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Supercoiled DNA: Structure

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Advanced Article

(Advanced articles are aimed at advanced undergraduates, graduate students, postgraduates, and researchers reading outside their field of expertise.)

Abstract

Supercoiling is introduced into DNA molecules when the double helix is twisted around its own axis in three-dimensional space. Experimental techniques that are sensitive to molecular shape can be used to analyse the topological states of DNA, but the approaches used most successfully are high-speed centrifugation, high-resolution microscopy and gel electrophoresis. Generally, DNA molecules are negatively supercoiled inside cells, although the level of supercoiling is not equal throughout the genome and many supercoils may be constrained by bound proteins. Supercoiling increases the free energy of DNA and influences DNA metabolism by promoting or hindering specific enzymatic processes. DNA topoisomerases are the main enzymes that regulate DNA topology and several different types of enzymes are present in all cells.

Keywords: DNA; linking number; supercoiling; topology; twist; writhe

Key Concepts

- Double stranded DNA helices can wind in three-dimensional space to form further helices of higher order, forming *supercoiled* DNA.
- Since the early 1960s the importance of DNA supercoiling to cellular processes has been apparent, with its most obvious consequence being that it aids compaction of large DNA molecules into the relatively small volume of cells.
- The extent of supercoiling in a DNA molecule is influenced by environmental conditions, such as ionic strength and temperature; since supercoiling of DNA influences the biological pathways in which it is involved, the level of DNA supercoiling inside cells is tightly regulated.
- Supercoiling provides a significant amount of free energy to DNA molecules and, inside cells, this can be used to drive structural transitions and other metabolic processes that would normally be thermodynamically unfavourable, such as opening of the DNA helix during replication and transcription.

- Mathematical and modelling studies have provided insight for quantitative analyses of DNA supercoiling, leading to definitions for *twist*, which describes how the individual strands of DNA coil around its axis, and *writhe*, which describes how the helix axis coils in three-dimensional space.

- DNA inside cells contains supercoils of two types: interwound, which is when circular DNA winds around its own axis; toroidal supercoiling occurs when the DNA helix forms a series of spirals around an imaginary ring.

- Any technique that is sensitive to molecular shape will be useful for experimental analysis of supercoiled DNA, but the large size of the molecules mean few techniques have been used successfully; those that have been widely used include high-speed centrifugation, high-resolution microscopy (e.g. electron microscopy and scanning-force microscopy) and agarose gel electrophoresis.

- A wide variety of proteins that bind to DNA alter the local geometry of its helix and influence DNA topology; an important characterised example of this effect is the winding of DNA around the eukaryotic histone octamer to form the nucleosome.

- A fundamental feature of closed domains of DNA, such as a circular molecule, is that the two strands of DNA are topologically linked and strand separation can be achieved only by breakage of one of the strands; the main enzymes that regulate DNA topology are DNA topoisomerases and they may act to remove or introduce negative supercoils or they may remove both positive and negative supercoils.

- Cellular processes that move macromolecular assemblies along DNA may generate localized DNA supercoiling since, as the large protein complex moves along the DNA, its rotation around the DNA may be inhibited.

Introduction

Normally, DNA occurs as a helical, double-stranded molecule in which the two strands pair up in antiparallel fashion; this is the classical B-type helical structure first solved in 1953 by Watson and Crick (Watson and Crick, 1953) using a range of experimental data obtained by many other scientists (Chargaff *et al*, 1950; Franklin and Gosling, 1953; Wilkins *et al*, 1953). The DNA helix is usually visualized in a linear form, but, frequently, the helix axis is curved and numerous ‘unusual DNA structures’ form under specific sequence and environmental conditions. See also: DNA Structure, DOI: 10.1002/9780470015902.a0006002.pub2; DNA Structure: A-, B- and Z-DNA Helix Families, DOI: 10.1038/npg.els.0003122; DNA Structure: Sequence Effects, DOI: 10.1002/9780470015902.a0002976.pub2; Non-B DNA Structure and Mutations Causing Human Genetic Disease, DOI: 10.1002/9780470015902.a0022657; Crick, Francis Harry Compton, DOI: 10.1038/npg.els.0002392; Watson, James Dewey, DOI:

10.1038/npg.els.0002445; Franklin, Rosalind Elsie, DOI: 10.1038/npg.els.0003559; Wilkins, Maurice Hugh Frederick, DOI: 10.1038/npg.els.0002954.

Notably, since the B-form of DNA is a configuration of minimum energy, any bending or twisting of the DNA molecule will increase its free energy. In addition to varying secondary structures, the DNA helix can wind in three-dimensional space to form further helices of higher order. DNA in this conformation is termed supercoiled and changes to this tertiary structure of a DNA molecule have dramatic consequences for the free energy and biology of the molecule. See also: Nucleic Acids: General Properties, DOI: 10.1038/npg.els.0001335; DNA Topology: Fundamentals, DOI: 10.1038/npg.els.0001038; DNA Topology: Supercoiling and Linking, DOI: 10.1038/npg.els.0003904.

In a linear double-stranded DNA molecule, the two strands of the helix are free to rotate around each other and, indeed, may unwind completely to give two separate strands. Complete separation is unlikely to happen inside cells because of the large number of base pairs contained within genomic DNA. Complete separation of the DNA helix may also be prevented because the molecule may exist within closed domains, for example by covalent joining of the DNA strands to give a circular molecule. The binding of proteins may also separate the DNA molecule into different domains, particularly if a loop of DNA is formed due to the same protein complex binding at two distinct sites on the DNA. A fundamental feature of closed domains in DNA is that the strands are topologically linked and strand separation can be achieved only by breakage of one of the strands. Unlike the open-ended DNA molecule, within closed domains of DNA the three-dimensional conformation of any base pair cannot be changed without influencing the structure of the remainder of the domain.

Studies of topological isoforms (topoisomers) of DNA began during the early 1960s with the demonstration that polyomavirus DNA was consistently isolated in linear and closed-circular forms. When Vinograd's laboratory showed that DNA extracted from cells was negatively supercoiled, the importance of DNA supercoiling to cellular processes became apparent (Lebowitz, 1990). Supercoiling of DNA has dramatic consequences for the biological pathways in which it is involved and, thus, the level of DNA supercoiling inside cells is tightly regulated.

