Helical Repeat and Linking Number of Surface-Wrapped DNA

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The geometric properties of duplex DNA are systematically altered when the DNA is wrapped on a protein surface. The linking number of surface-wrapped closed circular DNA is the sum of two integers: the winding number, Φ, a function of the helical repeat; and the surface linking number, SLk, a newly defined geometric constant that accounts for the effects of surface geometry on the twist and writhe of DNA. Changes in the helical repeat, *h*, and in the winding number can be deduced solely from surface geometry and superhelix density, σ. This treatment relates the theoretically important properties twist and writhe to the more experimentally accessible quantities Φ, *h*, SLk, and σ. The analysis is applied to three biologically important cases: interwinding of DNA in a plectonemic superhelix, catenated DNA, and minichromosomes.

THE HELICAL REPEAT IS A FUNDAMENTAL STRUCTURAL property of duplex DNA. This quantity, *h*, is commonly expressed as the number of base pairs per 360° rotation along the helix axis. In order to determine *h*, it is essential to define the local duplex rotation angle. This requires, in turn, specification of a local reference frame (*1, 2*). For example, DNA may be fixed to a plane surface, which serves as the reference frame, and the pattern of endonuclease scissions can then be determined (*3*). This procedure discriminates strand positions lying next to the surface from those away from the surface, and the nuclease digestion periodicity may be used to calculate *h*. The precise value of *h* for a linear or nicked circular DNA depends on base sequence (*4, 5*), on solution properties such as salt concentration (*6, 7*) and composition (*8*), and on the temperature (*6, 7, 9*). For a relaxed closed circular DNA, *h* can be deduced from the shifts in electrophoretic mobility that accompany small known changes in the DNA length (*10*). This method implicitly assumes that the axis of the circular DNA is nearly planar.

The axis of a superhelical DNA does not, however, lie in a plane, and neither does the axis of a DNA wrapped around a protein (*11*). In these cases, the wrapping surface itself may be used as reference frame for definition of *h*. Even in the absence of a physical surface, closed DNA may often be described by considering it to wind on a suitably chosen virtual surface, and *h* may then be defined via this virtual surface. Tightly interwound superhelical DNA can, for example, be considered to wrap on the surface of a spheroid (such as a capped cylinder). Unlike free linear or nicked circular DNA, *h* for surface-wrapped closed DNA is independent of solvent composition and temperature, provided that the surface is subject only to smooth deformations (*12*).

In this article we first show in general how the helical repeat of surface-wrapped DNA depends on surface geometry. We then derive specific relationships that predict the variation of *h* with superhelix density for a closed DNA wrapped around various surfaces. These results follow from the demonstration that the linking number can be written as the sum of two experimentally accessible integers. These are the surface linking number, SLk, and the winding number, Φ. SLk is a newly defined surface geometric constant that accounts for the effects of surface configuration on twist and writhe. Φ, which is also defined in terms of the surface geometry, is inversely proportional to the helical repeat. We apply these results to obtain expressions for the helical repeat of interwound superhelical DNA, of catenated DNA, and of minichromosomal DNA.

Surface geometry and the DNA winding number. We first consider the geometry of a closed DNA whose axis traces out a curve on a surface (*13*), as described in Fig. 1. The best known example of such a structure is the nucleosome core, around which the DNA wraps nearly twice, as a left-handed helix (curve A), on the surface of a cylinder (surface M). We take the curve C to be either of the backbone strands of the DNA. Strand C winds about the DNA axis A and lies alternately above and below the surface M.

We next obtain a precise definition of the winding number, Φ, of C about A. If the DNA is sliced with a plane P that is perpendicular to A, we obtain a cross-sectional piece containing a unique point a of

