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DNA wrapping in nucleosomes. The linking number problem re-examined

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ABSTRACT

Chromatin was assembled in vitro from relaxed closed circular DNA (SV40) and core histones at histone to DNA ratios of 0.2 to 0.3 (g/g) and incubated with topoisomerase I to relax supercoils in DNA regions not constrained by protein. Addition of histones H1 + H5 to the chromatin at an ionic strength of 0.1 M, in the presence of the solubilizing agent, polyglutamic acid, and topoisomerase I, increased the magnitude of the DNA linking number change, relative to protein-free DNA. No change in the linking number distribution occurred for relaxed protein-free DNA under these conditions. Control experiments indicated that the increase in the absolute value of the DNA linking number change in the chromatin could not be attributed to an increase in the number of nucleosomes per DNA molecule. These data suggest a solution to the linking number problem associated with models of chromatin structure.

INTRODUCTION

In order to understand the details of the higher levels of DNA compaction in chromatin, it is first necessary to know how the DNA is coiled in a contiguous chain of chromatin subunits. It is here, however, where a bothersome apparent inconsistency exists. Briefly stated, the problem is that many structural (1-5) and biochemical (6-10) studies suggest that DNA is coiled twice around each histone octamer, whereas measurements with both reassembled (11) and native, H1 associated (12), SV40 minichromosomes indicate that there is nearer to one topological turn (linking number change of -1) of DNA per nucleosome. This apparent conflict could be resolved by postulating that the periodicity of DNA in nucleosomes differs from that in solution (13,1). However, the periodicity of the lengths of single strand DNA fragments produced by DNAase I digestion of chromatin

(14) or core particles (15), thought to reflect the periodicity of nucleosomal DNA (16), is not significantly different from the periodicity of protein-free DNA in solution (17). Alternatively, chromatin models having less than two topological turns of DNA around each histone octamer, under certain circumstances could be proposed (18).

Although SV40 minichromosomes can be isolated associated with histone H1 (19), and the DNA linking number for these molecules has been found to be the same as in minichromosomes devoid of H1 (12), it has not been established that the chromatin structure in SV40 is the same as in H1-containing cellular chromatin. In fact, micrococcal nuclease digestion studies indicate that the "linker regions" in SV40 minichromosomes are highly variable, in contrast with the very ordered arrangement of nucleosomes in cellular chromatin (20,21). Therefore, I have re-examined the effect of histones H1 + H5 on the topology of DNA in nucleosomes; H1 does not impose torsional constraints on protein-free relaxed closed circular DNA (22).

MATERIALS AND METHODS

Simian virus 40 (SV40) DNA I was obtained from Bethesda Research Laboratories (Rockville, Md.), SV40 DNA component Ir was prepared as described by Bina and Singer (22), salt extracted core histones were prepared from chicken erythrocyte chromatin as previously described (23), poly (L-glutamic acid) of average molecular weight 100,000 was obtained from Miles-Yeda, and the relaxing extract containing topoisomerase I, prepared from LA 9 cells by the method of Germond et al. (11), was a gift from Dr. M. Bina. Histones H1 + H5 were extracted from chicken erythrocyte nuclei, which had been washed several times with 0.3 M NaCl, with 5% perchloric acid, adjusted to 0.1 N HCl, precipitated with acetone, dried under vacuum and dissolved in 0.1 M NaCl, 10 mM Tris-HCl, pH 8.0, 0.1 mM phenylmethyl sulfonyl fluoride. These histones were found to be intact and free of other proteins by sodium dodecyl sulfate gel electrophoresis. The H1 + H5 concentration was measured by absorbance at 280 nm, using an $A_{280}^{1\%}$ value of 2.2 (24). Fluorography was performed as described by Laskey et al. (25). Chromatin was assembled from

relaxed SV40 DNA (Ir) and core histones by a salt-step procedure from 2 M NaCl (26), the final DNA concentration was 5 μg/ml, and the final buffer contained 0.1 M NaCl, 10 mM Tris-HCl, pH 8.0, 0.1 mg/ml bovine serum albumin (Miles Pentex). This chromatin was incubated at 37°C for 30 min with relaxing extract containing topoisomerase I (2 μl/μg DNA) and then divided into aliquots for incubations with additional components. Histones H1 + H5 were premixed with polyglutamic acid before addition to chromatin solutions; the final polyglutamic acid concentration was 25 μg/ml and the molar ratio of H1 + H5 to core histone octamer was 10 in all samples to which these components were added. Chromatin samples were incubated at 37°C for 1 hr ± polyglutamic acid ± (H1 + H5) ± additional core histones, and the DNA was purified by sodium dodecyl sulfate-phenol extraction and precipitation with ethanol.

