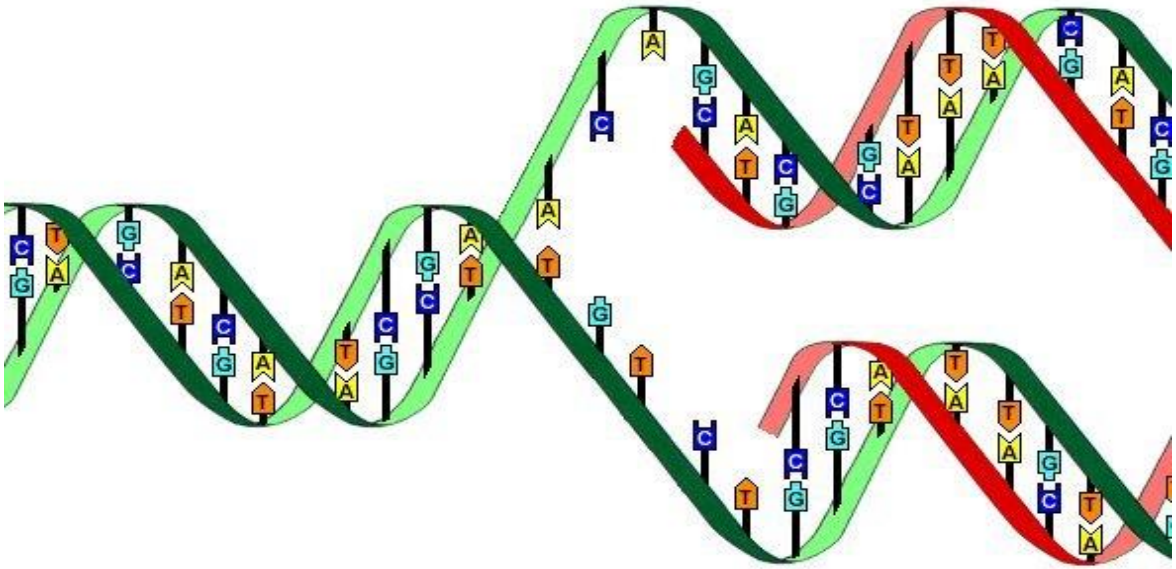
DNA REPLICATION



For B.Sc. Part I (Subs.) (Group B)

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Lecture No. 31

DNA Replication ??

DNA replication is the process, where an entire double-stranded DNA is copied to produce a second, identical DNA double helix.

During which phase of the cell cycle DNA replication occurs?

✓ During S phase of cell cycle DNA replication occurs.

- DNA replication is a biological process that occurs in all living organisms.
- DNA replication is the reaction in which daughter DNAs are synthesized using the parental DNAs as the template.

Central dogma

Three possible patterns of DNA replication:

1. Semiconservative replication

2. Conservative replication

3. Dispersive replication

1. **Semiconservative replication :** Semi-conservative DNA replication produces two helices that contain one old and one new **DNA** strand.

