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## Chromatin Reconstitution on Small DNA Rings

### II. DNA Supercoiling on the Nucleosome

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DNA supercoiling on the nucleosome was investigated by relaxing with topoisomerase I mono- and dinucleosomes reconstituted on small DNA rings. Besides 359 base-pair (bp) rings whose linking differences were integers, two additional series of rings with fractional differences, 341 and 354 bp in size, were used. Mononucleosomes reconstituted on 359 bp rings were found to relax into a single mononucleosome form. In contrast, 341 and 354 bp mononucleosomes relaxed into a mixture of two forms, corresponding to two adjacent topoisomers. The observation that the ratio between these two forms was, within each ring series, virtually independent of the initial linking number of the topoisomer used for the reconstitution suggested that each partition reflected an equilibrium. Comparison with the equilibria observed for the same rings in the absence of histones showed that the formation of a single nucleosome is associated with a linking number change of  $-1.1(\pm 0.1)$  turn. Dinucleosomes, in contrast, were not relaxed to completion and do not reach equilibria. The corresponding linking number change per nucleosome was, however, estimated to be similar to the above figure, in agreement with previous data from the literature obtained with circular chromatins containing larger numbers of nucleosomes.

DNA structure in mononucleosomes was subsequently investigated by means of high-resolution electron microscopy and gel electrophoresis. It was found that the above linking number reduction could be ascribed to a particle with a large open extranucleosomal DNA loop and with no more than 1.5 turns of a superhelix around the histone core. A theoretical model of a nucleosome on a small ring was constructed in which one part of the DNA was wrapped around a cylinder and the other part was free to vary both in torsion and flexion. The linking number reduction predicted was found to be most consistent with experimental data when the twist of the DNA in the superhelix was between 10.5 and 10.65 bp per turn, suggesting that wrapping on the nucleosome does not alter the twist of the DNA significantly. A lower estimate of the linking number reduction associated with a two-turn nucleosome was also derived, based on an analysis of recent data obtained upon treatment of reconstituted minichromosomes with gyrase. The value, 1.6 turns, set a lower limit of 10.44 bp per turn for the twist of nucleosomal DNA, in agreement with the above estimate. Reasons for the discrepancy between these results and the sequence periodicity of 10.2 bp recently found for nucleosomal DNA are discussed in terms of the different reference frames, absolute and local, respectively used for the two measurements.

#### 1. Introduction

A detailed knowledge of the superstructure of the chromatin fibre and of the way nucleosomes

interact with each other is essential for understanding the dynamics of the chromosome and, in particular, the process of chromatin decondensation, a prerequisite to gene expression. The simian

virus 40 (SV40) minichromosome, and circular chromatins in general, may be convenient models to address these questions because they permit determination of the linking number of the DNA double helix, a topological parameter that is expected to depend both on the path of the DNA inside each nucleosome and on the arrangement of nucleosomes relative to one another.

In this respect, an enduring problem of the SV40 minichromosome has been its linking number paradox. Form I SV40 DNA is known to have, relative to the relaxed form, a deficit of its linking number of about 24 to 26 turns; that is, it contains 24 to 26 negative titratable superhelical turns (Keller, 1975; Shure & Vinograd, 1976). At the same time, the native minichromosome from which the DNA originates contains 21 to 26 nucleosomes (Griffith, 1975; Germond *et al.*, 1975; Saragosti *et al.*, 1980). Such an approximately one-to-one relationship, which implies that each nucleosome reduces, on average, the linking number by about one turn, is in apparent contradiction with the DNA in an H1-containing nucleosome making two turns of a left-handed DNA superhelix (Finch *et al.*, 1977; Richmond *et al.*, 1984; Thoma *et al.*, 1979). In one hypothesis, the origin of the discrepancy has been thought to originate from the DNA that connects nucleosomes to each other. Linker DNA may indeed be bent or twisted *in vivo* or be arranged in a particular way in one nucleosome relative to the other (Worcest *et al.*, 1981), so as to increase the linking number by one turn. This increase should be subtracted from the putative two-turn reduction per nucleosome, which would result in the observed figure. In a second hypothesis, the DNA wrapped around the histone octamer has been believed to be overtwisted. Such overtwisting, if resulting in one full helical overturn along the approximately 160 bp<sup>†</sup> of nucleosomal DNA, would indeed be sufficient for the two turns of the left-handed DNA superhelix to reduce the linking number by only one turn (Finch *et al.*, 1977).

Early attempts to confirm the possibility that nucleosomal DNA might be overwound were made by measuring its cleavage periodicity with nucleases: 10.4 and 10.1 bp were obtained with DNase I (Prunell *et al.*, 1979; Lutter, 1979) and exonuclease III (Prunell, 1983), respectively. The latter estimate, when compared to a twist of free DNA in solution of 10.5 to 10.6 bp per turn, supported the overtwist hypothesis. That the former estimate did too was argued by Klug & Lutter (1981) on the basis of a restricted accessibility of the DNA to the DNase. As these experiments made it clear, however, unknown features of the nucleosome surface could influence the cleavage. Fortunately, an alternative route was opened more recently by the direct confirmation (Drew & Travers, 1985) of an older theoretical

model (Trifonov & Sussman, 1980) proposing that sequence-dependent unidirectional bending or bendability of the DNA double helix directs the placement of the histone core (for a review, see Trifonov, 1985). Since signals responsible for such bending appear to be sets of di- or trinucleotides repeating along the DNA sequence, the periodicity of this repetition may provide an estimate of the true periodicity of nucleosomal DNA in a local frame. While a computer search on published sequences of eukaryotic DNA led to a figure close to 10.5 bp for this sequence periodicity (Trifonov & Sussman, 1980; Trifonov, 1980), DNase digestion data obtained from naked bulk nucleosomal DNA, as well as direct sequence analysis of a large number of cloned core DNA fragments, rather pointed to 10.2 bp, again in apparent support of the overtwist hypothesis (Drew & Travers, 1985; Satchwell *et al.*, 1986). Interestingly, a similar figure was recently obtained for the average DNase I digestion periodicity of nucleosomal DNA in a reconstituted five-nucleosome complex (Drew & Calladine, 1987).

On the other hand, early measurements on nucleosomes devoid of H1, as reconstituted from DNA and core histones, also pointed to a linking number reduction of approximately one turn per nucleosome (Germond *et al.*, 1975), similar to that obtained for H1-containing native chromatin (see above). The meaning of this apparent coincidence has, however, remained unclear in the absence of a firm knowledge of the actual number of turns of the DNA superhelix in these nucleosomes. The figure has nevertheless been further refined recently into 1.0(+0.1) turn per nucleosome by Simpson *et al.* (1985), who reconstituted chromatin on circularized tandem repeats of an approximately 200 bp 5S RNA gene fragment able to position the nucleosome near its centre (Simpson & Stafford, 1983). In the accompanying paper, a series of small DNA rings was used to investigate the influence of the topoisomer linking difference on the nature of the reconstituted products. This, together with peculiar features of chromatin reconstituted on the nicked ring, have also suggested a linking number reduction of approximately one and two turns, respectively, in mono- and dinucleosomes (Goulet *et al.*, 1988).

In this paper, the linking number change associated with single nucleosome formation was carefully investigated by relaxing, with DNA topoisomerase I, mononucleosomes reconstituted on small DNA rings with either integral or fractional linking differences relative to their relaxed configuration. A figure of -1.1(+0.1) turns was found. These topological studies were correlated with a structural investigation of the DNA path inside and outside the nucleosome by means of electron microscopy and gel electrophoresis. It was found that the above linking number reduction could be ascribed to a particle with, at the most, 1.5 turns of a superhelix around the histone core and with a large open extranucleosomal loop. A theoretical model of a particle on a small ring, in

<sup>†</sup> Abbreviation used: bp, base-pair(s).

which the DNA superhelix was wrapped around a cylinder, is described in the Appendix. The linking number reduction predicted was found to be most consistent with experimental data when the twist of the DNA in the superhelix was between 10.5 and 10.65 bp per turn. The notion that the DNA wraps around the histone core in the nucleosome without significant alteration of its twist was found to be consistent with the overall behaviour of dinucleosomes both under the electron microscope and under relaxation with the topoisomerase, and also with recent data obtained upon treating reconstituted minichromosomes with gyrase (Garner *et al.*, 1987). Finally, the explanation for the present twist estimate being larger than that derived from the sequence or digestion periodicity (see above) is shown to lie in the different reference frames used for the two measurements, the laboratory frame and the local nucleosome frame, respectively. The actual sequence (digestion) periodicity is, however, smaller than that derived in the case of the above model, suggesting that the overall shape of the histone core, which is expected to influence the local periodicity but not the absolute one, may somewhat differ from that of a cylinder.

## 2. Materials and Methods

Materials and Methods were as described in the accompanying paper (Goulet *et al.*, 1988), except for the following.

### (a) DNA fragments

Two new fragments were used in addition to the 359 bp fragment previously described. The 341 bp fragment originated from the same *Mbo*I digest of plasmid pBR322 as the 359 bp fragment (Sutcliffe, 1978), while the 354 bp fragment came from a *Cla*I digest of phage  $\lambda$  DNA and spans nucleotides 34,696 to 35,050 (New England Biolabs catalogue). These fragments were purified, labelled with  $^{32}\text{P}$  and circularized as indicated for the 359 bp fragment.

### (b) Relaxation

Chromatin was relaxed by incubation with 300 to 600 units of DNA topoisomerase I/ml from calf thymus (BRL Laboratories) at a total DNA concentration of about 15  $\mu\text{g}/\text{ml}$  in buffer R (50 mM-Tris-HCl (pH 7.5), 0.1 mM-EDTA, 50 mM-KCl, 5 mM-MgCl<sub>2</sub>, 0.5 mM-dithiothreitol, 0.1 mg bovine serum albumin/ml). After 1 h at 37°C, the reaction was stopped by submitting the chromatin to gel electrophoresis or to sedimentation in sucrose gradients, or by extracting the DNA. Naked topoisomers were relaxed under identical conditions, except that the enzyme concentration was 80 units/ml. The reaction was stopped by deproteinization.

### (c) Linking differences of 341 and 354 bp topoisomers

These were calculated as indicated for 359 bp topoisomers in Materials and Methods of the accompanying paper, taking as the helical repeat of the DNA that measured under reference conditions (DNA gel electrophoresis buffer and 25°C):  $h^\circ = 10.56 \text{ bp/turn}$  (Goulet *et al.*,

1987).  $\alpha^\circ$  was first calculated from  $h^\circ$  using eqn (4) of the accompanying paper. One obtains for 341 and 354 bp rings,  $\alpha^\circ = 341/10.56 = 32.3$  and  $354/10.56 = 33.5$  turns, respectively. Eqn (2) of the accompanying paper then indicates that 341 bp topoisomers with  $\alpha = 33, 32, 31$  and 30 turns have linking differences  $\tau(\tau = \alpha - \alpha^\circ)$  of +0.7, -0.3, -1.3 and -2.3 turns, respectively. Similarly, 354 bp topoisomers with  $\alpha = 34, 33, 32$  and 31 turns correspond to  $\tau = +0.5, -0.5, -1.5$  and -2.5 turns, respectively. These numbers will identify the topoisomers.

