Equilibrium distributions of topological states in circular DNA: Interplay of supercoiling and knotting

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Two variables define the topological state of closed double-stranded DNA: the knot type, K, and ∆Lk, the linking number difference from relaxed DNA. The equilibrium distribution of probabilities of these states, P(∆Lk, K), is related to two conditional distributions: P(∆Lk|K), the distribution of ∆Lk for a particular K, and P(K|∆Lk) and also to two simple distributions: P(∆Lk), the distribution of ∆Lk irrespective of K, and P(K). We explored the relationships between these distributions, P(∆Lk, K), P(∆Lk), and P(K|∆Lk) were calculated from the simulated distributions of P(∆Lk|K) and of P(K). The calculated distributions agreed with previous experimental and theoretical results and greatly advanced on them. Our major focus was on P(K|∆Lk), the distribution of knot types for a particular value of ∆Lk, which had not been evaluated previously. We found that unknotted circular DNA is not the most probable state beyond small values of ∆Lk. Highly chiral knotted DNA has a lower free energy because it has less torsional deformation. Surprisingly, even at ∆Lk > 12, only one or two knot types dominate the P(K|∆Lk) distribution despite the huge number of knots of comparable complexity. A large fraction of the knots found belong to the small family of torus knots. The relationship between supercoiling and knotting in vivo is discussed.

Topological properties of DNA are essential for life. It is simplest to consider the topological properties of circular DNA in which both strands are intact, called closed circular DNA, but linear DNA in vivo is also topologically constrained (1, 2). The topological state of closed circular DNA can be described by two variables. One is the knot type, K, formed by the double helix axis. In particular, a molecule may be unknotted (unknot, trivial knot) or form a non-trivial knot. The second variable, the linking number of the complementary strands, Lk, describes the winding of the strands of the double helix about each other. It is more convenient to use the difference between Lk and that of relaxed DNA (Lk0), ∆Lk = Lk − Lk0, than Lk itself. Circular DNA extracted from cells has negative ∆Lk (3).

Random cyclization of linear DNA molecules results in an equilibrium distribution of topological states, P(∆Lk, K). Studies of the components of this distribution have greatly advanced our understanding of DNA conformational properties. The measurement in 1975 of the equilibrium distribution of ∆Lk for unknotted circular DNAs, the conditional distribution P(∆Lk|Unknot), led to elegant determinations of the free energy of supercoiling (4, 5). These textbook experiments were elaborated later to include the effect of DNA length, solvent, temperature, and ionic conditions (6–10). A theoretical analysis of P(∆Lk|Unknot) allowed the determination of the torsional rigidity of DNA (11–15). The conditional distribution for the simplest knot, P(∆Lk|Trefoil), has also been studied theoretically (16) and experimentally (17).

The value of Lk is not defined in nicked circular DNA, whose topological state is specified by knot type only. The corresponding equilibrium distribution of knots in torsionally unstrained molecules, P(K), has been the subject of many theoretical studies (16, 18–23). Experimental measurements of P(K) for different DNA lengths were performed for the first time in 1993 and allowed an accurate determination of the electrostatic repulsion between DNA segments under different ionic conditions (10, 17, 24).

To provide a more complete description of DNA topology, we evaluated the general relationships between P(∆Lk, K) and the four derivative distributions, P(∆Lk|K), P(K|∆Lk), P(K), and P(∆Lk). There is no known method for measuring P(∆Lk, K) directly, but it can be calculated from a pair of derivative distributions that can be measured or simulated, P(K) and P(∆Lk|K). P(∆Lk, K) can also be simulated directly.

We focused particularly on the conditional distribution P(K|∆Lk), the equilibrium distribution of knot types in DNA molecules with a particular value of ∆Lk. We computed P(K|∆Lk) in two different ways and obtained the same distributions. These computations showed, in agreement with (25), that beyond very small values of ∆Lk, the lowest energy form of DNA for a particular ∆Lk is knotted and not platonemically supercoiled. This preference arises because formation of highly chiral knots minimizes torsional deformation of DNA. Unexpectedly, we found that only a few knots dominated the distribution for a particular ∆Lk value and a large fraction of these knots belongs to the small family of torus knots. We discuss the relationship between supercoiling and knot formation inside the cell.

