Supercoiling in DNA

Introduction

Cellular DNA is highly compacted to fit in the limited volume of the cell. This indicates that it has very high degree of structural organization and its folding mechanisms allow it to pack itself as well as provide access to the information present within it.

Supercoiling means coiling of the coil. The DNA double helix is in the coiled form as both its strands coil around the central axis. When this axis coils upon itself, we say that DNA is supercoiled. In contrast to it when there is no net bending of the DNA axis upon itself, the DNA is said to be in relaxed state.

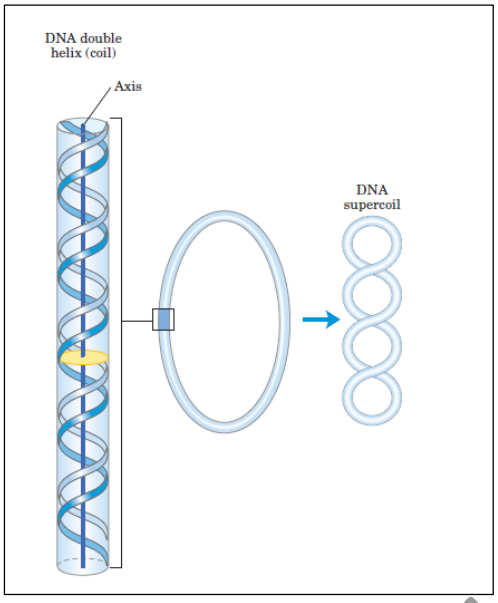


Figure: Supercoiled DNA

DNA supercoiling is an intrinsic property of the DNA tertiary structure. It takes place in all the cellular DNAs and is highly regulated by each cell. Study of supercoiling has mainly relied on the concepts of topology, a branch of mathematics.

Topology is defined as study of the properties of an object that do not change under continuous deformations.

In case of DNA, continuous deformations involve conformational changes due to thermal motion or an interaction with proteins or other molecules. Discontinuous deformations include DNA strand breakage. Therefore, in case of circular DNA molecules, a topological property is one that is unaffected by deformations of the DNA strands as long as no breaks are introduced. Thus, we can also conclude that topological properties only change when there is breakage and rejoining of the DNA backbone of one or both the DNA strands.

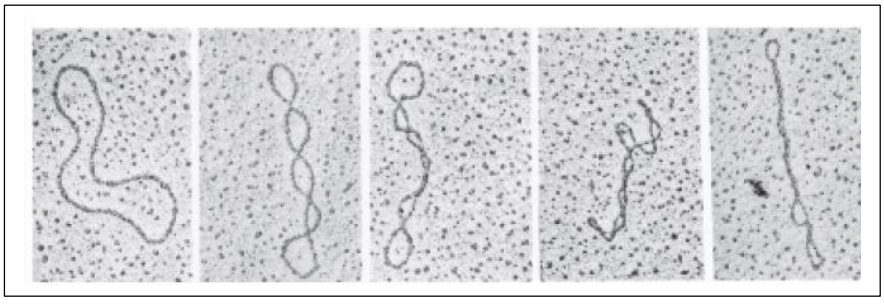


Figure: Relaxed & Supercoiled DNA

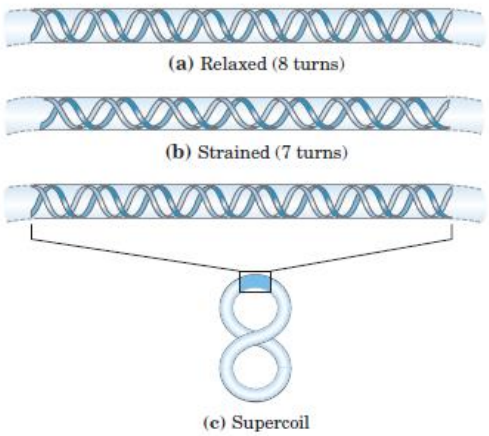
Leftmost is relaxed & as we move right, supercoiling is increasing.

Most of the cellular DNA is underwound:

This discussion is with respect to small circular DNAs so as to keep the discussion simple.