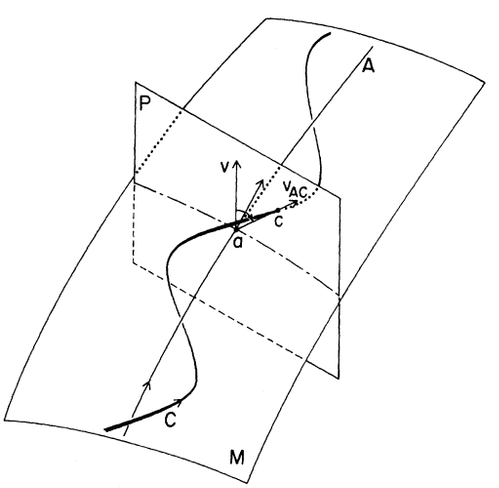

Fig. 1. Correspondence between a strand of duplex DNA and the DNA axis when the axis lies on a surface. The backbone chain (C) passes in a right-handed sense, alternatively above and below the surface (M). An imaginary plane (P), perpendicular to the axis curve (A) at point a, moves along A. The plane intersects backbone curve C at successive points c. The vector **v** is the surface normal to M at a. The vector $\mathbf{v}_{ac}$ is a unit vector along the correspondence line that joins a to c. The winding number of C about A is given by the number of revolutions of $\mathbf{v}_{ac}$ about **v** as P advances along curve A.

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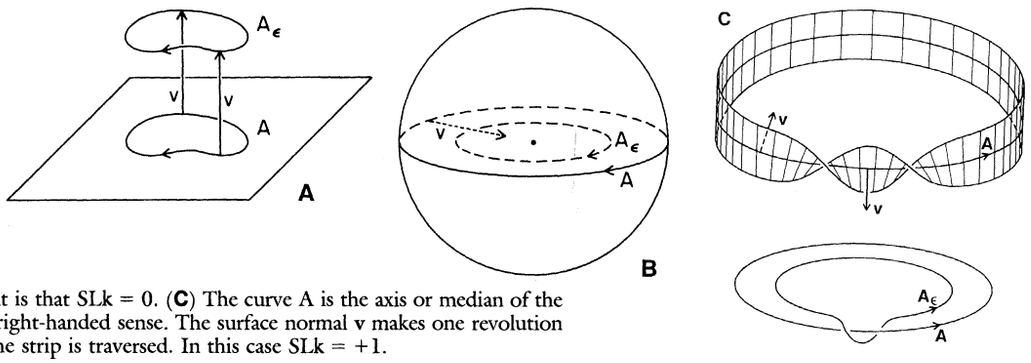

Fig. 2. The value of the surface linking number, SLk, for three common surfaces. (**A**) The axis curve A lies on a plane with surface normal **v** pointing upwards. A_ϵ is the curve obtained from A by a small translational displacement, ϵ, along **v**. SLk = Lk(A_ϵ, A), and in this case SLk = 0. (**B**) The curve A forms the equator of a sphere and the surface normal **v** is chosen to point inwards. The curve A_ϵ lies entirely inside the sphere, and is thus unlinked to A. Again, the result is that SLk = 0. (**C**) The curve A is the axis or median of the strip surface that is twisted once in a right-handed sense. The surface normal **v** makes one revolution about the axis as the twisted part of the strip is traversed. In this case SLk = +1.

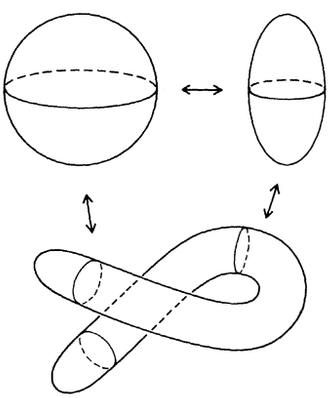

Fig. 3. Examples of spheroidal surfaces. The two distorted surfaces can be obtained from the sphere by smooth deformations. Spheroidal surfaces contain neither handles nor holes. Any two such surfaces are topologically equivalent.

A and a unique point c of C. Since both points a and c lie in plane P, the unit length vector along the lines that connect them, $\mathbf{v}_{ac}$, also lies in P. Proceeding along axis A, $\mathbf{v}_{ac}$ remains perpendicular to A and rotates about it. To measure the extent of this rotation we need a reference vector, directly related to the surface M, that also lies in plane P. The unit length vector **v** along the surface normal provides such a reference, since it is everywhere perpendicular to M (and hence to A). We define Φ formally as the number of times that $\mathbf{v}_{ac}$ rotates about **v** as A is traversed exactly once. Φ is positive if the direction of the rotation is that specified by the right-hand rule and negative if the rotation is opposite. Since for any closed DNA both $\mathbf{v}_{ac}$ and **v** begin and end at the same place, Φ is necessarily an integer. The (average) helical repeat, h, may now be defined as the number of base pairs per DNA, N, divided by the winding number: $h = N/\Phi$. The helical repeat is positive for right-handed DNA and negative for left-handed (for example, Z) DNA. For a relaxed DNA, the winding number is denoted Φ_0 and the helical repeat is $h_0 = N/\Phi_0$.

