Topological Process of Splitting DNA-links: Do enzymes dream of knots and links?

Abdul Adheem Mohamad[1] Tsukasa Yashiro [2]

[1] University of Nizwa, Nizwa, Oman

[2]Independent Mathematical Institute, Nagano, Japan

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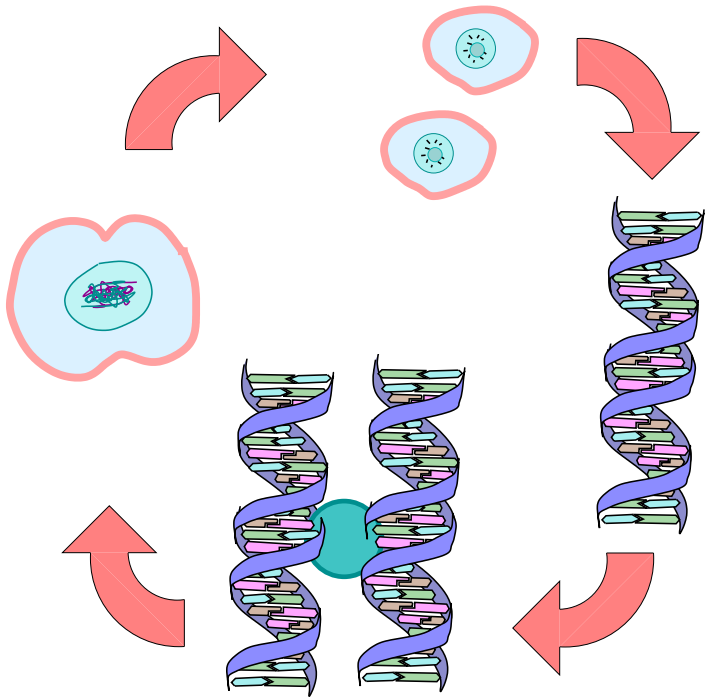
Contents

Cell Cycle

The eukaryotic cell cycle has two phases,

- Mitosis/cytokinesis and interphase, and also,
- During the interphase, DNA (deoxyribonucleic acid) is replicated.

DNA is a long molecule with radius 2 nm ($1\,\text{nm} = 1 \times 10^{-9}\text{m}$). The total length of human DNA is about 1.8 m

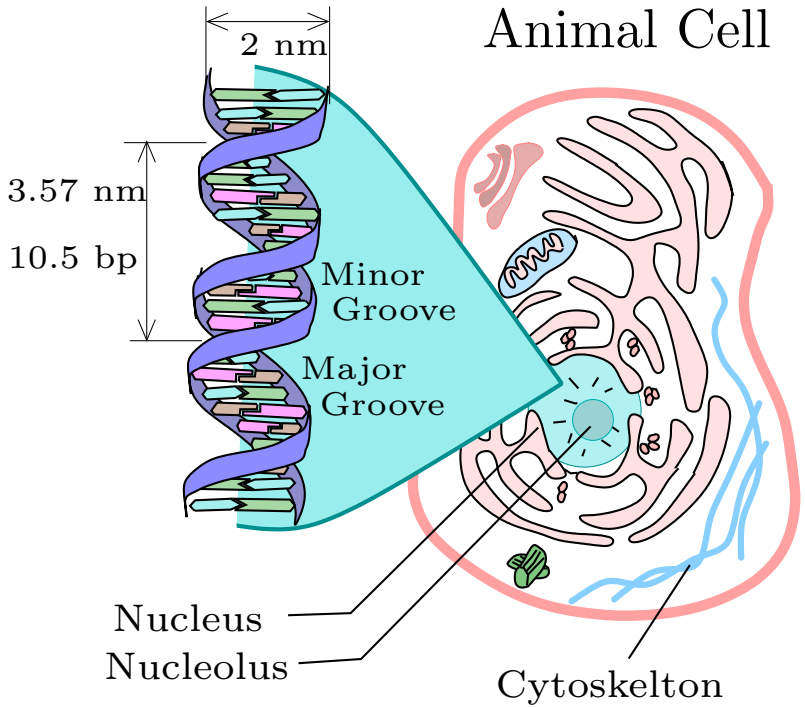


DNA synthesis

DNA has:

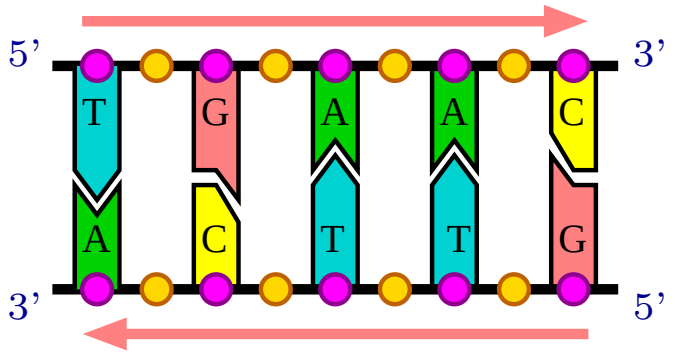
- a right-handed double helical backbones on the outside and base pairs lined up on the inside, and
- antiparallel orientation.

There are four bases,
A =Adenine, T =Thymine, C = Cytosine, and G =Guanine.
They form specific base pairs, A with T, and C with G.



