



DNA REPLICATION

Dr. A. D. NAVEEN KUMAR
Asst. Professor in Biochemistry
College of Medical and HEALTH Sciences
ADIGRAT University
Ethiopia

DNA Replication

➤ Types of DNA replication

Semi-conservative model of DNA replication

Prokaryotic DNA replication

Eukaryotic DNA replication

Inhibitors of DNA replication

(Analogues, Intercalation, Polymerase Inhibitors)

➤ DNA damage

Types and agents of mutations

Spontaneous, Radiation, Chemicals.

➤ Repair mechanisms

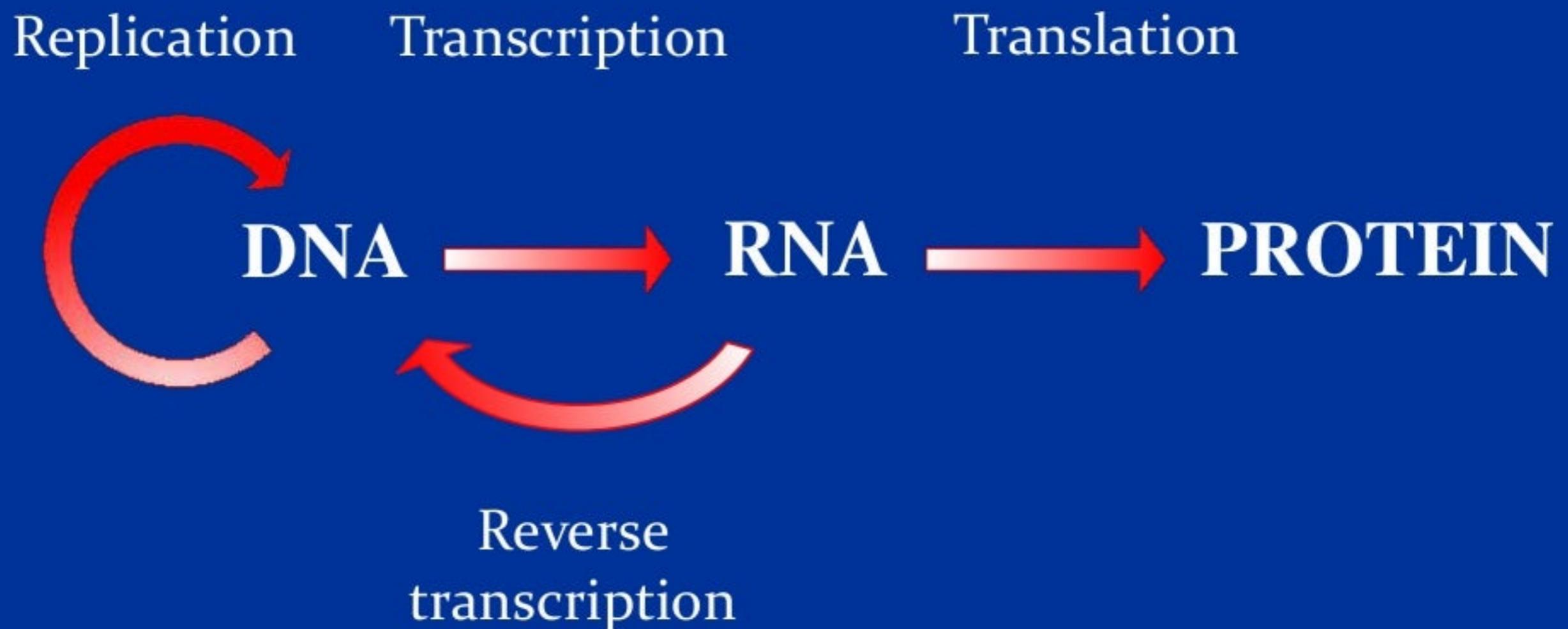
Base Excision, Nucleotide Excision, Mismatch Repair.

➤ DNA-recombination

In meiosis

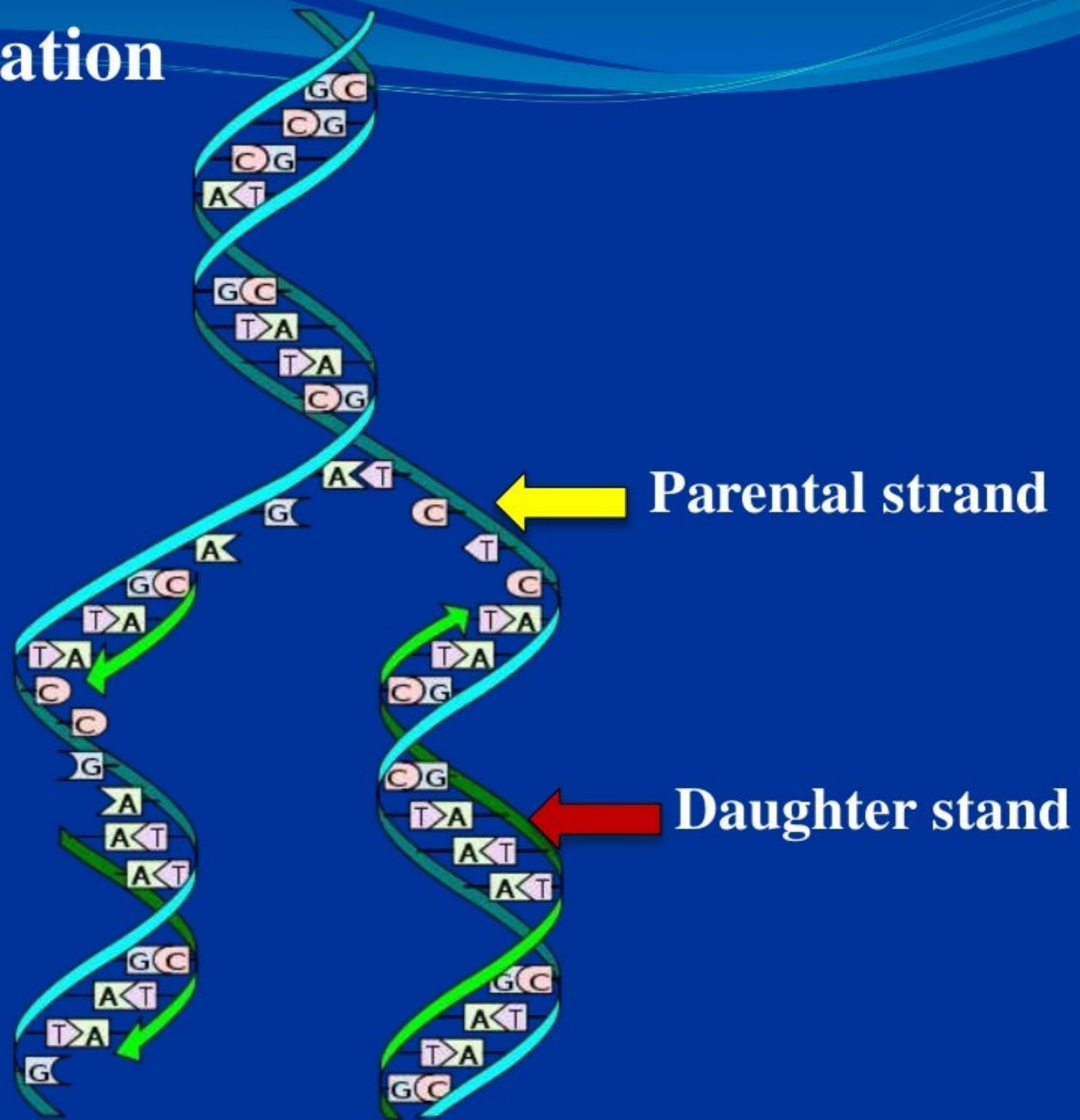
Transposition

Central dogma



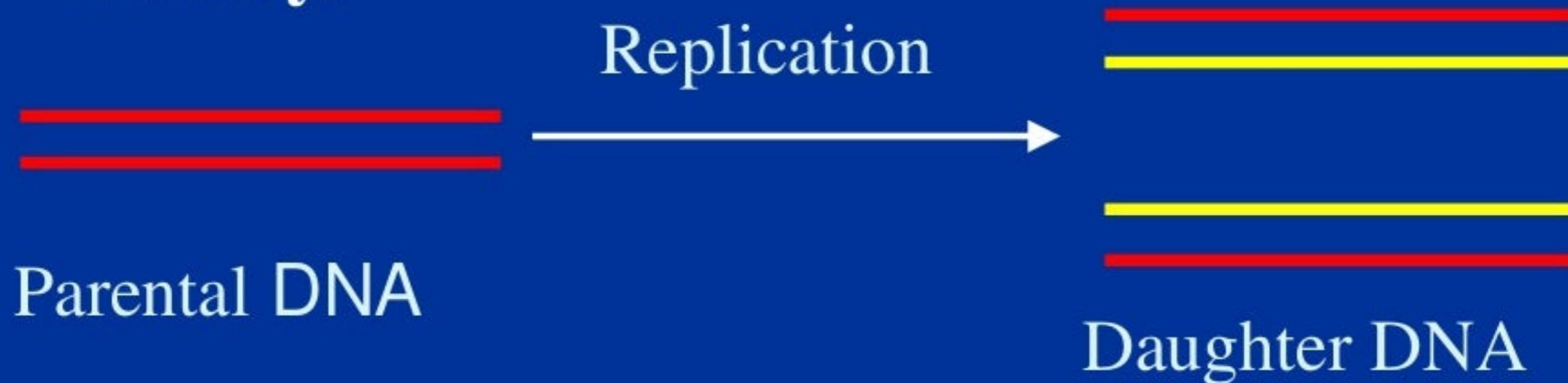
- DNA replication is a biological process that occurs in all living organisms and copies their exact DNA. It is the basis for biological inheritance.
- **Replication** is the process of synthesis of daughter DNA from parental DNA by the enzyme DNA Polymerase.
- $$\text{DNA} \quad \xrightarrow{\hspace{10em}} \quad \text{Lengthened DNA}$$
$$(\text{dNMP})_n + \text{dNTP} \longrightarrow (\text{dNMP})_{n+1} + \text{PPi}$$

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.



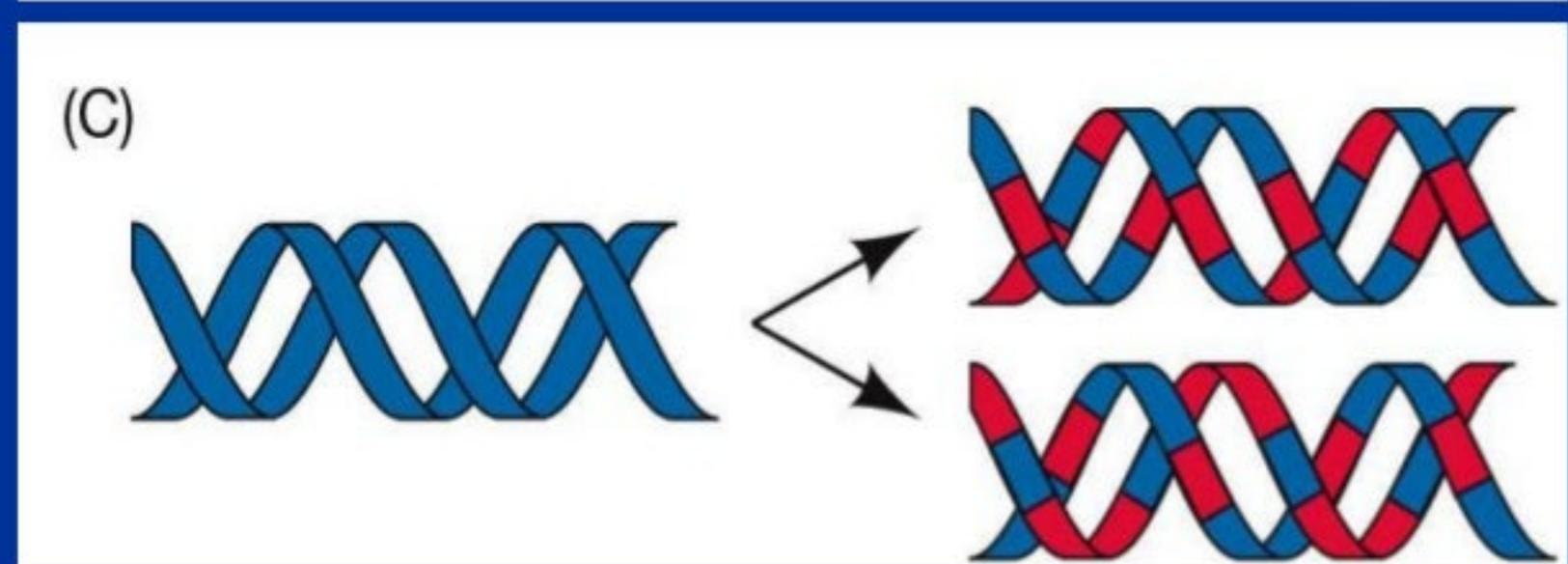
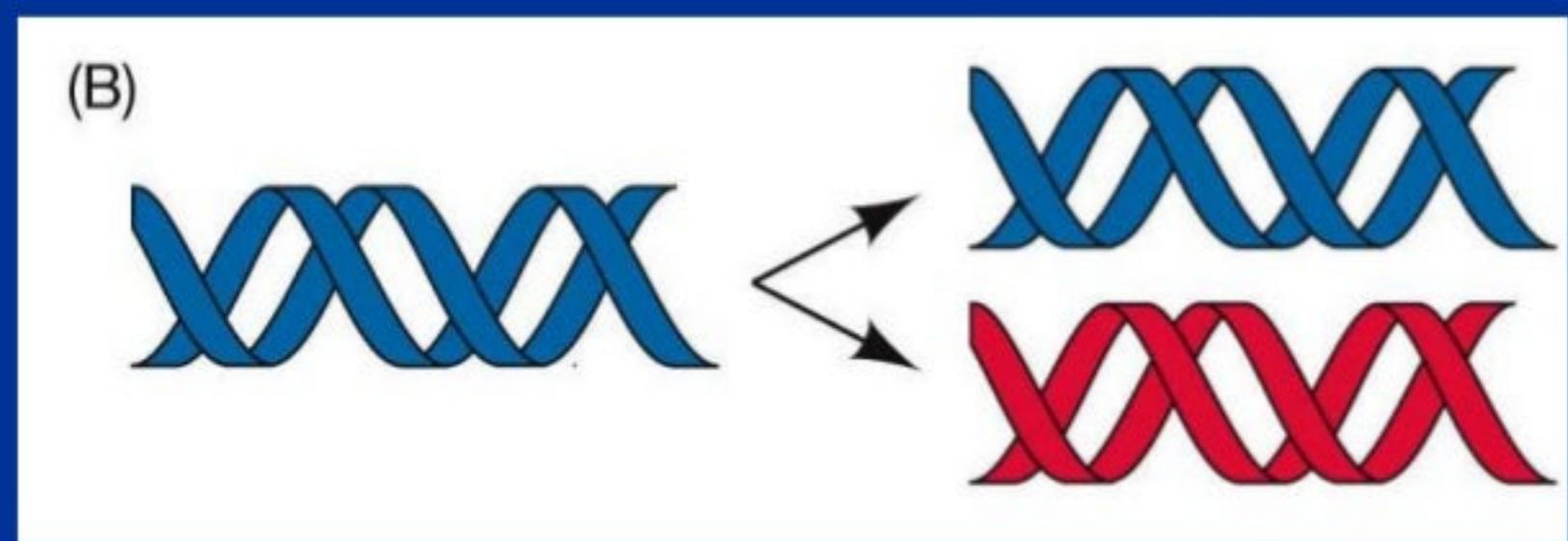
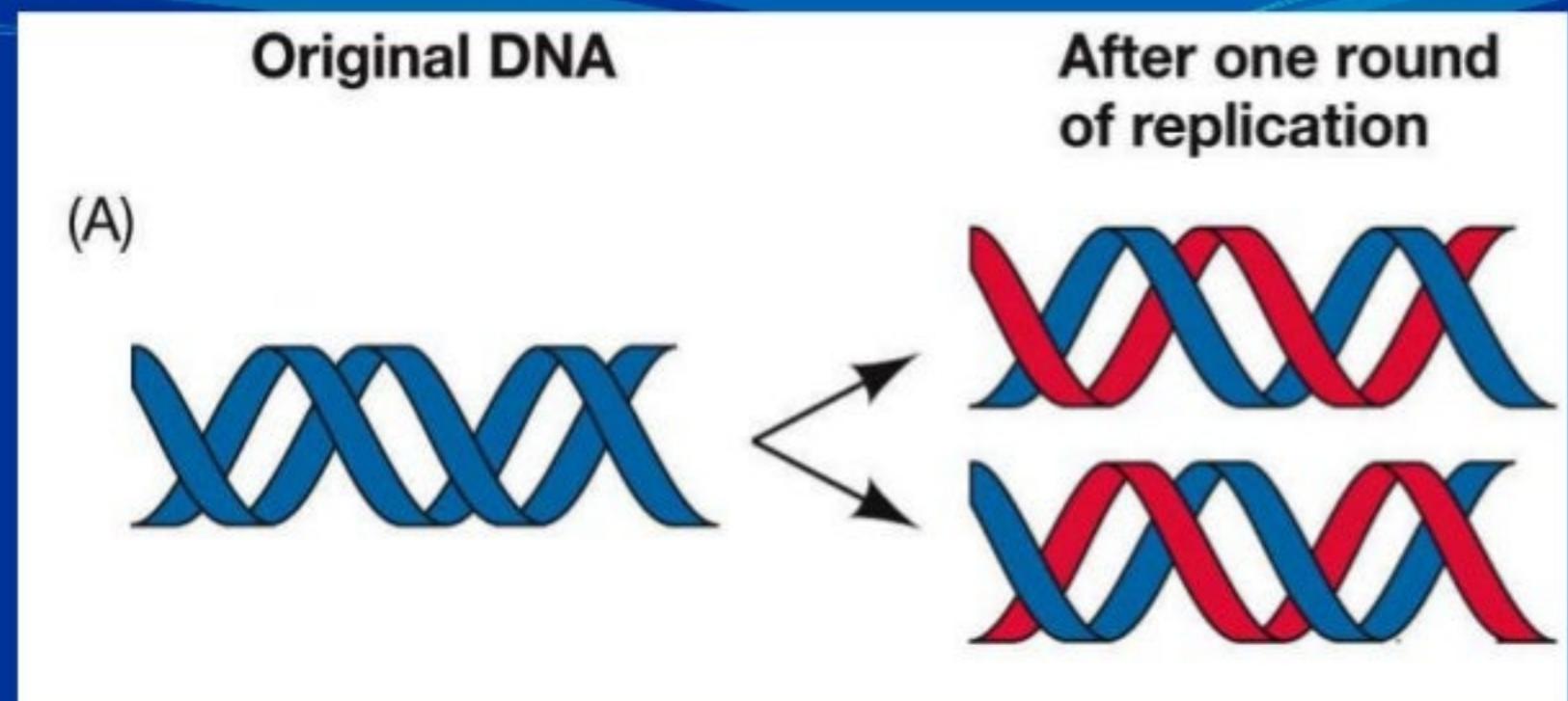
Three possible replication patterns:

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

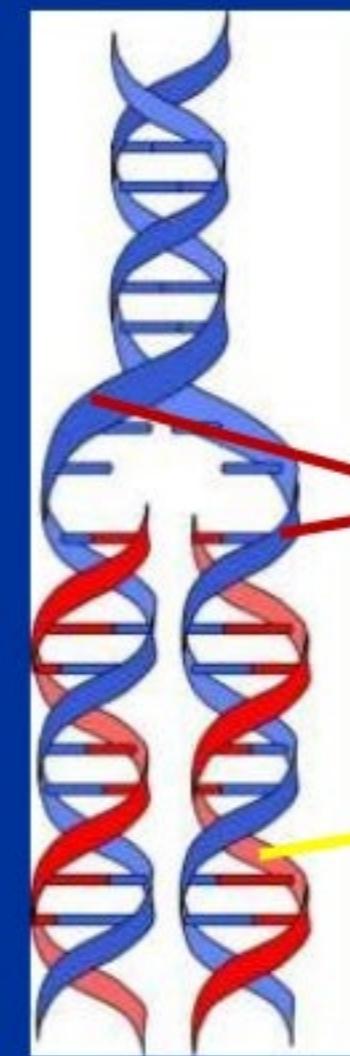
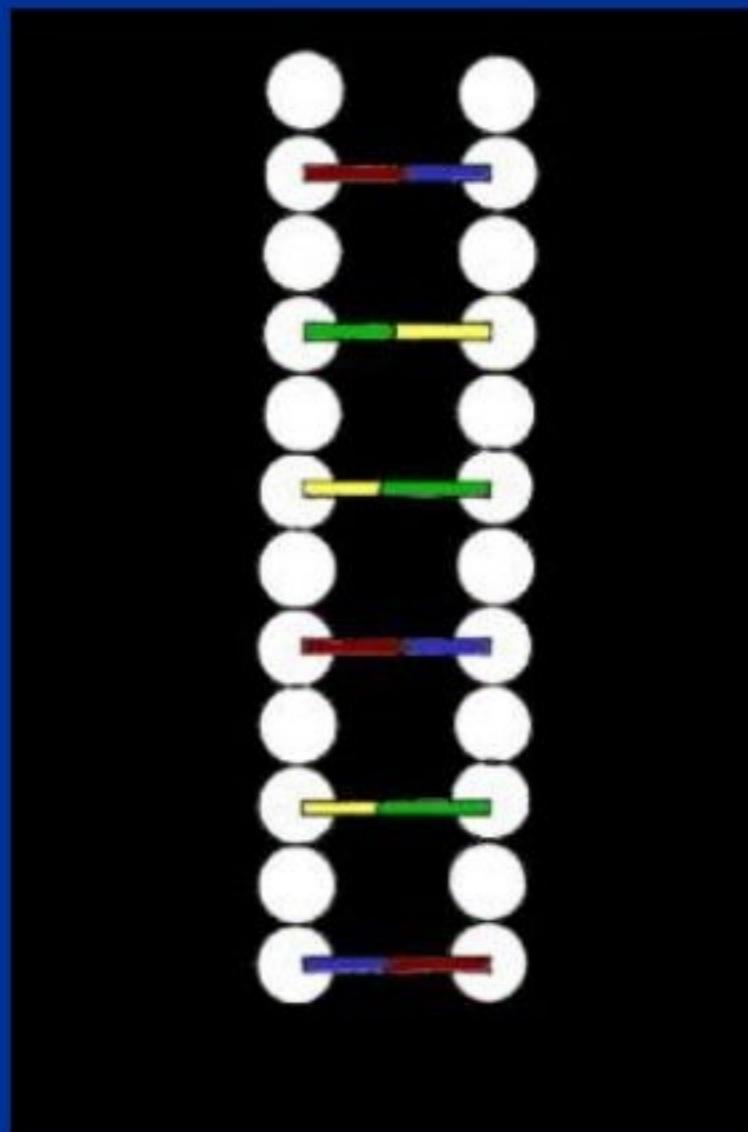
Semiconservative replication

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

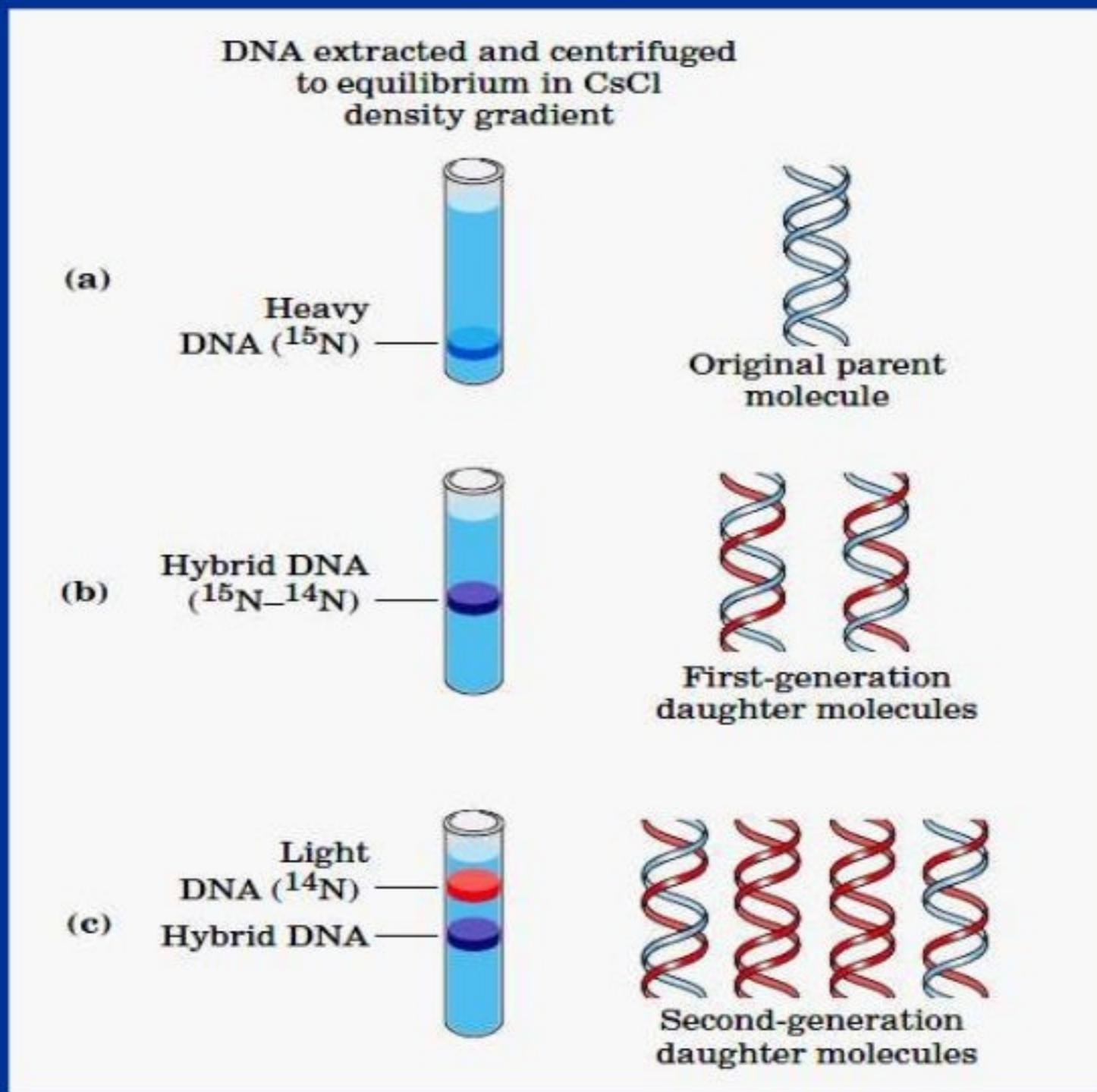


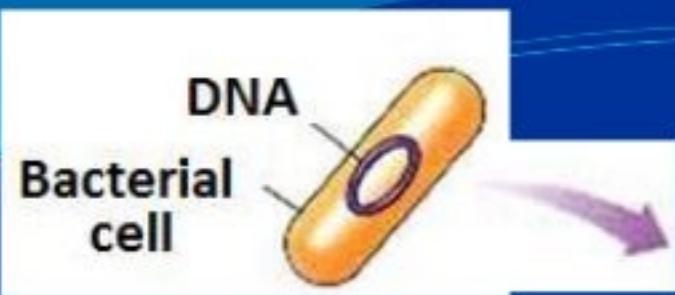
Parent strands
New / Daughter strand

Characteristics of Replication

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

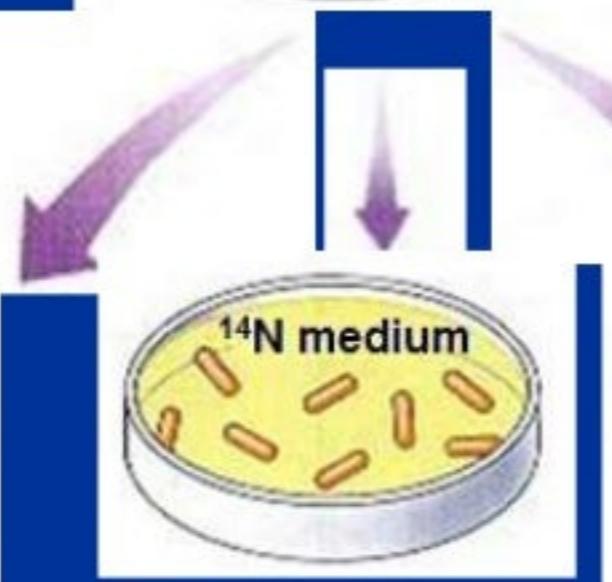
Meselson and Stahl experiment [1958] demonstrated Semiconservative replication





1

***E. coli* grown in the presence of ^{15}N (a heavy isotope of Nitrogen) for many generations**



- Cells get heavy-labeled DNA

Sampled at:
0 min

20 min

40 min

2

***E. coli* placed in medium containing only ^{14}N (a light isotope of Nitrogen)**



3

Cells broken open to extract DNA

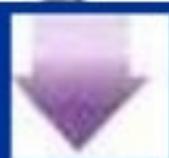
4

Suspended DNA in Cesium chloride (CsCl) solution.



5

CsCl density gradient centrifugation



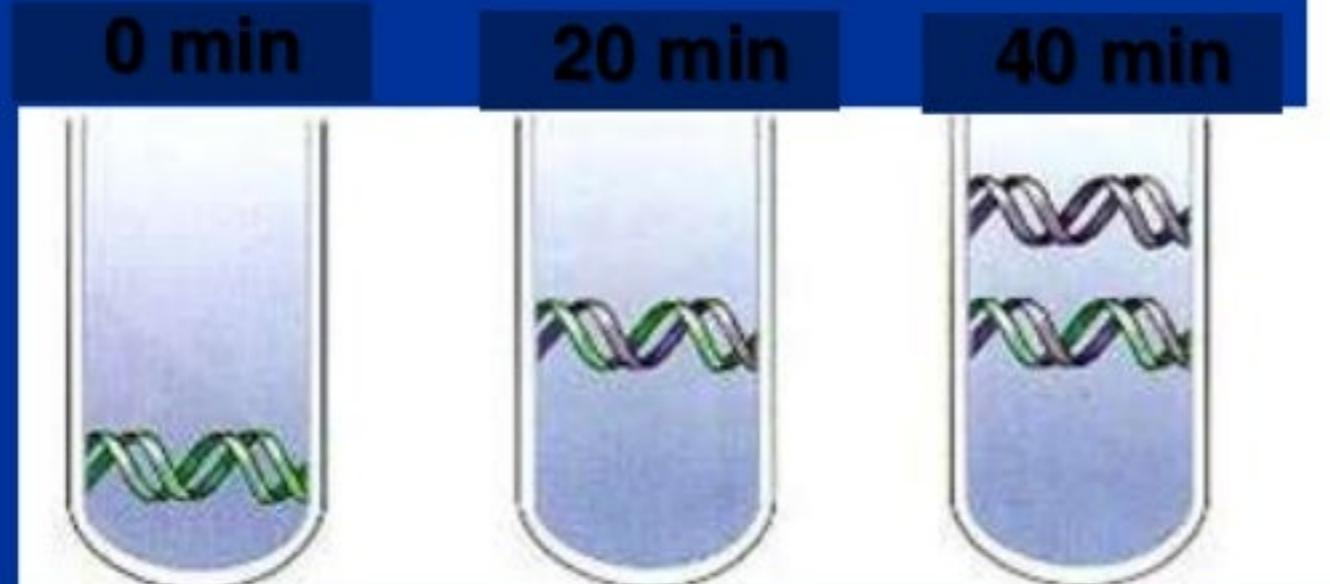
Lower Cs⁺
concentration



Higher Cs⁺
concentration

Less dense

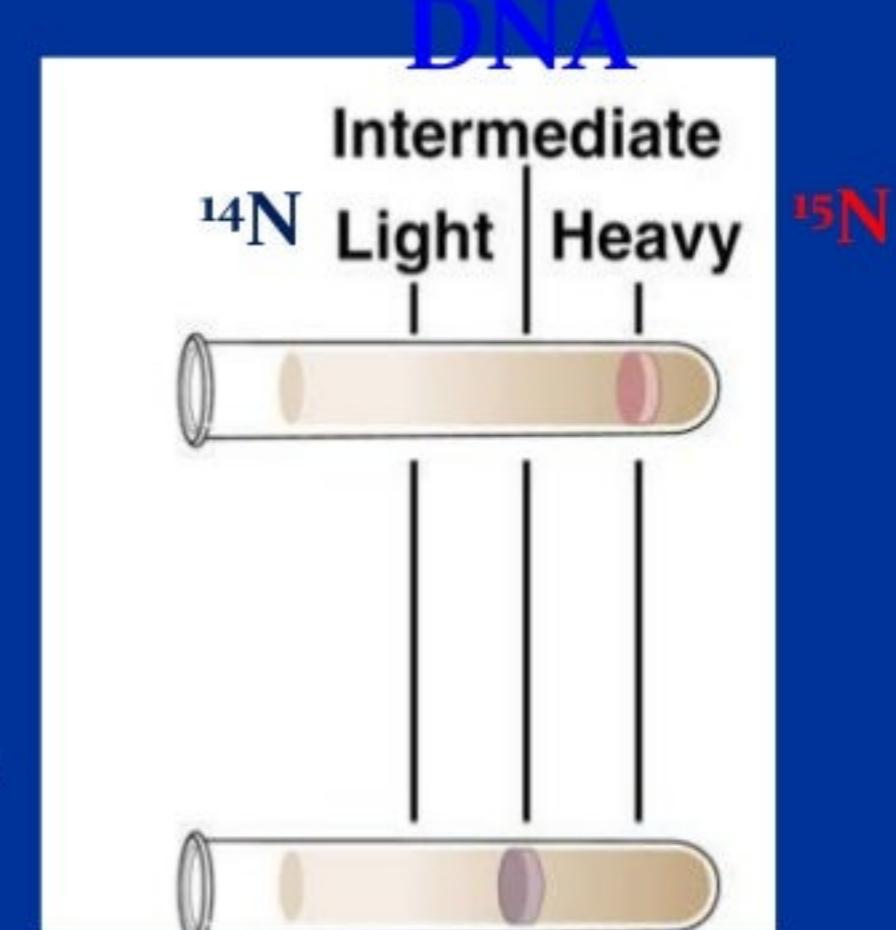
More dense



Both
strands
heavy

F1
generation
DNA (one
heavy/one
light strand)

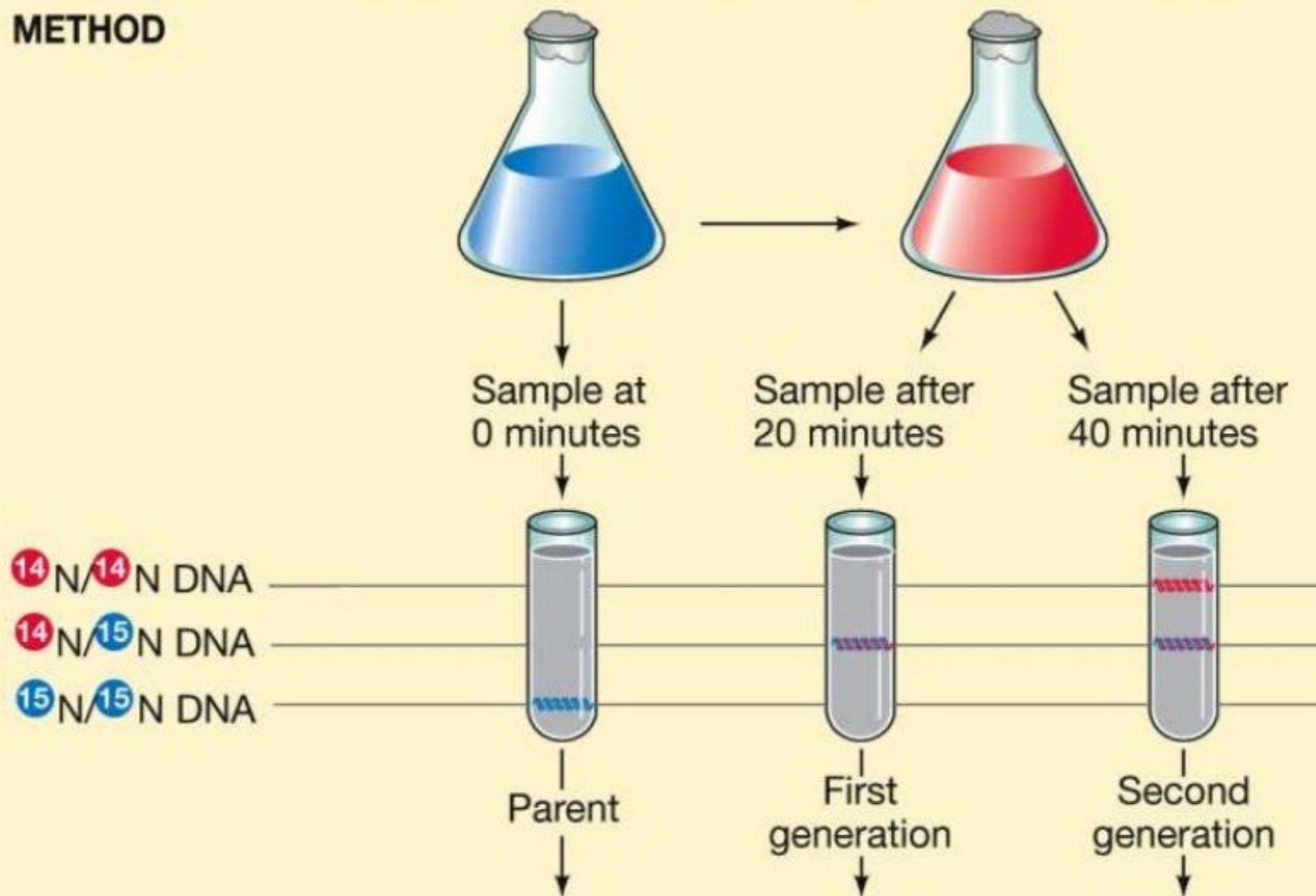
- Two light strands
- One heavy/One light strand



EXPERIMENT

HYPOTHESIS: Each DNA strand replicates semiconservatively.

