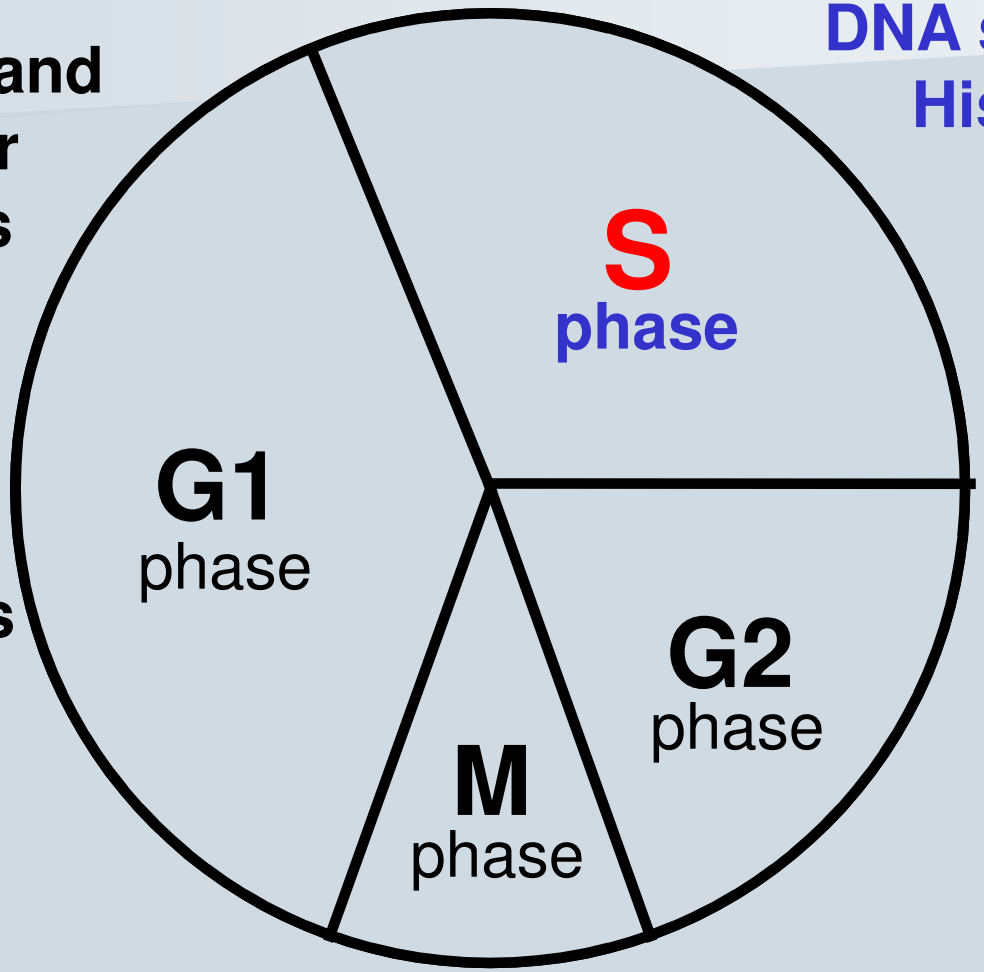
DNA Replication
Mutation during Replication
&
It's Repair

DR PIYUSH B. TAILOR
Associate Professor
Department of Biochemistry
Govt. Medical College
Surat

The mammalian cell cycle



Rapid growth and preparation for DNA synthesis

DNA synthesis and Histone synthesis

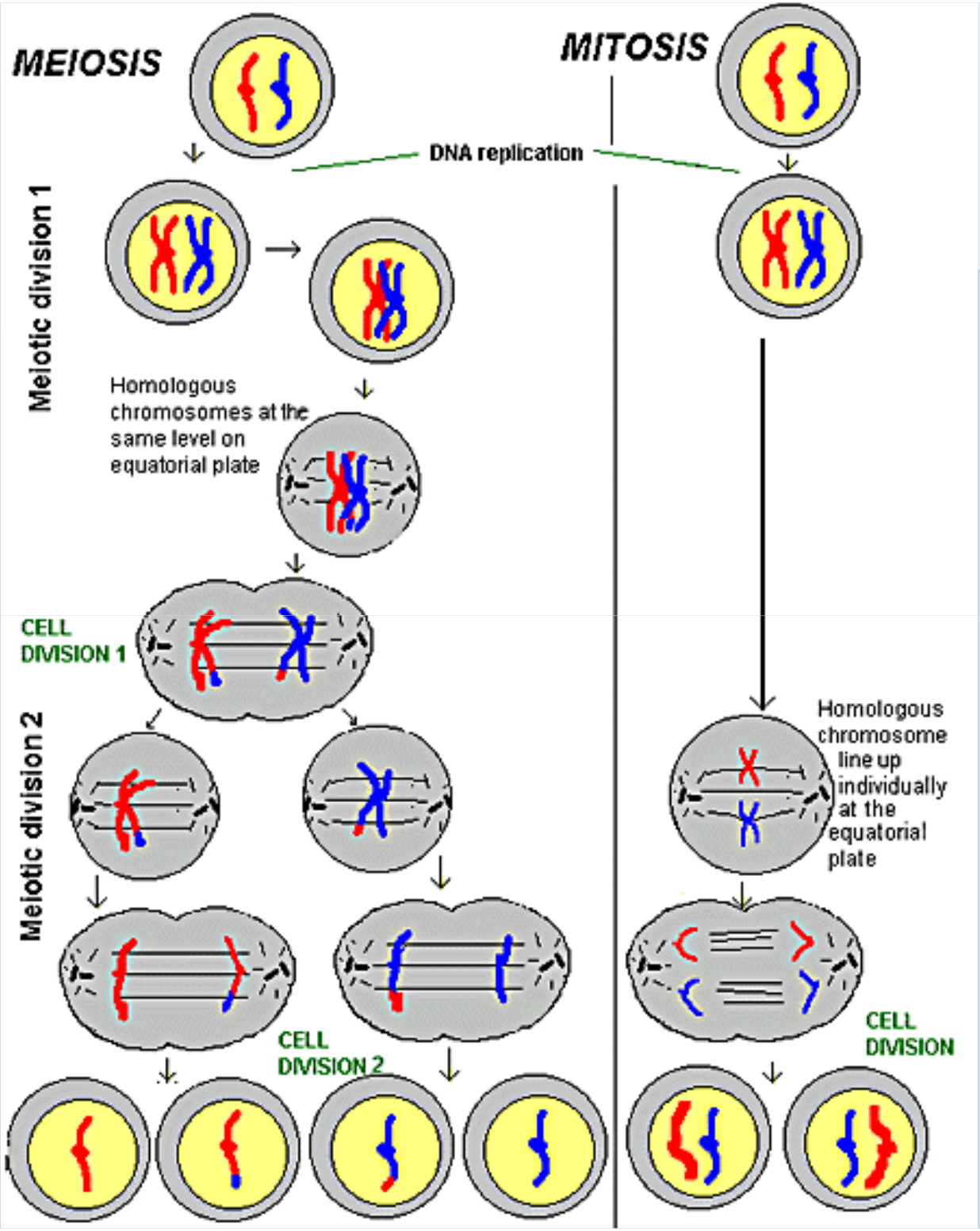
G0

Quiescent cells

After division, Cell either go into G0 or G1

Before division Cell Grow & preparation for cell division

Mitosis

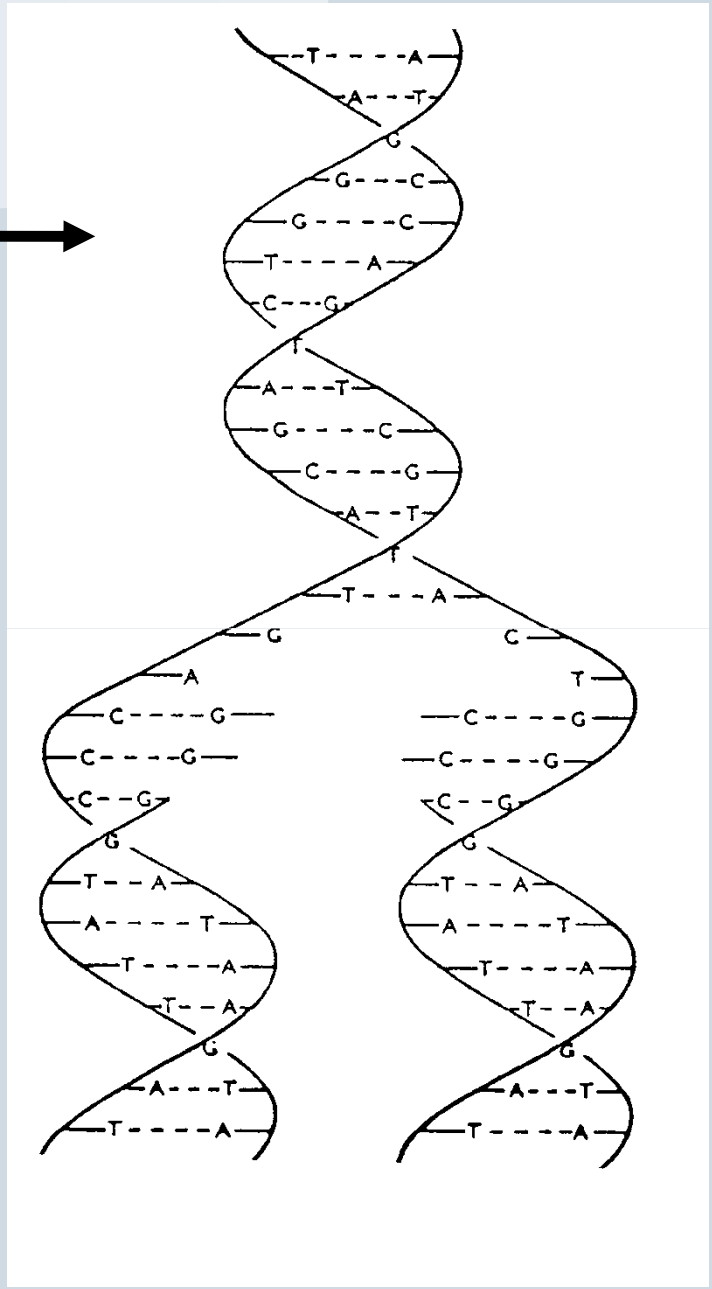

MEIOSIS
MITOSIS
DNA replication
Meiotic division 1
Homologous chromosomes at the same level on equatorial plate
CELL DIVISION 1
Meiotic division 2
Homologous chromosome line up individually at the equatorial plate
CELL DIVISION 2
CELL DIVISION

DNA replication is semi-conservative

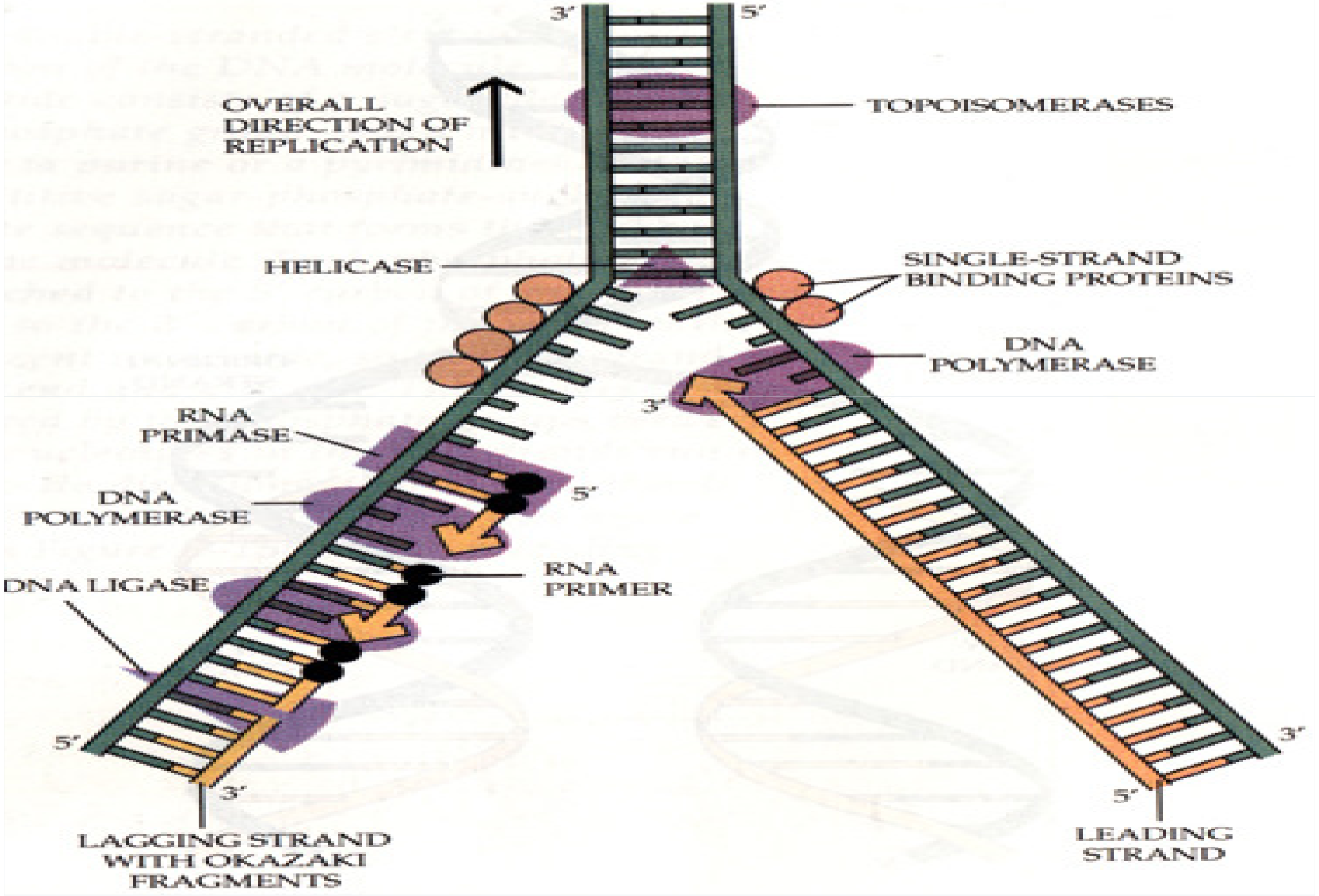
Parental DNA strands →

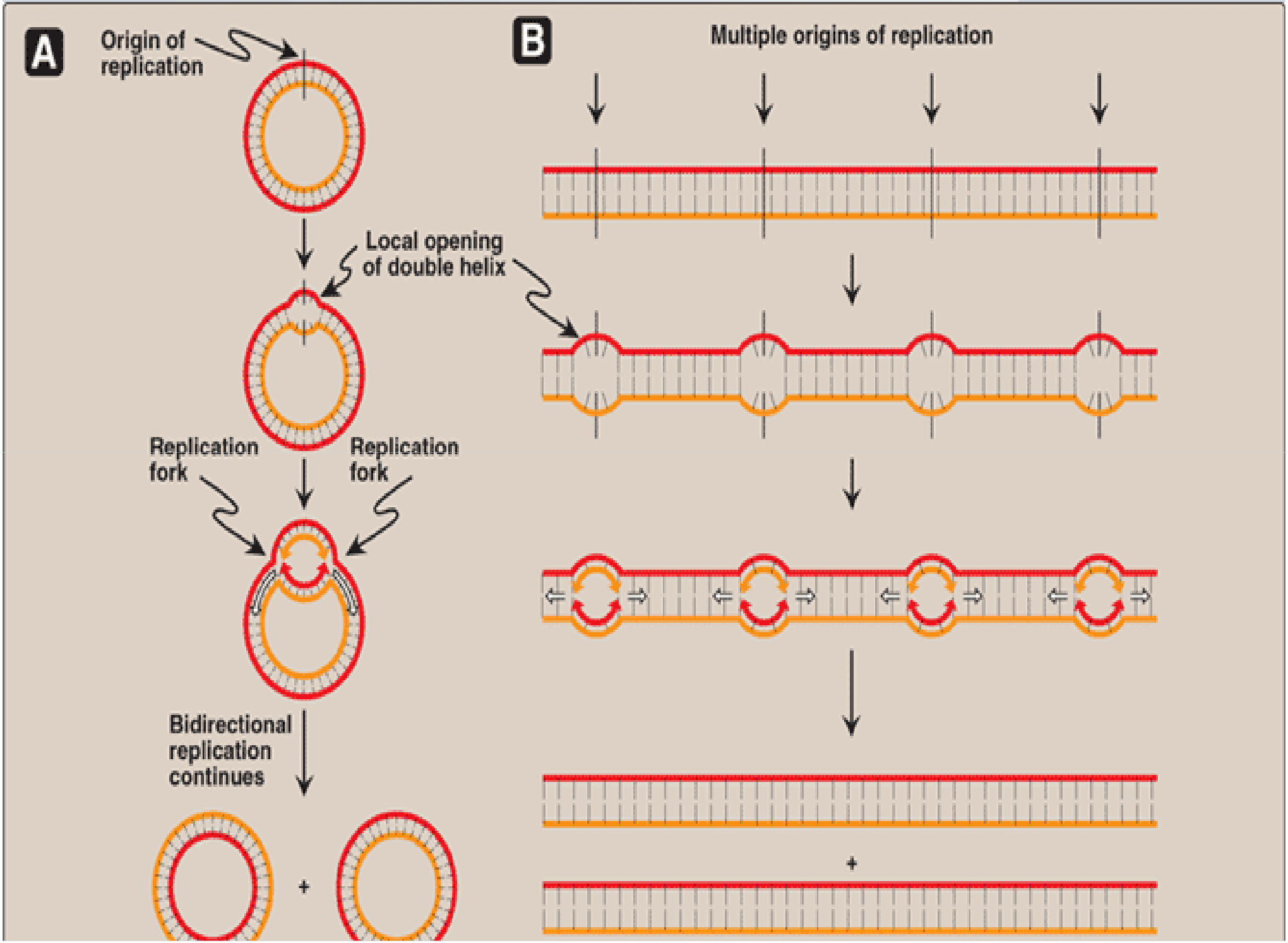
Each of the parental strands serves as a template for a daughter strand

Daughter DNA strands →

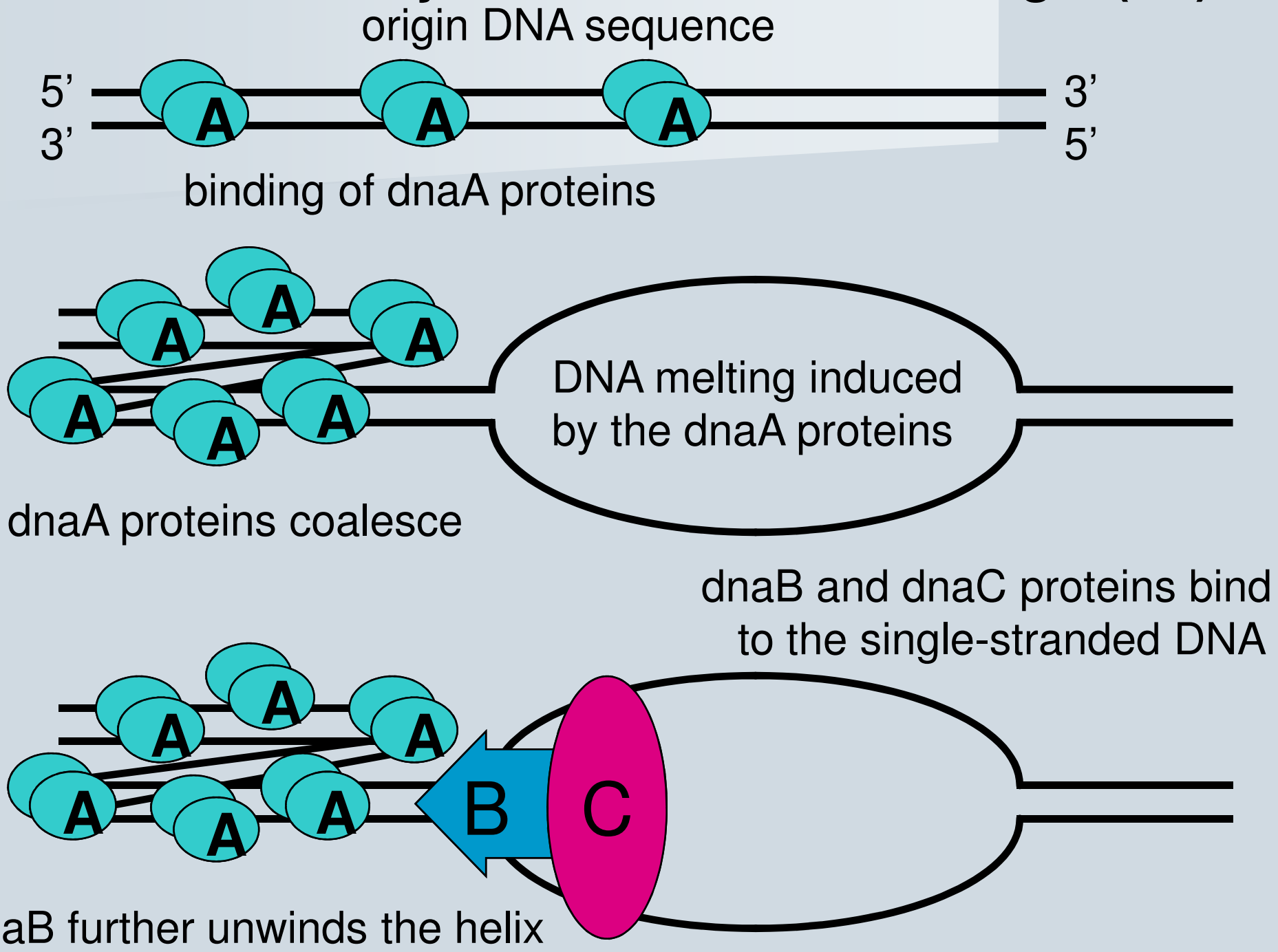


Replication




A
Origin of replication
Local opening of double helix
Replication fork
Replication fork
Bidirectional replication continues
+
B
Multiple origins of replication
+

