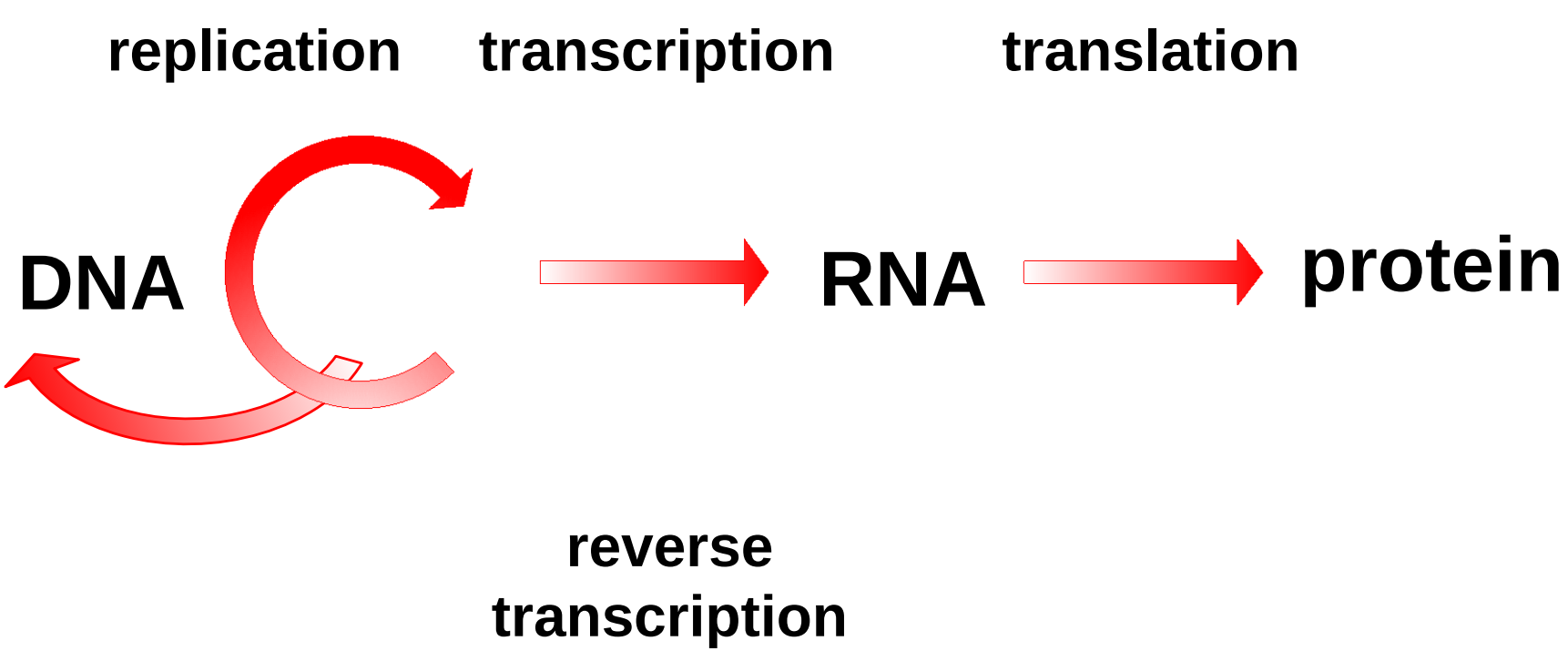
REPLICATION OF DNA

By

Sumit sharma
Assistant Professor
HMV jalandhar

- **To impart the basic concepts of DNA replication process**
- **To demonstrate the functions of various enzymes involved in the replication process**
- **To highlight on the different stages and mechanism of the replication process**

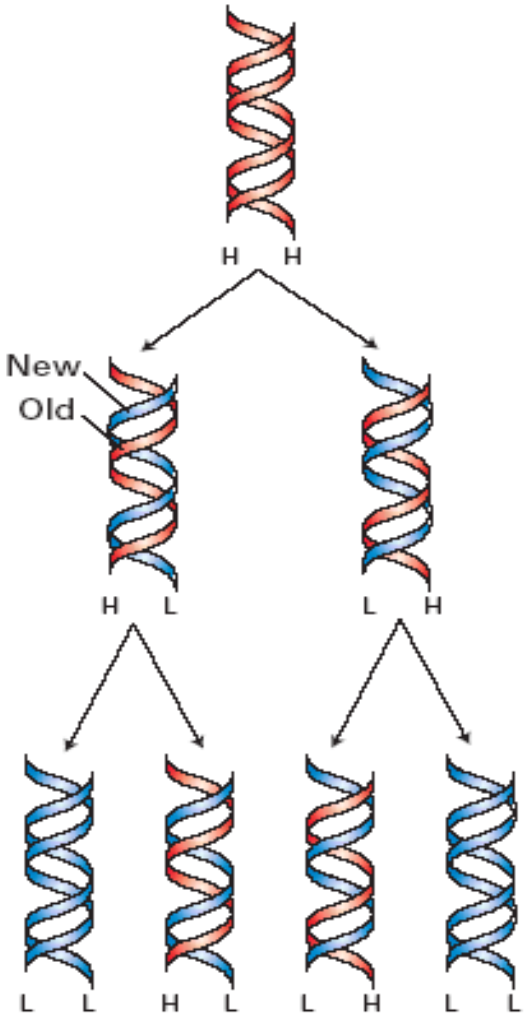
Central dogma



CENTRAL DOGMA

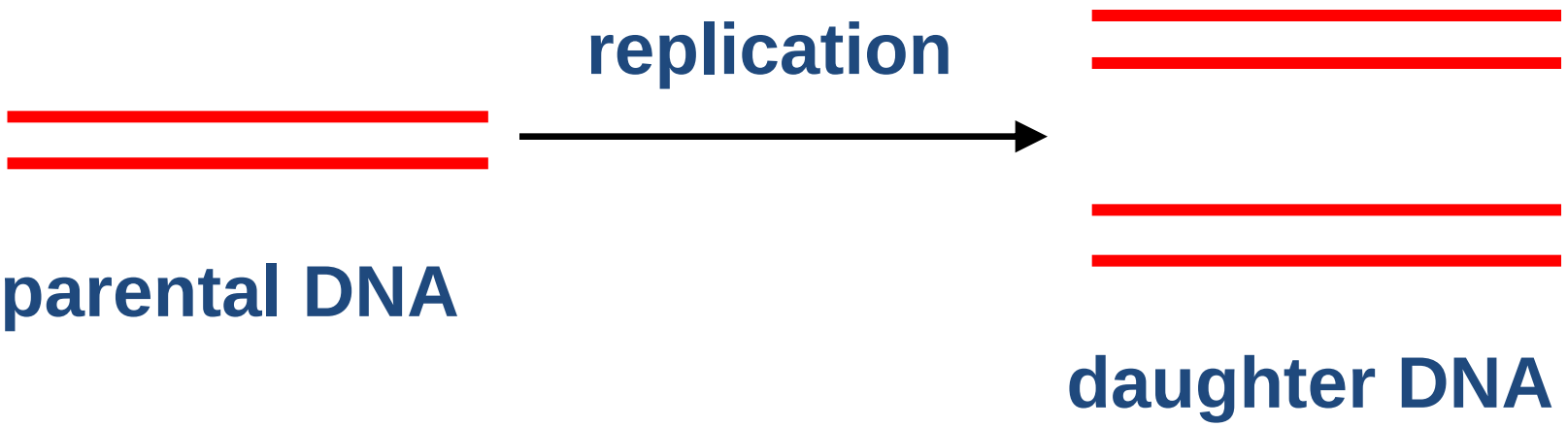
- **Replication:** synthesis of daughter DNA from parental DNA
- **Transcription:** synthesis of RNA using DNA as the template
- **Translation:** protein synthesis using mRNA molecules as the template
- **Reverse transcription:** synthesis of DNA using RNA as the template