Definition of Supercoiling

Quantitative measurements and analyses of DNA supercoiling have been defined. Mathematical studies have provided enormous insight for these definitions and are

discussed in more detail in other reviews and monographs (Bates and Maxwell, 2005; Bauer *et al*, 1980; Benham and Mielke, 2005; Schlick, 1995). The basic ideas are described below (Table 1) in relation to covalently closed-circular DNA (cccDNA) molecules, but similar principles apply to any closed domain of DNA. See also: DNA Topology: Fundamentals, DOI: 10.1038/npg.els.0001038; DNA Topology: Supercoiling and Linking, DOI: 10.1038/npg.els.0003904.

<Table 1 near here>

Linear double-stranded DNA molecules can be closed into a circle by the formation of 5′–3′ phosphodiester bonds to seal each strand. Due to the helical nature of the DNA backbone, after circularization the two strands of the helix cannot be separated without breaking one of them; the backbone strands are linked topologically. The number of links between the strands corresponds to the number of double-helical turns (twists) in the original DNA molecule (Figure 1). Upon circularization, this number must be an integer and is known as the linking number of the cccDNA molecule, abbreviated as *Lk*. (Note that earlier literature refers to the topological winding number of DNA, α, which is identical to *Lk*. Previously this term has also been abbreviated to *L*.) *Lk* is a topological property of cccDNA that does not depend on its particular conformation.

<Figure 1 near here>

Linking number is a fundamental property of any two closed curves in three-dimensional space and is equal to the number of times that one strand intersects the plane of the other. An intersection may act to increase or decrease the number of links between the two strands. By convention, positive values are given to links arising from forming a closed circle with a completely right-handed double helix (such as B-DNA). Consequently, links with a negative value arise from the formation of a cccDNA molecule from a left-handed DNA helix (such as Z-DNA). The overall *Lk* of a DNA molecule is equal to the sum of the sign-dependent intersections.

A given length of DNA has an inherent number of double-helical turns, which is equivalent to the length of the DNA (defined as *N* base pairs) divided by the number of base pairs per turn of the helix (defined as *h*). Values of *h* depend upon environmental conditions and sequence, but an average value is specified from standard conditions and is usually taken to be 10.5 bp per turn for B-DNA. The linking number corresponding to an unconstrained state is termed Lk^0 and, for any DNA:

- $$Lk^0 = N/h \qquad (1)$$

Since N/h need not necessarily be an integer, Lk^0 is not a true linking number (it is sometimes called the 'hypothetical linking number'). For DNA with N/h that is not an integer, some deformation of the molecule's conformation will be required to line up the strands to allow their closure into a circle, which leads to torsional stress within the cccDNA. In this situation, the most unconstrained DNA circle is referred to as the relaxed topoisomer, defined Lk_m. Note that, if N/h is an integer, when the linear DNA is bent to form a simple, planar circle, the strands will line up precisely and $Lk = Lk^0 = Lk_m$.

It is also possible to add or remove turns to the DNA helix before it is closed into a circle, leading to the molecule having Lk that deviates from Lk_m (Figure 1). Since the average conformation of DNA (B-type helix) is defined to have positive Lk, twisting up of the helix before closure leads to an increase in linking number above Lk_m and is defined as positive supercoiling. Analogously, unwinding of the helix before closure is defined as negative supercoiling. DNA molecules with positive and negative supercoiling may also be referred to as helices that are over- or underwound, respectively. Note that an underwound helix has an increased value of h.

It is clear that Lk is related to the number of turns of the helix, but these two parameters are not equivalent. This can be shown using mathematical analysis, which defines supercoiling in the form of topological and geometric parameters. Lk is a topological property and its value can only be applied to the complete DNA molecule. The twist (Tw) of DNA is a geometric parameter and its values have importance for local regions of the molecule; indeed, the value of Tw of the whole molecule is equal to the sum of individual sections of the molecule. There is a geometrical significance to any difference between Lk and Tw and this is named the writhe (Wr) of the molecule.

In terms of cccDNA, Tw and Wr are complementary geometric parameters, and each may be defined and described (Table 1): Tw describes how the individual strands of DNA coil around the axis of the DNA helix and Wr describes how the helix axis coils in space. Both are complex geometric functions whose values need not be an integer. The important finding in relation to studies of DNA supercoiling is that:

- $Lk = Tw + Wr$ (2)

The main consequence of this equation is that because Lk is invariant for a given cccDNA, any change in Tw of the molecule must be accompanied by an equal and opposite change in Wr, and vice versa.

Supercoiling of DNA can occur in two forms that produce different overall shapes for the molecule. Circular DNA that winds around itself, as shown in Figure 1, is called interwound (also referred to as plectonemic supercoils) and purified cccDNA in solution usually has this type of supercoiling (Benham and Mielke, 2005). Note that the sense of interwinding is right-handed in the case of negatively supercoiled DNA. Supercoiling can also be achieved if the DNA helix forms a series of spirals around an imaginary ring, taking a shape similar to a telephone flex. This kind of supercoiling is known as toroidal (Hud and Vilfan, 2005; Schlick, 1995). It corresponds most closely to the term 'superhelix' since a left-handed untwisting of the DNA helix (i.e. negative supercoiling) is manifested as a left-handed helix of higher order wound around the torus. Toroidal supercoiling is formed when DNA is wrapped around proteins, as in nucleosomes (Luger and Richmond, 1998; Richmond and Davey, 2003). In reality, DNA inside cells contains supercoils of both interwound and toroidal geometries. See also: Cell Biophysics, DOI: 10.1038/npg.els.0001271; DNA Topology: Fundamentals, DOI: 10.1038/npg.els.0001038; DNA Topology: Supercoiling and Linking, DOI: 10.1038/npg.els.0003904.

Specific Linking Difference, Superhelical Density

Changes in *Lk* of a DNA produce alterations to the level of supercoiling of the molecule. The change in linking number from Lk_m is a measurement of the extent of supercoiling of the molecule and is termed the linking difference of the DNA, or Δ*Lk*. The addition of turns to the DNA helix (positive supercoiling) leads to an increase in *Lk* over Lk_m, giving a positive Δ*Lk*. Conversely, the removal of turns from the DNA helix (negative supercoiling) gives a value of *Lk* lower than Lk_m, giving a negative Δ*Lk*.