When small circular DNAs have no breaks in either of the strands, we call them covalently closed circular DNA or cccDNA. If this DNA is in the relaxed form and conforms to B-DNA structure, then it will have 10.5bp per turn of the helix. If some sort of strain is introduced, then it will undergo supercoiling to accommodate the strain.

In most of the cases, the strain is introduced due to underwinding of the DNA double helix in a closed circle. In this case, DNA will have less helical turns than would be expected for the B-form. Consider the figure and its explanation given below:



Effects of DNA underwinding:

(a) A segment of DNA within a closed-circular molecule, 84 bp long, in its relaxed form with eight helical turns.

(b) Removal of one turn induces structural strain.

(c) The strain is generally accommodated by formation of a supercoil.

In the figure given above, 84bp segment is in relaxed state and contains 8 helical turns i.e. 10.5 bp/turn. When one of the turns is removed then there are 7 turns for 84 bp i.e. 12 bp/turn and it is a deviation from the most stable form of the DNA.

This deviation introduces a thermodynamic strain in the molecule and most part of this strain is accommodated by coiling the axis of the DNA on itself to form a supercoil. In theory, this strain can also be accommodated by separating the two DNA strands over a distance of 10bp.

But in isolated, closed circular DNA molecule, strain introduced by the underwinding is generally accommodated by supercoiling rather than strand separation. This is because coiling of the axis requires less energy than breaking the hydrogen bonds that stabilize paired bases.

However, in vivo underwinding of DNA makes it easier to separate the DNA strands so that information in them can be accessed. Every cell uses enzymes to underwind the DNA and the resulting strain represents a form of stored energy which is used to facilitate the compaction by supercoiling.

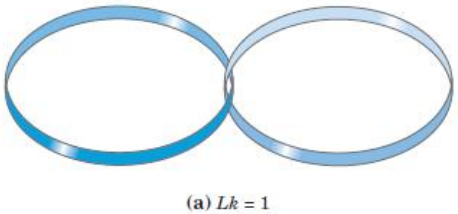
The underwound state can only be maintained if DNA is a closed circle or if it is bound and stabilized by proteins so that strands are not free to rotate around each other.

If there is a break in one of the strands and solution is protein free then there will be free rotation around the point of break and due to this underwound DNA will revert to the relaxed state. But if the DNA is cccDNA then to change the number of helical turns we need to break at least one of the strands.

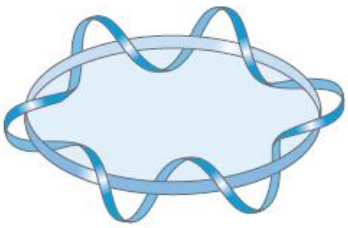
Linking number:

It is a topological property of the dsDNA because it does not vary when the DNA is bent or deformed, as long as both the strands remain intact.

In a cccDNA which is double stranded, linking number cam be defined as the number of times the second strand pierces the surface of the first strand. This definition is explained below.



(a) $Lk = 1$



(b) $Lk = 6$

Linking number, *Lk.* Here, as usual, each blue ribbon represents one strand of a double-stranded DNA molecule. For the molecule in **(a),** *Lk* = 1.

For the molecule in **(b),** *Lk*= 6.

One of the strands in **(b)** is kept untwisted for illustrative purposes, to define the border of an imaginary surface (shaded blue).

The number of times the twisting strand penetrates this surface provides a rigorous definition of linking number.

By convention, if strands are interwound in a right handed helix then Lk is defined as positive (+) and if strands are interwound in a left handed helix then Lk is negative (-). However, negative Lk is not encountered in the DNA.

Let us discuss it using example of a 2100bp closed circular DNA.

When molecule is relaxed, Lk = Number of bp / Number of bp per turn.

So, in our case Lk = 2100/10.5 = 200.

Now, let us assume underwinding and the linking number for relaxed form be Lk_0.

Lk_0 = 200 and if two turns are removed from this molecule then Lk = 198.

$\Delta Lk = Lk - Lk_0 = 198-200 = -2$.

Now, we can find out specific linking difference or superhelical density (σ).

$\sigma = \Delta Lk / Lk_0 = -2/200 = -0.01$. Negative (-) sign indicates that DNA is underwound.

Superhelical density is independent of the length of the DNA molecule and value of σ means that 1% of the helical turns in the B-form of the DNA have been removed. The degree of underwinding in cellular DNA is in the range of σ = -0.05 to -0.07 i.e. 5% to 7% of the cellular DNA is underwound.

