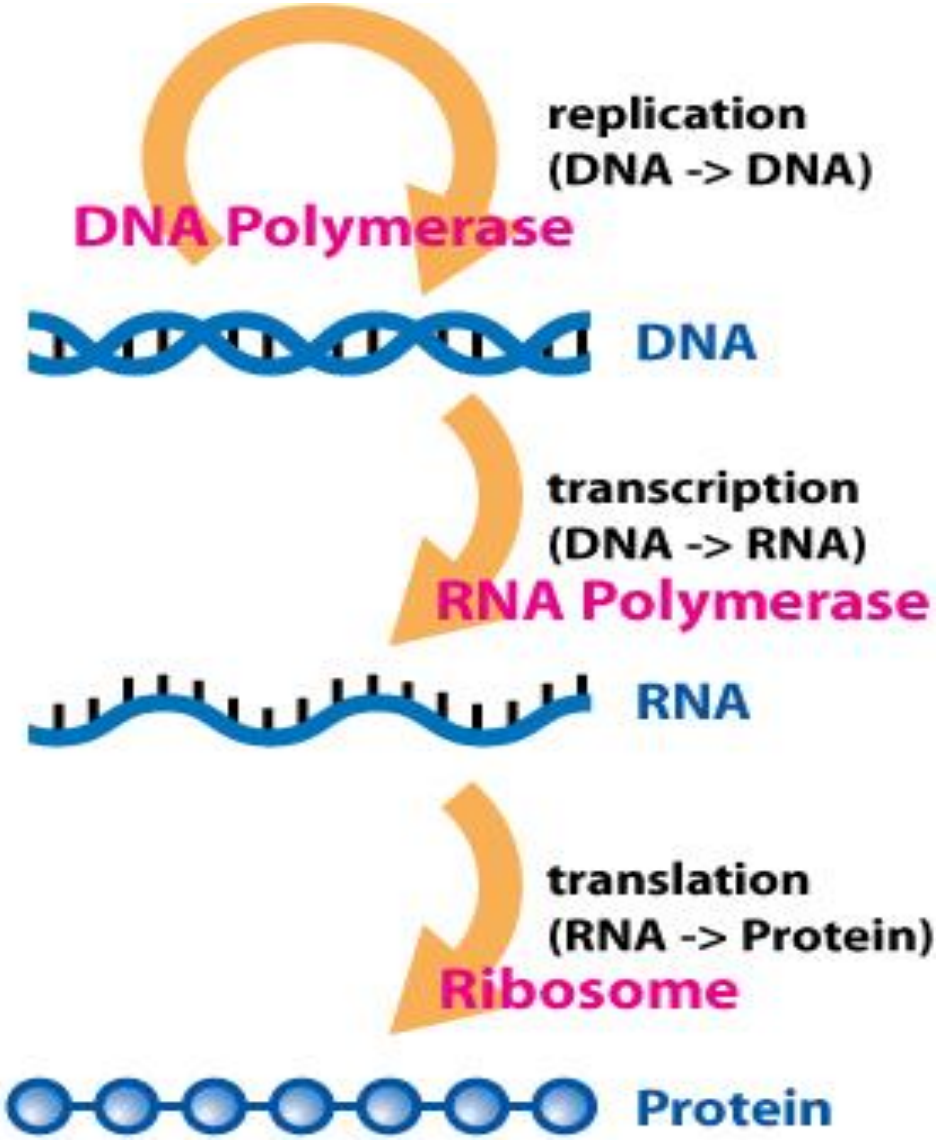
DNA REPLICATION

Specific Learning Objectives

At the end of lecture, student should able to

- enumerate various rules and steps of replication
- enumerate different DNA polymerase in eukaryote and prokaryote
- explain DNA polymerization on leading and lagging strands
- enumerate various inhibitors of DNA replication

Central Dogma of Molecular Biology



Replication

- DNA copies itself to produce identical daughter DNA –

synthesis of DNA

DNA Replication

Process of duplication of the entire genome prior to cell division

Biological significance:

- Basis for inheritance
- Fundamental process occurring in all cells for copying DNA to transfer the genetic information to daughter cells
- extreme accuracy of DNA replication , to preserve the integrity of the genome in successive generations
- In eukaryotes, replication : S phase of the cell cycle.
- Replication rate in eukaryotes is slower resulting in a higher fidelity/accuracy of replication in eukaryotes

