Genetic information

Part 1

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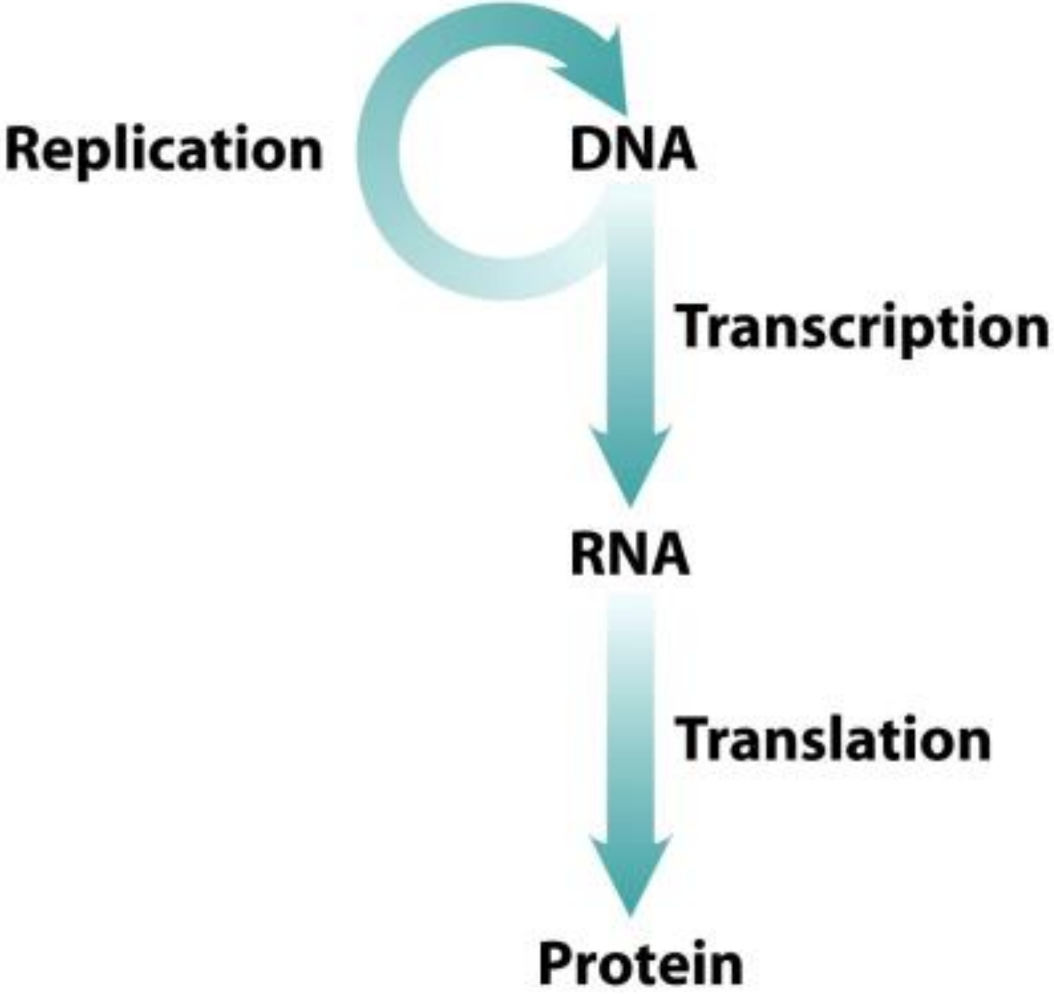
ภาควิชาชีวเคมี

คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

Outline

- DNA replication
  - DNA polymerase and other proteins in DNA replication
  - Telomere and telomerase
  - Damage to DNA and mutation
  - DNA repair
- RNA synthesis
- Protein synthesis

Central dogma



Replication

DNA

Transcription

RNA

Translation

Protein

Figure III-1
Lehninger Principles of Biochemistry, Fifth Edition
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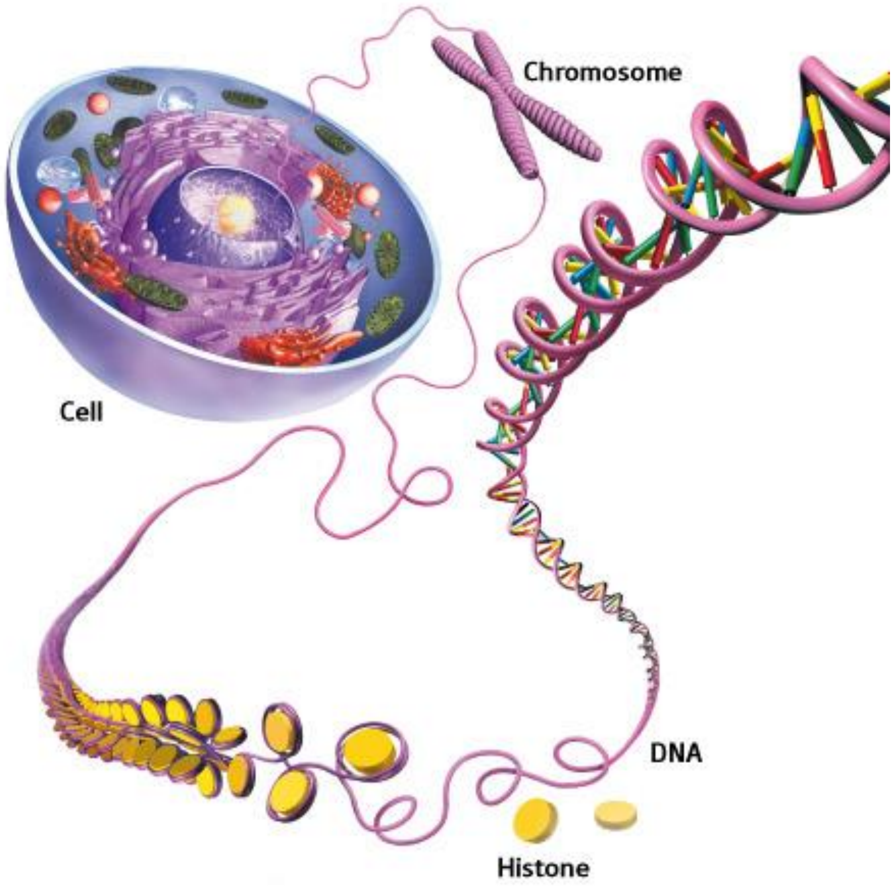
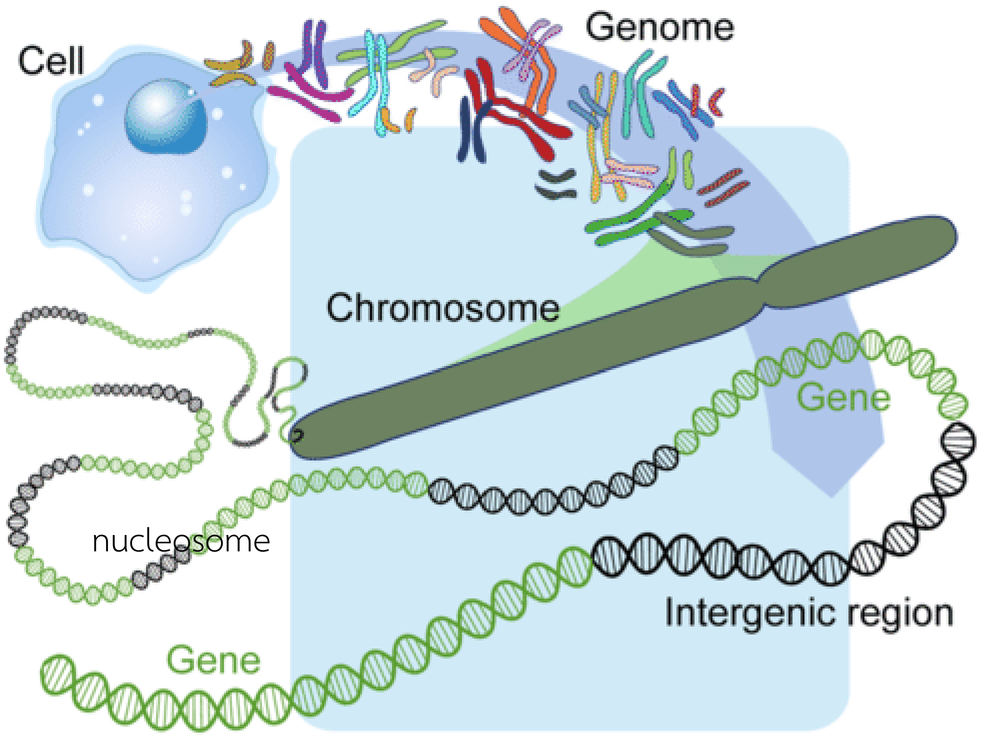
Terminology

- Deoxyribonucleic acid (DNA)
  - Stores biological information
  - Carrying the genetic instructions
  - Normally found as a double-stranded structure
- Ribonucleic acid (RNA)
  - Various biological roles in decoding, coding, regulation and expression of genes
  - Often found as a single-strand folded onto itself
- Protein
  - Various roles in catalyzing metabolic reactions, DNA replication, responding to stimuli, providing structure to cells and organisms, and transporting molecules

Terminology

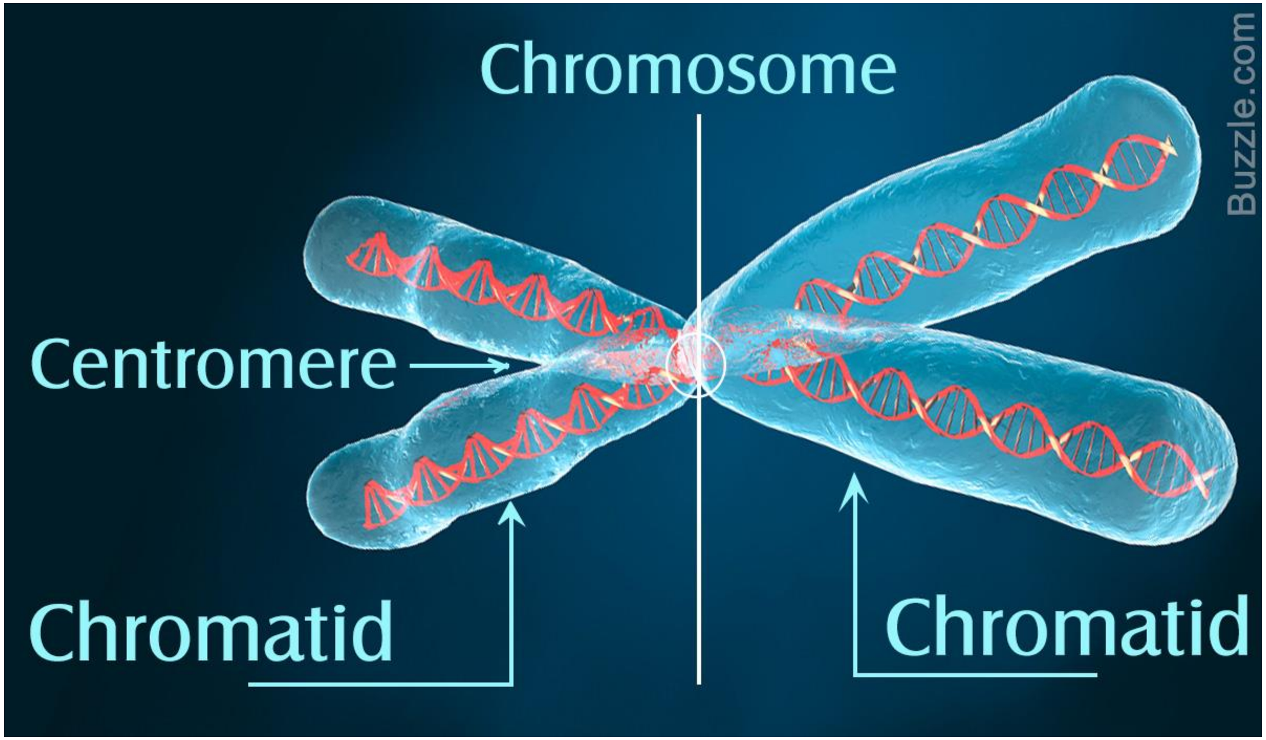
Chromosome, Gene, Intergenic region, Genome



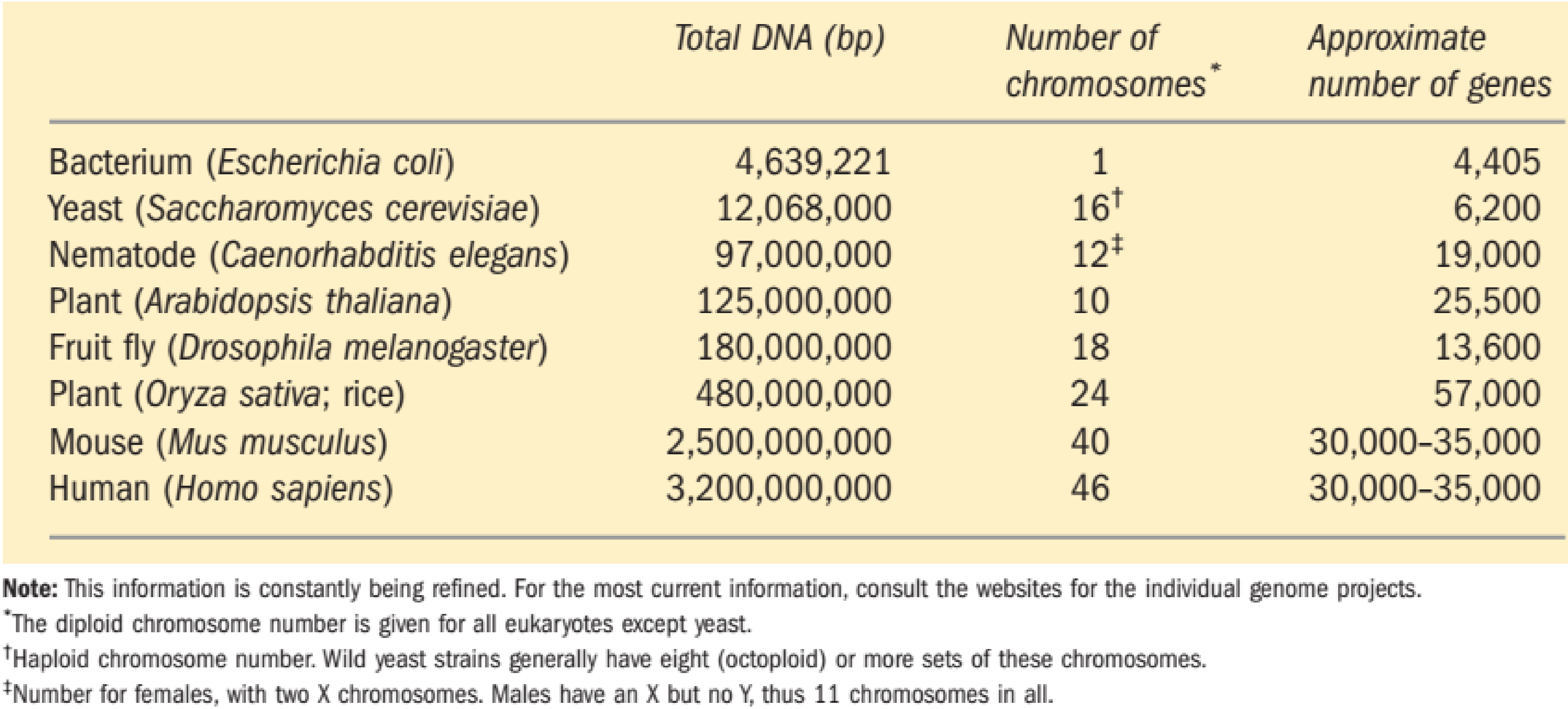


Chromosome

- Chromatin packing → Chromosome
- 23 pairs of human chromosome (22 autosomes plus 1 pair → sex chromosome)



DNA, Gene and Chromosome content in some genomes



	Total DNA (bp)	*Number of chromosomes*[*]	*Approximate number of genes*
Bacterium (*Escherichia coli*)	4,639,221	1	4,405
Yeast (*Saccharomyces cerevisiae*)	12,068,000	16[†]	6,200
Nematode (*Caenorhabditis elegans*)	97,000,000	12[‡]	19,000
Plant (*Arabidopsis thaliana*)	125,000,000	10	25,500
Fruit fly (*Drosophila melanogaster*)	180,000,000	18	13,600
Plant (*Oryza sativa*; rice)	480,000,000	24	57,000
Mouse (*Mus musculus*)	2,500,000,000	40	30,000–35,000
Human (*Homo sapiens*)	3,200,000,000	46	30,000–35,000

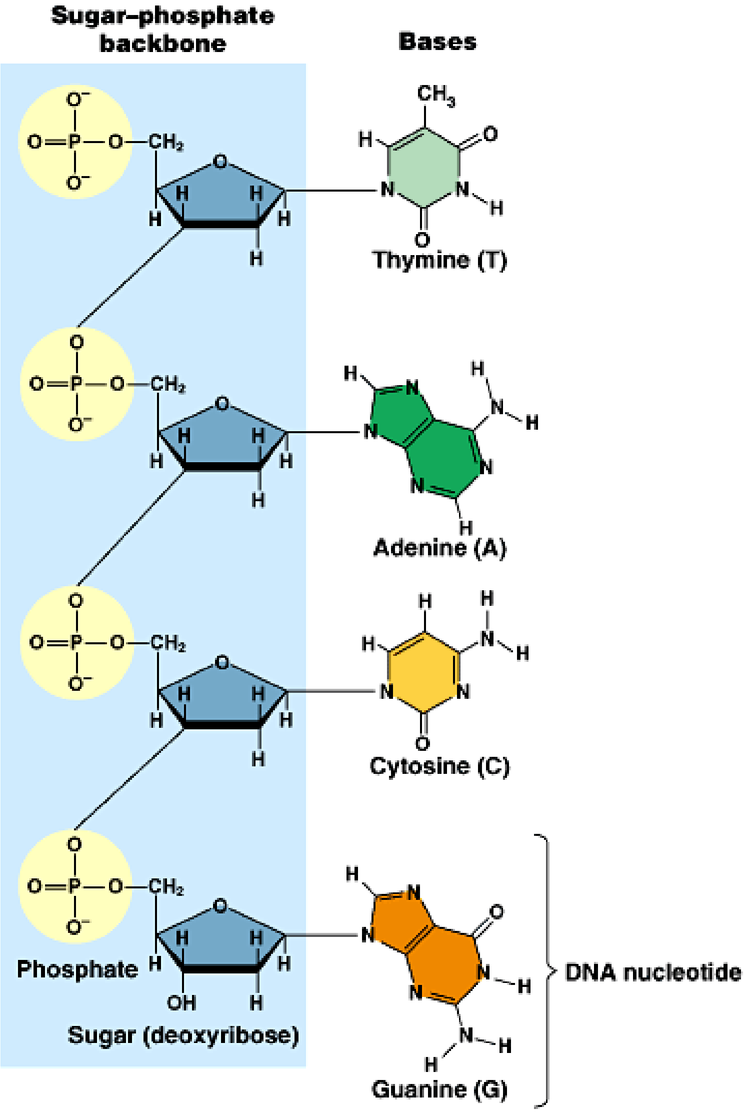
Note: This information is constantly being refined. For the most current information, consult the websites for the individual genome projects.

[*] The diploid chromosome number is given for all eukaryotes except yeast.