Methods of Calculations

DNA Model. We modeled DNA as a discrete analog of a worm-like chain and accounted for intersegment electrostatic repulsion. A DNA molecule composed of a Kuhn statistical length is modeled as a closed chain of kn rigid cylinders of equal length. Replacement of a continuous worm-like chain with kn hinged rigid segments is an approximation that improves as k increases. The bending energy of the chain, Eb, is given by

\[ E_b = 
\frac{\alpha k_B T}{2} \sum_{i=1}^{kn} \theta_i^2, \]  

where the summation extends over all the joints between the elementary segments, \( \theta_i \) is the angular displacement of segment i relative to segment \( i-1 \), \( \alpha \) is the bending rigidity constant, \( k_B \) is the Boltzmann constant, and T is the absolute temperature. The value of \( \alpha \) is defined so that the Kuhn statistical length corresponds to k rigid segments (12). We used k = 10, which has been shown to be large enough to obtain accurate results for supercoiled DNA (26). The Kuhn length was set equal to 100 nm (27).

In the simulation of closed circular DNA, we also accounted for the energy of torsional deformation, E:

\[ E_t = (2\pi^2 C/L)(\Delta Tw)^2, \]  

where C is the torsional rigidity constant of DNA, L is the length of the DNA chain, and \( \Delta Tw \) is the difference in double helical twist from relaxed DNA (26). The value of \( \Delta Tw \) was not specified in the model directly but was calculated for each...
conformation using White’s equation (28–30), which connects $\Delta Lk$ and writhe of the DNA axis, $W_r$, to $\Delta Tw$:

$$\Delta Tw = \Delta Lk - W_r.$$

Eq. 3 allows us to use our DNA model to simulate the properties of closed circular DNA with a specified value of $\Delta Lk$. The calculation of $W_r$ for a particular conformation was based on Le Bret’s algorithm (16).

The excluded volume effect and the electrostatic interactions between DNA segments are taken into account in the model via the concept of effective diameter, $d$. This is the actual diameter of the impenetrable cylindrical segments of the model chain. We used $d = 5$ nm throughout this work, which corresponds to a NaCl concentration of 0.2 M (24, 31).

**Monte Carlo Simulation Procedure.** We used the Metropolis Monte Carlo procedure (32) to generate an equilibrium set of conformations as described in detail elsewhere (33).

**Control of Topological Variables.** Since the chain segments are allowed to pass through each other during successive deformations in the Metropolis procedure, the knot type of the chain can change. The constructed equilibrium set of chain conformations specifies the equilibrium distributions of knots, $P(K)$. To calculate $P(K)$, one needs only to know the topology of each conformation. In the simulations, this is done by calculating the value of the Alexander polynomial, $\Delta(t)$ at $t = -1$ and $t = -2$ (18). Although the values of $\Delta(-1)$ and $\Delta(-2)$ distinguish all knots obtained in this work, to identify complex knots, we also calculated the more powerful invariant, the Jones polynomial (see ref. 34, for example), using a program written by Jenkins (35).

To calculate $P(\Delta Lk|K)$, we prevented a change of knot type during the simulation by rejecting trial conformations for which the values of $\Delta(-1)$ or $\Delta(-2)$ had changed. We calculated first $P(W_r|K)$, the distribution of $W_r$ for a particular knot type. The torsional and bending deformations of DNA are independent to a good approximation (36). This allowed us to calculate $P(\Delta Lk|K)$ as a convolution of $P(W_r|K)$ and the distribution of the torsional fluctuations, $P(\Delta Tw)$. Namely,

$$P(\Delta Lk|K) = \int P(W_r|K)P(\Delta Tw)dW_r$$

$$= \int P(W_r|K)P(\Delta Lk - W_r)dW_r. \quad [4]$$

We assumed that $P(\Delta Tw)$ is specified by a Gaussian distribution with variance $\langle (\Delta Tw)^2 \rangle$ given by ref. 15:

$$\langle (\Delta Tw)^2 \rangle = k_BT L/4\pi^2C. \quad [5]$$

We used a value of $3 \times 10^{-18}$ erg-cm (1 erg = 0.1 $\mu$J) for $C$ (10, 15, 27). This way of calculating $P(\Delta Lk|K)$ was first suggested by Benham (37) and was realized in ref. 11.

