Circular DNA

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Circular DNA

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In 1963 by Dulbecco and Vogt (1963) and Weil and Vinograd (1963) discovered that double-stranded DNA of the polyoma virus exists in a closed circular form. At present it is generally acknowledged that this form is typical of bacterial DNA and of cytoplasmic DNA in animals. Furthermore, giant DNA molecules in higher organisms form loop structures held together by protein fasteners in which each loop is largely analogous to closed circular DNA. The distinctive feature of closed circular molecules is that its topological state cannot be altered by any conformational rearrangement short of breaking DNA strands. This topological constraint is the basis for the characteristic properties of closed circular DNA which have fascinated biologists, physicists and mathematicians for the past 40 years. In this chapter we concentrate our attention on physical properties of circular DNA which understanding is necessary for the successful analysis of the biological effects connected with this form of the molecule.

1 DNA SUPERCOILING

Basic Concepts

Closed circular DNA and linking number

Two forms of circular DNA molecules are extracted from the cell; they were designated as form I and form II. The more compact form I was found to turn into form II after a single-stranded break was introduced into one chain of the double helix. Subsequent studies performed by Vinograd et al. (1965) linked the compactness of form I, in which both DNA strands are intact, to supercoiling. Form I came to be called the closed circular form. In this form each of the two strands that make up the DNA molecule is closed in on itself. A diagrammatic view of closed circular DNA is presented in Fig. 1.

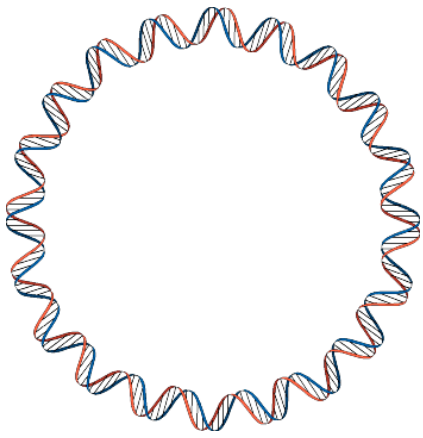

FIGURE 1 Diagram of closed circular DNA. The linking number, Lk, of the complementary strands is 20.

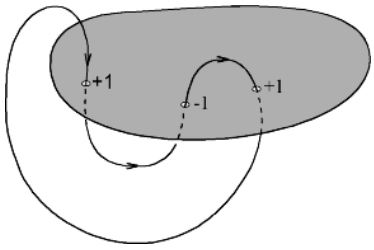

FIGURE 2 Definition of the linking number of two closed contours. An imaginary surface is drawn up on one contour, then one calculates the algebraic number of intersections between this surface and the other contour. $Lk = 1$ for this example.

The two strands of the double helix in closed circular DNA are linked. In topological terms, the link between two strands of the double helix belongs to the torus class. The quantitative description of such links is called the linking number, *Lk*, which may be determined in the following way (Fig. 2). One of the strands defines the edge of an imaginary surface (any such surface gives the same result). The *Lk* is the algebraic (i.e., sign-dependent) number of intersections between the other strand and this spanning surface. By convention, the *Lk* of a closed circular DNA formed by a right-handed double helix is positive. *Lk* depends only on the topological state of the strands and hence is maintained through all conformational changes that occur in the absence of strand breakage. Its value is always an integer. *Lk* can be also defined through the Gauss integral,

$$Lk = \oint_{C_1}\oint_{C_2} \frac{(d\mathbf{r}_1 \times d\mathbf{r}_2)\cdot \mathbf{r}_{12}}{r_{12}^3}, \qquad (1)$$

where $\mathbf{r}_1$ and $\mathbf{r}_2$ are vectors whose ends run, upon integration, over the first and second contours, C_1 and C_2 respectively, $\mathbf{r}_{12} = \mathbf{r}_2 - \mathbf{r}_1$ (see Fig. 3).

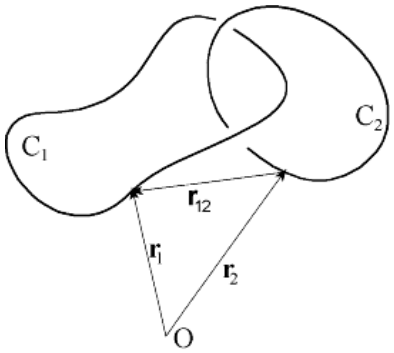

FIGURE 3 Notation of vectors in the calculation of the Gauss integral. O is the beginning of the coordinate system, C_1 and C_2 are the circular contours.

Quantitatively, the linking number is close to N/γ, where N is the number of base pairs in the molecule and γ is the number of base pairs per double-helix turn in linear DNA under given conditions. However, these values are not exactly equal one to another, and the difference between *Lk* and N/γ (which is also denoted as Lk_o) defines most of the properties of closed circular DNA.

Linking number difference and superhelix density. Topoisomers

The fact that closed circular DNA has a topological invariant gives rise to a new physical parameter which characterizes this form and determines many of its properties. This parameter, ΔLk, is called *the linking number difference* and is defined as

$$\Delta Lk = Lk - N/\gamma \qquad (2)$$

There are two inferences to be made from the above definition.

1. The value of ΔLk is not a topological invariant. It depends on the solution conditions which determine γ. Even though γ itself changes very slightly with changing ambient conditions, these changes may substantially alter ΔLk, as the right-hand part of Eq. (2) is the difference between two large quantities that are close in value.

2. The linking number Lk is by definition an integer, whereas N/γ should not be an integer. Hence ΔLk is not an integer either. However, the values of ΔLk for a closed circular DNA with a particular sequence can differ by an integer only. This simply follows from the fact that, whatever the prescribed conditions, all changes in ΔLk can only be due to changes in Lk, since the value of N/γ is the same for all molecules (Of course any change of Lk would involve a temporary violation of the integrity of a double-helix strand). Molecules that have the same chemical structure and differ only with respect to Lk are called *topoisomers*.

It often proves more convenient to use the value of *superhelix density*, σ, which is ΔLk normalized for Lk_o:

$$\sigma = \Delta Lk / Lk_o = \gamma \cdot \Delta Lk / N \,. \qquad (3)$$

Whenever $\Delta Lk \neq 0$, closed circular DNA is said to be supercoiled. Clearly, the entire double helix is stressed in supercoiled condition. This stress can either lead to a change in the actual number of base pairs per helix turn in closed circular DNA or cause a regular spatial deformation of the helix axis. It is this deformation of the helix axis gave rise to the term "superhelicity" or "supercoiling" (Vinograd et al., 1965), since DNA axis forms in this case a helix of a higher order (Fig. 4). Circular DNA extracted from cells turns out to be always (or nearly always) negatively supercoiled and has a σ between -0.03 and -0.09, but typically near the middle of this range (Bauer et al., 1980).

Twist and writhe

Supercoiling can be structurally realized in two ways: by deforming the molecular axis and by altering the twist of the double helix. This can be ascertained by means of a simple experiment involving a rubber hose. Take a piece of hose and a short rod that can be pushed into the hose with some effort. The rod can be used to join together the ends of the hose and thus rule out their reciprocal rotation around the hose axis. If before joining the two ends one turns one of them several times around the axis, i.e. if one twists the hose, it will shape itself into a helical band once the ends are joined. If one draws longitudinal stripes on the hose prior to the experiment, it will be clear hat reciprocal twisting of the ends also causes the hose's torsional deformation.

There is very important quantitative relationship between the deformation of the DNA axis, its torsional deformation and Lk of the complementary strands of the double helix. The first mathematical treatment of the problem was presented by Calugareanu (1961), who found the basic relationship between the geometrical and topological properties of a closed ribbon. In its current form the theorem was first proved by White (1969). Two years later, Fuller (1971) specifically suggested how the theorem can be applied to the analysis of circular DNA.

According to the theorem, the *Lk* of the edges of the ribbon is the sum of two values. One is the twist of the ribbon, *Tw*, a well known concept, and the second, a new concept, writhe, *Wr*. Thus,

$$Lk = Wr + Tw\,. \tag{4}$$

Tw is a measure of the number of times one of the edges of the ribbon spins about its axis. The value of *Tw* of the entire ribbon is the sum of the *Tw* of its parts. The value of *Wr* is defined by the spatial course of the ribbon axis; i.e., it is a characteristic of a single closed curve, unlike *Lk* and *Tw* which are properties of a closed ribbon. Thus *Lk* can be represented as a sum of two

FIGURE 4 A typical conformation of supercoiled DNA. The picture obtained by computer simulation of supercoiled molecule 3500 base pairs in length, σ =-0.06, for physiological ionic conditions (Vologodskii et al., 1992).

values that characterize the available degrees of freedom: torsional deformation around the ribbon axis and deformation of this axis. To apply the theorem to circular DNA, the two strands of the double helix are considered as edges of a ribbon.

Let us consider the most important properties of *Wr* (Fuller, 1971). *Wr* is completely specified by the geometry of DNA axis. It can be expressed through the Gauss integral (Eq. (1)), in which integration is performed both times along the same contour, the ribbon axis. *Wr* can be thought as a measure of a curve's net right-handed or left-handed asymmetry, i.e., its chirality, and is equal to zero for a planar curve. Unlike *Lk*, which can only be an integer, a curve's *Wr* can have any value. It changes continuously with the curve's deformation that does not involve the intersection of segments. A curve's *Wr* does not change with a change in the curve's scale and depends solely on its shape.

Consider *Wr* of an almost flat 8-shaped curve (Fig. 5).

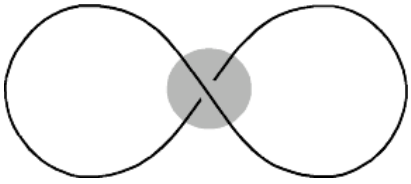

FIGURE 5 The nearly flat "eight" figure. Only the gray-circled part of the contour juts out of plane. For such a curve *Wr* approaches -1.

As it turns out, the *Wr* of such a curve is -1 if the curve is a fragment of a right-handed helix (the case in Fig. 5), and +1 if the crossing corresponds to a left-handed helix. This result does not depend on the shape or size of the two loops of the "8", or on the angle between the curve segments in the crossing area. In general, for nearly flat curves *Wr* is close to the algebraic sum of the contributions of all "crossings" (Fuller, 1971).

Another important property of *Wr* holds not only for the "quasi-flat" curve but for an arbitrary curve as well. When the curve is deformed in such a way that one of its parts passes through another, the writhe value experiences a leap by 2 (or -2 for the opposite direction of the pass). This property helped greatly in the analysis of the action mechanisms of DNA topoisomerases.

The main result of the ribbon theory, expressed in Eq. (4), is that *Lk* can be structurally realized in two different ways. The first way consists in changing the twist of the double helix, the second lies in the deformation of the helix axis, giving rise to a certain writhe.

Fuller was the first who made simple theoretical analysis of the shape of supercoiled DNA (Fuller, 1971). He calculated the *Wr* of two possible form, simple and interwound helices (Fig. 6).

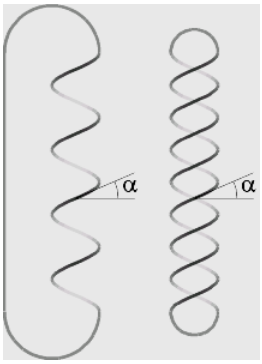

FIGURE 6 Diagram of simple (left) and interwound helices (right). The helix winding angles (a) are shown. The number of helical turns equals 4 and 8, correspondingly.

By neglecting the ends of the helices, he concluded that the *Wr* of a simple right-handed helix is given by

$$Wr = n(1 - \sin\alpha) \tag{5a}$$

and that the *Wr* of an interwound right-handed helix has the form

$$Wr = -n\sin\alpha \tag{5b}$$

where *n* is the number of helical turns and α is the winding angle. As either helix is extended, the value of α increases thereby decreasing the curvature of the rod and its bending energy. It follows from Eqs. (5), that as α increases, the $|Wr|$ of a simple helix diminishes, whereas that of an interwound helix increases. Thus, for a particular value of *Lk*, the torsional deformation will be less for the interwound form. Therefore, the interwound superhelix should be favored over a

simple one from an energetic point of view. Indeed, all available experimental and theoretical data indicate interwound conformations for supercoiled DNA (Vologodskii and Cozzarelli, 1994).

