamide gels to see whether any new information might be gained on the generation of the early and late digestion products, but the pattern on these gels was too similar to that of the intact histones to be informative.

When trypsinized reconstituted minichromosomes were digested with micrococcal nuclease, the size of the protected DNA fragment remained constant at about 150 bp (Figure 2). This is consistent

Table I. Effect of trypsinization on thermal untwisting and topology of DNA in nucleosomes.

Mean number of nucleosomes	Length of trypsin treatment (hr)	% protein remaining	$\frac{\bar{L}+ - \bar{L}-}{\bar{L}-}$ [a]	$\Delta\bar{L}$ [b]	% of bp protected [c]
12.5	--	100	--	2.0	100
	0.5	69	0.04	1.95	102
	0.5, d	69	0.06	2.15	94
	2.0	65	0.07	1.9	104
	2.0, d	65	0.11	2.5	79
	6.0	69	0.06	1.9	104
13	--	100	--	1.5	100
	2.0	72	0.05	1.55	98
	2.0, d	72	0.05	1.7	93
	6.0	59	n.d.	1.8	90
	6.0, d	59	n.d.	1.66	94
15	--	100	--	0.7	100
	0.5	67	0.03	1.2	86
	0.5, d	67	0.05	1.0	92
	2.0	55	0.02	n.d.	--
	2.0, d	55	0.06	0.7	100
	6.0	60	0.02	n.d.	--
	6.0, d	60	0.11	0.6	103
	Average for undialyzed:		0.04 ± 0.02		98 ± 6.8%
	Average for dialyzed:		0.07 ± 0.03		94 ± 7%

[a] $\bar{L}-$ = mean linking number at 37 C for the untrypsinized starting material relative to naked pBR322 relaxed at 37 C; $\bar{L}+$ = mean linking number at 37 C for the same sample after trypsinization.
[b] Absolute difference in mean linking number between samples relaxed with nicking-closing extract at 4 C and 37 C.
[c] This is defined as (bp protected against thermal untwisting after trypsinization)/(bp protected against thermal untwisting before trypsinization) x 100. The number of bp protected against thermal untwisting is calculated as [(4.4- $\bar{L}$)/4.4] x 4362.
[d] Sample dialyzed after trypsinization.

with the results obtained on trypsinization of core particles (25) and chromatin (26), and shows that the core particle structure does not significantly rearrange upon trypsinization, at least as assayed by micrococcal nuclease.

Minichromosomes digested with trypsin to 55-72% of the original histone content were treated with nicking-closing extract at 4 C and 37 C and the DNA analyzed on topoisomer-resolving gels. A typical gel is shown in Figure 3. The distribution of topoisomers is due both to heterogeneity in the

number of nucleosomes and thermal fluctuations in the DNA at the time of closure. By averaging measurements made from several such gels, we determined the mean linking number of the topoisomer distributions at 37 C, $\bar{L}$, and the absolute difference in linking number for each sample when relaxed at 4 C and 37 C, $\Delta\bar{L}$, as described previously (10). The results from three reconstitutions are given in Table I.

Since each nucleosome takes up one negative supercoil at 37 C in 0.2 M NaCl (9, 27, 28), the average number of nucleosomes in the untrypsinized control is given directly by $\bar{L}$, which is measured relative to naked pBR322. For trypsinized minichromosomes, the number of supercoils released per nucleosome when the histones are removed is decreased by 0.04 $\pm$ 0.02 (Table I). When the trypsinized minichromosomes are dialyzed to remove the smaller trypsinized fragments (24), the measured change in linking number per nucleosome becomes 0.07 $\pm$ 0.03. Thus, there is at most only a small loss of DNA supercoiling per nucleosome after trypsinization.

