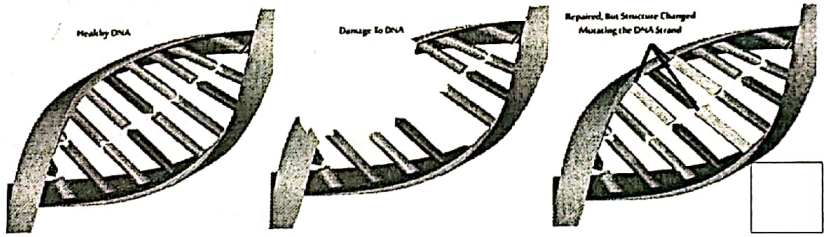
* DNA molecule are susceptible to damage numerous way

3/22/2019

DNA Damage, Mutations and Repair Mechanisms



Dr. Nesrin Mwafi
Biochemistry & Molecular Biology Department
Faculty of Medicine, Mutah University

* Why is can repair?
Because is a
① storge site of our genetic data
② have a system. callde Backup

DNA Damage

- DNA molecules like all other biomolecules can be damaged in numerous ways
- DNA damage occurs at a rate of $10^4 - 10^6$ molecular lesions per cell per day
- What are the sources and types of this damage?
- Can our cells recognise and repair this damage?
- What are the consequences of unrepaired damage on the cell fate?

* Time of repair: Befor cell division
If the damage occurs and the cell divisi
the damage Become
① Permanent
② inherited
③ propagate in subsequent generation
mutation
الورم للسرطان
Apopt
Cance
genetic disease

* Damage
no affect of Damage why??
Bec DNA molecule is repariable
We have repair system

induce تلقائي او

Classification of DNA Damage

- DNA damage can be classified according to the causative agents into two main types:

A. **Spontaneous damage (Endogenous):** arising naturally and in the absence of known causative agents. Spontaneous DNA lesions are random events " we can't predict when and in which type of cell will occure

B. **Induced damage (Exogenous):** occurs in the presence of known causative agents (external factors)

X-ray UV chemical

endogenous

Spontaneous DNA Damage

- Although DNA is highly stable, nevertheless it is susceptible to the following spontaneous changes under normal cell conditions:-

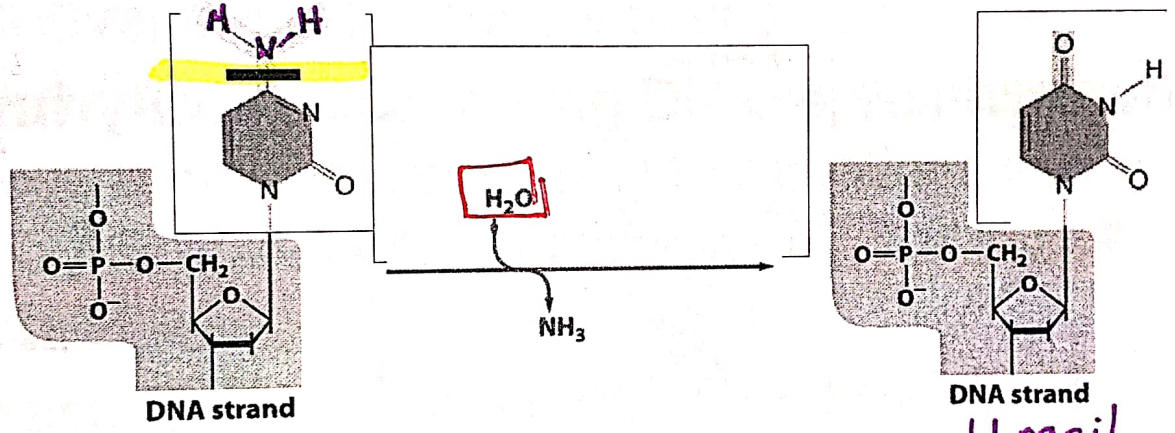
1. Deamination
2. Depurination
3. Replication errors
4. Base tautomers
5. Oxidative DNA damage

1 & 2 → The most type → Bcz both need just molecule of water (hydrolysis reaction)

Spontaneous DNA Damage

amino → keto

1. Deamination: the most common type is the spontaneous deamination of cytosine to uracil which occurs at a rate of about 100 bases/cell/day