RESULTS

Both the 165 base pair micrococcal nuclease digestion barrier (27,28) and the "zig-zag" morphology (4) characteristic of native chromatin can be resotred to H1-depleted cellular chromatin by addition of H1. This suggests that H1 can correctly interact with nucleosomes *in vitro*. Whether nucleosomes that are associated with H1 (or H5) in a way similar to that in cellular chromatin have an increased absolute linking number change, $|\Delta L|$, can be tested by adding H1 + H5, in the presence of topoisomerase I, to amply spaced nucleosomes contained in a closed circular DNA. Closed circular DNA molecules extracted from nucleohistone complexes are topoisomers, with linking numbers reflecting the number of nucleosomes per DNA molecule and the linking number change per nucleosome (11). These topoisomers can be resolved by agarose gel electrophoresis, and the relative amount of each topoisomer can be measured (29, 30).

There are several reasons, however, why the mere addition of H1 (or H5) and topoisomerase I to the nucleoprotein might not be expected to efficiently alter the topological state of the DNA. First, the nicking-closing activity of topoisomerase I (31) and, possibly, proper H1 (or H5)-nucleosome interactions (4,28)

require an ionic strength in the physiological range (about 0.1 M), and at this ionic strength, reassembled chromatin generally aggregates and precipitates in the presence of H1 (or H5). Additionally, the presence of H1 (or H5) substantially hinders the relaxation of even naked superhelical DNA (22) and may form intramolecular crosslinks in DNA (22) and in nucleoprotein complexes (32). Therefore, to increase the efficiency of the postulated reaction, I have added the additional component, polyglutamic acid, to serve as a solubilizer. Polyglutamic acid greatly facilitates the assembly of chromatin from DNA and core histones at physiological ionic strength (33). Moreover, small amounts of this acidic polypeptide, less than that required to extract H1 (or H5) histones from chromatin, render soluble H1 + H5 containing chromatin in 0.1 M NaCl (Künzler & Stein, unpublished observation).

Fig. 1 shows the results of such an experiment. As described above, a single chromatin sample was assembled from relaxed SV40 DNA and core histones (0.2-0.3 g/g DNA), incubated with topoisomerase I at an ionic strength of 0.1 M, and divided into portions for incubations with additional components. Scan (a) indicates that the chromatin sample contained a distribution of nucleoprotein species with a mean ΔL value per DNA molecule of approximately -7, consistent, within experimental uncertainties, with the amount of histone added to the DNA (0.25 g/g DNA). Thermal fluctuations in the DNA backbone at the time of closure also influence the observed distribution in a random way (29,34). Scan (b) shows that further incubation of this chromatin with polyglutamic acid results in a slight shift in the distribution to lower absolute ΔL values. In contrast, scan (c) shows that upon incubation of the chromatin with both polyglutamic acid and H1 + H5 histones, the distribution shifts, significantly, to higher absolute ΔL values; compare, for example, the relative intensities of bands 12 and 4 in (c) with those in either (a) or (b).

Fig. 2 shows the H1 + H5 induced shifts in the distribution of topoisomers from two additional independent experiments, one at a slightly lower and the other at a slightly higher histone to DNA ratio. At still lower histone to DNA ratios,