**Results**

**Definitions of $Lk$ and $\Delta Lk$, and the Classification of Knots.** We consider here the equilibrium probability distributions of the linking number difference, $\Delta Lk$, and knot type, $K$. The value of $Lk$ for two closed contours $C_1$ and $C_2$ can be defined as (30, 38):

$$Lk = \frac{1}{4\pi} \oint_{C_1} \oint_{C_2} \frac{[dr_1 \times dr_2]r_{12}}{r_{12}^3}, \quad [6]$$

where $r_1$ and $r_2$ are vectors that start at a point O and move, upon integration, over $C_1$ and $C_2$, respectively; $r_{12} = r_1 - r_2$. This definition using the Gauss integral can be applied equally to knotted and unknotted contours. $\Delta Lk$ can be calculated as

$$\Delta Lk = Lk - N/\gamma,$$

where $N$ is the number of base pairs in the DNA and $\gamma$ is the number of base pairs per turn of the unstressed double helix. Because the value of $N/\gamma$ is not integral, it is more convenient to consider $\Delta Lk$ as a continuous variable even though for any particular DNA its value can differ only in integral amounts. The distribution of discrete values of $\Delta Lk$ is obtained from the corresponding continuous distribution by simple renormalization. Although most of our calculations were for negative $\Delta Lk$, there is no internal chirality in the DNA model used in the simulations, and thus the results can be easily generalized to positive values of $\Delta Lk$.

Knots are classified according to the minimum number of intersections in their plane projection. We will refer to such presentation of knots as standard forms. The simplest knot has three intersections in standard form, and there are only four different types of knots with less than six intersections (Fig. 1A). As the number of intersections in the standard form increases, the number of knot types grows very fast: there are 1,701,936 knots with less than 17 crossings (39). A knot and its mirror image are rarely the same, and for that reason knots are divided into three groups: right-handed, left-handed, and achiral. For achiral knots, the mirror image is equivalent to the knot itself. balloons are topologically equivalent (only the knot 41 is equivalent to its mirror image among the four simplest knots shown in Fig. 1A), but only one representative of a pair is accounted in the classification (see ref. 34, for example, for more details).
Contributions from both mirror images of the chiral knots $3_1$, $5_1$, and $5_2$ are shown. Each peak is a Gaussian distribution over $\Delta L_k$. The distribution $P(\Delta L_k)$, the sum of $P(\Delta L_k, K)$ over $K$, is shown by the black line. The simulations were performed for DNA molecules 4 kb in length.

General Relationships Between $P(\Delta L_k, K)$ and the Derivative Distributions. We use in our calculations four general relationships among $P(\Delta L_k, K)$ and the derivative distributions $P(\Delta L_k|K)$, $P(K|\Delta L_k)$, $P(K)$, and $P(\Delta L_k)$. These relationships, valid for any two-dimensional distribution, are:

\[ P(\Delta L_k, K) = P(\Delta L_k|K)P(K); \]
\[ P(\Delta L_k, K) = P(K|\Delta L_k)P(\Delta L_k); \]
\[ P(K) = \sum_{\Delta L_k} P(\Delta L_k, K); \]
\[ P(\Delta L_k) = \sum_{K} P(\Delta L_k, K). \]

The Distribution of Knots and $\Delta L_k$, $P(\Delta L_k, K)$. Fig. 1B illustrates typical conformations of the simplest knots obtained in the simulation of DNA molecules 4 kb in length. We calculated the equilibrium distribution, $P(\Delta L_k, K)$, using Eq. 8 and simulated distributions of $P(\Delta L_k|K)$ and $P(K)$ (Fig. 2). Because $P(K)$ decreases sharply as knot complexity grows, we were able to calculate $P(\Delta L_k)$ with reasonable accuracy only for the four simplest knots, $3_1$, $4_1$, $5_1$, and $5_2$. Knots $3_1$, $5_1$, and $5_2$ are chiral, and therefore both of their mirror images are shown in Fig. 2; knot $4_1$ is achiral. The values of $P(K)$ are shown in Table 1. In agreement with experimental data (4, 5, 17), we found that for all these knots $P(\Delta L_k|K)$ is approximated well by the Gaussian distribution:

\[ P(\Delta L_k|K) = \frac{1}{\sigma_K \sqrt{2\pi}} \exp\left[-\left(\Delta L_k - \mu_K\right)^2/2\sigma_K^2\right]. \]

The values of the distribution variance, $\sigma_K^2$, and of $c_K$ are shown in Table 1. The simulation data did not deviate by more than 15% from the best fitted Gaussian curve in the interval $[-4\sigma_K, 4\sigma_K]$. The values of $c_K$ correspond to the average values of writhes, $\langle Wr\rangle$, over the distribution of equilibrium conformations of nicked circular DNA forming a knot $K$. Our values of $c_K$ are in full agreement with those calculated by Katritch et al. (40).