Initiation of DNA synthesis at the E. coli origin (ori)



Dna A protein:

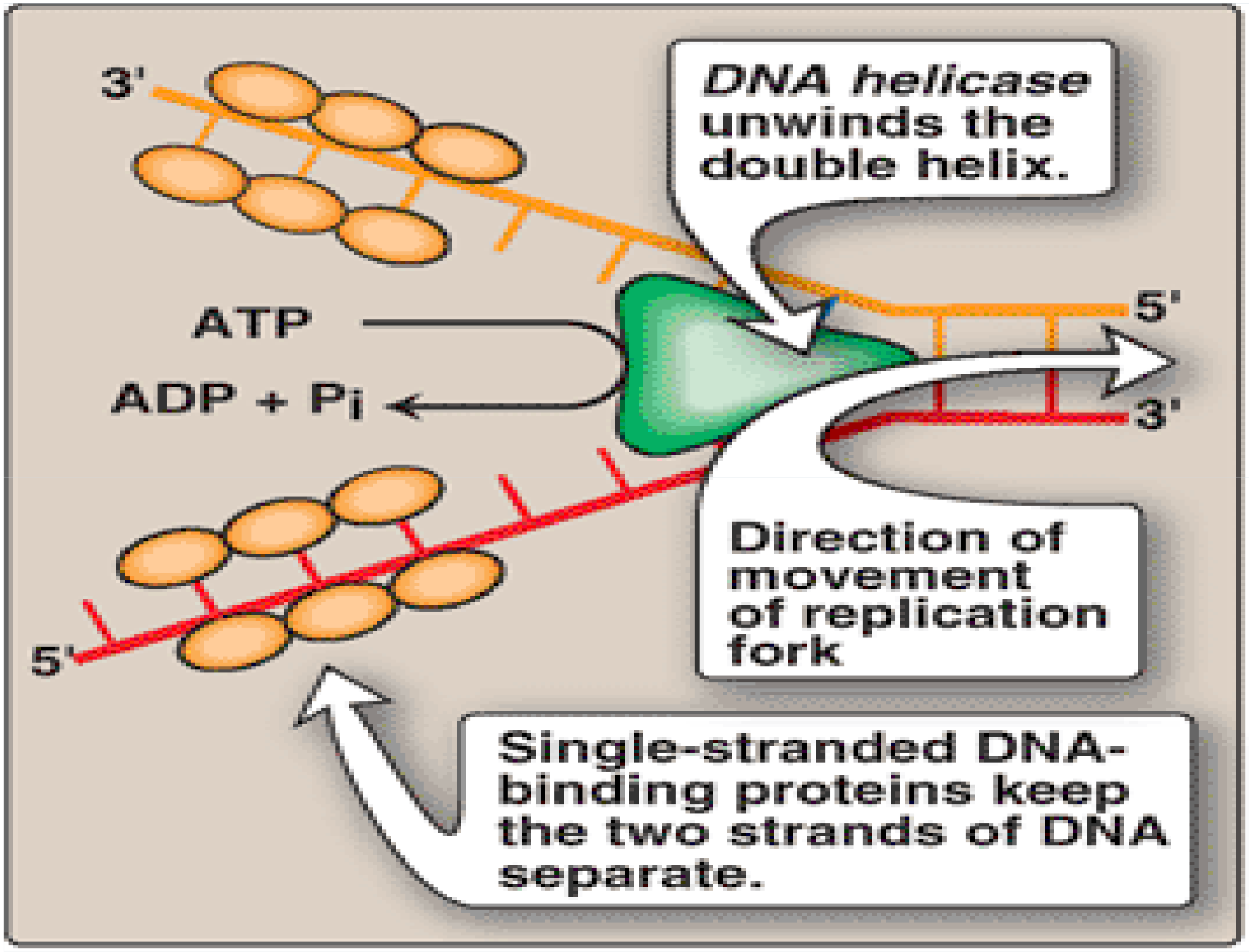
- Bind at the origin of replication
- Binds to specific nucleotide sequences
  - at AT-rich regions.
- ATP-dependent
- Strand separation
- Formation of localized ssDNA.

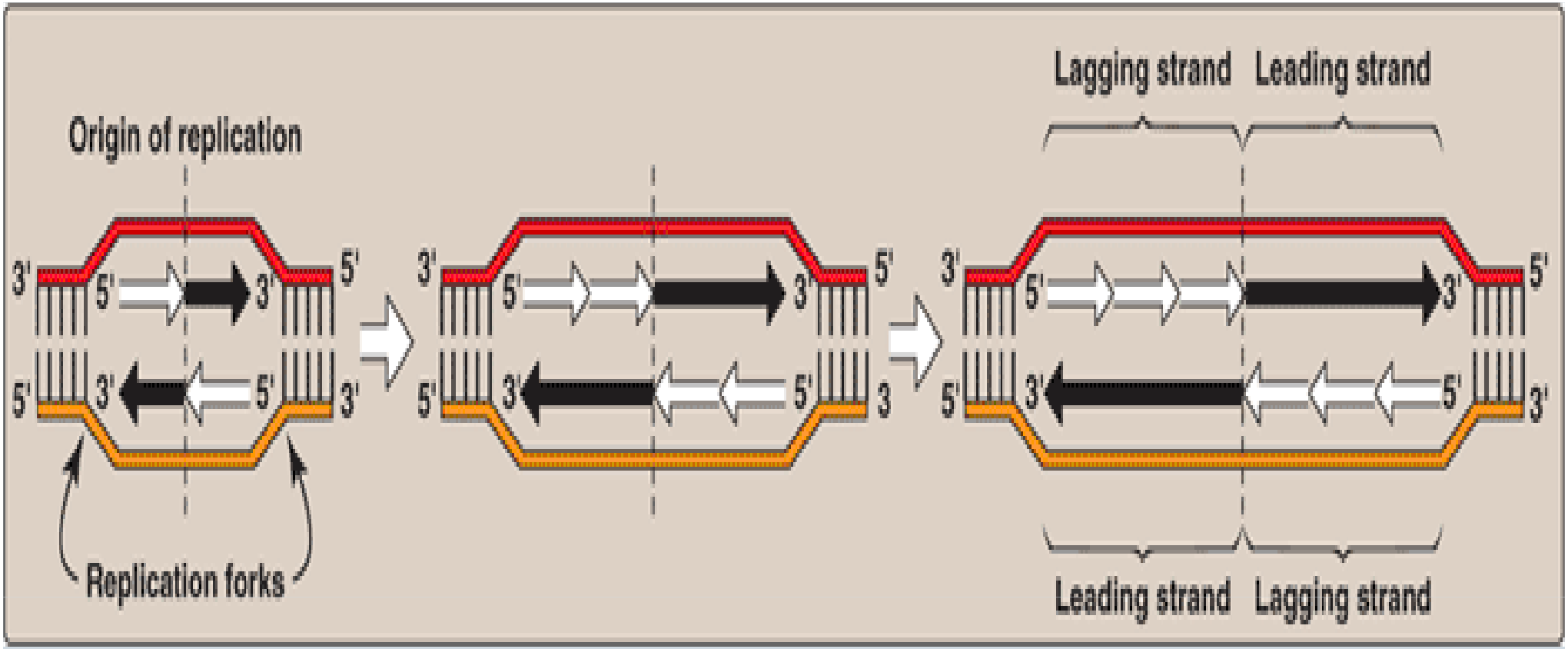
DNA helicases:

- Bind to ssDNA near replication fork
- Unwind double helix.
- ATP energy dependent

Single-stranded DNA-binding (SSB) proteins:

- Bind to the ssDNA
- Bind cooperatively
  - binding of one SSBP makes easier for another SSBP to bind tightly .
- Keep two strands of DNA separated
- Protect DNA from nucleases activity that cleave ssDNA.


3'
DNA helicase unwinds the double helix.
ATP
ADP + Pi
5'
3'
5'
Direction of movement of replication fork
Single-stranded DNA-binding proteins keep the two strands of DNA separate.



The DNA polymerases

= Copying the DNA templates

= Read parental sequences in the 3′→5′ direction

= Synthesize new DNA strands in the 5′→3′ (antiparallel)

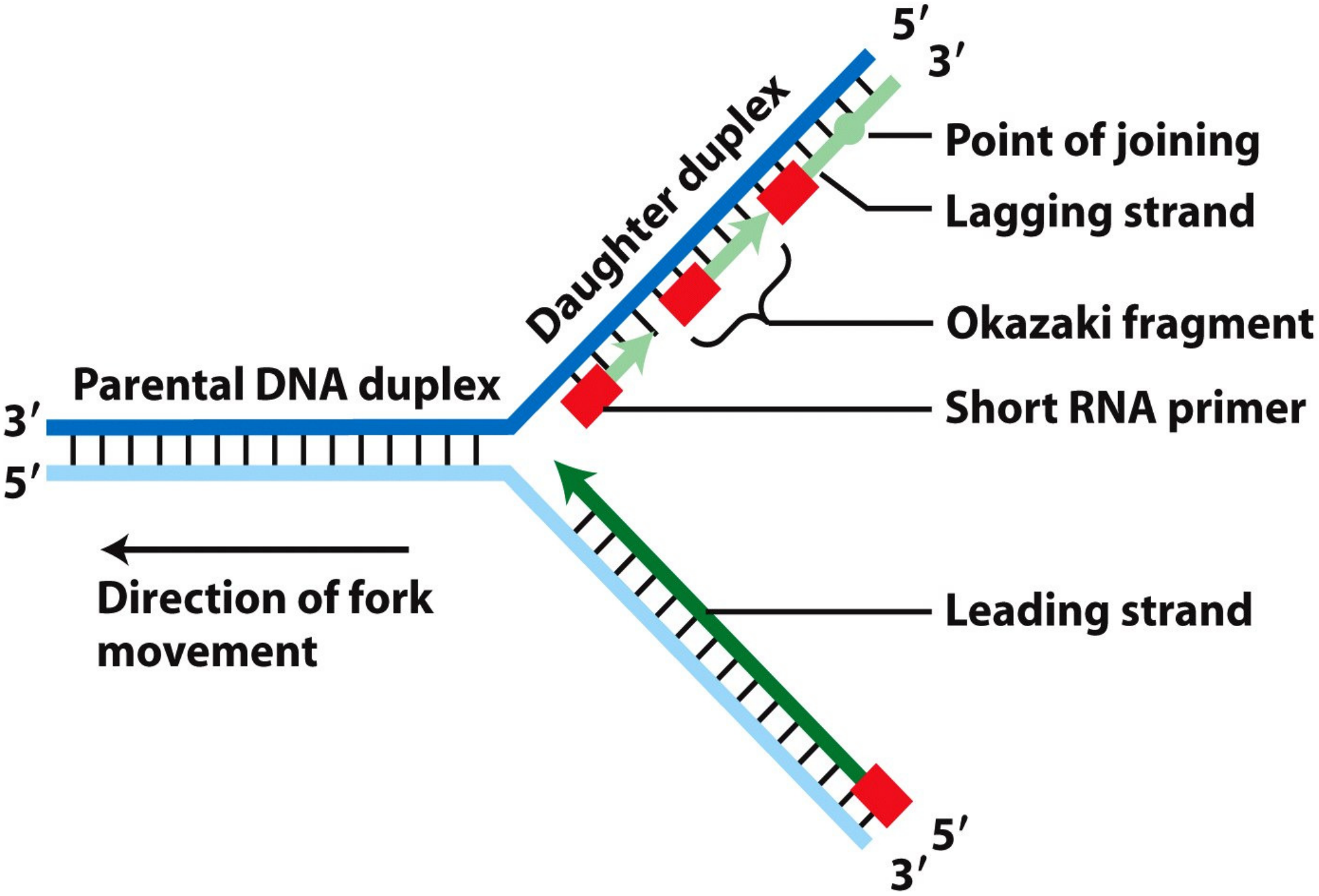
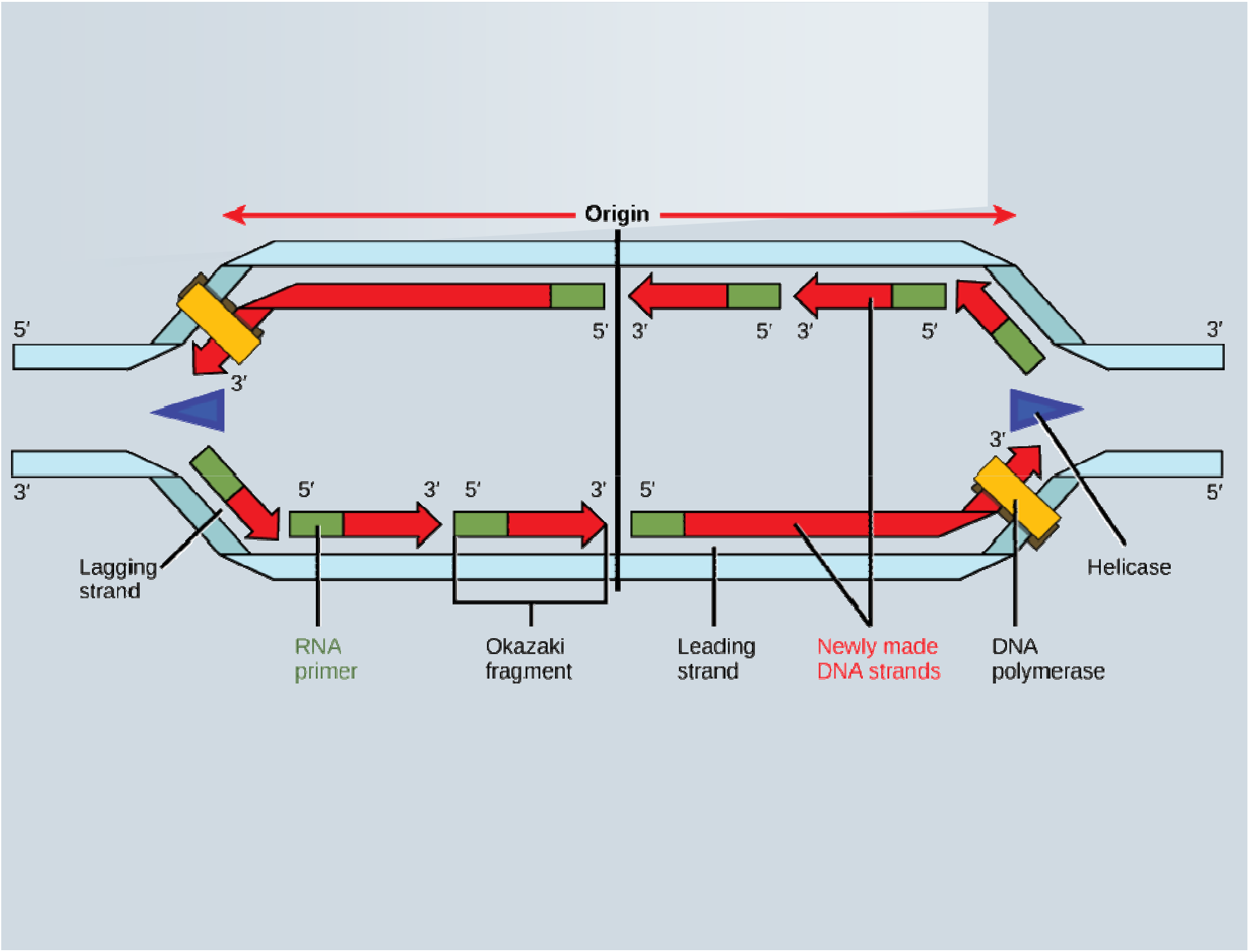
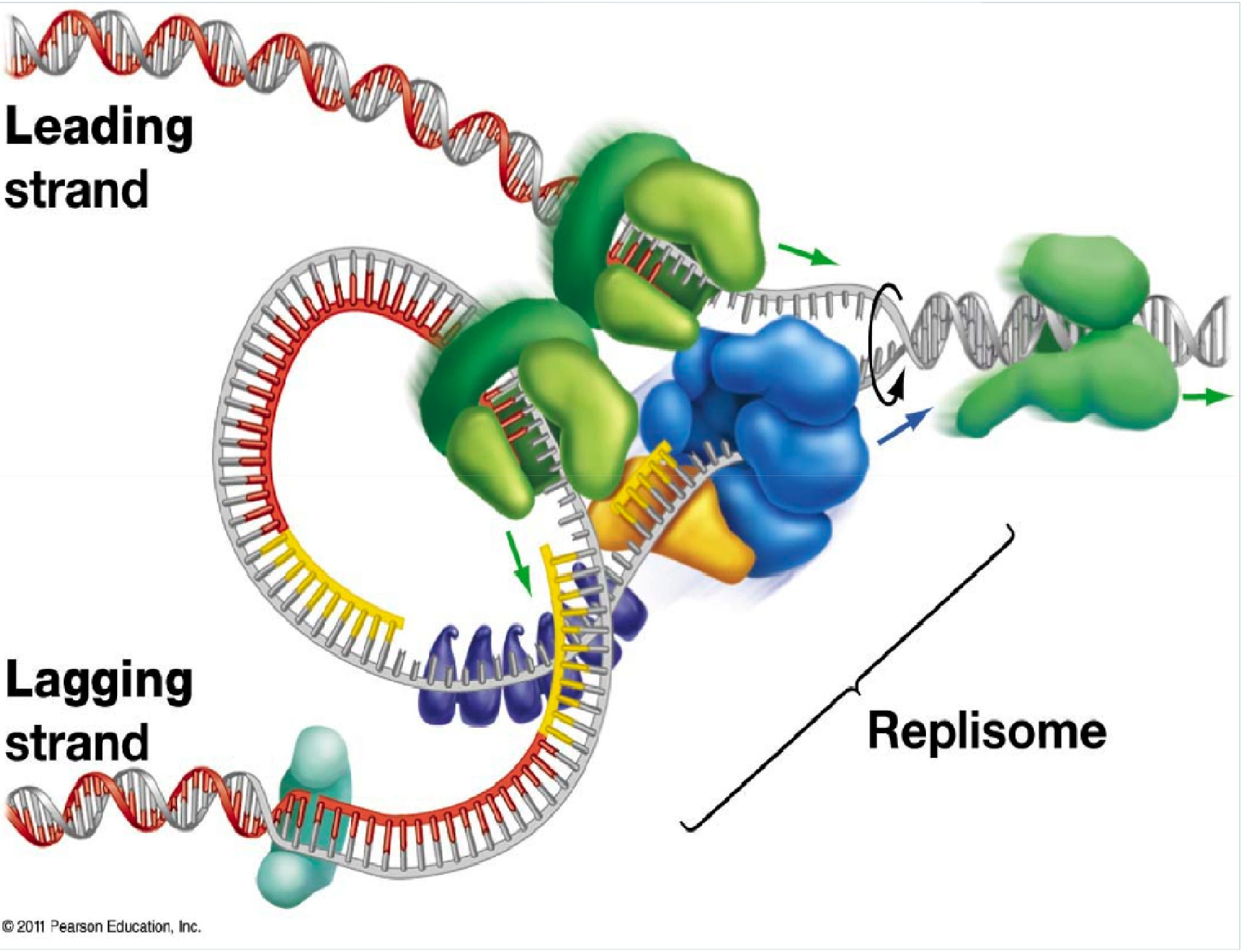


Figure 4-30
Molecular Cell Biology, Sixth Edition


Origin
5′
3′
5′
3′
5′
3′
5′
3′
5′
3′
5′
3′
5′
3′
5′
3′
Lagging strand
Helicase
RNA primer
Okazaki fragment
Leading strand
Newly made DNA strands
DNA polymerase

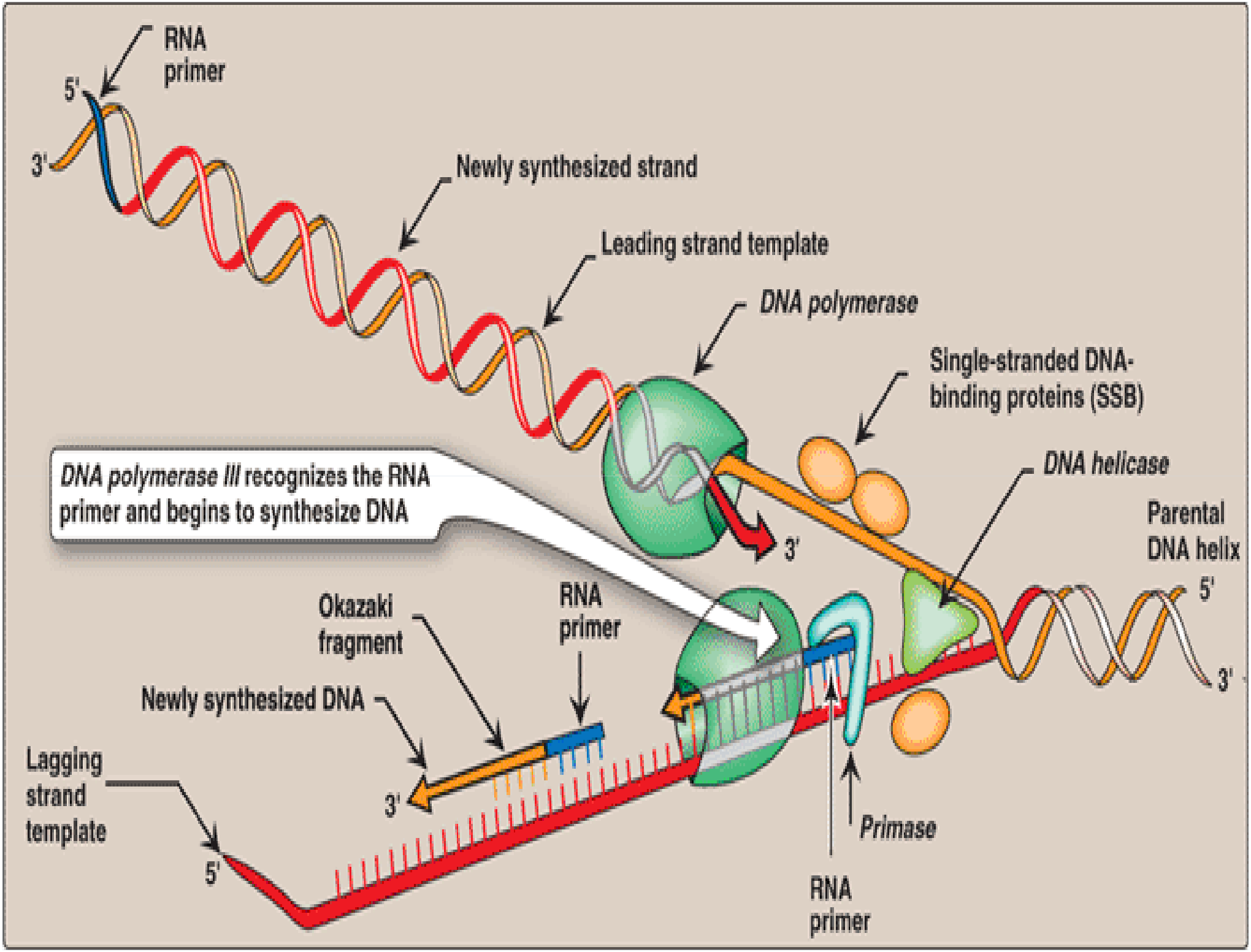


- **Leading strand:**
  - Synthesized in direction of replication fork.
  - Synthesized *continuously*.
- **Lagging strand:**
  - Strand that synthesized in the direction away from the replication fork.
  - Synthesized *discontinuously*
  - Synthesized in small fragments of DNA
  - "*Okazaki fragments*"
  - joined to become a single, continuous strand.

