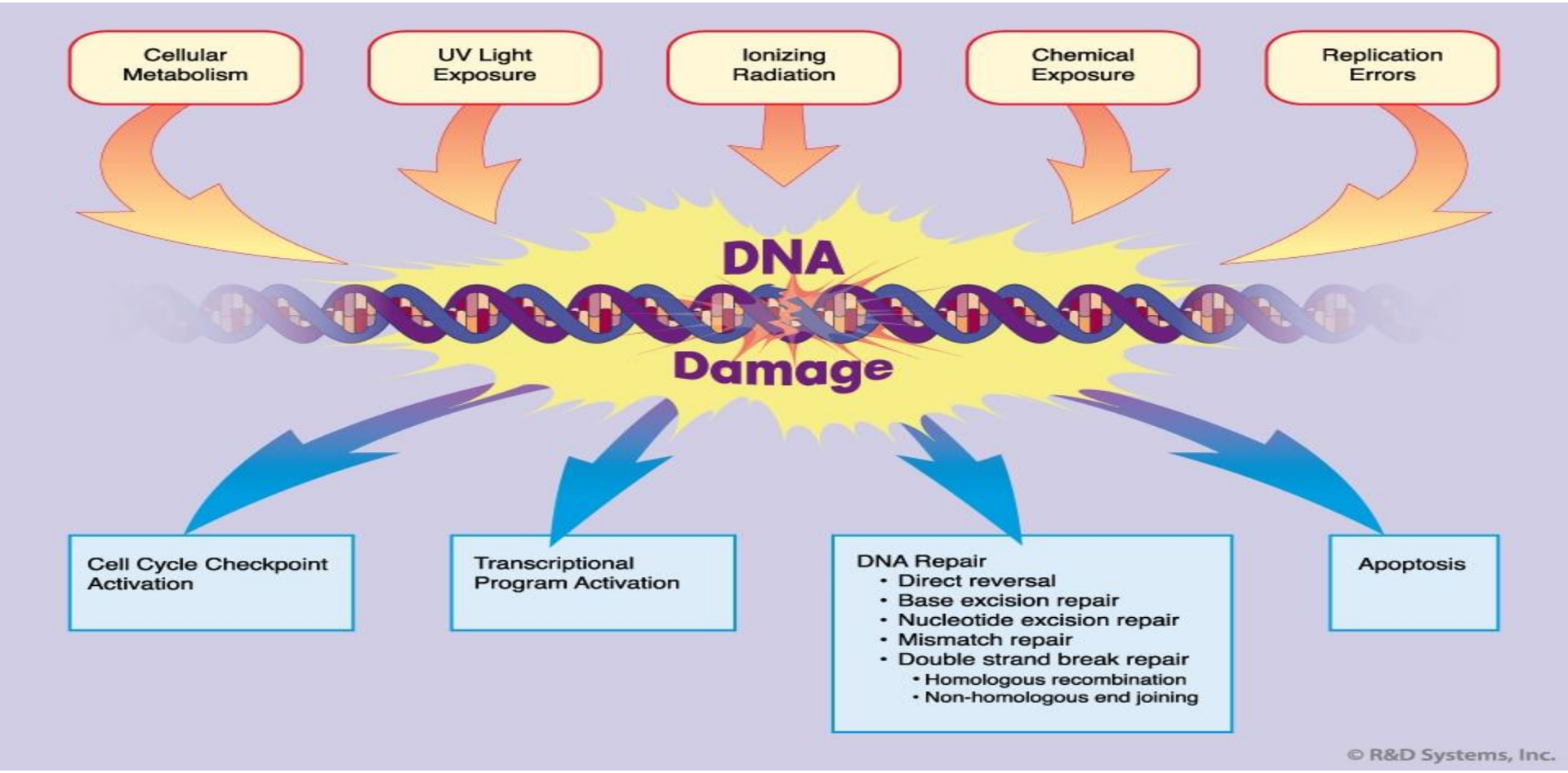
DNA Damage, Mutations and Repair Mechanisms



DNA Damage

- DNA molecules like all other biomolecules are subjected to be damaged endogenously or exogenously.
- Most of the damage can be repaired by different repair mechanisms
- Can be classified into:

A. Endogenous (spontaneous) damage:

Random and spontaneous DNA lesions arises naturally without known causes.

B. Exogenous (Induced) damage:

Occurs due to various external factors.

Endogenous DNA Damage

- DNA is subjected to be damaged by spontaneous changes under normal cell conditions including:
  - A. Deamination
  - B. Depurination
  - C. Replication errors
  - D. Oxidative DNA damage

A. **Deamination**:

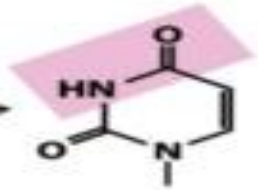
e.g. cytosine $\xrightarrow{\text{deamination}}$ uracil

- It occurs at a rate of about 100 bases/cell/day

Deamination of DNA Bases

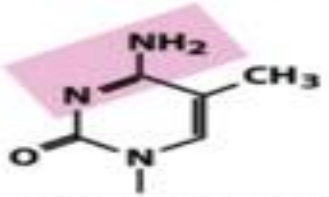
Deamination of C→U and 5-meC→T occur *spontaneously* in a human cell.

Cytosine

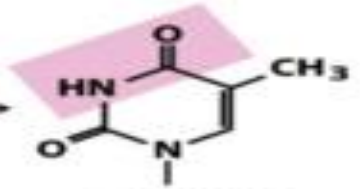


Uracil

Deamination of A and G also occurs spontaneously, but at a slower rate.



5-Methylcytosine

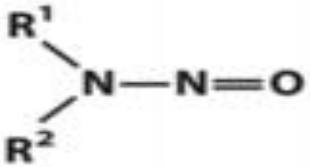


Thymine

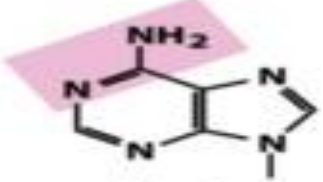
Some common chemicals can accelerate the rate of deamination.

$NaNO_2$ Sodium nitrite

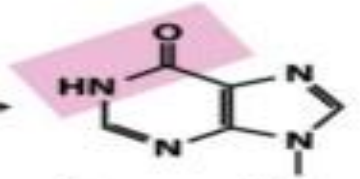
$NaNO_3$ Sodium nitrate



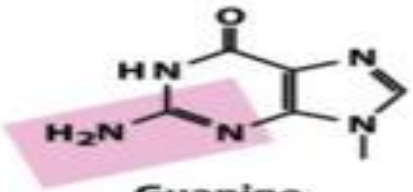
Nitrosamine



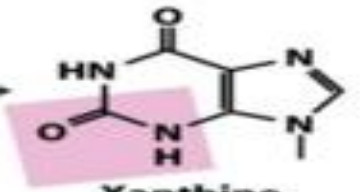
Adenine



Hypoxanthine



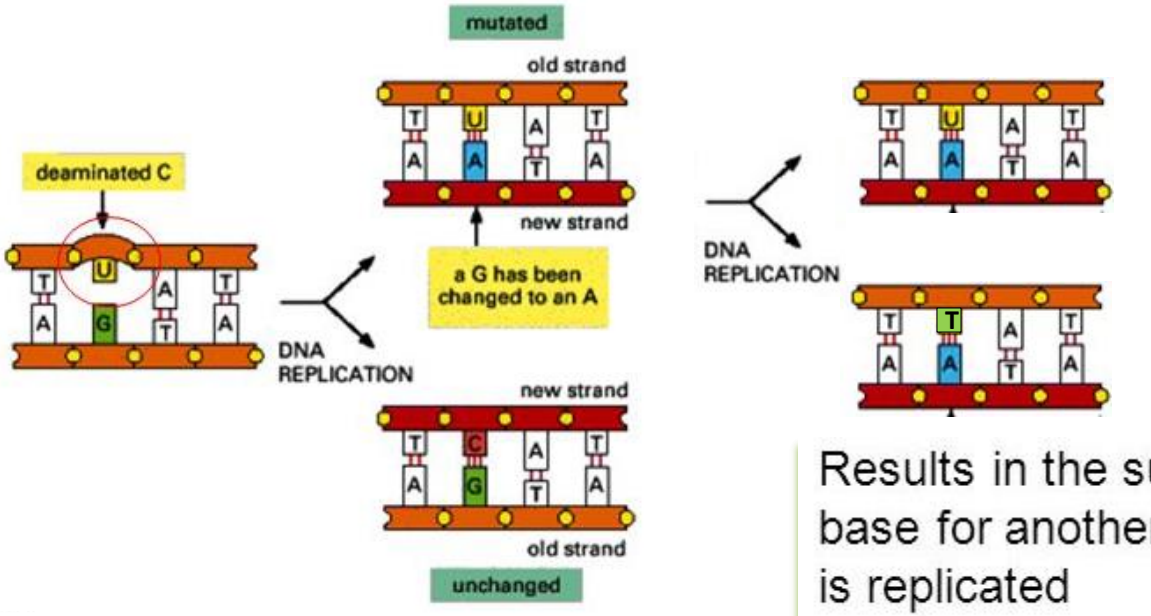
Guanine



Xanthine

Lehninger 8-29 and 8-31a

Deamination of cytosine produces uracil



Results in the substitution of one base for another when the DNA is replicated

Nitrogenous base	Original base pair	Deamination product which base pairs with ( ) 1st round	Substituted base pair 2nd round
Cytosine	C-G	Uracil (A)	T-A
Adenine	A-T	Hypoxanthine (C)	G-C
Guanine	G-C	Xanthine (T)	A-T
5-Me cytosine	C-G	Thymine (A)	T-A
Thymine	T-A	Thymine	T-A

