DNA Damage, Mutations and Repair Mechanisms

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* DNA repair لازم يكون قبل الـ cell division (قبل DNA duplication) لانه إذا صار damage بعد الانقسام
• mutation وبنسمية بهاي الحالة permanent damage (دائم) يكون بال daughter cell

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 (بالوضع الطبيعي)
- 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?

DNA is repairable molecule unlike other molecule like Proten and lipid.

DNA → reversable damage

other molecule → irreversable damage.

DNA Damage

Classification of DNA Damage

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

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

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

Spontaneous DNA Damage

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

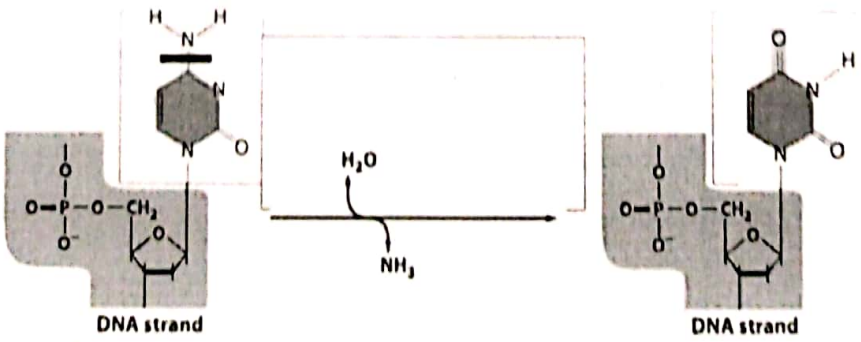
1. Deamination
2. Depurination

→ the most common type of spontaneous damage

3. Replication errors
4. Base tautomers
5. Oxidative DNA damage

Spontaneous DNA Damage

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

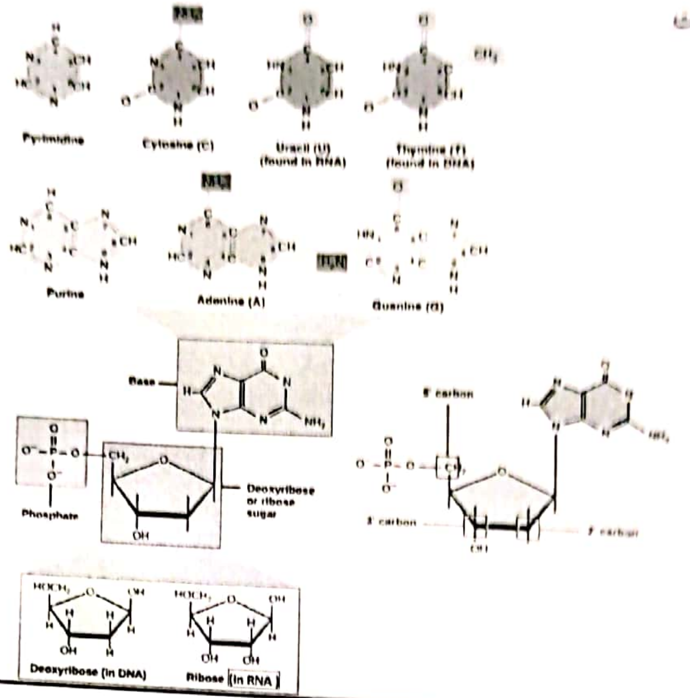


* deamenated product of thyamin is thyamin because it doesn't have amino group.
يعني ال thyamin ما بصيرله deamenation لأنه ما عليه amino group.

