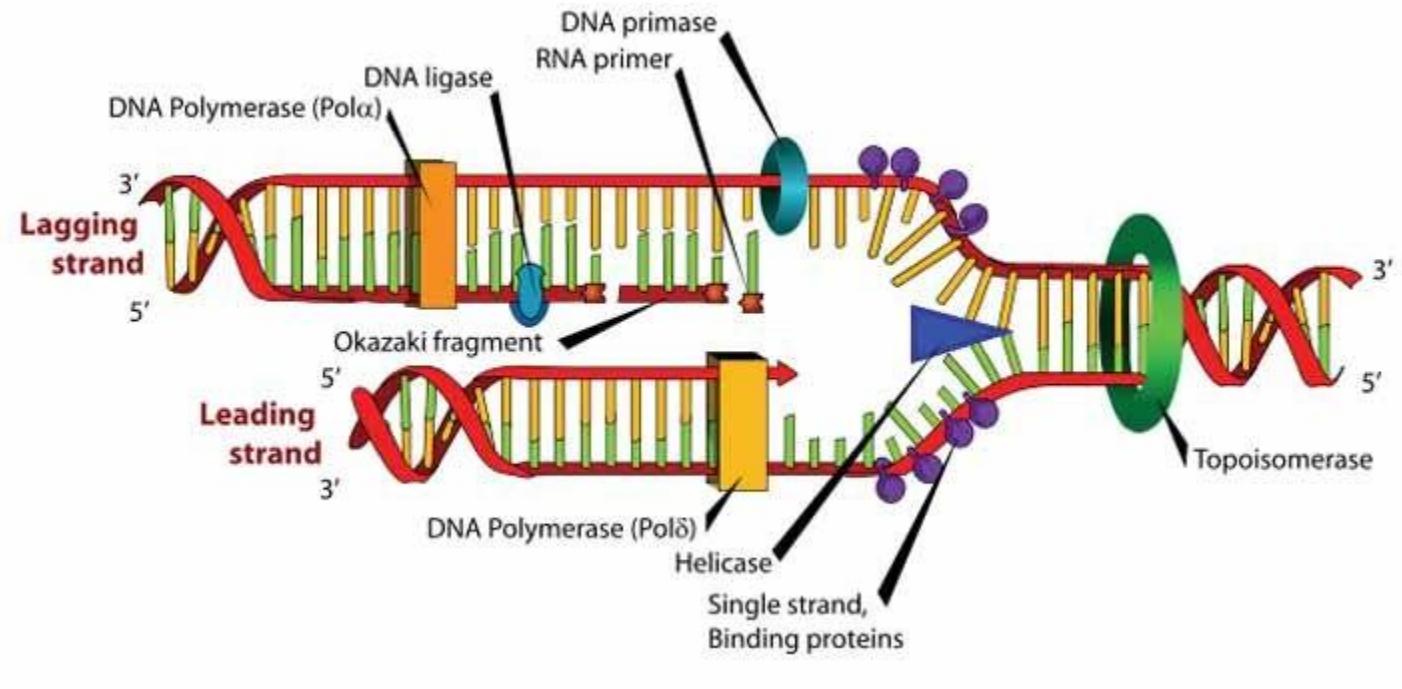
Bhagalpur National College, Bhagalpur

(A Constituent unit of Tilka Manjhi Bhagalpur University, Bhagalpur)