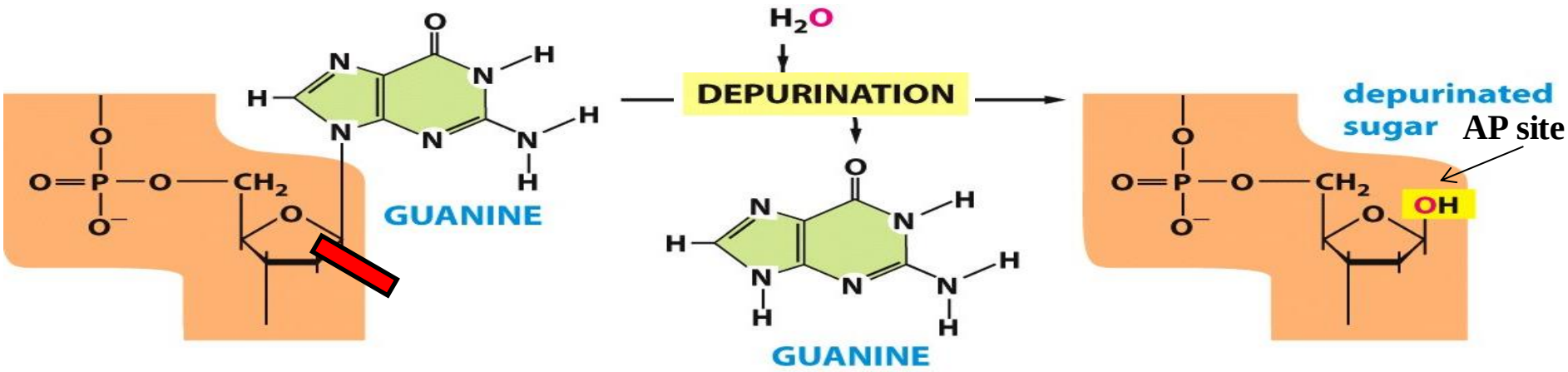
- The mismatched base pairs in DNA molecule helps in the recognition of the damage and enzymatic removal of the unusual bases (such as DNA N-glycosylase enzyme)

- If the damage is not corrected, during DNA replication most of these changes would lead to mutations in the daughter strand of DNA mainly in the form of base pair substitution

- These mutations will become permanent and finally will be inherited

B. Depurination:

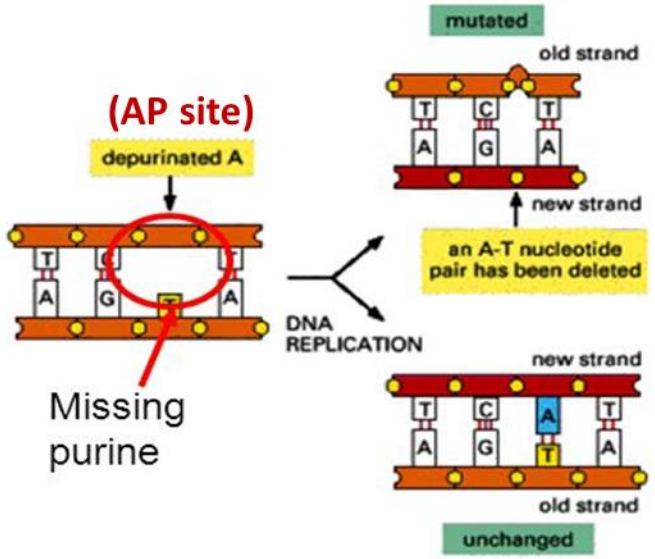
- The loss of a purine base by spontaneous hydrolysis of the N-glycosidic bond that links it to deoxyribose C1' ⟶ resulting in apurinic site (AP site)
- Under physiological conditions, depurination occurs at a rate of about 5000 bases/cell/day
- AP site can be recognized and repaired by specific repair mechanisms



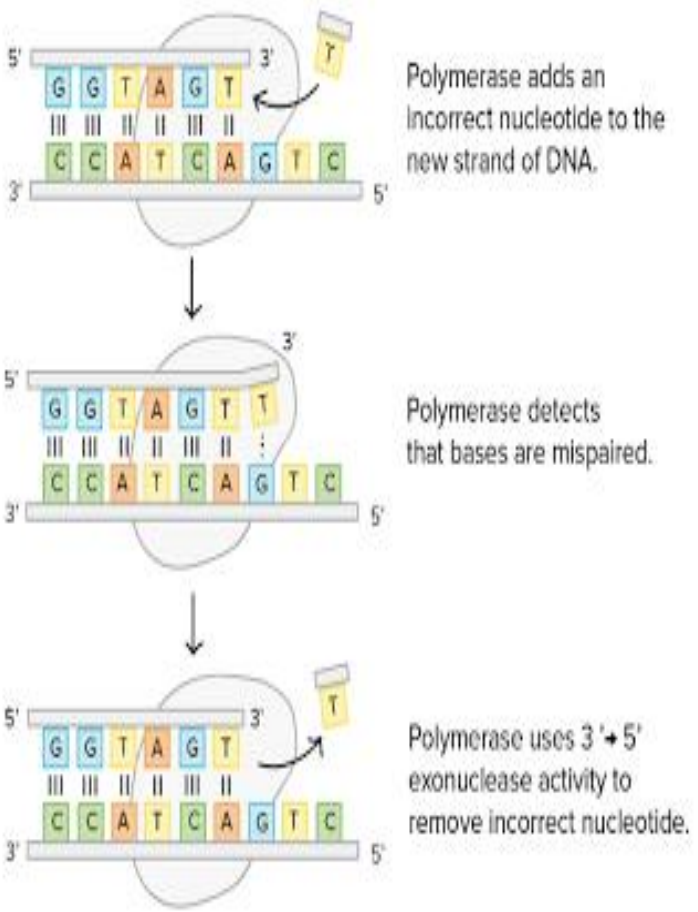
- If left uncorrected, during DNA replication these changes would lead to mutations in the daughter DNA chain (base pair deletion)

C. **Replication errors**

- Spontaneous lesions may occur during DNA replication in which a wrong base is add to the newly synthesized strand (base substitution), a DNA base is skipped (base deletion) or extra base is added (base insertion)

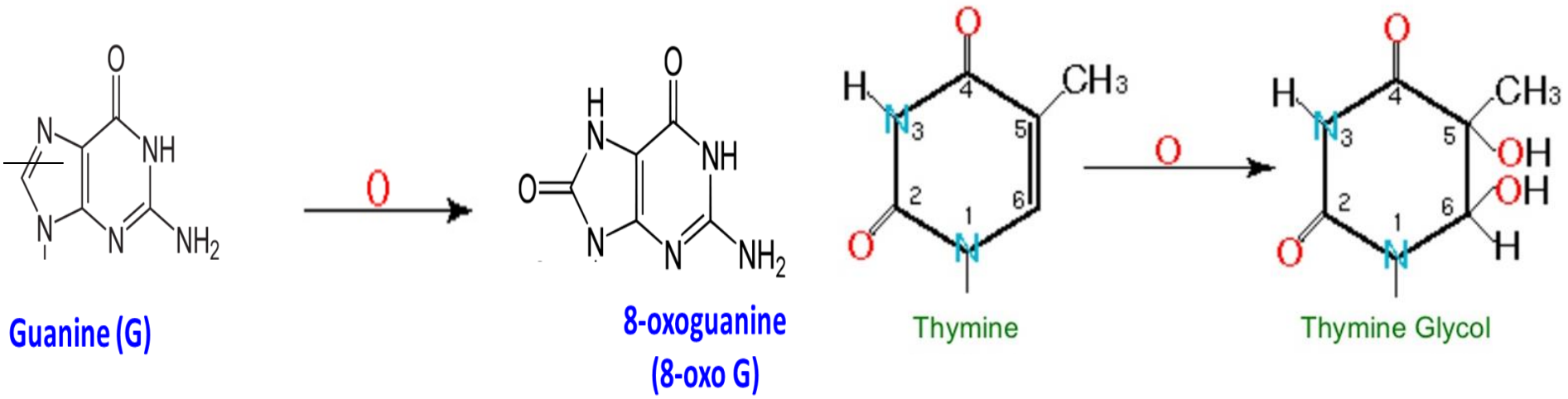


- Such errors are normally detected and repaired immediately by the proofreading/editing activity of DNA polymerase enzyme (3'-5' exonuclease activity)
- Otherwise, DNA repair enzymes will recognize the mismatched base pairs and repair them