Basic Questions

Eq. (4) expresses that there are at least two ways of structural realization of supercoiling. So the questions arises:

1. How is DNA supercoiling distributed between different possible ways of its structural realization?

2. How does this partitioning depend on σ and on solution conditions?

3. How does DNA sequence affect the partitioning?

Mathematical analysis of supercoiling described in the previous section suggested that supercoiling causes only elastic torsional and bending deformation of the double helix. However, sufficiently high negative supercoiling can also cause disruptions of the regular DNA structure, (nonelastic deformation). Such disruptions come out as opened base pairs, cruciforms, Z and H forms of DNA. We will consider the issue later in this chapter.

Today we know a lot about conformational changes caused in DNA by supercoiling. Essential part of this knowledge was obtained by experimental methods specific for supercoiled DNA. These methods are very powerful and often open unique opportunities to study general properties of the double helix. Therefore we first consider specific experimental approaches to study DNA supercoiling.

Experimental Study of DNA Supercoiling

Measuring ΔLk

The value of DNA supercoiling is specified by ΔLk and thus experimental determination of ΔLk is the first problem to be solved. Two different experimental approaches to the problem are based on the fact that supercoiling makes DNA more compact, increasing its mobility in gel or the sedimentation constant. The first method developed in the pioneering studies of Vinograd and coworkers uses the titration of supercoiling by an intercalating dye (Bauer and Vinograd, 1968). This approach is applicable only to negatively supercoiled DNA. The molecules of some dyes have the ability to intercalate themselves between base pairs in the double helix on binding to DNA, thus reducing the helical rotation angle between the base pairs. As an increasing number of these molecules bind themselves to negatively supercoiled DNA, the tensions within the DNA molecule are gradually removed and conformations of the double-helix axis becomes less compact. This causes a decrease in the molecule's mobility, which can be registered experimentally (Fig. 7). Then, after the number of ligand molecules per base pair, ν, exceeds the value

$$\nu = 360 \cdot \Delta Lk / (\phi N) \qquad (6)$$

where ϕ corresponds to the change of the angle, in degrees, between adjacent pairs upon the integration of a ligand molecule between them, the tensions in DNA will start growing again. This will increase the molecule's mobility. As a result, DNA mobility has a minimum. Having

found the value of ν at the minimum one can find ΔLk from Eq. (6) if the value of ϕ is known. For ethidium bromide, which is most often used for the titration of supercoiled DNA, ϕ = -26°. The number of bound ligand molecules can be found with the help of spectroscopic methods.

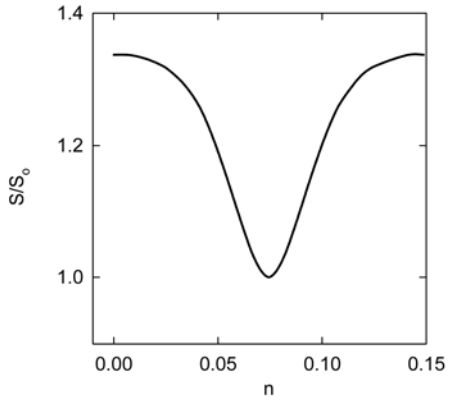

FIGURE 7 Dependence of the sedimentation constant for closed circular DNA on the amount of bound ligand molecules. The ratio of the sedimentation constants of supercoiled and open circular forms is plotted.

Keller (1975) proposed the second approach to determining the linking number difference in closed circular DNA. This approach is based on the fact that the values of ΔLk in any mixture of DNA topoisomers can differ by an integer only. The electrophoretic mobility of DNA is so sensitive to conformational changes, that under appropriate experimental conditions molecules with a difference in ΔLk of 1 produce separate bands in the electrophoretic pattern. If DNA sample contains all possible topoisomers with ΔLk from 0 to some limiting value and they are all well resolved with respect to mobility, one can find the value of ΔLk corresponding to each band simply by band counting (Fig. 8).

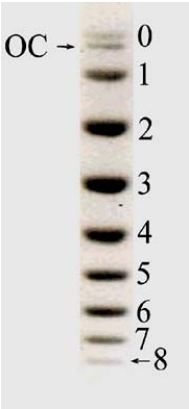

FIGURE 8 Gel electrophoretic separation of topoisomers of pUC19 DNA. The mixture of topoisomers covering the range of ΔLk from 0 to -8 was electrophoresed from a single well in 1% agarose from top to bottom. The topoisomer with $\Delta Lk = 0$ has the lowest mobility: it moves slightly slower than the opened (nicked) circular DNA (OC). The value of (-ΔLk) for each topoisomer is shown. (Illustration provided by M. Vologodskaia.)

One can then scan the gel to find the relative amount of DNA at each band. The band that corresponds to $\Delta Lk \approx 0$ (since ΔLk is not an integer we should talk about the closest to 0 possible value) can be identified through a comparison with the band for the nicked circular form. One should bear in mind, however, the fact that topoisomer mobility is determined by the absolute

value of ΔLk only, so the presence of topoisomers with both negative and positive ΔLk can make interpreting the electrophoresis more difficult. Another restriction of the method lies in the saturation of molecular mobility growth with increasing $|\Delta Lk|$.

A very elegant and effective way to overcome these shortcomings, two-dimensional gel electrophoresis, was proposed by Lee et al. (1981). A mixture of DNA topoisomers is loaded into a well at top left corner of a slab gel, and electrophoresed along left side of the gel. The bands corresponding to topoisomers with large ΔLk values merge into one spot. After that the gel is transferred in a buffer containing the intercalating ligand chloroquine and electrophoresed in the second, horizontal direction (Fig. 9). The mobility of topoisomers in the second direction is no longer determined by ΔLk, but by the value $(\Delta Lk - N\gamma\phi/360)$, which can be regarded as the effective linking number difference $(\phi < 0)$. We can say that the distribution of ΔLk for the second direction is shifted on a positive value. As a result, the mobilities of topoisomers with opposite values of ΔLk are different in the second direction. Also, the topoisomers with large negative ΔLk, which had identical mobility in the first direction, move with different speed in the second direction. The number of topoisomers that can be resolved almost doubles in the case of two-dimensional electrophoresis.

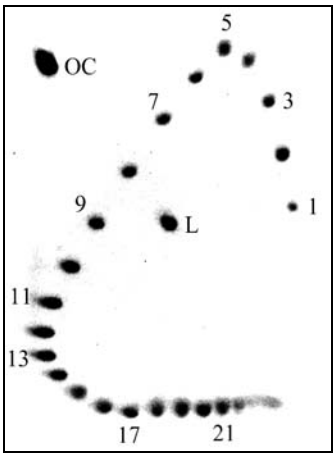

FIGURE 9 Separation of pUC19 DNA topoisomers by two-dimensional gel electrophoresis. Topoisomers 1-4 have positive supercoiling, the rest have negative supercoiling. After electrophoresis was performed in the first direction, from top to down, the gel was saturated with ligand intercalating into the double helix. Upon electrophoresis in the second direction, from left to right, the 12th and 13th topoisomers turned out to be relaxed. The spot in the top left corner corresponds to the open circular form (OC), the spot in the middle of the gel corresponds to the linear DNA (L). (Illustration provided by M. Vologodskaia.)

The separation of DNA topoisomers in a gel has proved to be one of the most potent techniques in DNA research. This method and its two-dimensional version have led to a whole series of remarkable experimental studies (reviewed in (Wang, 1986; Vologodskii, 1992)).

It is important to keep in mind that ΔLk depends on the ambient conditions in solution. A temperature increase of 30° causes σ to increase by approximately 0.01, since the double helix unwinds with rising temperature (Bauer et al., 1980). The increase constitutes about 20% of the characteristic σ for a closed circular DNA isolated from the cell. The double helix winds itself up with increasing the ion concentration, and a change of σ resulting of changing ionic conditions can be as large as 0.01 (Bauer et al., 1980; Rybenkov et al., 1997b).

Obtaining DNA with a preset ΔLk

To obtain a DNA with a preset superhelix density, closed circular DNA molecules are treated with an enzyme called topoisomerase I. The action of this enzyme can alter the value of *Lk* in closed circular DNA as it introduces a single-stranded break into the double helix and then, after a while, reseals it (Wang, 1996). In between these two events the DNA may undergo changes in axial twist and shape, so that *Lk* gradually relaxes to its equilibrium value for the given conditions, i.e. the value corresponding to the minimum stress in closed circular DNA. This equilibrium value of *Lk* can be changed within a wide range by adding various ligands which alter the helical rotation angle of the double helix upon binding to it. Thus, by adding varying amounts of an intercalating dye (usually ethidium bromide), one can obtain, after enzyme treatment and removing both ligand and enzyme from the solution, closed circular DNA with a desired negative supercoling. It was difficult to obtain positively supercoiled DNA in this way until recently, when a protein was found which increases Lk_o upon binding with the double helix (LaMarr et al., 1997).

It is important that by such a way we always obtain a distribution of DNA topoisomers with *Lk* distributed around a mean value rather than one topoisomer (if DNA is longer than 500 base pairs). This distribution is analyzed in details in the next section.

Equilibrium Distribution of Topoisomers

Free energy of supercoiling

Closed circular DNA molecules with a Δ*Lk* value other than zero have additional free energy, which is called supercoiling free energy, *G*(Δ*Lk*). Precise knowledge of *G*(Δ*Lk*) is very important for successful analysis of many problems related with DNA supercoiling. Here we will consider one of two known ways of the experimental determination of *G*(Δ*Lk*). In 1975 the equilibrium thermal distribution of topoisomers in closed circular DNA was studied simultaneously by two groups (Depew and Wang, 1975; Pulleyblank et al. 1975). The distribution was obtained by the same treatment of circular closed DNA with topoisomerase I, in the absence of any ligands. As it was said above, the enzyme can alter the value of *Lk* in closed circular DNA by breaking and then restoring one strand in the double helix. This treatment causes the distribution of molecules with respect to *Lk* to relax towards the equilibrium form. Large DNA molecules adopt a lot of different conformations in solution with comparable probability and these conformations correspond to different values of *Tw* and *Wr*, and consequently Δ*Lk*. Therefore the equilibrium distribution of topoisomers contains in comparable amounts more than one topoisomer if DNA length exceeds 500 bp.

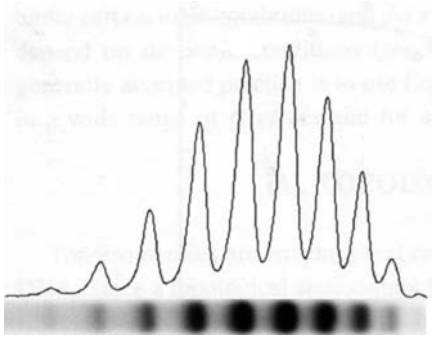

FIGURE 10 Equilibrium topoisomer distribution of circular DNA 10,000 base pairs in length. A photograph of the gel is shown together with its quantitative scan. Adjacent peaks correspond to topoisomers that differ by 1 in the *Lk* value.

The distributions were analyzed by gel electrophoresis. Fig. 10 presents typical result of such experiment. The maximum of the equilibrium distribution always corresponds to $\Delta Lk = 0$. It is also clear that molecules with close absolute values of ΔLk must have close mobility, hence be next to each other in the gel. Distribution of the kind presented in Fig. 10, where molecules having positive and negative ΔLk values are separated, is the result of electrophoresis conditions that differ from those used for the topoisomerase reaction. The change in conditions means that γ in Eq. (2) needs to be replaced by a different value, γ', while Lk remains unchanged. As a result the entire distribution shifts on the value $\Delta Lk - \Delta Lk' = N(1/\gamma - 1/\gamma')$, and for a large enough value of this difference all the topoisomers will be well separated.