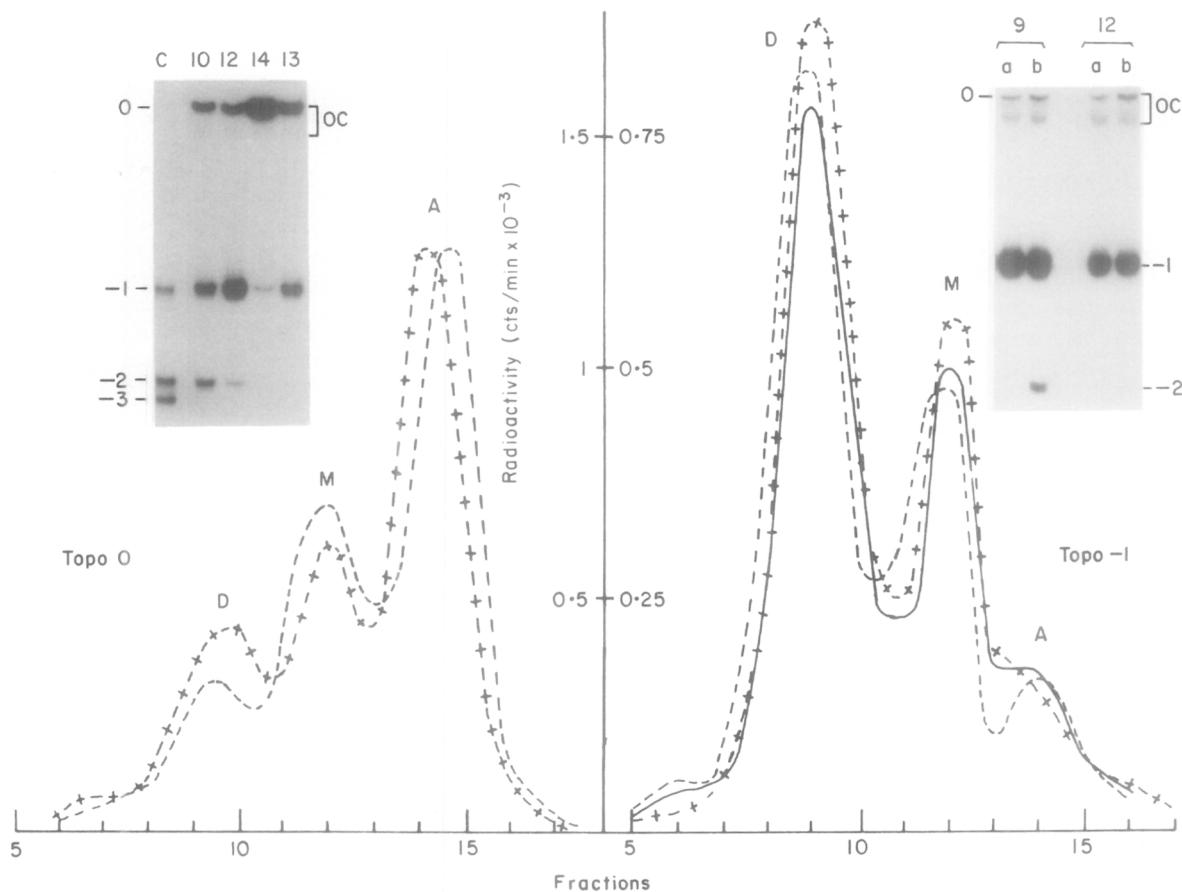
There are 2 different circumstances under which the helical repeat of the DNA will be different from that indicated above. These are the relaxation by topoisomerase I, and the electrophoresis in "chromatin" gels at low ionic strength. Corresponding  $h^\circ$  increments were estimated using the *V* curve method with topoisomers of 3 different fragments of size 506, 641 (methylated form) and 665 bp, which were electrophoresed under appropriate conditions (for a description of the method and of the fragments, see Goulet *et al.*, 1987). The mean  $h^\circ$  value of these fragments was found to vary from 10.56 bp/turn under the above reference conditions to 10.53 bp/turn in buffer R (from which dithiothreitol and bovine serum albumin were omitted) at 37°C and 10.59 bp/turn in TE buffer (10 mM-Tris-HCl (pH 7.5), 1 mM-EDTA) at 27.5°C (see Materials and Methods in the accompanying paper for "chromatin" gel conditions). Taking these periodicities into account, it turns out that all topoisomer linking differences are either decreased or increased (in algebraic terms) by approximately 0.1 turn relative to those described above under relaxation and low ionic strength gel electrophoresis conditions, respectively.

## 3. Results

### (a) Relaxation of chromatin reconstituted on 359 bp topoisomers

#### (i) Topoisomers 0 and -1

Chromatin was reconstituted on each topoisomer and treated with topoisomerase I from calf thymus. Sedimentation profiles of treated and untreated chromatins (Fig. 1) are very similar and show three peaks, A, M and D, which have been identified in the accompanying paper (Goulet *et al.*, 1988) as unreacted DNA, nucleosome monomers and nucleosome dimers, respectively. DNAs were extracted from fractions of gradients and their topoisomer composition determined by gel electrophoresis (Fig. 1). Relative amounts of different topoisomers were estimated by visual inspection of the autoradiographs and, when required for a better accuracy, from peak areas in densitometric tracings. The results showed: (1) in peak A of chromatin reconstituted on topoisomer 0 (fraction 14), a small relaxation into topoisomer -1; (2) in mononucleosomes (fractions 12 in peaks M), a conversion of approximately two-thirds of topoisomer 0 into topoisomer -1 (a trace amount of topoisomer -2 can be seen), and virtually no conversion of topoisomer -1, even at the higher enzyme concentration (b); and (3) in dinucleosomes (fractions 10 and 9, respectively, in peaks D), a conversion of approximately two-thirds of



**Figure 1.** Relaxation of chromatin reconstituted on 359 bp topoisomers 0 and -1. Chromatin were reconstituted using the salt dialysis procedure with linear DNA as a carrier (see Materials and Methods of Goulet *et al.*, 1988) at histone to DNA weight ratios  $r_w = 1$  (topoisomer 0) and 0.75 (topoisomer -1). Reconstitution mixtures were sedimented in sucrose gradients as indicated (Goulet *et al.*, 1988) after incubation with 0 (—), 300 (—) and 600 units (—+) of DNA topoisomerase I/ml (see Materials and Methods), and the radioactivity in the fractions was measured. DNA was purified from chromatin fractions by addition of NaCl to 1 M and SDS to 1%, shaking with 1 vol. chloroform/isoamyl alcohol (24:1, v/v) and precipitation with ethanol, and was electrophoresed in 5% polyacrylamide gels as described in the accompanying paper. Autoradiographs are shown. OC, open circular DNA. a (—) and b (—+), 300 and 600 units topoisomerase I/ml, respectively.

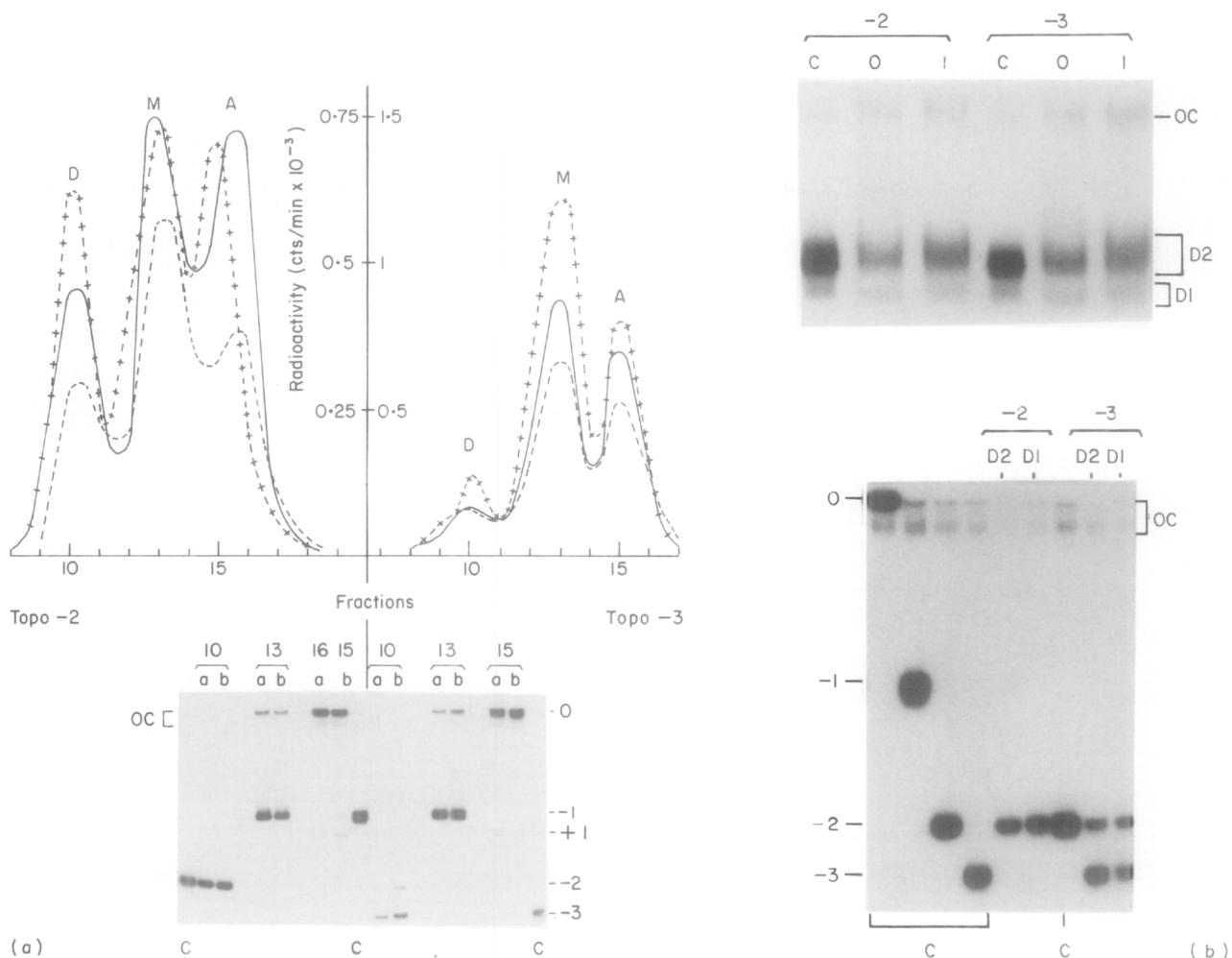
topoisomer 0 into topoisomers -1 and -2, and a slight conversion of topoisomer -1 into topoisomer -2 at the higher enzyme concentration (b). Noteworthy is the amount of topoisomer 0, which is significant in M and D peaks of topoisomer 0 chromatin but becomes small in topoisomer -1 chromatin. Even this small amount is likely to be overestimated, since the nicked ring, which is present in trace quantity in the sample, partly comigrates with topoisomer 0 (Fig. 1).

#### (ii) Topoisomers -2 and -3

Because of their larger negative supercoiling, these topoisomers reconstitute chromatin faster than topoisomers 0 and -1, so that mononucleosomes have been observed to have a rather transient existence when the salt-dialysis method is used as described above with linear DNA as a carrier. One way to slow down the process is to replace the linear DNA with the unlabelled topoisomer. Chromatin so reconstituted were sedimented in sucrose gradients before and after

treatment with the topoisomerase (Fig. 2(a), top). The topoisomer composition of the peaks (Fig. 2(a), bottom) is very similar at the two concentrations of enzyme used (a and b). Results show: (1) in the A peaks (fractions 15 or 16), a complete relaxation into topoisomer 0, with a trace of topoisomers -1 and +1; (2) in the M peaks (fractions 13), a virtually complete conversion of both topoisomers into topoisomer -1 (a small amount of topoisomer 0, estimated to about one-fifth of that of topoisomer -1, is also visible for both chromatin); and (3) in D peaks (fractions 10), no conversion of topoisomer -2 and a relaxation of one-third to one-quarter of topoisomer -3 into topoisomer -2.

It has been shown in the accompanying paper that two distinct forms of dimers, D1 and D2, differing by their sedimentation and gel electrophoretic properties, are sequentially generated in the course of the reconstitution. Although the exact nature of their difference with respect to their protein content is not clear (see Discussion in the accompanying paper), it was interesting to test

