A Topological Approach to Nucleosome Structure and Dynamics: The Linking Number Paradox and Other Issues

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ABSTRACT The linking number paradox of DNA in chromatin (two negative crossings around the octamer, associated with a unit linking number reduction), which is 21 years old this year, has come of age. After stirring much debate in the past, the initially hypothetical explanation of the paradox by DNA overtwisting on the nucleosome surface is now presented as a hard fact in recent textbooks. The first part of this article presents a historical perspective of the problem and details the numerous attempts to measure DNA local periodicity, which in one remarkable example sowed the seeds for the discovery of DNA bending. The second part is devoted to the DNA minicircle system, which has been developed in the author's laboratory as an alternative to the local-periodicity-measurement approach. It offers a simple proposal: a unit linking number reduction associated with a single crossing. This conclusion is contrasted with the latest high-resolution crystallographic data of the nucleosome in the third part of the article, and the fourth part examines the available evidence supporting an extension of these results to nucleosomes in chromatin. The last part addresses another basic question pertaining to nucleosome dynamics, the conformational flexibility of the histone tetramer.

INTRODUCTION

DNA in the cell is not naked, but is complexed with basic proteins, the histones, to form chromatin (chromatin also contains numerous nonhistone proteins, each of which is present in much smaller amounts than individual histones). Chromatin is made of a structural repeat unit, the nucleosome, composed of a nucleo-proteic core containing 146 bp of DNA wrapped around an octamer of two copies each of the four core histones H2A, H2B, H3, and H4, and of ~50-bp-long linker DNAs. One copy of a fifth histone, known as the linker histone, H1 or H5 (H5 is H1's counterpart in nuclei of bird erythrocytes) binds to this assembly of approximately equal masses (100,000 Da) of DNA and histones. Chromatin not only ensures the compaction necessary for DNA packaging inside the cell nucleus, but also provides the framework for gene-regulated expression, owing to its structural flexibility at both higher order structure and nucleosome levels.

DNA supercoiling on the nucleosome

DNA supercoiling (see Bauer et al., 1980, for a review) was first described by Vinograd and co-workers more than 30 years ago (Vinograd et al., 1965), but its origin in eukaryotes was understood to lie in the nucleosome structure only 10 years later (Germond et al., 1975). Nucleosomes are indeed also present in the circular DNAs of eucaryotic viruses, such as SV40, which use host histones to form a minichromosome. Minichromosomes are in contact with topoisomerases in vivo and are relaxed, but after extraction of the proteins, the DNA is found to be superhelical. DNA supercoiling can be quantitated through the use of a topological parameter, the linking number (Lk), which measures the number of times one DNA strand goes around the other when the molecule is unfolded and put flat on a surface. This number is therefore an integer. In practice, because of thermal fluctuations during the ligation step in forming the double-stranded circular molecules, one has to deal not with molecules of unique Lk, but with a population of molecules of Lk differing by one unit. These molecules are topological isomers, called topoisomers. As a consequence, it is rather the mean Lk of the population, $\langle Lk \rangle$, which is measured. This mean is in general fractional. Supercoiling is then defined as the change in $\langle Lk \rangle$ (ΔLk) relative to a reference population obtained upon relaxation with topoisomerase I. ΔLk can be conveniently measured by gel electrophoresis through the fractionation of the different topoisomers of the population. By this means, $\langle Lk \rangle$ was found to decrease by ~26 ($\Delta Lk = -26$) in SV40 minichromosome (Shure and Vinograd, 1976). This figure turned out to be approximately equal to the number of nucleosomes, 24–27, which could be counted under electron microscopy either directly (Saragosti et al., 1980) or after psoralen-cross-linking and protein extraction (Sogo et al., 1986). Each nucleosome of the SV40 minichromosome therefore appeared to reduce Lk by 1 ($\Delta Lk = -1$ per nucleosome). This result, which applies to the H1-containing native minichromosome, also holds for the H1-free minichromosome reconstituted in vitro from naked DNA and the four core histones (Germond et al., 1975; Simpson et al., 1985; Norton et al., 1989).

On the other hand, the core of the nucleosome, which could be obtained in large amounts and in relatively homo-

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geneous form through digestion of linker DNAs by micrococal nuclease, was crystallized. The first structure obtained by x-ray diffraction at relatively low resolution (Finch et al., 1977) and the successive refinements (Richmond et al., 1984, 1988; Struck et al., 1992; see below for the most recent one by Luger et al., 1997) showed that the 146 bp were wrapped around the histone octamer into 1¾ turns of a left-handed superhelix of pitch 27 Å and diameter (measured on the double helix axis) of 86 Å. This structure let researchers suppose that the linkers would continue the trajectories defined by the entering and exiting DNAs in the core particle, to form with 166 bp two full turns of the superhelix and one additional crossing. This crossing could then be sealed by H1. So far this model has not been confirmed by x-ray diffraction, although crystals of H1-containing nucleosomes, known as chromatosomes, have been produced (Richmond et al., 1993).

A two-turn particle, and two negative DNA crossings, therefore appeared to reduce *Lk* by 1 ($\Delta Lk = -1$), whereas common sense suggested a reduction of 2 ($\Delta Lk = -2$). This contradiction, known as the "linking number paradox," soon elicited two contradictory explanations (see Wang, 1982, for a fair account of the two models). For the pioneers of the nucleosome crystal structure, an overtwisting of the double helix occurred upon wrapping around the histones (Finch et al., 1977; Klug and Lutter, 1981), although this overtwisting could not at that time be directly measured, because of insufficient resolution. The required overtwisting was about one turn ($\Delta Tw = +1$), as it could be calculated from the differential equation

$$\Delta Lk = \Delta Tw + Wr \quad (1)$$

which relates ΔLk to the changes in twist (ΔTw) and writhing ($\Delta Wr = Wr$, because the writhe of unconstrained DNA is zero; White, 1969; Fuller, 1971; Crick, 1976). This equation, valid for the whole minichromosome, can also be applied to individual nucleosomes if the minichromosome is a random juxtaposition of independent topological domains with no net contribution of linker DNAs to total ΔTw and Wr. Equation 1 is then verified for $\Delta Lk = -1$, $\Delta Tw = +1$, and Wr (the writhe of a two-turn left-handed superhelix) $= -2$. In fact, $Wr = -2$ would be obtained only for a flat superhelix with a zero pitch, but with the actual parameters of the superhelix, Wr is closer to -1.7 (see below). This leads to $\Delta Tw = +0.7$. Such an increase in twist in $N_n = 166$ bp of wrapped DNA in two superhelical turns should reduce its helical periodicity, h, relative to the value of DNA free in solution, by ~0.5 bp/turn, as shown by the equation

$$\Delta h = -h^2 \cdot \Delta Tw/N_n \quad (2)$$

in which

$$h = N_n/Tw \quad (3)$$

In 1977, the DNA helical periodicity on the nucleosome had already been estimated from in situ digestion with DNase I (the principle of this measurement is explained below). The value obtained, 10.0 bp/turn (Noll, 1974; this periodicity was later found to be underestimated and to be closer to 10.3–10.4 bp/turn; see below), coincided exactly with the periodicity measured by x-ray diffraction on dehydrated fibers. Based on these premises, Finch et al. (1977) predicted that the periodicity of DNA free in solution should be larger than 10.0 bp/turn and close to 10.5 bp/turn. Surprisingly enough, such a value, also supported by energy calculations (Levitt, 1978), turned out to be confirmed by experimental measurements (Wang, 1979).

In the alternative explanation of the paradox, the DNA was thought to wrap without twist alteration ($\Delta Tw = 0$), but linkers would contribute to Wr, owing to a peculiar spatial arrangement of nucleosomes relative to one another (Worcel et al., 1981). However, this model fell rapidly into disgrace, to the advantage of the "overtwisting" model, which has stirred heated debate (see Morse and Simpson, 1988; Klug and Travers, 1989; and White and Bauer, 1989, for recent discussion) and stimulated much work aimed at the measurement of DNA helical periodicity in situ.

