Replication

- **Replication overview:**

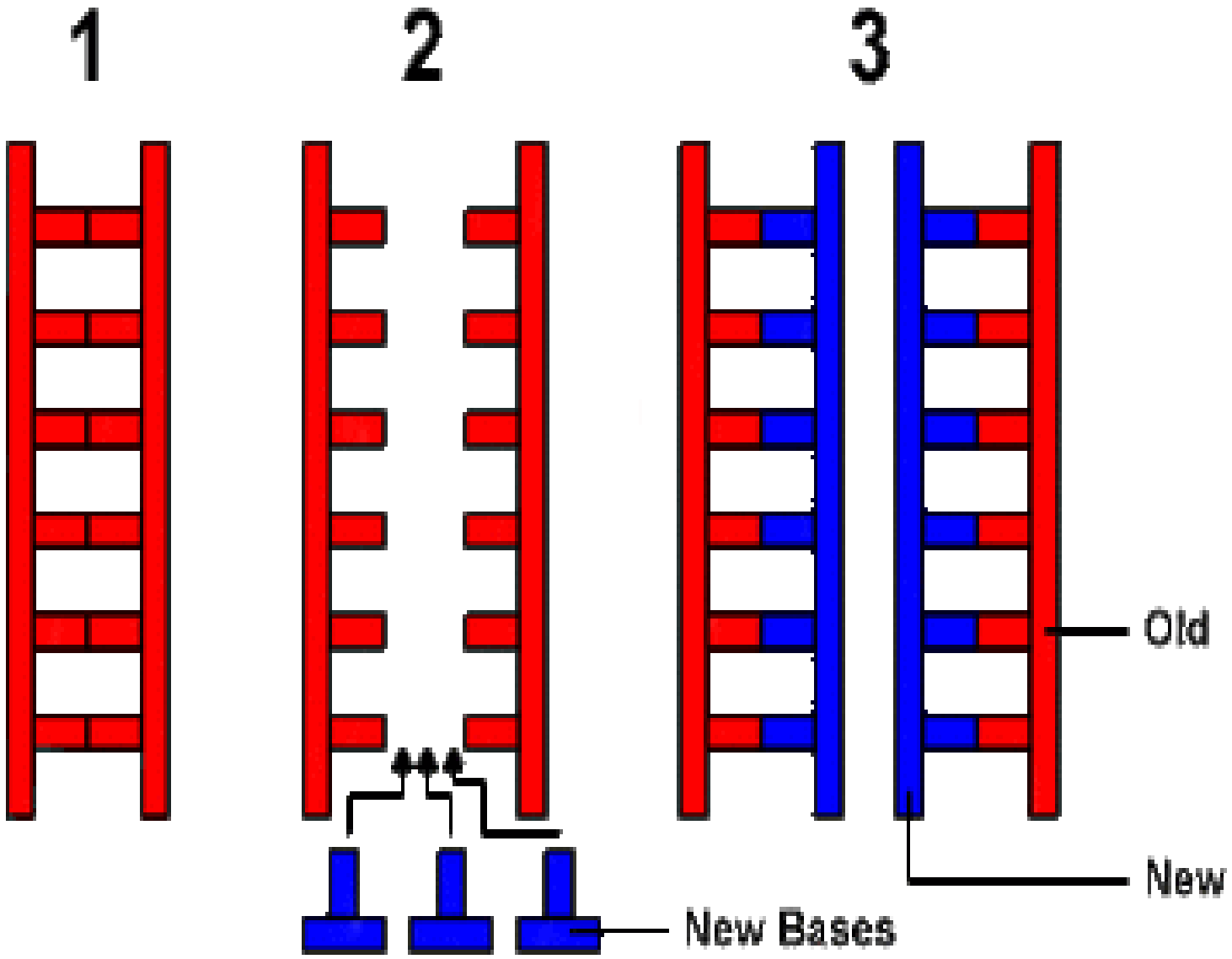
-Double–stranded DNA unwinds.