METHOD



Three
rounds of
replication:

Original
DNA



0 min

1st Round:



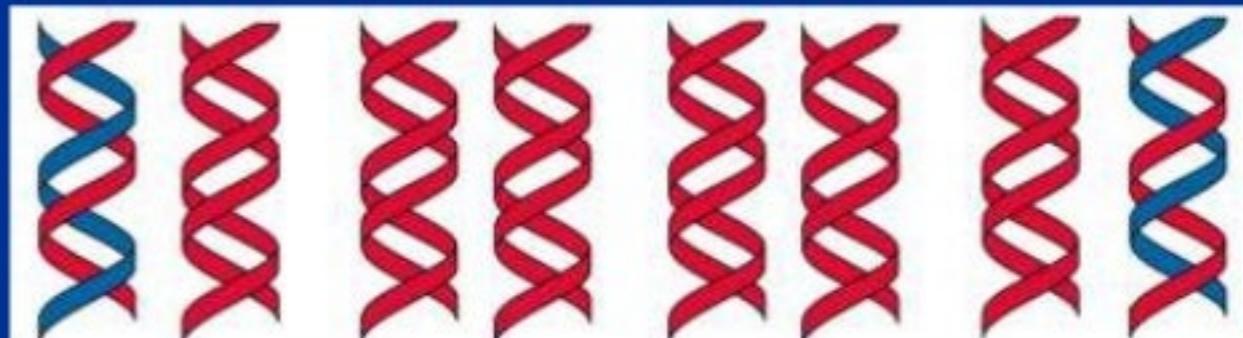
20 min

2nd
Round:

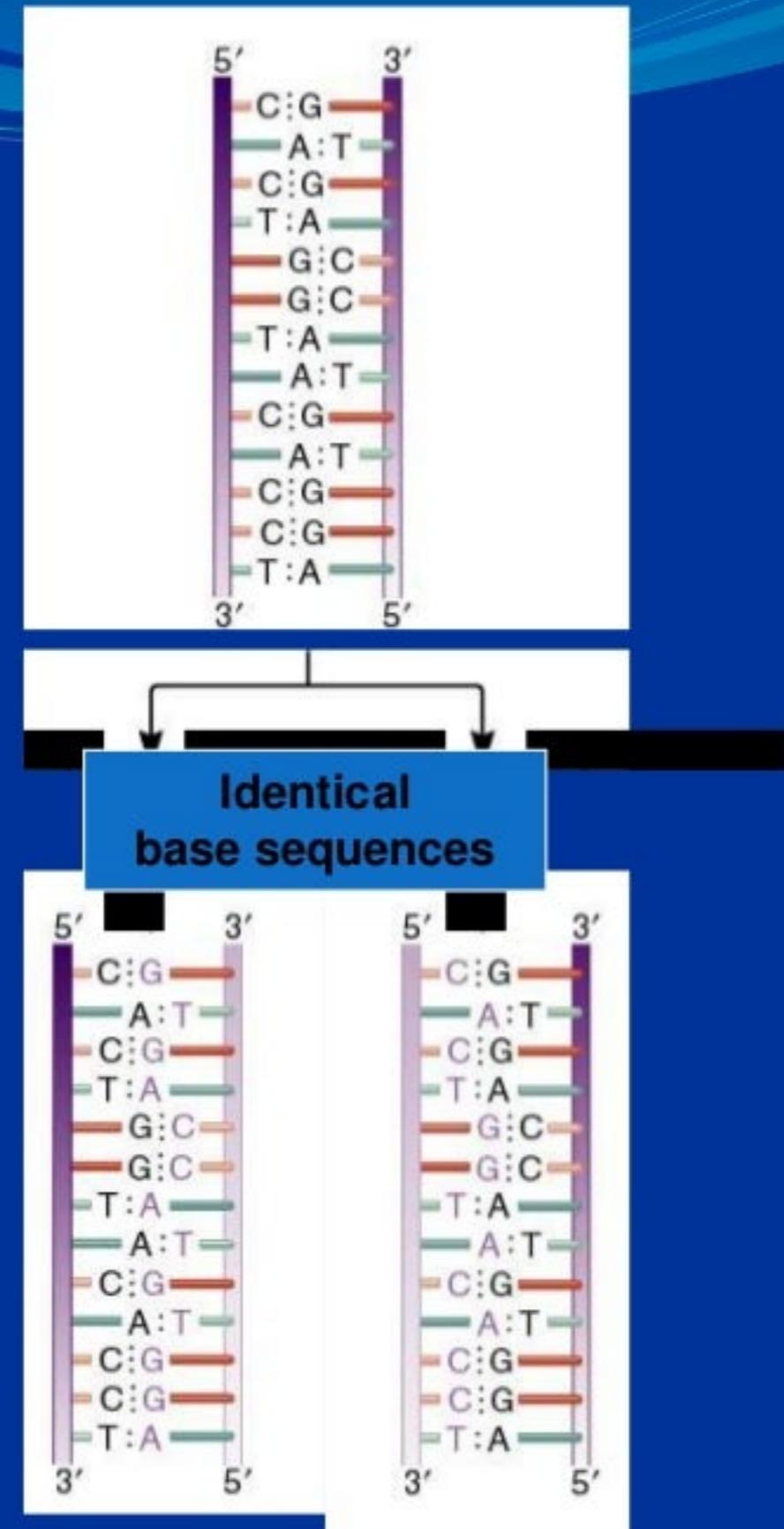
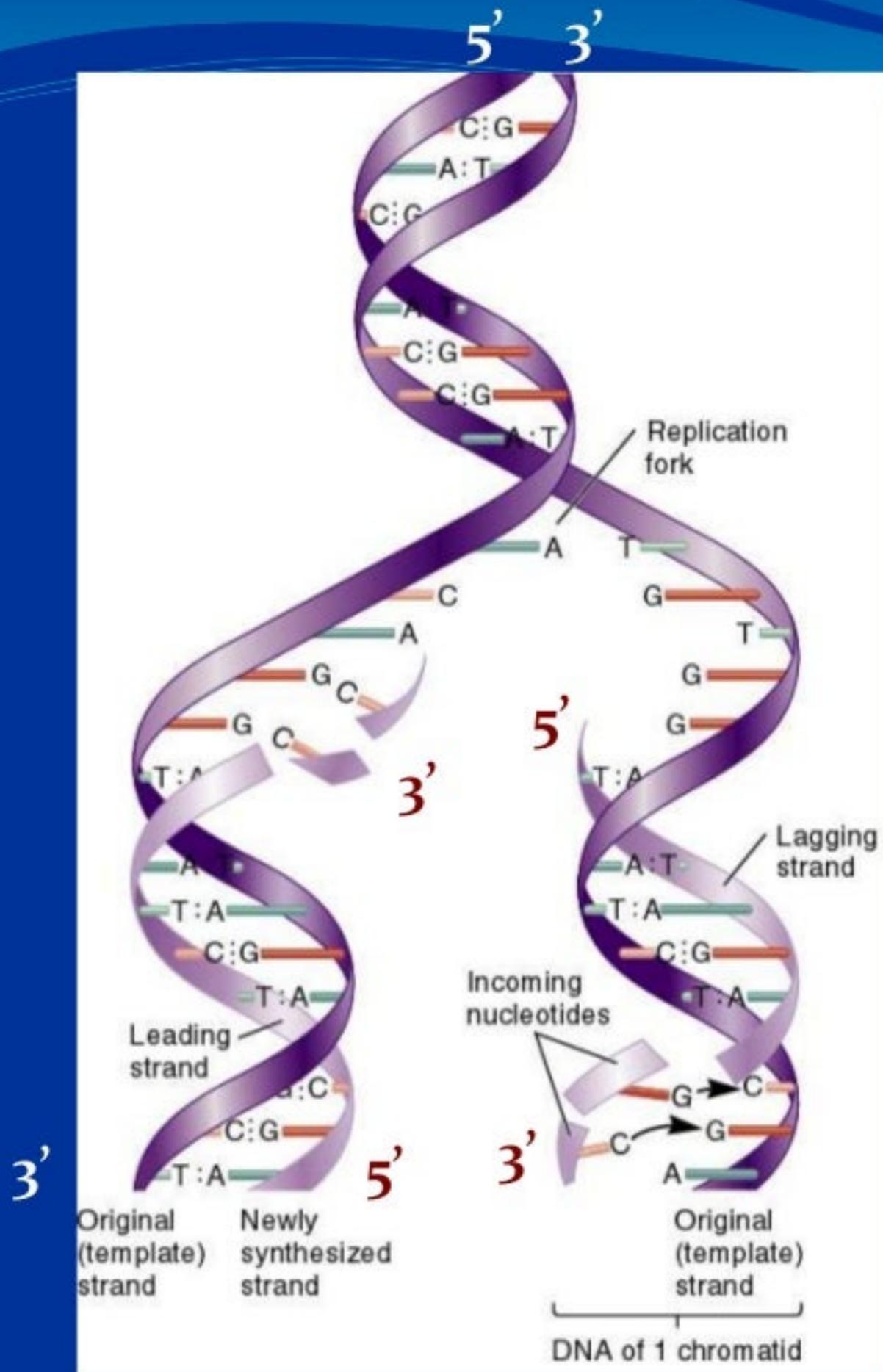


40 min

3rd
Round:



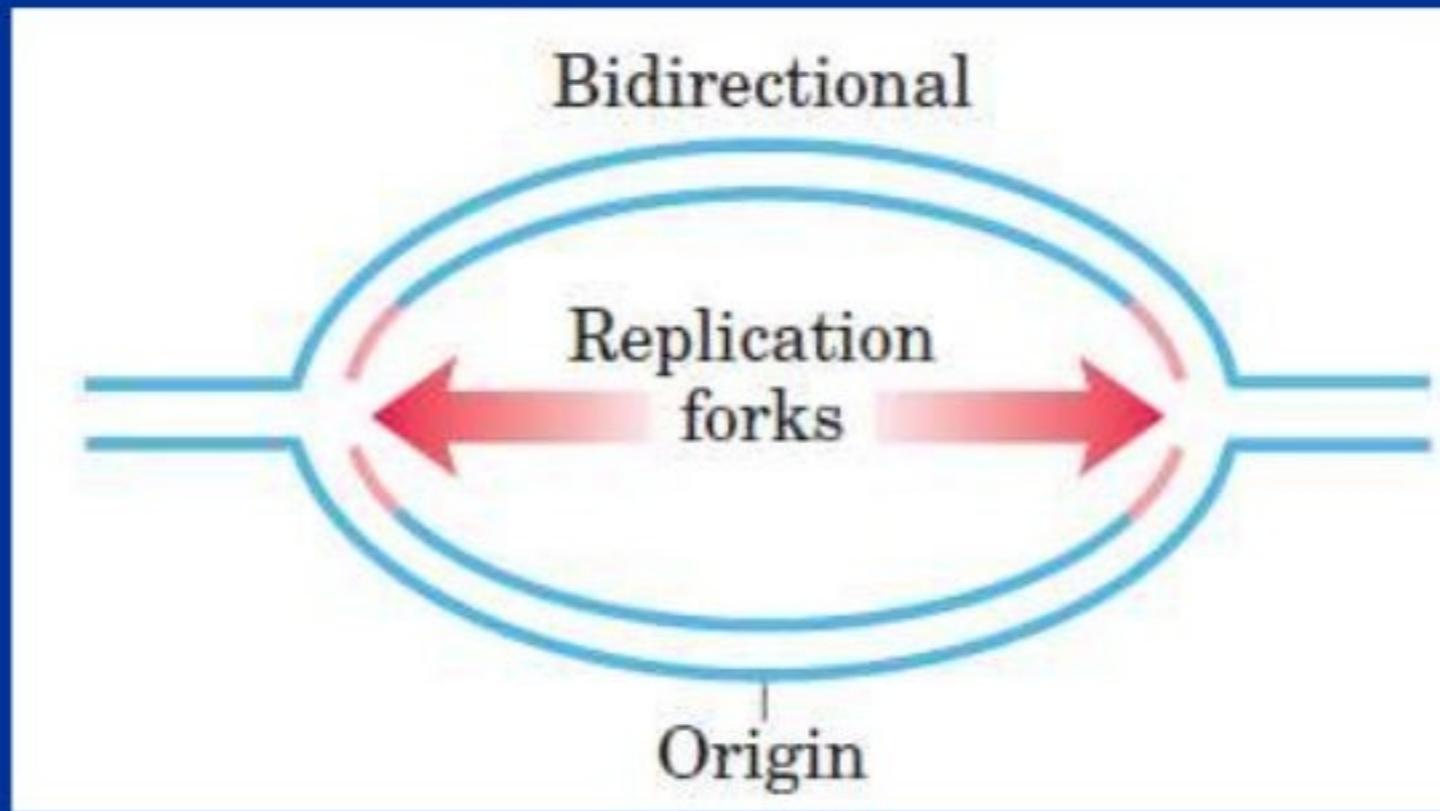
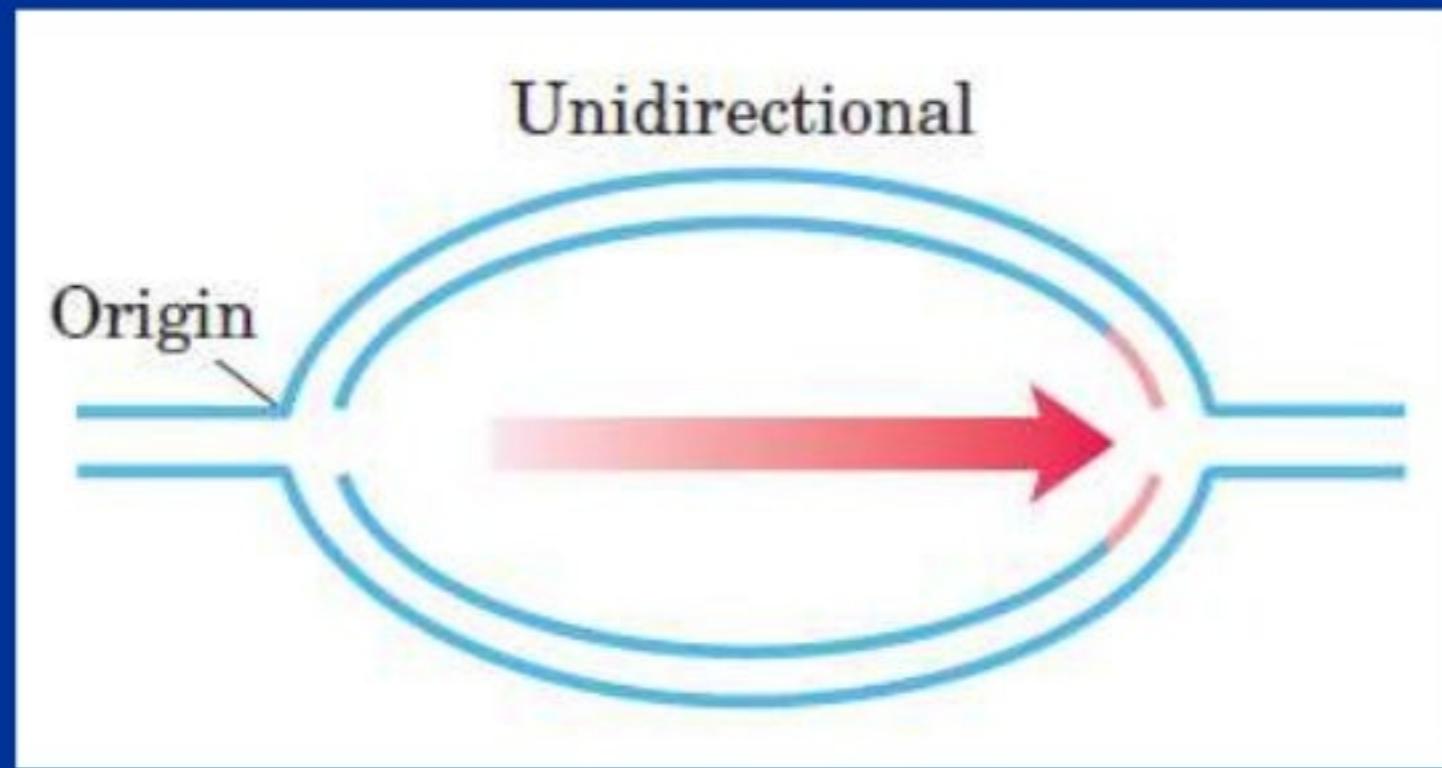
60
min?



Semiconservative Replication

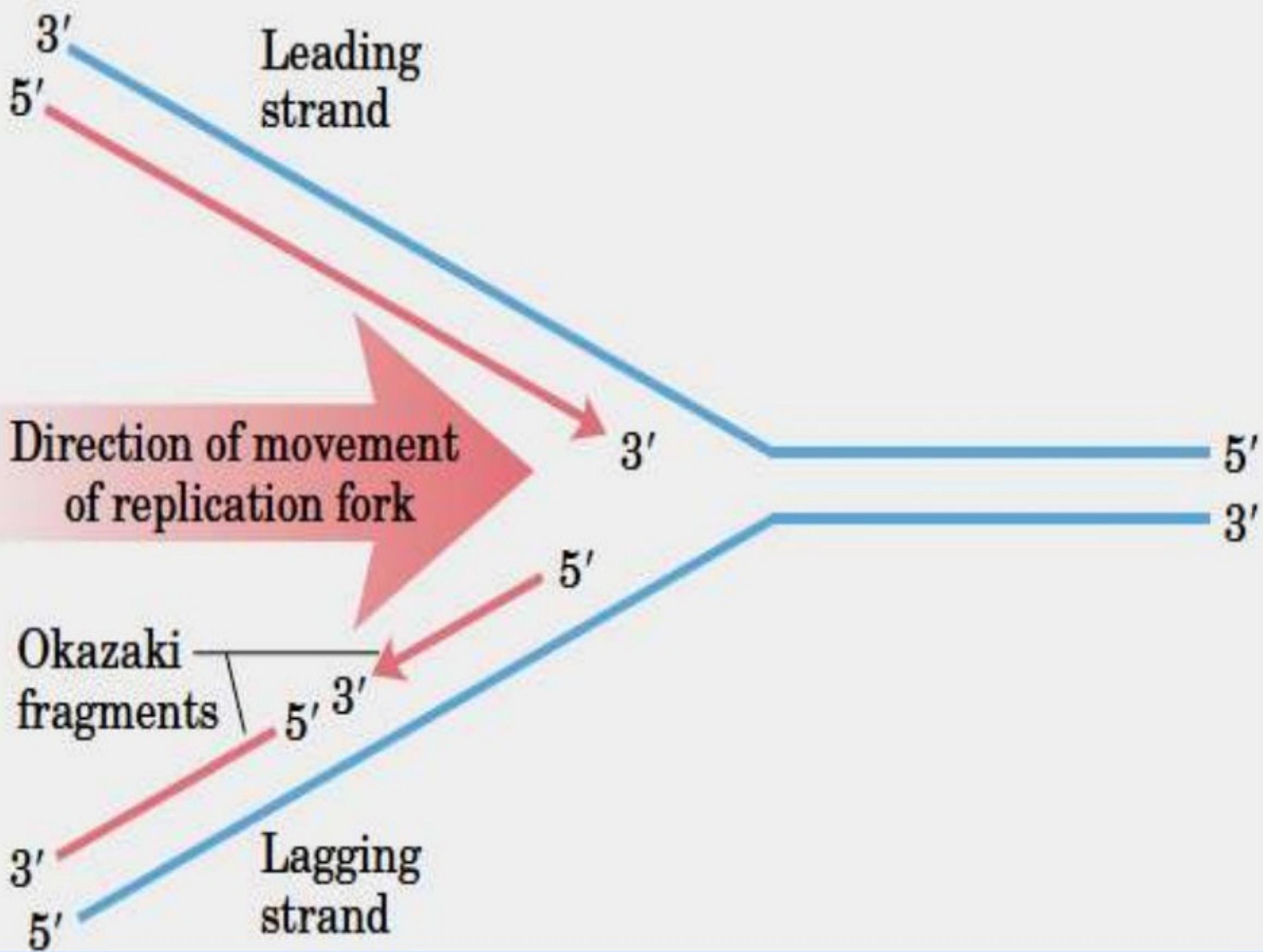
Half of the parental DNA molecule is conserved in each new double helix, paired with a newly synthesized complementary strand. This is called semiconservative replication.

Direction of the DNA Replication

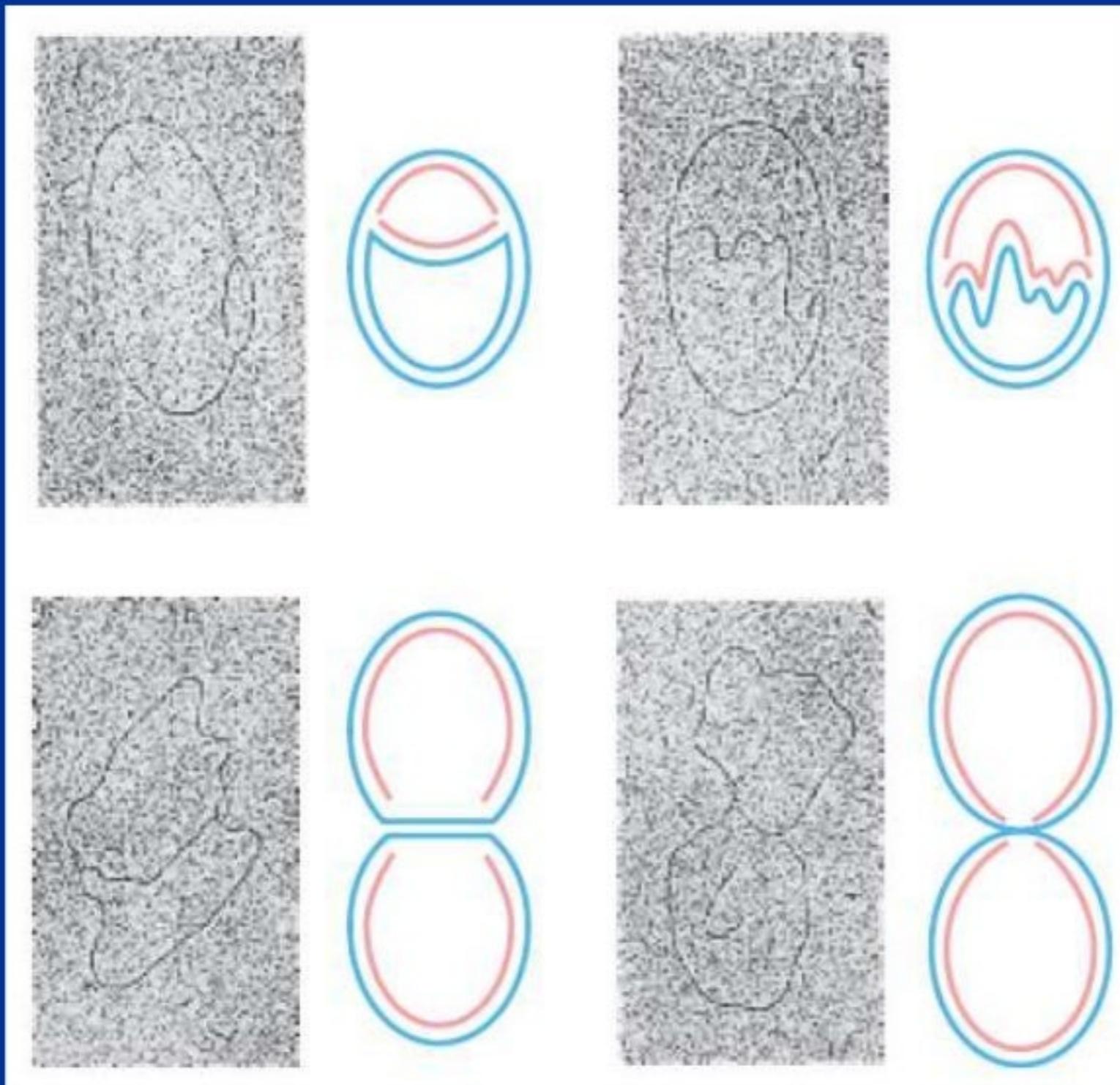


Bidirectional Replication

- 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**.



Replication of Prokaryotes



The replication process starts from the origin, and proceeds in two opposite directions.

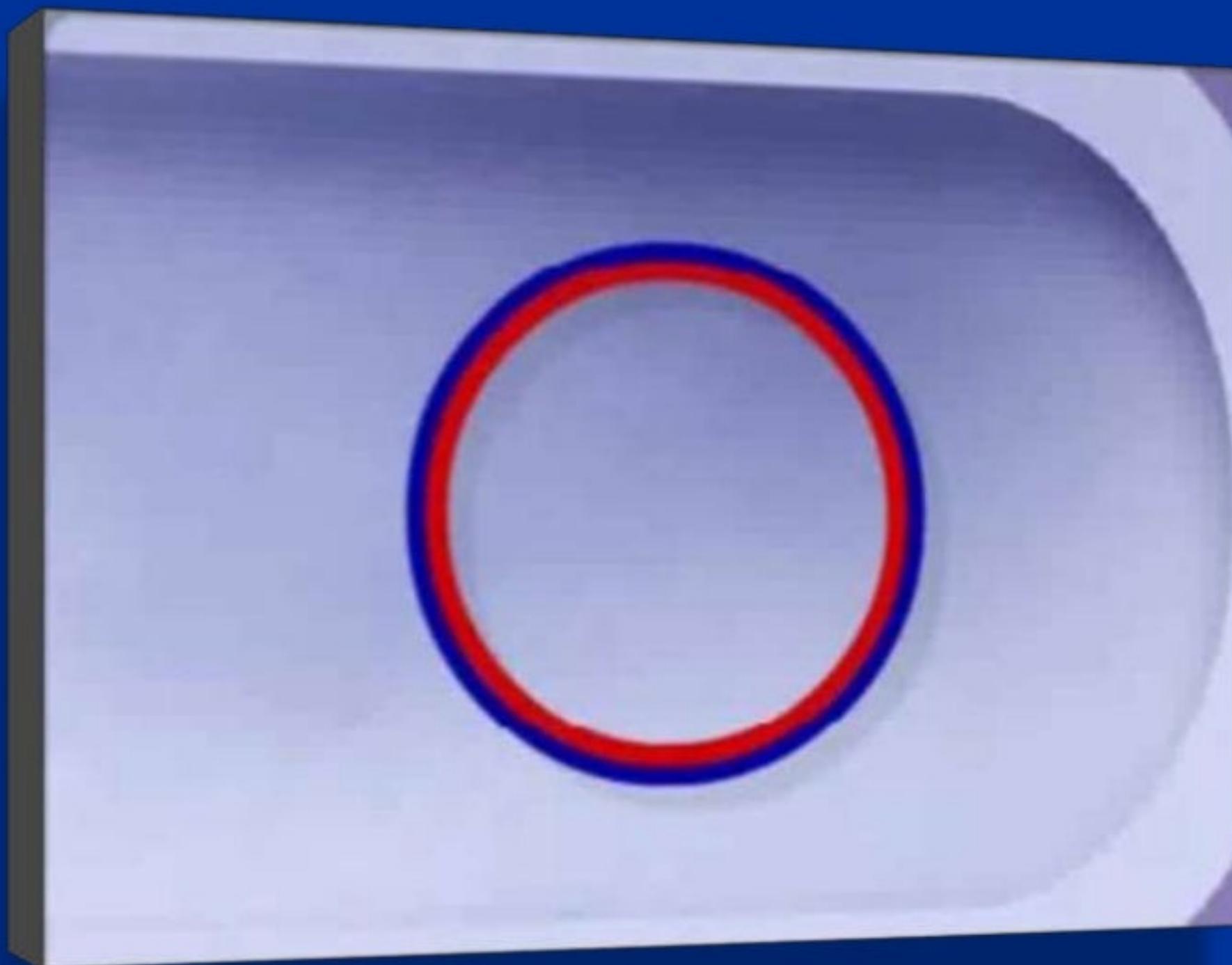
It is named **θ- Replication**.

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 topoisomerase 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 REPLICATION



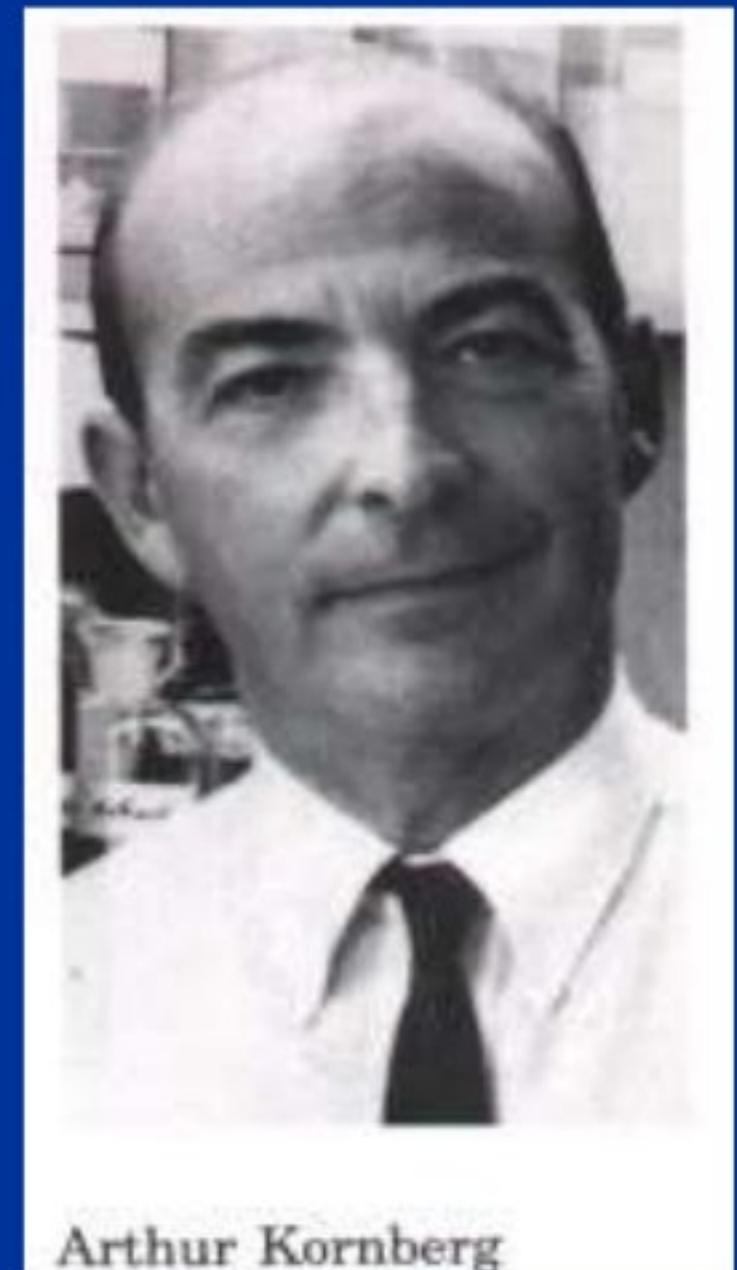
Enzymes and proteins of DNA Replication

Protein	M _r W	Sub units	Function
Dna A protein	50,000	1	Recognizes <i>ori</i> sequences
Dna B protein (DNA Helicase)	300,000	6	Unwinds/opens dsDNA
Dna C protein	29,000	1	Assists Dna B to bind at <i>ori-site</i>
DNA polymerases			Synthesizes the new DNA strands
Dna G protein (DNA Primase)	60,000	1	Synthesize RNA primer
Single Strand Binding Proteins (SSB)	75,600	4	Binds single-stranded DNA
DNA Gyrase (DNA Topoisomerase)	400,000	4	Relieves torsional strain generated by unwinding

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. $5' \rightarrow 3'$ Polymerase activity
 2. Exonuclease activity

Comparison of *DNA Polymerases of E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	<i>polA</i>	<i>polB</i>	<i>polC (dnaE)</i>
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	$\geq 500,000$

Exonuclease functions

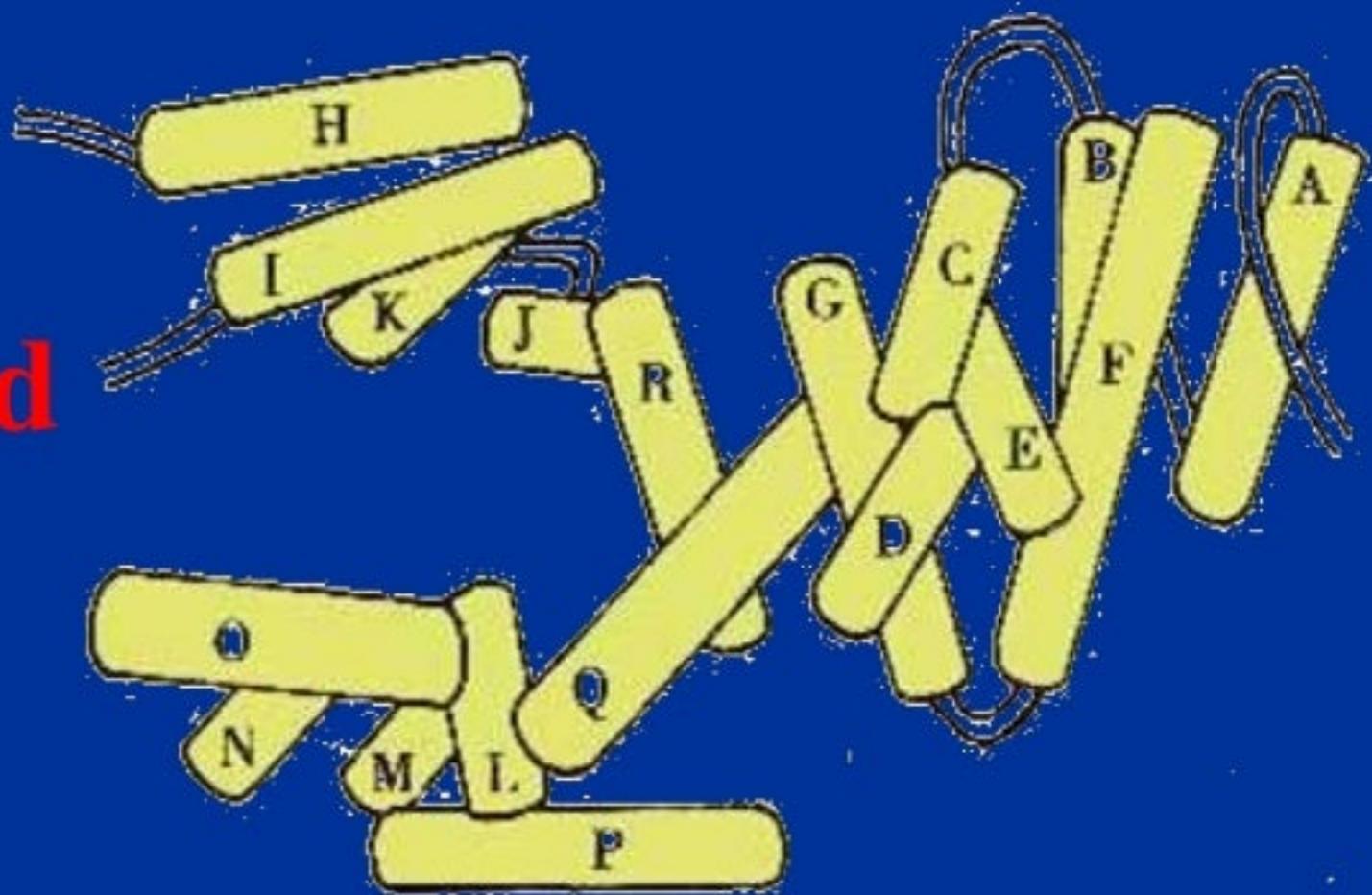
$5' \rightarrow 3'$
exonuclease
activity
removes primer or
excise mutated
segment

