1.6: DNA Supercoiling and Topoisomerases

Unwinding of the helix during DNA replication (by the action of helicase) results in supercoiling of the DNA ahead of the replication fork.

- This supercoiling increases with the progression of the replication fork.
- If the supercoiling is not relieved, it will physically prevent the movement of helicase.

The topology of DNA can be described by three parameters:

1. **Linking Number** (L) An integer value. "Positive" is referenced as right-handed.
2. **Twist** (T) A real number (the "apparent" linkage number)
3. **Writhe** (W) A real number ("supercoils" in the DNA structure)

Consider closed circular DNA:

- **Linking number** is an integer value.
- It refers to the number of times the two strands of the duplex make a complete 360 degree turn.

For circularly closed DNA, like the *E. coli* genome, the linking number can only be changed if we do the following:

1. **physically break the duplex**
2. introduce (or remove) a 360 degree turn
3. ligate (covalently close) the break.
Rubber tubing "helix" experiment

Cut two lengths of 1/8" rubber tubing, each about 20” long. Insert a smaller piece of tubing, or piece of pipette tip in the ends to allow the ends to be connected. These two pieces of rubber tubing represent each strand of a DNA duplex. DNA can be ligated, or joined, when we have 5’ (phosphate) and 3’ (hydroxyl) ends. So we need a way to keep track of which end is which for each piece of tubing.

1. You can either write "5" and "3" on opposite ends of each piece and align them in opposite directions, or
2. On one piece of tubing, mark both ends with a sharpie. Only similarly colored ends can be ligated (see diagram above)

This will allow you to maintain correct strand "orientation" when you "ligate" the strands of the duplex.

- Introduce a Linking number = +2 (two 360° right handed twists into the duplex)
- then "ligate" the ends (make sure you maintain strand orientation, i.e. you connect the appropriate two strands).

Confirm that the correct linking number has been introduced by "melting" the duplex on one side and forcing all turns into a small region of the duplex (easy to count this way).

- Confirm that looking down the helix at the turns that they are **right handed** (does not matter which way you look down the helix).

Note that the "duplex" when held between thumb and forefinger and allowed to hang, prefers a "supercoiled" topology, as opposed to "relaxed" [Note: this is usually seen with very skinny tubing, larger diameter tubing may not readily adopt this topology].

- Confirm that the "supercoils" are actually **left handed** as you look down the supercoils (regardless of direction down the supercoils).
- The "supercoil" is most likely a full 360°, rather than 180°. In any case, hold the ends of the duplex so that a left handed 360° "supercoil" is present.

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*Figure 1.6.1: Turns to change the linking number*
• Now, count how many times the strands of the "duplex" cross each other. In this conformation, the strands of the "duplex" will **not** actually cross each other (Note: you may have one strand crossing and then later uncrossing, for a net result of no crossing).

Thus, in response to the introduction of +2 Linking number, the "duplex" can adopt +2 (180°) "supercoils", such that the resulting **apparent linkage number** (i.e. "twist" value) of the two strands is zero.

Note

A supercoil is considered positive, if it is "left handed").

• Remove one "positive" supercoil by unwinding by 180°.

• Now hold the ends of the "duplex" and count the **apparent linkage number** (i.e. "twists"). There will be a single right-handed "twist".

• **Thus, a single 180° "positive" supercoil has the effect of removing a single "positive" twist (i.e. reduces the apparent linkage number by 1).**

The **Writhe** number refers to the number of supercoils present.

• Although it may seem that the consequence of introducing supercoiling (Writhe) is changing the Linking number, it **is not**.

• The consequence of Writhe is that the Twist (**apparent linkage number**) is altered (increased or decreased).

**DNA has a preferred "Twist" value** (preferred apparent linkage number) **for a specified length** of DNA:

• Watson and Crick's model of the DNA duplex had 10 basepairs per turn.

• Under physiological conditions of salt (0.15 M NaCl) and temperature, DNA **prefers to adopt about 10.6 bp/turn**.