If Lk_m is not equal to Lk^0, the 'relaxed' topoisomer will contain a small amount of torsional strain, which should really be counted towards the total supercoiling of the DNA. Thus, an exact definition of Δ*Lk* is:

- $$\Delta Lk = Lk - Lk^0 \qquad (3)$$

Furthermore, since changes in *Lk* produce corresponding changes in *Tw* and/or *Wr*,

- $$\Delta Lk = \Delta Wr + \Delta Tw \qquad (4)$$

Specific values of Δ*Lk* produce more torsional stress in small DNA molecules than in large ones because they comprise a larger proportion of the overall *Lk*. To allow comparison of the

degree of supercoiling in molecules of different sizes, it is useful to normalize measurements of supercoiling to give the specific linking difference (σ); frequently, this is referred to as superhelical density (Muskhelishvili and Travers, 2003). Since cccDNA molecules of Lk^0 do not contain supercoils, this serves as a good reference point for such normalization:

- $$\sigma = \frac{Lk - Lk^0}{Lk^0} = \frac{\Delta Lk}{Lk^0} \quad (5)$$

The specific linking difference allows meaningful comparison between DNA molecules. For example, natural cccDNA molecules, such as bacterial plasmids, vary widely in size, but, when isolated *in vitro*, the majority have values for σ of −0.05 to −0.06.

Energetics of Supercoiled DNA

Like all molecules, DNA will assume a configuration of minimum energy, and this is usually a helix of the B-form. Upon bending or twisting of the molecule, its energy is increased. For a cccDNA with a surplus or deficit in *Lk*, conformational modifications introduce specific changes to the free energy of the molecule. For example, to accommodate the same length of DNA in fewer helical turns, the double helix must be untwisted, leading to a substantial increase in the deformation energy of the molecule. By taking an appropriate writhed configuration, the cccDNA minimizes the amount by which it departs from the B configuration and reduces its deformation energy. On the other hand, writhing always introduces some curvature, and so it increases the bending contribution to the energy of the molecule. Since *Wr* and *Tw* are interconvertible, it is apparent that the underwound DNA molecule will assume a configuration that optimizes twist while introducing the smallest possible amount of bending (Benham and Mielke, 2005; Vologodskii and Cozzarelli, 1994a).

Experimental studies during the 1970s established that the free energy of a supercoiled DNA sample (ΔG_{sc}) has a quadratic dependence on ΔLk:

- $$\Delta G_{sc} = K.\Delta Lk^2 \quad (6)$$

where *K* is a DNA length-dependent constant. Thus, samples of cccDNA exist in a normal (Gaussian) distribution of topoisomers (i.e. molecules have a continuous, symmetrical distribution of *Lk* around the most intensely populated topoisomer – see Figure 2). Theoretical simulations suggest it is likely that ΔG_{sc} varies with ionic conditions and, in fact, it may not be a quadratic function of ΔLk under all conditions (Schlick, 1995; Vologodskii and Cozzarelli, 1994a). The influence of environmental conditions on DNA supercoiling is due, at

least in part, to the fact that ionic strength and temperature alter *Tw* of double-helical DNA. Effects of ionic environment on the three-dimensional structure of DNA are to be expected because DNA is a polyelectrolyte with a net negative charge at every nucleotide residue.

<Figure 2 near here>

The free energy of supercoiling can be normalized to circle size in the same manner as described for Δ*Lk*. Hence:

- $\Delta G_{sc}/N = NK(\Delta Lk/N)^2$ (7)

For DNA circles of 3–10 kb, it was shown that *NK* is independent of DNA circle size, and that *NK* ≈ 1100*RT*, where *R* is the gas constant and *T* is the temperature. Since Δ*Lk*/*N* is proportional to σ, the free energy of supercoiling per base pair is proportional to σ^2, and is independent of circle size. Studies with small DNA molecules showed that *NK* increases gradually with decreasing DNA size, with a value of about 4000*RT* for a 300-bp circle. The interpretation of this finding was that, for small molecules, writhing of the DNA becomes increasingly unfavourable relative to twisting. Due to these energy considerations, it is thought that a higher proportion of supercoiling is partitioned into twisting rather than writhing for small DNA circles.

Intercalation

Intercalators contain a planar, usually polycyclic, aromatic ring structure, which allows them to insert between two base pairs of a double-stranded DNA helix. The best-known examples of intercalating molecules are chloroquine and ethidium bromide (EtdBr) (Wu *et al*, 1988). While these molecules bind similarly to most DNA sequences, other intercalators prefer to bind to specific sequences. For example, actinomycin D binds most avidly between neighbouring G–C base pairs. A consequence of intercalators binding to DNA is that they will increase the distance between the adjacent base pairs. This causes a local unwinding of the DNA helix, resulting in an overall increase in the helical repeat and a decrease in *Tw*. For a closed domain of DNA, this will produce a corresponding increase in *Wr*.

Intercalators have been of enormous value in the experimental measurements of plasmid supercoiling (Liu and Wang, 1975; Wang, 1974). EtdBr exhibits a large increase in fluorescence upon binding to DNA, making it particularly useful to molecular biologists through its use as a stain for DNA. Furthermore, as outlined in Figure 2, this intercalator has been widely used during the preparation of topoisomers at different levels of supercoiling

and in agarose gel electrophoresis analysis of *Lk* (Bowater *et al*, 1992). See also: Staining, Viewing and Photography of Gels and Estimation of Fragment Sizes, DOI: 10.1038/npg.els.0003777.

Intercalation of one molecule of EtdBr to DNA causes a local unwinding of adjacent base pairs of 26°. Some classes of intercalator affect the helix in the opposite manner, leading to a localized increase in the twist of the helix. The best-characterized example of such a molecule is netropsin, which binds to the minor groove of AT-rich DNA and increases winding of the helix by approximately 9° for each molecule bound (Schlick and Olson, 1992).

Assays

In principle, any technique that is sensitive to molecular shape will be useful for experimental analysis of supercoiled DNA. However, because of the large size of these molecules, few techniques have been used successfully to provide direct structural information.

Of central importance to the discovery and initial characterization of supercoiled DNA was the use of high-speed centrifugation (Lebowitz, 1990). The sedimentation velocity and buoyant density of polyoma viral DNA was monitored after various treatments that induce strand separation or cleavage of the DNA helix. These experiments clearly identified that DNA molecules could exist in a variety of forms that differed only by their shape, and the concept of supercoiled DNA was founded.