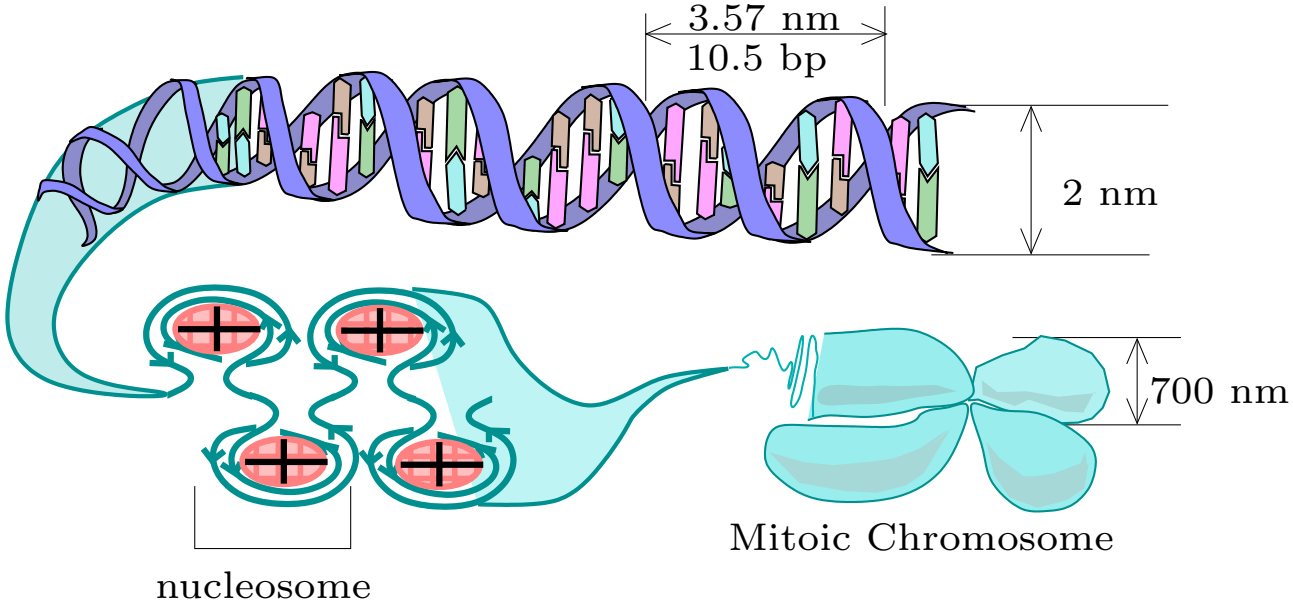
Topological orientation

Topologically, we assume that DNA has a parallel orientation.



Chromosomes

The double strand DNA (ds-DNA) forms a winding structure around a histone core to make a bead structure called a **nucleosome**. [1] [2]



[1] Andrew D Bates, Anthony Maxwell, et al. DNA topology. Oxford University Press, USA, 2005.

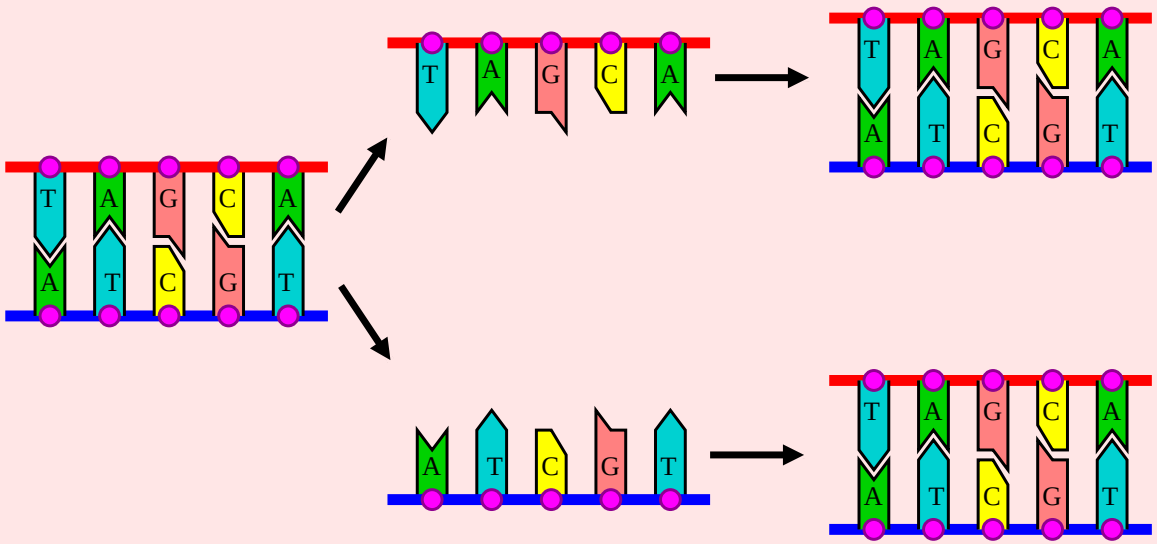
[2] Richard R Sinden. DNA structure and function. Gulf Professional Publishing, 1994.

Semi-Conservative Replication

In 1958 Meselson and Stahl did an experiment to show that DNA is replicated by **semi-conservative** replication.

Semi-Conservative Scheme

The sequence of bases along each backbone is preserved in copied DNA.

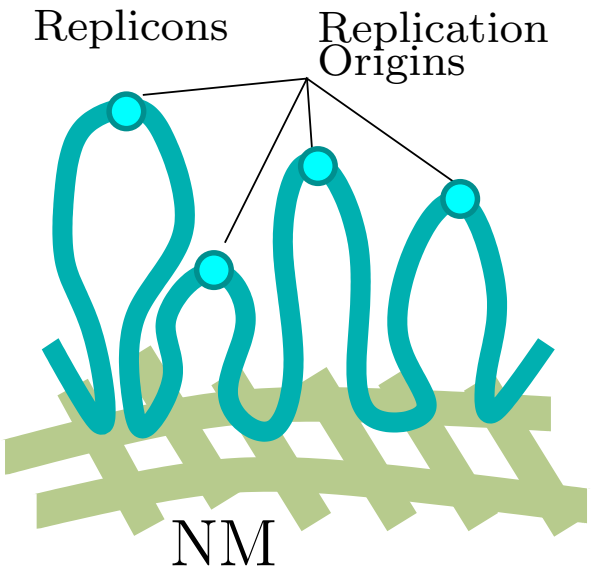


Replicons

The replication is done on each looped segment called a **replicon**. It is believed that the ends of replicon are anchored at the **nuclear matrix (NM)**.