DNA LOCAL PERIODICITY ON THE NUCLEOSOME

This measurement was first performed with DNase I, an enzyme capable of cleaving one strand at a time. When DNA is adsorbed to a surface, only one side of the double helix is exposed, and the DNase tends to cut along directions perpendicular to the surface (Fig. 1). The lengths of

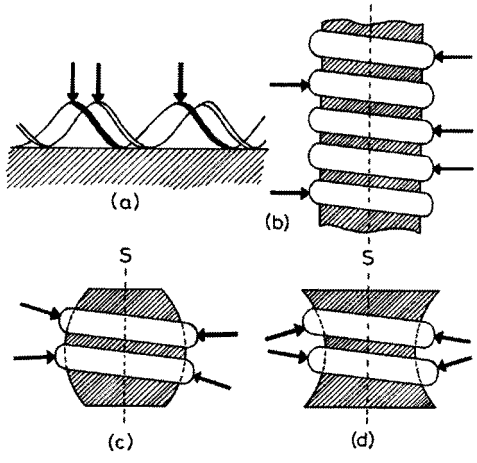

FIGURE 1 DNase cleavage patterns of wrapped DNA. DNA with a ~10.5 bp/turn helical periodicity when free in solution is adsorbed onto a flat surface (*a*), or wrapped into a left-handed superhelix around an infinite cylinder (*b*), or convex (*c*) or concave (*d*) revolution surfaces of axes *S*. Directions of cleavage by the DNase (*arrows*) remain perpendicular to the surface. These directions are in the plane containing the *S* axis, and are perpendicular to this axis in *b*, but not in *c* or *d*. When the DNA is adsorbed or wrapped free of torsional constraint, cleavage generates single strands of lengths that are either exact multiples of the helical periodicity (*a*) or are smaller by 0.15 nucleotide (10.5 − 0.15 = 10.35) when the superhelix has the diameter and the pitch of the nucleosomal superhelix (*b*). Cleavage periodicities may also depart from 10.35 nucleotides, and be larger (*c*) or smaller (*d*).

the single-stranded fragments obtained after histone extraction, which can be measured by gel electrophoresis under denaturing conditions, are then multiples of a unit length representing the digestion periodicity. If the surface is a plane (Fig. 1 *a*), digestion periodicity and helical periodicity are equal. In other words, in the absence of twist alteration upon adsorption, and taking the helical periodicity of DNA free in solution as 10.5 bp/turn under physiological conditions, single strands of lengths that are multiples of 10.5 nucleotides should be generated. (Accurate measurements of DNA helical periodicity have produced figures from 10.56 bp/turn (Goulet et al., 1987) at room temperature in the absence of divalent cations, to 10.53 (Zivanovic et al., 1988), 10.45 (Shore and Baldwin, 1983), and 10.54 bp/turn (Horowitz and Wang, 1984) in the presence of 5–10 mM Mg^{2+} at 20–37°C.) Such digestion products have indeed been obtained using hydroxylapatite crystals as the adsorbing surface (Rhodes and Klug, 1981). In contrast, digestion periodicity and helical periodicity are no longer equal if DNA is wrapped around a cylinder into a superhelix. The reason is that such wrapping not only bends DNA, but also twists it (Ulanovsky and Trifonov, 1983). In the case of a left-handed superhelix (Fig. 1 *b*), the digestion periodicity is smaller than the helical periodicity. (The reverse would be observed with a right-handed superhelix.) The former (digestion) periodicity is the *local* periodicity, h_{loc}, and the latter, h_{intr} (h in Eq. 3), is termed the *intrinsic* periodicity.

Defining h_{loc} physically is straightforward once the wrapping surface is known. It is the spacing between the most external regions of each strand, which are maximally exposed to external attack (Fig. 1), or between the most internal regions that are in contact with the surface. h_{loc} is therefore a strict measure of the twist of the double helix around its axis relative to the surface. h_{intr}, in contrast, measures not only this twist, but also the continuous change in the direction of the double helix axis as DNA wraps around the surface (Cozzarelli et al., 1990). h_{intr} is a convenient parameter in problems of wrapping surfaces because, as classical mechanics teaches, it is an invariant, i.e., it does not depend on the surface, as long as no torsional constraint is applied. This property, however, requires a perfectly elastic rod, which is only an approximation for DNA. In contrast, as is clear from its definition, h_{local} depends on the surface. It is noteworthy that the same conclusions would be reached by using the formalism of the "surface linking number" theory (White et al., 1988).

The relation between h_{loc} and h_{intr} can be precisely calculated for simple geometries. For a superhelix of radius r and pitch p wrapped around a perfect cylinder, one has (Le Bret, 1988)

$$1/h_{loc} = 1/h_{intr} + 3.4\ p/[(2\pi r)^2 + p^2] \qquad (4)$$

with 3.4 being the double helix rise per base pair (in Å). For the nucleosomal superhelix (r = 43 Å and p = 27 Å), Eq. 4 gives $h_{loc} - h_{int} = -0.15$ (the same figure was obtained by Ulanovsky and Trifonov, 1983), which brings h_{loc} to $10.5 - 0.15 = 10.35$.

Building on Noll's (1974) result, subsequent DNase I digestions of chromatin in nuclei led to h_{loc} = 10.3–10.4 nucleotides (Prunell et al., 1979; Lutter, 1979) instead of 10.0. This value is therefore that predicted if nucleosomal DNA has h_{intr} = 10.5 bp/turn. However, unique nucleosomes reconstituted on specific DNA fragments showed h_{loc} ranging from 9.9 to 10.5 nucleotides (see Table 1), a dispersion 10 times larger than the ±0.03 bp/turn observed for a collection of unique DNA sequences free in solution (Goulet et al., 1987). This raised the possibility that h_{loc} could vary from one nucleosome to the other, although the respective contributions of a sequence effect on nucleosome structure and/or DNase cleavage specificity in these variations were unclear. Regardless of these uncertainties, the 10.3–10.4 figure could still be meaningful, because it was obtained for a large spectrum of DNA sequences.

TABLE 1 Local periodicities (h_{loc}) of nucleosomal DNA

Technique	Probe	Sequence	h_{loc} (bp/turn)	Reference
Cleavage	DNase I	Mixed	10.35 (±0.05)	Prunell *et al.* (1979) Lutter (1979)
	Exonuclease III	Mixed	11 (edges) 10.1 (middle)	Prunell (1983)
	DNase I	Unique	9.9–10.5	Drew and Calladine (1987) Drew and McCall (1987)
		Unique	10.3; 10.5	Duband-Goulet *et al.* (1992)
	OH radicals	Unique	10.2 (±0.05)	Hayes *et al.* (1990)
		Mixed	10.2 (±0.05)	Hayes *et al.* (1991)
Sequencing	Statistical sequencing	—	10.2	Drew and Travers (1985)
	Trinucleotide repeat	—	10.2 (±0.1)	Satchwell *et al.* (1986)
	Dinucleotide repeat	—	9.7	Lowman and Bina (1990)
		—	10.26; 10.0	Bina (1994)
		—	10.3 (±0.2)	Ioshikhes *et al.* (1996)
UV illumination	Pyrimidine dimers	Mixed	10.3 (±0.1)	Gale *et al.* (1987) Gale and Smerdon (1988)
Competitive reconstitution	Repeat of flexible motifs	—	10.1 (±0.1)	Shrader and Crothers (1990)

Alternative approaches to h_{loc} involved digestions with exonuclease III and OH radicals, pyrimidine dimer formation, and competitive reconstitution with sequences made of flexible DNA motifs (see Table 1). Another approach, which deserves special comment because of its wider impact, started from the observation of a periodicity in the occurrence of some dinucleotides, especially AA and TT, along eukaryotic DNA but not procaryotic DNA (Trifonov and Sussman, 1980). Because this periodicity of occurrence was in approximate phase with the helical periodicity, these dinucleotides were supposed to deflect the DNA helical axis or be more easily compressed in the major or minor groove, so as to lead to a permanent, or easily induced unidirectional curvature of the DNA. This curvature, in turn, was thought to facilitate wrapping around the nucleosome (see Trifonov, 1985, for a review). Subsequently, the existence of bent DNA was experimentally demonstrated (Marini et al., 1982), as were Trifonov and Sussman (1980) predictions (Drew and Travers, 1985). From this it became clear that the exact spacing between AA and TT dinucleotides, respectively, and more generally between short (A, T) and (G, C) runs (Drew and Travers, 1985), was to provide another estimate for h_{loc} in the nucleosome. Such "sequence" periodicity is in essence identical to the above "digestion" periodicity: the (G, C) runs that were found to face out just replace the arrows in Fig. 1.