• Writhe is introduced in the DNA to achieve this value for the "Twist" (apparent linkage number)

For a given (fixed) Linkage number over a given length of DNA, the DNA can adopt either positive or negative supercoils to achieve a "twist" (apparent linkage number) such that there will be 10.6 basepairs/turn.

**Linkage number does not change with supercoiling** (it can only change by breaking the duplex)

• Writhe has the effect of changing the apparent Linkage number.

• One supercoil is defined as being able to change the apparent linkage number by +/- 1.

The twist value (apparent linkage number) for a given length of DNA is related to the number of base pairs per turn that the DNA wants to adopt:

**Linkage Number = (size of DNA in base pairs)/(basepairs/turn) + Writhe**

or

**Linkage Number = #of Twists + Writhe**

this is usually abbreviated as...
**Linkage = Twist + Writhe**

\[ L = T + W \]

For example, if we have a circularly closed DNA molecule with a length of 5300 base pairs, and a preferred conformation of 10.6 basepairs per turn, can it achieve this conformation without having to introduce any supercoiling (i.e. writhe)?

Apparent linkage number (Twist) = \( \frac{5300 \text{ base pairs}}{10.6 \text{ base pairs/turn}} \)

Apparent linkage number (Twist) = 500

In other words, in order to achieve the desired conformation of 10.6 bp/turn in the helix, exactly 500 turns are required over the length of 5300 base pairs.

Linkage number = 500 + Writhe

We can have integral values for the linkage number, and we can certainly introduce 500, which would require no Writhe at all:

500 = 500 + 0

What is the DNA molecule was 5200 base pairs?

Apparent linkage number (Twist) = \( \frac{5200 \text{ base pairs}}{10.6 \text{ base pairs/turn}} \)

Apparent linkage number (Twist) = 490.6

We can introduce either 490 or 491 as a linkage number, but not 490.6. What happens if there is a linkage number of 490 in the DNA molecule?

Linkage number = Twist + Writhe

490 = 490.6 + Writhe

Writhe = -0.6

In this case, the DNA adopts a negative 0.6 supercoil (about 108° of a right-handed supercoil) which will increase the apparent linkage number from 490 to 490.6 (and achieve 10.6 basepairs per turn in the duplex).

How many basepairs per turn would there be in the DNA if the DNA was not able to adopt any supercoil structure for this length of DNA with a linkage number of 490?

Linkage number = Twist + Writhe

490 = Twist + 0

Twist = 490 turns
Twist = (5200 base pairs) / (bp/turn) = 490 turns

bp/turn = 5200 base pairs / 490 turns

bp/turn = 10.61

There are slightly more than 10.6 basepairs per turn in the DNA

A small circularly closed genome

The Simian Virus 40 (SV40) genome is a circular, closed, double stranded DNA genome. For the purposes of this discussion, it has 5300 bases. We expect that under physiological conditions the DNA will exhibit 10.6 base pairs per turn (i.e. one Twist = 10.6 bp/turn). In this case, with no Writhe, the Linking number would be:

Linking number = 5300 bp/(10.6 bp/turn) + 0

Linking number = 500 turns

i.e. we would expect 500 360° turns of the DNA strands over the length of the circular genome.

- This form (with 10.6 base pairs per turn) with no Writhe represents the "standard", or undistorted, DNA helix.
- This is also known as the "relaxed" form of DNA, and the duplex could physically be laid out flat on a surface because it needs no Writhe to achieve the preferred value of 10.6 basepairs per twist:

![Figure 1.6.2: Standard DNA helix](https://bio.libretexts.org/Bookshelves/Biochemistry/Supplemental_Modules_(Biochemistry)/1%3A_DNA/1.6%3A_DNA_Super...)

However, when the replication of SV40 is initially completed it is observed that there remains an open duplex region in the DNA:
The result is that there are about 475 turns of the helix within the duplex DNA (i.e. the Linking number = 475).