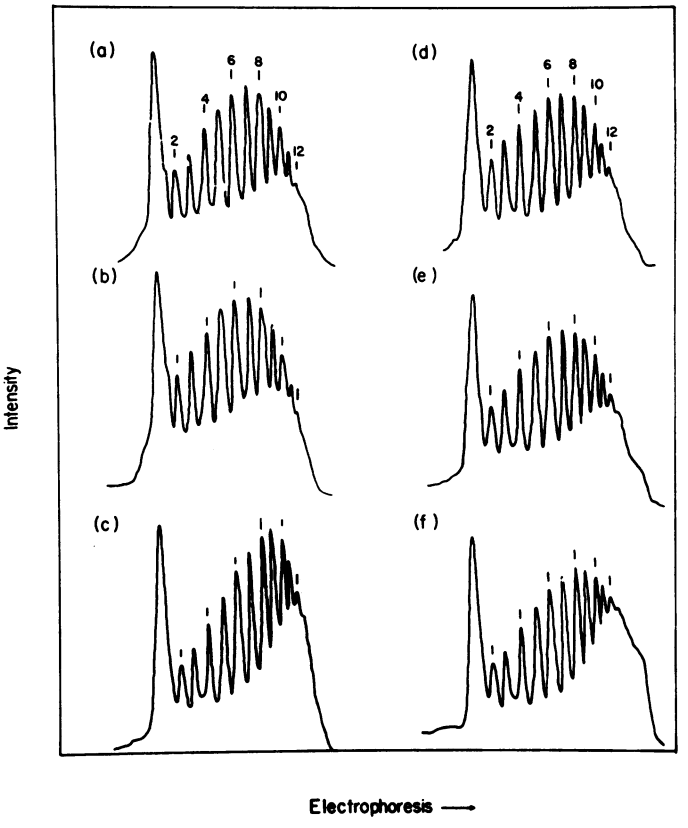

Figure 1. Alteration of the linking number distribution of SV40 DNA extracted from in vitro assembled chromatin that was incubated with H1 + H5 and polyglutamic acid in the presence of topoisomerase I, and control experiments. The densitometer scans of agarose gels show linking number distributions for (a) untreated chromatin that was assembled at a core histone to DNA ratio of 0.25 (g/g), (b) this chromatin incubated with polyglutamic acid, (c) incubated with H1 + H5 and polyglutamic acid, (d) incubated with 25% additional core histones, (e) 50% additional core histones, (f) 100% additional core histones. The numbers (and marks) above bands denote $|\Delta L|$ values, which were determined as described in the text.

topoisomers resulting from thermal fluctuations around $\Delta L = 0$ (naked closed circular DNA) interfere with the experiment. Also, at higher histone to DNA ratios, the magnitude of the shift in the distribution decreased (see below for an explanation). Moreover, in the absence of polyglutamic acid, the

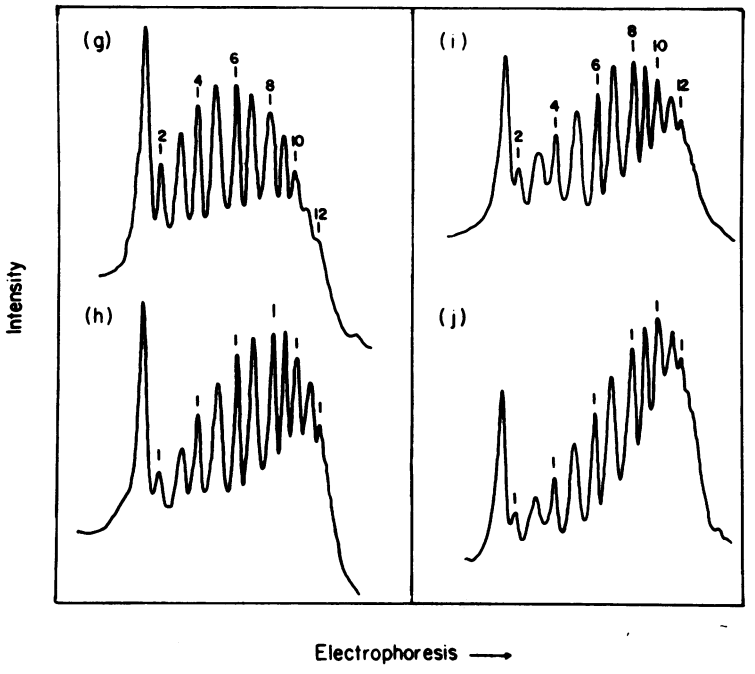

Figure 2. Additional experiments showing the shift in the distribution of DNA topoisomers resulting from the addition of H1 + H5 to chromatin assembled from core histones and SV40 DNA. The samples are (g) untreated chromatin that was assembled at a core histone to DNA ratio of 0.20 (g/g), (h) this chromatin incubated with H1 + H5 and polyglutamic acid; (i) untreated chromatin that was assembled at a core histone to DNA ratio of 0.3 (g/g) and incubated 1 hr at 37°C, (j) this chromatin incubated with H1 + H5 and polyglutamic acid. The low mobility band of greatest intensity arises from nicked DNA (form II).

shift was barely discernible.

The results of a numerical analysis of distributions a - c, f, and g - j are presented in Table 1. $|\Delta L|$ values were measured relative to the linking number of naked closed circular SV40 DNA, relaxed under identical conditions. This reference DNA ($\Delta L = 0$) electrophoreses, under the conditions chosen here, with a mobility slightly lower than that of Form II (nicked) DNA (Fig. 4 and unpublished observations). Thus, these electrophoreses conditions do not alter the twist number of DNA to a significant extent, relative to the incubation conditions.

