Nanobiotechnology and Nanomedicine
Course code: 601104

Centre for Nanoscience and Technology
Aryabhatta Knowledge University, Patna
Mithapur, Patna
Bihar

Content developed by: Dr. Vijay Kumar Ravi

DNA present in Bacteria

- *E. coli* K-12 has a genome of approximately 4.6 million nucleotide pairs which is contained in a single circular molecule of DNA.
- It codes for about 4300 different kinds of proteins.
- Bacteria lack a special nuclear compartment, and carry their genes on a single DNA molecule, which is often circular.
- This DNA is also associated with proteins that package and condense it.
- Protein associated with it are different from the proteins that carry these functions in eukaryotes.
- The bacterial DNA with its attendant proteins is often called the bacterial “chromosome.”
- It does not have the alike structure as eukaryotic chromosomes.
- Not much studied how the bacterial DNA is packaged.

Lecture-2 continued

Genome of *E. coli* strain K-12

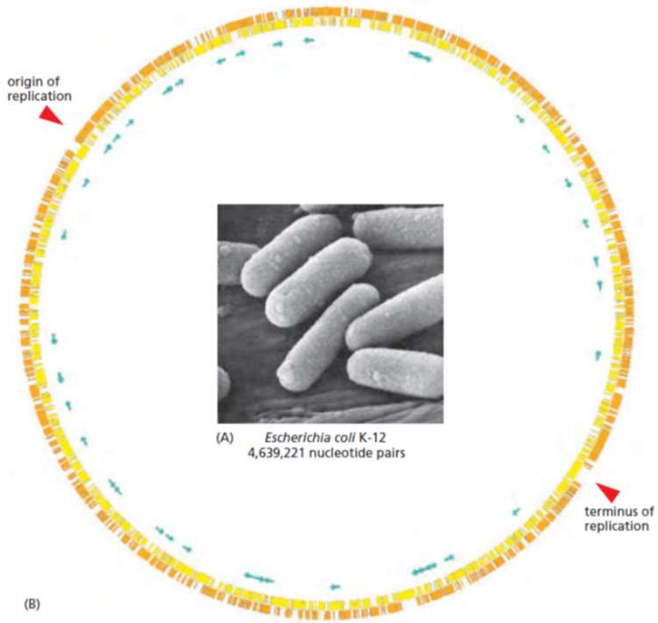


Figure : **The genome of *E. coli.* (A) A cluster of *E. coli* cells. (B) A diagram of the genome of *E. coli* strain K-12. The Image is circular because the DNA of *E. coli*, like other prokaryotes, forms a single, closed loop. Protein-coding genes are shown as *yellow* or *orange bars*, depending on the DNA strand from which they are transcribed; genes encoding only RNA molecules are indicated by *green arrows*. (A, courtesy of Dr. Tony Brain and David Parker/Photo Researchers; B, adapted from F.R. Blattner et al., *Science* 277:1453–1462, 1997.)**

DNA replication in Bacteria

- *E. coli takes* about 30 minutes to duplicate its genome of 4.6 × 10^6 nucleotide pairs.
- No Okazaki fragments are shown on the lagging strand.
- What happens as the two replication forks approach each other and collide at the end of the replication cycle is not well understood.

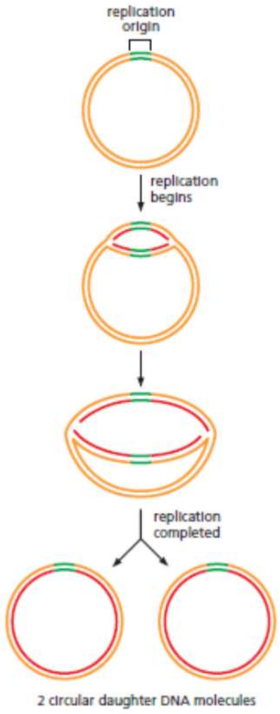


Figure: DNA replication of a bacterial genome.

DNA supercoiling in Bacteria

- **Supercoiling of DNA is process of over or under-winding of a DNA strand.**
- **DNA supercoiling is important for DNA packaging inside all cells and also play a role in gene expression.**
- **DNA molecules are negatively supercoiled inside cells.**
- **The degree of supercoiling is not equal throughout the genome.**
- **Majority of 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.**

- **Enzymes such as topoisomerases enable to change DNA topology to facilitate functions such as DNA replication or transcription. Mathematical expressions are used to describe supercoiling by comparing different coiled states to relaxed B-form DNA.**