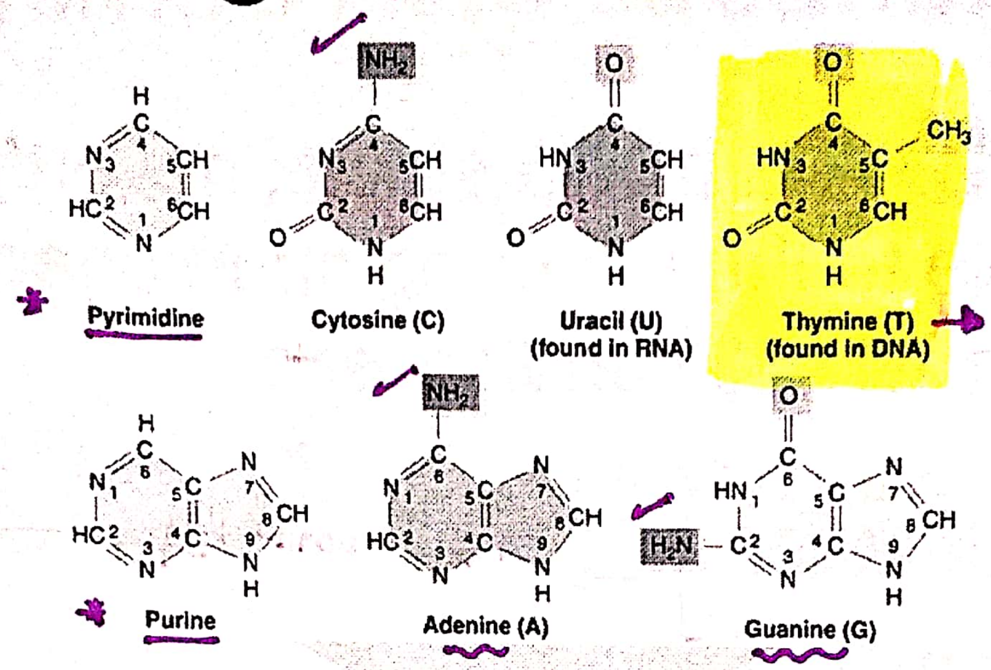
Ketogroup

Cytosine

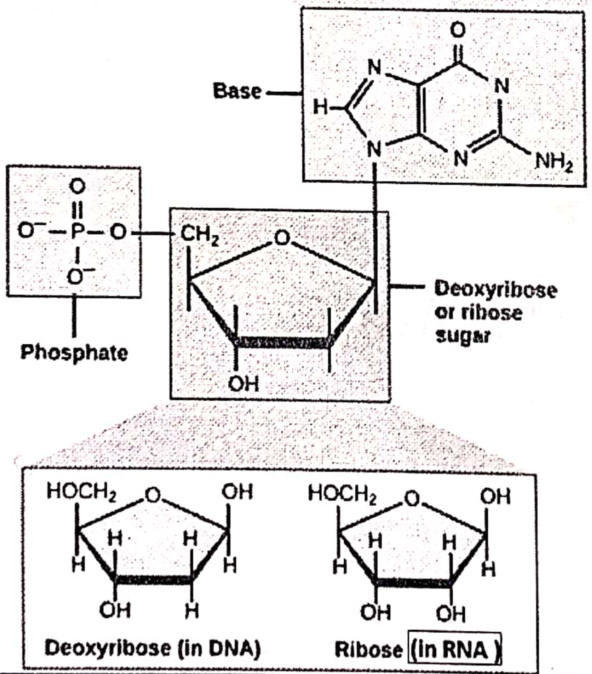
Uracil

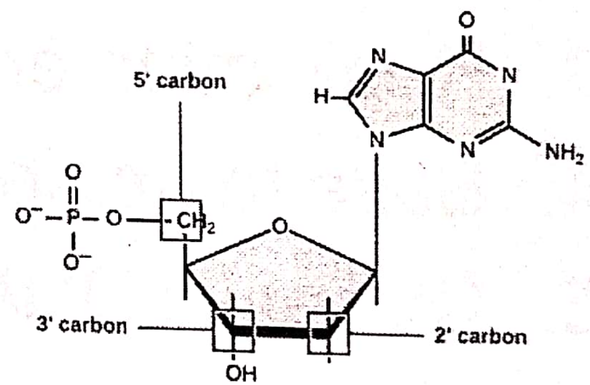
→ Is unusual to see it in DNA

Nitrogenous Bases

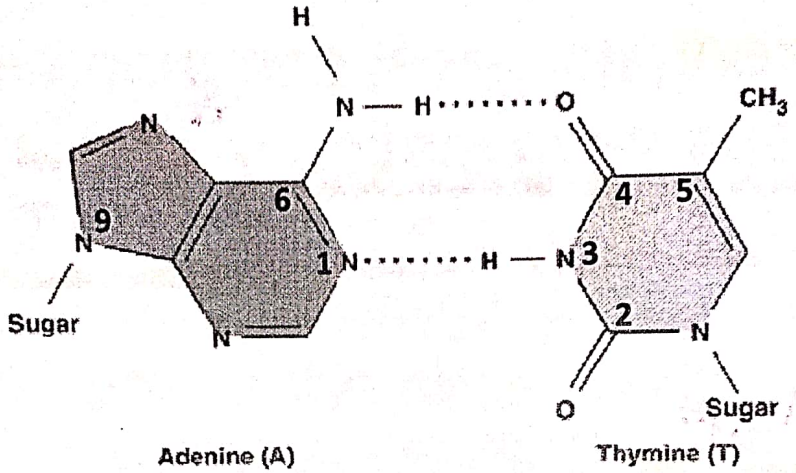


Deamination





Nitrogenous Bases

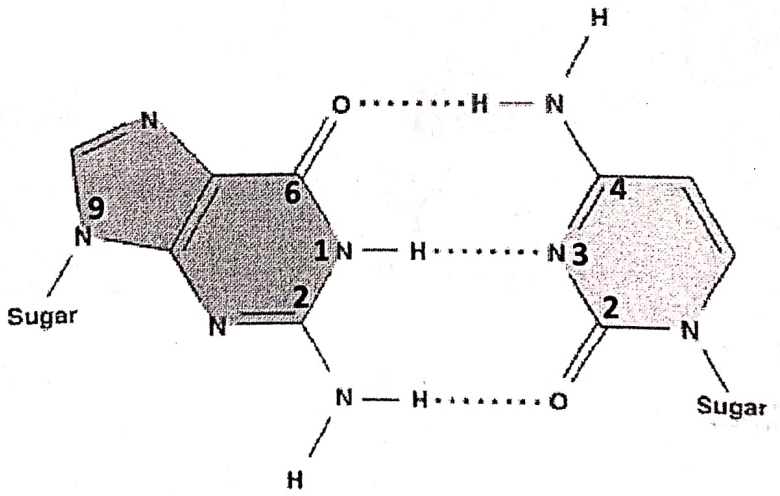


Adenine (A) Thymine (T)

2 Bond

Purines

Pyrimidines

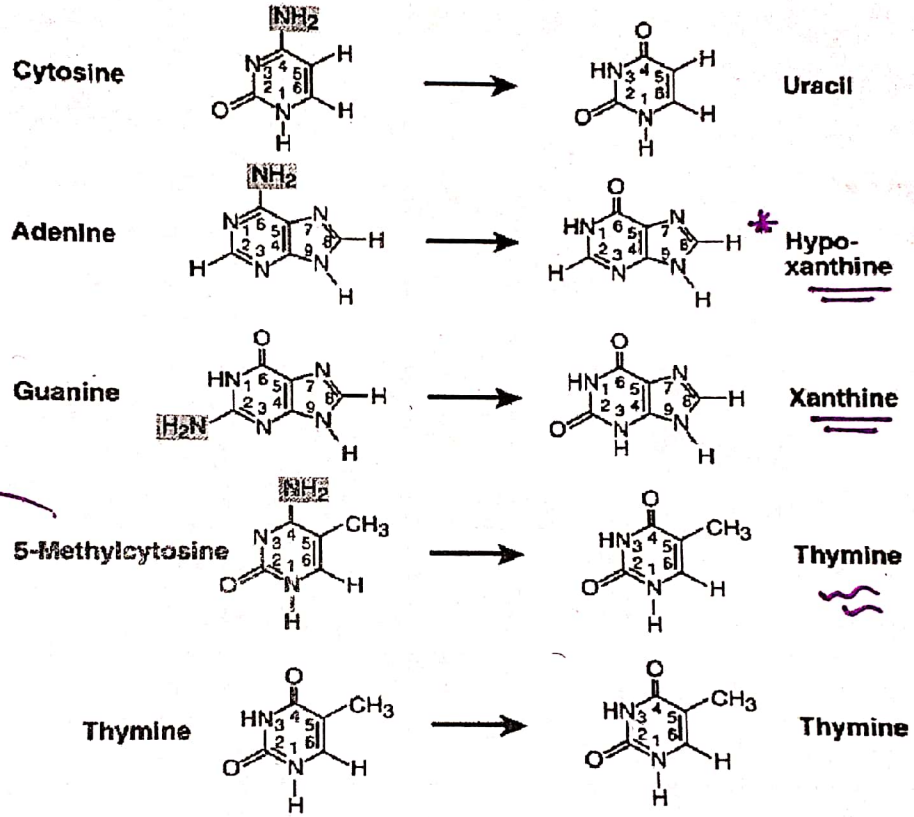


Guanine (G) Cytosine (C)

3 Bond

Deamination

- Other possible deamination events in DNA:



We use it in gene silencing

* mismatch
x mispair

لا تغير *

Deamination

- The chemistry of DNA four bases facilitates the damage detection through mismatched base pairs
- Specific DNA repair enzymes (i.e. DNA N- glycosylases) are capable of detection and removal of such unusual bases
- If left uncorrected, during DNA replication most of these changes would lead to mutations in the daughter DNA chain (**particularly base pair substitution**)
- These mutations will propagate throughout subsequent cell generations (inherited)