Basic rules of replication

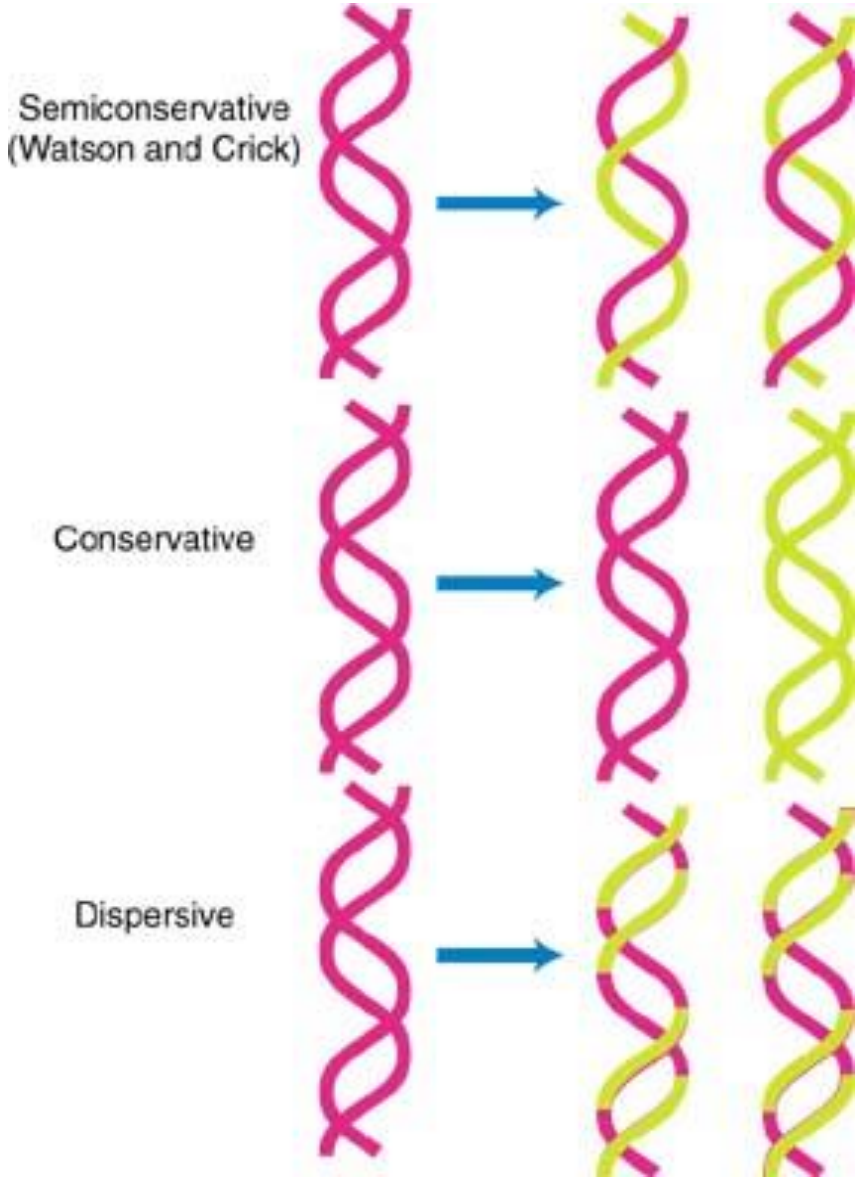
A. Semi-conservative
B. Starts at the ‘origin’
C. Synthesis always in the 5-3’ direction
D. Can be uni or bidirectional
E. Semi-discontinuous
F. RNA primers required

Replication: Mechanism

- **Semiconservative** : (accepted)
  Half of the parental (original) DNA is conserved in the daughter DNA
- Proved by: Meselson & Stahl

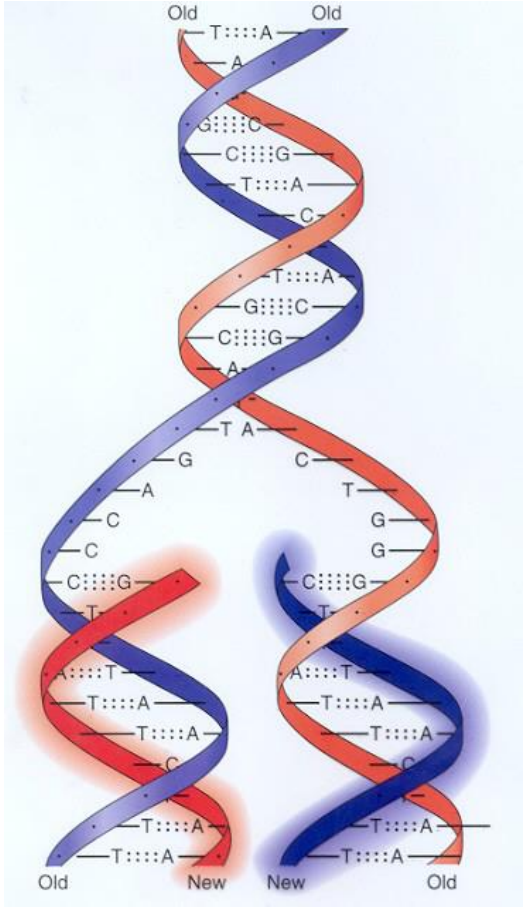
- **Conservative**: (not accepted)
  after the replication, both the strands of the daughter DNA are newly synthesized. Where as, parent-DNA retains both the original strands.

- **Dispersive**: (hypothetical)
  parent-strand is randomly scattered in the Newly synthesized double helical DNA.