DNA Replication



General Concepts of 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**



DAUGHTER STRAND SYNTHESIS

- **Chemical formulation:**

$$(dNMP)_n + dNTP \longrightarrow (dNMP)_{n+1} + PPi$$

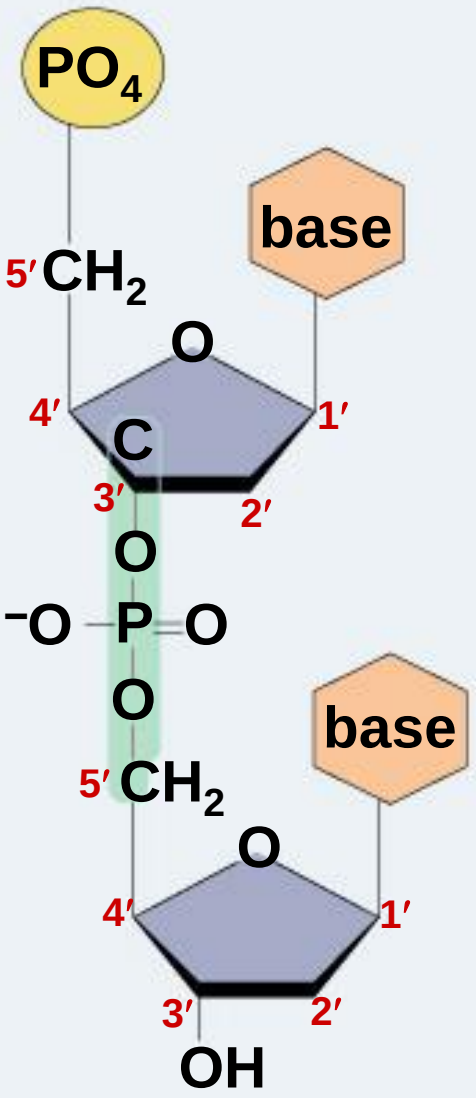
DNA strand substrate

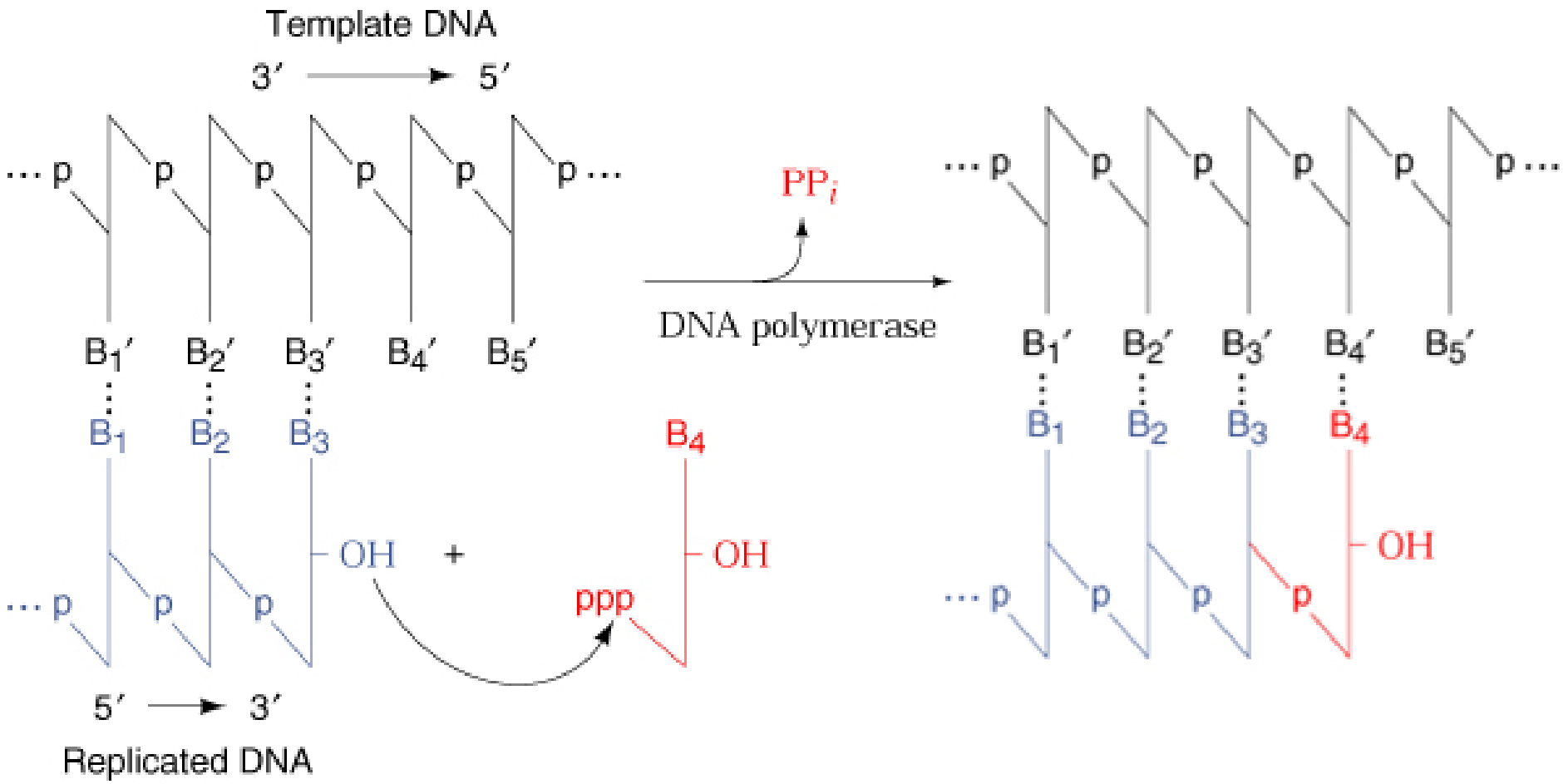
elongated DNA strand

- **The nature of DNA replication is a series of 3´- 5´ phosphodiester bond formation catalyzed by a group of enzymes.**

THE DNA BACKBONE

- Putting the DNA backbone together
  - refer to the 3′ and 5′ ends of the DNA




Template DNA
3′
5′
··· p p p p p p ···
B1′ B2′ B3′ B4′ B5′
B1 B2 B3
OH
+
B4
OH
ppp
··· p p p
5′
3′
Replicated DNA
PPi
DNA polymerase
··· p p p p p p ···
B1′ B2′ B3′ B4′ B5′
B1 B2 B3 B4
OH
··· p p p p