$3' \rightarrow 5'$ exonuclease
activity
excise mismatched
nuleotides



DNA Polymerase - I

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



N-end

DNA-pol I

C-end



- Small fragment (323 AA): having $5' \rightarrow 3'$ exonuclease activity
- Large fragment (604 AA): called Klenow fragment, having DNA polymerization and $3' \rightarrow 5'$ exonuclease activity

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**

Subunits of DNA Polymerase III of *E. coli*

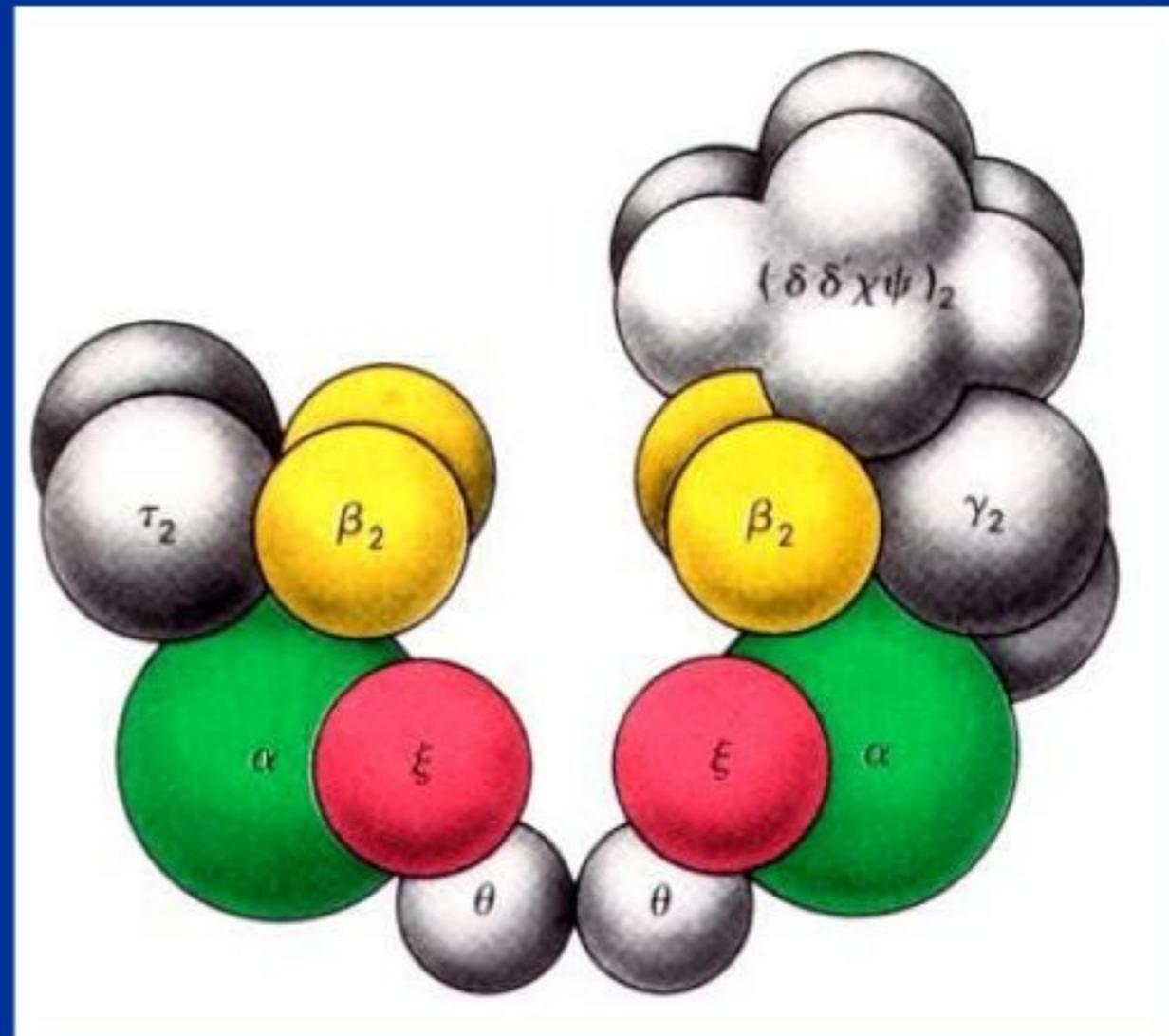
Subunit	Number of subunits per holoenzyme	M_r of subunit	Gene	Function of subunit	
α	2	129,900	<i>polC</i> (<i>dnaE</i>)	Polymerization activity	Core polymerase
ε	2	27,500	<i>dnaQ</i> (<i>mutD</i>)	3' \rightarrow 5' Proofreading exonuclease	
θ	2	8,600	<i>holE</i>		
τ	2	71,100	<i>dnaX</i>	Stable template binding; core enzyme dimerization	Clamp-loading (γ) complex that loads β subunits on lagging strand at each Okazaki fragment
γ	1	47,500	<i>dnaX</i> *	Clamp loader	
δ	1	38,700	<i>holA</i>	Clamp opener	
δ'	1	36,900	<i>holB</i>	Clamp loader	
χ	1	16,600	<i>holC</i>	Interaction with SSB	
ψ	1	15,200	<i>holD</i>	Interaction with γ and χ	
β	4	40,600	<i>dnaN</i>	DNA clamp required for optimal processivity	

Structure of DNA-pol III

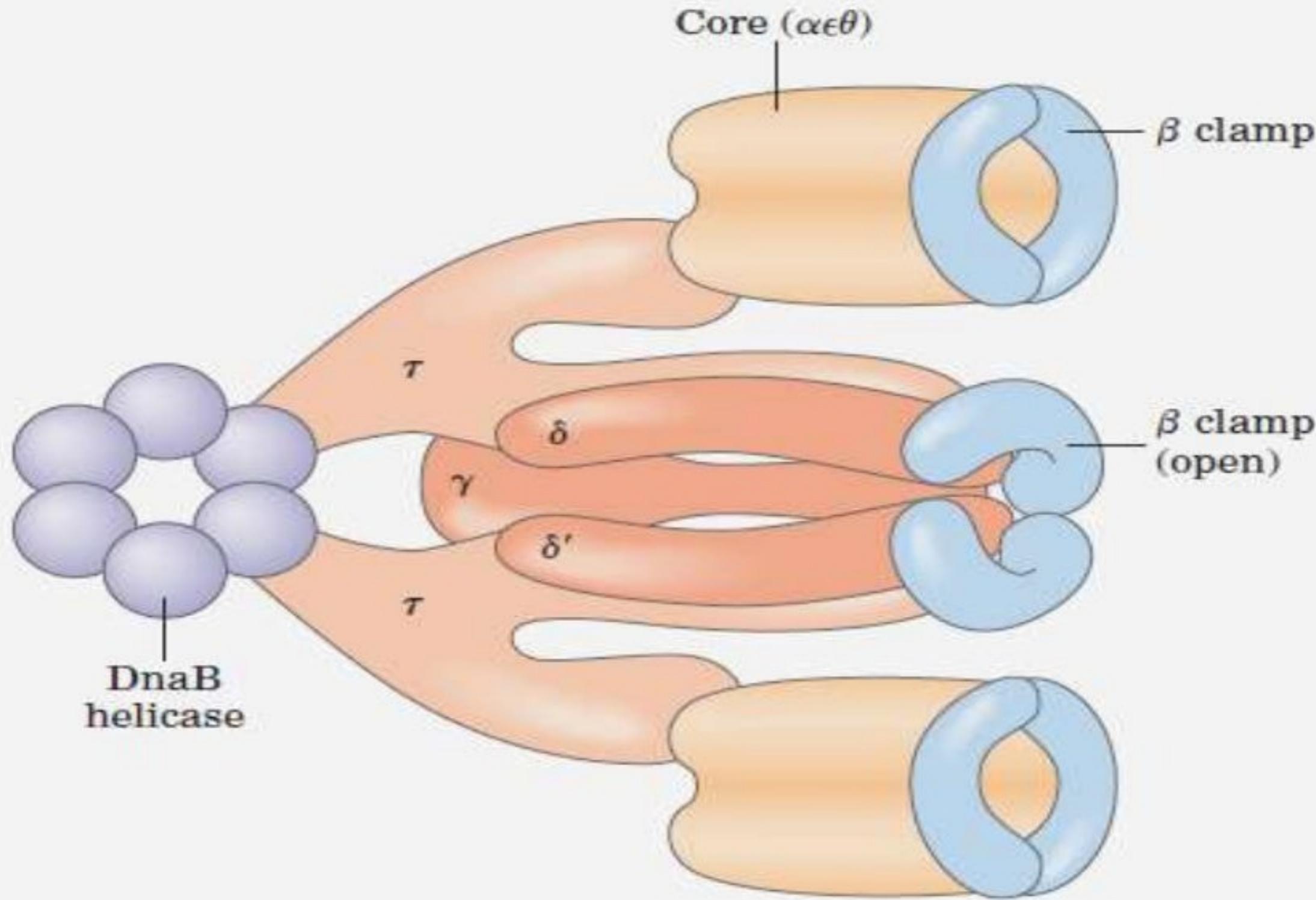
α : has $5' \rightarrow 3'$ polymerizing activity

ξ : has $3' \rightarrow 5'$ exonuclease activity and plays a key role to ensure the replication fidelity.

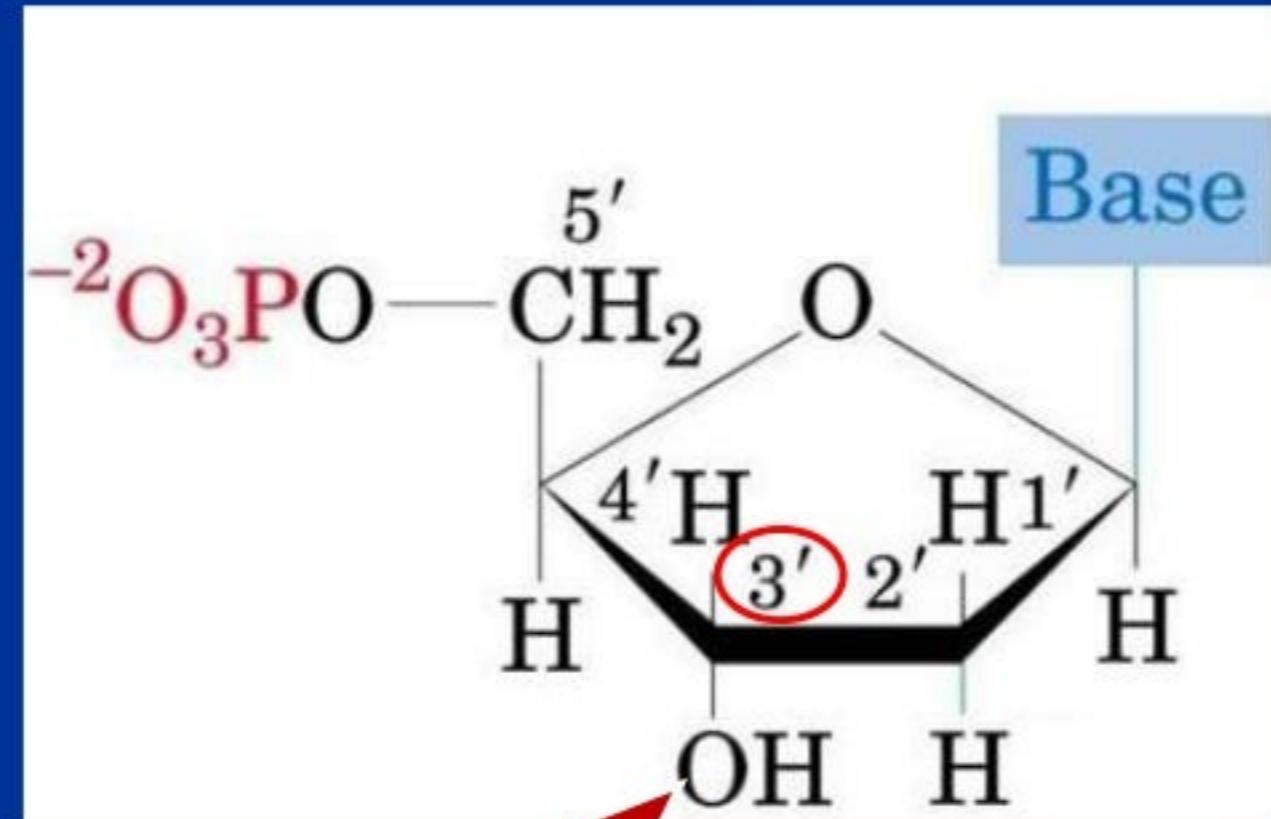
θ : maintain heterodimer structure







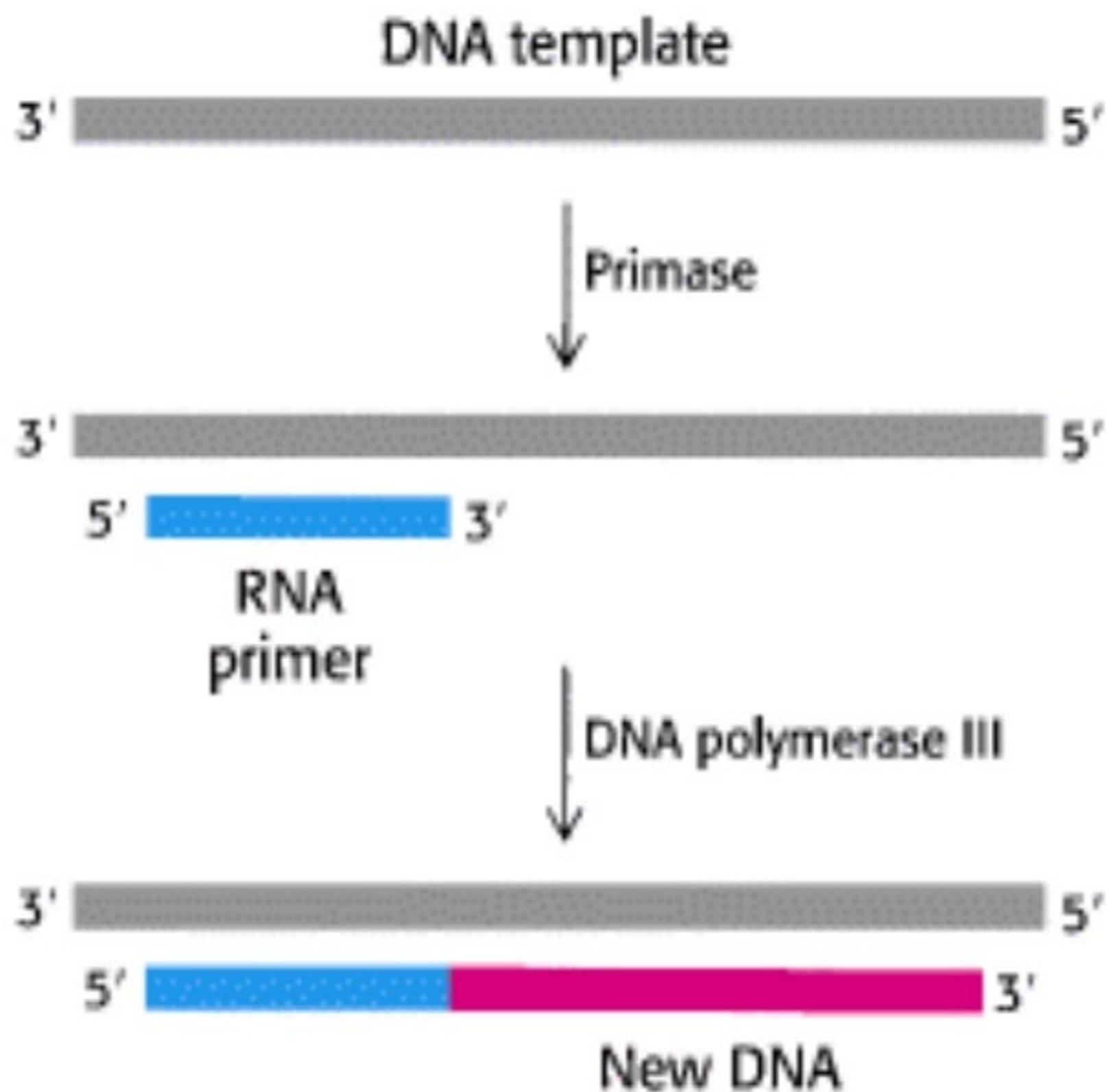
Nucleotides are always added to the growing strand at the 3' end – the end at which the DNA strand has a **free** –OH group on the 3' carbon of its terminal deoxyribose



Free 3'- hydroxyl group

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.



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

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

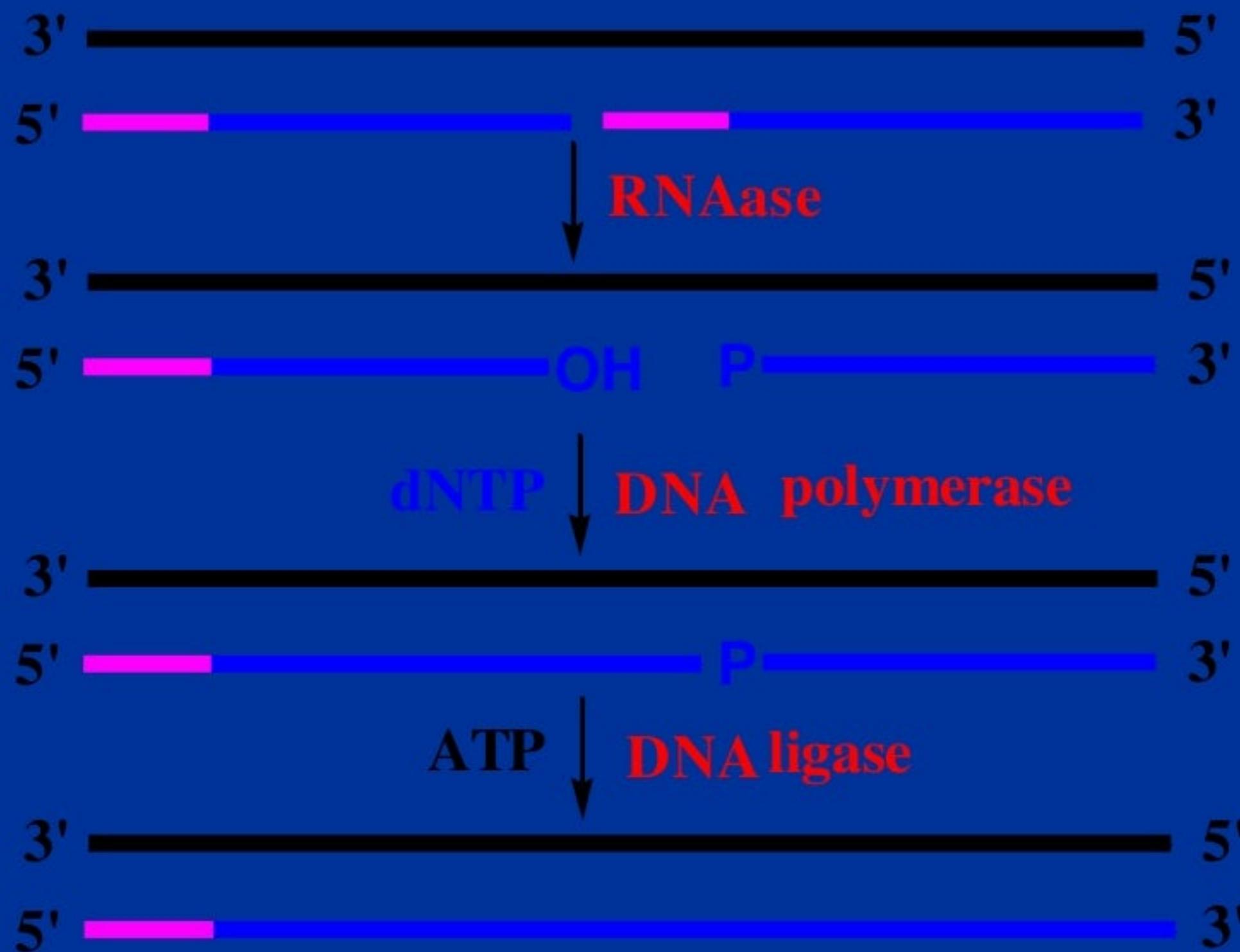
SSB protein

- 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.

DNA Gyrase

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

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.

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

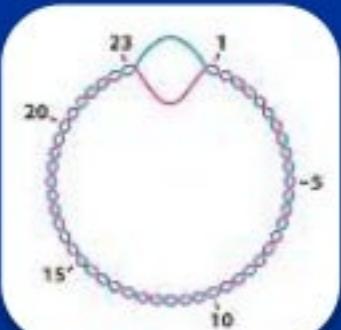
Proofreading and Correction

- 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.

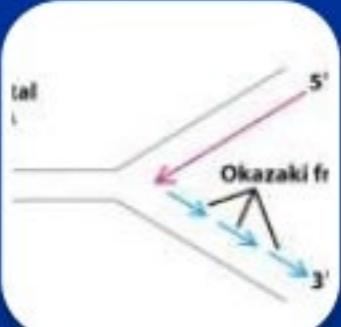
DNA Replication Process

DNA REPLICATION

STAGES



Initiation



Elongation



Termination

Three Stages of replication

1). Initiation

- ✓ occurs at the **origin of replication**
- ✓ separates dsDNA, primer synthesis

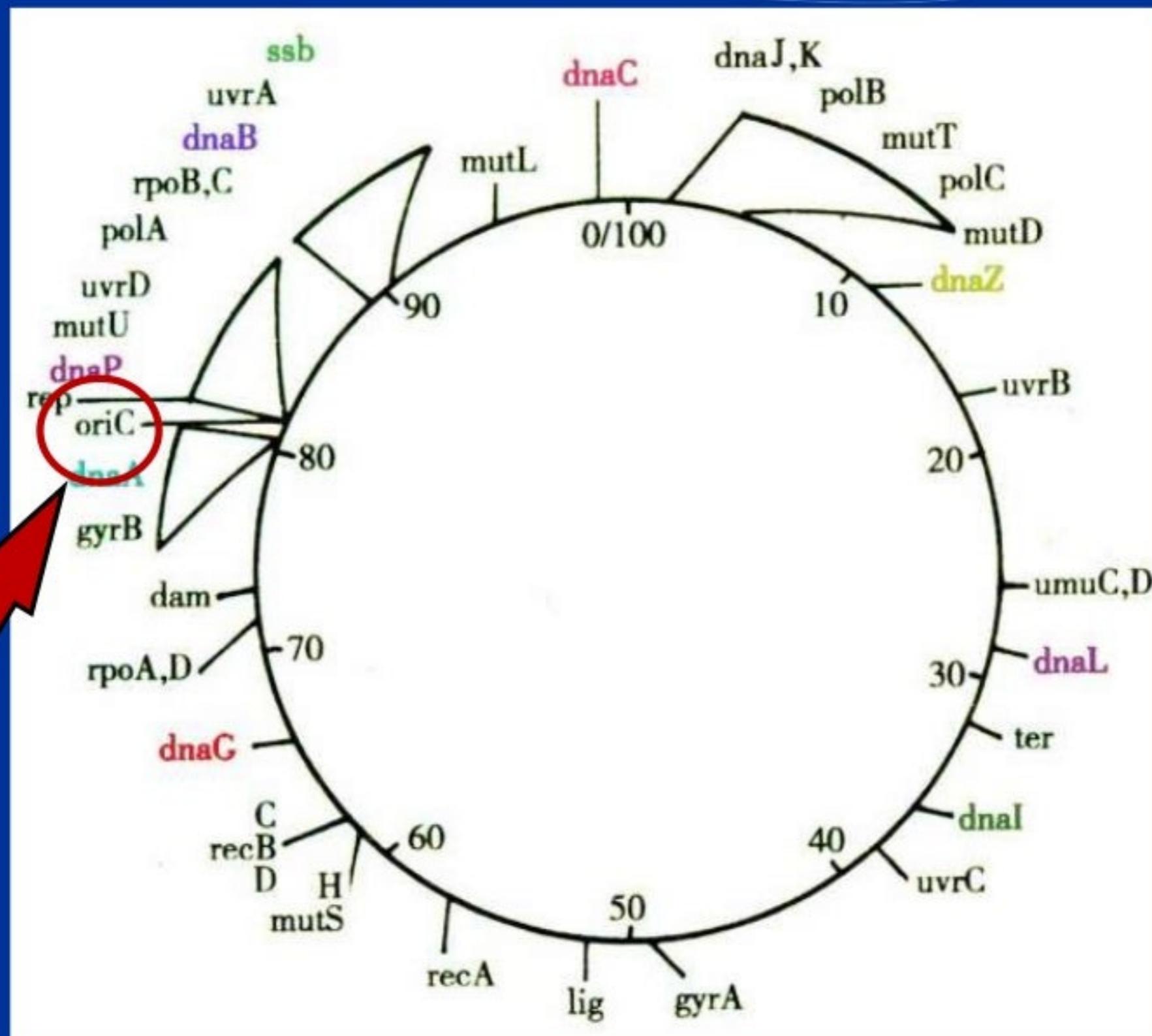
2). Elongation

- ✓ involves the *addition of new nucleotides* (dNTPs) **based on complementarity 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*

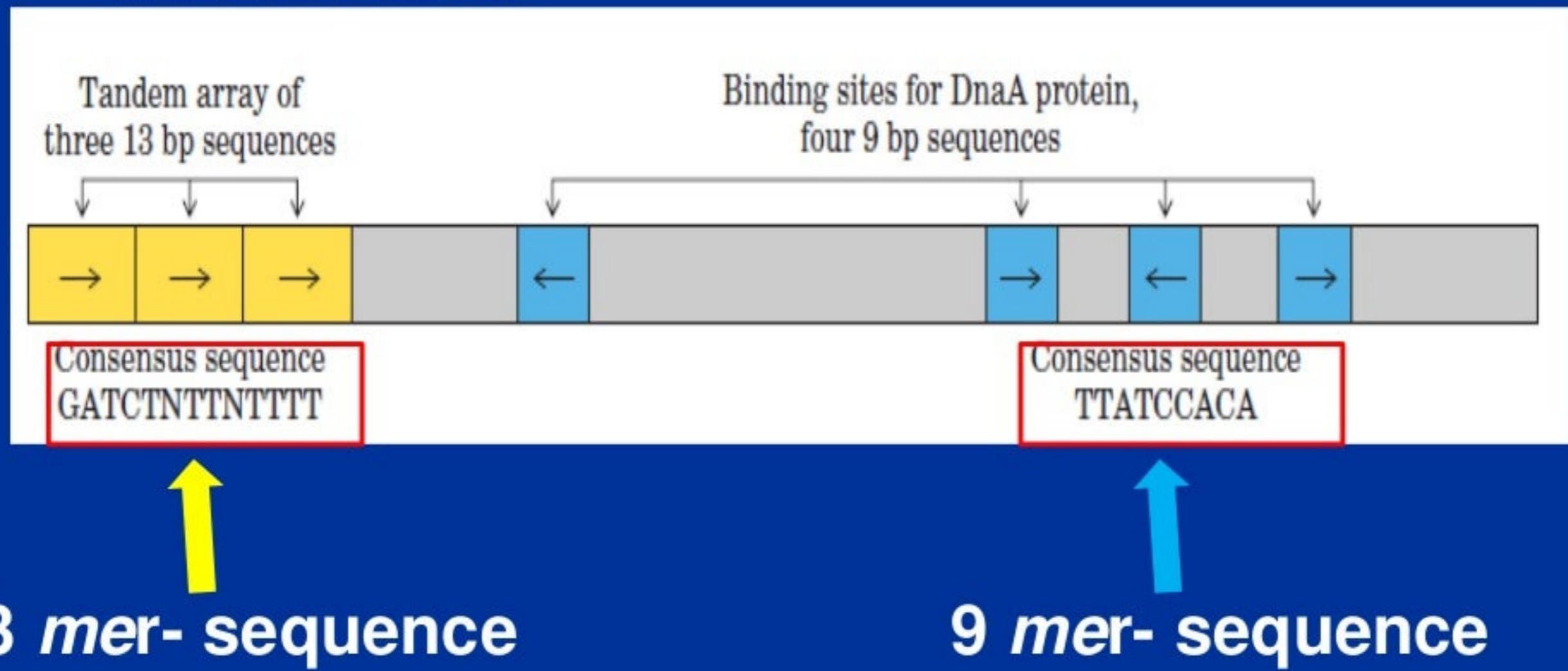
Genome of *E. coli*



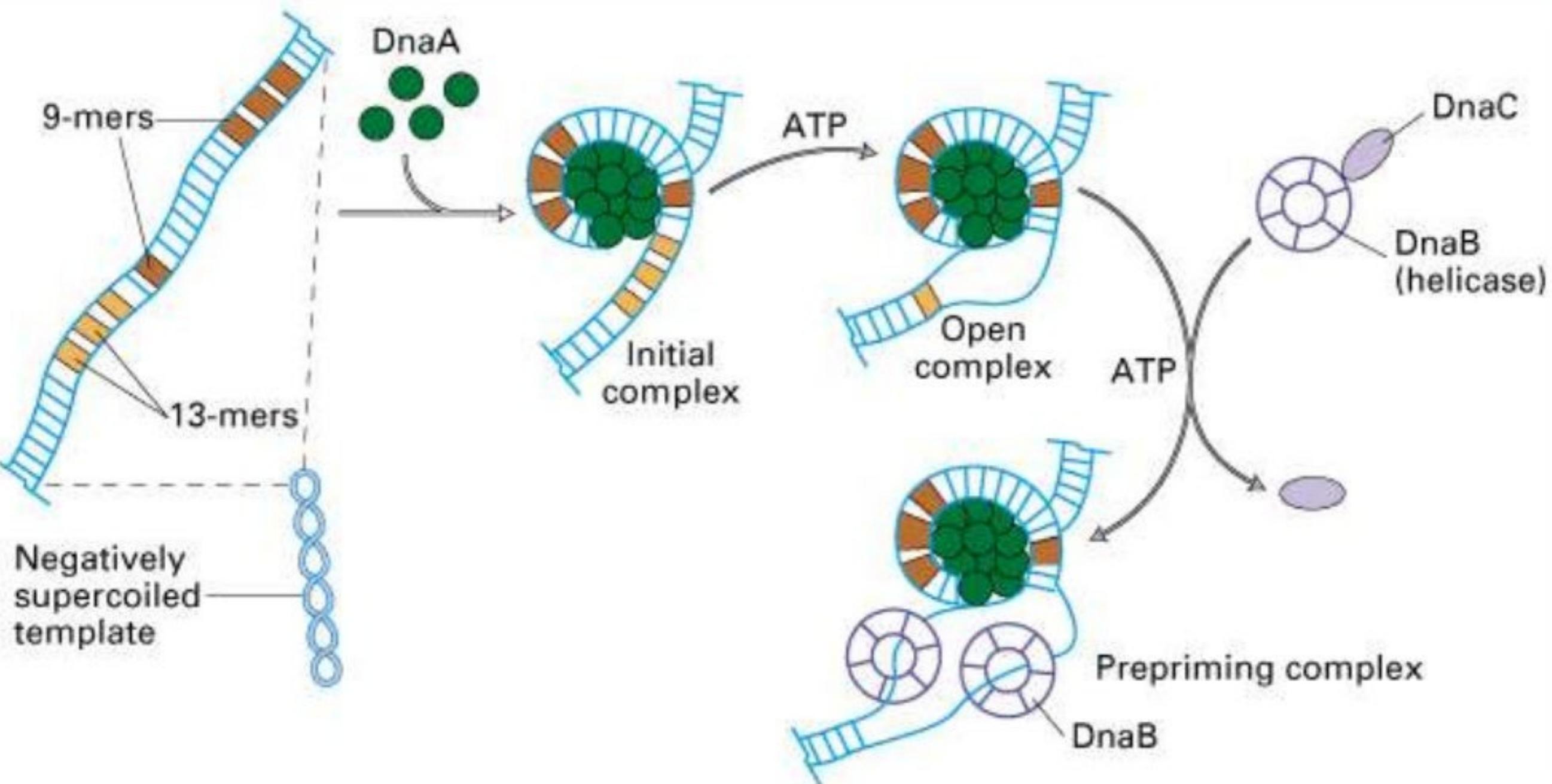
ori-Site

Initiation

- The replication starts at a particular point called **origin of Replication (or) ori-Site.**
- The structure of the origin is 248 bp long and AT-rich.

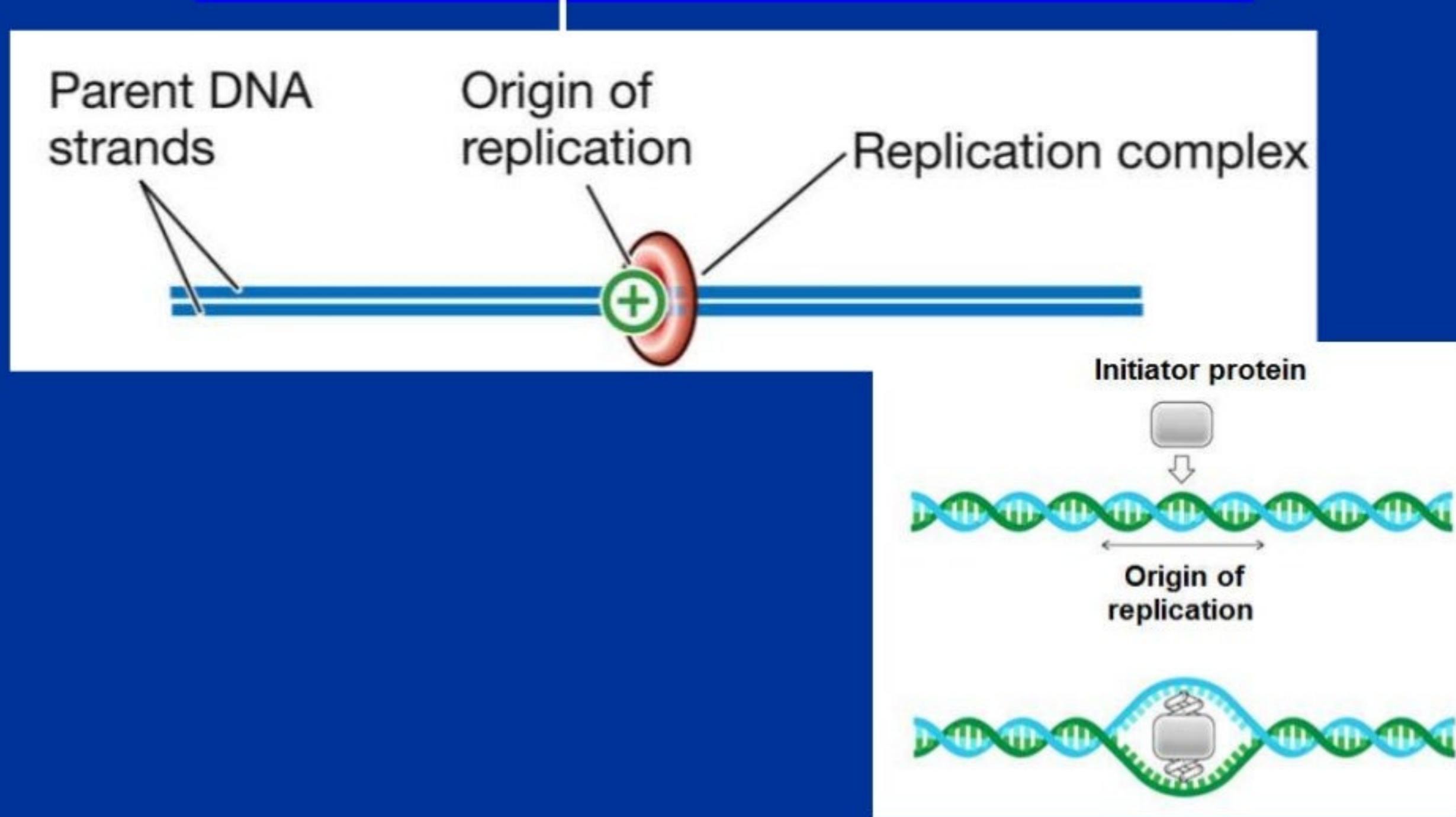


Formation of Preprimosome



Origin of Replication

Site where DNA synthesis starts



Formation of Replication fork

- DnaA recognizes ori C.
- DnaB (Helicase) and DnaC join the DNA-DnaA complex, open the local AT-rich region, and move on the template downstream further to separate enough space.
- DnaA is replaced gradually.
- SSB protein binds the complex to stabilize ssDNA.