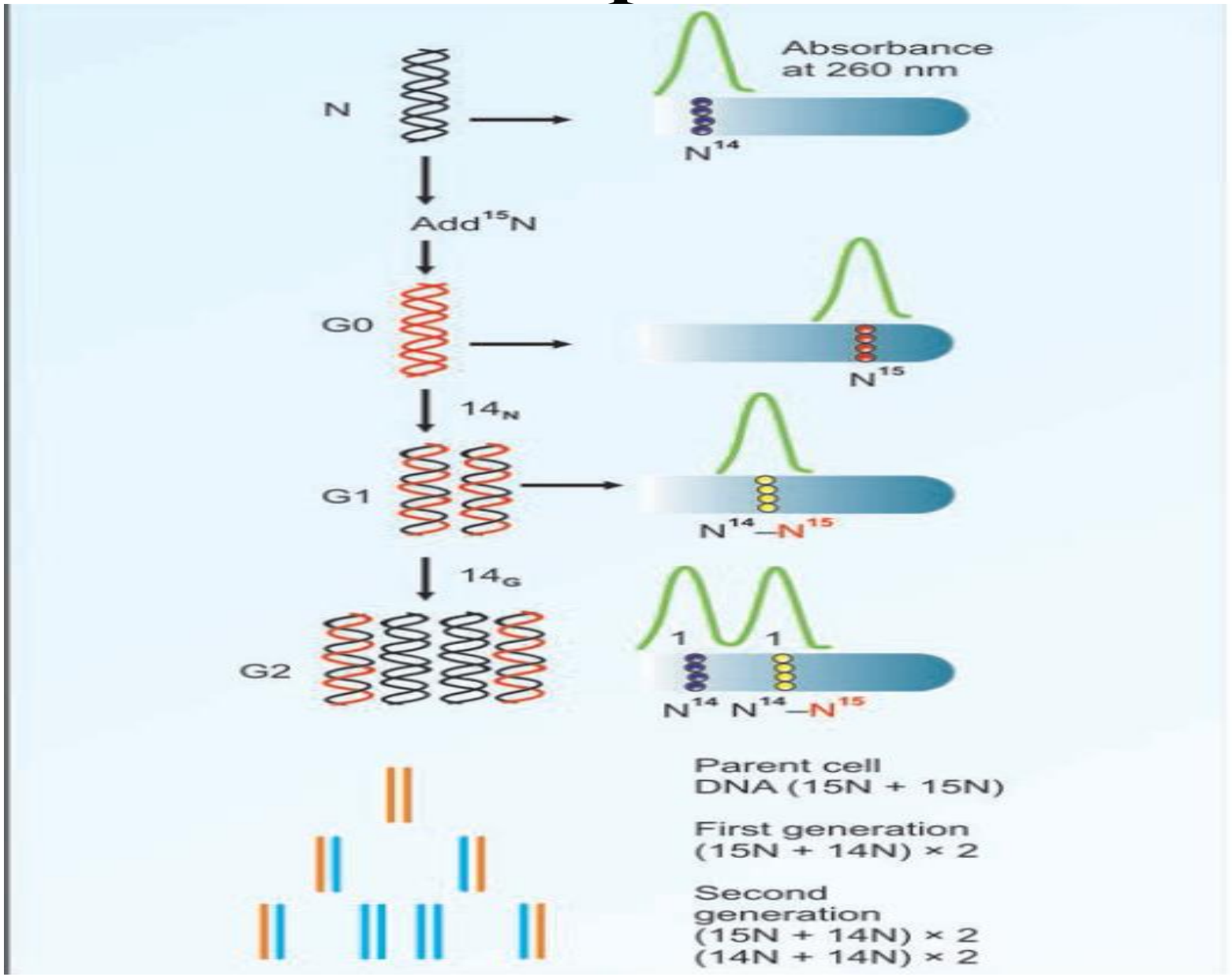


Semi-conservative

- Parental strands are not degraded
- Base pairing allows each strand to serve as a template for new strand
- New duplex is 1/2 parent template & 1/2 new DNA



Meselson & Stahl experiment



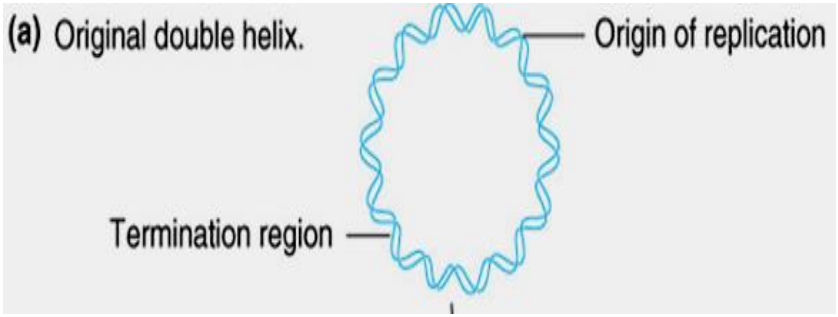
Origin of Replication

Initiator proteins identify specific base sequences on DNA called sites of origin (ori)

Prokaryotes – single origin site E.g *E.coli* - *oriC*

Eukaryotes – multiple sites of origin (replicator)

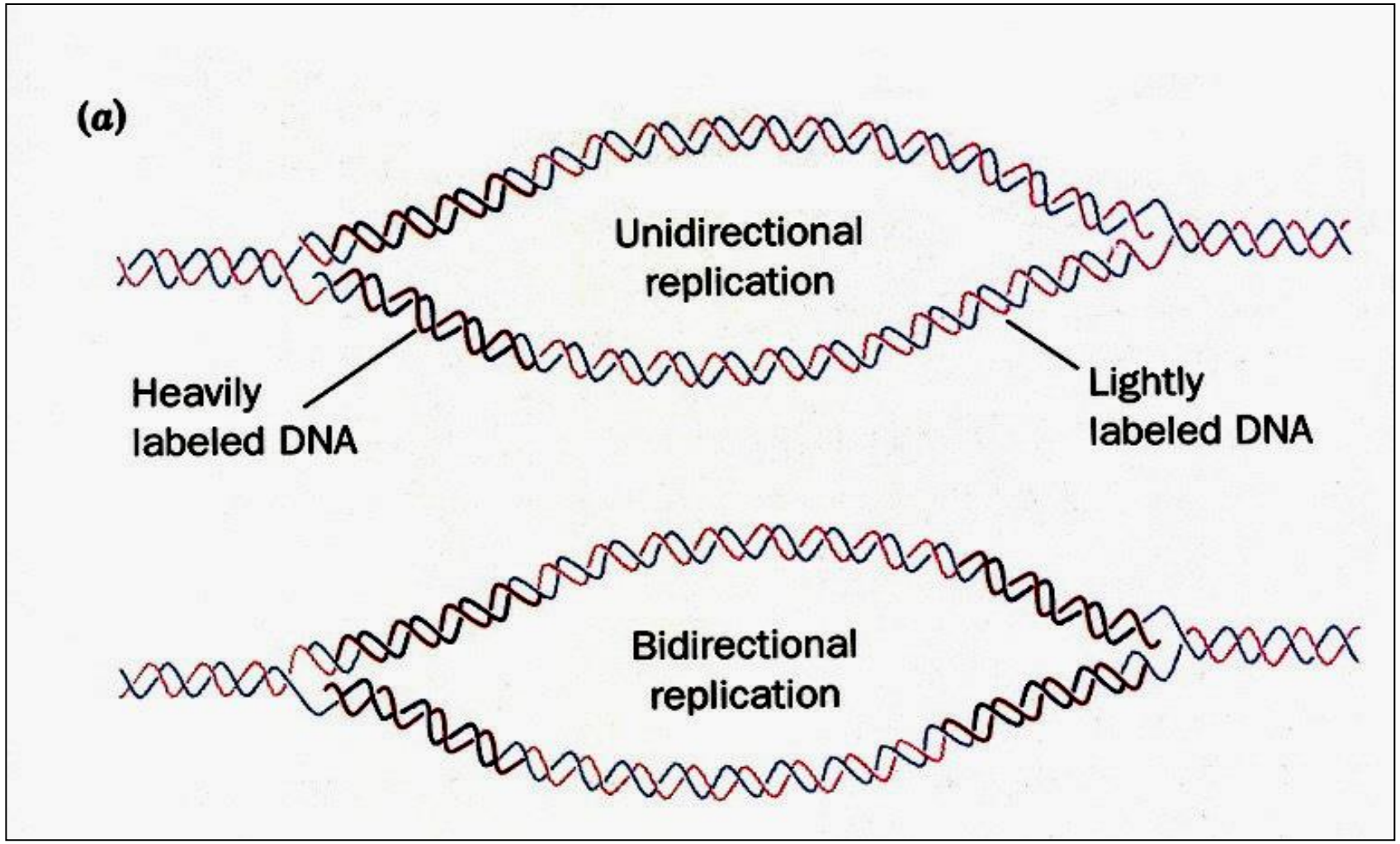
E.g. yeast - ARS (autonomously replicating sequences)