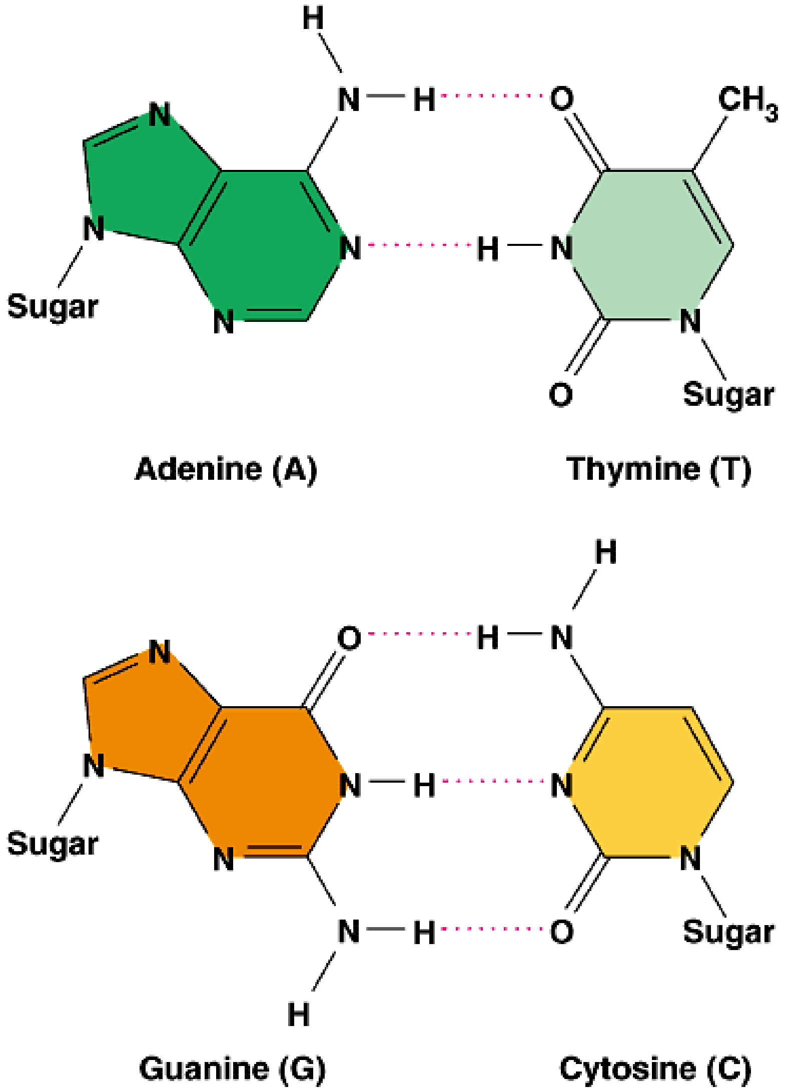
[†] Haploid chromosome number. Wild yeast strains generally have eight (octoploid) or more sets of these chromosomes.

[‡] Number for females, with two X chromosomes. Males have an X but no Y, thus 11 chromosomes in all.

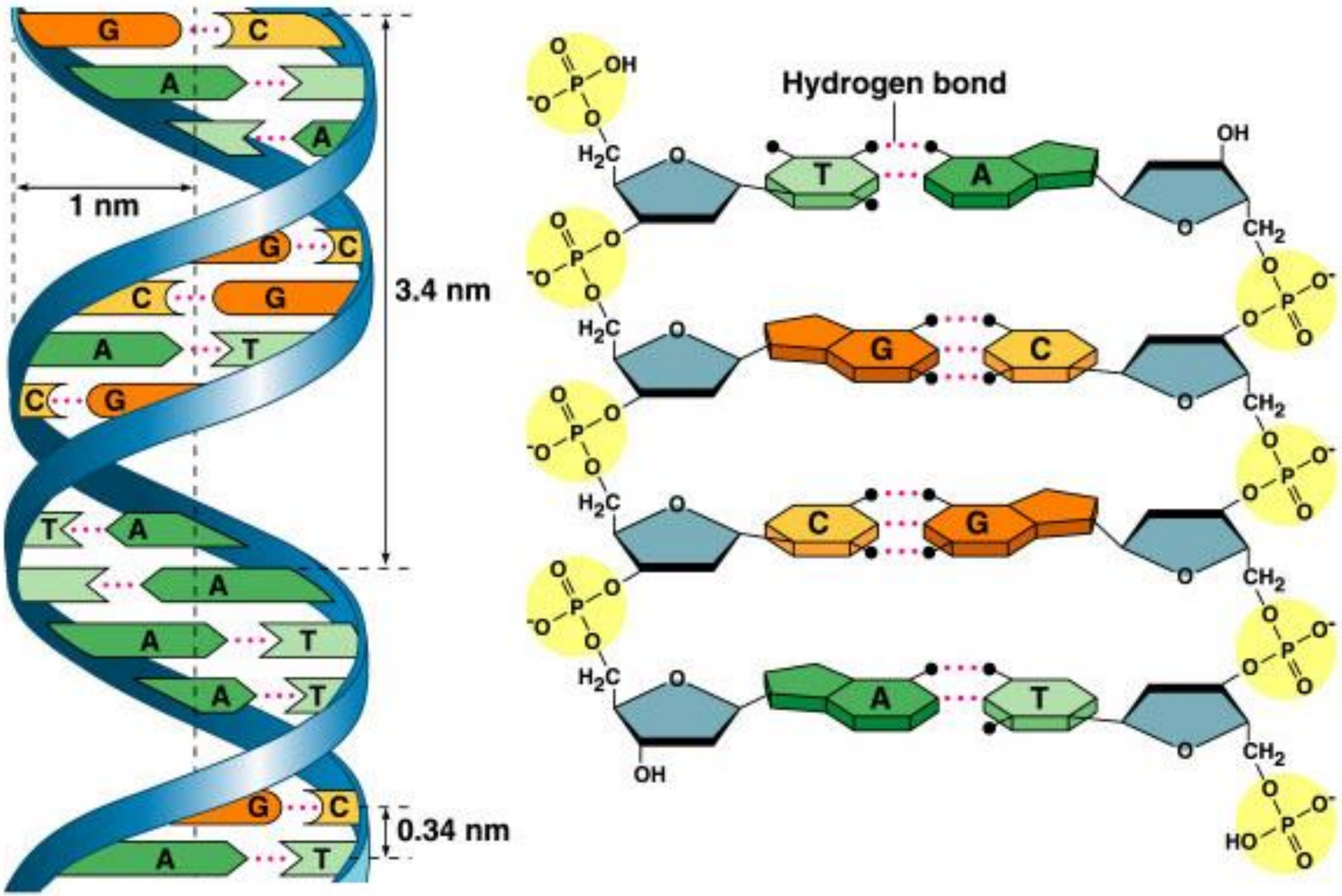
The structure of a DNA stand



Base pairing in DNA



The double helix



(a) Key features of DNA structure

(b) Partial chemical structure

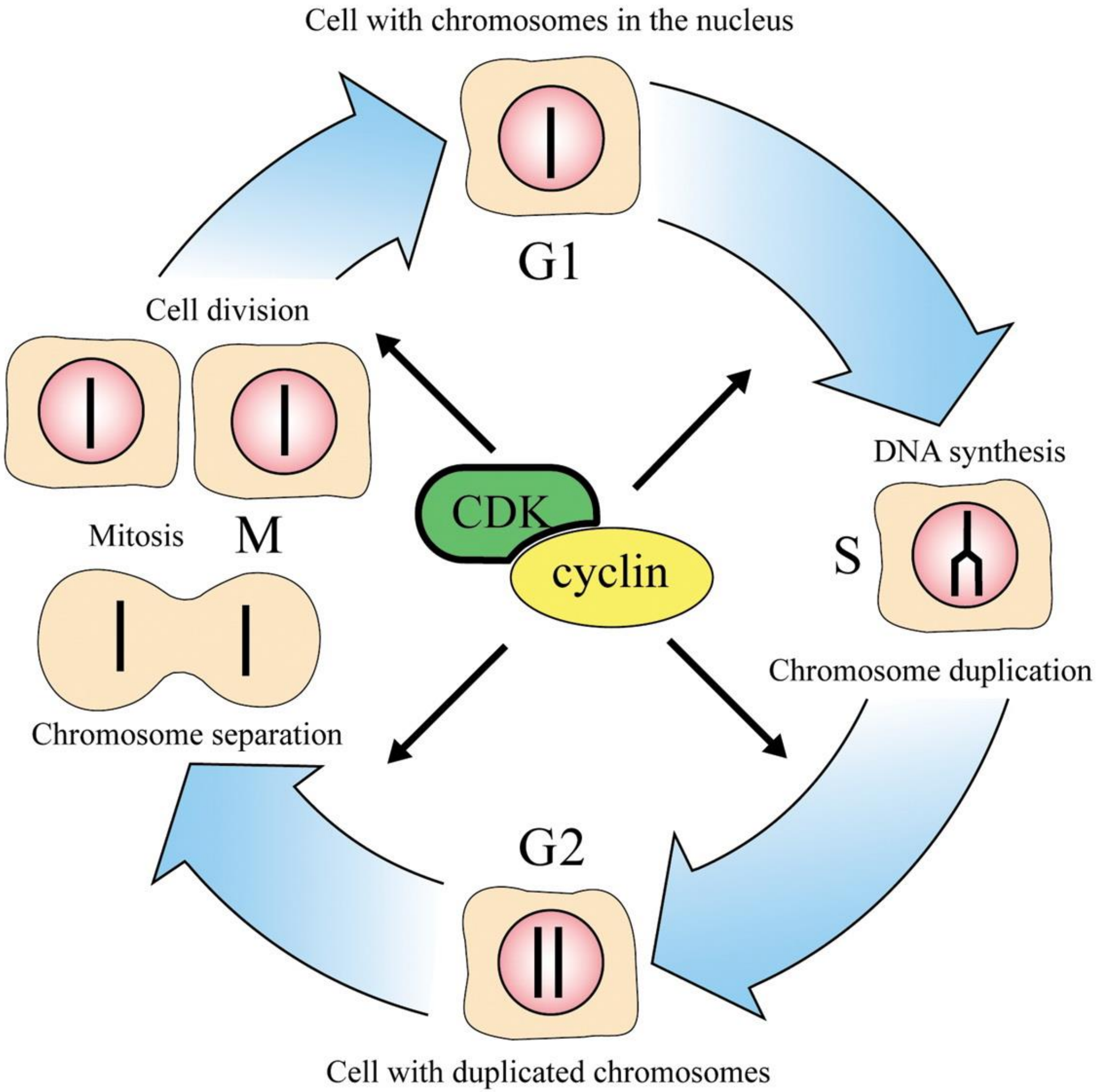
DNA replication - Benefits

1. Transfer same biological information to a new cell (daughter cell)

parental cell (diploid) → daughter cell (diploid)