DNA REPLICATION SYSTEM

Template: double stranded DNA

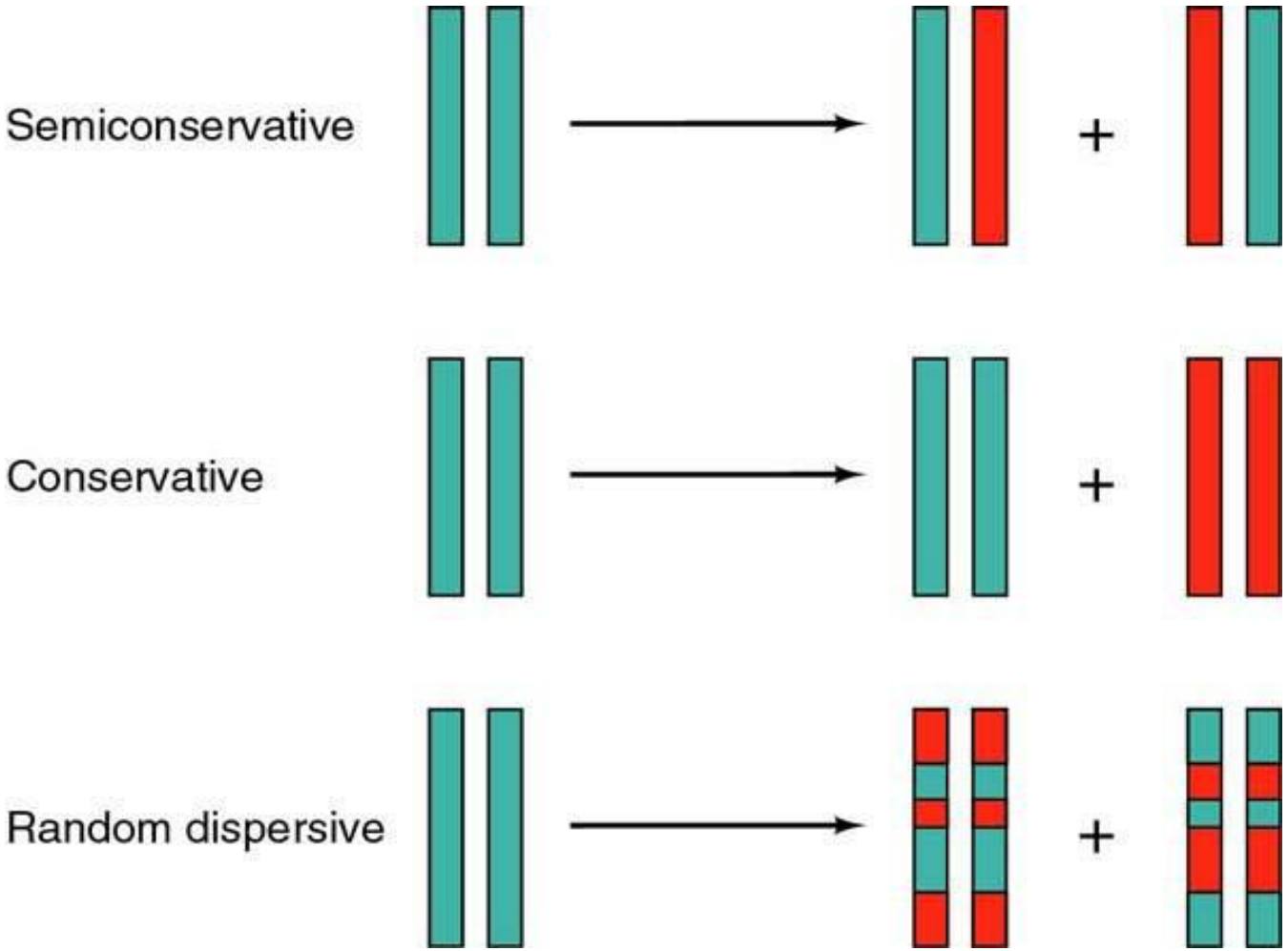
Substrate: dNTP

Primer: short RNA fragment with a free 3′-OH end

Enzyme: DNA-dependent DNA polymerase (DDDP),
other enzymes,
protein factor

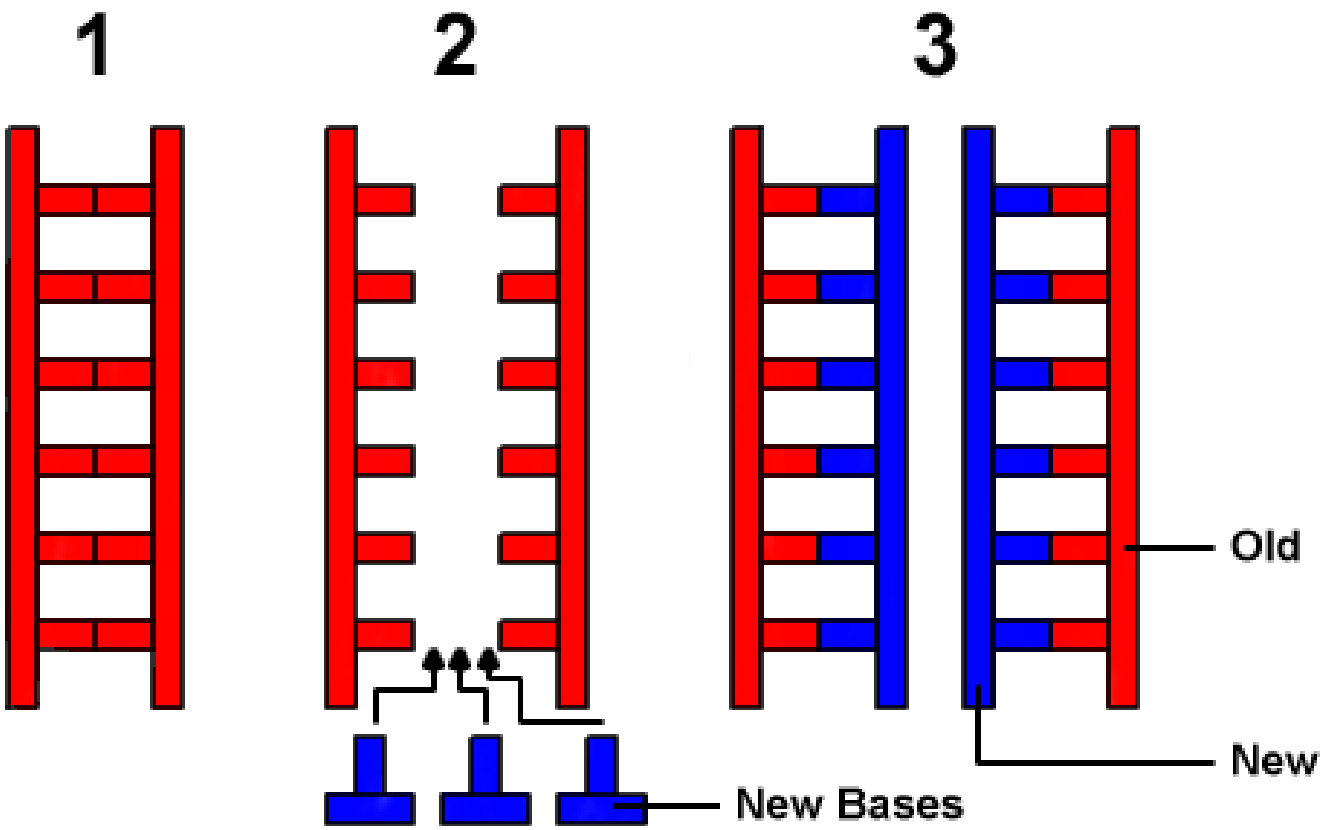
Characteristics of replication

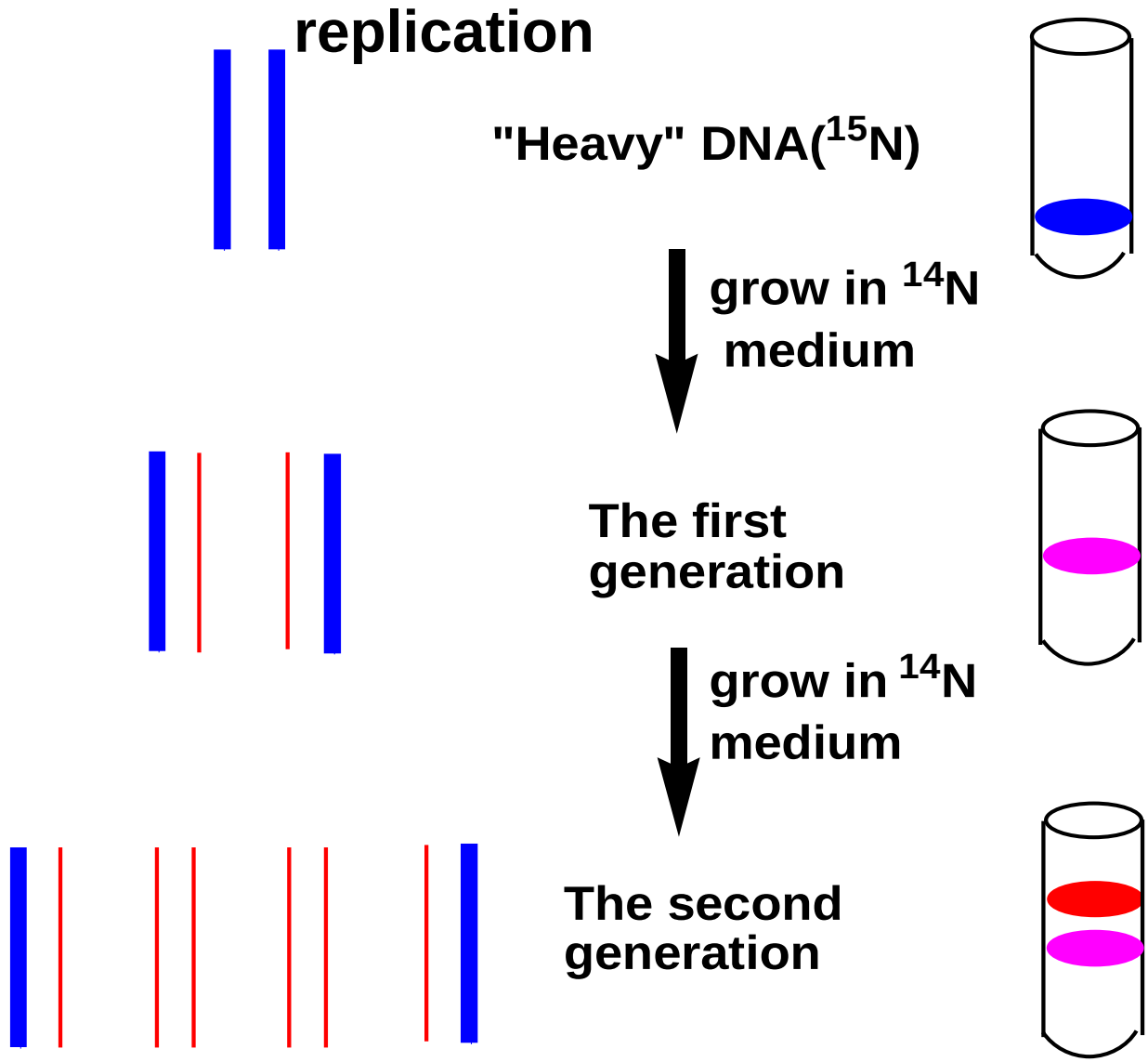
- **Semi-conservative replication**
- **Bidirectional replication**
- **Semi-continuous replication**
- **High fidelity**


Semiconservative
Conservative
Random dispersive

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

Semiconservative replication




replication
"Heavy" DNA(15N)
grow in 14N medium
The first generation
grow in 14N medium
The second generation