The winding number, Φ, is directly related to the linking number, Lk. This is most easily seen with a perfectly relaxed closed DNA, which has no net writhe because its axis lies in a plane, on the average. In this case the normal vector **v** always points in the same direction and thus defines a constant reference vector by which to measure Φ. For this reason the number of times that C revolves about A is exactly equal to the linking number; that is, Φ = Lk (a rigorous proof of this is presented below). When DNA wraps on a nonplanar surface, the direction of the reference vector **v** is not generally constant, nor is the writhe of the DNA generally zero. In this case the linking number is not necessarily equal to Φ but is also a function of the change in the reference vector. We next derive the general expression of the linking number of DNA on an arbitrary surface.

The linking number of surface wrapped DNA. We begin with the conservation condition for closed DNA (*14*) that expresses the linking number in terms of strand-axis twist, Tw(C,A), and the axis writhe, Wr.

$$\text{Lk} \equiv \text{Lk(C,A)} = \text{Tw(C,A)} + \text{Wr} \quad (1)$$

We have shown previously (*15*) that Tw(C,A) divides into two parts: the winding number, Φ; and the surface twist, STw, a term that measures how the reference vector changes.

$$\text{Tw(C,A)} = \text{STw} + \Phi \quad (2)$$

Incorporating Eq. 2 into Eq. 1, we obtain

$$\text{Lk} = \text{STw} + \Phi + \text{Wr} \quad (3)$$

The meaning of STw may be stated in terms of a displacement curve, A_ϵ. This is the curve obtained from A by moving it a distance $\epsilon \neq 0$ along the surface normal vector **v**. The choice of ϵ is arbitrary provided that A_ϵ never intersects A during the displacement. Examples of displacement curves are shown in Fig. 2. We showed earlier (*15*) that these considerations lead to the equality

$$\text{STw} = \text{Tw}(A_\epsilon, A)$$

Since the direction of the displacement is everywhere perpendicular to the surface, the twist of A_ϵ about A reflects the contribution of the surface geometry to the total twist. As is true for all closed curves, the linking number of A_ϵ with A is the sum of the twist of A_ϵ about A and the writhe of A (*14*).

$$\text{Lk}(A_\epsilon, A) = \text{Tw}(A_\epsilon, A) + \text{Wr} \quad (4a)$$

Because Lk(A_ϵ, A) is determined by the surface geometry, we term this quantity the surface linking number of the curve A and denote it SLk.

$$\text{SLk} = \text{Lk}(A_\epsilon, A)$$

Rewritten in terms of the surface-related quantities, Eq. 4a becomes

$$\text{SLk} = \text{STw} + \text{Wr} \quad (4b)$$

Examples that illustrate the concept and methods of calculation of SLk are presented in Fig. 2.

The linking number of the surface-wrapped DNA may now be expressed as the sum of quantities that can be independently determined. Combining Eqs. 3 and 4b leads to the result

$$\text{Lk} = \text{SLk} + \Phi \quad (5)$$

This important formula states that the linking number of closed DNA wrapped on a surface can be written as the sum of two integers: SLk and Φ. SLk is the linking number of A_ϵ and A and may be calculated from the surface geometry. Φ is the winding number of DNA about the surface normal and can be measured with chemical and enzymatic probes. Φ can also be interpreted as ± one-

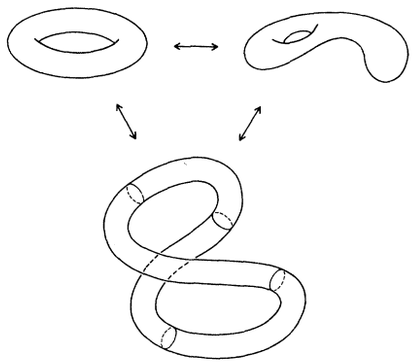

Fig. 4. Examples of toroidal surfaces. The lower two surfaces can be obtained from the undistorted torus by smooth deformations. A torus can be thought of as a sphere with one handle (see legend to Fig. 3).

