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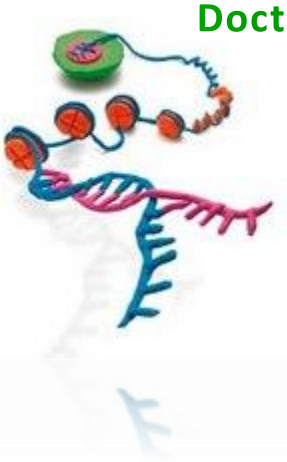
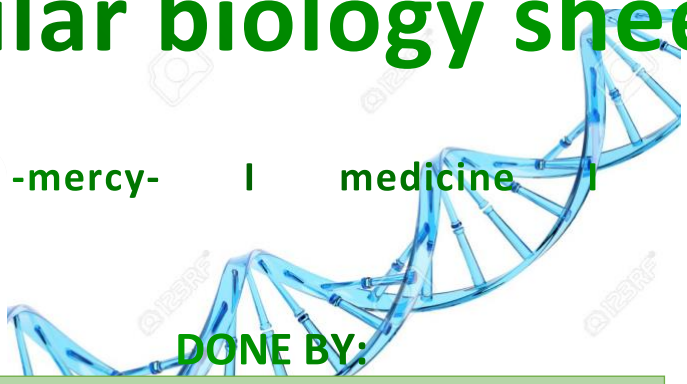
Molecular biology sheet

Doctor 2021 -mercy- I medicine I MU





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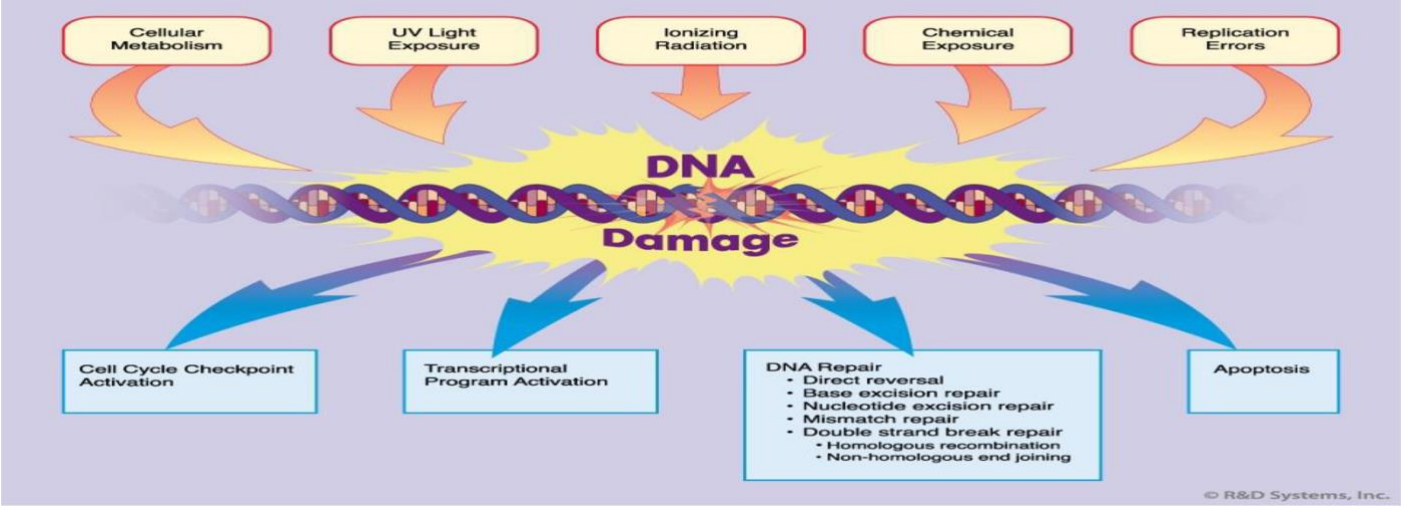
DNA Damage, Mutations and Repair Mechanisms



***DNA→ polar or non polar? polar because of hydroxyl group.**

***DNA is negatively charged due to phosphate groups.**

***importance of major and minor grooves? Regulatory proteins bind to major grooves.**

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.

*** 3.5*10^9 deoxyribonucletides (number of nucleotide in each human cell).**

*** Millions of errors occur during DNA replication, yet 99% of these faults are repaired, and another 99% of the rest 1% faults occur in non-coding regions of DNA.**

*** Non-coding regions of DNA (do not code for proteins) include:**

1) 5' & 3' non translated parts of genes.