The size of replicon

If the diameter of DNA is 2 cm, then the length of a replicon is about 357 m



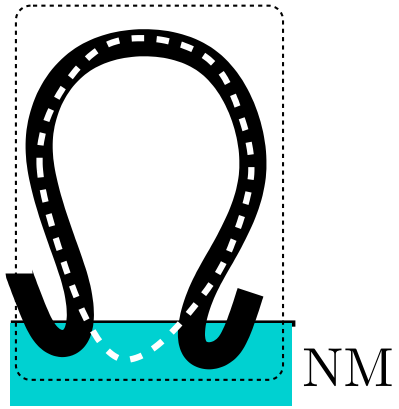
DNA-link

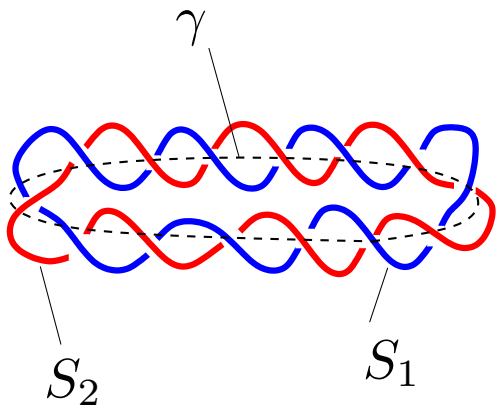
Topologically, the replicon is viewed as a special 2-component link $L(S_1, S_2; \gamma)$, which has the following properties.

A1 γ is a **trivial** oriented knot.

A2 L is a 2-component link, and S_1 and S_2 form a double helix along γ.

We call this link a **DNA-link**.

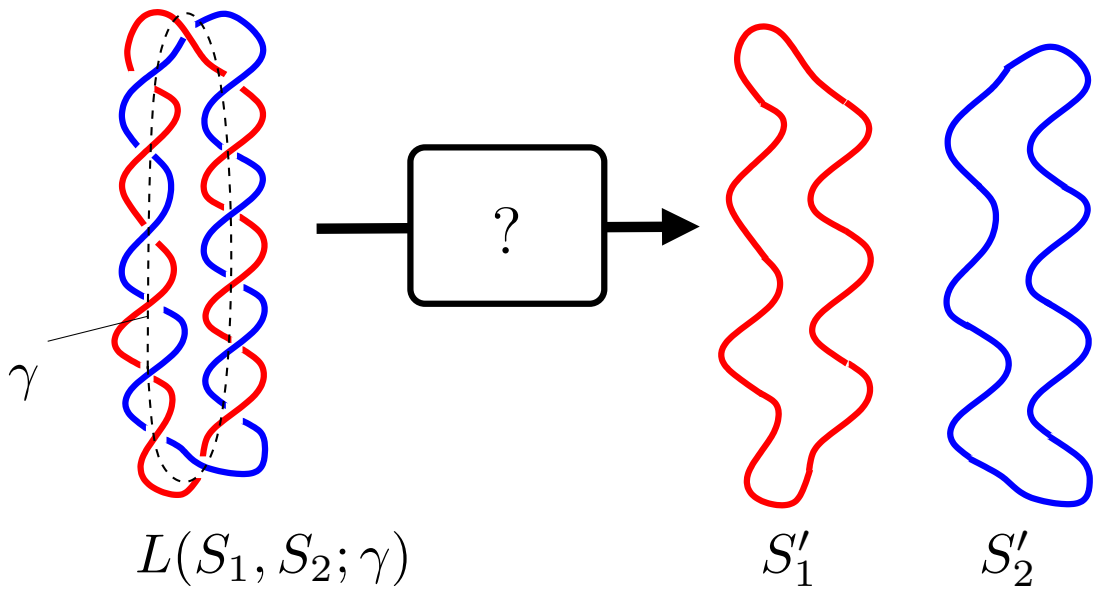




Topological Semi-conservative scheme

The semi-conservative scheme (Topological version)

The semi-conservative scheme is interpreted as such: the DNA-link $L(S_1, S_2; \gamma)$ is deformed into a split link $\{S_1', S_2'\}$; that is, the linking number of L will become zero.



Linking Number Formula for DNA

It is known that the following formula[3] holds:

Proposition 2.1 (White(1969))

For a DNA-link $L(S_1, S_2; \gamma)$,

$$Lk(L) = Lk(S_1, S_2) = Tw(S_1, S_2) + Wr(\gamma),$$

where Tw *is the number of full twists along* γ, *and* $Wr(\gamma)$ *is the writhe of* γ.

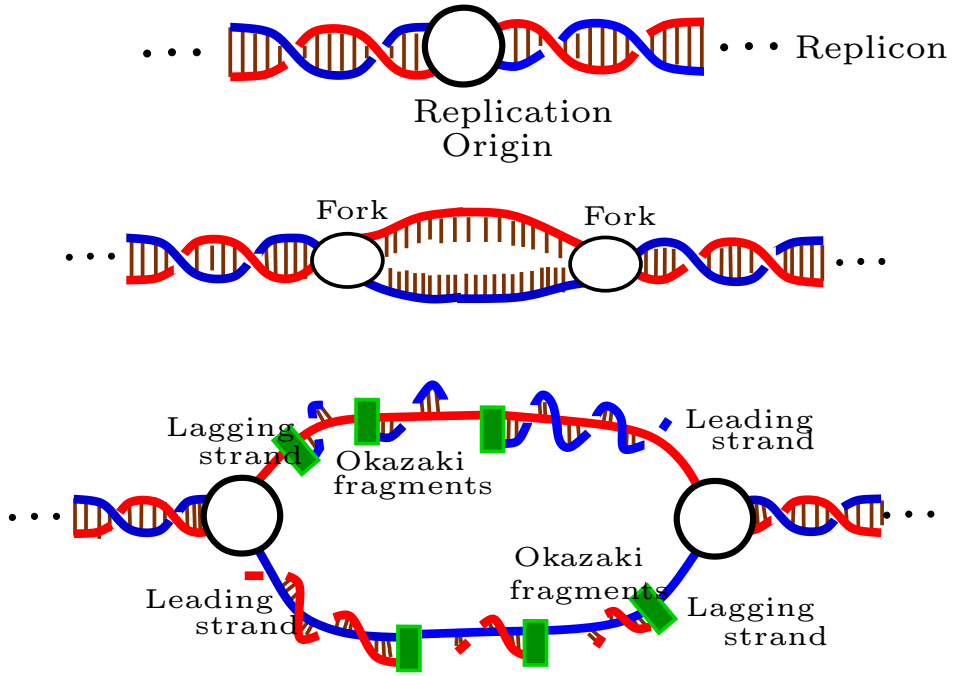
Corollary 2.1

For a DNA-link $L(S_1, S_2; \gamma)$, $Lk(L) = 0$ *if and only if* L *is split.*

[3] J. H. White,Self-linking and Gauss integral in higher dimensions, Amer. J. of Math.,(1969), 693-728