D. Oxidative damage of DNA:

- Reactive Oxygen Species during normal metabolic processes such as superoxide radical $\cdot O_2^-$ can attack DNA leading to its damage.
- If the level of ROS is beyond the antioxidant activity of a cell, this will cause oxidative stress resulting in chemical modification of nitrogenous bases and mispairing.
- 8-oxoguanine (8-oxo G) is one of the major product of DNA oxidation. Another modified base is thymine glycol



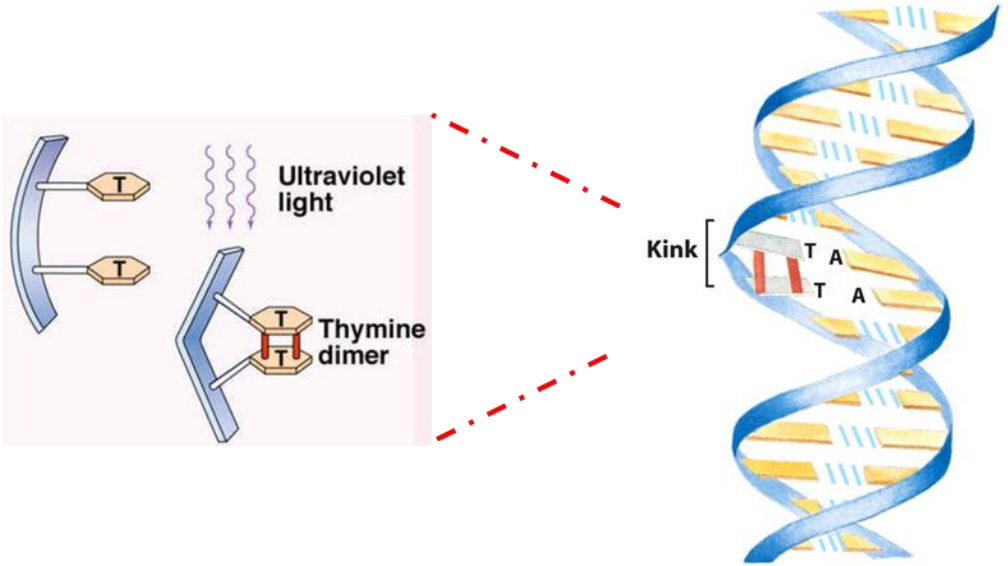
Exogenous DNA damage

A. Radiation damage:

- By UV light and ionizing radiation

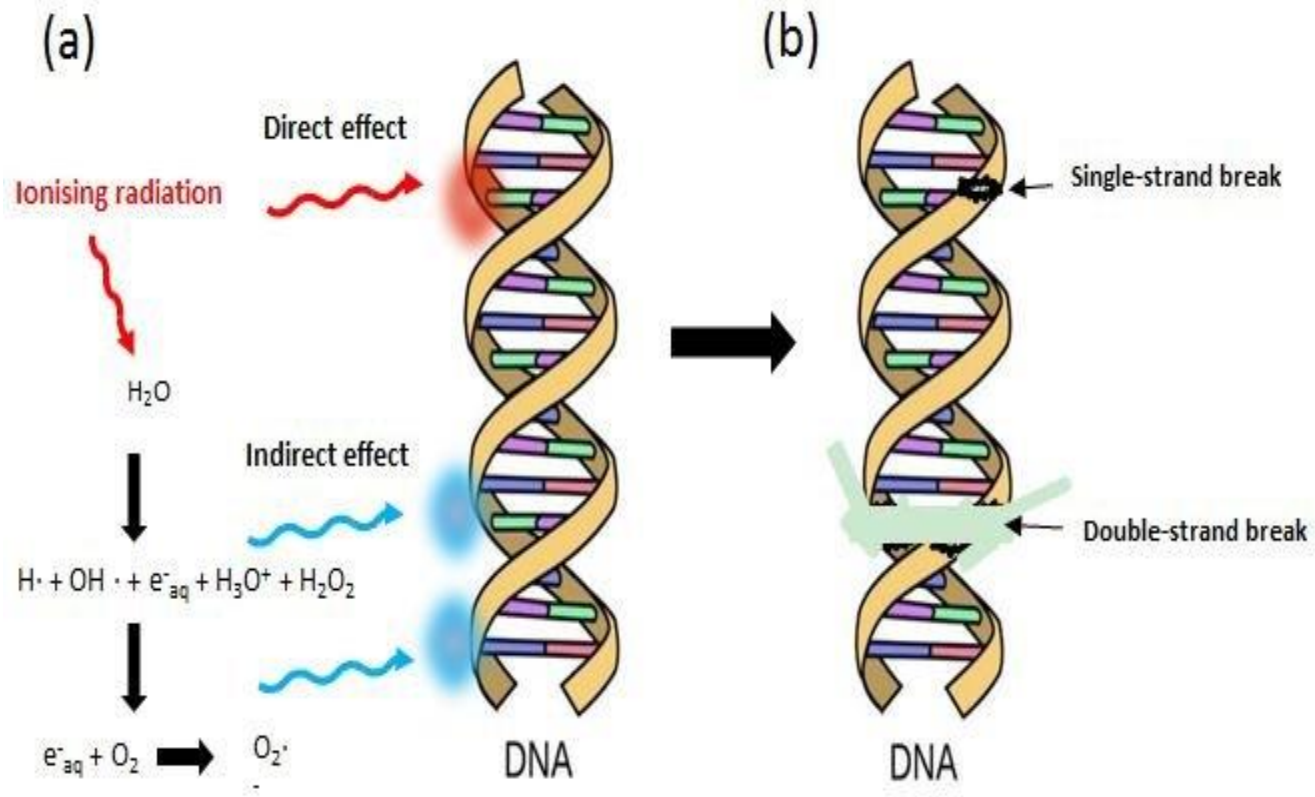
UV rays

- Pyrimidines are highly sensitive to UV light. They form pyrimidine dimer (thymine dimer) by forming intra-strand crosslinking (T-dimer)
- Dimers alter DNA structure causing a kink or a knot in DNA strand)
- Thymine dimers prevent proper replication.
- The cell either undergoes an apoptosis or malignancy
- T dimer types: cyclobutane pyrimidine dimer and pyrimidine 6-4 pyrimidone photoproduct



Ionizing Radiation:

- Like cosmic rays, X-rays and gamma rays can damage DNA molecules in 2 ways:
- Direct DNA damage by producing single strand break (SSB) and the more severe double strand break
- Indirect DNA damage by production of free radicals (exogenous ROS) which alter the structure of bases



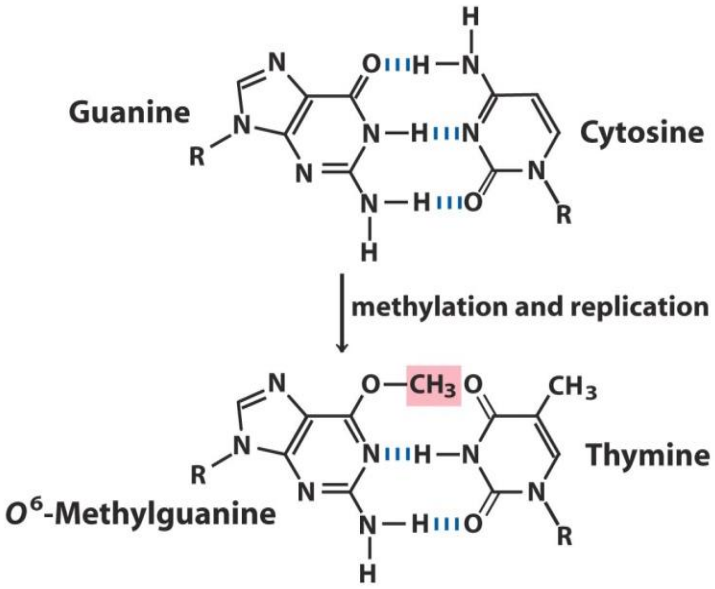
B. Chemical mutagens:

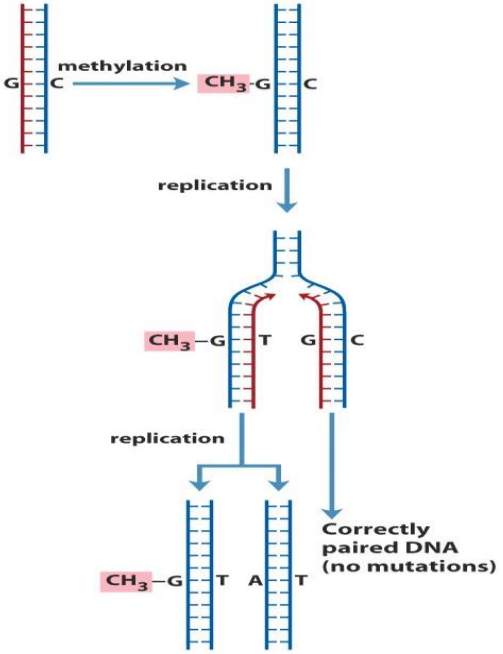
- Agents that can induce mutations if the repair system can not recognize their damaging effects and not repaired, they include:

1- Base modifying agents
2- Base analogs
3- Intercalating agents

1. Base Modifying Agents

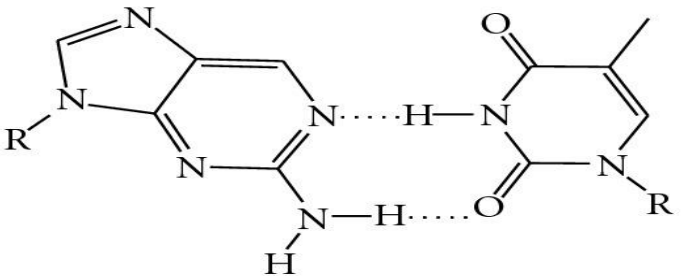
- Change or modify the chemical structure of DNA bases resulting in mispairing and other problems
- These includes alkylating agents such as SAM (S-adenosyl methionine) which adds methyl group to guanine leading to the formation of O^6 methylguanine
- If not repaired, this lesion can lead to a base pair substitution (base pair changers)





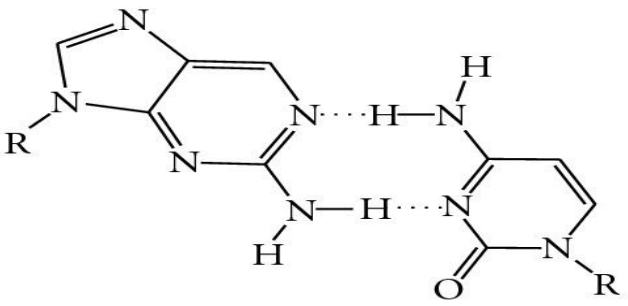
2. Base analogs

- Chemicals with similar structures to that of any of the four standard bases of DNA like 2-amino purine the base analog of adenine (6-amino purine).
- They replace them in DNA strand but do not always pair with normal bases leading to base pair substitution (e.g. AT bp is replaced with GC bp)



2-Aminopurine Thymine

(a)

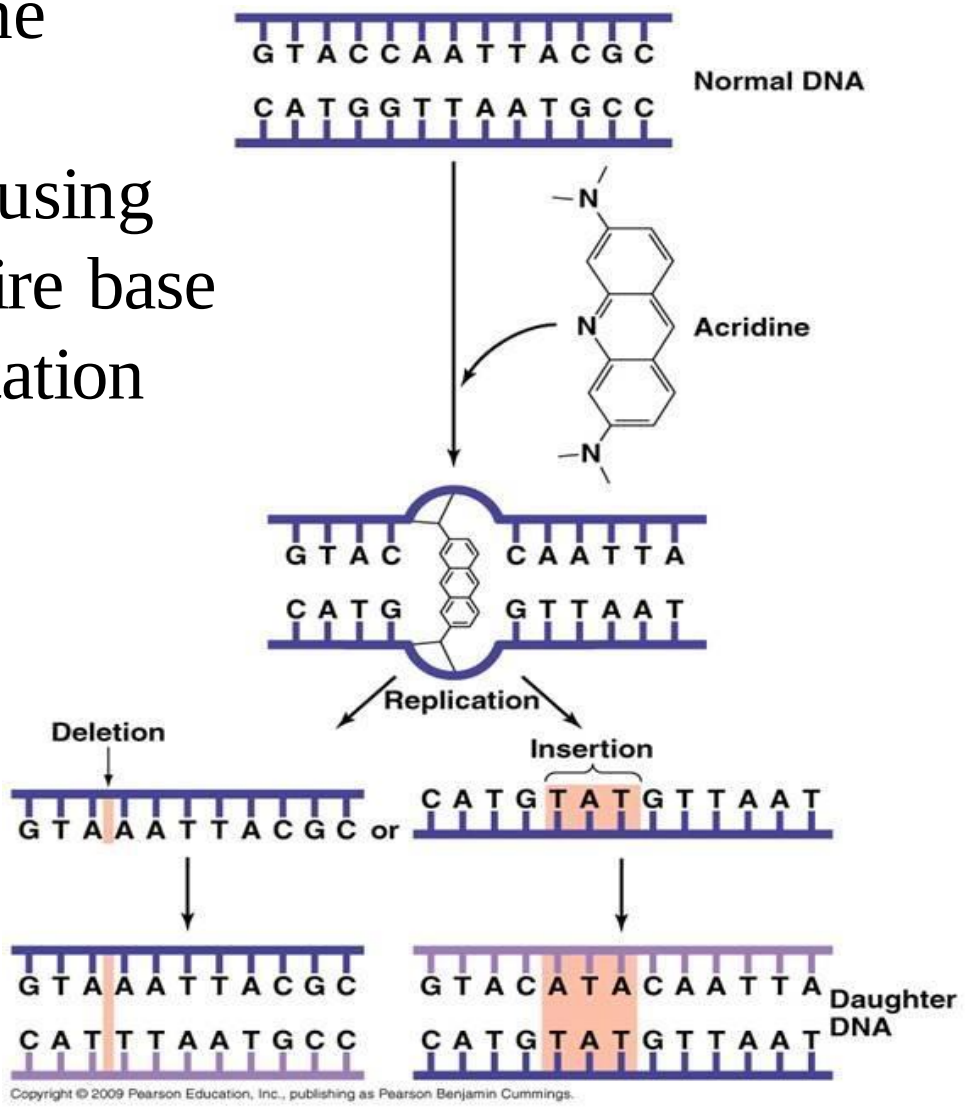


2-Aminopurine Cytosine

(b)

3. Intercalating Agents

- Sandwich themselves between adjacent DNA bases like acridine orange, benzopyrene (cigarette smoke), aflatoxin B1 (mycotoxins produced by some fungi)
- They affect DNA structure causing insertion or deletion of an entire base pair leading to frameshift mutation



DNA Repair Pathways

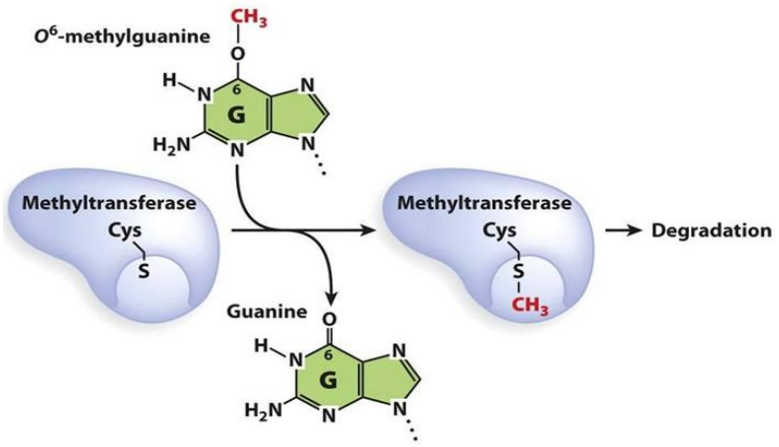
DNA repair mechanisms

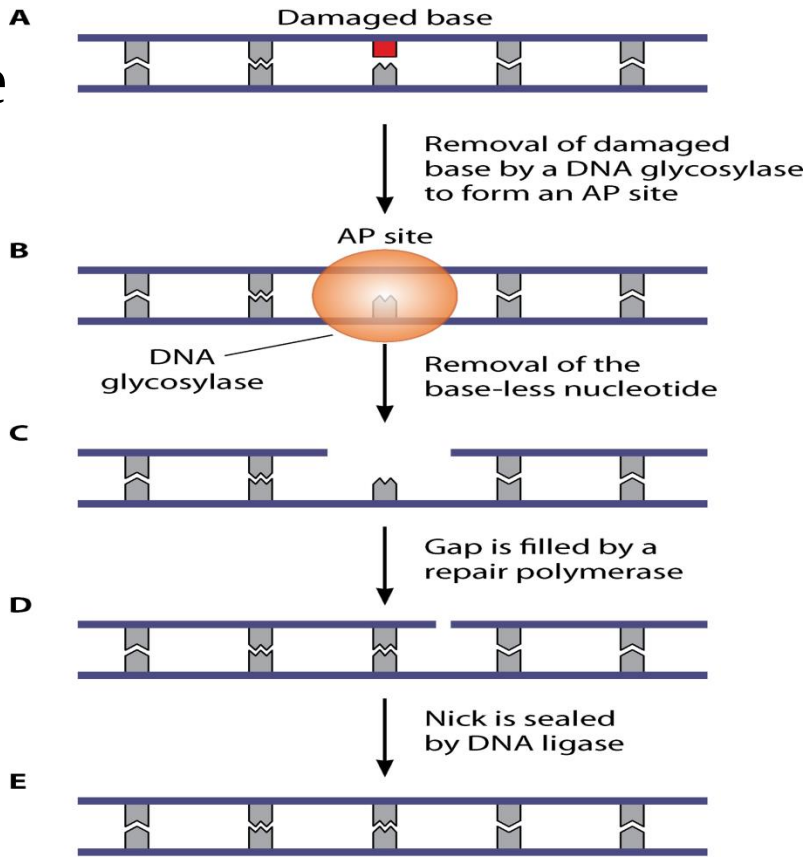
- DNA repair system is a collection of processes by which a cell identifies and corrects various DNA lesions
- Several repair strategies are available:
  A. Direct/reversal repair
  B. Base excision repair (BER)
  C. Nucleotide excision repair (NER)
  D. Strand-directed Mismatch repair (MMR)
  E. Double strand breaks repair (DSB)