This supercoiling which is induced by underwinding of the DNA is called as negative supercoiling. In some conditions, the DNA helix is overwound leading to positive (+) σ and the resulting supercoiling is called as positive supercoiling.

Note: If there is a break in one of the strands in a circular DNA then Lk is undefined.

Two forms of circular DNA that differ only in a topological property such as linking number are called as topoisomers.

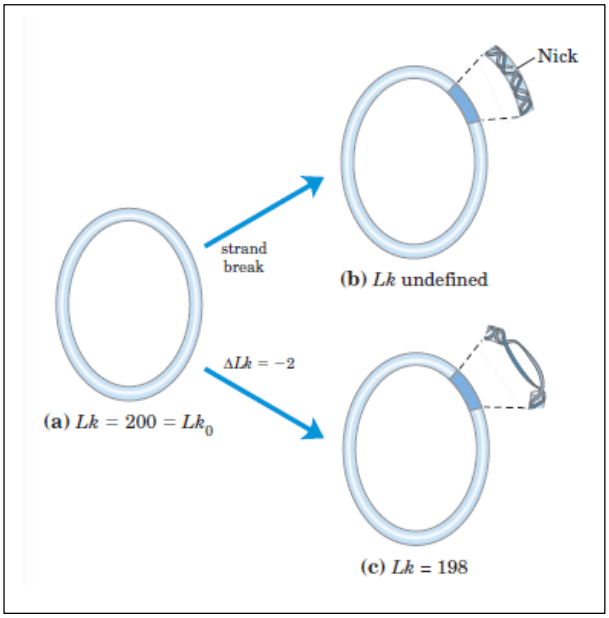


Figure: Linking number

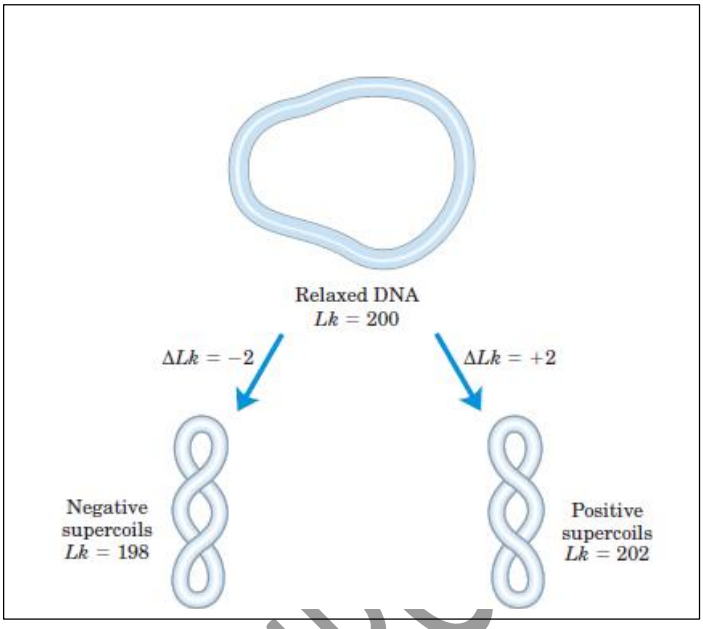


Figure: Negative and Positive supercoiling

DNA Topoisomerase

These are the enzymes that can increase or decrease the DNA underwinding and the property of DNA they change is the linking number.

These enzymes are of two classes:

- **Type I topoisomerases:** They transiently break one of the DNA strands, pass the unbroken strand through break and rejoin the broken ends. **They change linking number in increments of 1 and DO NOT require ATP.**
- **Type II topoisomerases:** They transiently break both the DNA strands, pass the duplex through break and then rejoin the broken ends. **They change linking number in increments of 2 and they REQUIRE ATP.**

Topoisomerases in *E. coli*:

- *E. coli* has 4 different topoisomerases and are labelled as I-IV.
- Topoisomerase I and III are example of **type I topoisomerase.** They relax the DNA by removing the negative supercoils. Thus, they increase the Lk too.
- Topoisomerase II also called as DNA gyrase is example of **type II topoisomerase.** It depends on ATP for its function and leads to introduction of negative supercoils which leads to reduction in Lk.
- The degree of supercoiling in bacterial DNA is maintained by regulation of the net activity of topoisomerases I and II.