- The DNA is said to be **underwound**.
- An open area is energetically unfavorable.
- *The covalently closed molecule cannot adjust for this by increasing the Linking number.* That is, it cannot **spontaneously** break one or both strands of the duplex, introduce another 25 turns into the duplex (increase the Linking number by 25) and re-ligate the duplex.

The DNA has **three** choices:

1. It can adjust the number of **basepairs per turn** throughout the molecule from a desired 10.6 bp/turn to 11.2 bp/turn (i.e. 5300 bp/475 turns). (NOTE: an **increase** in the number of basepairs per turn will **decrease** the twist value; **underwound** DNA has a greater number of basepairs per turn).
2. The DNA can coil up into a "supercoil" topology and maintain the desired twist value (10.6) with the given linking number (475 in this case).
3. The duplex can exist with a twist of 10.6 bp/turn for most of the structure, and then have **a region with zero twist** (not necessarily a melted duplex). This is quite unfavorable due to the geometry required of bond angles.

Thus for the 5300 bp SV40 genome, with a Linking number of 475, to maintain a value of 10.6 bp/twist, a total of 25 negative supercoils (Writhe=25) are needed:

\[
475 = \frac{5300}{10.6} + \text{Writhe}
\]

\[-25 = \text{Writhe}\]

- That is, **25 negative supercoils** (twenty five 180° turns of the DNA duplex, **right handed** as you look down the supercoiling).
Topoisomerases

The enzymes that control DNA topology are critical to DNA replication and transcription.

- As the replication fork opens up, the region of the duplex in front of the fork becomes overwound - i.e. it has fewer basepairs per turn.
- The linking number has not changed, but the length of DNA which contains all the turns is effectively shorter.
- To maintain 10.6 bp/turn in that region, the DNA will adopt positive supercoils.

For example, during the early stages of SV40 replication, the duplex around the origin of replication may initially melt (open up) a region of 750 bases. Since the Linkage number (500) is unchanged, it is effectively distributed over only:

$$5300 - 750 = 4550 \text{ bases.}$$

Assuming no supercoiling has been introduced:

$$500 = \frac{4550}{X} + 0$$

$$= 9.1 \text{ basepairs/twist}$$

Thus, if no supercoiling is introduced, the DNA must adopt a conformation of 9.01 base pairs/twist of the helix within the region ahead of the replication fork.

- This is energetically unfavorable, and one option for the DNA is to adopt a supercoiled configuration to achieve 10.6 bp/twist:

$$500 = \frac{4550}{10.6} + \text{Writhe}$$

$$70.8 = \text{Writhe}$$

- Thus, movement of the growing fork causes the DNA to adopt positive supercoils.
- In this case the DNA has adopted 70.8 left handed supercoils (180° each).
- Twist (=basepairs * [twist/basepair]) and Writhe are both real numbers.

Type I Topoisomerase

- Type I topoisomerases cut one strand of the DNA (i.e. it "nicks" the DNA duplex).
- The 5’ phosphate of the nicked strand is covalently attached to a tyrosine in the protein.
- The 3’ end of the nick then passes once through the duplex.
- The nick is then resealed, and the linkage number is change by a value of +1.
- This can therefore result in the removal of a single negative supercoil.

In E. coli, type I topoisomerase can only relieve negatively supercoiled DNA (negative supercoiling is the end result of newly replicated DNA genome). In eukaryotes, type I topoisomerase can also relieve positively supercoiled DNA.
The net result of *E. coli* topol can be diagrammed as follows:

![Diagram](image)

Figure 1.6.4: *E. Coli Topoisomerase I*

**Type II Topoisomerases**

- Type II topoisomerases actually cleave the duplex DNA in changing the linkage number.
- Type II topoisomerases can **convert a single positive supercoil into a negative supercoil**.
- **Thus the linkage number is reduced by two (-2) in a single step.**
- Type II topoisomerases are involved in both decatenation of daughter chromosomes, and **relieving the positive supercoiling ahead of the replication fork.**
- *E. coli* DNA **gyrase** is an example of a type II topoisomerase.
Figure 1.6.5: Type II Isomerase activity