Primer synthesis

- **Primase** joins and forms a complex called **primosome**.
- Primase starts the **synthesis of primers** on the ssDNA template using NTP as the substrates in the **5' - 3'** direction at the expense of ATP.
- The short RNA fragments provide free **3' -OH** groups for DNA elongation.

INITIATION

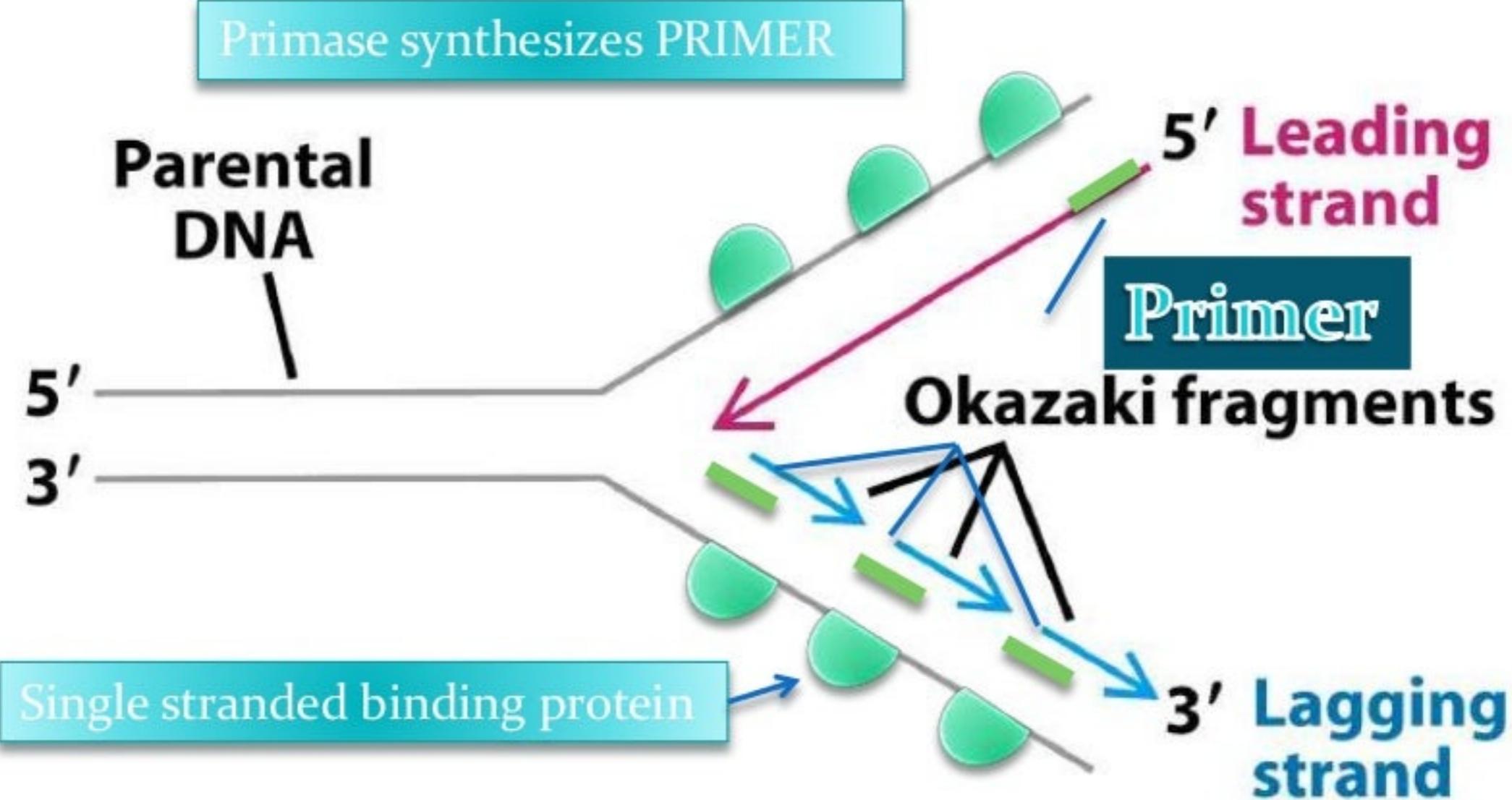
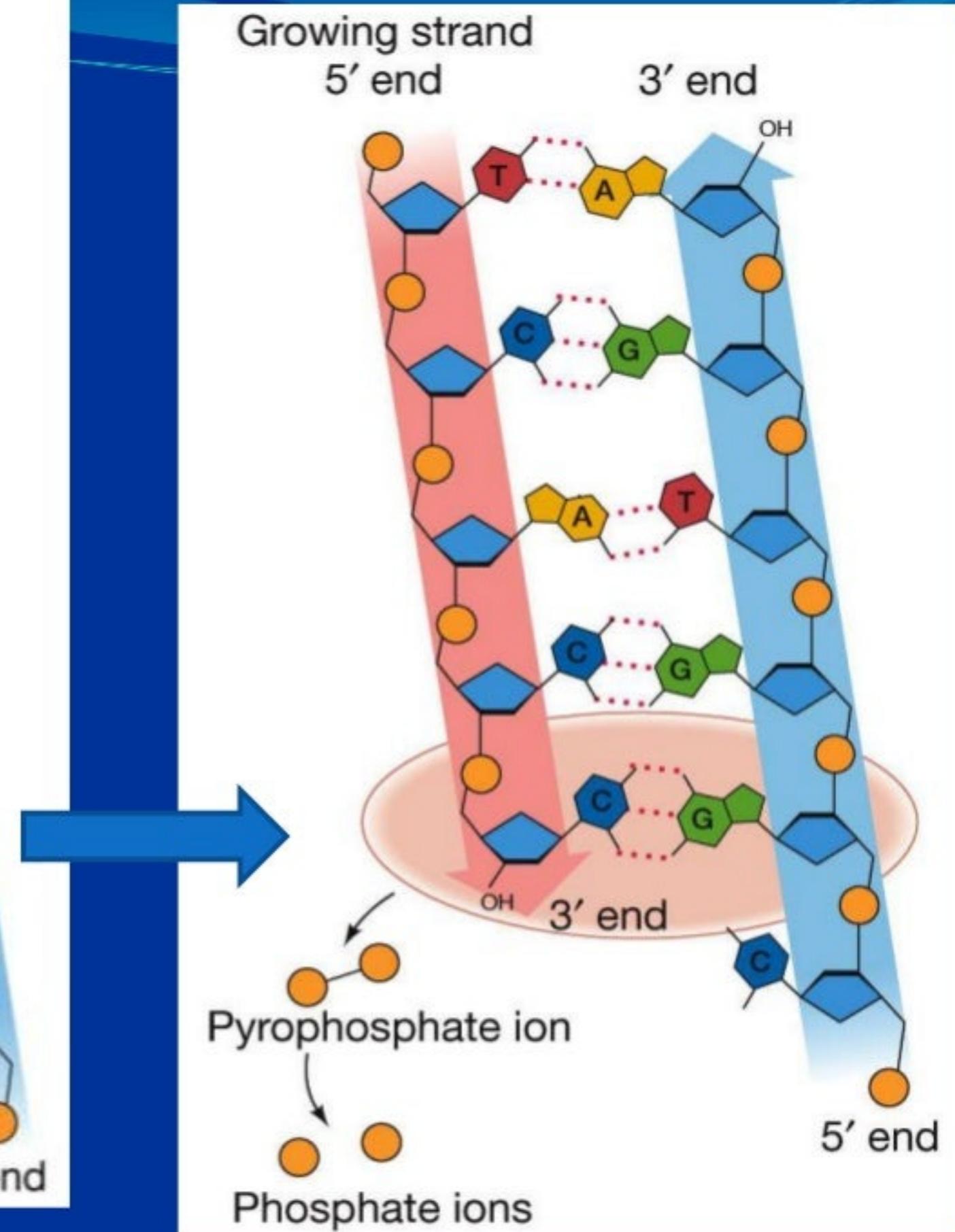
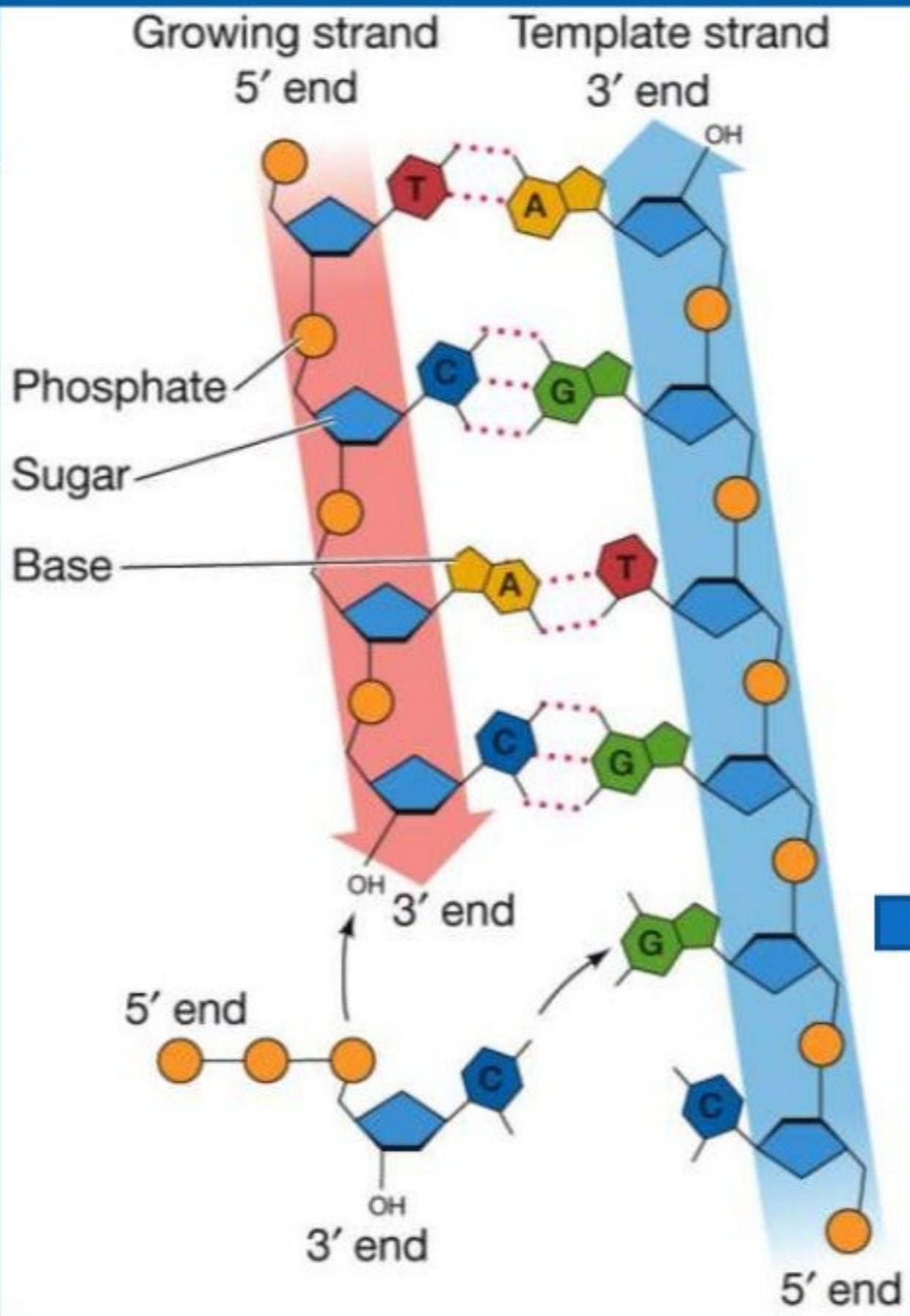


Figure 28-21

Elongation

- dNTPs are **continuously connected** to the primer or the nascent DNA chain by **DNA-pol III**.
- The core enzymes (α , ϵ , and θ) catalyze the synthesis of leading and lagging strands, respectively.
- The nature of the chain elongation is the series formation of the **phosphodiester bonds**.



ELONGATION

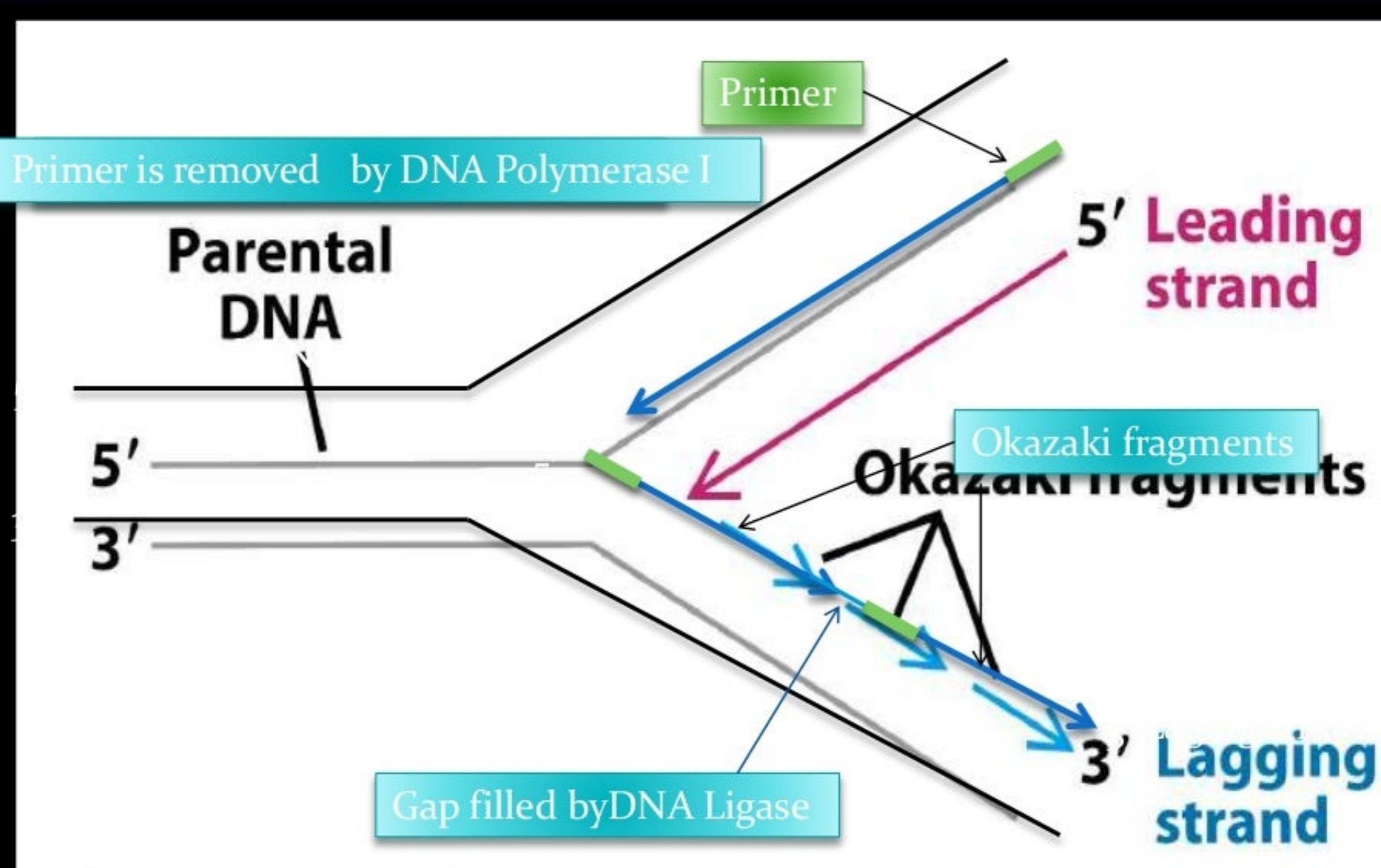


Figure 28-21

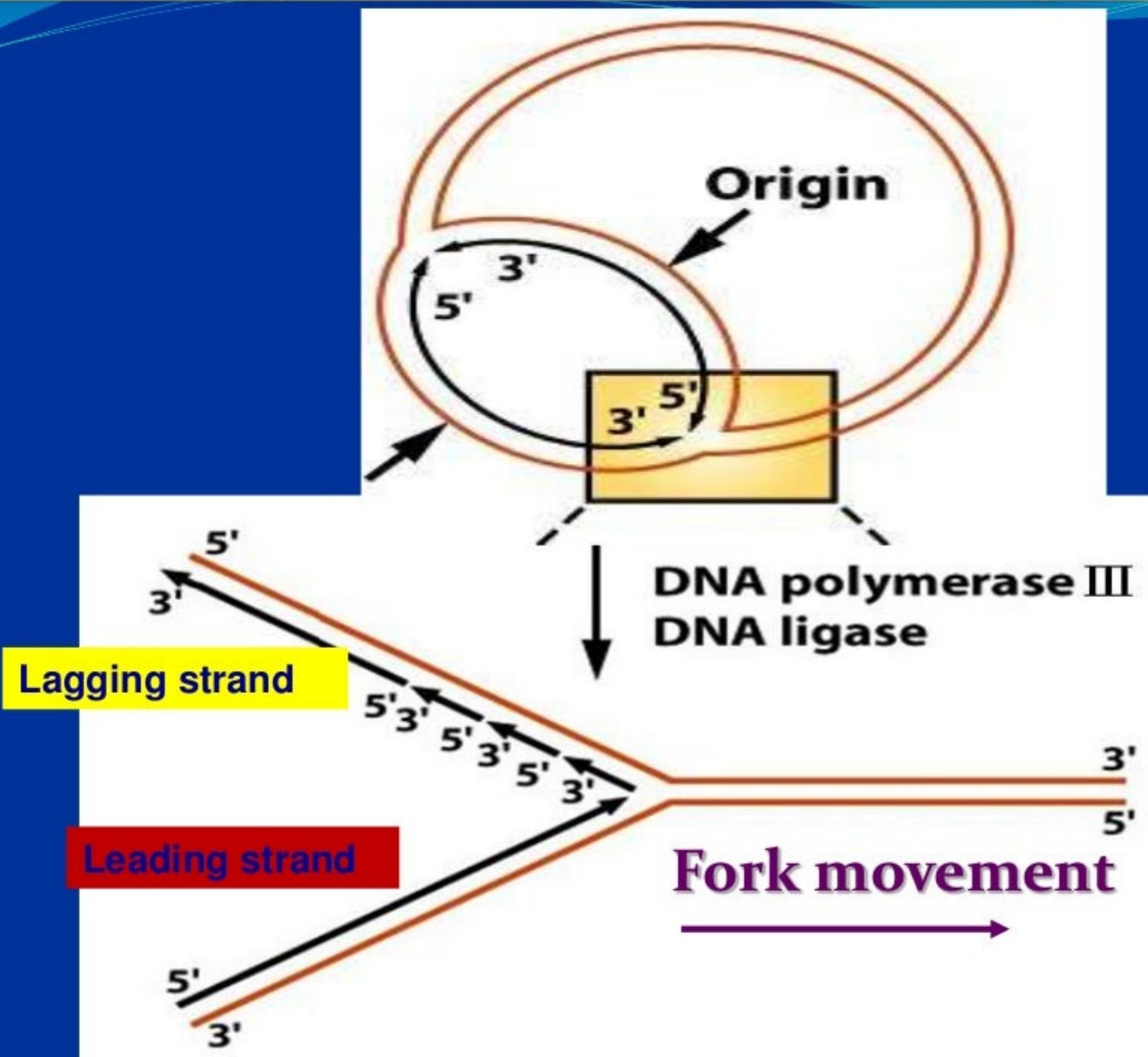
Lagging strand synthesis

- RNA Primers on Okazaki fragments are digested by the enzyme RNase.
- The gaps are filled by DNA-pol I in the $5' \rightarrow 3'$ direction.
- The nick between the $5'$ end of one fragment and the $3'$ end of the next fragment is sealed by ligase.

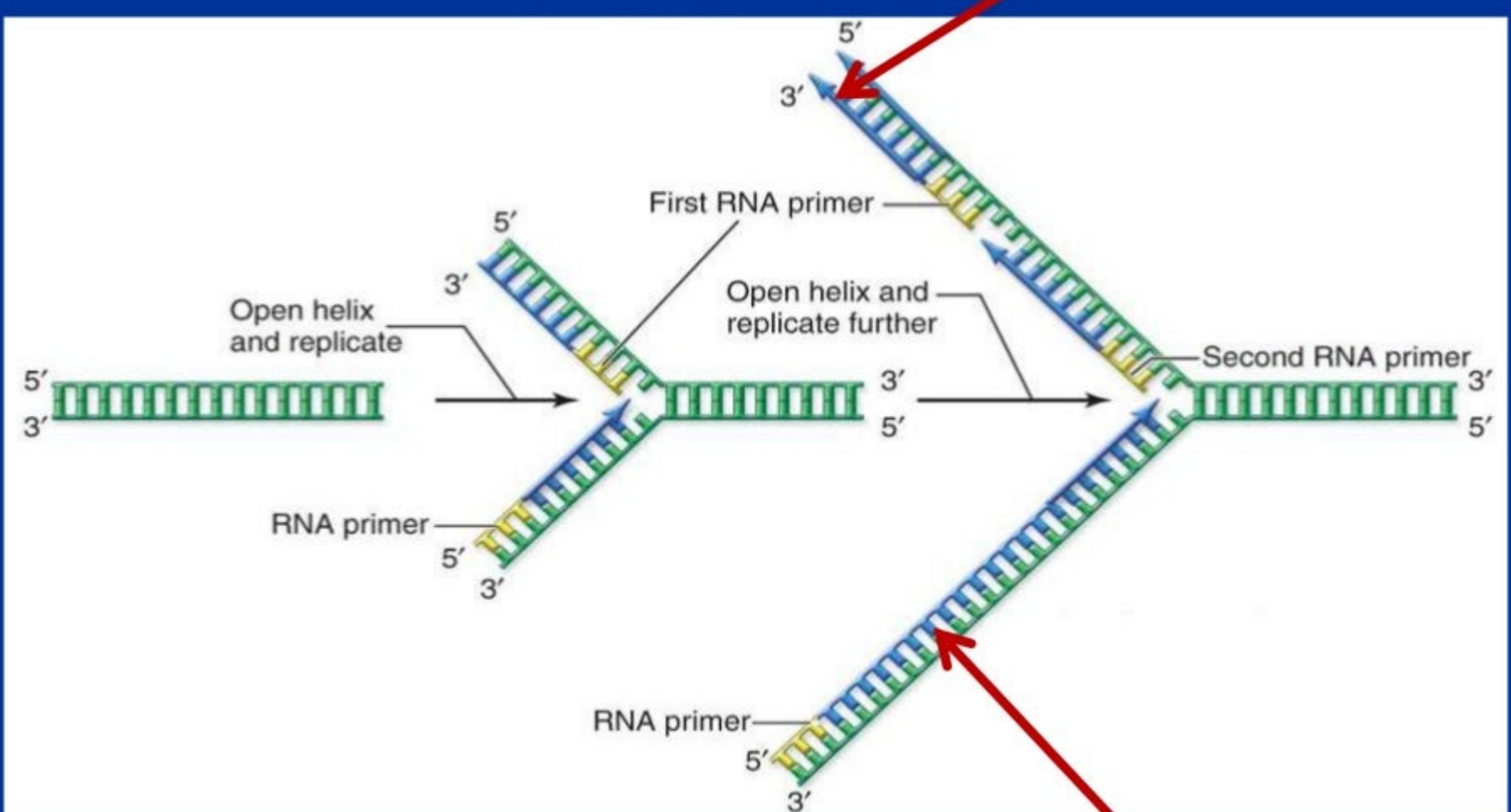
Okazaki fragments

- 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 in prokaryotes and **100-150 nt** long in eukaryotes.
- The daughter strand consisting of **Okazaki fragments** is called the **lagging strand**.

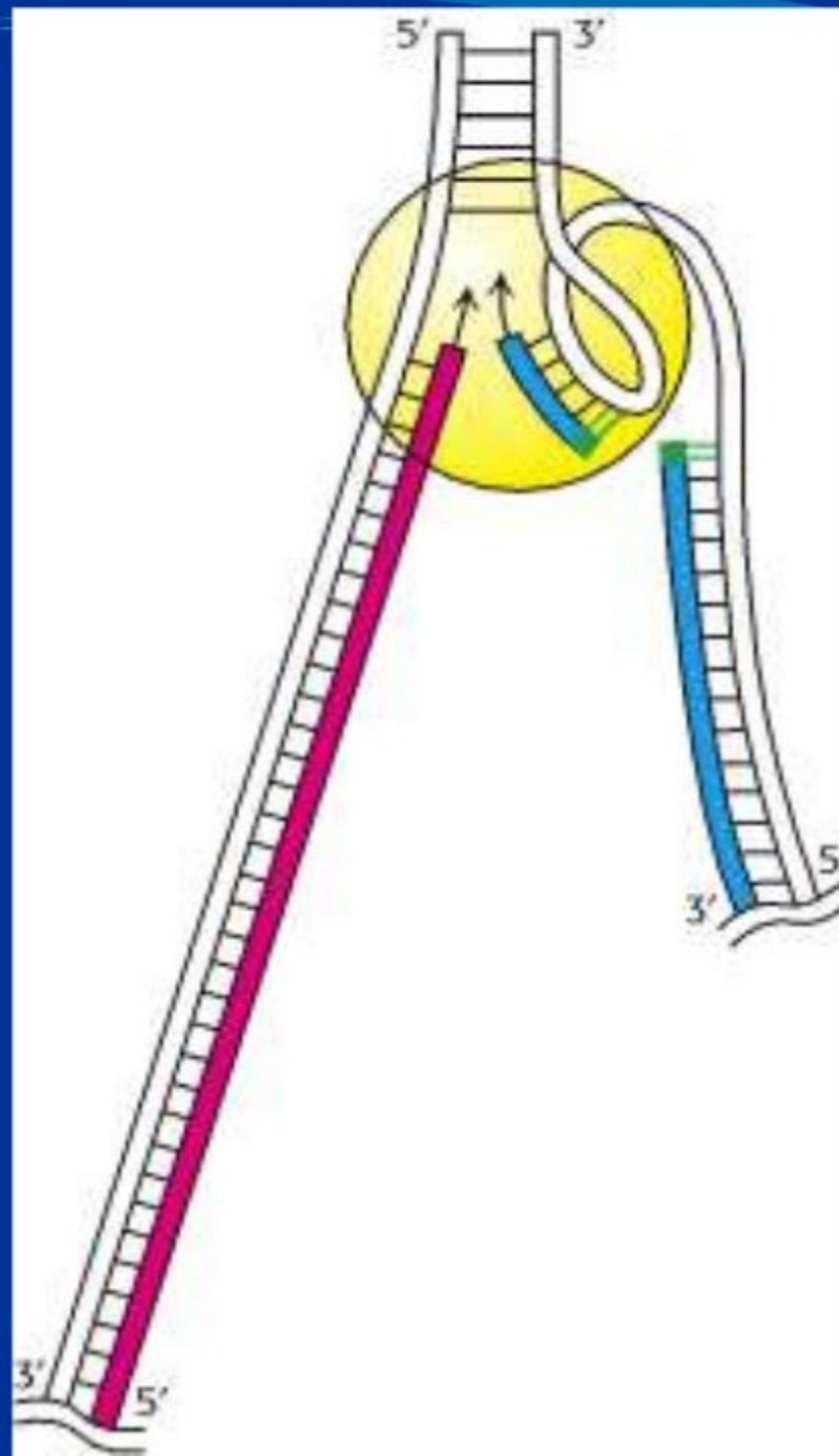
Directionality of the DNA strands at a replication fork

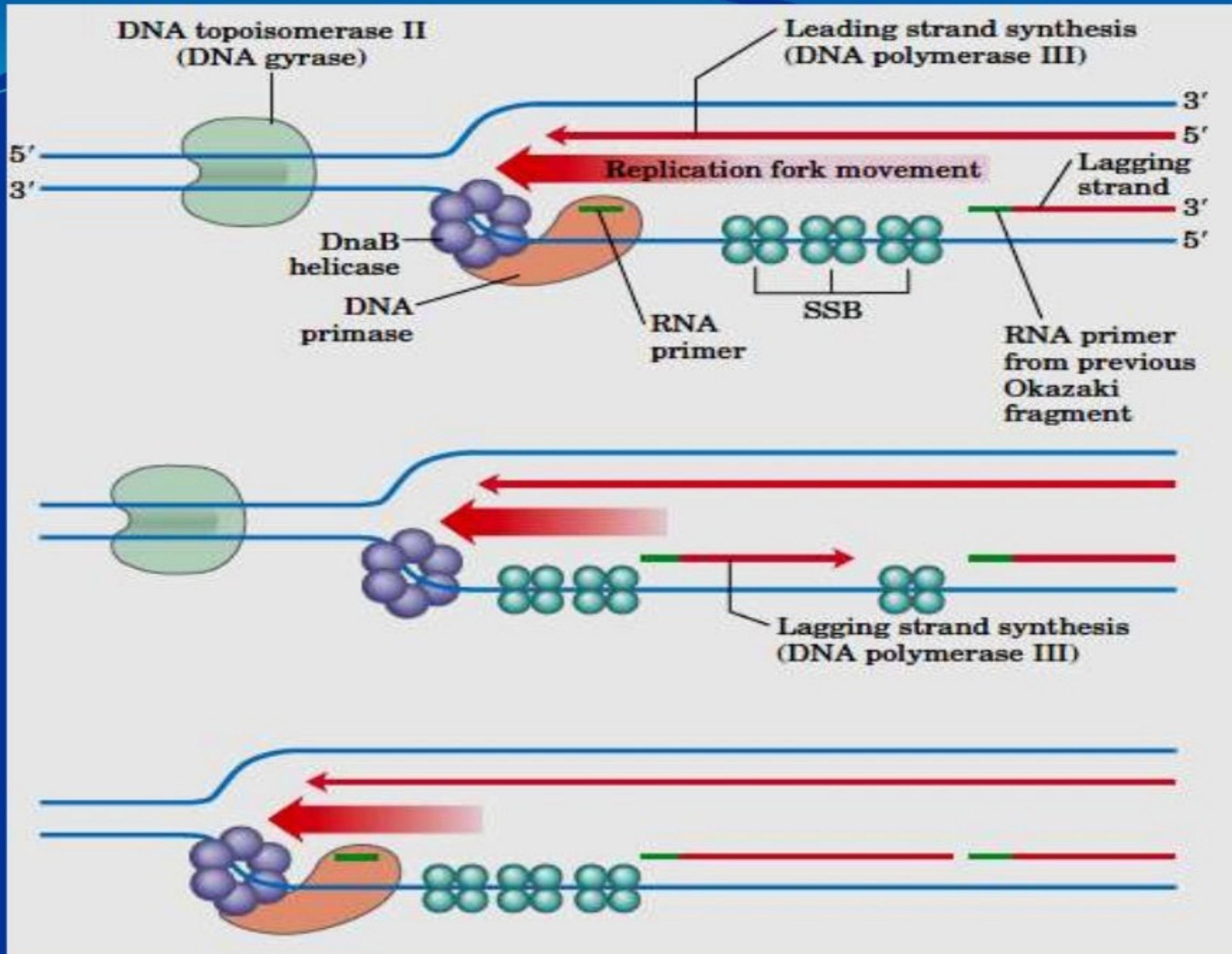


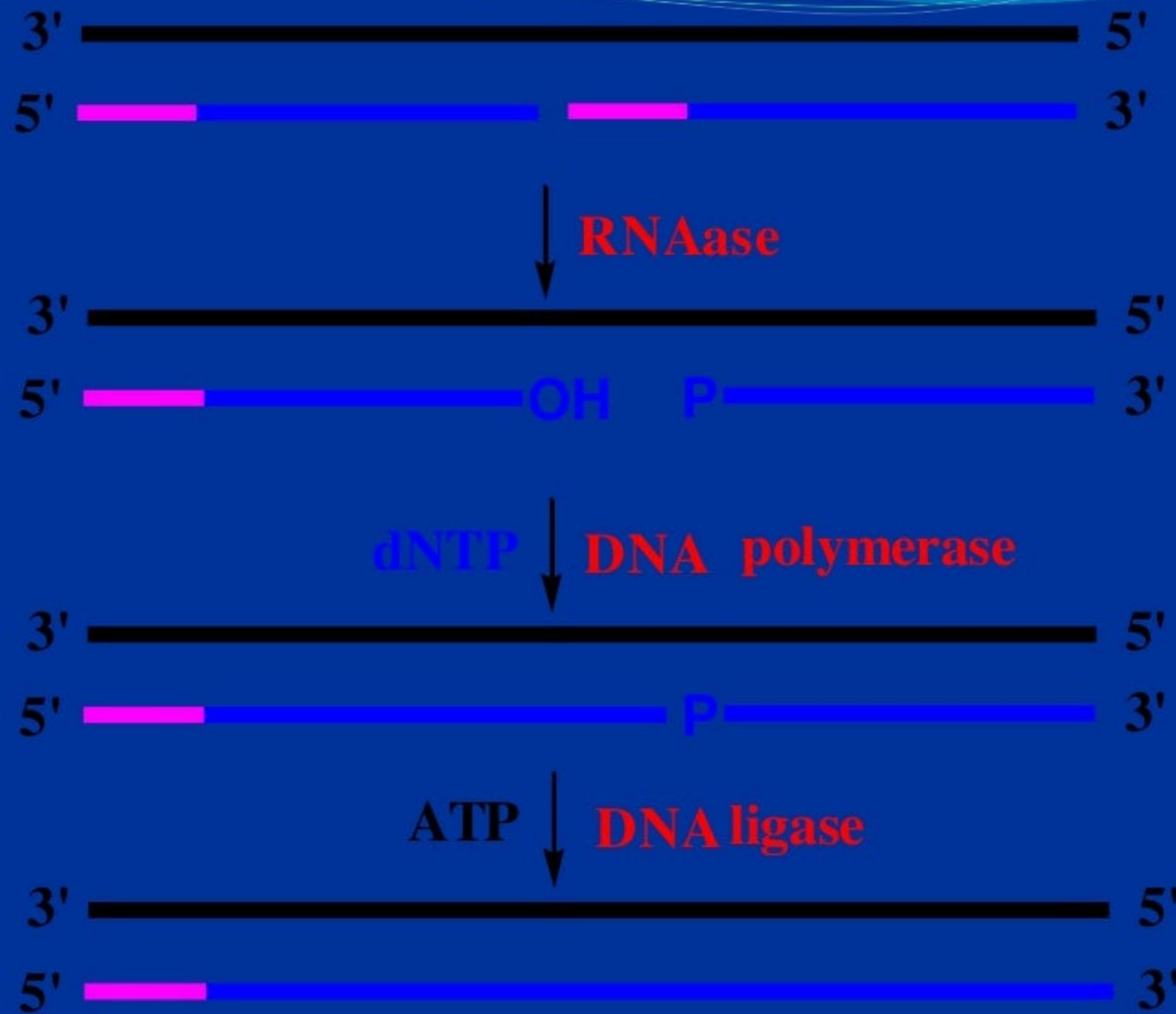
Lagging strand (discontinuous)



Leading strand (continuous)

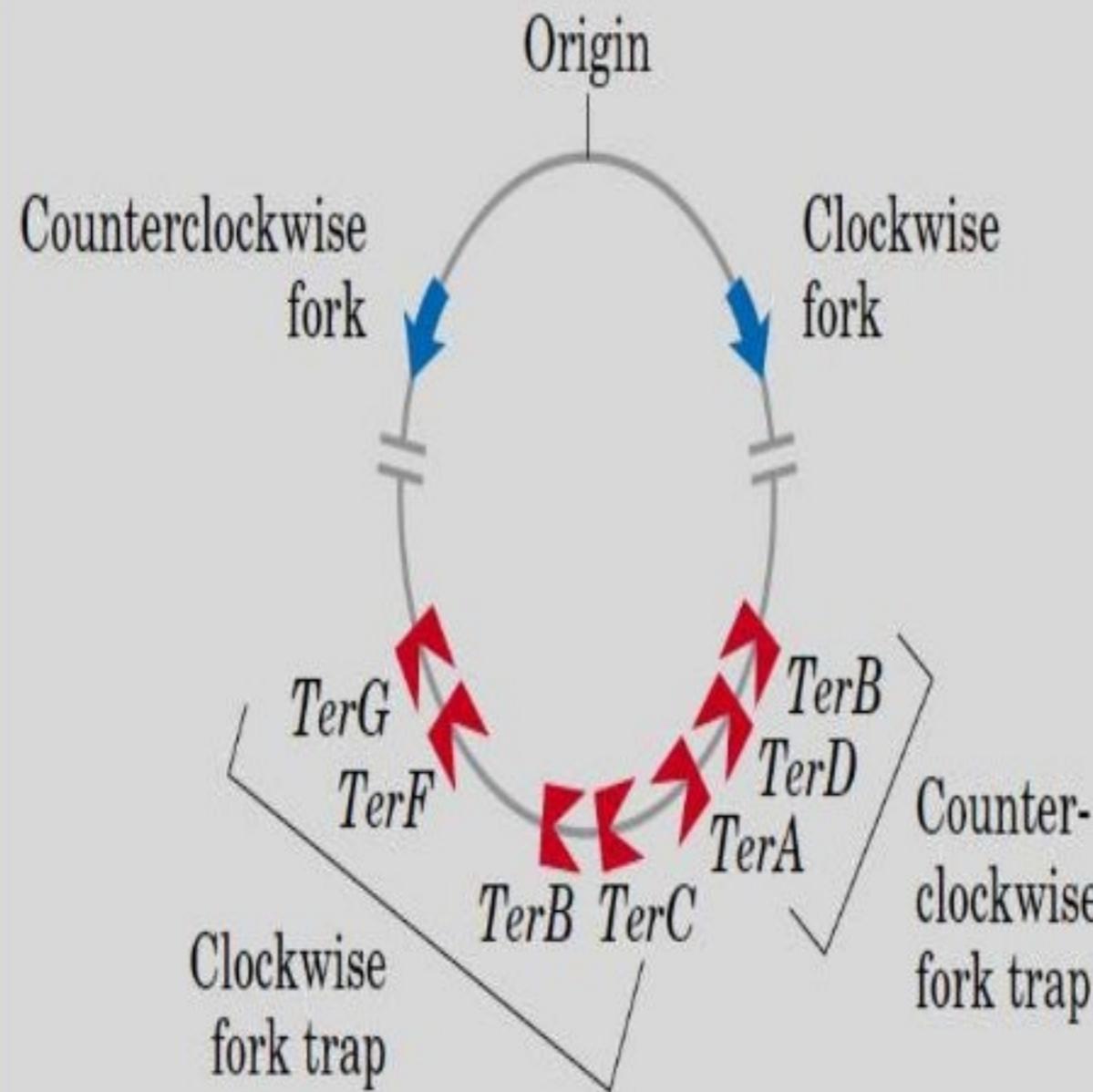






Termination

- The replication of *E. coli* is bidirectional from one origin, and the **two replication forks must meet** at one point called ter at 32.
- All the primers will be removed, and all the **fragments** will be **connected** by DNA-pol I and ligase.