High-resolution microscopy provides explicit analysis of molecular structure and electron microscopy (EM), cryo-EM and scanning-force microscopy have been used to analyse supercoiled forms of DNA (Vologodskii and Cozzarelli, 1994a). Each of these techniques has confirmed that supercoiled DNA has a compact shape and that the interwound form predominates in naked DNA. Moreover, high-resolution microscopy clearly shows that supercoiled DNA is often branched and that its conformational and thermodynamic properties depend on ionic conditions (Vologodskii and Cozzarelli, 1994a).

The other main technique that has been used to analyse DNA supercoiling is agarose gel electrophoresis. Smaller and/or more compact molecules migrate more rapidly during electrophoresis and, thus, DNA molecules that are linear, nicked circles or supercoiled circles can be separated (Figure 2) (Bowater *et al*, 1992; Keller, 1975). Compared with high-speed centrifugation, this technique provides a higher resolution for distinction between molecules with different shape and it is cheaper and easier to use. Incorporation of intercalators into electrophoresis running buffers allows topoisomers of high σ to be resolved

(Figure 2a,b) and their exploitation in two-dimensional gel electrophoresis allows a wide range of topoisomers to be analysed on a single gel (Figure 2c) (Bowater *et al*, 1992). See also: Gel Electrophoresis, DOI: 10.1002/9780470015902.a0005335.pub2; Staining, Viewing and Photography of Gels and Estimation of Fragment Sizes, DOI: 10.1038/npg.els.0003777.

Other experimental techniques have been used to provide less direct information about the structure of supercoiled DNA, including a variety of spectroscopic methods (circular dichroism, static and dynamic light scattering) (Lilley and Dahlberg, 1992; Vologodskii and Cozzarelli, 1994a). Utilization of complementary techniques, particularly in combination with theoretical methods, has provided significant information on the three-dimensional structure of supercoiled DNA.

Catenanes and Knots

Knots and catenanes (Figure 3) occur frequently in DNA, primarily as a consequence of the complex biochemical reactions that take place within closed topological domains. These structures can influence processes occurring on the DNA molecule, such as replication and transcription, and they are also utilized as intermediates in some types of genetic recombination (Wasserman and Cozzarelli, 1986). See also: DNA Topology: Fundamentals, DOI: 10.1038/npg.els.0001038; DNA Topology: Supercoiling and Linking, DOI: 10.1038/npg.els.0003904.

<Figure 3 near here>

Knots were first detected in DNA treated with topoisomerases *in vitro* (Wasserman and Cozzarelli, 1986). They have also been observed in native DNA, although they are not particularly common. Catenanes are more prevalent and are utilized in a number of diverse biological systems. Catenated molecules were first observed in mitochondrial DNA from human cells (Wasserman and Cozzarelli, 1986) and their most common occurrence is as intermediates during the replication of circular DNA.

Topological knots of a wide degree of complexity can be formed (Wasserman and Cozzarelli, 1986; Witz and Stasiak, 2010). Mathematical methods allow unique description of different knots and catenanes, although these descriptions become complex for highly knotted structures and for catenanes involving many loops. Theoretical analyses of these types of DNA conformations have been particularly valuable in the development of molecular models of supercoiled DNA (Benham and Mielke, 2005; Schlick, 1995; Vologodskii and Cozzarelli, 1994b).

Protein Binding and DNA Topology

A wide variety of proteins that bind to DNA alter the local geometry of its helix and influence DNA topology. The best-known example of this effect is the winding of DNA around the eukaryotic histone octamer to form the nucleosome (Luger and Richmond, 1998; Richmond and Davey, 2003). Histone proteins are positively charged and have no enzymatic activity, but they allow extremely large DNAs to be compacted to fit within each eukaryotic cell. Each histone core envelops approximately 1.8 left-handed turns of DNA and stabilizes negative writhing within the complex. Supercoiling of this type is constrained because it is not available to influence the structure of the remainder of the DNA. See also: Chromosomes and Chromatin, DOI: 10.1002/9780470015902.a0005766.pub2; Chromosome Structure, DOI: 10.1002/9780470015902.a0001486.pub2; DNA Coiling and Unwinding, DOI: 10.1038/npg.els.0005967; Nucleosomes: Structure and Function, DOI: 10.1038/npg.els.0001155.

Prokaryotes do not contain histone proteins, but they do harbour proteins that influence DNA architecture. The two most abundant of these in the nucleoid of *Escherichia coli* are H-NS (H1) and HU (Drlica and Rouviere-Yaniv, 1987). These proteins constrain supercoils and have highly pleotropic effects, affecting genome stability, and recombination- and transcription-related events (Dillon and Dorman, 2010). Proteins that have more specific cellular functions also exhibit differential binding to DNA templates at various levels of supercoiling. Included among these are polypeptides that bind to specific DNA sequences, such as integration host factor (IHF), which is involved in site-specific recombination (Swinger and Rice, 2004), and factor for inversion stimulation (FIS), which influences transcription at certain promoters (Travers *et al*, 2001). Other proteins that bind to DNA independent of its sequence become abundant under specific growth conditions. For example, Dps is induced upon starvation of *E. coli* and is important for coordinating cellular responses to such stress (Chiancone and Ceci, 2010). See also: Chromosomes: Nonhistone Proteins, DOI: 10.1038/npg.els.0001158.

Enzymatic Modulations of DNA Topology: Global and Local DNA Supercoiling

As a fundamental component of the three-dimensional structure of DNA, it is essential that cells regulate the overall amount of supercoiling that persists within chromatin – frequently referred to as the global level. The main enzymes involved in control mechanisms are the DNA topoisomerases, which can alter *Lk* of DNA (Schoeffler and Berger, 2008; Vos *et al*,

2011). General DNA topoisomerases function with little regard for DNA sequence. Additional enzymes that are involved in site-specific recombination reactions have considerable amino acid homology to some topoisomerases and have similar reaction mechanisms (Wasserman and Cozzarelli, 1986).

Topoisomerases with a wide variety of activities have been identified (Corbett and Berger, 2004; Schoeffler and Berger, 2008): they may act to remove or introduce negative supercoils or they may remove both positive and negative supercoils. In some cases these topological changes are coupled to the hydrolysis of ATP, as is the case for DNA gyrase and reverse gyrase (Gubaev and Klostermeier, 2014; Lulchev and Klostermeier, 2014). DNA gyrases are well-characterised essential enzymes in bacteria that are able to add negative supercoils to DNA. By contrast, reverse gyrases are able to positively supercoil a circular DNA; these atypical topoisomerases are present in some hyperthermophilic organisms (Forterre *et al*, 2007). See also: DNA Coiling and Unwinding, DOI: 10.1038/npg.els.0005967; Topoisomerases, DOI: 10.1038/npg.els.0001039.