Prokaryotes

Uni or bidirectional

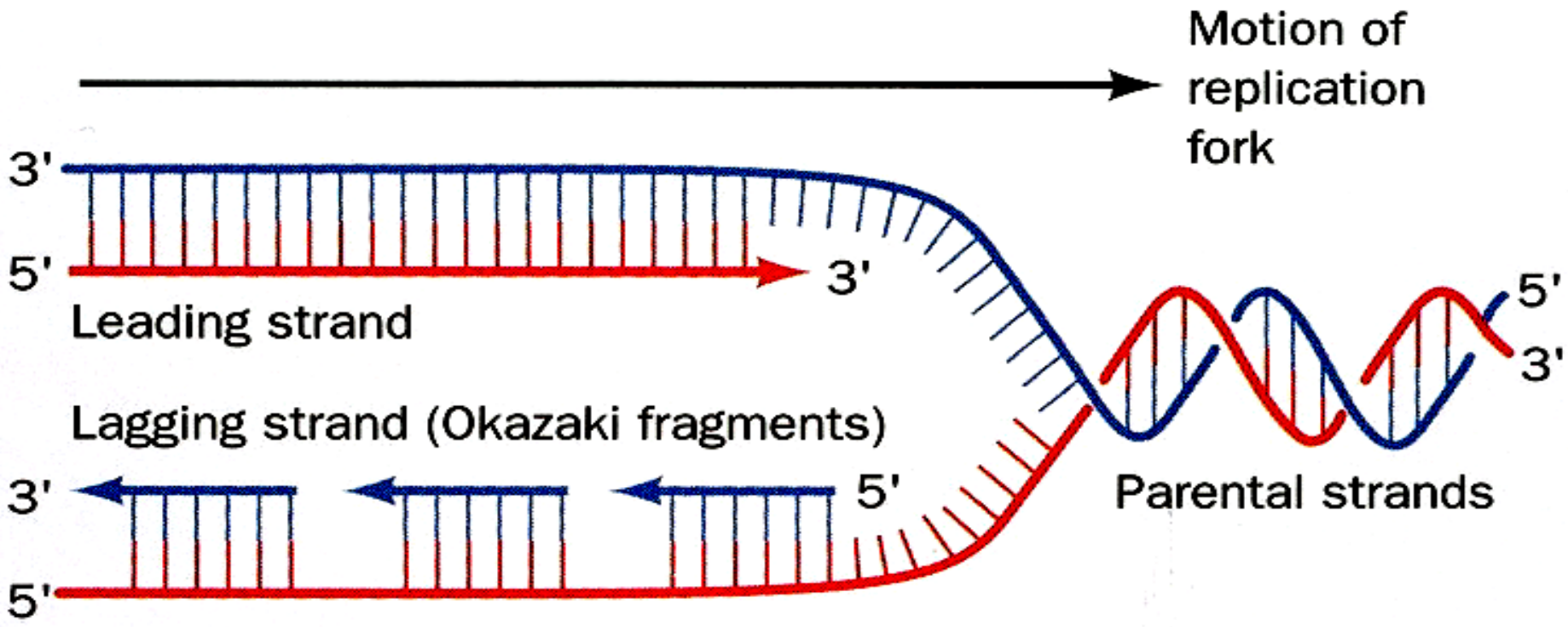
- Replication forks move in one or opposite directions