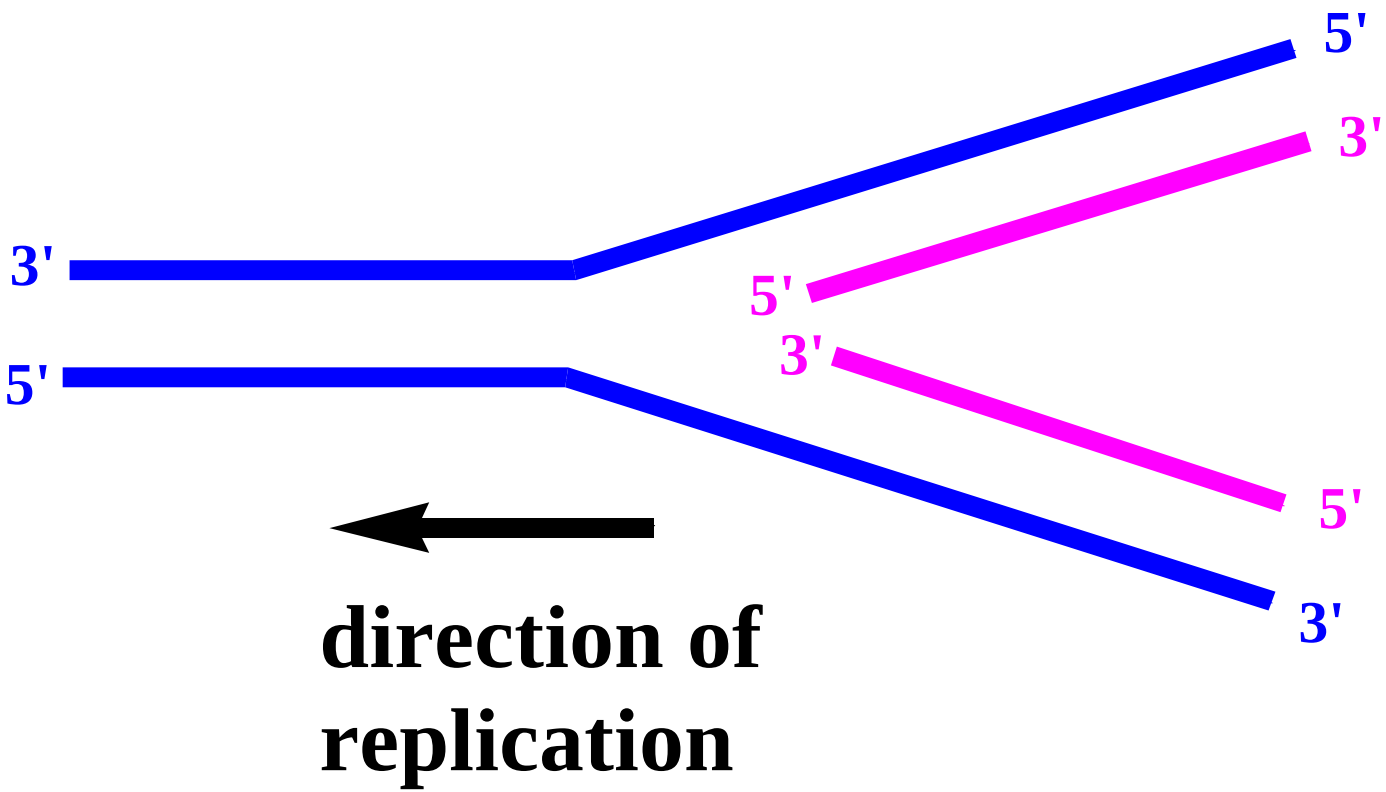
Significance

The genetic information is ensured to be transferred from one generation to the next generation with a high fidelity.

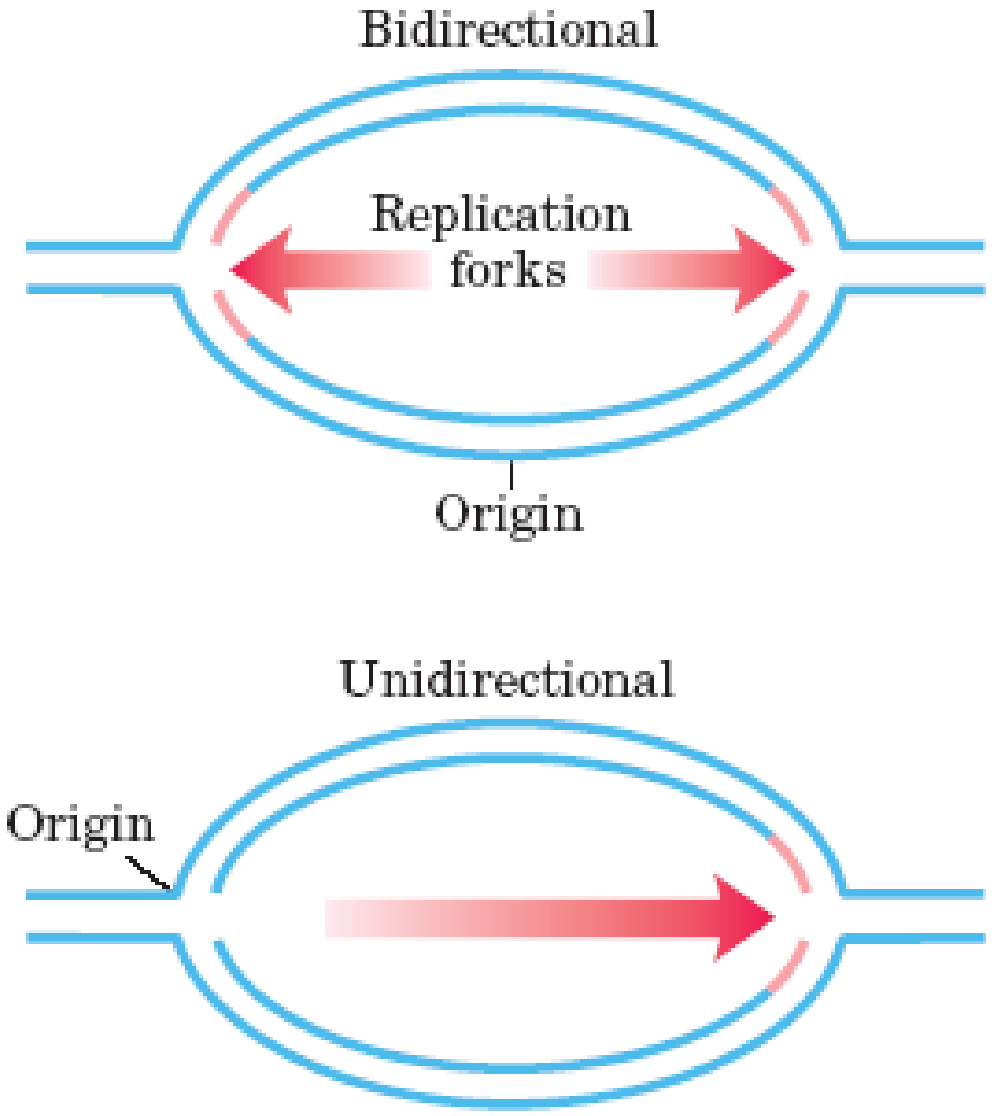
Bidirectional replication

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

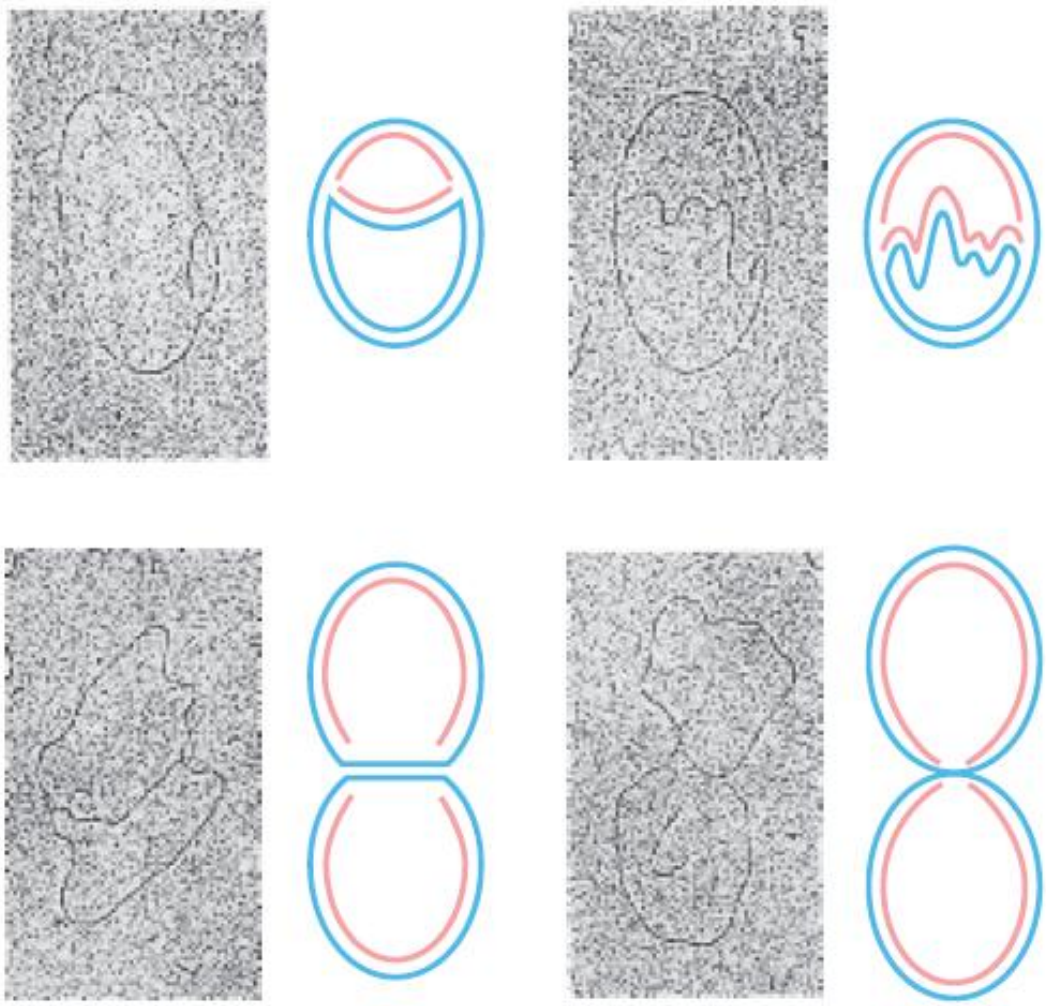
Replication Fork



- **Once the dsDNA is opened at the origin, two replication forks are formed spontaneously.**
- **These two replication forks move in opposite directions as the syntheses continue.**