As shown in Table 1, h_{loc} estimates are spread over a wide range (9.7–11 bp/turn), even if most of them are between 10.2 and 10.3 (the mean of all values is 10.24) and do suggest some amount of overtwisting ($h_{loc} < 10.35$ and $h_{intr} < 10.5$). But there is an additional uncertainty at the level of the histone surface, which has no reason a priori to be the perfect cylinder depicted in Fig. 1 *b*. The surface could be convex (Fig. 1 *c*) or concave (Fig. 1 *d*), in which cases different h_{loc} values would be obtained for a unique $h_{intr} = 10.5$ bp/turn of wrapped DNA (see legend to Fig. 1). At the time the data were collected, no structure, including the octamer structure of Arents et al. (1991) and the subsequent core particle reconstruction by simulated DNA docking (which only required the number of base pairs between adjacent DNA binding sites to be an integer, 10 or 11; Arents and Moudrianakis, 1993), could clarify this dilemma (but see below).

A NUCLEOSOME ON A DNA MINICIRCLE

In this chromatin system, which was initially developed in the author's laboratory as an alternative to h_{loc} measurements, a single nucleosome is reconstituted on a DNA minicircle of ~350 bp (Goulet et al., 1988). The associated ΔLk (ΔLk_n) is measured as described above for the minichromosome (Zivanovic et al., 1988). A naked topoisomer is first relaxed with topoisomerase I to give no more than two adjacent topoisomers at the most in an equilibrium distribution. (The small number of topoisomers is due to the low flexibility of a minicircle under thermal fluctuations.) $\langle Lk \rangle = Lk_o$ can be calculated from the relative amounts of these topoisomers. Lk_o measures the linking number of the most probable conformation of the naked minicircle under the conditions used. Lk_o is also equal to N/h, in which N is the minicircle size. The linking number difference of any given topoisomer is

$$\Delta Lk = Lk - Lk_o \tag{5}$$

The minicircle reconstituted with a nucleosome is similarly relaxed under the same conditions. $\langle Lk \rangle$ of the topoisomer distribution obtained after histone extraction is the linking number of the most probable conformation of the minicircle partially wrapped around the nucleosome, Lk_o^n. ΔLk_n is then given by

$$\Delta Lk_n = Lk_o^n - Lk_o \tag{6}$$

Four DNA minicircles have led to a mean $\Delta Lk_n = -1.1$ (± 0.1) (Zivanovic et al., 1988; Hamiche et al., 1996a). Interestingly, whereas the other minicircles gave rise to equilibria between two mononucleosome forms, the 359-bp minicircle generated a single form corresponding to topoisomer -1 (topo -1, of $\Delta Lk = -1$; see Eq. 5). Consistently, the nucleosome reconstituted on topo -1 (mono -1) remained unaffected by incubation with the topoisomerase. This indicated the complete relaxation of the mono -1 external loop, consistent with a linking number difference in the loop $\Delta Lk_l = \Delta Lk - \Delta Lk_n = 0$, and therefore with ΔLk_n (in the open state) $= -1$ (Zivanovic et al., 1988).

Such a result was not significantly different from that obtained with the SV40 minichromosome ($\Delta Lk = -1$ per nucleosome). However, this result could now be correlated with the exact DNA path within the particle and in the external loop. Electron microscopic examination of chromatin reconstituted on 359-bp topo -2 (Fig. 2) revealed a nucleosome occupying two alternative states. In the open state (*inset b*), the loop does not cross and $\mu \approx 1.4$; in the closed state (*inset c*), the loop crosses almost at a right angle, and $\mu \approx 1¾$. These two states were found to be in a salt-dependent equilibrium. The chromatin shown in Fig. 2 is in 10 mM monovalent salt, and mononucleosomes of the two species are in approximately equal proportions. In 100 mM NaCl, in contrast, most of the nucleosomes showed the closed conformation. (The same behavior was observed for dinucleosomes (*insets d* and *e* in Fig. 2).) This presumably originates from the correlative decrease in the electrostatic repulsion between entering and exiting DNAs. The increase in $|\Delta Lk_l|$ as a consequence of the salt-induced increase in the twist of loop DNA should also favor the closed conformation, but its actual value, ~10%, is too small to explain the effect observed. Interestingly, this dual conformation was not found for mono -1, which remained in the open state under both salt conditions (Zivanovic et al., 1988).

Higher salt conformations of 359-bp mono -1 and -2, together with the conformation of mono 0, are schematized in Fig. 3. Mono 0 and -2 show the closed state, with $\mu \approx 1¾$ expected from the core particle crystal structure (see above). In contrast, mono -1 is in the open state, with $\mu \approx$

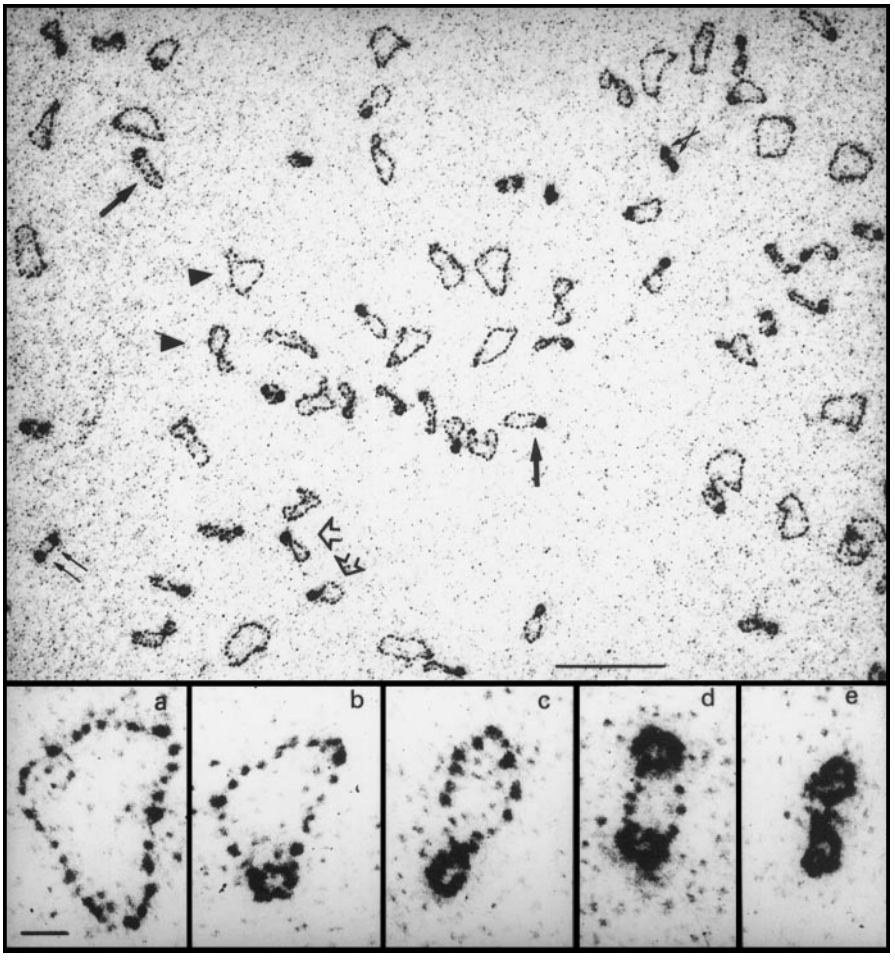

FIGURE 2 Electron micrographs of 359-bp topo −2 reconstituted with histone octamers and spread at low ionic strength. Unreacted naked DNA (*arrowheads*), nucleosome monomers (*single arrows*), and dimers (*arrow doublets*) are marked. Insets display enlargements of naked DNA (*a*), crossed (*c* and *e*) and uncrossed (*b* and *d*) nucleosome monomers and dimers, respectively. Bars = 100 nm and ~300 bp (*top*) and 10 nm and ~30 bp (*insets*). Negatives are shown.