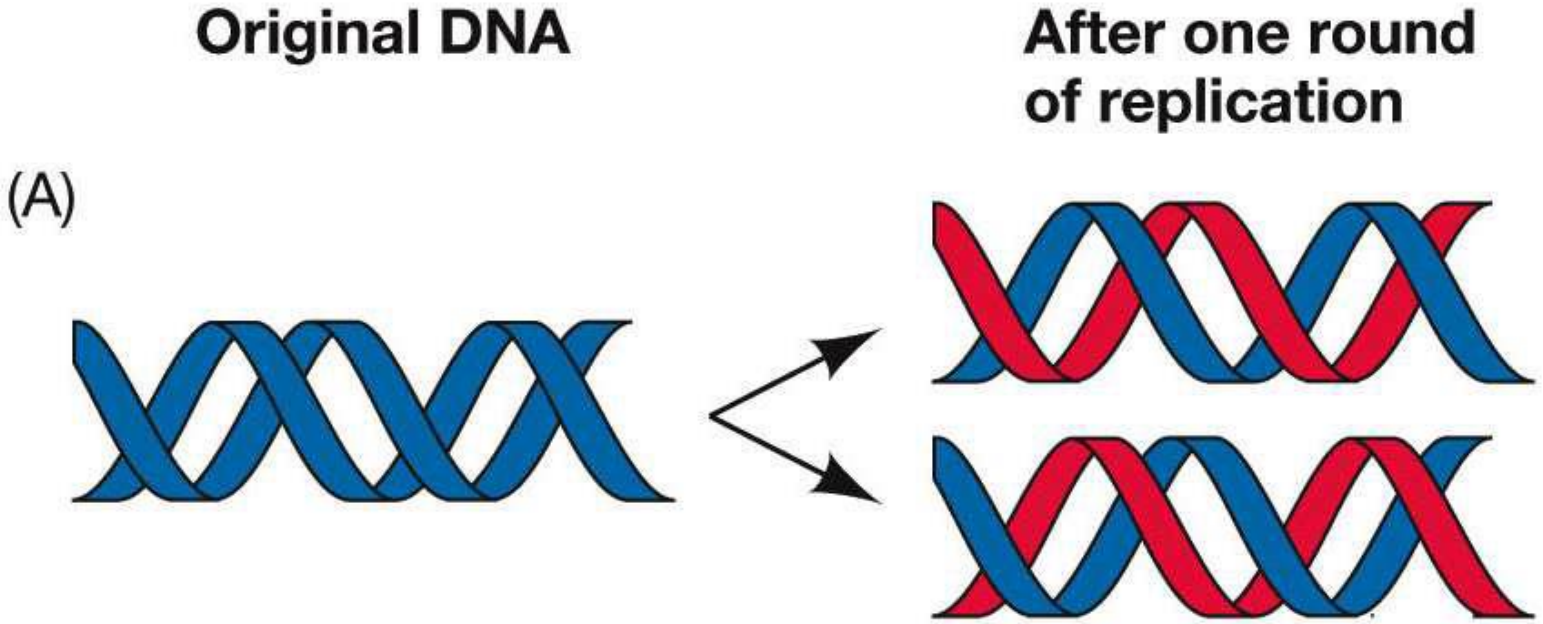


Figure: Semiconservative DNA replication

2. Conservative replication: Conservative DNA replication produces one helix made entirely of old DNA and one helix made entirely of new DNA.