A similar situation was found for the measured change in $\Delta\bar{L}$, the difference in linking number between minichromosomes relaxed at 4 C and 37 C, for intact versus trypsinized nucleosomes. Before dialysis, there is essentially no change in $\Delta\bar{L}$ for trypsinized versus intact nucleosomes. After dialysis, the length of DNA constrained from thermal untwisting is 94 $\pm$ 7% of the starting value, corresponding to the release of 15 $\pm$ 16 bp per nucleosome. Thus, there is little if any change in the length of DNA constrained from thermal untwisting in core particles after limit trypsinization. Further trypsinization, such that the histone mass is decreased to less than 50% of the starting value, results in larger changes in $\bar{L}$ and $\Delta\bar{L}$ relative to the intact minichromosomes. This is most probably due to loss of entire nucleosomes as the core histones are further degraded by trypsin.

Role of histone H5

The effect which adding histone H5 had on supercoiling and thermal untwisting of DNA in reconstituted minichromosomes was also investigated. Histone H5 from chick erythrocytes was added to reconstituted minichromosomes in the presence of

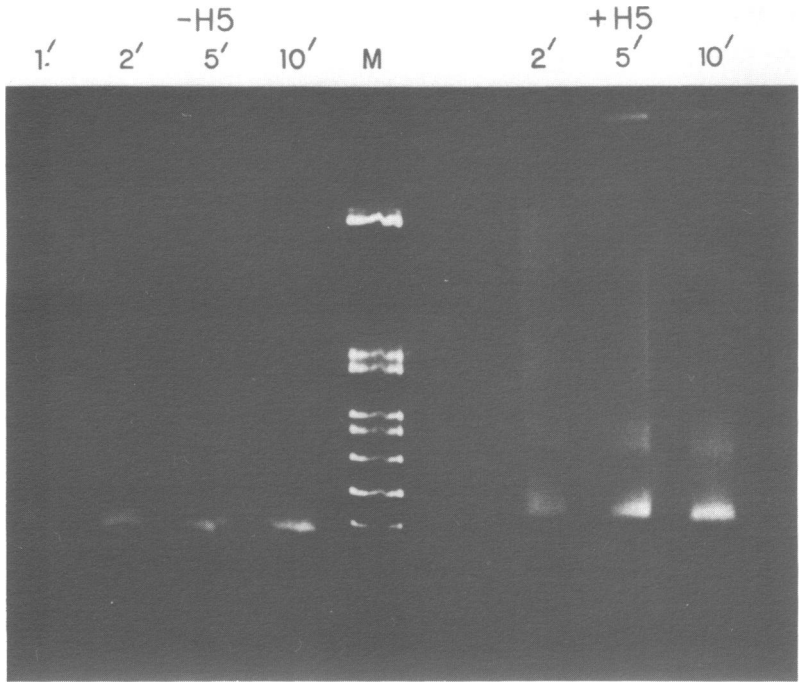

Figure 4. Micrococcal nuclease digestion of reconstituted minichromosomes before and after addition of histone H5. Samples (about 4 ug each) were digested with 0.4 units of micrococcal nuclease for -H5 samples, 0.8 units for +H5 samples, each in 160 ul total volume. Aliquots of 40 ul were withdrawn at the indicated times and the DNA purified and electrophoresed on a 6% polyacrylamide gel at 150 V for 90 min. Lane M is pBR322 DNA cut with Hinf I.

polyglutamic acid (14). These minichromosomes, as well as minichromosomes lacking histone H5, were then relaxed with nicking-closing extract at 37 C, and also at 4 C. In this way any change in linking number brought about by addition of histone H5 to core particles could be measured, and the change in twist between 4 C and 37 C for minichromosomes containing histone H5 could be determined.

The reconstitution of histone H5 onto minichromosomes was assayed by digestion with micrococcal nuclease, and by column chromatography of the reconstituted minichromosomes followed by electrophoresis of the proteins on triton-acid-urea containing polyacrylamide gels. Figure 4 shows the analysis by gel

electrophoresis of the DNA fragments protected from micrococcal nuclease digestion in nucleosomes with and without histone H5. It can be seen from the size of both the monomer and dimer fragments that, within the resolution of the gel, the results are consistent with protected fragment sizes of 145 bp and 160 bp for nucleosomes without and with histone H5, respectively. It is worth noting that the low intensity of the dimer band relative to the monomer, and the absence of fragments corresponding to higher order oligomers, argues against significant close-packing of nucleosomes in this particular sample. Since this sample was reconstituted at a relatively high histone:DNA ratio (0.8 g histones: g DNA), it is unlikely that close-packing of nucleosomes contributed to any of the results discussed below (for example, by changing the topology of DNA on the nucleosome).