2) Cis regulatory elements (promoters, enhancers & silencers)

3) introns

Endogenous DNA Damage

- DNA is subjected to be damaged by spontaneous changes under normal cell conditions including:

A. Deamination

B. Depurination

C. Replication errors

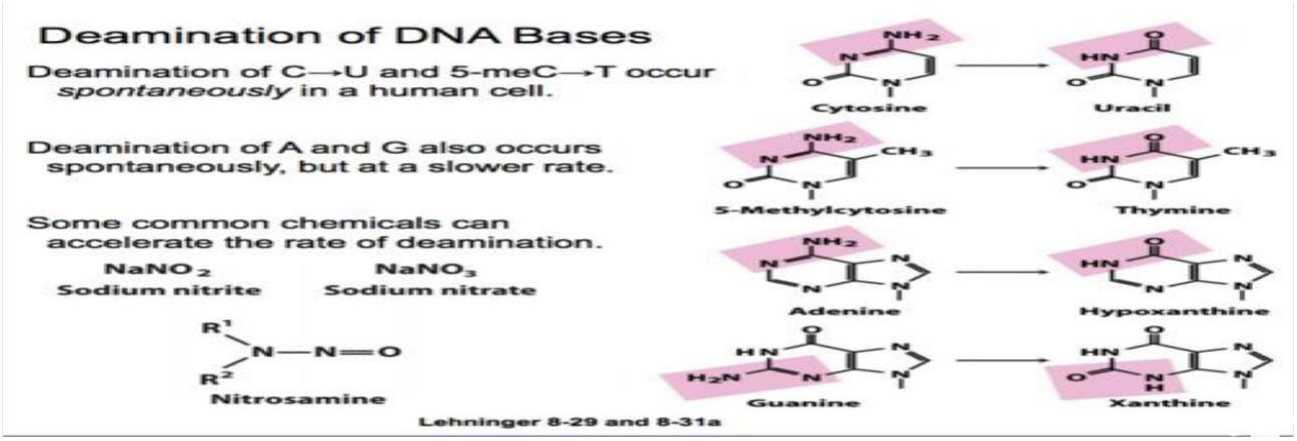
D. Oxidative DNA damage

A. Deamination:

Deamination

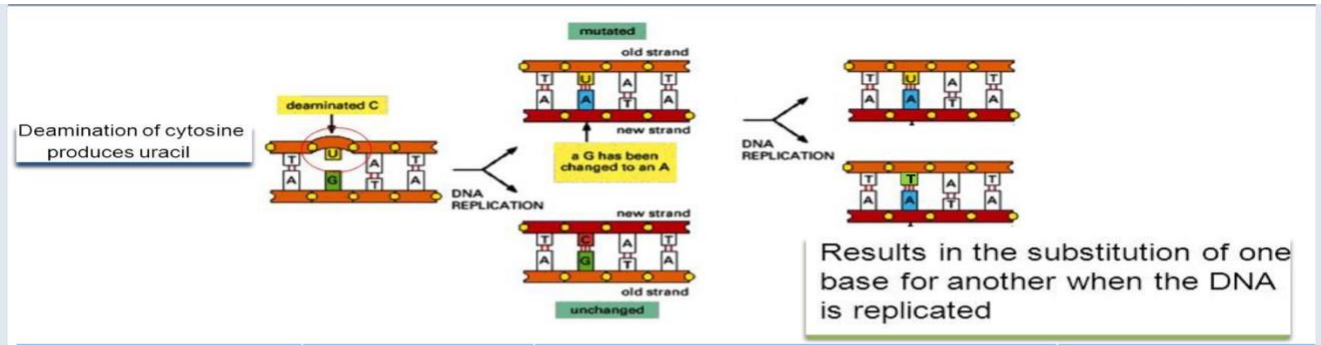
e.g. cytosine. ——————> uracil

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



NaNO2 and NaNO3 are mutagens and carcinogenic.

In normal metabolic pathways in liver or lungs, Adenine and guanine are converted into hypoxanthine and xanthine respectively, and their final product is uric acid, which causes gout.



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

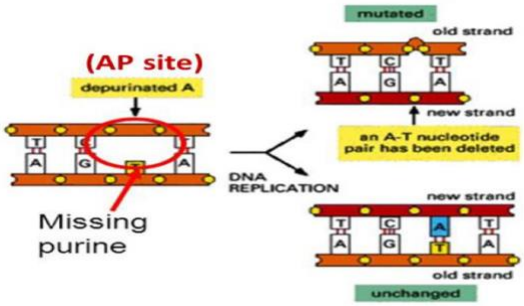
B. Depurination: **And may cause carcinogenic disease**

- Purines are less stable than pyrimidines.
- 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) (this site lacks purine)

- 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)