Bidirectional
Replication
forks
Origin
Unidirectional
Origin

REPLICATION OF PROKARYOTES

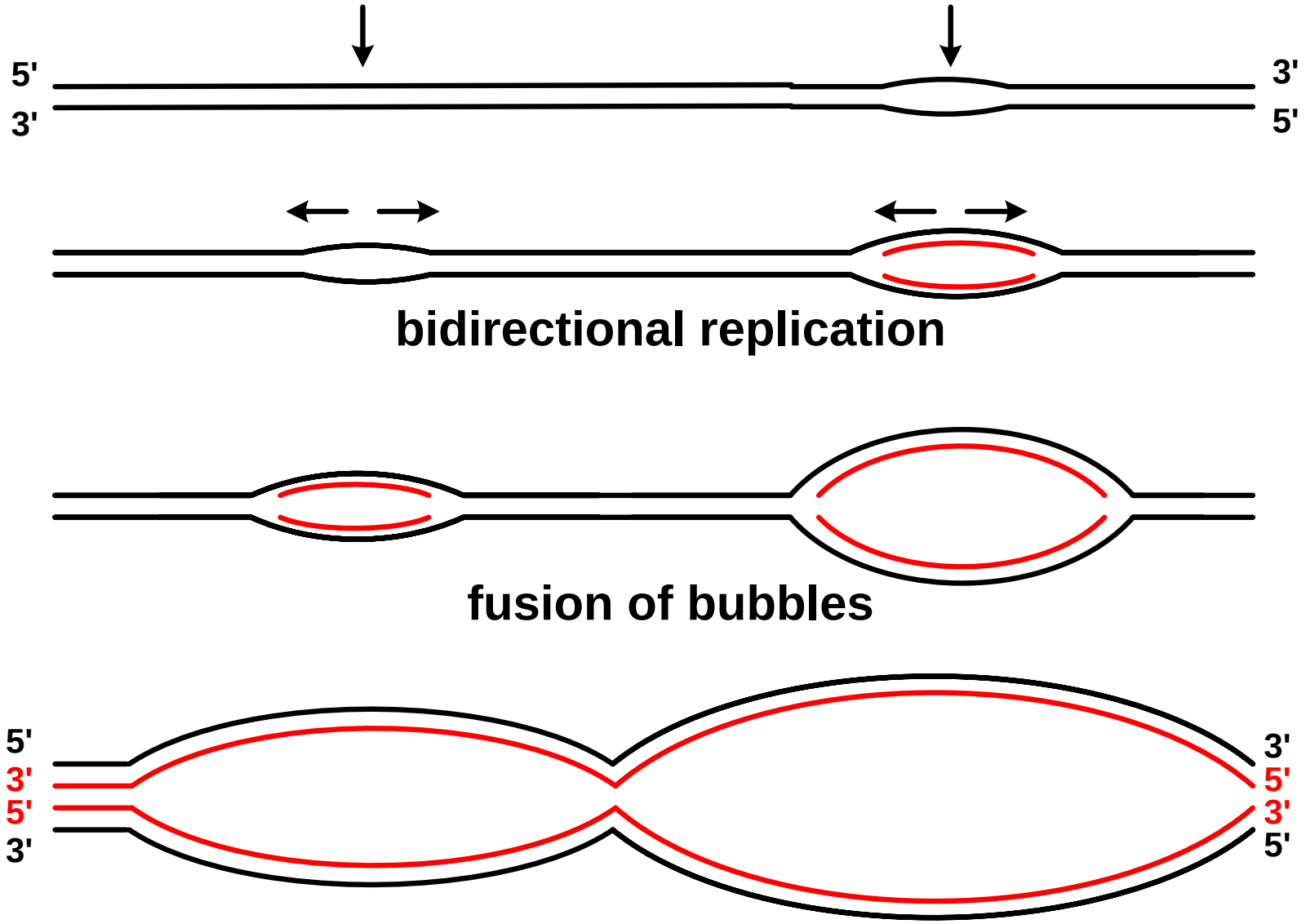


The replication process starts from the origin, and proceeds in two opposite directions. It is named θ replication.

Replication in eukaryotes

- **Chromosomes of eukaryotes have multiple origins.**
- **The space between two adjacent origins is called the replicon, a functional unit of replication.**

origins of DNA replication (every ~150 kb)

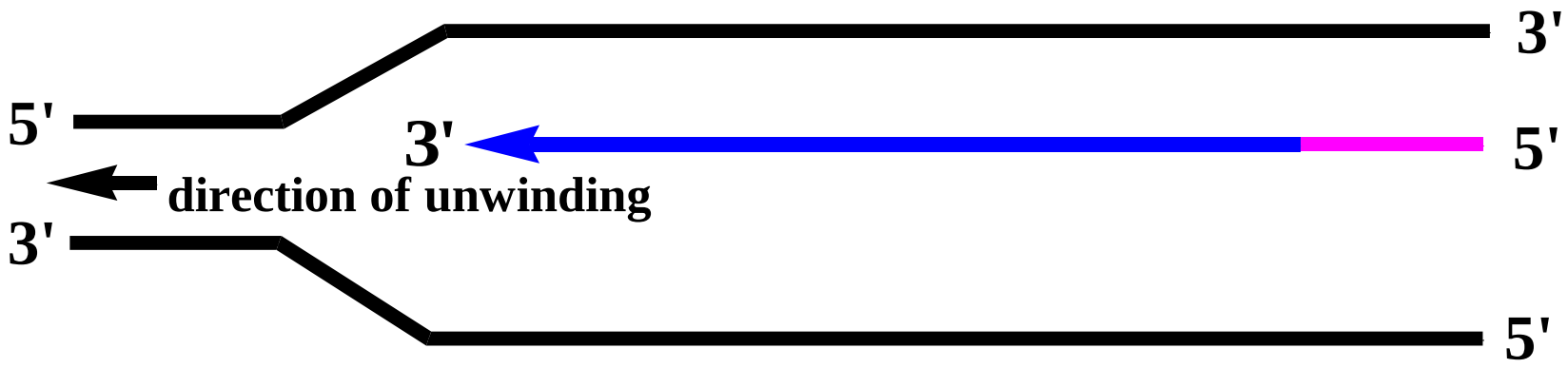


§ 1.3 Semi-continuous Replication

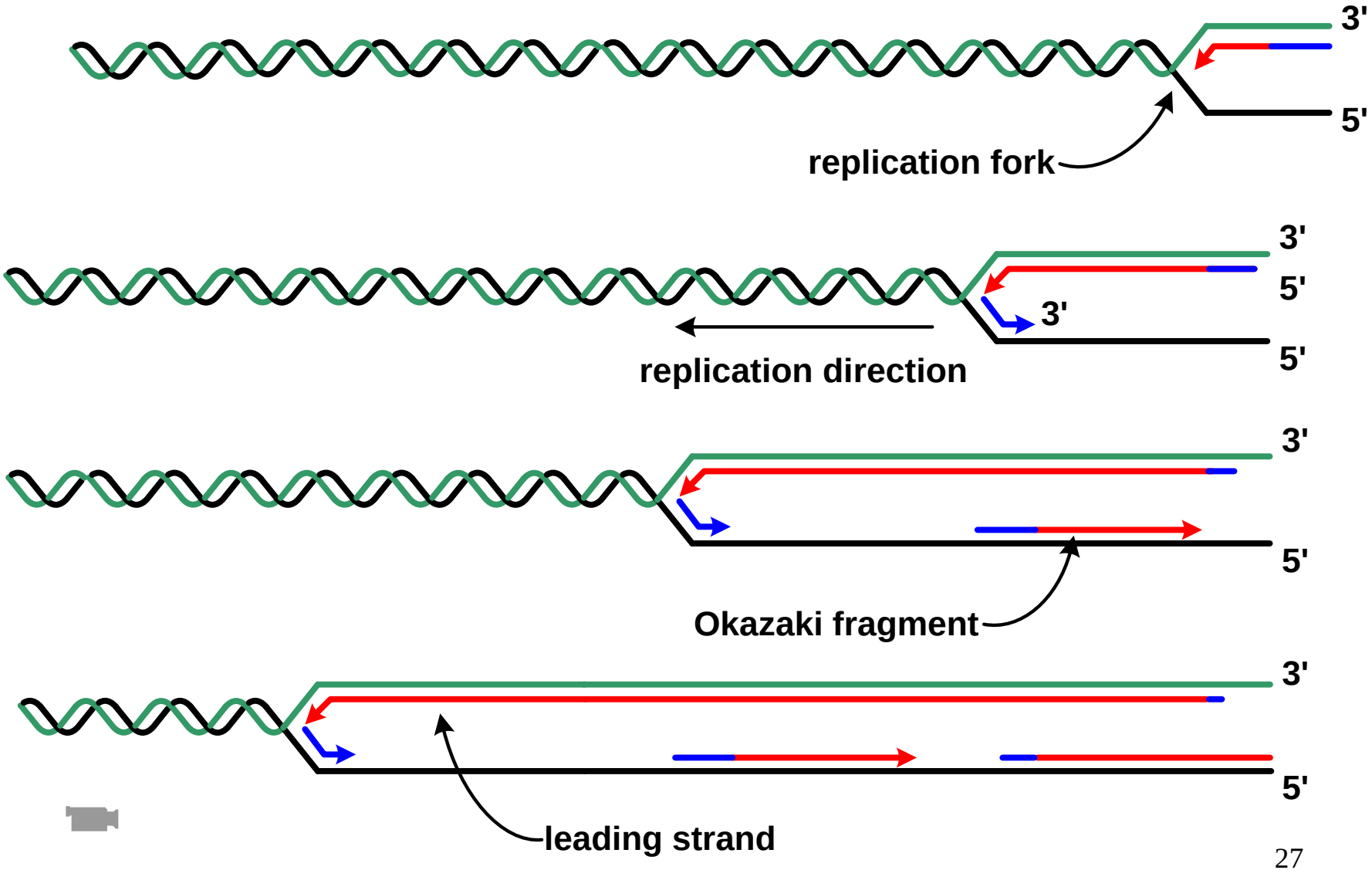
The daughter strands on two template strands are synthesized differently since the replication process obeys the principle that DNA is synthesized from the 5′ end to the 3′ end.

