Molecular biology

Replication

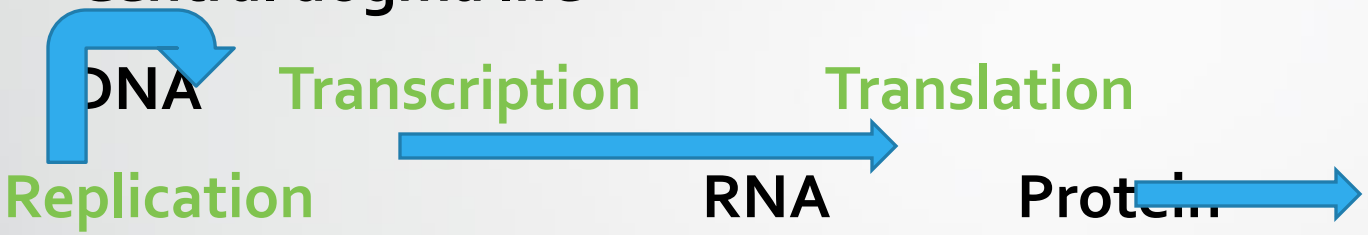
Transcription

Translation

Bluepen online

www.bluepenonline.com

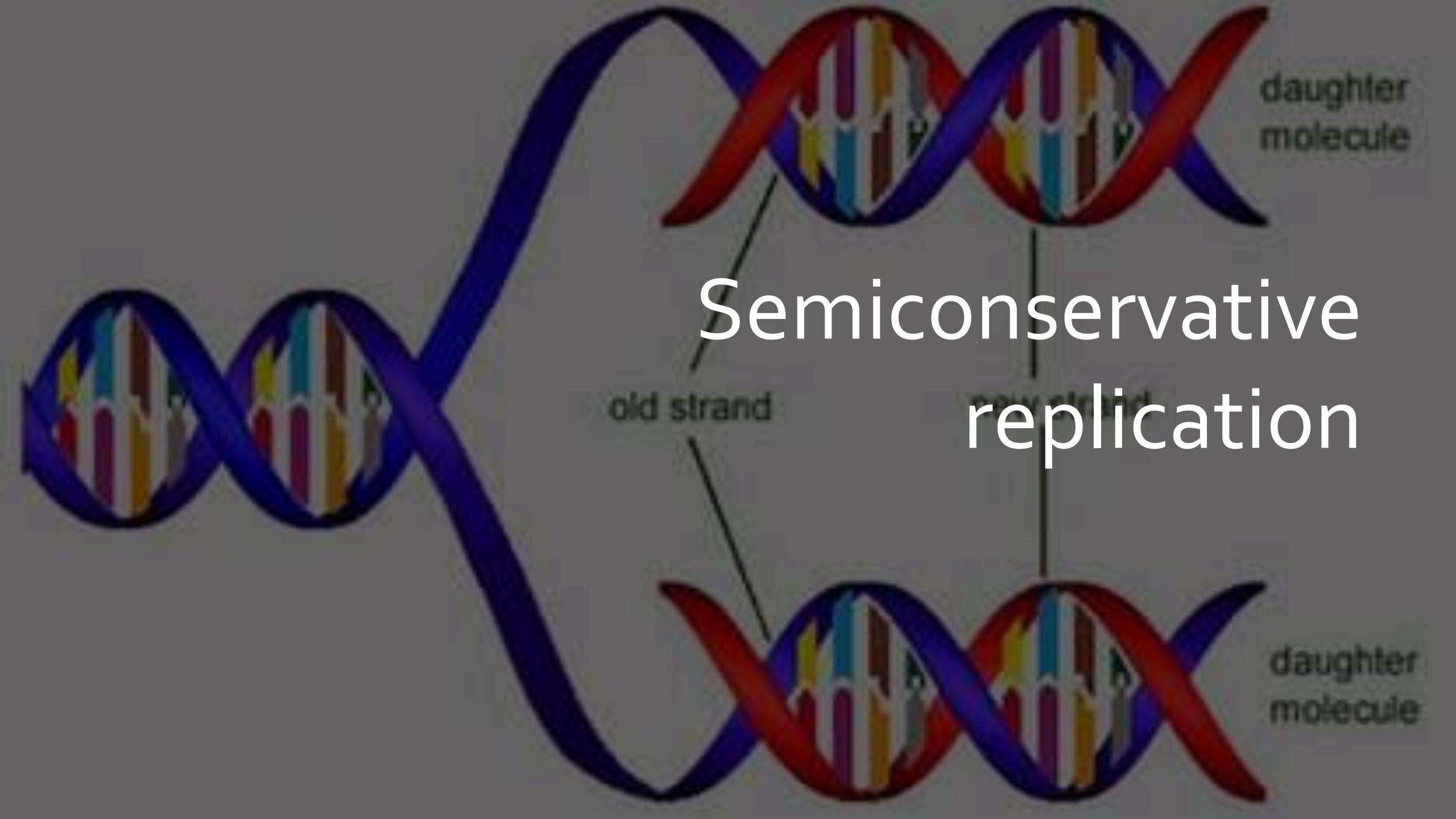
- **Deoxyribonucleic acid (DNA)** is a macromolecule that carries genetic information from generation to generation
- **Central dogma life**