Fig. 3. Simulated distribution of $P(K|\Delta L_k)$ for 4-kb DNA. The distribution was obtained by using Eqs. 9 and 10 and the data from Table 1. Each curve corresponds to a particular knot: $3_1$ (red), $3_1$ (blue), $5_1$ (light green), and $5_2$ (turquoise).

The Distribution $P(K|\Delta L_k)$. Without extra simulation, two other derivative distributions, $P(K|\Delta L_k)$ and $P(\Delta L_k)$, can be obtained from $P(\Delta L_k, K)$ by using Eqs. 9 and 11. $P(K|\Delta L_k)$, the calculated distribution of knots as a function of $\Delta L_k$, is shown in Fig. 3. Although only unknotted circular molecules ($0_1$) and four knots, $3_1$, $4_1$, $5_1$, and $5_2$, were taken into account during the calculation, further results showed (see Fig. 4) that only these knots compete for appearance in the range of $\Delta L_k$ between 0 and $-7.5$. It is easy to understand why knot $4_1$ does not appear in this distribution. The average value of $Wr$ for the amphichiral $4_1$ equals zero, and thus it competes for appearance with the trivial knot in the range of $\Delta L_k$ around zero but loses out because $P(\Delta L_k, 0_1) \gg P(\Delta L_k, 4_1)$. It is more interesting that there is a very small amount of the knot $5_2$ in Fig. 3. This is because $P(\Delta L_k, 3_1) \gg P(\Delta L_k, 5_2)$, even though the absolute value of $\langle Wr\rangle$ is lower for $3_1$ than for $5_2$ (see Fig. 2), for all values of $-\Delta L_k$ less than 8. For $-\Delta L_k > 7.2$, knot $5_2$ makes a major contribution to the conditional distribution.

It is difficult to obtain $P(K|\Delta L_k)$ for larger values of $\Delta L_k$ using Eqs. 9 and 11, because we are not able to calculate $P(\Delta L_k)$ for more complex knots. We can, however, simulate $P(K|\Delta L_k)$ directly for a range of values of $\Delta L_k$. The algorithm we use allows us to restrict the equilibrium set of conformations to certain values of $K$ or $\Delta L_k$ to one or both of these restrictions (see Methods of Calculations). To calculate $P(K|\Delta L_k)$, we constructed equilibrium sets of conformations for particular values of $\Delta L_k$ but allowed any change of knot type during the simulation.

The distributions $P(K|\Delta L_k)$ obtained by direct simulation for DNA molecules 4 kb in length are shown in Fig. 4. The distribution for 4-kb DNA in Fig. 4B is nearly identical with the calculated distribution in Fig. 3 over the same range of $\Delta L_k$ and for DNA of the same size. This agreement demonstrates the consistency of the computations. A remarkable feature of the distribution is readily
seen in Fig. 4: for any particular value of $D_Lk$, only very few knots dominate. Indeed, there are 1,701,936 different knots with less than 17 crossings in their standard form (39), but only 12 of them appear in the conditional distributions with probability more than 0.1! Four other knots that appear in Fig. 4 have more than 16 crossings in the standard form and therefore are not among these 1,701,936.

Comparison of the distributions calculated for the two DNA lengths shows that the same knots make the major contributions in both cases, although they appear at slightly different values of $D_Lk$. Also slightly more knots contribute to the distribution for longer DNA. This weak dependence of $P(K|D_Lk)$ on DNA length is due to the fact that the average Wr of knotted molecules is nearly length-independent (16, 40). The knots that make the major contribution to $P(K|D_Lk)$ are shown in Fig. 5A in standard form. Typical conformations for some of these knots are shown in Fig. 5B; they are quite similar to the standard presentations and contain barely any extraneous crossings.

Why do so few knots make the major contribution to $P(K|D_Lk)$? To address this question, we calculated the average $Wr$ for these knots in the absence of the torsional stress. The results presented in Table 2 show that the values of $|Wr|$ of the represented knots are very large and exceed the number of crossings in their standard form.