The experiments demonstrated (Depew and Wang, 1975; Pulleyblank et al., 1975; Horowitz and Wang, 1984) that the resulting distribution is always a normal one and thus can be specified by its variance, $\langle(\Delta Lk)^2\rangle$:

$$P(\Delta Lk) = A\exp\left[-\frac{(\Delta Lk)^2}{2\langle(\Delta Lk)^2\rangle}\right], \qquad (7)$$

where A is the normalization factor, and the brackets $\langle\ \rangle$ means the averaging over the equilibrium set of DNA conformations. On the other hand, there is general relationship between the probability of a state and the corresponding free energy. The equilibrium distribution of ΔLk can be expressed as

$$P(\Delta Lk) = A\exp[-G(\Delta Lk)/RT)]. \qquad (8)$$

Experiments showed (Fig. 11) that $\langle(\Delta Lk)^2\rangle$ is proportional to DNA length if $N > 2500$ (Depew and Wang, 1975; Pulleyblank et al., 1975; Horowitz and Wang, 1984) :

$$\langle(\Delta Lk)^2\rangle = N/2K, \qquad (9)$$

where K is a constant. Comparing Eqs. (7), (8) and (9) one concludes that

$$G(\Delta Lk) = KRT(\Delta Lk)^2/N \qquad \text{if} \qquad N > 2500. \qquad (10)$$

Fig.11 shows that $\langle(\Delta Lk)^2\rangle$ diminishes faster when $N < 2500$.

It was predicted theoretically (Klenin et al., 1989) and recently shown experimentally (Rybenkov et al., 1997) that the value of K depends on ionic conditions. For ionic conditions close to physiological ones (0.2 M NaCl or 10 mM MgCl2) K = 1100 if N > 2500 (Depew and Wang, 1975; Pulleyblank et al., 1975; Horowitz and Wang, 1984; Rybenkov et al., 1997c).

In spite of its intrinsic elegance, the method of finding $G(\Delta Lk)$ based on the equilibrium distribution of topoisomers has a shortcoming. The method allows $G(\Delta Lk)$ to be determined only for $|\sigma| < 0.01$, whereas it is the -0.07 -0.03 range that is of the greatest interest in physical and

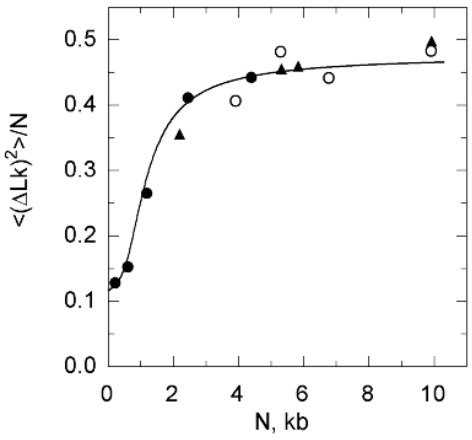

FIGURE 11 Dependence of the variance, $\left\langle (\Delta Lk)^2 \right\rangle$, of the equilibrium topoisomer distribution on the DNA length, N, measured in thousands of base pairs. The variance values have been normalized for N. The experimental data are from refs. (Depew and Wang, 1975) (○), (Pulleyblank et al., 1975) (▲), (Horowitz and Wang, 1984) (●).

biological terms. Recent theoretical calculations of $G(\Delta Lk)$ showed that although the quadratic dependence on ΔLk is kept until $|\sigma| < 0.06$ for physiological ionic conditions, it is not the case for lower ion concentrations (Vologodskii et al., 1992; Vologodskii and Cozzarelli, 1994). Another approach to $G(\Delta Lk)$ determination based on titration of supercoiling by an intercalating ligand, pioneered by Bauer and Vinograd (1970), seems more appropriate in this case (see also (Hsieh and Wang, 1975)).

$$\left\langle (\Delta Tw)^2 \right\rangle = NlkT/(4\pi^2 C), \qquad (12)$$

where l is the separation between adjacent base pairs along DNA axis (l = 0.34 nm), and k is the Boltzmann constant (Vologodskii et al., 1979) .

Further analysis of the equilibrium distribution of topoisomers

It is important to understand well the nature of the equilibrium distribution of topoisomers considered above, so we are continuing its discussion. The experimentally observed equilibrium fluctuations in the $\left\langle (\Delta Lk)^2 \right\rangle$ are made up of fluctuations in the twist, Tw, and writhe, Wr, of the double helix. The enzyme-assisted rejoining of the DNA strand at the break point occurring at a random moment in time fixes the momentary value of the sum $Tw + Wr$, i.e. Lk. In a circular DNA with a single-stranded break torsional (changing of Tw) and bending (changing of Wr) fluctuations occur independently from each other (this is an assumption which should be considered as a good first approximation). Therefore, the variances, $\left\langle (\Delta Lk)^2 \right\rangle$, $\left\langle (\Delta Tw)^2 \right\rangle$, and $\left\langle (Wr)^2 \right\rangle$, follow the equation:

$$\left\langle (\Delta Lk)^2 \right\rangle = \left\langle (\Delta Tw)^2 \right\rangle + \left\langle (Wr)^2 \right\rangle. \qquad (11)$$

Since the value of $\langle(\Delta Lk)^2\rangle$ has been found experimentally (see above), and $\langle(Wr)^2\rangle$ can be calculated (see below), Eq. (11) makes it possible to determine $\langle(\Delta Tw)^2\rangle$. On the hand, the value of $\langle(\Delta Tw)^2\rangle$ depends only on DNA torsional rigidity, C:

$$\langle(\Delta Tw)^2\rangle = NlkT/(4\pi^2 C) \qquad (12)$$

where l is the separation between adjacent base pairs along DNA axis (l = 0.34 nm), and k is the Boltzmann constant (Vologodskii et al., 1979) .

Although DNA double helix is very rigid molecule in the scale of a few base pairs, long DNA molecules are flexible regarding their thermal fluctuations (see (Cantor and Schimmel, 1980) , for example). Thus a circular DNA longer than a few hundreds base pairs adopts very different conformations in solutions (Fig. 12). Each of these conformations has a

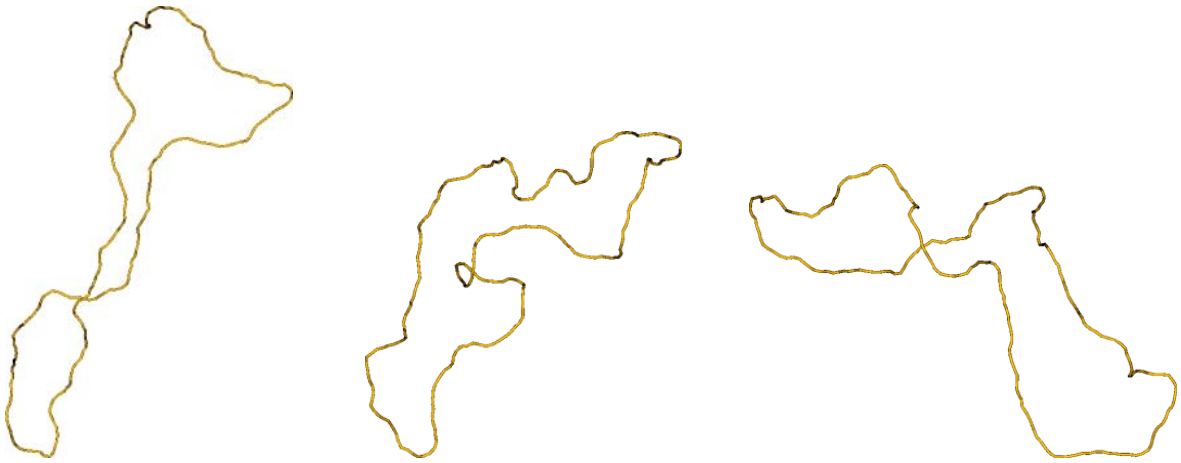

FIGURE 12 Typical conformations of nicked circular DNA 3500 base pairs in length. The illustration was obtained by computer simulation of the equilibrium conformational set (Klenin et al., 1989). The values of *Wr* for each simulated conformation are shown.

certain value of *Wr*. To evaluate $\langle(Wr)^2\rangle$ we can simulate the equilibrium distribution of DNA conformations for a particular DNA length and calculate $\langle(Wr)^2\rangle$ over the simulated set of conformations (Benham, 1978; Vologodskii et al., 1979) . Such simulation allows to compute $\langle(Wr)^2\rangle$ for different DNA lengths with good accuracy (see (Klenin et al., 1989) and refs. therein). The simulation results showed that $\langle(Wr)^2\rangle$ is proportional to N if $N > 2500$. However, $\langle(Wr)^2\rangle$ decreases faster for shorter DNA molecules, since their typical conformations are

becoming more and more flat as the length decreases (*Wr* of a flat conformation equals zero). The difference between the experimentally determined value $\langle(\Delta Lk)^2\rangle$ and the calculated value $\langle(Wr)^2\rangle$, however, is proportional to *N* over the whole range of *N* studied, 250 < *N* < 10000, in full agreement with Eq. (12). The values of $\langle(\Delta Tw)^2\rangle$ found in this way made it possible to determine the torsional rigidity of the double helix on the basis of Eq. (12). The obtained value of *C* equals $3\cdot10^{-19}$ erg·cm (Klenin et al., 1989).

Conformations of Supercoiled DNA

Electron microscopy (EM) is the most straightforward way to study conformations of supercoiled DNA. This method has been used extensively since the discovery of DNA supercoiling by Vinograd and coworkers in 1965 (Vinograd et al., 1965). They found that supercoiled DNA has a compact, interwound form. This conclusion was conformed in many EM studies which also brought quantitative information about DNA supercoiling (Laundon and Griffith, 1988; Adrian et al., 1990; Boles et al., 1990). The data obtained include the measurements of $\langle Wr\rangle / \Delta Lk$ and the average superhelix radius as a function of σ, and the branching frequency of the superhelix axis. It became clear, however, that labile DNA conformations may change during sample preparation for EM (see (Vologodskii and Cozzarelli, 1994) for details). A serious problem for interpreting EM results is that the ionic conditions on the grid are not specified. The DNA is exposed to several different solutions and dried before being viewed, and it is unknown when in the procedure the DNA is "fixed". Cryoelectron microscopy and atomic force microscopy are free from some of the disadvantages of more conventional EM since in these methods DNA is viewed in solution or in a thin layer of vitrified water without shadowing or staining. It was shown by these methods that conformations of supercoiled DNA depend greatly on ionic conditions (Adrian et al., 1990; Bednar et al., 1994; Lyubchenko and Shlyakhtenko, 1997). Still, independent solution studies are required to conform conclusions of the methods about this very flexible object.

The solution methods, like hydrodynamic and optical ones, do not give direct, model-independent information about three-dimensional structure of supercoiled DNA. These methods do, however, measure structure-dependent features of the molecules for well-defined solution conditions while perturbing conformations only minimally. The methods turned to be very productive in the studies of DNA supercoiling when used in combination with the computer simulation of supercoiled molecules. The approach is based on comparison of simulated and measured properties of supercoiled DNA (Gebe et al. 1996; Rybenkov et al., 1997c, 1997d; Hammermann et al. 1998). If the results are in agreement one can assume that simulated and actual conformations look similar as well.

The efficiency of this approach originates from the fact that DNA conformational properties can be accurately described in terms of a simple model (Vologodskii et al., 1992). A DNA molecule is modeled as a chain consisting of *m* rigid segments that are cylinders of equal length. The length the cylinders does not affect the simulation results as soon as it corresponds to less than 30 bp. The energy of the chain consists of the energy of bending and torsional deformations and the energy of electrostatic repulsion between DNA segments. Correspondingly, there are only three parameters in the model which specify its bending and torsional rigidity and the

intersegment repulsion. The values of all these parameters are known from numerous independent studies.

FIGURE 13 DNA model that can be used to describe large-scale conformational properties of the double helix. The model chain consists of the straight cylinders. The picture illustrates the correspondence between the model cylinders (shown by black lines) and DNA molecule (shown by gray).