- **The biological information flows from DNA to RNA and there to proteins .**
- **This is the central dogma of life**
- **DNA controls every function of the cell through protein synthesis**

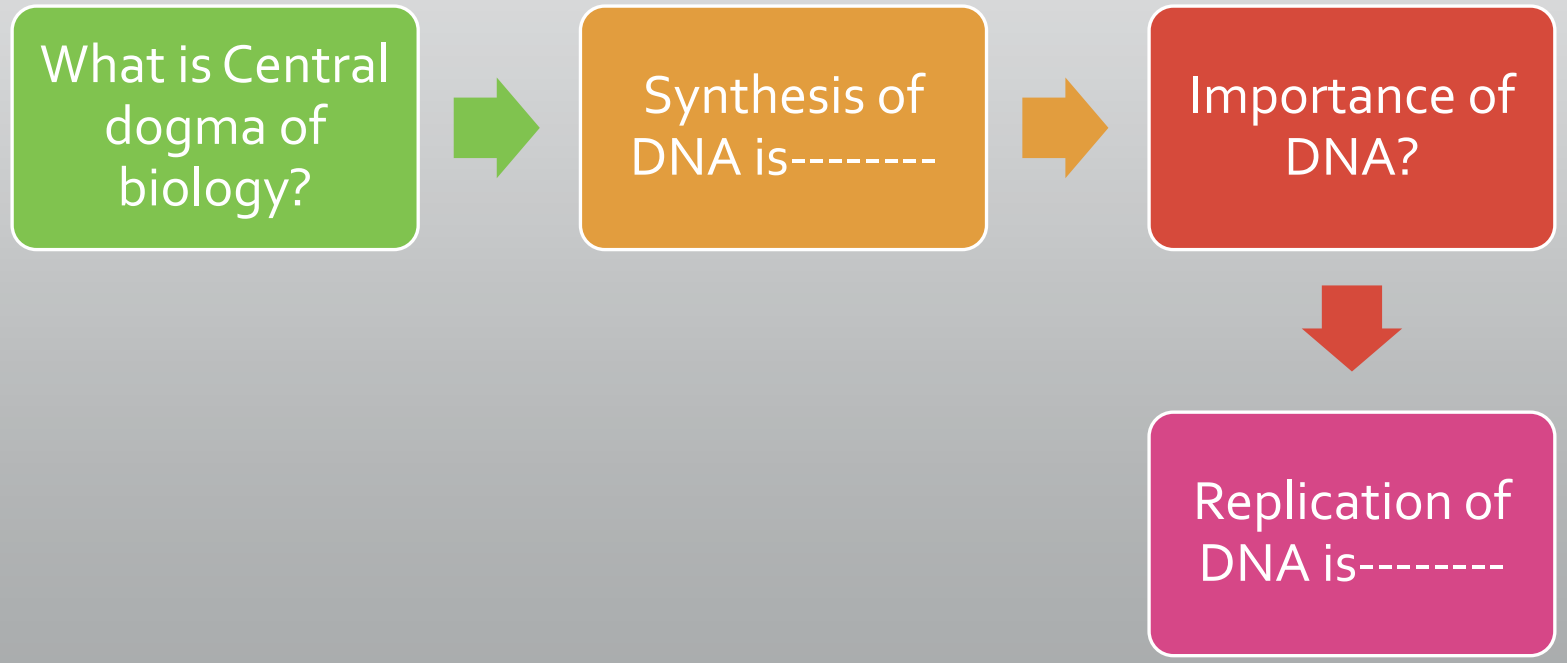
Replication

- DNA is a major store of genetic information
- To transfer genetic information from a parent cell to daughter cell during cellular reproduction, the DNA must be duplicated.
- **The duplication or synthesis of DNA is called replication**
- The method of replication results in **semi -conservative mechanism**, in which each replicated duplex daughter DNA molecule contains one parent strand and one newly synthesized strand



Semiconservative replication

Interaction no 1



Introduction

1)DNA synthesis is catalyzed by enzyme called **DNA dependent DNA polymerase.** They are called **DNA dependent** as they require DNA template

- Each strand of DNA serves as template strand over which a new strand is synthesized

2)**The base pairing rule (Chargaff's rule**) is always maintained

- The new strand is joined to the old strand by hydrogen bonds between base pairs(A withT and G with C)

3)Each daughter cell gets only one strand of parent DNA

4)Old DNA is not degraded ,but conserved for the daughter nuclei(semi conservative process)

5)DNA polymerase/Kornberg enzyme involved in the synthesis of new complementary strand in 5′ to 3′ direction