Table 1. Analysis of Topoisomer Distributions

	Mode	Mean	Degree of Skewness	Fraction of Topoisomers with |ΔL| greater than 4,6,8 and 10			
a	7.0	7.3	+0.11	0.75	0.53	0.32	0.15
b	6.5	7.0	+0.07	0.73	0.48	0.26	0.11
c	9.0	7.9	+0.83	0.81	0.62	0.42	0.21
f	8.2	9.9	-0.11	0.86	0.69	0.53	0.35
g	5.6	5.6	-0.30	0.72	0.51	0.30	0.14
h	9.0	7.9	+0.98	0.81	0.63	0.40	0.20
i	8.0	7.9	+0.29	0.77	0.61	0.40	0.22
j	10.0	9.3	+1.72	0.88	0.74	0.50	0.20

ΔL values were assigned by counting the bands, starting from the position of the reference DNA. Only closed circular DNA molecules with $|\Delta L| \geq 1$ were included in the analysis. The fraction of nicked DNA was estimated to be 7% from the experiments performed at high core histone to DNA ratios (not shown). An additional correction of 8.7% was applied in distributions a - f to account for the amount of tracer DNA added.

The mode, mean and degree of skewness of the distributions were measured. The mode is the interpolated maximum peak intensity. The mean was calculated as $\sum_{i>1} i\, I_i / \Sigma I_i$, where I_i is the intensity of the band with $|\Delta L| = i$; mobilities of the poorly resolved peaks with $|\Delta L|$ values greater than 13 were determined from linear plots of log $|\Delta L|$ versus mobility. The degree of skewness is defined as $\frac{A_L}{A_R} - 1$ where A_L and A_R are the areas under the curve to the left and right of the mode of the distribution. Additionally, the number fractions of topoisomers with $|\Delta L|$ values greater than 4, 6, 8 and 10 were calculated from the curve areas.

This analysis indicates that in the absence of additional histones the distributions are nearly symmetrical (approximately Gaussian). Upon addition of H1 + H5, the distributions shifted to higher $|\Delta L|$ values and became significantly skewed to the right. These changes are characterized numerically in Table 2.

Table 2. Change in Initial Distribution Upon Incubation with Additional Histones

	Mode(%)	Mean(%)	Degree of Skewness
(c) with respect to (b)	+38	+13	+0.76
(f) with respect to (b)	+26	+41	-0.18
(h) with respect to (g)	+60	+41	+1.3
(j) with respect to (i)	+25	+18	+1.4

The largest shift in the mode (60%) and the mean (41%) occurred for distribution (h) relative to (g), the experiment with the lowest core histone to DNA ratio. A slightly larger shift in the mean $|\Delta L|$ value (50%) was obtained when distribution (h) was compared with that of the chromatin sample incubated in the presence of polyglutamic acid (not shown). Although the mean $|\Delta L|$ value for distribution (c) increased only by 13%, the ΔL value of the topoisomer most abundant (given by the mode of the distribution) increased by 38%.

In order to provide direct evidence that, under these conditions, H1 + H5 interacts with nucleosomes, the interaction was probed by micrococcal nuclease digestion. Since considerably larger amounts of material were required, chromatin was assembled from core histones and high molecular weight chicken erythrocyte DNA at the same histone to DNA ratio (0.25 g/g) as with SV40 DNA. H1 + H5 was then added to the chromatin under the same conditions as used above, except that topoisomerase I was not included (since the DNA was not closed circular). Fig. 3 shows that a distinct pause occurred in the digestion with a lower cut-off of about 170 base pairs for the sample that contained H1 + H5. The control sample, not containing H1 + H5, was digested rapidly to 145 base pairs, and does not shows a pause at 170; lanes 3 and 5 were digested for the same length of time. This suggests that many of the nucleosomes were converted to "chromatosome"-like particles, characteristic of cellular chromatin (10,27,28), upon addition of H1 + H5. The greater amount of higher molecular weight DNA remaining, at equivalent digestion times, for the H1 + H5 containing sample probably results, in part, from DNA