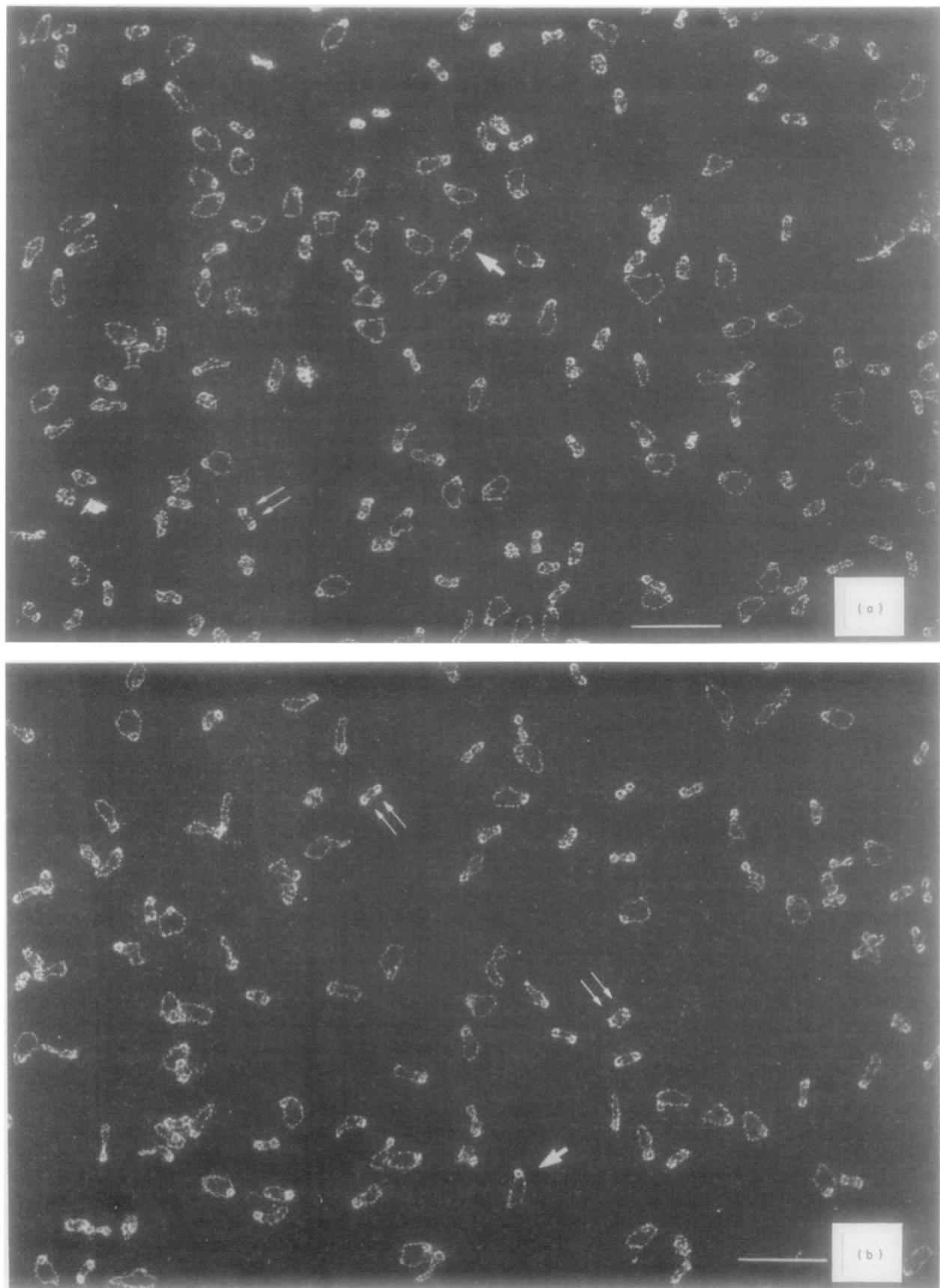


**Figure 2.** Relaxation of chromatins reconstituted on 359 bp topoisomers  $-2$  and  $-3$ . (a) Reconstitutions were performed at  $r_w = 1$  as described in the legend to Fig. 1, except that the linear DNA carrier was replaced by the same weight of the unlabelled topoisomer (see Materials and Methods in the accompanying paper). Incubations with DNA topoisomerase I, centrifugations, and topoisomer composition analysis of gradient fractions were as described in the legend to Fig. 1 (a, —; b, + + + +). (b) Chromatins were reconstituted using the salt dialysis method and a linear DNA carrier at  $r_w = 1$ , and electrophoresed in a 4% polyacrylamide gel (top) at low ionic strength as described in the accompanying paper (see Materials and Methods), before (C) and after incubation with 0 (0) or 600 units topoisomerase I/ml (I). Dimer bands D1 and D2 were cut out of the gel, DNAs were extracted according to Maxam & Gilbert (1977), and electrophoresed in the bottom gel, together with control topoisomers (C).

whether these particles would also differ in their DNA topology. The obtention of D2, however, requires the use of linear DNA as a carrier, otherwise maturation of D1 into D2 does not occur (see Results in the accompanying paper). Figure 2(b), top, shows the electrophoretic fractionation of chromatins so reconstituted on topoisomers  $-2$  and  $-3$ , before and after treatment with the topoisomerase. The strong upper band in the gel has been identified as D2 in the accompanying paper (see  $r_w = 1$  patterns in Fig. 2(c) and (d) of Goulet *et al.*, 1988), and the weaker and lower band is D1. Some release of open circular DNA is observed upon chromatin incubation even without enzyme (compare lanes C and 0 in Fig. 2(b), top). This is presumably a consequence of a preferential destabilization of the particles formed on that DNA, consistent with its

smaller amount in D1 and D2 as compared to starting DNAs seen in Figure 2(b), bottom. This Figure reveals that D1 and D2 dinucleosomes are not modified by the enzyme when on topoisomer  $-2$ , but are converted to approximately one-half and one-quarter to topoisomer  $-2$ , respectively, when on topoisomer  $-3$ . It may therefore be concluded that D1 and D2 are topologically equivalent.

In conclusion, relaxation of mononucleosomes  $0$ ,  $-2$  and  $-3$ , essentially leads to mononucleosome  $-1$ . Consistently, mononucleosome  $-1$  appears not to be affected by the enzyme treatment. On the other hand, dinucleosomes  $0$ ,  $-1$  and  $-3$  relax toward dinucleosome  $-2$ , which is again consistently not modified by the treatment. Interestingly, the two dinucleosome subtypes, D1 and D2, behave similarly upon relaxation. In



**Figure 3.** Visualization of chromatin reconstituted on 359 bp topoisomer -1 before and after salt addition. The reconstitution was carried out at  $r_w = 1$  as described in the legend to Fig. 2(a). Samples were diluted before adsorption to the grids either in (a) TE buffer (10 mM-Tris-HCl, 1 mM-EDTA (pH 7.5)), or in (b) TE buffer plus 0.1 M-NaCl (see the accompanying article for methods). Note the abundance of mono- (single arrow) and dinucleosomes (arrow doublet) without crossing, both before and after salt addition. The bars represent 0.1  $\mu$ m or about 300 bp.

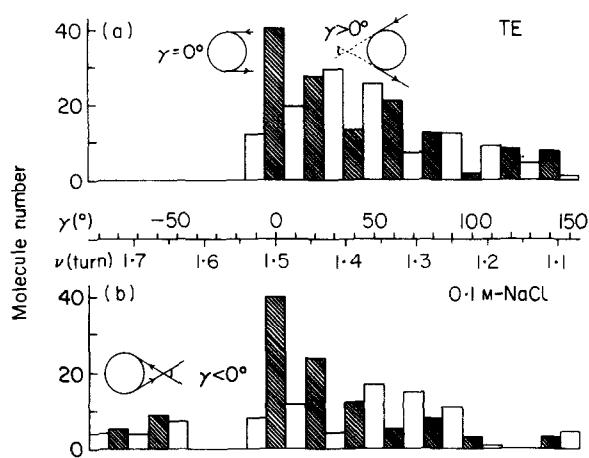
contrast to the cases of mononucleosomes -2 and -3, for which none of the initial topoisomer remains after the reaction, the relaxation of dinucleosomes 0, -1 and -3 is incomplete, concerning at the most about two-thirds of the material in the particular example of dinucleosome 0 and at the enzyme concentrations used.

(b) *Further properties of 359 bp mono- and dinucleosomes*

(i) *Electron microscopy of topoisomer -1 chromatin. Effect of salt. Number of turns of the DNA superhelix*

It is shown in the accompanying paper that if no more than 45% of mononucleosomes and 18% of dinucleosomes in topoisomer -2 chromatin exhibit a crossing in their loop or linker DNA in TE buffer, most of them (87 and 94%, respectively) do in the presence of 0.1 M-NaCl (see Fig. 6 and Table 1 of Goulet *et al.*, 1988). Figure 3 shows the same experiment performed with topoisomer -1 chromatin. In striking contrast with the above situation, few crossings are observed under either salt condition (Fig. 3(a) and (b)). Only two mononucleosomes were found to cross in TE buffer out of a total of 244 molecules, and 32 (15%) in 0.1 M-NaCl out of 212. For dinucleosomes, these figures were 13% and 20%, respectively. Crossing frequencies were not found to change significantly relative to the values in 0.1 M-NaCl when the chromatin was in buffer R (not shown). Chromatin reconstituted on topoisomer 0 at  $r_w = 1$  was also examined. Crossings in nucleosome monomers appeared to be rare in TE buffer, but to increase to approximately 40% in 0.1 M-NaCl. A small amount of particles with a dinucleosome appearance were found in this chromatin, along with a large number of naked rings and structures involving two or three DNA molecules apparently brought together through histone linkage.

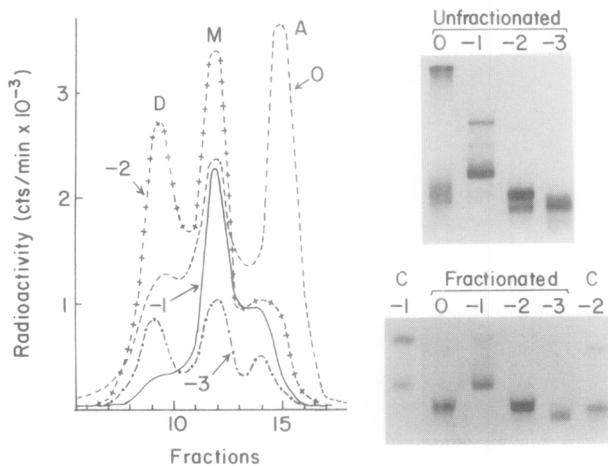
Because nucleosomes appear to be mostly adsorbed with their DNA superhelix axis perpendicular to the grids (see Discussion in the accompanying paper), the number of turns of this superhelix around the histone core in mononucleosomes,  $v$ , may be estimated by measuring the angles between entering and exiting DNAs,  $\gamma$ , in the preparation shown in Figure 3. The tangents to these DNAs may cross opposite the loop relative to the core, and  $\gamma$  is taken as positive, or toward the loop (which usually results into a crossing) and  $\gamma$  is negative. Figure 4 shows histograms of  $\gamma$  obtained in TE buffer and in 0.1 M-NaCl, respectively. These histograms show scattered distributions with a relative accumulation of molecules around  $\gamma = 0^\circ$  ( $v = 1.50$  turns), less pronounced for chromatin in TE buffer, and no molecule beyond  $\gamma = -10^\circ$  ( $v = 1.53$  turns), except for those that cross in 0.1 M-salt. Mean values of  $v$  are 1.38 turns in TE buffer and 1.41 turns in 0.1 M-salt when the molecules with a crossing are put into the  $\gamma = 0^\circ$  class.



**Figure 4.** Histograms depicting the distributions of the angles between entering and exiting DNAs in mononucleosomes reconstituted on 359 bp topoisomer -1. Angles ( $\gamma$ ) were measured manually on enlarged negatives at a final magnification of 700,000 $\times$  for 244 (a) and 212 (b) molecules from the chromatin showed in Fig. 3(a) and 3(b), respectively. The sign of the angle was specified as indicated. The number of turns of the DNA superhelix ( $v$ ) was calculated from the angle using the equation  $v = (540 - \gamma)/360$ . A few molecules had outranged angles and were not included in the histograms.

(ii) *Electrophoretic mobility*

Further wrapping of the DNA around the histone core beyond  $v = 1.5$  turns, as observed for the crossed configuration of mononucleosome -2 (inset (c) in Fig. 5 and Fig. 6(b) of the accompanying paper), is expected to lead to an additional compaction of the structure. Such compaction may be detectable by gel electrophoresis. Figure 5, bottom right, shows a comparison between the four different mononucleosomes previously purified by sedimentation in sucrose gradients (see profiles in Fig. 5). Their identical electrophoretic mobility before and after purification (compare the 2 gels in Fig. 5) ensured that they were not damaged at this step. Interestingly, mononucleosome -1 migrates the least, consistent with an absence of crossing in this mononucleosome, and with its occurrence in the others. The symmetrical behaviour of mononucleosomes 0 and -2 relative to mononucleosome -1 (Fig. 5) further suggests that loop crossings in mononucleosomes 0 and -2 occur in reverse directions. Moreover, the larger mobility of mononucleosome -3 relative to -2 indicates that this mononucleosome achieves a larger compaction, presumably through a further supercoiling of the loop. To investigate a potential effect of salt, the electrophoresis was repeated after addition of 50 mM-NaCl to the electrode and gel buffer. The gel (not presented) showed bands of similar relative mobilities, suggesting that the structure of mononucleosomes 0 and -2 in the gel was salt-independent, in contrast to their behaviour under the electron microscope (see above). Finally, it is noteworthy that dinucleosomes, in contrast to



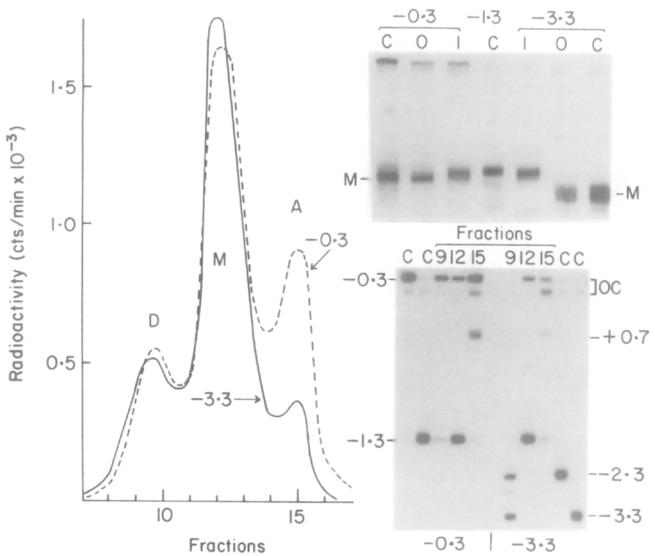
**Figure 5.** Comparative electrophoretic properties of mononucleosomes reconstituted on different 359 bp topoisomers. Reconstitutions were carried out with linear DNA as a carrier according to either the salt-dialysis method at  $r_w = 1$  (topoisomer 0) or the low-salt procedure (this procedure uses polyglutamic acid as a cofactor (see Materials and Methods of Goulet *et al.*, 1988) at  $r_w = 0.65$  (topoisomers -1, -2 and -3). Reconstituted chromatins were both sedimented through sucrose gradients (see profiles) and electrophoresed at low ionic strength (top gel). Purified nucleosome monomers from gradient fractions 12 were subsequently electrophoresed in the bottom gel, together with unfractionated chromatins as controls (C).

mononucleosomes, are not significantly discriminated from each other by gel electrophoresis (see Fig. 2(b), top; see also Fig. 2(b), bottom, of the preceding paper).