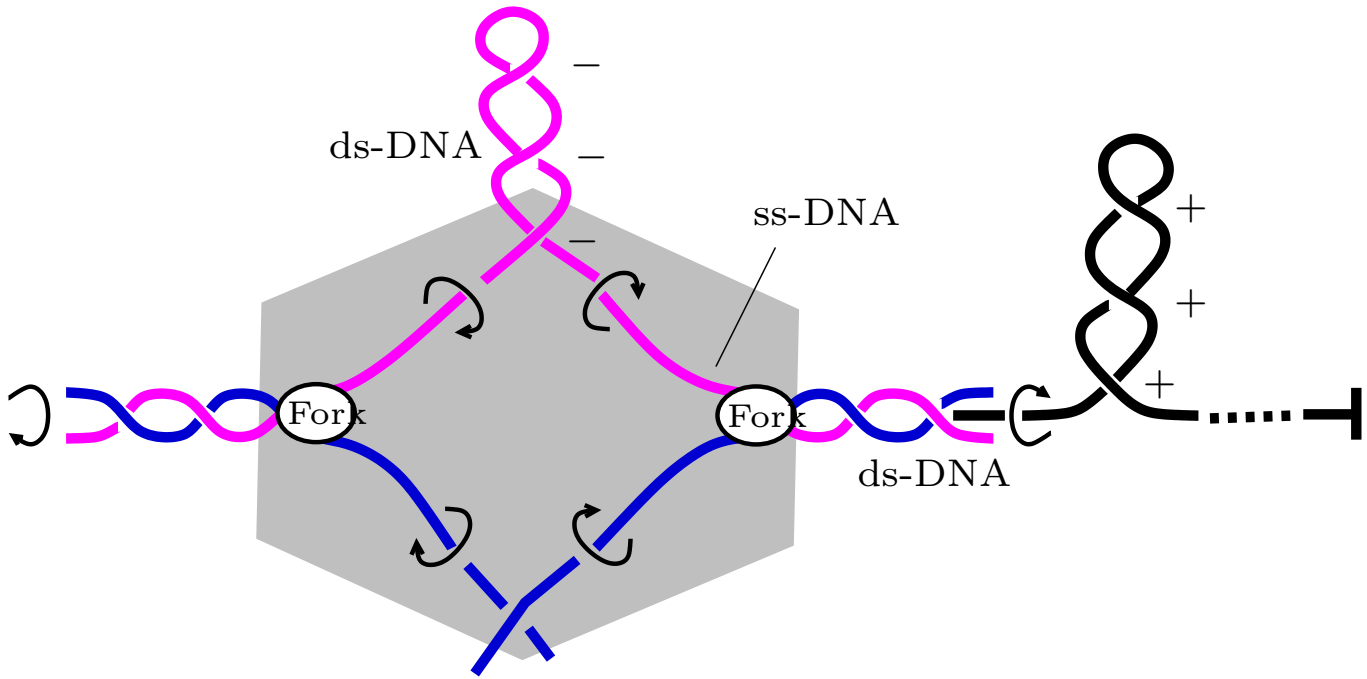
At the replication origin,

1. the ds-DNA is relaxed and split into two ss-DNAs, and
2. new nucleotides and double helix are constructed.



Unwinding and Supercoils

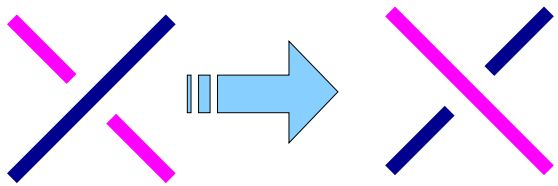
As the forks move away from the replication origin, both single strand DNA (ss-DNA) and ds-DNA are rotated. Some positive and negative **supercoils** are introduced ahead of and behind the fork respectively.



The supercoil becomes an obstruction against the replication process.

Topological Unknotting Operation

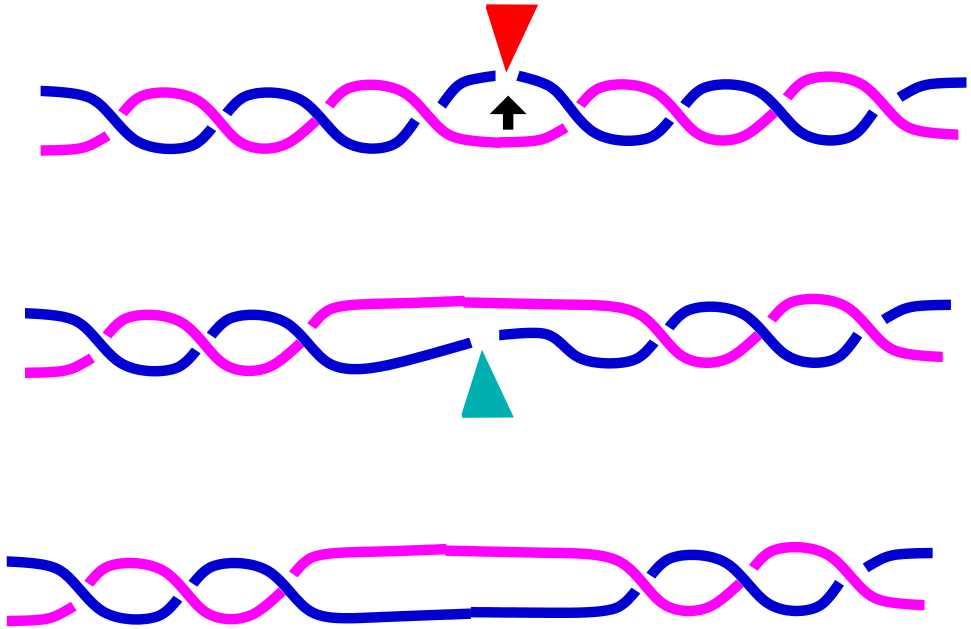
To resolve the supercoils and linking of the DNA strands, we need to apply some unknotting operations.