- **RNA primer**
  - DNA polymerases cannot initiate replication on a totally single-stranded template.
  - Require an RNA primer
  - Short chain of RNA base-paired.
  - With free hydroxyl group on 3’-end of RNA strand.
  - This hydroxyl group serves as the first acceptor of a nucleotide by action of DNA polymerase.

- **Primase:**
  - Synthesizes short of RNA (approx. 10 nucleotides)
  - Complementary and antiparallel to DNA template.
  - U in RNA pairs with A in DNA.
  - On lagging strand = Multiple RNA primers
  - On leading strand = Only one RNA primer require.

- **Primosome:**
  - The primosome makes the RNA primer.
  - As with DNA synthesis, the direction of synthesis of the primer is 5′→3′ (antiparallel to the template strand).


RNA primer
5'
3'
Newly synthesized strand
Leading strand template
DNA polymerase
Single-stranded DNA-binding proteins (SSB)
DNA helicase
DNA polymerase III recognizes the RNA primer and begins to synthesize DNA
Parental DNA helix
5'
3'
3'
Okazaki fragment
RNA primer
Newly synthesized DNA
Lagging strand template
3'
5'
Primase
RNA primer

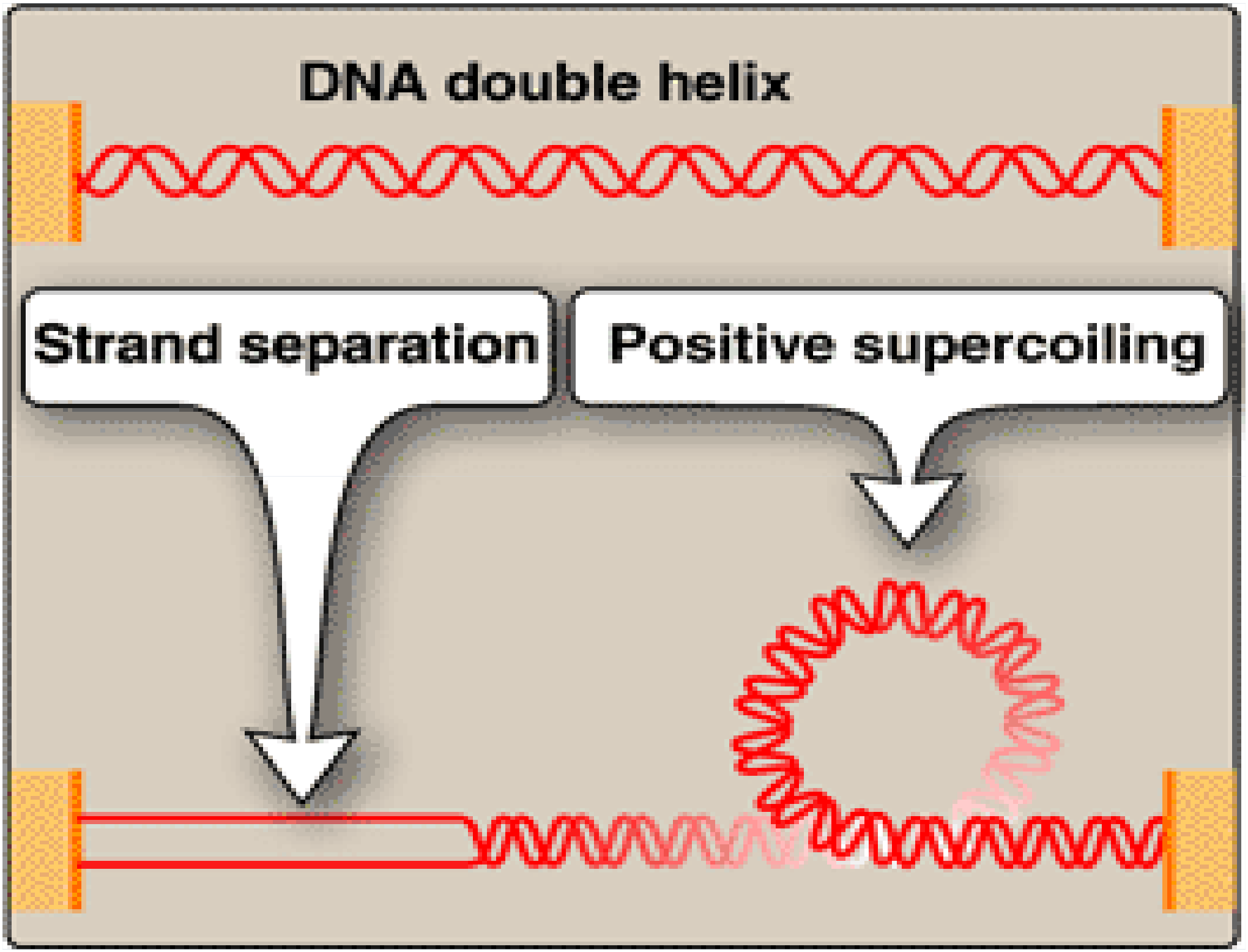
Chain Elongation

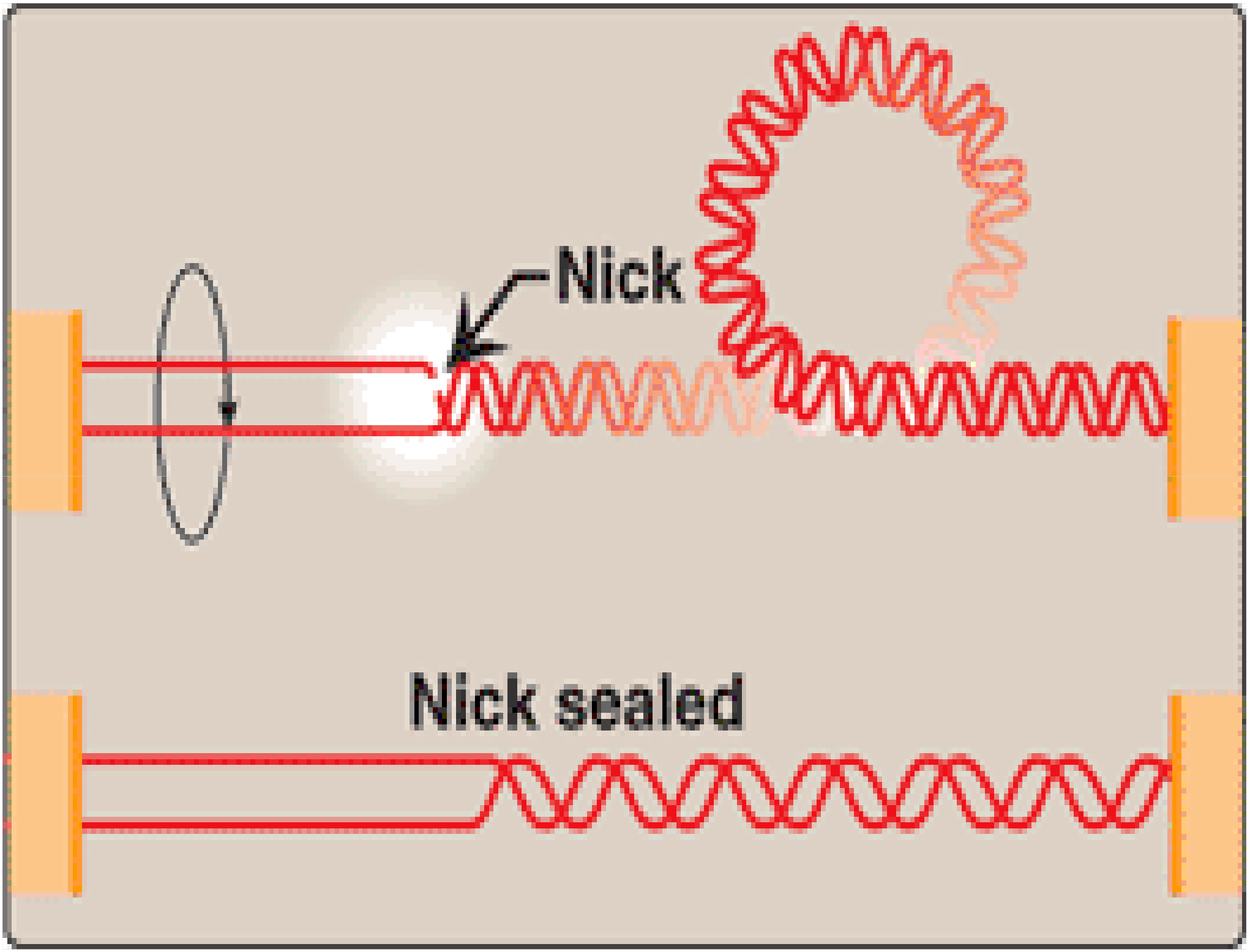
- DNA polymerases 5′→3′ direction elongate a new DNA strand
- Add deoxyribonucleotides, one at a time, to the 3′-end .
- New strand grows in the 5′→3′ direction, antiparallel
- DNA polymerase III is a highly "processive" enzyme
  - Remains bound to template strand as it moves along
  - β subunit forming a ring with template strand
  - As a sliding DNA clamp.

- With each nucleotide add Pyrophosphate (PP_i) is released
- All four deoxyribonucleoside triphosphates (dATP, dTTP, dCTP, and dGTP) are require.

Proof-Reading of new DNA

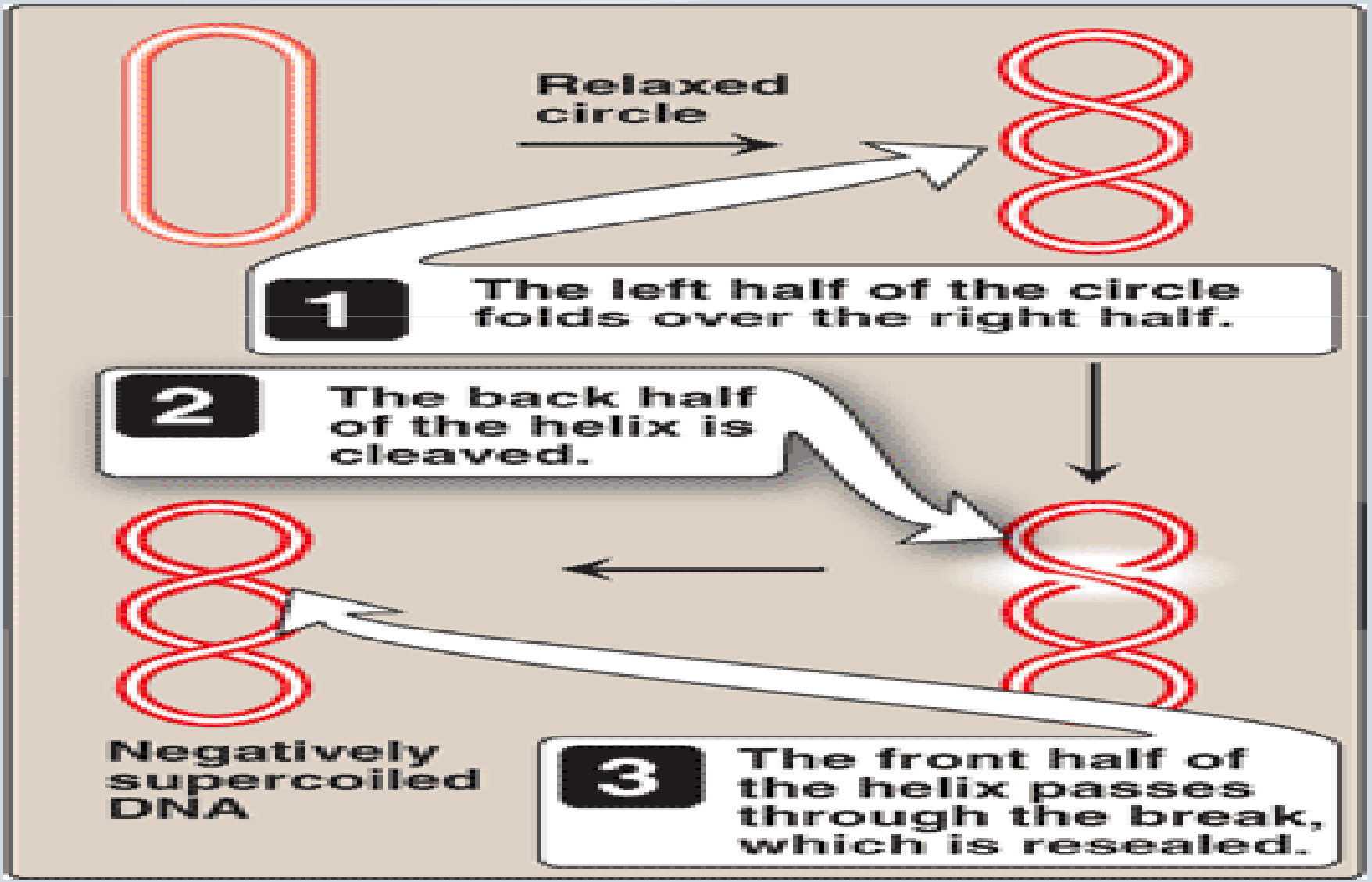
- Misreading of template sequence make in deleterious or mutations.
- To ensure replication fidelity,
- DNA polymerase III 3′→5′ exonuclease has addition "**Proofreading**" activity.
- 3′→5′ exonuclease removes misplaced nucleotide.
- Than 5′→3′ polymerase then replaces it with correct nucleotide.

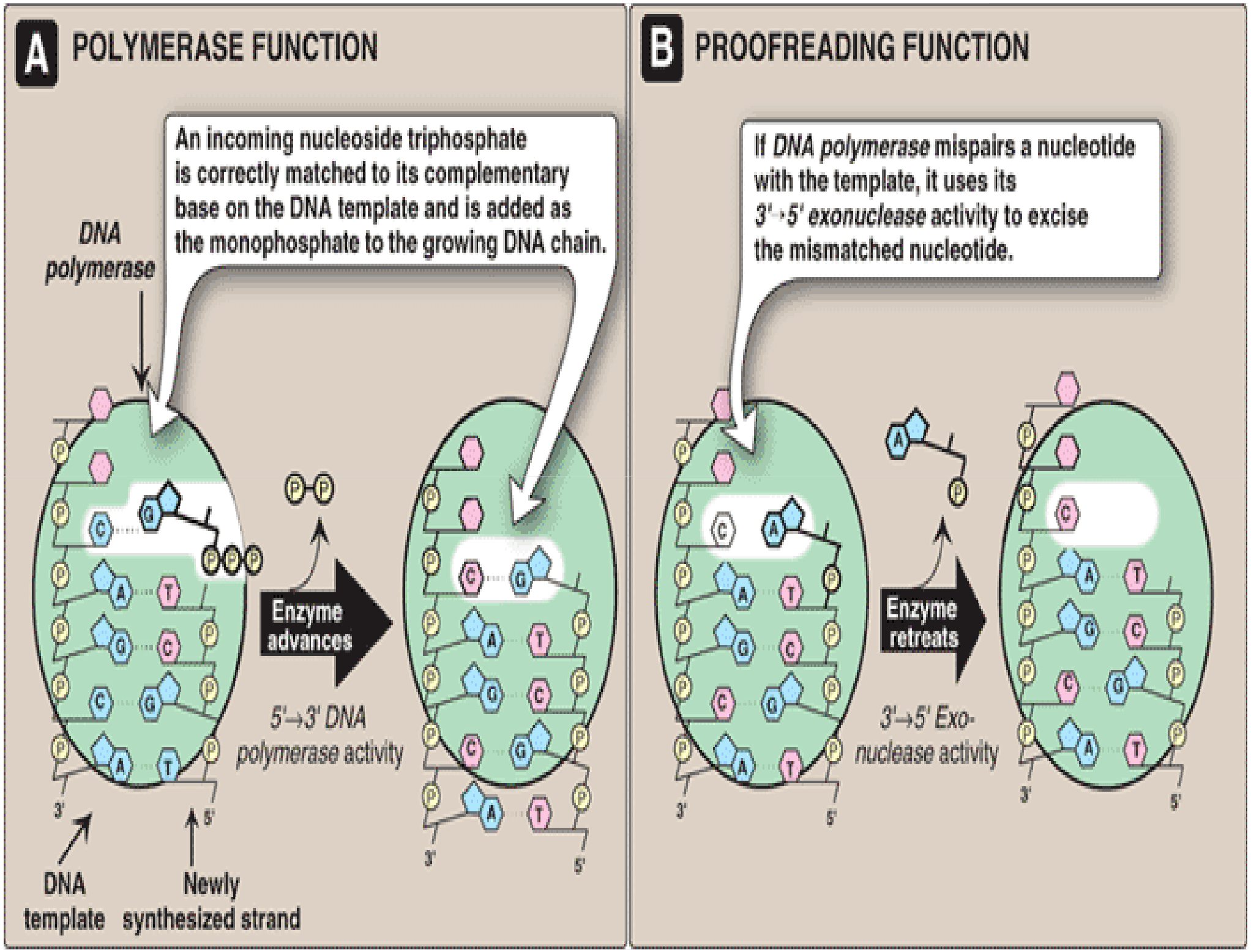

DNA double helix
Strand separation
Positive supercoiling


Nick
Nick sealed

Properties of Topo-isomerase (Gyrase)

- Relieve supercoiling in downstream of DNA during replication by making break in strand & again reseal it.
- Have both action of Nuclease & Ligase
- Type – I = act by making break in one strand
  = Break require energy, resealing does not require energy
- Type – II = act by making break in both strands.
  = Breaking & Resealing both require energy.
- **Antibiotics =** Ciprofloxacin, Nalidixic acid inhibits bacterial Gyrase.
- **Anti-tumour agents =** Etoposide, Adriamycin ,Doxorubicin inhibits eukaryotics topo-isomerase.


Relaxed circle
1
The left half of the circle folds over the right half.
2
The back half of the helix is cleaved.
Negatively supercoiled DNA
3
The front half of the helix passes through the break, which is resealed.