PPT Presentation for B.Sc. III- DNA Replication



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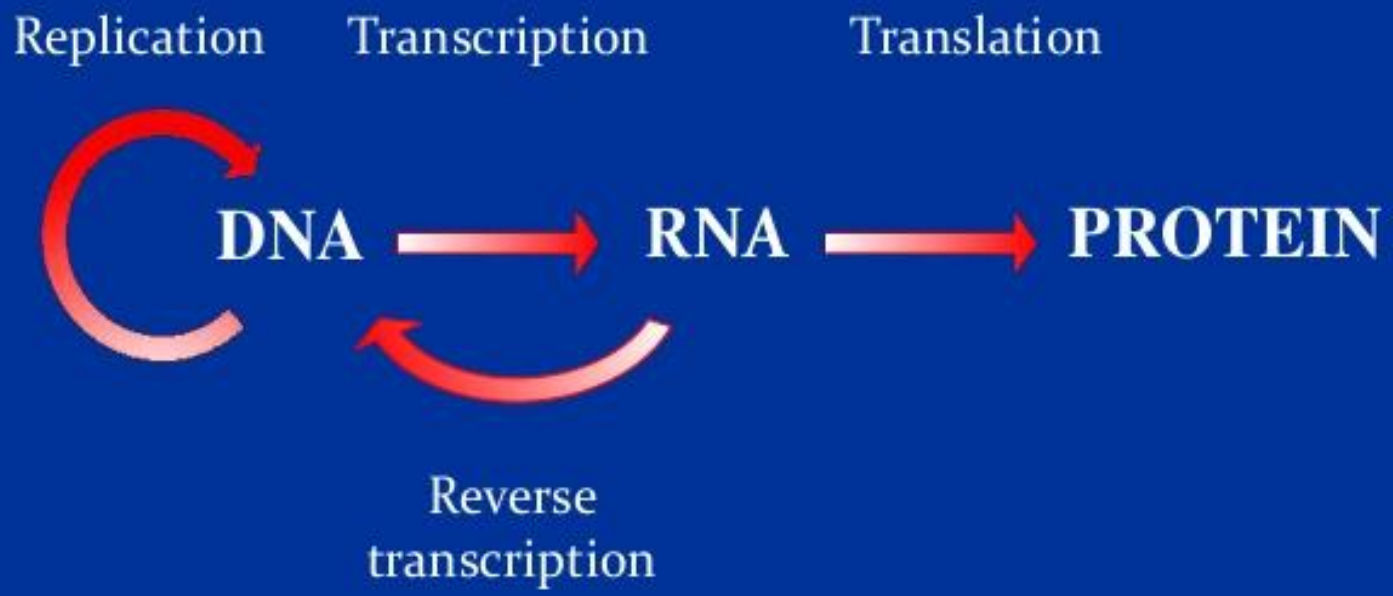
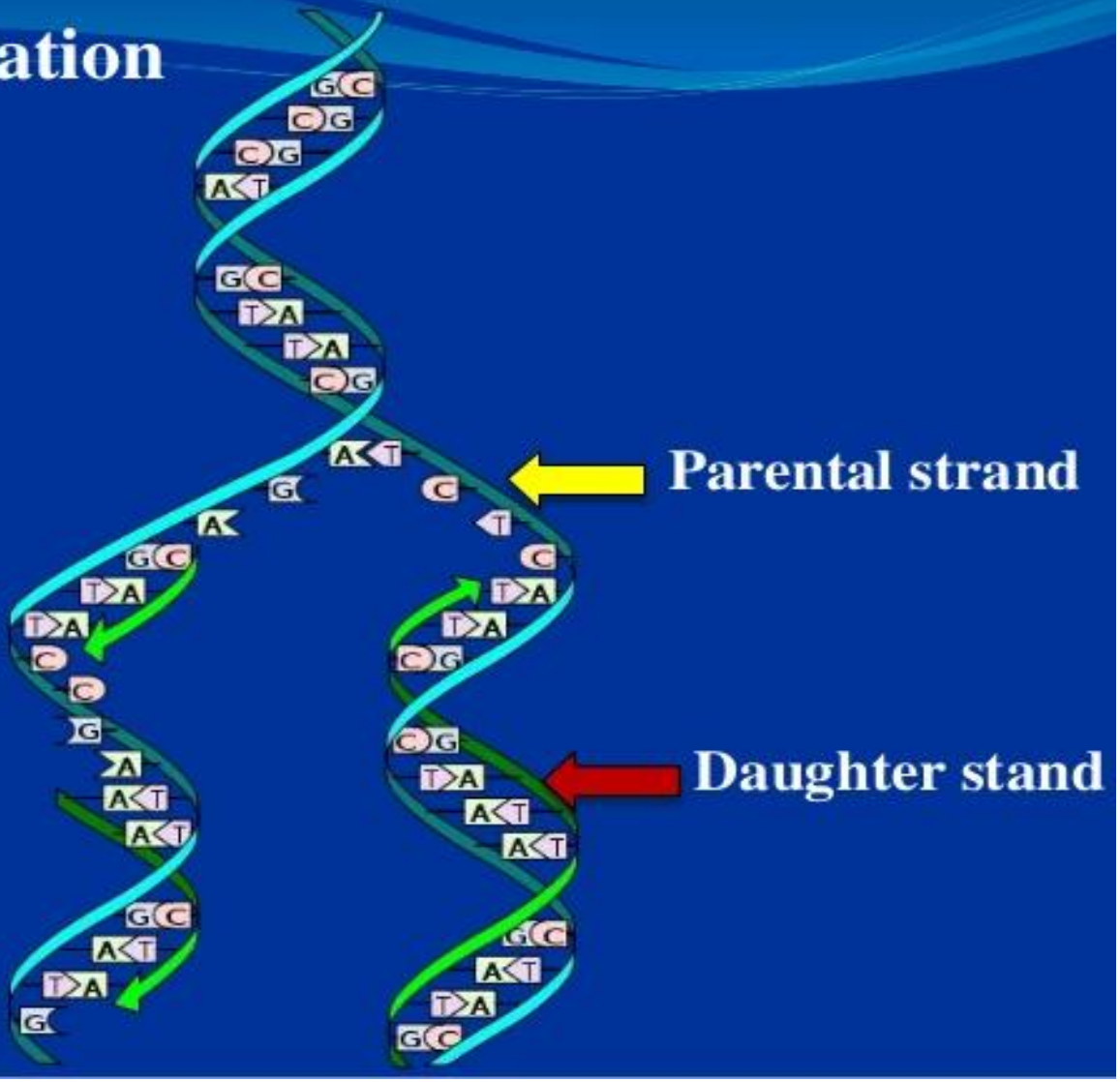
Central dogma



- DNA replication is a biological process that occurs in all living organisms and copies their exact DNA. It is the basis for biological inheritance.
- Replication is the process of synthesis of daughter DNA from parental DNA by the enzyme DNA Polymerase.
- $(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1} + PPi$

  DNA → Lengthened DNA

DNA Replication



DNA Replication

- **A reaction in which daughter DNAs are synthesized using the parental DNAs as the template.**
- **Transferring the genetic information to the descendant generation with a high fidelity.**