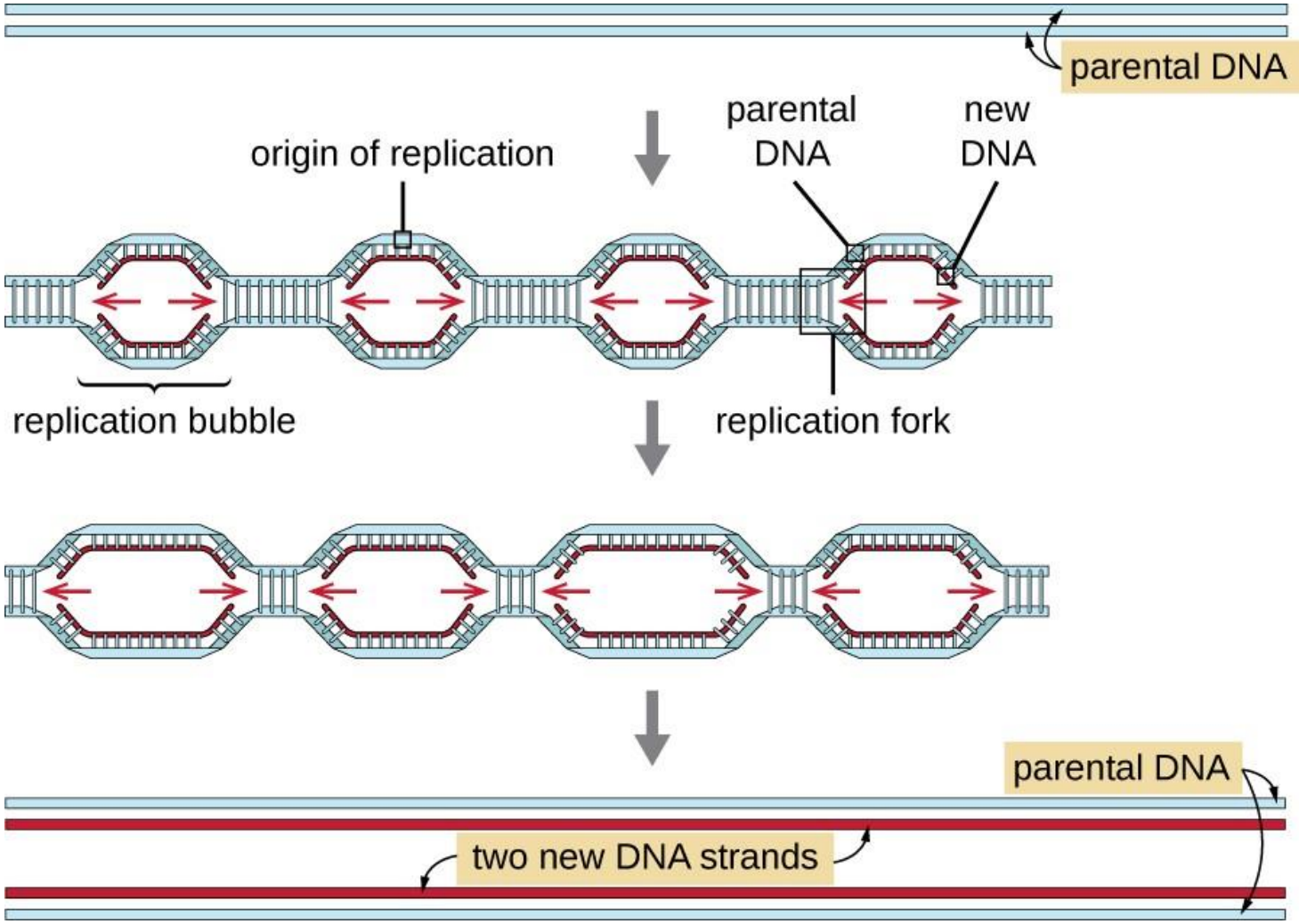
6)Substrates-dATP,dTTP,dCTP,dGTP

Stages of replication

1)Initiation

2)Elongation

3)Termination


parental DNA
origin of replication
parental DNA
new DNA
replication bubble
replication fork
parental DNA
two new DNA strands

1)Initiation

Initiation of DNA replication involves unwinding or separation of two complimentary DNA strands and formation of replication fork

- Unwinding occurs at a specific site is called **origin of replication,(ORI/ Ori)where active synthesis occurs**. This region is called replication fork.
- **Prokaryotes-single site**
- **Eukaryotes-Multiple site**

These sites Are **AT rich** regions

Specific protein **dnaA** (20-50 monomers)binds with the site of origin of replication, This causes double stranded DNA to separate

Replication of double stranded DNA is **bidirectional**

In eukaryotes replication begins at multiple sites composed of AT rich regions is referred to as **consensus sequence**

Multiple Ori in eukaryotes are essential for **rapid replication**

Interaction Time

ii)Replication bubble

Two complementary strands of DNA separate at the site of replication to form a bubble

Multiple replication bubbles are formed in eukaryotes

iii) Repliosome/DNA replicase system

The complex of enzyme proteins and other factors required for DNA replication is called Repliosome

Interaction no2

- Chargaff's rule
- Repliosome
- Ori C
- Reason for multiple Ori in eukaryotes
- Dna protein
- Kornberg’s enzyme

Steps involved in initiation/ Components of Repliosome

1. **Dna A protein** ,binds at 'Ori' and opens the duplex.
2. **DnaB /Helicase-**Separate the strands, using energy from ATP.Helicase move in both directions unwinding the strands in advance of replication. This forms replication bubble with two replication forks
3. **Topoisomerases-**The stress produced due to unwinding by helicase is released by cutting either one or both DNA strands.