A
POLYMERASE FUNCTION
An incoming nucleoside triphosphate is correctly matched to its complementary base on the DNA template and is added as the monophosphate to the growing DNA chain.
DNA polymerase
Enzyme advances
5'→3' DNA polymerase activity
DNA template
Newly synthesized strand
B
PROOFREADING FUNCTION
If DNA polymerase mispairs a nucleotide with the template, it uses its 3'→5' exonuclease activity to excise the mismatched nucleotide.
Enzyme retreats
3'→5' Exo-nuclease activity

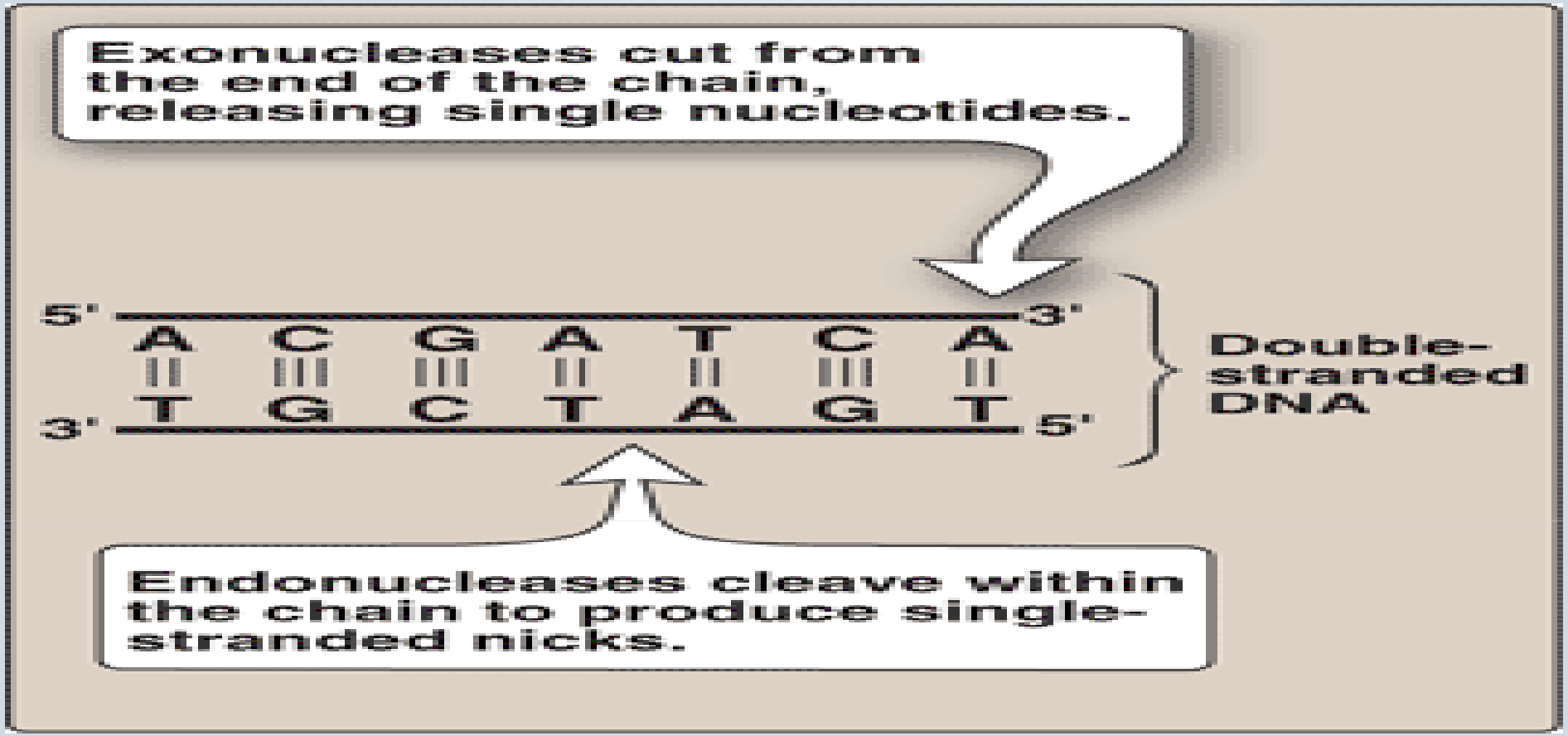
Excision of RNA primers and their replacement by DNA

- DNA polymerase III continues to synthesize DNA on the lagging strand until it is blocked by proximity to an RNA primer.
- DNA polymerase I excise RNA and filll the gap.

- DNA polymerase III = 5′→3′ polymerase activity that synthesizes DNA
  = 3′→5′ exonuclease activity that proofreads
- DNA polymerase I = 5′→3′ exonuclease activity, hydrolytically remove the RNA primer.
  = 5′→3′ polymerase activity.
  = 3′→5′ exonuclease activity that proofreads

- ***DNA polymerase I***
  - locates space
  - between 3′-end of New DNA & 5′-end of adjacent RNA primer.
  - Hydrolytically removes RNA .
  - Make 5′→3′ exonuclease activity.
  - Than, 5′→3′ polymerase activity to fill Gap by synthesis of new DNA.
  - 3′→5′ exonuclease activity to make "proofreads" .

Endonuclease versus exonuclease activity

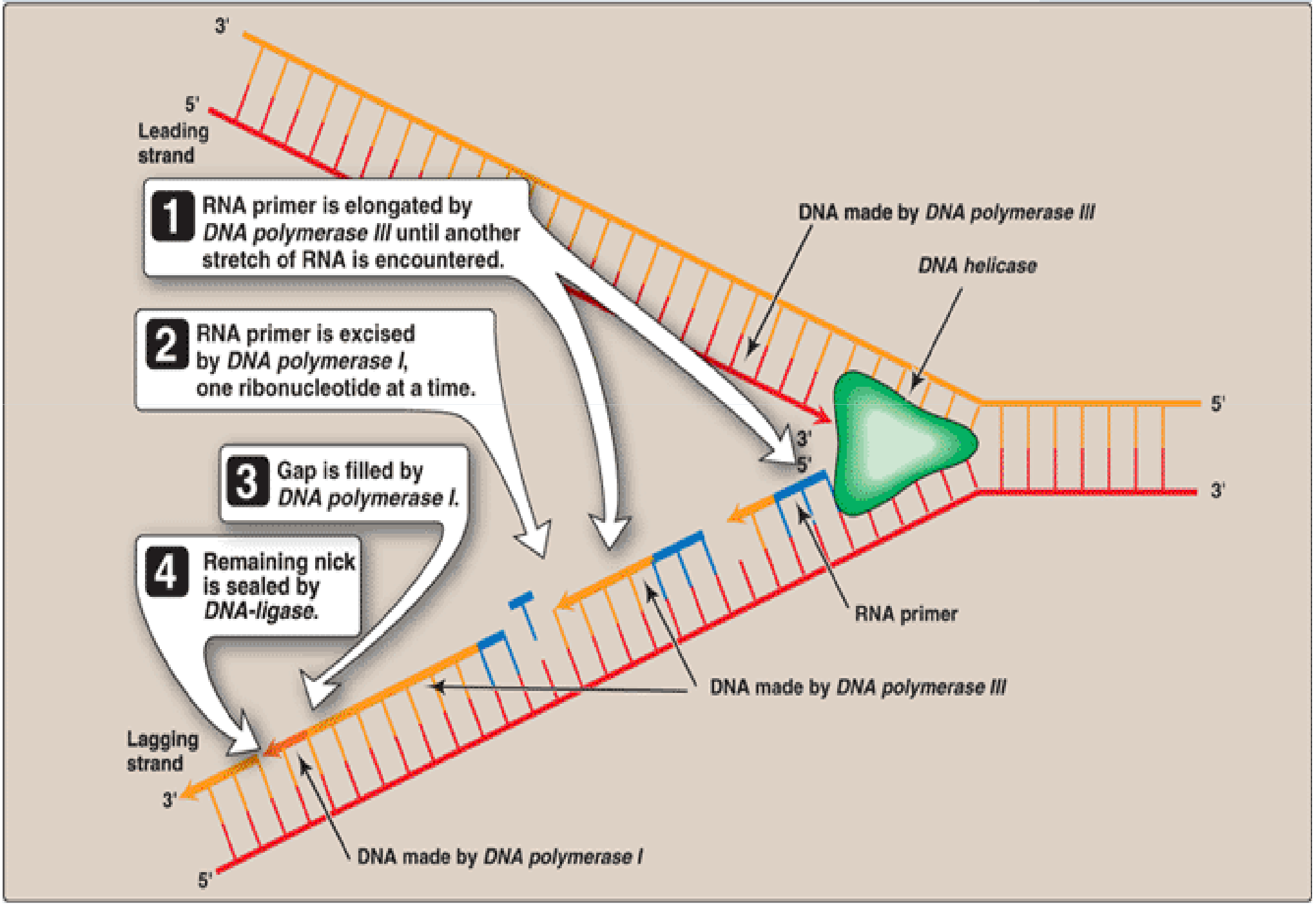


- Exonuclease = Remove one nucleotide at a time from the end of the DNA chain
- Endonuclease = Remove the chain Internally.

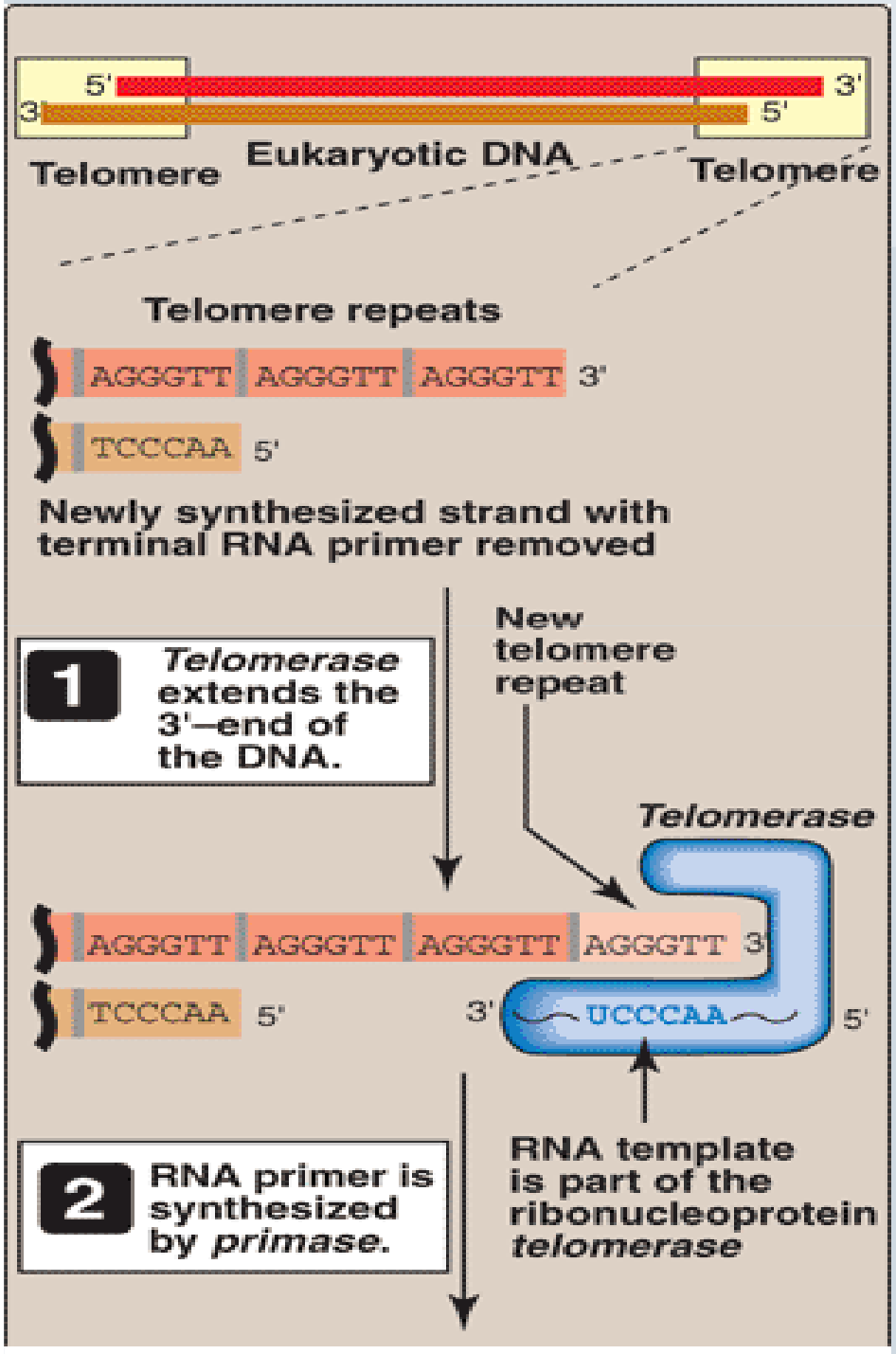
Differences between 5′→3′ & 3′→5′ exonucleases

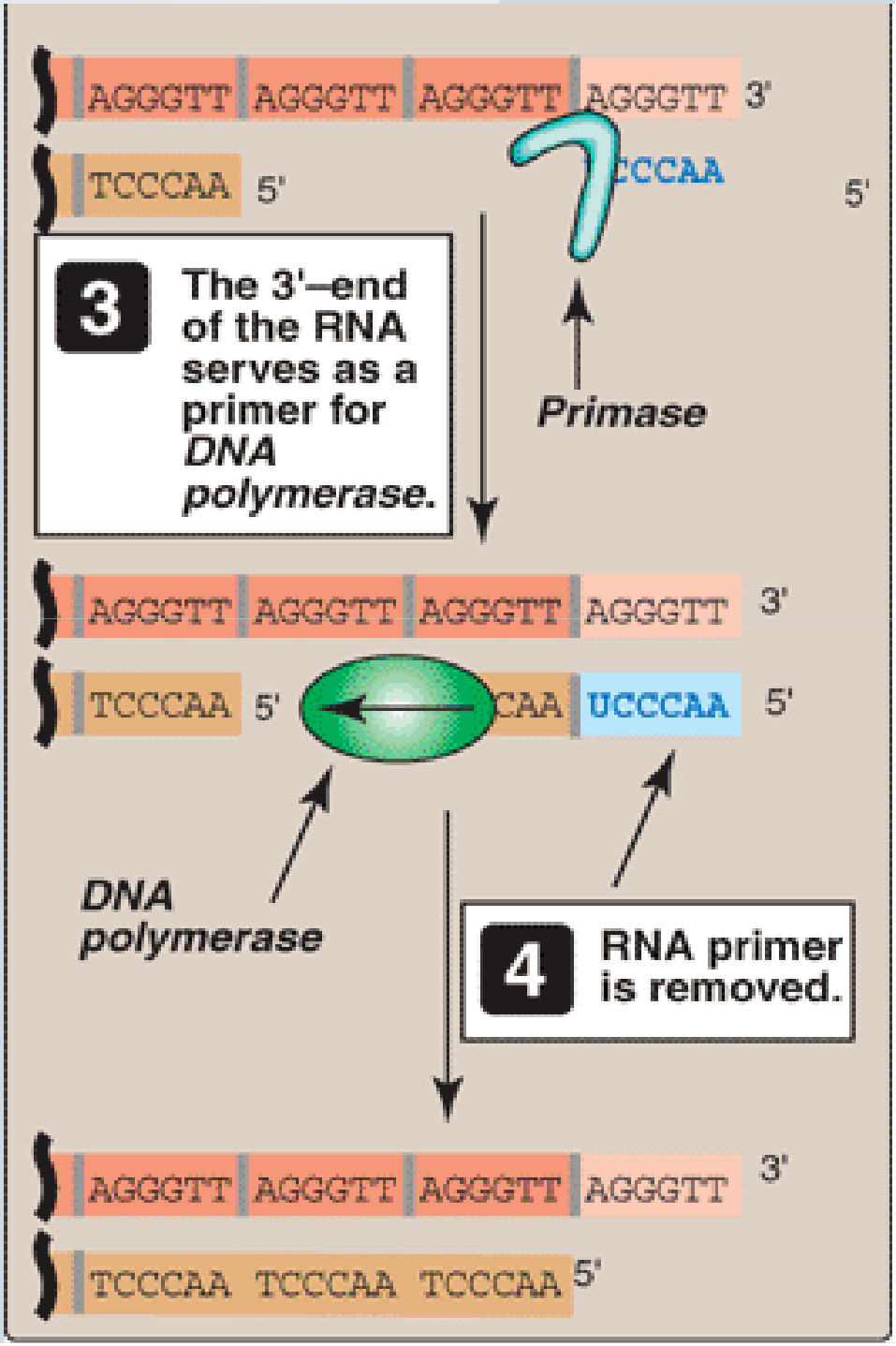
- 3′→5′ exonuclease
  - Remove nucleotides in the 3′→5′ direction
  - Remove one nucleotide at a time.
  - Important in proof reading
- 5′→3′ exonuclease
  - Remove groups of altered nucleotides in the 5′→3′ direction
  - Removing from one to ten nucleotides at a time.
  - Important in repair of damaged DNA

Removal of RNA primer and filling of the resulting “gaps” by DNA polymerase I.



Telomere & Telomerase





Telomere

- **Gap at extreme 5’-end of the lagging strand**
- After removal of RNA primer
- This End is protect with proteins.
- **The DNA–protein complex is termed “Telomere”.**
- Consists of tandem repeats of AGGGTT.

Telemere

- In normal somatic cells, telomeres shorten with each successive cell division.
- if shortened beyond some critical length, the cell can not servive.
- In germ cells, other stem cells & in cancer cells
  - telomeres do not shorten
  - so the cells survival is longer.

Telemerase

- Enzyme = Ribonucleoprotein (Telomerase)
- Maintain lenghth.
- Reverse transcriptase.
- Make RNA template to DNA 5′→3′
- Lengthen GT-rich strand
- Than Primase can synthesize an RNA primer.
- Than RNA primer is extended by DNA polymerase and make de novo DNA synthesis

Telomere Significant

- Mitotic clock.
- Providing information of aging and cancer.

Reverse transcriptase

- Replication of retroviruses
- Human Immunodeficiency Virus (HIV).
- Viruses carry their genome in form of ssRNA.
- Following infection of a host cell,
- Viral enzyme,uses the viral RNA as a template for the 5′→3′ synthesis of viral DNA
- Than Viral DNA integrated into host chromosomes.
- In eukaryotes, such elements are transcribed to RNA.

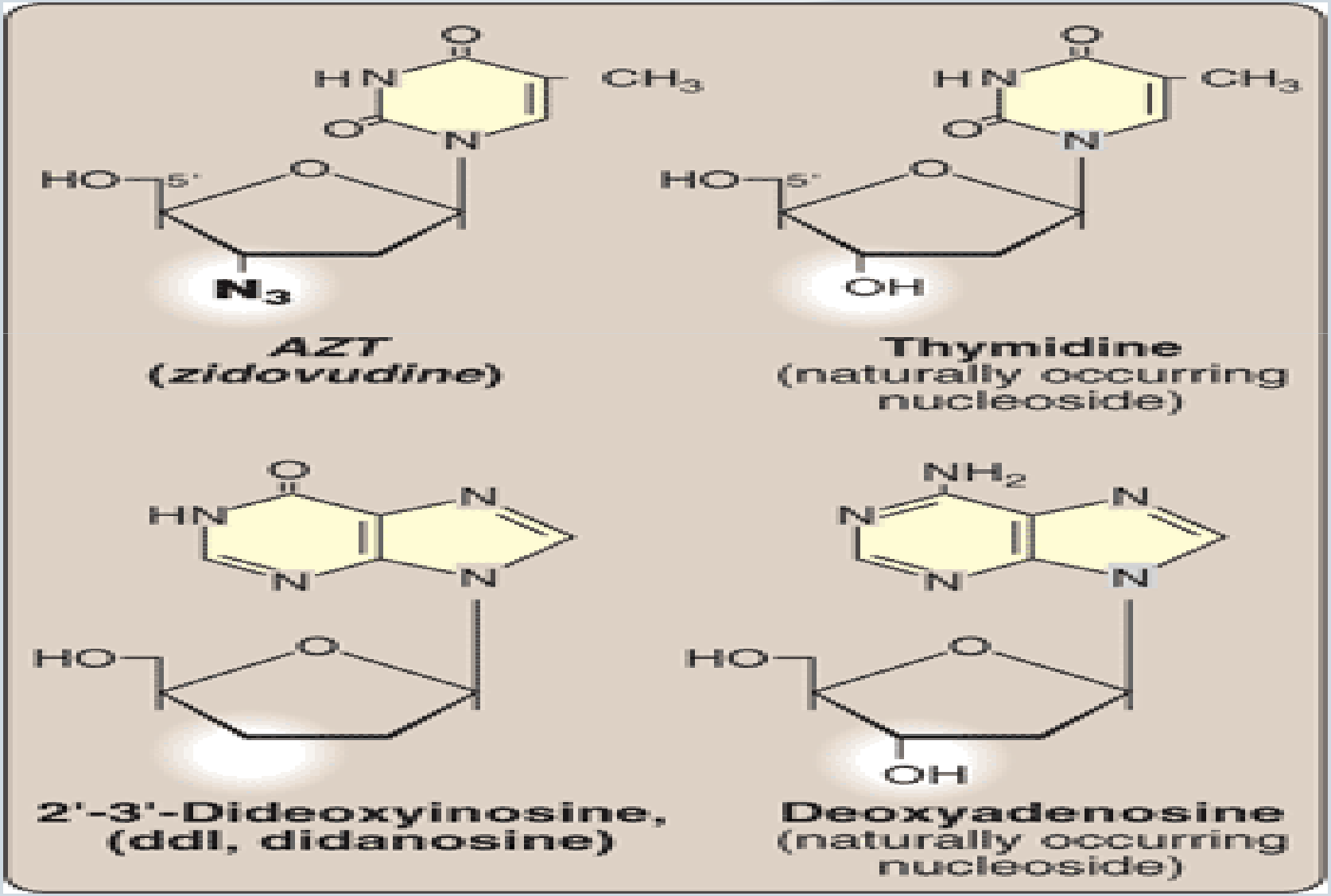
Eukaryotic DNA polymerases

- Five key eukaryotic DNA polymerases identified.
- Pol α and pol Δ:
- **Pol α** is a multisubunit enzyme.
  - One subunit has primase activity,
- **Pol Δ**
  - Elongation of DNA on the leading strand and elongate
  - 3′→5′ exonuclease activity to proofread the newly synthesized DNA.
  - Associates with the protein, proliferating cell nuclear antigen, which serves as a sliding DNA clamp in much the same way the β subunit of DNA polymerase III does in E. coli.
- **Pol β** and **pol ε** are involved in **DNA repair**.
- **Pol γ** replicates mitochondrial DNA.