#### (c) Relaxation of chromatin reconstituted on other DNA rings

##### (i) 341 bp rings

Chromatin was reconstituted on topoisomers -0.3 and -3.3 using the salt-dialysis method, and treated with topoisomerase I. These chromatins were sedimented in sucrose gradients (Fig. 6; left) and electrophoresed together with untreated chromatins and chromatin reconstituted on topoisomer -1.3 as controls (Fig. 6, top right). Sedimentation profiles reveal that, in both cases, most of the material consists of mononucleosomes, which is true also for topoisomer -1.3 chromatin (not shown). This in turn permits identification of the main bands in the electrophoretic patterns. The topoisomer composition of fractions from the gradients is shown in the gel of Figure 6, bottom. (1) The unreacted DNA (fractions 15 in peaks A) is converted into a mixture of topoisomers -0.3 and +0.7. The large amount of open relative to closed circular DNA for chromatin reconstituted on topoisomer -3.3 may be explained by their differential nucleosome uptake, which enriches peak A in the less-reacting species. In contrast, nucleosomes form to similar extents on the open circular DNA and on topoisomer -0.3, which explains its

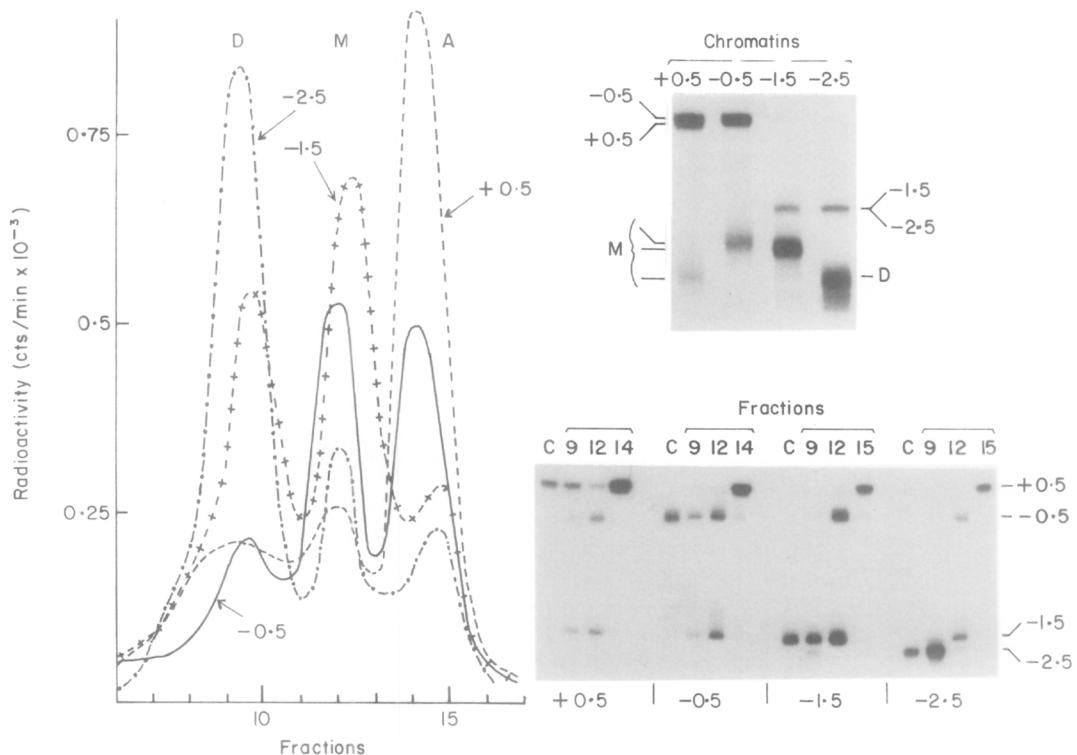


**Figure 6.** Relaxation of chromatins reconstituted on 2 different 341 bp topoisomers. Reconstitutions used the salt-dialysis method at  $r_w = 0.5$  with linear DNA (topoisomer -0.3) or supercoiled from I pBR322 DNA (topoisomers -1.3 and -3.3) as carriers. Chromatins were electrophoresed in the top gel before (C) or after incubation with 0 (0) or 600 units topoisomerase I/ml (1). Enzyme-treated chromatins were also sedimented in sucrose gradients (see profiles). DNAs were extracted from gradient fractions and electrophoresed in the bottom gel, together with control topoisomers (C).

smaller relative amount for chromatin reconstituted on that topoisomer. (2) Mononucleosomes (fractions 12 in peaks M) show a large conversion of topoisomer -0.3 into topoisomer -1.3, and a complete conversion of topoisomer -3.3 into topoisomers -1.3 and -0.3. Interestingly, those conversions lead in both cases to very similar proportions of topoisomers -0.3 and -1.3, approximately 1 : 3 and 1 : 4 for topoisomer -0.3 and -3.3 chromatins, respectively. (3) In dinucleosomes (fractions 9 in peaks D), about one-quarter of topoisomer -0.3 is relaxed into topoisomer -1.3, whilst approximately half of topoisomer -3.3 is converted into topoisomer -2.3.

This relaxation of mononucleosomes into mostly topoisomer -1.3 explains the relative positions of the M bands observed in the "chromatin" gel of Figure 6, top. Before the topoisomerase treatment, mononucleosome -3.3 indeed migrated faster than -0.3, which in turn migrated ahead of -1.3. In contrast, topoisomerase-treated mononucleosomes -0.3 and -3.3 essentially migrated like mononucleosome -1.3. The bands, however, show a trailing on their faster moving side clearly due to the presence of a minor amount of mononucleosome -0.3, and consistent with the presence of topoisomer -0.3, together with topoisomer -1.3, in peaks M of the sucrose gradients after relaxation.

Chromatin was also reconstituted on topoisomers -1.3 and -2.3, treated with the topoisomerase and sedimented in sucrose gradients (results not shown). The topoisomer analysis of peak fractions from



**Figure 7.** Relaxation of chromatins reconstituted on 4 different 354 bp topoisomers. Chromatins were reconstituted using the low-salt procedure (see Materials and Methods of the accompanying paper) with linear DNA as a carrier, at  $r_w = 0.65$ , and were electrophoresed in the top gel. The chromatins were also treated with 600 units topoisomerase I/ml and sedimented through sucrose gradients (see profiles). DNAs purified from gradient fractions were electrophoresed in the bottom gel, together with control topoisomers (C).

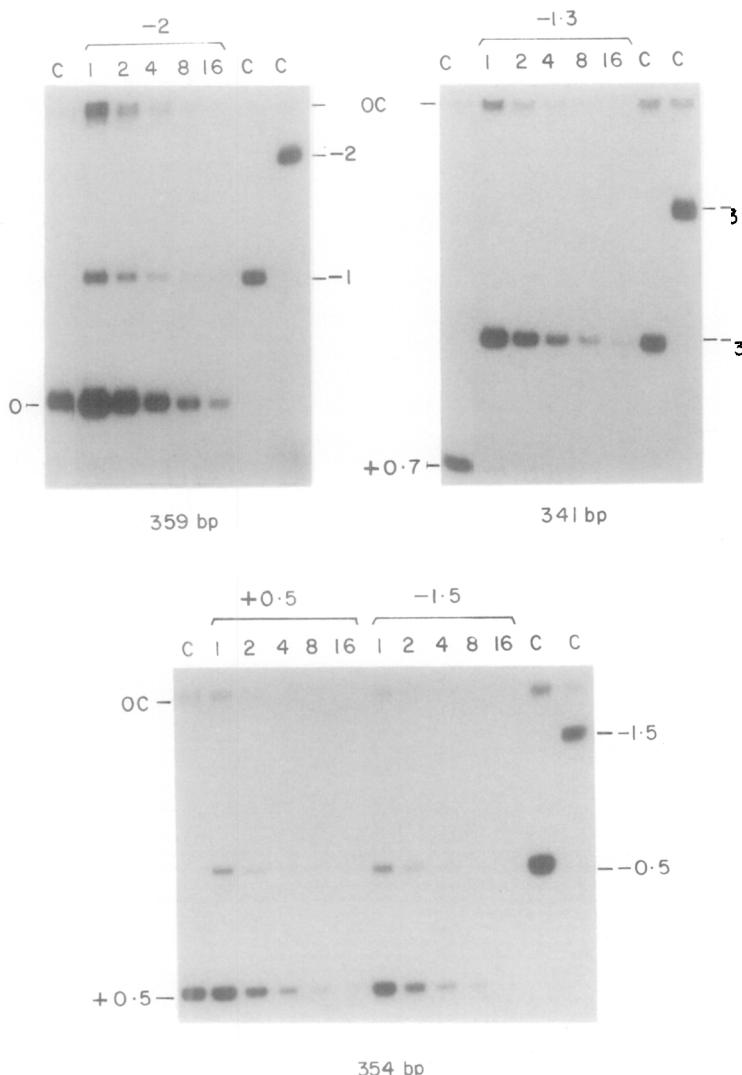
gradients revealed a partial relaxation of mononucleosome  $-1.3$  into  $-0.3$  and a complete relaxation of mononucleosome  $-2.3$  into mononucleosomes  $-0.3$  and  $-1.3$ . Ratios between these two mononucleosomes ( $-0.3$  and  $-1.3$ ), in both cases about  $1:4$ , were found to be similar to those obtained above from relaxation of topoisomer  $-0.3$  and  $-3.3$  chromatins. In dinucleosome peaks, a conversion of approximately one-third of topoisomer  $-1.3$  into topoisomer  $-2.3$  and a slight conversion, estimated to be less than 10%, of topoisomer  $-2.3$  into topoisomer  $-1.3$  were observed.

In conclusion, it appears that mononucleosomes reconstituted on 341 bp rings are relaxed into a mixture of  $-0.3$  and  $-1.3$ . Interestingly, their proportions, about  $1:4$ , respectively, do not appear to depend much on which mononucleosome is relaxed. This suggests that relaxations went to virtual completion in all cases, and not only in those of topoisomer  $-2.3$  (not shown) and  $-3.3$  chromatins (Fig. 6), where the initial topoisomers have completely disappeared after the reaction. As observed for 359 bp dinucleosomes, relaxation of 341 bp dinucleosomes is incomplete, since it concerns, at most, about half of the particles in the case of dinucleosome  $-3.3$  (Fig. 6). This relaxation apparently tends toward an intermediate point between topoisomers  $-1.3$  and  $-2.3$ , as demonstrated by the mutual interconversion

observed between corresponding dimers (results not shown).