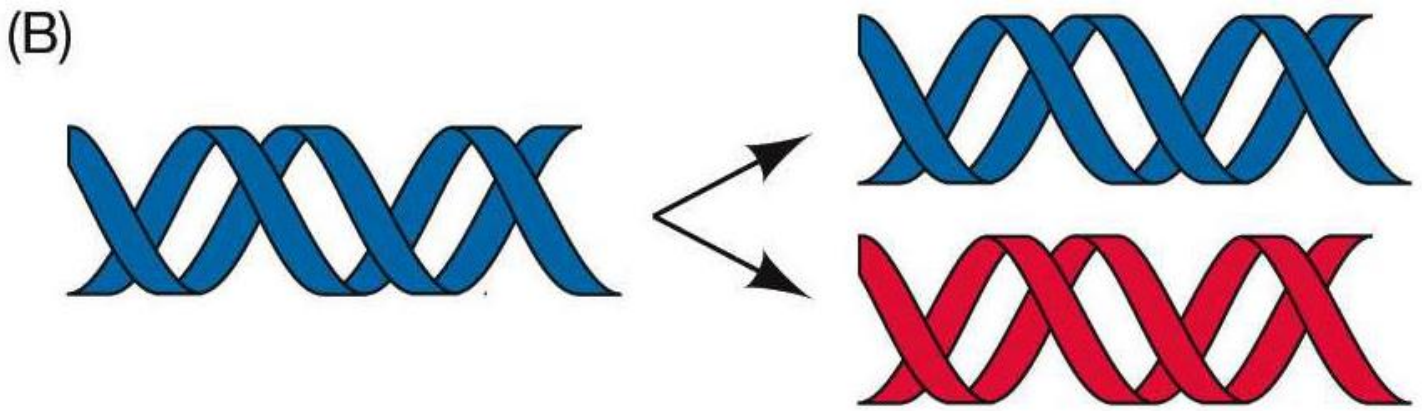


Figure: Conservative DNA replication

3. Dispersive replication

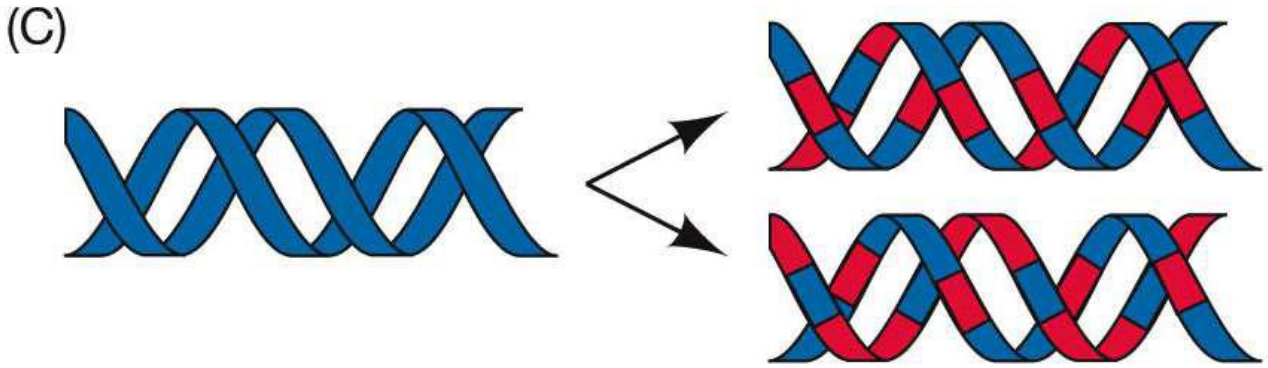
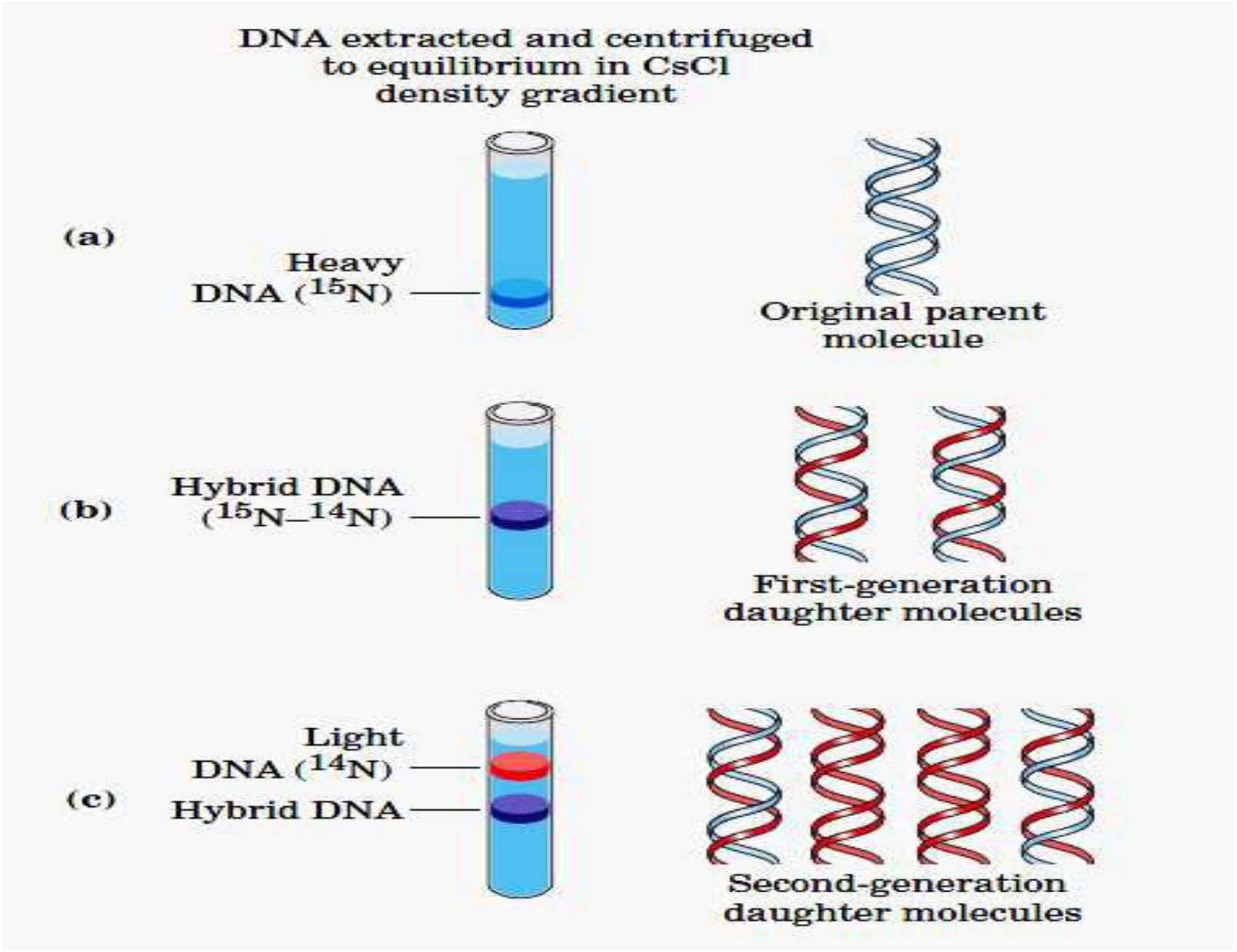