Topoisomerases are classed as type I or II, according to the mechanism by which they produce topological changes of DNA (Schoeffler and Berger, 2008). Type I topoisomerases are further subdivided into two groups, types IA and IB, which exhibit dissimilar structures and distinct reaction characteristics. Type I enzymes transiently cleave one strand of the helix, pass the intact strand through and seal the break. Type II enzymes also make transient breaks in the helix, but they cleave both complementary strands of the molecule before passing another intact double-stranded molecule through the break. A consequence of these different reaction mechanisms is that type I enzymes change *Lk* in steps of 1, whereas type II enzymes change *Lk* in multiples of two.

Global DNA supercoiling varies for different cell types and growth conditions and DNA topoisomerases maintain levels within strict physiological boundaries (Baranello *et al*, 2012; Gilbert and Allan, 2014; Roca, 2011). Cellular processes that involve movement of macromolecular assemblies along DNA may also generate localized DNA supercoiling. As a large protein complex moves along the DNA, its rotation around the DNA may be inhibited (Liu and Wang, 1987). Instead, the DNA will rotate upon its axis, causing an increase in twist ahead of the complex and a reduction in twist behind; these twist changes are equivalent to positive and negative DNA supercoiling, respectively. This process is named ‘twin domains of supercoiling’, in recognition of the two regions of supercoiling that border the large protein complex. This phenomenon is best characterized for transcription (Figure 4), although it is also likely to occur during the action of DNA polymerases and DNA helicases. Such local topological changes *in vivo* have been shown to have a significant impact on the

conformation and function of important DNA sequence elements, such as promoters and DNA replication origins (Travers and Muskhelishvili, 2005; Wu and Fang, 2003).

<Figure 4 near here>

Since DNA strands are not broken during processes generating twin domains of supercoiling, there is no overall change of *Lk*. In a linear molecule such transient supercoiling will diffuse away and on a circular molecule the negative and positive supercoils will cancel out by diffusion around the circle. However, since chromatin is organized into discrete domains that are topologically independent, the diffusion of supercoils may be blocked and elevated levels of DNA supercoiling may build up (Gilbert and Allan, 2014). The relative orientation of neighbouring promoters may also influence the formation of transcription-induced supercoiling. For example, highly negatively supercoiled DNA may form between two divergent promoters that transcribe away from each other, whereas DNA that is between two convergent promoters may be positively supercoiled. DNA topoisomerases prevent increases in localized DNA supercoiling: in bacteria, negative and positive supercoils are removed by topoisomerase I and DNA gyrase, respectively (Corbett and Berger, 2004; Schoeffler and Berger, 2008). Inhibition of the activity of either of these enzymes, for example, by mutation, can lead to significant changes to cellular DNA topology (Hatfield and Benham, 2002; Wu and Fang, 2003). See also: DNA Coiling and Unwinding, DOI: 10.1038/npg.els.0005967; DNA Topology: Fundamentals, DOI: 10.1038/npg.els.0001038; DNA Topology: Supercoiling and Linking, DOI: 10.1038/npg.els.0003904; Topoisomerases, DOI: 10.1038/npg.els.0001039.

Biological Functions

The unconstrained σ of chromatin is believed to be about −0.02 to −0.03 in prokaryotic cells and is probably less negatively supercoiled in eukaryotes (Drlica, 1992). When localized variations to DNA topology are considered, it is clear that supercoiling provides a significant amount of free energy to DNA molecules inside cells. This increase in free energy can be used to drive structural transitions and other metabolic processes that would normally be thermodynamically unfavourable. For example, DNA can adopt a wide range of “unusual” structures that are different to the standard B-form helix, and many of these are more likely to form in molecules that negatively supercoiled (Kouzine and Levens, 2007). Importantly, some of these non-B-DNA structures have been linked with physiological consequences, including some types of human diseases. See also: Base Pairing in DNA: Unusual Patterns, DOI: 10.1038/npg.els.0003127; DNA Structure, DOI:

10.1002/9780470015902.a0006002.pub2; Non-B DNA Structure and Mutations Causing Human Genetic Disease, DOI: 10.1002/9780470015902.a0022657.

The most obvious consequence of DNA supercoiling is that it aids compaction of very large DNA molecules into the relatively small volume of cells. The most efficient form of length reduction arises from toroidal winding. Notably, size problems are particularly acute in eukaryotic cells and are overcome by the binding of DNA into toroids (nucleosomes and higher order structures). In addition to these effects, DNA supercoiling has a direct influence on many aspects of DNA metabolism *in vivo*. The binding of proteins to DNA is often influenced by supercoiling. Conversely, the binding of proteins that remove DNA supercoils can be used to relieve excess energy associated with supercoiling and prevent unfavourable deformations within the DNA. See also: Cell Biophysics, DOI: 10.1038/npg.els.0001271; Protein–DNA Interactions: Structure and Energetics, DOI: 10.1038/npg.els.0001349.

DNA topology plays a fundamental role in facilitating site-specific recombination reactions. Furthermore, analysis of the topology of reaction products has provided significant information towards understanding the recombination reaction, particularly for processes involving IHF and resolvases (Swinger and Rice, 2004; Wasserman and Cozzarelli, 1986).

Increased free energy associated with negative supercoiling can also be used to separate the strands of the DNA helix (Figure 1), which is usually unfavourable under physiological conditions. Thus, negatively supercoiled DNA templates assist processes that require opening of the DNA helix, such as replication and transcription. In general, these processes are increased at higher levels of negative supercoiling, but the relationship between σ and efficiency of transcription is complex. Some promoters are inhibited by increases in negative DNA supercoiling, suggesting that sequence or chromatin context are also important (Gilbert and Allan, 2014).

Current Research Topics

The influence of supercoiling upon the three-dimensional structure of DNA is well understood *in vitro*. The relationship of these observations to the structure of DNA *in vivo* is less clear. For example, many experiments show that twin domains of supercoiling can be generated when macromolecular protein assemblies translocate along DNA, and these could have profound effects on DNA metabolism inside cells. Although localized levels of DNA supercoiling are observed to vary *in vivo*, it seems that DNA topoisomerases normally keep these variations within well-defined limits (Baranello *et al*, 2012; Gilbert and Allan, 2014; Roca, 2011). Evidence is growing to show that variable levels of supercoiling may

impact on specific reactions that involve DNA metabolism. Ongoing research is evaluating the extent by which DNA topology exerts regulatory influences over DNA metabolism. Developments of new scientific technologies have been particularly useful for visualising how proteins influence DNA topology inside cells and at the single-molecule level *in vitro* (De Vlaminck and Dekker, 2012; Koster *et al*, 2010; Neuman, 2010). See also: Magnetic Tweezers, DOI: 10.1002/9780470015902.a0023173.