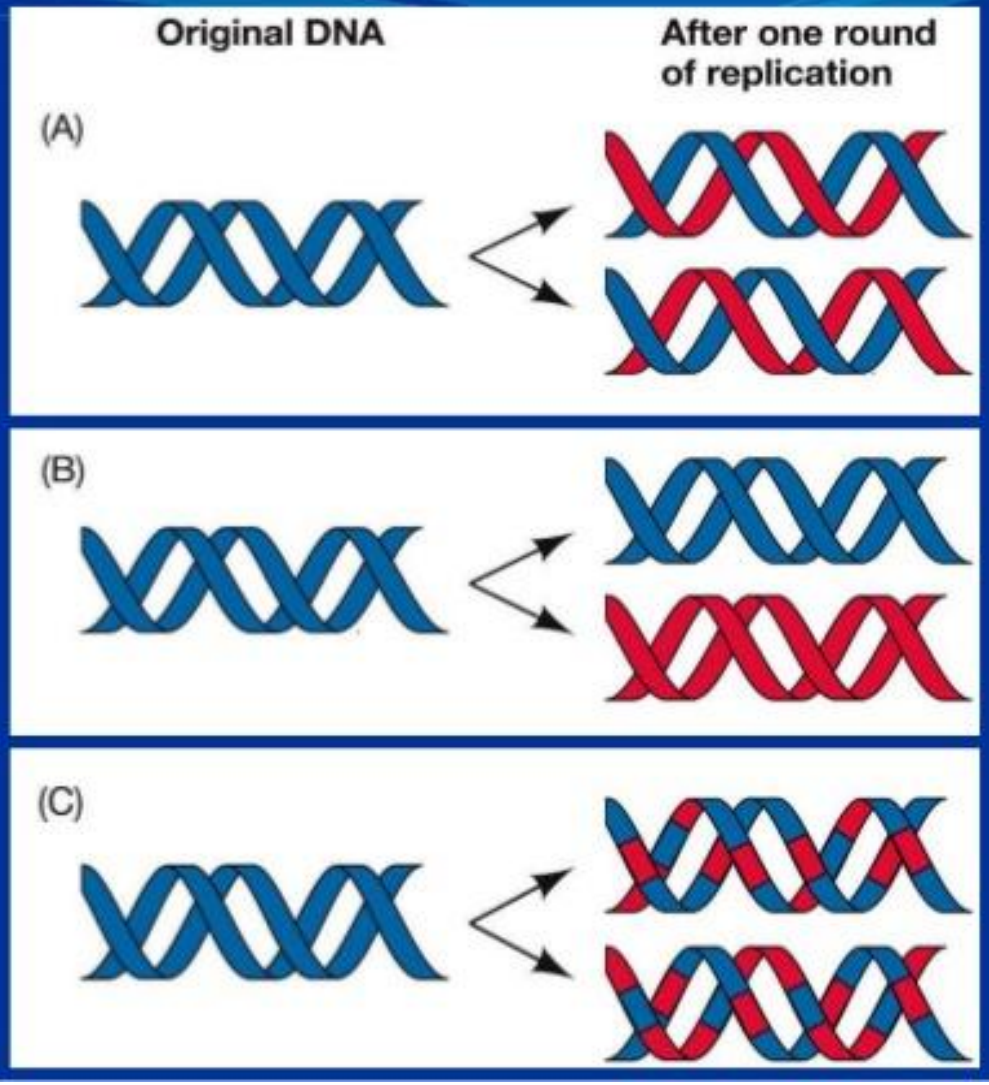
Three possible replication patterns:

1. ***Semiconservative replication***
2. ***Conservative replication***
3. ***Dispersive replication***

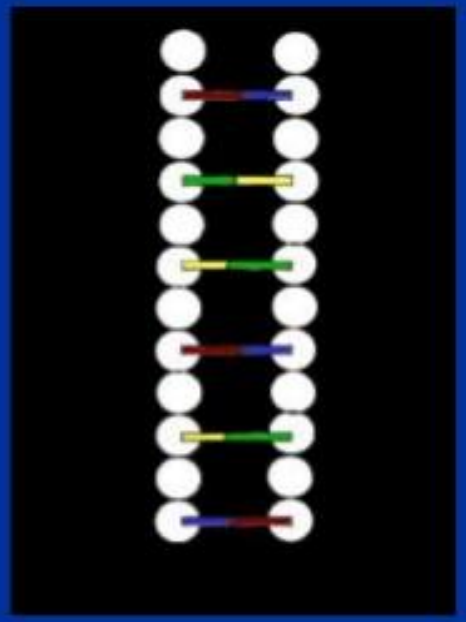
Semiconservative replication

Conservative replication

Dispersive replication



Each parent strand serves as a template for a new strand and the two new DNA strands each have one old and one new strand

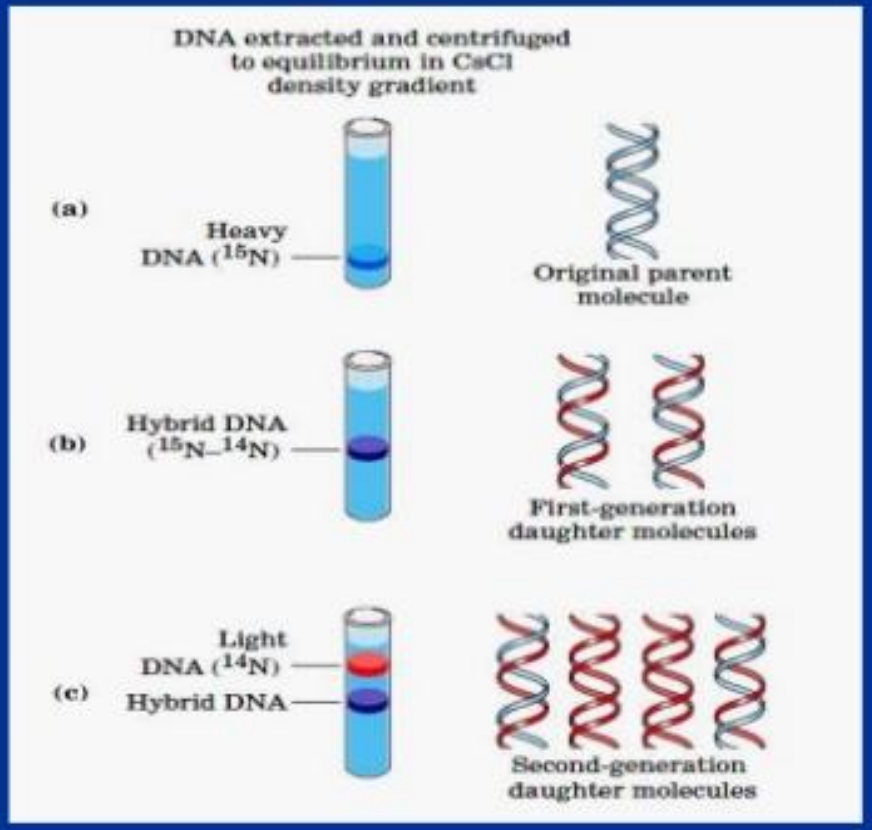


Characteristics of Replication

- Semi-conservative replication
- Bidirectional replication
- Semi-continuous replication
- High fidelity