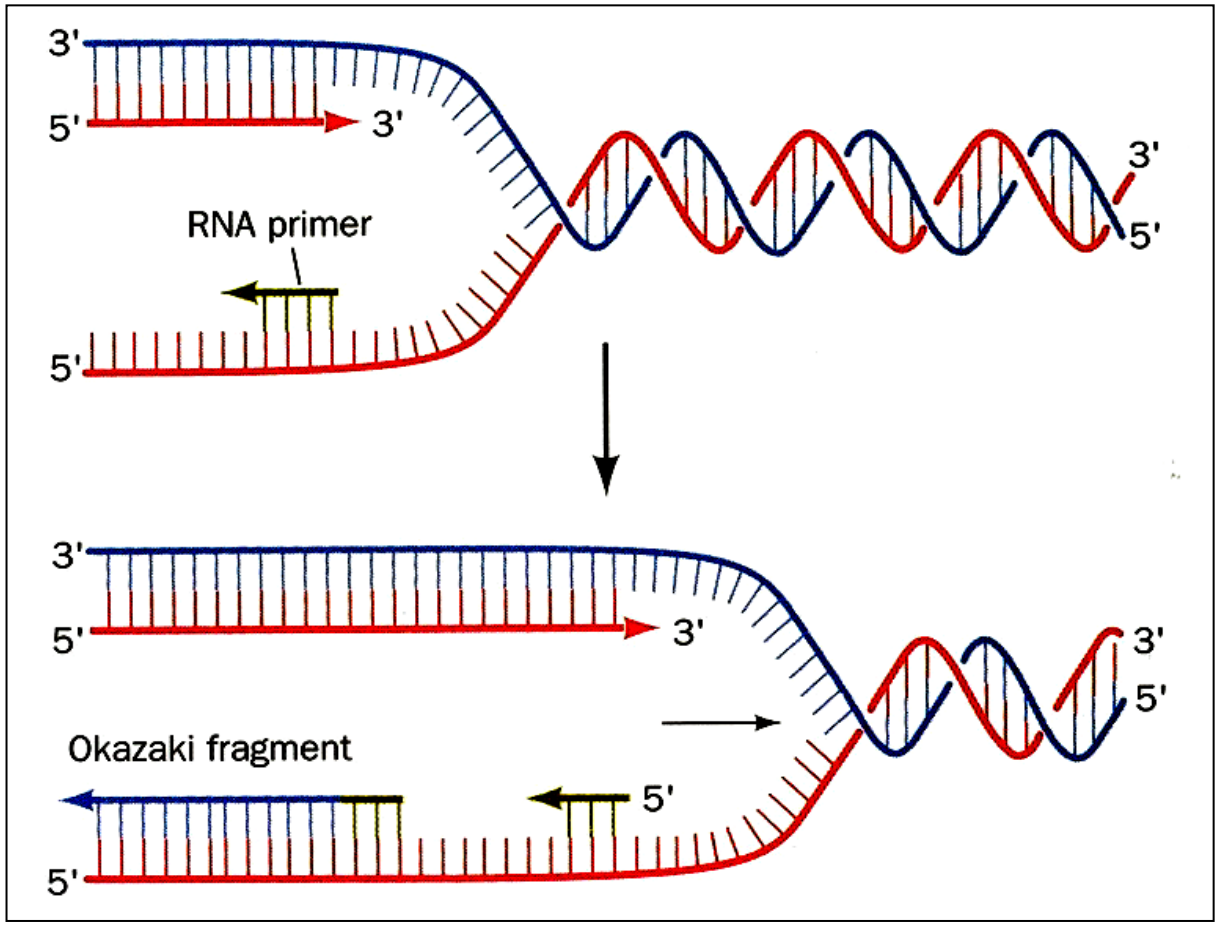
Semi-discontinuous replication

Anti parallel strands replicated simultaneously

- Leading strand synthesis **<u>continuously</u>** in 5'– 3'
- Lagging strand synthesis in **<u>fragments</u>** in 5'-3'



RNA Primers required



- DNA polymerase can only add nucleotides
- to 3' end of a growing DNA strand
- need a "starter" nucleotide to make a bond
- Strand only grows 5' → 3'
- Template is read in the 3' → 5' direction while polymerization takes place in the 5' → 3' direction

RNA PRIMERS

- Synthesized by Primase
- Serves as a starter sequence for DNA polymerase III
- Only one RNA Primer-required for the leading strand
- RNA Primers for the lagging strand depend on the number of "OKAZAKI FRAGMENTS"
- RNA Primer has a free 3'OH group to which the first Nucleotide is bound.

Steps of DNA Replication

- Identification of the origins of replication
- Unwinding (denaturation) of dsDNA to provide an ssDNA template
- Formation of the replication fork
- Initiation of DNA synthesis and elongation
- Primer removal and ligation of the newly synthesized DNA segments
- Reconstitution of chromatin structure

Core proteins at the replication fork

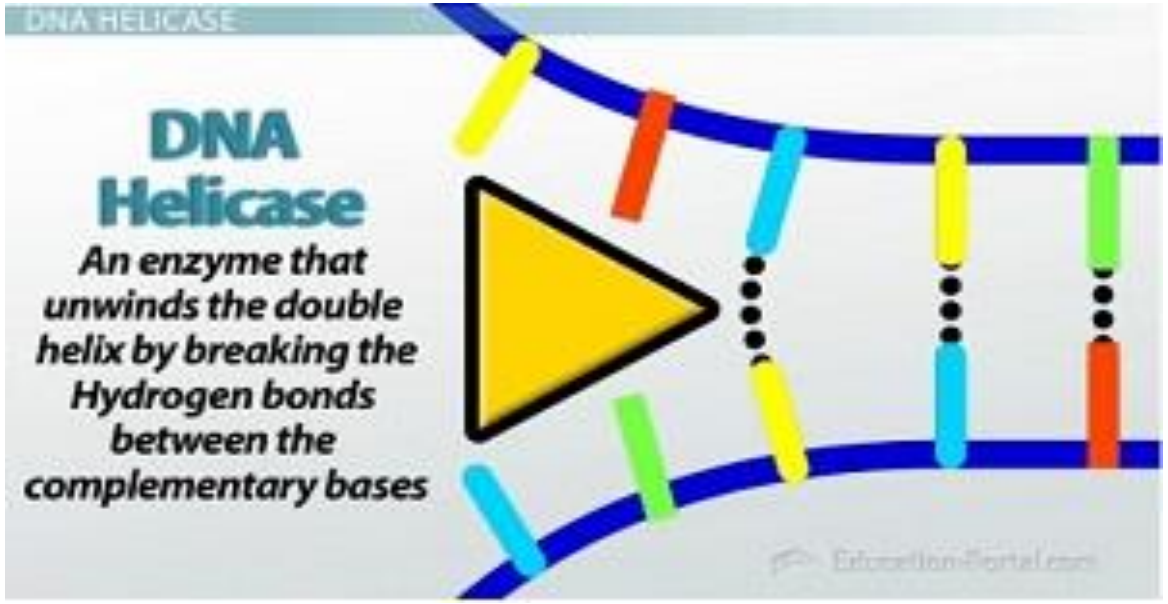
Topoisomerases	- prevents torsion by DNA breaks
Helicases	- separates 2 strands
Primase	- RNA primer synthesis
Single strand binding proteins	- prevent reannealing of single strands
DNA polymerase	- synthesis of new strand
Tethering protein	- stabilises polymerase
DNA ligase	- seals nick via phosphodiester linkage

Origin of Replication

- Replication starts at the specific site on DNA - **origin of replication**
- Origin of replication is recognized by specific proteins - **origin recognition complex** (ORC)
- Origin of replication in prokaryotes - ***ori*** (single)
- Origin of replication in Eukaryotes - **replicators** (multiple)