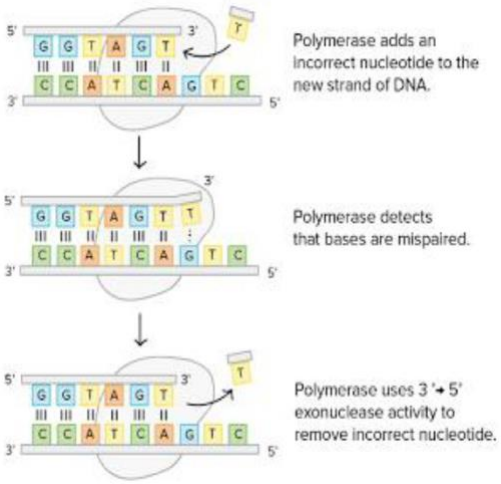


C. Replication errors

- 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



DNA polymerase has 3 activities:-

1) 5' → 3' polymerase activity
2) 3' → 5' proof-reading activity
3) 3' → 5' exonuclease activity

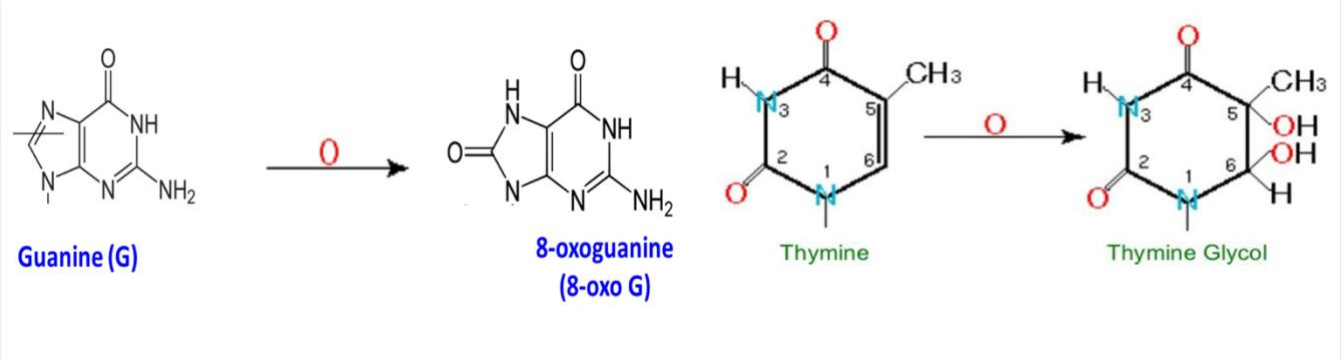
D. Oxidative damage of DNA:

- Reactive Oxygen Species during normal metabolic processes such as superoxide radical .O2 - 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

***oxidative stress : some biological reactions release reactive oxygen species ROS (free radicals). Imbalance of free radicals to antioxidants is called oxidative stress, which causes mutations, cancers or even cell death.**



Exogenous DNA damage

A. Radiation damage:

- By UV light and ionizing radiation

UV rays

UV rays are required in synthesis of Vit D

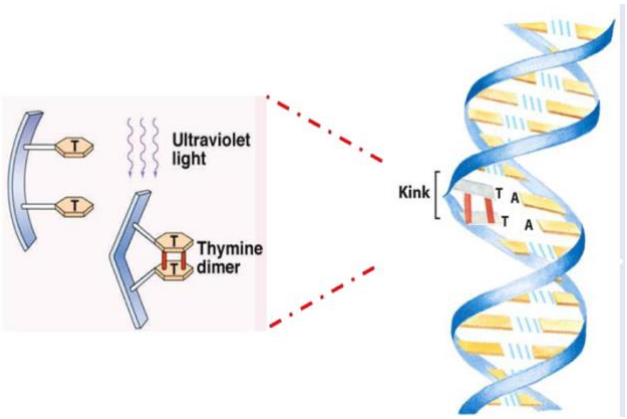
- Pyrimidines are highly sensitive to UV light. They form pyrimidine dimer (thymine dimer) by forming intra-strand crosslinking (T-dimer)

- Dimers alter DNA structure causing a kink or a knot in DNA strand)

- Thymine dimers prevent proper replication.