Type of Mutation

If the 2 double strand mutated the (DNA re) work: From the homologous chromosome

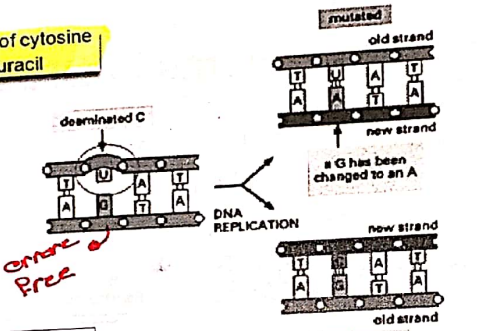
How the DNA replication enzyme was Know in this area we have a
- mutation :
* mismatch
- we need C :
* the 2nd complementary strand

C≡A
G≡T

Deamination

we can't repine in this site

Deamination of cytosine produces uracil



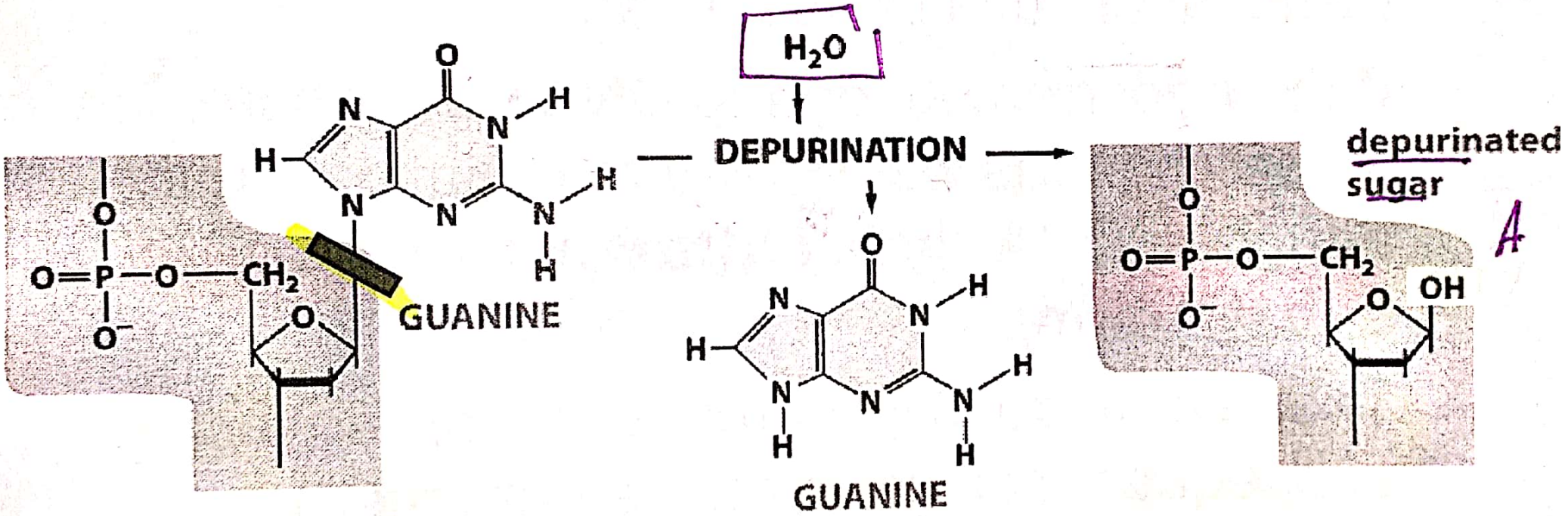
Results in the substitution of one base for another when the DNA is replicated

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

دليل
mismatch

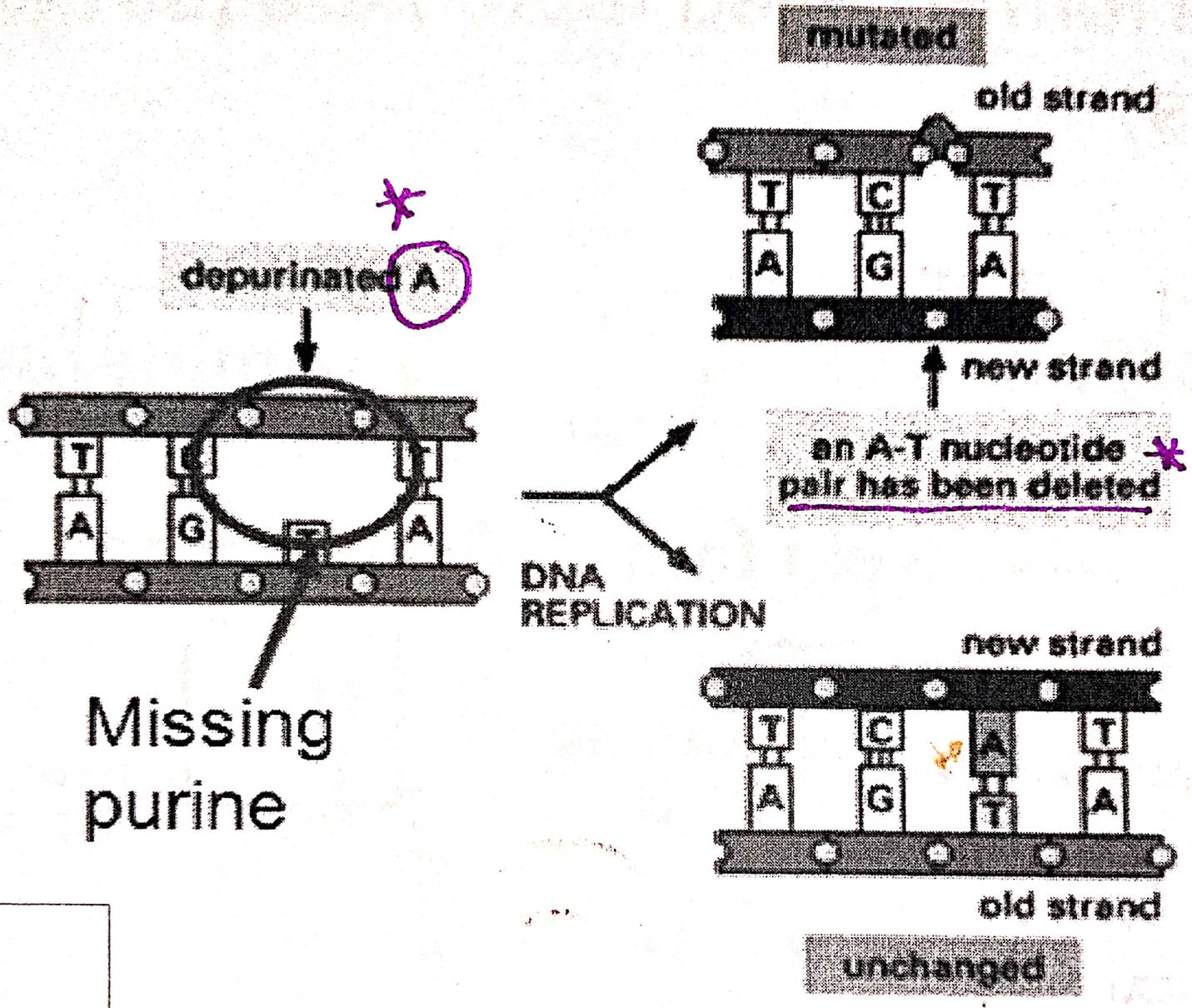
Spontaneous DNA Damage

2. Depurination: the loss of a purine base by spontaneous hydrolysis of the N-glycosidic bond that links it to deoxyribose C1'