Meselson and Stahl experiment [1958]

demonstrated Semiconservative replication

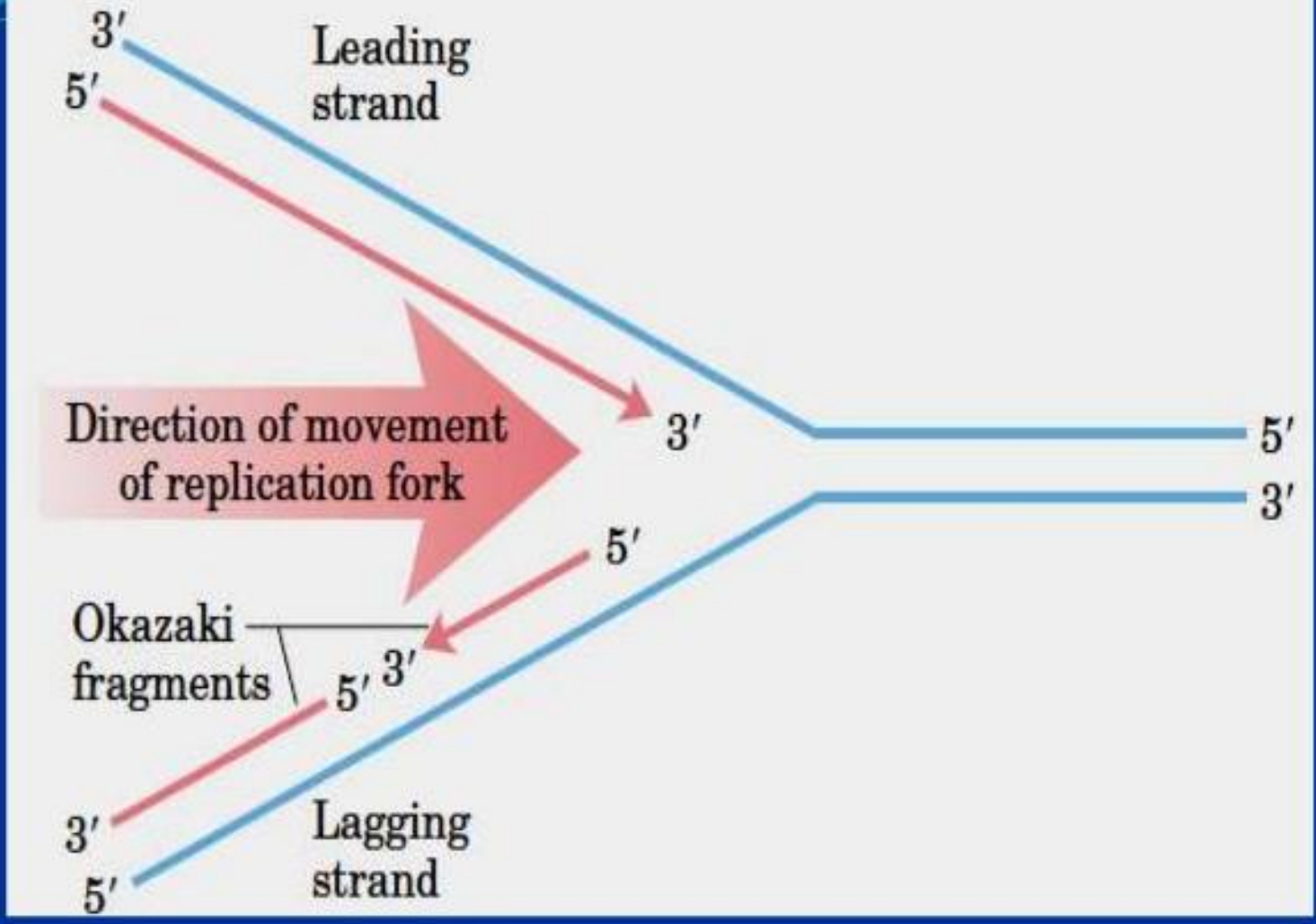


Semiconservative Replication

Half of the parental DNA molecule is conserved in each new double helix, paired with a newly synthesized complementary strand. This is called semiconservative replication.

Bidirectional Replication

- Replication starts from unwinding the dsDNA at a particular point (called **origin / *ori* site**), followed by the synthesis on each strand.
- The parental dsDNA and two newly formed dsDNA form a Y-shape structure called Replication fork.


3′
5′
Leading strand
Direction of movement of replication fork
3′
5′
3′
5′
Okazaki fragments
5′
3′
3′
5′
Lagging strand

Replication Enzymes & Proteins

- **DNA Polymerase** - Matches the correct nucleotides then joins / polymerizes adjacent nucleotides to each other.
- **Helicase** - Unwinds the DNA and melts it.
- **Primase** - Provides an RNA primer to start polymerization.

- **Single Strand Binding Proteins** - Keep the DNA single stranded after it has been melted by helicase
- **Gyrase** - A topisomerase that Relieves torsional strain in the DNA molecule.
- **Ligase** - Joins adjacent DNA strands together (fixes "nicks")
- **Telomerase** - Finishes off the ends of DNA strands in Eukaryotes

DNA Polymerases of Prokaryotes

DNA Polymerase-I

- The first DNA- dependent DNA polymerase (DNA Pol -I) was discovered in 1958 by Arthur Kornberg who received Nobel Prize in physiology & medicine in 1959.
- DNA Polymerase is considered as Kornberg Enzyme.

Arthur Kornberg

- Later, DNA-Pol II and DNA-Pol III were identified.
- All of them possess the following biological activity.

  1. 5′→3′ Polymerse activity
  2. Exonuclease activity

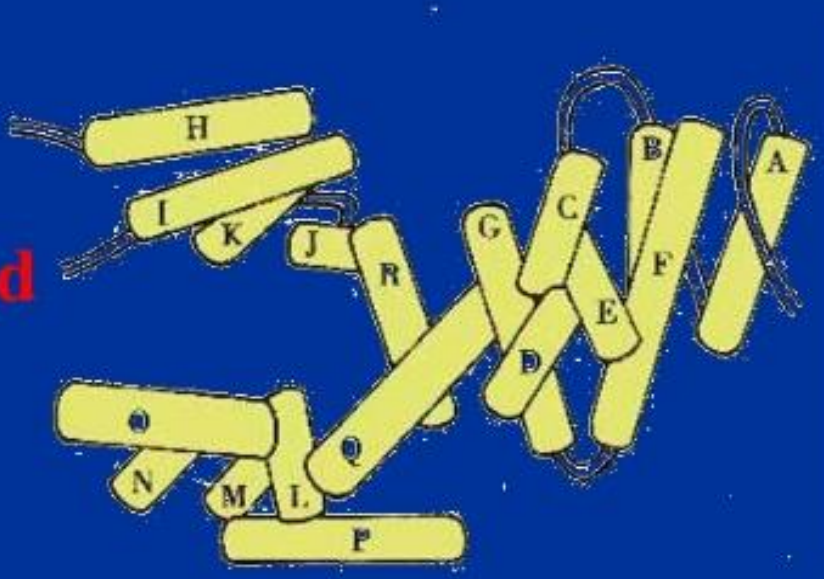
Exonuclease functions

5′ →3′ exonuclease activity removes primer or excise mutated segment

3′ →5′ exonuclease activity excise mismatched nuleotides

DNA Polmerase - I

- Mainly responsible for **proofreading and filling the gaps**, repairing DNA damage