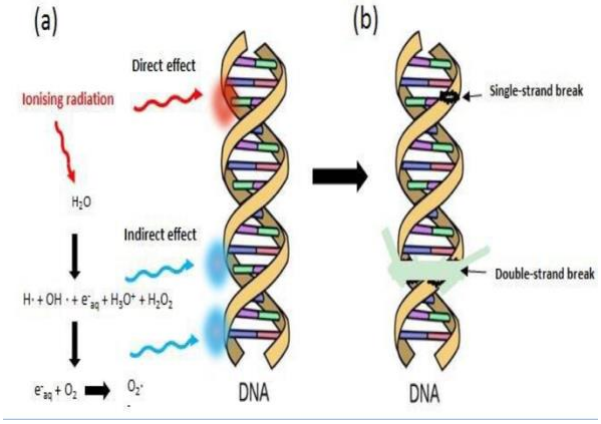
- The cell either undergoes an apoptosis or malignancy

- T dimer types: cyclobutane pyrimidine dimer and pyrimidine 6-4 pyrimidone photoproduct



*** UV light causes thymine dimer and induces the activity of photolyases as repair mechanism.**

*** Thymine dimers form kinks in the DNA, which interferes with the replication.**



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:

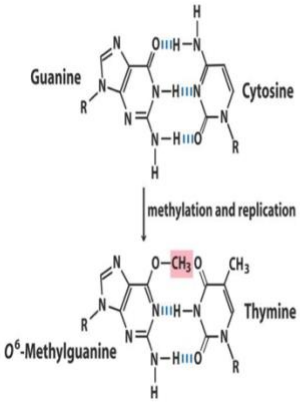
1- Base modifying agents
2- Base analogs
3- Intercalating agent

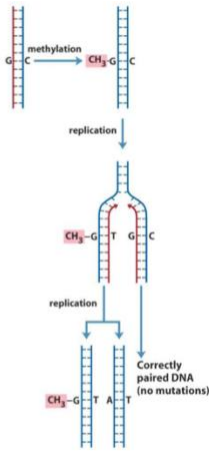
1. Base Modifying Agents

- 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 O6 methylguanine

- If not repaired, this lesion can lead to a base pair substitution (base pair changers)

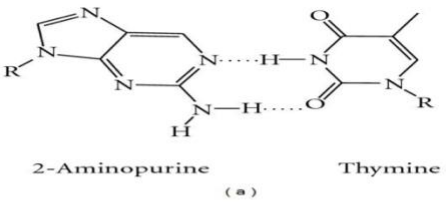


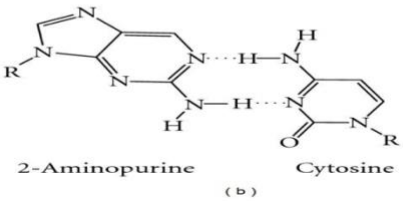


2. Base analogs

- 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)

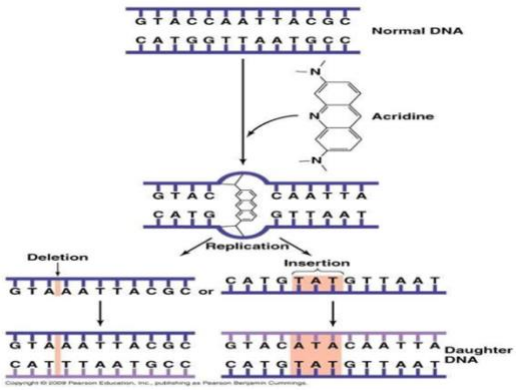




3. Intercalating Agents

- 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



Frameshift mutation is caused as one nucleotide from the adjacent codon is added to this mutated genetic codon.

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)

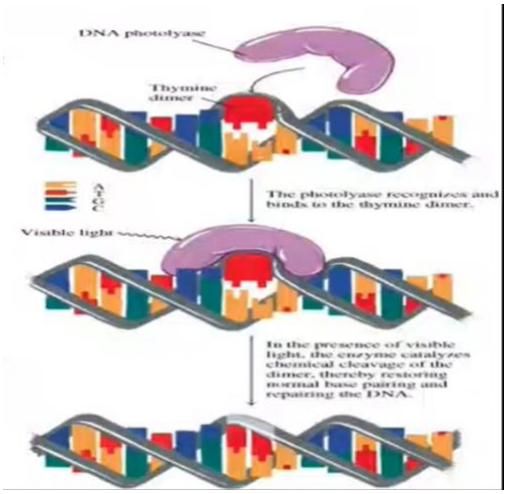
C. Nucleotide excision repair (NER)

D. Strand-directed Mismatch repair (MMR)

E. Double strand breaks repair (DSB)

A. Direct repair system

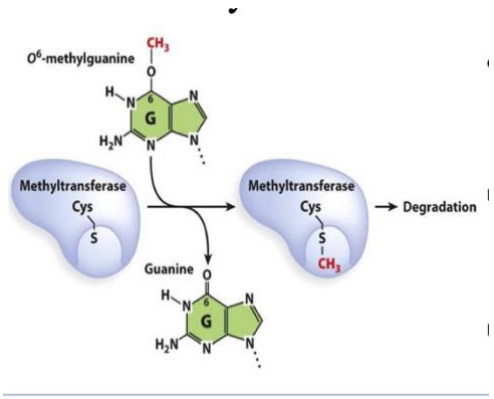
*** it is a reversal repair, usually for thymine dimers.**