Figure: Dispersive DNA replication

Evidence in support of semiconservative mode of DNA replication (Meselson and Stahl's experiment):

- Meselson and Stahl (1958) cultured (Escherichia coli) bacteria in a culture medium containing NH4Cl (N15 is the heavy isotopes of nitrogen) as the only nitrogen source for many generations. This result was that N15 was incorporated into newly synthesized DNA (as well as other nitrogen containing compounds). This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a cesium chloride (CsCl) density gradient.
- N15 is not a radioactive isotope, and it can be separated from N14 only based on densities.
- When these bacteria with N15 were transferred in cultural medium containing N14, it was found that DNA separated from fresh generation of bacteria possesses one strand heavier than the other. The heavier strand represents the parental strand and lighter one is the new one synthesized from the culture indicating semiconservative mode of DNA replication.


DNA extracted and centrifuged to equilibrium in CsCl density gradient
(a)
Heavy DNA (^{15}N)
Original parent molecule
(b)
Hybrid DNA (^{15}N–^{14}N)
First-generation daughter molecules
(c)
Light DNA (^{14}N)
Hybrid DNA
Second-generation daughter molecules

Process of DNA Replication

➢The separation of 2 chains of DNA is termed as unzipping. And it takes place due to the breaking of H bonds. The process of unzipping starts at a certain specific point which is termed as initiation point or origin of replication.

➢ In prokaryotes there occur only one origin of replication but in eukaryotes there occur many origin of replication i.e. unzipping starts at many points simultaneously.

✓ **Helicase:** The enzyme responsible for unwinding/unzipping (breaking the hydrogen bond with the help of energy of ATP). In the process of unzipping Mg2+ act as cofactor.

✓**Topoisomerase:** The unwinding creates tension in the uncoiled part by forming more supercoils. Tension is released by enzymes toposiomerases. They causes nicking of one strand of DNA to relax the two strand of DNA and resealing the same. (**Gyrase is a type of topoisomerase in *E.coli)***

✓ A protein “Helix destabilizing protein” or “SSB (single stranded DNA binding protein)” prevents recoiling of two separated strands during the process of replication.