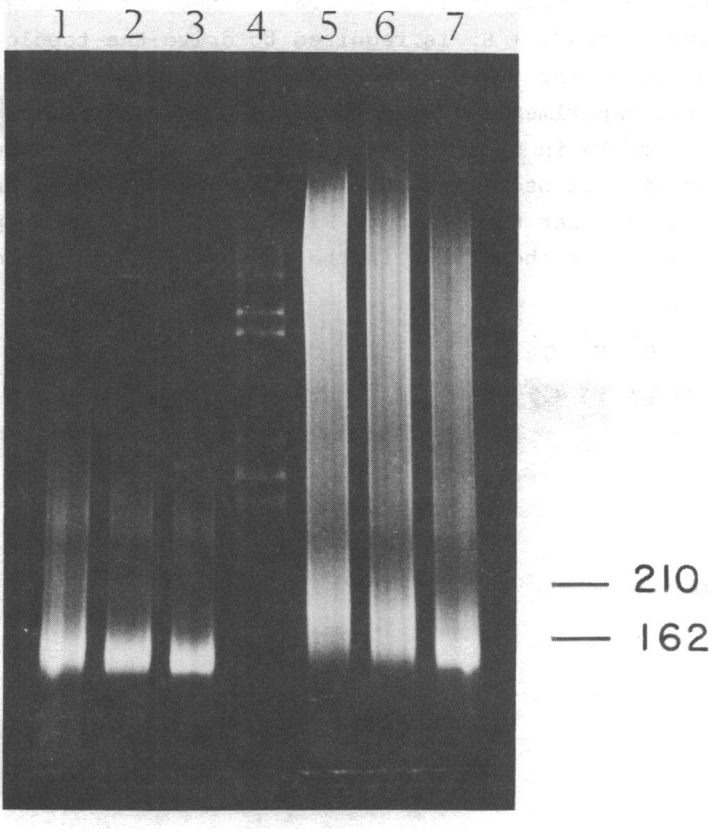

Figure 3. DNA fragments remaining after micrococcal nuclease digestion of in vitro assembled chromatin (0.25 g histones/g DNA) in the presence of polyglutamic acid, 1-3, and in the presence of H1 + H5 and polyglutamic acid, 5-7. Samples were digested at room temperature in the incubation buffer containing, in addition, 2 mM $CaCl_2$ and 0.4 units enzyme (Worthington)/µg DNA for: (1) 30 sec, (2) 1 min, (3) 2 min, (5) 2 min, (6) 4 min, (7) 8 min. Lane (4) shows a Hin c II (New England Biolabs) digest of ϕX174 RF DNA (Bethesda Research Labs). The DNA was electrophoresed on a 5% polyacrylamide gel as described in reference (10).

interactions with excess H1 + H5. A ratio of about 10 moles of H1 + H5 per mole of inner histone octamers was found to produce the largest shift for the polyglutamic acid concentration used here. Presumably, both polyglutamic acid and regions of naked DNA compete with nucleosomes for H1 (or H5). Even in native chromatin, H1 (and H5) readily exchanges onto naked DNA at an

ionic strength of 0.1M at 37°C (35,28). Thus, it is reasonable that an excess of H1 + H5 is required to drive the topological alteration reaction.

Control experiments were performed to assess whether H1 + H5 addition could in some indirect way lead to an increase in the number of nucleosomes per DNA molecule, rather than an increase in $|\Delta L|$ per nucleosome. First, the gel photograph on the left in Fig. 4 shows that in the presence of topoisomerase