deaminated product

Nitrogenous Bases

#خليك بالبيت

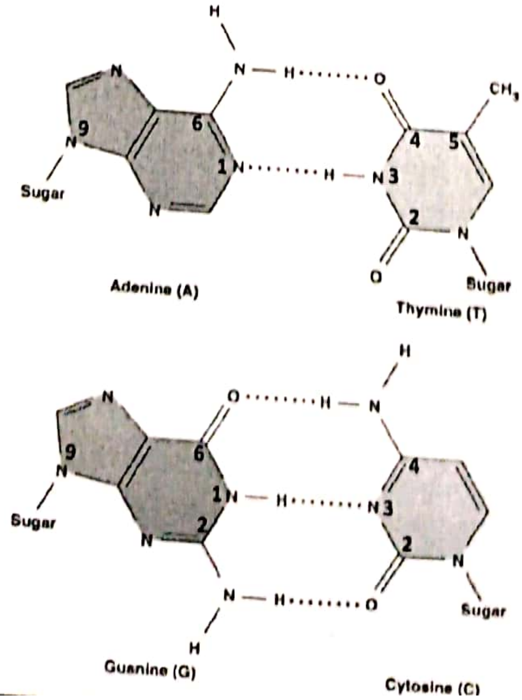


Nitrogenous Bases

#خليك بالبيت

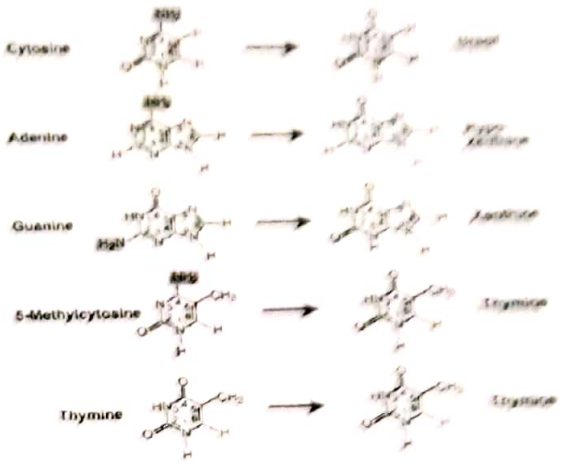
Purines

Pyrimidines

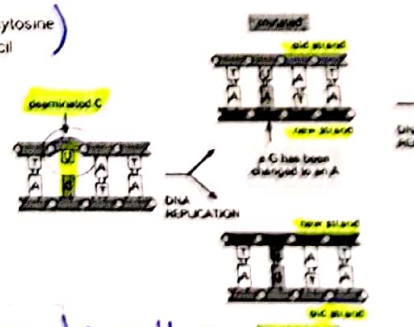


Deamination

- Other possible deamination events in DNA



Deamination



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

* الجدول حفظ

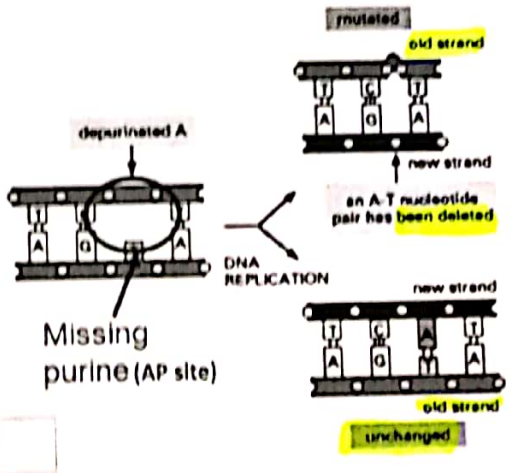
Nitrogenous base	Original base pair	Deamination product which base pairs with ( ) 1st round	Substituted base pair 2nd round
Cytosine	C-G	Uracil (A)	T-A
Adenine	A-T	Hypoxanthine (C)	G-C
Guanine	G-C	Xanthine (T)	A-T
5-Me cytosine	C-G	Thymine (A)	T-A
Thymine	T-A	Thymine	T-A

the consequences of this deamination event is the occurance of mismatch base pairs.

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)** mutation تؤدي الى
- This error will propagate throughout subsequent generations (inherited)

Depurination



DNA is repairable molecul because there are 2 types of backup system in DNA:

1- second complementary strand: guide the DNA repair enzyme to add original base pair

2- homologus chromosome: (2 strands of DNA واذا كان الخلل باد

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)

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

replication errors: spontaneous errors which escaped from proofreading activity if they aren't recoghise by DNA polymerase enzyme.

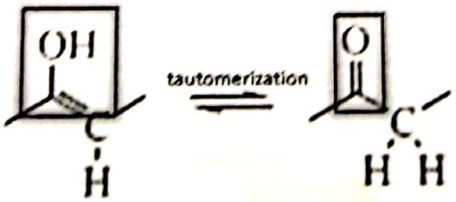
Spontaneous DNA Damage

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

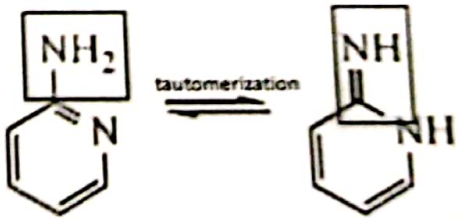
1. Keto/Enol pair **2. Amine/Imine pair**