Inhibition of DNA synthesis by nucleoside analogs

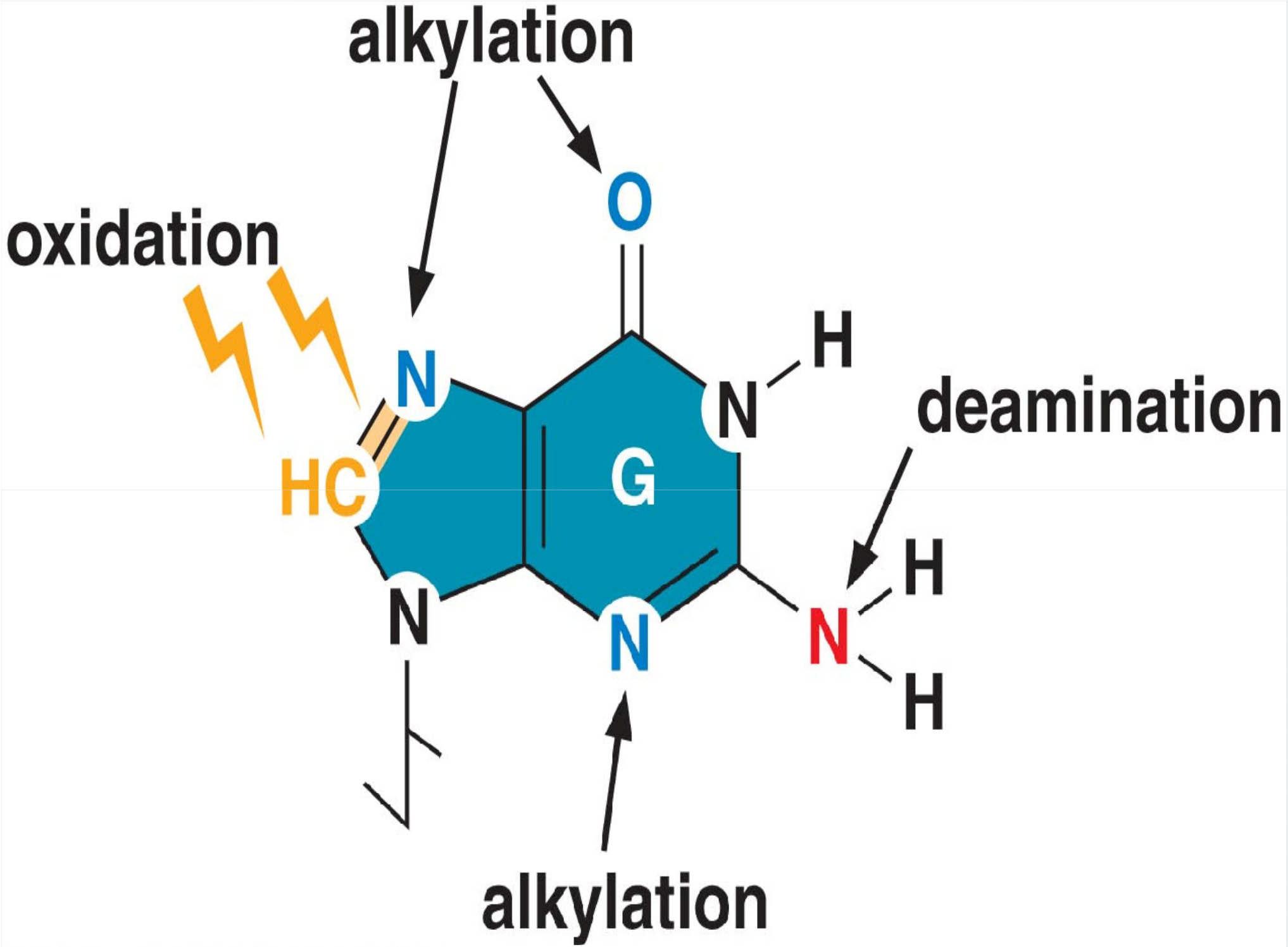
- Conversion of the deoxyribose to another sugar as in Arabinose , prevents further chain elongation.
- Cytosine arabinoside = Anticancer chemotherapy.
- Adenine arabinoside = Antiviral agent.
- Zidovudine (AZT) = Modifying the sugar.
  = termination of DNA elongation.
  = Use in AIDS

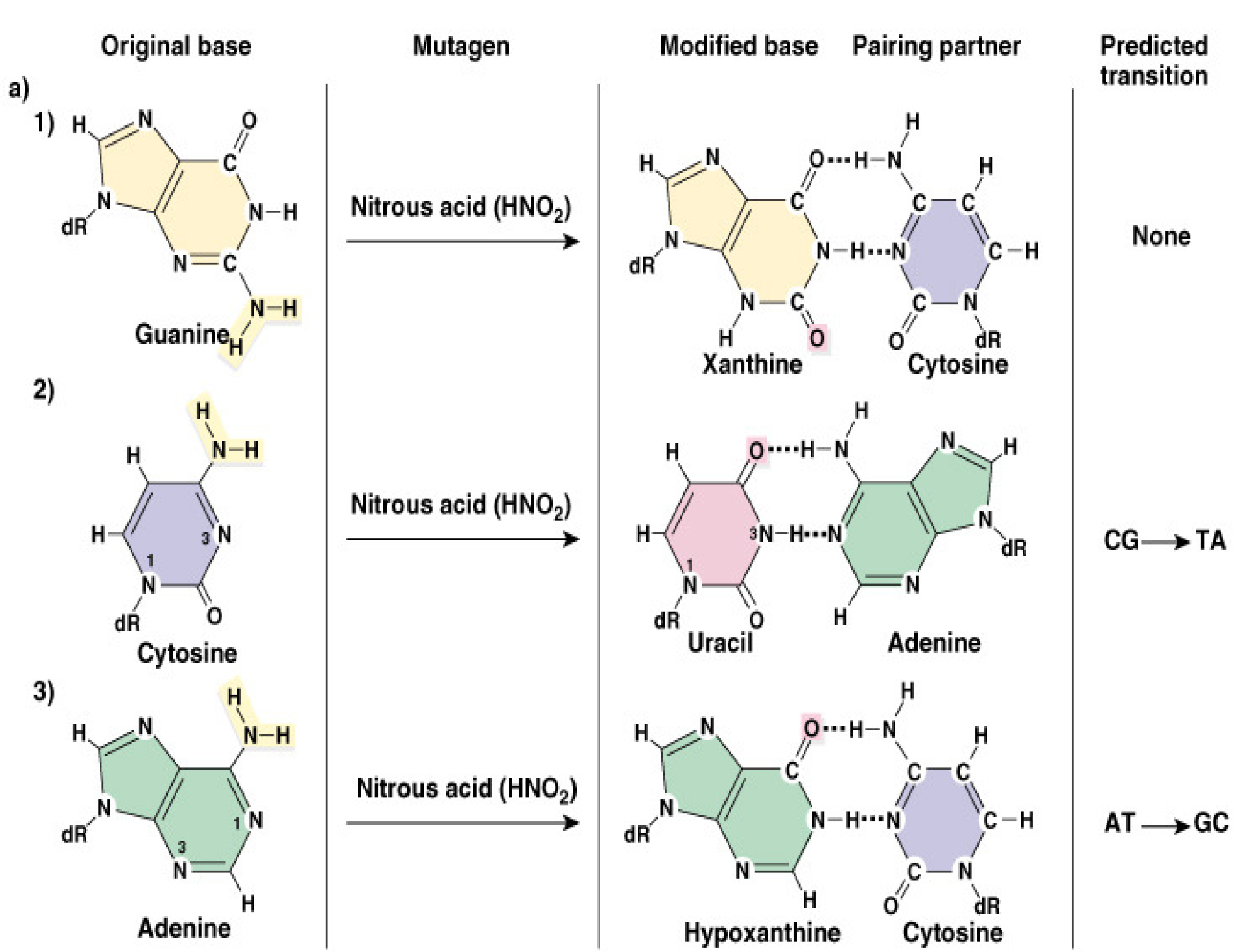
Drugs Structural Analogue to Nitrogen base

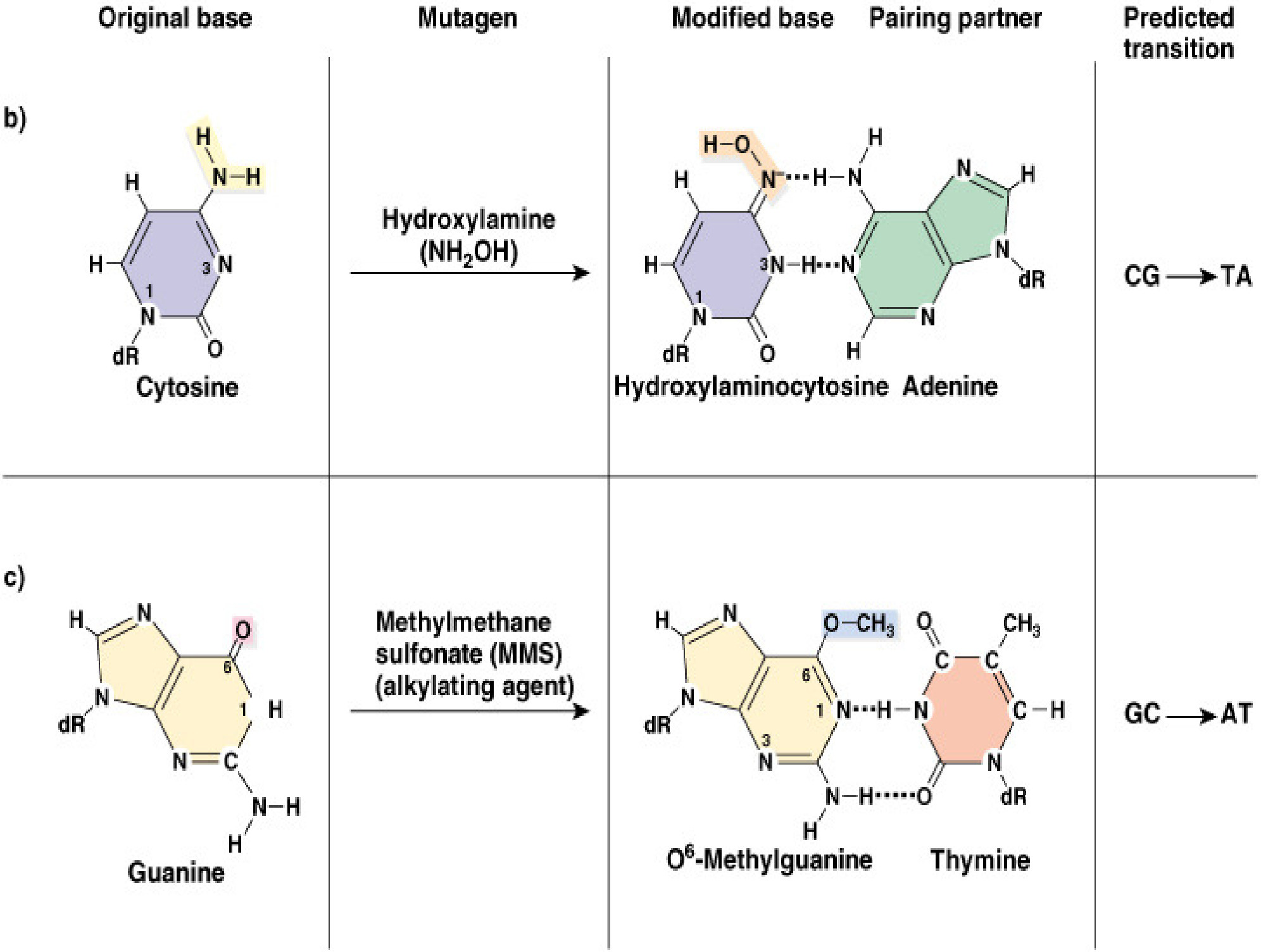


Can Zidovudine affect human cellular DNA replication ?

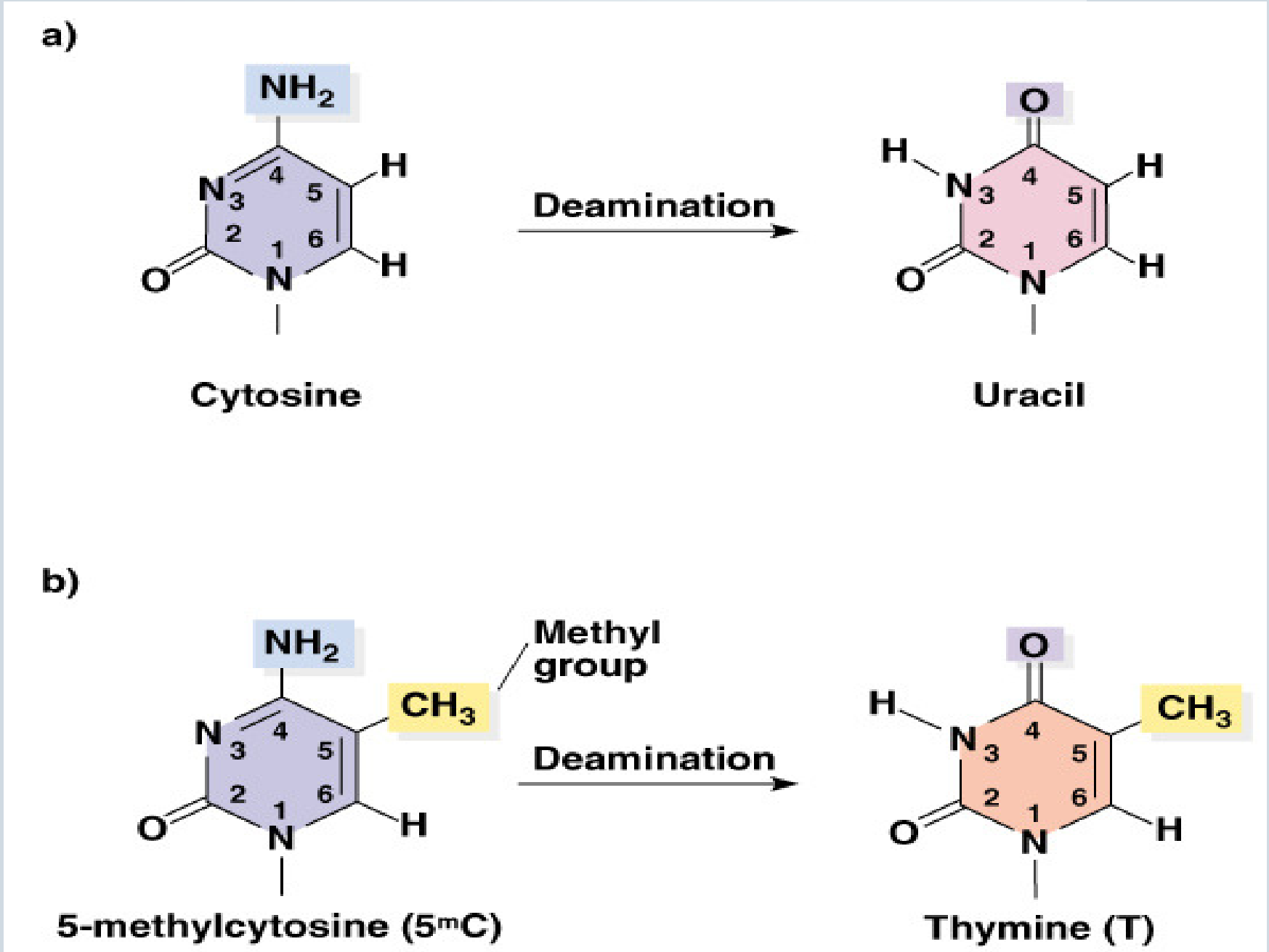
DNA Damage
&
DNA Repair



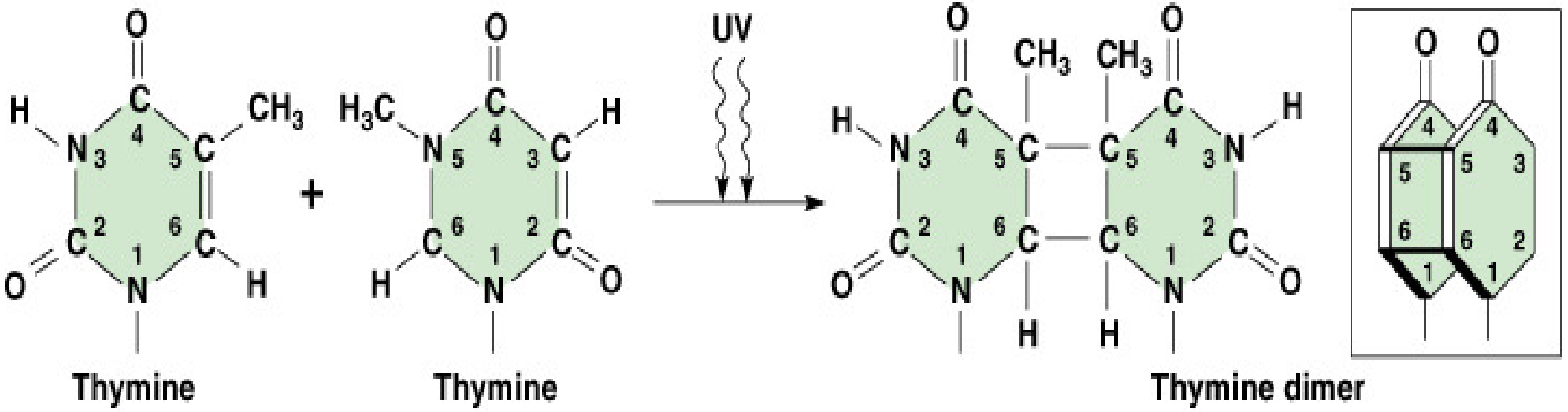

Original base
Mutagen
Modified base
Pairing partner
Predicted transition
a)
1)
Guanine
Nitrous acid (HNO_2)
Xanthine
Cytosine
None
2)
Cytosine
Nitrous acid (HNO_2)
Uracil
Adenine
CG → TA
3)
Adenine
Nitrous acid (HNO_2)
Hypoxanthine
Cytosine
AT → GC


Original base
Mutagen
Modified base
Pairing partner
Predicted transition
b)
Cytosine
Hydroxylamine
(NH2OH)
Hydroxylaminocytosine
Adenine
CG → TA
c)
Guanine
Methylmethane
sulfonate (MMS)
(alkylating agent)
O6-Methylguanine
Thymine
GC → AT

Deamination



Thymine Dimer



DNA Damage

I. Single-base alteration
   A. Depurination
   B. Deamination of cytosine to uracil
   C. Deamination of adenine to hypoxanthine
   D. Alkylation of base
   E. Insertion or deletion of nucleotide
   F. Base-analog incorporation

II. Two-base alteration
   A. UV light–induced thymine-thymine (pyrimidine) dimer
   B. Bifunctional alkylating agent cross-linkage