#### (ii) 354 bp rings

Figure 7, top right, shows an electrophoretic fractionation of chromatins reconstituted using the low-salt method and the same histone to DNA mass ratio on four topoisomers,  $+0.5$ ,  $-0.5$ ,  $-1.5$  and  $-2.5$ . It can be observed that naked topoisomers  $+0.5$  and  $-0.5$  nearly comigrate, which provides a direct control of their linking difference in the gel. An exact comigration, i.e. equal and opposite linking differences, would mean  $\alpha^\circ = 354/h^\circ = 33.5$  turns (see Materials and Methods), and therefore  $h^\circ = 10.57$  bp per turn. Because topoisomer  $+0.5$  actually migrates slightly ahead of topoisomer  $-0.5$ ,  $h^\circ$  should be slightly larger than 10.57 bp per turn, and possibly equal to 10.58 bp per turn. This value is close to the 10.59 bp per turn estimated in Materials and Methods for the DNA average helical repeat under these conditions. Reconstituted chromatins were treated with the topoisomerase and sedimented in sucrose gradients. The profiles (Fig. 7) show that the proportion of dimers (peak D) increases with the negative constraint of the topoisomers, whilst that of the unreacted DNA (peaks A) decreases. These features parallel similar observations made in the accompanying paper with chromatin reconstituted on 359 bp rings. The smaller proportion of dimers, relative to monomers,



**Figure 8.** Relaxation of naked topoisomers: 359 bp topoisomer  $-2$ , 341 bp topoisomer  $-1.3$  and 354 bp topoisomers  $+0.5$  and  $-1.5$  were treated with topoisomerase I as indicated in Materials and Methods. DNAs were subsequently purified as described in the legend to Fig. 1 and serial dilutions (numbers) were loaded on to the gels, together with control topoisomers (C). The gels and the electrode buffer contained chloroquine (diphosphate salt; Sigma) to  $125 \mu\text{g}/\text{ml}$ . Autoradiographs are shown. OC, open circular DNA.

in profiles corresponding to topoisomers  $+0.5$ ,  $-0.5$  and  $-1.5$  indicates that the strong bands visible in the electrophoretic patterns (Fig. 7, top right) can be ascribed to mononucleosomes. For chromatin reconstituted on topoisomer  $-2.5$ , in contrast, the strong band apparently corresponds to dinucleosomes, probably of the D2 subtype, while mononucleosomes may migrate in the minor band visible immediately below. Mononucleosomes  $+0.5$  and  $-2.5$ , therefore, appear to migrate more and consequently to be more compact than mononucleosomes  $-0.5$  and  $-1.5$ , which nearly comigrate in the gel.

The topoisomer composition of peak fractions from the gradients (Fig. 7, bottom right) shows: (1) in peaks A (fractions 14 or 15), a virtually complete relaxation into topoisomer  $+0.5$ , with a trace of topoisomer  $-0.5$ ; (2) in mononucleosomes (fractions 12), a large relaxation of topoisomers  $+0.5$  and  $-2.5$  into topoisomers  $-0.5$  and  $-1.5$ ,

with relative proportions between them of about  $1:1$  and  $1:1.5$ , respectively, and conversions of approximately half each of topoisomers  $-0.5$  and  $-1.5$  into topoisomers  $-1.5$  and  $-0.5$ , respectively; and (3) in dinucleosomes (fractions 9), conversions of approximately one-third to one-half of topoisomer  $+0.5$  into topoisomers  $-0.5$  and  $-1.5$ , one-third of topoisomer  $-0.5$  into topoisomer  $-1.5$ , and one-tenth of topoisomer  $-1.5$  into topoisomer  $-2.5$ . A slight conversion of topoisomer  $-2.5$  into topoisomer  $-1.5$  is also observed, although the weak  $-1.5$  band is somewhat obscured by the intense  $-2.5$  band.

In summary, it appears that 354 bp mononucleosomes relax into a mixture of mononucleosomes  $-0.5$  and  $-1.5$ . Their ratio remains close to 1 regardless of which mononucleosome is relaxed. This again suggests that relaxations are not far from completion, even for topoisomer  $-0.5$  and  $-1.5$  chromatins, in which large amounts of the

initial topoisomers are still observed after the reaction. Similarly, the relaxation of 354 bp dinucleosomes appears to be rather ineffective, at least at the enzyme concentration used, with at most a conversion of less than half of the particles in the case of dinucleosome +0.5. Finally, the interconversion observed between dinucleosomes -1.5 and -2.5 indicates that this relaxation tends toward an intermediate point between the corresponding topoisomers.

#### (d) Relaxation of the naked topoisomers

Because unreacted DNAs in peaks A of the sucrose gradients were not necessarily completely naked, 359, 341 and 354 bp topoisomers -2, -1.3 and -1.5, respectively, were relaxed with the topoisomerase under buffer and temperature conditions identical with those used for chromatin (see Materials and Methods). Serial dilutions of the products were electrophoresed in chloroquine-containing gels (Fig. 8). The Figure shows that all topoisomers equilibrate between two topoisomers, 0 and -1, +0.7 and -0.3, and +0.5 and -0.5, respectively. Ratios between these topoisomers appear by visual inspection of the autoradiographs to be approximately 8, 1:16 and 3, respectively. The observation that none of the starting topoisomers remains after the treatment indicates that the reactions went to completion, and suggests that the distributions observed may reflect the equilibria. This is actually supported, in the particular case of the 354 bp ring, by a relaxation of topoisomer +0.5, which showed an equilibration between topoisomers +0.5 and -0.5 similar to that observed with topoisomer -1.5 (Fig. 8).

### 4. Discussion

#### (a) Linking number reduction associated with single nucleosome formation

##### (i) Mononucleosome equilibration

Upon topoisomerase treatment, mononucleosomes reconstituted on 341 and 354 bp rings appeared to partition into two forms, mononucleosomes -0.3 and -1.3 (Fig. 6), and mononucleosomes -0.5 and -1.5 (Fig. 7), respectively. The relative amount of those products was found not to depend much on which mononucleosome was relaxed, which has been interpreted as meaning that relaxations went close to completion in all cases. These partitions may therefore reflect an equilibration between the two species similar to that observed with the naked rings (Fig. 8).

The 359 bp mononucleosomes may similarly equilibrate between a major species, mononucleosome -1, and a minor one, mononucleosome 0. The ratio of topoisomer 0 over topoisomer -1 observed after relaxation in peaks M of the sucrose gradients is, however, highly variable, since it decreases from about 0.5 in topoisomer 0 chromatin to virtually 0 in topoisomer -1 chromatin (see fractions 12 in the

gels of Fig. 1). In this hypothesis, such variability would necessarily reflect an incomplete relaxation of mononucleosomes 0 or -1, or of both. This would in turn be in contrast to the cases of topoisomer -2 and -3 chromatins in which the initial topoisomer disappearance indicates a complete relaxation of both mononucleosomes -2 and -3 (see fractions 13 in the gel of Fig. 2(a)). Such discrepancy, together with the observation that the variation of the ratio between topoisomers 0 and -1 (this ratio is equal to about 0.2 in the cases of topoisomer -2 and -3 chromatins) actually parallels the evolution of the relative sizes of peaks A and M (see Figs 1 and 2(a)), strongly argues in favour of another possibility; that topoisomer 0 originates from a contamination of mononucleosomes by unreacted DNA. The 359 bp mononucleosomes therefore appear to relax into a single form, mononucleosome -1, and not two as do the 341 and 354 bp mononucleosomes.

##### (ii) Calculation

The linking number change associated with the formation of the nucleosome,  $\tau_n$ , was given in equation (6) in the accompanying paper (Goulet *et al.*, 1988) as  $\tau_n = \alpha_n^0 - \alpha^0 \cdot \alpha^0$  and  $\alpha_n^0$  are the mean linking numbers of the topoisomer distributions obtained at the equilibrium upon relaxation of naked DNAs and mononucleosomes, respectively. Table 1 gives the ratios,  $r$ , between the two topoisomers in these distributions (see Results) and the values of  $\alpha^0$  and  $\alpha_n^0$  derived from these ratios.  $\alpha^0$  was also estimated from the DNA helical periodicity,  $h^0$ , under relaxation conditions (see Materials and Methods).  $\tau_n$  was subsequently calculated (see above), using for  $\alpha^0$  its two different estimates which, as shown in the Table, are sometimes quite distinct. Such discrepancies may result from an influence of the nucleotide sequence on the twist of the fragments (Goulet *et al.*, 1987), although their importance indicates that other unknown factors are involved. The two values of  $\langle \tau_n \rangle$ , the mean for the three different rings, however, appear quite consistent with each other if the larger error of the first one is taken into account. The second estimate, rounded to the first decimal place, gives  $\langle \tau_n \rangle = -1.1$  turns. Its overall error will be taken as  $\pm 0.1$  turn.

##### (iii) Relation to other work and comments

The present value of  $\langle \tau_n \rangle$  appears to be close to the linking number increment of  $-1.0(\pm 0.1)$  turn per nucleosome found by Simpson *et al.* (1985) from minichromosomes reconstituted with 5 to 20 nucleosomes. In this case, however, the DNA path within and between the nucleosomes was unknown, and no topological information at the level of a single nucleosome could be derived. The consistency between the two results is, however, supported by the analysis by Stein (1980), who showed that a circular chromatin involving 1.5-turn particles with random relative orientations would indeed lead to such a figure.

**Table 1**  
*Linking number increment associated with single nucleosome formation*

Mononucleosome				DNA					$\alpha^\circ$ (turns)	
Rings (bp)	Topo.	$\alpha$ (turns)	$r\left(\frac{\alpha+1}{\alpha}\right)$	$\alpha_n^\circ$ (turns)	Topo.	$\alpha$ (turns)	$r\left(\frac{\alpha+1}{\alpha}\right)$	Mean	$N/h^\circ$	
359	-1	33		33.00	0	34	8	33.89	34.09	
					-1	33				
341	-0.3	32	1/4	31.20	+0.7	33	1/16	32.06	32.38	
	-1.3	31			-0.3	32				
354	-0.5	33	1	32.50	+0.5	34	3	33.75	33.62	
	-1.5	32			-0.5	33				
$\tau_n$ (mean)		$\tau_n (N/h^\circ)$								
		-0.89	-1.09							
		-0.86	-1.18							
		-1.25	-1.12							
$\langle \tau_n \rangle$	-1.0	-1.13								
(turn)	$\pm 0.2$	$\pm 0.04$								

The Table shows the topoisomers (Topo.) involved in the equilibrium populations observed after topoisomerase treatment of mononucleosomes or naked DNA, and their linking numbers ( $\alpha$ ) taken from Materials and Methods in the accompanying (359 bp ring) and present paper (341 and 354 bp rings), and their ratios ( $r$ ) measured in Results. The mean of  $\alpha$  in those populations ( $\alpha_n^\circ$  and  $\alpha^\circ$ , respectively) is given by  $((\alpha+1)r+\alpha)/(r+1)$ .  $\alpha^\circ$  can also be calculated from  $N/h^\circ$ , where  $N$  is the ring size and  $h^\circ = 10.53$  bp/turn is the average helical repeat of the DNA under relaxation conditions (see Materials and Methods).  $\tau_n$  (mean) and  $\tau_n (N/h^\circ)(\tau_n - \alpha_n^\circ - \alpha^\circ)$  measure the linking number change upon nucleosome formation (see Discussion), using for  $\alpha^\circ$  its 2 different estimates, respectively.  $\langle \tau_n \rangle$  is the mean of  $\tau_n$  for the 3 different rings.

$\tau_n$  should not depend on the enzyme used. This has been demonstrated directly by the very similar figure obtained in our laboratory upon sealing of the nick in nucleosome monomers reconstituted on 359, 341 and 354 bp nicked rings with ligase under temperature and ionic conditions identical with those used with topoisomerase I, and quantification of the subsequent topoisomers (unpublished results). In contrast,  $\tau_n$  is expected to depend on the environment, since both  $\alpha^\circ$  and  $\alpha_n^\circ$  do. Equation (A36) in the Appendix actually shows how  $\tau_n$  depends on the DNA helical twists in the nucleosome,  $h_n$ , and in the loop (in its relaxed state),  $h_l$ . The differential of this equation gives the increment of  $\tau_n$  from conditions 1 to 2:

$$\Delta\tau_n = N_n(1/h_l^1 - 1/h_l^2), \quad (1)$$

in which  $N_n$  is the length of the histone-bound DNA. Equation (1) indicates, after replacing  $h_l^1$  and  $h_l^2$  by their values (see Materials and Methods), that  $\tau_n$  increases from -1.1 turns in the relaxation reaction (Table 1) to approximately -1.0 turn in the "chromatin" gel ( $N_n$  was taken to be equal to 110 bp, corresponding to  $110/80 \approx 1.4$  turns of the DNA superhelix; see Results and below).