Depurination

- Under physiological conditions, depurination occurs at a rate of about 5000 bases/cell/day
- Depurination results in apurinic site (AP site) which 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 **(particularly base pair deletion)**
- This error will propagate throughout subsequent generations (inherited)



DNA replicating
high Fidelity

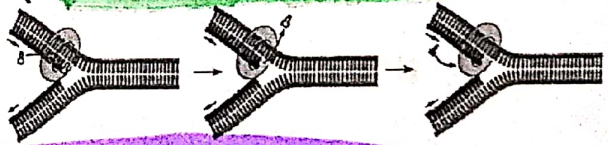
3/22/201

DNA polymerase enzyme
5'–3' →
· elongation
3'–5' →
Proofreading
(exonuclease)

Spontaneous DNA Damage

3. Replication errors: spontaneous lesions may occur during DNA replication in which the 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)

DNA proofreading



- Otherwise, DNA repair enzymes will recognize the mismatched base pairs and repair them

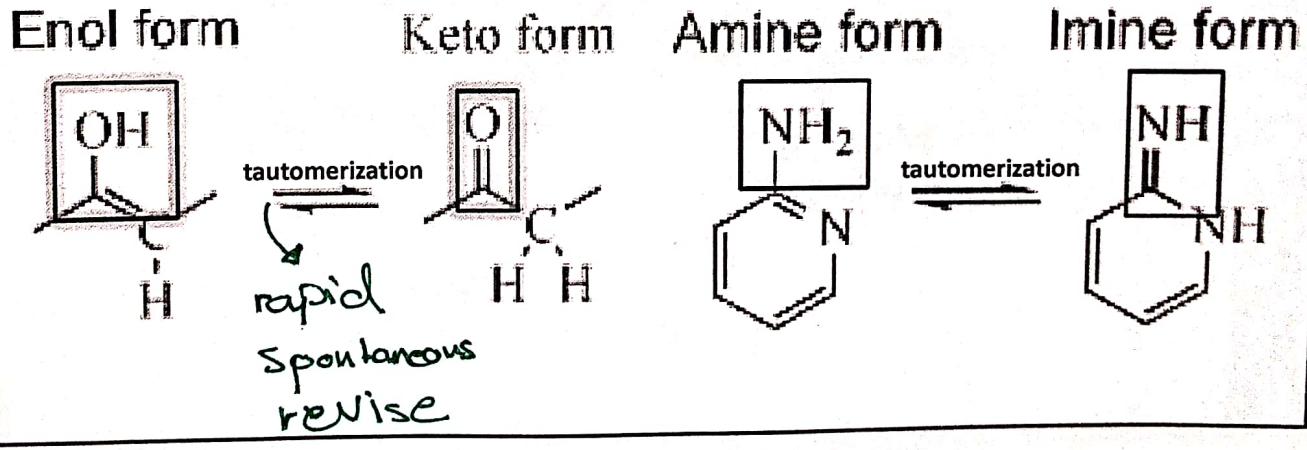
(constitutional) structural isomers

Spontaneous DNA Damage

4. Base tautomers: DNA bases exist in one of several forms called tautomers (structural isomers)

1. Keto/Enol pair (rare) * تذكر