DNA Damage

III. Chain breaks

A. Ionizing radiation

B. Oxidative free radical

IV. Cross-linkage

A. Between bases in same or opposite strands

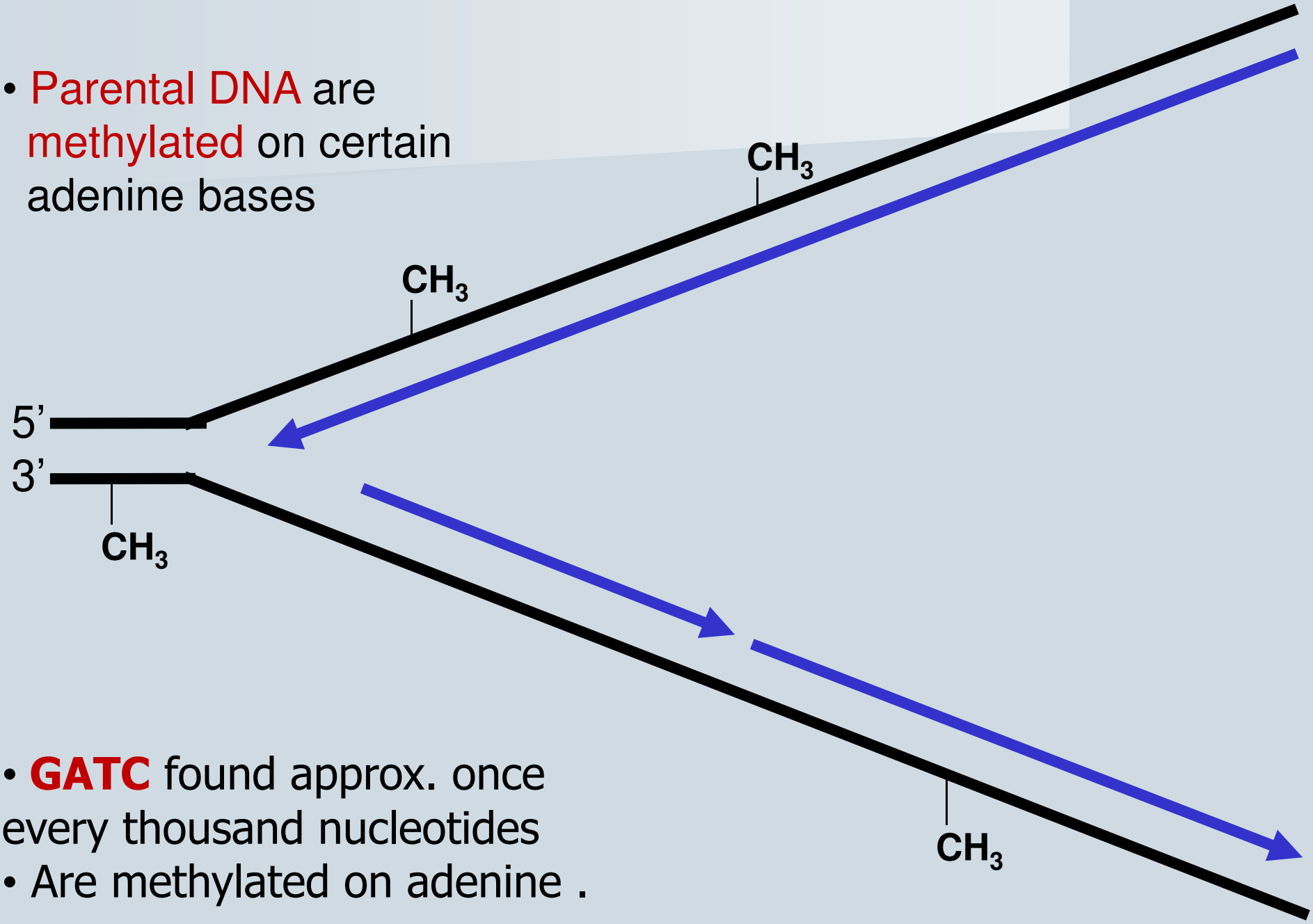
B. Between DNA and protein molecules (eg histones)

Mechanisms of DNA Repair

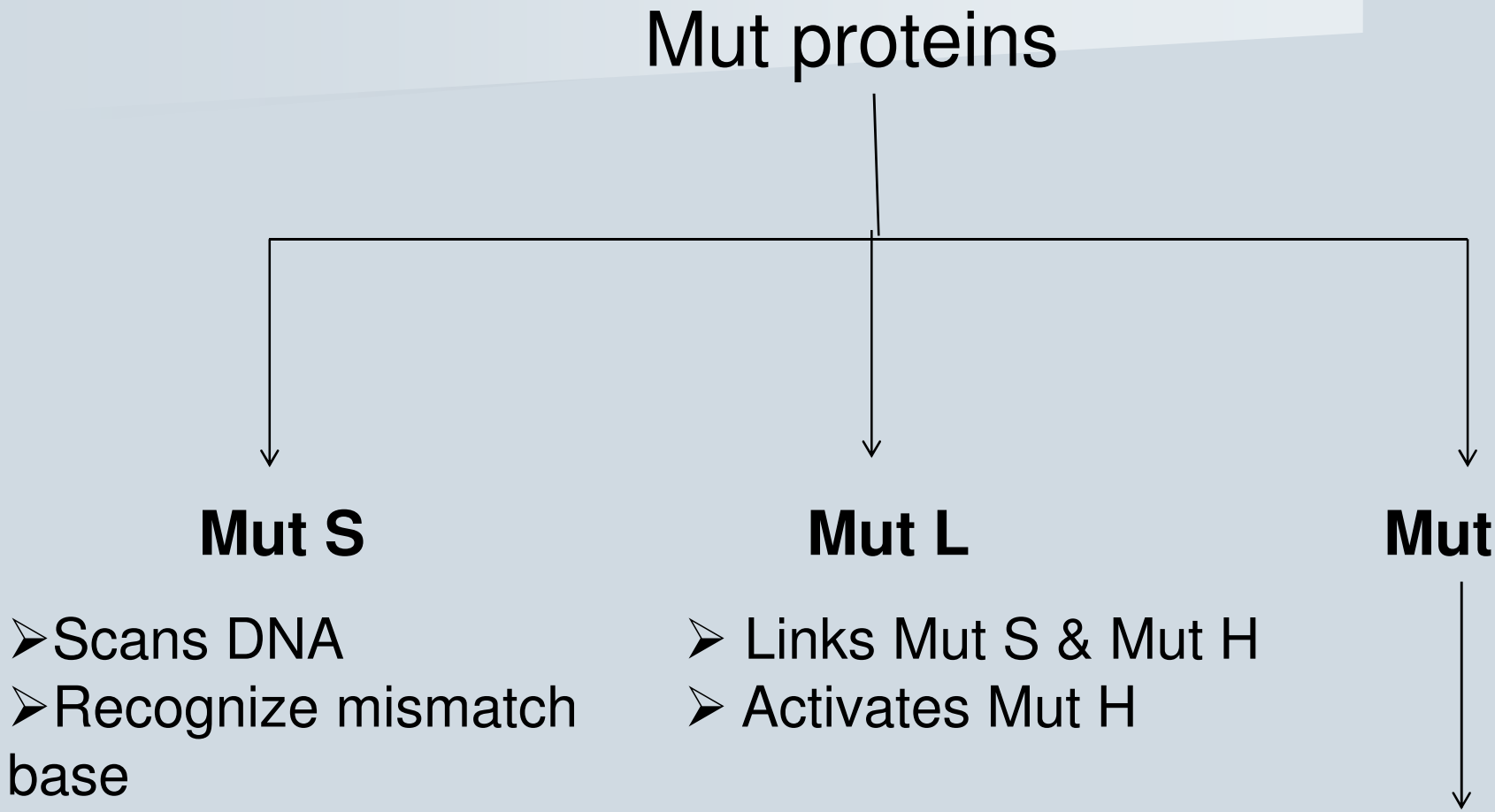
1. Proofreading by the DNA polymerases
2. Mismatch (post-replication) repair
3. Base Excision repair
4. Nucleotide Excision repair

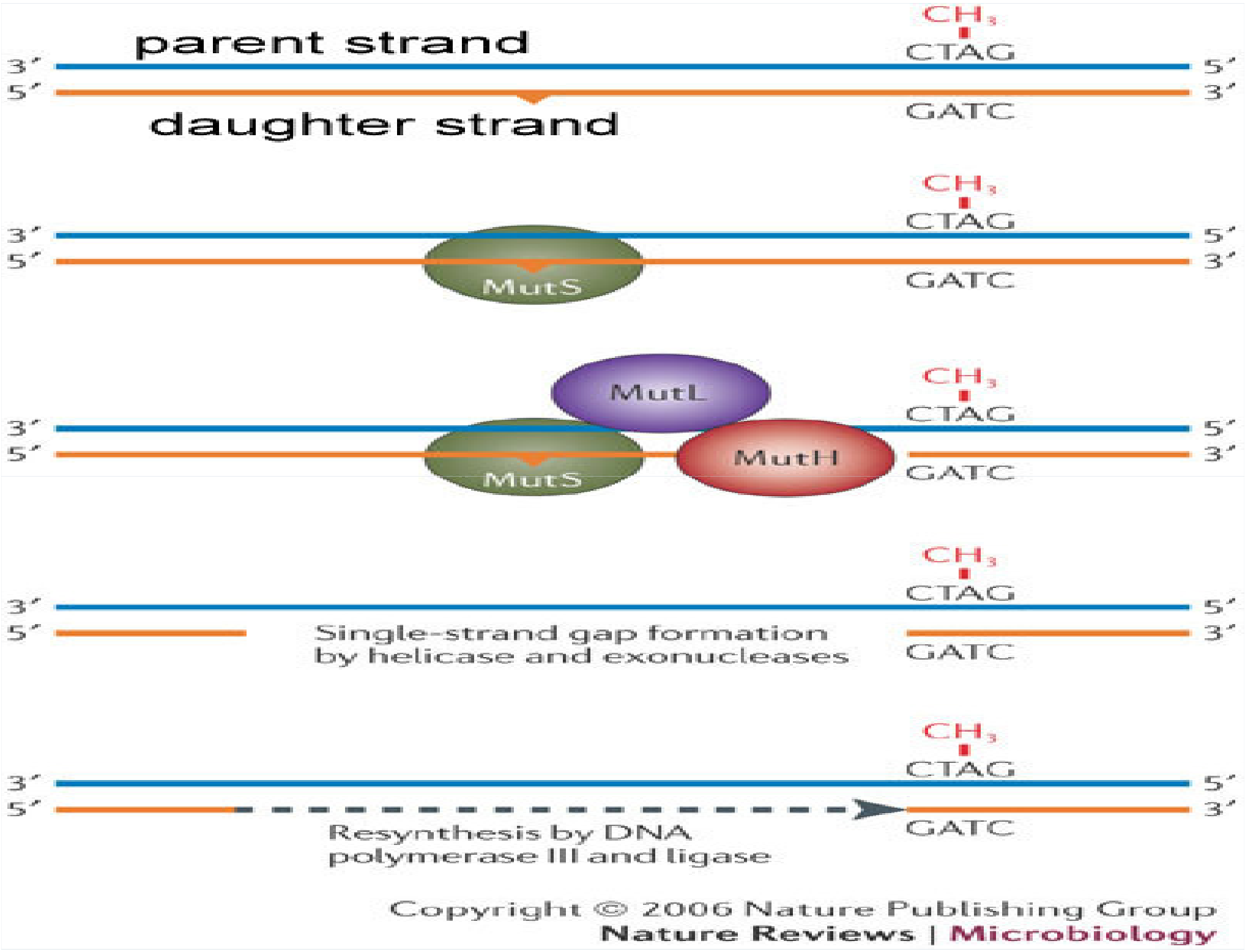
Mismatch (Post-replication) repair

- Parental DNA are methylated on certain adenine bases



- **GATC** found approx. once every thousand nucleotides
- Are methylated on adenine .


Mut proteins
Mut S
Mut L
Mut H
➢Scans DNA
➢Recognize mismatch base
➢ Links Mut S & Mut H
➢ Activates Mut H
➢Binds to hemi methylated GATC sequence



Mismatch Repair

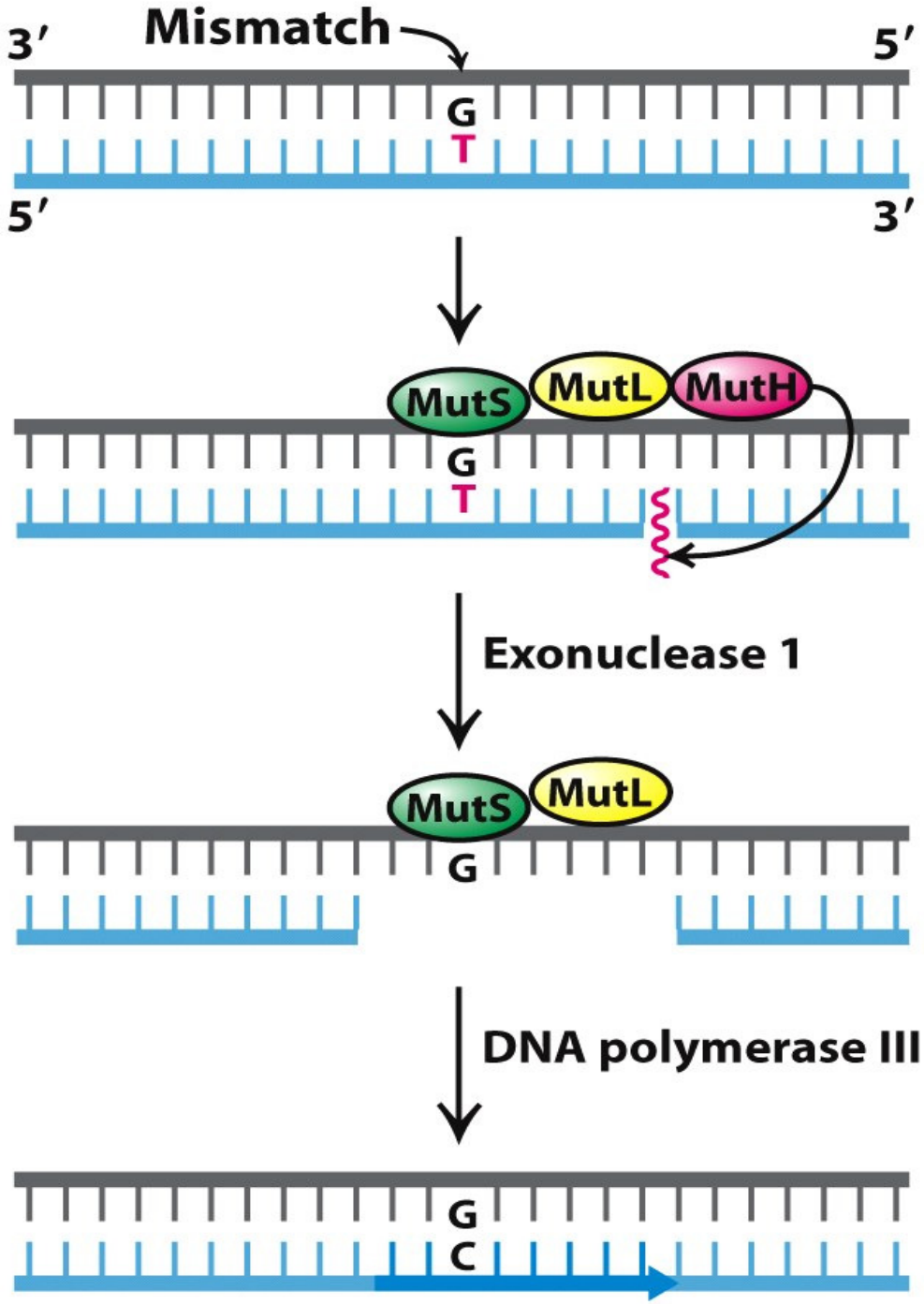


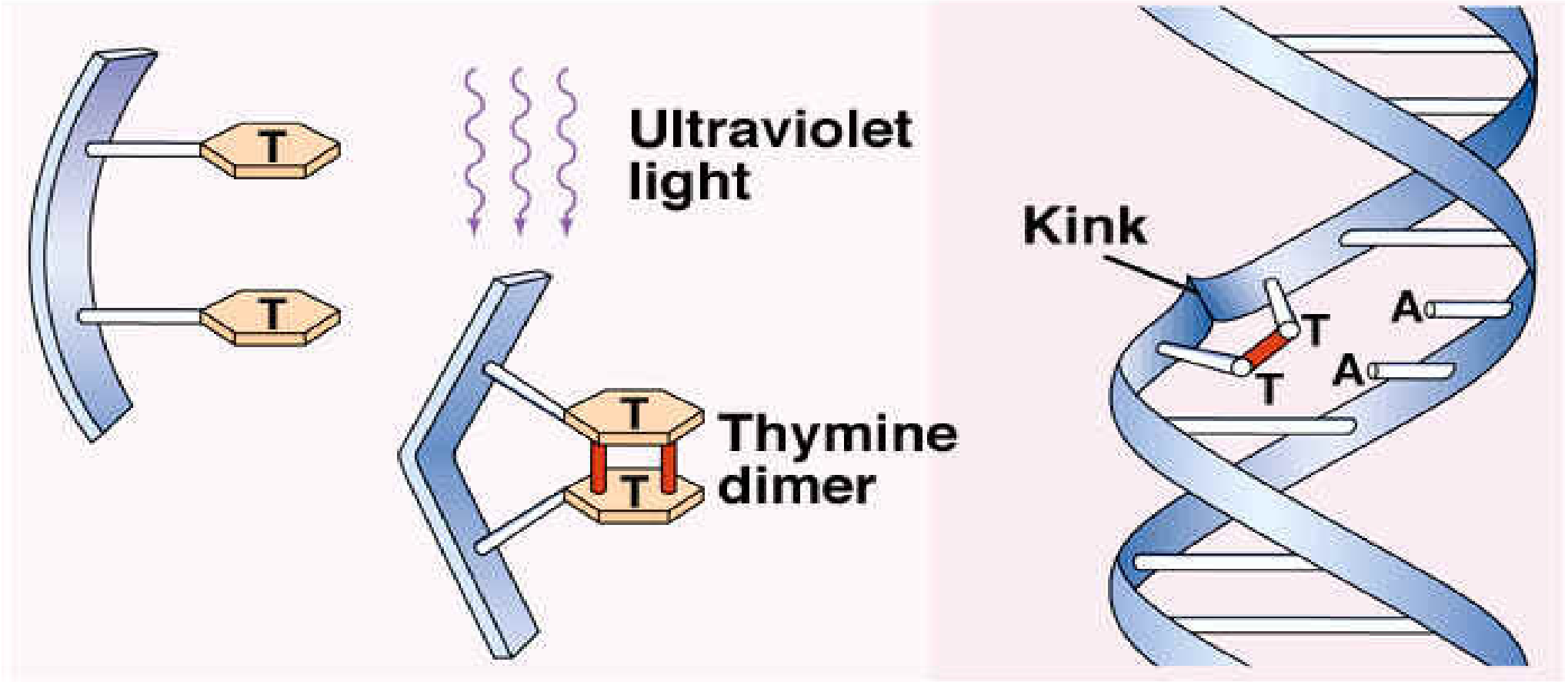
Figure 28.36
***Biochemistry*, Seventh Edition**

Repair of Mismatch DNA damage

- Mismatch is identified
- Endonuclease nicks the strand
- Exonuclease remove Mismatched nucleotide(s).
- Additional nucleotides at the 5′- and 3′-ends are also removed.
- DNA polymerase & DNA ligase fill the gap.
- E.g. = Hereditary Nonpolyposis Colorectal Cancer (HNPCC) (Lynch syndrome).

Thymine Dimer due to UV light

Pyrimidine Dimer

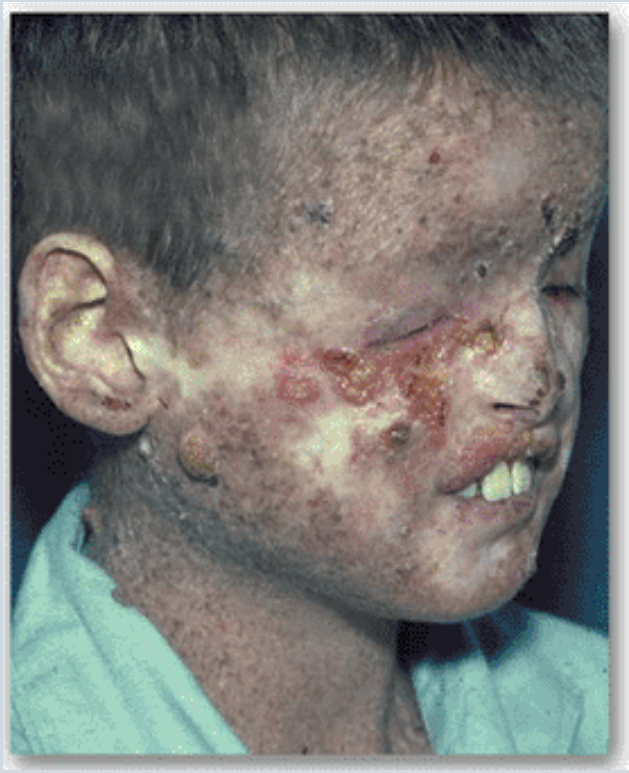


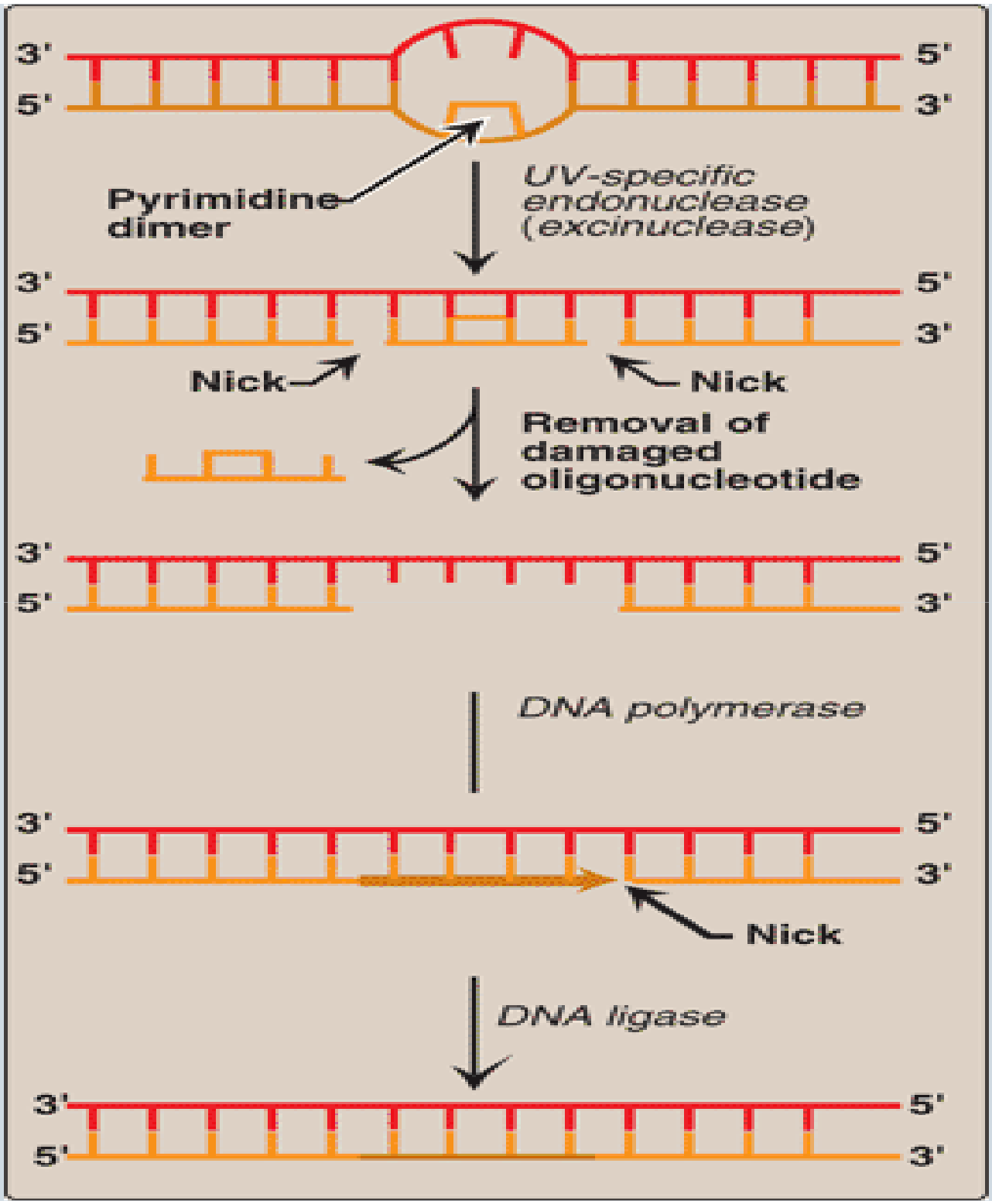
Mismatch repair for Thymine Dimer due to UV light

- **Dimer = Thymine dimer**
- Obstruct DNA polymerase
- Inhibit DNA replication
- **UV-specific endonuclease (uvrABC excinuclease)**
- **Recognition and excise dimer**
- **Dimer containing short oligonucleotide removed**.
- Gap is filled same repair as mismatch repair.