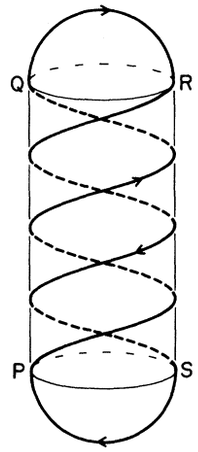

Fig. 5. Representation of an interwound helix on a cylinder having hemispherical caps. This is a spheroid, of the type illustrated in Fig. 3. The DNA axis is continuous, winding in a right-handed fashion along the cylindrical section, from P to Q and from R to S. The connections between Q and R and between P and S lie along the spherical caps.

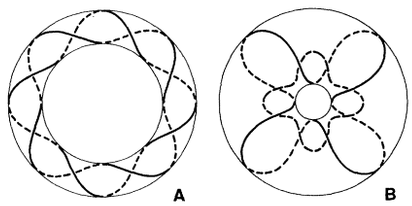

Fig. 6. Representation of catenated DNA and the associated torus surfaces. The toroidal surface depicted here is regular (undistorted), but topologically equivalent wrappings may be constructed about distorted toroidal surfaces, such as those shown in Fig. 5. (**A**) The catenated rings are of equal length, and each winds four times around the torus in a right-handed sense. Both rings lie on the surface of the same torus. (**B**) One of the two rings is depicted three times longer than the other. The associated torus surfaces are shown for the case in which the helical winding of each submolecule is uniform. The larger DNA lies on the surface of the torus and the smaller, which is also shown toroidally wrapped, lies completely inside.

half the number of times the backbone curve C intersects the surface. Relaxed closed DNA is nearly planar, hence in this case SLk = 0 and the linking number is $Lk_0 = \Phi_0$.

Principal applications of Eq. 5 are to DNA wrapped on mathematically closed surfaces, such as spheroids and toroids. Thus, many enzymes are globular proteins, well-represented by spheroids; and the collection of core nucleosomes and linker regions in minichromosomes can be regarded as generating a toroidal structure. In such cases, the integer numbers SLk and Φ are unchanged under smooth deformations. Examples of such smooth deformations are the distortion of a sphere into an ellipsoid and the distortion of a circular torus into an elongated torus, as shown in Figs. 3 and 4. In particular, SLk remains constant if no breaks, tears, or other discontinuities are introduced. Since Lk is constant for an unbroken closed circular DNA, it follows from Eq. 5 that Φ is also constant. This constancy of the winding number is important in biochemical terms, particularly when the DNA is wrapped on the surface of an enzyme (*16*).

The relation between the helical repeat and the superhelix density. We next show how to evaluate the helical repeat of surface wrapped DNA as a function of the superhelix density for various values of the surface linking number. The linking difference of a closed circular DNA is given by $\Delta Lk = Lk - Lk_0$, where $Lk_0 = \Phi_0 = N/h_0$. Combining this expression with Eq. 5,

$$\Delta Lk = SLk + \Phi - Lk_0 \tag{6}$$

The superhelix density (or specific linking number) is commonly used to characterize closed DNA. This quantity is defined as $\sigma = \Delta Lk/Lk_0$. Additional insight may be gained by analyzing σ in terms of its winding and surface linking components. To do this, Eq. 6 is divided by Lk_0 to obtain

$$\sigma = \frac{SLk}{Lk_0} + \frac{(\Phi - Lk_0)}{Lk_0}$$

Now Lk_0 is identical to Φ_0 and, putting $\Delta\Phi = \Phi - \Phi_0$, the superhelix density may be written as

$$\sigma = (SLk/Lk_0) + (\Delta\Phi/\Phi_0)$$

Thus σ consists of two terms: the first, SLk/Lk_0, arises from the contribution of the surface linking; and the second, $\Delta\Phi/\Phi_0$, arises from the change in winding. Alternatively, since $\Phi = N/h$ and $Lk_0 = N/h_0$, we obtain a relation between the superhelix density and the other quantities defined above:

$$\sigma = \frac{SLk}{Lk_0} + \frac{h_0}{h} - 1$$

The above equation may be solved for the helical repeat to give

$$h = \frac{h_0}{\sigma - \dfrac{SLk}{Lk_0} + 1} \tag{7}$$

This very general relation makes possible the calculation of the helical repeat of any closed DNA on any surface M. It also follows from the discussion immediately above that h is unchanged if the structure is smoothly deformed.

