MOLECULAR BIOLOGY
LECTURE 7
DNA DAMAGE AND REPAIR MECHANISMS
NOTES

Sohaib S. Maitah

Slide (2): DNA Damage

• DNA molecules like all other biomolecules can be damaged in numerous ways.

• DNA damage occurs at a rate of 104 – 106 molecular lesions per cell per day.

-Fortunately, DNA molecules are repairable molcules unlike other molecules such as Proteins and Lipids, because the cell is equipped with efficent repair system to repair the DNA.

• 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?

- The repairing process should occur before the cell divison, because if not so this damage will be inherited and will propgate to subsequent generations and there will be mutations as a final result:
1.Cancerous cell.
2. Inherited diseases.
3.Apoptosis.

Slide (3): DNA Damage "An empty slide"

Slide (4): 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.

- This type of damage does occur ranomly, we can't when or where does it occur!
- Endogenous: Internally.

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

- Exogenous: Externally.

Slide (5): 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. Oxidative DNA damage.

- These are the most common conditions, but not the only occuring ones, and keep in mind that the very first 2 conditions are the most occuring among the mentioned conditions above.

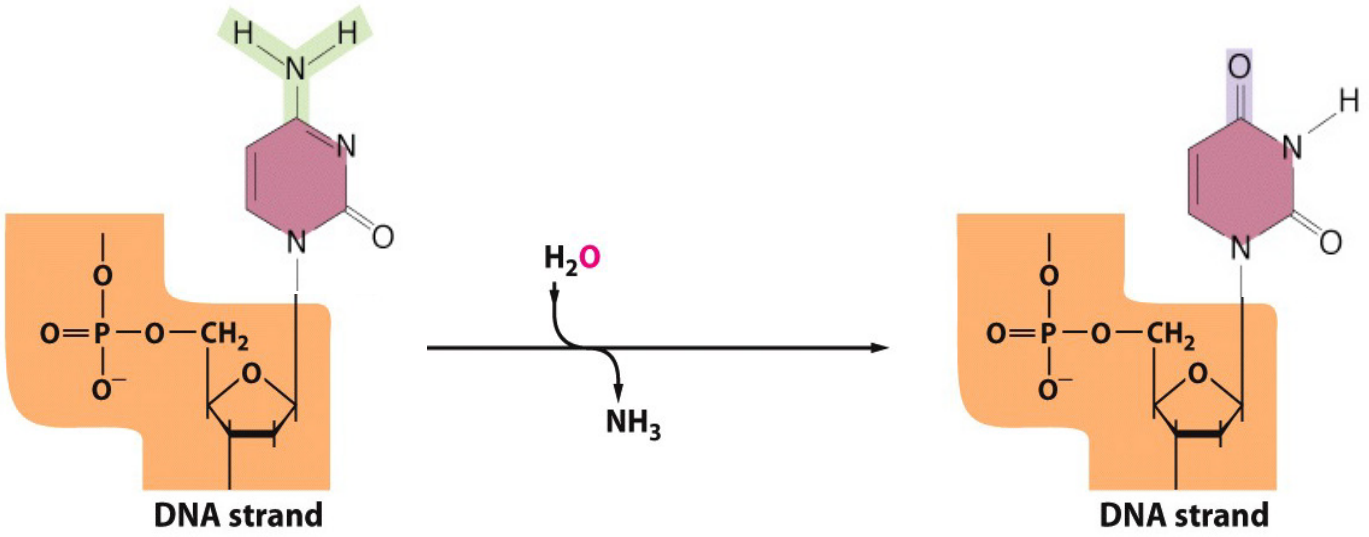
Slide (6): Spontaneous DNA Damage

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

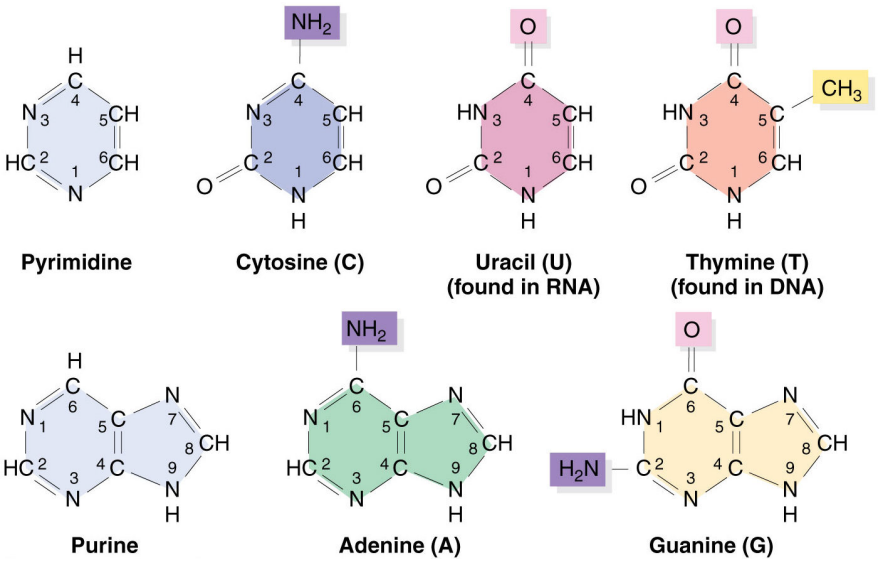
- "De" as a prefix means "Removal of" so Deamination means the removal of Amine group.
-The Amino group located on the 4 nitrogenous bases is quite unstable, and it's enogh for 1 water molecule to break it's bond.