The values of all these parameters are known from numerous independent studies. Using this model one can simulate a random set of conformations that corresponds to the equilibrium conformational set for actual DNA molecules. Monte Carlo method is usually used to prepare the set (Vologodskii et al., 1992). This set allows one to determine mean values and distributions of many superhelix properties such as *Wr*, the number of branches, or sedimentation coefficient. There is an analogy between this computer approach and EM that can also be considered as a Monte-Carlo method, because conclusions are drawn from a limited statistical sampling. Of course, a much larger set can be generated computationally.

The study of the equilibrium formation of DNA catenanes between supercoiled and nicked circular DNA gives a good example of this approach (Rybenkov et al., 1997d). In this work catenanes were formed by cyclizing linear DNA with long cohesive ends in the presence of supercoiled molecules.

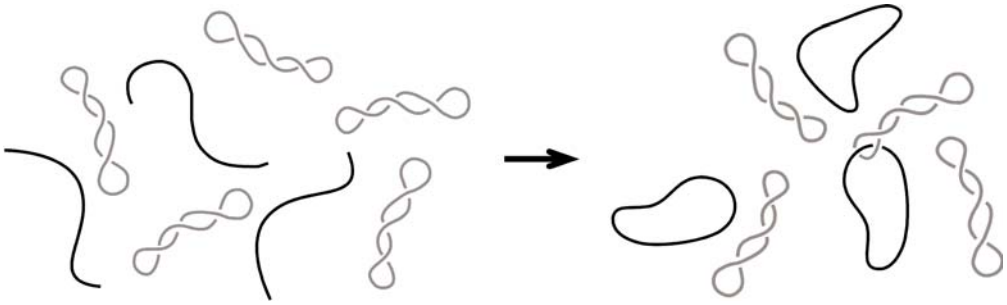

FIGURE 14 Probing conformational properties of supercoiled DNA by catenation. The diagram shows formation of catenanes between supercoiled and cyclizing linear molecules. The cyclization occurred via long cohesive ends of the linear molecules. The concentration of linear molecules was much lower than the concentration of the supercoiled DNA, so there were no links between them.

The efficiency of the catenation depends on the distance between opposing segments of DNA in the interwound superhelix. Thus, the fraction of cyclizing molecules that becomes topologically linked with the supercoiled DNA is the product of the concentration of the supercoiled DNA and a proportionality constant, B , that depends on conformations of supercoiled DNA. The values of B were measured for different ionic conditions and supercoiling. In parallel with the

experiments, the same values were calculated using Monte Carlo simulations of the equilibrium distribution of DNA conformations. Very good agreement between measured and simulated values of *B* was found in this study over a broad range of σ and ionic conditions (Fig. 15).

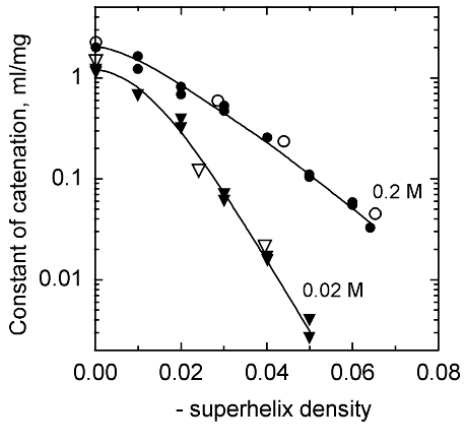

FIGURE 15 Probing conformations of supercoiled DNA by catenation. Measured and simulated equilibrium constants of catenation, , are shown as a function of supercoiling at NaCl concentration of 0.2 M (circles) and 0.02 M (triangles). The experimental values of B (open symbols) are shown together with calculated results (filled symbols). The solid lines are the best fits to the calculated data.

The large effect of ionic conditions on the value of *B* is due to the changes of the electrostatic repulsion between DNA segments. Taking into account that there were no adjustable parameters in the simulation, the agreement between experimental and simulation results is striking. Very good agreement between experimental and simulated results was obtained in the other studies of supercoiled DNA based on the comparison of solution measurements and computer simulations. This allowed us to conclude that the simulations predict conformations of the supercoiled DNA with good accuracy and can be used to study properties of the supercoiled molecules that are hard to measure directly. Fig. 16 shows typical simulated conformations of supercoiled molecules for two different ionic conditions and σ = -0.06. Although the conformations for both

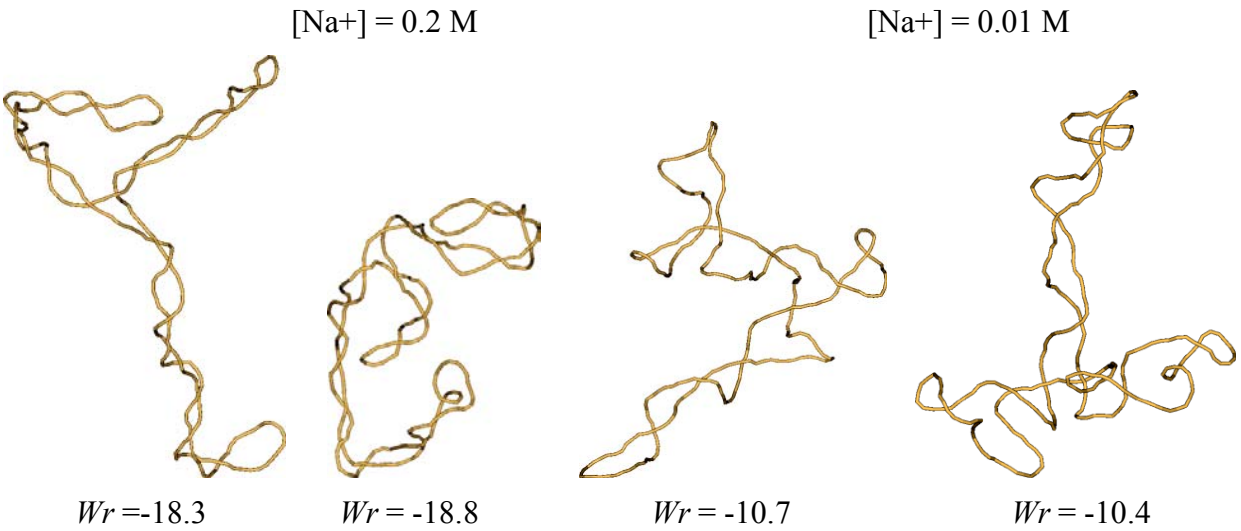

FIGURE 16 Typical simulated conformations of supercoiled DNA in solution containing 0.2 M NaCl (left) and 0.01 M NaCl (right). The conformations of the model chains correspond to DNA 3.5 kb in length and = –0.06.

ion concentrations clearly correspond to branched interwound superhelices, there is a large difference between them. This difference explains a big effect of ionic conditions on the equilibrium constant of catenation shown in Fig. 15.

Table 1. Average conformational parameters of supercoiled DNA.

Superhelix density, σ	Wr/Δ*Lk*	Superhelix diameter, nm	(Number of superhelix turns)/ Δ*Lk*	Number of superhelix branches
-0.03	0.73 ± 0.01	19 ± 1	0.9 ± 0.02	-
-0.06	0.73 ± 0.01	10 ± 1	0.9 ± 0.02	1 per 1500 bp

The parameters are listed for supercoiled DNA molecules larger than 2500 bp in length, for (Vologodskii et al., 1992, 1994, Hammermann et al. 1998).

Formation of Noncanonical Structures

General consideration

So far we considered properties of circular DNA which do not involve disruptions of the regular structure of the double helix. However, with sufficiently high negative supercoiling such disruptions become inevitable. In effect, such disruptions is a way of structural realization of supercoiling. In contrast to torsional and bending deformations, which are distributed along the whole circular DNA molecule, disruptions in the regular structure have a local character, while their type and location depend on the DNA sequence. All these alternative structures have a smaller (compared with the canonical B-form of DNA) interwinding of one strand of the DNA relative to the other.

The simplest type of structures induced by negative supercoiling are melted or opened regions where DNA strands are not interwoven in the relax state (Fig. 17). Such regions appear first of all in the DNA sections enriched with AT base pairs because melting of AT pairs requires less free energy than does the melting of GC pairs.

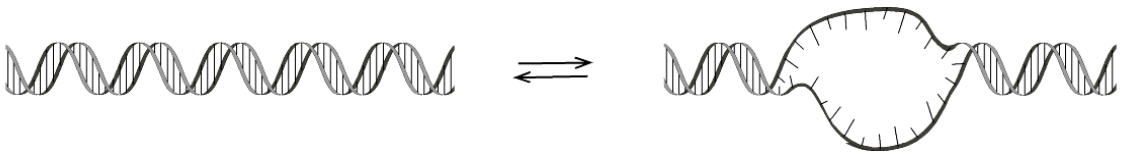

FIGURE 17 Formation of the opened region in double-stranded DNA. Complementary strands are not interwoven, in average, in such region.

In palindromic regions of DNA, cruciform structures may appear (Fig. 18). Again, the complementary strands of DNA are not twisted relative to each other in the cruciform structures. At the same time, a large part of nucleotides of the cruciforms are involved in the helical

structure, which means that their free energy corresponds to the free energy of a regular linear double helix. That is why formation of a long enough cruciform structure must be preferable to the formation of opened sections of the same size.

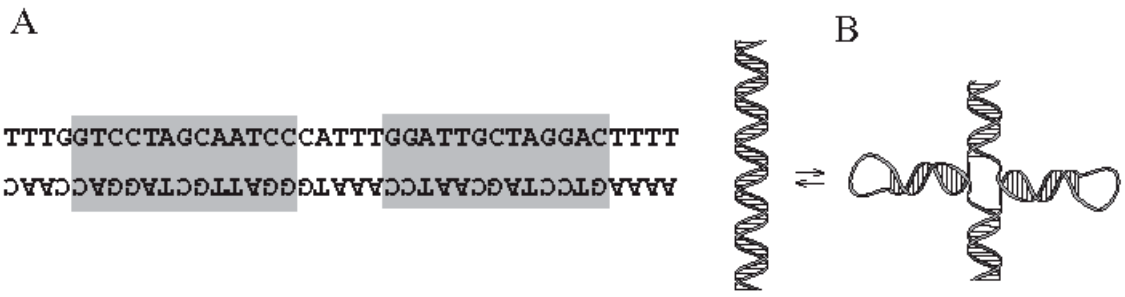

FIGURE 18 Formation of cruciform in double-stranded DNA. (A) Example of a palindromic sequence. The symmetrical parts of the sequence are marked. (B) Diagram of the transition between B-DNA and cruciform.

The maximum release of negative superhelix stress per base pair causes formation of the Z form (Rich et al., 1984), where the strands are twisted into a left-handed helix with a helical repeat of 12 base pairs per turn (Fig. 19). Z form predominantly occurs in DNA regions with a regular alternation of purines and pyrimidines, first of all G and C.

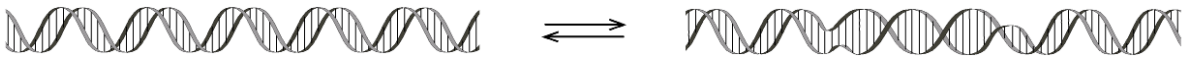

FIGURE 19 Formation of the left handed Z form inside B-DNA segment.

Another type of noncanonical structure occasioned by negative supercoiling is the H form, which may occur in DNA sections where one strand comprises only purine and the other only pyrimidine bases (Mirkin and Frank-Kamenetskii, 1994). The main element of the H form is a

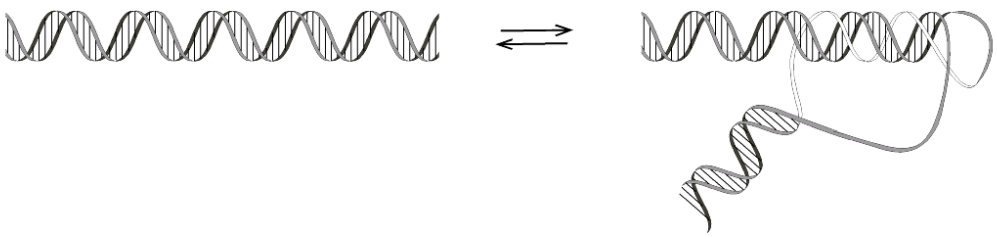

FIGURE 20 Formation of the H form inside B-DNA segment. The pyrimidine strand and half the purine strand make up a triple helix; the other half of the purine strand is free. Only Watson-Crick base pairs are shown. The CGC triplets are protonated in this structure.

triple helix stabilized by Watson-Crick and Hoogsteen base pairs (Fig. 20). Topologically, the H form is equivalent to an open section or a cruciform structure: the complementary strands of DNA in the H form are not twisted relative to each other. Occurrence of the H form is stimulated by the reduction of pH of the solution.