Reconstituted minichromosomes with and without histone H5 were also passed over spin columns of Sephadex G50 and the proteins present in the eluted complexes analyzed by electrophoresis in triton-acid-urea containing polyacrylamide gels. Densitometric scans of the gels revealed that all four core histones were present in equimolar ratio in both cases, and histone H5 was found in a ratio of 1-2 molecules per nucleosome in the complexes to which it was added. There was no histone H5 visible in the complexes in which it was not added back, demonstrating that this histone was effectively removed by the passage of the reconstituted minichromosomes over G50 spin columns after dialysis against 0.6 M NaCl.

The effect that adding histone H5 to reconstituted minichromosomes had on linking number is shown in Table II. We find, in agreement with earlier reports (18), that when supercoiled pBR322 is incubated with histone H5 and polyglutamic acid in the absence of core histones and relaxed at 37 C, the same value of $\bar{L}$ is obtained as for relaxed, naked pBR322 (data not shown). This observation holds true for ratios varying from 0.1-0.4 g H5/g DNA, which covers the range used for reconstitution onto nucleosomes. Reconstituted minichromosomes, on the other hand, appear always to suffer a slight increase in negative supercoiling (a decrease in linking number) upon addition of histone H5. However, this increase has no apparent

Table II. Change in mean linking number of minichromosomes relaxed at 37 C after addition of histone H5.

Histone:DNA ratio (g/g)	Mean number of nucleosomes	$\bar{L}_{-H5}-\bar{L}_{+H5}$ [a]	$\frac{\bar{L}_{-H5}-\bar{L}_{+H5}}{\bar{L}_{-H5}}$
0	0	0.0	--
0.2	1.8	2.65 ± 0.15	1.2 ± 0.4
0.3	2.5	0.18 ± 0.44	0.07 ± 0.18
0.45	6.6	0.85 ± 0.43	0.13 ± 0.07
0.6	12.5	0.47 ± 0.24	0.04 ± 0.02
0.75	14.7	1.45 ± 0.15	0.10 ± 0.01
0.75	17.0	0.32 ± 0.29	0.02 ± 0.02
0.8	17.0	0.30 ± 0.00	0.02 ± 0.00
0.9	17.0	0.35 ± 0.04	0.02 ± 0.00
1.1 [b]	18.2	0.35 ± 0.35	0.02 ± 0.02
0.2 [b]	5.6	2.3	0.41
0.25 [b]	7.3	0.6	0.08
0.3 [b]	7.9	1.4	0.18

[a] Measured at 37 C in 0.2 M NaCl.
[b] From ref. 13; histones were reconstituted onto closed circular SV40 DNA, which is 5243 bp in length.