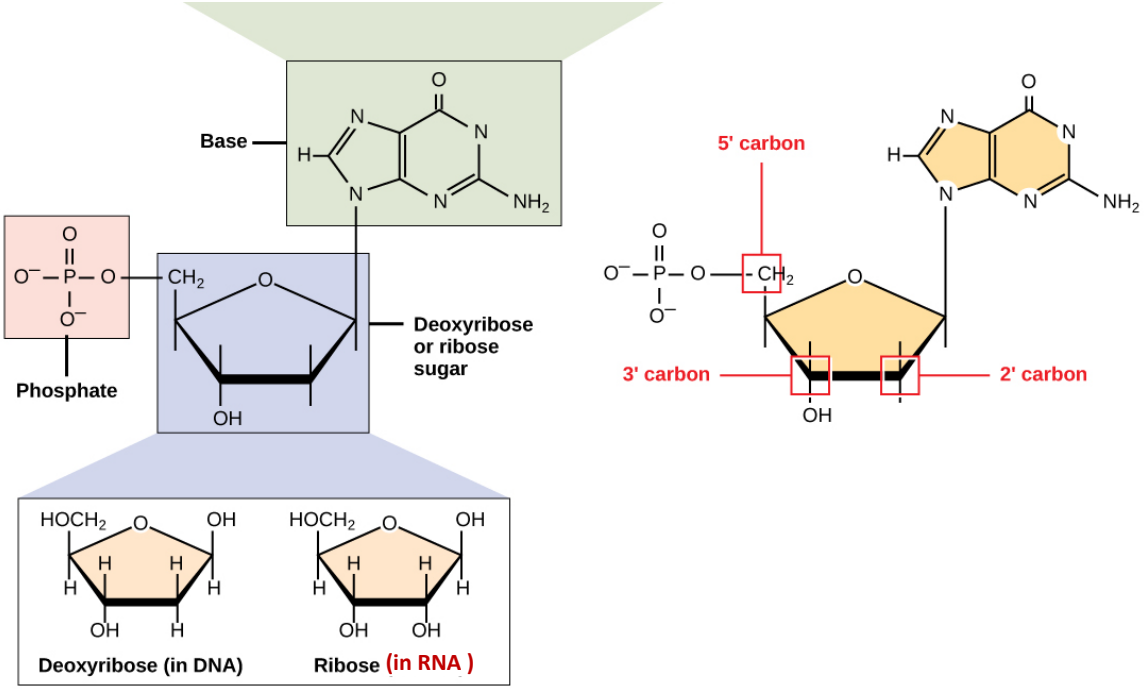
- A Hydrolysis reaction in presence of water, removes the Amino group and converts it into Ketone.

- The nitrogenous base with the highest deamination rate is Cyto-sine.



Slide (7): Nitrogenous Bases

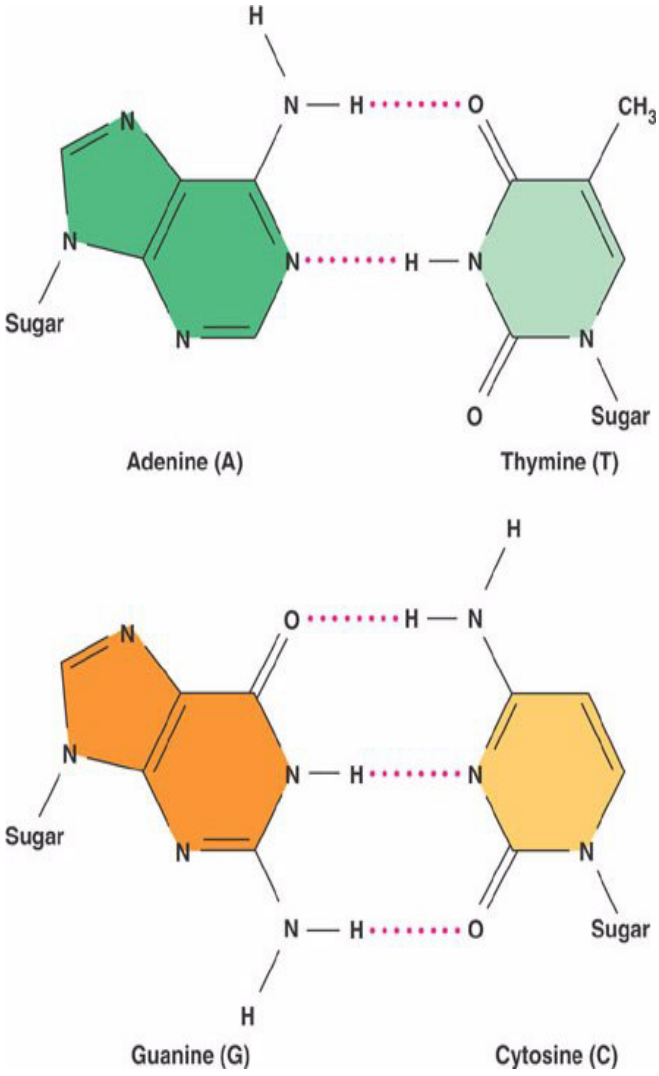




- Uracil is deaminated Cytosine.

- Thymine is Methylated Uracil.