Leading strand

On the template having the 3´- end, the daughter strand is synthesized continuously in the 5’-3’ direction. This strand is referred to as the leading strand.



Semi continuous replication



Okkazaki fragments

- **Many DNA fragments are synthesized sequentially on the DNA template strand having the 5´- end. These DNA fragments are called Okazaki fragments. They are 1000 – 2000 nt long for prokaryotes and 100-150 nt long for eukaryotes.**
- **The daughter strand consisting of Okazaki fragments is called the lagging strand.**

Semi continuous replication

Continuous synthesis of the leading strand and discontinuous synthesis of the lagging strand represent a unique feature of DNA replication. It is referred to as the semi-continuous replication.

Enzymology of DNA Replication

Enzyme and Protein factors

protein	M_r	#	function
Dna A protein	50,000	1	recognize origin
Dna B protein	300,000	6	open dsDNA
Dna C protein	29,000	1	assist Dna B binding
DNA pol			Elongate the DNA strands
Dna G protein	60,000	1	synthesize RNA primer
SSB	75,600	4	single-strand binding
DNA topoisomerase	400,000	4	release supercoil constraint

DNA Polymerase
DNA-pol of prokaryotes

- **The first DNA-dependent DNA polymerase (short for DNA-pol I) was discovered in 1958 by Arthur Kornberg who received Nobel Prize in physiology or medicine in 1959.**

Arthur Kornberg

- **Later, DNA-pol II and DNA-pol III were identified in experiments using mutated *E.coli* cell line.**
- **All of them possess the following biological activity.**

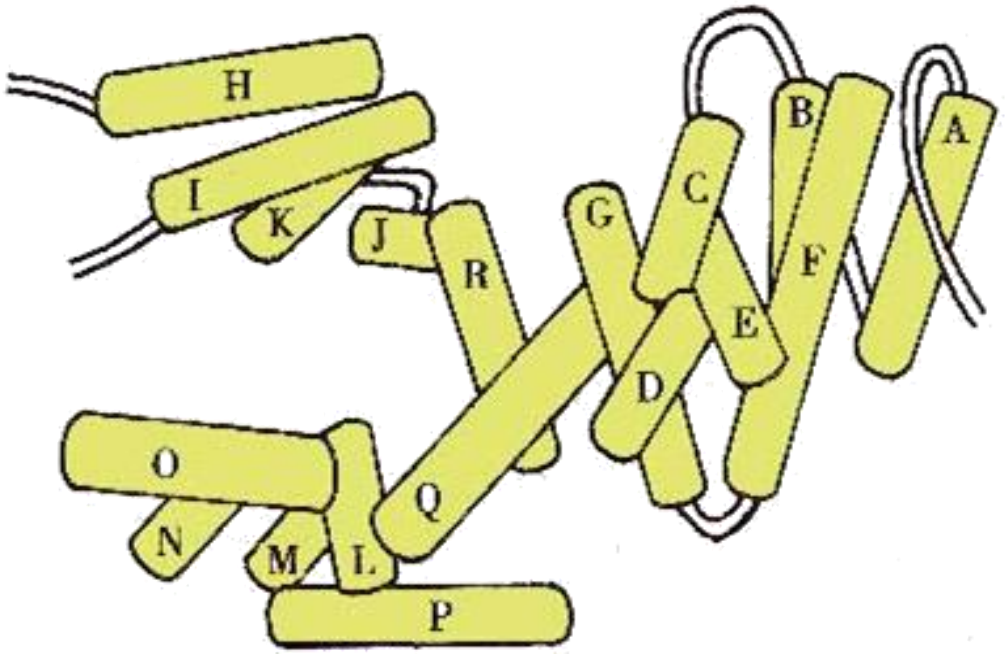
  1. 5′→3′ polymerizing

  2. exonuclease

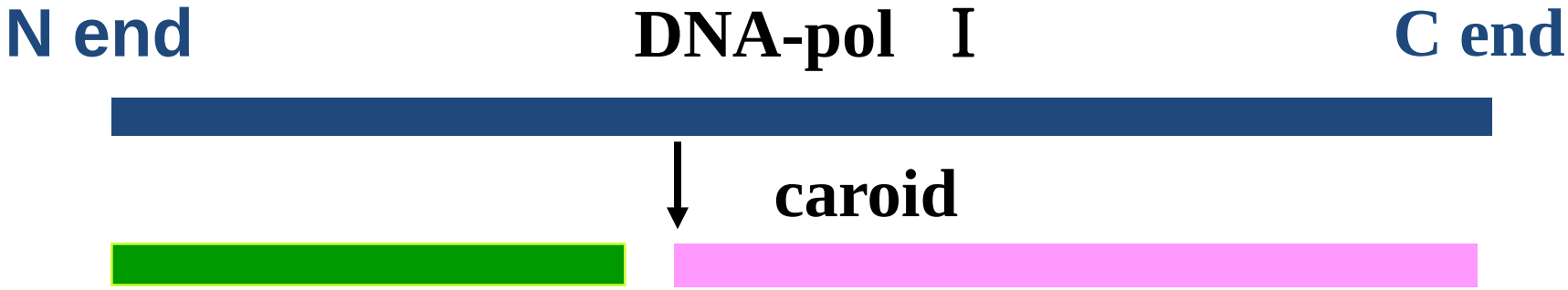
	DNA polymerase		
	I	II	III
Structural gene*	*polA*	*polB*	*polC* (*dnaE*)
Subunits (number of different types)	1	7	≥10
M_r	103,000	88,000†	791,500
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

DNA-pol I

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



Klewno fragment



- **small fragment (323 AA): having 5′→3′ exonuclease activity**
- **large fragment (604 AA): called Klenow fragment, having DNA polymerization and 3′→5′ exonuclease activity**

- **Temporary functional when DNA-pol I and DNA-pol III are not functional**
- **Still capable for doing synthesis on the damaged template**
- **Participating in DNA repairing**