Separation (unwinding) of double helical DNA

- **Protein A** (DnaA) binds at the specific site of origin & opens the double helical structure
- **Helicase** (DnaB): unwinds the double helical DNA & separates the two strands by breaking the H-bonds



Separation (unwinding) of double helical DNA

- **Topoisomerase**: relieves the super coiling

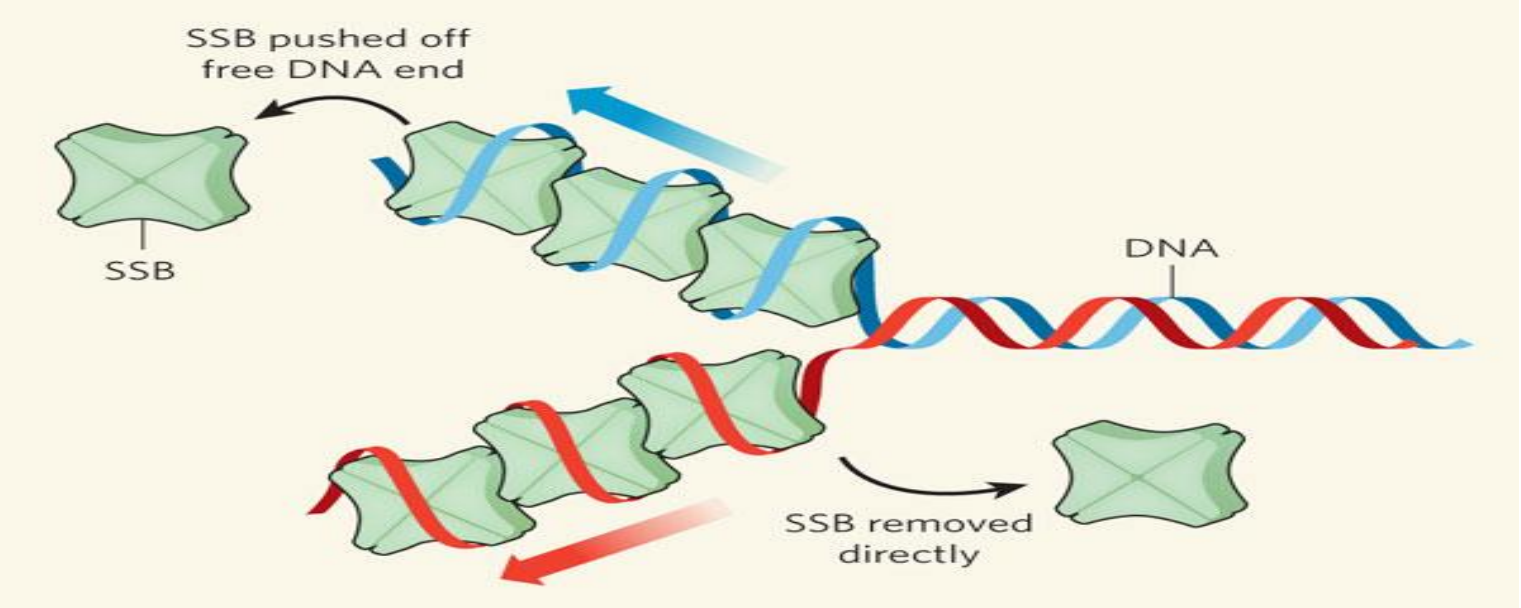
  Type I: makes break in one strand & relieves super coiling and sealing the break.

  Type II: makes break in both the strands, rotates, relieves the super coiling and sealing the break.

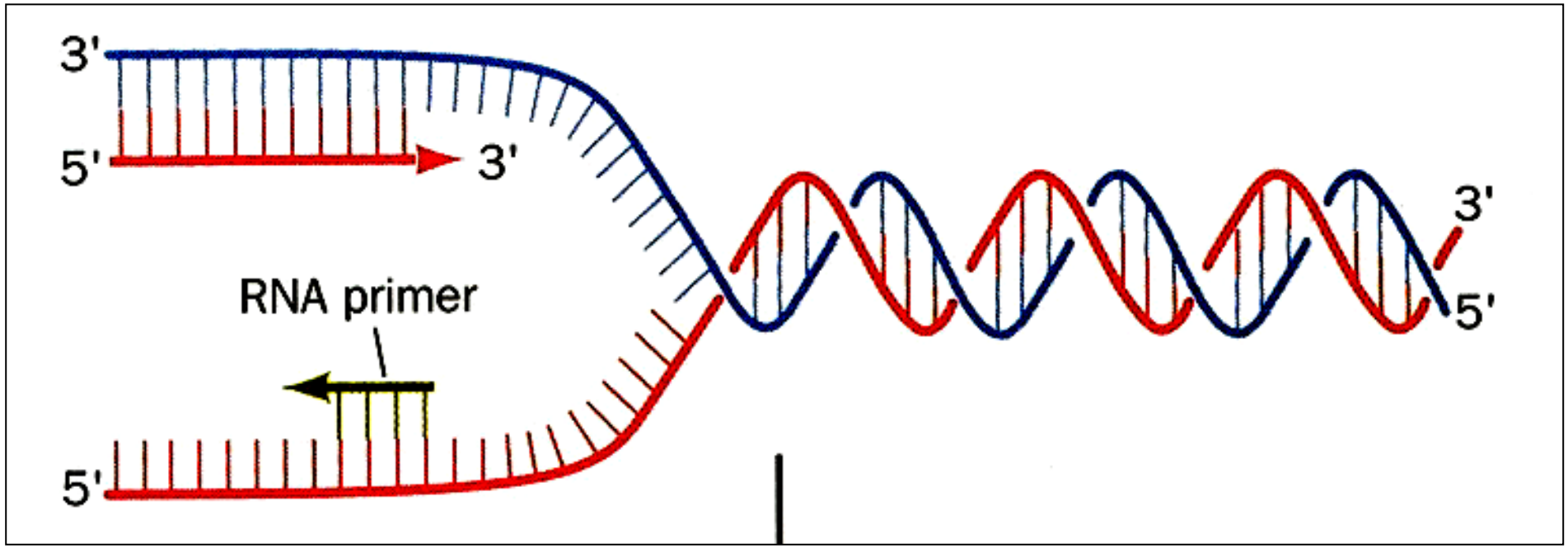
- **Gyrase**: Topoisomerase Type II in prokaryotes