2. Biological inheritance
3. DNA repair

The Cell Cycle



Cell with chromosomes in the nucleus

G1

DNA synthesis

S

Chromosome duplication

G2

Cell with duplicated chromosomes

Chromosome separation

Mitosis

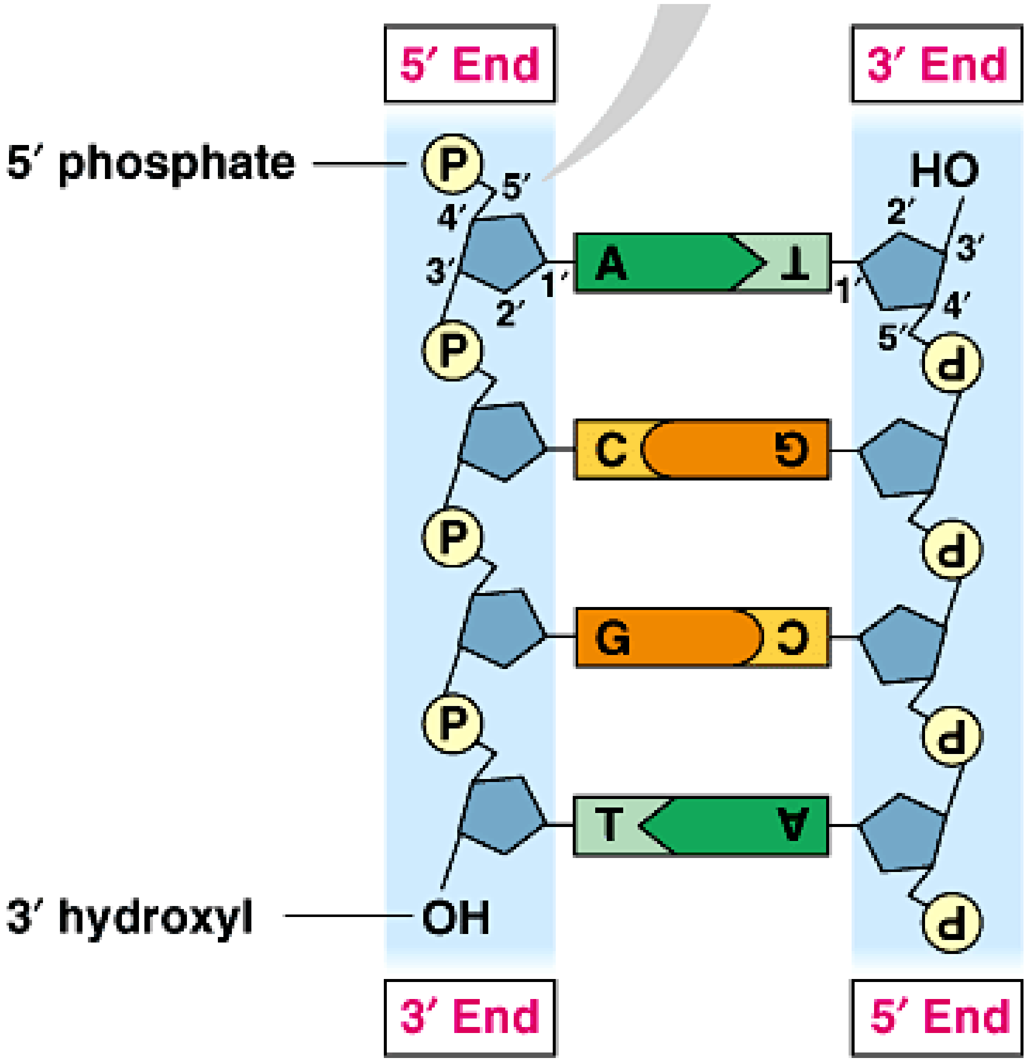
M

Cell division

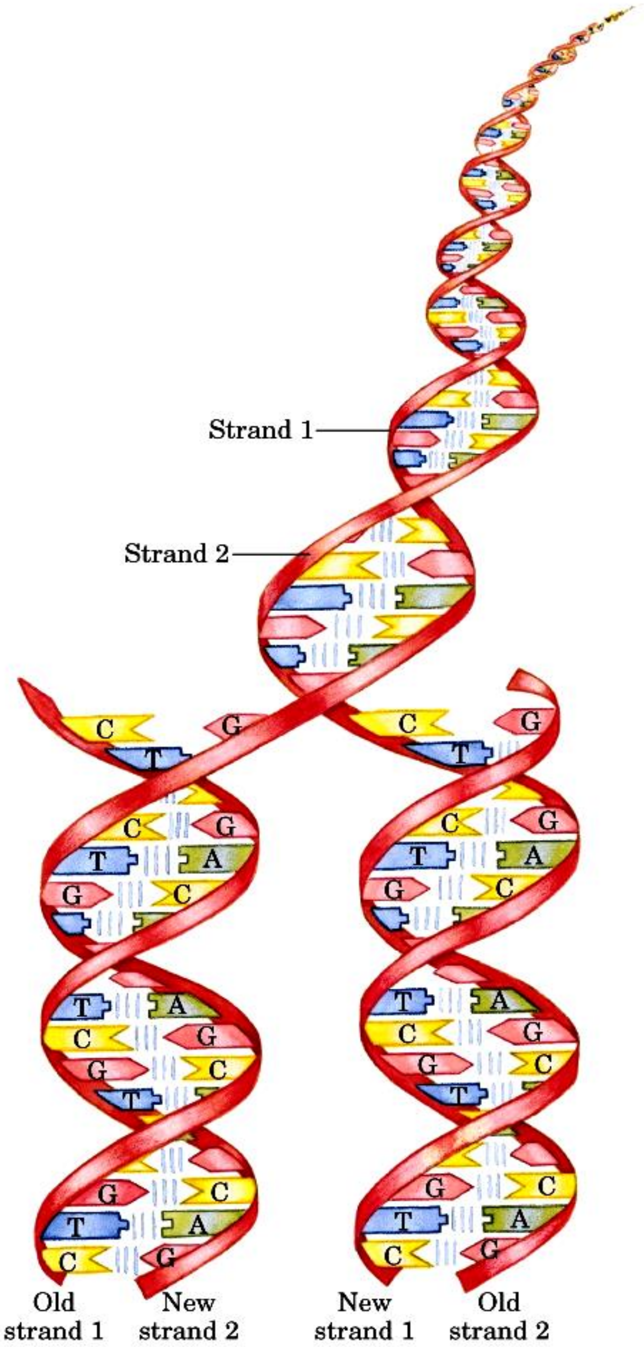
CDK

cyclin

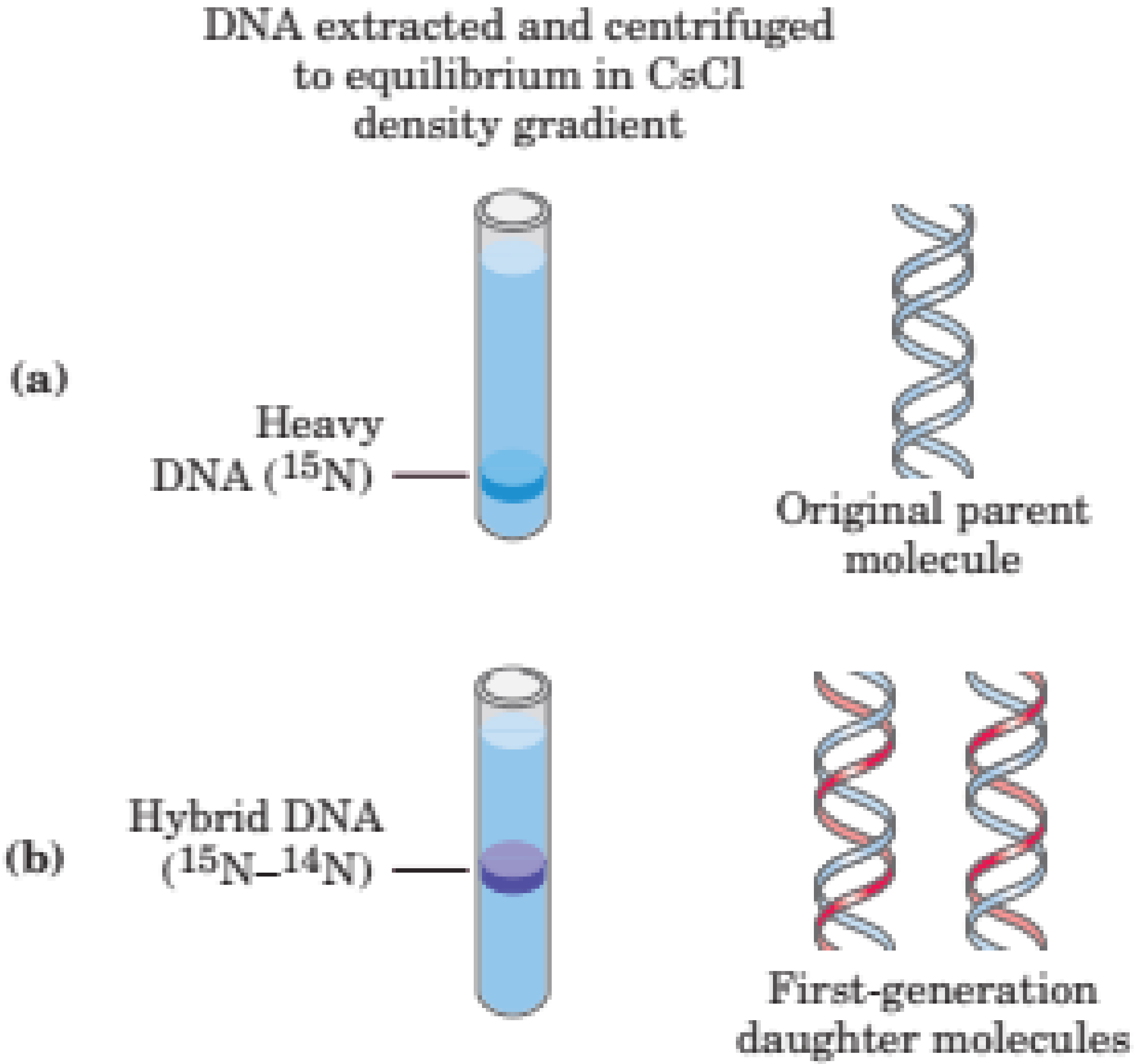
The two strands of DNA are antiparallel



Semiconservative replication

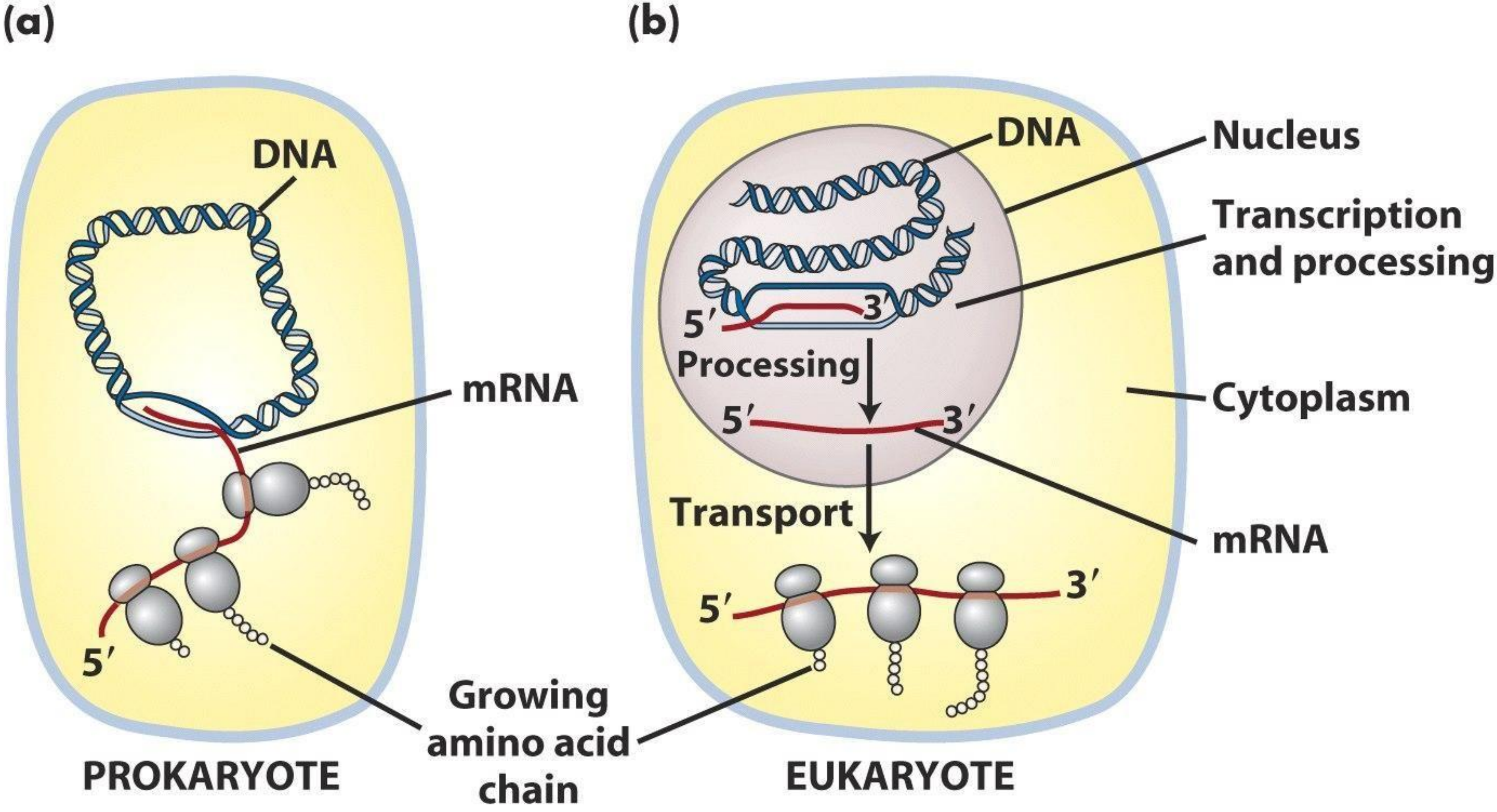


The Meselson-Stahl experiment



DNA is degraded by Nucleases

- **Exonucleases** removing nucleotides only from the 5’ or the 3’ end
- 5’→3’ : removing nucleotides only from the 5’ end
- 3’→5’ : removing nucleotides only from the 3’ end
- **Endonucleases** degrade at specific internal sites


(a)
(b)
DNA
mRNA
5′
Growing amino acid chain
PROKARYOTE
DNA
Nucleus
Transcription and processing
5′
3′
Processing
5′
3′
Cytoplasm
Transport
mRNA
5′
3′
EUKARYOTE

Ingredients of DNA replication

- DNA template
- dNTP : dATP, dGTP, dCTP และ dTTP
- RNA primer
- Enzymes proteins : ex. DNA polymerase, DNA ligase, DNA gyrase, primase, SSB, DnaC
- Energy : ATP

Origin of replication

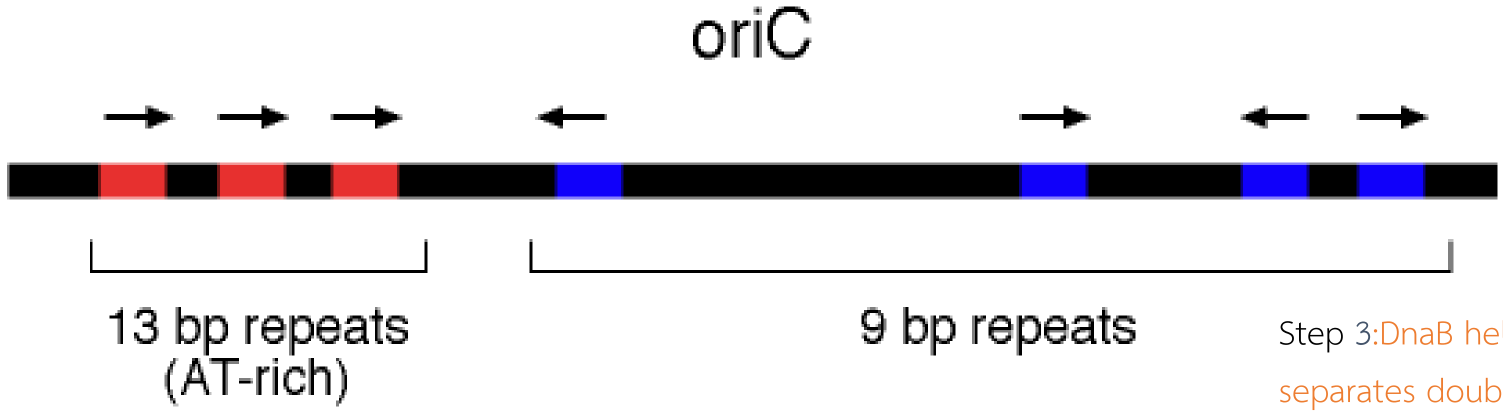
- Origin of replication

- Bidirectional

- Prokaryotic cells : one point of origin of replication

- Eukaryotic cells : multiple origins of replication → multiple fragments of DNA replication

Example: Replication in *E.coli* that start at ORI named oriC



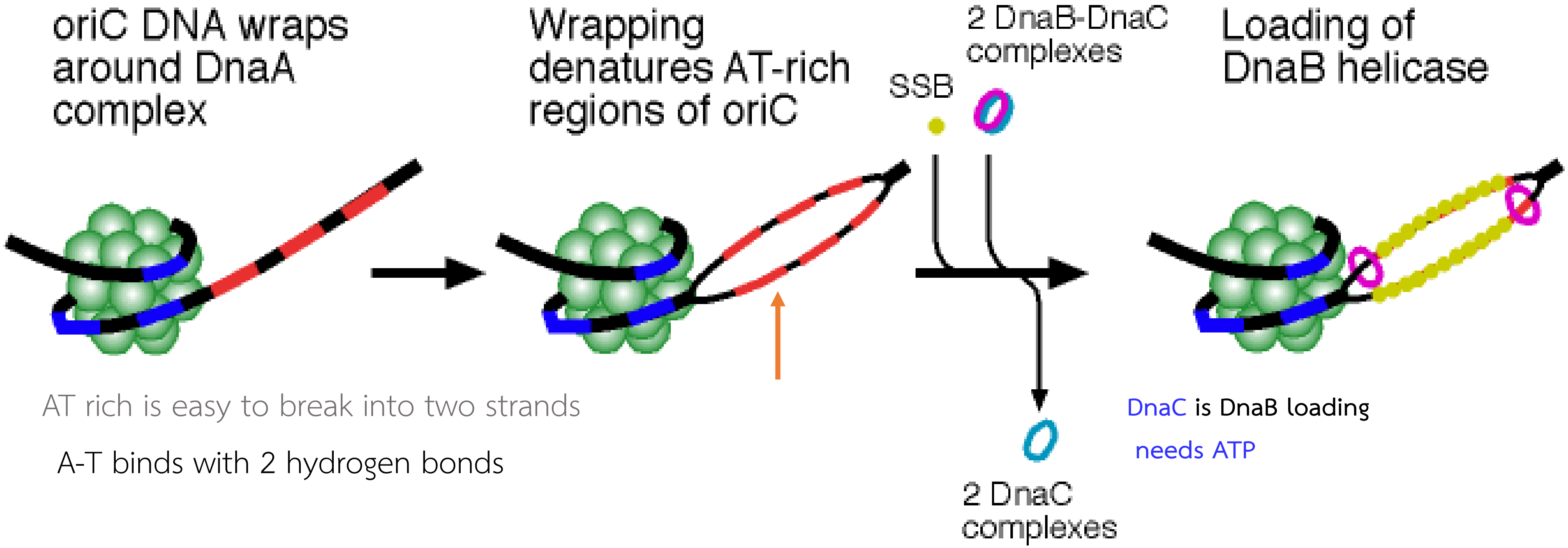
oriC

13 bp repeats
(AT-rich)

9 bp repeats

Step 3:DnaB helicase

separates double-stranded DNA



Step 1

oriC DNA wraps around DnaA complex

Step 2

Wrapping denatures AT-rich regions of oriC

SSB

2 DnaB-DnaC complexes

Loading of DnaB helicase

2 DnaC complexes

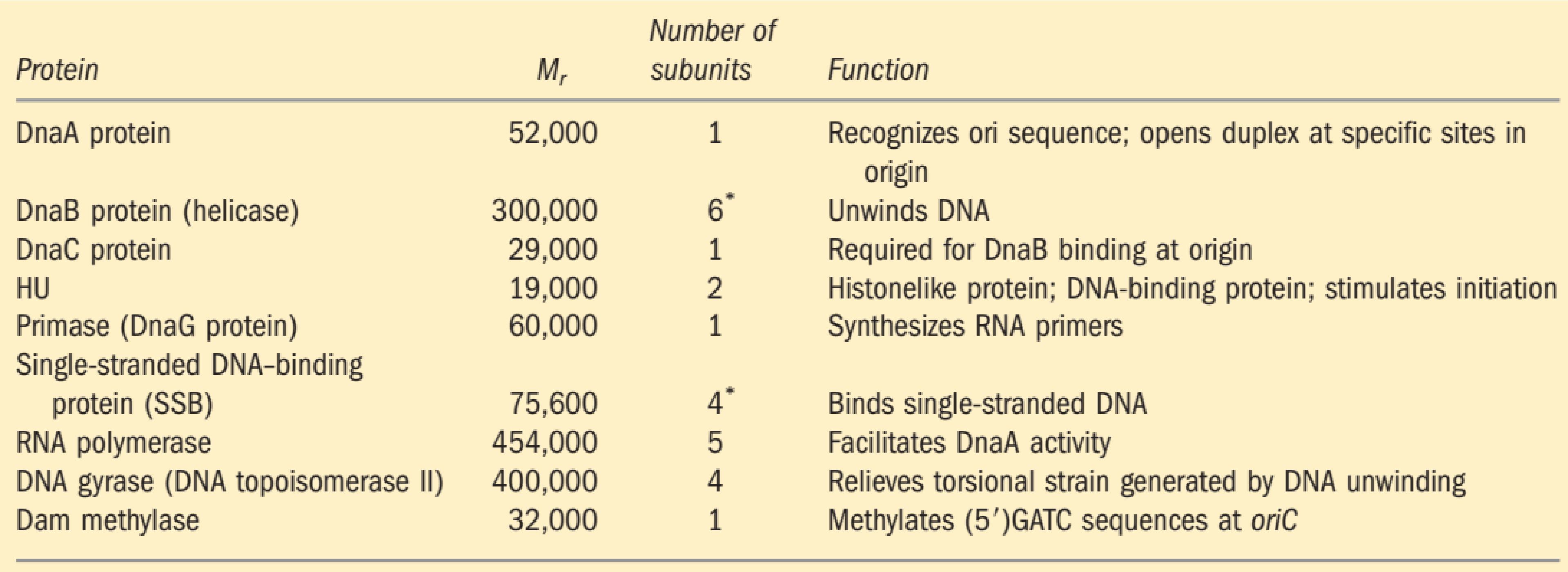
DnaC is DnaB loading

needs ATP

AT rich is easy to break into two strands

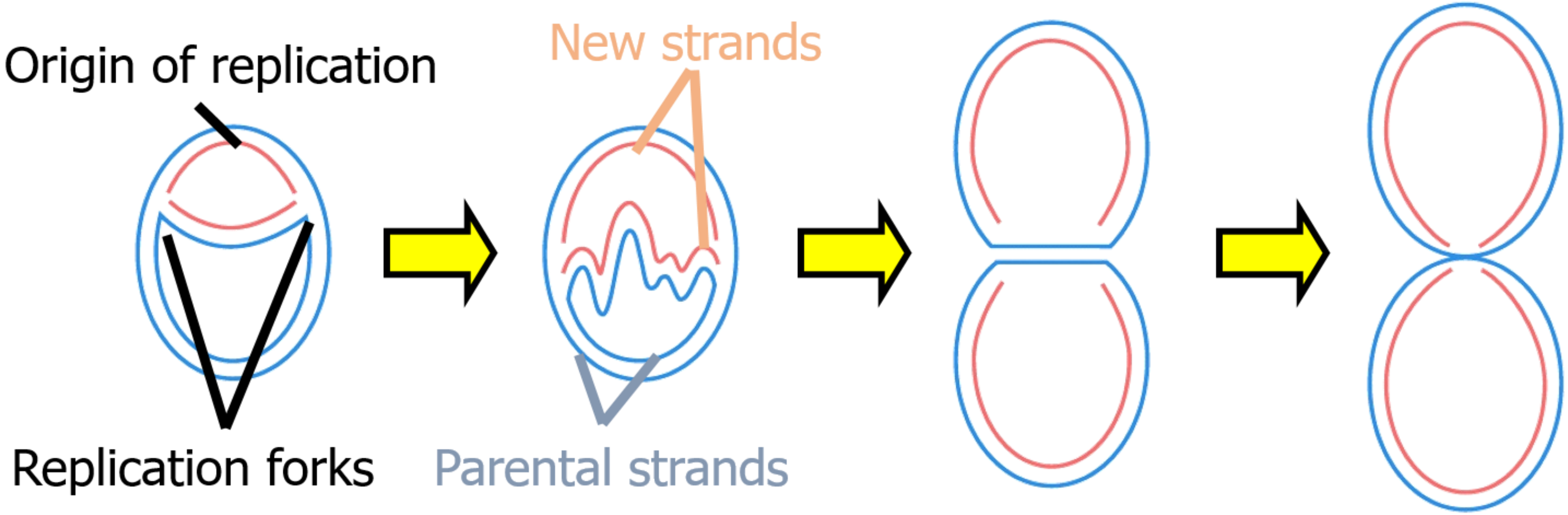
A-T binds with 2 hydrogen bonds

Proteins Required to Initiate Replication at the *E. coli* Origin



Protein	M_r	*Number of subunits*	*Function*
DnaA protein	52,000	1	Recognizes ori sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	29,000	1	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA–binding protein (SSB)	75,600	4*	Binds single-stranded DNA
RNA polymerase	454,000	5	Facilitates DnaA activity
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5′)GATC sequences at *oriC*

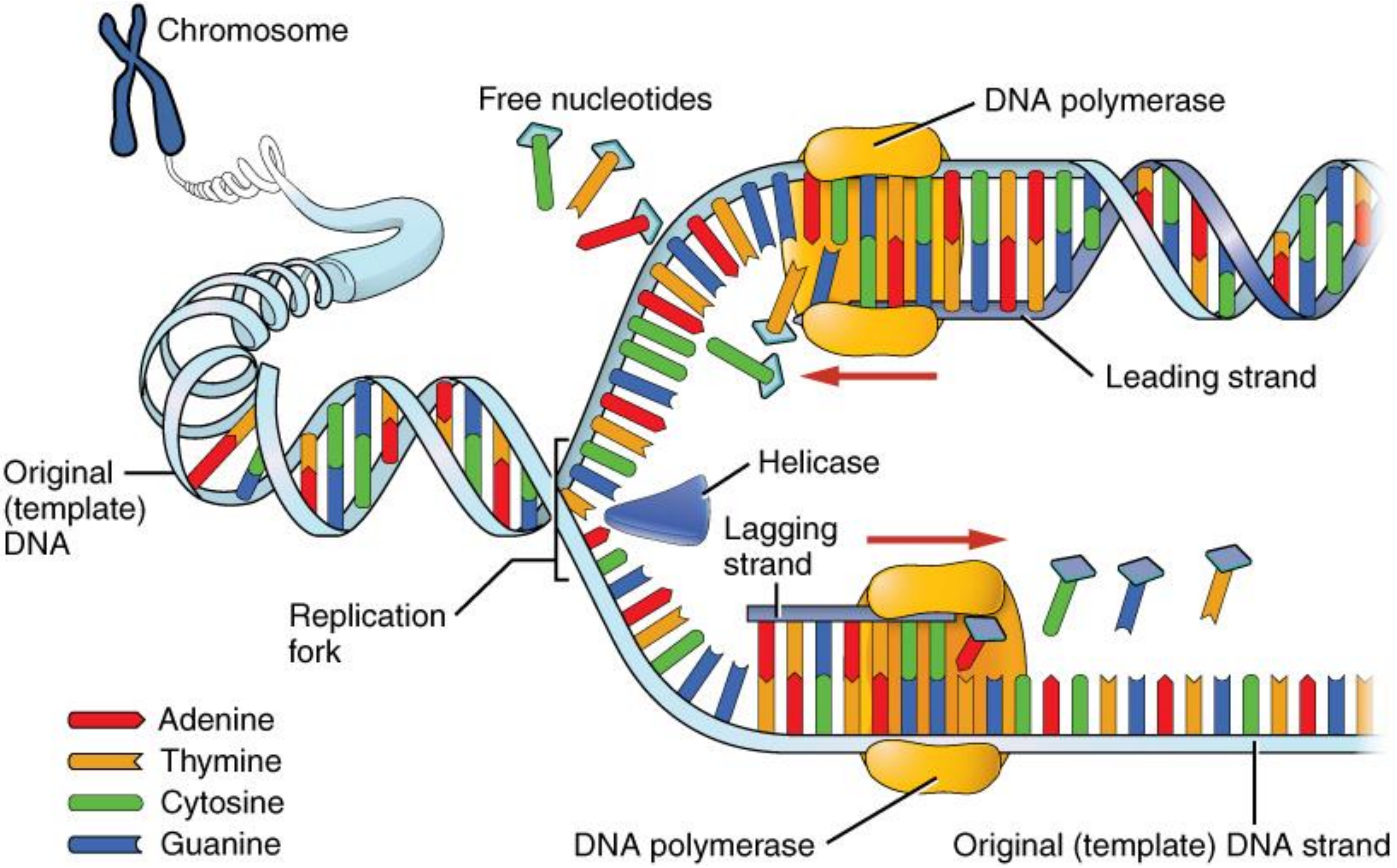
Bidirectional replication of a circular chromosome



Shape of replicating circular chromosome θ = “theta”