OH مجموعة الكيتون

Enol form ⇌ Keto form



Amine form ⇌ Imine form (rare)



enol form is rare

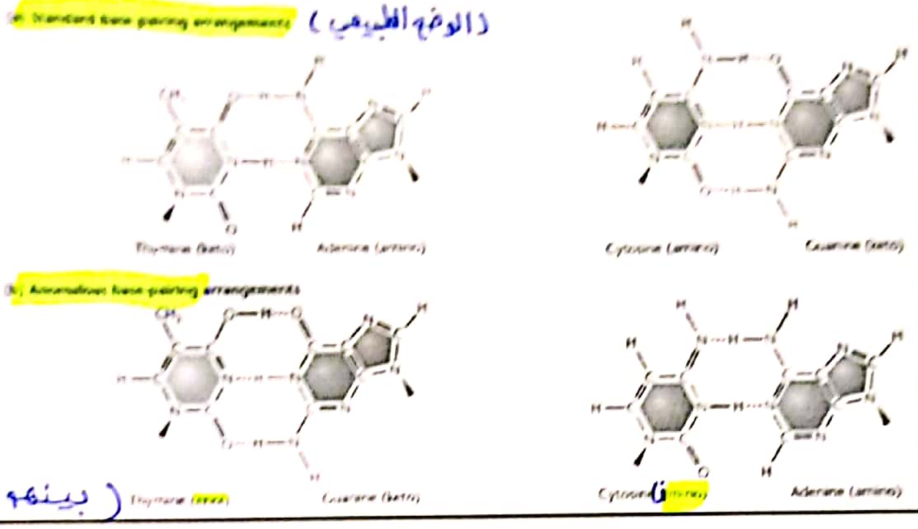
* ال adinine و ال cytosine موجودين بال amino form
* ال thymine و ال guanine موجودين بال keto form.

Base Tautomers

#خليك بالبيت

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

(a) Standard base pairing arrangements (الوضع الطبيعي)



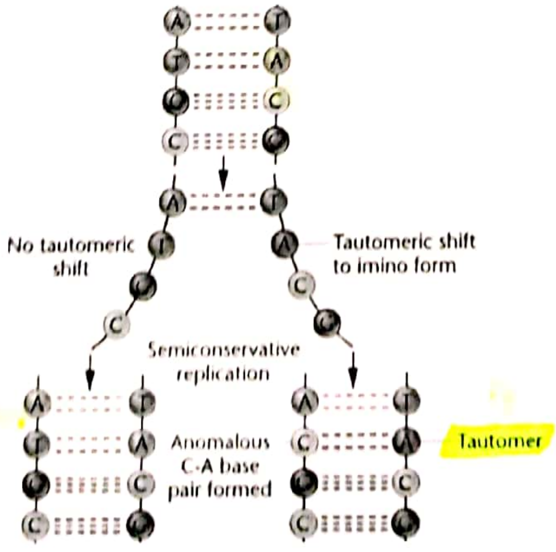
3 H bond (بينهم)

(2 H bond بينهم)

Base Tautomers

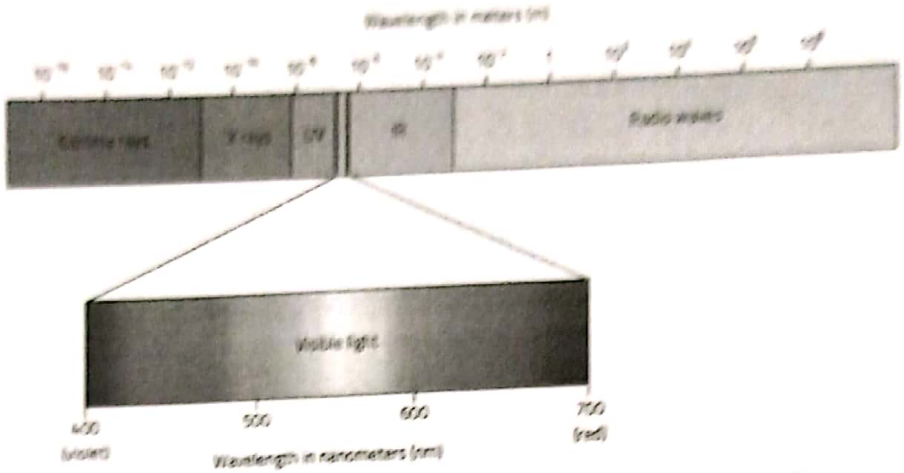
#خليك بالبيت

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



Induced DNA damage

1. **Radiation damage:** which includes both UV light and ionizing radiation like x-rays