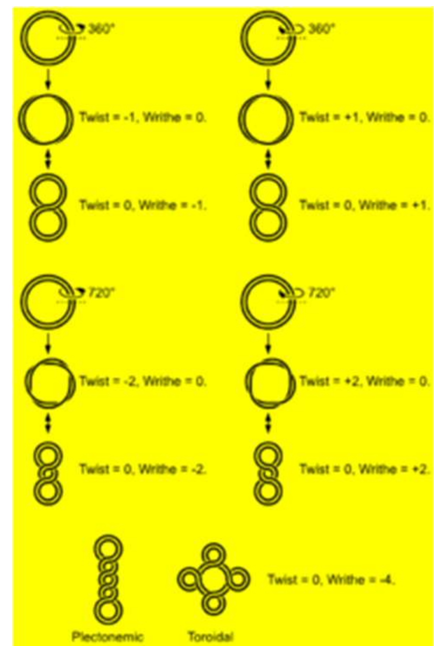
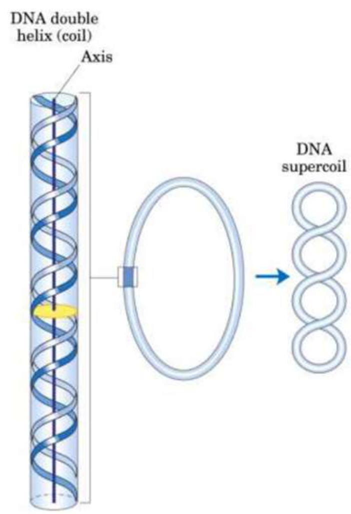


Fig: DNA supercoiling

Supercoiled structure of linear DNA molecules with constrained ends



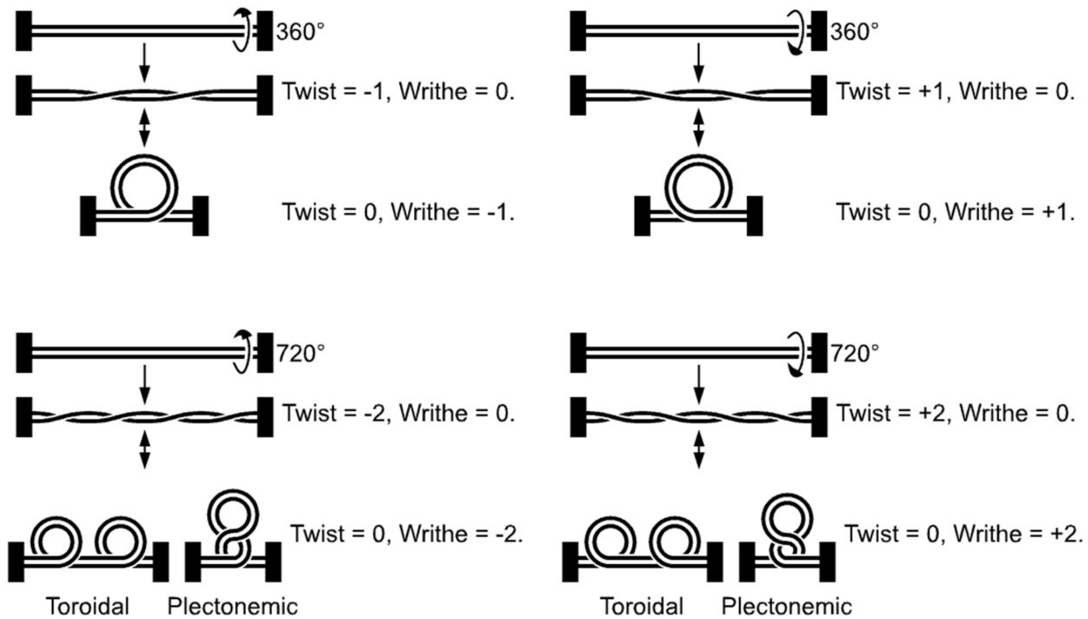
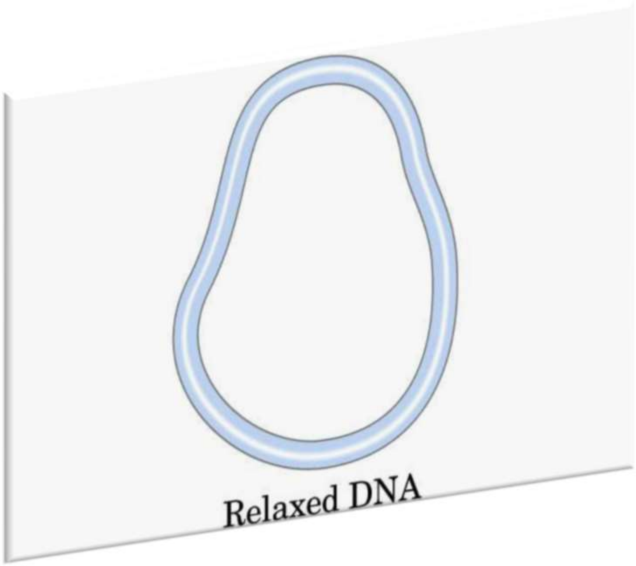


Fig: Supercoiled structure of linear DNA molecules with constrained ends. The helical nature of the DNA duplex is omitted for clarity.