1.4. As already mentioned, $\Delta Lk_l = 0$ in mono −1, which means that all of the topoisomer linking difference ($\Delta Lk = -1$) is taken up by the nucleosome. In contrast, ΔLk_l of +1 and −1 remain in the loop of mono 0 and −2, respectively, as confirmed by their conversion into mono −1 upon incubation with the topoisomerase (Zivanovic et al., 1988). ΔLk_l helps the loop to rotate around the dyad axis *D*, which results in a positive or negative node (see legend to Fig. 3). ΔLk_l, in fact, appears to act in concert with histone-DNA interactions at the core position to stabilize the closed state of the nucleosome, consistent with an increase in loop DNA bending forces in that conformation. This is in contrast to the case of a "linear" nucleosome, in which histone-DNA interactions alone suffice to maintain the closed conformation (see below). (An insight into the probable nature of the differences in histone-DNA interactions between open and closed states will be given below, when we consider the high-resolution crystal structure of Luger et al. (1997).) A crossed loop and larger wrapping in mono 0 and −2 are confirmed by their faster migration upon gel electrophoresis compared to mono −1 (Zivanovic et al., 1988). In conclusion, these results demonstrate that the most probable loop conformation is open, and that $\Delta Lk_n = -1.1$ is associated with $\nu \approx 1.4$.

This result alone—a single negative crossing associated with a unit linking number reduction—provided the simplest possible outcome, i.e., the paradox did not exist in the first place. However, reviewers of the paper submitted at that time were not happy with it, and required a more quantitative answer. For this, the nucleosome on its minicircle was simulated by a model in which one part of the DNA was wrapped around a cylinder into a left-handed superhelix, and the other part, the loop, was free to vary in both flexion and torsion (Le Bret, 1988). Fig. 4 shows the total writhe, *Wr*, computed for the most probable conformation of the minicircle, as a function of ν. When ν increases from 1 to 1.4, *Wr* remains equal to −1 and the loop keeps an open conformation (the open state; *inset a*). Upon further increase in ν, *Wr* decreases rapidly to reach a plateau region at $Wr = -1.7$. At the same time, the loop rotates by a negative angle around the dyad, *D*, and eventually crosses into a negative node (the closed state; *inset c*). Note that the loop is relaxed in all of these conformations, and that its rotation is driven by the increase in ν and not by ΔLk_l, as in mono −2 in Fig. 3. The midtransition ($Wr = -1.35$) is reached at $\nu = 1.6$ when the loop plane is parallel to the superhelix axis, *S* (*inset b*). Interestingly, this *Wr*-versus-*u* curve, which was later confirmed by Zhang et al. (1994),

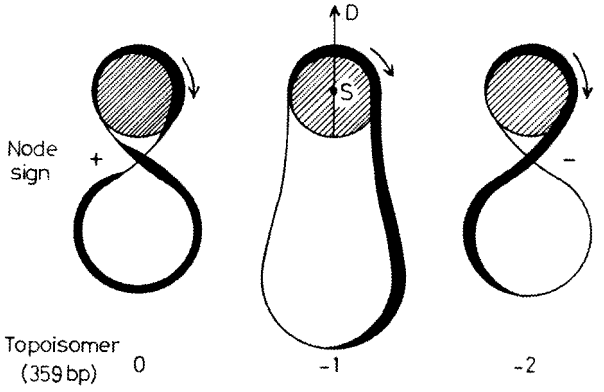

FIGURE 3 Schemes of loop conformations in circular mononucleosomes. In mono −1, the loop is relaxed and its plane is approximately perpendicular to the DNA superhelix axis, *S*. In monos −2 and 0, the loop has rotated around the dyad axis, *D*, by a negative or positive angle, forming a negative or positive node. These rotations are triggered by the residual linking differences in the loops (see text). Note that exiting DNA (*arrow*) is above and remains above in the negative crossing (mono −2), whereas it goes below in the positive crossing (mono 0). The rotation angle shown is close to 180°. The loop and the DNA superhelix are considered as two independent topological domains delimited by the clamping of the DNA to the histones at the 1.4-turn positions. A second clamping close to the 1.75-turn positions must occur in the closed conformation in mono −2, and perhaps in mono 0.

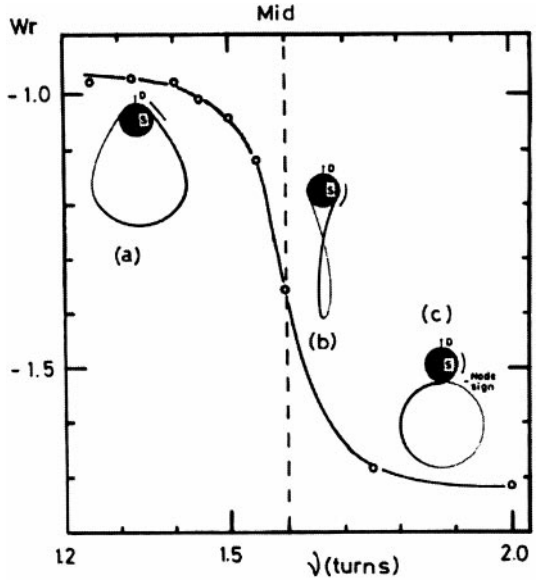

FIGURE 4 Computed writhe (*Wr*) of the most probable conformation of the DNA minicircle partially wrapped into ν turns of a superhelix around the nucleosome. Data points were taken from Le Bret (1988). Loop conformations at $\nu \sim 1.4$ (*a*), ~ 1.6 (*b*), and ~ 2 (*c*) are shown. The loop plane is perpendicular to the *S* axis in *a* and *c*, and parallel to this axis in *b*.

was not found to depend much on the particular values used for the DNA persistence length or the twisting flexibility coefficient (Le Bret, 1988), and the curve would presumably not be much disturbed by deformations of the histone surface such as those depicted in Fig. 1. For $\nu \approx 1.4$, one obtains $Wr \approx -1.0$ (Fig. 4) and ΔTw (the potential twist alteration upon wrapping) $= \Delta Lk_n - Wr$ (see Eq. 1) $= -0.2$ to -0.1. (Note that ΔTw is for the whole minicircle, but that the contribution of the relaxed loop to ΔTw is zero.)

The lower part of the curve in Fig. 4 (the closed state of the nucleosome) has also been explored by measuring ΔLk_n for a two-turn H5-containing nucleosome (Zivanovic et al., 1990). Although mono −1 showed virtually no crossing under electron microscopy (see above), a crossing occurred after H5 binding. At the same time, the gel electrophoretic mobility increased, consistent with a larger DNA wrapping. Topoisomerase relaxation now led to ΔLk_n between −1.6 and −1.65. From Eq. 1, and with a theoretical $Wr = -1.7$ for $\nu = 1.75$ (Fig. 4), one obtains $\Delta Tw = +0.05$ to $+0.1$.