Applications of surface winding analysis. We next describe specific applications of surface winding analysis to DNA wrapped on spheroidal and on toroidal surfaces. A spheroidal surface is either an undistorted sphere or a sphere that has been deformed in a smooth manner. In particular, spheroidal surfaces contain neither holes nor handles (*17*) (Fig. 3). We choose A as a closed curve representing the DNA axis wrapped on such a surface and **v** as the inward pointing normal. In this case A_ϵ lies entirely inside the surface and thus may be deformed even to a single point without crossing A. Since SLk is unchanged under such a deformation, it follows that SLk = 0. Therefore, from Eqs. 5 and 7,

$$Lk = \Phi$$

$$h = \frac{h_0}{\sigma + 1}$$

Thus the linking number of DNA on a spheroidal surface is equal to the winding number, and the helical repeat is a simple function of the relaxed helical repeat and the superhelix density.

These results are next applied to plectonemically interwound DNA, the form adopted by superhelical DNA in solution (*18*). This case is readily generalized to DNA wrapped on any other spheroidal surface, however, because of the invariance of Φ under smooth

deformation. In Fig. 5 we illustrate a model of DNA winding about a particular spheroidal surface, here a cylinder with spherical caps. Beginning at the point P, the DNA winds n times in a right-handed helix along a cylinder of radius r with pitch $2\pi p$, ending at the point Q. The DNA next crosses the top spherical cap from Q to R, winds helically n times down the cylinder to point S, and finally completes its path by crossing the lower spherical cap from S to P. Since the surface is spheroidal, it satisfies the equations immediately above. A direct consequence of this result is that for interwound DNA, $h > h_0$ if $\sigma < 0$ and $h < h_0$ if $\sigma > 0$. As a particular example, σ for naturally occurring plasmid DNA is typically -0.06 (*19*). Taking h_0 to be 10.6 ± 0.1 base pairs per turn (*20*), it is predicted that h should increase to 11.3 ± 0.1 for a DNA of native superhelix density. Since SLk = 0 for interwound DNA, it further follows from Eq. 4b that Wr = −STw. This surprising result shows that for interwound DNA of constant linking number, the writhe may change without changing either the winding number or the helical repeat. This is because any change in writhe is entirely offset by an equal and opposite change in the surface twist, leaving Φ and h unaltered (compare with Eq. 3).

The second special type of surface is the torus-like surface. This class includes the classical round torus as well as its smooth deformations (Fig. 4). We assume that the axial path A of the DNA winds n times around the handle as it traverses the length of the torus. If we let **v** be the inward pointing surface normal, and ϵ be the inner radius of the torus, then A_ϵ is the central axis of the torus. Then SLk, being Lk(A_ϵ, A), is +n if the axial winding is right-handed, and SLk = −n if the axial winding is left-handed. (An example of right-handed toroidal winding is given by either ring shown in Fig. 6a, where n = 4.) Combining this result with Eqs. 5 and 7, for the right-handed case

$$\mathrm{Lk} = +n + \Phi$$

$$h = \frac{h_0}{\sigma - \dfrac{n}{\mathrm{Lk}_0} + 1}$$

and for the left-handed case

$$\mathrm{Lk} = -n + \Phi$$

$$h = \frac{h_0}{\sigma + \dfrac{n}{\mathrm{Lk}_0} + 1}$$

Examples of DNA that can be considered to lie on torus-like surfaces are the catenanes generated by phage λ int-mediated recombination (*21*). Under the assumption that the DNA rings of the catenane lie on a virtual torus, then a closed helical trajectory on the torus will be traversed as one proceeds along the axis of either DNA molecule. In this case the trajectory is known to be right-handed. Examples of such catenanes on toroidal surfaces are shown in Fig. 6. In Fig. 6a both submolecules are of equal length and the same virtual torus is generated by either component. In Fig. 6b the rings are of unequal length. Although both rings are of interest, we focus our discussion here on the larger ring. Thus we use the virtual surface generated by the larger submolecule. The smaller ring lies entirely inside this toroidal surface.