A. Direct repair system

- Direct repair also called direct reversal because the damage can be directly recognized and reversed
- Two specific enzymes are involved in direct repair:

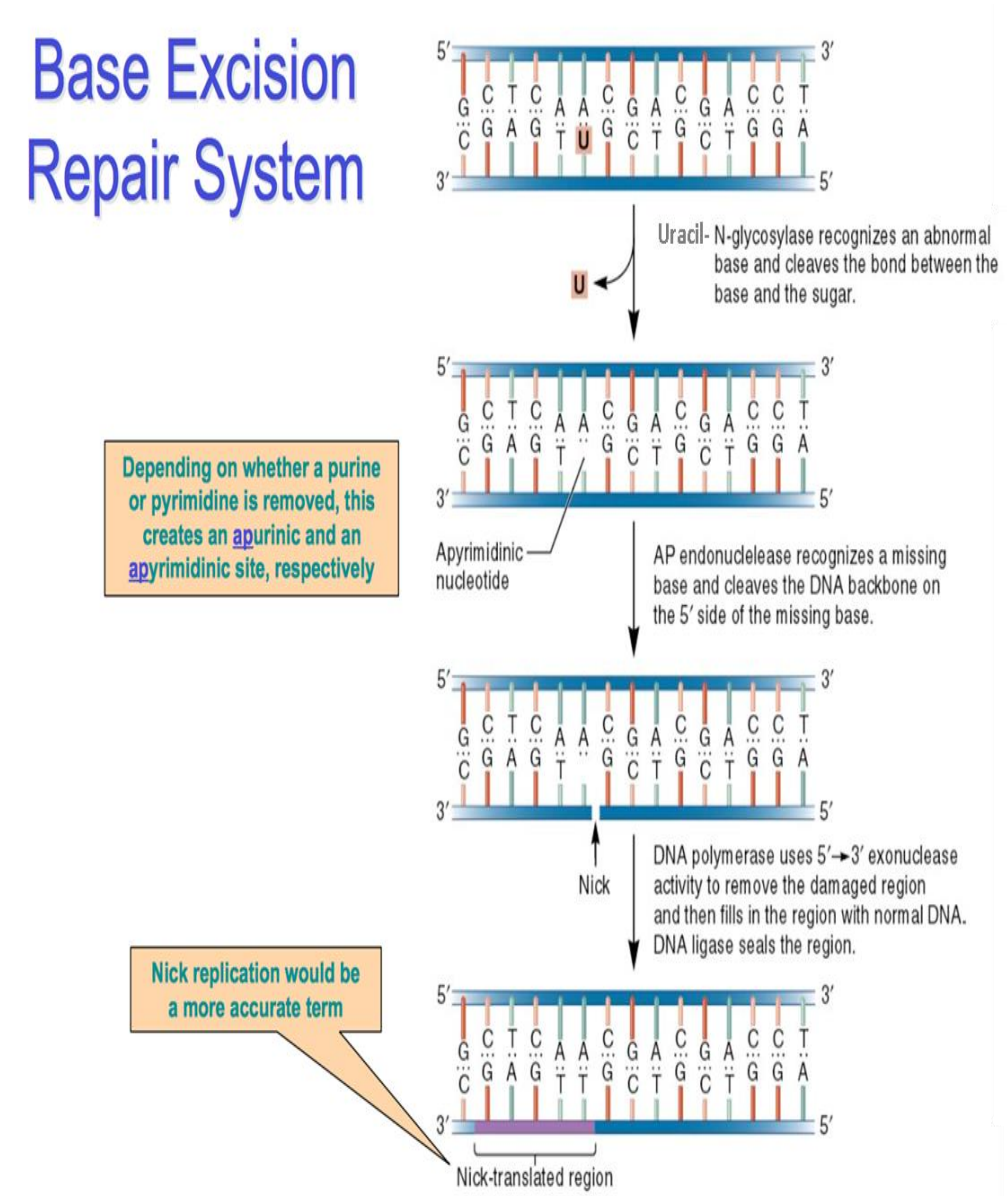
1. Photolyases which repair UV induced damage in plants, bacteria and some animals (excluding humans) by splitting the dimers
2. O6-methylguanine methyltransferase which transfers methyl group from G to a cysteine residue within the enzyme itself (suicide)





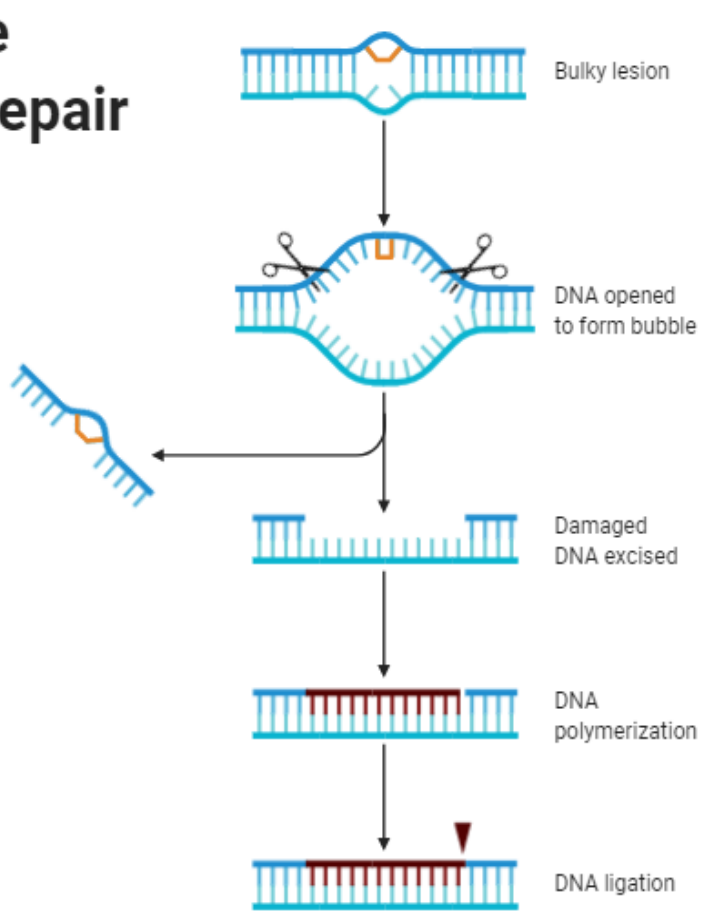
B. Base excision repair

- It involves a category of enzymes known as DNA-N-glycosylases like uracil DNA glycosylase
- Glycosylases recognize damaged bases and remove them resulting in apurinic or apyrimidinic (AP) site
- AP endonucleases nick the damaged backbone at 5' end of AP site
- DNA polymerase removes the damaged region using its 5' to 3' exonuclease activity and correctly synthesizes the new strand. Finally, DNA ligase seals the strand.