- 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

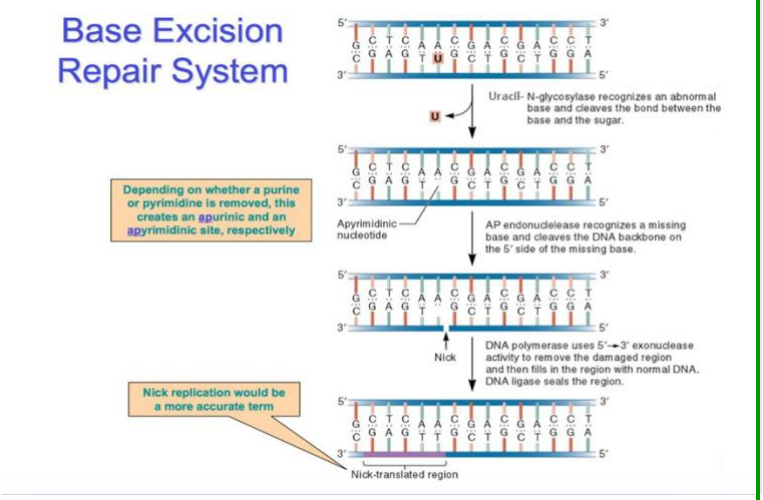


2. O6-methylguanine methyltransferase which transfers methyl group from G to a cysteine **(so cysteine is converted into methionine)** residue within the enzyme itself (suicide) **(photolyase depends on suicidal inhibition)**

B. Base excision repair

- It involves a category of enzymes known as DNA-N-glycosylases like uracil DNA glycosylase **(specific for uracil)**

- Glycosylases recognize damaged bases and remove them resulting in apurinic or apyrimidinic (AP) site **(uracil is removed)**



- AP endonucleases nick the the damaged backbone at 5' end of AP site (**will make a cut in the strand of AP to allow the DNA polymerase for repairing)**

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.

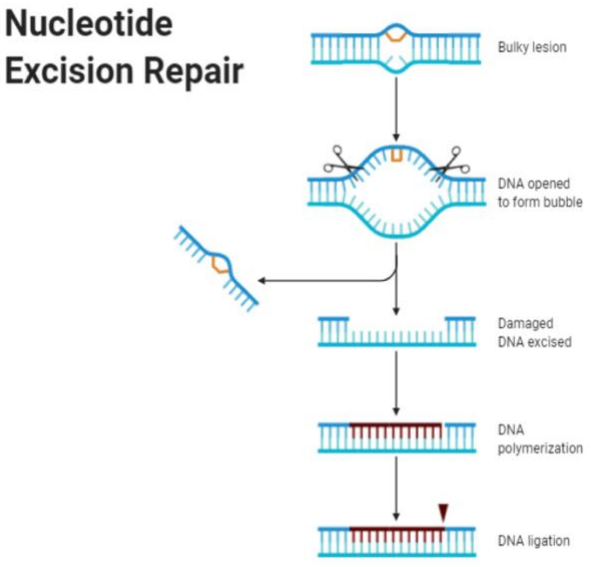
C. Nucleotide excision repair (NER)

*** not only for thymine dimers.**

- 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) **(of one strand)** by endonucleases

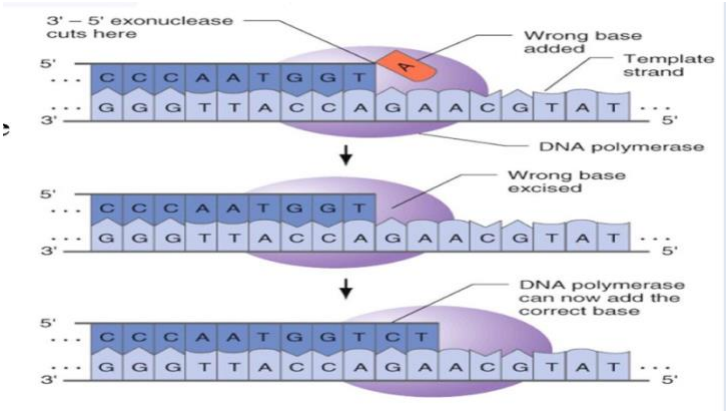
3. Resynthesis of the removed DNA region by DNA polymerase followed by ligase to seal the region