DNA replication

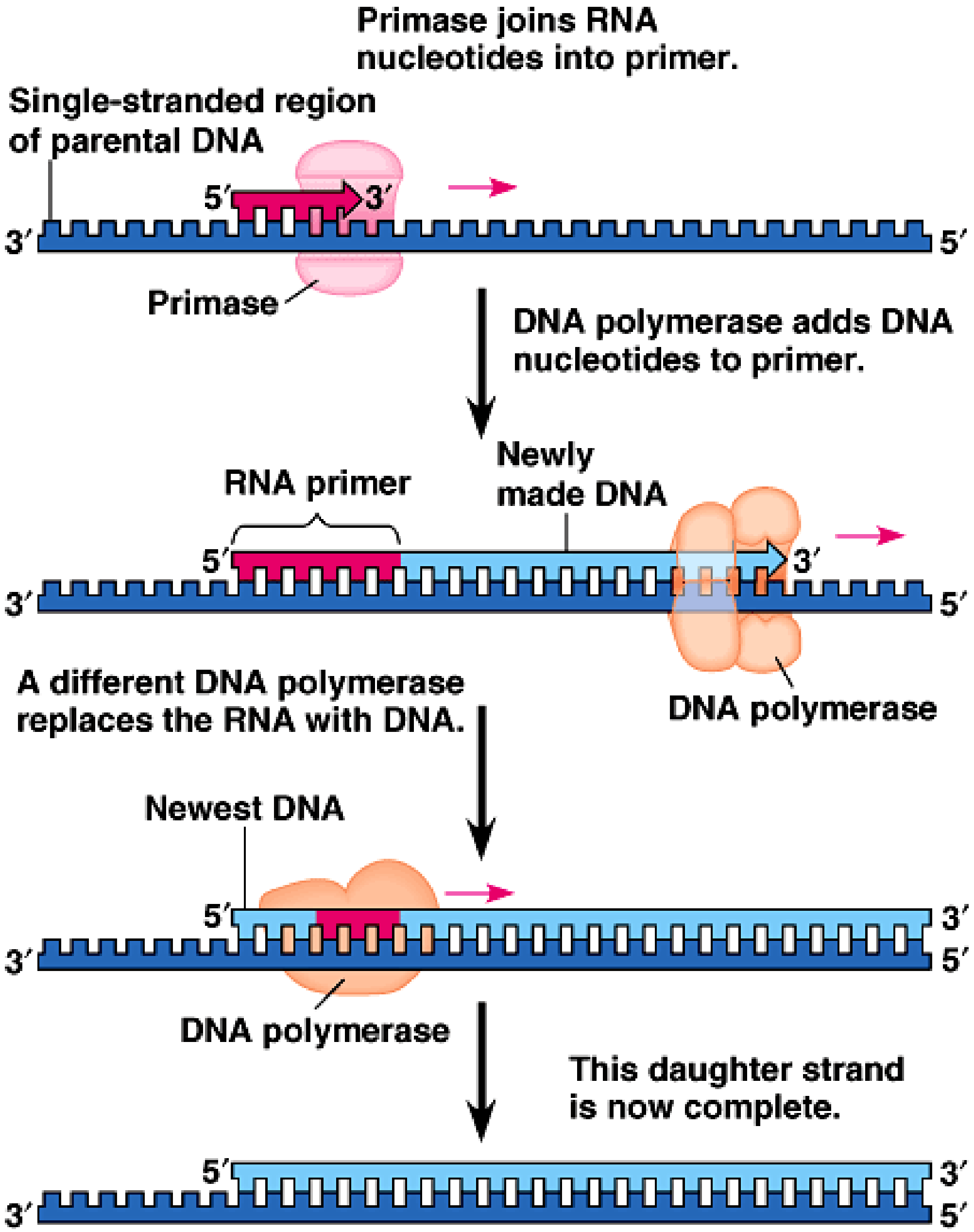
- In S-phase (synthesis phase) of cell cycle
- Begins with unwinding of DNA helix
- Each DNA strand → template of the new strands
- Product : two identical double stranded DNA
- Semiconservative replication


Chromosome
Free nucleotides
DNA polymerase
Leading strand
Original
(template)
DNA
Helicase
Lagging
strand
Replication
fork
Adenine
Thymine
Cytosine
Guanine
DNA polymerase
Original (template) DNA strand

DNA Replication

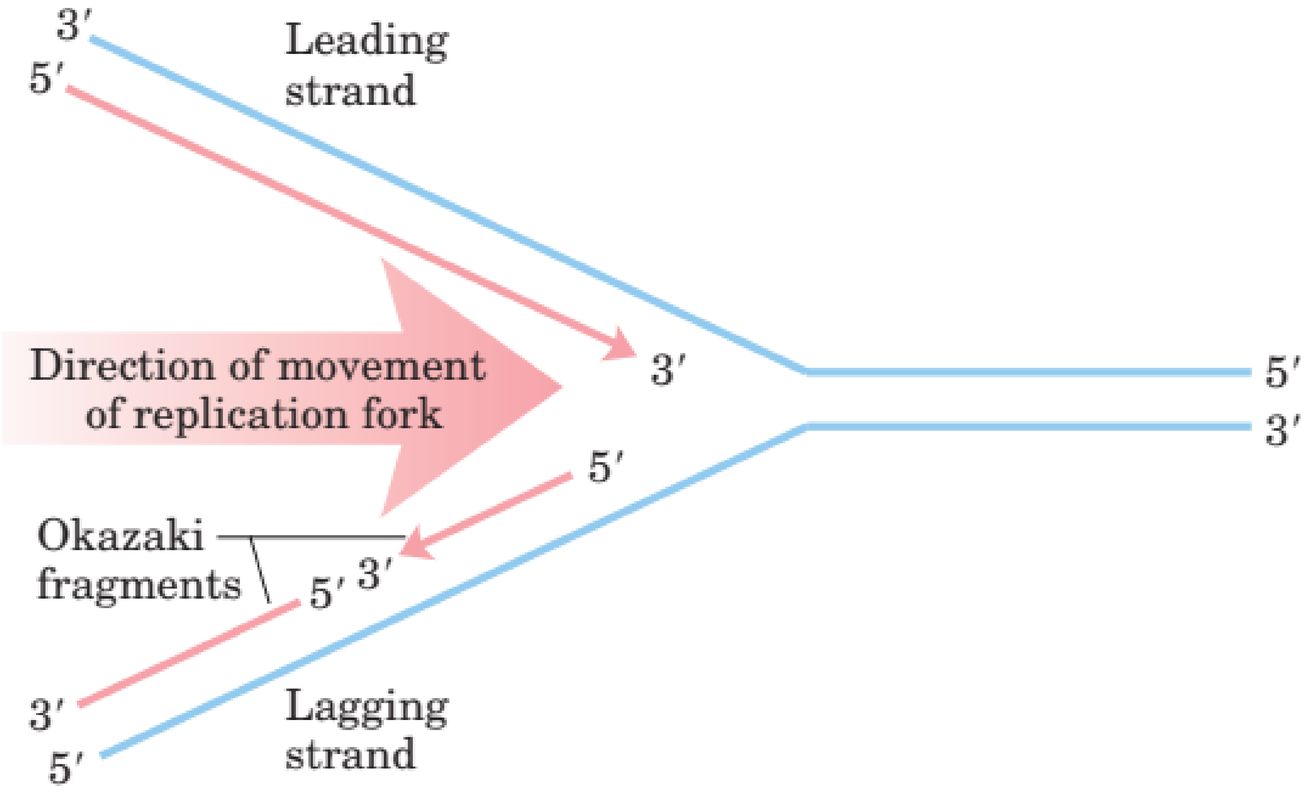
- DNA synthesis always starts with RNA primer (10 bases) laid first, later replaced with DNA
- Leading strand is continuous
- Lagging strand is discontinuous, in Okasaki fragments (100-200 bases long)
- Ligase joins the fragments

Priming DNA synthesis with RNA

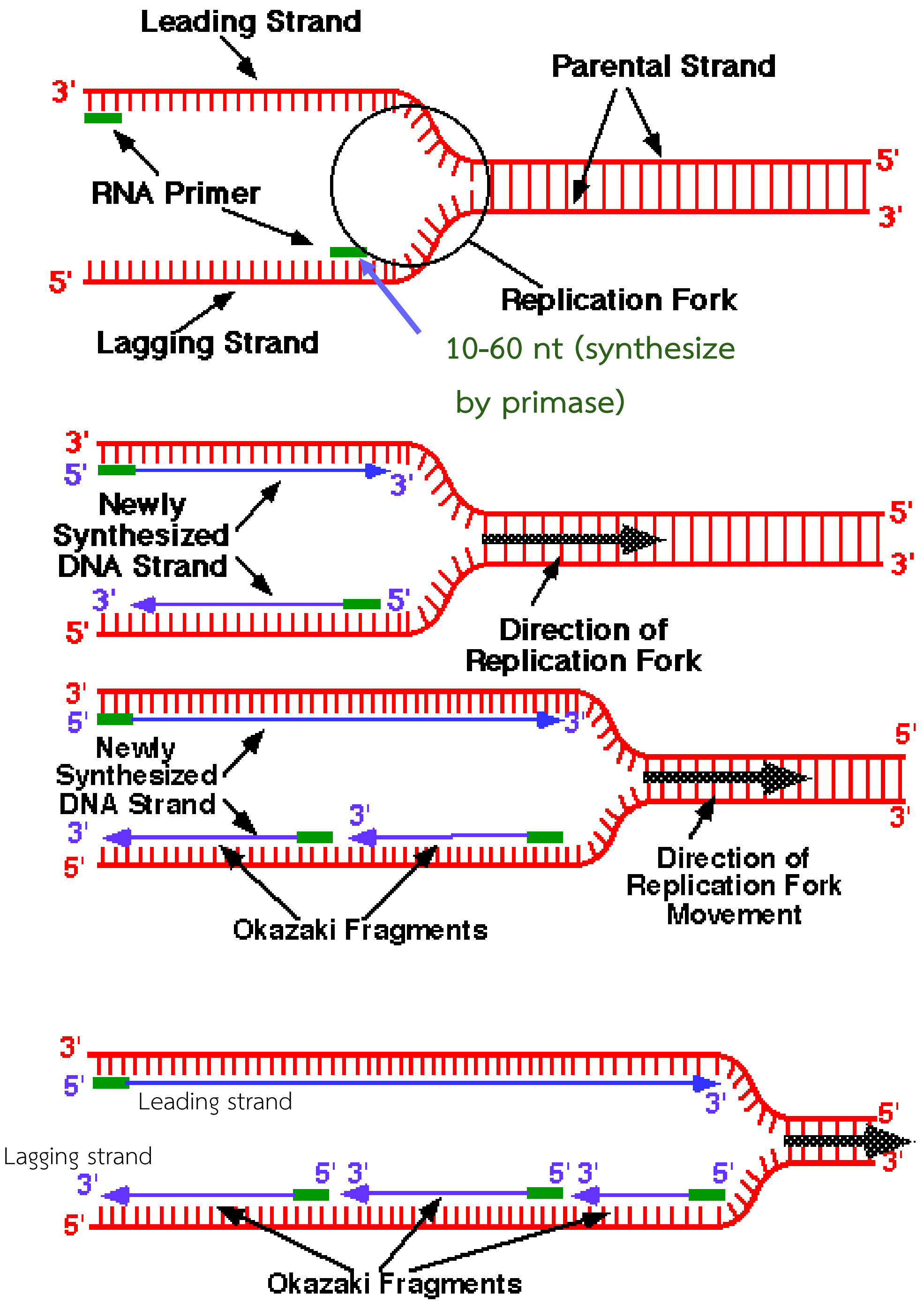


Defining DNA strands at the replication fork

DNA Synthesis Proceeds in a 5’ → 3’ Direction



Replication



10-60 nt (synthesize by primase)

Leading strand

Lagging strand

1. Primase synthesizes the RNA primers (green)

2.Enzyme DNA polymerase III synthesize DNA

3. DNA Polymerase then continue synthesis by 5' to 3' direction

4. Okazaki is synthesized as a short ones due to the space

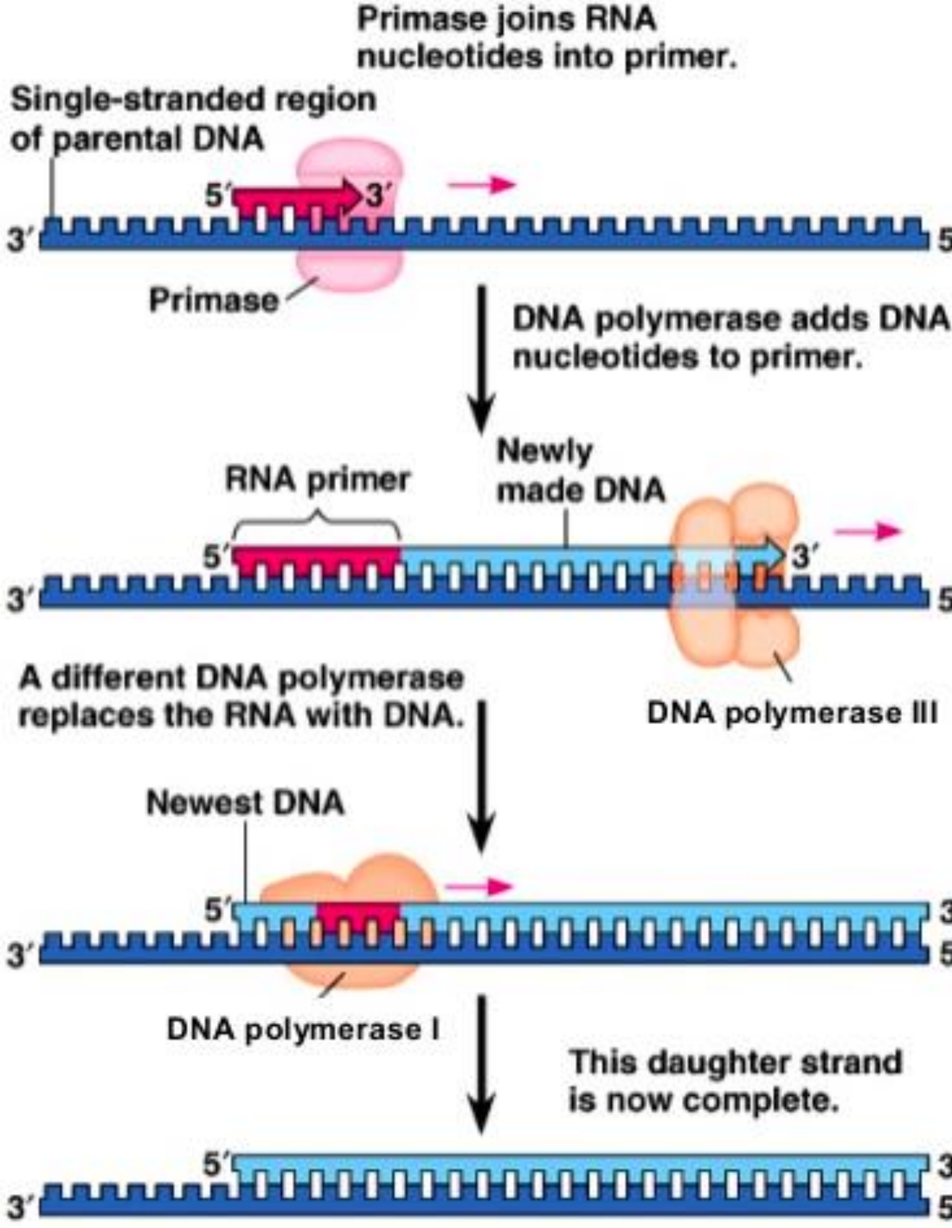
5. DNA Pol I remove RNA primer and replace with DNA

6. Enzyme ligase links all gaps

DNA elongation

DNA pol III :

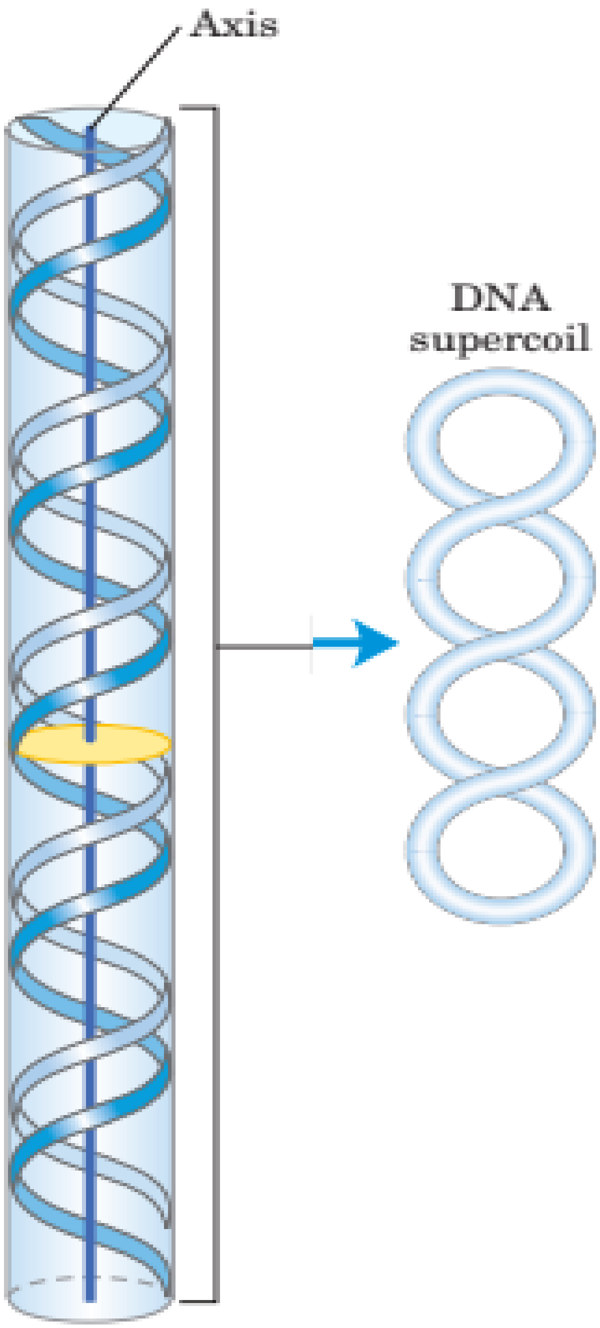
Elongate DNA strand



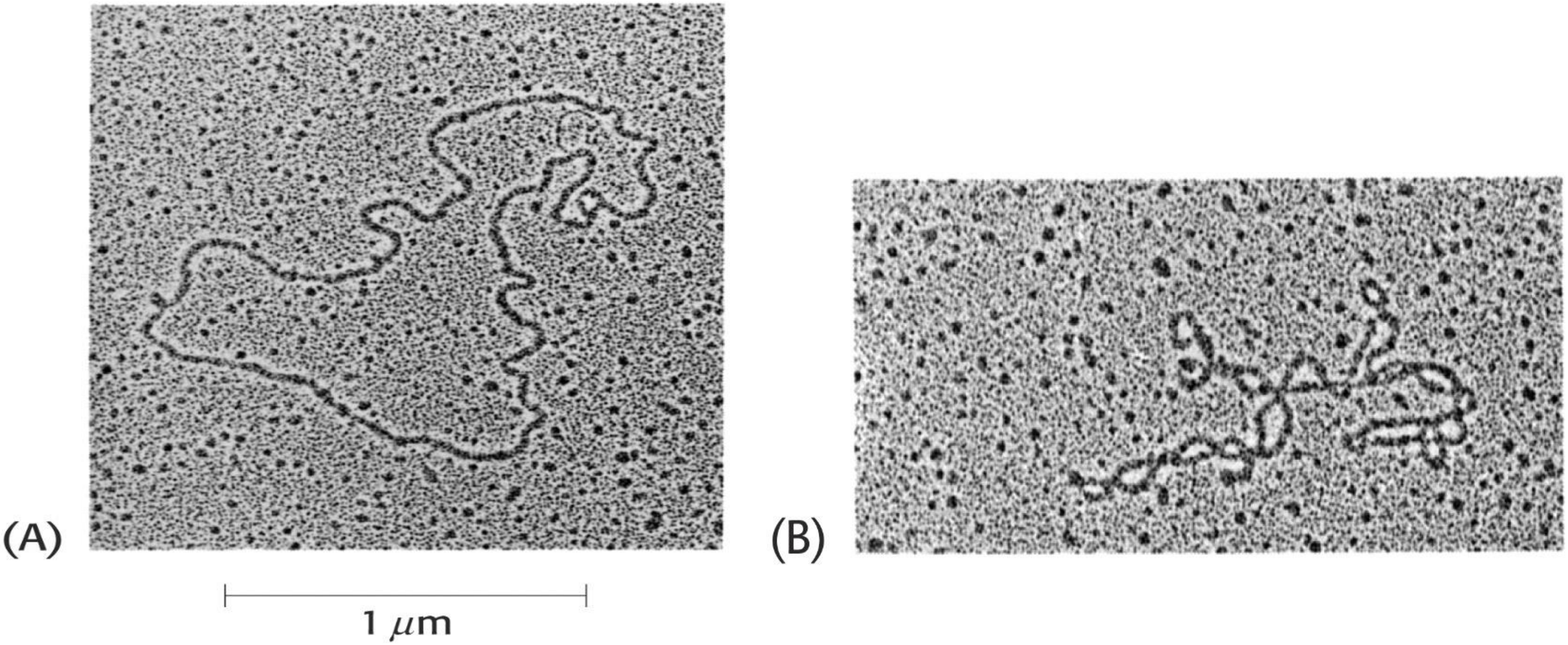
DNA pol I :

Replace RNA with DNA

Supercoils

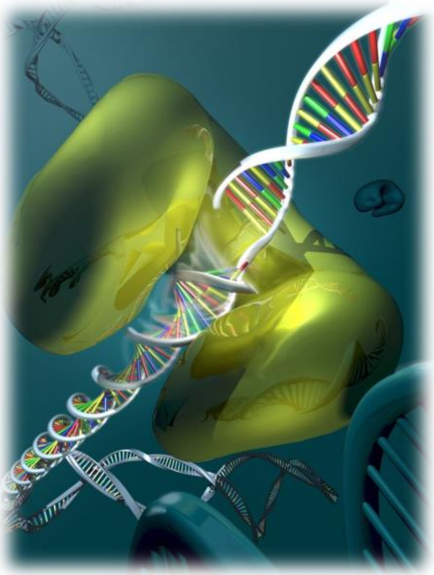


Supercoiled DNA



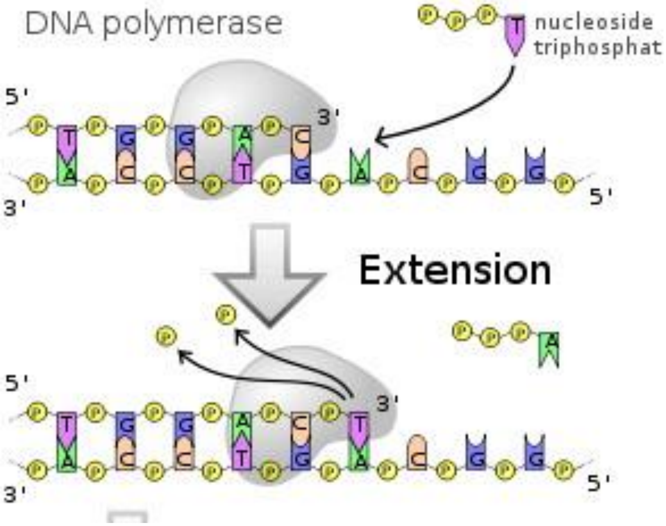
Topoisomerase

- **Type II Topoisomerase** enzyme in human or called **gyrase** in plants and bacteria can cut DNA on both strand while holding. DNA spin to release the twist and ligate.
- The enzyme requires ATP.
- Fluoroquinolone inhibit gyrase. It can be used as a drug to kill bacteria.