C. Nucleotide excision repair (NER)

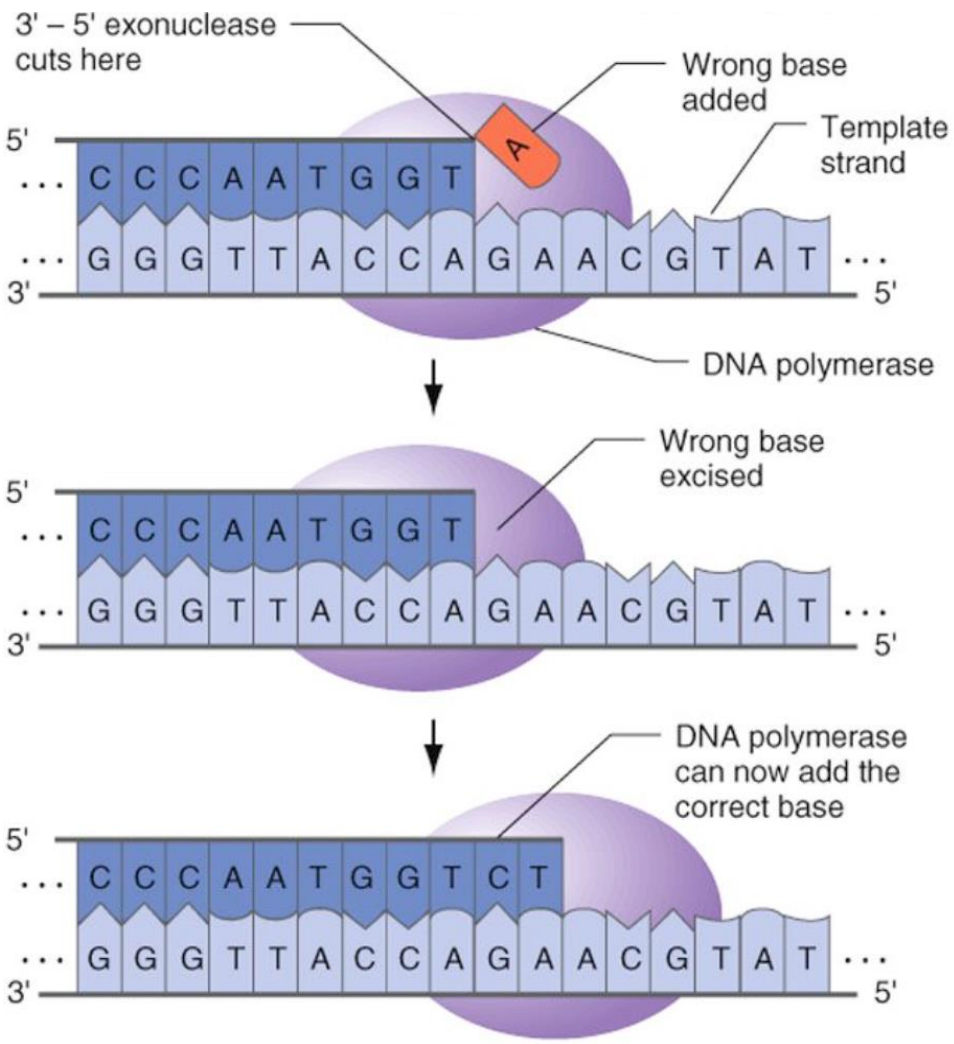
- The system corrects lesions which commonly cause bulk distortions in DNA helix like UV-induced pyrimidine dimers.
- It is highly conserved used in both eukaryotes and prokaryotes
- The damaged region is removed in a process of three steps:
  1. Recognition of the damage by enzymes of the system
  2. Excision of damaged DNA (12-24 nucleotides long) by endonucleases
  3. Resynthesis of the removed DNA region by DNA polymerase followed by ligase to seal the region



- Xeroderma pigmentosum is an autosomal recessive disorder due to lacking the normal UV repair enzymes (NER genes).
- It creates hypersensitivity to sunlight and a tendency to develop cancer skin.

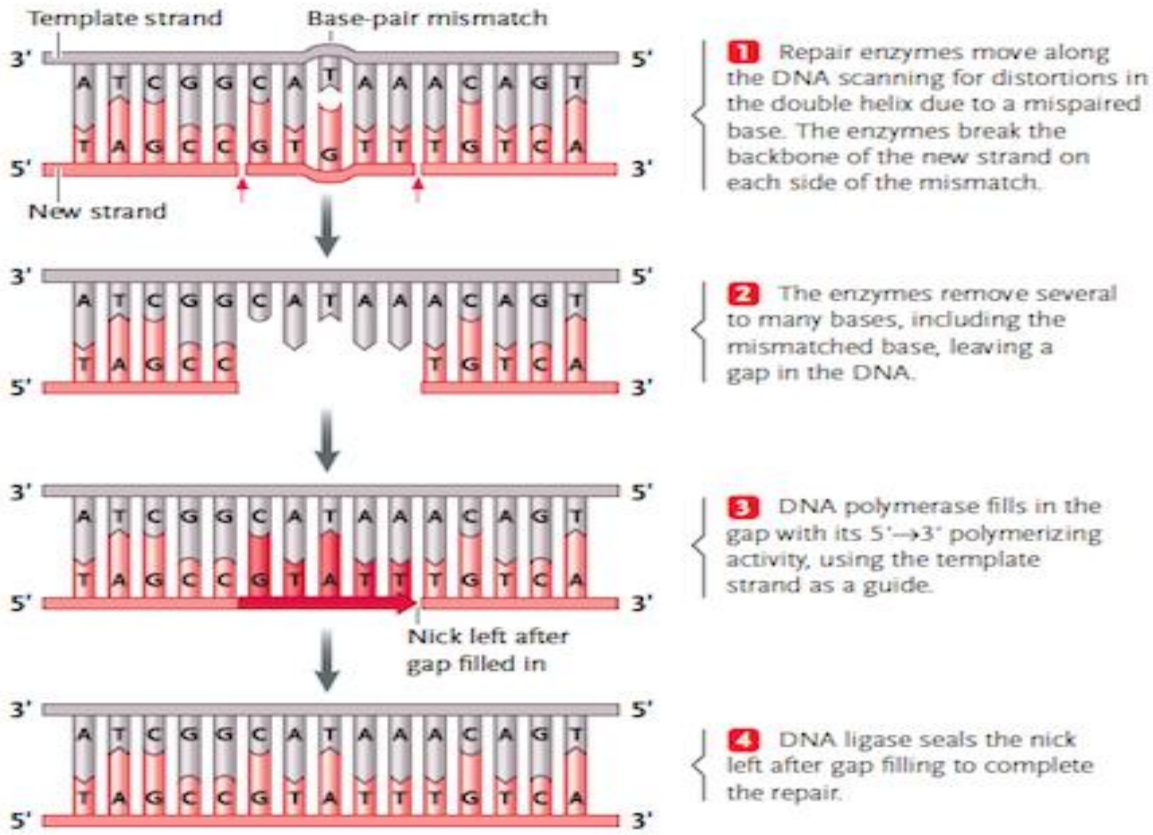
D. Strand-directed mismatch repair

- This system corrects errors introduced during DNA replication (e.g. base substitution, deletions or insertions)
- Replication errors are rare due to high fidelity of DNA replication process
- DNA polymerases have proofreading 3'-5' exonuclease (reverse) activity which recognizes mismatched bases and excises them
- Mismatch system recognizes and corrects errors that escaped from DNA polymerase proofreading machinery
- In a process of three steps:



1. Mismatched base pair is recognized
2. Excision of DNA segment containing the mismatched nucleotide from the newly synthesized strand
3. Resynthesis of the excised segment

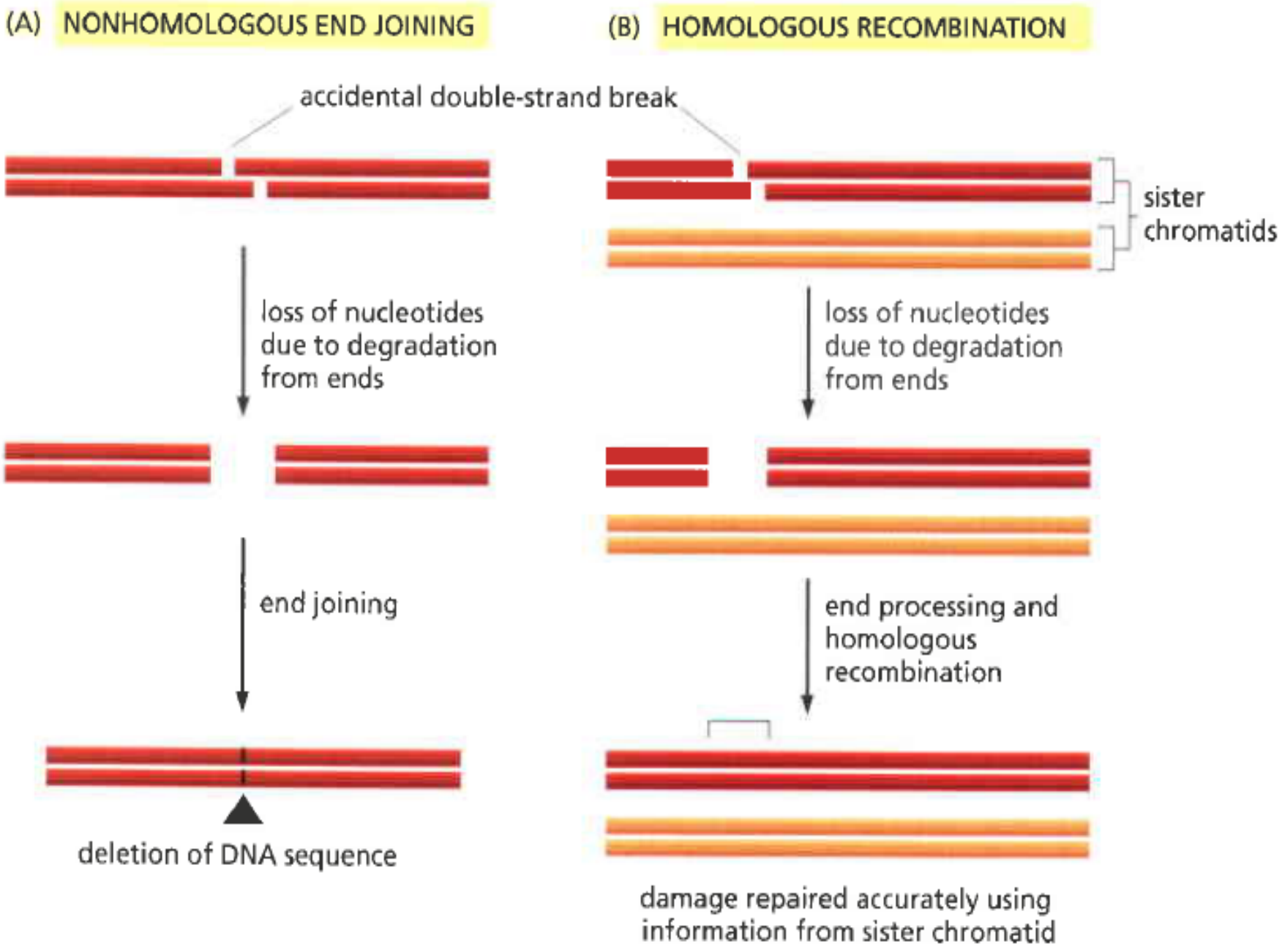
- It is called strand-directed MMR because MMR enzymes are selectively directed to the newly synthesized strand rather than to the old strand