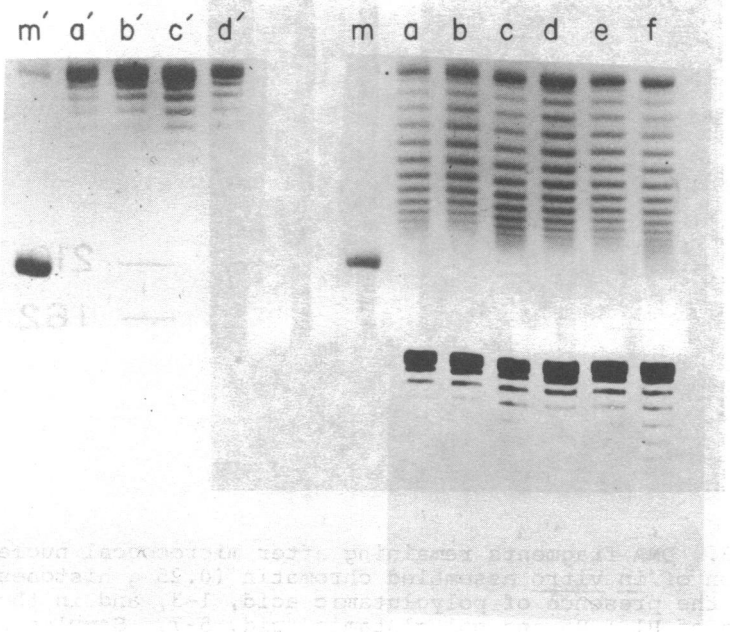

Figure 4. Agarose gels of DNA topoisomers. The upper photograph on the right shows the ethidium bromide stained agarose gel corresponding to scans (a)-(f); (m) is a SV40 DNA I marker. The lower photograph on the right shows a fluorogram prepared from the above gel. Electrophoresis was from top to bottom. The photograph on the left shows an agarose gel with samples: (m') untreated SV40 DNA I, (a') SV40 DNA I was incubated with topoisomerase I for 1 hr at 37°C in 0.1 M NaCl, 10 mM Tris-HCl, pH 8.0, 0.1 mg/ml bovine serum albumin, (b') relaxed SV40 DNA, prepared as in (a'), was incubated an additional hr to show that 1 hr was sufficient for complete relaxation, (c') relaxed SV40 DNA, prepared as in (a'), was incubated 1 hr with topoisomerase I, H1 + H5 (0.6 g/g DNA) and polyglutamic acid, (d') Form I SV40 DNA, to which was added H1 + H5 (0.6 g/g DNA) and polyglutamic acid, was incubated 1 hr with topoisomerase I.

I and polyglutamic acid, H1 + H5 does not induce supercoiling of relaxed SV40 DNA. Additionally, under these conditions, supercoiled SV40 DNA (form I) is efficiently relaxed, showing that the presence of polyglutamic acid allows the topological state of the DNA to be changed in the presence of H1 + H5, and that H1 + H5 does not stabilize pre-existing superhelical turns in naked DNA. These results indicate that the linking number shift induced by H1 + H5 cannot be attributed to direct interactions with the protein-free regions of DNA in the chromatin.

To determine whether small amounts of additional core histones could produce a shift in the distribution comparable to that in Fig. 1, scan (c), additional core histones were added to the chromatin in place of H1 + H5; the concentrations of all other components were the same as when H1 + H5 was used. Fig. 1, scans (d) - (f) show the linking number distribution shifts resulting from 25, 50 and 100% additional core histones. The scans (Fig. 1) and the gel photograph (Fig. 4, upper right) show that additional core histones result in a broadening of the distribution of topoisomers, in marked contrast to the rightward skewing observed with H1 + H5 (compare c with f). Approximately 35% of the topoisomers in (f) have $|\Delta L|$ values greater than 10, as compared with 15% in (a), 11% in (b), or 21% in the skewed distribution (c) (see Table 1). In fact, distribution (f) is skewed slightly to the left (Table 1). The appearance of distribution (f) is easily explained if core histones strongly prefer to assembly on DNA molecules that have greater numbers of nucleosomes. A cooperative mode of assembly, in the presence of polyglutamic acid, was observed previously (33). Also, since 10 times the amount of additional core histones are required to produce a topoisomer with $|\Delta L| = 20$ from one with $|\Delta L| = 10$ than to produce a topoisomer with $|\Delta L| = 2$ from one with $|\Delta L| = 1$, the initial distribution appears insensitive to small additions of core histones (Fig. 1 and 4, d and e) when observing only DNA. This control strongly suggests that the H1 + H5 induced shift does not result from an increased availability of small amounts of unassembled core histones, possibly in the sample tube.

In order to test whether core histones participate in a

dissociation-reassociation equilibrium, which could be influenced by histones H1 + H5, a ^{3}H-labeled SV40 DNA (form I) tracer was added to the assembled chromatin after the ionic strength had been lowered to 0.1 M and just before the incubation with topoisomerase I. Thus, if core histones transiently dissociate from DNA, exchange should occur, and the tracer DNA should acquire a distribution of linking numbers similar to that of the unlabeled DNA. On the other hand, if the histones are irreversibly bound to the DNA in the assembled chromatin (until the deproteinization step), the tracer DNA should appear relaxed. Comparison of the fluorogram (Fig. 4, lower right) with the stained gel (upper right) clearly indicates that the latter is the case; core histone exchange does not occur at 0.1 M ionic strength, consistent with the findings of others (36), and polyglutamic acid (with or without H1 + H5) does not induce exchange of nucleosomal histones. Also, the tracer DNA served as an internal control that only DNA molecules associated with core histones undergo topological alteration in the presence of H1 + H5. The lack of detectable supercoiling of the tracer DNA induced by low amounts of additional core histones (fluorogram, d and e) is again consistent with the strong preference of additional core histones for DNA molecules containing greater numbers of nucleosomes, discussed above. Essentially complete supercoiling of the tracer DNA occurred at a total core histone to DNA weight ratio of 1.0, a 300% excess (not shown).