Topoisomerases in eukaryotes:

- Example of type I topoisomerases are Topoisomerase I and III.
- Example of type II topoisomerases are Topoisomerase IIα and Topoisomerase IIβ.
- Eukaryotic type II topoisomerases can`t introduce negative supercoils i.e. can`t underwind the DNA. However, they can relax positive as well as negative supercoils.

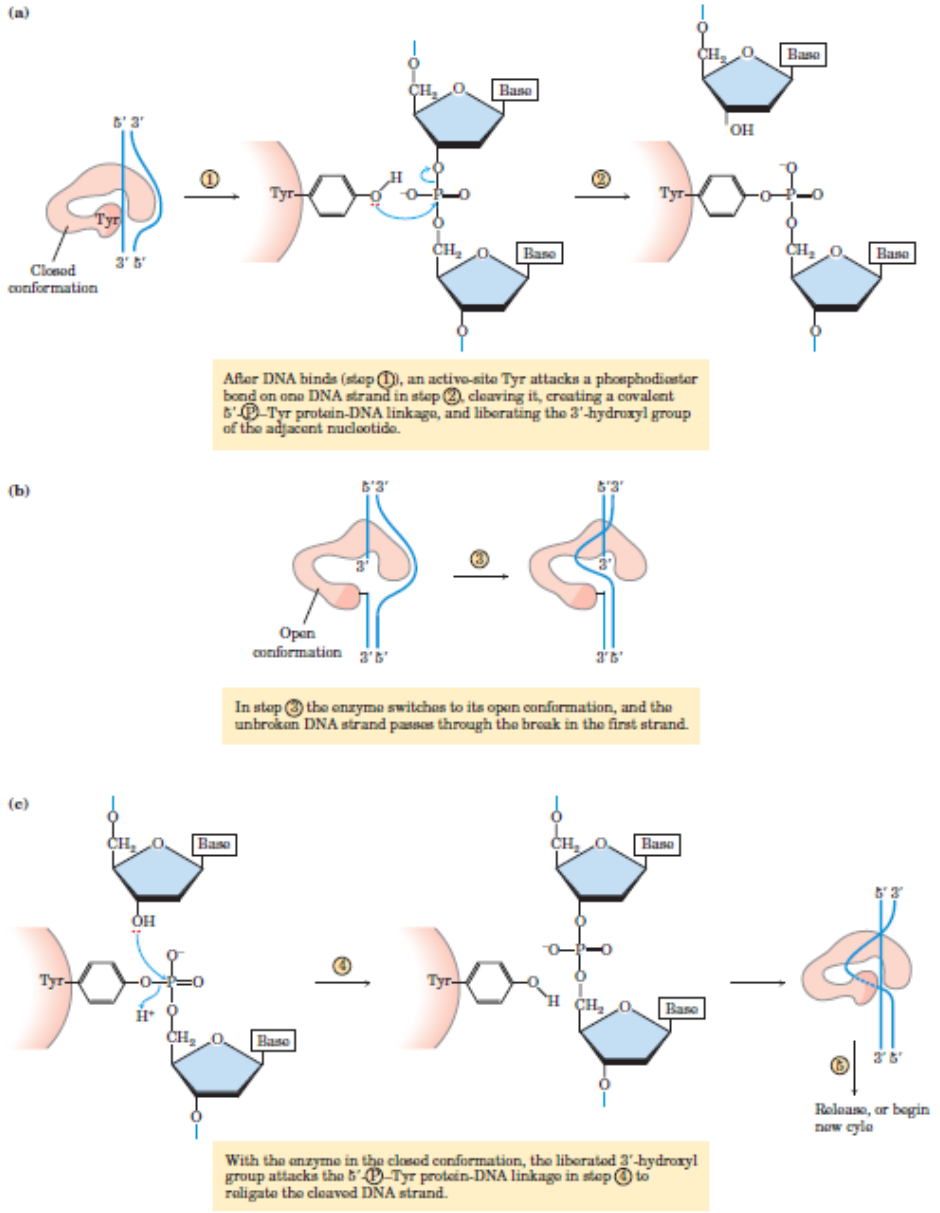


Figure: *E. coli* type I topoisomerase working mechanism. Firstly, DNA binds to the closed conformation of the enzyme and undergoes cleavage of one DNA strand. After this, enzyme changes to open conformation and intact DNA strand passes through the break created earlier. Now, enzyme switches back to closed conformation again and the strand is sealed again.