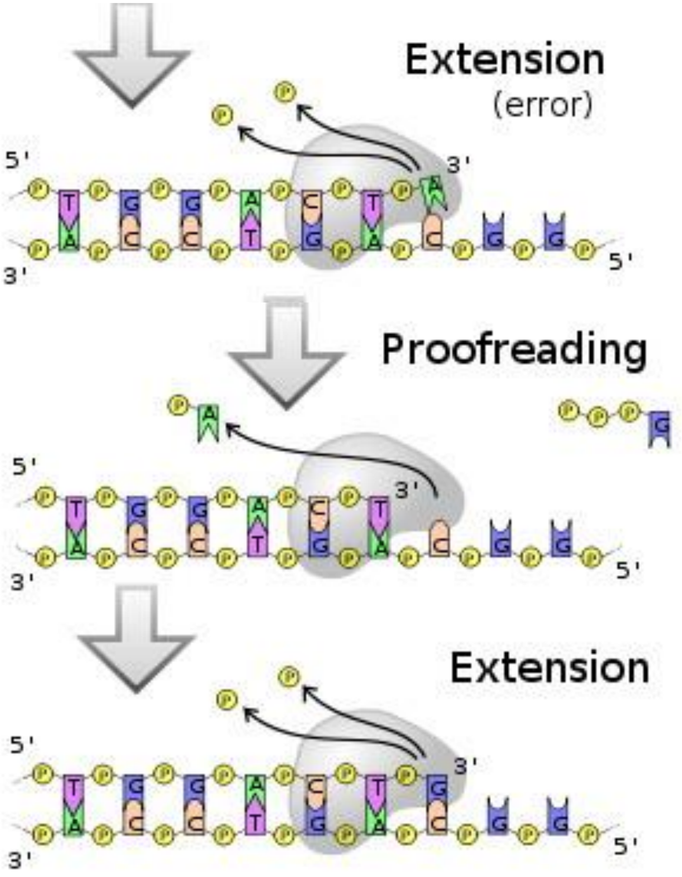
Gyrase, type II topoisomerase

Fidelity of DNA Replication

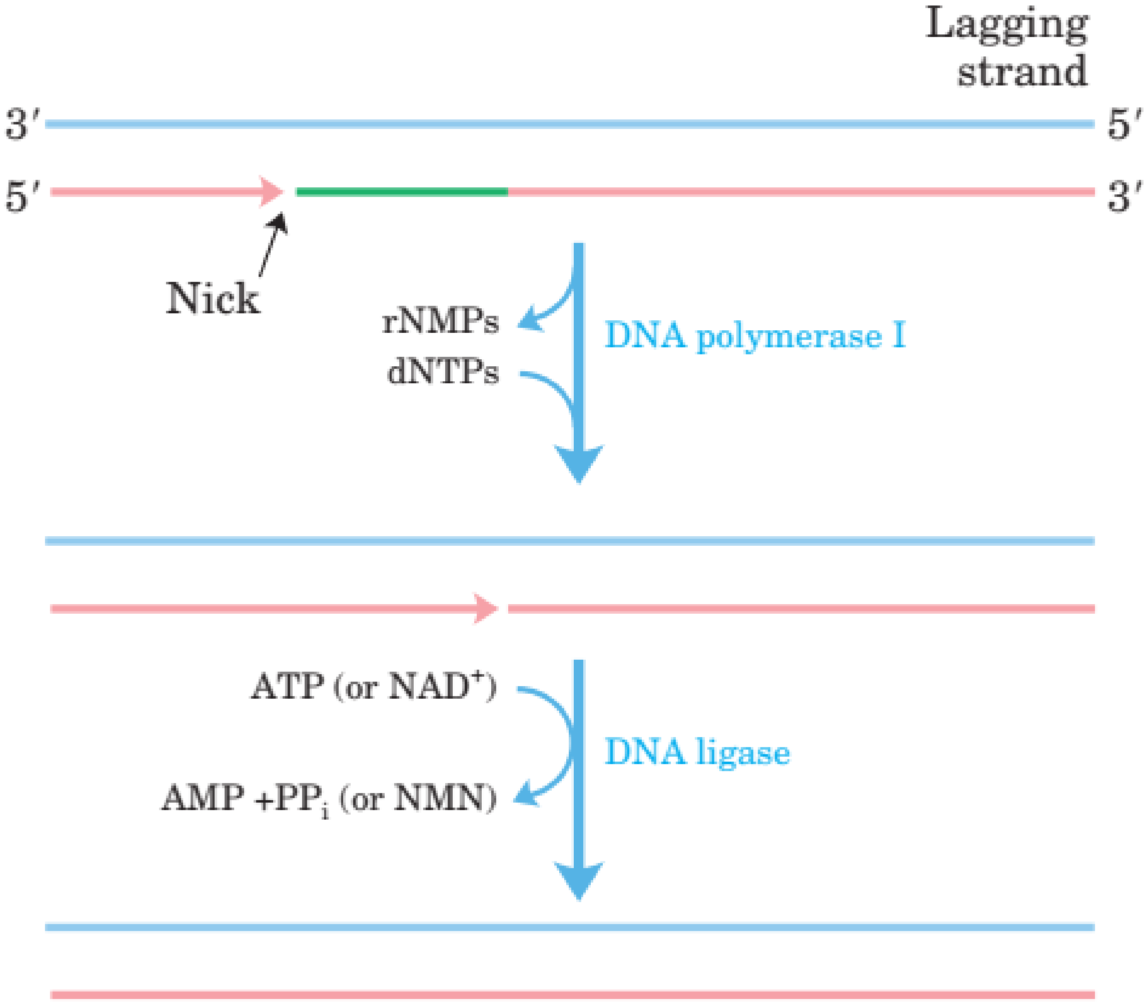


1. Correct bases selection by DNA polymerase III
2. Pol III has proofreading activity which means it go backward (3’→ 5’ exonuclease) to remove wrong nucleotides and extension again.
3. Specific mismatch repair after replication

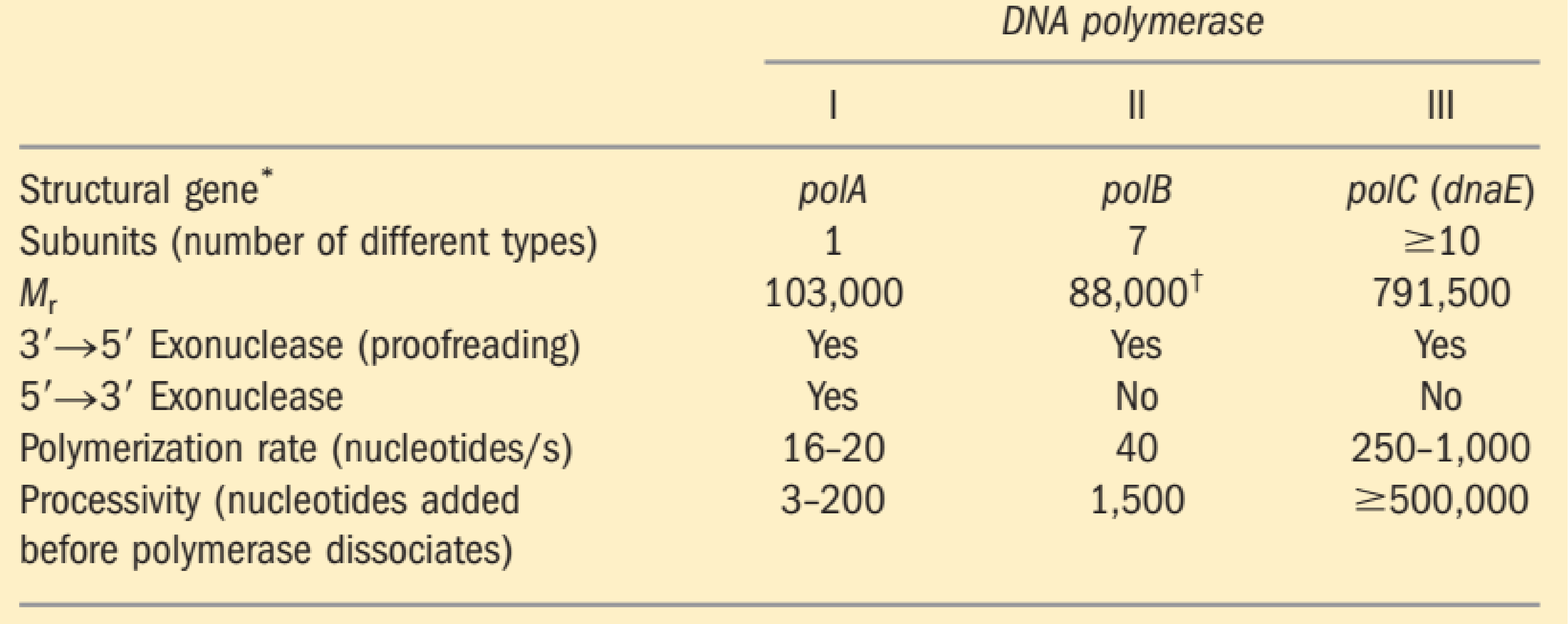
Error can happen 1 in 10^{9}-10^{10} bases.



Final steps in the synthesis of lagging strand

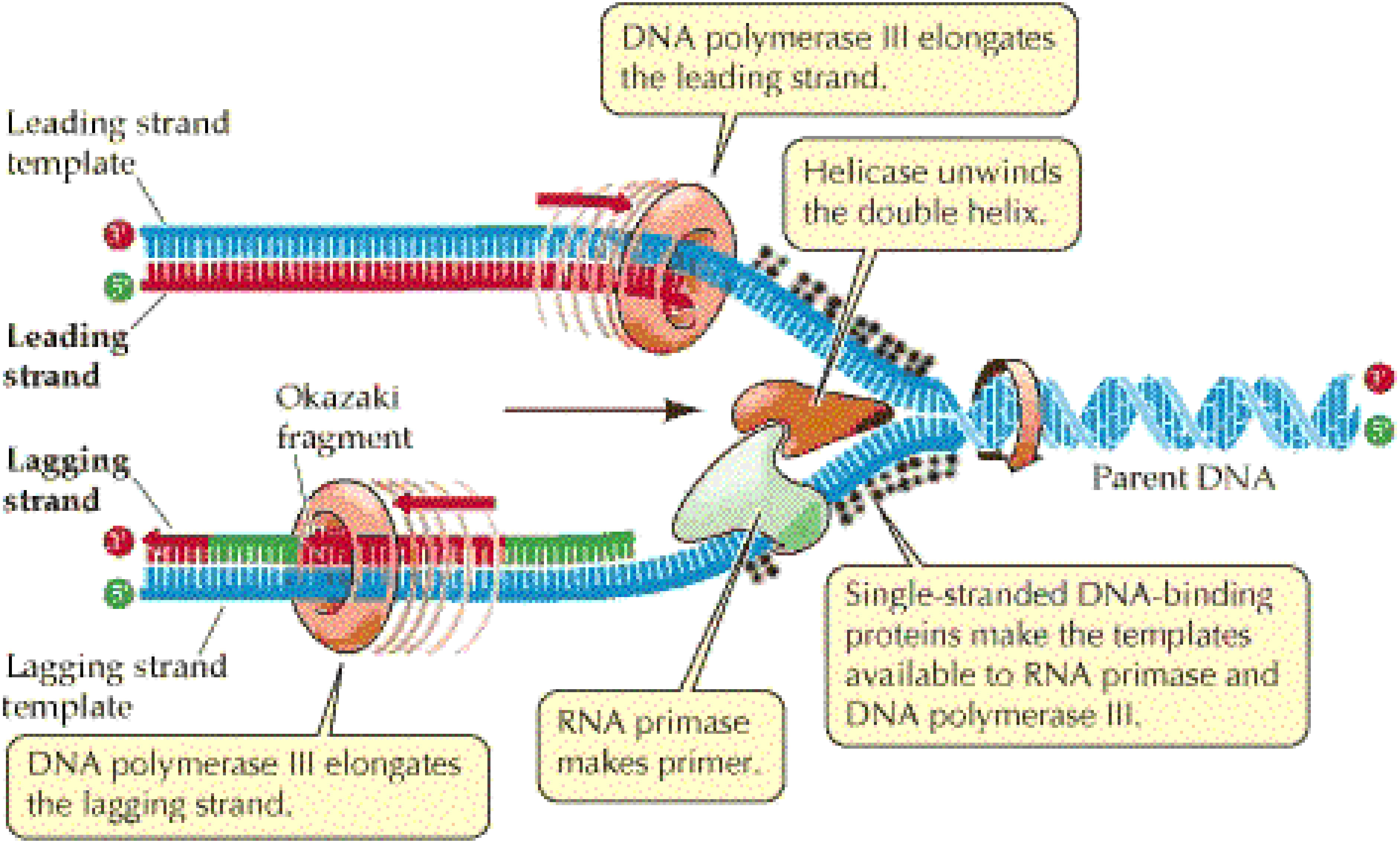


Comparison of DNA Polymerases of Prokaryote



	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

Replication of DNA (Prokaryote)



Replication in Eukaryote

- Multiple origin of replication in one chromosome to make it faster.
- Shorter Okazaki fragments
- histones-DNA complex dissociated before replication fork (That is way replication in eukaryote is slower than bacteria)

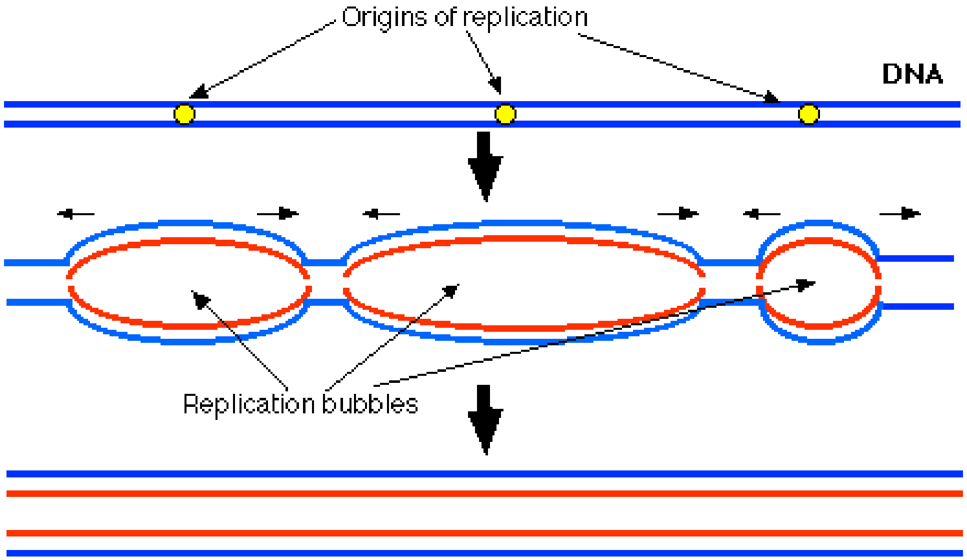
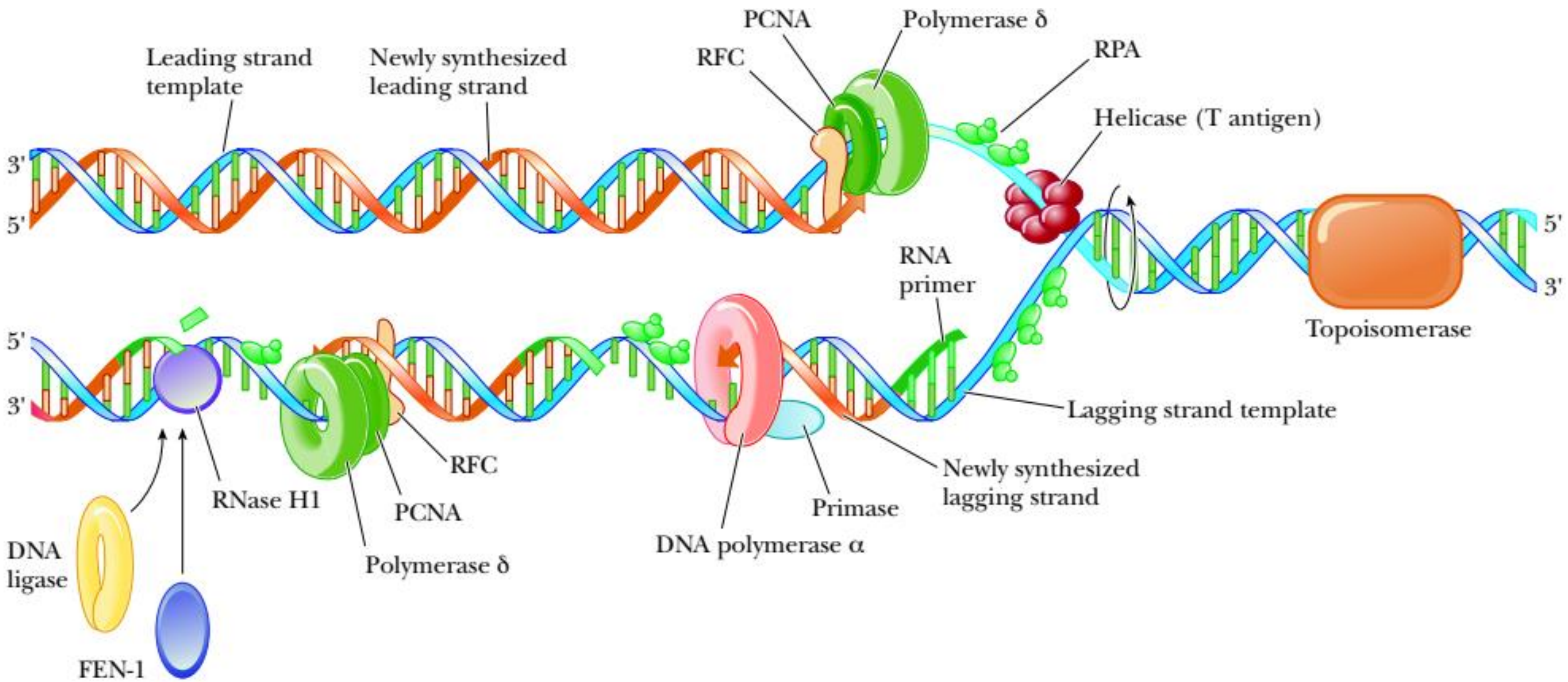