✓ **RNA Priming:** To start the synthesis of new chain, special type of RNA is required which is termed as **RNA primer**. The formation of RNA primer is catalysed by an enzyme RNA polymerase (primase). Synthesis of RNA primer (50-100 neucleotides) takes place in 5' → 3' direction.

✓ **Formation of DNA on RNA primers**: The new strands of DNA are formed in the 5' → 3' direction. from the 3' → 5' template DNA by the addition of deoxyribonucleotides to the 3' end of primer RNA. Nucleotides are obtained from Nucleoplasm. In the nucleoplasm, Nucleotides are present in the form of triphosphates like dATP, dGTP, dCTP, dTTP etc.

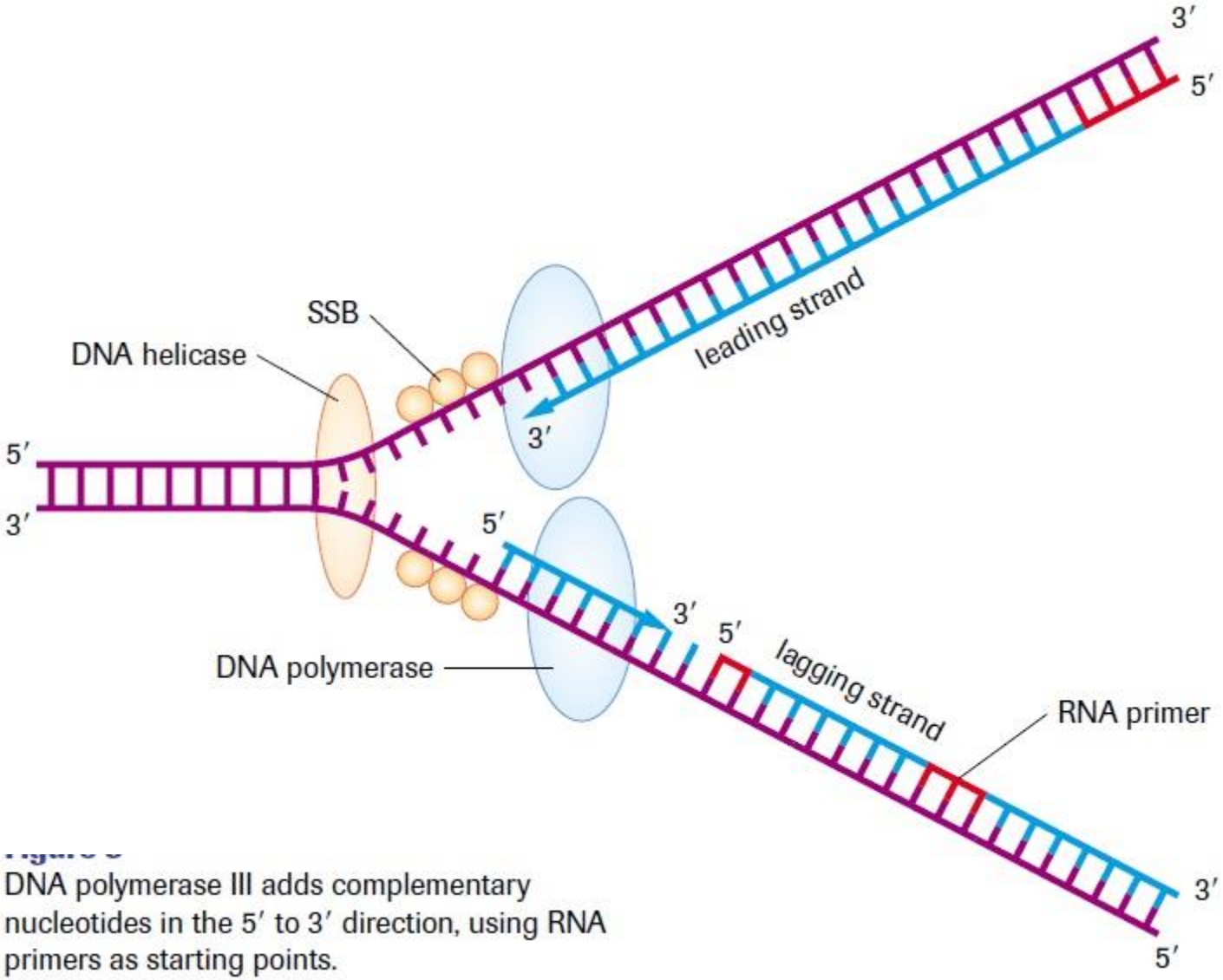
✓ During replication the 2 phosphate groups of all Nucleotides are separated. In this process energy is yielded which is consumed in DNA replication. So it is clear that DNA does not depend on mitochondria for its energy requirements.