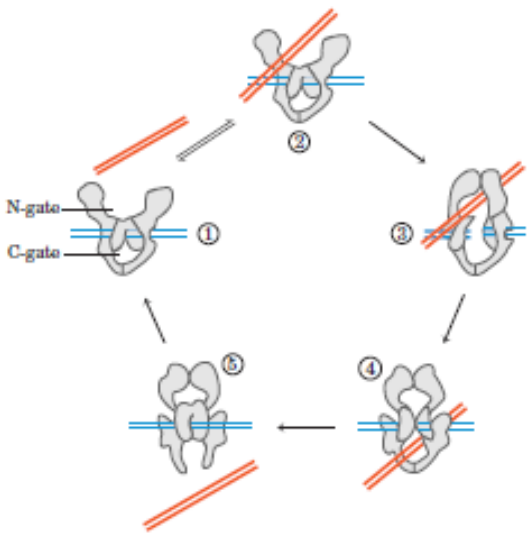


Figure: Working of eukaryotic type IIA topoisomerase. In step 1, multisubunit enzyme binds to one DNA molecule. There are gated cavities and the one above DNA is N-gate and below DNA is called as C-gate.

In step 2, second segment of the same DNA is bound at N-gate then trapped as shown in step 3. In step 4, both the strands of the first DNA are cleaved and second DNA is made to pass through the break.

In step 5, broken DNA is religated & second DNA segment is released through the C-gate.

During this whole process, two ATP molecules are utilized.

Demonstrating the effect of Topoisomerase on the DNA:

The effects of the DNA topoisomerases on the Lk can be shown using the Agarose gel electrophoresis. If a sample of plasmids has same Lk then they will move together as a distinct band. However, if there is difference in the Lk even by value of 1 then they will have different mobility in the gel electrophoresis.

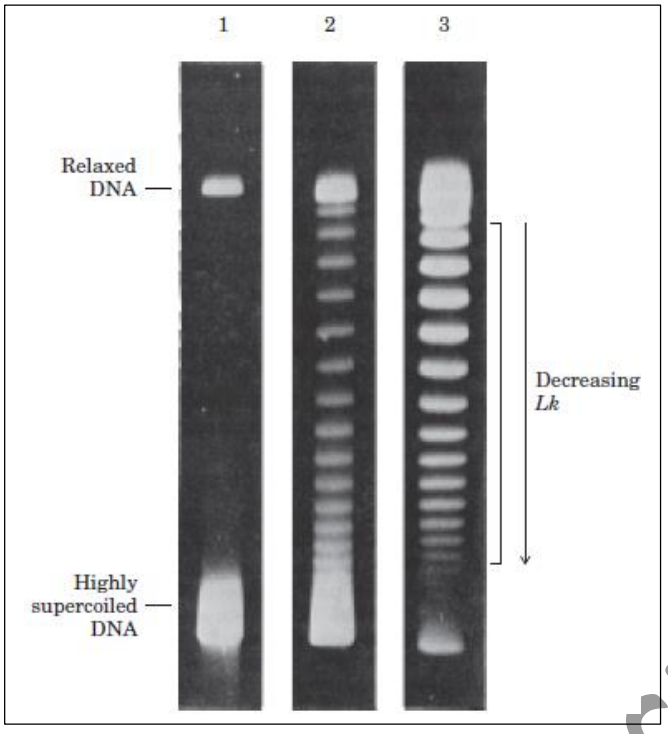


Figure: Visualization of topoisomers

Lane 1 shows supercoiled and relaxed DNA of the same molecular size.

Lane 2 & 3 are showing the effect of treating the supercoiled DNA from lane 1 with type I topoisomerase.

DNA in lane 3 was treated for a longer time than the one in lane 2.

Individual bands in the region marked by bracket in lane 3 is showing individual topoisomers and each varying just by Lk 1.