Slide (8): Nitrogenous Bases

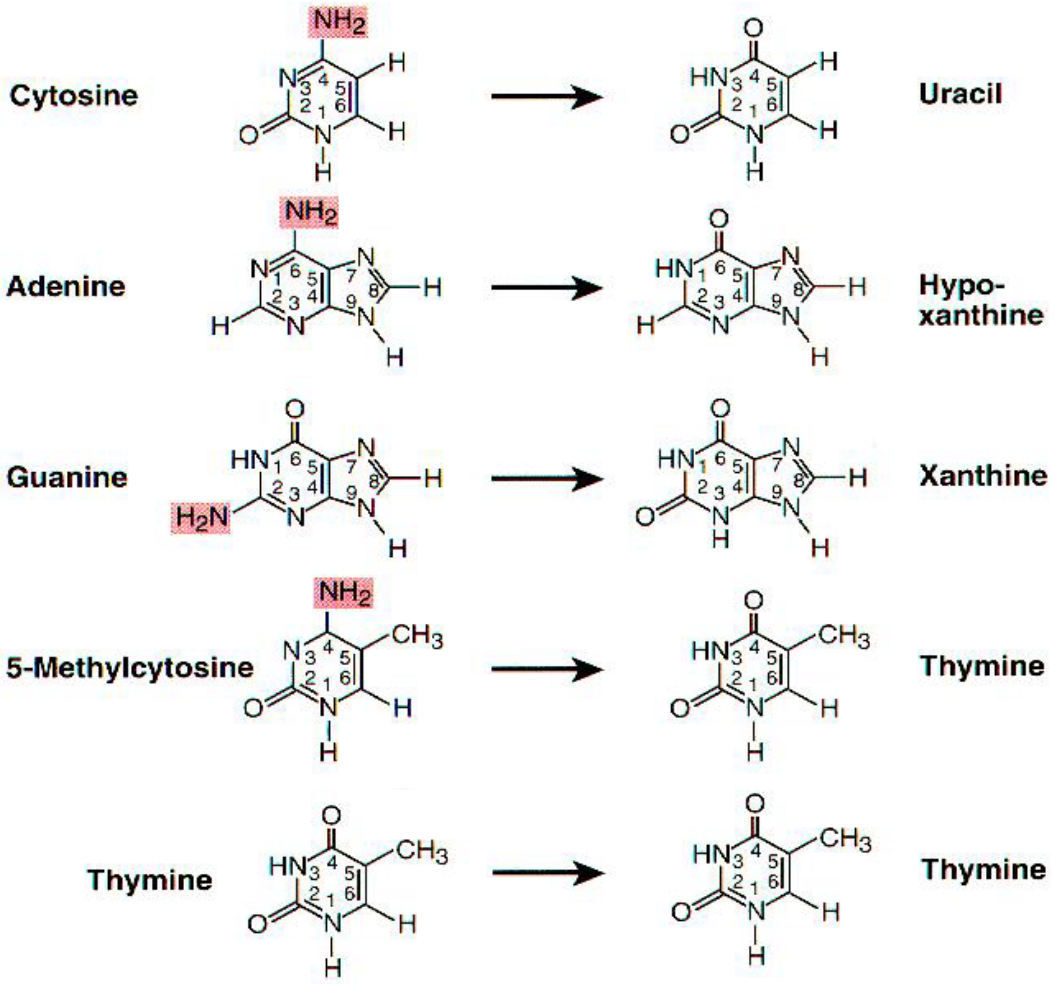


Purines **Pyrimidines**

- According to Watson-Crick Model, Purine always bonds to Pyrimidine

Slide (9): Deamination

• Other possible deamination events in DNA:

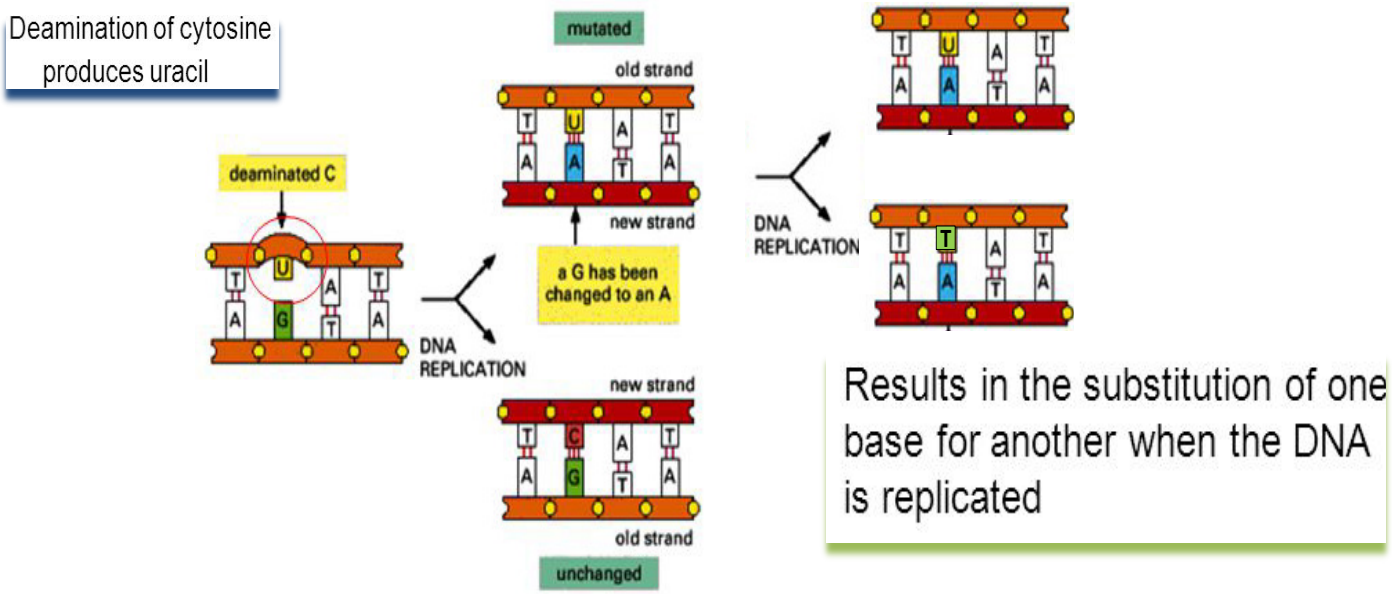


- Thymine cann't be deaminated, since it does have no Amino group in it's own structure.

- 5-Methylcytosine:
2-3 % of the Cytosine in our genomic material is methylated to produce 5-Methylcytosine as a part ot Gene expression regulation, to be more specific the regulation occurs as silencing regulation.

- The Resulted bases are either:
1. Unusual: like Uracil should not be found in DNA.
2. Unnatural: Xanthine and Hypo-Xanthine.

Slide (10): Deamination



- The figure above shows what happens if the Divison occurs without repairing the damage.

- Note: the still be a chance to repair the damage after the first round of replication by enzymes, but after the second round there is no chance.

- The table below should be memorized!

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

Slide (11): 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.