This operation is necessary to split a DNA-link.

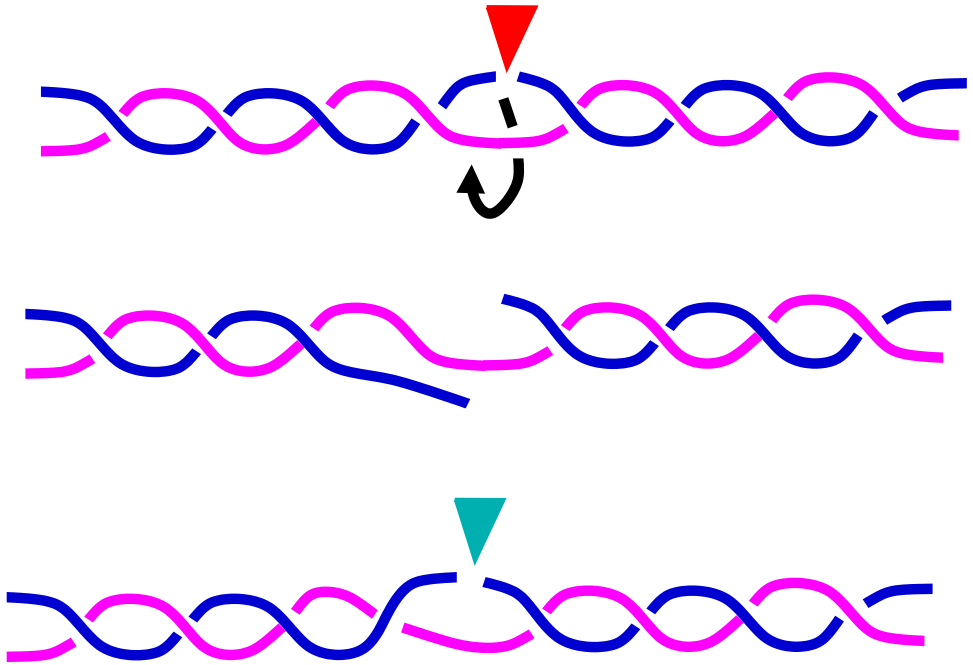
Biological Unknotting Operations

Type I decreases the twisting number of ss-DNA.
Topoisomerase IA. The topoisomerase IA nicks one single strand to make a gap to let another single strand pass through the gap, and reseal the gap.



Biological Unknotting Operations

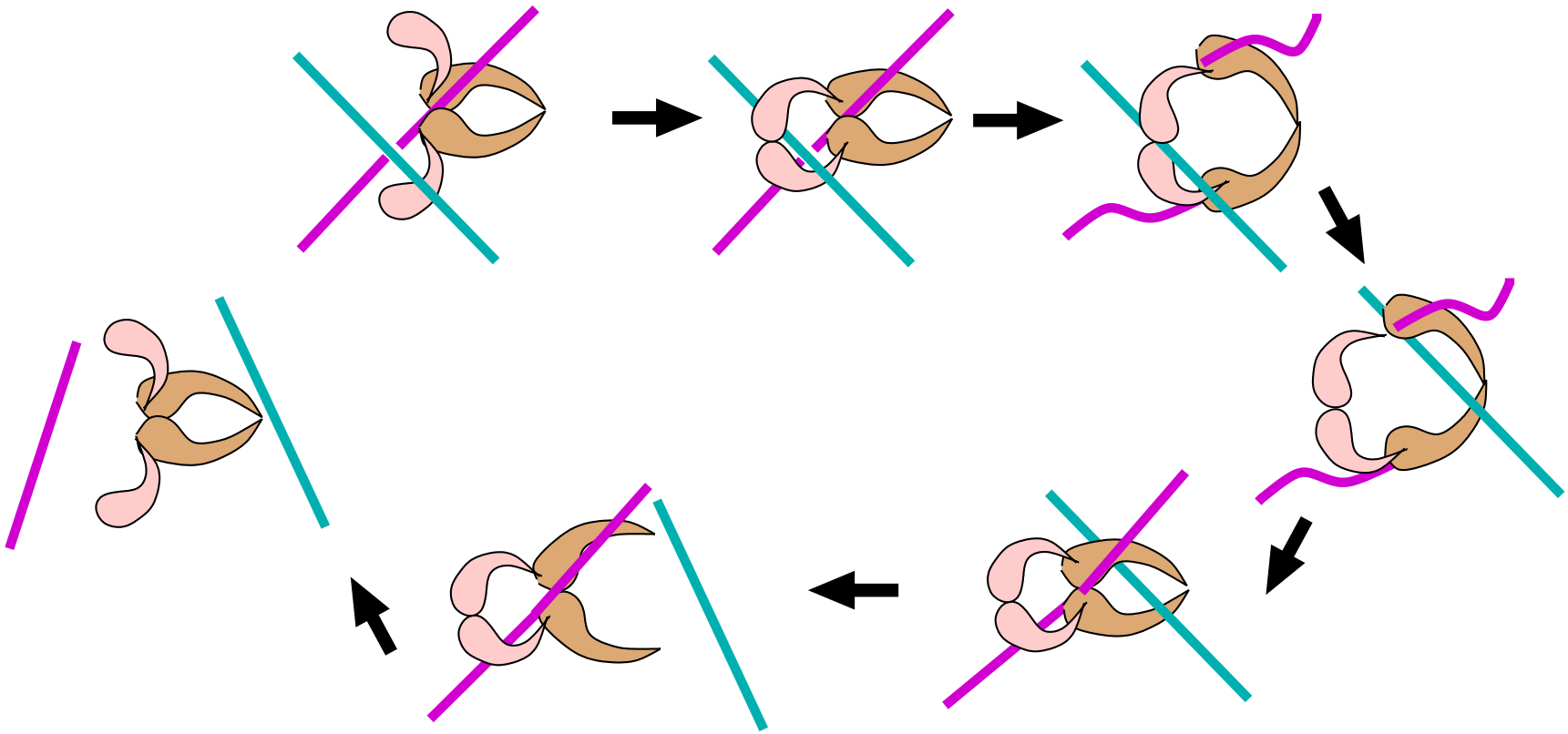
Topoisomerase IB. The topoisomerase IB nicks one single strand to make a pair of free ends and let one of the free ends rotate around the complete single strand.