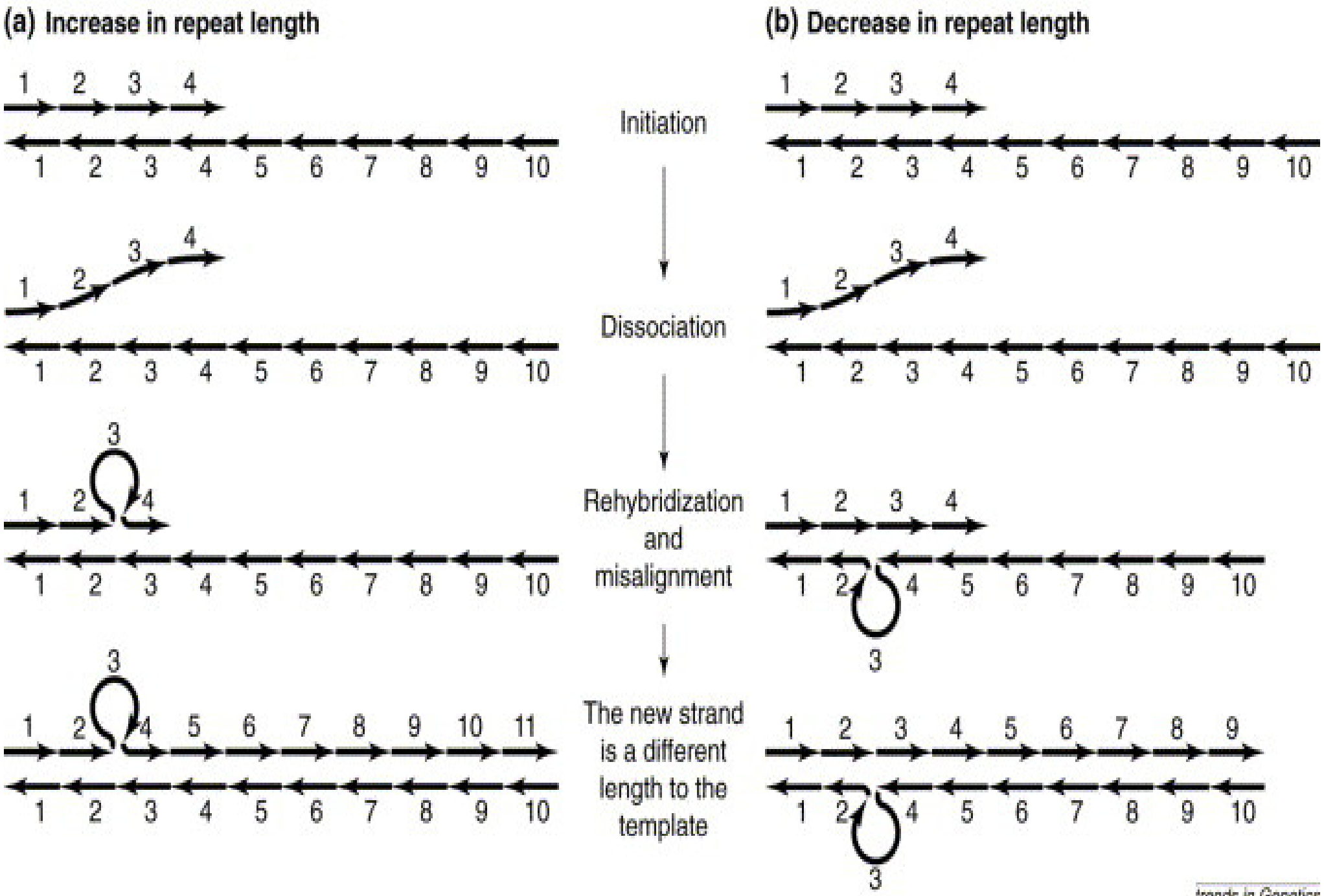
UV radiation and cancer

- **Xeroderma Pigmentosum**
- **Skin cancer**
- Due to exposer to unfiltered sunlight.
- Defect in "UV-damage repair mechanism.




3'
5'
5'
3'
Pyrimidine dimer
UV-specific endonuclease (excinuclease)
Nick
Nick
Removal of damaged oligonucleotide
DNA polymerase
Nick
DNA ligase

Microsatellite instability (MSI)



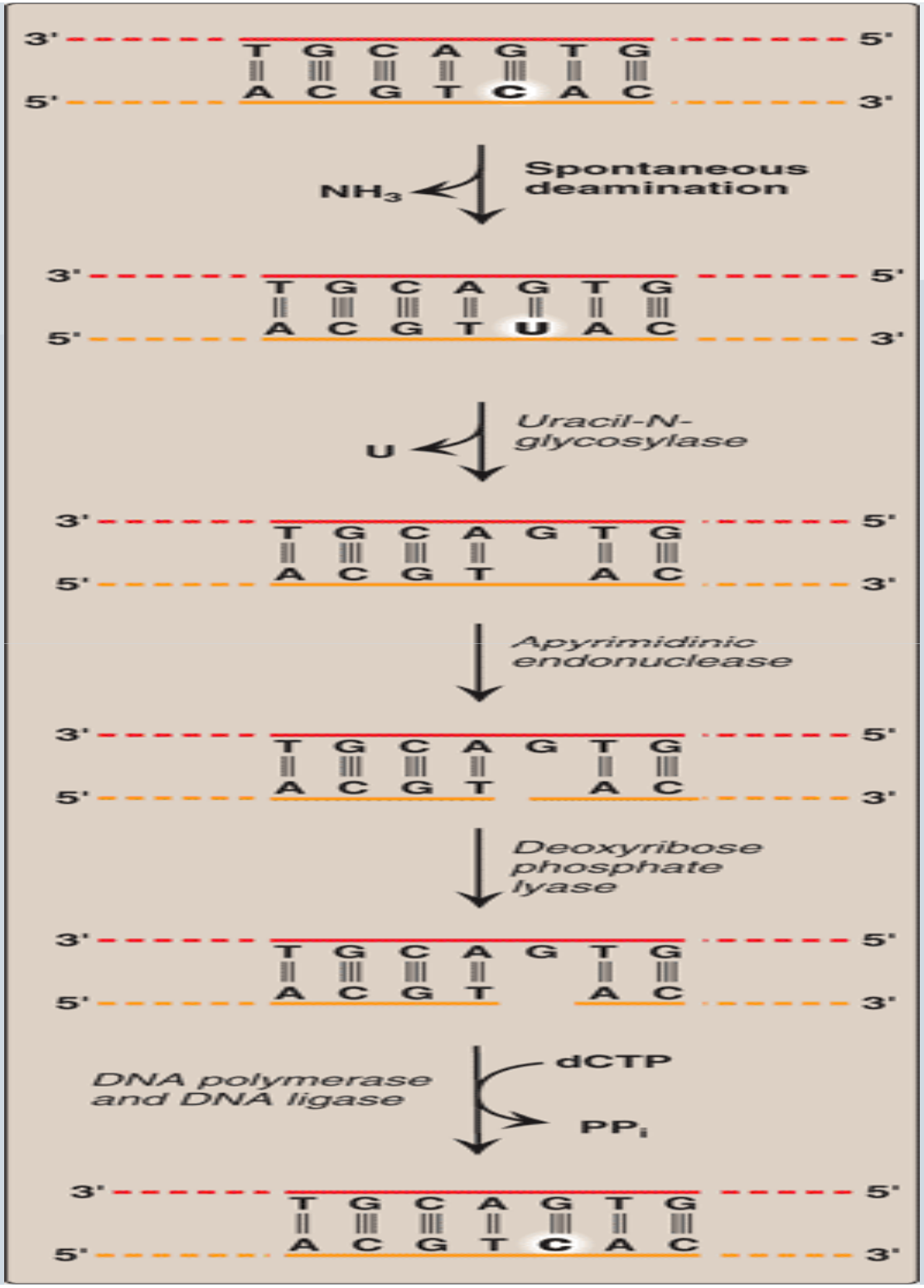
Mismatch Repair for Microsatellite instability (**MSI**)

- Microsatellites = repeated dinucleotide “CA”
- DNA polymerase slips out these sequences
- Forms loop
- If defects in MMR repair process
  - Increase in length of DNA
  - Decrease In length of DNA

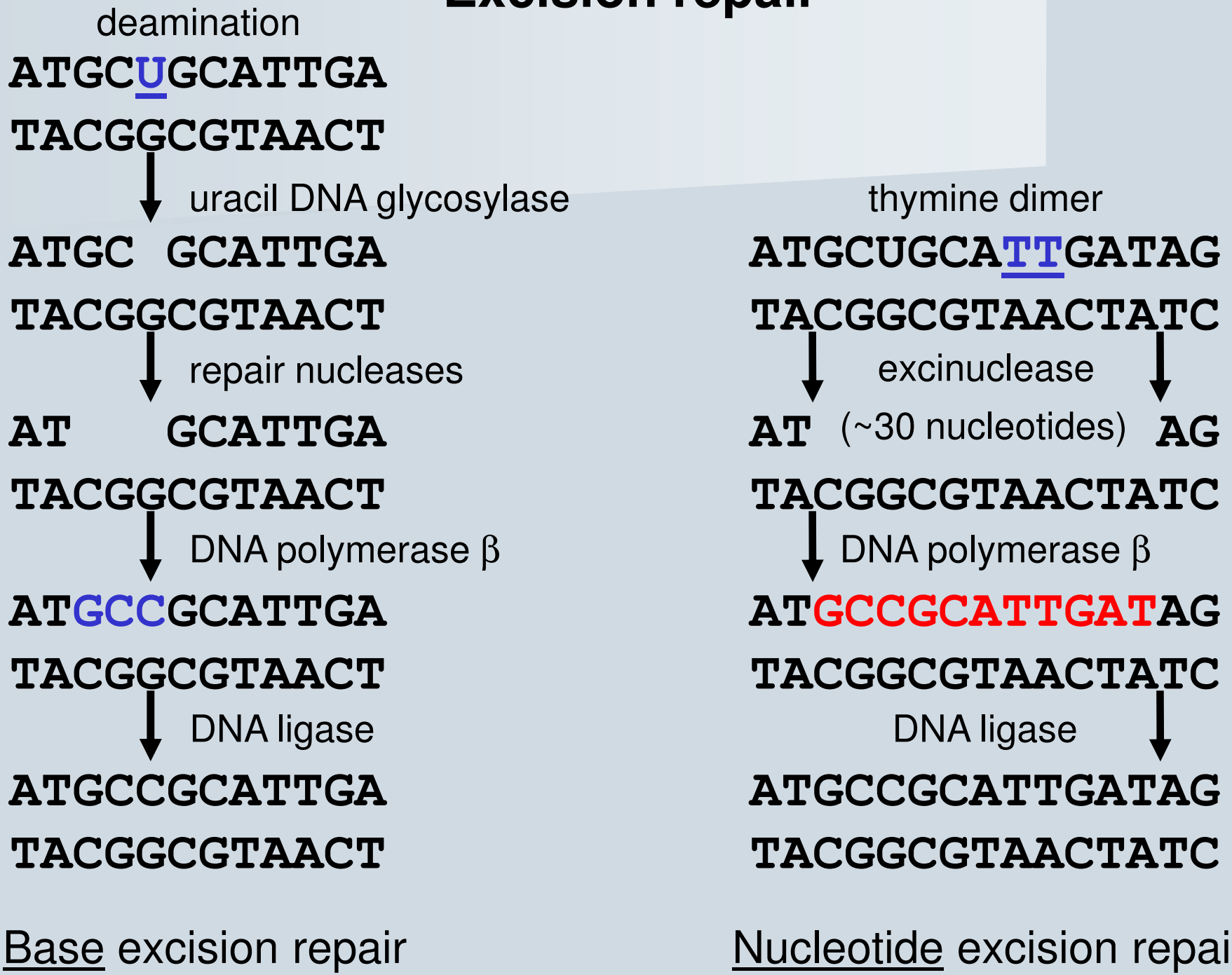
- Corrected by MMR and NER mechanism

Base Excision Repair

- **Deamination** type of damage is repaired by Base excision repair .
- **Removal of abnormal bases only :**
  - Deaminaion convert Cytosine = Uracil
  - N-Glycosidic bond break first
  - Specific AP-endonucleases
  - Recognition AP site = Missing base
  - Hydrolytically cleave nitrogen base.
  - Initiate the process of excision.
  - Remove Deoxyribose phosphate
  - Than Polymerse & Ligase complete repair


3' T G C A G T G 5'
5' A C G T C A C 3'
NH3
Spontaneous deamination
3' T G C A G T G 5'
5' A C G T U A C 3'
U
Uracil-N-glycosylase
3' T G C A G T G 5'
5' A C G T A C 3'
Apyrimidinic endonuclease
3' T G C A G T G 5'
5' A C G T A C 3'
Deoxyribose phosphate lyase
3' T G C A G T G 5'
5' A C G T A C 3'
DNA polymerase and DNA ligase
dCTP
PPi
3' T G C A G T G 5'
5' A C G T C A C 3'