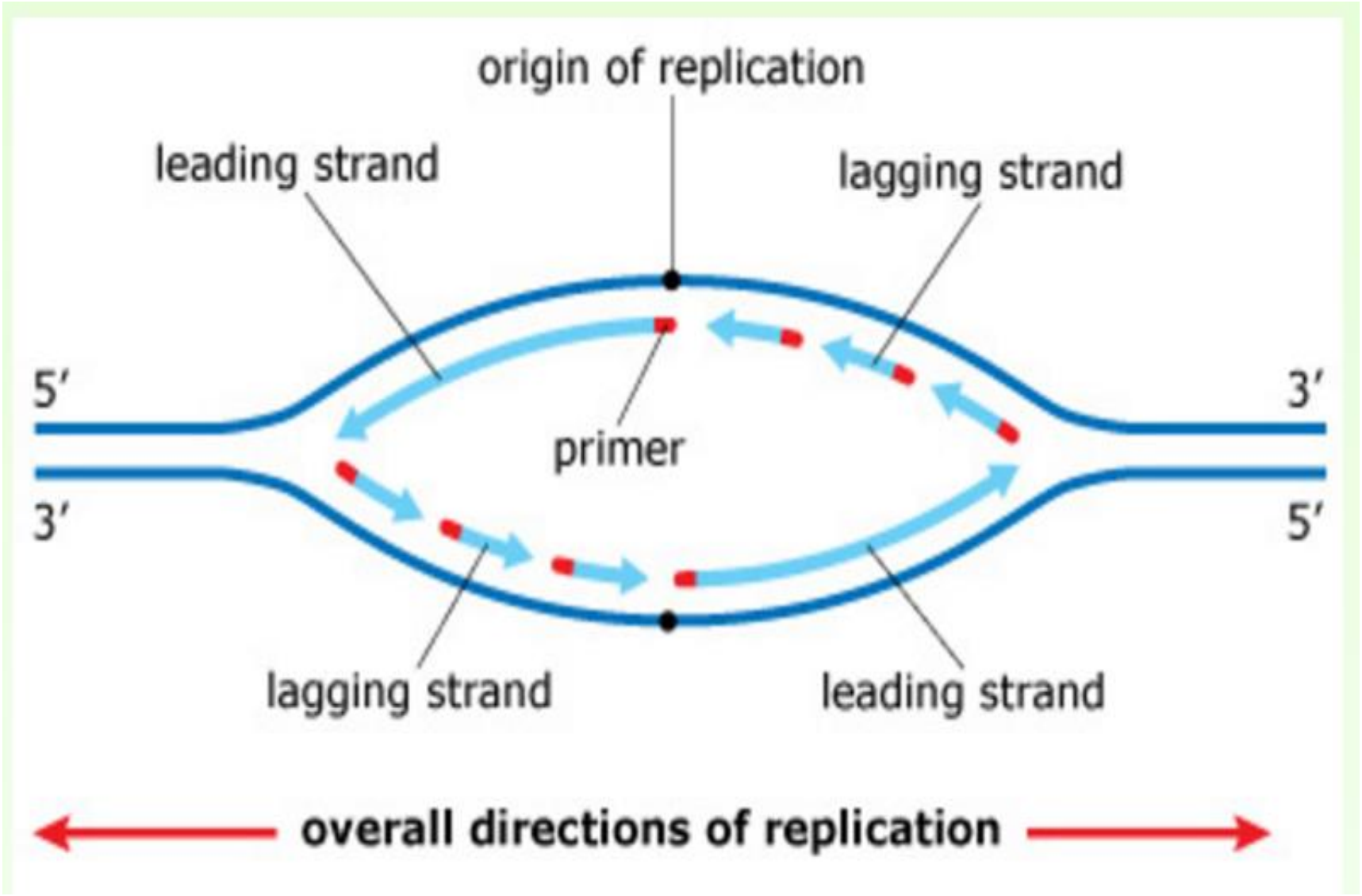
-The junction of the unwound molecules is a replication fork.

-A new strand is synthesized according to base pairing rule with the parent strand.

-Two molecules of DNA are synthesized (daughters), each has one new and one old DNA strand.

-Replication is bidirectional: this means that the replication forks move in both direction away from the origin.