![Fig. 4. Simulated distribution of $P(K|D_Lk)$ for 2.4-kb (A) and 4-kb (B) DNAs. The data were obtained by direct simulation of this conditional distribution. Each curve corresponds to a particular knot. Curves are shown only for those knots for which $P(K|D_Lk)$ exceeds 0.1 (with the exception of knot $5_2$). The standard form of these knots is shown in Fig. 5, and some of their features are listed in Table 2.](image)

![Fig. 5. Knots that make the major contributions to $P(K|D_Lk)$.) Shown are the standard forms of these knots (A) and four examples of simulated conformations of knots (B). Knot notations are explained in the legend to Table 2.](image)

### Table 2. Knots that make the major contributions to $P(K|D_Lk)$

<table>
<thead>
<tr>
<th>Knot type</th>
<th>Alexander polynomial, $\Delta(−1)$, $\Delta(−2)$</th>
<th>$Wr$</th>
<th>$p, q$ for torus knots</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3_1$</td>
<td>3, 7</td>
<td>-3.42</td>
<td>3, 2</td>
</tr>
<tr>
<td>$5_1$</td>
<td>5, 31</td>
<td>-6.23</td>
<td>2, 5</td>
</tr>
<tr>
<td>$7_1$</td>
<td>7, 127</td>
<td>-9.03</td>
<td>2, 7</td>
</tr>
<tr>
<td>$8_{19}$</td>
<td>3, 91</td>
<td>-8.59</td>
<td>3, 4</td>
</tr>
<tr>
<td>$10_{124}$</td>
<td>1, 331</td>
<td>-11.17</td>
<td>3, 5</td>
</tr>
<tr>
<td>$10_{139}$</td>
<td>3, 259</td>
<td>-11.38</td>
<td></td>
</tr>
<tr>
<td>$12_{242}$</td>
<td>1, 1291</td>
<td>-13.57</td>
<td></td>
</tr>
<tr>
<td>$12_{25}$</td>
<td>5, 1147</td>
<td>-13.68</td>
<td></td>
</tr>
<tr>
<td>$15_{31}185$</td>
<td>5, 6355</td>
<td>-15.74</td>
<td>4, 5</td>
</tr>
<tr>
<td>$14_{1881}$</td>
<td>1, 5419</td>
<td>-15.93</td>
<td>3, 7</td>
</tr>
<tr>
<td>$14_{6022}$</td>
<td>3, 5131</td>
<td>-16.14</td>
<td></td>
</tr>
<tr>
<td>$K_1$</td>
<td>11, 26611</td>
<td>-18.07</td>
<td></td>
</tr>
<tr>
<td>$K_2$</td>
<td>19, 29059</td>
<td>-18.24</td>
<td></td>
</tr>
<tr>
<td>$K_3$</td>
<td>16, 23154</td>
<td>-18.39</td>
<td>3, 8</td>
</tr>
<tr>
<td>$K_4$</td>
<td>13, 106483</td>
<td>-20.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21, 115843</td>
<td>-20.50</td>
<td></td>
</tr>
</tbody>
</table>

Notations for knots $3_1$, $5_1$, $7_1$, $8_{19}$, $10_{124}$ and $10_{139}$ are explained in ref. 42; those for $12_{242}$, $12_{25}$, $15_{31}185$, $14_{1881}$, $14_{6022}$, $15_{31}185$, and $16_{23154}$ are presented in ref. 39. Knots $K_1$–$K_4$ have more crossings than knots in any tables available to us; their structure is shown in Fig. 5. The largest odd numbers that divide $|\Delta(−2)|$ rather than the values of $|\Delta(−2)|$ themselves are shown in the table.
The most interesting and most unexpected results were obtained for the conditional distribution \( P(K|\Delta Lk) \). The simulations showed that only a few highly chiral knots make a major contribution to the distribution at any particular value of \( \Delta Lk \). Only 12 of more than 1.7 million knots with fewer than 17 crossings in standard form appear in the distribution with probability larger than 0.1, but for these knots the probability approaches 1 at particular values of \( \Delta Lk \) (Fig. 4). The major feature of these knots is a high value of average \( Wr \) when torsionally unstressed. A large fraction of the knots belongs to the torus family, although not all torus knots contribute to the distribution. Increasing the DNA length from 2.4 to 4 kb does not change \( P(K|\Delta Lk) \) substantially.