One more structure where the double helix is somewhat unwound compared to B-DNA is A-DNA. The difference in the angles of helical rotation for these two forms is so small, however, that negative supercoiling cannot by itself induce B→A transition of any DNA section in usual solution conditions.

It worth to emphasize once again that the formation of all the above noncanonical structures is thermodynamically wasteful in linear DNA in near-physiological conditions, and it is only negative supercoiling that allows them to evolve under such conditions.

Experimental detection of noncanonical structures

All the existing methods of studying noncanonical DNA structures occasioned by negative supercoiling can be divided into two groups. The first group brings together the methods based on the registration of local changes in DNA sections where a structural modification has taken place. In principle, these methods can be used just as effectively to register local structural changes in linear molecules, too. We shall refer to this group as methods of localization of structural transitions. The second group of methods is based on the measurement of integral properties of DNA. As it is, only one method in this group, which is based on the use of the specific properties of closed circular DNA, is truly effective. On the formation of a noncanonical structure under the impact of negative supercoiling, superhelical stress itself decreases and so does the absolute value of writhe, which determines the electrophoretic mobility of DNA molecules in gel. This change in the mobility lies at the core of the second approach. The method does not provide any information on where exactly the conformational change has happened, but allows one to obtain quantitative characteristics of the transition. The method offers a unique opportunity of reliable quantitative registration of conformational changes in a region that accounts for about 1% of the total length of the molecule. No other physical method registering integral properties of DNA (UV-spectroscopy, circular dichroism, infra-red spectroscopy, NMR) makes it possible to monitor changes affecting such a small fraction of DNA base pairs.

Methods of localization of structural transitions Most methods of localization of structural transitions are based on the breaking DNA at the site of the formation of a noncanonical structure with the subsequent mapping of these breaks. The breaks can be introduced by special enzymes, endonucleases, that specifically hydrolyze single-stranded DNA. These endonucleases are sensitive even to minor irregularities in the helical structure of DNA. In cruciform structures they cause breaks in the loops of the hairpins (Lilley, 1980; Panayotatos and Wells, 1981). There are sites sensitive to these endonucleases in the areas of Z and H forms and, naturally, in opened regions of DNA. In supercoiled DNAs there are also other areas of increased sensitivity to such endonucleases, though the nature of structural changes in such areas is not clear yet. Although after the application of the first single-strand break noncanonical structures in DNA soon disappear as a result of the torsional relaxation, the enzymes often go on to cut the second strand of the DNA, opposite the first break. That is why a considerable fraction of the molecules end up in the linear form after the treatment with the endonucleases.

Another way of making breaks in the areas of the formation of noncanonical structures is a chemical modification of DNA bases at the spots which in the regular B form are screened off by

the double helix structure. Further chemical treatment of such selectively modified DNA leads to a break-up of the sugar-phosphate chains at the sites of primary modification.

Restriction analysis is used for rough preliminary mapping of the breaks. DNA molecules converted into the linear form by way of nuclease or chemical cutting are treated with some restriction endonuclease to obtain a set of restriction fragments. This set is compared by way of electrophoretic separation with a reference set obtained by treatment of the original circular DNA with the same restriction endonuclease. If the cutting at the point of structural deformation occurred in one DNA region, two additional bands should appear in the electrophoresis of the first set of fragments, compared with the reference set. Besides, one of the lines of the reference set should be missing in the first set of fragments or become much less intensive. By measuring the length of the additional fragments one can find two possible localizations of the structural deformation. After the preliminary localization of the specific cleavage, one can map the cuts at the nucleotide level. By this method Lilley and Panayotatos and Wells discovered the formation of cruciform structures in natural supercoiled DNAs (Lilley, 1980; Panayotatos and Wells, 1981).

Method of two-dimensional gel electrophoresis This very elegant and effective method of analyzing local conformational changes in supercoiled DNA was first suggested by Wang and coworkers (Wang et al., 1983). The method allows one to obtain, as a result of a single experiment, the complete dependence of the probability of an alternative structure formation on ΔLk. Besides, it yields important information on the DNA unwinding associated with this transition. Clearly, the two-dimensional electrophoresis is the most informative and accurate method of quantitative analysis of local conformational transitions in supercoiled DNA.

The electrophoretic mobility of DNA molecules increases monotonously with the growth of the absolute value of ΔLk. This is due to the fact that topoisomers with a larger $|\Delta Lk|$ must have a greater $|Wr|$ and, consequently, a more compact conformation. This, however, is true only as long as the molecule undergoes no conformational changes which reduce superhelical stress and, consequently, $|Wr|$. DNA topoisomers that have undergone the transition will have lower mobility compared to what it would have been in the absence of the transition. As a result, the monotonous rise in the mobility of molecules with the growing $|\Delta Lk|$ may be disrupted. An one-dimensional electrophoresis of the mixture of topoisomers will show a pattern of irregular bands by which it will be hard to identify specific topoisomers. Two-dimensional electrophoresis helps to identify the bands with specific topoisomers. Electrophoresis in the first direction is performed under ordinary conditions, along one of the edges of a flat gel. This results in the distribution of topoisomers that was described above. Before the electrophoresis in the second direction the gel is soaked in a buffer containing an intercalating ligand. As a result of the ligand binding, the torsional stress in DNA is no longer determined by ΔLk but by the value $(\Delta Lk - \nu\phi N/360)$, where ν is the number of bound ligand molecules per base pair, ϕ is the angle of unwinding of the double helix upon the binding of a single ligand molecule $(\phi < 0)$, and N is the number of base pairs in DNA. The concentration of the ligand is selected in such a way that the remaining superhelical stress should be insufficient for the formation of a noncanonical structure in the topoisomer set. In this case, the mobility of topoisomers monotonously depends on $\left|\Delta Lk - \nu\phi N/360\right|$. That is why after the completion of electrophoresis in the second direction, correlating spots with specific topoisomers is not difficult at all (Fig. 21). One can see in the figure that the mobility of the 17th topoisomer is close to that of the 12th during the first

direction of the electrophoresis. It means that these topoisomers have the same *Wr* and, consequently, the same torsional stress. Thus, the elastic torsional deformations for the topoisomers have to be same as well. This is possible only if the difference in ΔLk for these topoisomers is compensated by the change of nonelastic torsional deformation during the transition, δTw , which equals 5 in this case (For some reasons for cruciform structures the observed value of δTw is often 1 to 1.5 turns larger than the estimated value).

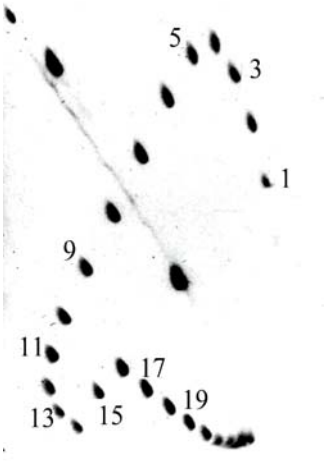

FIGURE 21 Cruciform extrusion in palindromic region d[CCC$(AT)_{16}$GGG] of circular DNA 3000 bp in length. The mobility of topoisomers in the course of the first, vertical electrophoresis leaps sharply between the 14th and 15th, what becomes clear after the completion of electrophoresis in the second, horizontal direction. This mobility leap is associated with the cruciform formation in the palindromic region. (Illustration provided by M. Vologodskaia.)

Thermodynamic analysis

Let us consider a simple thermodynamics of the formation of noncanonical structures in closed circular DNA (more advanced version of this analysis can be found in ref. (Vologodskii, 1992)). The analysis is very useful for comprehending the main regularities of the formation of noncanonical structures in supercoiled DNA. It is based on the generally accepted assumption that the free energy of the formation of noncanonical structures can be presented in the form of two terms (Benham, 1979; Vologodskii et al., 1979; Wang et al., 1983). One of these terms corresponds to the transition-related change in the free energy of supercoiling, and the second is the change in the free energy of linear DNA upon the conformational transition. As long as DNA remains in regular B form, it torsional stress and, consequently, the free energy of supercoiling is defined by ΔLk (see Eq. (10)). We assume that the energy of supercoiling will continue to depend only on the DNA's elastic deformation after the formation of a of noncanonical structure. The magnitude of elastic deformation will be defined now by $\Delta Lk - \delta Tw$ rather than by ΔLk, where δTw is the change of DNA twist resulting from the transition of m base pairs to the alternative structure. The value of δTw can be expressed through the number of base pairs per helix turn for B form of DNA, γ_B, and the number of base pairs per turn of one strand around the other in the alternative structure, γ_{alt}:

$$\delta Tw = m(1/\gamma_{alt} - 1/\gamma_B) \qquad (13)$$

Thereby the energy of supercoiling can now be expressed in the form:

$$G = KRT(\Delta Lk + m\kappa / \gamma_B)^2 / N , \tag{14}$$

where we introduced $\kappa = 1 - \gamma_B / \gamma_{alt}$. The value of κ specifies the change of DNA twist per base pair associated with a particular transition. Accordingly, the change in the free energy of supercoiling, δG, equals:

$$\delta G = KRT[(\Delta Lk + m\kappa / \gamma_B)^2 - (\Delta Lk)^2] / N . \tag{15}$$

It is often more convenient to express δG as a function of σ:

$$\delta G = AN[(\sigma + m\kappa / N)^2 - \sigma^2], \tag{16}$$

where $A = KRT / \gamma_B^2$. This equation formally implies that the formation of any structure with a lesser interwinding of the complementary strands than in B-DNA, reduces the energy of negative supercoiling and, consequently, must be stimulated by such supercoiling. This, of course, is true only under the condition that this structure forms in a comparatively short DNA fragment, so that the $m\kappa / \gamma_B$ value does not exceed $|\Delta Lk|$.

The change in the free energy of linear DNA upon the conformational transition can be calculated as $\sum_{i=1}^{m} \Delta F_i + 2F_j$, where ΔF_i is the free energy change associated with base pair *i*, and F_j is the free energy of each boundary between B form and the alternative structure. At the transition point, σ_{tr}, the full free energy change must be zero:

$$\delta G + \sum_{i=1}^{m} \Delta F_i + 2F_j = 0 . \tag{17}$$

Let us consider application of this analysis to two types of alternative structures.

Cruciform structures Of the energy parameters, ΔF_i and F_j, in the case of cruciforms the ΔF_i values must be assumed to be zero. Indeed, the cross grows in size at the expense of the destruction of two base pairs in the main helix and the formation of two identical pairs in the hairpins. This should not change the free energy of the structure, because the number of paired bases and all border elements of the structure remain as they were. This means that the formation of cruciform structures must be characterized by a single energy parameter F_j. Thus, Eq.(17) is reduced to

$$-\sigma_{tr} = F_j / (Am) + m / (2N) . \tag{18}$$

We accounted here that for cruciform structures the parameter κ is equal to 1. Indeed, in cruciform structures the complementary strands are not twisted relative to each other ($\gamma_{alt} = \infty$). It follows from Eq. (18) that the absolute value of $\sigma_{tr.}$ decreases when *m* increases, since the last term in Eq. (18) is relatively small. Experimental studies show that cruciform extrusion at a typical palindromic regions of 25-30 bp in length takes place at $\sigma_{tr.}$ of (-0.04) ÷ (-0.06) (Murchie and Lilley, 1992). Taking $A = 10RT$, one can evaluate F_j from these data which is around 10 kcal/mol.