***Ter*-binding proteins** - will recognizes the Termination sequences and helps to achieve the termination process.

Nucl.

www.FreeScienceLectures.com

INHIBITORS OF DNA REPLICATION

Nalidixic acid

Novobiocin

Ciprofloxacin

INHIBITORS OF DNA REPLICATION

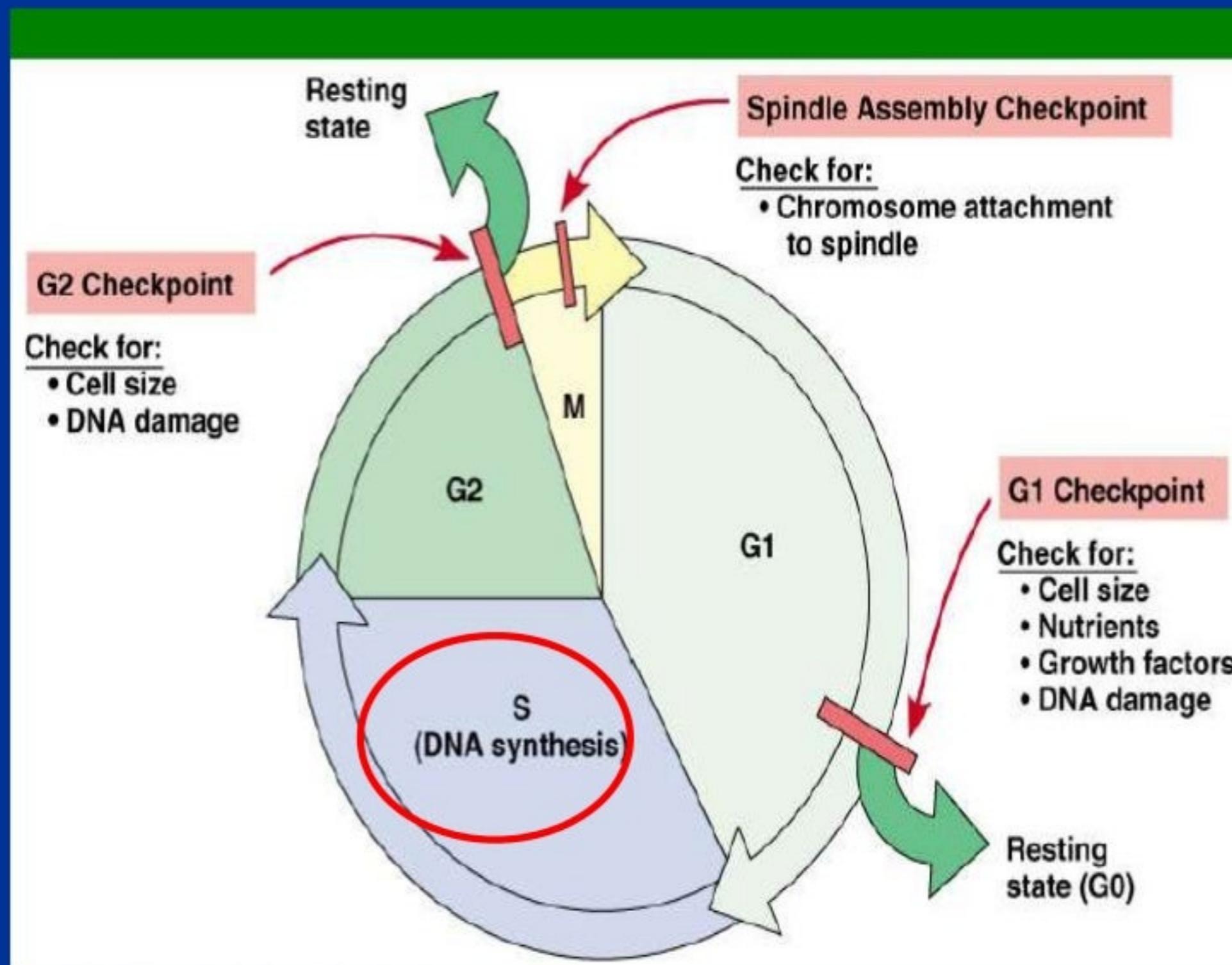
Adriyamycin

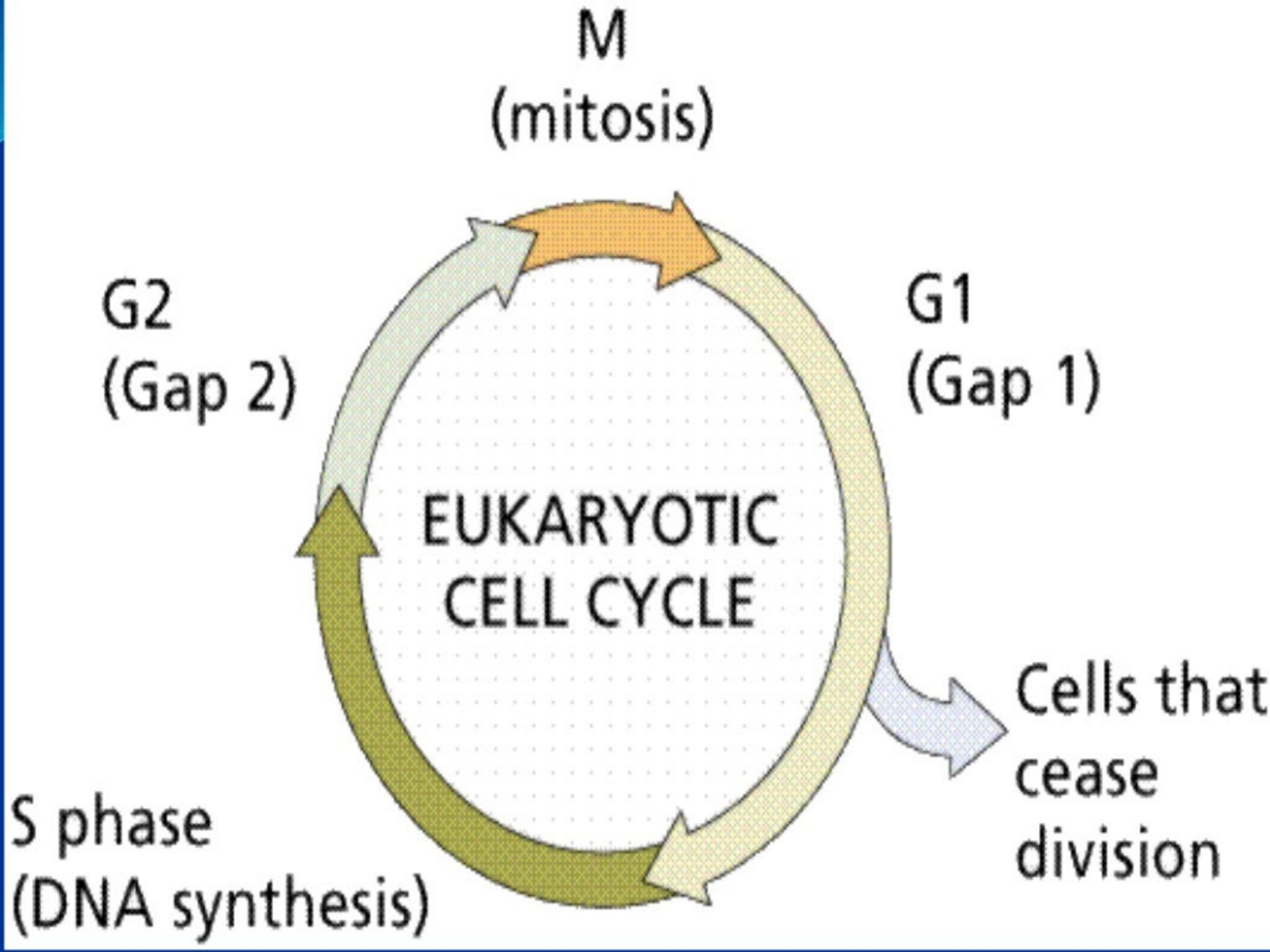
Etoposide

Doxorubicin

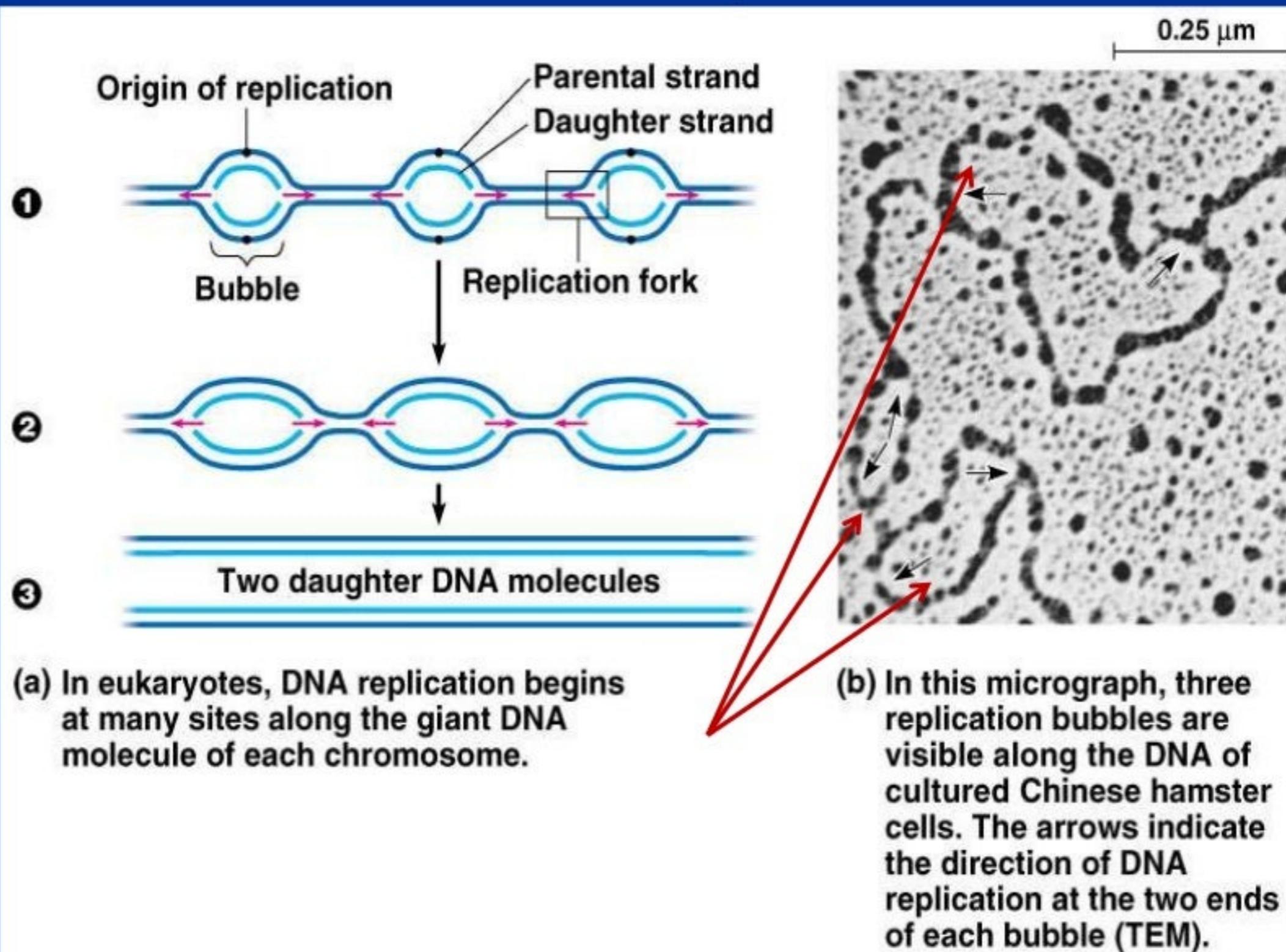
Eukaryotic DNA Replication

- DNA replication is closely related with cell cycle.





Multiple origins on one chromosome, and replications are activated in a sequential order rather than simultaneously.



DNA Polymerase of Eukaryotes

Eukaryotic Enzyme

Prokaryotic Enzyme

DNA-pol α : initiate replication and synthesize primers

→ DnaG, primase

DNA-pol β : replication with low fidelity

→ Repair

DNA-pol γ : → Mitochondrial DNA synthesis

DNA-pol δ : elongation

→ DNA-pol III

DNA-pol ϵ : lagging strand synthesis, proofreading and gap filling

→ DNA-pol I

Initiation

- The eukaryotic replication origins are **shorter** than that of *E. coli*. The *ori*-sites in Eukaryotes called **ARS (Autonomously Replicating Sequences) (or) Replicators.**
- Requires **DNA-pol α** (primase activity) and **DNA-pol δ** (polymerase activity and helicase activity).
- **DNA-pol δ** requires a protein called for its activity **Proliferating Cell Nuclear Antigen (PCNA)**.
- Needs **Topoisomerase** and **Replication factors (RF)** to assist.

Elongation

- DNA replication and nucleosome assembling occur simultaneously.
- Overall replication speed is compatible with that of prokaryotes.

Termination

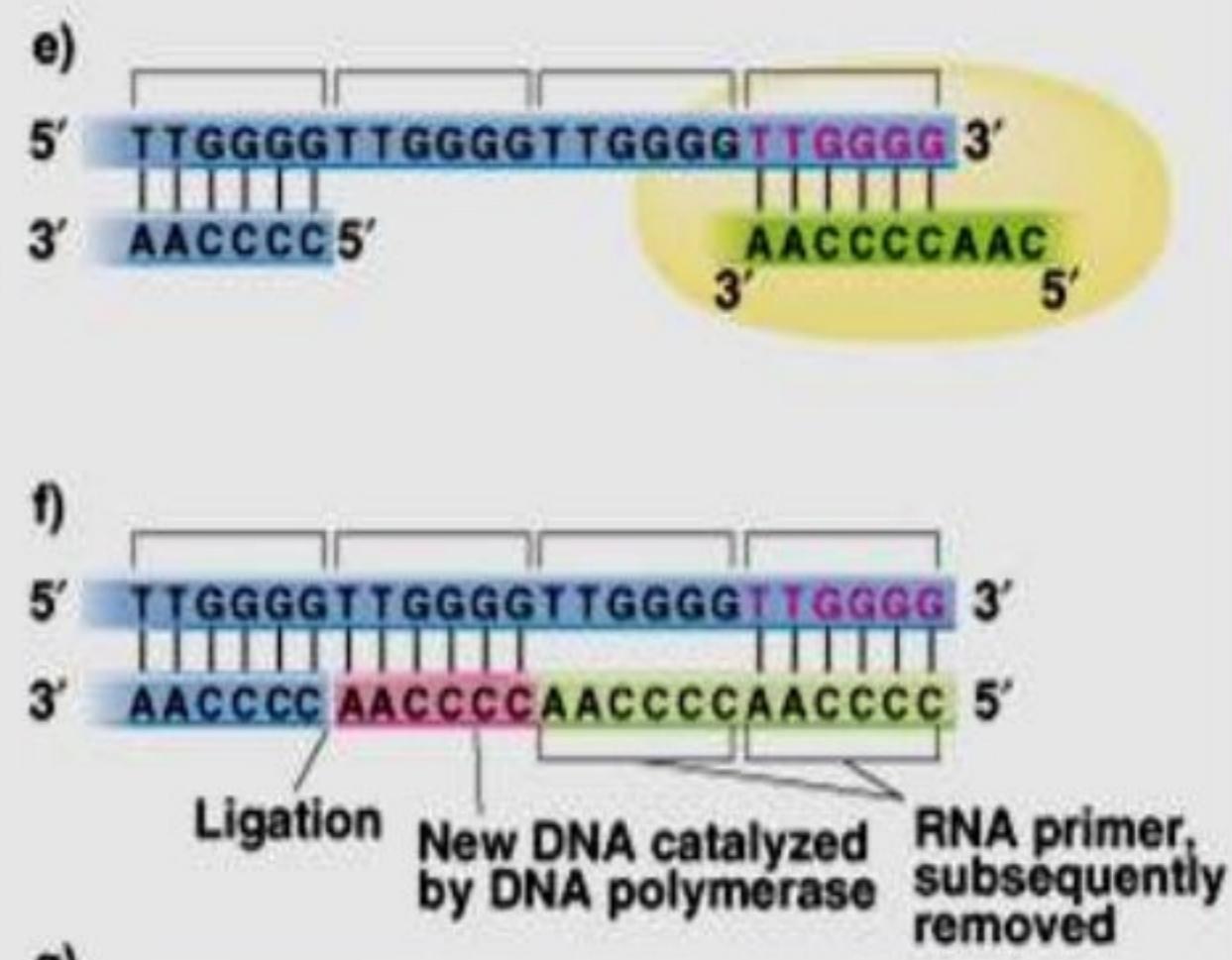
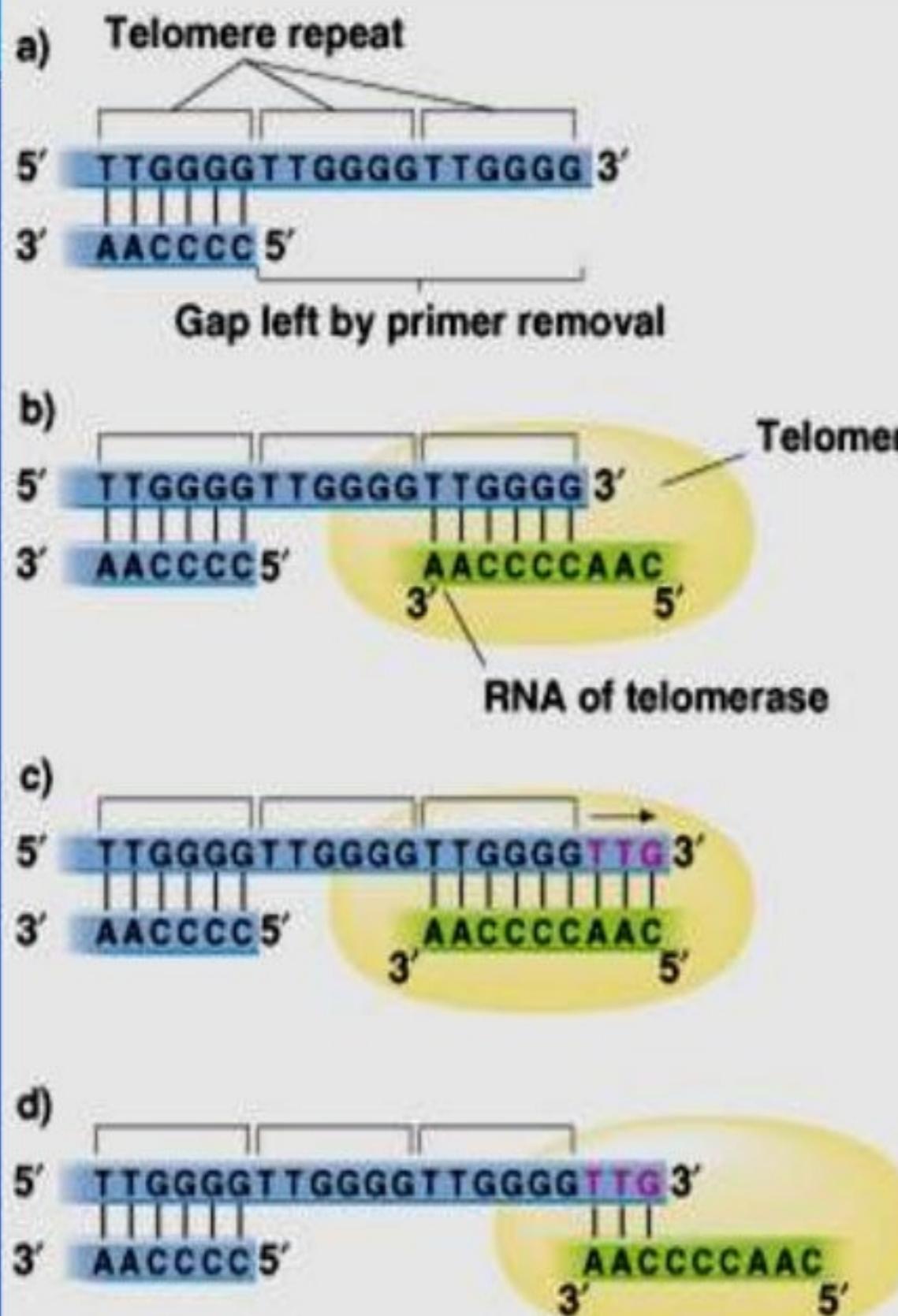


Telomere

- The terminal structure of eukaryotic DNA of chromosomes is called **telomere**.
- Telomere is composed of **terminal DNA sequence and protein**.
- The sequence of typical telomeres is rich in **T** and **G**.
- The telomere structure is crucial to keep the termini of chromosomes in the cell from becoming entangled and sticking to each other.

Telomerase

- The eukaryotic cells use **telomerase** to maintain the integrity of DNA telomere.
- The telomerase is composed of
 - telomerase **RNA**
 - telomerase association **protein**
 - telomerase **reverse transcriptase**
- It is able to **synthesize** DNA using **RNA** as the template.



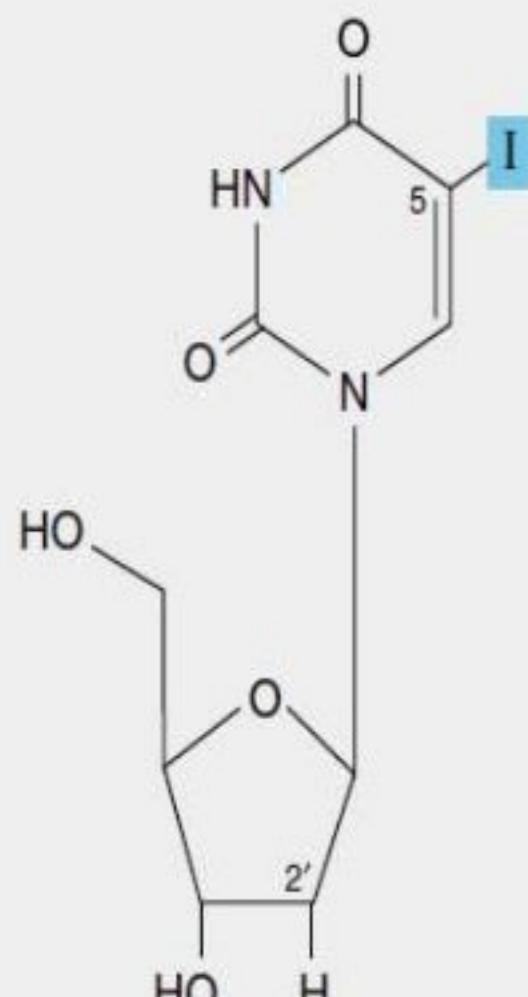
Step in Replication	Prokaryotic cells	Eukaryotic cells
Recognition of origin of replication	Dna A protein	RpA (Replication Protein-A)
Unwinding of DNA double helix	Helicase (requires ATP)	Helicase (requires ATP)
Stabilization of unwound template strands	Single-stranded DNA-binding protein (SSB)	Single-stranded DNA-binding protein (SSB)
Synthesis of RNA primers	Primase	Primase
Synthesis of DNA	DNA polymerase III	DNA polymerase δ
Leading strand		
Lagging strand	DNA polymerase III	DNA polymerase ϵ
Removal of RNA primers	DNA polymerase I ($5 \rightarrow 3'$ exonuclease)	RNase-H
Replacement of RNA with DNA	DNA polymerase I	Unknown
Joining of Okazaki fragments	DNA ligase (requires NAD)	DNA ligase (requires ATP)
Removal of positive supercoils ahead of advancing replication forks	DNA topoisomerase II (DNA gyrase)	DNA topoisomerase II

BASE ANALOGUES

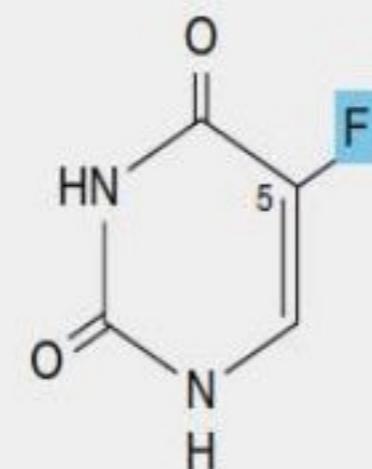
A **base analog** is chemical that can substitute for a normal nitrogen base in Nucleic acids.

They are categorized in two separate groups, **purine analogues** and **pyrimidine analogues**.

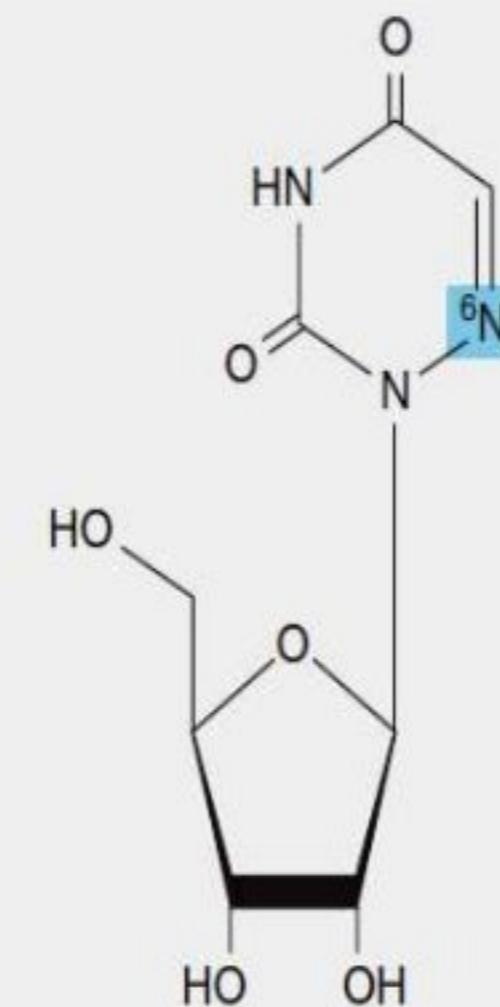
Oncologists employ **5-fluoro- or 5- iodouracil**, **3-deoxyuridine**, **6-thioguanine and 6-mercaptopurine**, **5- or 6-azauridine**, **5- or 6-azacytidine** and **8-azaguanine** which are incorporated into DNA prior to cell division.



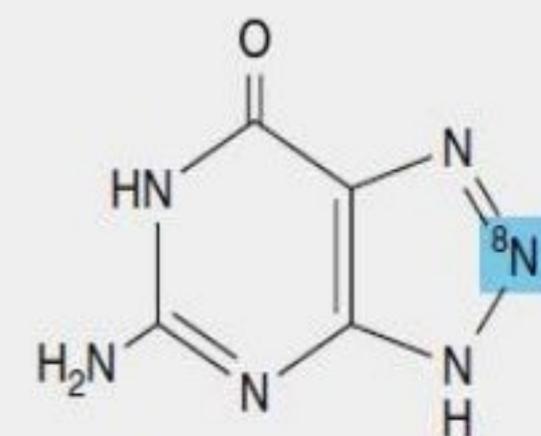
5-Iodo-2'-deoxyuridine



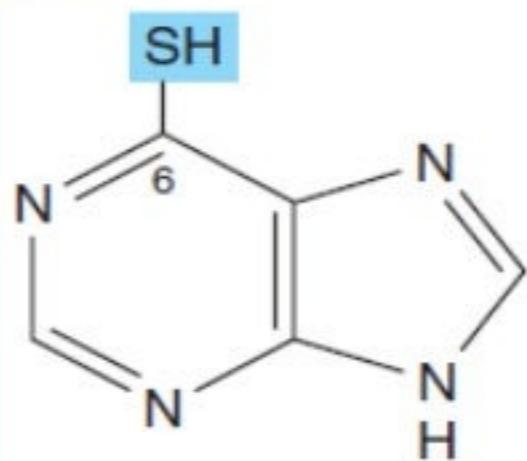
5-Fluorouracil



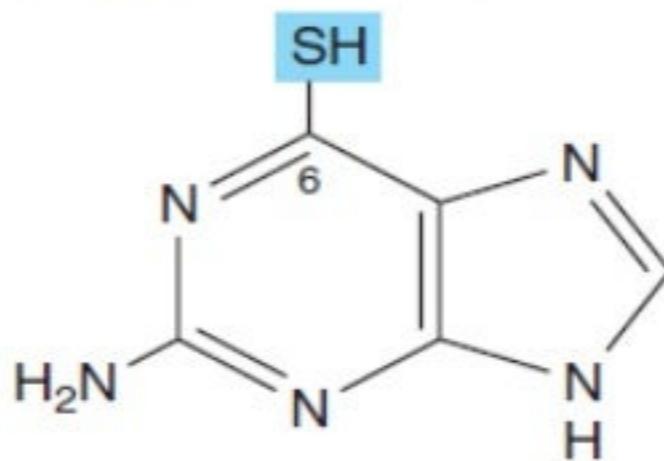
6-Azauridine



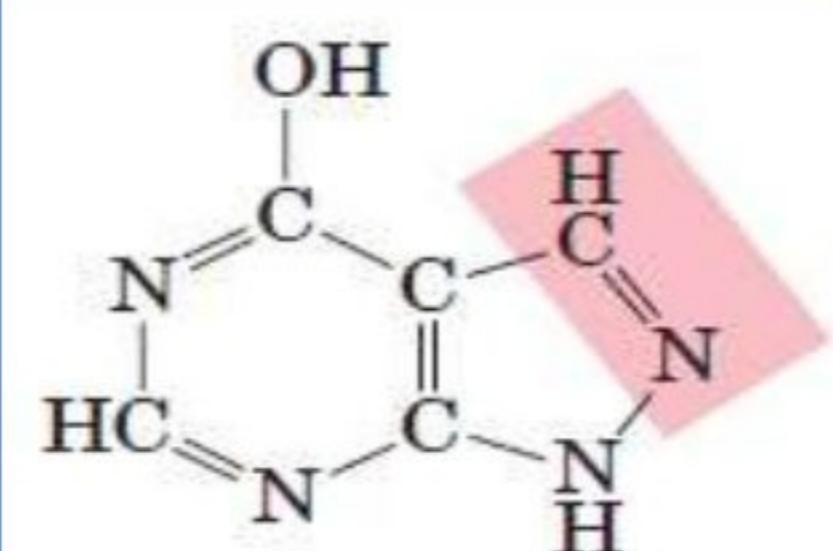
8-Azaguanine



6-Mercaptopurine



6-Thioguanine

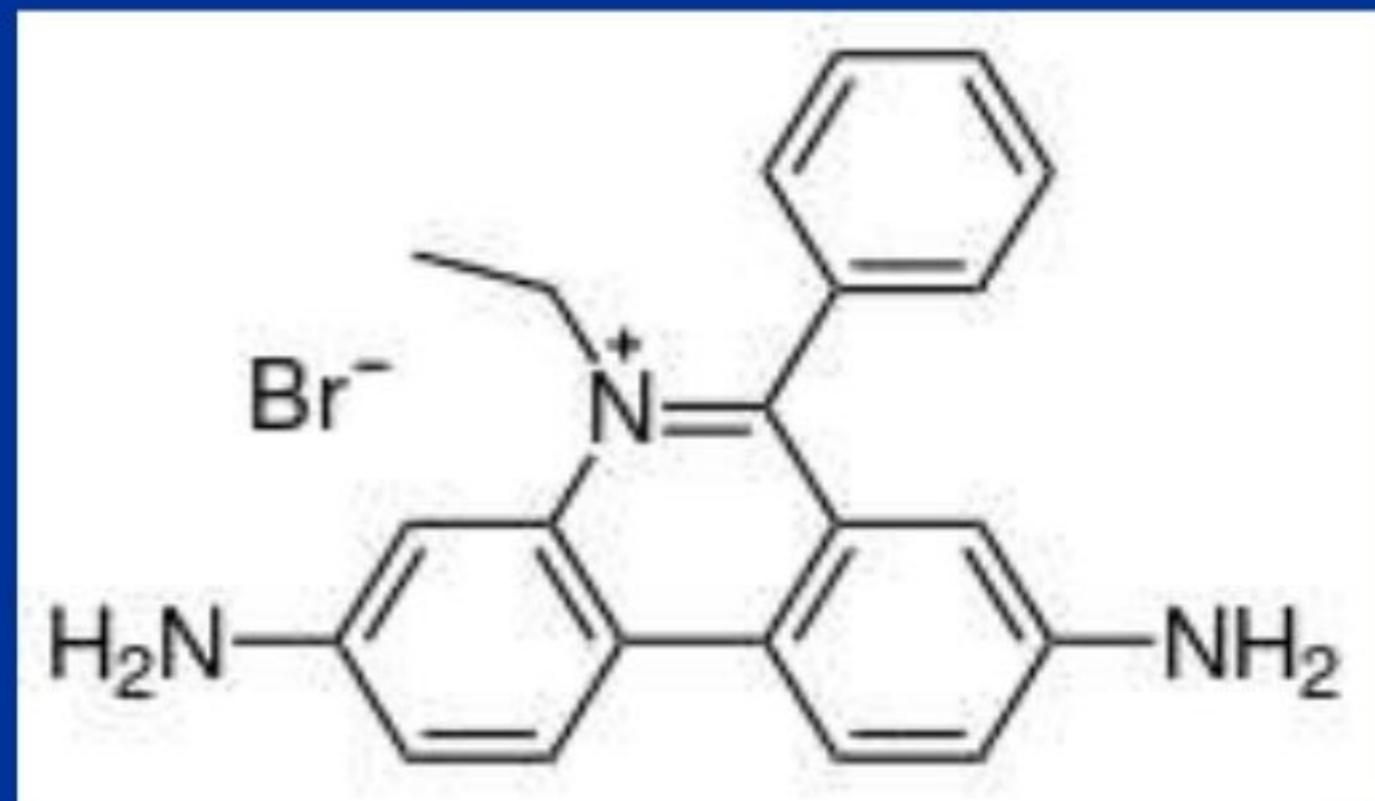


Allopurinol

Intercalating agents

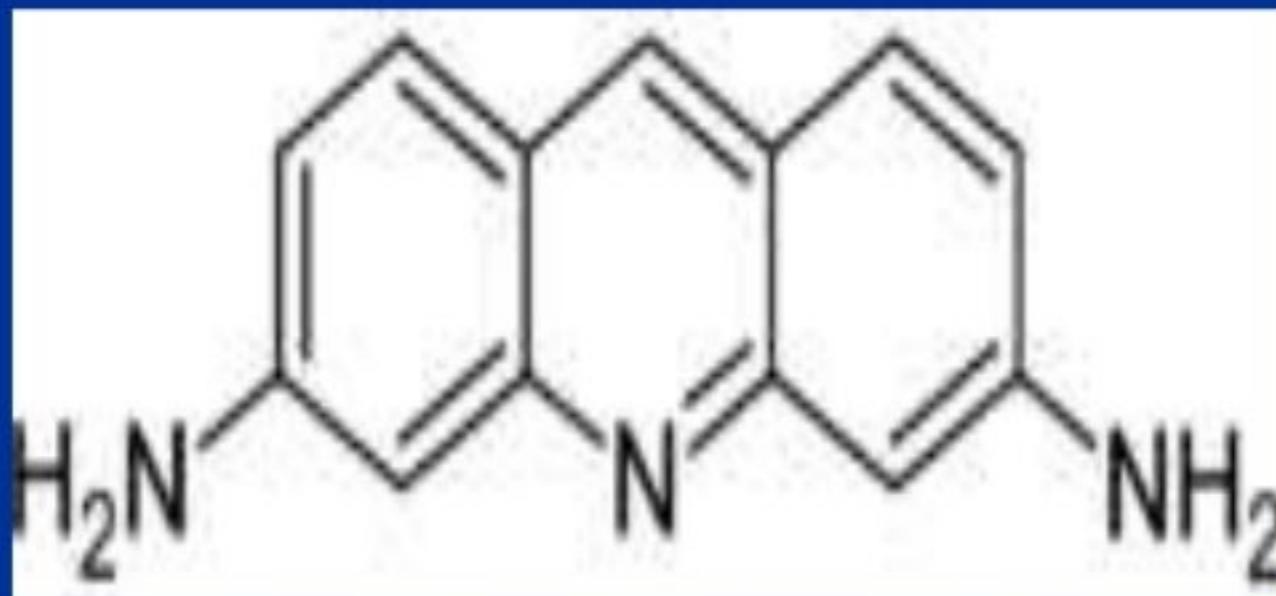
These are the molecules that can insert between bases in DNA base pairs, causing mutation during replication.