1
2
3
Old
New
New Bases


origin of replication
leading strand
lagging strand
5′
3′
primer
3′
5′
lagging strand
leading strand
overall directions of replication

❖Replication

- The **synthesis of DNA**. It is the copying and transformation of genetic information found in DNA to daughter cells. It occurs during the **S phase** of cell cycle.
- **DNA replication is Semiconservative:**

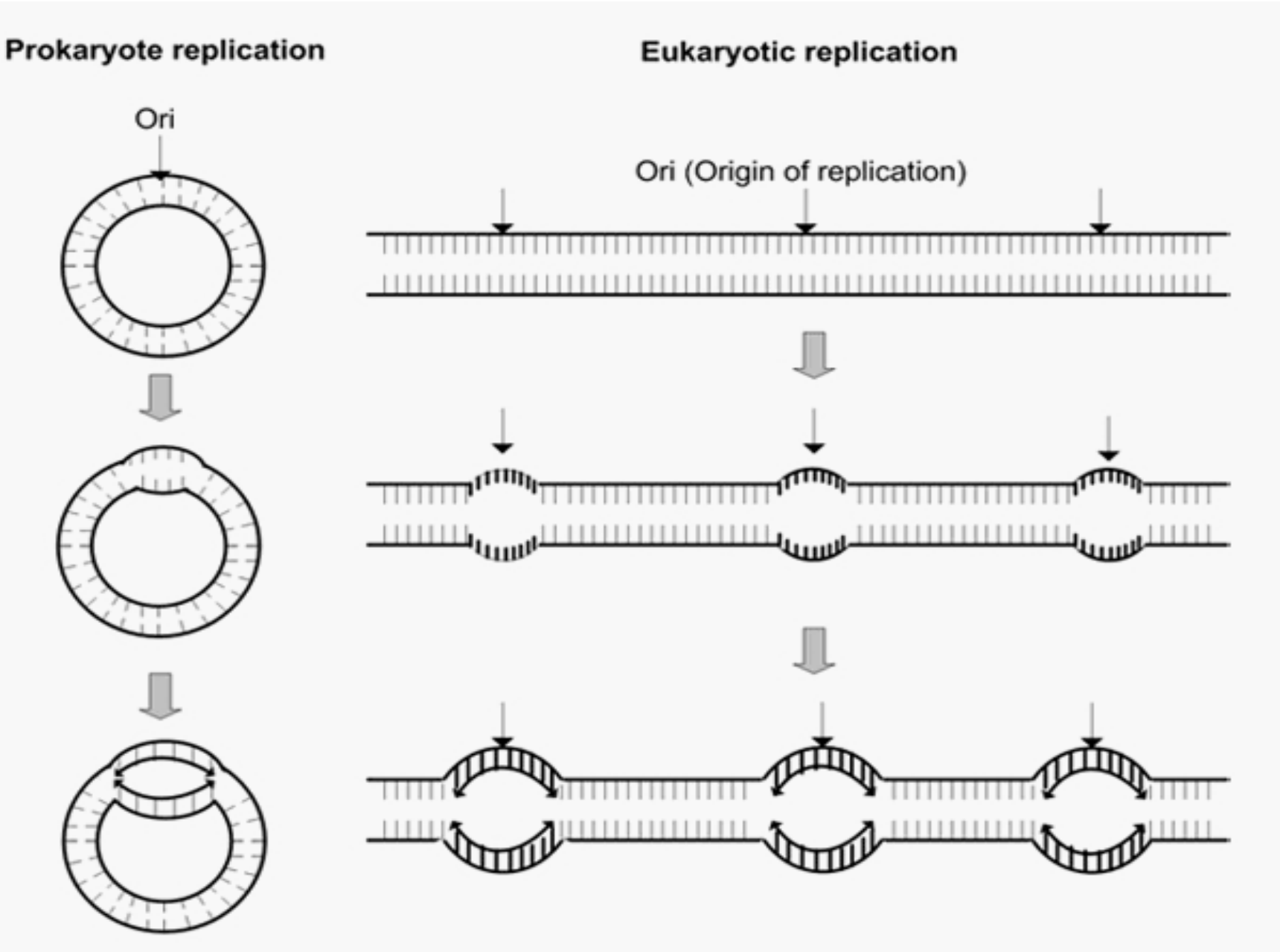
-During DNA replication the old strands separate from each other, and every strand acts as a template for the formation of new complementary strand according to the **base –pairing rule**.