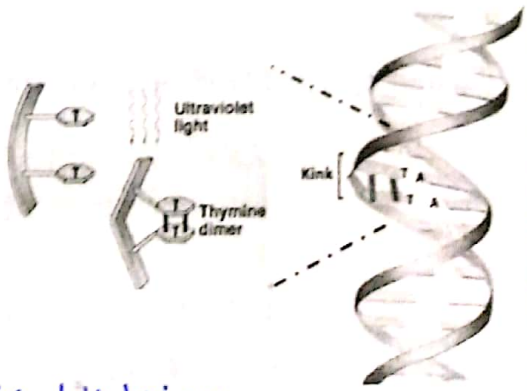


Induced DNA damage

خليك بالبيت

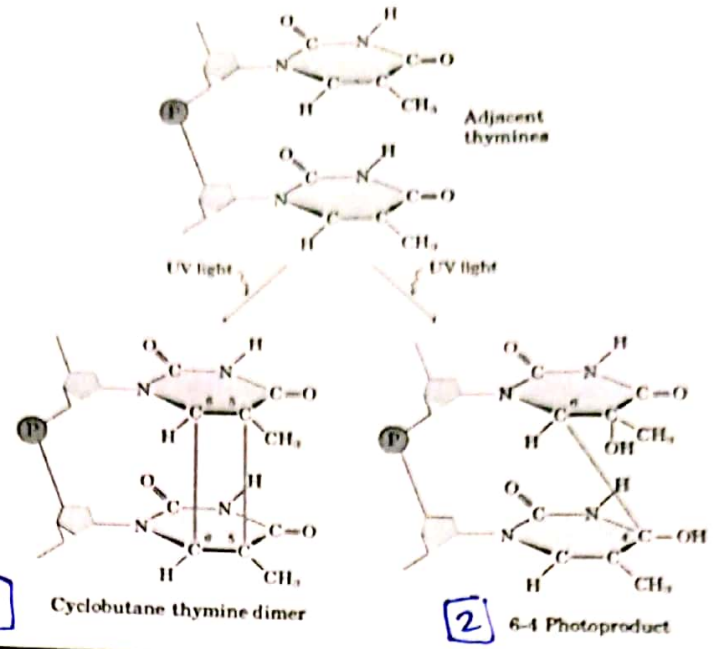
A. Ultraviolet Radiation

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



this type of damage is called intra strand cross linking.

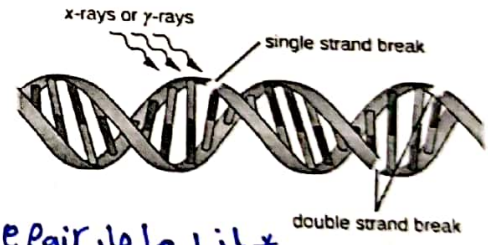
T-Dimer Types



Induced DNA damage

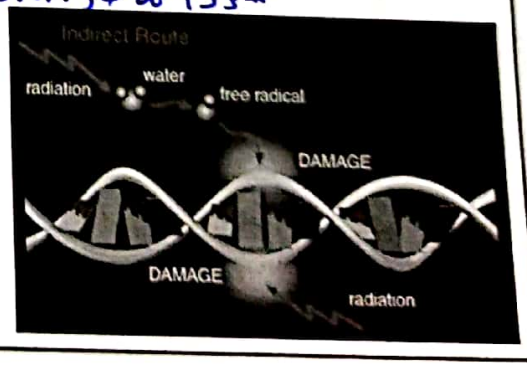
B. Ionizing Radiation: like cosmic rays, X-rays and gamma rays can damage DNA molecules in 2 ways:

- Direct DNA damage by producing single strand break (SSB) and the more severe double strand break (DSB)



(loss of genes) chromosmal aberration حيصير damage الـ repair لهذا الـ * إذا ما صار

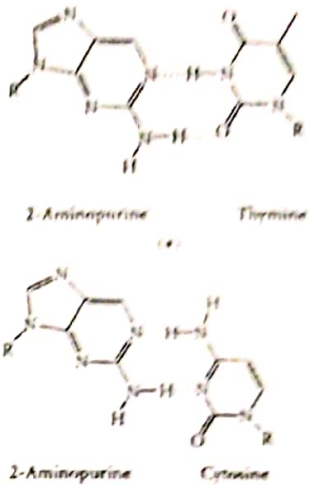
- Indirect DNA damage by production of free radicals which alter the structure of bases by generation of exogenous Ros.



* اذا تعرضنا لـ x-ray بشكل متكرر وعلى فترات متقاربة حيصير من الصعب إصلاح هذا الـ damage بالتالي حيحصل cancer.