✓The formation of new chain always takes place in 5'→3' direction. As a result of this one chain of DNA is continuously formed and it is termed as **Leading strand.** The formation of second chain begins from the centre and not from the terminal points, so this chain is discontinuous and is made up of small segments called **Okazaki fragments.** This discontinuous chain is termed as **Lagging strand.**

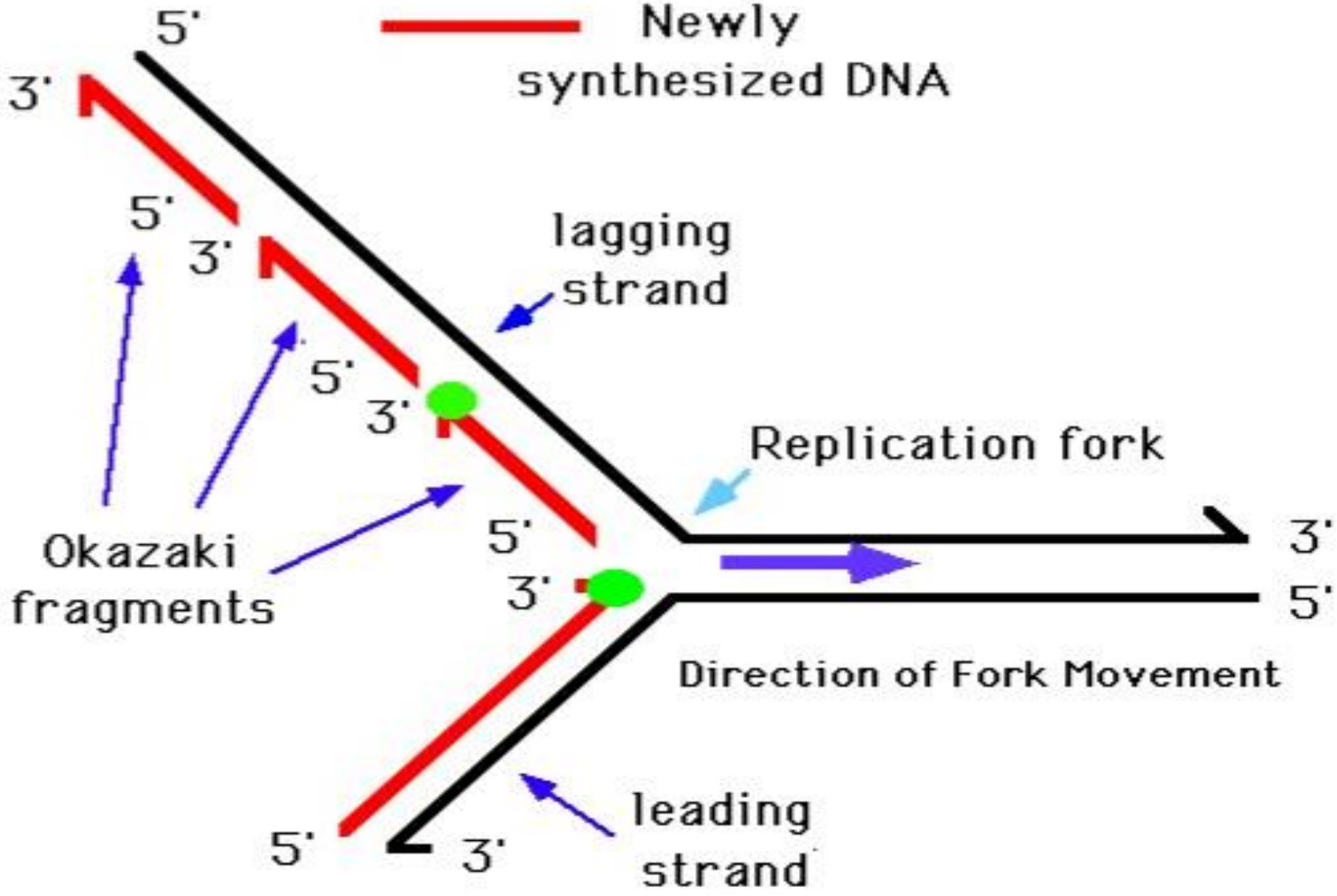
✓ **Excision of RNA primers:** Once a small segment of an okazaki fragment has been formed. The RNA primers are removed by the activity of **DNA polymerase I.**