DNA Polymerase - II

- Temporarily functional when DNA-pol I and DNA-pol III are not functional.
- Still capable for doing synthesis on the damaged template.
- Participates in DNA repair process.

DNA Polymerase - III

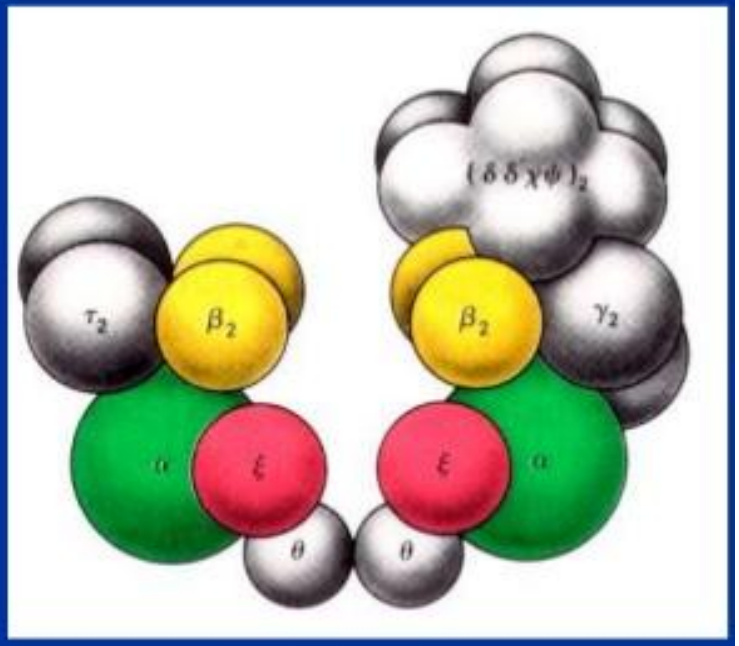
- A heterodimer enzyme composed of ten different subunits
- Having the highest polymerization activity (10^5 nt/min)
- The true enzyme responsible for the elongation process

Structure of DNA-pol III

α: has 5′ → 3′ polymerizing activity

ε: has 3′ → 5′ exonuclease activity and plays a key role to ensure the replication fidelity.

θ: maintain heterodimer structure



Replication process

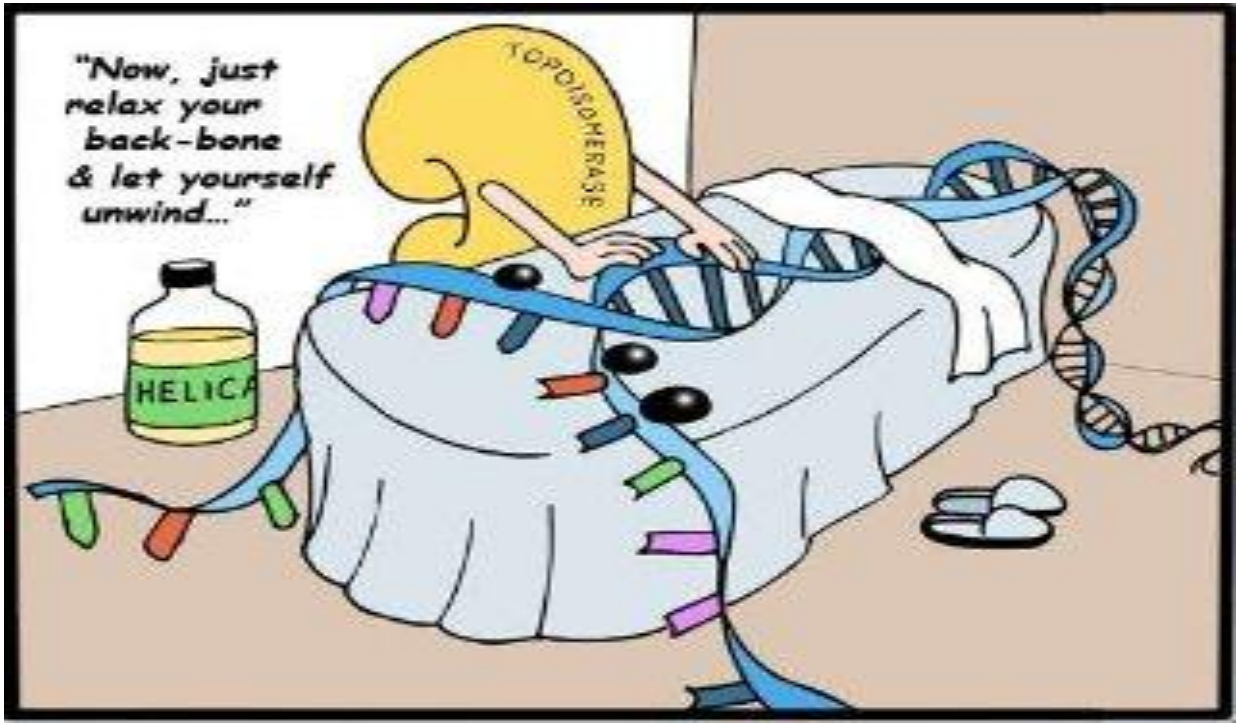
- DNA Replication, like all biological polymerization processes, proceeds in three enzymatically catalyzed and coordinated steps:
- initiation
- elongation and
- termination