2. Amine/Imine pair (rare)

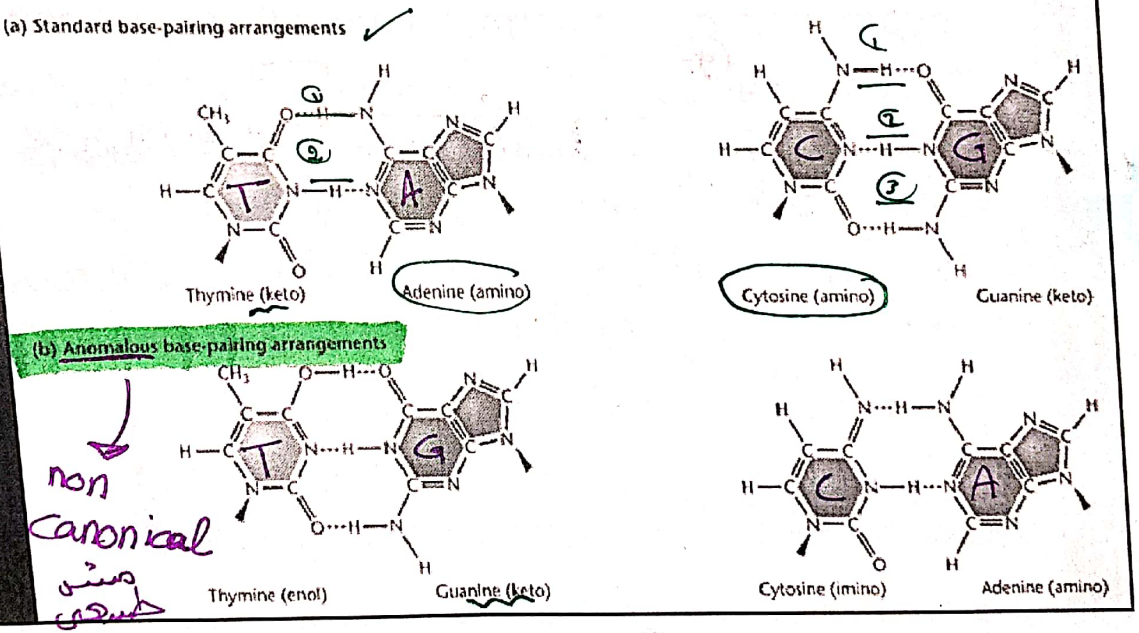


T = Keto, G = Keto
A = amino, C = amino
normal

3/22/2019

Base Tautomers

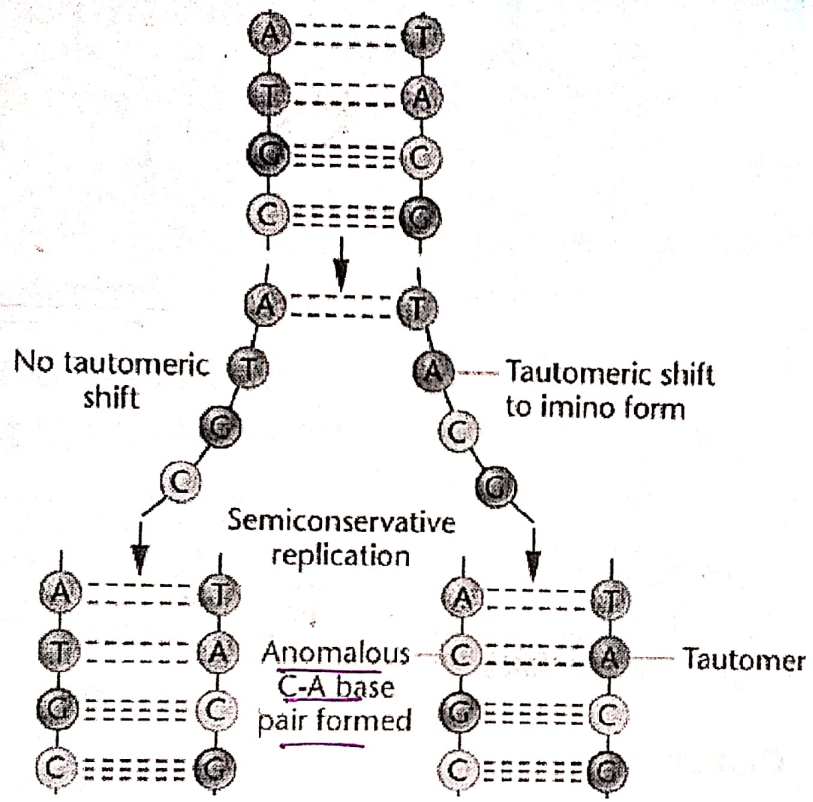
- Actually enol/imine forms are rare and tend to cause mispairing



non canonical

Base Tautomers

- If not repaired, it can lead to tautomeric shift mutation

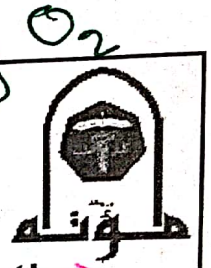


شو الفرق بين Subst و tautomers

Sub → stander = stand

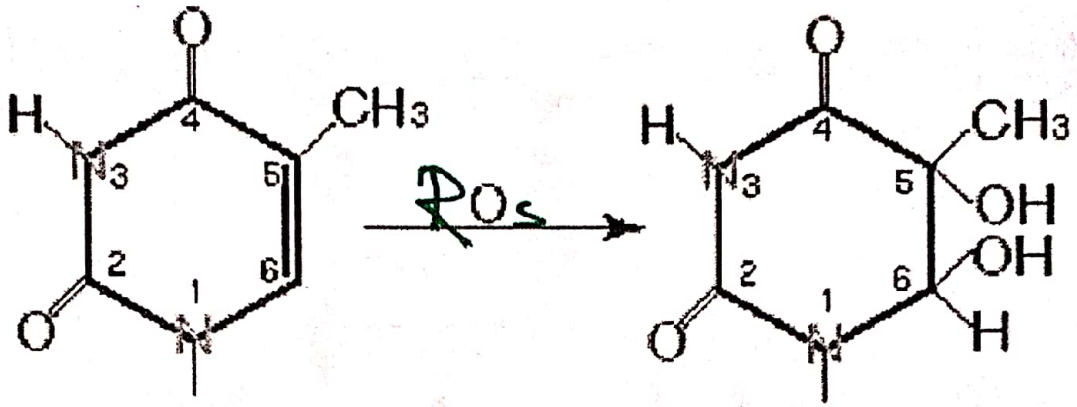
→ stande = non stander (Anomalous)

Spontaneous DNA Damage



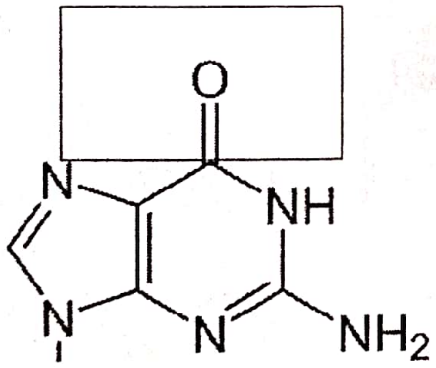
5. Oxidative DNA Damage: Endogenous Reactive Oxygen Species (ROS) are produced as byproducts during normal metabolic processes
   - ROS such as superoxide radical $\cdot O_2^-$ attack DNA leading to damage. They can chemically modify nitrogenous bases leading to mispairing
   - 8-oxoguanine (8-oxo G) is one of the major product of DNA oxidation. Another modified base is thymine glycol