Base analogs

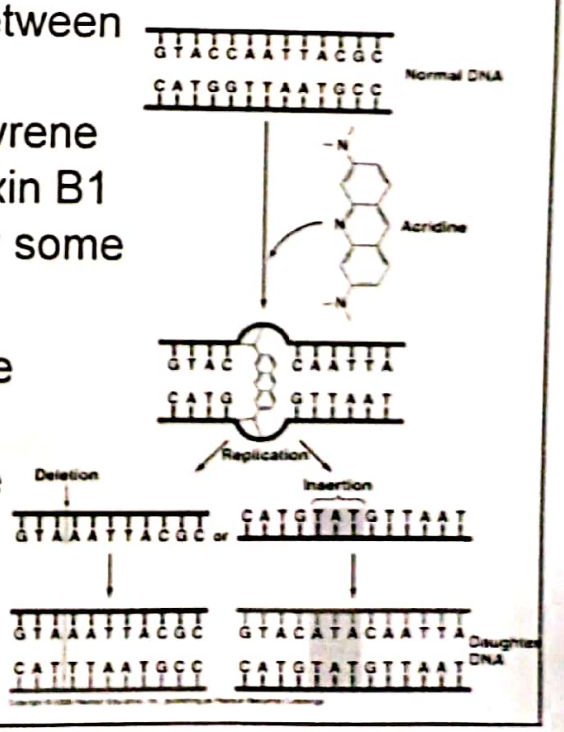
- Chemicals with structures similar to that of any of the four standard bases of DNA like 2-amino purine the base analog of adenine (6-amino purine). They replace them in DNA strand but do not **always** pair with normal bases leading to **base pair substitution** (e.g. AT bp is replaced with GC bp)



* ال 2 amino purin عند مقابلة للاربتا بدل بال cytosine
بالتالي حيأدي لـ DNA damaged

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

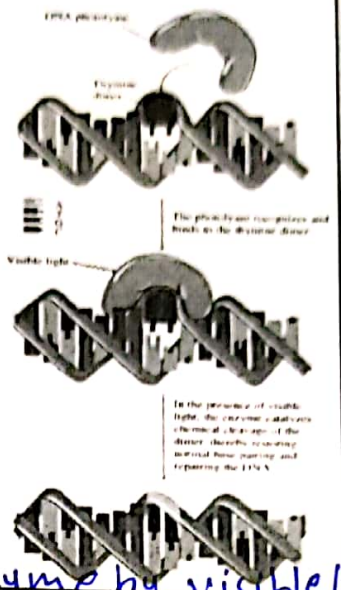
- DNA repair system : is a collection of processes by which a cell identifies and corrects various DNA lesions
- Several repair strategies are available:
  A. Direct/reversal repair
  B. Base excision repair (BER)
  C. Nucleotide excision repair (NER)
  D. Strand-directed Mismatch repair (MMR)
  E. Double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

* حفظ الاختصارات مهم.

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



* mechanis of repair

1- activation of photolyase enzyme by visible light (Photoactivation)

2- cleavage the T-dimer

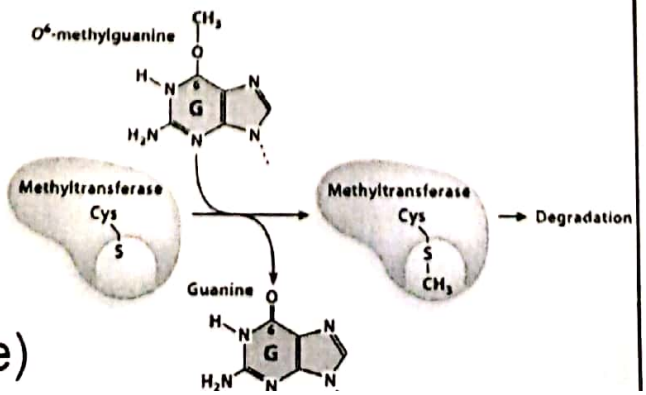
Direct Repair system

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

حفظ الاختصار

* repair the damage result from base modifying agent.