Xeroderma pigmentosum is an autosomal recessive disorder **(hereditary disease)** 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

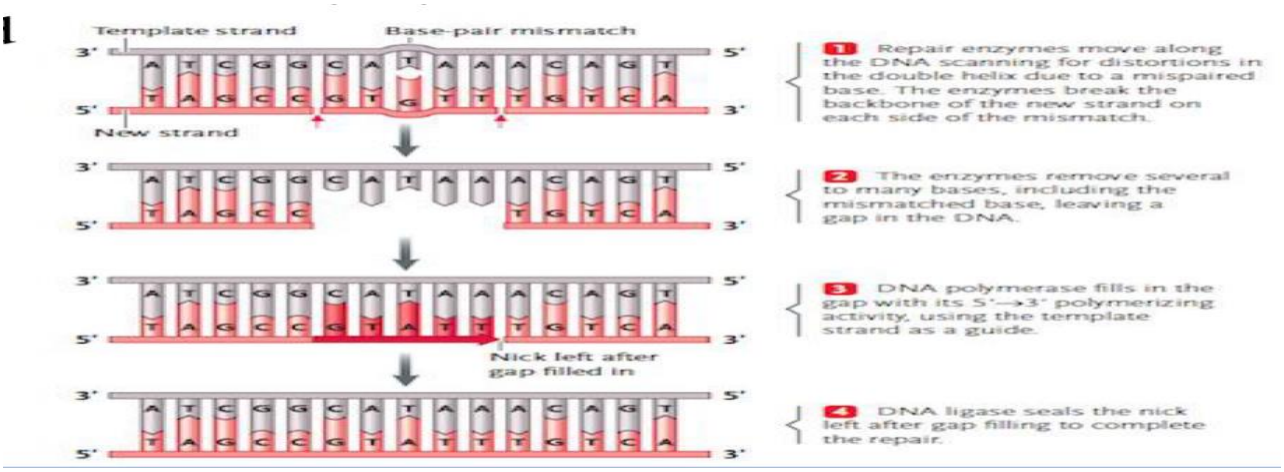
- In a process of three steps:

1. Mismatched base pair is recognized

2. Excision of DNA segment containing the mismatched nucleotide from the newly synthesized strand **(in order to ensure correct complementarity)**

3. Resynthesis of the excised segment - It is called strand-directed MMR **(miss match repair)** because MMR enzymes are selectively directed to the newly synthesized strand rather than to the old strand

*** it cuts a whole segment around the altered nitrogenous base, to ensure that there are no mistakes**



E. Double strand breaks repair

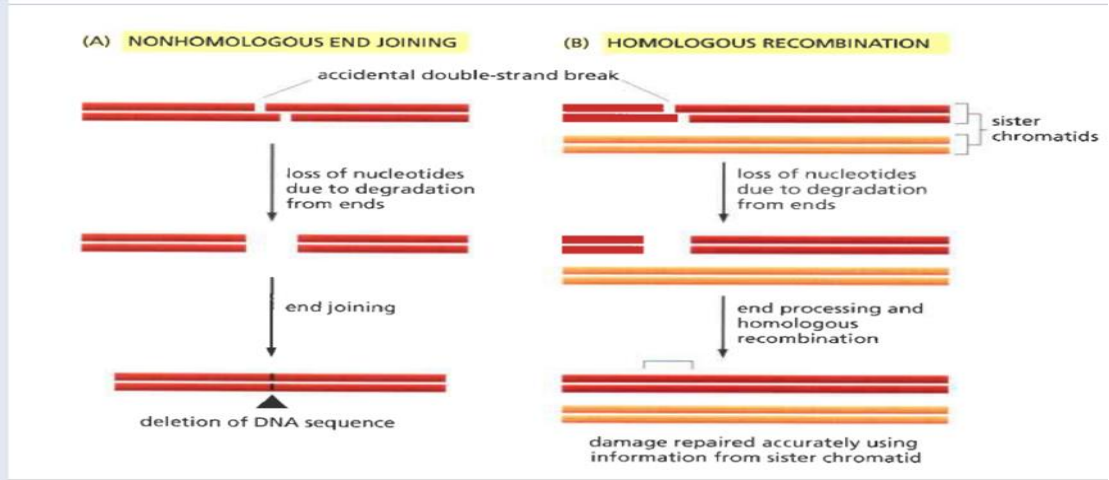
- A dangerous type of DNA damage which can lead to chromosomes fragmentation and consequently loss of genes (chromosomal aberration) if left unrepaired

- 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 * * , **it involves shortening of DNA, as the sister chromatid is not used as a template for repairing the lost segment**