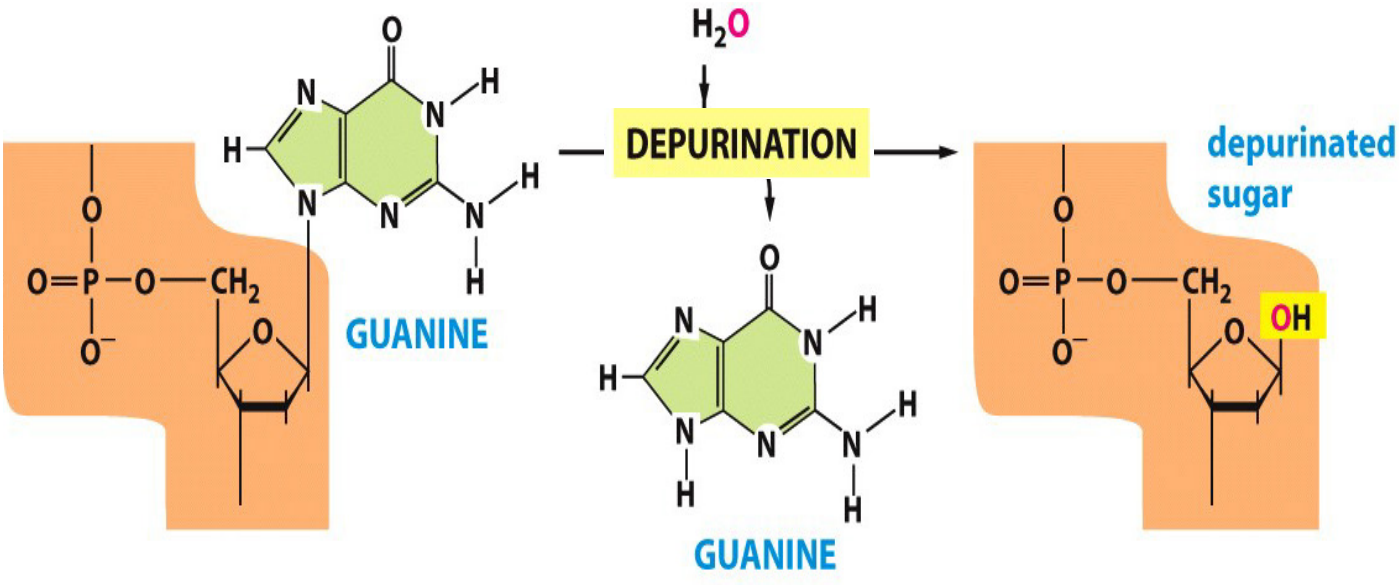
• If left uncorrected, during DNA replication most of these changes would lead to mutations in the daughter DNA chain (particularly base pair substitution).

•These mutations will propagate throughout subsequent cell generations (inherited).

Slide (12): 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′ apurinic site (AP site).

• Under physiological conditions, depurination occurs at a rate of about 5000 bases/cell/day.



Slide (13): Depurination

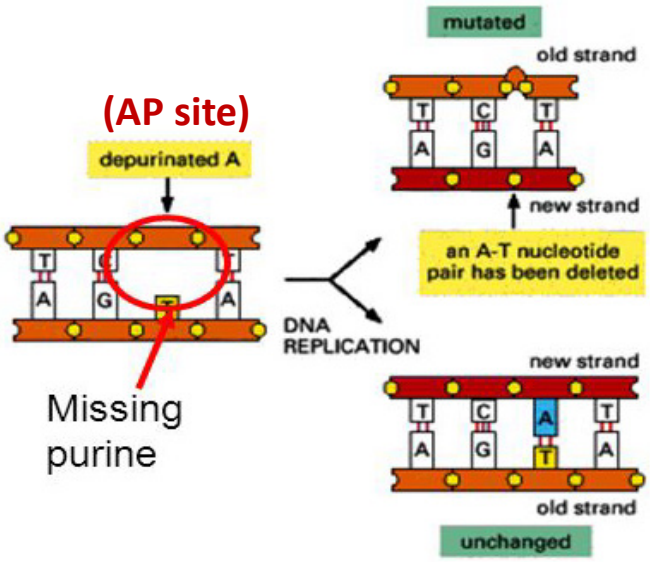
• Depurination results in apurinic site (AP site) which can be recognized and repaired by specific repair mechanisms.

• If left uncorrected, during DNA replication these changes would lead to mutations in the daughter DNA chain (base pair deletion) “See the figure below”.

• This error will propagate throughout subsequent generations (inherited).

• DNA is repairable due to presence of two backup systems: DNA 2nd complementary strand, homologous chromosomes.

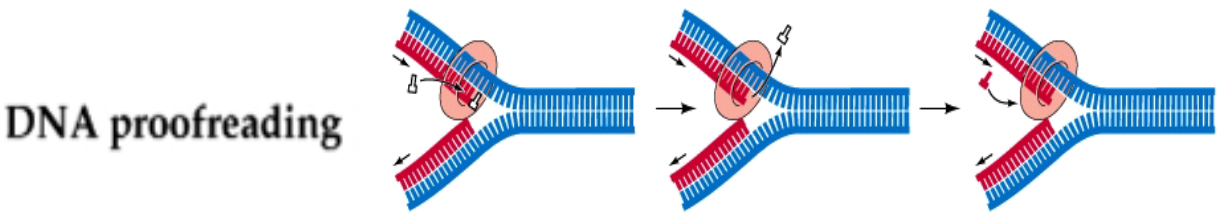
- DNA goes for the second backup system “Homologous Chromosomes” when the two strands are damaged, so when the 2 strands are damaged we depend on the other copy of the gene on the homologous chromosome.



Slide (14): Spontaneous DNA Damage

3. Replication errors: "DNA Polymerase enzyme has 3 activities: 1. 5'-3' elongation activity, and it always adds to the 3' end, the following 3 errors (A,B and C) are results to an error in the first activity by this enzyme" spontaneous lesions may occur during DNA replication in which the wrong base is add to the newly synthesized strand (A.base substitution), a DNA base is skipped (B.base deletion) or extra base is added (C.base insertion).

• Such errors are normally detected and repaired immediately by the proofreading/editing activity of DNA polymerase enzyme (3'-5' exonuclease activity and this is the 2nd activity of DNA polymerase enzyme, it's also called "Proofreading activity or editing").



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

Slide (15): Spontaneous DNA Damage

4. Oxidative DNA Damage: Endogenous Reactive Oxygen Species (ROS) are produced as "Endogenous ROS" byproducts during normal metabolic processes.

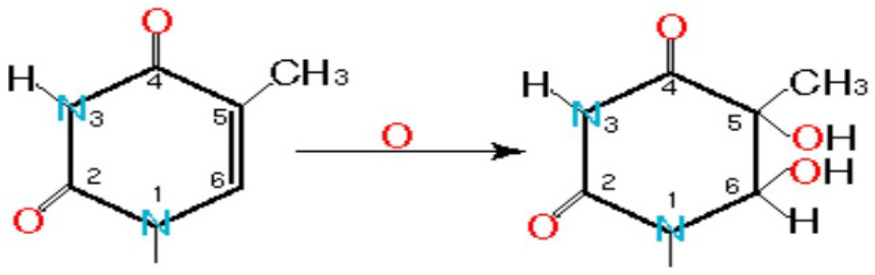
- ROS are Oxygen containing molecules, highly reactive and they have damaging effect to Biomolecules "DNA, Lipid or Protein".
- ROS are free radicals.