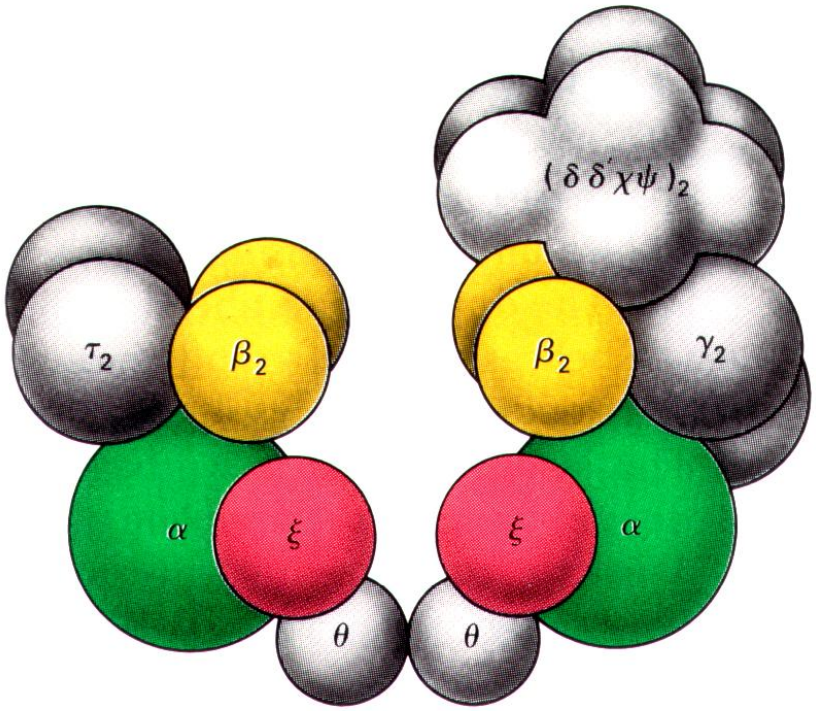
DNA-ppl III

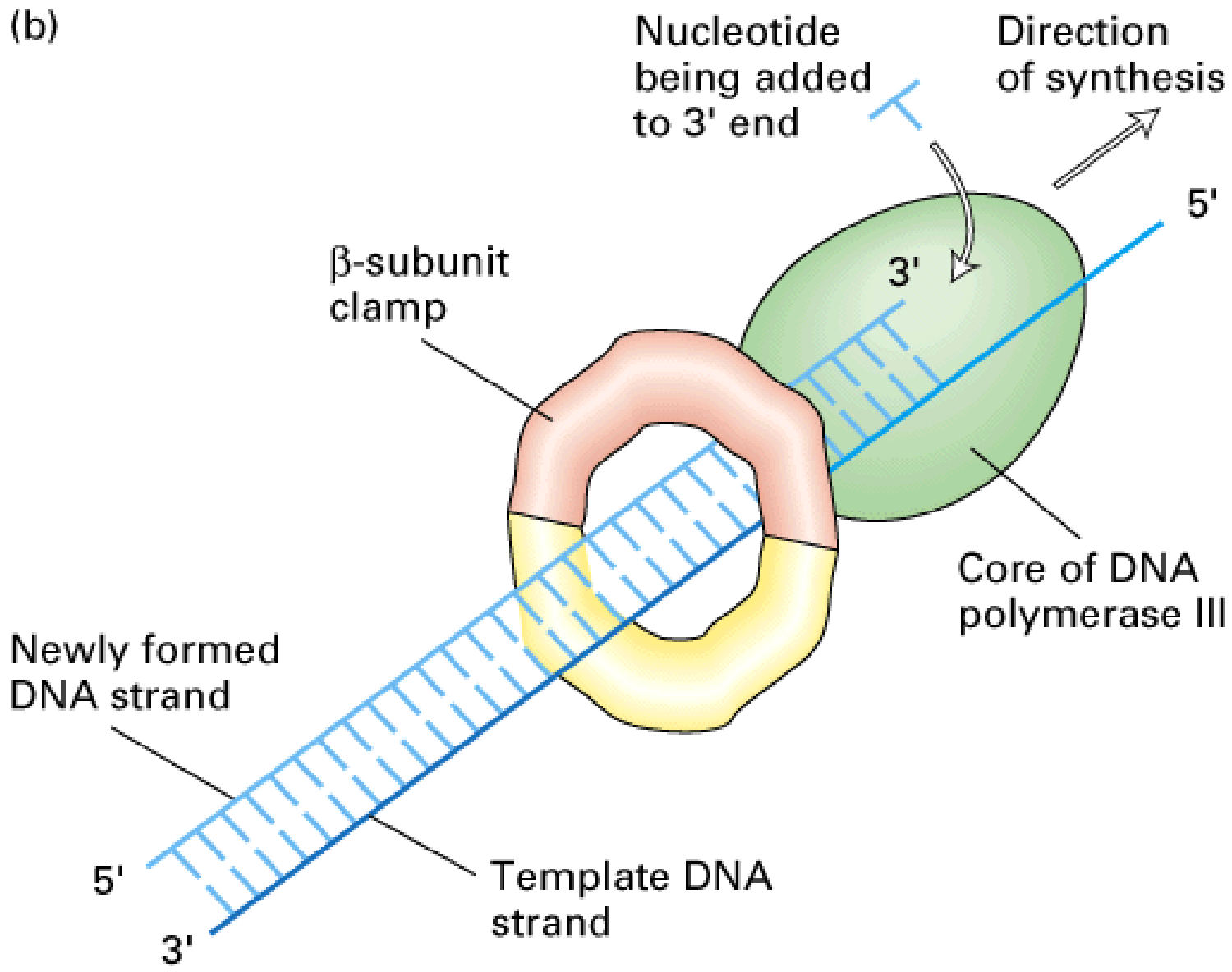
- **A heterodimer enzyme composed of ten different subunits**
- **Having the highest polymerization activity (10^5 nt/min)**
- **The true enzyme responsible for the elongation process**

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




(b)
Nucleotide being added to 3' end
Direction of synthesis
T
5'
3'
β-subunit clamp
Core of DNA polymerase III
Newly formed DNA strand
5'
3'
Template DNA strand

DNA-pol α: initiate replication and synthesize primers → **DnaG, primase**

DNA-pol β: replication with low fidelity → **repairing**

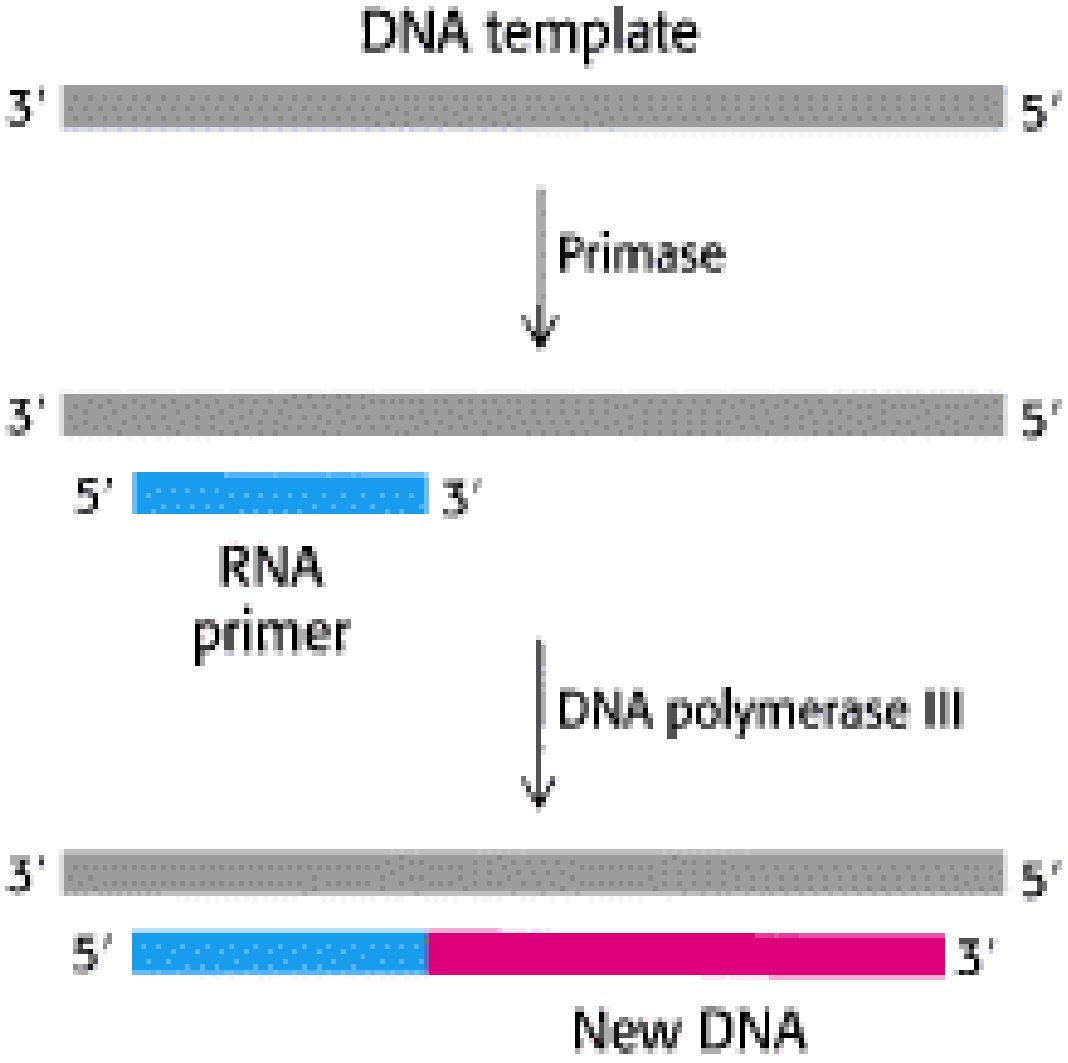
DNA-pol γ: polymerization in mitochondria

DNA-pol δ: elongation → **DNA-pol III**

DNA-pol ε: proofreading and filling gap → **DNA-pol I**

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 decades of nucleotides long.**


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

- **Primers provide free 3′-OH groups to react with the α-P atom of dNTP to form phosphoester bonds.**
- **Primase, DnaB, DnaC and an origin form a primosome complex at the initiation phase.**

§ 2.3 Helicase

- **Also referred to as DnaB.**
- **It opens the double strand DNA with consuming ATP.**
- **The opening process with the assistance of DnaA and DnaC**

§ 2.4 SSB protein

- **Stand for single strand DNA binding protein**
- **SSB protein maintains the DNA template in the single strand form in order to**
  - **prevent the dsDNA formation;**
  - **protect the vulnerable ssDNA from nucleases.**

§ 2.5 Topoisomerase

- **Opening the dsDNA will create supercoil ahead of replication forks.**
- **The supercoil constraint needs to be released by topoisomerases.**