-Semiconservative means that the newly formed two daughters DNA contain **one original old** strand and **one newly formed complementary strand**. **This is important to transfer genetic information in the correct sequence.**

Steps of DNA replication:

- **A-Separation of the two DNA strands:**

1-DNA replication starts at a specific DNA sequence called the **origin of replication**. In prokaryotes it is **single** and termed **oriC.** While in eukaryotes there are **multiple** origins of replication that contain AT base pairs and called **autonomous replication sequences (ARS).**


Prokaryote replication
Ori
Eukaryotic replication
Ori (Origin of replication)

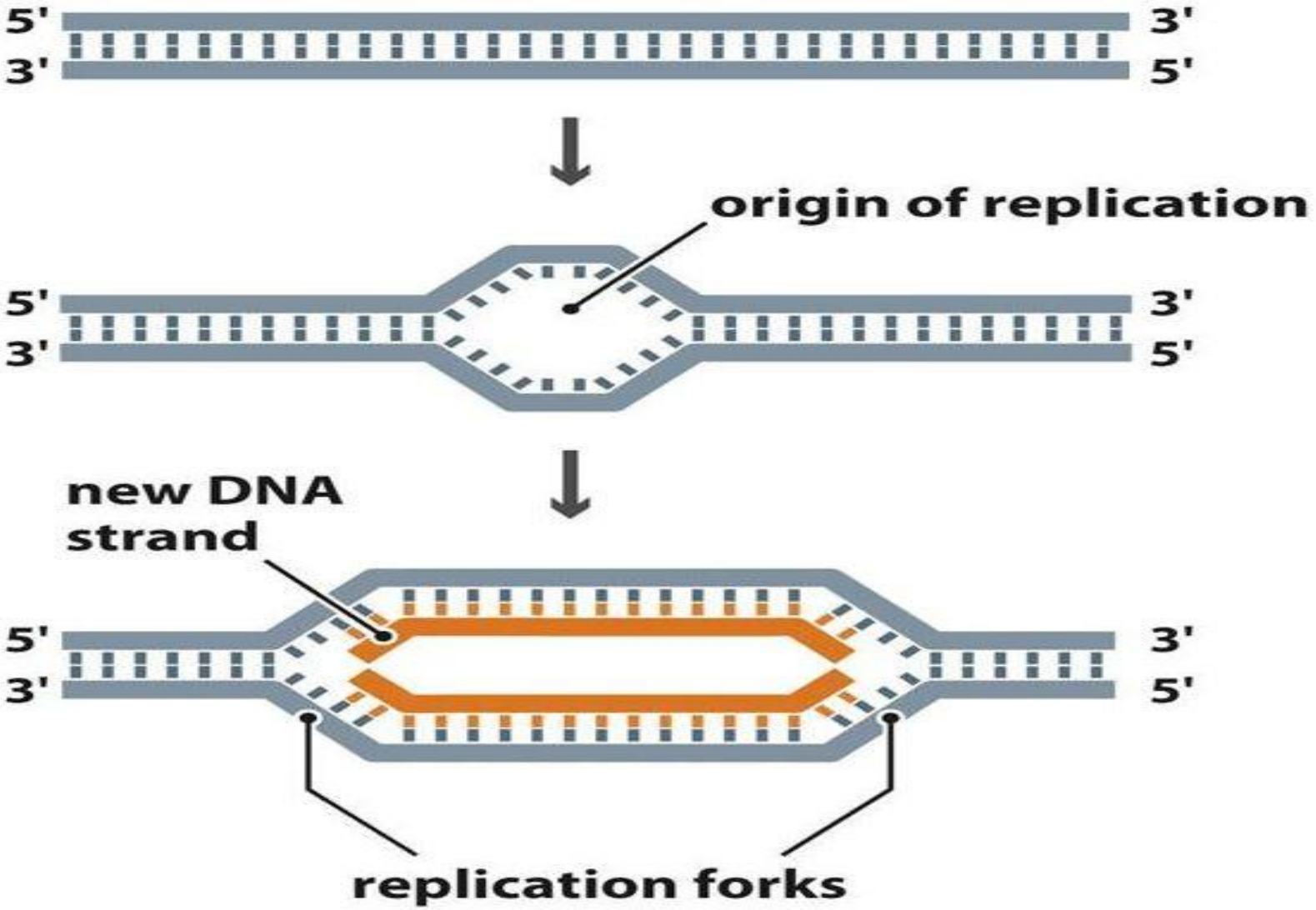
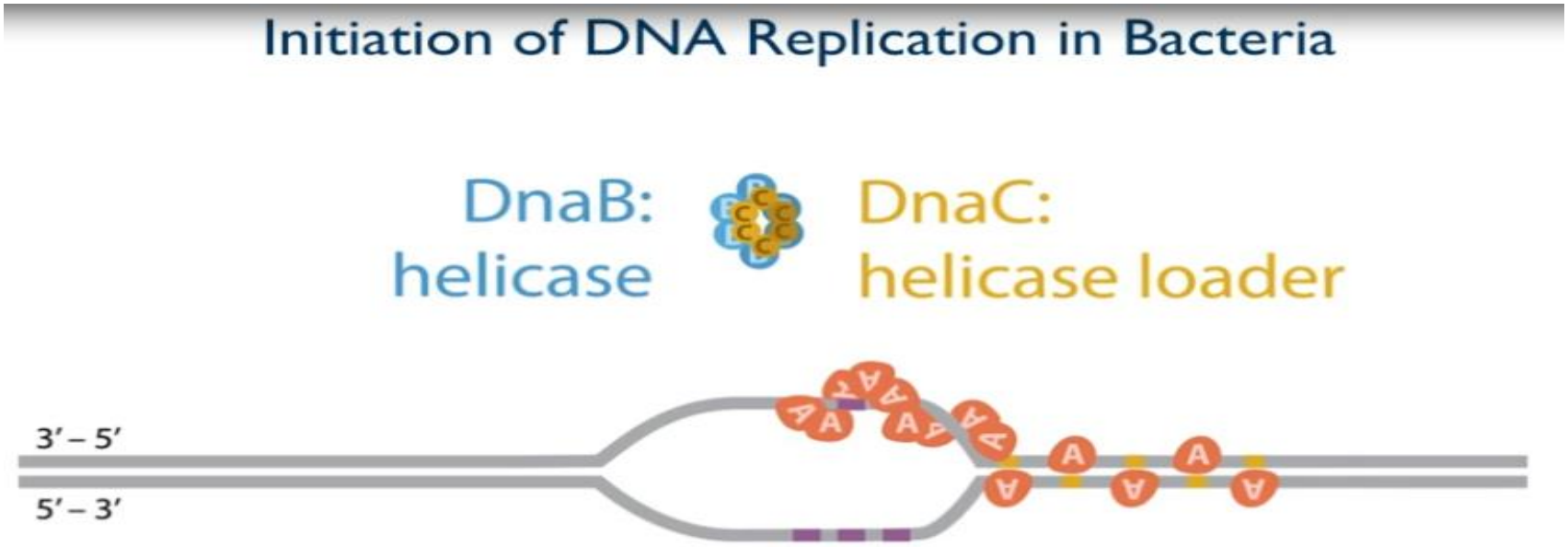