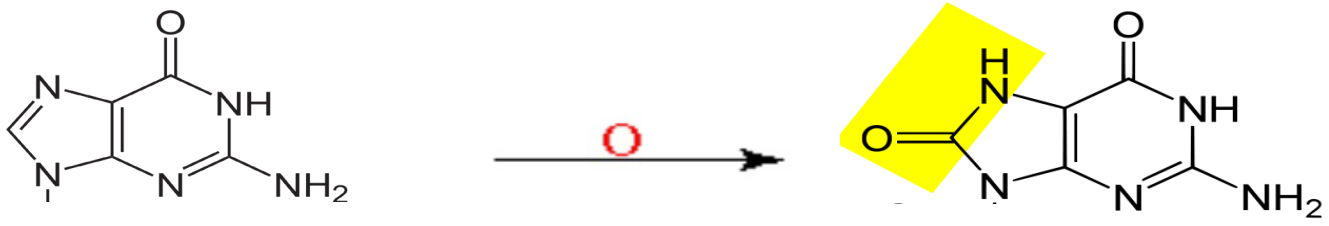
• ROS such as superoxide radical .O2- attack DNA leading to damage.

• When ROS levels exceed the antioxidant capacity of a cell, a deleterious condition known as oxidative stress occurs

• 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.

Slide (16): Oxidative DNA Damage



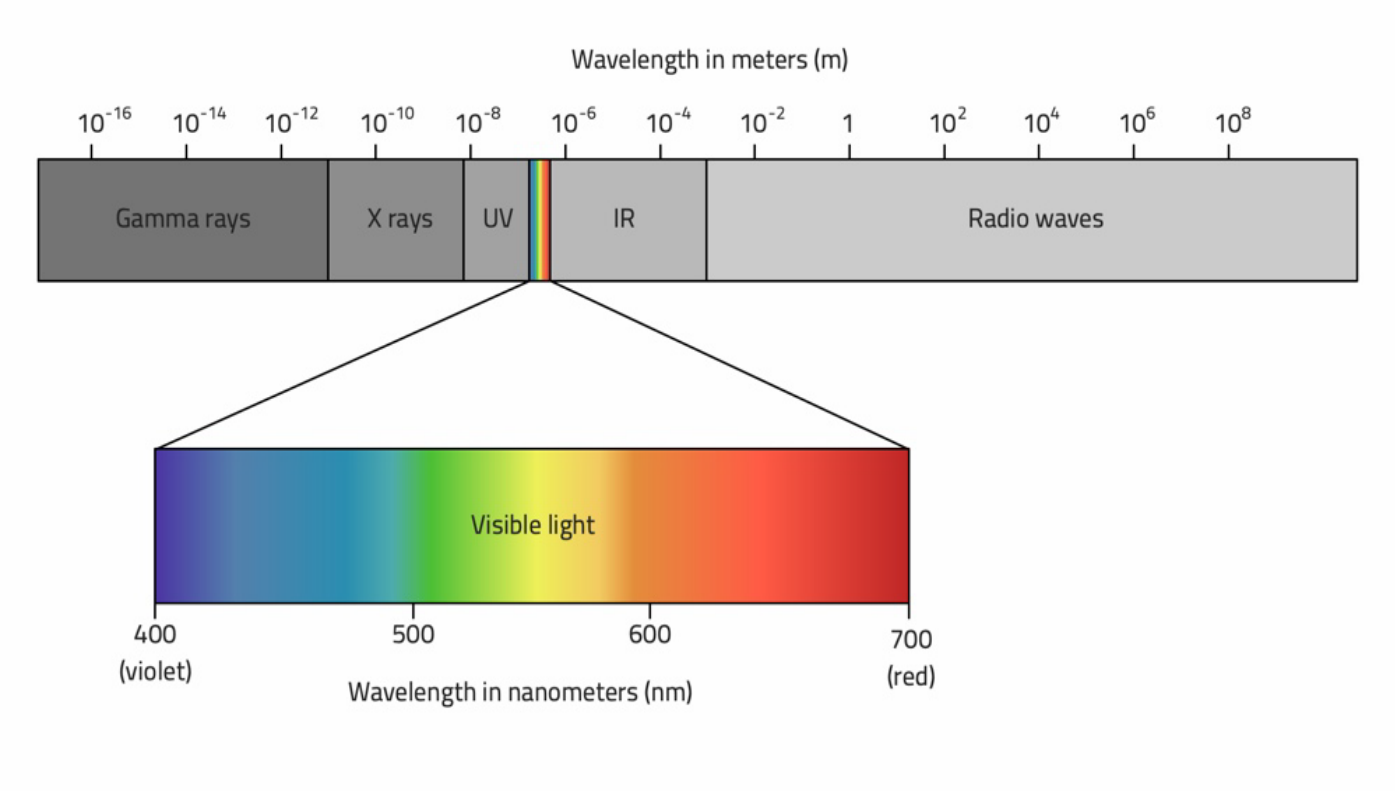


Guanine (G)

8-oxoguanine (8-oxo G)

Slide (17): Induced DNA damage

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



Slide (18): Induced DNA damage

A. Ultraviolet Radiation:

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

- This type of damage is called intra-strand crosslinking, Intra means in the very same strand.

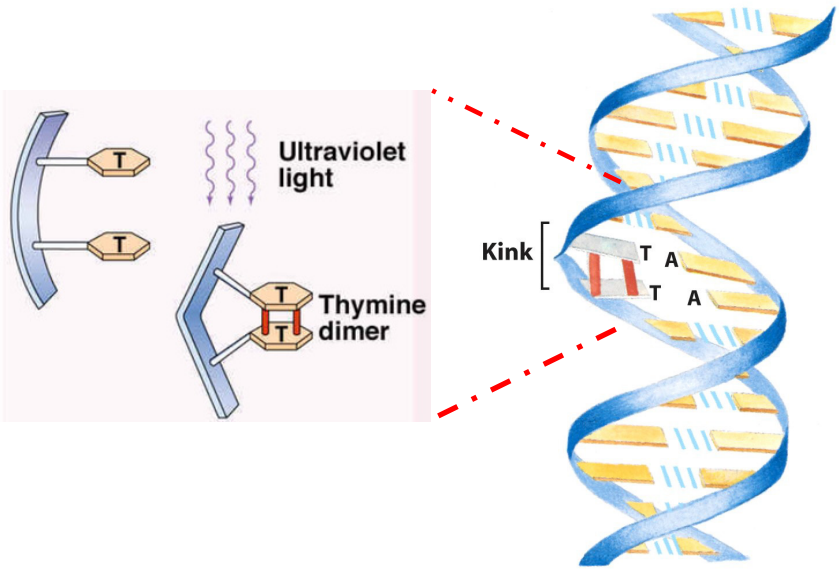
- Mostly occurs when two Thymine bases are next door neighbours.