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



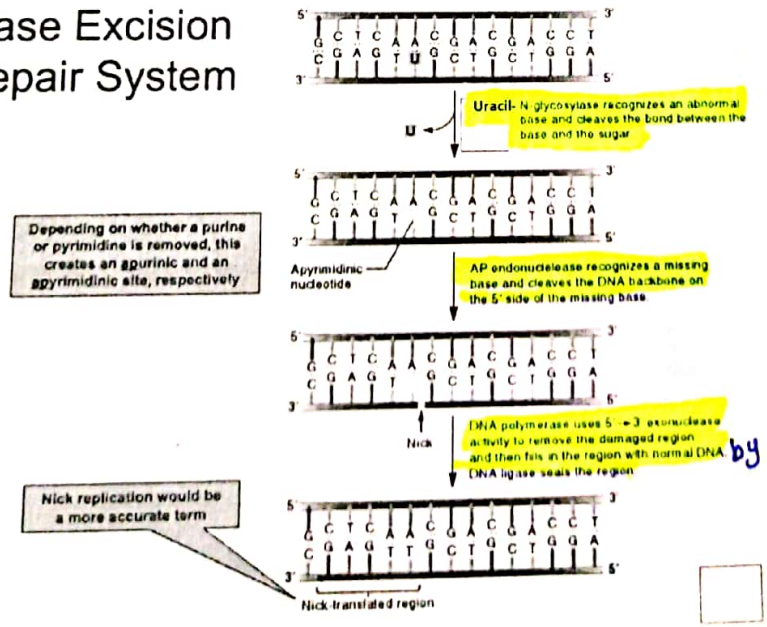
This repair is specific to remove the modified pairs such as depurination or deamination or oxidative damage.

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



by DNA Polymerase elongation activity.

Nucleotide Excision Repair

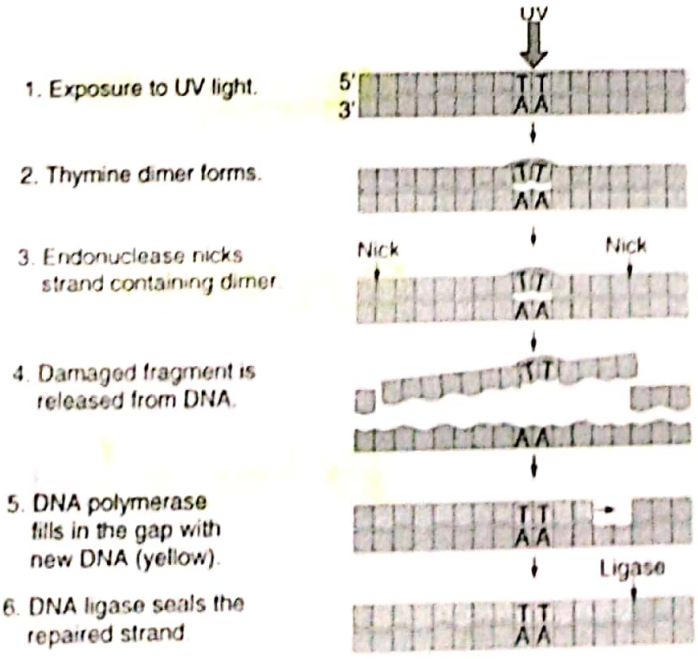
- Nucleotide excision repair system **(NER)** corrects lesions which commonly cause bulk distortions in DNA helix like UV-induced pyrimidine dimers. NER is highly conserved used in both eukaryotes and prokaryotes
- The damaged region is removed in 3 steps process:

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

اللي يا لصندوق الازرق
للإطلاع فقط

Nucleotide Excision Repair

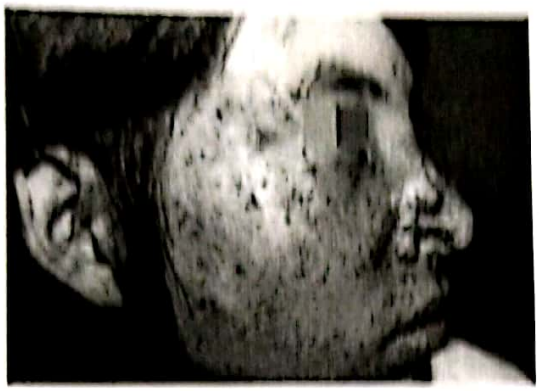
* السلايد كامل للاطلاع فقط



- Excision repair enzymes release damaged regions of DNA.
- Single strand released
- Repair is then completed by DNA polymerase and DNA ligase