Figure 10.16 Introduction to Genetics (© Garland Science 2012)

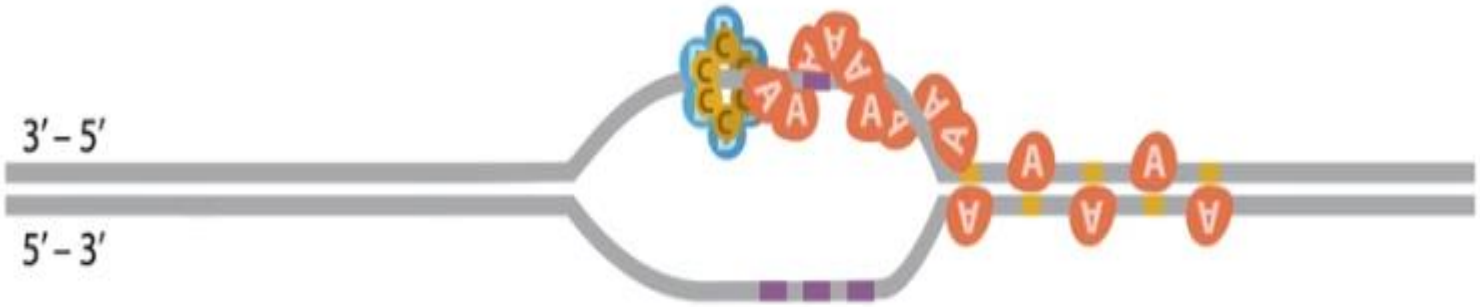
- **2-A protein called dnaA protein recognizes the origin of replication and separates the two DNA strands at a very small region in origin of replication site by ATP hydrolysis.(i.e. unwinding is initiated by binding of dnaA to the origin of replication and this allows helicase enzyme to have access to DNA strands.)**

- **It is hypothesized that DNA stretching by DnaA bound to the origin promotes strand separation which allows more DnaA to bind to the unwound region.**

- **The DnaC helicase loader then interacts with the DnaA bound to the single-stranded DNA to recruit the DnaB helicase.**