Table 10.4 The Biochemical Properties of Eukaryotic DNA Polymerases

	α	δ	ε	β	γ
Mass (kDa)					
Native	>250	170	256	36–38	160–300
Catalytic core	165–180	125	215	36–38	125
Other subunits	70, 50, 60	48	55	None	35, 47
Location	Nucleus	Nucleus	Nucleus	Nucleus	Mitochondria
Associated functions					
3' → 5' exonuclease	No	Yes	Yes	No	Yes
Primase	Yes	No	No	No	No
Replication	Yes	Yes	Yes	No	Yes
Repair	No	?	Yes	Yes	No

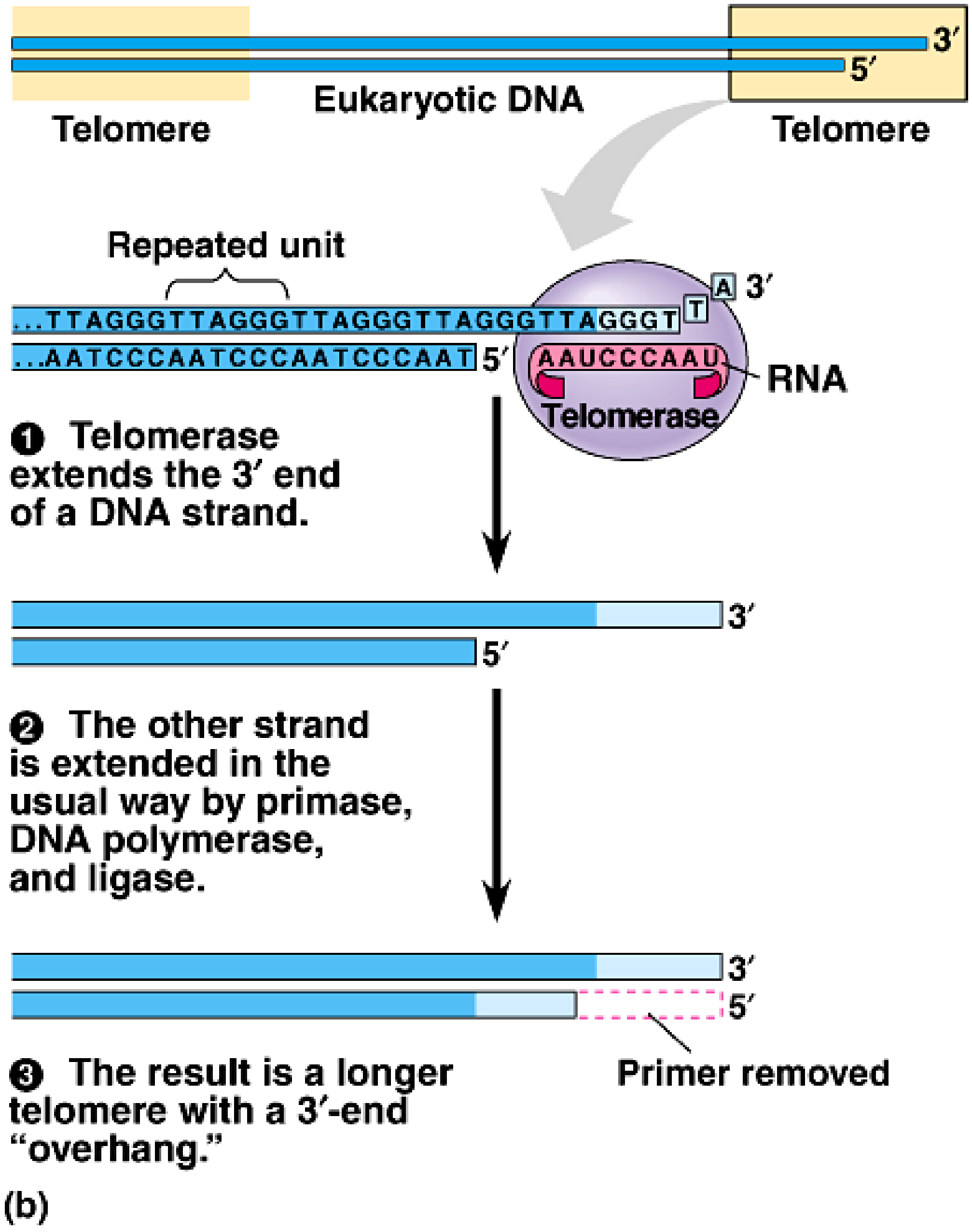
Source: Adapted from Kornberg, A., and Baker, T. A., 1992. *DNA Replication*, 2nd ed. New York: W. H. Freeman and Co.

Replication of DNA (Eukaryote)



Biochemistry, Campbell 9th ed.

Telomeres and telomerase



❶ Telomerase extends the 3′ end of a DNA strand.

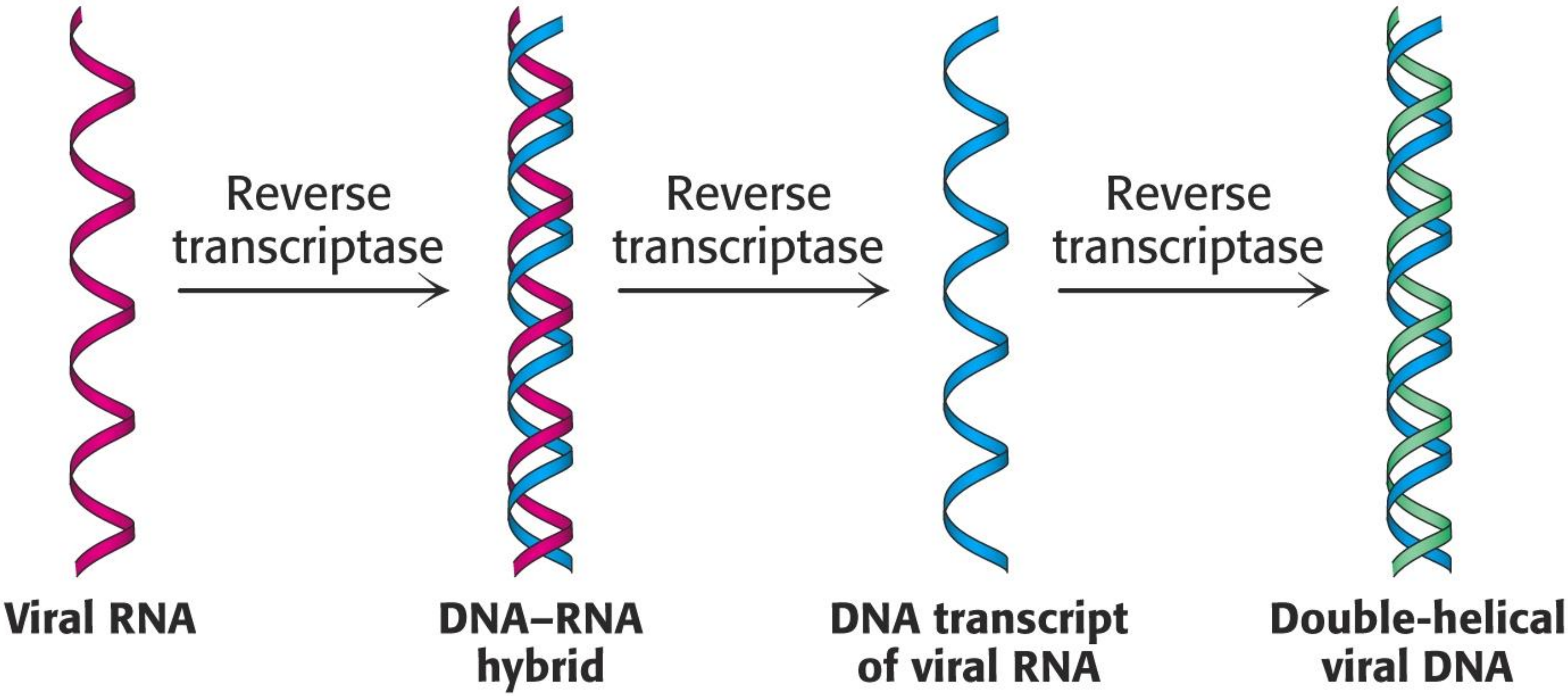
❷ The other strand is extended in the usual way by primase, DNA polymerase, and ligase.

❸ The result is a longer telomere with a 3′-end "overhang."

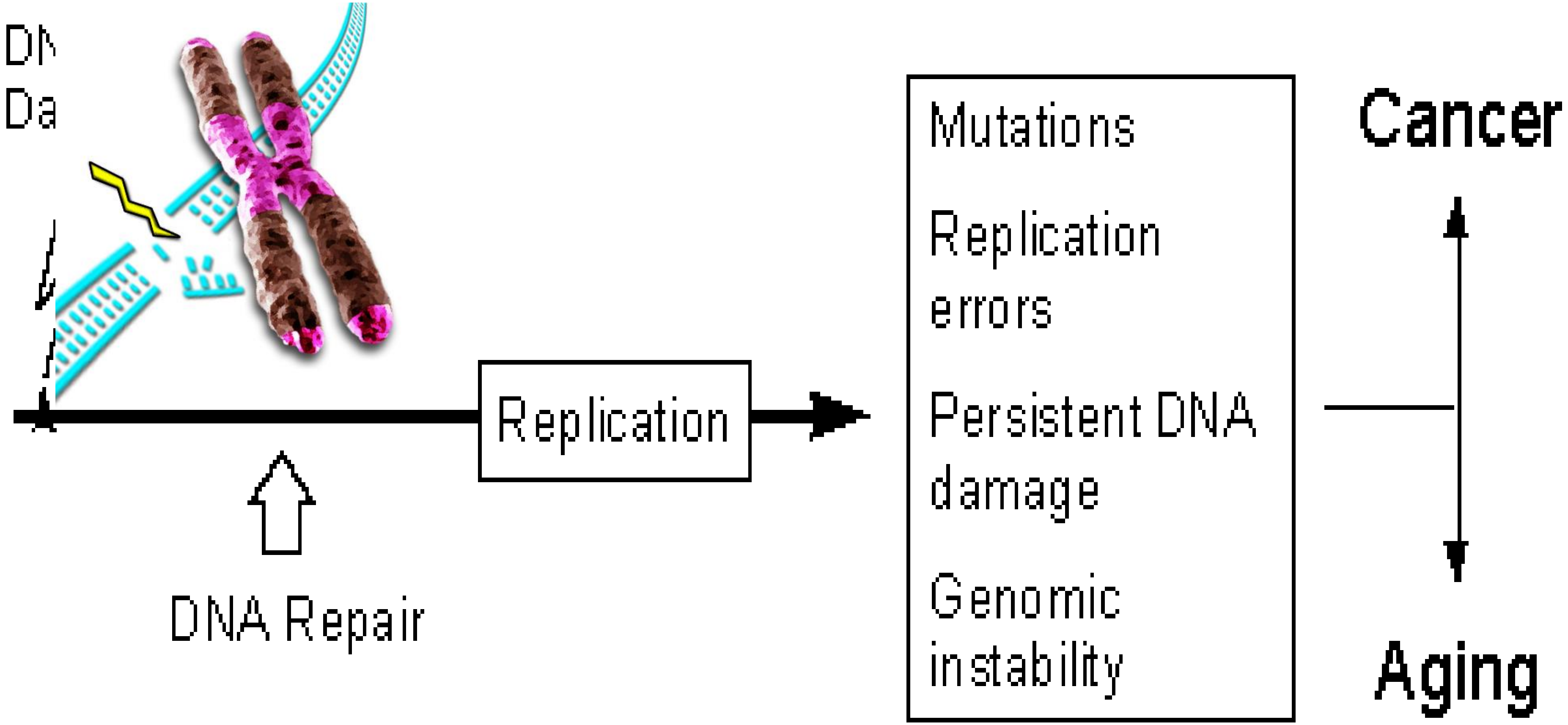
End-replication & telomeres

- DNA polymerase can’t finish the 5’ ends
- DNA would get 10 bases shorter each time replicated
- Telomeres are “expendable” repetitive (TTAGGG) sequences at end of chromosomes
- Telomerase restores telomers, present in germ-line cells and cancerous cells

Replication of Retrovirus



DNA damages & repair



DN
Da

Replication

DNA Repair

Mutations

Replication errors

Persistent DNA damage

Genomic instability

Cancer

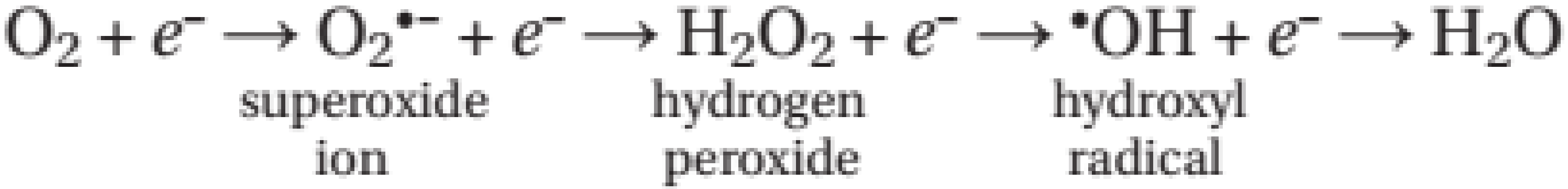
Aging

Mutation = Damage - Repair

DNA damage

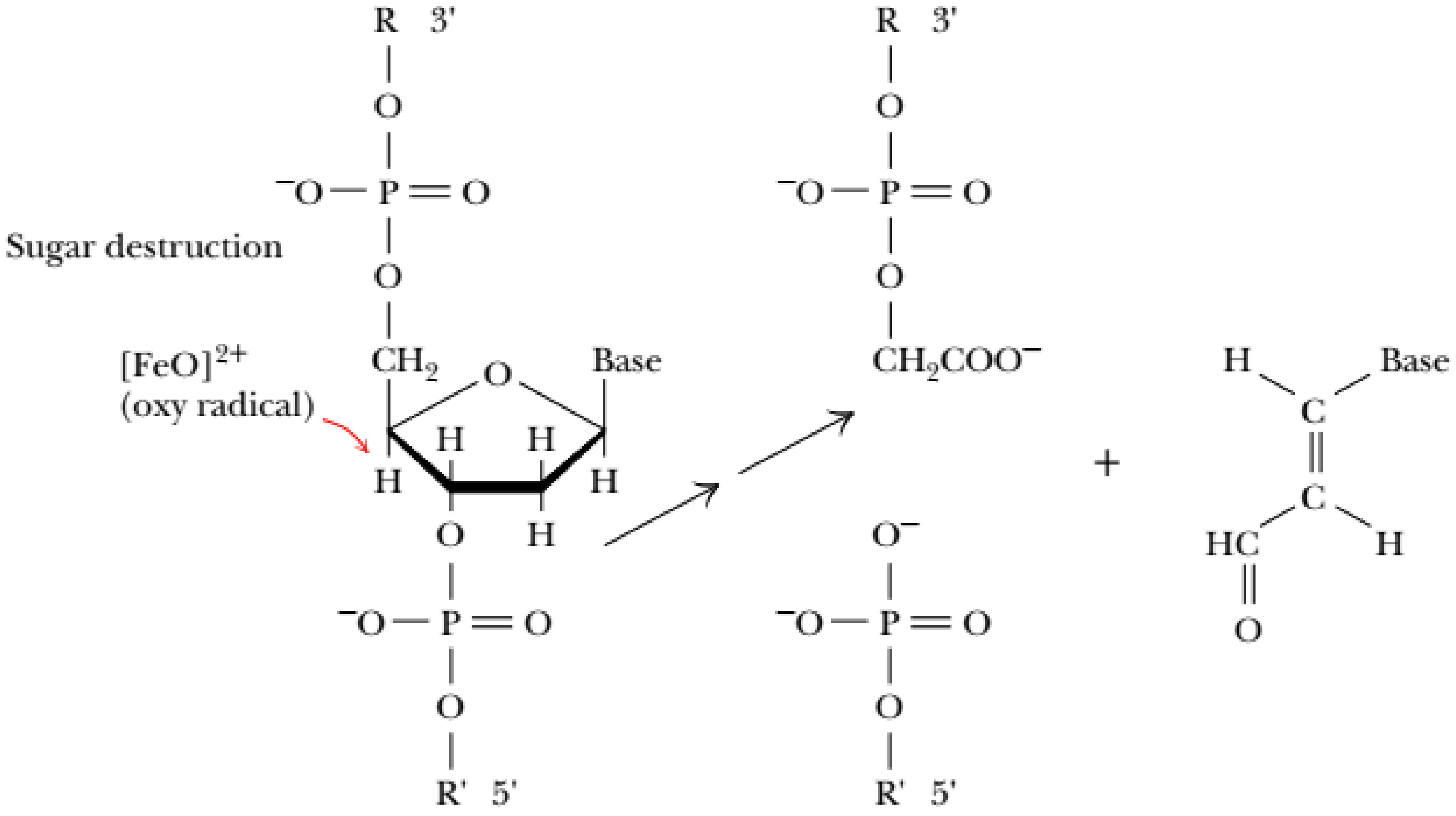
1. Endogenous biochemical processes
2. Exogenous mutagens

Reactive oxygen species (ROS)



$$O_2 + e^- \rightarrow \underset{\text{superoxide ion}}{O_2^{\bullet -}} + e^- \rightarrow \underset{\text{hydrogen peroxide}}{H_2O_2} + e^- \rightarrow \underset{\text{hydroxyl radical}}{{}^{\bullet}OH} + e^- \rightarrow H_2O$$