Biological Unknotting Operations

Type II decreases the rotational stress of ds-DNA.

Topoisomerase II. The topoisomerase II makes a gap on the double strand DNA and let other piece of double strand pass through the gap, and reseal the gap.



Topological Questions

Biological Scenario

During the process of the DNA replication, if the ds-DNA is supercoiled or knotted, then topoisomerases make it simple.

However, we topologists might have the following questions.

Topologists' Questions

1. Do enzymes know if the ds-DNA is knotted?
   Not likely.
2. Do enzymes know which crossings should be changed?
   Not likely. It is not known how a knotted circle is deformed into a trivial circle with only local information.

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Possible Scenario

We propose a possible scenario:

Possible Scenario

1. The imaginary core curve γ keeps its triviality during the replication process.
2. Topoisomerases are allocated to the right place by a conformation of DNA.

There are mainly two topological problems.

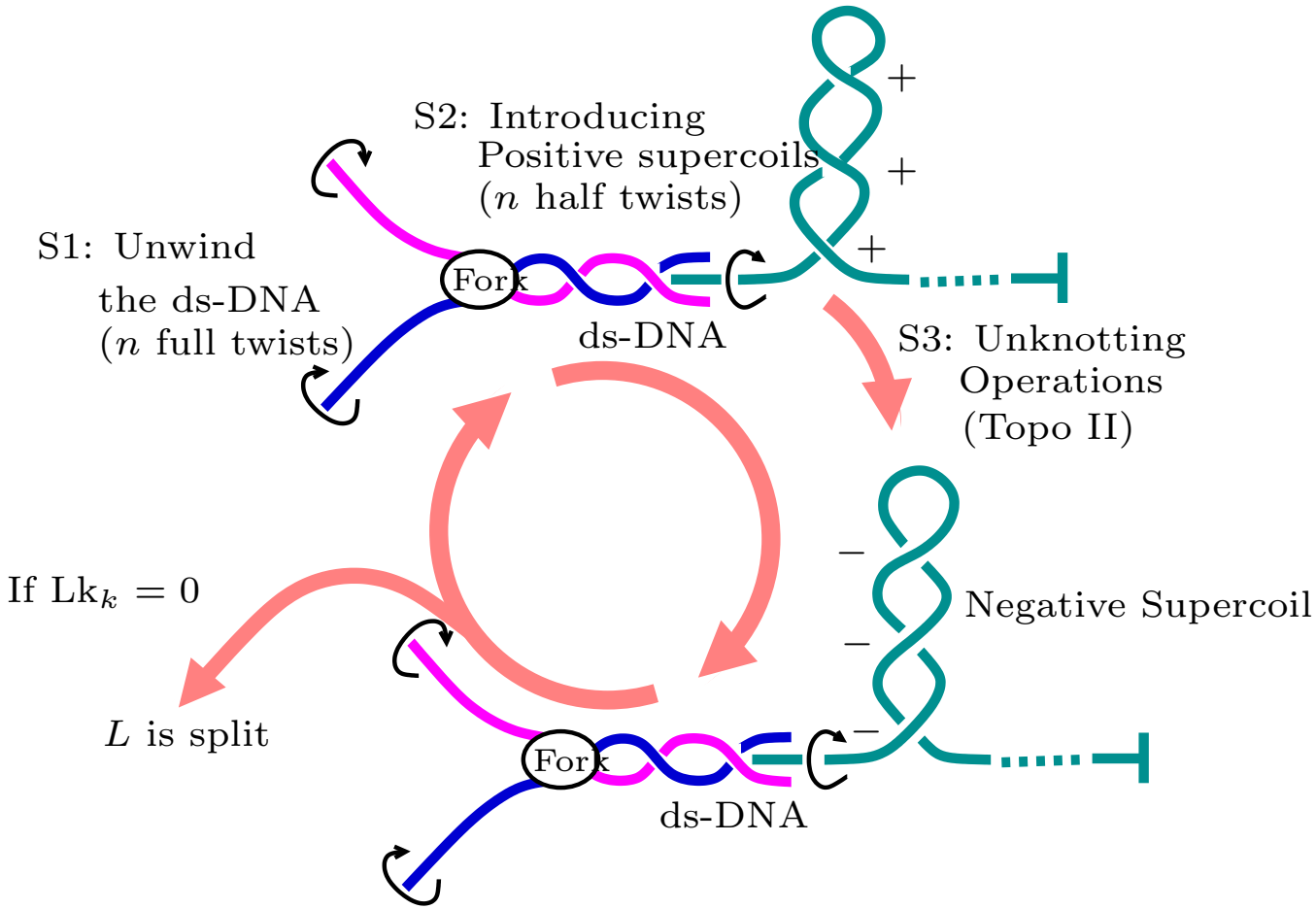
Problems

P1. How is the linking number of the DNA-link reduced?

P2. How are topoisomerases allocated to the right crossings that need to be changed to resolve supercoils?

Reduction Process

To solve P1: Reduction Problem, we propose the following procedure.



Proposition 3.1

Let L be a DNA-link with the initial twists Tw_0 and the initial writhe Wr_0. If the reduction process is applied to L k times, then the linking number Lk_k is given by the following.

$$\mathrm{Lk}_k = Tw_0 \left[2\left(1-c\right)^k - \left(1+\alpha c\right) \right], \tag{1}$$

where α is the writhe contribution to each nucleosome, and c is the rate of unwound full twists to Tw_0.

The number of repetition

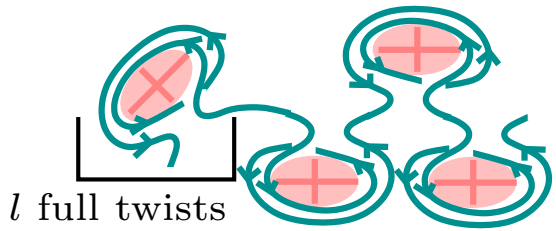
Assertion

The number n of unwound full twists is equal to the number of nucleosomes ahead of the fork.