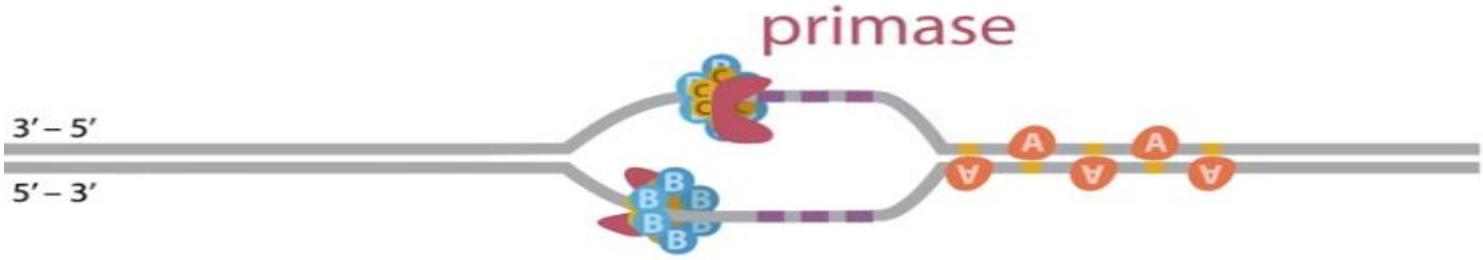


After the initiator (DnaA) has bound to oriC, the combination of ssDNA and DnaA recruits a complex of two proteins:
the DNA helicase (DnaB) and helicase loader (DnaC). Importantly, binding to the helicase loader inactivates the DNA helicase, preventing it from functioning at inappropriate sites (non origin regions).



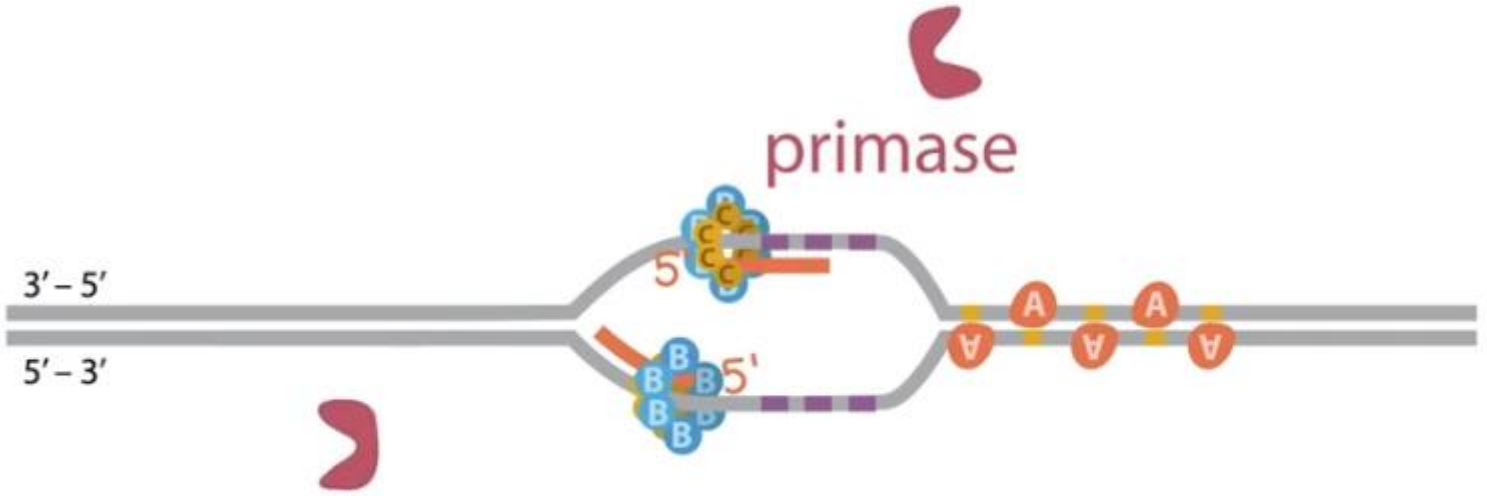
Although the mechanism of loading is not understood in detail, the process requires the opening of the DNA helicase hexameric ring to allow it to encircle the targeted ssDNA