There are reciprocal interactions between virtually every reaction involving DNA and DNA topology. In other words, DNA topology influences its metabolism and DNA metabolism influences its topology (Fogg *et al*, 2012). Thus, there is obvious potential for DNA topology to be used in the regulation of gene expression (Ptacin and Shapiro, 2013). There is significant evidence that this occurs under some physiological conditions, but the extent to which this happens remains unclear. It seems likely that because global DNA supercoiling is an intrinsic property of the DNA template, it may be too universal to provide the fine control of expression of all genes. Perhaps the cell has evolved mechanisms that sever the links between DNA topology and transcription in some circumstances? The situation could be dramatically different for local DNA supercoiling where the surrounding DNA determines the topological changes. The manner by which these interactions take place are still unresolved. Well-characterized experimental systems are now available to monitor reactions such as replication, transcription and recombination, from both pro- and eukaryotes. Continued development and application of *in vitro* and *in vivo* approaches will provide significant advances in our understanding of how the three-dimensional structure of DNA integrates within cellular metabolism.

Glossary

Catenane

Interlinked double-stranded DNA circles, i.e. that cannot be unlinked without breakage of both strands of one double-stranded helix.

Closed-circular DNA

Double-helical DNA with no free ends, i.e. both strands are closed circles with no discontinuities in their phosphodiester backbones.

Linking difference

Difference between the linking number of a particular topoisomer of closed-circular DNA and the average linking number of relaxed DNA; can have positive or negative values.

Linking number

Number of times two strands of closed-circular DNA are connected. It is distributed between the two geometric parameters twist and writhe.

Open-circular DNA

Double-helical DNA with one strand containing a broken phosphodiester bond; also referred to as ‘nicked DNA’.

Relaxed DNA

Closed-circular DNA formed with minimal torsional strain of the DNA helix.

Supercoiled DNA

DNA containing coiling in addition to its normal helical path; closed-circular DNA molecules formed under torsional stress have average linking difference not equal to zero – can be positive or negative.

Topoisomer

(from *topo*logical *isomer*) Closed-circular DNA molecule of unique linking number.

Topoisomerase

Enzyme that catalyses changes in the linking number of closed-circular DNA.

Twist

Number of double-helical turns in a given length of DNA, measured relative to the DNA helix axis.

Writhe

Geometric parameter that describes the path of a DNA helix in three-dimensional space.

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Table 1. Definitions of abbreviation of DNA topological parameters

Abbreviation	Term	Definition
cccDNA	Covalently closed circular DNA	Double-helical DNA without free ends, i.e. both strands are closed circles
N	Length	Total number of base pairs in DNA
h	Helical repeat (helical pitch)	Number of base pairs per turn of DNA helix
Lk	Linking number	For cccDNA, number of times one DNA strand intersects the plane of the other
Lk^0	Hypothetical linking number	cccDNA without torsional strain (equal to *N*/*h*)
Lk_m	Relaxed linking number	Linking number of topoisomer with least torsional strain (may not be equal to *N*/*h*)
ΔLk	Linking difference	For supercoiled DNA, difference between *Lk* and Lk^0
σ	Specific linking difference	ΔLk normalized to length of DNA (equal to $\Delta Lk/Lk^0$)
Tw	Twist	Number of turns within DNA double helix
Wr	Writhe	Number of times DNA double helix crosses its own path
ΔG_{sc}	Free energy of supercoiling	Additional free energy contained within DNA due to presence of supercoils