Excision repair

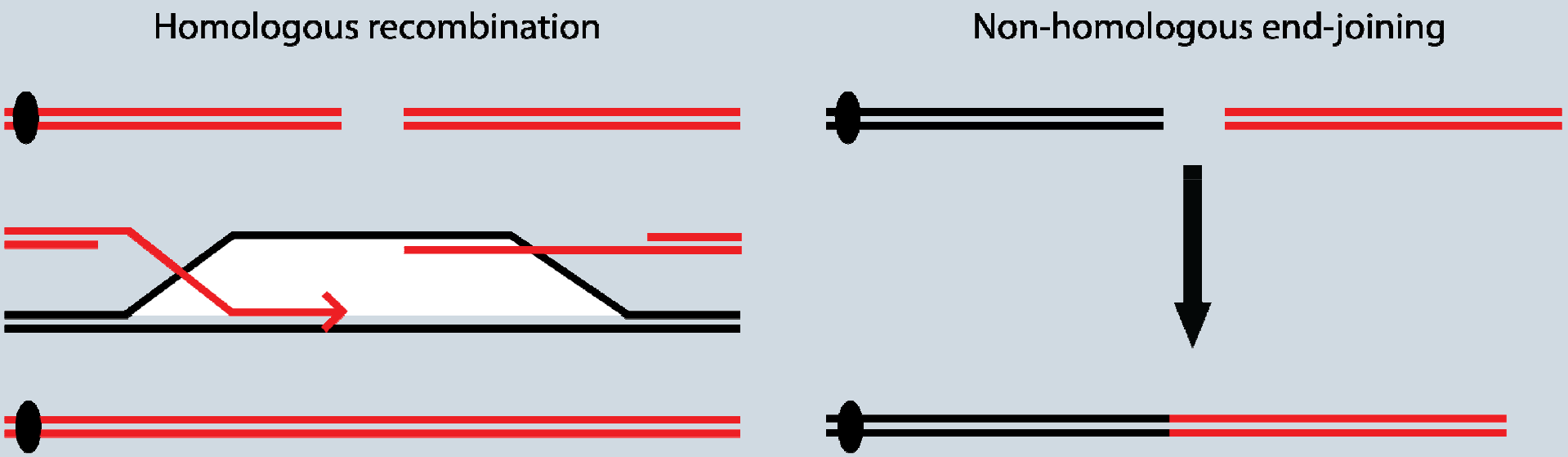


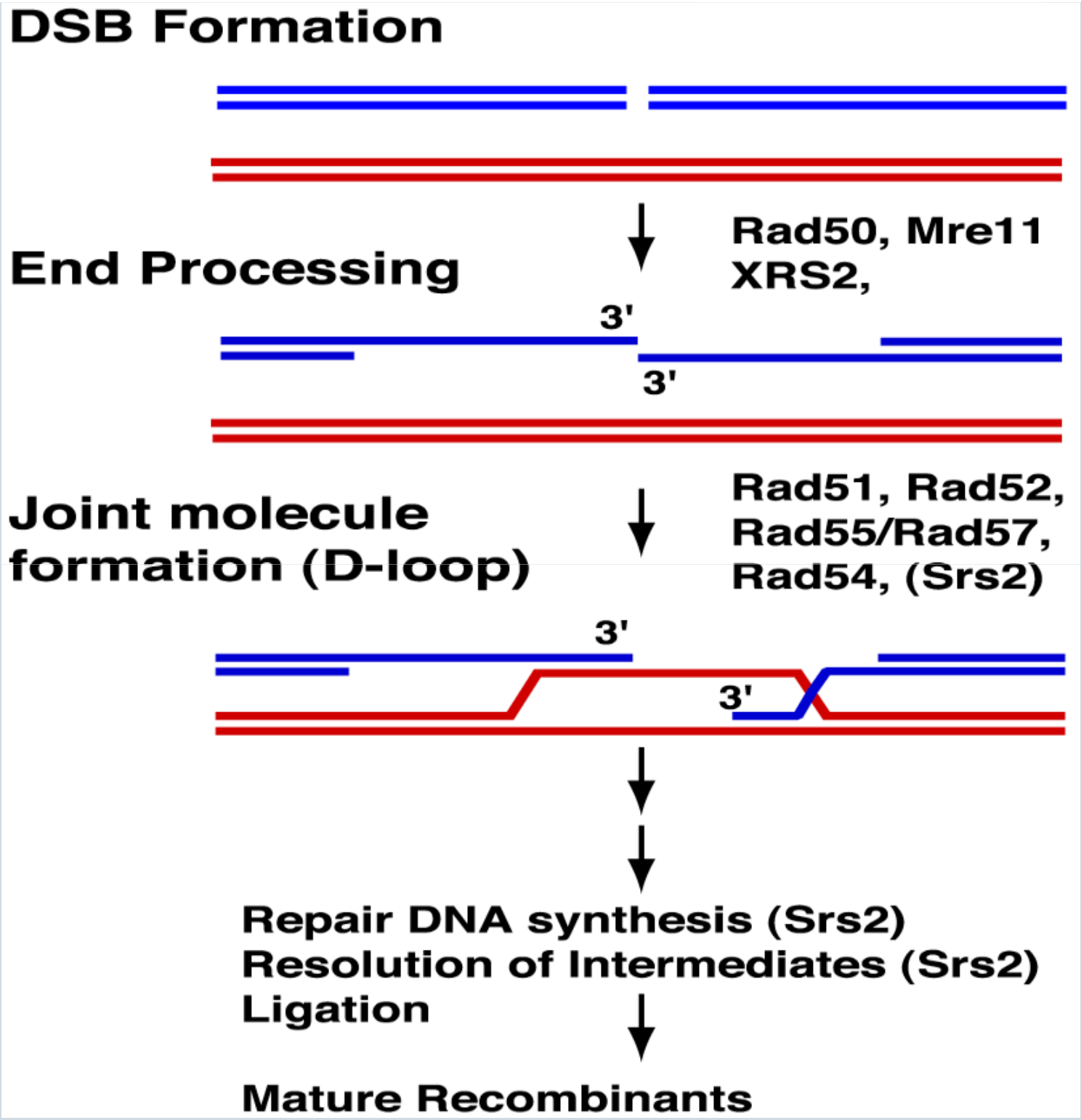
Base excision repair

Nucleotide excision repair

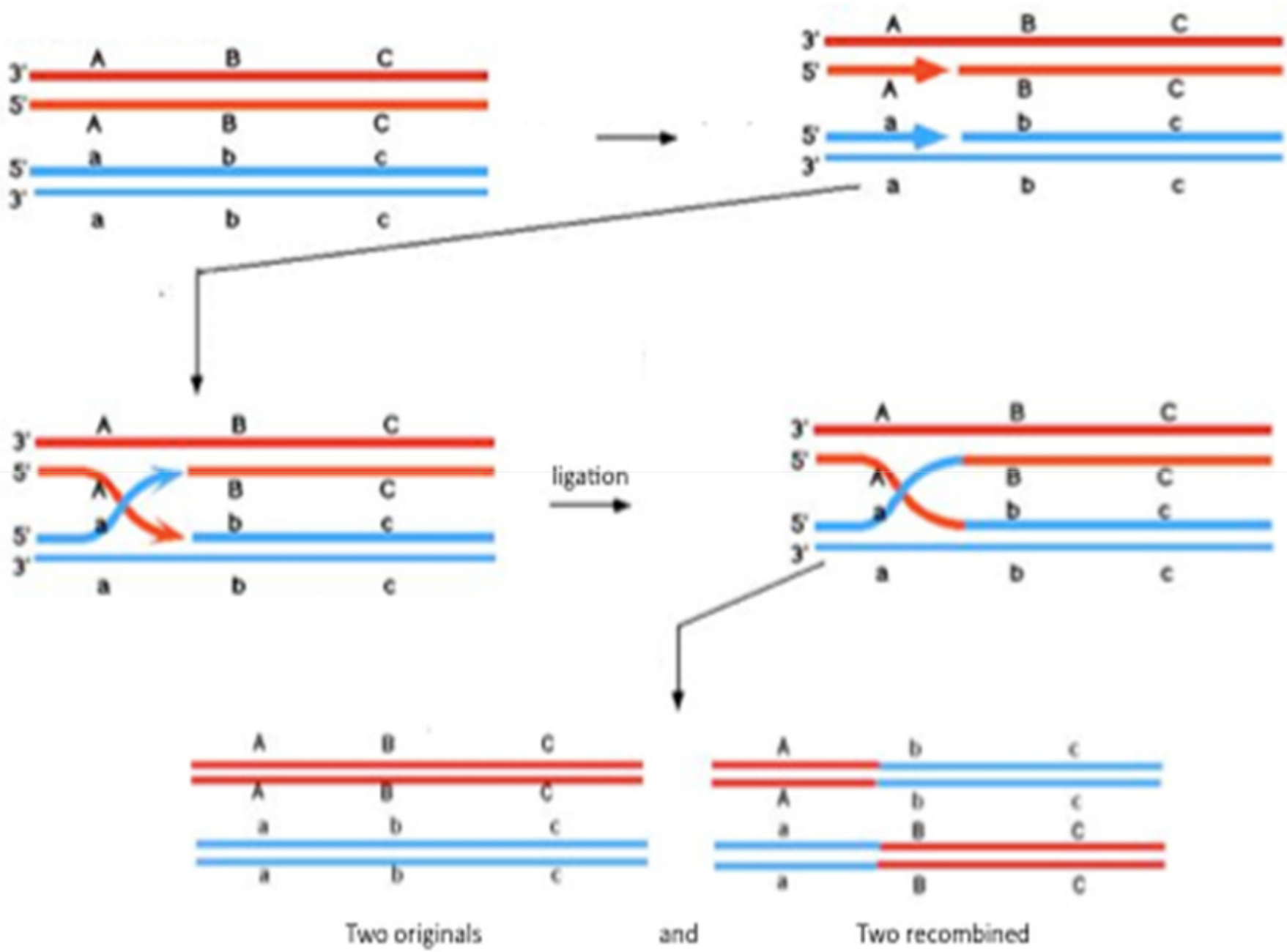
Repair Of Double Strand Break

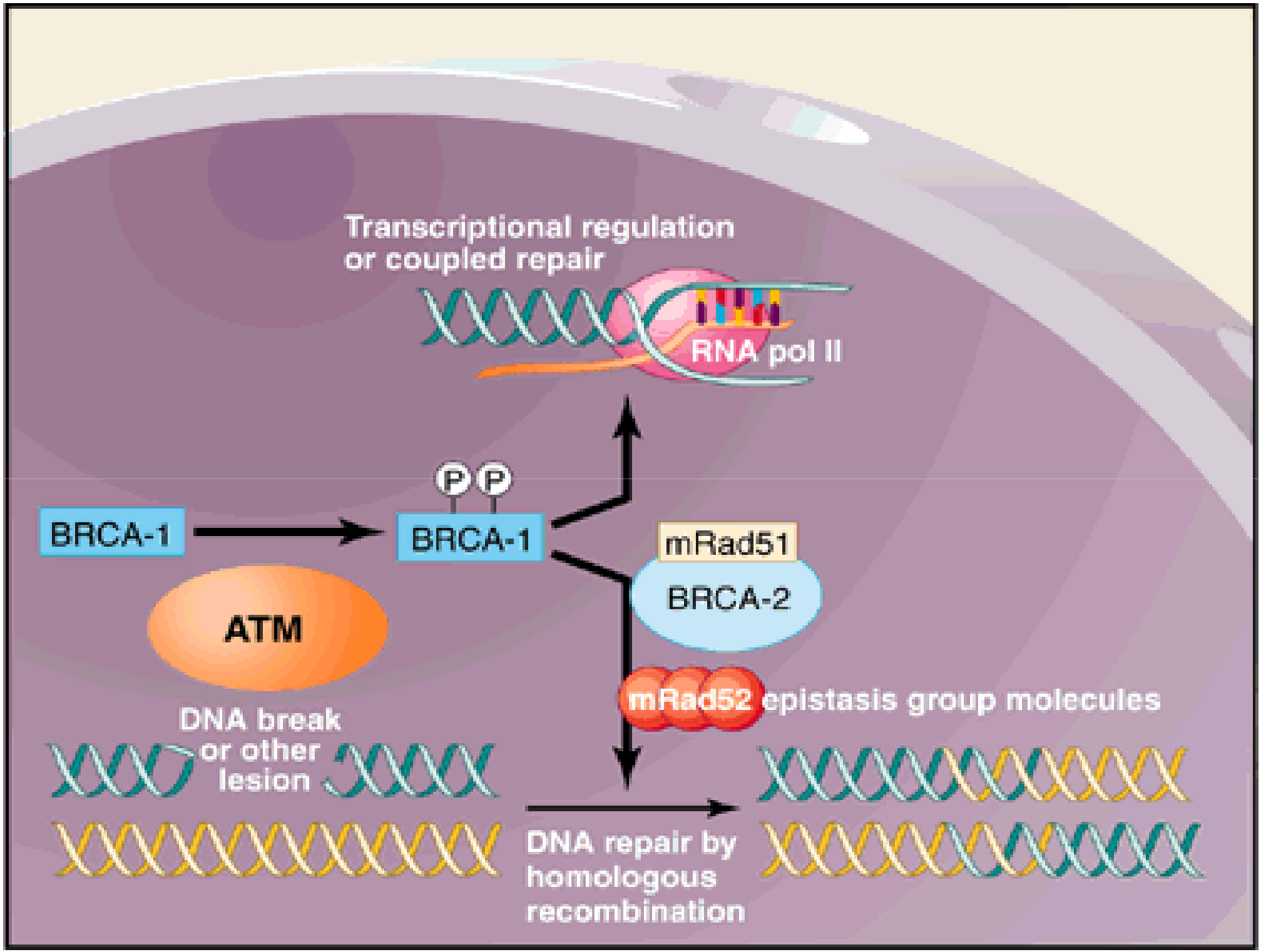
- Occur due to High-energy radiation or oxidative free radicals
- Potentially lethal
- **Non-Homologous End-joining Repair (NHER)**
  - Error prone and mutagenic.
  - Very low fidelity
  - Defects in this repair system
    - Severe immunodeficiency syndromes & Cancer
- **Homologous recombination repair (HR)**
  - Less error
  - Higher fidelity


Homologous recombination
Non-homologous end-joining


DSB Formation
End Processing
Rad50, Mre11
XRS2,
3'
3'
Joint molecule
formation (D-loop)
Rad51, Rad52,
Rad55/Rad57,
Rad54, (Srs2)
3'
3'
Repair DNA synthesis (Srs2)
Resolution of Intermediates (Srs2)
Ligation
Mature Recombinants

Homologous Recombination




Transcriptional regulation
or coupled repair
RNA pol II
P P
BRCA-1
BRCA-1
mRad51
BRCA-2
ATM
mRad52 epistasis group molecules
DNA break
or other
lesion
DNA repair by
homologous
recombination

Defects in DNA repair or replication

Xeroderma pigmentosum

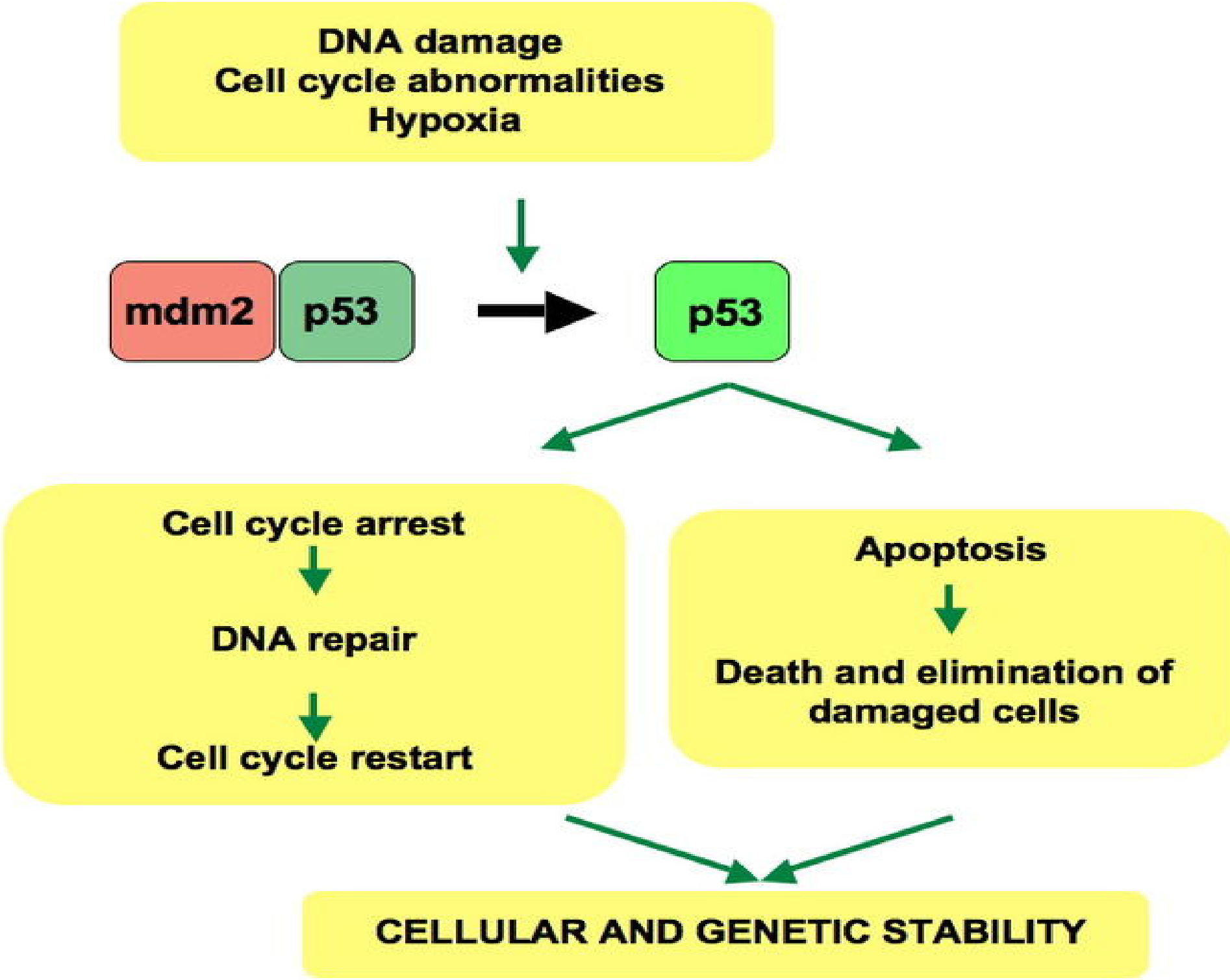
- Mutations in genes in nucleotide excision repair
- >1000-fold increase of sunlight-induced skin cancer

Ataxia telangiectasia

- Defect ingene that detects DNA damage
- Increased with exposer to X-ray

Defects in DNA repair or replication

- **Fanconi anemia**
  - caused by a gene involved in DNA repair
  - increased risk of X-ray and sensitivity to sunlight
- **Bloom syndrome**
  - caused by mutations in a a DNA helicase gene
  - increased risk of X-ray
  - sensitivity to sunlight
- **Cockayne syndrome**
  - caused by a defect in transcription-linked DNA repair
  - sensitivity to sunlight
- **Werner's syndrome**
  - caused by mutations in a DNA helicase gene
  - premature aging


DNA damage
Cell cycle abnormalities
Hypoxia
mdm2
p53
p53
Cell cycle arrest
DNA repair
Cell cycle restart
Apoptosis
Death and elimination of damaged cells
CELLULAR AND GENETIC STABILITY

p53

Function

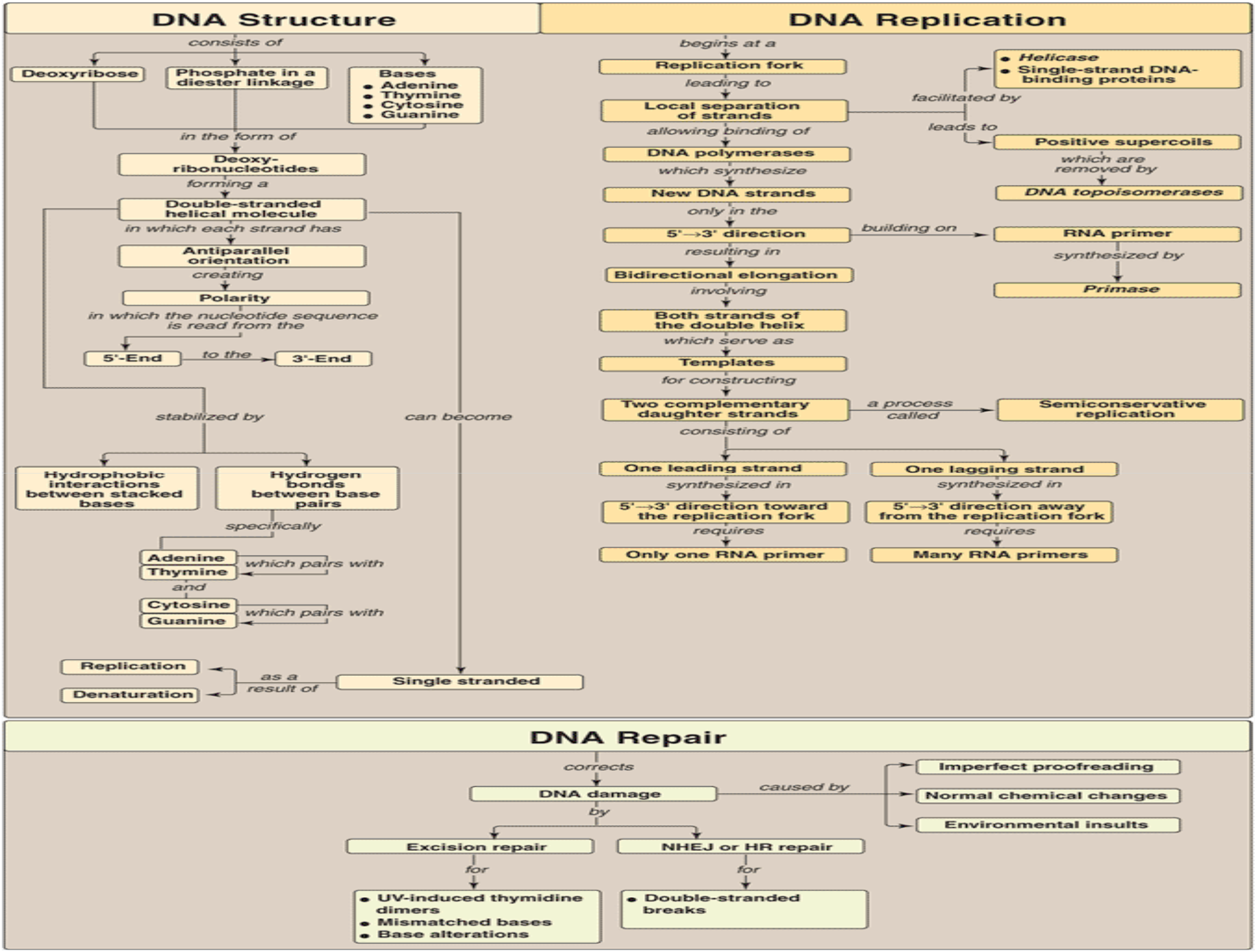
- Role in apoptosis, genomic stability
- Anti-cancer role

Mechanism

- Activate DNA repair proteins
- Arrest growth by holding the cell cycle at G_1/S
- Hold cell here for long enough
- DNA repair proteins get time to repair
- Otherwise
- Initiate apoptosis, the programmed cell death, if DNA damage proves to be irreparable.

p53

- p21 (WAF1) binds to the G1-S/CDK (CDK2)
- CDK important for the G1-S transition in the cell cycle
- p21 + G1-S/CDK (CDK2) complex inhibiting their activity.
- Cell cannot continue to the next stage of cell division.


DNA Structure
consists of
Deoxyribose
Phosphate in a diester linkage
Bases
Adenine
Thymine
Cytosine
Guanine
in the form of
Deoxy-ribonucleotides
forming a
Double-stranded helical molecule
in which each strand has
Antiparallel orientation
creating
Polarity
in which the nucleotide sequence is read from the
5'-End
to the
3'-End
stabilized by
can become
Hydrophobic interactions between stacked bases
Hydrogen bonds between base pairs
specifically
Adenine
Thymine
which pairs with
and
Cytosine
Guanine
which pairs with
Replication
Denaturation
as a result of
Single stranded
DNA Replication
begins at a
Replication fork
leading to
Local separation of strands
facilitated by
Helicase
Single-strand DNA-binding proteins
leads to
Positive supercoils
which are removed by
DNA topoisomerases
allowing binding of
DNA polymerases
which synthesize
New DNA strands
only in the
5'→3' direction
building on
RNA primer
synthesized by
Primase
resulting in
Bidirectional elongation
involving
Both strands of the double helix
which serve as
Templates
for constructing
Two complementary daughter strands
a process called
Semiconservative replication
consisting of
One leading strand
synthesized in
5'→3' direction toward the replication fork
requires
Only one RNA primer
One lagging strand
synthesized in
5'→3' direction away from the replication fork
requires
Many RNA primers
DNA Repair
corrects
DNA damage
caused by
Imperfect proofreading
Normal chemical changes
Environmental insults
by
Excision repair
NHEJ or HR repair
for
for
UV-induced thymidine dimers
Mismatched bases
Base alterations
Double-stranded breaks

- The RNA polymerase that produces the primer necessary for DNA synthesis is called .
  a. polymerase
  b. helicase
  c. primase
  d. ligase

- An enzyme that form a covalent bond between adjacent 5'-P and 3'-OH termini of separate fragments of DNA is
  a. convertase
  b. primase
  c. ligase
  d. topoisomerase

- An enzymes that breaks & than seal the break of DNA strand to remove underwinding or overwinding of the DNA helix is
  a. helicases
  b. DNA polymerase
  c. topoisomerases
  d. ligases

- Proof reading activity of DNA polymerase refers to
  a. 5′ to 3′ exonuclease activity
  b. 5′ to 3′ polymerase activity
  c. 3′ to 5′ exonuclease activity
  d. 3′ to 5′ polymerase activity

- What is false about DNA Polymerase I?

a. 5’ to 3’ polymerase activity
b. 5’ to 3’ exonuclease activity
c. 5’ to 3’ proof reading activity
d. None.

- Arabinose (analogue of deoxyribose) is
  a. Use as antiviral and anticancer drug
  b. Use to inhibit replication.
  c. Use as anti- diabetic agent.
  d. a & b.

- Which of the following is true about DNA topoisomerase
  a. It unwinds DNA.
  b. It always break both strand of DNA
  c. It produces positive supercoiling.
  d. None

- The 3' end of each Okazaki fragment is joined to the 5' end of the next fragment by
  a. DNA Polymerase I & DNA ligase
  b. DNA Polymerase III & DNA ligase
  c. DNA ligase
  d. DNA Polymerase I

- Topo isomerase enzyme is inhibited by antibiotic
  a. Ciprofloxacin
  b. Adriamycin
  c. Doxorubicin
  d. Amoxycillin

- During mismatch repair , parent DNA strand is identify by it’s
  a. Ribosylation
  b. Hydroxylation
  c. methylation
  d. phosphorylation

- Error during DNA replication can be corrected by
  a. DNA ligase
  b. Primase
  c. DNA Polymerase
  d. Topoisomerase

- All of the following is a tumor suppressor protein, EXCEPT

a. p53

b. mdm2

c. BRCA

d. UV specific endonuclease

- About “Non homologous end joining”, what is incorrect out of following?

a. higher chance of gene loss.

b. higher fidelity of fidelity

c. higher chance of gene exchange

d. higher chance of immunodeficiency syndrome.