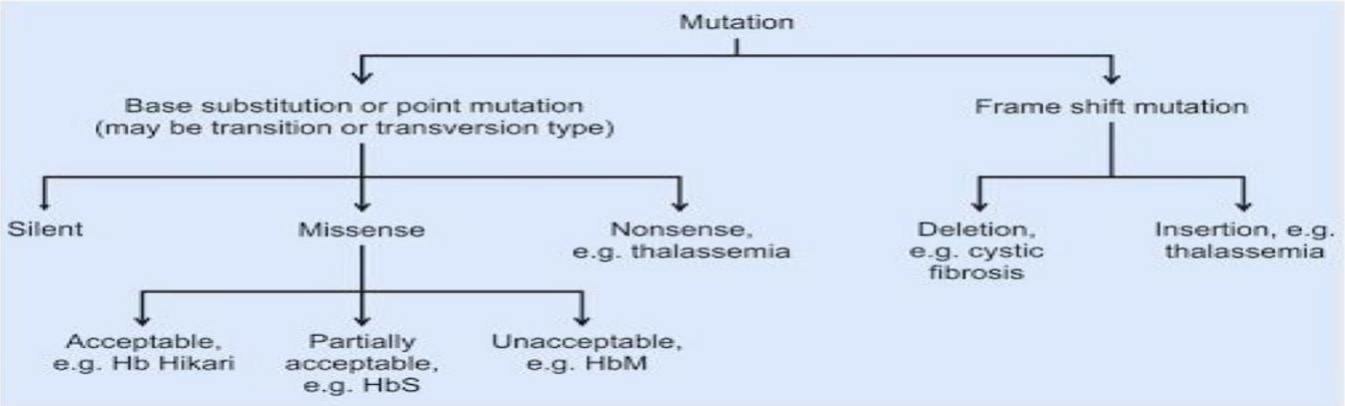
2. Homologous recombination: is an error-free mechanism of repair because the damage is accurately repaired using information from sister chromatid

(sister chromatid works as a template for repairing the lost segment)



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.

***Acceptable point mutation like Hb Hikari gives us a Hb which resembles the structure of original Hbb.**

***Partially acceptable mutation such as Hbs is normal unless the patient is exposed to deoxygentation.**

*** Unacceptable mutations such as HbM has Fe+3 instead of Fe+2. Fe+3 (ferric) can't carry oxygen. Dangerous effect**

Frameshift mutation

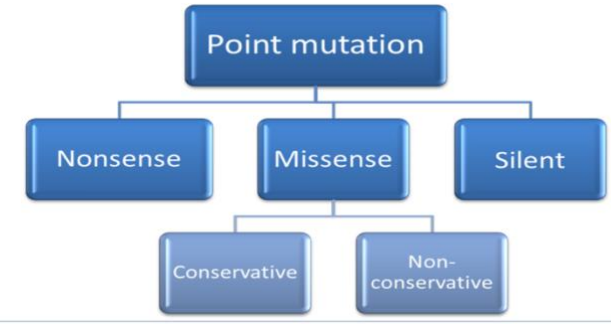
- It occurs with the insertion or deletion of a number of nucleotides (not the multiple of three) causing the alteration of the reading frame **beyond point of mutation.**

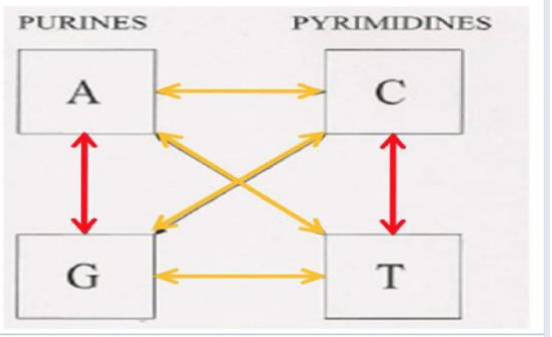
Types of mutations at the DNA level	Results at the molecular level
No mutation — Wild type	Thr Lys Arg Gly; Codon 1 Codon 2 Codon 3 Codon 4; A C A A A G A G A G G T — Codons specify wild-type protein.
Base insertion — Frameshift mutation	Thr Glu Glu Arg ... ; A C A G A A G A G A G G T ...
Base deletion — Frameshift mutation	Thr Arg Glu Val ... ; A C A A G A G A G G T ...