E. Double strand breaks repair

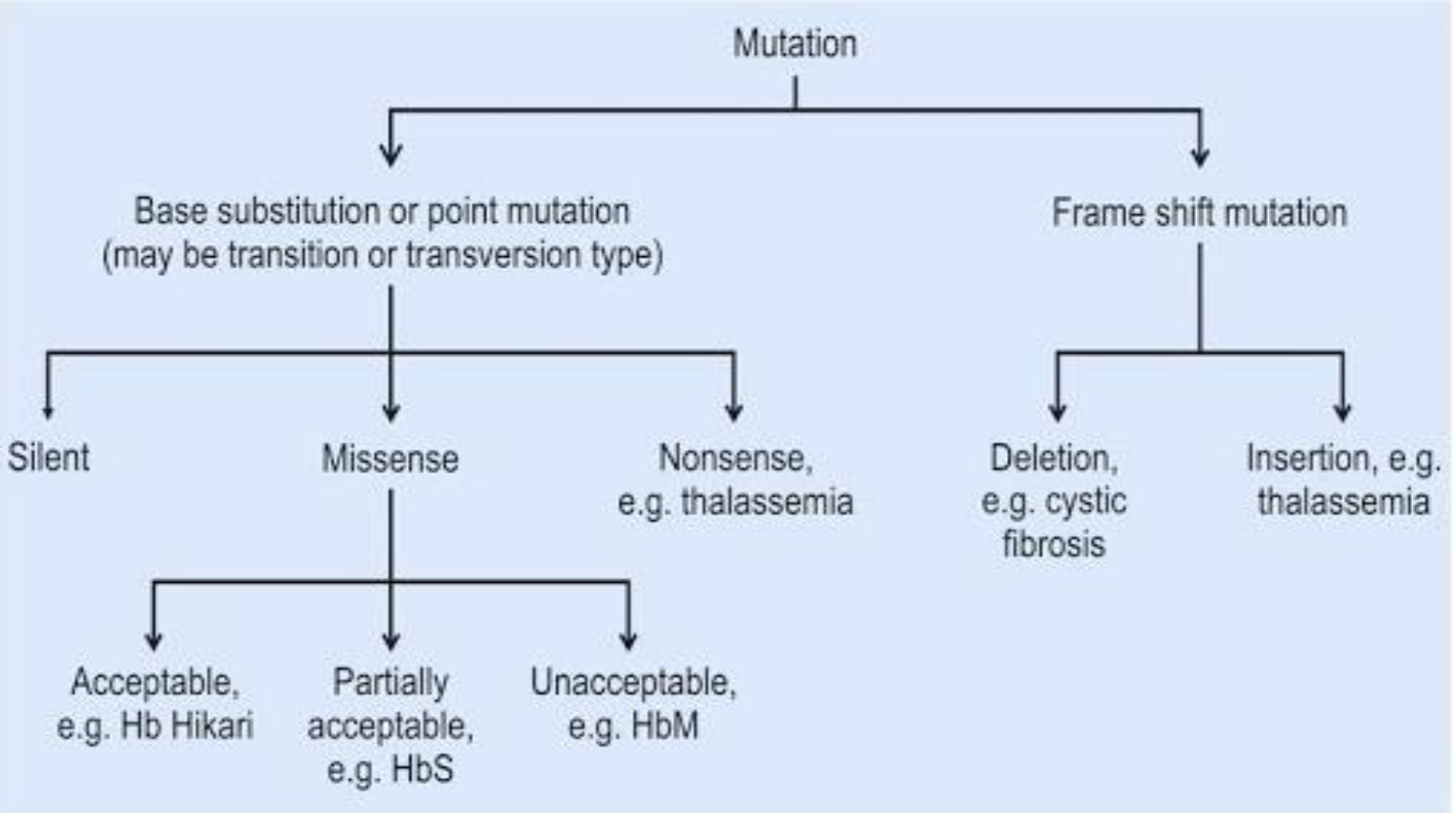
- A dangerous type of DNA damage which can lead to chromosomes fragmentation and consequently loss of genes (chromosomal aberration) if left unrepaired
- Two types of repair mechanisms:

1. Non-homologous end Joining: it is an error-prone mechanism of repair because it results in a change of DNA sequence at the site of breakage
2. Homologous recombination: is an error-free mechanism of repair because the damage is accurately repaired using information from sister chromatid


(A) NONHOMOLOGOUS END JOINING
(B) HOMOLOGOUS RECOMBINATION
accidental double-strand break
sister chromatids
loss of nucleotides due to degradation from ends
loss of nucleotides due to degradation from ends
end joining
end processing and homologous recombination
deletion of DNA sequence
damage repaired accurately using information from sister chromatid

Mutations

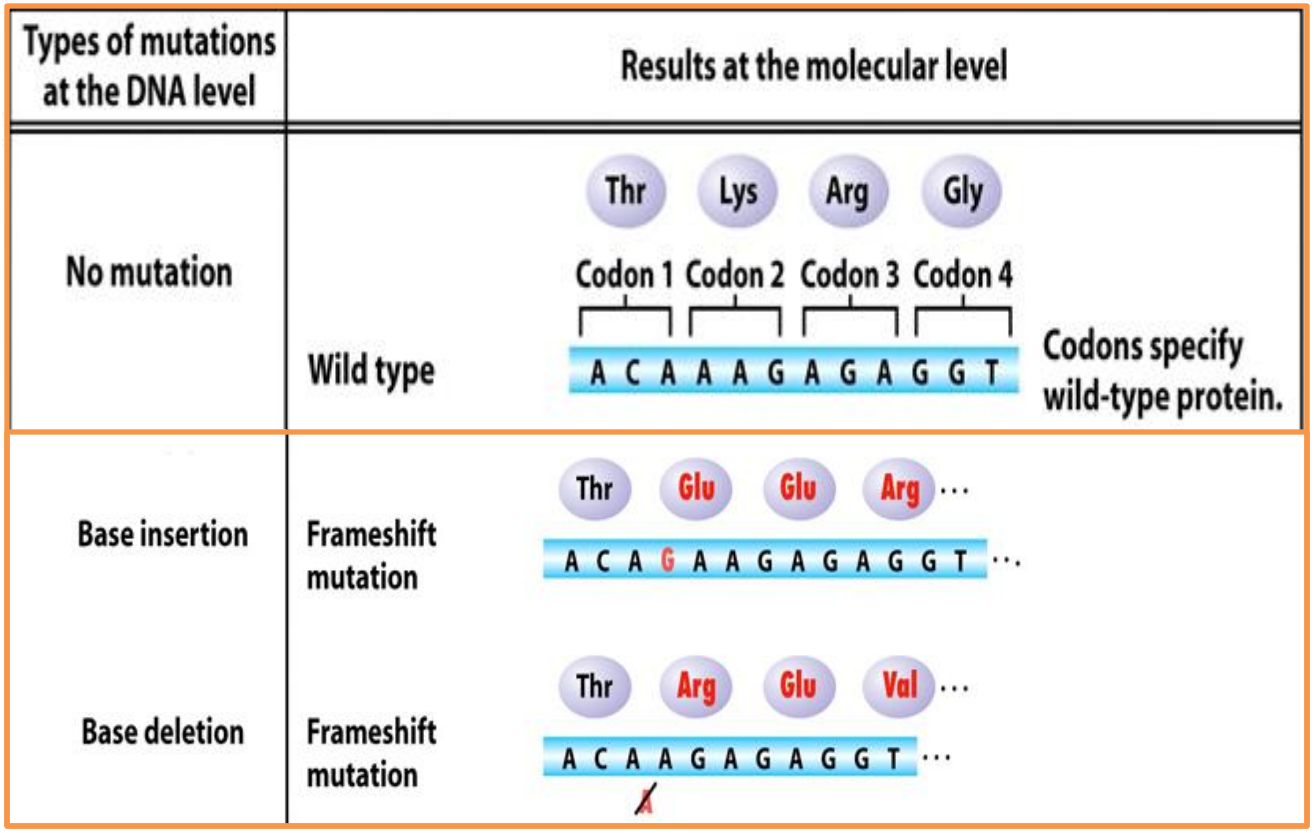
DNA Damage and Mutation



- Mutations are alterations in the sequence of genome to be targeted by the DNA repair systems and if not corrected, will be replicated, become permanent and inherited.