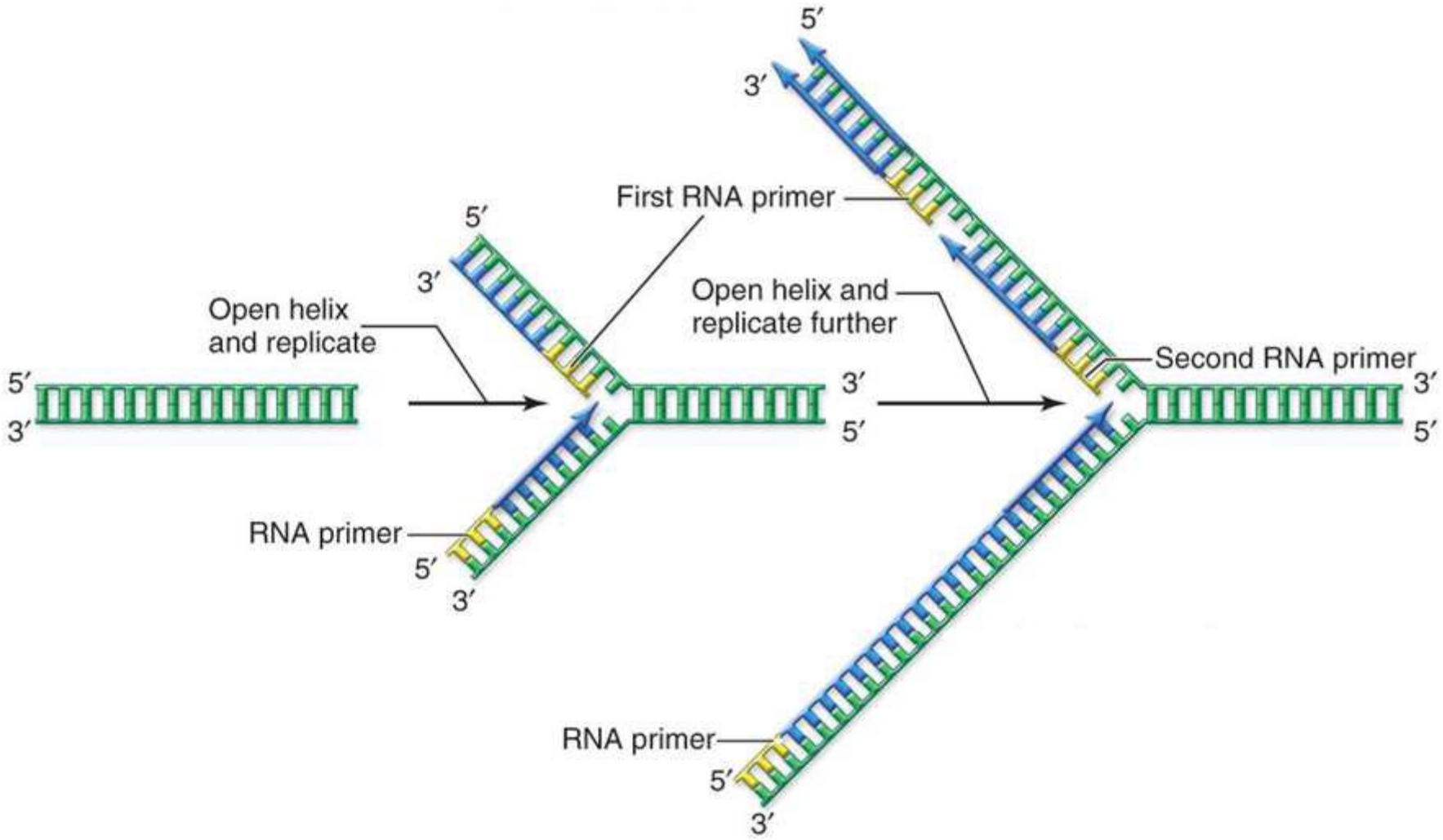
✓ **Joining of okazaki fragments:** The gaps left between Okazaki fragments are filled with complimentary deoxyribonucleotide residues by DNA polymerase-I. Finally, the adjacent 5' and 3' ends are joined by **DNA ligase (Khorana).**