Such $\Delta Tw = -0.2$ to $+0.1$, when compared to an expected ΔTw of $+0.7$ (see above) or $+0.56$ (White and Bauer, 1989), makes a strong case against the "overtwisting" model. They actually indicate that DNA wraps around the octamer with little, if any, positive torsional constraint, that is, with an h_{intr} close to that of DNA free in solution (10.5_3 bp/turn under relaxation conditions; see above). This conclusion does not exclude local twist variations, but requires these variations to approximately compensate over the length of nucleosomal DNA.

A CORE PARTICLE STRUCTURE AT HIGH RESOLUTION

This long-awaited crystal structure (Luger et al., 1997) offers an opportunity to bring the above model to a test. The DNA fragment used was an inverted repeat of a 73-bp sequence with a dyad axis of symmetry going through the middle of the two central base pairs. Such a palindromic sequence apparently helped in growing better diffracting crystals. However, the twofold symmetries of the sequence and of the octamer did not perfectly match each other, because the octamer preferred to position itself with its dyad on one of the two central base pairs rather than in between. This led to the two halves being of unequal lengths, 72 and 73 bp, respectively (excluding the base pair on the dyad). As a consequence, a given sequence motif in the 72-bp half would have a 1-bp deficit in its distance from the dyad and in its positive twist around the double-helix axis, compared to the same motif in the 73-bp half. It turned out that a stretching-overtwisting occurred, spread over a 12-bp region of the 72-bp half, which compensates for those deficits. As a result, the twofold symmetry was essentially restored beyond 23 bp from the dyad and down to the DNA terminus.

An objective way of measuring local periodicity in this structure is by considering the bases that are in closest proximity to an arginine side chain inserted into the minor groove ("colored bases" in figure 1 *b* in Luger et al., 1997). These bases can be conveniently identified by reference to the nearest crossing in the small groove (for the superhelix viewed along its axis), i.e., by a fractional superhelix location (SHL) number. In the SHL terminology, integral numbers correspond to crossings in the large groove, with SHL0

on the dyad. Table 2 gives the spacings separating these bases on the two strands and the cumulated spacings between opposite SHL numbers. h_{loc} are then obtained by dividing cumulated spacings by the corresponding segment numbers. h_{loc} are the same for the two strands and decrease regularly from 10.43 at SHL ± 3½ to 10.23 at SHL ± 6½ (Table 2). Very similar h_{loc} values were obtained when spacings were measured instead between integral SHL numbers (figure 1 *d* in Luger et al., 1997; not shown). This identity shows that the angular orientation of "colored bases" about the double helix axis does not vary along the path of the superhelix, and therefore that no "concave" bias of the type schematized in Fig. 1 *d* takes place, at least in this particular nucleosome.

In the structure of Luger et al. (1997), most distal histone-fold contacts with DNA occur with H2A-H2B at SHL ± 5½. These SHLs encompass a single-stranded DNA length of 113 nucleotides (Table 2), i.e., a double-stranded length of 113 + 4 = 117 bp when the staggering of "colored bases" in one strand relative to the other is taken into account. This 117-bp figure is close to estimates of the length of wrapped DNA in the open state, 115 and 109 bp, which were obtained, respectively, by electron microscopy from the distribution of the angles between entering and exiting DNAs in 359-bp mono −1 (Zivanovic et al., 1988), and from DNA thermal flexibility measurements (Hamiche and Prunell, 1992). This, in turn, strongly suggests that the open state results from the breakage of DNA interactions at SHL ± 6½ with the so-called histone-fold extensions of H3 (see Luger et al., 1997). h_{loc} figures to be considered in open and closed states are therefore 10.27 (at SHL ± 5½) and 10.23 (at SHL ± 6½).

Entering the new dimensions of the DNA superhelix (r = 41.8 Å and p = 23.9 Å in Luger et al. (1997); against 43 and 27 Å, respectively, reported by Richmond et al. (1984) and used by Le Bret (1988)) in Eq. 4, one obtains h_{intr} = 10.40 and 10.36 in open and closed states, respectively. (This represents an increase of 0.13 over h_{loc}, compared to 0.15 with former superhelix parameters. The new parameters would also alter Wr values in Fig. 4, but only very slightly; D. Swigon, personal communication.) This results in ΔTw = +0.14 and +0.22 in open and closed states, as calculated from Eq. 2 with $\Delta h = h_{int} - 10.53$ and N_n = 117 and 133 + 4 = 137 bp, respectively. If these overtwistings fall short of that needed in the "overtwisting" explanation of the paradox (ΔTw = +0.56 to +0.7; see above), they are also larger (by ~0.1) than the maximum value of +0.1 permitted in the minicircle system. It is interesting to note that this excess overtwisting is the result of the 1-bp stretching-overtwisting in the 72-bp half referred to above.

The 73-bp half clearly has a lower free energy than the 72-bp half, because the 72-bp half tends to adopt the 73-bp half conformation, rather than the other way around. A lower stability of the 72-bp half is also evident in view of the high energy cost of stretching-overtwisting, because stretching is rather the consequence of undertwisting (as observed with intercalating drugs, such as ethidium bromide; Coury et al., 1996). As a consequence, a palindromic

TABLE 2 Local periodicities (h_{loc}) measured from the core particle high-resolution crystal structure of Luger *et al.* (1997)

	72-bp half								73-bp half						
72-plus 73-bp halves*															
Brown strand															
SHL	−6½	−5½	−4½	−3½	−2½	−1½	−½	**0**	+½	+1½	+2½	+3½	+4½	+5½	+6½
Spacing (bp)	10	10	10	11	10	9	**4**	**7**	10	10	12	10	10	10	
SHL		±½		±1½		±2½		±3½		±4½		±5½		±6½	
Spacing (bp)		11		30		50		73		93		113		133	
h_{loc} (bp/turn)		11		10		10		10.43		10.33		10.27		10.23	
Turquoise strand															
SHL	−6½	−5½	−4½	−3½	−2½	−1½	−½	**0**	+½	+1½	+2½	+3½	+4½	+5½	+6½
Spacing (bp)	10	10	10	12	9	10	**7**	**4**	9	10	12	10	10	10	
SHL		±½		±1½		±2½		±3½		±4½		±5½		±6½	
Spacing (bp)		11		30		49		73		93		113		133	
h_{loc} (bp/turn)		11		10		9.8		10.43		10.33		10.27		10.23	
2 × 73-bp halves#															
Brown strand															
SHL	−6½	−5½	−4½	−3½	−2½	−1½	−½	**0**	+½	+1½	+2½	+3½	+4½	+5½	+6½
Spacing (bp)	10	10	10	12	10	9	**4**	**7**	10	10	12	10	10	10	
SHL		±½		±1½		±2½		±3½		±4½		±5½		±6½	
Spacing (bp)		11		30		50		74		94		114		134	
h_{loc} (bp/turn)		11		10		10		10.57		10.44		10.36		10.31	

*Spacings were measured by counting the number of base pairs between bases closer to arginine side chains inserted into the minor groove ("colored bases" in figure 1 *b* in Luger et al., 1997). These bases are referred to by fractional superhelix location (SHL) numbers (see text). SHL0 (bold) corresponds to the base at the dyad position. For the counting, bases delimiting the segments are shared equally between adjacent segments. h_{loc} values are obtained by dividing cumulated spacings by the corresponding number of segments.