DISCUSSION

Evidence has been presented that histones H1 + H5 can alter the topology of a closed circular DNA that contains nucleosomes (in the presence of topoisomerase I). The stabilization of 2 complete turns of DNA around the inner histone core by histone H1 (or H5) has been suggested by Simpson (10), based upon the results of DNAase I mapping studies; nucleosomes with H1 (or H5) bound at the coincident DNA entry and exit points were termed chromatosomes. This role for H1 (or H5) was also proposed by Thoma et al., (4) based upon morphological comparison studies of H1 - containing and H1 - depleted chromatin. H1 - containing chromatin appeared to have coincident DNA entry and exit points

in nucleosomes, whereas, H1 - depleted chromatin did not (4). Also, Nöll et al., (38) have shown that chromatin assembled in vitro from DNA and core histones appears, morphologically, to have less than 2 full turns of DNA per octamer. Thus, an additional coiling of DNA around each octamer from about 1 3/4 turns to 2 full turns could provide the mechanism for the topological alteration induced by H1 (or H5), although a few other mechanisms could be imagined.

If DNA in adjacent nucleosomes is coiled in a continuous helical fashion, the increase in the mean $|\Delta L|$ value of the topoisomer distribution, upon completion of 2 full turns (in the presence of topoisomerase I), would be expected to be approximately 14% (0.25/1.75). A smaller increase in the mean $|\Delta L|$ would be expected if not every nucleosome interacted correctly with H1 (or H5). Since increases in the mean $|\Delta L|$ as high as 40 - 50% were observed (Table 2), this model is not completely satisfactory.

There is another way of wrapping DNA in adjacent nucleosomes, however, which is very consistent with the observations made here and by others. Fig. 5 shows a rope model depicting the DNA path in 3 nucleosomes devoid of H1 (or H5) and in 2 "chromatosomes" (marked with arrows). Note that if the coils are placed such that their cylindrical axes are perpendicular to the plane of the paper (as in the pair at the lower right), and the stretches of DNA between nucleosomes are not twisted, the DNA winds alternatively downward and upward in adjacent nucleosomes. Such models have been proposed by others (4,18,37,38). Of interest here is the fact that for such nucleosomes, devoid of H1 (or H5), with about 1 3/4 turns of DNA around each octamer, the value of ΔL per nucleosome is close to -1 even though the DNA is wrapped 1 3/4 turns around each octamer. This can be easily verified using a ribbon (39). As pointed out by Crick (39), the value of ΔL per nucleosome is strongly influenced by the way DNA exits from each core. Additionally, completion of an additional 1/4 turn (for 2 complete turns) around each core, as in H1 - associated nucleosomes, must now be accompanied by the introduction of one negative superhelical turn per nucleosome, for a closed circular DNA. Consequently, in the presence

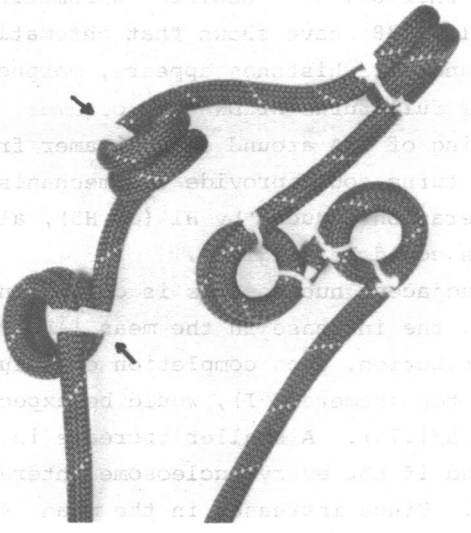

Figure 5. Model illustrating the DNA wrapping in chromatosomes (the 2 nucleosomes denoted by arrows) and in nucleosomes not associated with H1 (or H5) (other 3). The spacing and orientation of the nucleosomes are arbitrary.

of topoisomerase I, this will lead to a ΔL value close to -2 per nucleosome.