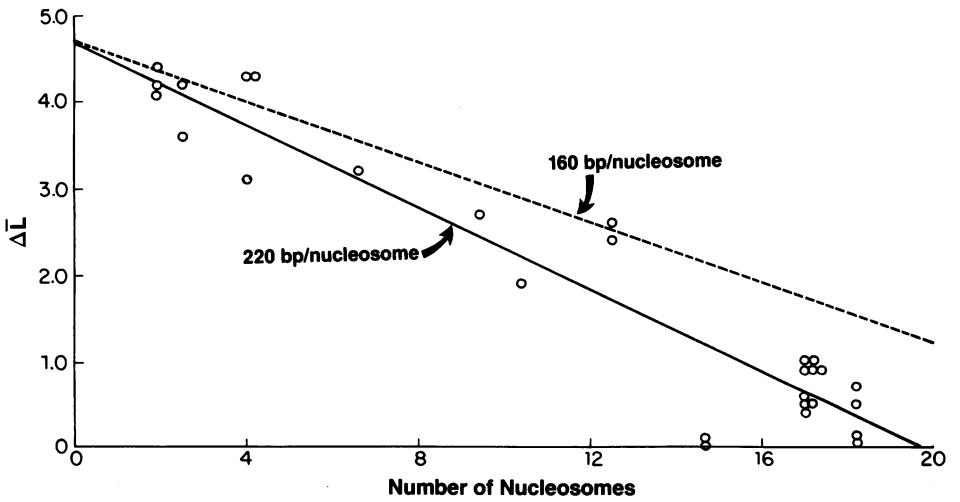

Figure 5. Difference in linking number, $\Delta\bar{L}$, at 20 C, between minichromosomes containing histone H5 relaxed at 4 C and 37 C, plotted against the mean number of nucleosomes on the minichromosome. Each circle represents combined data from two or more gel electrophoretic analyses of a single reconstituted minichromosome.

correlation with the number of nucleosomes present on the minichromosome.

The length of DNA constrained from thermal untwisting in nucleosomes containing histone H5 was determined in parallel experiments. Figure 5 shows the difference in mean linking number $\Delta\overline{L}$ between H5 containing minichromosomes relaxed with nicking-closing extract at 4 C and 37 C as a function of the mean number of nucleosomes present. The data fit well to the indicated straight line, with $P < 0.001$. The slope of the line leads to a value of 222 bp of DNA constrained from thermal untwisting per nucleosome, with an error of ± 0.025, corresponding to ± 25 bp. The length of DNA constrained from thermal untwisting in nucleosomes containing histone H5 is therefore considerably greater than the length protected from digestion by micrococcal nuclease, as was also found to be the case for core particles, where the thermally constrained length was measured to be 230 ± 20 bp.

DISCUSSION

Role of the histone tails

Previously, we have shown that 230 ± 20 bp of DNA is prevented from thermal untwisting in the nucleosome. This implies that not only the 146 bp of core DNA is torsionally constrained, but also up to 84 bp of linker DNA is held at constant twist, possibly by virtue of association with the histone proteins (10). We have attempted to address the nature of that association by measuring the thermal untwisting of DNA in minichromosomes following trypsinization. When nucleosome core particles or chromatin are treated with trypsin, cleavage at the histone amino termini (and the C-termini of H2A and H3) results in the generation of a discrete set of limit peptides, comprising about two-thirds of the original histone mass, which still allow the basic structure of the nucleosome core particle to be maintained (24; reviewed in 29). Our expectation was that trypsinization of minichromosomes would lead to an increase in $\Delta\overline{L}$, reflecting release of the linker from the constraint against thermal untwisting. If all the linker DNA were freed to thermally untwist in a plasmid minichromosome, 84 bp of

additional free DNA would be observed per nucleosome. However, what we actually observed was that at most, about 15 bp of DNA per nucleosome were released to thermally untwist at limit trypsinization.

These results indicate that the histone termini are not implicated in the constraint of linker DNA against thermal untwisting. This is not to say that contacts between the histone termini and the DNA are non-existent, but only that those contacts are not essential to the constraint of nucleosomal DNA against thermal untwisting. Recent X-ray data on the nucleosome core particle suggest that the DNA helix beyond the 145 bp core is likely to interact with histone H2A (30). Crosslinking experiments indicate that histone H3 also interacts with the linker DNA (31). These results, in concert with our own findings, suggest that the interaction which constrains the linker from thermal untwisting must involve the central, globular portions of the core histones. Footprinting of positioned nucleosomes or crystal structures of nucleosomes containing DNA longer than 145 bp would seem to be the best way to characterize these interactions.