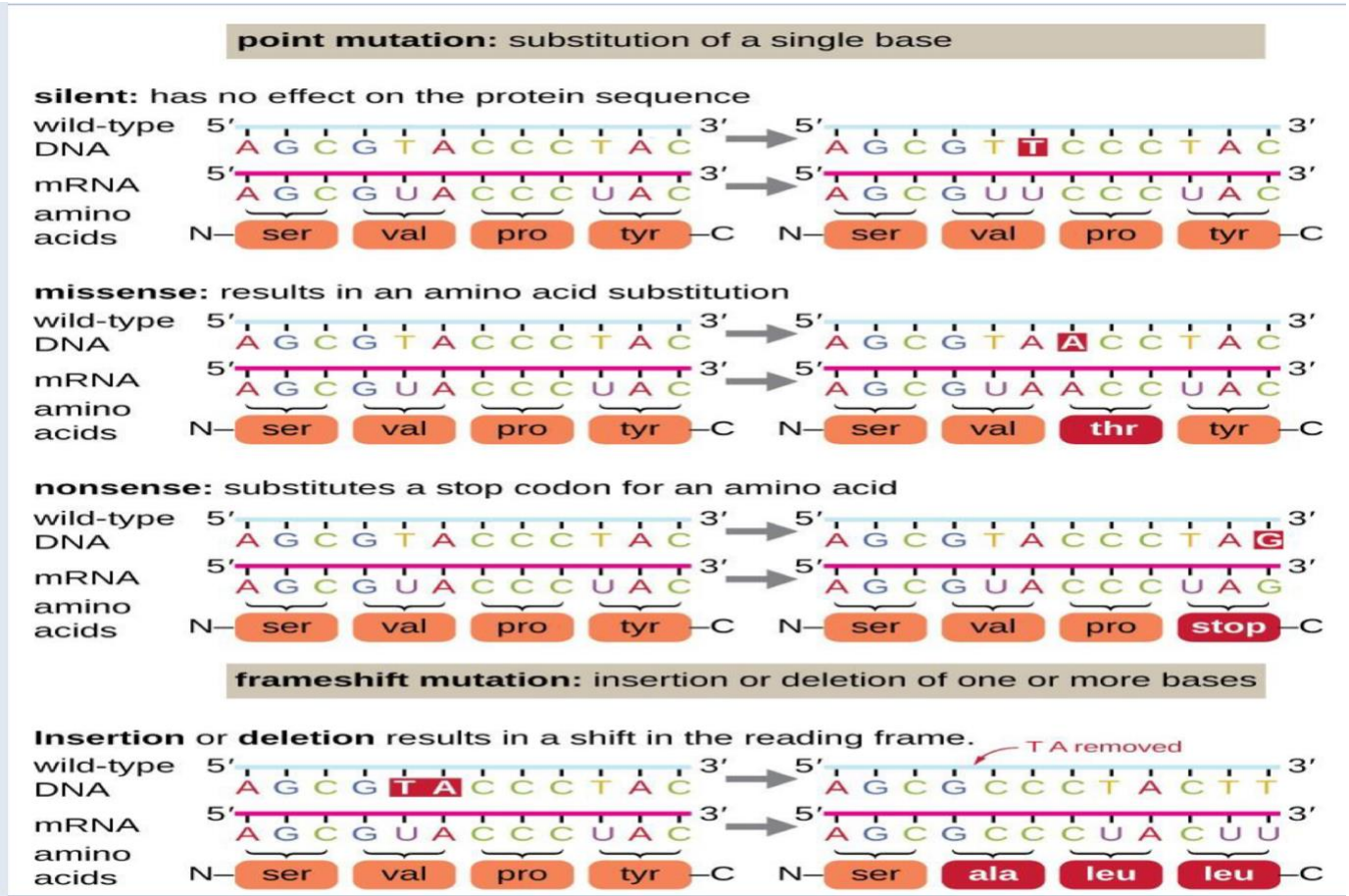
Point Mutation

- 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







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)

***for instance UCA → UAA (stop)**

Which results in premature termination of protein synthesis forming non-functional protein

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

*** as a result of alteration of a nitrogenous base, which converts the codon to encode for different amino acid.**

***Results in pathogenicity of disease→ sickle cell=non conservative**

Sickle cell —> Glutamic acid is converted into valine.

****serine(hydroxyl containing AA) → after point mutation→ threonine(hydroxyl containing AA) → a conservative missense mutation**

The behavior of this amino acid will not be change because serine and theronine polar and the dangerous effect will be on the function of the produced protein not in phathogenicity of the disease

- **In conclusion, either non-coservative and conservative missense mutations produce completely different proteins.**

تم بحمد الله ، اخر شيت لهذا الفصل ♡
بالتوفيق رَوْح 💪
#لجنة-الطب-والجراحة
لا تنسونا من صالح دعائكم

وهيك بنكون أنهينا المادة مع أطيب الأمنيات بالتوفيق والنجاح

Translate from Arabic