Examples: **Ethidiumbromide, Proflavine and Daunorubicin.**



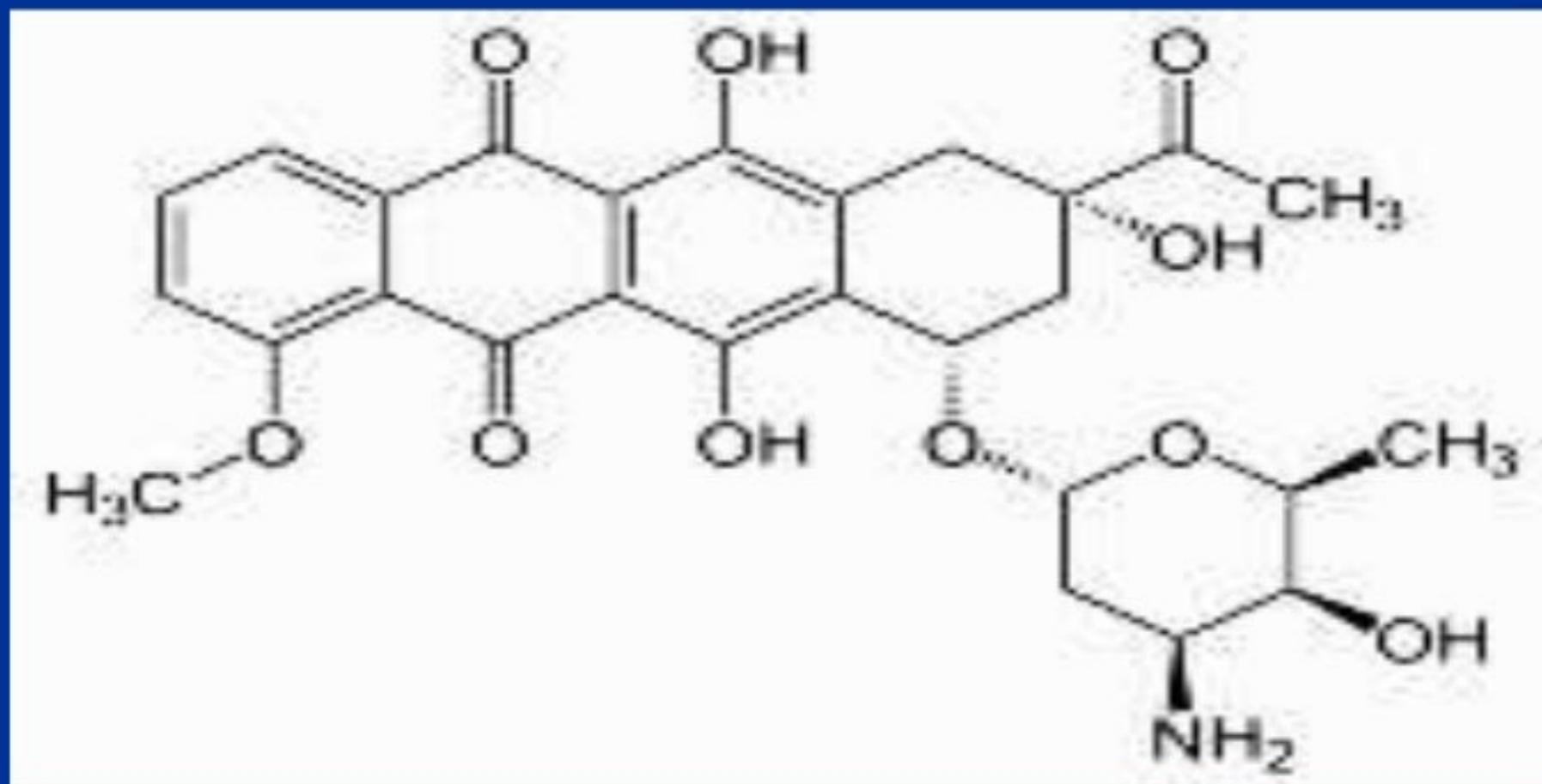
Ethidiumbromide

Proflavine



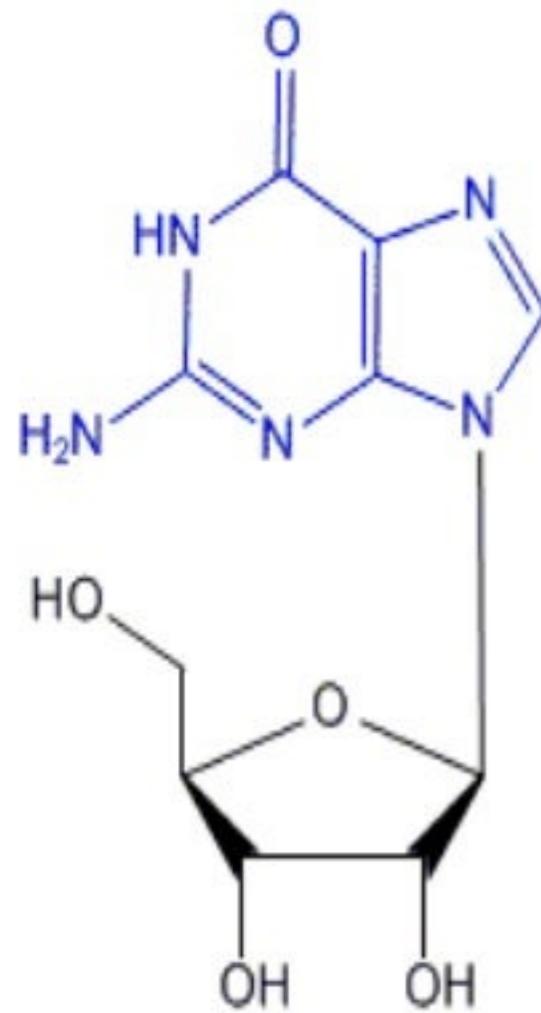
It also called **Proflavin** and **Diaminoacridine** , is an acriflavine derivative, a disinfectant bacteriostatic against many **gram-positivebacteria**.

Daunorubucin is most commonly used to treat specific types of **leukemia** such as **Acute myeloid leukemia** , **Acute lymphocytic leukemia**) and also for the treatment of **Neuroblastoma**.

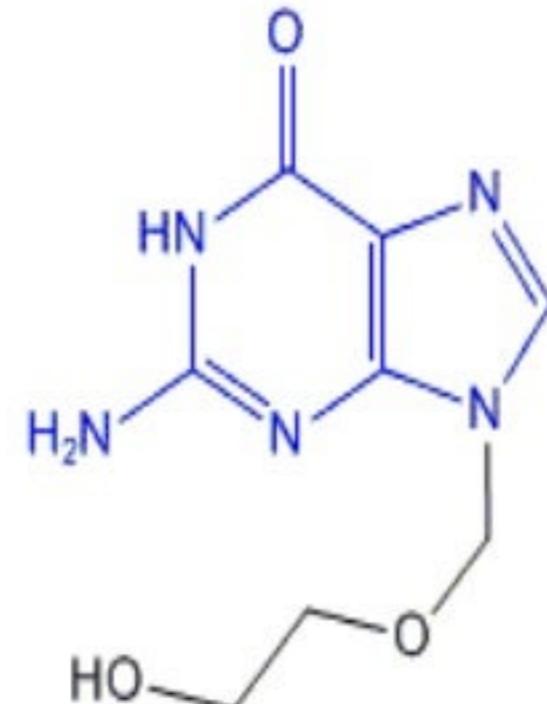


Daunorubicin

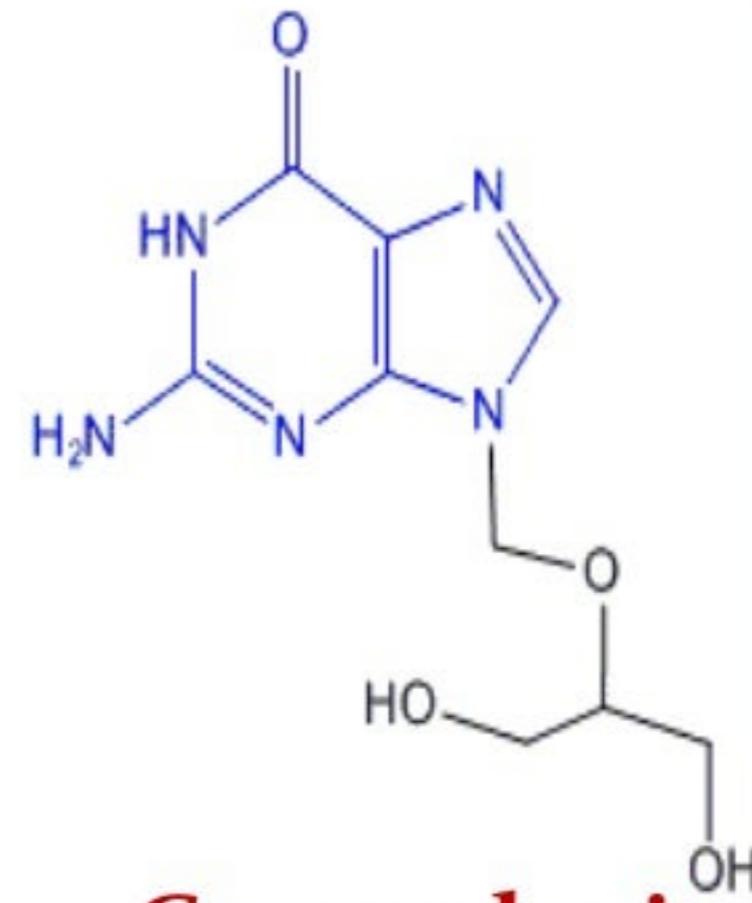
DNA Polymerase Inhibitors



Guanosine



Acyclovir

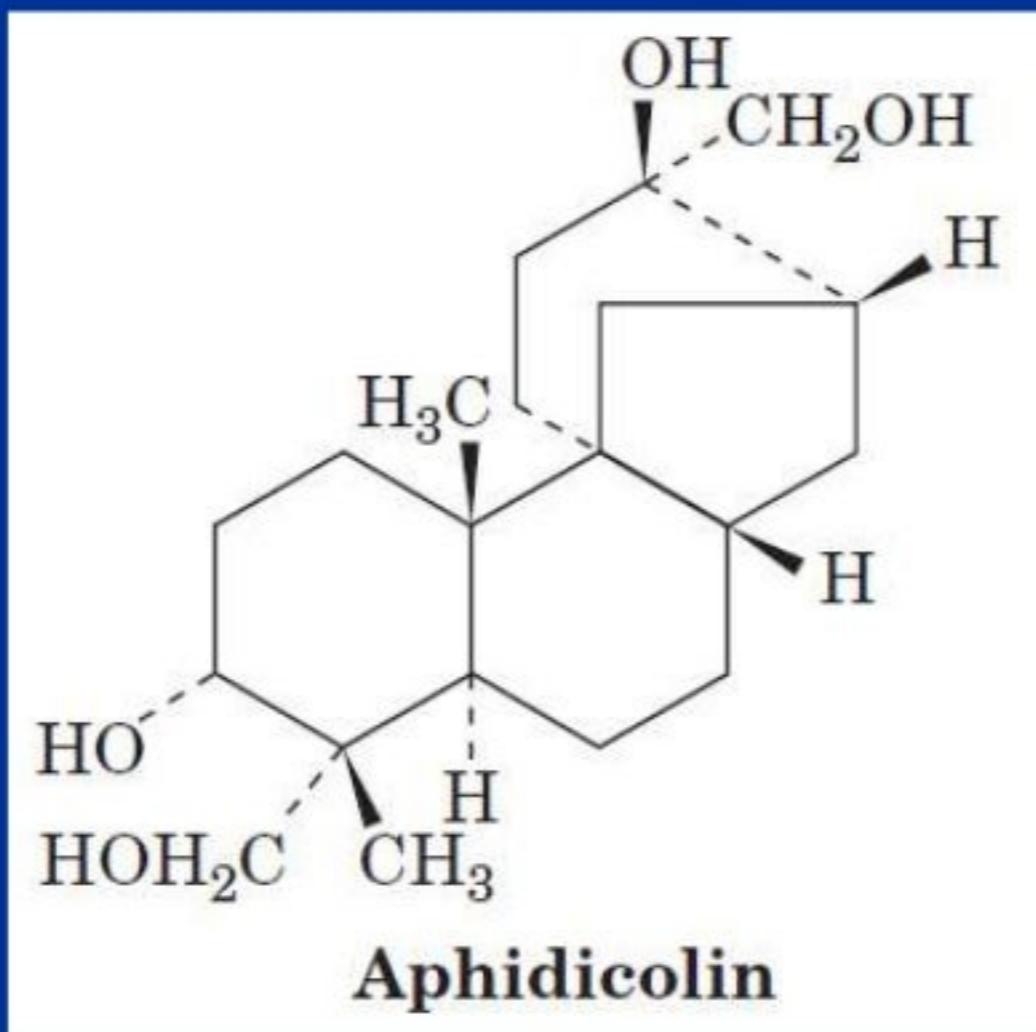


Gancyclovir

Natuarlly occurring
Nitrogen base
essential in DNA
Replication

Inhibitors of Viral DNA Polymerase

Aphidicolin



Inhibits DNA Polymerase- ϵ in Eukaryotes

DNA DAMAGE

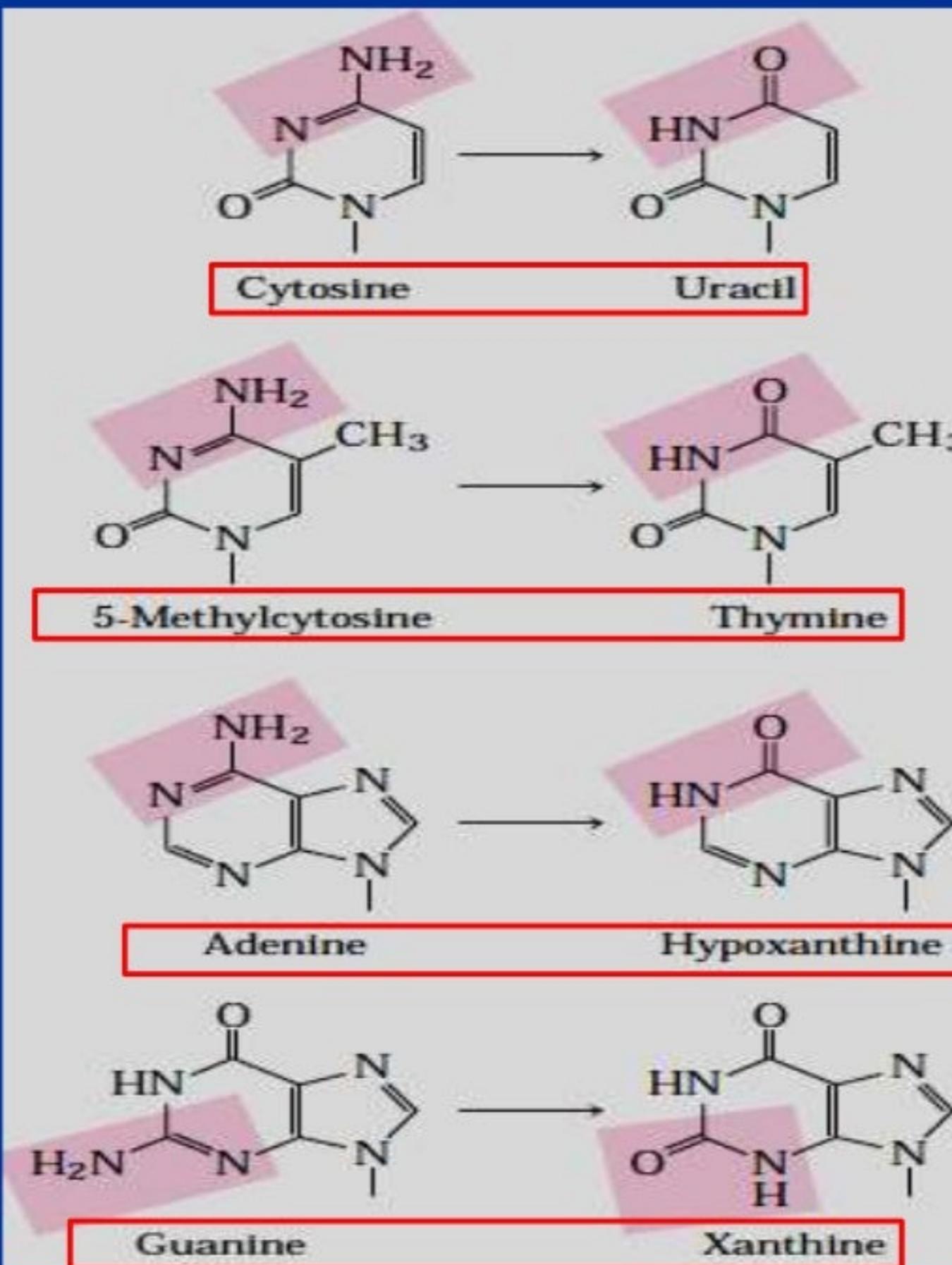
- DNA is easily damaged under normal physiological conditions.
 - The return of damaged DNA to its normal sequence and structure is called **Repair**.
 - Many different kinds of physical & chemical agents damage DNA. Some of these are:-
 - 1) **Endogenous agents**
 - 2) **Exogenous agents**
- Agents that damage DNA can be mutagenic, cytotoxic or both.
- DNA damaging agents that cause mutations are called **Mutagens**.

Types of DNA Damage

The damages done to DNA by physical, chemical and environmental agents can be broadly classified into four categories with different types.

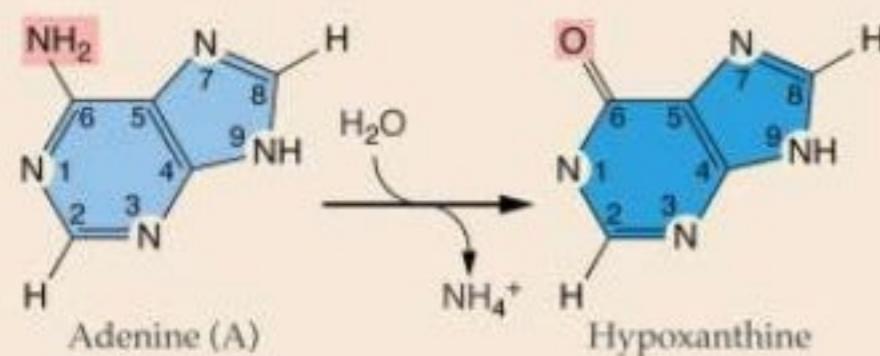
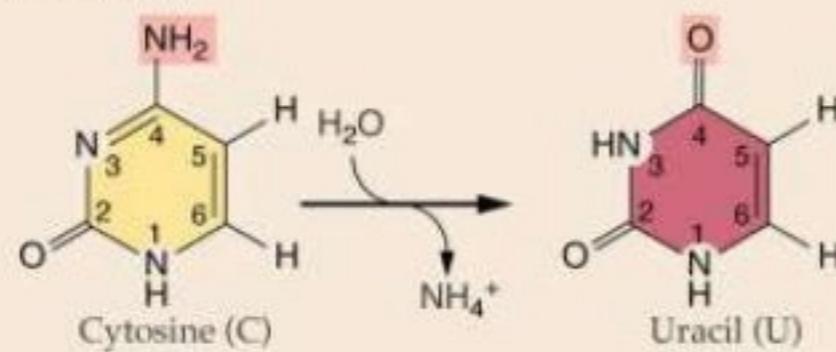
Category	Types
Single-base alteration	Deamination (C→U; A→hypoxanthine) Depurination Base alkylation Insertion or deletion of nucleotides Incorporation of base analogue
Two-base alteration	UV light induced pyrimidine dimer alteration (T-T)
Chain breaks	Oxidative free radical formation Ionizing radiation
Cross-linkage	Between bases in the same or opposite strands Between the DNA and protein molecules

Single Base Alterations



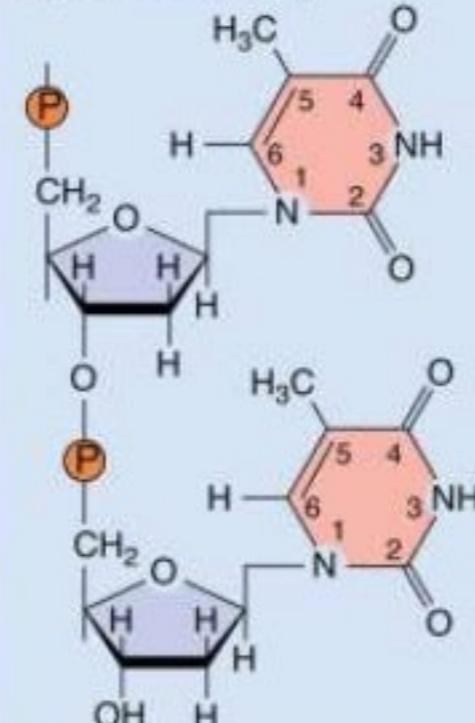
Spontaneous

Deamination

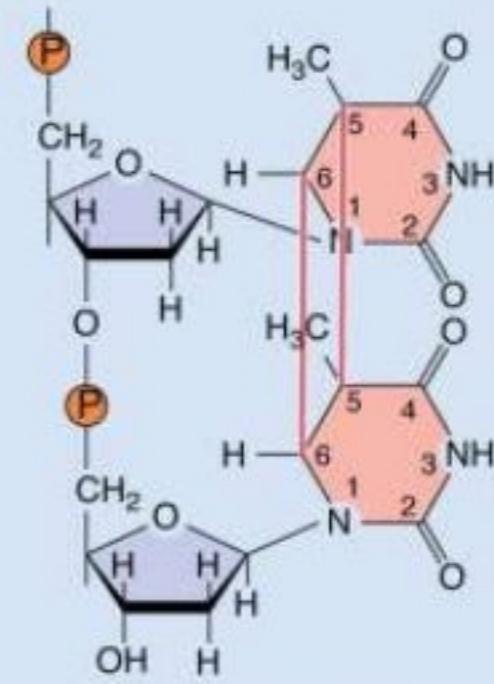


Induced

Exposure to UV light

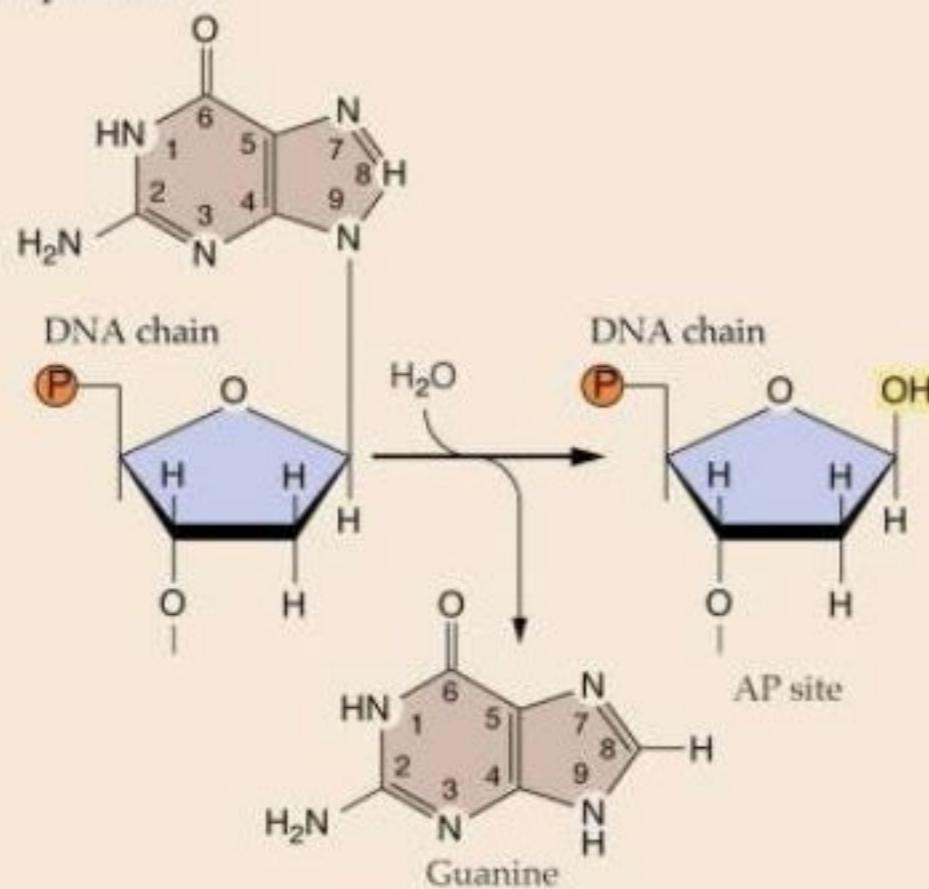


Adjacent thymines in DNA

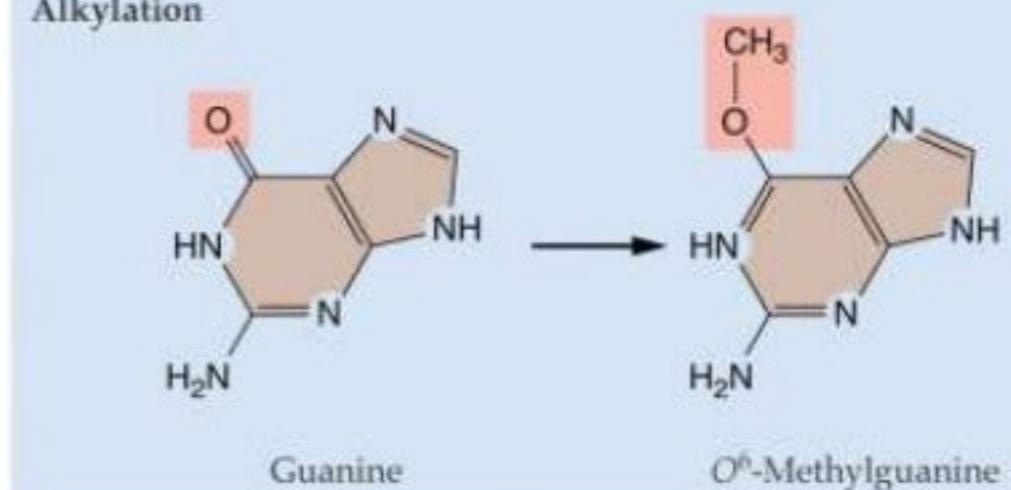


Thymine dimer

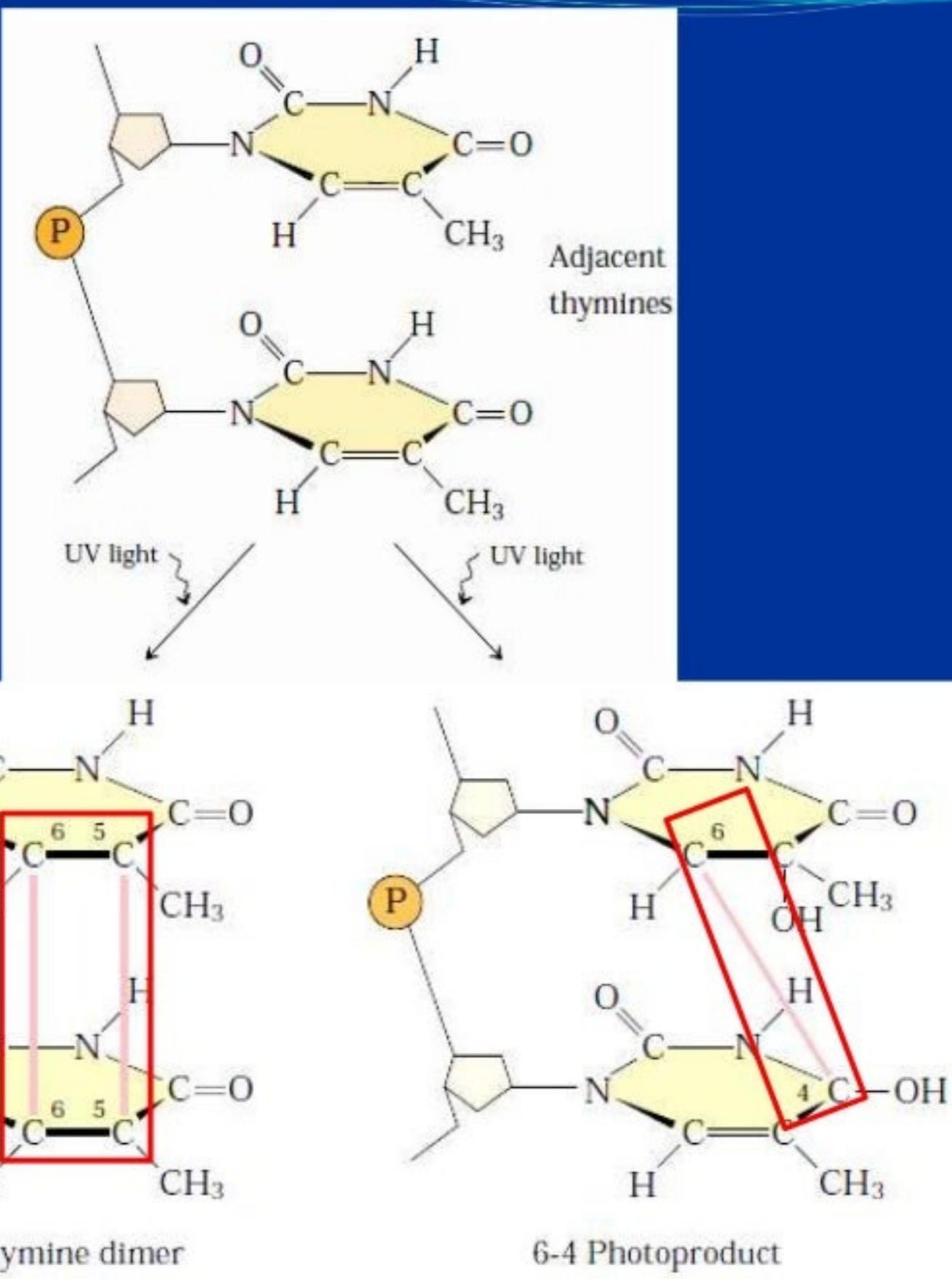
Depurination



Alkylation



Double Base Alterations



DNA damaging Agents

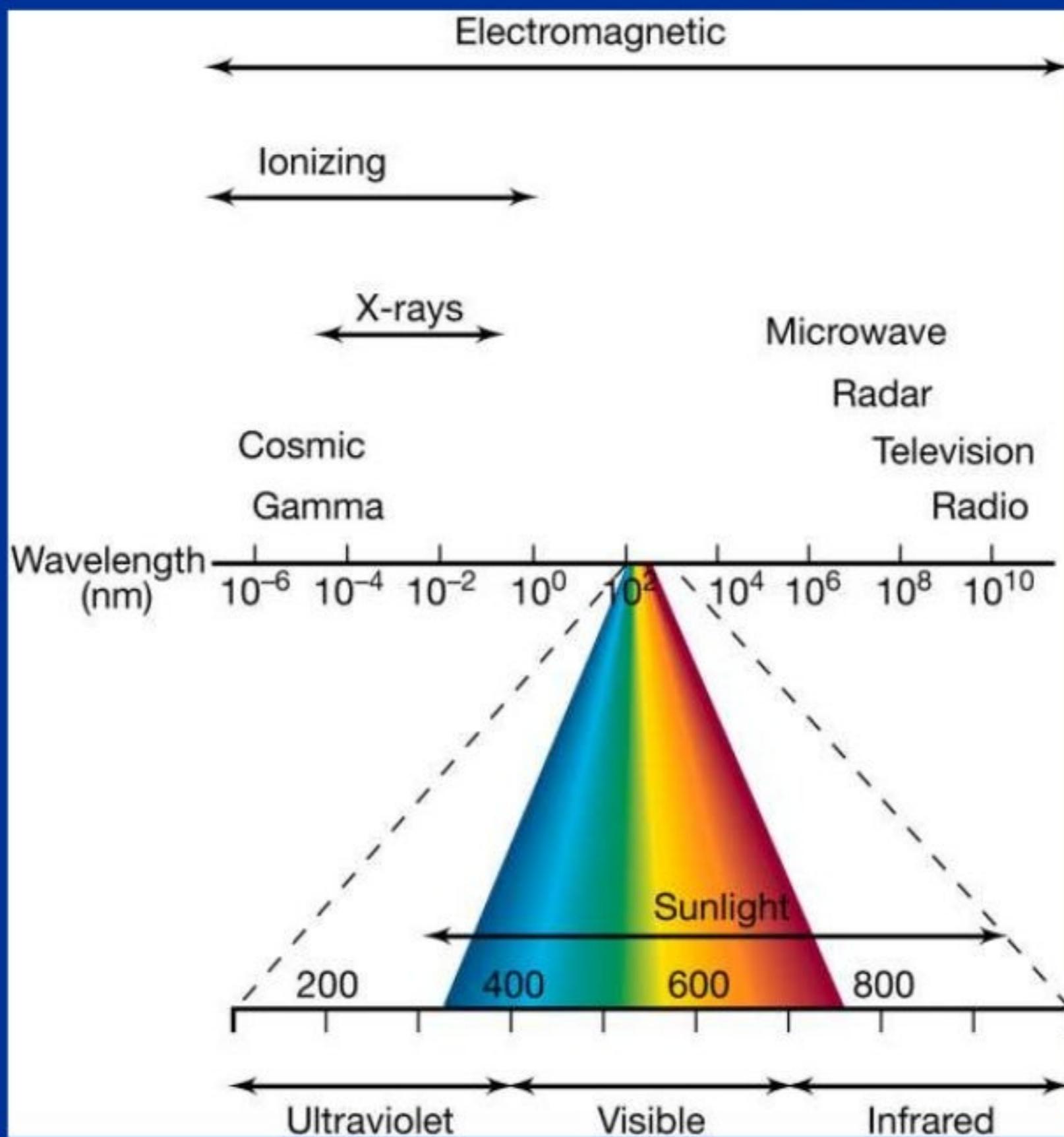
Spontaneous Agents

Highly reactive oxygen radicals produced as a by products during normal cellular respiration as well as by other biochemical pathways.