4. **Single stranded binding proteins (SSB)** -Stabilizes the separated strands and prevents their reassociation

5.RNA primer

For the synthesis of new DNA ,a short fragment of RNA(100-200nucleotides long)is required as a primer

The enzyme RNA polymerase/ primase in association with SSB forms a complex called primosome and produce RNA primer.

A short stretch of RNA that is complementary to one of the template strand

- After DNA synthesis has been initiated ,the RNA primer is removed by DNA polymerase(DNAP)by using exonuclease activity and is replaced with deoxyribonucleotides by DNAP.

6)DNA polymerase

DNA synthesis is catalyzed by enzymes called **DNA dependent DNA polymerase.**

<u>These polymerases are required for</u>

-DNA chain elongation

-DNA repair(5′-3′ exonuclease activity)

-Proof reading(3′-5′ exonuclease activity)

There are three types of polymerase in Prokaryotes

1)DNA polymerase I (Pol I)

Completes chain synthesis between okazaki fragments on the lagging strand

2) DNA polymerase II(Pol II)

Concerned with proof reading and DNA repair

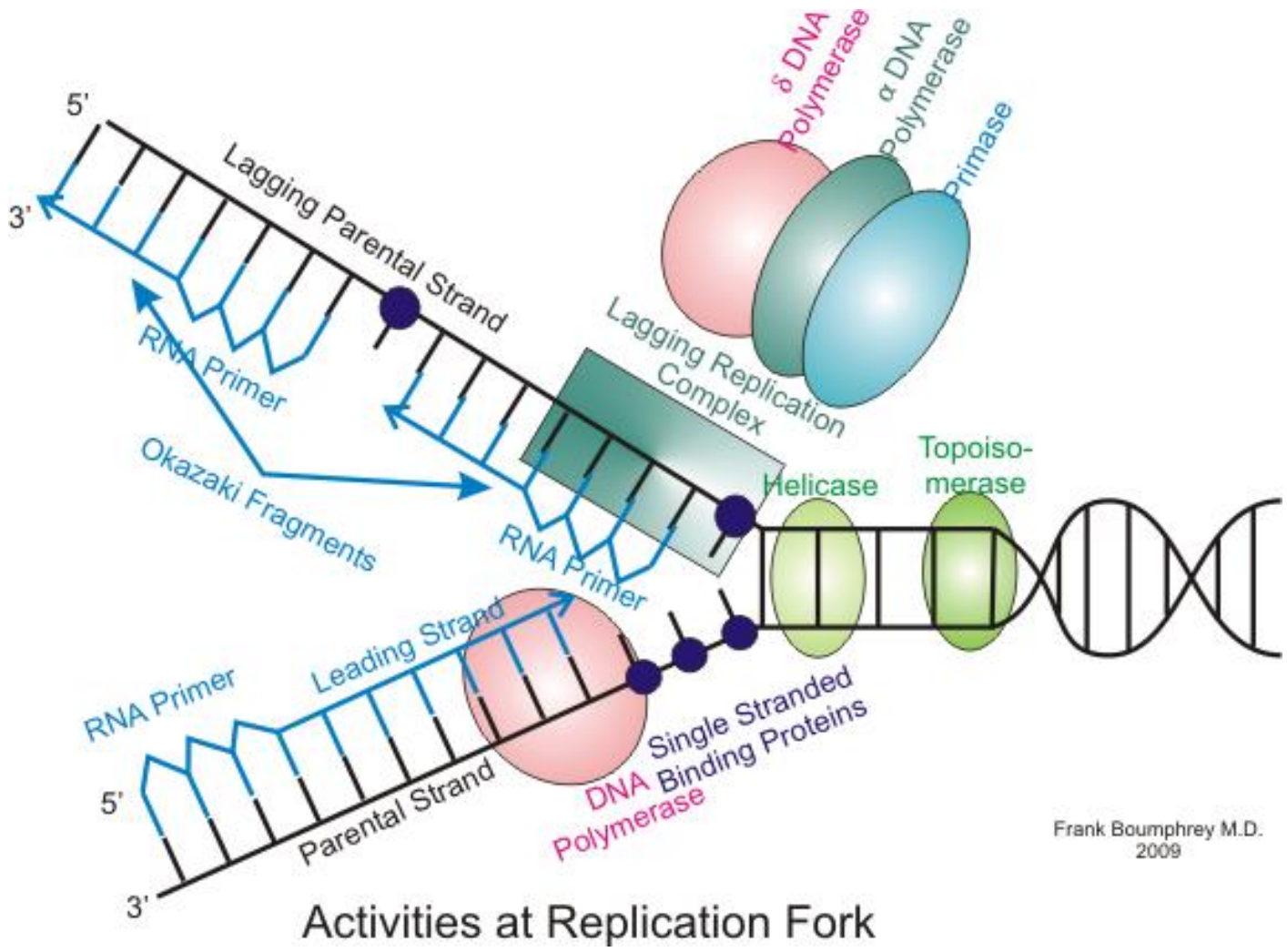
3)DNA polymerase III (Pol III)