Left-handed Z form. The transition of a DNA fragment from a right-handed B-helix into a left-handed Z-helix ( ) provides the maximum release of superhelical stress compared to other noncanonical structures:

$$\kappa = 1 - 10.5/(-12) = 1.87 .$$

Although the transition was initially observed for $d(CG)_n \cdot d(CG)_n$ and $d(AC)_n \cdot d(GT)_n$ inserts in supercoiled DNA, many others sequences can be converted into the Z form as long as the superhelical stress is high enough. For DNA regions with a regular alternation of purines and pyrimidines ΔF_{GC} =0.33 kcal/mol and ΔF_{AT} =1.15 kcal/mol, F_j is close to 5 kcal/mol (Peck and Wang, 1983; Ellison et al., 1985; Mirkin et al., 1987). More general analysis of Z DNA formation for an arbitrary DNA sequence can be found in (Anshelevich et al., 1988).

There is strong mutual influence between different transitions in closed circular DNA, caused by negative supercoiling. The primary reason for this is that any such transition reduces the superhelical stress in DNA, a driving force for other transitions. This mutual influence has to be taken into account in a quantitative analysis of conformational changes in supercoiled DNA. The phenomena has been studied in details both experimentally and theoretically (Benham, 1983; Kelleher et al., 1986; Ellison et al., 1987; Aboul-ela et al., 1992; Vologodskii, 1992). Here we only illustrate it by one graphic example, B-Z transitions in a plasmid containing two $d(CG)_{12} \cdot d(CG)_{12}$ inserts separated by a certain distance. With the growth of negative supercoiling in that DNA, two consecutive cooperative transitions are observed, corresponding to B-Z transitions in both inserts (Fig. 22). Since the inserts are identical, it is impossible to say which of them undergoes the transition earlier and which later, but the transition in one of them

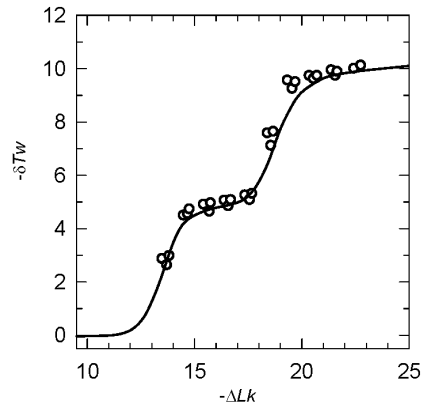

FIGURE 22 Changing equilibrium twist of DNA upon the B-Z transition in two identical inserts, $d(CG)_{12} \cdot d(CG)_{12}$, within the 4400-bp plasmid (Kelleher et al., 1986). The theoretical curve is shown alongside the experimental data (O).

reduces the superhelical stress and holds back the formation of the Z form in the other insert. Such a picture of conformational transitions was observed experimentally and obtained theoretically (Kelleher et al., 1986). It is important to emphasize that this kind of behavior is essentially impossible in linear DNA where two such transitions occur independently and simultaneously.

How Supercoiling Can Affect DNA Biological Functions

Supercoiling and DNA-protein interaction

Important biological feature of DNA supercoiling is its ability to affect binding of proteins with the double helix. There are two ways how such effect can occur. First, a protein can unwind the double helix locally upon binding with DNA. It is hardly possible that a protein can overtwist the double helix since the B form of DNA is already strongly winded. So, the value of the local change of DNA twist, δtw, can be only negative. Second, a protein can also introduce a local chirality into the bound DNA segment. This creates a local writhe, δwr, which can be either negative or positive. In both cases, the Lk of unstressed double helix, Lk_o, is changed on some value, δlk, which is equal to either δtw or δwr. This change results in the change of the supercoiling free energy, similar to the formation of local noncanonical structures. We can analyze this quantitatively. In a good approximation the free energy of supercoiling depends only on the DNA's elastic deformation. The magnitude of this deformation will be defined now by $Lk-(Lk_o+\delta lk)$ rather than by $(Lk-Lk_o)$. Accordingly, the change in the free energy of supercoiling upon the protein binding, δG, equals (see Eq. (10)):

$$\delta G = KRT[(Lk-Lk_o-\delta lk)^2-(Lk-Lk_o)^2] \approx -2KRT/N\cdot\Delta Lk\cdot\delta lk \qquad (19)$$

We can also express δG as a function of σ:

$$\delta G = -2KRT\sigma/\gamma_B\cdot\delta lk\,. \qquad (20)$$

The value of δG affects DNA-protein biding constant, which is changing by the multiplier

$$k_s = \exp[-\delta G/RT] = \exp[2K\sigma/\gamma_B\cdot\delta lk]\,. \qquad (21)$$

Since the sign of δG can be either positive or negative, negative supercoiling can both increase and decrease the binding constant.

Thermodynamics and kinetics of DNA juxtaposition

The second way by which DNA supercoiling influences biological reactions is based on global changes of DNA conformations. These global changes can affect the probability of juxtaposition of DNA sites that are far apart along the chain contour. Such consequences of supercoiling are important for the processes where two or more DNA sites are joined by protein bridges. Numerous examples of such multi-site complexes are found in DNA replication, transcription, site-specific recombination, and transposition.

If we take a look on a typical conformation of supercoiled DNA (Fig. 4), is becomes clear that supercoiling increases the probability of site juxtaposition. Indeed, there are many pairs of juxtaposed sites at each conformation of supercoiled DNA, while the juxtapositions are rare in typical conformations of the relaxed molecules (Fig. 12). Results of the computer simulation show that supercoiling magnifies the probability of juxtaposition by more than two orders of value (Fig. 23). Such increase of the juxtaposition probability can accelerate greatly enzymatic processes that involve simultaneous interaction with two or more DNA sites. Supercoiling also changes preferable relative orientations of the juxtaposed sites, and this can be very important for the efficiency of some enzymatic reactions (Vologodskii and Cozzarelli, 1994; Stone et al., 2003).

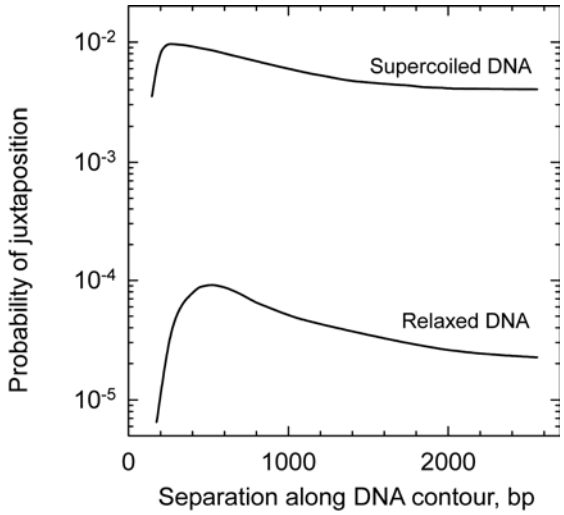

FIGURE 23 Probability of juxtaposition of two sites, separated along DNA contour, for supercoiled and relaxed molecules. The data were obtained by the analysis of simulated ensembles of DNA conformations (Vologodskii and Cozzarelli, 1994) for molecules of 5600 bp in length. The analysis assumed that two sites are juxtaposed if the distance between them does not exceed 10 nm.

2 KNOTS AND LINKS IN DNA

As a linear DNA molecule links into a circle, it ends up in a specific topological state. Until now we examined different topological states associated with the linking number of the complementary strands of the double helix. The strands' linking number, however, is not the only topological characteristic of circular DNA. As it closes into a circle, any polymeric chain may finish up in an unknotted state or form a knot of a certain type. In the case of the DNA double-helix, we should talk about knots formed by its axis (an unknotted closed chain is considered as a trivial knot). With all conformational rearrangements of the chains the type of the knot must remain unchanged. This factor must influence the conformational properties of circular DNA molecules, that have to depend on the topology of the axis.

Just as different types of knots may form through the ring closure of single chains, a closed pair or a larger number of polymeric chains may form links of different types, or catenanes. We have already considered special type of links formed by the complementary strands of a double helix in a closed circular DNA. In this section, however, the double helix will be treated as a simple polymer chain. Linked DNA molecules occur quite frequently in nature and can be also obtained *in vitro*. Links of two chains, as well as knots formed by isolated molecules, come in an

infinitely large number of topologically non-equivalent types. The notion of linking number that was extensively used above is good only for characterizing links of a certain class (torus links) formed by complementary strands of circular DNA. The overall picture is much more complicated.

Knots and links often form in living cells and in the course of various laboratory manipulations with DNA. Different aspects of these issues are discussed in reviews (Wasserman and Cozzarelli, 1986; Stark and Boocock, 1995; Ullsperger et al., 1995).

Types of Knots and Links

For the classification knots and links have to be deformed to the standard form of their projection on a plane. The standard form of a knot (link) projection is such an image thereof when the minimum number of intersections on the projection is achieved. One must be always careful, though, that there should be no self-intersection of chains in the course of such reduction. Deformations of this kind are known in topology as isotopic deformations. Two knots (or links) which can be transformed into each other by way of isotopic deformation belong to the same isotopic type.

Knots can be simple or composite. A knot is composite if there is an unlimited surface crossed by the knot at two points only that divides it into two knots. This definition is illustrated in Fig. 24. The simplest knot has three intersections in the standard form and is called a trefoil. Fig. 25 shows the initial part of the table of simplest knots – namely, all knots with less than six intersections in the standard form.

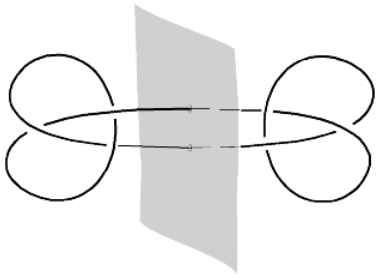

FIGURE 24 A composite knot. The shaded unbounded surface intersects this knot at two points only. By contracting these points into one along the surface, we obtain two trefoils.

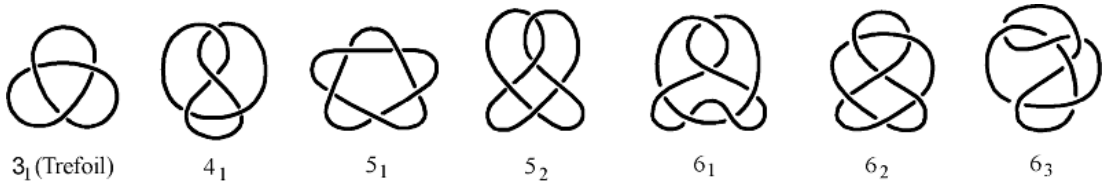

FIGURE 25 Table of simple knots with less than six intersections in the standard form.

A knot and its mirror image are considered to belong to the same type of knot, although they may belong to the same or different isotopic types. In particular, the trefoil and its mirror image cannot be transformed into each other by way of continuous deformation without self-intersection, and therefore belong to different isotopic types. The figure eight knot (4_1) and its mirror image belong to the same isotopic type. The table of knots is, in fact, a table of knot types, for it features only one representative of mirror pairs. With the growth of the number of intersections the number of types of simple knots grows very fast. As it turns out, there are 49 types of simple knots with nine intersections, 165 with ten and about 552 types of knots with eleven intersections (Rolfsen, 1976; Adams, 1994).

The tables of two-contour links are based on the same principle as the table of knots (Rolfsen, 1976). The initial part of the table of link types is shown in Fig. 26. Although three links in this table, 2_1, 4_1 and 6_1, belong to the torus class which corresponds to the links of the complementary strands of the double helix in closed circular unknotted DNA, the fraction of torus links between more complex links is very small.

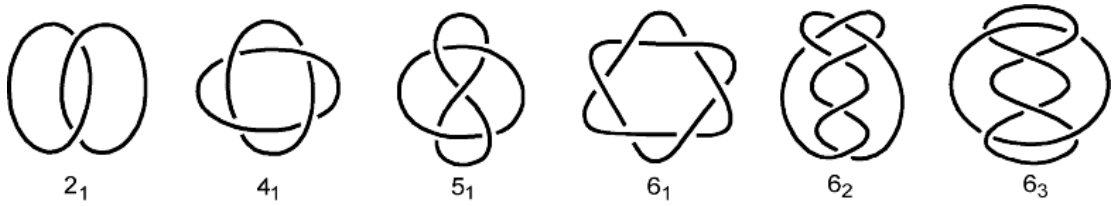

FIGURE 26 Table of links with less than seven intersections in the standard form.