Reactive Oxygen Species (ROS) :

- Hydrogen peroxide (H_2O_2)
 - Hydroxyl radicals ($OH\cdot^-$) – Most potent
 - Superoxide ($O_2\cdot^-$)
-
- ✓ ROS causes DNA damage such as **Oxidation of Nitrogen Bases, deoxy Ribose and Strand breaks.**

Radiation can cause mutations



Radiation

- The high energy electromagnetic radiation to the exposure of which cell experience considerable damage to their DNA are:
 1. **Ultraviolet light:**
- The major type of damage caused by UV light is divided into three bands:
 - I. **UV-A (321-400 nm)**
 - II. **UV-B (296-320 nm)**
 - III. **UV-C (100-295 nm)**

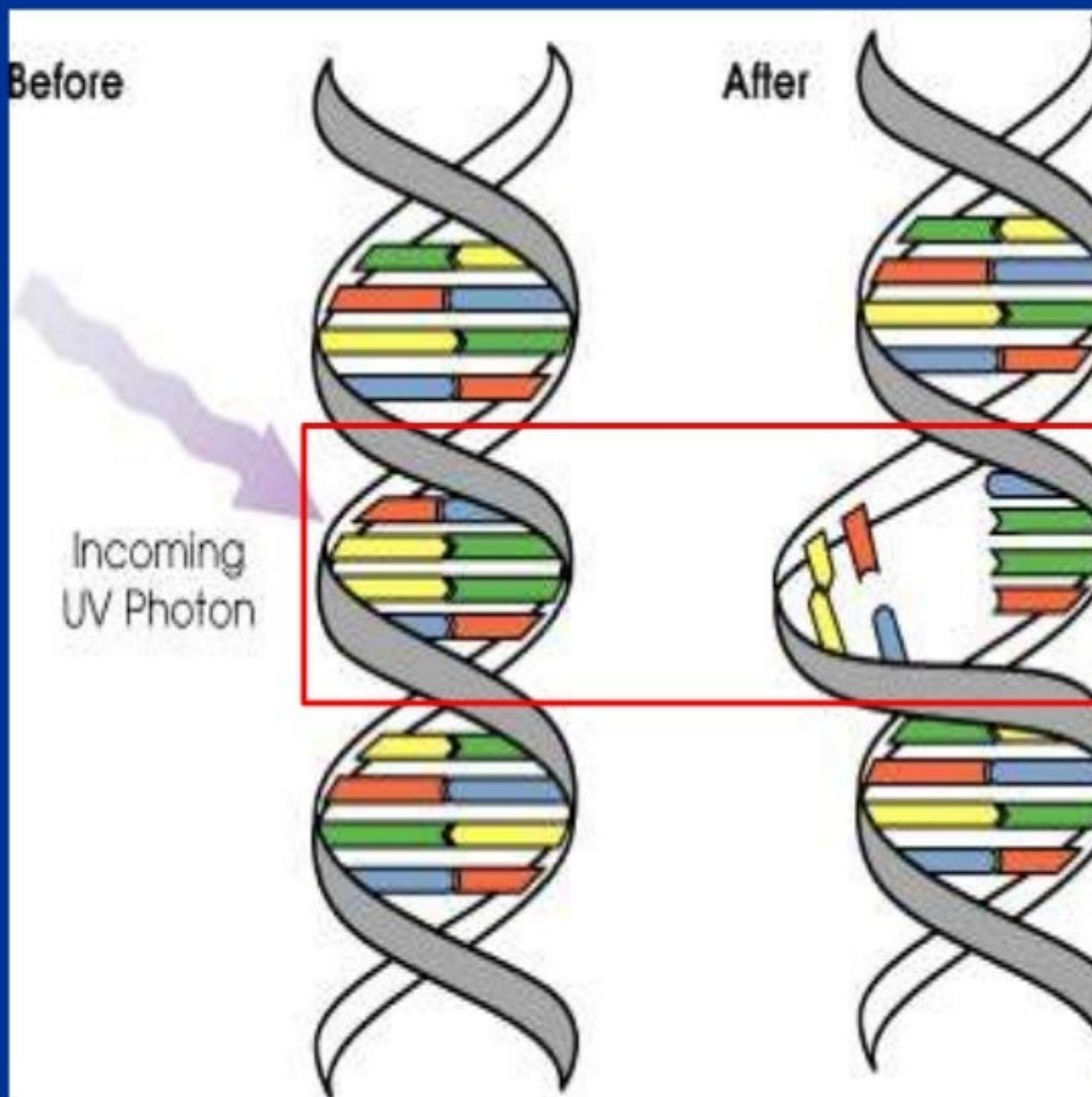
2. X- Rays

3. Gamma Rays

- Through these direct damage takes place when DNA or water tightly bound to it absorbs the radiation.

Indirect damage takes place when water or other molecules surrounding the DNA absorbs the radiation & form reactive species that then damage DNA.

Effect of UV on DNA structure



Chemicals Agents

1) Deaminating Agents:

Sodium Nitrite (NaNO₂)

Sodium Nitrate (NaNO₃)

Nitrosamine

Nitrous Acid (HNO₂)

2) Alkylating Agents:

Dimethyl sulfate (DMS)

Dimethyl nitrosamine

Nitrogen mustard

Mutations

- Mutation refers to a change in the DNA structure of a gene. The substances (chemicals) which can induce mutations are collectively known as mutagens.
- The changes that occur in DNA on mutation are reflected in **Replication, Transcription and Translation** .
- Mutations occur in 2 ways:
 - 1) **Spontaneous mutations:** Mistakes in DNA replication.
 - 2) **Induced mutation:** Caused by Mutagens.

1) Point mutations : A point mutation or single base substitution, is a type of mutation that causes the replacement of single base nucleotides with another nucleotides of DNA .

Substitutions

(a) Transitions : In this case, a purine (or) a pyrimidine) is replaced by another.

(b) Transversions : These are characterized by replacement of a purine by a pyrimidine or vice versa.

Substitution

DNA: TAC G**C**A TGG AAT

mRNA: AUG C**GU** ACC UUA

Amino acids:

Met — **Arg** — Thr — Leu



Substitution

DNA: TAC G**T**A TGG AAT

mRNA: AUG C**AU** ACC UUA

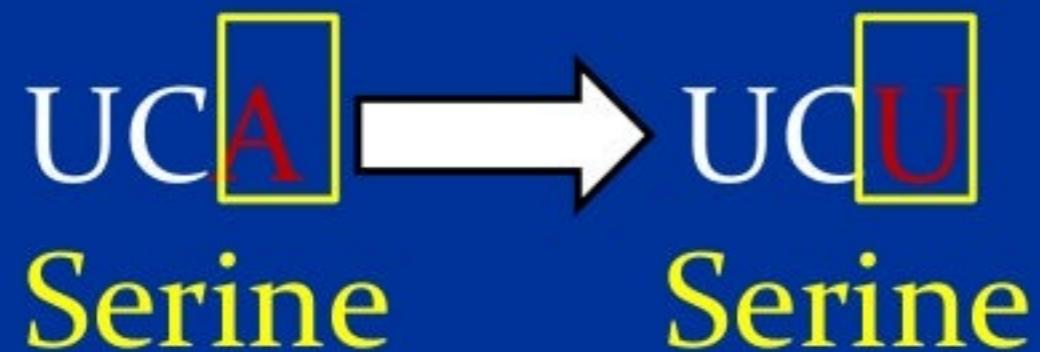
Amino acids:

Met — **His** — Thr — Leu

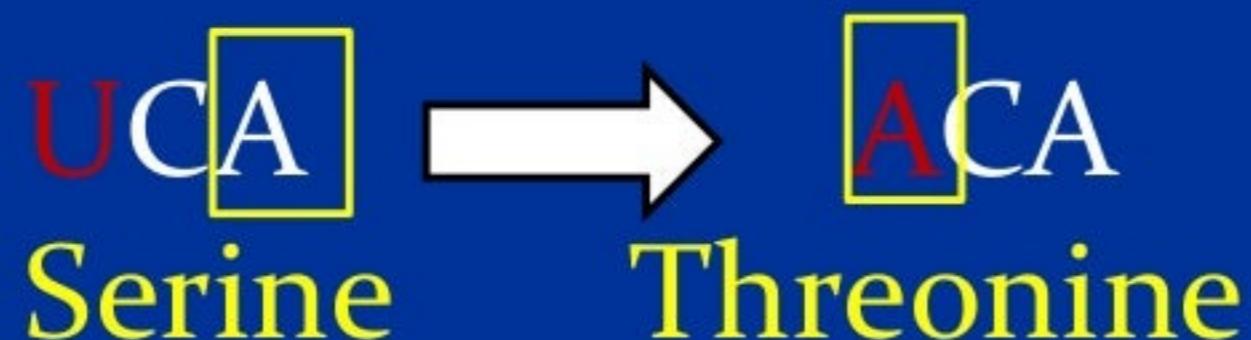
U		C		A		G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys			
C	UUC Phe	UCC Ser	UAC Tyr	UGC Cys			
A	UUA Leu	UCA Ser	UAA Stop	UGA Stop			
A	UUG Leu	UCG Ser	UAG Stop	UGG Trp			
C	CUU Leu	CCU Pro	CAU His	CGU Arg			
C	CUC Leu	CCC Pro	CAC His	CGC Arg			
	CUA Leu	CCA Pro	CAA Gln	CGA Arg			
A	CUG Leu	CCG Pro	CAG Gln	CGG Arg			
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser			
A	AUC Ile	ACC Thr	AAC Asn	AGC Ser			
	AUA Ile	ACA Thr	AAA Lys	AGA Arg			
A	AUG Met	ACG Thr	AAG Lys	AGG Arg			
G	GUU Val	GCU Ala	GAU Asp	GGU Gly			
G	GUC Val	GCC Ala	GAC Asp	GGC Gly			
	GUA Val	GCA Ala	GAA Glu	GGA Gly			
G	GUG Val	GCG Ala	GAG Glu	GGG Gly			

Point Mutations

Silent Mutation :



Missense Mutation :



Nonsense Mutation :

UGG



UGA

Tryptophan

Stop Codon

UAU



UAU

Tyrosine

Stop Codon

UAC



UAG

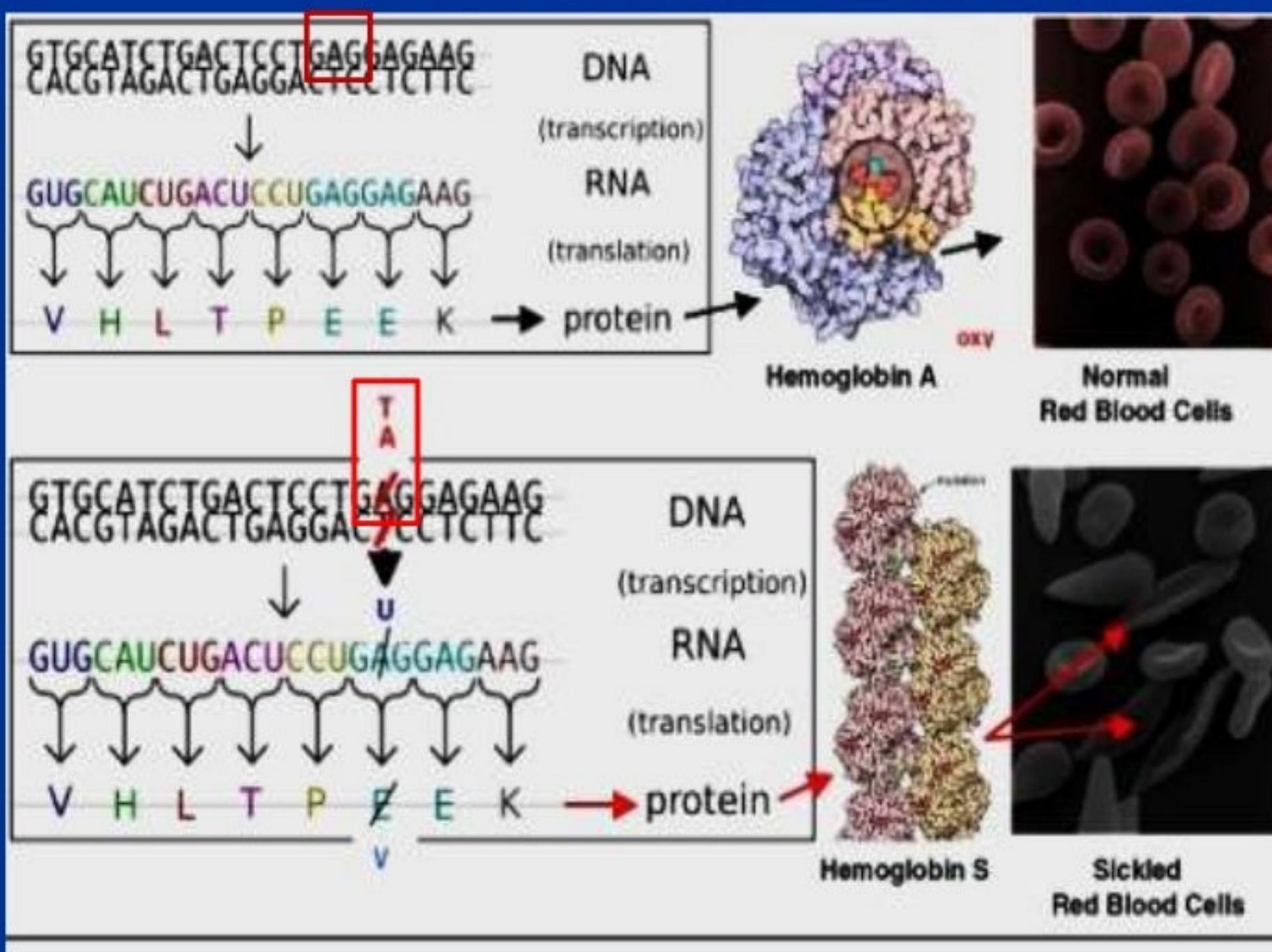
Tyrosine

Stop Codon

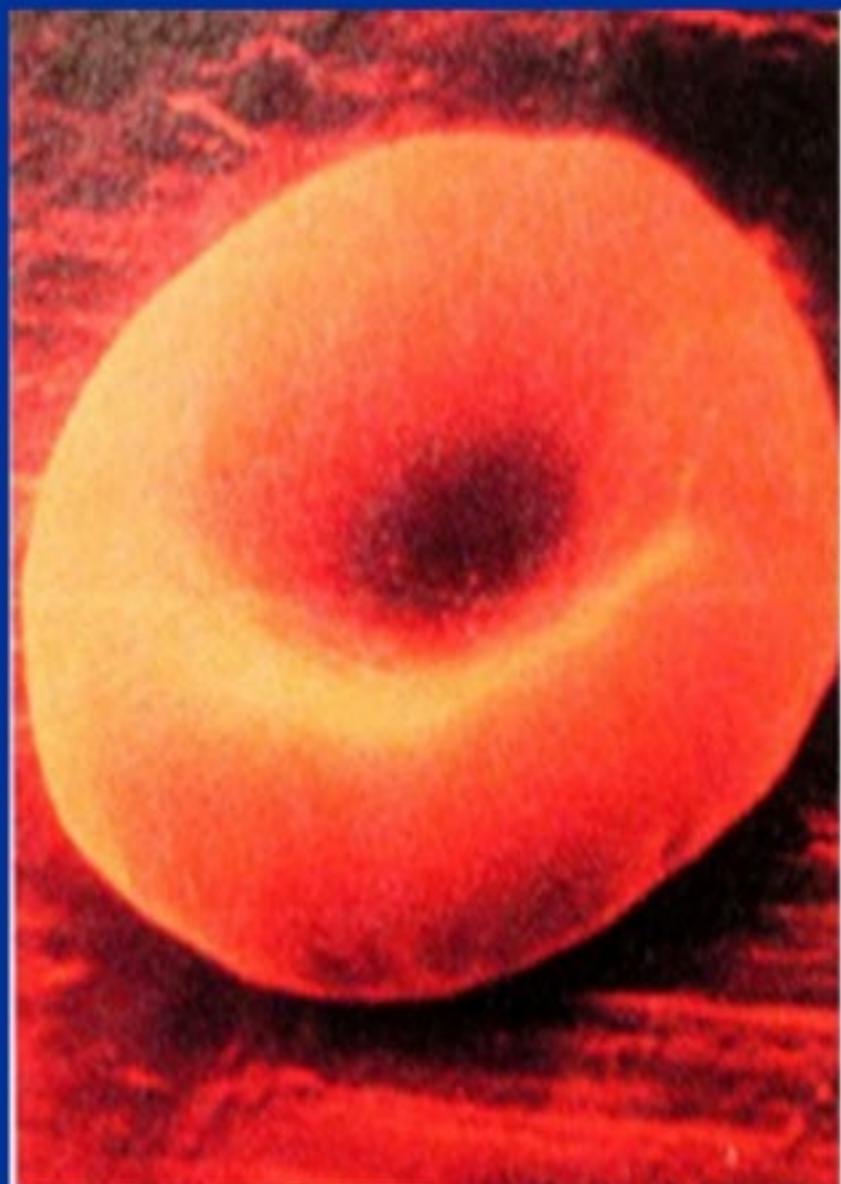
Missense mutation

A point mutation can also change a codon so that a **different protein** is specified, a non synonymous change.

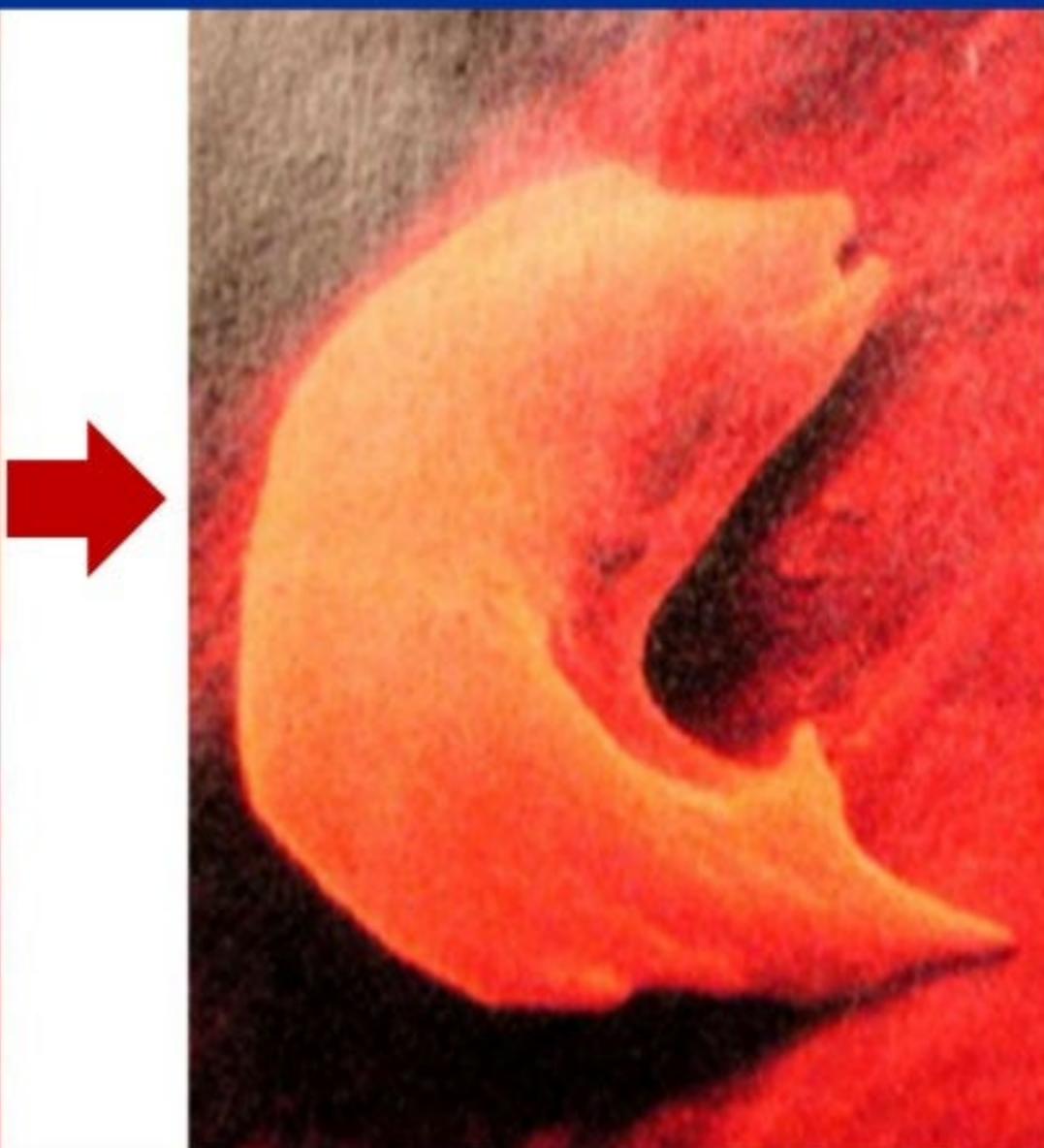
Sickle Cell Anemia



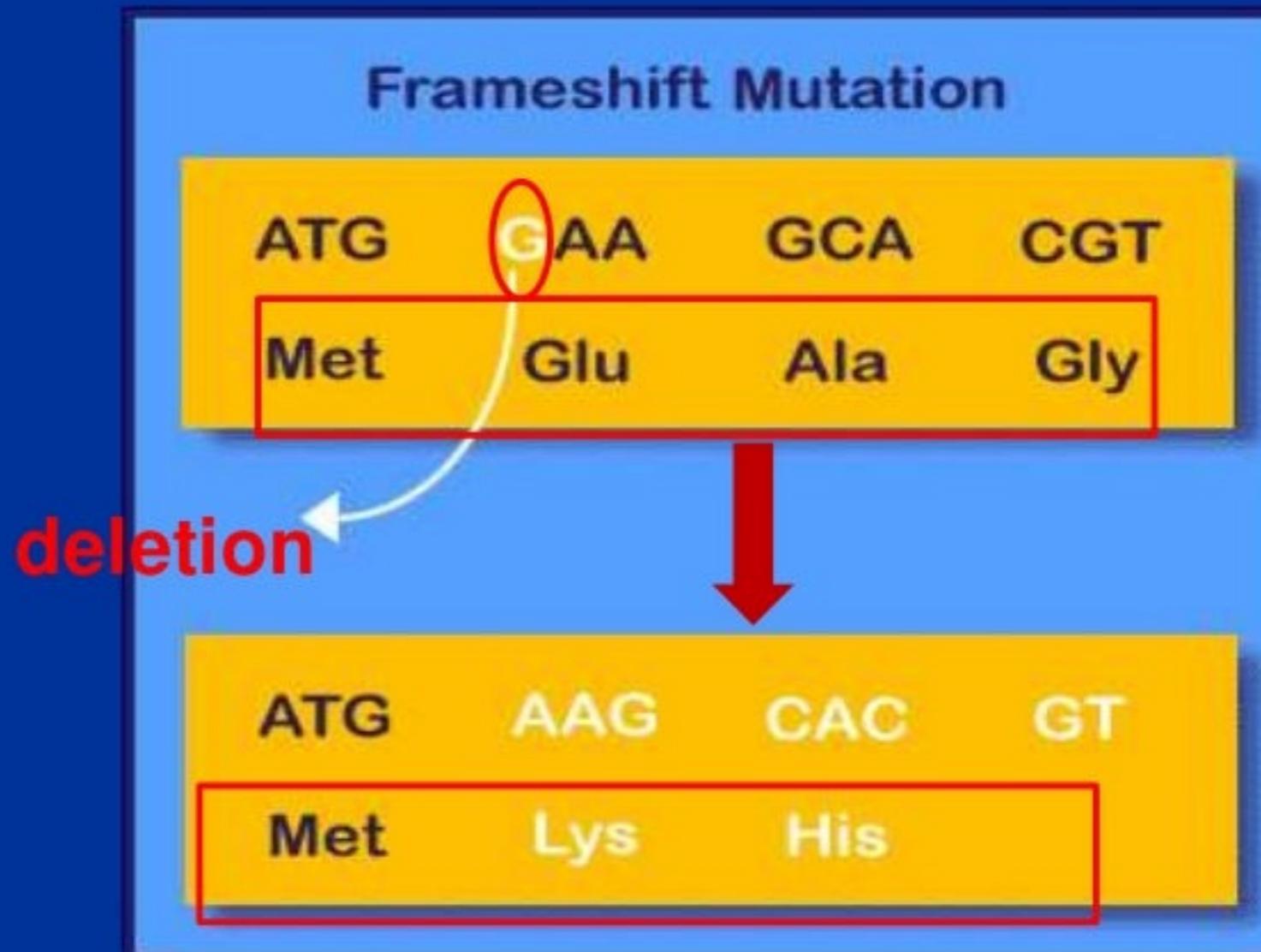
Normal red blood cell



Sickle cell RBC



2). Frameshift mutations : These occur when one or more base pairs are inserted in or deleted from the DNA, respectively, causing insertion (or) deletion mutations.



DNA REPAIR MECHANISMS

Base Excision Repair

- For correction of specific Chemical damage in DNA
 - Uracil
 - Hypoxanthine
 - 3-methyl Adenine
 - Formamido pyrimidine
 - 5,6 - Hydrated Thymine

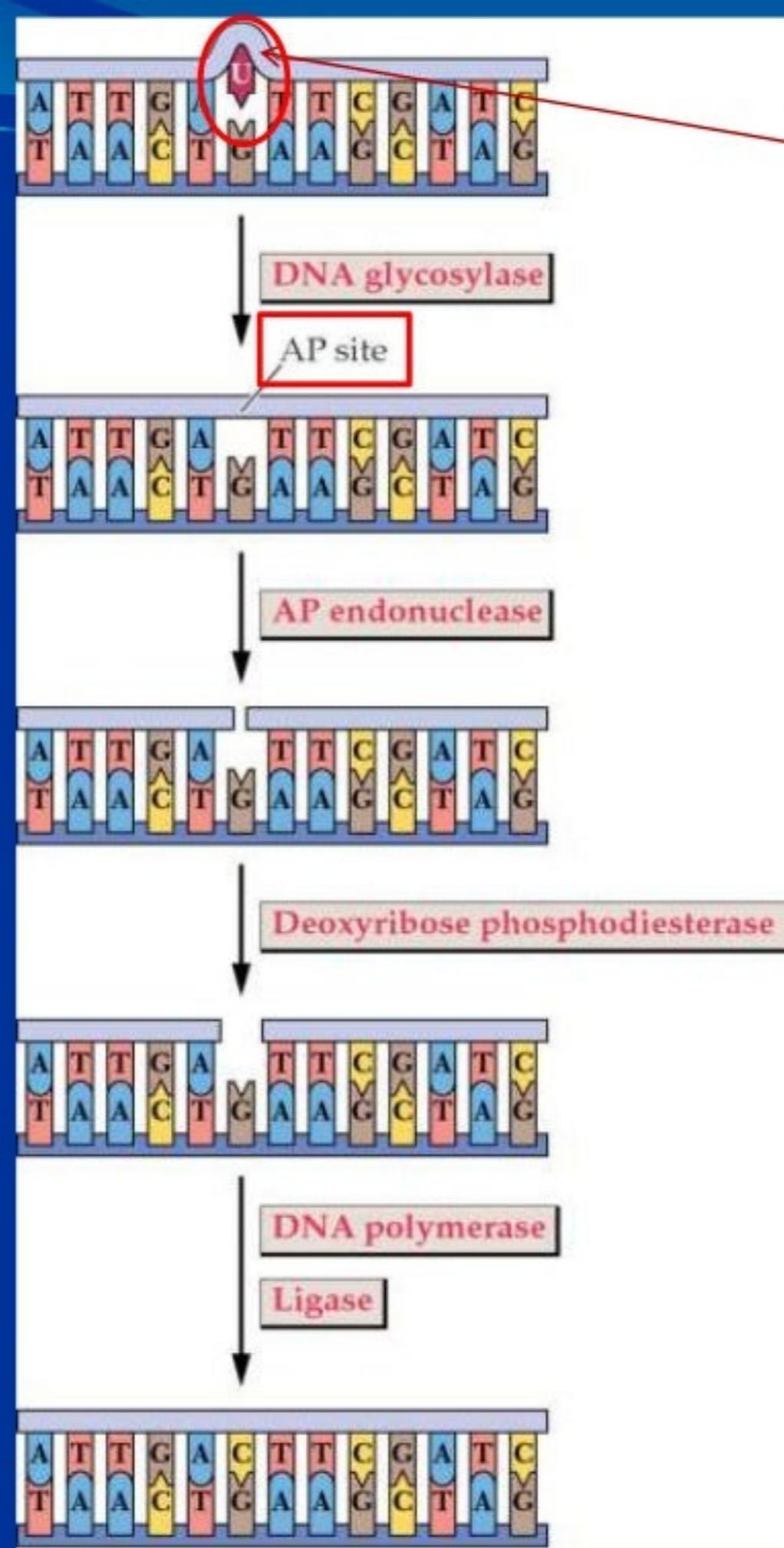
Enzymes/proteins	Type of damage
Base-excision repair	
DNA glycosylases	Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; in some other organisms, pyrimidine dimers
AP endonucleases	
DNA polymerase I	
DNA ligase	

Base Excision Repair (BER)

Variety of **DNA glycosylases**, for different types of damaged bases.

AP endonuclease recognizes sites with a missing base; cleaves sugar-phosphate backbone.

Deoxyribose phosphodiesterase removes the sugar-phosphate lacking the base.



Deaminated Cytosine

Nucleotide Excision Repair (NER)

- Used by the cells to repair bulky DNA damages
- Non specific DNA damage
 - Chemical adducts ...
 - UV photoproducts

Enzymes/proteins

Type of damage

Nucleotide-excision repair

ABC excinuclease

DNA lesions that cause
large structural changes

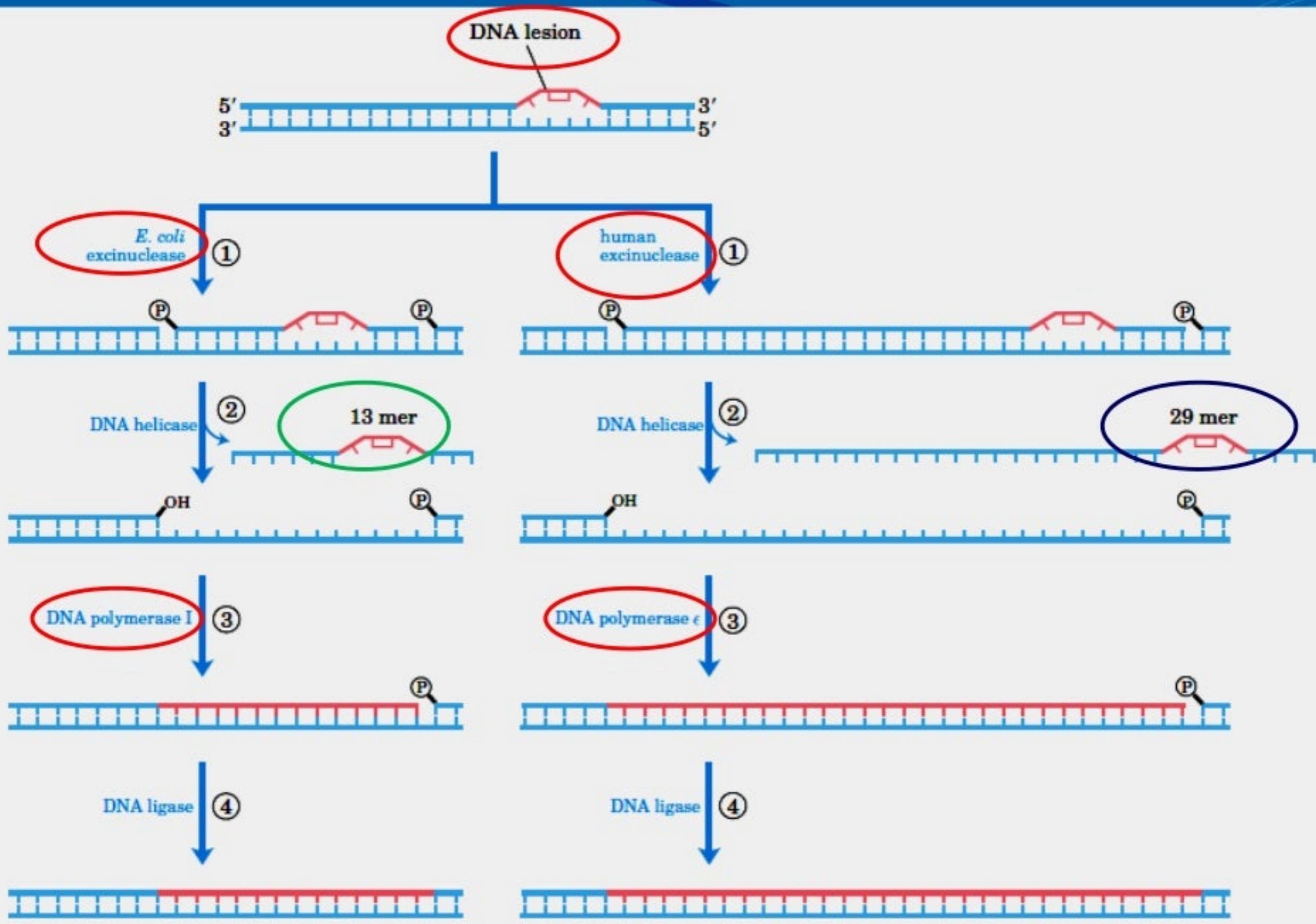
(e.g., pyrimidine dimers)

DNA polymerase I

DNA ligase

Xeroderma pigmentosum (XP) is a rare autosomal recessive disease. The affected Patients are photosensitive and susceptible to Skin cancers.

It is due to a defect in the **Nucleotide Excision Repair** of the damaged D NA.