(b) *Properties of mononucleosomes on small DNA rings*

(i) *Number of turns of the DNA superhelix in 359 bp mononucleosomes - 1*

Histograms shown in Figure 4 indicate that this number is smaller than, or at the most equal to, 1.5. That this is true also in the gel is suggested by the

small electrophoretic mobility of this mononucleosome as compared to that of mononucleosomes 0 and -2, consistent with the expanded DNA configuration conferred by an uncrossed loop, and therefore with  $v \leq 1.5$  turns. These histograms show that mononucleosomes -1 in the  $v = 1.5$  turn class are relatively more frequent, especially in 0.1 M-NaCl. The existence of a major species in the gel, presumably with the same  $v = 1.5$  turns, may appear to be consistent with the sharpness of the band obtained in Figure 5 (see also Fig. 2(b) of the accompanying paper), since a significant amount of particles with  $v < 1.5$  turns (such particles are less compact and should migrate less) would be expected to generate a smear above that band, which is not observed. It is noteworthy, however, that a polydisperse population would also lead to a sharp band if  $v$  fluctuates rapidly within a particle, compared to the electrophoresis time. The possibility nevertheless remains that  $v < 1.5$  turn particles do not exist in any significant amount in solution and are generated by artefacts of electron microscopy. A common artefact involves the shearing forces that develop during the spreading of the sample. The procedure used, however, does not involve a spreading but rather a direct adsorption on to the grid, which is less likely to cause the particles to unravel.

A value of 1.5 turns or less for  $v$  implies that the DNA is no longer attached to the two potential binding sites present at the core positions, which are known to otherwise immobilize 1.8 turns and 145 bp of the DNA superhelix (Richmond *et al.*, 1984). The nearest binding sites, approximately

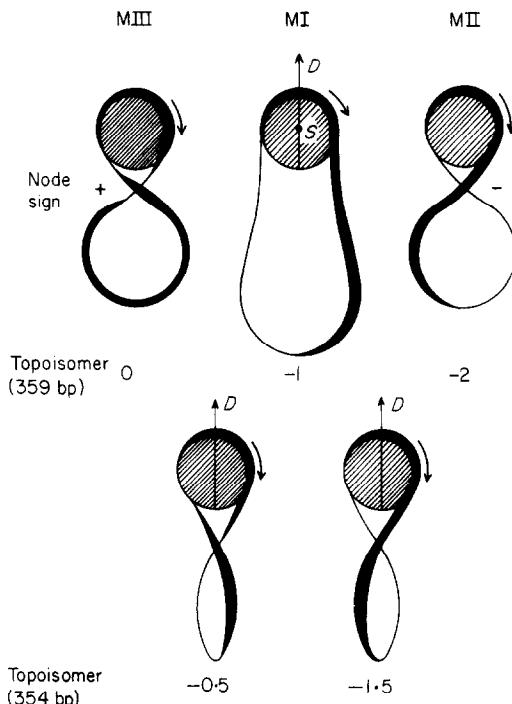
10 bp further inside the particle, may then be expected to hold about 125 bp of DNA. 121 bp have been found upon digestion with exonuclease III plus S<sub>1</sub> nuclease (Prunell, 1983), which leads to a value of  $v = 121/80 = 1.51$  turns, close to the more frequent  $v = 1.50$  turn value of Figure 4. The breaking of one or both of these sites may subsequently occur, leading to about 110 and about 100 bp of immobilized DNA. Such a change in the DNA length by about 10 bp and therefore of  $\gamma$  by about 45°, however, is not apparent in the histograms. In contrast to mononucleosome -1, mononucleosome -2 in higher salt, at least as seen in the electron microscope (see Results), appears to be more in agreement with the crystal structure, since  $v$  is then found to be close to two turns. This configuration of the DNA superhelix is nevertheless unstable, since mononucleosome -2 is readily relaxed into -1 by treatment with topoisomerase (Fig. 2).

Such lability of histone-DNA binding sites may result from the small size of the loop together with the intrinsic stiffness of the DNA, since flexural strain is smaller in the uncrossed configuration when the binding sites at the "core" positions are broken and still smaller after further breaking of those at the "120 bp" positions. Consistent with this interpretation may be the observation of pauses at the core positions when mononucleosomes -1 are digested with micrococcal nuclease (see Fig. 3 of the accompanying paper), since this suggests a restoration of the binding sites after DNA cleavage. It remains to be explained why such breakage of histone-DNA binding sites may be facilitated by low salt. This is indeed indicated by the smaller number of mononucleosomes -1 and -2 with a crossed configuration in TE buffer as compared to 0.1 M-salt (see Results), and by the correlative alteration of the tangent angle distribution from Figure 4(b) to (a). A first factor may be the increase in the DNA bending rigidity, which is known to accompany a decrease in the salt concentration (Kam *et al.*, 1981; Le Bret, 1982). This increase is modest, however, and a more important factor is presumably the increased repulsion between the two duplexes in the node region, as discussed in the accompanying paper.

In conclusion, it appears that a relatively favoured value of  $v$  in 359 bp mononucleosome -1 is 1.5 turns, but that particles with  $v$  of less than 1.5 turns also exist, whose relative amount depends on the salt concentration. These features, which differ from those of the crystallized 1.8-turn core particle, may be explained by the difficulty of histone-DNA binding sites to maintain the loop DNA highly curved, together with an electrostatic repulsion between the duplexes in the node region should a crossing occur.

#### (ii) Loop structure

Higher electrophoretic mobilities of mononucleosomes 0 and -2 as compared to mononucleosome -1 (Fig. 5) suggest that most of these mononucleo-



**Figure 9.** Schemes showing the loop configurations in nucleosome monomers reconstituted on small DNA rings as a function of the topoisomer linking difference. Top: In configuration M I, corresponding to 359 bp topoisomer -1, the extranucleosomal DNA loop is open and its plane is approximately perpendicular to the DNA superhelix axis,  $S$ . In the M II and M III configurations, which are for topoisomers -2 and 0, the plane of the loop has rotated around the nucleosome dyad axis,  $D$ , either by a negative or by a positive angle, forming either a negative or a positive node, respectively. Note that exiting DNA (arrow) is above and remains above in the negative crossing (M II), while it goes below in the positive crossing (M III). The rotation angle shown is close to 180°. Bottom: The 354 bp mononucleosomes are represented after a rotation of the loop plane around the dyad axis,  $D$ , either by a positive (left) or negative angle (right) relative to configuration M I of 359 bp mononucleosomes. The angle shown is about 90°.

somes cross in the gel. Such a situation appears to differ from that of the same particles on the grids, since not more than half of mononucleosomes -2 and only a small proportion of mononucleosomes 0 do in TE buffer (see Results). This discrepancy may arise from the tendency for the gel matrix to compact circular DNA molecules (Zivanovic *et al.*, 1986). This effect, when exerted on the particles, should indeed displace their equilibrium toward more condensed, crossed configurations ( $v > 1.5$  turns). Such configurations would in turn be expected to be largely insensitive to an increase in salt concentration, in keeping with the observation that particle relative mobilities remained virtually unchanged after addition of salt to the gel, and in contrast to the strong salt-dependence of the crossing frequency observed under the electron microscope (see Results).

The expected structure of mononucleosomes 0 and -2 in the gel is illustrated by Figure 9, and

that of mononucleosome -1, for which  $\nu$  has been taken to be equal to 1.5 turns and the loop plane is approximately perpendicular to the superhelix axis. This configuration of the loop is in agreement with its near relaxation, as shown by equation (6) in the accompanying paper. One indeed obtains  $\tau_l^{-1}$  (the linking difference in the loop of mononucleosome -1 relative to its fully relaxed state)

$$= \tau^{-1} - \tau_n = -0.9 + 1.0 = 0.1 \text{ turn}$$

(see values of  $\tau^{-1}$  and  $\tau_n$  under low ionic strength gel electrophoresis conditions in Materials and Methods and above, respectively). Crossings in mononucleosomes 0 and -2 may then form through a rotation of the loop around the molecular dyad, D, in reverse directions, resulting in a positive node (mononucleosome 0; configuration M III in Fig. 9) or a negative one (mononucleosome -2; configuration M II in Fig. 9). It is noteworthy, however, that rotation angles may be smaller than the 180° angles shown in Figure 9. These angles may even not be exactly equal and opposite, since the particles are not symmetrical. Angles may also fluctuate around their average value, since rapid fluctuations, as compared to the electrophoresis time, will still result in sharp bands in the gel (a similar process may be involved in the case of mononucleosome -1; see above). As an additional consequence of the asymmetry of the two particles, one expects that similar overall compactations (as shown by their comigration in the gel; Fig. 5) require a larger  $|\tau_l|$  difference in mononucleosome 0 than in -2, since larger flexural strain must be involved in the positive, as compared to the negative, node. One indeed gets

$$\tau_l^0 = \tau^0 - \tau_n = 0.1 + 1.0 = 1.1 \text{ turns}$$

and

$$\tau_l^{-2} = \tau^{-2} - \tau_n = -1.9 + 1.0 = -0.9 \text{ turn.}$$

A  $\tau_n$  figure close to -1 turn is also consistent with the electrophoretic behaviour of 354 bp and 341 bp mononucleosomes. The near-comigration observed with 354 bp mononucleosomes -0.5 and -1.5 in Figure 7 clearly results from the need for the loop to accommodate opposite and approximately equal linking differences. One indeed has

$$\tau_l^{-0.5} = \tau^{-0.5} - \tau_n = -0.4 + 1.0 = +0.6 \text{ turn}$$

and

$$\tau_l^{-1.5} = \tau^{-1.5} - \tau_n = -1.4 + 1.0 = -0.4 \text{ turn}$$

(see  $\tau$  values in Materials and Methods). That mononucleosome -0.5 does not migrate faster but rather slightly slower than mononucleosome -1.5 may be due to the same kind of anisotropy as that found above in the case of 359 bp mononucleosomes 0 and -2. A scheme of these mononucleosomes with the plane of the loop approximately parallel to the superhelix axis is shown in Figure 9. The same calculation performed for 341 bp mononucleosomes -0.3 and -1.3 leads to  $\tau_l^{-0.3} = +0.8$  turn and  $\tau_l^{-1.3} = -0.2$  turn, in agreement with the faster mobility of mononucleosome -0.3 (Fig. 6).

### (iii) Loop relaxation

The conversion of mononucleosomes 0, -2 and -3 into mononucleosome -1 upon treatment with topoisomerase can be simply explained in terms of free energy if the contribution of the DNA interaction with potential histone binding sites at the core positions (see above) is small relative to the free energy of DNA supercoiling in the loop, as suggested from the above discussion. This free energy is indeed larger in mononucleosomes 0, -2 and -3 than in mononucleosome -1 ( $\tau_l = +1.0$ , -1.0 and -2.0 turns against 0.0 turn, respectively, under relaxation conditions). This explanation does not hold, however, for the partial conversion of 341 bp mononucleosome -1.3 into mononucleosome -0.3 (see Results), since the free energy is now larger in mononucleosome -0.3 ( $\tau_l = +0.7$  turn) than in -1.3 ( $\tau_l = -0.3$  turn).  $\tau_l$  is known to partition into twisting and writhing. It is twisting, that is torsional stress, to which the enzyme is believed to be sensitive. The effective occurrence of the conversion suggests that writhing fluctuations of the loop upon thermal motions in mononucleosome -1.3 are sufficient for the torsional stress to reach transient peak values capable of activating the topoisomerase even with a linking difference  $|\tau_l|$  of less than 0.5 turn. Mononucleosome -0.3 will then be converted back into -1.3. It is this dynamic process that presumably leads to the equilibrations observed between these mononucleosomes as well as between 354 bp mononucleosomes -0.5 and -1.5 (Fig. 7), and between naked topoisomers (Fig. 8).

## (c) Helical twist of nucleosomal DNA

### (i) The model

What do the present results imply for the helical twist of the DNA on the nucleosome,  $h_n$ ? To answer that question, a nucleosome on a small ring was simulated by wrapping one part of the DNA around a cylinder into a left-handed superhelix of pitch 27 Å and diameter 86 Å (1 Å = 0.1 nm; Richmond *et al.*, 1984) and allowing the other part to vary both in flexion and torsion (see Appendix). A concrete result of this simulation is shown in Figure A3 of the Appendix as a stereoscopic view of the DNA conformation obtained with  $\nu = 1.45$  turns in the case of the 359 bp ring. The loop is relaxed and its shape minimizes the bending energy. This bending contribution to the energy of the loop is shown in Figure A1 as a function of the total writhing of the DNA conformation,  $w$ , for  $\nu = 1.5$  and 1.6 turns. The curves are not symmetrical and reflect a larger energy for conformations of the loop tending to form a positive node (configuration M III in Fig. 9) than for those tending to form a negative node (M II in Fig. 9). Such anisotropy is due to the left-handed orientation of the superhelix and was already apparent from the approximately 20% discrepancy in loop linking differences ( $|\tau_l|$ ) required for the electrophoretic comigration of mononucleosomes 0 and -2 (see above). The curves

in Figure A1 permit calculation of the writhing,  $w_0$ , that minimizes the bending energy ( $w_0$  was taken at the middle of the valley region for  $v = 1.6$  turns).  $w_0$  does not depend on the ring size nor on the rigidity parameters used for the double helix. Values of  $w_0$  are given in the Appendix (Table A1) for  $v$  values of between 1.25 and 2 turns. The Table shows that  $w_0$  remains close to -1.0 turn between  $v = 1.25$  and 1.5 turns and then decreases abruptly for  $v$  of greater than 1.5 turns as a consequence of a crossing occurring in the loop. It is in the middle of that transition of  $w_0$  at  $v = 1.6$  turns ( $w_0 = -1.35$  turns; Table A1) that the energy curve shows the flattest bottom (Fig. A1), reflecting equal energies for the negatively crossed and uncrossed loops. It has to be emphasized that this crossing takes place in a relaxed loop (the loop is nicked and DNA strands are free to rotate) at  $\tau_1 = 0$ . This is in contrast to the case of mononucleosomes -2 and -3, where the crossing is driven by  $\tau_1$ . Above  $v$  of 1.75 turns and up to  $v$  equal to two turns,  $w_0$  remains again virtually constant and close to -1.7 turns (Table A1).