We next apply Eq. 7 to obtain the effect of catenation on the DNA helical repeat. We showed above that SLk for DNA on a torus is the linking number of the DNA axis with the central axis of the torus. If the component rings are of equal length, the axis of either one can be deformed into the central axis without intersecting the axis of the other. If the submolecules are of unequal length, the axis of the smaller of the two can always be deformed into the central axis without intersecting the axis of the larger (because the smaller ring lies entirely inside the toroidal surface). In either case, SLk is therefore equal to the linking number of the two DNA axes, the catenation number. The catenation number is easily measured by electrophoresis or by electron microscopy (*21*).

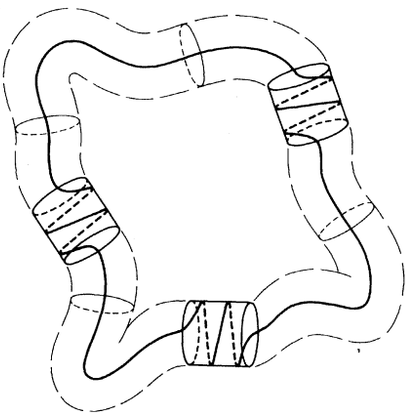

Fig. 7. Cartoon of a minichromosome. Three cylinders representing histone octamers are wound by DNA so as to form three nucleosomes. The nucleosomes are connected by linker DNA segments. Successive nucleosomes are connected by deformable cylinders; the deformations are determined by the coiling of the linker DNA.

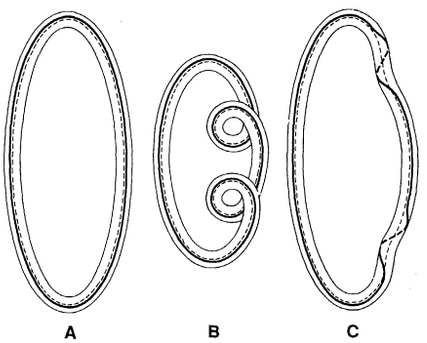

Fig. 8. Winding of the DNA axis on a toroidal surface. (**A**) A circular torus is shown, with the DNA axis lying always on the surface (solid line) and never winding about the torus central axis (dashed line). (**B**) The same torus is shown after the introduction of two left-handed coils by cutting, winding, and resealing. (**C**) The torus shown in (B) has been deformed so that the central axis is nearly planar. The DNA axis now winds twice in a left-handed sense about the central axis of the torus. The geometric and topological quantities are discussed in the text.

The variation of SLk with σ for both an equal length (e) and an unequal length (u) catenane has been determined (*22*). For the particular equal length case examined, both rings were 3.5 kb; and for the unequal length case, the larger rings was 2.9 kb and the smaller ring was 0.9 kb. It was found that $\sigma_e = (0.32\ \mathrm{SLk} - 0.30)\mathrm{Lk}_{0,e}$ and $\sigma_u = (0.76\ \mathrm{SLk} - 0.91)/\mathrm{Lk}_{0,u}$. Combining these results with Eq. 7, the expected values of h are

$$h_e = \frac{h_0}{1 - (0.68\ \mathrm{SLk} + 0.30)/\mathrm{Lk}_{0,e}}$$

$$h_u = \frac{h_0}{1 - (0.24\ \mathrm{SLk} + 0.91)/\mathrm{Lk}_{0,u}}$$

In both cases the helical repeat increases with SLk at constant Lk_0, as expected for right-handed intertwining. The effect of intertwining on the helical repeat of the larger ring in the unequal length case is less than when the rings are equal, as indicated by the smaller coefficient of SLk. This may reflect the fact that the larger ring is less constrained in this case and hence more nearly resembles a free DNA molecule.

Finally, we address the question of left-handed coiling in the nucleosome, such as occurs in the SV40 virion minichromosome. Each core nucleosome may be described as a cylinder, the histone octamer, around which the DNA wraps approximately 1.8 times

(*23*). The DNA linker regions lie between the individual nucleosomes. We construct a closed toroidal surface connecting these nucleosome cylinders as shown in cartoon form in Fig. 7. Each pair of successive cylinders is connected by a deformed cylindrical section or piece, all of the same radius, on which the linker DNA is constrained to lie. The specification of each piece is arbitrary, so long as the linker DNA lies on it, and the piece takes into account any coiling of the linker. One can, in particular, think of the linker as forming a generating curve for the cylindrical region. It is especially important that the linker DNA not wind about the cylindrical piece. These conditions insure that all contributions to SLk due to winding about the torus handle come only from intranucleosome winding. All other contributions to SLk must therefore come from the coiling of that portion of the torus associated with the linkers.