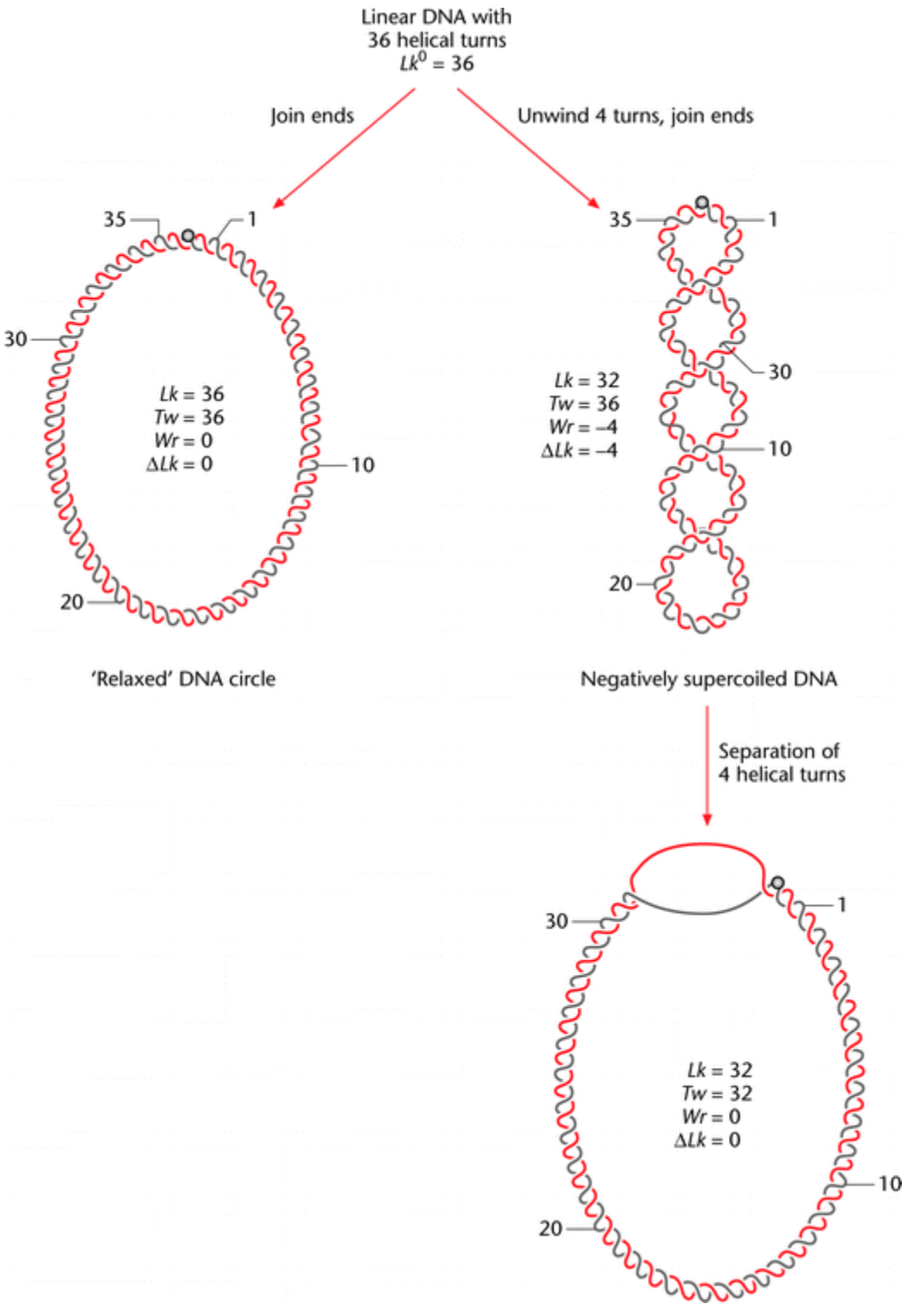

Figure 1. Relationship of linking number, twist and writhe of closed circular forms of DNA. Closed DNA circles can be made by formation of covalent 5′–3′ phosphodiester bonds on each strand of a linear molecule. For a linear molecule with 36 helical turns, the linking number of this unconstrained state (Lk^0) is 36.

Closure into an unconstrained planar circle, as shown on the left side of the figure, produces a molecule with twist (*Tw*) = 36 and writhe (*Wr*) = 0. If the number of helical turns is altered before closure, the DNA molecule adopts a supercoiled conformation. On the right side of the figure, four helical turns are removed from the molecule, reducing the linking number (*Lk*) to 32. For simplicity, the figure shows all unwinding partitioned as *Wr*, although such changes are usually partitioned between *Tw* and *Wr*. Unwinding of helical turns produces negatively supercoiled DNA (or $-\Delta Lk$) as shown, whereas the inclusion of additional turns produces positively supercoiled DNA. For DNA with $-\Delta Lk$ in the interwound form, the superhelical turns are right-handed. Note that separation of DNA strands removes negative supercoils (equivalent to the addition of positive supercoils).

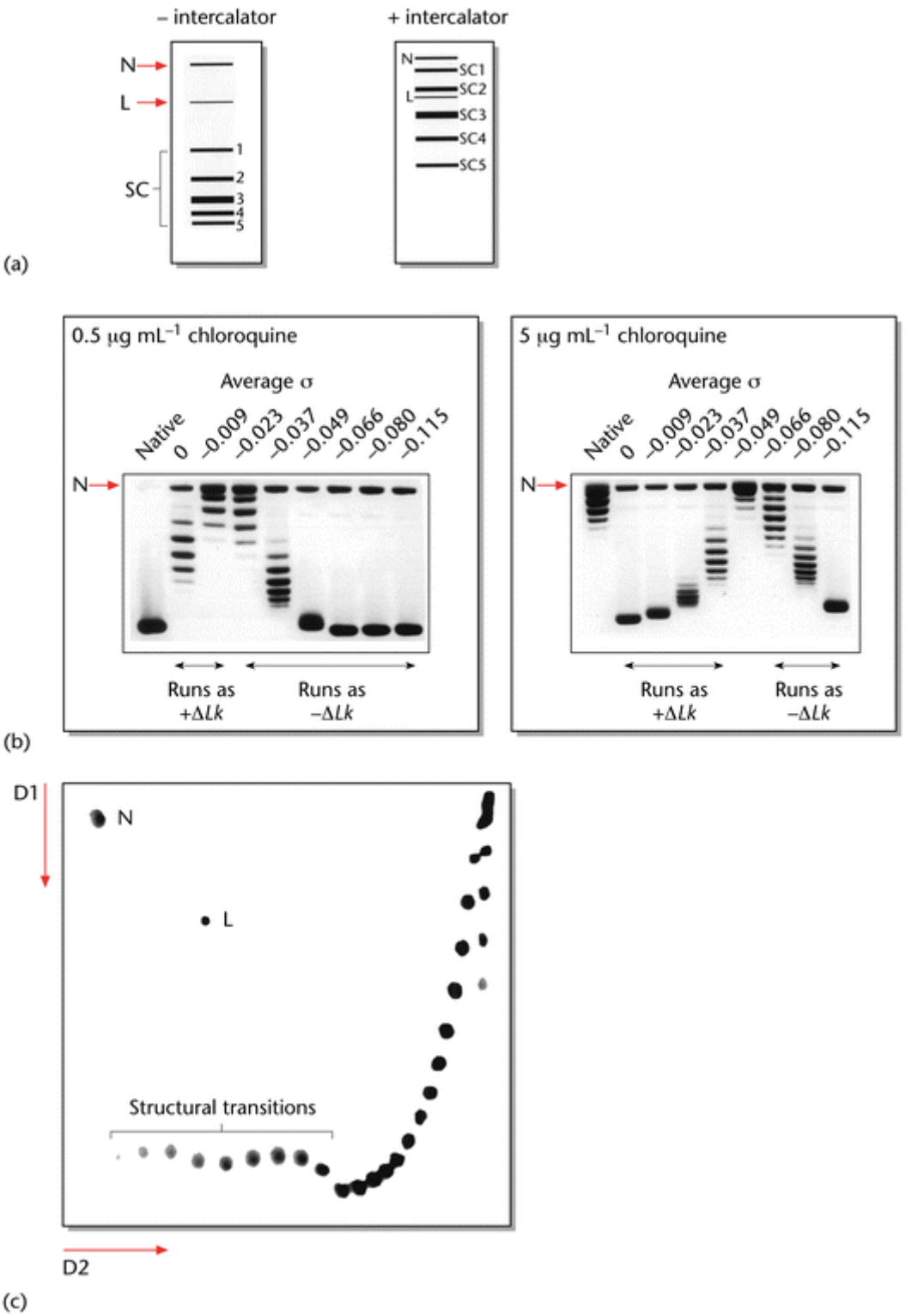

Figure 2. Measurement of linking number by gel electrophoresis. (a) Schematic illustration of a DNA sample separated by electrophoresis through an agarose gel with and without an intercalator. DNA isolated from bacterial cells contains molecules with different topology: some have their backbones unbroken and are negatively supercoiled (SC), some have one strand broken and referred to as ‘nicked’ (N) and