Oxidation damage



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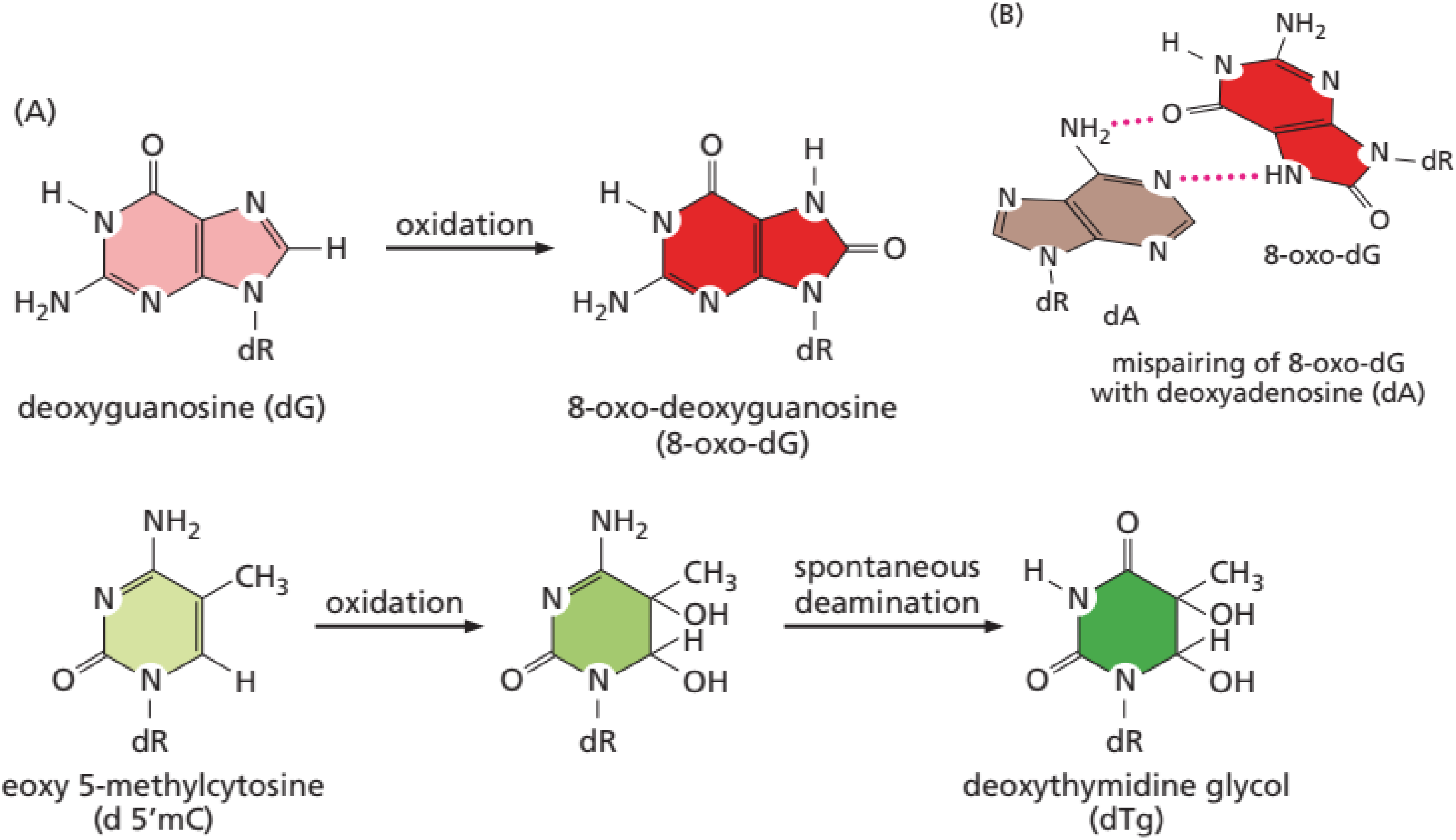
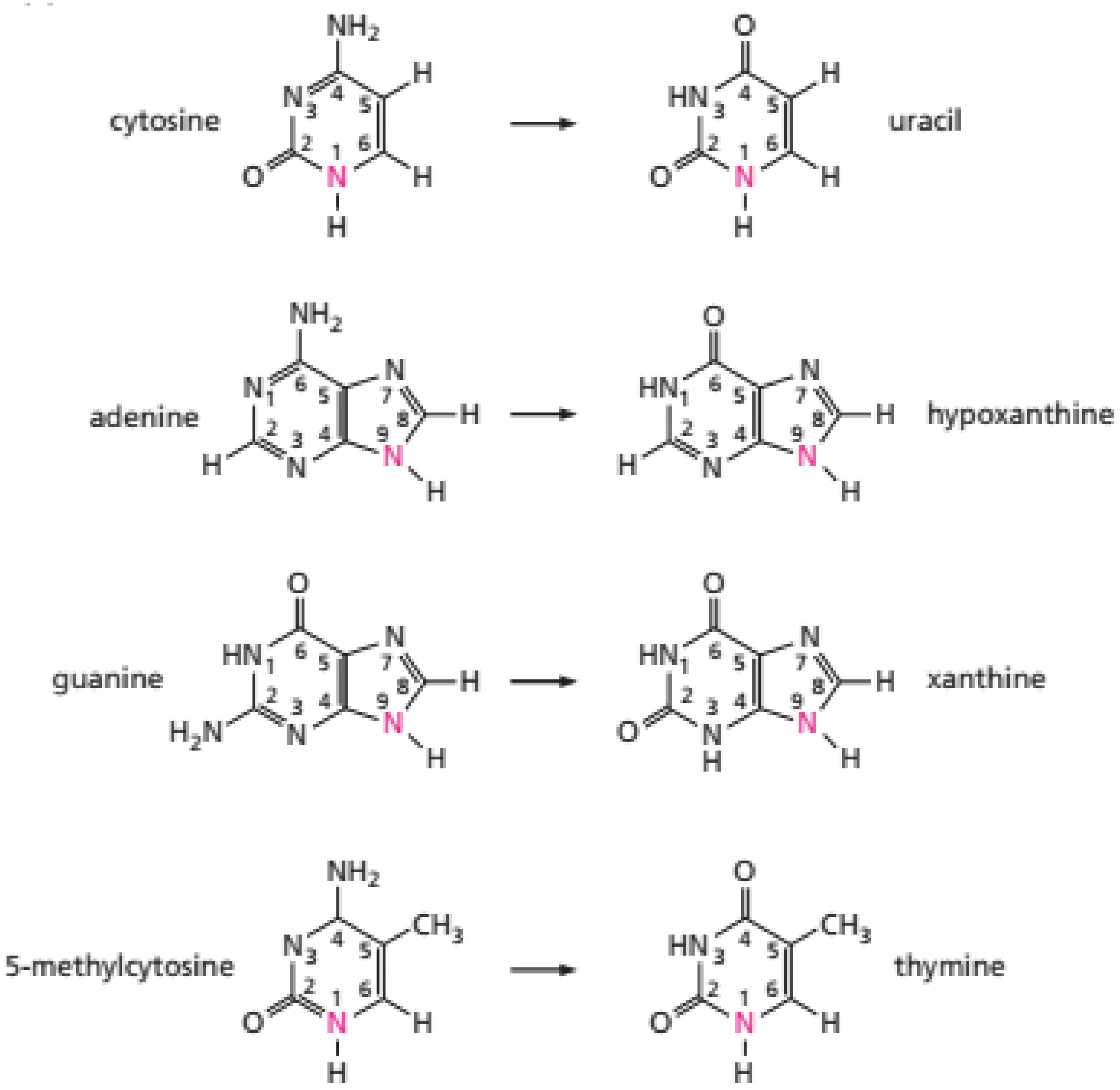
Oxidation damage



The Biology of cancer, Weinberg 2nd ed

Base deamination



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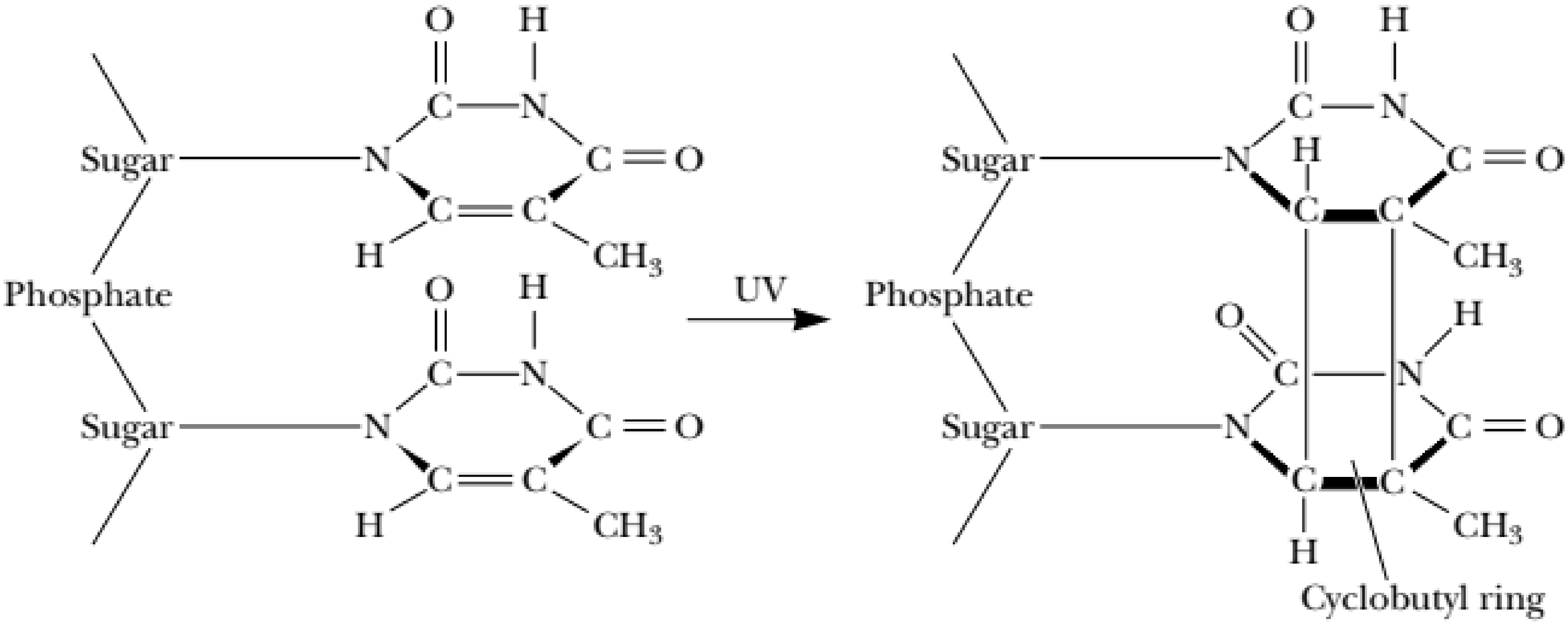
DNA damage

1.Endogenous biochemical processes

2. Exogenous mutagens

UV irradiation



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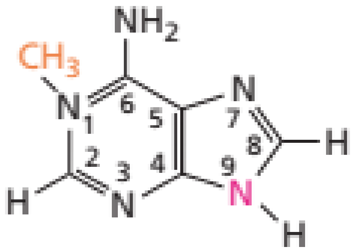
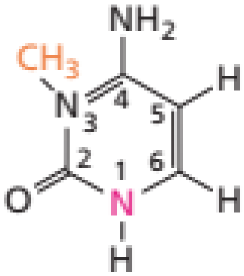
Alkylation

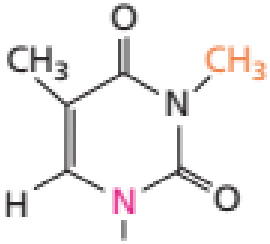
(C)



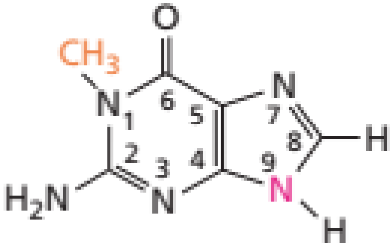
1-methyladenine



3-methylcytosine



3-methylthymine



1-methylguanine

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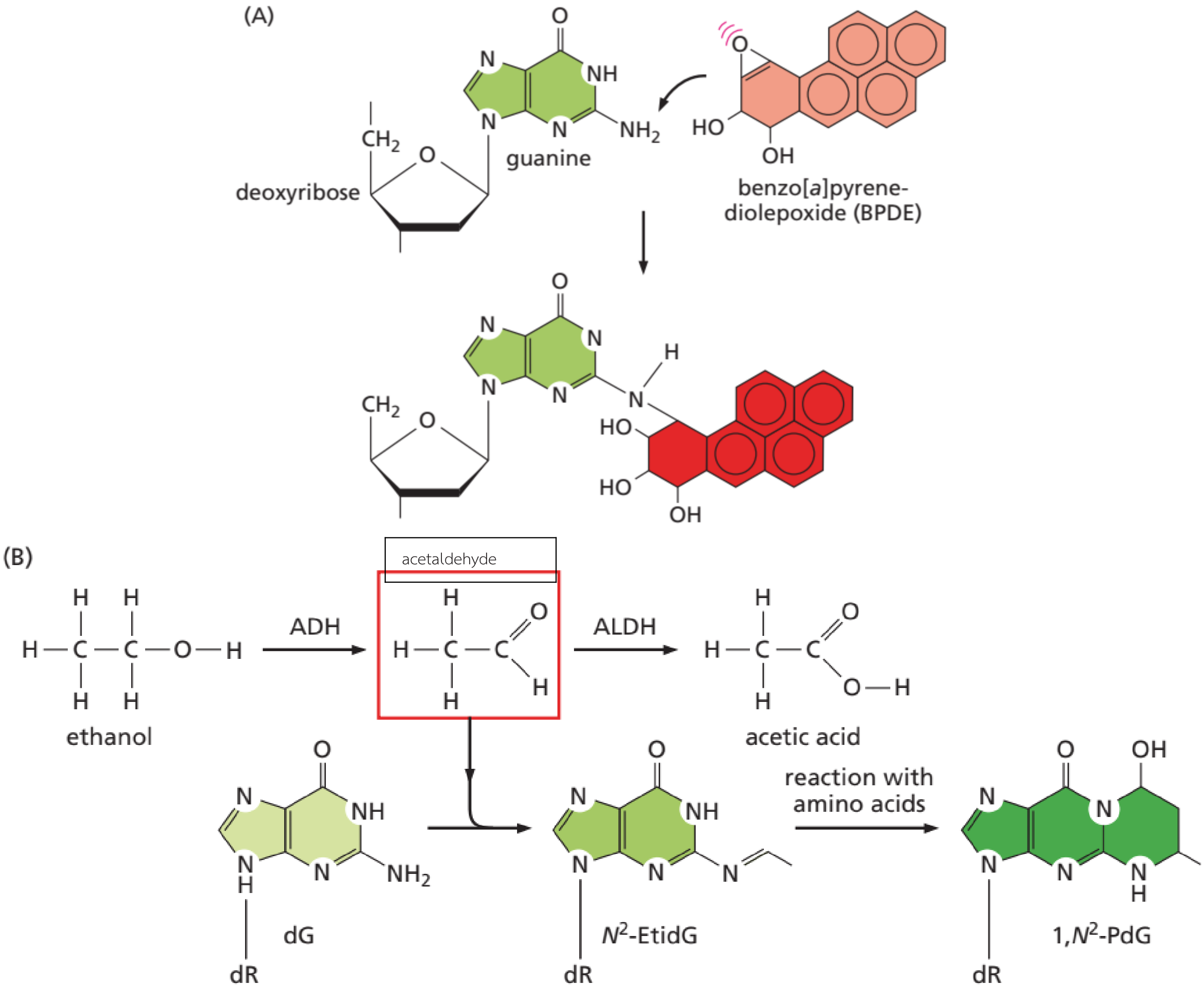
DNA adducts



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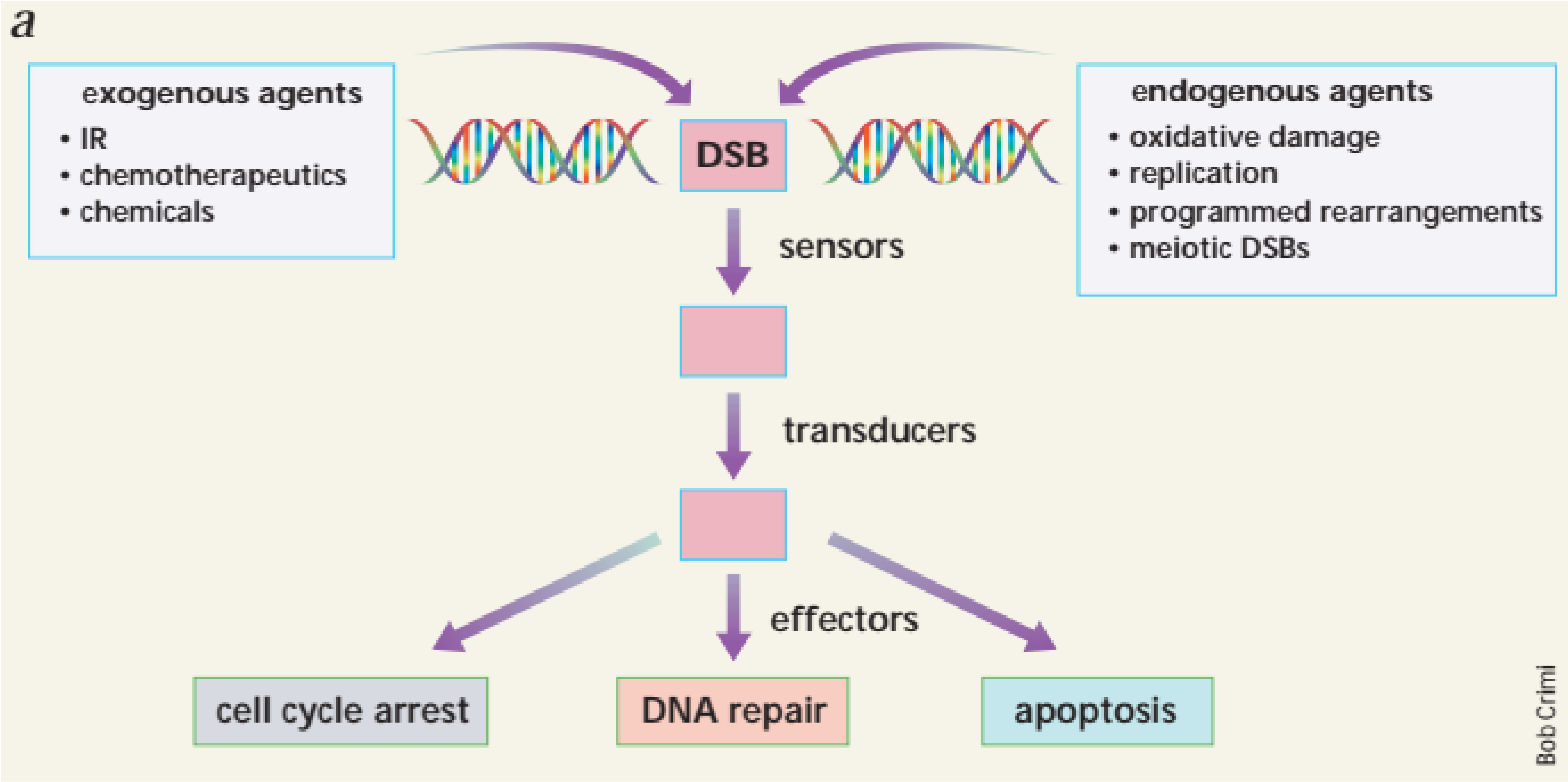
Signaling of DSBs

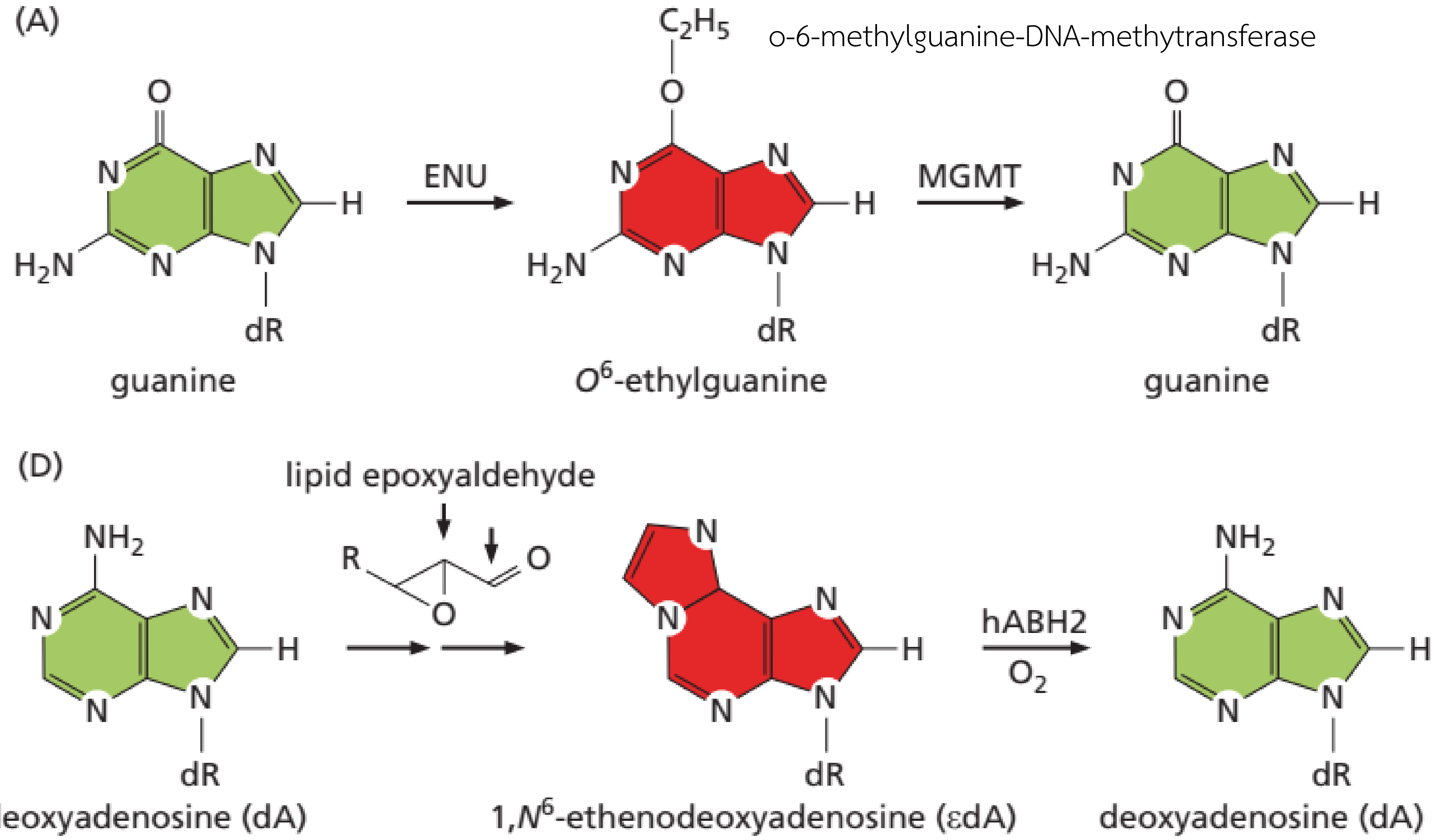


nature genetics • volume 27 • march 2001

DNA repair

- Direct reversal
    - Dealkylating repair enzymes
- Excision repair
    - Proofreading
    - Nucleotide-excision repair
    - Base-Excision Repair
    - Mismatch Repair
- Double-stranded breaks (DBS)
    - Nonhomologous DNA end-joining
    - Homologous recombination