Catalyzes leading and lagging strand synthesis

- **5 DNA polymerases exist in eukaryotic cells** (α,β,γ,δ and ε)

Interaction no3

- During replication ,unwinding of double helix is initiated by--------
- The unwound strands of DNA are held apart by-------
- RNA primer is formed by the enzyme-----
- DNA polymerase activities?
- Types of DNA polymerase?



Activities at Replication Fork

2)Elongation

RNA primer synthesized at each of replication forks,**DNA polymerase III** initiates synthesis of new DNA strand by adding deoxyribonucleotides to the 3′ end of the RNA primer

DNA polymerase III can synthesize new chain only in 5′-3′ direction

Both DNA strands are synthesized simultaneously but in opposite direction, one is in direction towards the replication fork,the other in a direction away from the replication fork

The DNA chain which runs in the 3′-5′ direction is copied by polymerase III as a **continuous** strand ,**requiring one primer.**This new strand is known as **leading strand.**

DNA chain run in 5′-3′ direction is copied by polymerase III as a **discontinuous** manner **because synthesis can proceed only** in the

5′ to 3′ direction. This new strand is known as lagging strand.

-This requires numerous RNA primers,as the replication fork moves RNA primers are synthesized at specific intervals

These RNA primers are extended by DNA polymerase III into short pieces of DNA called **Okazaki fragments**

After the completion of lagging strand synthesis RNA primers are removed by DNA polymerase I

DNA polymerase I also fills the gap that are produced by removal of primer leaving only a nick ,it cannot join polynucleotide chain together .

An additional enzyme **DNA ligase** catalyzes the formation of a phosphodiester bond to seal the okazaki fragments.

Interaction no4

- Leading strand
- Lagging strand
- Importance of Okazaki fragments
- Role of DNA ligase
- Direction of DNA synthesis

Proof reading

- DNA is copied by DNA polymerase with high fidelity (accuracy).Incorrect nucleotides are incorporated with a frequency of one in 10^8 to

  10^{12} bases, which could lead to **mutation.**
- Error ratio during replication is kept at very low level by **proof reading .**
- All the three polymerases have proof reading activity
- DNA pol I known to excise mismatched nucleotides before the introduction of next nucleotide

3)Termination

- Termination sequences ,e.g.'ter' direct termination of replication
- A specific protein **' ter' binding protein binds these sequence and prevents helicase** from further unwinding of DNA and facilitates the termination of replication.

Interaction no 5

Explain Proof reading mechanism?

Termination of replication

Interaction Time & Summary

Thank You

- Give us your feedback
- Please let's know what do you want
- We open discussion forum
- Knowledge is an ocean
- We focus on your continuous improvement.
- We assess right time with right faculty and create success
- 100% Students Engagement + Personalized Learning

Email

info@bluepenonline.com

Call Us

+91-81 0504 2828

+91-63 6631 1818

+44-7514973149

Reach Us

www.bluepenonline.com