To examine whether this hypothesis is really consistent with the topological alteration observed, simulated experiments were performed. Fig. 6, A, shows the type of topoisomer shift expected if every nucleosome had undergone a change in ΔL from -1 to -2. The reaction is shown in two steps, for the purpose of illustration. The first step ignores thermal fluctuations at the time of DNA closure, and the second step simulates the effect of these fluctuations by the conversion of 25% of each topoisomer into the topoisomers with ΔL differences of ± 1. The net effect is a shift in mode and mean of the distribution by a factor of 2, along with a substantial broadening, and necessarily, a decrease in the intensity of the new maximum relative to that in the initial distribution. This differs from what was observed.

It is very reasonable, however, to assume that not every nucleosome ($\Delta L = -1$) was converted into a "chromatosome"

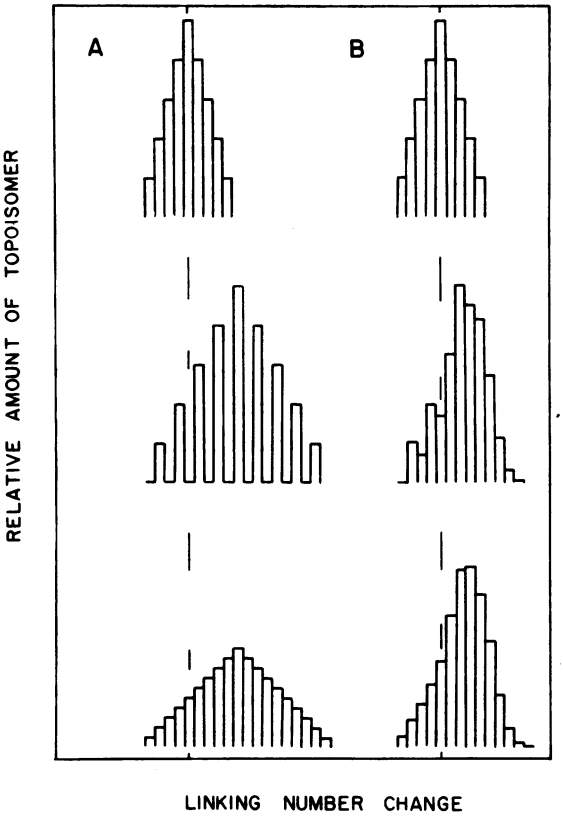

Figure 6. Simulated experiments. (A) Topoisomer distribution shift expected if every nucleosome undergoes a change in ΔL from -1 to -2. (B) Topoisomer distribution shift expected if the probability of the completion of 2 full DNA turns per nucleosome decreases with the number of nucleosomes per DNA molecule.

(ΔL = -2). In vitro assembled nucleosomes tend to close pack (40-42) and it seems reasonable to suppose that this would hinder the completion of two full turns of DNA around inner histone cores. Also, since a greater fraciton of the nucleosomes should be close packed on DNA molecules that contain greater numbers of core histone octamers, the probability for a successful H1 (or H5) - nucleosome interaction should decrease with the number of nucleosomes per DNA molecule. Fig. 6, B, shows the topoisomer shift that would occur if the probability of a successful H1 (or

H5) - nucleosome interaction decreased, for example, linearly, from unity, for a DNA molecule with a single nucleosome, to zero, for a DNA molecule with 10 nucleosomes. With these hypothetical single nucleosome probabilities, the probability of the conversion of one topoisomer into another was calculated using elementary probability theory. Analysis of the distributions in Fig. 6, B, shows that the mode increased from 5 to 8 (60%), the mean increased from 5 to 7.3 (46%), and the degree of skewness increased from zero to +0.36. These values are similar to those measured for distribution (h) relative to (g) (see Table 2). Also, a probability for the completion of 2 full DNA turns around inner histone cores that decreases with the number of nucleosomes present is consistent with the observation that the topoisomer shift by H1 + H5 is very inefficient at high core histone to DNA ratios, such as those in native SV40 minichromosomes. Moreover, in addition to this effect, it is likely that some nonspecific interactions involving H1 (or H5) persist which could hinder the topological alteration. Therefore, this simulated experiment affirms the plausibility of the model proposed and suggests an explanation for the skewing of the distributions.

The hypothesis proposed here for the topological alteration of nucleosomal DNA by H1 (or H5) provides a solution to the linking number problem; the value of ΔL per nucleosome in H1 (or H5) - containing cellular chromatin is approximatly -2, whereas, the ΔL value for nucleosomes devoid of H1 (or H5) is close to -1. Also, a necessary implication of this hypothesis is that the mode of association of H1 with SV40 chromatin likely differs from that in cellular chromatin.

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