Oxidative DNA Damage

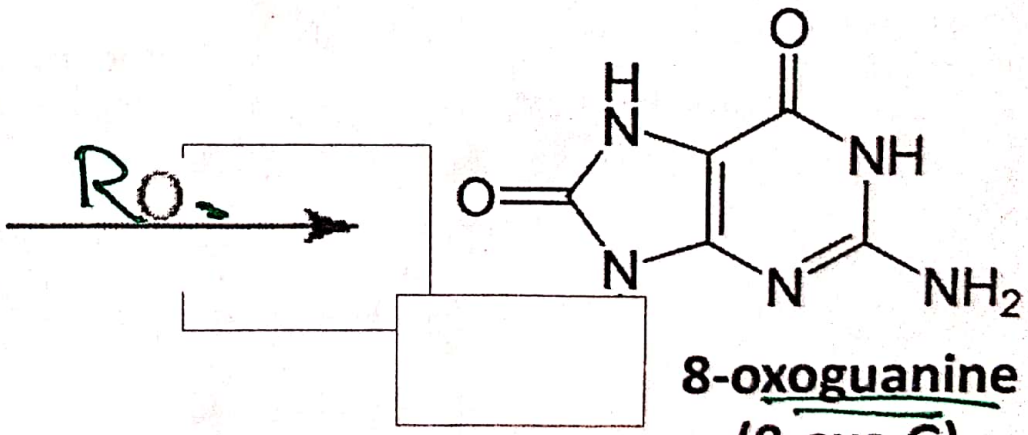


Thymine

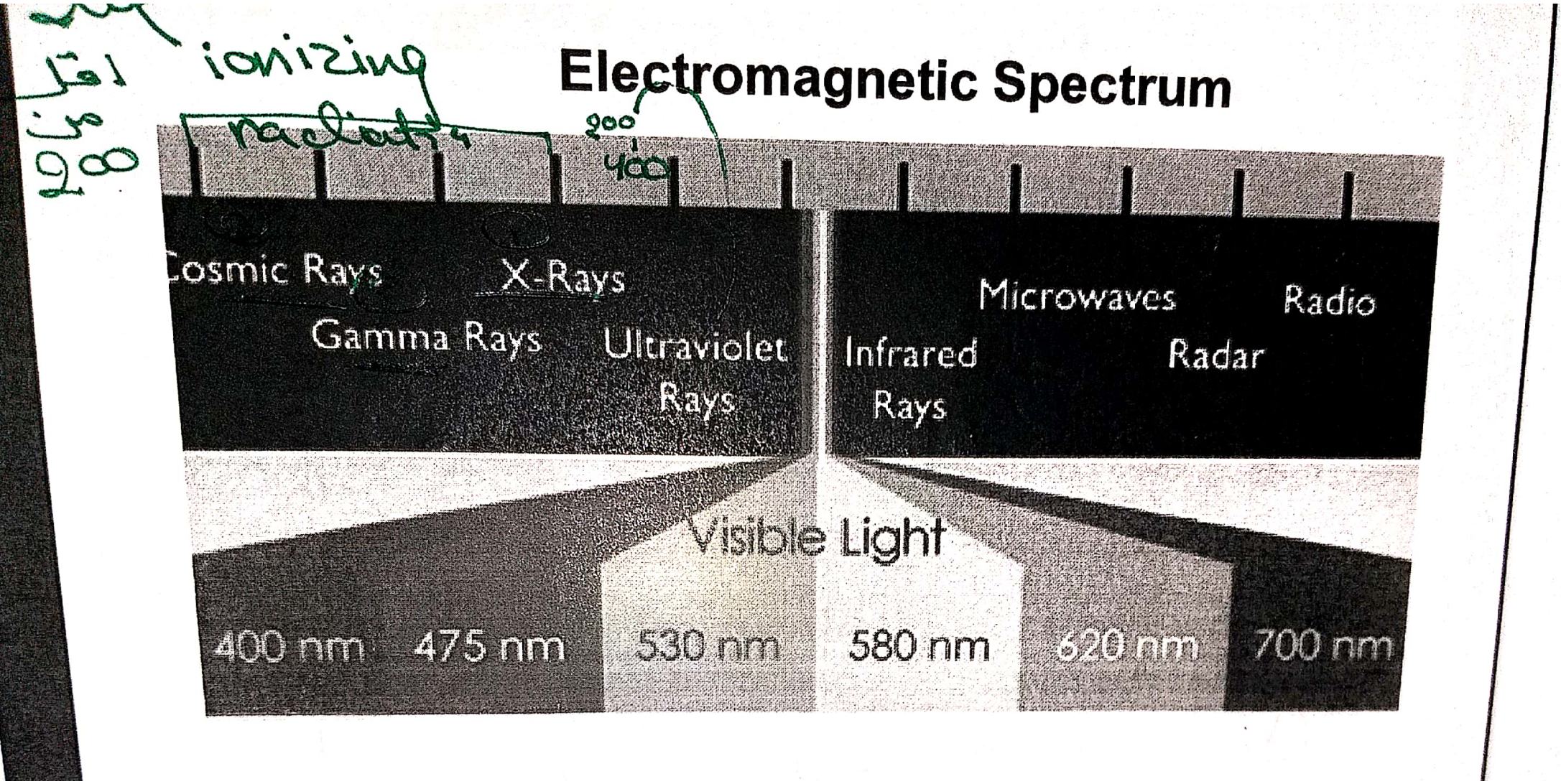
Thymine Glycol



Guanine (G)



8-oxoguanine
(8-oxo G)



Induced DNA damage

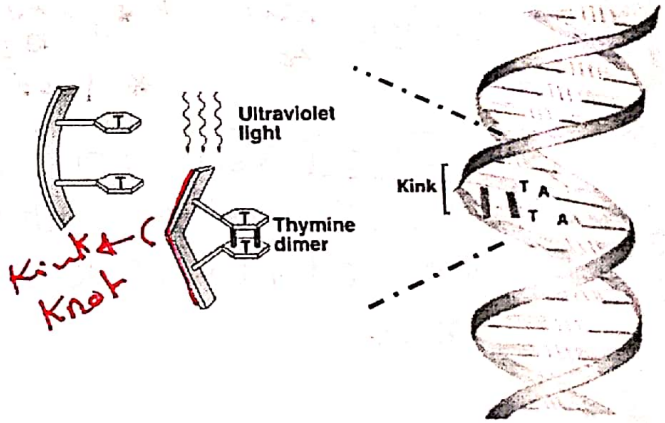
1. **Radiation Damage:** which includes both UV light and ionizing radiation

"especially 2T which are next door neighbor" to each other

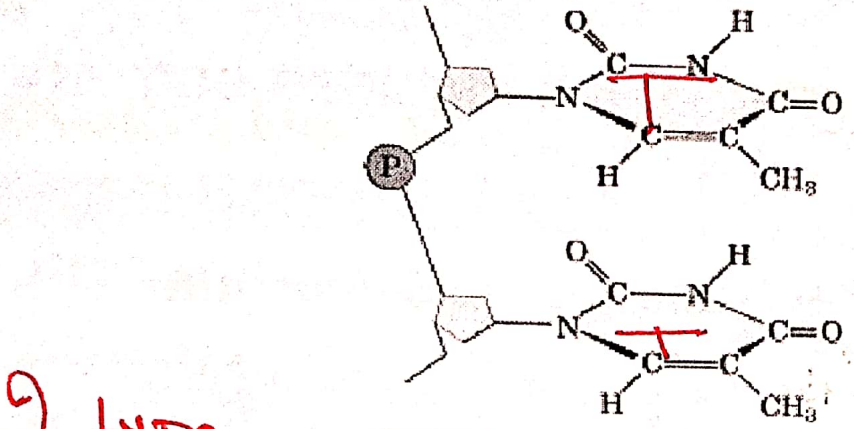
A. Ultraviolet Radiation

- Pyrimidines are highly sensitive to UV light. They form pyrimidine dimer (intra-strand crosslinking) particularly thymine dimer
- Dimers alter DNA structure (kink or knot in DNA strand)
- Thymine dimers prevent proper replication. The cell either dies (apoptosis) or forms a malignant tumour (cancer)

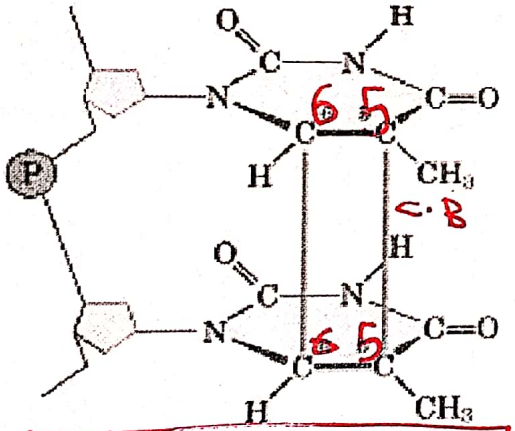
Block replecitio



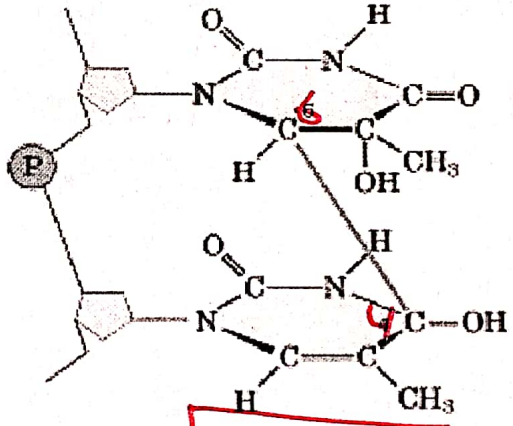
A. Ultraviolet Radiation



2 type dimer



Cyclobutane thymine dimer



6-4 Photoproduct

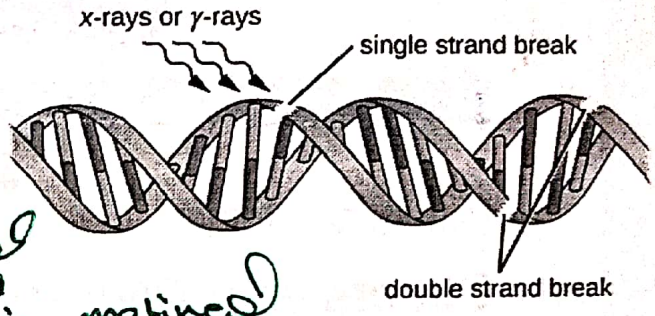
2 covalent Bond

1 covalent Bond.