Topological Equilibrium

It was discussed earlier that long DNA molecules adopt many different conformations in solution and their properties have to described in terms of the probability distribution (1.4.2). Among many possible distributions one is very important, the distribution which corresponds to thermodynamic equilibrium. We have considered the equilibrium distribution of ($Tw + Wr$) in open circular DNA. Another example is the distribution of the end-to-end distance in linear molecules. As a result of thermal motion the distributions of these and other properties always relax to their equilibrium form. So after some time we will always have an equilibrium distribution of conformational properties of our molecules in solution.

Distributions of topological states of circular molecules are different. These distributions are not changed as long as the molecule backbones stay intact. This is true for the distributions simply because it is true for each circular molecule. The system of circular molecules does not relax to the equilibrium distribution of topological states. The concept of the equilibrium distribution is very useful, however, in this case as well.

A formal definition of the equilibrium distribution of topological states of circular molecules is not different from the equilibrium distributions of properties of linear molecules. This is the most probable distribution which has to reached by random exchange between different states in solution. This distribution minimizes the free energy of the molecule set. We would get the

equilibrium distribution of topological states if our circular molecules have phantom backbones, so their segments could pass one through another during the thermal motion. Thus, to calculate the distribution we should forget about forbidden exchange between different topological states and use usual rules of statistical mechanics.

Although exchange between different topological states is impossible in solution of circular DNAs, we can obtain the topological equilibrium by slow cyclization of linear molecules (reviewed in (Wang, 1986; Vologodskii, 1992)). The cyclization can be performed by joining cohesive ends of DNA. If the cohesive ends are long enough (about dozen of nucleotides or longer), a circular form is stable at room temperature; for shorter cohesive ends ligation is required to seal at least one of two single stranded brakes. Sometime we can also use enzymes, DNA topoisomerases, which catalyze the strand passage reaction between DNA segments, to obtain the equilibrium distribution. The second approach can shift the distribution from its equilibrium form, however, if the enzyme consume the free energy of ATP hydrolysis catalyzing strand passage reaction (Rybenkov et al., 1997a).

Equilibrium Probabilities of Knots in Circular DNA

Let us first consider very diluted solution of opened circular DNA so the equilibrium probability of links between them is negligible. In this case the equilibrium distribution of topological states is reduced to the probabilities of unknotted circles and various knots. The first question is what is probability of any knot for a circular DNA of a particular length? To solve the problem we have to learn how to distinguish between unknotted circular molecules and knots of various types. Let us first consider theoretical aspect of this problem.

How to analyze the topology of a particular closed chain configuration, or how to determine the knot type to which this configuration belongs? Let us imagine that we have a heavily entangled circular cord and we want to find out whether it is knotted or not. The fact that persistent attempts to untangle the cord have produced no result cannot be taken as a proof that we are dealing with a knot. What is needed for resolving this problem is a single-valued algorithm of verification of the topological identity of the configuration in question. The construction of such algorithms belongs to the realm of a branch of mathematics known as topology (Adams, 1994). Topologists developed invariants of the topological states, characteristics thereof which remain unchanged with any deformations of the chains performed without selfintersections. The simplest invariant of topological state is the Gauss integral, which defines the linking number of two chains (reviewed in (Frank-Kamenetskii and Vologodskii, 1981; Vologodskii, 1992). Of course, for classifying the state of chains with a topological invariant, the latter must assume different values for different topological states. Not a single topological invariant meets this requirement in full measure, but there are very powerful ones among them, which help identify many elementary types of knots (links) and distinguish them from more complex ones. The Gauss integral is a fairly weak topological invariant and is of no use for distinguishing many linked states of chains from unlinked states. Still the integral is very useful for analysis of DNA supercoiling, since it distinguishes all links of the torus class. Another very useful invariant is the Alexander polynomial which is convenient in computer simulation (reviewed in (Frank-Kamenetskii and Vologodskii, 1981)). It is a polynomial of one variable in the case of knots and of two variables in the case of links of two circular chains. The invariant was used in all studies dealing with the computer calculation of the equilibrium distribution of topological states.

Starting from 1974, about a dozen studies have been devoted to computer calculations of the equilibrium probability of knots (reviewed in (Frank-Kamenetskii and Vologodskii, 1981; Michels and Wiegel, 1986); see also (Deguchi and Tsurusaki, 1997) and refs. therein). The results show that equilibrium probability of knotted circular molecules increases with their length (Fig. 27). The figure shows that for DNA molecules a thousands base pairs in length the equilibrium fraction of knots is rather small, although the fraction of the simplest knots, trefoils,

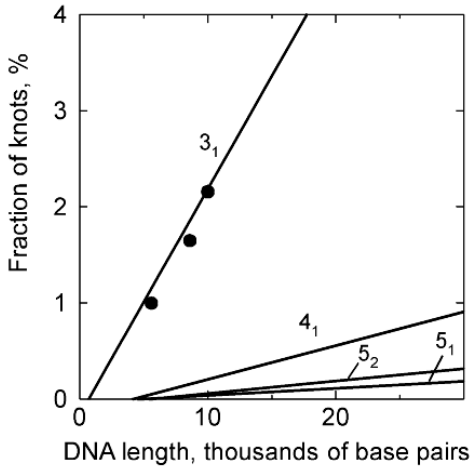

FIGURE 27 Equilibrium probability of the simplest knots in circular DNA. Results of computer simulations (Rybenkov et al., 1993), solid lines, are shown together with experimental measurements (Rybenkov et al., 1993; Shaw and Wang, 1993), symbols. The data correspond to the physiological ionic conditions.

can be measured with good accuracy. Computer calculations of the equilibrium probabilities of knots showed, that these probabilities reduce dramatically when the thickness of the molecule increases (Klenin et al., 1988). In case of DNA, the effective thickness is defined not only by the geometrical diameter of the double helix, but also by electrostatic repulsion of the DNA segments. Therefore comparison between calculated and measured equilibrium fraction of trefoils allowed to determine electrostatic repulsion between DNA segments for different ionic conditions (Rybenkov et al., 1993; Shaw and Wang, 1993; Rybenkov et al., 1997b).

Knots and links of different types formed by circular DNA molecules have different electrophoretic mobility in gel, which makes it possible to separate them by electrophoresis (Fig. 28). The method requires special calibration, since it is impossible to say in advance what position a particular topological structure must occupy relative to the unknotted circular DNA form. A sufficiently large body of experimental data on the mobility of various topological structures has been accumulated by now (Wasserman and Cozzarelli, 1986; Vologodskii et al., 1998). To separate knotted and linked DNA molecules by gel electrophoresis they must have single-strand breaks, for otherwise the mobility will also depend on the double helix's linking number.

Equilibrium Probabilities of Links

If the concentration of DNA molecules during their random cyclization in solution is not too small, some fraction of the molecules will form catenanes at thermodynamic equilibrium. Let us

suggest that the concentration is still small enough so most of circular molecules are not linked with others at equilibrium; in this case links of 3 and more molecules can be neglected. Thus, we

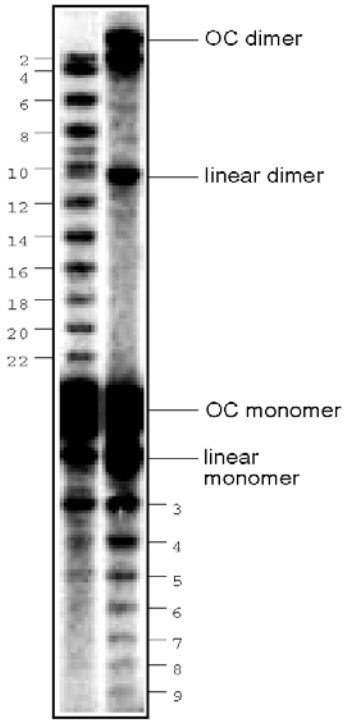

FIGURE 28 Electrophoretic separation of knotted (right lane) and linked (left lane) DNA molecules 4363 bp length. Each band corresponds knots or links with a specific number of intersections in the standard form. These numbers are shown next to each band. All links belong to the torus family. OC dimer is open circular DNA molecule of double length. (Illustration provided by E. M. Shekhtman and D. E. Adams.)

can consider formation of catenanes as bimolecular reaction which is characterized by its equilibrium constant, B. Equilibrium concentration of linked DNA molecules, c_{cat}, can be found from the equation

$$c_{cat} = B(c - c_{cat})^2 \approx Bc^2 , \qquad (19)$$

where c is the total concentration of circular molecules. The value of B depends on DNA length and ionic conditions in solutions. Dependence B on DNA length for physiological ionic conditions is shown in Fig. 29.

We can also consider topological equilibrium for a system which includes two types of circular DNAs (A and B). Such system was used to probe conformations of supercoiled molecules (described in 1.5). It consisted of supercoiled molecules of one length (molecules A) and cyclizing molecules of another length (molecules B). The system was prepared by such a way that there was only partial topological equilibrium: there were no AA catenanes although concentration of molecules A was rather high but there was equilibrium regarding AB and BB links. Since the goal was to measure the equilibrium constant for AB links, such partial

equilibrium simplified the analysis of various products in this system. It is interesting that similar approach to measure equilibrium catenation was used by Wang and Schwarz more than 35 years ago (Wang and Schwartz, 1967).

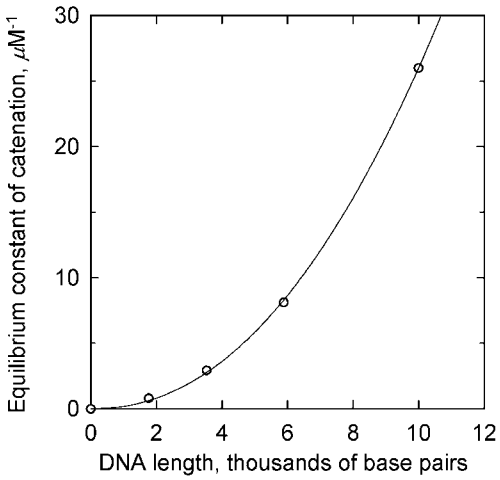

FIGURE 29 Equilibrium constant of catenation of two identical circular DNAs. The data obtained by computer simulation for the physiological ionic conditions (Klenin et al., 1988).

We have considered here different distributions of topological states: distributions of topoisomers of supercoiled DNA, distributions of knots and links. All these distributions have one very important advantage in comparison with distributions of other DNA conformational properties. They are not changed during all manipulations with DNA which are required for quantitative analysis of these distribution. Conditions under which the distributions are analyzed are not important in this case. This contrasts distributions of topological states from the distribution of many other conformational properties that are so easy to disturb. This advantage of topological distributions was widely used in studies of conformational properties DNA molecules per se and action of enzymes which are capable to change DNA topology (see (Wasserman and Cozzarelli, 1986; Stark and Boocock, 1995) for reviews of some biological applications).

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References

Aboul-ela, F., R. P. Bowater, and D. M. J. Lilley. 1992. Competing B-Z and helix-coil conformational transitions in supercoiled plasmid DNA. *J. Biol. Chem.* 267:1776-1785.

Adams, C. C. 1994. The knot book. Freeman, New York.

Adrian, M., t. H.-B. B., W. Wahli, A. Z. Stasiak, A. Stasiak, and J. Dubochet. 1990. Direct visualization of supercoiled DNA molecules in solution. *EMBO J.* 9:4551-4554.

Anshelevich, V. V., A. V. Vologodskii, and M. D. Frank-Kamenetskii. 1988. A theoretical study of formation of DNA noncanonical structures under negative superhelical stress. *J. Biomol. Struct. Dyn.* 6:247-259.

Bauer, W. and J. Vinograd. 1968. The interaction of closed circular DNA with intercalative dyes. I. The superhelix density of SV40 DNA in the presence and absence of dye. *J. Mol. Biol.* 33:141-71.