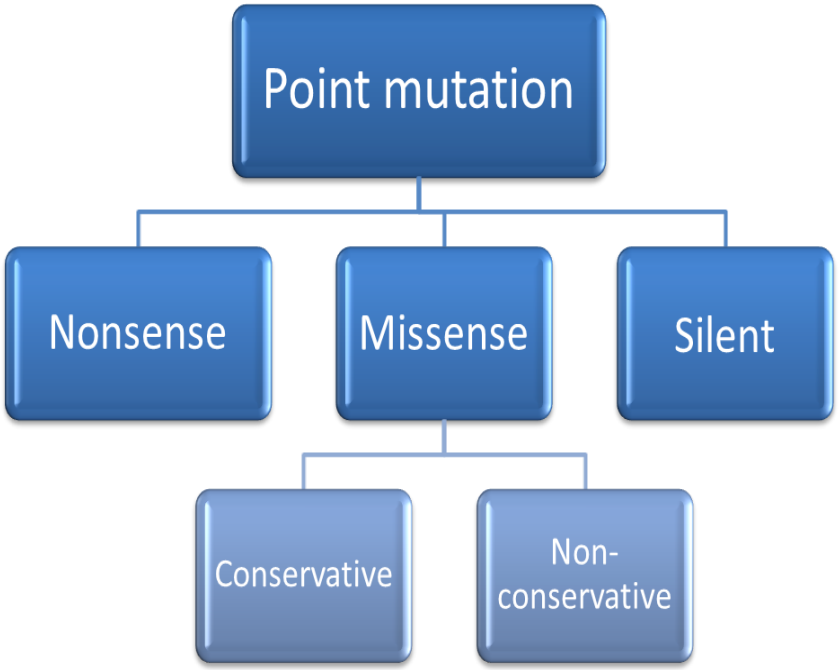
Frameshift mutation

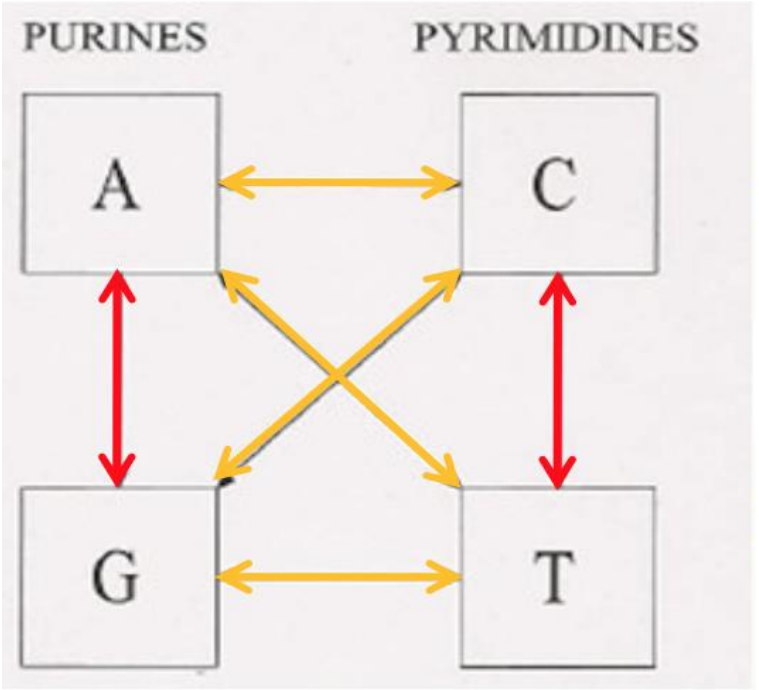
- It occurs with the insertion or deletion of a number of nucleotides (not the multiple of three) causing the alteration of the reading frame



Point Mutation

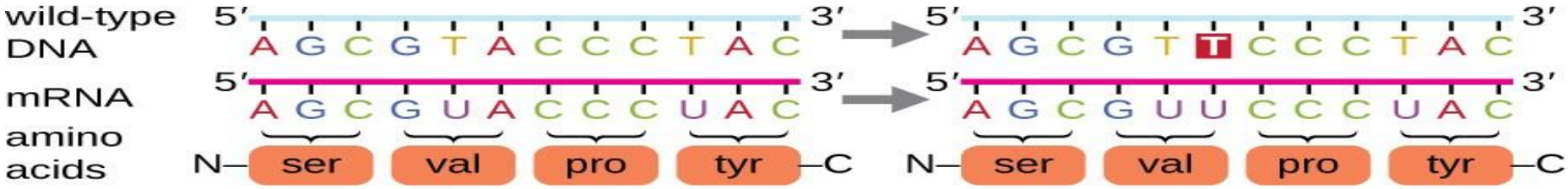
- Point mutation: an alteration in DNA sequence by a single nucleotide base and consequently a change in single base pair (substitution)
- Substitution at a point is called Transition if one purine is replaced with another purine or one pyrimidine with another pyrimidine and it is called Transversion if one purine is replaced with one pyrimidine or vise versa



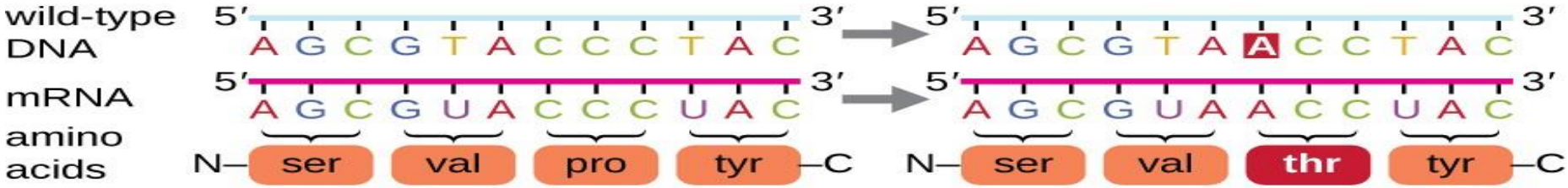


point mutation: substitution of a single base

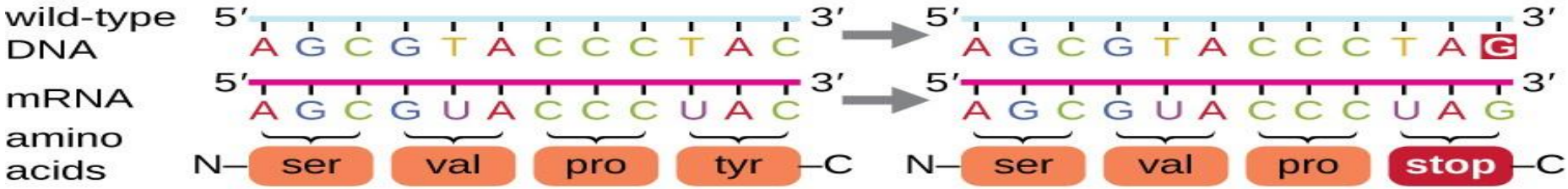
silent: has no effect on the protein sequence



missense: results in an amino acid substitution

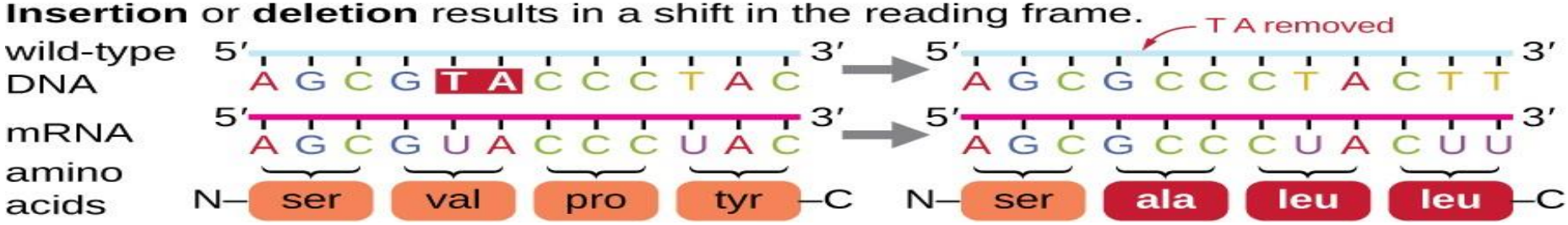


nonsense: substitutes a stop codon for an amino acid



frameshift mutation: insertion or deletion of one or more bases

Insertion or **deletion** results in a shift in the reading frame.



1. Silent mutation: a change in triplet codon without a change in the encoded amino acid. Thus, it has no effect on the protein sequence
2. Nonsense mutation: the codon changes from amino acid codon to stop codon resulting in truncated protein (mostly non-functional)
3. Missense mutation: codon change alters the amino acid encoded. It could be conservative if the new amino acid is chemically similar to the original one or non-conservative if it is chemically dissimilar