- The dimer is formed be a covalent bond.

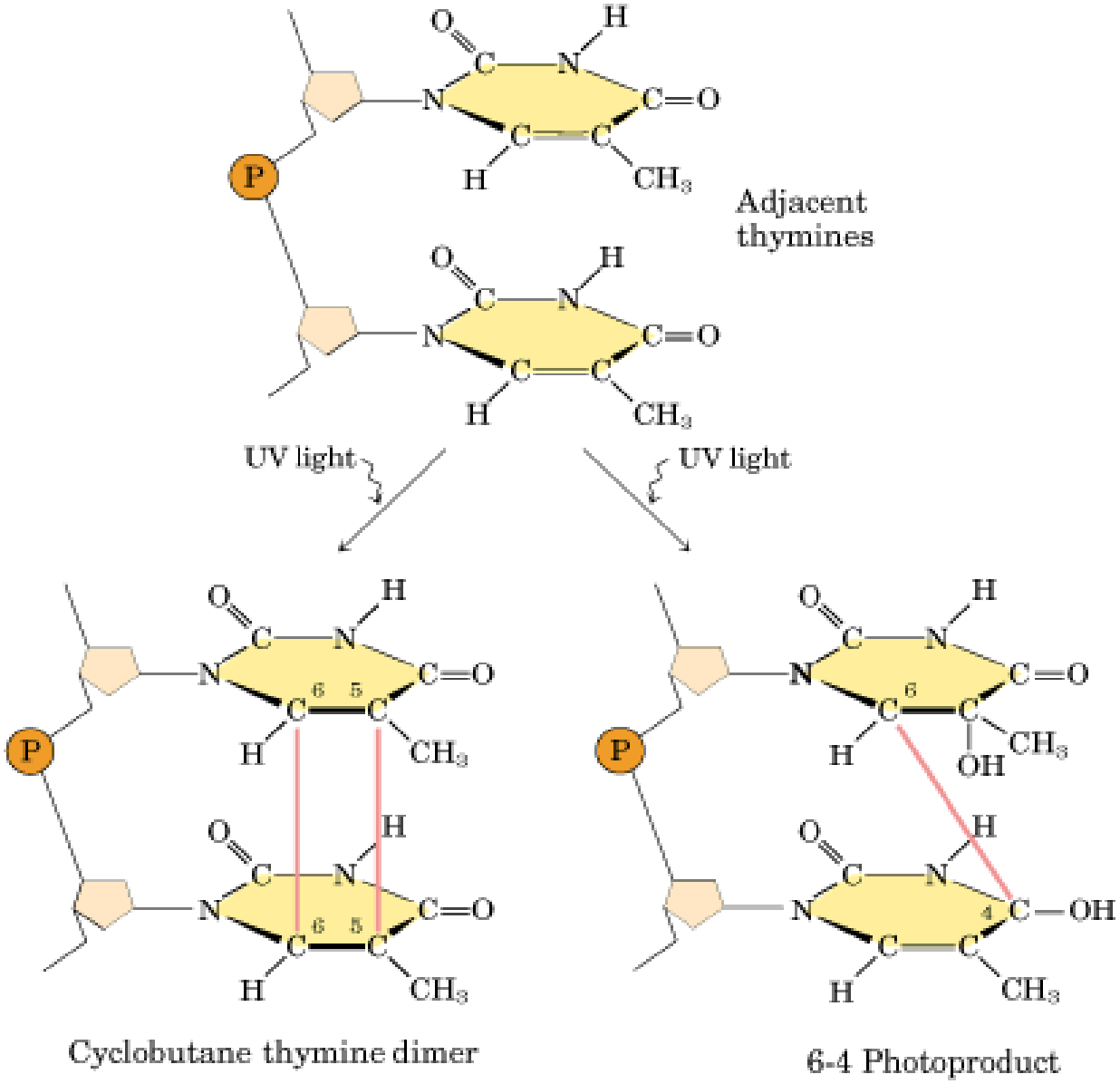
• Dimers alter DNA structure (kink or knot in DNA strand)

• Thymine dimers prevent proper replication, because the DNA Polymerase cann't bind and process.

• The cell either dies (apoptosis) or forms a malignant tumour (cancer).



Slide (19): T-Dimer Types



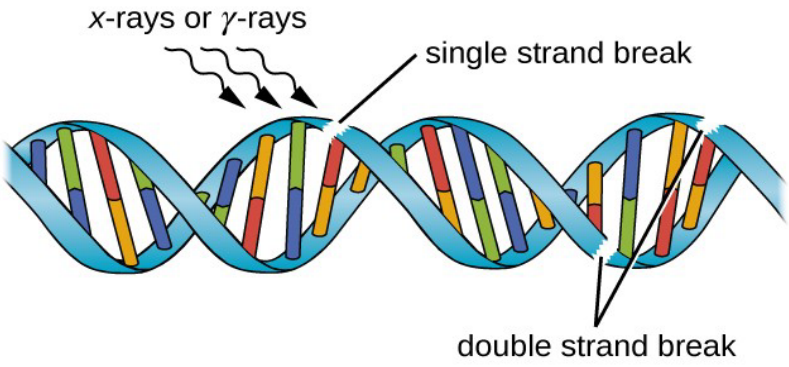
- T-Dimer types is divided into types:
1. Cyclobutane Thymine Dimer.
2. 6-4 Photoproduct.
and these 2 dimers vary by the number of covaleny bond, Cyclobutane Thymine Dimer has 2 covalent bond, whereas the 6-4 Photoproduct has only one covalent bond.