#The brown strand of the symmetrized 2 × 73-bp superhelix is obtained by juxtaposing brown and turquoise strands of the 73-bp half. The turquoise strand can be similarly obtained, and obviously leads to the same h_{loc} as the brown strand (not shown).

sequence composed of the two 73-bp halves plus 1 bp in the middle (total length = 147 bp) is expected to form a stable nucleosome with the h_{loc} figures of the symmetrized particle (10.36 and 10.31 in open and closed states, respectively; Table 2). This would lead to $\Delta Tw = +0.04$ and $+0.11$, respectively, in good agreement with predictions of the minicircle system. In contrast, the symmetrized particle made of the two 72-bp halves (145 bp total) should be less stable, and may not form if given the choice between different sequences in competitive reconstitution experiments. Consistent with these notions, Luger et al. (1997) report that the location of the region of stretching-overtwisting may not be attributed to histone-DNA interactions within the flanking sequence, but rather to the strength of the interparticle contacts between DNA termini. The authors do not mention whether this qualification could also apply to the very existence of that region. A failure of stretching-overtwisting to occur in solution nevertheless remains an open possibility.

In summary, even with stretching-overtwisting, this first high-resolution structure shows a DNA overtwisting that is markedly too small (2.5- to 3-fold) to explain the paradox according to the "overtwisting" hypothesis. The confirmation of stretching-overtwisting in solution would leave an excess overtwisting of ~0.1 relative to higher overtwisting estimates in the minicircle system. A straightforward explanation for this discrepancy could be a true variation in h_{loc} from one nucleosome to another, as scattered figures in Table 1 would suggest (see above). However, recent progress in our understanding of the system will probably lead to a reevaluation of DNA overtwisting in minicircle nucleosomes. The use of a series of minicircles with unique sequence and N increasing by 1-bp increments from 351 to 366 bp has uncovered a significant dependence of ΔLk_n on N. ΔLk_n periodically oscillates between extremes of -1.0 around $N = 350$ and 360 bp, and -1.45 around 355 and 365 bp. Modeling of these oscillations, which presumably reflect the nucleosome ability to thermally fluctuate between open and closed states, will allow us to further refine ΔLk_n estimates for the two states (F. De Lucia, M. Alilat, A. Sivolob, J. Cohen-Solal, and A. Prunell, manuscript in preparation).

NUCLEOSOMES IN CHROMATIN

Before concluding that $\Delta Lk = -1$ per nucleosome in chromatin (see above) can similarly be explained by a failure of the linkers to cross, it is useful to separately address the issues of H1/H5-free and H1/H5-containing chromatins.

H1/H5-free chromatin

If the open state of the nucleosome does reflect an attempt to minimize the bending free energy within the topological constraints of the minicircle, then this open state should not be observed in mononucleosomes reconstituted on linear 250–350-bp fragments. Such mononucleosomes, when visualized by scanning transmission (Hamiche et al., 1996b) and cryoelectron microscopies (followed by 3D reconstruction in this latter case) (Furrer et al., 1995), showed a closed state-like wrapping of ~1.7 turns, as expected. However, entering and exiting DNAs did not cross, but rather bent away from each other and from the nucleosome surface at the entry-exit point (Fig. 5 *a*), presumably as a consequence of DNA-DNA electrostatic repulsion. The extension of this result to nucleosomes in a fiber seems natural, especially in view of the numerous observations of such uncrossed linkers in H1/H5-free chromatin (see, for example, Thoma et al., 1979; Noll et al., 1980). At the time of these observations, however, it was not clear whether the stretching forces exerted during chromatin adsorption on the grid were responsible for these uncrossed conformations.

H1/H5-containing chromatin

In the presence of H1/H5, chromatin undergoes a strong compaction, which has made the nucleosome arrangement as well as the DNA path hard to define. As a consequence, it is not clear why the linker histone-induced increase in $|\Delta Lk|$ observed for nucleosomes on DNA minicircles does not occur in minichromosomes ($\Delta Lk = -1$ per nucleosome, regardless of the presence or absence of the linker histone; Germond et al., 1975; Stein, 1980; Morse and Cantor, 1986). In the hope that a clue to this discrepancy lay in the fine structure of entering and exiting DNAs, linear mononucleosomes were reconstituted with engineered H5s that had variously trimmed C-terminal tails. The resulting par-

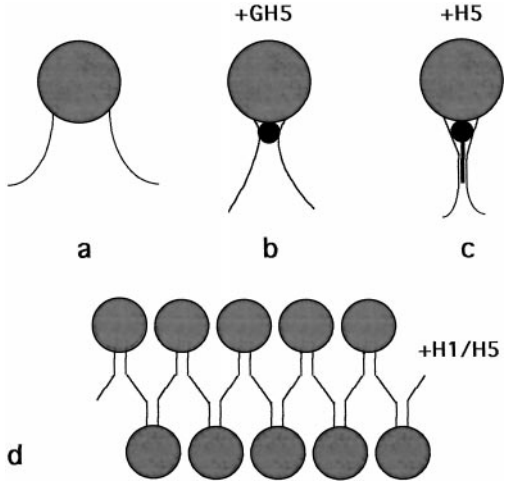

FIGURE 5 Schemes of linker histone-dependent DNA structure in mononucleosomes and the chromatin fiber. In the absence of H5, DNA wraps ~1.7 turns around the octamer, but exiting and entering DNAs do not cross, because of their bending away from each other (*a*). In the presence of H5 globular domain (GH5), wrapping increases to almost two turns, but DNAs still do not cross, because of an accentuation of the bends (*b*). An H5 C-terminal tail connects the DNAs, forming a ~30-bp-long stem (*c*). Linker DNA parallel arrangement in the zigzag model of the H1/H5-containing 30-nm chromatin fiber (*d*). No effective crossing of the linkers occurs.

ticles were visualized by electron microscopy, and the H5-induced shift in their electrophoretic mobility, which was found to depend on nucleosome position relative to the fragment ends, was carefully analyzed (Hamiche et al., 1996b). The following conclusions could be reached: 1) H5 globular domain (GH5) increased ν from 1.7 to 1.8–1.9, but entering and exiting DNAs still failed to cross, because of an accentuation of the bends (Fig. 5 *b*). Only the H5 C-terminal tail was able to efficiently counteract the repulsion of the DNAs, bridging them together into a stem over a distance proportional to its length, ~30 bp for the full-length tail (Hamiche et al., 1996b) (Fig. 5 *c*). A subsequent study showed that the H5 tail also induces a stem in circular mononucleosomes, which was accompanied by an increase in $|\Delta Lk|$ proportional to its length (Hamiche et al., unpublished data). These results indicate that the two DNA duplexes in the stem wind negatively around each other, the half-turn winding, and therefore the effective crossing, being reached only with the full-length H5.

The observation of a stem in native H1-containing trinucleosomes (Bednar et al., 1995) suggests its ubiquitous character. A stem is consistent with zigzag models of chromatin superstructure in which H1 is located in the fiber interior (Graziano et al., 1994; Zlatanova et al., 1994) and straight linkers project out from the nucleosomes (Staynov, 1983; Williams et al., 1986; Bordas et al., 1986; Woodcock et al., 1993; Horowitz et al., 1994; Pehrson, 1995; van Holde and Zlatanova, 1996). The question, then, is: Do stem DNAs also wind around each other in the superstructure? Two lines of evidence suggest that they do not, but are instead simply juxtaposed parallel to each other, as depicted in Fig. 5 *d*.

First, an H5-induced increase in $|\Delta Lk|$ similar to that observed with mononucleosomes does take place in minichromosomes, but only at lower nucleosome density (Stein, 1980). This indicates that such nucleosomes can rotate around their dyad axis relative to one another upon H5 binding, whereas that rotation is hindered at higher density because of nucleosome interactions. (The reality of these interactions is supported by the low thermal flexibility of DNA in H1/H5-free chromatin (or the poor ability to untwist upon an elevation of the temperature) (Morse and Cantor, 1985), as opposed to a high DNA thermal flexibility in nucleosomes on DNA minicircles (Hamiche and Prunell, 1992).) The second piece of evidence is provided by a direct measurement by flow linear dichroism of the angle of the nucleosome flat faces relative to the fiber axis. Complete trypsin digestion of H1/H5 tails in native fibers from chicken erythrocytes was found not to significantly modify this orientation (the dichroism remained positive), although the expected decompaction was observed by light scattering. In contrast, nucleosome orientation changed (the dichroism turned negative) and presumably became random, when H3 tails began to be digested (Makarov et al., 1984; Dimitrov et al., 1986). In addition to raising interesting questions regarding the exact nature and geometry of such nucleosome interactions, these observations suggest that the stem in chromatin, contrary to expectations from the minicircle system, can assemble without winding, and disassemble without unwinding of the two constitutive duplexes around each other. These features are simply explained if, as already mentioned, the two duplexes in the stem remain unwound, because of the specificity of H3 tail-mediated nucleosome interactions.