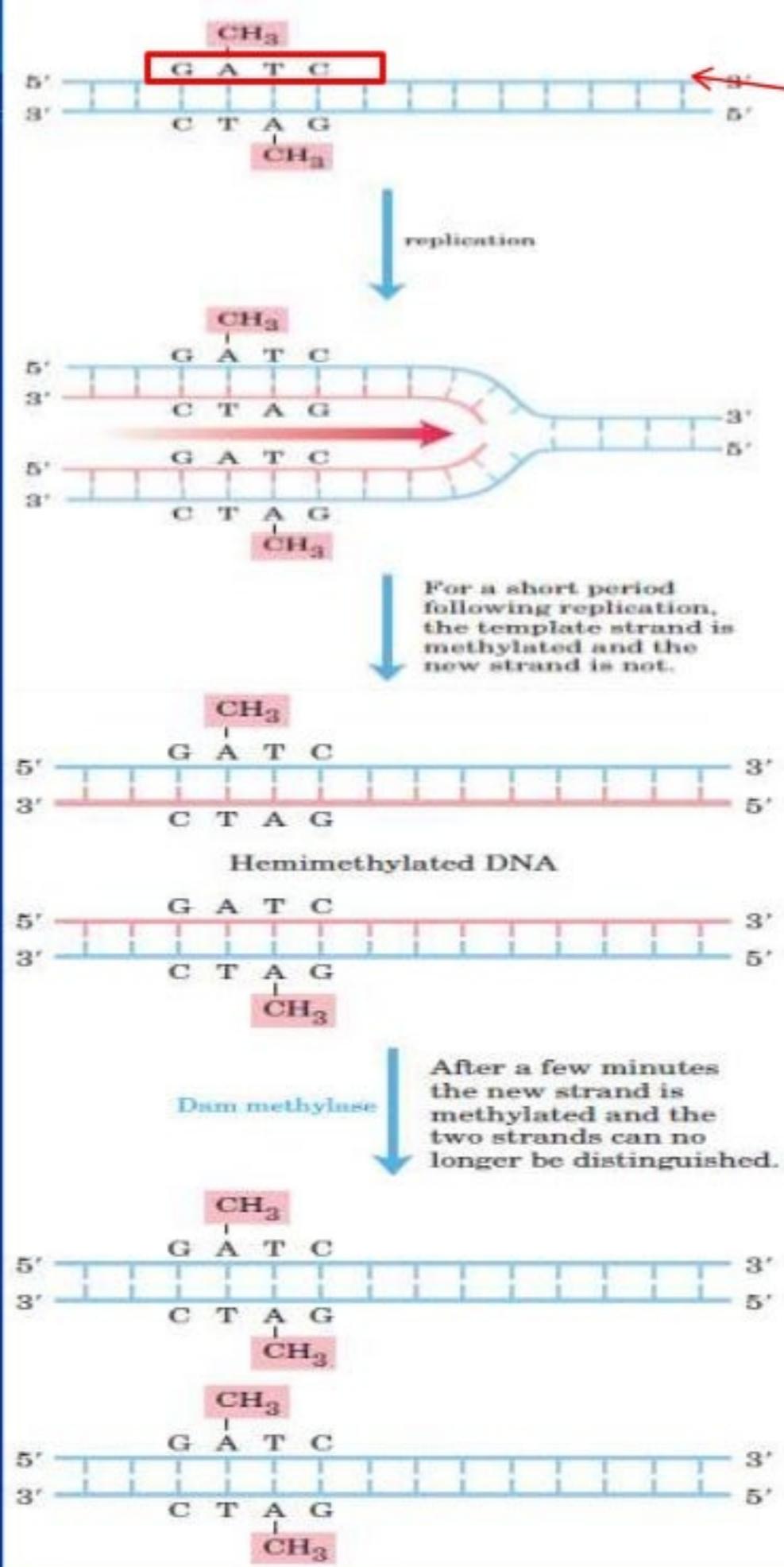
Mismatch repair

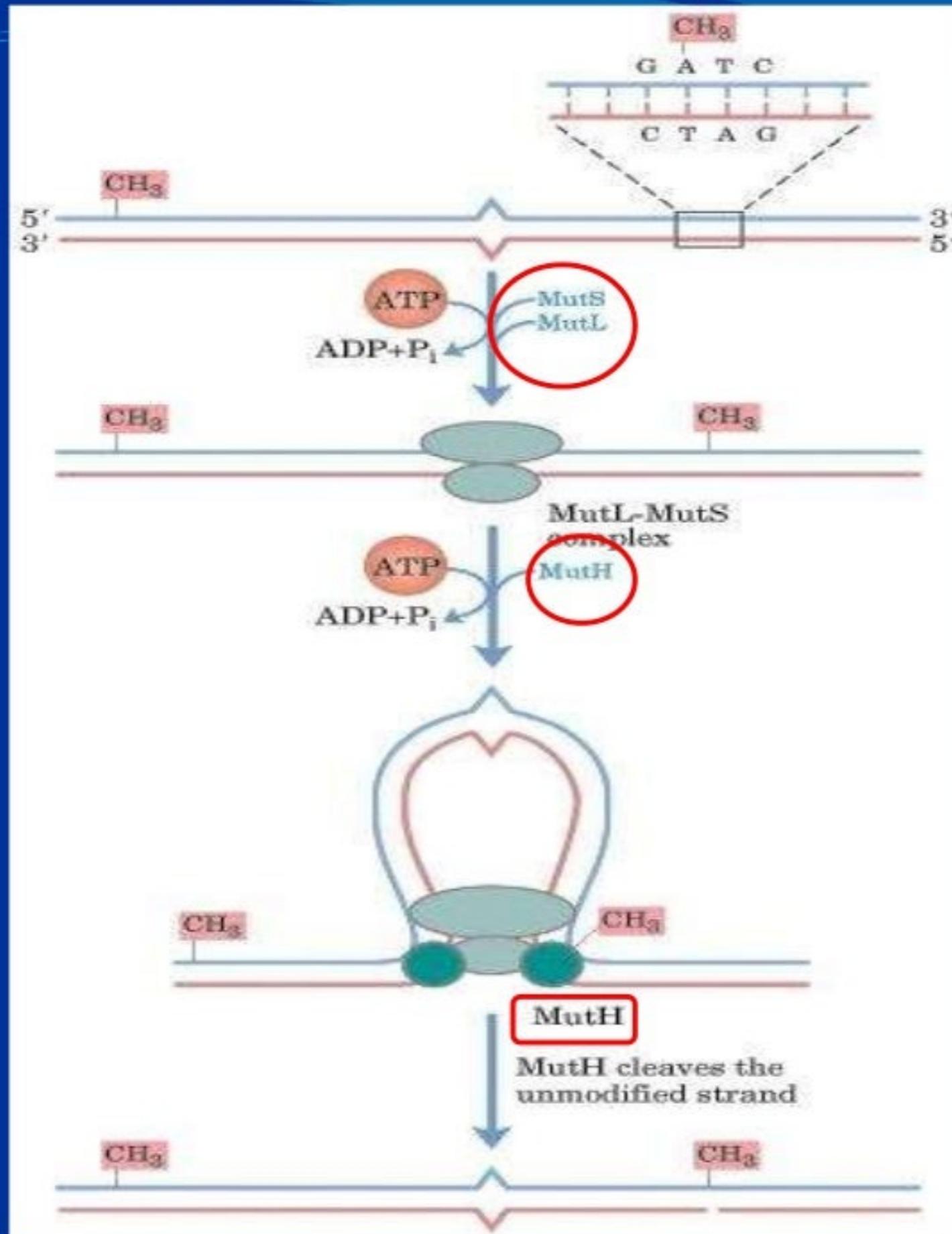
<i>Enzymes/proteins</i>	<i>Type of damage</i>
Mismatch repair	
Dam methylase	Mismatches
MutH, MutL, MutS proteins	
DNA helicase II	
SSB	
DNA polymerase III	
Exonuclease I	
Exonuclease VII	
RecJ nuclease	
Exonuclease X	
DNA ligase	

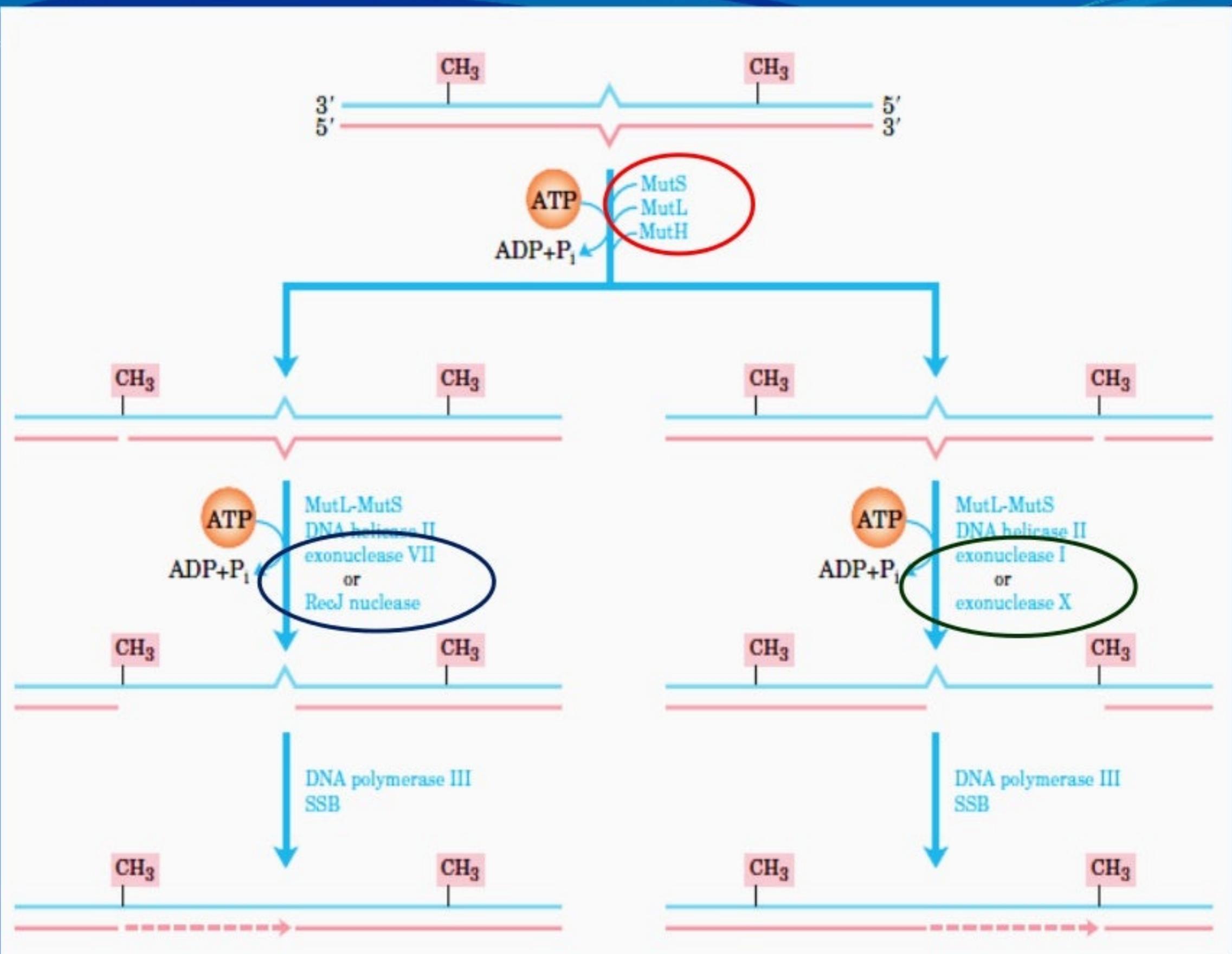
Mismatch repair system is an excision/resynthesis system that can be divided into 4 phases:

- (i) recognition of a mismatch by MutS proteins**
- (ii) recruitment of Repair enzymes**
- (iii) excision of the incorrect sequence**
- (iv) resynthesis by DNA polymerase using the parental strand as a template.**

Parental Strand







DNA REPAIR DISORDERS

Xeroderma Pigmentosum

- Transmitted as autosomal recessive disorder.
- **Genetic defect:** DNA repair mechanisms are defective.
 - DNA damage produced by UV irradiation specially thymine dimers, cannot be incised. Results from inborn deficiency of the enzyme “**nicking endonuclease**”.

Clinical Manifestations :

- Increased cutaneous sensitivity to UV rays of sunlight.
- Produces blisters on the skin.
- Dry keratosis, hyperpigmentation and atrophy of skin.
- May produce corneal ulcers.

Ataxia telangiectasia :

- A familial disorder.
- **Inheritance:** Autosomal recessive
- Increased sensitivity to X-rays and UV rays is seen.

Clinical manifestations :

- Progressive cerebellar ataxia.
- Oculocutaneous telangiectasia.
- Frequent sin pulmonary infections.
- **Lymphoreticular neoplasms** are common in this condition.
- IgE deficiency has been demonstrated in 67 per cent of cases.

Bloom's Syndrome

Chromosomal breaks and rearrangements are seen in this condition.

- **Genetic defect:** Defective DNA-ligase.

Clinical Manifestations

- Facial erythema
- Photosensitivity

Fanconi's Anaemia :

- An autosomal recessive anemia. Defective gene is located in **chromosomes 20q** and **9q**.
- **Defect:** Defective repair of cross-linking damage.
- Characterized by An increased frequency of cancer and by chromosomal instability.

Hereditary Nonpolyposis Colon Cancer (HNPCC)

- Most common inherited cancer.
- Defect: Faulty mismatch repair.
- Genetic defect has been located in **chromosome 2**,
The located gene is called **hMSH-2**.
- Mutations of **hMSH-2** account for 50 to 60 per cent
of **HNPCC** cases.

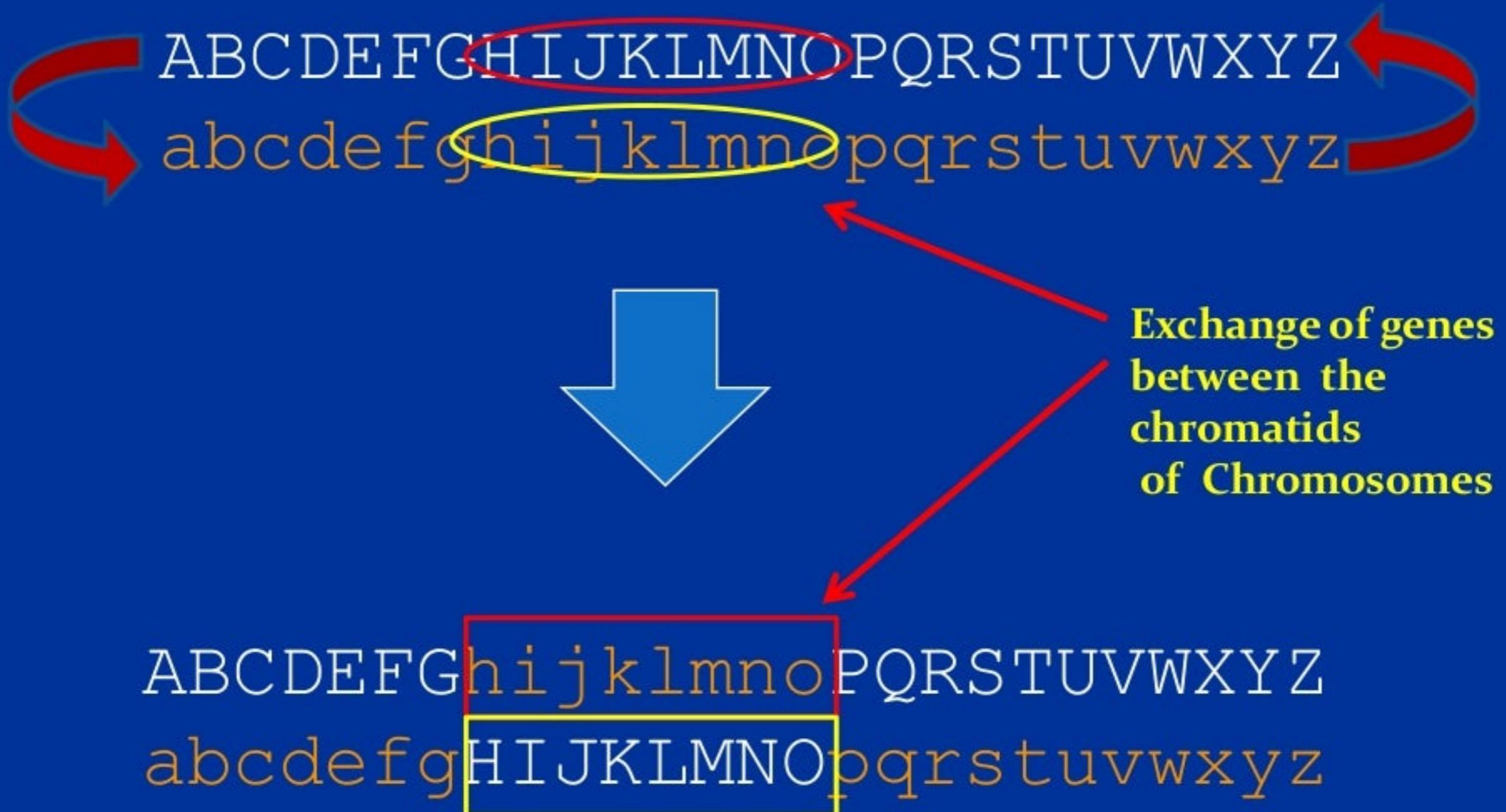
Recombination

Genetic diversity in a species is maintained through both **mutation** and **recombination**.

Mutation alters single genes or small groups of genes in an individual, whereas **recombination redistributes the contents of a genome** among various individuals during reproduction.

- Recombination basically involves the exchange of genetic information.
- There are mainly two types of recombination.
 - Homologous Recombination (Meiosis).
 - Transposition.
- Recombination is mediated by the breakage and joining of DNA strands.

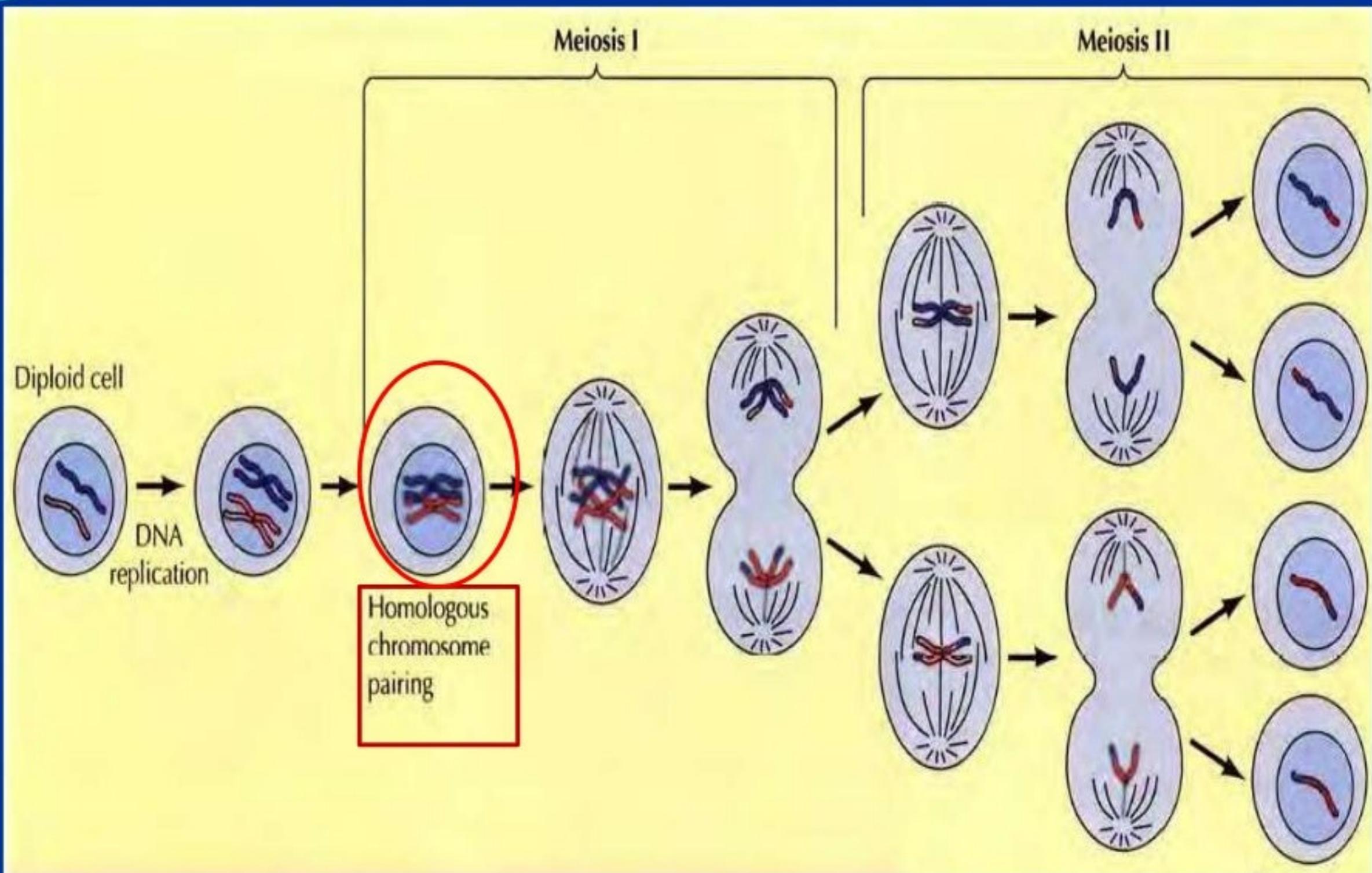
Recombination

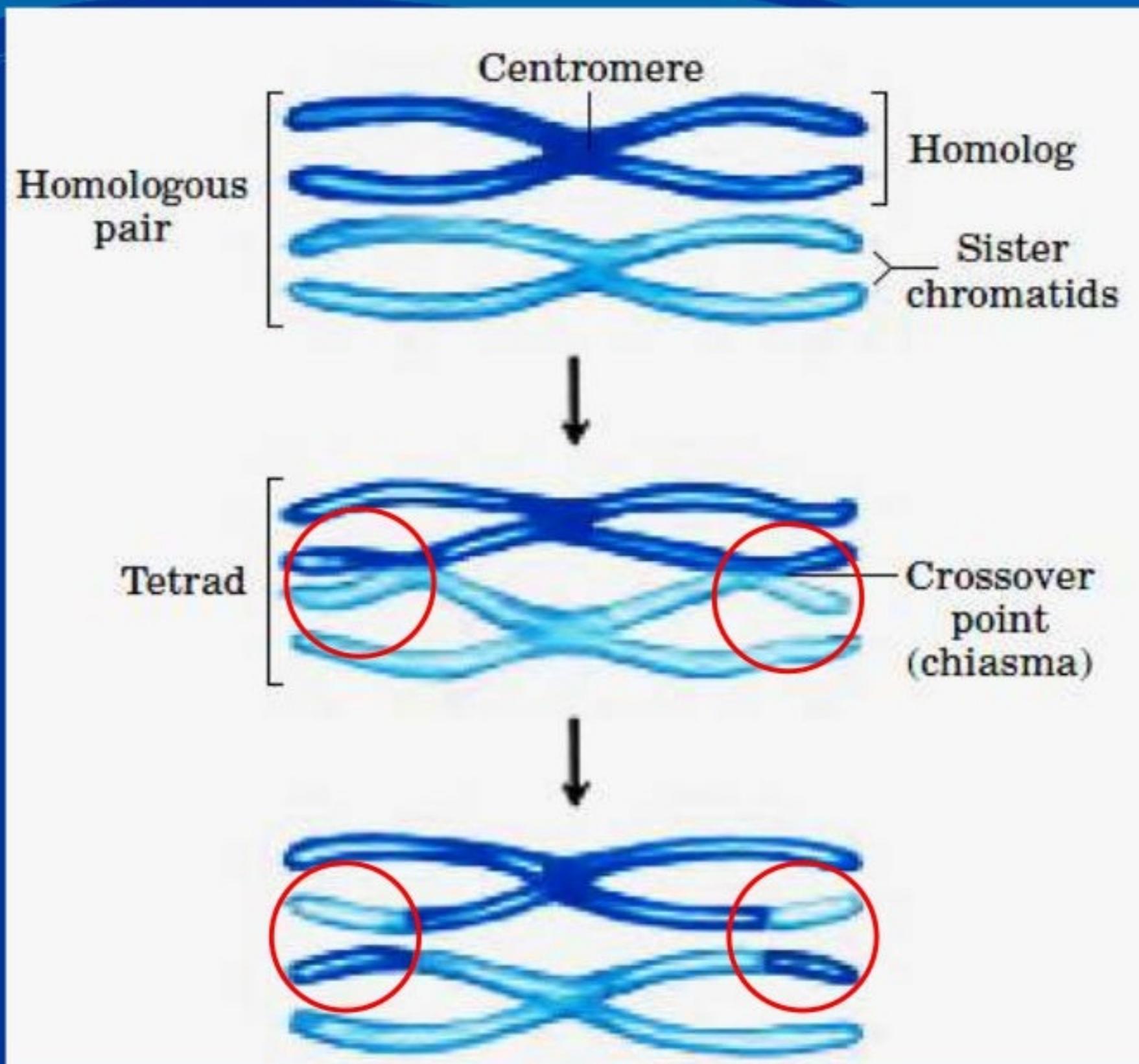


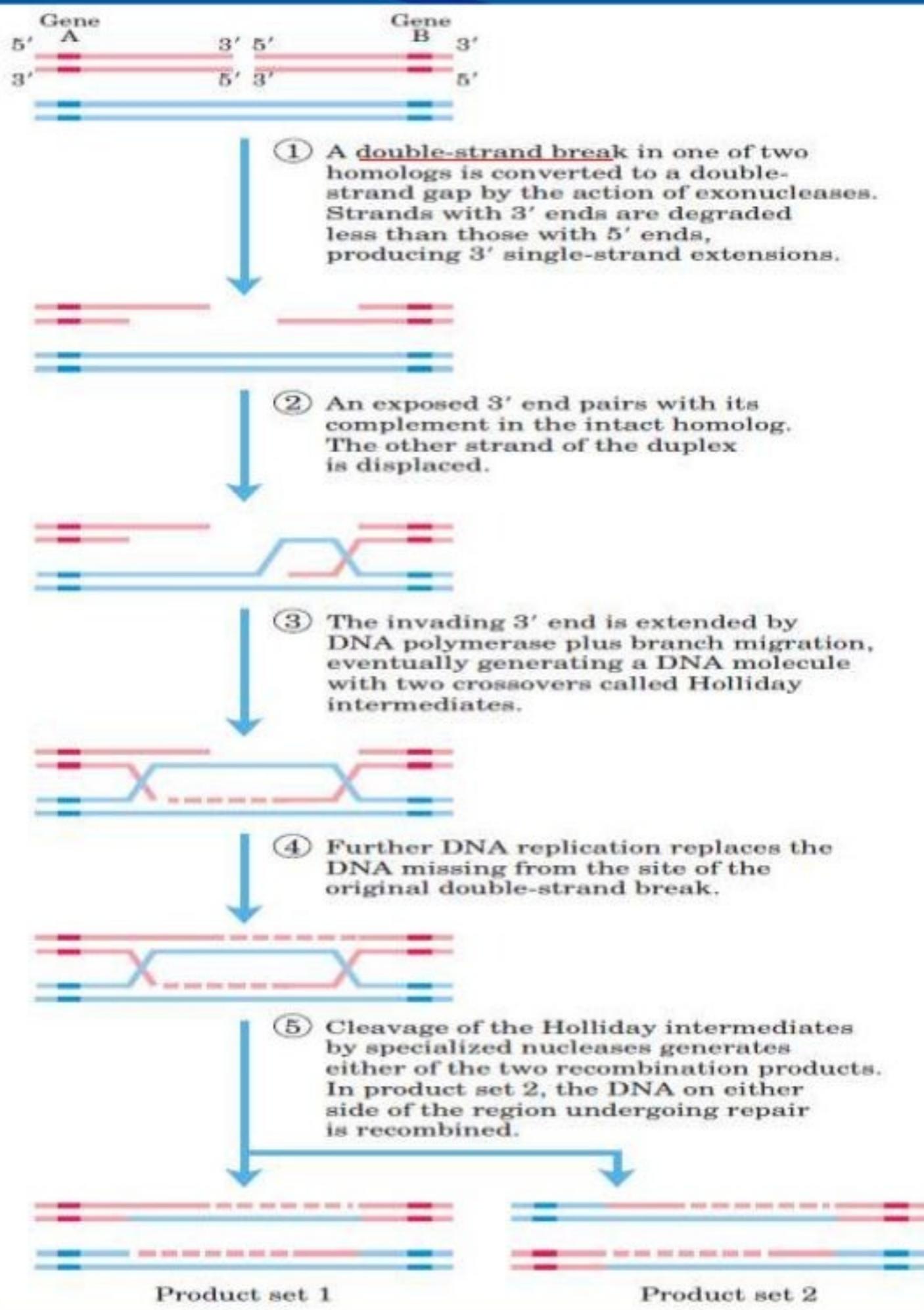
Homologous Recombination

In eukaryotes, **homologous genetic recombination** can have several roles in **replication** and **cell division**, including the repair of stalled replication forks.

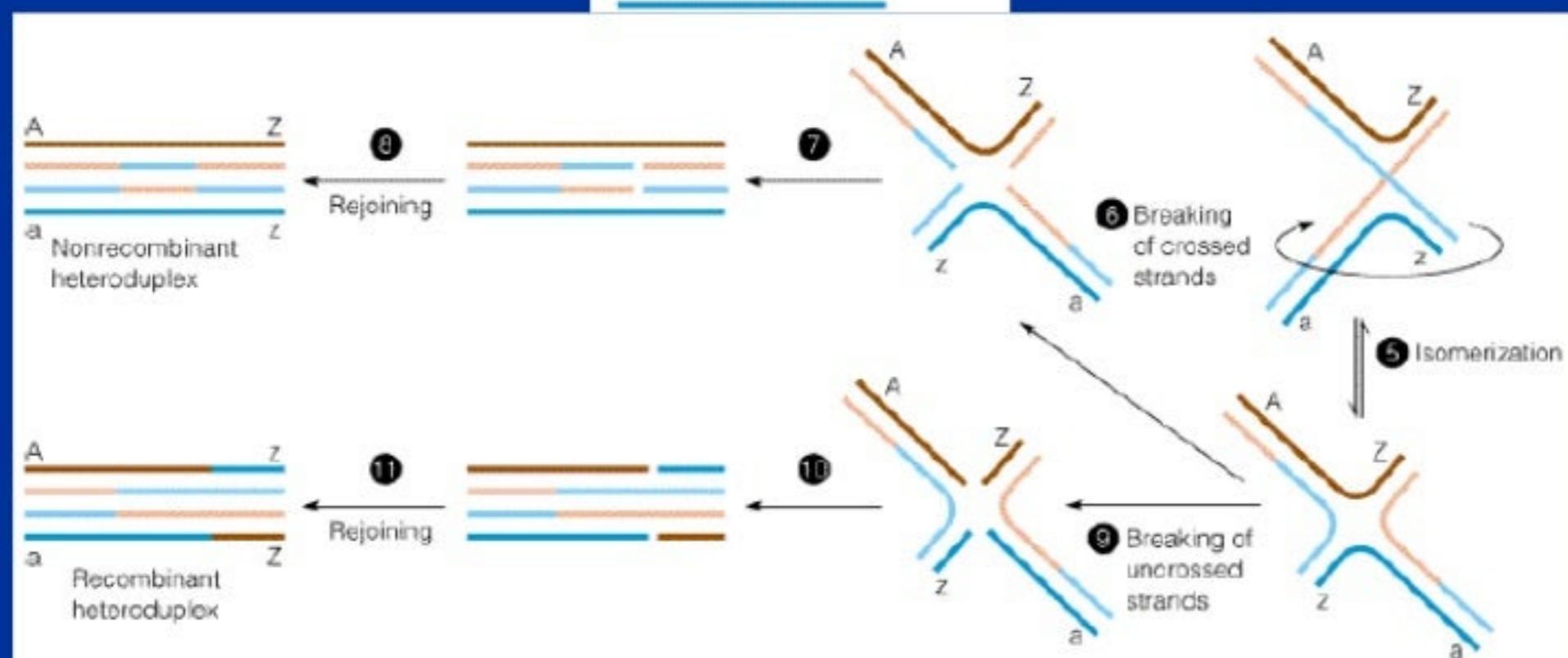
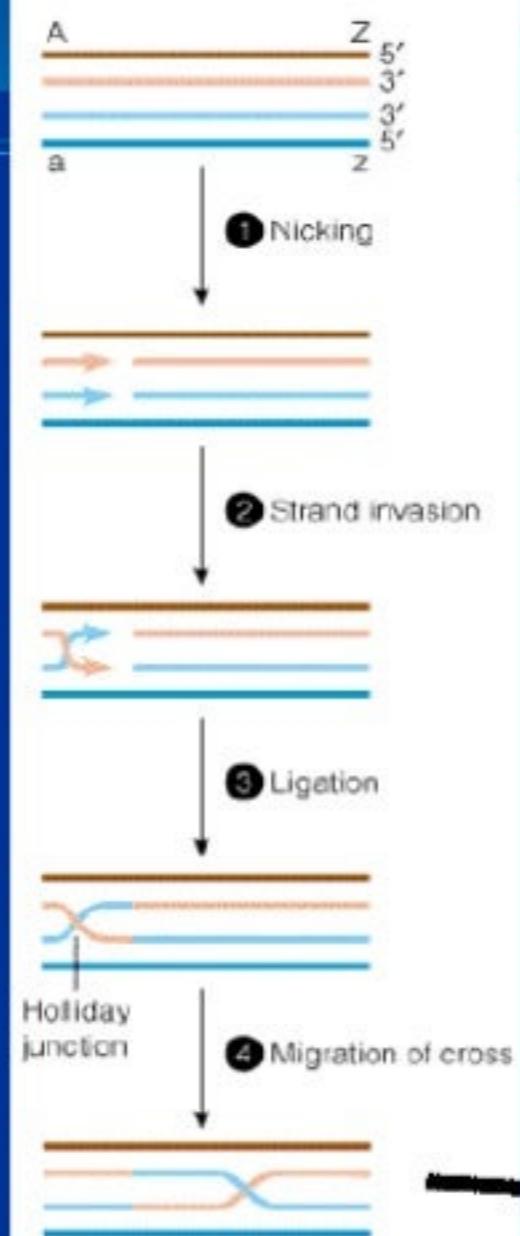
Recombination occurs with the highest frequency during **meiosis**, the process by which diploid germ-line cells with two sets of chromosomes divide to produce **haploid gametes**— **sperm cells** or **ova** in higher eukaryotes—each gamete having only one member of each chromosome pair.







Holliday Junction Model for Homologous Recombination



Transposition

Transposition primarily involves the movement of specific pieces of DNA in the genome.

The mobile segments of DNA are called **transposons (or) transposable elements**.

Types of Transposition : Two types

- 1). DNA transposition
- 2). Retrotransposition

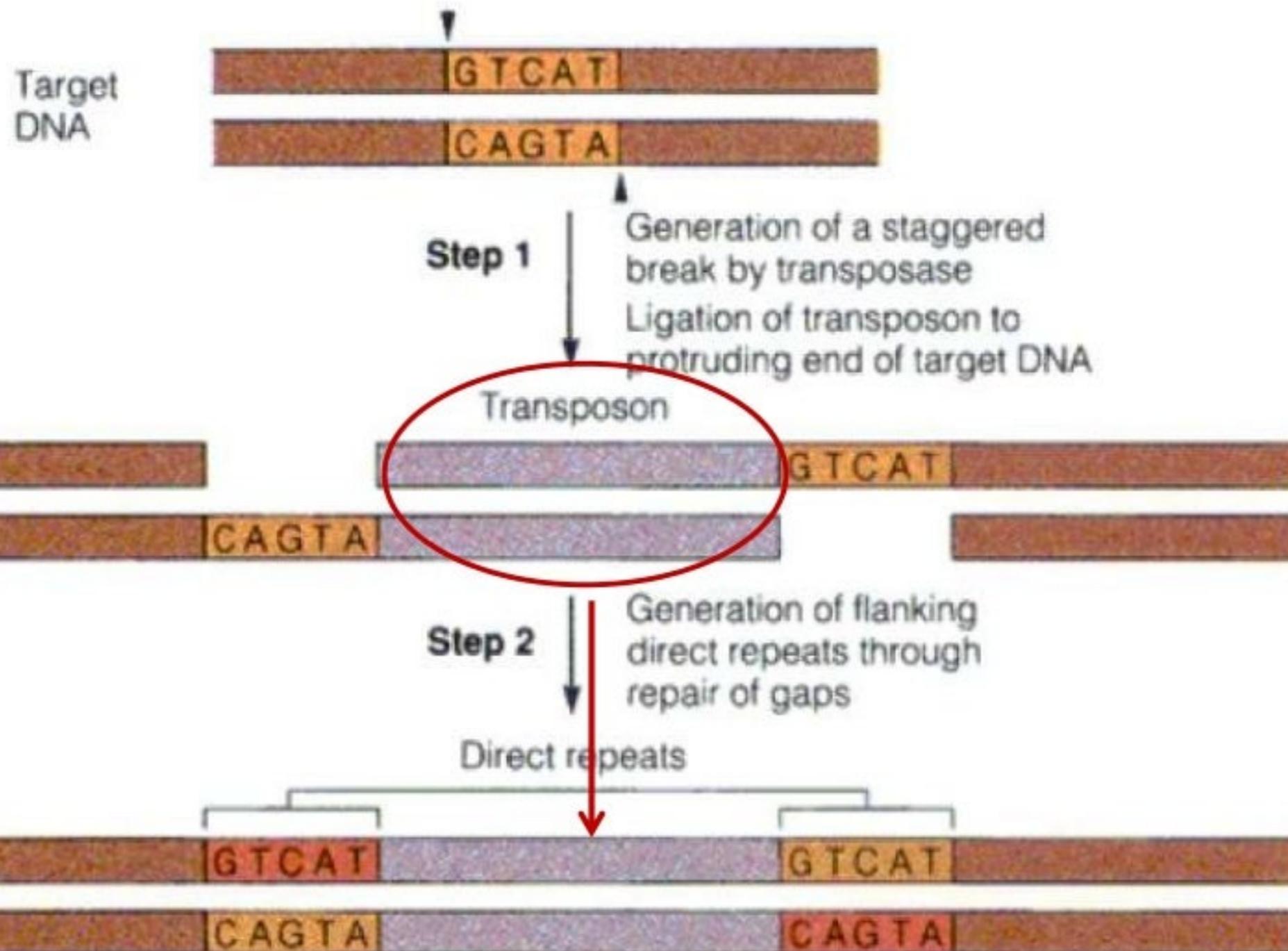
DNA transposition :

Some transposons are capable of direct transposition of DNA to DNA.

This may occur either by replicative transposition or conservative transposition .

DNA transposition is less common than retro transposition in case of eukaryotes.

However, in case of prokaryotes, DNA transposons are more important than RNA transposons.



Retrotransposition

Transposition involving RNA intermediate represents **Retrotransposition**.

A copy of RNA formed from a transposon(also called as retro transposon).

Then by the enzyme **Reverse transcriptase**, DNA is copied from the RNA.

The newly formed DNA which is a copy of the transposon gets integrated into the genome.

This integration may occur randomly on the same chromosome or/ on a different chromosome.