some have both strands broken to produce a linear molecule (L). Note that the supercoiled DNA consists of a Gaussian distribution of different topoisomers. Upon addition of intercalator, the migration of intact molecules is altered, but that of nicked and linear molecules is not changed. (b) Enzymatic relaxation of plasmid DNA in the presence of varying concentrations of intercalator produces samples containing topoisomers at different levels of supercoiling. Utilization of multiple gels with different concentrations of intercalator allows measurement of ΔLk. For each sample, average superhelical density (σ) is shown above the lane. Note that in each gel, samples can have positively or negatively supercoiled topoisomers. The inclusion of intercalator in the running buffer alters the electrophoretic mobility of all topoisomers equivalently. Superhelical density can be measured for experimental samples ('native') by comparison with those of known σ. (c) Two-dimensional agarose gel electrophoresis of topoisomers ranging from high negative σ to moderate positive σ. A DNA sample is loaded in a single well in a large agarose gel and electrophoresis is performed under specific conditions (usually without intercalator) in direction D1. After soaking of the gel in buffer containing intercalator, electrophoresis is continued in direction D2 (90° to D1). The gel shown contained 20 μg mL^{-1} chloroquine during the second electrophoresis, resulting in all topoisomers having positive σ. Deviation of topoisomers from a smooth curve indicates that structural transitions in the DNA molecules reduced their negative σ during the first direction of electrophoresis. Spots marked 'N' and 'L' indicate the position of migration of 'nicked' and 'linear' DNA molecules, respectively.

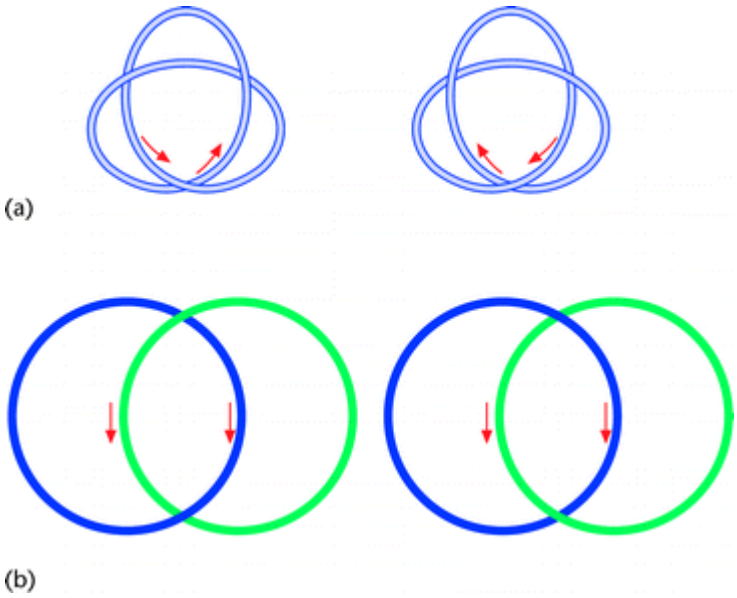

Figure 3. Representation of knots and catenanes. (a) Topological knots may be formed in closed circles of DNA. The simplest knot that can be formed is called a trefoil because there are three lobes to the structure when it is laid flat. Two isomers of the trefoil knot are shown. Many other more complex knots may be formed within cccDNA molecules. (b) Catenanes are formed when two circular DNA molecules are interlocked. Catenanes containing complex, multiple links and involving many DNA circles have been observed in naturally occurring DNA. The arrows indicate that the polarity of a knot or catenane is influenced by the directionality of the sequence in the DNA molecule.

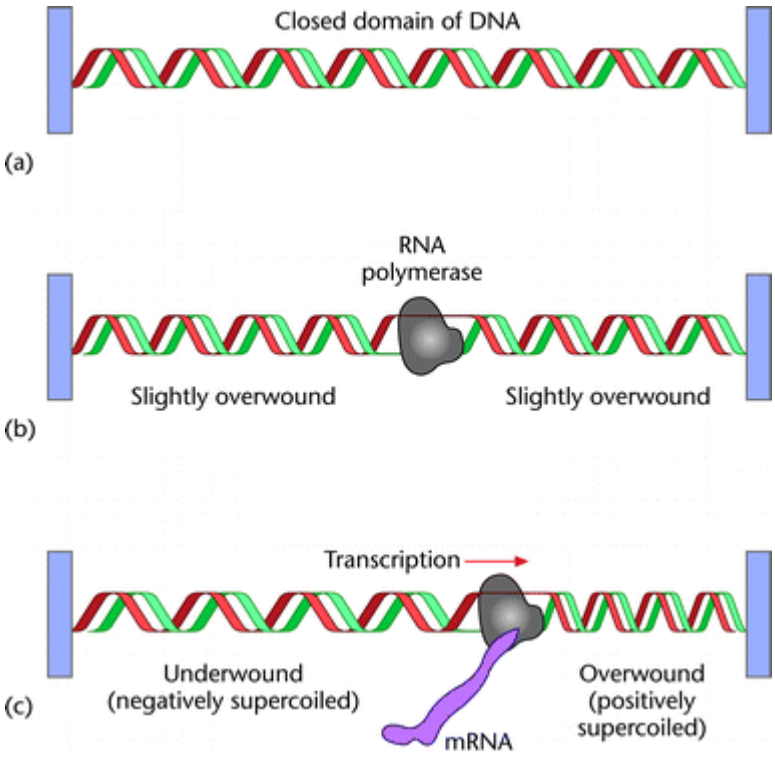

Figure 4. Twin domains of supercoiling are generated during transcription. (a) The shaded cylinders flank a closed domain of DNA containing eight helical turns. (b) To accommodate the transcriptional complex, some unwinding of the DNA helix occurs producing slight overwinding of the remaining DNA within each closed domain. (c) During transcription elongation, rotation of the large transcriptional complex around the DNA is hindered and positive and negative supercoiling are generated ahead and behind the polymerase, respectively. In this diagram, positive and negative supercoiling is represented by the presence of the same number of helical turns over a shorter and longer distance of DNA, respectively. Several biological mechanisms exist to remove these supercoils.

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