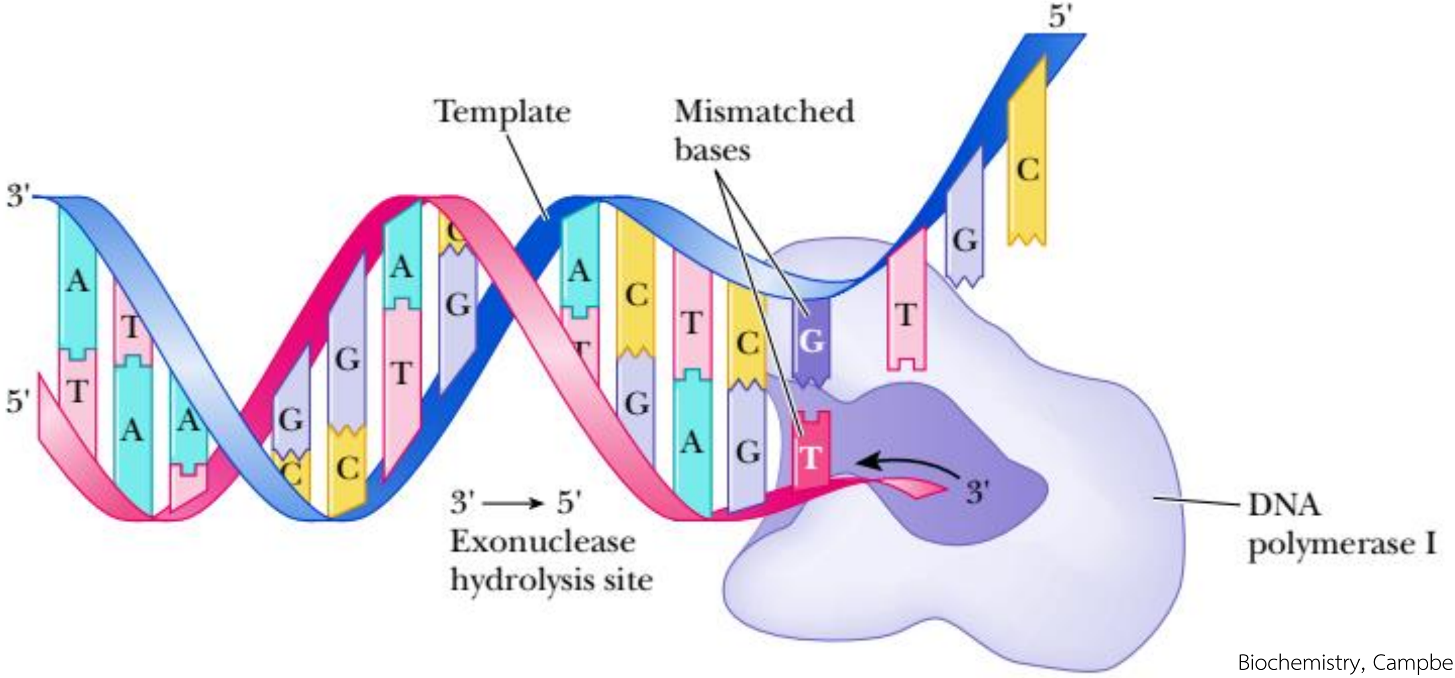
Dealkylating repair enzymes



o-6-methylguanine-DNA-methytransferase

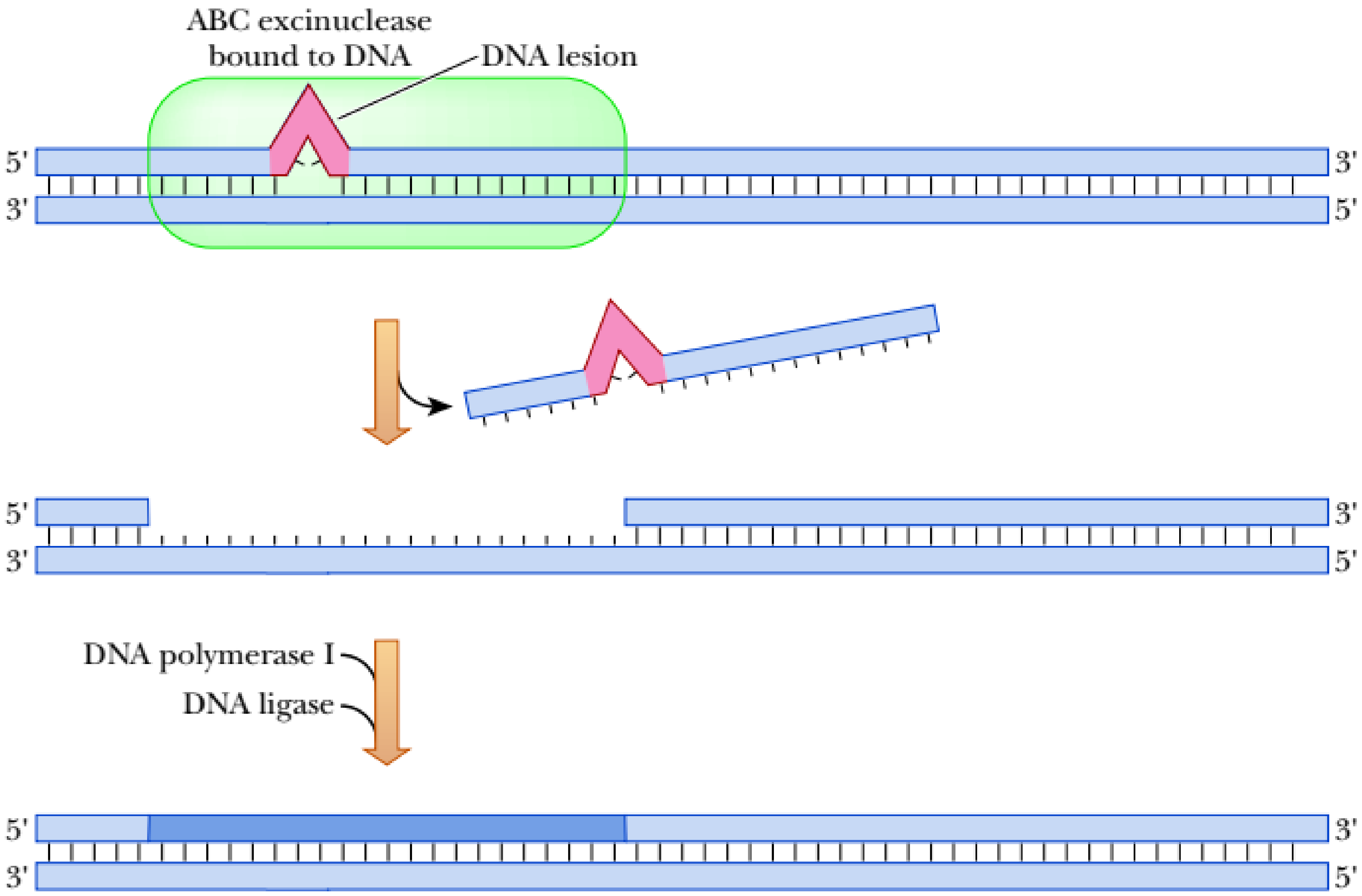
Proofreading

- Errors in replication occur spontaneously only once in every 10^9 to 10^{10} base pairs.
- *Proofreading* -> removal of incorrect nucleotides immediately during the replication process.

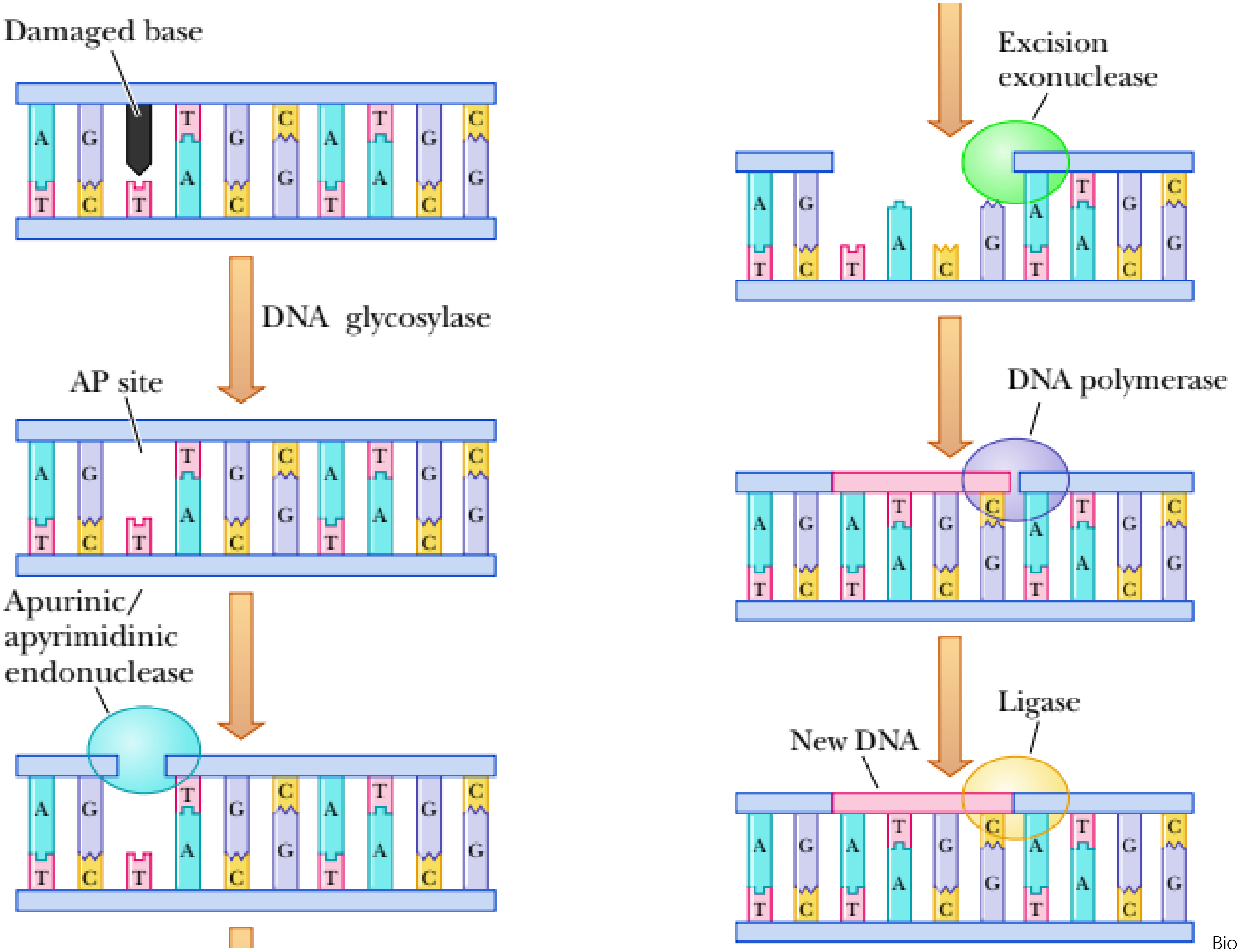


Biochemistry, Campbell 9th ed.

Nucleotide-excision repair



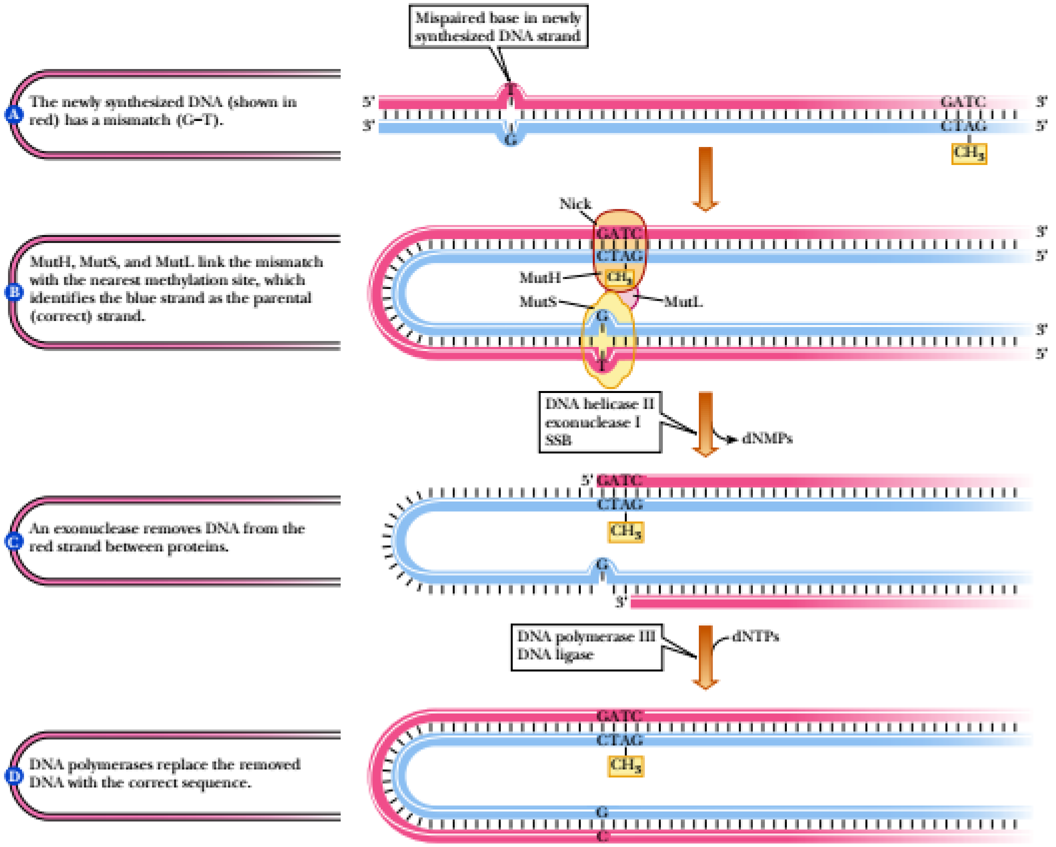
Base-Excision Repair



Biochemistry, Campbell 9th ed.

Mismatch Repair

- Enzymes recognize that two bases are incorrectly paired. The area with the mismatch is removed, and DNA polymerases replicate the area again.



A The newly synthesized DNA (shown in red) has a mismatch (G–T).

B MutH, MutS, and MutL link the mismatch with the nearest methylation site, which identifies the blue strand as the parental (correct) strand.

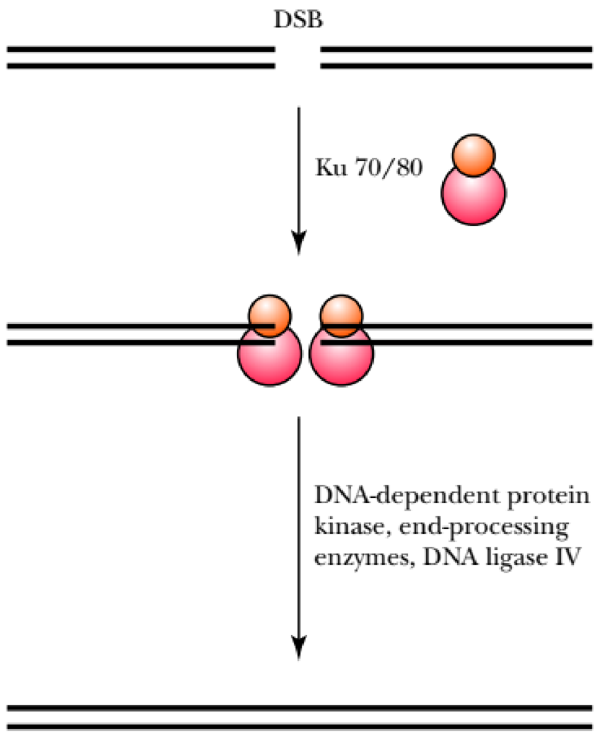
C An exonuclease removes DNA from the red strand between proteins.

D DNA polymerases replace the removed DNA with the correct sequence.

Biochemistry, Campbell 9th ed.

Double-Stranded Breaks (DSB)

- When both strands of DNA are broken
- repair mechanism that exists to handle a DSB is called *nonhomologous DNA end-joining (NHEJ)*

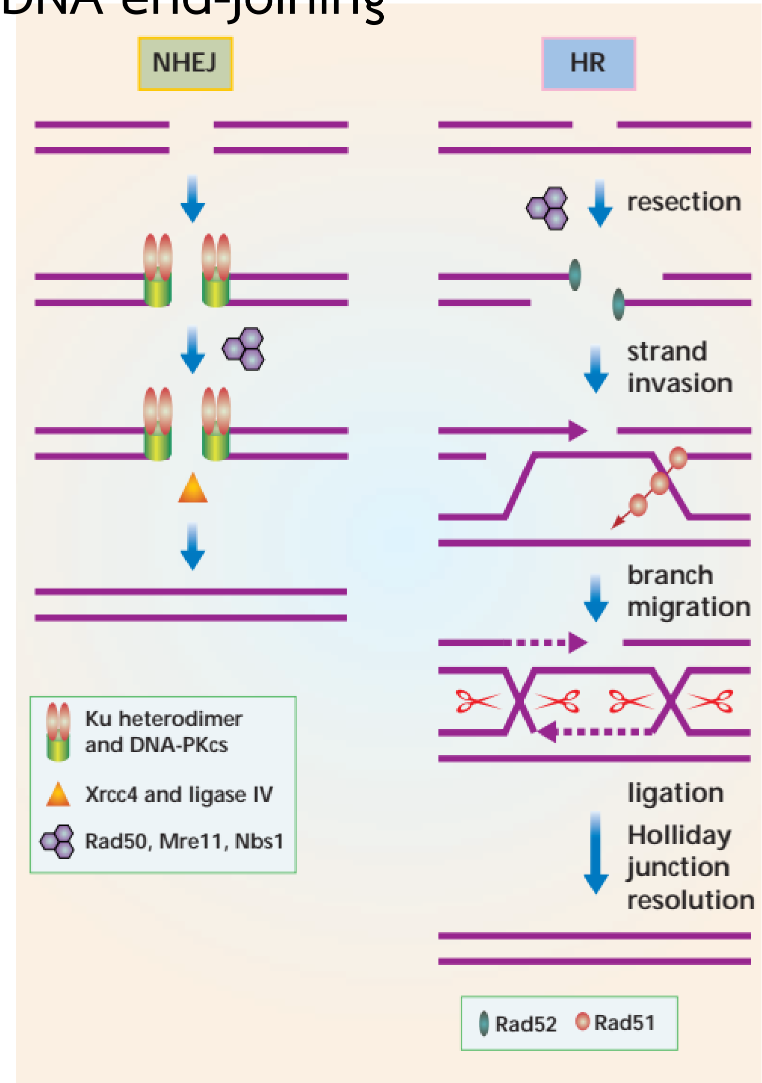


Biochemistry, Campbell 9th ed.

Pathways of DSB repair

Nonhomologous DNA end-joining

Homologous recombination



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Suggested reading