Slide (20): Induced DNA damage

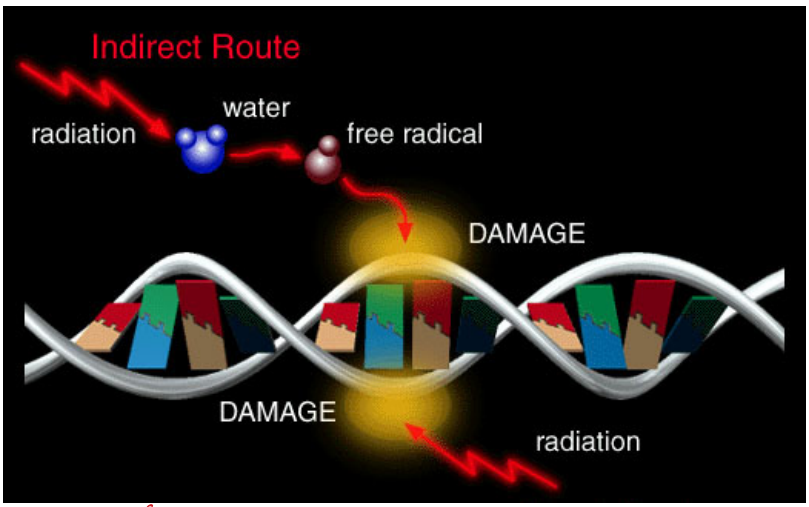
B. Ionizing Radiation: like cosmic rays, X-rays and gamma rays can damage DNA molecules in 2 ways:

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

- SSB is less dangerous than DSB, DSB causes chromosomal aber-rations.
- Enzymes can repair the damage, but this repair is limited, and when we are exposed to high amounts of X-rays the enzymes can't repair the damage.



• Indirect DNA damage by production of free radicals (exogenous ROS) which alter the structure of bases.



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Slide (21): Induced DNA damage

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

-Base modifying agents.

-Base analogs.

-Intercalating agents.

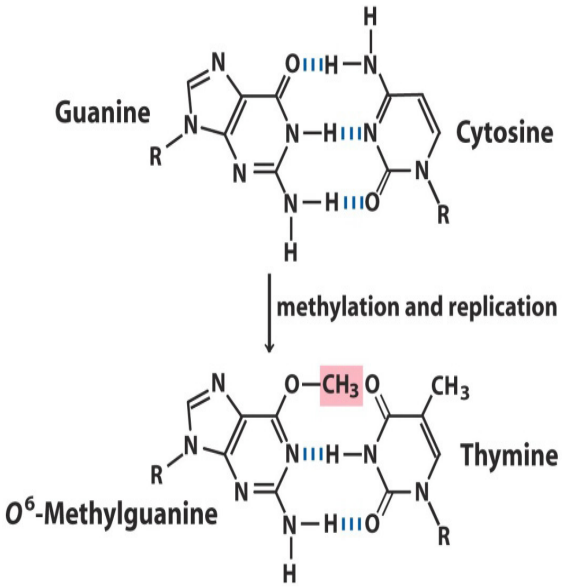
- Only Base modifuing agent is required and the remaining two for reading only...

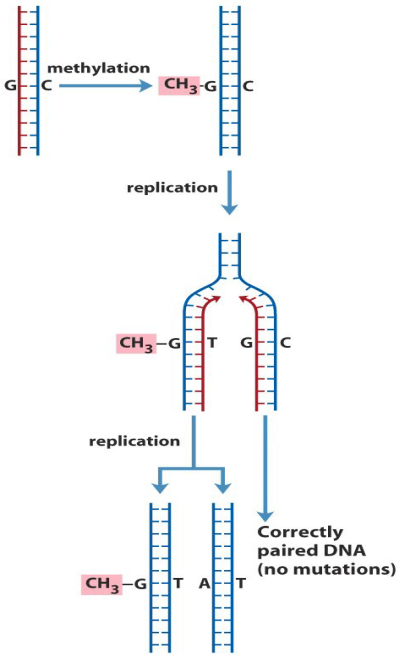
Slide (22): Base Modifying Agents

• Change or modifiy 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 O6 methylguanine (O6 MeG).

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

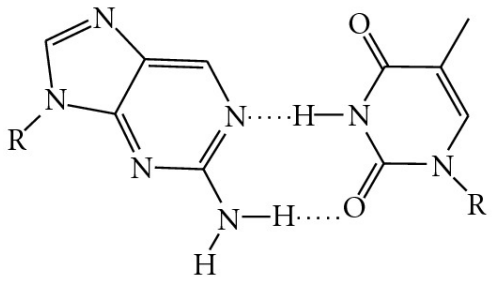




Slide (23): Base Analogs "Skip it"

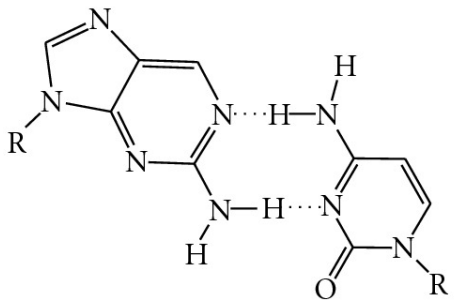
• Chemicals with structures similar to that of any of the four standard bases of DNA like 2-amino purine the base analog of adenine (6-amino purine).

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).



2-Aminopurine Thymine

(a)