Figure A2 of the Appendix shows the theoretical estimate of  $\alpha_n^\circ$  (see above and Table 1) as a function of the ring size,  $N$ , for  $v = 1.45$  turns. Interestingly,  $\alpha_n^\circ$  tends to remain integral when  $N$  varies by a few base-pairs around the sizes for which the loop is expected to be relaxed, approximately 338, 348 to 349, and 359 bp. Such an increase of  $\alpha_n^\circ$  by steps of 1 contrasts with the linear increase (broken line in Fig. A2) that would be observed if the energy curves in Figure A1 were symmetrical around  $w_0$ , in which case  $\alpha_n^\circ$  and  $\tau_n$  ( $\tau_n = \alpha_n^\circ - \alpha^\circ$ ; see above) would become simple linear functions of  $w_0$  (eqns (A35) and (A36), respectively). The fit with the experimental values of  $\alpha_n^\circ$  (Table 1), however, is similar in both cases and is obtained for almost identical values of  $h_n = h_1$  (see the legend to Fig. A2). This suggests, in particular, that equation (A36) is a fair approximation of  $\tau_n$  and justifies its use to derive equation (1) (see also below), but does not permit the determination of whether the real variation of  $\alpha_n^\circ$  with  $N$  is stepwise or linear. Additional rings of different sizes would obviously be necessary to settle that point.  $h_n = h_1$  values, which provide the best fit between the step curve (Fig. A2) and experimental data, were similarly calculated for other values of  $v$ .  $h_n$  values expected for nucleosomal DNA were subsequently derived by incorporating the measured value of  $h_1$  (see Materials and Methods) and are listed in Table A1, column 3. As shown in that Table, the overall variation of  $h_n$  parallels that observed for  $w_0$ . Between  $v = 1.25$  and 1.5 turns,  $h_n$  decreases slightly between 10.71 and 10.58 bp per turn, whilst it decreases abruptly below 10.58 bp per turn for  $v$  larger than 1.5 turns.

Electron microscopic data, together with the known positions of the DNA binding sites on the octamer surface, have pointed to a maximum value of  $v$  very close to 1.50 turns (see above). An independent estimate of  $v$  based on the small

crossing frequency observed for mononucleosomes -1 under the electron microscope can be obtained as follows. A value of  $v$  equal, for example, to 1.6 turns would lead to  $w_0 = -1.35$  turns, the mid value between extreme  $w_0$  values of -1.0 and -1.7 turns. This means that the *relaxed* loop in 359 bp mononucleosome -1 would then be half on its way toward a crossing, as depicted in Figure 9 for 354 bp mononucleosome -1.5. Because  $\tau_1 = 0$ , the particle would remain insensitive to the topoisomerase. Its electrophoretic mobility would, however, be altered and would become similar to that of mononucleosome 0 ( $\tau_1 = +1$  turn), whose conformation would tend toward that of 354 bp mononucleosome -0.5 in Figure 9. Moreover, fluctuations of the loop about its average position would be expected to make equal the numbers of nucleosomes with and without a crossing on the grids. This is clearly inconsistent with the data, supporting the conclusion that  $v$  is smaller than 1.6 turns. The same reasoning may now be made in the reverse way, starting from the 15% crossings observed experimentally. Assuming no crossing for  $v = 1.5$  turns and  $w_0 = -1.0$  turn and 100% crossings for  $v = 1.75$  turns and  $w_0 = -1.7$  turns, then 15% crossings would lead to  $w_0 = -1.0 - 0.15$  (1.7 to 1.0) = -1.1 turn. This value of  $w_0$  is intermediate between those obtained for  $v = 1.50$  and 1.55 turns (see Table A1) and gives, after linear interpolation,  $v = 1.525$  turns and  $h_n = 10.51$  bp per turn. Setting this figure as a higher limit for the mean value of  $v$ , and 1.4 turns (the mean under higher salt conditions; see Fig. 4(b) and Results) as a lower limit, one has  $10.5 < h_n < 10.65$  bp per turn (Table A1).

#### (ii) A putative two-turn nucleosome

Two-turn nucleosomes were probably involved in a recent experiment in which form I pBR322 DNA was reconstituted using the four core histones with a variable number of nucleosomes, and was subsequently further negatively supercoiled by treatment with DNA gyrase (Garner *et al.*, 1987). The basic observation was that the maximum level of supercoiling that was attainable was nearly identical for the naked plasmid ( $\tau = \alpha - \alpha^\circ = -40.2$  turns; see Table 1 of Garner *et al.*, 1987) and for the reconstituted complex (the supercoiling was in that case measured after deproteinization:  $\tau_c = -41.7$  turns with 15 nucleosomes):  $41.7/15 = 2.8$  negative superturns per nucleosome, as compared to one in the relaxed complex, are indeed likely to have the same effect as that observed above with 359 bp topoisomers -2 and -3 as compared to topoisomer -1; that is, to stabilize the two-turn nucleosome. If the portions of DNA between the nucleosomes take up the same superhelical density as naked DNA ( $\sigma = \tau/\alpha^\circ = -40.2 \times 10.5/4363 = -0.097$ ) upon treatment with gyrase, then  $\tau_c$  for the complex reconstituted with 15 nucleosomes is given by:

$$\tau_c = 15\tau_n^{(2)} + (4363 - 15s)\sigma/10.5, \quad (2)$$

in which  $\tau_n^{(2)}$  is the linking number change

associated with the two-turn nucleosome and  $s$  the number of base-pairs immobilized on it (a similar equation was derived by Garner *et al.*, 1987). Replacing  $\sigma$  by  $-0.097$ ,  $\tau_c$  by  $-41.7$  turns and  $s$  by 160 bp gives  $\tau_n^{(2)} = -1.57$  turns. This leads to an intrinsic superhelical density of the DNA in the nucleosome of  $-1.57 \times 10.5/160 = -0.103$ . This superhelix density is close to that achieved by gyrase acting on naked DNA, which explains in a simple way why nucleosomes are not "seen" by the enzyme. This shows in particular that there is no need for nucleosomal DNA to untwist, a conclusion at variance with that reached by Garner *et al.* (1987).

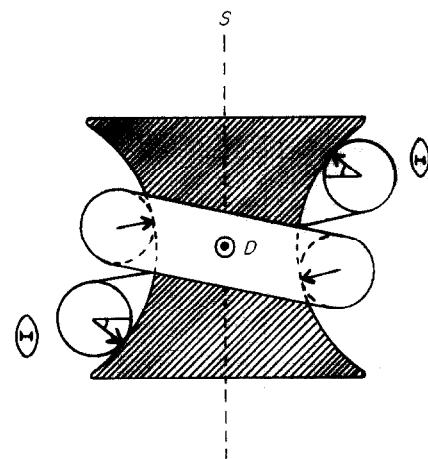
$\tau_n^{(2)}$  can subsequently be used to calculate  $h_n$  from equation (A36). With  $N_n = 160$  bp,  $w_0 = -1.7$  turns (see Table A1) and  $h_1 = 10.53$  bp per turn,  $h_n = 10.44$  bp per turn. Some nucleosomes are, however, likely to be closely packed and no longer to contain enough DNA to complete the two turns of the superhelix. In that case, only the central 1.5 turns of the superhelix ( $\sim 120$  bp, see above) may stay immobilized, while the remaining DNA on each side may undergo twist and writhe. The intrinsic superhelix density in such particles would be  $\tau_n^{(1.5)} \times 10.5/120$ . Replacing  $\tau_n^{(1.5)}$  by  $-1.1$  turns (Table 1), one gets a density of  $-0.096$ , slightly smaller than that obtained above for the two-turn nucleosome. This difference, however small, will significantly alter the estimate of  $\tau_n^{(2)}$ , as derived from equation (2). With eight two-turn particles and seven 1.5-turn particles, for example,  $|\tau_n^{(2)}|$  would be equal to 1.66 turns and  $h_n$ , as calculated from equation (A36), to 10.50 bp per turn. An additional bias will arise if the above condition to equation (2) is not entirely fulfilled; that is, if all linker DNAs do not achieve the same superhelical density as naked DNA under gyrase treatment due to a hindered rotation of some nucleosomes relative to one another. It is easy to show from equation (2) that a correction for this effect will also increase the estimates of  $|\tau_n^{(2)}|$  and  $h_n$  over the above values. It can therefore be concluded that data presented by Garner *et al.* (1987) are consistent with the above conclusion that  $h_n$  may be between 10.5 and 10.65 bp per turn.

### (iii) Relation to other estimates of the nucleosomal DNA periodicity

The present estimate of the twist is different from the values of  $10.21(\pm 0.05)$  and  $10.2(\pm 0.1)$  bp per turn derived from nucleotide sequencing (Satchwell *et al.*, 1986) and DNase I digestion (Drew & Calladine, 1987), respectively (see Introduction). As shown by equation (A36) in the Appendix,  $h_n = 10.2$  bp per turn would lead to  $w_0 \approx -1.4$  turns with  $\tau_n = -1.1$  turns (Table 1),  $N_n = 110$  bp and  $h_1 = 10.53$  bp per turn, which means that more than half ( $0.4/0.7 \approx 60\%$ ) of 359 bp mononucleosomes  $-1$  would be expected to cross. Alternatively, with  $w_0 = -1.0$  turn,  $\tau_n$  would be equal to  $-0.7$  turn. For the two-turn nucleosome, the expected value of  $\tau_n$  would be  $-1.2$  turns ( $N_n = 160$  bp and  $w_0 = -1.7$

turns). A value of 10.2 bp per turn for  $h_n$  appears, therefore, difficult to reconcile with the present data and also with those of Garner *et al.* (1987).

Part of this discrepancy probably lies in the fact that a twist estimate based on topological data, as the present one, is necessarily measured in an absolute frame of reference, whilst that derived from sequencing or digestion is not. An absolute frame indeed keeps the twist invariant regardless of the shape of the double helix axis as long as no torsional tension is generated. In a local frame, in contrast, this is not true in general. Ulanovsky & Trifonov (1983) considered the number of base-pairs separating adjacent DNA-cylinder contact points along one strand of the double helix. It is this periodicity,  $h_c$  (in bp), that may be thought to be accessible through these techniques. With  $h_n = 10.55$  bp per turn,  $h_c$  would be close to 10.4 bp (as obtained from equation (A38(i)) in the Appendix), still higher than 10.2 bp. A formal way to decrease  $h_c$  could be to wrap the DNA, not on a cylinder, but on a concave surface as shown in Figure 10. The angular orientation of the DNA-



**Figure 10.** Highly schematic representation of a nucleosome showing one possibility to reconcile a DNA cleavage (or sequence) periodicity of 10.2 bp ( $h_c$ ) with a helical twist of 10.55 bp/turn ( $h_n$ ). The histone core is the internal portion of a doughnut on which 1.5 turns of a left-handed DNA superhelix is wrapped with a 2-fold symmetry of axis  $D$ . A cross-section of the resulting particle perpendicular to the  $D$  axis and containing the superhelix axis,  $S$ , is shown.  $h_n$  is measured around the axis of the double helix in an absolute frame of reference, while  $h_c$  is the number of base-pairs separating adjacent DNA-surface contact points along 1 strand of the DNA. The angular orientation of these contacts about the double helix axis (arrows) varies continuously from the perpendicular to the superhelix axis ( $S$ ) at the superhelix mid position (where the  $D$  axis passes through the double helix) to a direction at an angle  $\theta$  with this perpendicular at the edges, i.e. at 60 bp (0.75 turn of the DNA superhelix) away from the mid position on either side.  $h_c$  and  $\theta$  are approximately related by the equation  $\theta/360 = 60/(1/h_c - 1/10.4)$ , where 10.4 is the  $h_c$  periodicity for a DNA with  $h_n = 10.55$  bp/turn wrapped around a cylinder into a superhelix of similar average pitch and diameter (see eqn (A38(ii)) in the Appendix). Replacing  $h_c$  gives  $\theta = 42^\circ$ .