Then n is proportional to Tw_0:

$$n = c\mathrm{Tw}_0 = \frac{\mathrm{Tw}_0}{l}, \tag{2}$$

where l is the number of full twists within the DNA around a nucleosome and its linker DNA.



After applying the unknotting operations to the chromatin fibre at the first stage, the number of full-twists Tw_1 is given by

$$\mathrm{Tw}_1 = \mathrm{Tw}_0 \left(1 - c\right)$$

At the kth stage,

$$\mathrm{Tw}_k = \mathrm{Tw}_0 \left(1 - c\right)^k , \tag{3}$$

where k is the number of repetition of the deformation cycles.

On the other hand, the initial writhe Wr_0 is given by the following.

$$\mathrm{Wr}_0 = -\alpha\tau_0 = -\alpha c \mathrm{Tw}_0$$

We obtain the following.

$$\mathrm{Wr}_k = -\mathrm{Tw}_0 \left(1 + \alpha c\right) + \mathrm{Tw}_0 \left(1 - c\right)^k \tag{4}$$

Therefore, the sum of (3) and (4) is the linking number after applying the procedure k times.

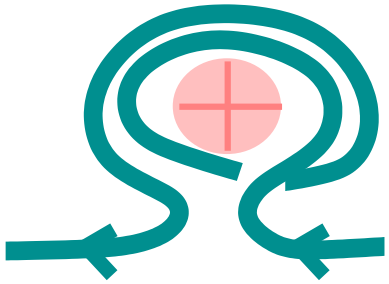
$$\begin{aligned} \mathrm{Lk}_k &= \mathrm{Tw}_k + \mathrm{Wr}_k \\ &= \mathrm{Tw}_0 \left[2\left(1 - c\right)^k - \left(1 + \alpha c\right)\right] \end{aligned} \tag{5}$$

Proposition 3.2

Suppose that the reduction system is applied to a DNA-link multiple times to obtain the linking number zero. The number of the repetition k is given by

$$k = \frac{\ln\left(\frac{1+\alpha c}{2}\right)}{\ln\left(1-c\right)} \tag{6}$$

Since the number α is a constant, k is determined by the parameter c, which is given by Tw_0.



$197\,\mathrm{bp}$
$= 18.8$ full twists

For a nucleosome, the DNA wraps around the histone core about 1.8 times. The total length of the DNA is $197\,\mathrm{bp}$, which is 18.8 full twists:

$$l = \frac{197}{10.5} \approx 18.8, \qquad c = \frac{1}{l}$$

A recent study[4] shows that the writhe contributed to each nucleosome is -1.26. Thus $k = 11.5$. This means we need to repeat the process 11.5 times to get the linking number zero.

[4] J. Segura et al. Intracellular nucleosomes constrain a DNA linking number difference of- 1.26 that reconciles the lk paradox. Nature communications, 9(1):1–9, 2018.

Reduction with Topo I

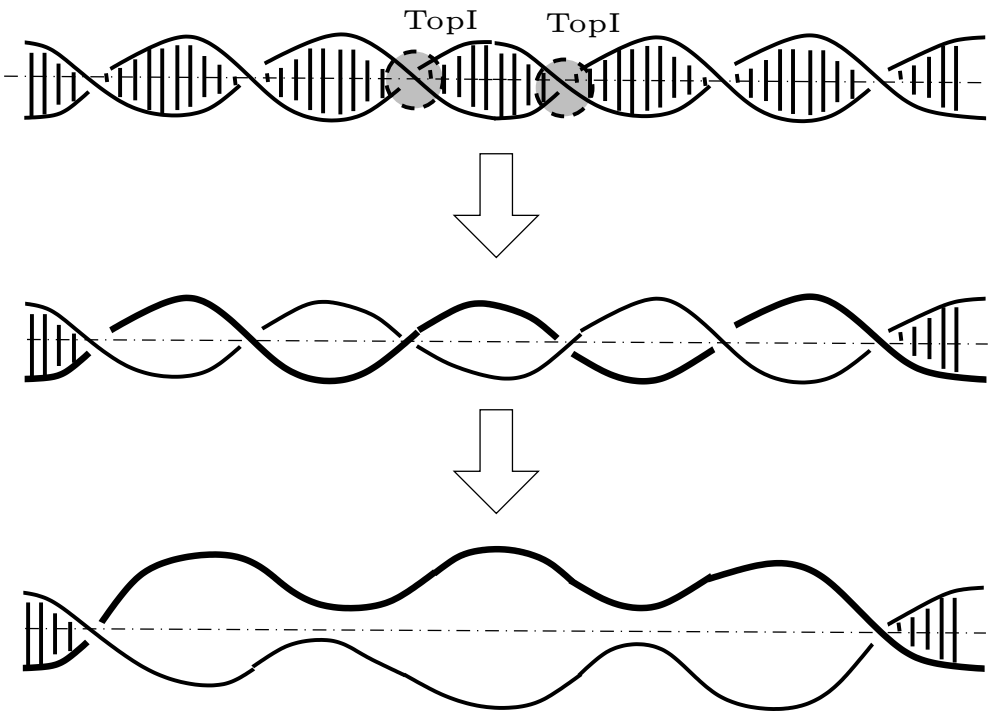


Figure 1:

This reduction reduces the twisting number Tw_0 to $0.2\mathrm{Tw}_0$.

This gives $c' = \frac{5}{l}$. With $\alpha = 1.26$, we obtain

$$k = 1.3$$

Topo II allocation

We assume that the chromatin fibre has the (juxtapositioned) shape.
There will be an ε-crossing near a nucleosome.