We also find no change in the linking number of minichromosomes following trypsinization, indicating that the supercoiling of DNA on the nucleosome is essentially unaffected by removal of the histone termini. This is in agreement with a variety of observations (micrococcal nuclease and DNase I digestion, sedimentation coefficient, and X-ray data (reviewed in 29)) which suggest that the core particle structure is preserved after trypsinization. However, evidence to the contrary also exists. For example, the CD spectrum of trypsinized core particles is intermediate between that of native core particles and free DNA in the 260-280 nm region (11, 23-25). This has been interpreted in one case as being due to a release of DNA supercoiling from the nucleosome (24). However, the CD spectrum is also likely to depend upon the helical twist of the DNA (32-33), the extreme bending of DNA around the histone octamer, and the close proximity of two DNA helices lying side by side. It therefore seems difficult to derive any unequivocal quantitative conclusions about nucleosome structure from the CD

spectrum. Other data which have interpreted to demonstrate changes in core particle structure following trypsinization include thermal denaturation measurements, which suggest exposure of a fraction of the DNA such that it melts similarly to free DNA (25), and NMR experiments, which indicate that the histone termini are associated with the core particle below 0.35 M NaCl (34). However, these data do not really conflict with an intact core particle structure, as the NMR only monitors a select group of histone amino acid residues which need not reflect overall nucleosome structure, and the thermal denaturation measurements are done at elevated temperatures not necessarily relevant to the structure at 37 C and below. In sum, the direct measurement of the invariance of linking number in closed circular minichromosomes following trypsinization provides compelling evidence, in spite of the observations just discussed, that the histone tails do not contribute to the supercoiling of DNA in core particles. Rather, the tails are probably involved in the higher order organization of chromatin (35, and as discussed in 29).

Role of histone H5

Our measurements of the change in linking number brought about by addition of histone H5, summarized in Table II, are in fairly good agreement with those of Stein (18), but our interpretation differs. Stein reports linking number increases of 13-41% upon addition of H1 + H5 to reconstituted SV40 minichromosomes at low nucleosome densities. At higher nucleosome densities, the observed shifts in $\overline{L}$ are smaller, and Stein argues that close packing of core particles may be interfering with correct reconstitution of H1 + H5. However, we rarely see DNA fragments corresponding to nucleosome dimers protected from micrococcal nuclease digestion at less than 10 nucleosomes per pBR322 molecule, and even at higher nucleosome densities and early times of digestion, dimer fragments are very weak if visible at all (see Fig. 4, for example). Furthermore, histone H5 can be added to close-packed nucleosomes in the presence of polyglutamic acid to regenerate chromatin containing linker of normal lengths (36). We therefore doubt that crowding would hinder the association of histone H5 with the core

particles of our minichromosomes. On the contrary, we believe that histone H5 reconstitutes correctly onto the majority of the core particles, on the basis of both the micrococcal nuclease protection pattern (Fig. 4) and the recovery of histone H5 from minichromosomes eluted over Sephadex G50 spin columns. Moreover, the results of Stein and Bina (14) indicate that the levels of histone H5 used in our procedure (3-4 mol histone H5 per nucleosome) should result in the addition of about one molecule of H5 per nucleosome.

From the data in Table II, then, it appears that the binding of histone H5 causes at most a slight increase in the uptake of negatively supercoiled DNA by the nucleosome. This would argue against the notion that the binding of histone H5 (or H1) to the nucleosome could cause a unit change in the linking number of the DNA (18). A smaller change in supercoiling, such as that which would result from histone H5 conferring an additional 1/4 turn in the DNA around the nucleosome, cannot be ruled out as the uncertainties in our data are too large.

The data summarized in Fig. 5 show that, when histone H5 is present, about 220 bp of DNA per nucleosome are constrained from thermal untwisting. This is a value not much different from that found for core particles (10), and is still considerably longer than the size of the fragment resistant to micrococcal nuclease. It therefore seems that the interaction between the histones and the linker DNA is not greatly altered by addition of histone H5. This suggests to us that the function of histone H5 (and H1) is not so much to organize the linker DNA as to catalyze the condensation of chromatin into higher order structures. This view seems to us entirely consistent with recently proposed models for the higher order structure of chromatin (37).

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