Single Stranded DNA binding protein

- bind with separated DNA Strands
- prevent them from annealing

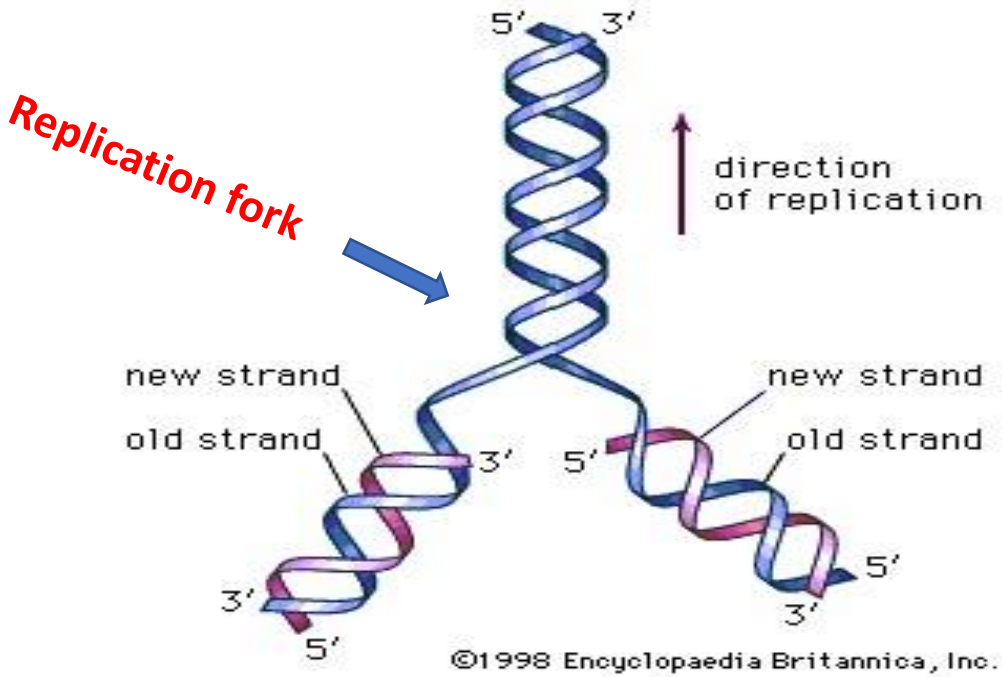


Primer synthesis



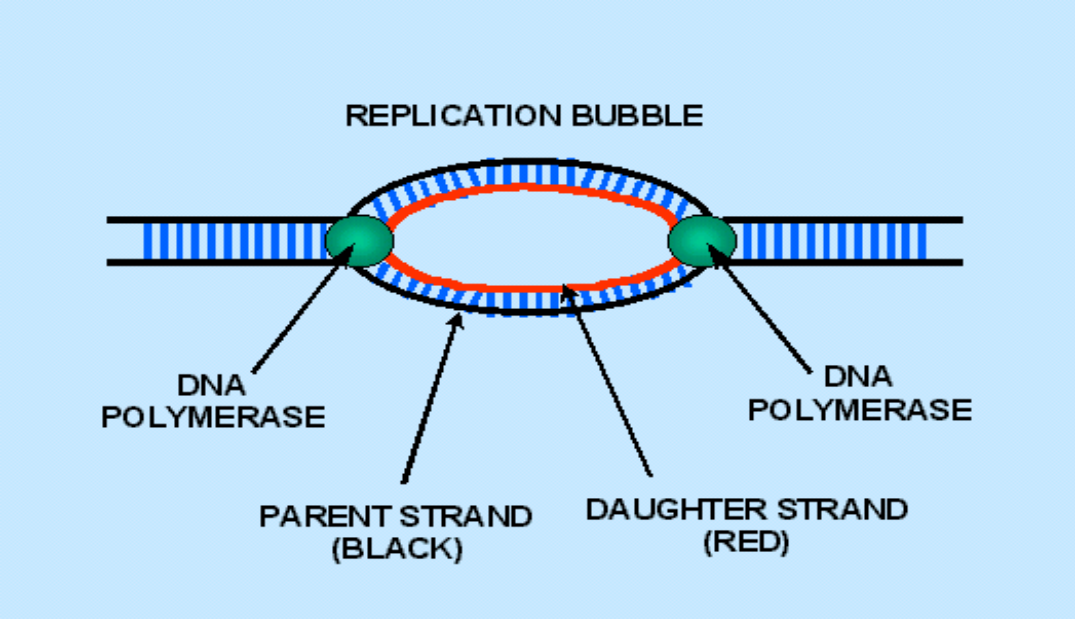
Replication fork

- Part of the parent double helical DNA unwinds and forms **replication fork** .
- Synthesis of DNA occurs at this site
- Replication fork slowly moves along the parent DNA as new daughter DNA is synthesized.



Replication bubble

- Separation of double helical structure of DNA by **Helicase** takes place on both directions (bidirectional) and thus forms bubble or eye – **replication bubble**.