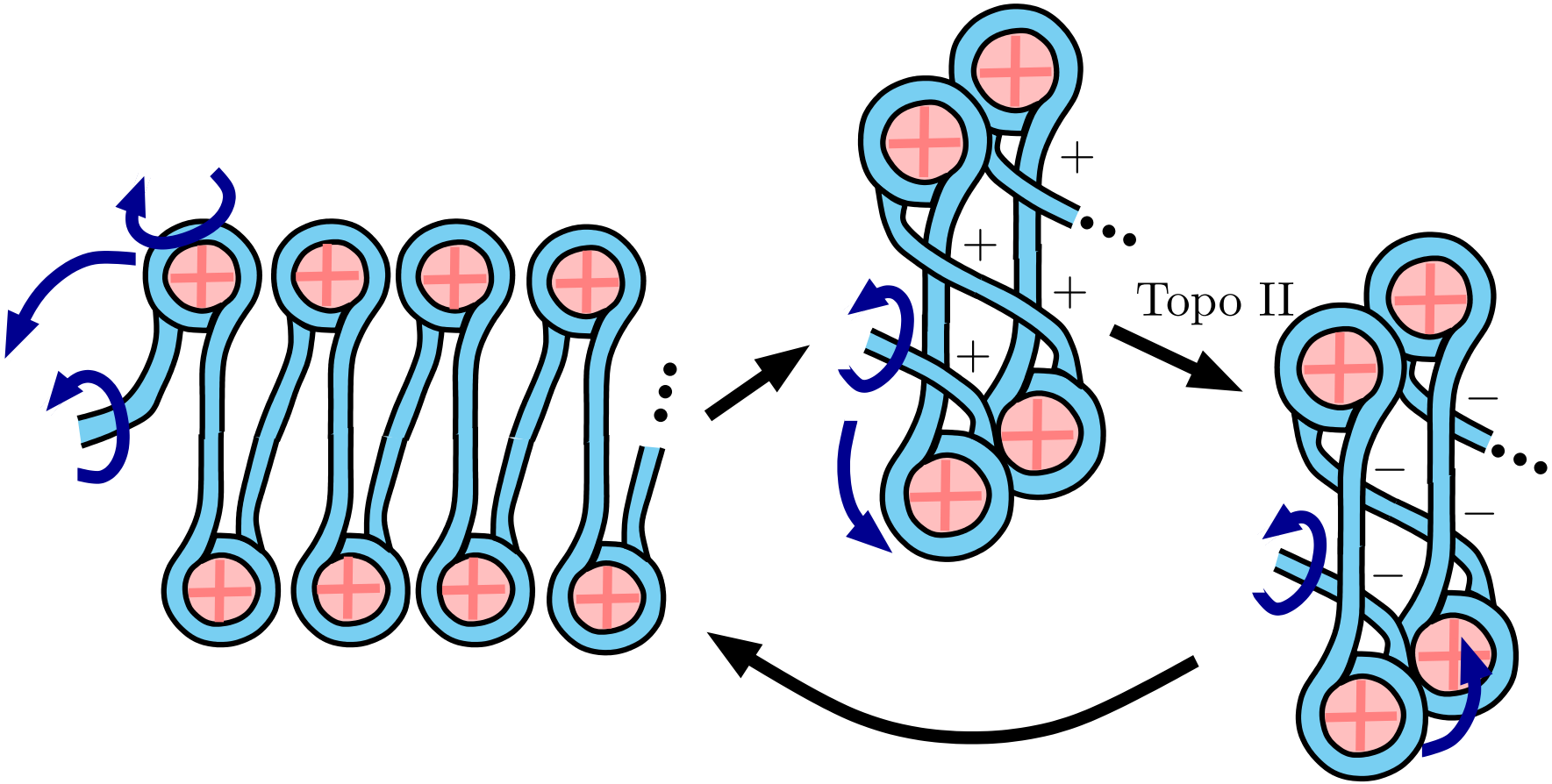
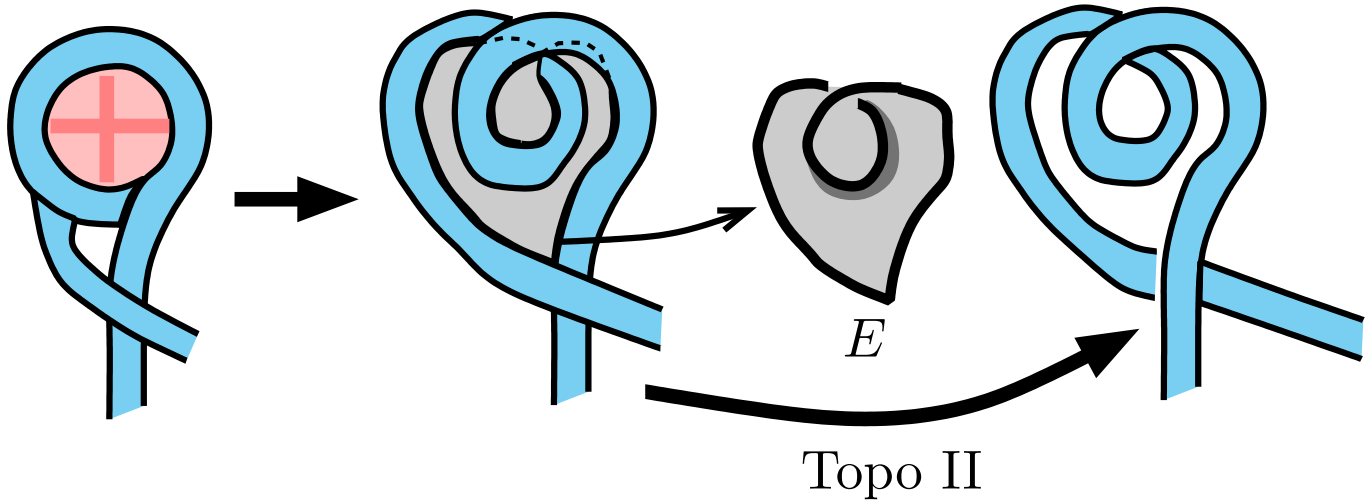


Figure 2: Unknotting operations near nucleosomes.

The unknotting operation does not change the knot type.



The histone core plays a role of an embedded disc bounded by the loop.

Conclusion and further study

We obtained the following conclusion.

Our splitting process of DNA-links shows:

1. Keeping the triviality of γ works well.
2. Combining type I and II topoisomerases efficiently simplify DNA.
3. Enzymes are allocated to right places by a special conformation of DNA.

The further investigation needs to be done on

1. supercoils behind the forks,
2. other types of topoisomerases (e.g. topoisomerase IB), and
3. time duration of the replication.

Possible application

For Anti-Cancer Medicines.

Thank You!