DNA Replication

DNA replication includes:

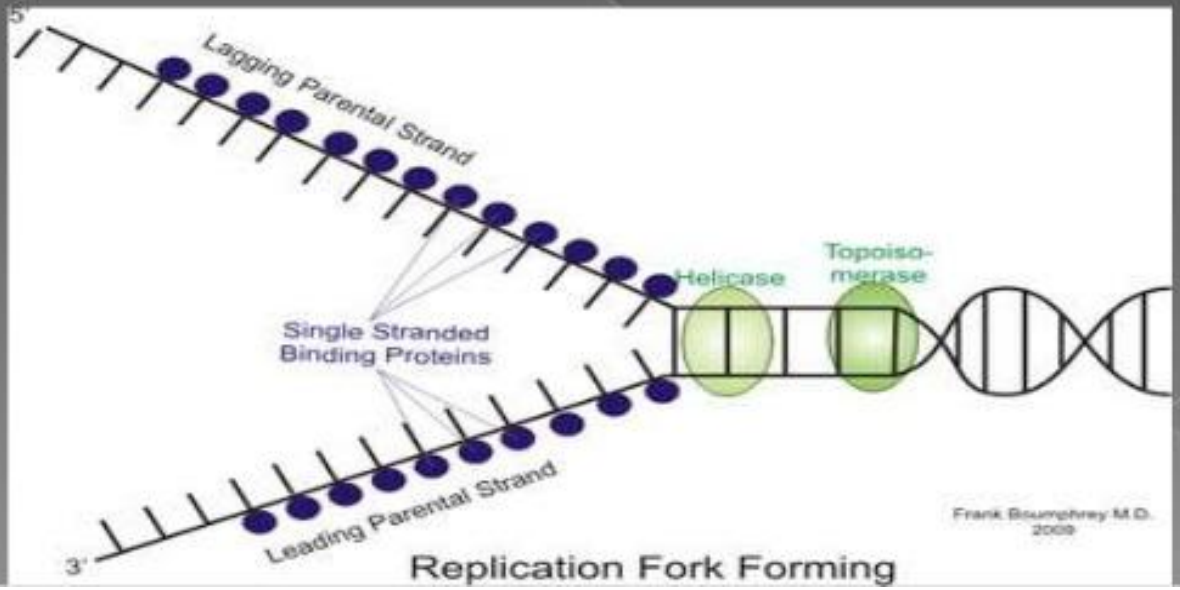
- **initiation** – replication begins at an **origin of replication**
- **elongation** – new strands of DNA are synthesized by **DNA polymerase**
- **termination** – replication is terminated differently in prokaryotes and eukaryotes

Initiation


"Now, just relax your back-bone & let yourself unwind..."
TOPOISOMERASE
HELICA

Cont…

- The separation of the two single strands of DNA creates a ‘Y’ shape called a replication ‘fork’.



RNA Primase

- Also called DnaG
- Primase is able to synthesize primers using free NTPs as the substrate and the ssDNA as the template.
- Primers are short RNA fragments of a several nucleotides long.