Nucleotide Excision Repair

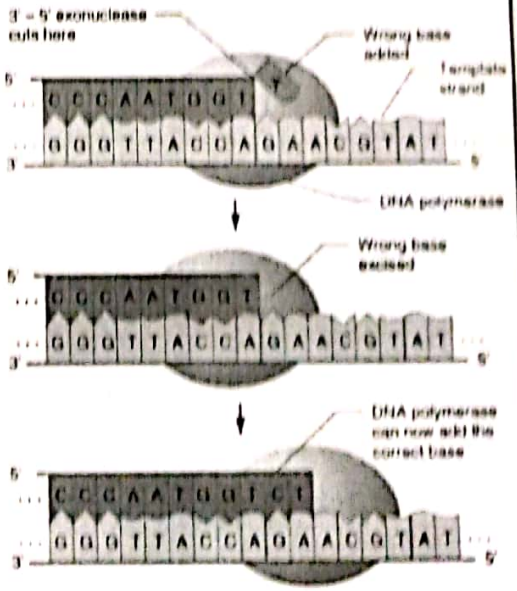
- Xeroderma pigmentosum (XP) is a recessive disorder in which victims lack the normal UV repair enzymes (DNA genes). This creates hypersensitivity to sunlight and a tendency to develop skin cancer



Strand-directed Mismatch Repair

* this repair occur when the error during DNA replication escaped from proofreading activity.

- Mismatch repair **(MMR)** 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



Strand-directed Mismatch Repair

- Mismatch system recognizes and corrects errors that escaped from DNA polymerase proofreading machinery
- 3 steps process:

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 (it can distanguag between old strand and new strand)

old strand ...

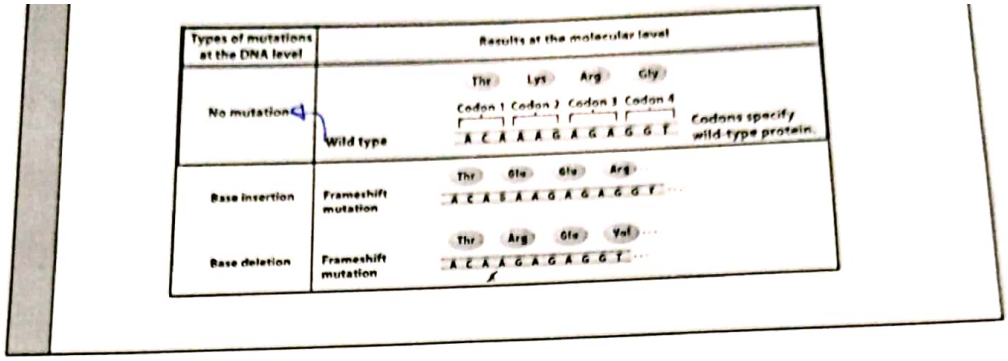
Double strand breaks repair (DSB)

- A dangerous type of DNA damage which can lead t chromosomes fragmentation and consequently los genes **(chromosomal aberration)** if left unrepaire
- Two types of repair mechanisms:

1. Non-homologous End Joining (NHEJ): it is an er prone mechanism of repair because it results in change of DNA sequence at the site of breakag
2. Homologous recombination (HR) is an error-free mechanism of repair because the damage is accurately repaired using information from siste chromatid