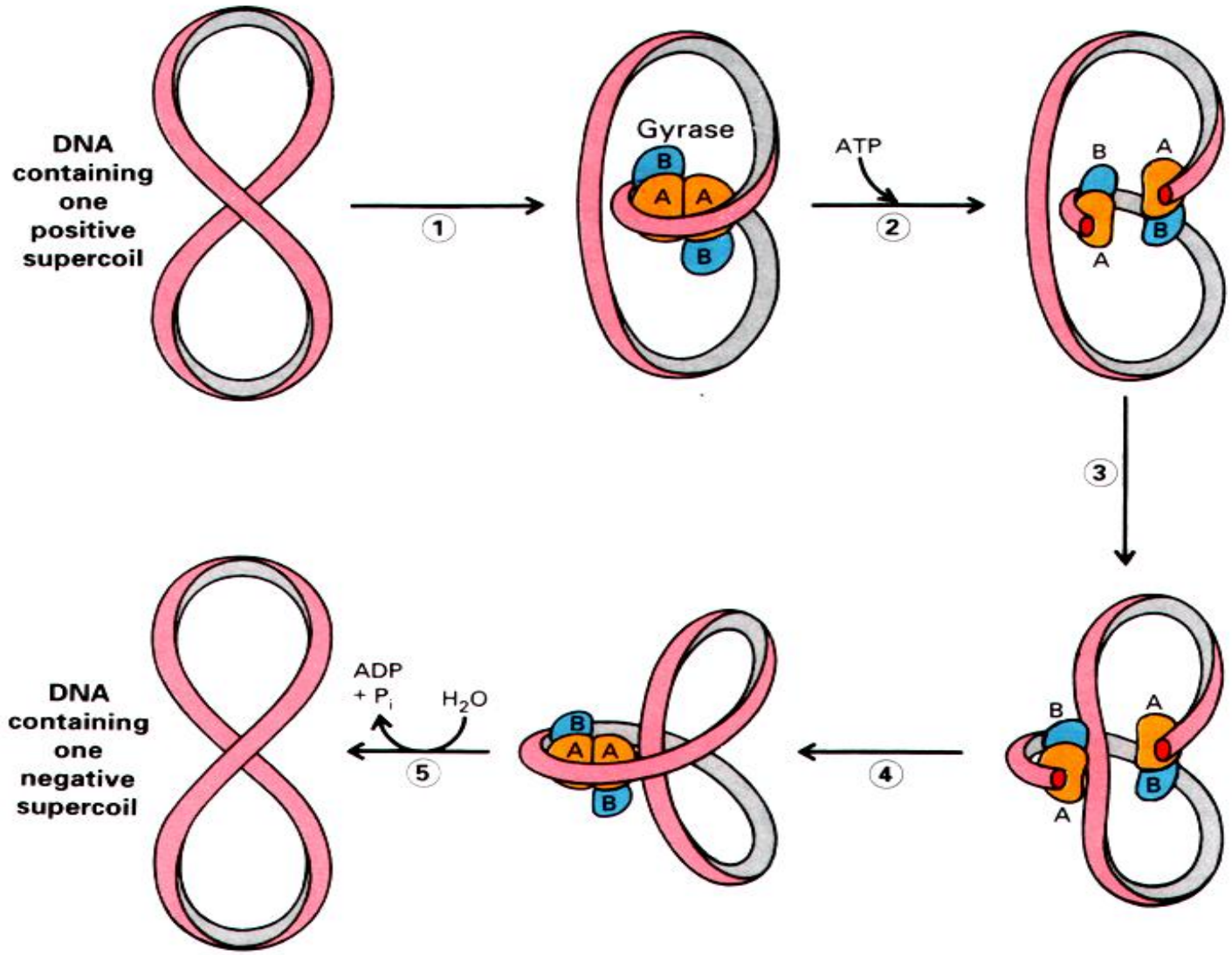
- **The interconversion of topoisomers of dsDNA is catalyzed by a topoisomerase in a three-step process:**
  - **Cleavage of one or both strands of DNA**
  - **Passage of a segment of DNA through this break**
  - **Resealing of the DNA break**

Topoisomerase I (topo I)

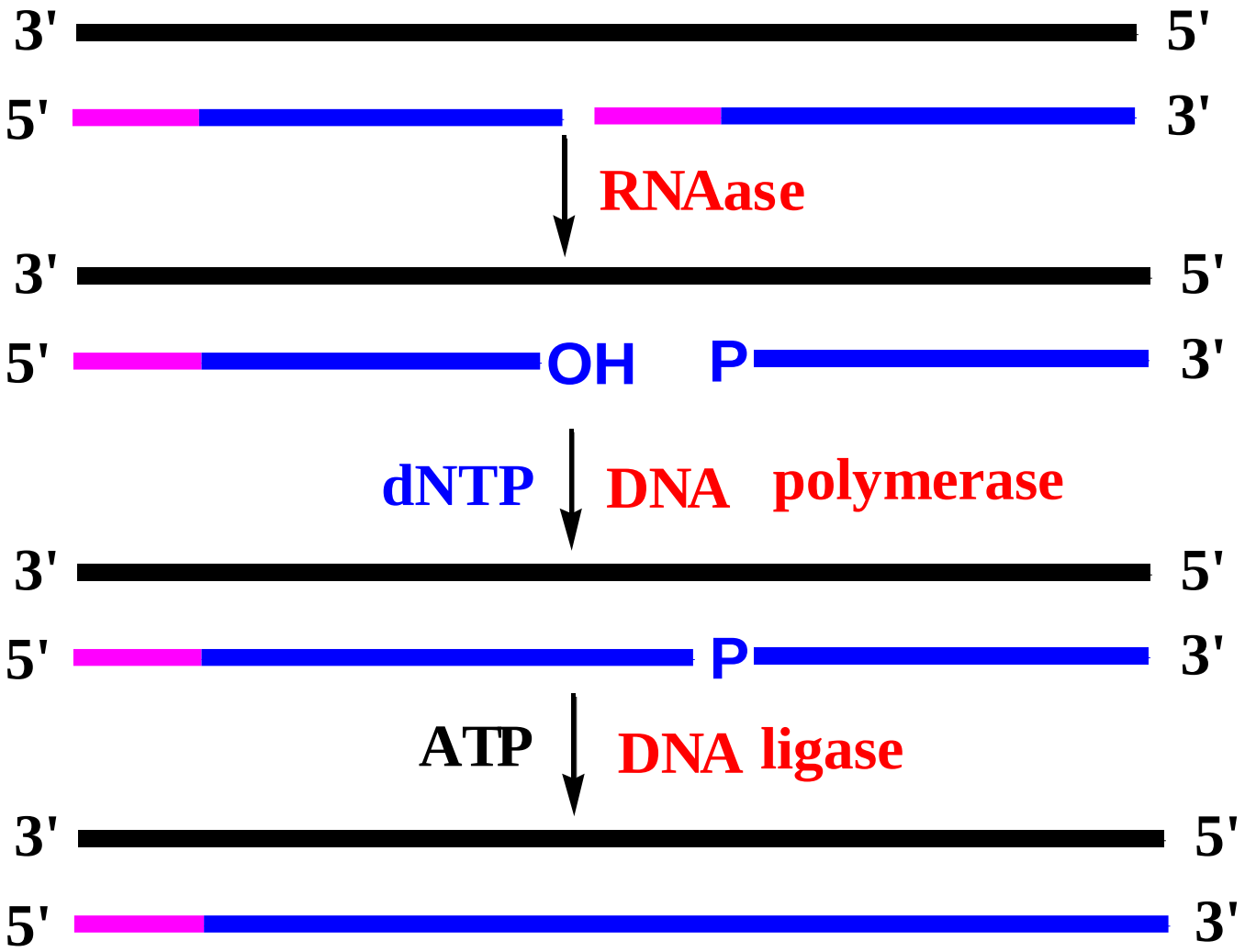
- **Also called ω-protein in prokaryotes.**
- **It cuts a phosphoester bond on one DNA strand, rotates the broken DNA freely around the other strand to relax the constraint, and reseals the cut.**

Topoisomerase II (topo II)

- **It is named gyrase in prokaryotes.**
- **It cuts phosphoester bonds on both strands of dsDNA, releases the supercoil constraint, and reforms the phosphoester bonds.**
- **It can change dsDNA into the negative supercoil state with consumption of ATP.**


DNA containing one positive supercoil
Gyrase
B
A
A
B
1
ATP
2
B
A
A
B
3
B
A
A
B
4
ADP + P_i
H_2O
5
DNA containing one negative supercoil

§ 2.6 DNA Ligase



- **Connect two adjacent ssDNA strands by joining the 3′-OH of one DNA strand to the 5′-P of another DNA strand.**
- **Sealing the nick in the process of replication, repairing, recombination, and splicing.**

§ 2.7 Replication Fidelity

- **Replication based on the principle of base pairing is crucial to the high accuracy of the genetic information transfer.**
- **Enzymes use two mechanisms to ensure the replication fidelity.**
  - **Proofreading and real-time correction**
  - **Base selection**

Proofreading and correction

- **DNA-pol I has the function to correct the mismatched nucleotides.**
- **It identifies the mismatched nucleotide, removes it using the 3´-5´ exonuclease activity, add a correct base, and continues the replication.**

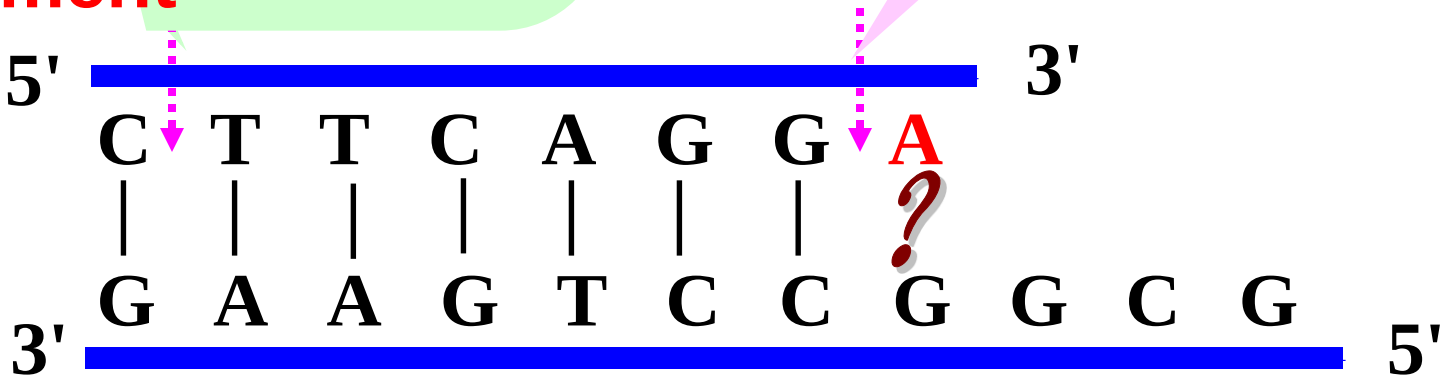
Exonuclease functions

5′→3′ exonuclease activity

cut primer or excise mutated segment

3′→5′ exonuclease activity

excise mismatched nuleotides



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