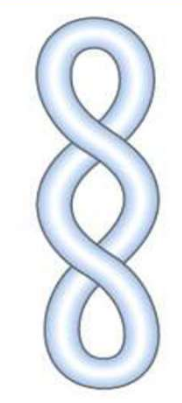
Positive and Negative supercoiling

- In the relax state the DNA helix has the normal number of base pairs per helical turn.
- When helix is over twisted, it becomes tighter as result the edges of the narrow groove move closer together.
- If the helix is under twisted, the edges of the narrow groove tends to move further apart.
- The changing the twist from the relaxed state requires additional and increases the stress along the molecule.



Positive supercoiling

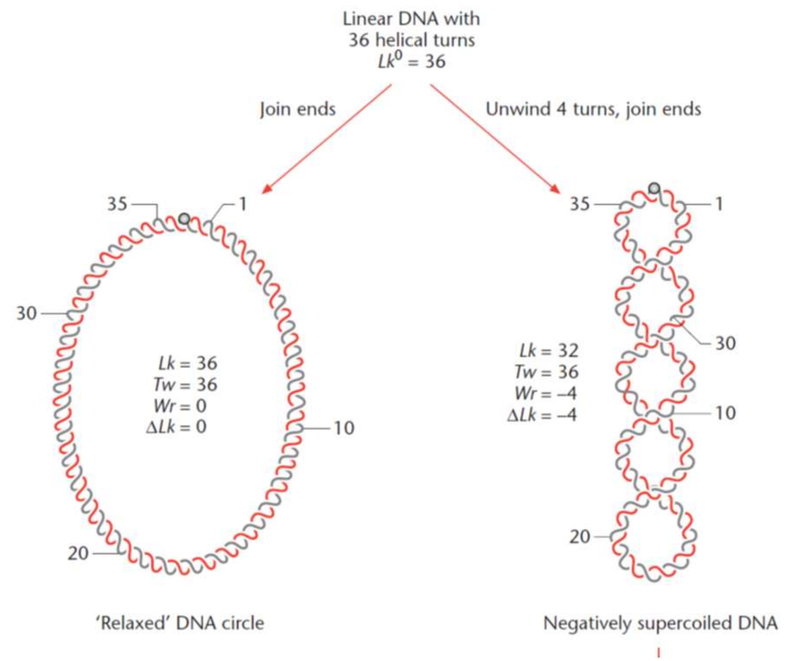
- Positive supercoiling is the right-handed and double helical form of DNA.
- It is twisted tightly in a right handed direction until the helix creates knot.
- The positive supercoiling is more condensed as the supercoil forms at the direction of DNA helix



Positive supercoils

Negative supercoiling

- The negative supercoiling is the left-handed and double helical form of DNA.
- Prokaryotes and Eukaryotes usually have negative supercoiled DNA.
- It is naturally prevalent as it prepares the molecule for processes that require separation of the DNA strands without the need of additional energy.



Courtesy: Richard Peter Bowater

Mathematical Expression of supercoiling

- If linear double-stranded DNA molecules is 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.
- At this condition 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.
- Upon circularization, this number must be an integer and is known as the linking number of the cccDNA (covalently closed circular DNA shown as figure in previous slide

Mathematical Expression of supercoiling

A given length of DNA has an inherent number of double-helical turns, 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

$$Lk^0 = N/h$$

In terms of cccDNA, Tw and Wr are complementary geometric parameters. Tw defines how the individual strands of DNA coil around the axis of the DNA helix, Wr defines 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:

$$Lk = Tw + Wr$$

Mathematical Expression of supercoiling

Experimental studies in 1970s (Vologodskii and Cozzarelli, 1994a) established that the free energy of a supercoiled DNA sample free energy of supercoiled has a quadratic dependence on linking difference shown below equation, where K is a DNA length-dependent constant.

$$\Delta G_{sc} = K.\Delta Lk^2$$

(ΔG_{sc}) **Free energy of super coiled DNA**

ΔLk: **Linking difference**

Tutorial-1

1. Write the function of DNA.
2. Describe the importance of DNA prospective to nanobiotechnology.
3. Write assay on DNA as future nanodevice.

References

- **J. D. Watson and F.H.C Crick, Nature 171, (1953), 737-738.**
- **B. Alberts, A. Johnson, J. Lewis, D. Morgan, M. Raff, K. Roberts and P. Walter, Molecular Biology of the Cell, 6th edition, Garland Science, Taylor and Francis group.**
- **R. P. Bowater Supercoiled DNA structure, DOI: 10.1038/npg.els.0001040.**
- **https://en.wikipedia.org/wiki/DNA**
- **https://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397/**

THANK YOU