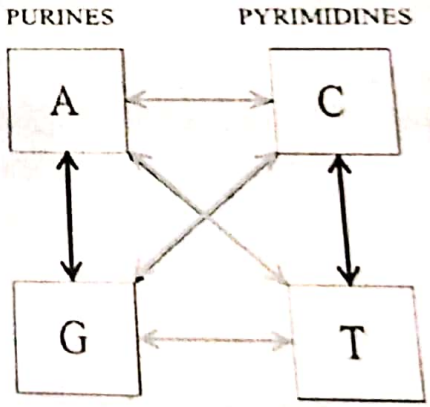
not completly efficient

completly efficent



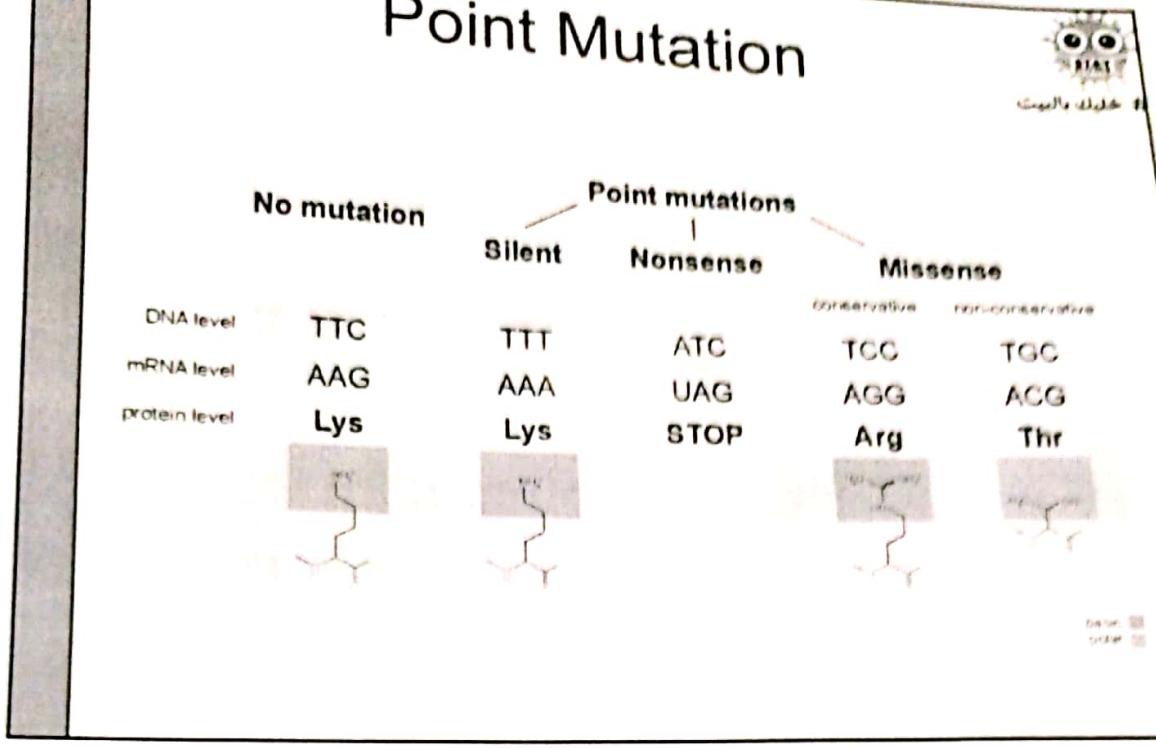
Point Mutation

- 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



ال transition الها اربع احتمالات للحدوث بينما ال transversion الها ثمن احتمالات

Point Mutation



Point Mutation

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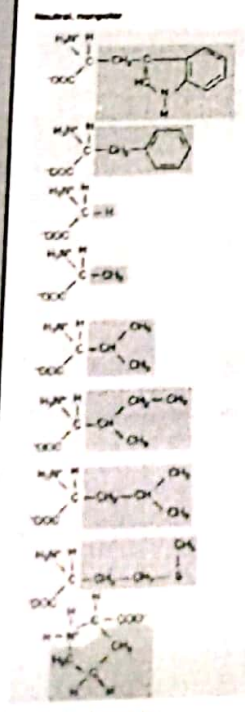

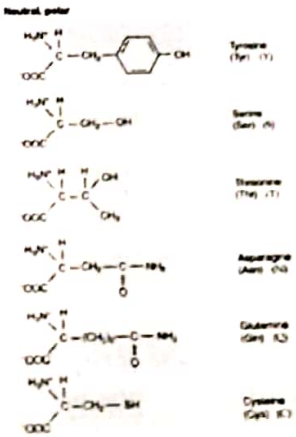

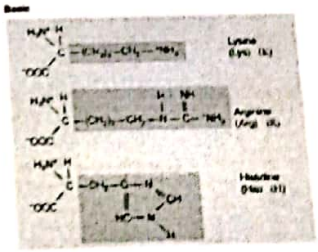

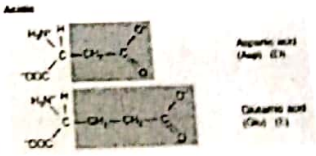

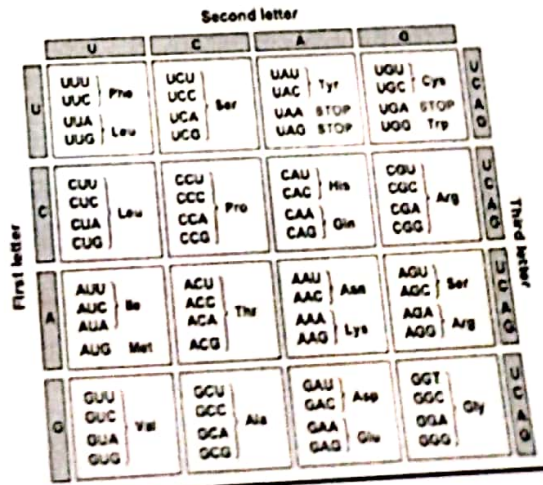

مطلوب معرفة كل aminoacid بأي ~~موجودة~~ ممسوحة موجودة