DNA Polymerase

- **DNA Polymerase:** Enzyme catalyzes synthesis of DNA
- Separated, parent- DNA strands act as **templates**
- Synthesis of new DNA-strands takes place on both the templates simultaneously.
- Direction of synthesis: **5' → 3'**
- **Requires RNA primer**

DNA Polymerase

- **DNA Polymerase** : ***prokaryotes***
- DNA Polymerase I
- DNA Polymerase II
- DNA Polymerase III

- **DNA Polymerase**: ***eukaryotes***
- α-DNA Polymerase
- β-DNA Polymerase
- γ-DNA Polymerase
- δ-DNA Polymerase
- ε-DNA Polymerase

DNA polymerase: Prokaryotes

DNA polymerase		
I	Repair & gap filling due to the removal of RNA primer	5'→3' exonuclease,it removes RNA primer & also filling the gap by synthesiing DNA chain. It also removes mismatched or damaged DNA (repair)
II	Proof reading & repair	
III	Elongation of the DNA chain	3'→5' exonuclease (proof reading)

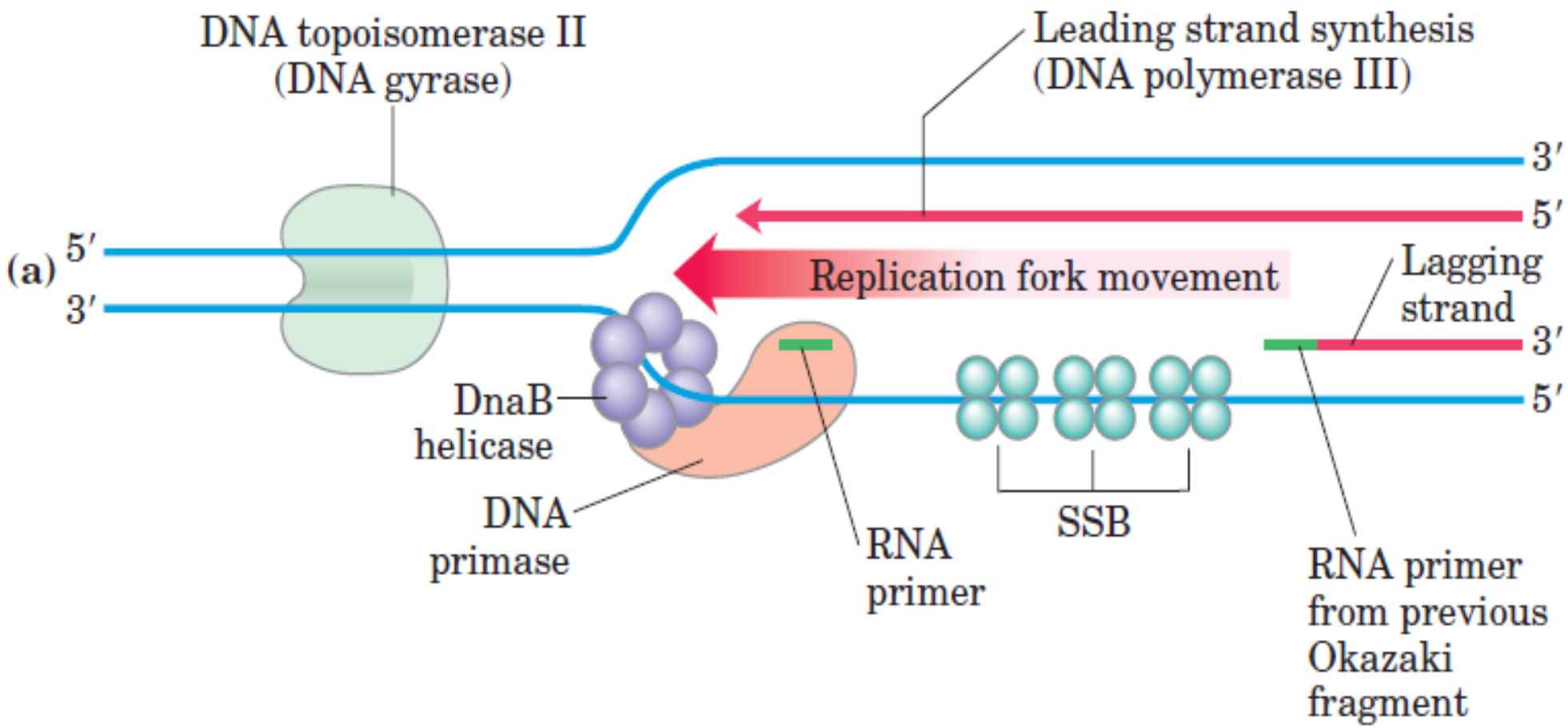
DNA Polymerase: eukaryotes

S.N.	DNA polymerase	Action
1	α-polymerase	Synthesis of RNA primer 5'-3'Polymerase activity
2	β-polymerase	Repair of DNA
3	γ-polymerase	Replication of mt DNA
4	δ-polymerase	Replication on leading strand & proof reading (3'-5' exonuclease)
5	ε-polymerase	Replication on lagging strand & proof reading

DNA polymerase III : Proof-reading activity

- checks every incoming deoxy nucleotide triphosphate
- allows only the complementary (to template strand) bases to be incorporated into the growing DNA strand.
- edits the newly synthesized DNA strand and any mismatched base incorporated, will be removed by it's **3'- 5' Nucleosidase** (exonuclease) activity.
- Misreading of template may occur at 10000: 1 ratio but it will be corrected by this proof reading activity.

Synthesis of new strands



1. Origin of replication is identified. Then unwinding of parental DNA to form a replication fork.
2. RNA primer complementary to the DNA template is synthesized by RNA primase.
3. DNA synthesis is continuous in the leading strand (towards replication fork) by DNA polymerase.
4. DNA synthesis is discontinuous in the lagging strand (away from the fork), as Okazaki fragments.
5. Elongation: In both strands, the synthesis is from 5' to 3' direction.
6. Then the RNA pieces are removed; the gaps filled by deoxynucleotides by DNAP and the pieces are ligated by DNA ligase.
7. Proof reading is done by the DNA polymerase.
8. Finally organised into chromatin.
9. Main enzymes involved in replication are: DNA polymerases; Helicases; Topoisomerases; DNA primase; Single strand binding proteins; and DNA ligase.

Replication: Inhibitors

S.N.	Inhibitor	Enzyme	Therapeutic use
1	Ciprofloxacin Novobiocin Nalidixic acid	Gyrase - prokaryotes	Antibiotic
2	Adriamycin/ Doxorubicin Daunorubicin Etoposide	Topoisomerase - eukaryotes	Anticancer