In conclusion, this discussion suggests that $\Delta Lk = -1$ per nucleosome in minichromosomes also reflects a failure of nucleosome entering and exiting DNAs (the linkers) to cross. The increase in wrapping over the 1.4 turns of the open state in the minicircle system, up to the bridging together of adjacent linkers into a stem in the H1/H5-containing fiber, is indeed expected to be topologically neutral, i.e., ΔLk remains equal to -1, as long as no effective crossing is produced.

NUCLEOSOME DYNAMICS: CONFORMATIONAL FLEXIBILITY OF THE $(H3\text{-}H4)_2$ TETRAMER

Genetic expression and replication and DNA repair in eucaryotic cells require the nucleosome to be a dynamic structure. It has long been thought that this dynamic should be mediated by the tripartite organization of the histone octamer made of a $(H3\text{-}H4)_2$ tetramer flanked by two H2A-H2B dimers (Eickbush and Moudrianakis, 1978). Thermodynamics studies have shown that the forces holding the tetramer and the dimers together were of a different nature and much stronger than the forces linking the dimers to the tetramer, despite the extensive dimer-tetramer interface (Baxevanis et al., 1991). This point is illustrated by octamer disassembly into a tetramer and two dimers below ~1 M salt. Two main observations suggested that this lability of H2A-H2B dimers played a physiological role: first, the existence of a deficit in H2A-H2B within nucleosomes originating from transcriptionaly active chromatin (Baer and Rhodes, 1983); second, the exchange of H2A-H2B, but not of H3-H4, with the intranuclear histone pool during in vivo transcription (Louters and Chalkley, 1985; Schwager et al., 1985; Jackson, 1990). These data prompted an investigation of the topological properties of the $(H3\text{-}H4)_2$ tetramer, using the concepts and methodology developed for the octamer (Hamiche et al., 1996a).

The observation

Fig. 6 compares the electrophoretic mobility of 10 topoisomers (ΔLk (see Eq. 3) = -1 to $+1.2$) originating from 351–359-bp minicircles, before (Fig. 6 *A*) and after (Fig. 6 *B*) reconstitution with the tetramer. Topoisomers closer to relaxation are slower, as expected, whereas more supercoiled topoisomers are faster, unlike the M_T particles, the mobility of which does not vary much. This, in turn, is consistent with a unique histone stoichiometry in the particle (a single tetramer, as estimated by histone quantitation and sedimentation velocity experiments). Reconstitution is

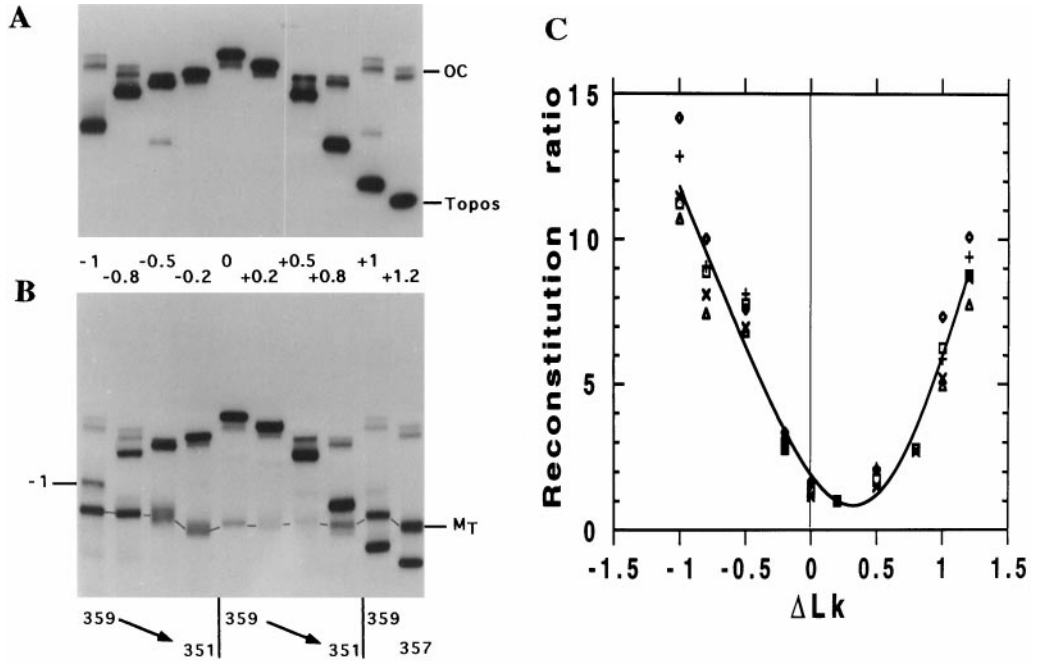

FIGURE 6 Supercoiling-dependent formation of the M_T particle. Autoradiograms of gel electrophoretic patterns of $\Delta Lk = -1$, 0, and +1; −0.8, +0.2, and +1.2; −0.5 and +0.5; and −0.2 and +0.8 topoisomers of 359, 357, 354, and 351 bp DNA minicircles, respectively, before (*A*) and after (*B*) reconstitution with $(H3\text{-}H4)_2$ tetramers. OC, Open circular DNA. (*C*) Topoisomer fractions in the M_T band, divided by the corresponding fraction obtained for topo +0.2, were plotted as a function of ΔLk. Data were derived from the gel in *B* and four additional, independent experiments. The curve is a fifth-degree polynomial.

minimal for topoisomers closer to relaxation, and increases with negative and positive ΔLk (Fig. 6 *C*). This increase with negative ΔLk was expected, because it is also observed with the octamer and simply reflects the relief of the free energy of supercoiling upon wrapping into a left-handed superhelix (Goulet et al., 1988). The increase in reconstitution with positive ΔLk, in contrast, is not observed with the octamer, and must originate from a major structural transition of the DNA on the particle.

The model

This transition could reflect a change either in the writhe or in the twist of the wrapped double helix (or a combination of the two). In the first hypothesis, the left-handed superhelix would become right-handed, whereas in the second, the double helix would overtwist within the frame of the left-handed superhelix. Although both types of transitions can relieve the topoisomer positive linking difference (and facilitate particle formation), a switch of the superhelix handedness is expected to be more efficient than overtwisting in doing this, particularly when it is realized that such overtwisting would have to be spread over only ~70 bp of DNA (the length wrapped around the tetramer; see below). A change in the superhelix handedness is also supported by the overall shape of the tetramer within the octamer crystal structure (Arents et al., 1991). This tetramer resembles a twisted horseshoe forming 0.75 turn of a proteinaceous left-handed molecular superhelix, with a large cavity in the center. The left-handedness of this superhelix is further described as resulting from the clockwise rotation of the two crescent-shaped H3-H4 dimers relative to each other around the H3-H3 interface (Fig. 7, *left*). The switch to the right-handed superhelix has been proposed to occur through a local deformation of the H3-H4 dimers, resulting in a rotation in the reverse, counterclockwise direction around the H3-H3 interface (Fig. 7, *right*) (Hamiche et al., 1996a).