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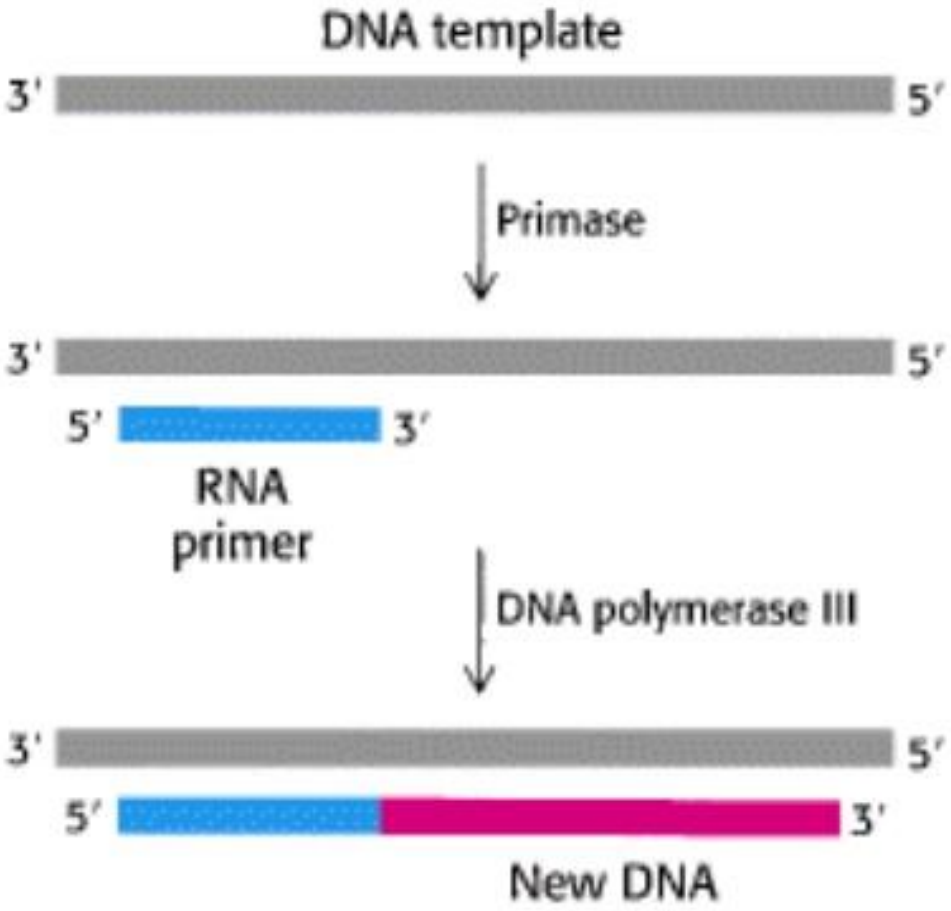
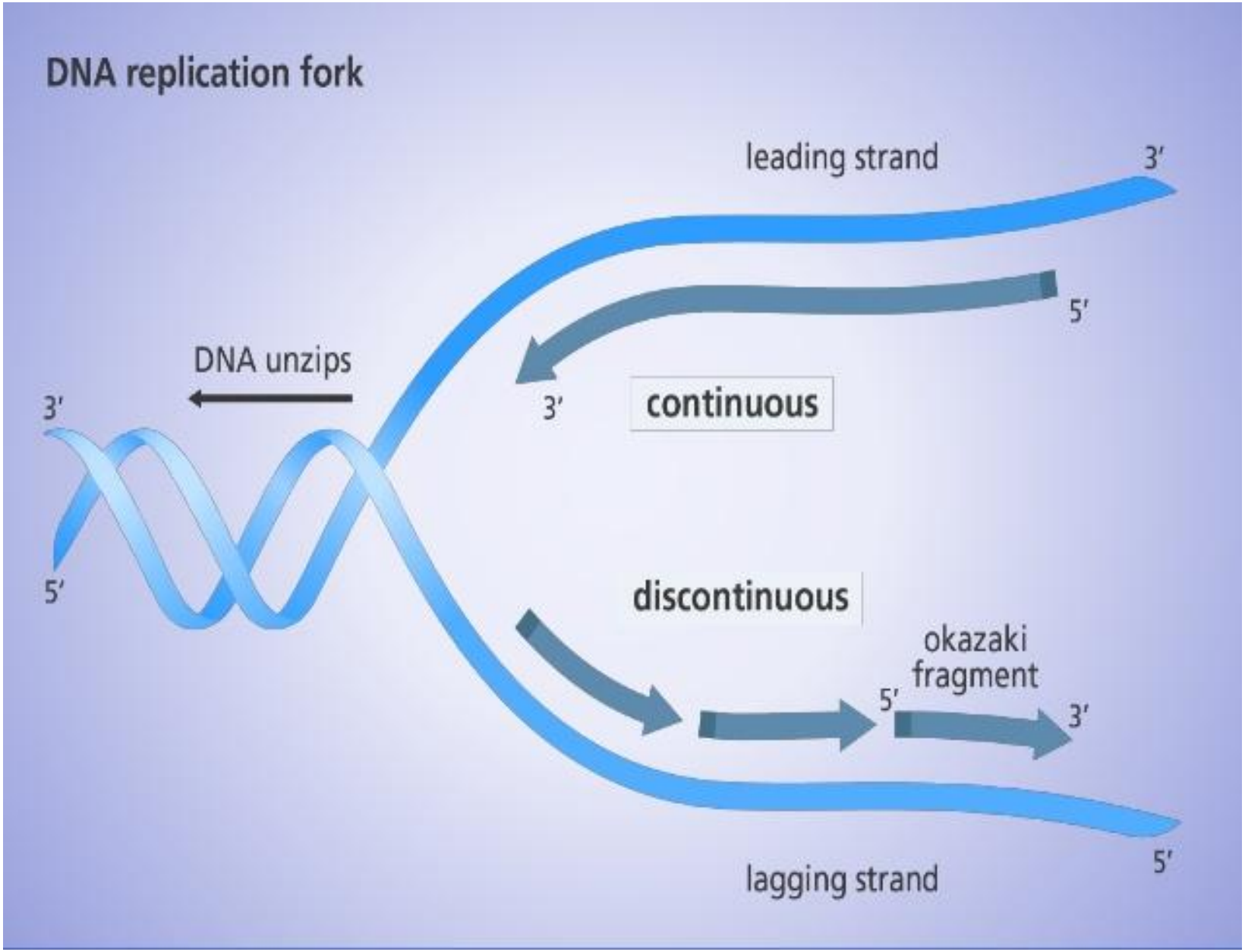
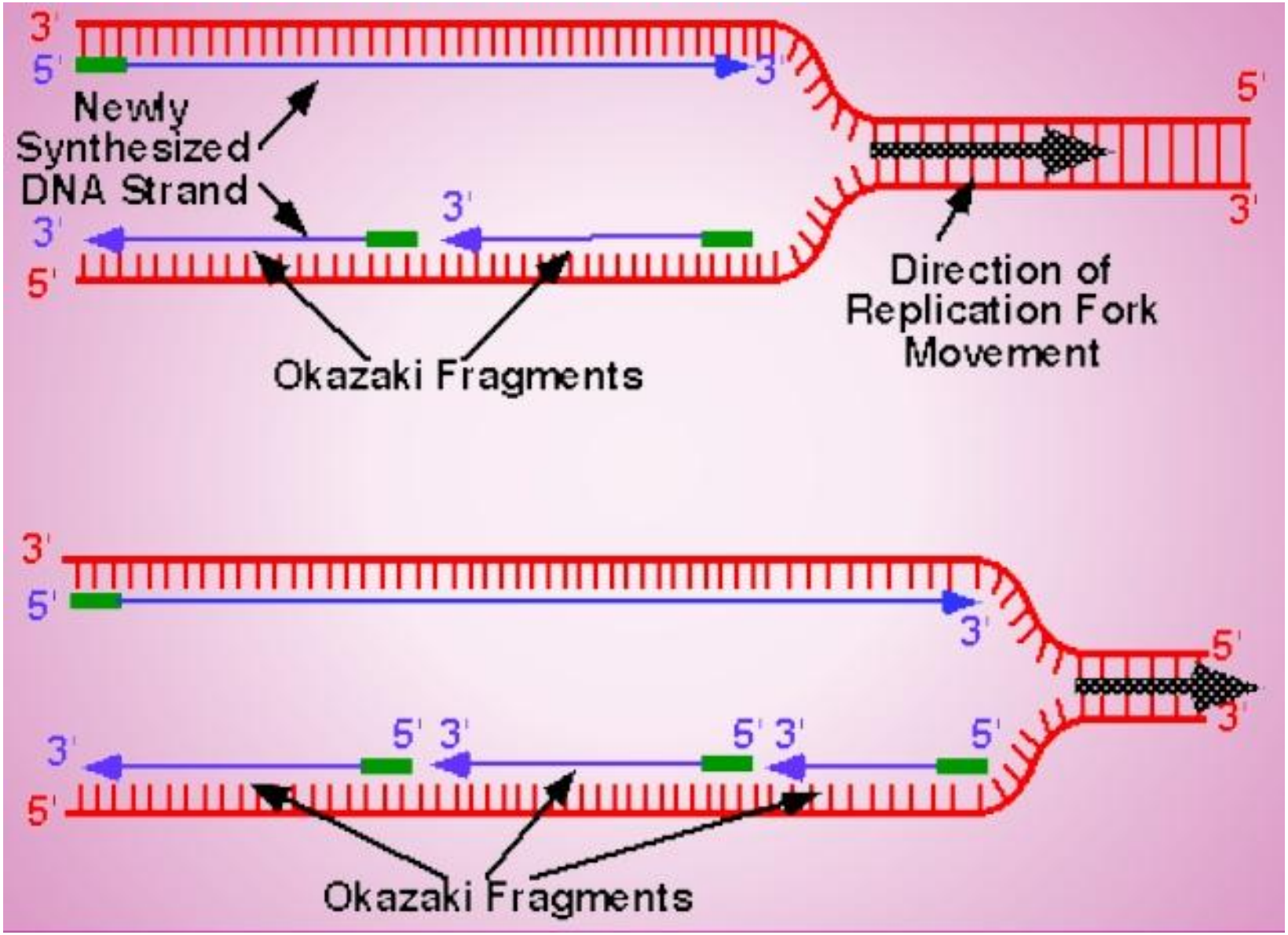
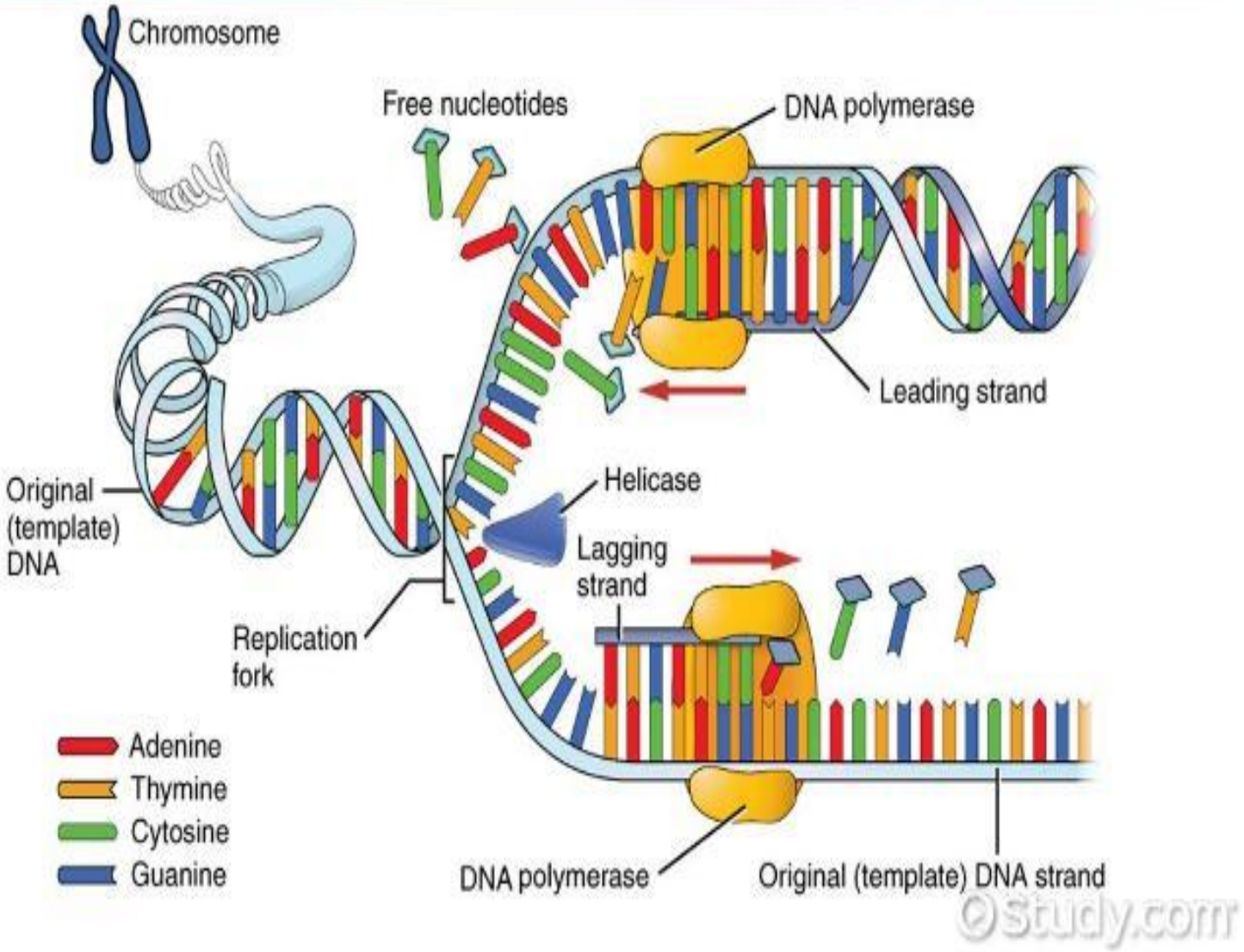

DNA template
3'
5'
Primase
3'
5'
5'
3'
RNA
primer
DNA polymerase III
3'
5'
5'
3'
New DNA


DNA replication fork
leading strand
3′
5′
DNA unzips
3′
3′
continuous
5′
discontinuous
okazaki
fragment
5′
3′
5′
lagging strand


3'
5'
Newly
Synthesized
DNA Strand
3'
3'
5'
3'
5'
3'
Direction of
Replication Fork
Movement
Okazaki Fragments
3'
5'
3'
5'
3'
5' 3'
5' 3'
5'
5'
3'
Okazaki Fragments

WHAT IS DNA POLYMERASE?



Cont…

- Once all of the bases are matched up (A with T, C with G), an enzyme called exonuclease strips away the primer(s).
- The gaps where the primer(s) were are then filled by yet more complementary nucleotides.


Thank you!