Initiation of DNA Replication in Bacteria

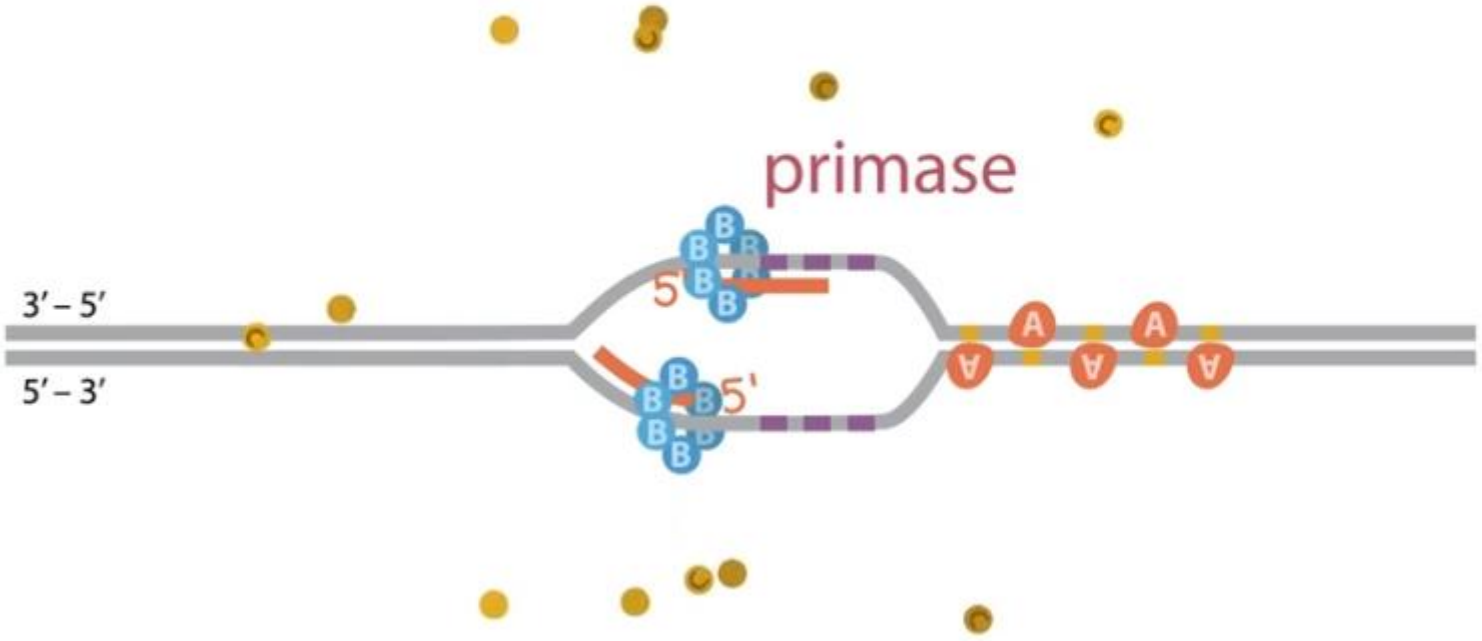


Helicase recruits DNA **primase** to the origin DNA, resulting in the synthesis of an RNA primer on each strand of the origin. In addition to generating the primers for the leading DNA strands, this event also causes the release of the helicase loader and, therefore, the activation of the helicase.