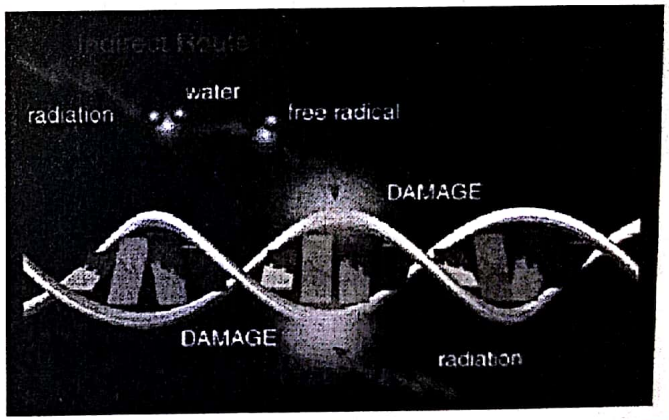
Induced DNA damage

B. 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 (DSB)



- Indirect DNA damage by production of free radicals which alter the structure of bases



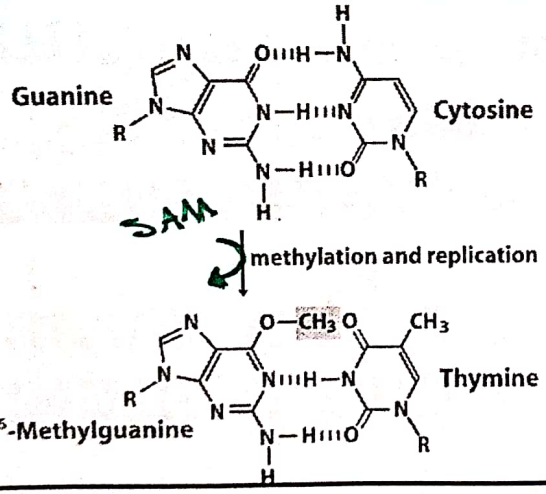
Induced DNA damage

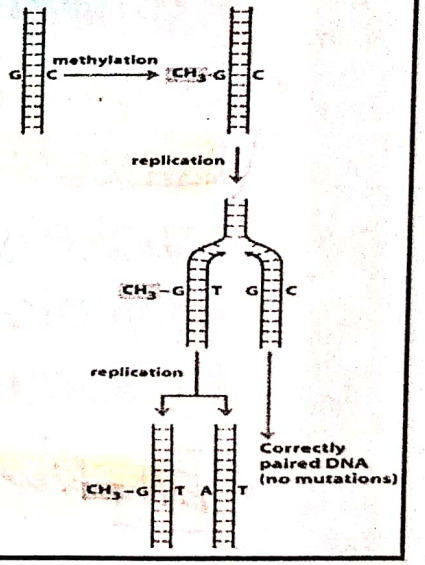
2. **Chemical mutagens:** are agents which induce mutations if their damaging effects on DNA have not been recognized and repaired

1. **Base modifying agents**
2. **Base analogs**
3. **Intercalating agents** → (Cancer مسرطن)

Base Modifying Agents

- Change 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 O^6 methylguanine (O^6 MeG)
- If not repaired, this lesion can lead to a **base pair substitution** (base pair changers)