Bauer, W. and J. Vinograd. 1970. The interaction of closed circular DNA with intercalative dyes. II. The free energy of superhelix formation in SV40 DNA. *J. Mol. Biol.* 47:419-435.

Bauer, W. R., F. H. C. Crick, and J. H. White. 1980. Supercoiled DNA. *Sci. Am.* 243:100-113.

Bednar, J., P. Furrer, A. Stasiak, J. Dubochet, E. H. Egelman, and A. D. Bates. 1994. The twist, writhe and overall shape of supercoiled DNA change during counterion-induced transition from a loosely to a tightly interwound superhelix. *J. Mol. Biol.* 235:825-847.

Benham, C. J. 1978. The statistics of superhelicity. *J. Mol. Biol.* 123:361-70.

Benham, C. J. 1979. Torsional stress and local denaturation in supercoiled DNA. *Proc. Natl. Acad. Sci. USA*. 76:3870-4.

Benham, C. J. 1983. Statistical mechanical analysis of competing conformational transitions in superhelical DNA. *Cold Spring Harbor Symp. Quant. Biol.* 47:219-227.

Boles, T. C., J. H. White, and N. R. Cozzarelli. 1990. Structure of plectonemically supercoiled DNA. *J. Mol. Biol.* 213:931-51.

Calugareanu, G. 1961. Sur las classes d'isotopie des noeuds tridimensionnels et leurs invariants. *Czech. Math. J.* 11:588-625.

Cantor, C. R. and P. R. Schimmel. 1980. Biophysical chemistry. W. H. Freeman and Company, New York.

Deguchi, T. and K. Tsurusaki. 1997. Universality of random knotting. *Phys. Rev. E* 55:6245-6248.

Depew, R. E. and J. C. Wang. 1975. Conformational fluctuations of DNA helix. *Proc. Natl. Acad. Sci. USA* 72:4275-4279.

Dulbecco, R. and M. Vogt. 1963. Evidence for a ring structure of polyoma virus DNA. *Proc. Natl. Acad. Sci. USA* 50:236-243.

Ellison, M. J., M. J. Fenton, P. S. Ho, and A. Rich. 1987. Long-range interactions of multiple DNA structural transitions within a common topological domain. *EMBO J.* 6:1513-22.

Ellison, M. J., R. J. Kelleher 3d., A. H. Wang, J. F. Habener, and A. Rich. 1985. Sequence-dependent energetics of the B-Z transition in supercoiled DNA containing nonalternating purine-pyrimidine sequences. *Proc. Natl. Acad. Sci. USA.* 82:8320-8324.

Frank-Kamenetskii, M. D. and A. V. Vologodskii. 1981. Topological aspects of the physics of polymers: The theory and its biophysical applications. *Sov. Phys.-Usp.* 24:679-696.

Fuller, F. B. 1971. The writhing number of a space curve. *Proc. Natl. Acad. Sci. USA* 68:815-819.

Horowitz, D. S. and J. C. Wang. 1984. Torsional rigidity of DNA and length dependence of the free energy of DNA supercoiling. *J. Mol. Biol.* 173:75-91.

Hsieh, T. S. and J. C. Wang. 1975. Thermodynamic properties of superhelical DNAs. *Biochemistry* 14:527-535.

Kelleher, R. J., 3d., M. J. Ellison, P. S. Ho, and A. Rich. 1986. Competitive behavior of multiple, discrete B-Z transitions in supercoiled DNA. Proc. Natl. Acad. Sci. USA 83:6342-6.

Keller, W. 1975. Determination of the number of superhelical turns in simian virus 40 DNA by gel electrophoresis. *Proc. Natl. Acad. Sci. USA* 72:4876-4880.

Klenin, K. V., A. V. Vologodskii, V. V. Anshelevich, A. M. Dykhne, and M. D. Frank-Kamenetskii. 1988. Effect of excluded volume on topological properties of circular DNA. *J. Biomol. Struct. Dyn.* 5:1173-1185.

Klenin, K. V., A. V. Vologodskii, V. V. Anshelevich, V. Y. Klisko, A. M. Dykhne, and M. D. Frank-Kamenetskii. 1989. Variance of writhe for wormlike DNA rings with excluded volume. *J. Biomol. Struct. Dyn.* 6:707-714.

LaMarr, W. A., K. M. Sandman, J. N. Reeve, and P. C. Dedon. 1997. Large scale preparation of positively supercoiled DNA using the archaeal histone HMf. *Nucl. Acids Res.* 25:1660-1661.

Laundon, C. H. and J. D. Griffith. 1988. Curved helix segments can uniquely orient the topology of supertwisted DNA. *Cell* 52:545-549.

Lee, C. H., H. Mizusawa, and T. Kakefuda. 1981. Unwinding of double-stranded DNA helix by dehydration. *Proc. Natl. Acad. Sci. USA* 78:2838-42.

Lilley, D. M. 1980. The inverted repeat as a recognizable structural feature in supercoiled DNA molecules. *Proc. Natl. Acad. Sci. USA* 77:6468-72.

Lyubchenko, Y. L. and L. S. Shlyakhtenko. 1997. Visualization of supercoiled DNA with atomic force microscopy in situ. *Proc. Natl. Acad. Sci. USA* 94:496-501.

Michels, J. P. J. and F. W. Wiegel. 1986. On the topology of a polymer ring. *Proc. Roy. Soc. London A* 403:269-284.

Mirkin, S. M. and M. D. Frank-Kamenetskii. 1994. H-DNA and related structures. *Ann. Rev. Biophys. Biomol. Struct.* 23:541-576.

Mirkin, S. M., V. I. Lyamichev, V. P. Kumarev, V. F. Kobzev, V. V. Nosikov, and A. V. Vologodskii. 1987. The energetics of the B-Z transition in DNA. *J. Biomol. Struct. Dyn.* 5:79-88.

Murchie, A. I. H. and D. M. J. Lilley. 1992. Supercoiled DNA and Cruciform Structures. *Methods in Enzymology* 211:158-180.

Panayotatos, N. and R. D. Wells. 1981. Cruciform structures in supercoiled DNA. *Nature* 289:466-470.

Peck, L. J. and J. C. Wang. 1983. Energetics of B-to-Z transition in DNA. *Proc. Natl. Acad. Sci. USA* 80:6206-10.

Pulleyblank, D. E., M. Shure, D. Tang, J. Vinograd, and H. P. Vosberg. 1975. Action of nicking-closing enzyme on supercoiled and nonsupercoiled closed circular DNA: Formation of a Boltzmann distribution of topological isomers. *Proc. Natl. Acad. Sci. USA* 72:4280-4284.

Rich, A., A. Nordheim, and A. H.-J. Wang. 1984. The chemistry and biology of left-handed Z-DNA. *Annu. Rev. Biochem.* 53:791-846.

Rolfsen, D. 1976. Knots and Links. Publish or Perish, Inc., Berkeley, CA.

Rybenkov, V. V., N. R. Cozzarelli, and A. V. Vologodskii. 1993. Probability of DNA knotting and the effective diameter of the DNA double helix. *Proc. Natl. Acad. Sci. USA* 90:5307-5311.

Rybenkov, V. V., C. Ullsperger, A. V. Vologodskii, and N. R. Cozzarelli. 1997a. Simplification of DNA topology below equilibrium values by type II topoisomerases. *Science* 277:690-693.

Rybenkov, V. V., A. V. Vologodskii, and N. R. Cozzarelli. 1997b. The effect of ionic conditions on DNA helical repeat, effective diameter, and free energy of supercoiling. *Nucl. Acids Res.* 25:1412-1418.

Rybenkov, V. V., A. V. Vologodskii, and N. R. Cozzarelli. 1997c. The effect of ionic conditions on the conformations of supercoiled DNA. II. Equilibrium catenation. *J. Mol. Biol.* 267:312-323.

Rybenkov, V. V., A. V. Vologoskii, and N. R. Cozzarelli. 1997d. The effect of ionic conditions on the conformations of supercoiled DNA. I. Sedimentation analysis. *J. Mol. Biol*. 267:299-311.

Shaw, S. Y. and J. C. Wang. 1993. Knotting of a DNA chain during ring closure. *Science* 260:533-536.

Stone, M. D., Z. Bryant, , N. J. Crisona, S. B. Smith, A. V. Vologodskii, C. Bustamante and N. R. Cozzarelli. 2003. Chirality sensing by Escherichia coli topoisomerase IV and the mechanism of type II topoisomerases. *Proc. Natl. Acad. Sci. USA* 100:8654-8659.

Stark, W. M. and M. R. Boocock. 1995. Topological selectivity in site-specific recombination. In Mobile genetic elements. D. J. Sherratt, editor. ARL Press/Oxford Univ. Press, Oxford. 101-129.

Ullsperger, C. J., A. V. Vologodskii, and A. V. Cozzarelli. 1995. Unlinking of DNA by topoisomerases during DNA replication. *Nucl. Acids and Mol. Biol.* 9:115-142.

Vinograd, J., J. Lebowitz, R. Radloff, R. Watson, and P. Laipis. 1965. The twisted circular form of polyoma viral DNA. *Proc. Natl. Acad. Sci. USA* 53:1104-1111.

Vologodskii, A. V. 1992. Topology and physics of circular DNA. CRC Press, Boca Roton.

Vologodskii, A. V., V. V. Anshelevich, A. V. Lukashin, and M. D. Frank-Kamenetskii. 1979. Statistical mechanics of supercoils and the torsional stiffness of the DNA. *Nature* 280:294-298.

Vologodskii, A. V. and N. R. Cozzarelli. 1994. Conformational and thermodynamic properties of supercoiled DNA. *Ann. Rev. Biophys. Biomol. Struct.* 23:609-643.

Vologodskii, A. V. and N. R. Cozzarelli. 1994. Supercoiling, knotting, looping, and other large-scale conformational properties of DNA. *Curr. Opin. Struct. Biol.* 4:372-375.

Vologodskii, A. V. and N. R. Cozzarelli. 1996. Effect of supercoiling on the juxtaposition and relative orientation of DNA sites. *Biophys. J.* 70:2548-2556.

Vologodskii, A. V., N. J. Crisona, B. Laurie, P. Pieranski, V. Katritch, J. Dubochet, and A. Stasiak. 1998. Sedimentation and electrophoretic migration of DNA knots and catenanes. *J. Mol. Biol.* 278:1-3.

Vologodskii, A. V., S. D. Levene, K. V. Klenin, M. D. Frank-Kamenetskii, and N. R. Cozzarelli. 1992. Conformational and thermodynamic properties of supercoiled DNA. *J. Mol. Biol.* 227:1224-1243.

Vologodskii, A. V., A. V. Lukashin, V. V. Anshelevich, and M. D. Frank-Kamenetskii. 1979. Fluctuations in superhelical DNA. *Nucl. Acids Res.* 6:967-982.

Wang, J. C. 1986. Circular DNA. In Cyclic Polymers. J. A. Semlyen, edt. Elsevier Appl. Sci. Publ. Ltd, Essex, England. 225-260.

Wang, J. C. 1996. DNA topoisomerases. *Ann. Rev. Biochem.* 65:635-695.

Wang, J. C., L. J. Peck, and K. Becherer. 1983. DNA supercoiling and its effects on DNA structure and function. *Cold Spring Harbor Symp. Quant. Biol.* 47:85-91.

Wang, J. C. and H. Schwartz. 1967. Noncomplementarity in base sequences between the cohesive ends of coliphages 186 and lambda and the formation of interlocked rings between the two DNA's. *Biopolymers* 5:953-66.

Wasserman, S. A. and N. R. Cozzarelli. 1986. Biochemical topology: applications to DNA recombination and replication. *Science* 232:951-960.

Weil, R. and J. Vinograd. 1963. The cyclic helix and cyclic coil forms of polyoma viral DNA. *Proc. Natl. Acad. Sci. USA* 50:730-739.

White, J. H. 1969. Self-linking and the Gauss integral in higher dimensions. *Am. J. Math.* 91:693-728.