In the simulations, we used only one particular value of DNA torsional rigidity, \( C \), but a wide range of values for \( C \) have been reported (8, 15, 25, 45–47). The effect of the torsional rigidity on the distributions studied is rather simple, as long as the torsional and bending deformations in DNA are energetically independent. In this case, \( P(\Delta Lk, K) \) can be expressed as a convolution of the twist distribution, \( P(\Delta Tw) \) and the distribution of writhe and knot types, \( P(Wr, K) \), similar to Eq. 4. Since \( P(Wr, K) \) gives the distribution for nicked circular DNA, it does not depend on \( C \). Thus, the dependence of \( P(\Delta Lk, K) \) on \( C \) depends only on \( P(\Delta Tw) \), which is a Gaussian distribution centered at \( \Delta Tw = 0 \) that broadens as \( C \) decreases (see Eq. 5). The conclusion of this analysis is that lowering \( C \) will not change the knots that appear in \( P(K|\Delta Lk) \), but particular knots will be found at higher values of \( \Delta Lk \) (see Fig. 4) and the peaks will be broader. We confirmed this in a special simulation in which \( C = 1 \times 10^{-19} \) erg/cm, \( P(K|\Delta Lk) \) was shifted by 2 in comparison with the results in Fig. 4B, which was calculated by using \( C = 3 \times 10^{-19} \) erg/cm.

It is important to emphasize that the distributions \( P(\Delta Lk|K) \) and \( P(K) \) can be measured experimentally and that all other distributions, \( P(\Delta Lk, K) \) and \( P(\Delta Lk) \), can be calculated from the measured ones. The distribution \( P(K) \) can be generated by cyclization of linear DNA molecules via cohesive ends (17, 24). Separation of knots by gel electrophoresis and measurement of their relative amounts allows the evaluation of \( P(K) \). The equilibrium distribution of \( \Delta Lk \) for a particular knot \( P(\Delta Lk|K) \) can be obtained by ligation of nicks in these knots (17) and measurement of the distribution of \( \Delta Lk \) topoisomers by gel electrophoresis. Recombinases, topoisomerases, and DNA replication can also be used to obtain specific knot types for similar analyses (48).

The distribution \( P(\Delta Lk|K) \) has been studied in great detail for unknotted molecules (4–10), and there is very good agreement between these measurements and the results of computer simu-
Relaxation is restricted by a particular value of $K$ due to the topological constraint.

In our computation of $P(K|\Delta Lk)$, we restricted topological relaxation in a reciprocal fashion and kept the value of $\Delta Lk$ constant but allowed $K$ to change. The most probable states found in this computer experiment correspond to the minima of $G(\Delta Lk, K)$, under the condition that $\Delta Lk$ is constant. Again, these minima are not, in general, the local minima of $G(\Delta Lk, K)$.

What would happen if we added a type II topoisomerase to closed circular DNA? These enzymes can change both topological variables, $\Delta Lk$ and $K$, by catalyzing the passages of one double-stranded segment through another. We might expect that these enzymes will yield unknotted molecules with $\Delta Lk = 0$, corresponding to the global minimum of $G(\Delta Lk, K)$. The situation is more complex, however. DNA gyrase introduces negative supercoils in circular DNA (51), and other type II topoisomerases unite knots in DNA molecules below equilibrium level (52). This is possible because the strand passage reactions catalyzed by the enzymes are coupled to ATP hydrolysis, which serves as a source of energy. Thus, it is difficult to predict the distribution of topological states of circular DNA in the presence of type II topoisomerases. The presence of type I topoisomerases inside prokaryotic cells, which relax negative supercoils, makes this dynamic picture even more complex.

We know that DNA molecules inside of cells adopt pleotonomically supercoiled unknotted conformations [see review (41) and references therein]. This is certainly the desired result for the cell because pleotomic (--) supercoils perform essential work in promoting double-helix opening and DNA compaction. The equilibrium distribution of topological states studied in this work shows that this result is far from obvious, because for DNA with even a modest $\Delta Lk$ the free energy of highly chiral knots is lower than that of an unknotted pleotomic superhelix. It is possible that changing the amounts and/or activity of topoisomerases or other DNA ligands could result in knotting of circular DNA in vivo.

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