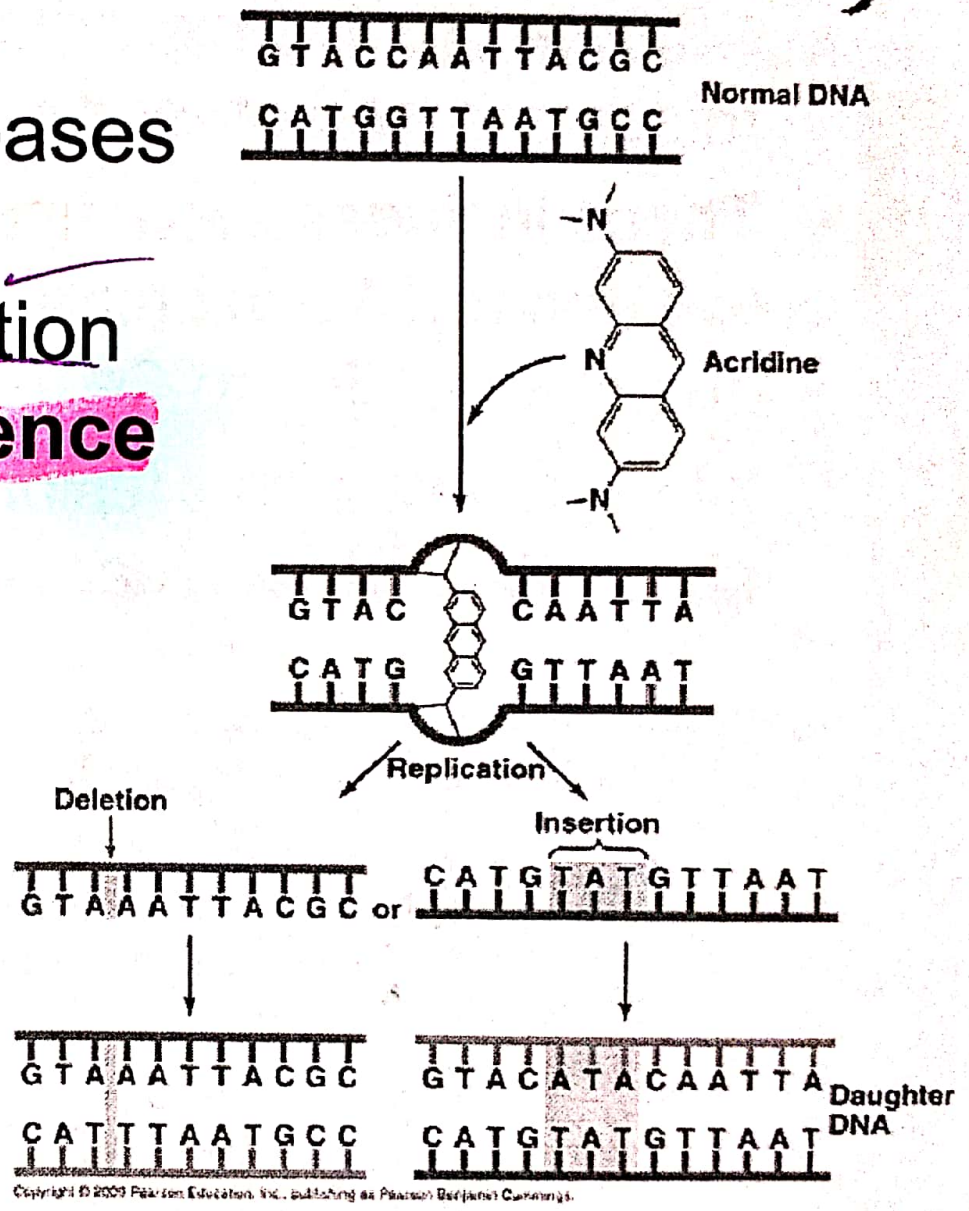




Intercalating Agents

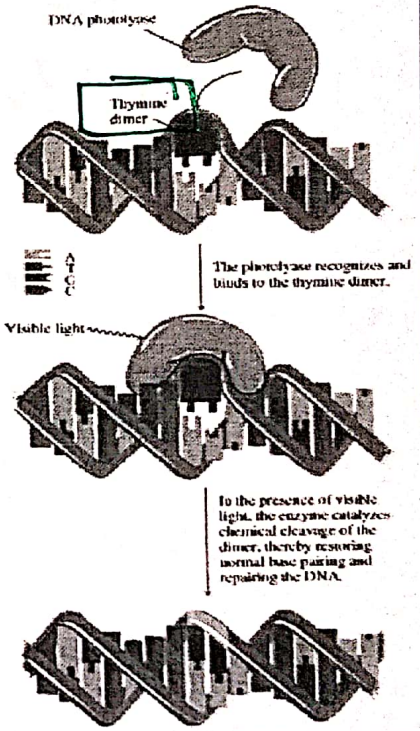
- Sandwich themselves between adjacent DNA bases so affect DNA structure causing insertion or deletion of an entire base pair **(hence frameshift mutation)**
- Like acridine orange, benzopyrene (cigarette smoke), aflatoxin B1 (mycotoxins produced by some fungi)

Ethidium bromide



Direct Repair system

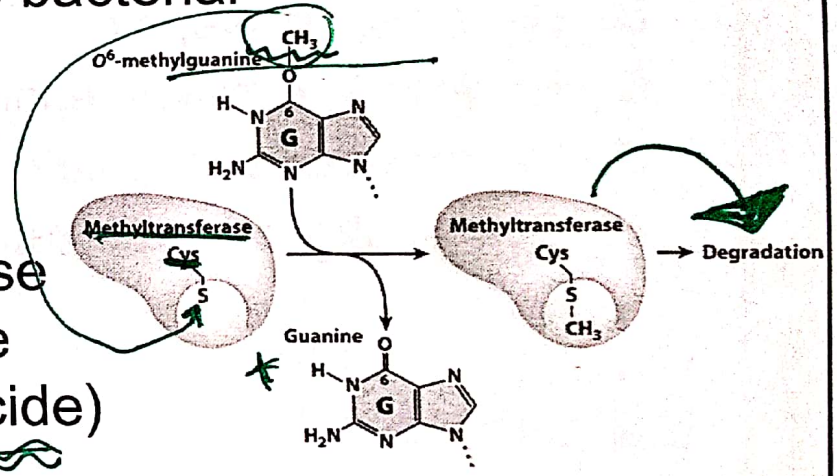
- Direct repair also called direct reversal because the damage can be directly recognized and reversed
- Two specific enzymes are involved in direct repair:
  1. Photolyases which repair UV induced damage in plants, bacteria and some animals (excluding humans) by splitting the dimers



Direct Repair system

2. O6-methylguanine methyltransferase (MGMT) which transfer methyl group from G to a cysteine residue within the enzyme itself. Ada and Ogt are the two bacterial isoforms of MGMT

- This reaction is stoichiometric rather than catalytic because each enzyme can be used only once (suicide)



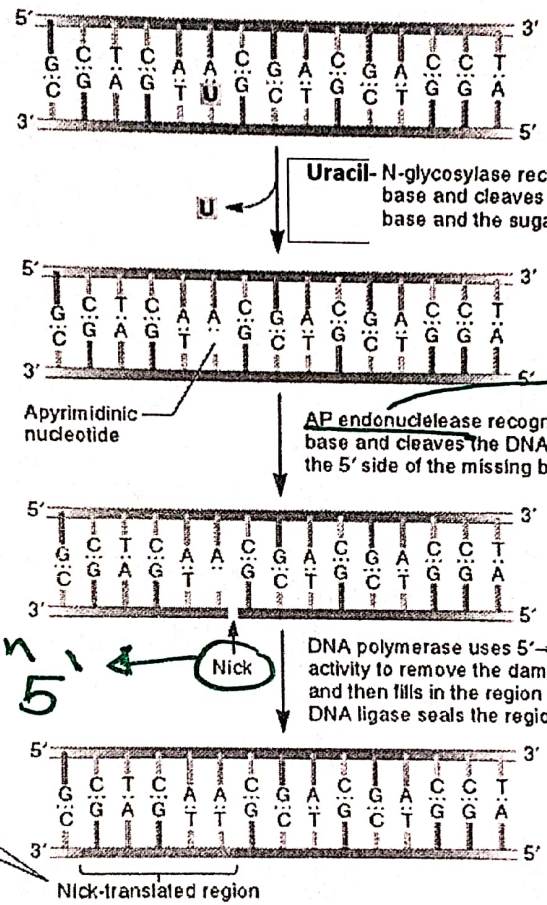
BER

Base Excision Repair

- Base excision repair **(BER)** 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 enzymes 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.

Base Excision Repair

Base Excision Repair System



Depending on whether a purine or pyrimidine is removed, this creates an apurinic and an apyrimidinic site, respectively

digests from middle not Terminal.

in 5′

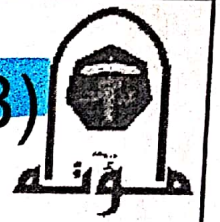
Nick replication would be a more accurate term

Nucleotide Excision Repair

- Nucleotide excision repair system **(NER)** corrects lesions which commonly cause bulk distortions in DNA helix like UV-induced pyrimidine dimers. NER is highly conserved used in both eukaryotes and prokaryotes
- The damaged region is removed in 3 steps process:

1. Recognition of the damage by NER enzymes
2. Excision of damaged DNA (12-24 nucleotides long) by endonucleases
3. Resynthesis of removed DNA region by DNA polymerase followed by ligase to seal the region

Double strand breaks repair (DSB)



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

1. Non-homologous End Joining (NHEJ): 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 (HR) is an error-free mechanism of repair because the damage is accurately repaired using information from sister chromatid

Frameshift Mutation

- Frameshift mutation: any addition (insertion) or deletion which alters the reading frame (i.e. not in a multiple of three)

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