Initiation of DNA Replication in Bacteria



Initiation of DNA Replication in Bacteria

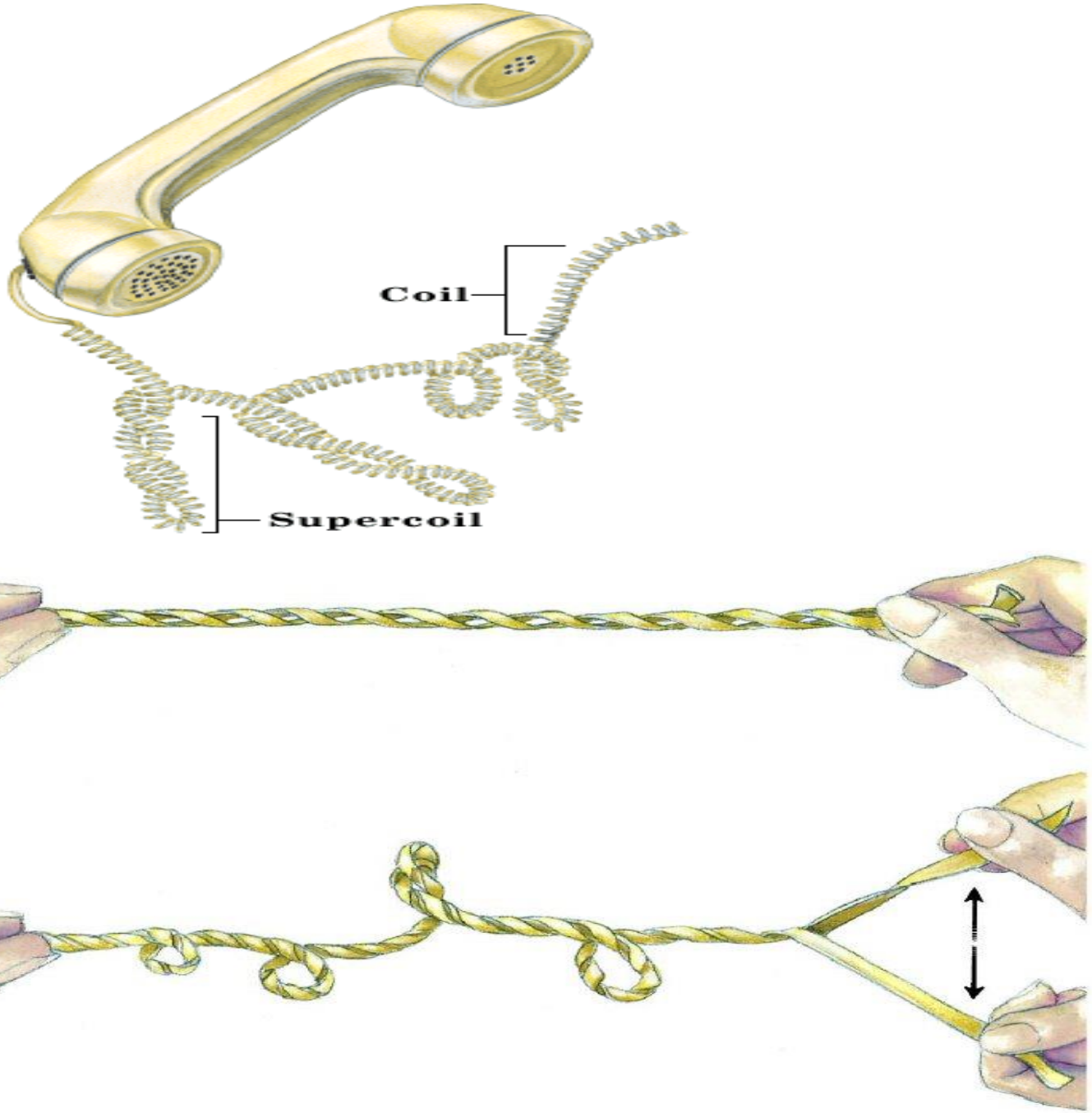


3-DNA helicase (dnaB protein) enzyme separates the double helix by breaking the hydrogen bonds between the two DNA strands using energy from ATP hydrolysis.

4-The 2 DNA strands are kept apart (unpaired) by special proteins known as single strand DNA binding proteins (SSB) ,which binds tightly to each separated strands preventing them from rejoining & protect the single stranded DNA from nucleases that cleave it.

5-Prepriming complex: formed from dnaA protein, dnaC protein, SSB proteins, and DNA helicases. This complex is responsible for replication initiation and maintaining the separation of the two DNA strands.

6-DNA Topoisomerases: are responsible for removing supercoils in the helix formed as the 2 strands are separated from each other ,this creates coils in front of the separated part (supercoils) which prevents further separation of the helix. Topoisomerases have both nuclease (strand cutting) and ligase (strand resealing) activities. Topoisomerases make transient cut (in the phosphodiester bond) in one strand (**topoisomerase I**) or both stands (**topoisomerase II**).


Coil
Supercoil

7-DNA gyrase, a Type II topoisomerase found in bacteria and plants, has the unusual property of being able to introduce negative supercoils into relaxed circular DNA using energy from the hydrolysis of ATP. This facilitates the future replication of DNA because the negative supercoils neutralize the positive supercoils introduced during opening of the double helix. It also aids in the transient strand separation required during transcription.

8-Now each relaxed single strand acts as a template to direct the synthesis of a new daughter DNA strand.

❖Clinical significance:

- **Quinolones** antimicrobial drugs e.g. nalidixic acid (Negram) act by inhibiting bacterial gyrase preventing bacterial replication and transcription.
- **Anticancer agents**, such as etoposide, target human topoisomerase II.