- In prokaryotes, there are 3 enzymes known to function in replication & repair (DNA polymerase I, II & III).
- In eukaryotes, there are 5 enzymes known to function in replication & repair DNA pol α, β, γ, δ, ε.



DNA polymerase III adds complementary nucleotides in the 5′ to 3′ direction, using RNA primers as starting points.


5'
3'
Newly
synthesized DNA
5'
3'
lagging
strand
5'
3'
Replication fork
Okazaki
fragments
5'
3'
3'
5'
Direction of Fork Movement
5'
3'
leading
strand


5′
3′
First RNA primer
5′
3′
Open helix
and replicate
Open helix and
replicate further
Second RNA primer
5′
3′
3′
5′
3′
5′
RNA primer
5′
3′
RNA primer
5′
3′

Three Stages of replication

1). Initiation:

✓occurs at the origin of replication

✓ separates dsDNA, primer synthesis

2). Elongation

✓ involves the *addition of new nucleotides* (dNTPs) based oncomplementarity of the template strand

✓forms phosphoester bonds, correct the mismatch bases, extending the DNA strand**,**

3). Termination

✓stops the DNA Replication occurs at a specific *termination site*

Eukaryotic DNA replication

✓Multiple origin site

✓Multiple replication bubbles

✓ **Enzymes – 5 types**

✓DNA polymerase - **α , β, γ, δ and ε**

✓DNA Poly α = Synthesis of RNA primer (both strand) Responsible for initiation

✓Poly , β = Repair of DNA

✓ Poly γ = Replication of mitochondrial DNA

✓Poly δ = Replication of leading and Proof reading

✓ Poly ε = Lagging strand synthesis

Step in Replication	Prokaryotic cells	Eukaryotic cells
Recognition of origin of replication	Dna A protein	RpA (Replication Protein-A)
Unwinding of DNA double helix	Helicase	Helicase
Stabilization of unwound template strands	Single-stranded DNA-binding protein (SSB)	Single-stranded DNA-binding protein (SSB)
Synthesis of RNA primers	Primase	Primase
Synthesis of DNA Leading strand Lagging strand	 DNA polymerase III DNA polymerase III	 DNA polymerase δ DNA polymerase Ɛ
Removal of RNA primers	DNA polymerase I (5→ 3' exonuclease	RNAse-H
Replacement of RNA with DNA	DNA polymerase I	Unknown
Joining of Okazaki fragments	DNA ligase (requires NAD)	DNA ligase (requires ATP)

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Thank You!!!