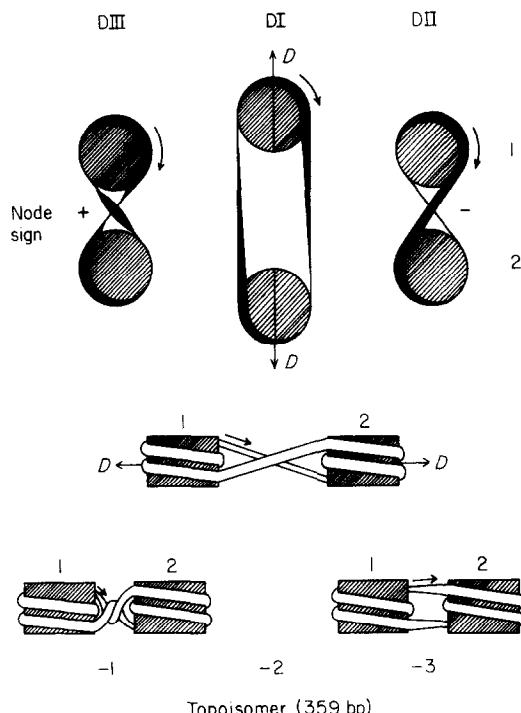
surface contact points relative to the axis of the double helix, indicated by arrows, will then vary continuously from the perpendicular to the superhelix axis at the mid position (where the molecular dyad axis D crosses the double helix) to a direction at an angle  $\theta$  with that perpendicular at both ends of the superhelix. Because the arrows and the double helix wind in opposite directions,  $h_c$  will be shorter than in the case of a cylinder. For a 1.5-turn particle,  $h_c = 10.2$  bp would lead to  $\theta = 42^\circ$ . While all this remains speculative, it is interesting to note that the 0.75 turn of the superhelix running in the model of Figure 10 from the upper end down to the mid position would have, when viewed down the S axis, a spiral shape somewhat reminiscent of that shown in Figure 2 of Richmond *et al.* (1984). It is noteworthy that the present model is somehow the reverse of that presented by Prunell *et al.* (1979), in which the histone core was instead barrel-shaped and  $h_c$  was consequently larger than  $h_n$ .

(d) Properties of dinucleosomes on small DNA rings

(i) Structure

Schemes in Figure 11 show the structures expected for dinucleosomes reconstituted on the different topoisomers when the DNA is wound around each histone core without torsional constraint, and when nucleosomes are arranged relative to one another for the connecting DNAs to be also free from torsional constraint. In configuration D I (dinucleosome -2), linkers are parallel when the particle is viewed from above (Fig. 11, top), but they cross when viewed from the side (Fig. 11, bottom); the opposite is true for configuration D II (dinucleosome -3). In configuration D III (dinucleosome -1), linkers cross from above and form a knot from the side (Fig. 11). D II and D III are actually derived from D I through an approximately  $-180^\circ$  and a  $+180^\circ$  rotation, respectively, of nucleosome 2 relative to nucleosome 1 around their common dyad axes. As a consequence, linkers form a negative (D II) or a positive node (D III). Note that DNA exiting from nucleosome 1 (arrow) is above and remains above in the negative crossing, while it goes below before coming back above in the positive crossing. The smaller distance between nucleosomes in D II and D III as compared to D I is intended to show the greater compaction achieved by these configurations.

As revealed by electron microscopy (see Results), a large majority of dinucleosomes -2 (82%; see Results) indeed appeared to be in configuration D I in TE buffer. In 0.1 M-salt, in contrast, most of them showed a crossing and presumably adopted configuration D II. The mechanism of this conversion is discussed in the accompanying paper in terms of an attraction between the octamers. In contrast, dinucleosomes -1 were only rarely found in a crossed configuration, presumably D III, even in 0.1 M-NaCl (Fig. 3). Their preference for configuration D I is likely to reflect the difficulty for two short DNA linkers to cross into a positive node, due to bending strain (Fig. 11). Such difficulty was in fact already apparent for mononucleosomes 0, despite the fact that DNA available for the node was much longer, as shown by the observation that only about 40% of mononucleosomes 0 were in configuration M III (Fig. 9) in higher salt, while more than 80% of mononucleosomes -2 were in configuration M II under the



**Figure 11.** Schemes showing the DNA configurations in nucleosome dimers reconstituted on 359 bp rings as a function of the topoisomer linking difference. Representations are from above (top) and from the side (bottom). In configuration D I (topoisomer -2), DNA is coiled in an ascending superhelix in nucleosome 1, exits to nucleosome 2 where it is coiled in a second ascending superhelix. This is in contrast to configurations D II and D III (topoisomers -3 and -1, respectively) where DNA is coiled in a descending superhelix in nucleosome 2. Configurations D II and D III are obtained from configuration D I after an approx.  $-180^\circ$  or a  $+180^\circ$  rotation, respectively, of nucleosome 2 relative to nucleosome 1 around their common dyad axes. As a consequence, linkers form a negative (D II) or a positive node (D III). Note that DNA exiting from nucleosome 1 (arrow) is above and remains above in the negative crossing, while it goes below before coming back above in the positive crossing. The smaller distance between nucleosomes in D II and D III as compared to D I is intended to show the greater compaction achieved by these configurations.

same conditions (see Results). The fact that dinucleosomes -1 still have nucleosomes approximately opposed on a diameter of the ring (Fig. 3), however, suggests that virtually all of them adopted the D III configuration at higher salt concentrations during reconstitution with the salt-dialysis procedure (see the accompanying paper for a discussion of the nucleosome spacing mechanism on small DNA rings). Finally, to what extent dinucleosomes -3 adopt configuration D II has not been checked.

(ii) Linking number reduction

In contrast to mononucleosomes, dinucleosomes are not relaxed to completion. One obvious reason for this discrepancy is that the residual linking

difference,  $\tau_l$ , is now distributed over two physically separated linkers instead of a single loop. If, for example, a loop with  $|\tau_l| = 0.6$  turn is expected to be easily relaxed, linkers with  $|\tau_l| = 0.3$  turn each are not. As a consequence, the topoisomer populations obtained do not reflect an equilibrium and the procedure in Table 1 for calculation of the linking number reduction is inapplicable. A simple alternative requires: (1) definition of the equation:

$$\tau^i = \tau_d + \tau_l^i, \quad (3)$$

equivalent to equation (6) in the accompanying paper, in which the  $i$  superscript refers to the topoisomer and  $\tau_d$  is the linking number change upon dinucleosome formation; and (2) the assumption that a linking difference  $|\tau_l|$  of greater than 0.5 turn is necessary for the topoisomerase to work. The observed relaxation of dinucleosomes  $-1$  and  $-3$  toward  $-2$  then implies  $|\tau_l^{-1}|$  and  $|\tau_l^{-3}|$  greater than 0.5 turn. Replacing  $\tau_l^{-1}$  and  $\tau_l^{-3}$  in equation (3) with  $\tau^i$  under relaxation conditions (see Materials and Methods) gives  $1.6 < |\tau_d| < 2.6$  turns. The interconversion of 341 bp dinucleosomes  $-1.3$  and  $-2.3$ , and 354 bp dinucleosomes  $-1.5$  and  $-2.5$ , respectively, on the other hand, implies  $1.4 < |\tau_d| < 2.4$  turns and  $1.6 < |\tau_d| < 2.6$  turns, and therefore  $1.6 < |\tau_d| < 2.4$  turns. It can be further noticed that many more dinucleosomes  $-1.3$  were relaxed into dinucleosomes  $-2.3$  than  $-2.3$  into  $-1.3$  (see Results), suggesting  $|\tau_d| > (1.4 + 2.4)/2 = 1.9$  turns. One finally gets  $1.9 < |\tau_d| < 2.4$  turns, and  $0.95 < |\tau_d/2| < 1.2$ .  $\tau_d/2$  therefore appears to be virtually identical with  $\tau_n$  (Table 1). This result, together with that of Simpson *et al.* (1985; see above), indicates that the linking number reduction per nucleosome is virtually independent of the number of nucleosomes. Such a value of about two turns for  $|\tau_d|$  also implies that  $|\tau_l^{-2}|$  is close to zero (see eqn (3)), which is consistent both with the failure of the topoisomerase to act on dinucleosome  $-2$  (Fig. 2(a)), and with the existence of relaxed linkers when these dinucleosomes adopt configuration D I (Fig. 11).

#### (e) Concluding remarks

Structural and topological data for nucleosome monomers reconstituted on small DNA rings were presented which appear, in the light of a theoretical simulation, most consistent with the DNA wrapped around the histone core having a twist close to that of the double helix free in solution. This conclusion was found to be supported by data obtained by others on a putative gyrase-induced, two-turn nucleosome, as well as by the overall behaviour of nucleosome dimers reconstituted on the same small DNA rings. This does not exclude the possibility for local departures from the *B*-form double helix, such as kinks, under- or overwindings (Richmond *et al.*, 1984), but may require them to make a negligible net contribution to the twist over the length of nucleosomal DNA. The data also argue against the possibility of a significant dependence of the twist

of the free double helix on flexure, even at such large curvature. Such dependence, as originally proposed by Levitt (1978) on theoretical grounds, has not been confirmed experimentally, at least for rings of between 665 and 359 bp (Goulet *et al.*, 1987) and 250 to 210 bp (Shore & Baldwin, 1983; Horowitz & Wang, 1984). This of course points to a flexure-independent  $h_1$  value in the approximately 250 bp (359–110) loop, which was an implicit assumption in the above simulation.

These results therefore make the nucleosomal DNA overtwist hypothesis unlikely to explain the linking number paradox of the SV40 minichromosome. The answer to this question in terms of a peculiar relative arrangement of the nucleosomes (see Introduction) will, however, require as a first step a better understanding of how H1 may seal the two turns of the DNA superhelix, and also the determination of the correlative increase in the linking number reduction. Work along these lines is in progress.

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## APPENDIX

### Computation of the Helical Twist of Nucleosomal DNA

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The aim of this Appendix is to simulate the partial wrapping of a small DNA ring around the histone core in order to get a theoretical estimation of the topoisomer distribution that has been experimentally measured for mononucleosomes after treatment of the chromatin with topoisomerase I (see Table 1 of the main paper).

#### 1. Summary of the Method

In this simulation, the DNA ring consists of two regions. In the first  $N_n$  bp† long region, the duplex axis of the DNA is wrapped around a cylinder of radius 43 Å (1 Å = 0.1 nm) and forms a left-handed superhelix of pitch 27 Å as measured in the crystal of the core particle (Richmond *et al.*, 1984). The number of turns,  $v$ , of that superhelix is varied between 1.25 and 2. In this region, the local twist (or helical repeat, or rate of twist) is  $h_n$ , and the total twist is  $N_n/h_n$ . In the second  $N_l$  bp long region, the DNA forms a loop immersed in the aqueous medium. The loop may take many different conformations, and the total twist of the covalently closed DNA can be deduced from the writhing of

the conformation and the linking number chosen for the whole DNA ring. When the loop DNA has at least one single-stranded scission, and when the DNA strands can freely rotate around the duplex axis, the loop DNA is in the relaxed state. The total twist of the relaxed loop DNA is taken as  $N_l/h_l$ , where  $h_l$  is the local twist of the unconstrained DNA free in solution under the conditions of the enzyme treatment. Both  $h_n$  and  $h_l$  are measured in an absolute reference frame and can, in the simulation, be arbitrarily fixed. The total twist of the entire DNA ring for the configuration with a relaxed loop is,  $\beta_0$ :

$$\beta_0 = N_n/h_n + N_l/h_l. \quad (A1)$$

At this point, we are facing many conformations of the loop that have been generated according to geometric considerations. These conformations contribute to the distribution of the topoisomers according to the value of their total energy. We assume that the main contribution to the total energy is the elastic energy of a homogeneous isotropic rod. In that case, the energy,  $E(w, \alpha, \beta_0)$ , depends on the DNA elastic parameters, the writhing,  $w$ , the linking number,  $\alpha$ , and the total twist,  $\beta_0$ . The population of the topoisomer of

† Abbreviation used: bp, base-pair(s).