Predictions

Because DNA crossing polarity is not usually recognized under classical electron microscopy, M_T particles formed on positive and negative topoisomers (i.e., their DNA path) should be undistinguishable. Electron micrographs in Fig. 8 show that naked topo −1 and +1 in higher and lower galleries, respectively, are open as expected, whereas $M_T(-1)$ and $M_T(+1)$ are crossed (median and lower galleries). Both particles exhibit a small hollow loop, which presumably contains all of the protein and a large external loop. Their contour length is the same as that of naked DNA, indicating the absence of the hidden turn observed in nucleosomes reconstituted on the same topo −1 (upper gallery). Given the circumference of the small loop and the mean DNA crossing angle, ~0.7 turn and ~60 bp of DNA were estimated to wrap around the tetramer, a value in keeping with the length (~70 bp) protected against micrococcal nuclease digestion (Read et al., 1985; Dong and van Holde, 1991). The DNA appears therefore to simply follow

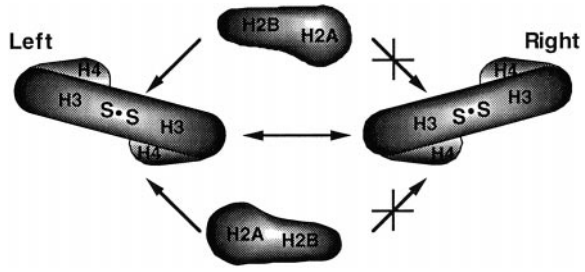

FIGURE 7 Model for tetramer conformational transition. The tetramer is shown as a left-handed ramp (*Left*), in which the sectors formed by H3-H4 dimers are off the dyad-containing plane by 15° each in the clockwise direction (Arents et al., 1991). The S-S disulfide on the dyad axis bridges Cys^{110} in the two H3s. Tetramer conformational transition is thought to occur through a local structural deformation of the two H3-H4 dimers, resulting in a rotation in a counterclockwise direction around the H3/H3 interface, or the S-S bridge, to give a right-handed molecular superhelix (*Right*). H2A-H2B dimers can cap the left-handed tetramer to assemble the octamer, but not the right-handed tetramer.

the helical ramp, either left-handed or right-handed, of the protein. Such a 0.7-turn wrapping is at variance with the ~1.5 turns obtained in other laboratories (Read et al., 1985; Dong and van Holde, 1991; Hayes et al., 1991), which may reflect the stacking of two tetramers into pseudooctamers (Baxevanis et al., 1991; Flaus et al., 1996). (Type II particles in median and lower galleries probably originate from such a stacking; see legend to Fig. 8.)

A second prediction of the model is that the transition may still occur after the cross-linking of the two H3s through a disulfide (S-S) bridge between two Cys^{110}. The reason is that the S-S bridge, which is known not to hamper nucleosome formation (Camerini-Otero and Felsenfeld, 1977), is located on the dyad axis (Daban and Cantor, 1982; Wang et al., 1994) right at the point of the putative rotation between the two H3-H4 dimers. The experiment showed that cross-linked tetramers resulting from oxidation in the presence of Cu(II) (1,10-phenanthroline) reconstitute $M_T(-1)$ and $M_T(+1)$ with an efficiency similar to that of nonoxidized tetramers (Hamiche et al., 1996a).

It is noteworthy that these observations, although consistent with the model in Fig. 7, do not bear on the issue of tetramer change in geometry, for which direct support has recently been obtained. The introduction of a steric hindrance in the dyad region of the tetramer by the linking of bulky adducts to Cys^{110} residues before reconstitution was found to block the DNA-tetramer complex in the right-handed conformation (unpublished results).

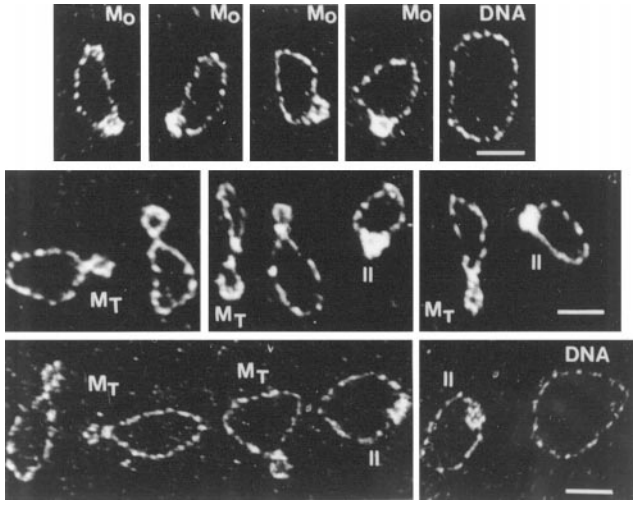

FIGURE 8 Electron micrographs of reconstitution products of 359-bp topos −1 and +1 with histone octamers and tetramers spread at low ionic strength. Representative molecules are shown. Higher, median, and lower galleries: topoisomer −1 reconstituted with octamers (M_O) and tetramers (M_T), and topoisomer +1 reconstituted with tetramers (M_T), respectively. DNA: Naked topoisomers. II: Nucleosome-resembling particles presumably made of two or more tetramers stacked on top of each other. Typical percentages were 30–35%, 50%, and 15–20% for DNA, M_T and type II particles, respectively, for both topos −1 and +1. Bars = 20 nm and ~60 bp.

Energetics of the transition

A close examination of the curve in Fig. 6 shows that reconstitution increases immediately with negative supercoiling, as expected, but that the increase with positive supercoiling is delayed and starts only at $\Delta Lk \approx +0.5$. This suggests that some energy is required to trigger the DNA-tetramer complex into the right-handed conformation. This energy can be calculated from the free energy of supercoiling of topo +0.5, ~1.5 kcal/mol (Horowitz and Wang, 1984). Subsequently, reconstitution increases at the same rate as with negative supercoiling, futher suggesting that all positive topoisomers use the same fraction of their energy to trigger the transition, the remaining energy being used for wrapping. These features therefore imply that the DNA-tetramer complex is more stable in the left-handed conformation. This was confirmed by the spontaneous conversion of $M_T(+1)$ and $M_T(+1.2)$ into $M_T(-1)$ and $M_T(-0.8)$, respectively, upon incubation with topoisomerase I. A remarkable aspect of the transition is its low energy (1.5 kcal/mol is only 2.5 times *RT*), which in turn implies that DNA thermal fluctuations should be able, at least at their peak value, to trigger it. In fact, the presence of minor, although significant amounts of positive topoisomers in relaxation equilibria of M_T particles reconstituted on negative topoisomers (Hamiche et al., 1996a) is a direct confirmation of this expectation.

CONCLUSIONS

Tetramer binding affinity for both negatively and positively supercoiled DNA had previously been observed with plasmids (Jackson, 1995). The affinity for negatively supercoiled DNA was explained by the DNA left-handed wrapping around the histones, whereas the affinity to positively supercoiled DNA was interpreted as a reflection of DNA overtwisting on the histone surface. It was argued that positive supercoiling, in overtwisting the DNA, facilitated

the reconstitution by relieving the histones from doing so (but see above). The advantage of a tetramer with an affinity for positively supercoiled DNA was nevertheless recognized. Such a tetramer could sustain the positive supercoiling wave pushed in front by the polymerase (Liu and Wang, 1987), whereas H2A-H2B would be destabilized and released (see above). The tetramer could then serve as a nucleation site for nucleosome regeneration under the negative supercoiling wave pulled behind by the polymerase (Jackson, 1993, 1995; Jackson et al., 1994). The model of Fig. 7 reinforces this view in explaining the basis for tetramer affinity for positively supercoiled DNA. Moreover, the observed loss of affinity of H2A-H2B dimers for the right-handed tetramer (see Fig. 7) directly supports the possibility that H2A-H2B successive release and reassociation is accompanied by a switch of the tetramer from one conformation to the other, the two events being possibly mechanically linked. Tetramer conformational flexibility may also have a role in transcriptional initiation and in replication, and more generally in many processes dependent on the dynamics of the nucleosome in vivo.

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