How SLk may alter when the torus is itself coiled is illustrated by the example in Fig. 8. A nearly circular torus is shown in Fig. 8A, where the DNA axis A is taken to be the curve lying on top of the torus. In this case, A is clearly not linked to the central axis of the torus, so that SLk = 0. If two left-handed coils are introduced by cutting, coiling and then resealing (a non-smooth deformation), the result is the coiled torus in Fig. 8B. In this case the writhing of the DNA axis A is approximately −2. Here STw ≈ 0, since A clearly does not twist about the central axis of the torus (always lying above it at corresponding points). Therefore, by Eq. 4B, the integer SLk = −2. An additional check that SLk = −2 is provided if we smoothly deform the coiled torus (Fig. 8B) into the nearly circular one (Fig. 8C). In this case, A is deformed into a curve that rotates twice around the central axis of the torus in a left-handed sense. Since the deformation is smooth, SLk remains −2.

If a minichromosome is torsionally relaxed in the linker DNA regions, all contributions to SLk must arise from the wrapping of DNA about the histone octamers. Thus, for a minichromosome of this type in which the DNA axis is wrapped left-handed about m histone octamers, SLk = $-1.8m$ and the (average) helical repeat for the entire minichromosome is given by

$$h = \frac{h_0}{\sigma + \frac{1.8m}{\mathrm{Lk}_0} + 1}$$

For the virion SV40 minichromosome $N = 5243$, $m = 26 \pm 2$ (*24*), and $\Delta\mathrm{Lk} = -26 \pm 0.5$ (*25*). We take h_0 to be 10.6 ± 0.1 for a random sequence DNA (*20*), and calculate the values $\mathrm{Lk}_0 = 495$, $\sigma = -0.053$, and $1.8m = 46.8$. Employing these data in the above equation, the calculated result is $h = 10.17$. It is well established that the helical repeat of DNA is reduced when the double helix is wound about a histone octamer to form a nucleosome (*26*). On the basis of deoxyribonuclease I digestion studies, the experimental value of h is 10.17 bp per turn for a random sequence DNA wound on a single histone octamer (*1*) and nearer 10.0 bp per turn when averaged over a reconstituted oligonucleosome structure containing five octamers (*2*). Our calculated value is based on a single nucleosome model and gives very good agreement with this experimental result. The closeness of the agreement might be fortuitous, however, in light of the various uncertainties in the experimental measurements. Thus, introducing the uncertainties in ΔLk and m stated above gives a calculated range in h of 10.10 to 10.26.

The model chosen here is the simple one in which no net change in SLk occurs in the linker regions. Some contribution of the linker DNA to SLk is, however, consistent with the available data. For example, additional left-handed linker DNA wrapping of 0.2 turn per histone octamer, such as might occur with the histone H1–containing intracellular SV40 minichromosome (*27*), reduces the calculated value of h by 0.1, to 10.07, a value still within the experimental range. We note in particular that the predicted values of h arise solely from considerations of the surface geometry and do not involve changes in the detailed nature of the physical or chemical interactions between the DNA and the protein.

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10. This is the band shift method [J. C. Wang, *ibid.* **76**, 200 (1979)] where DNA topoisomerase I is used to produce families of topoisomers nearly centered around the fully relaxed closed circular species.
11. A protein surface may be defined by moving a water-sized spherical probe around the van der Waals surface of each external atom in the protein. The solvent accessible surface is then taken to be the continuous sheet defined by the locus of the center of the probe. The consequences of choosing this definition have been described [F. M. Richards, *Annu. Rev. Biophys. Bioeng.* **6**, 151 (1977)]. This complex surface may be represented approximately by the surface of revolution most nearly having the same axial symmetry and minimizing the least-squares deviation from the envelope of external residues.
12. A smooth deformation in the present context is one that can be accomplished without removing any part of the DNA from the closed protein surface. In the absence of denaturation, biologically significant deformations of proteins are generally smooth.
13. We restrict the analysis to surfaces that are well-behaved mathematically; that is, the tangent plane exists at all points and varies smoothly, and the surface normal is well-defined and differentiable.
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