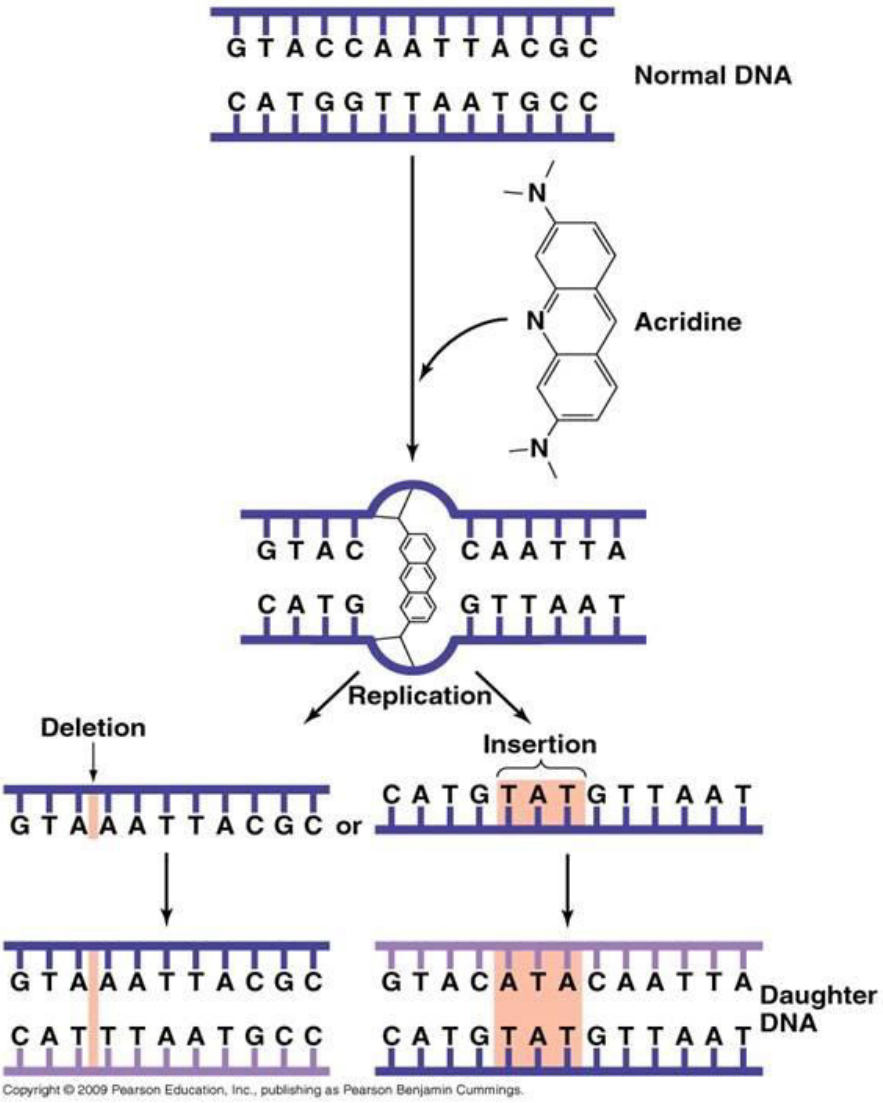
2-Aminopurine Cytosine

(b)

Slide (24): Intercalating Agents "Skip it"

• 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.



Slide (25): DNA Repair Pathways "An empty slide"

Slide (26): 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:

1. Direct/reversal repair.
2. Base excision repair (BER).
3. Nucleotide excision repair (NER).
4. Strand-directed Mismatch repair (MMR).
5. Double strand breaks repair (DSB).

- Memorize the abbreviations, you may be asked about them in the exam.

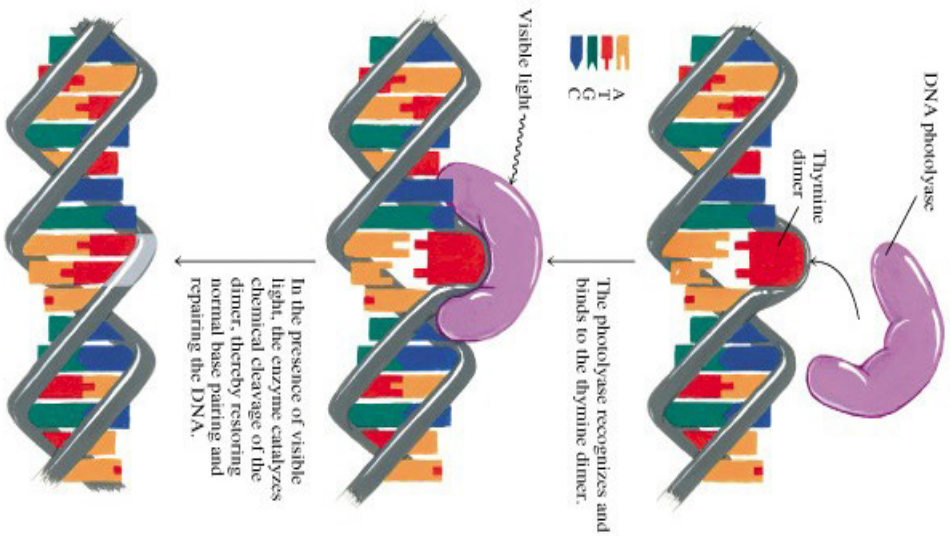
Slide (27): Direct Repair system

• Direct repair also called direct reversal because the damage can be directly recognized and reversed.

• Two specific enzymes are involved in direct repair:

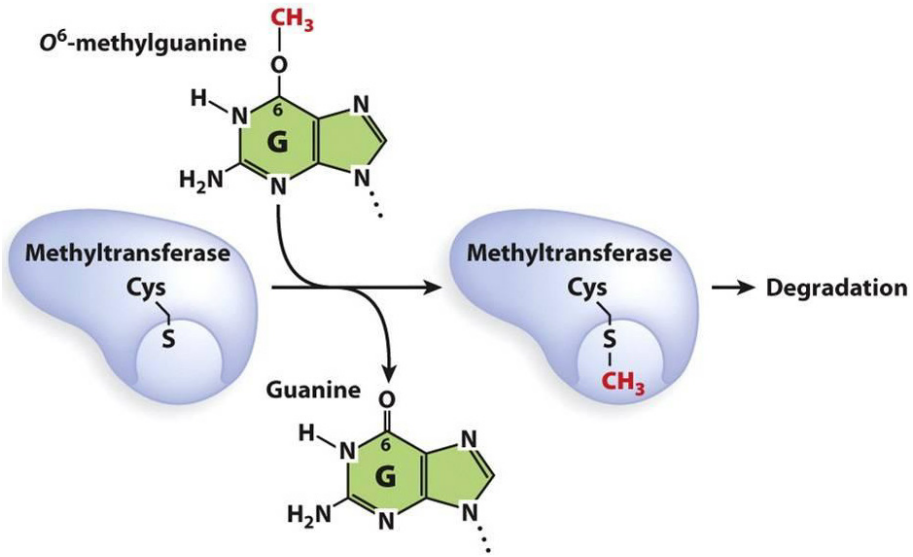
1. Photolyases which repair UV induced damage in plants, bacteria and some animals (excluding humans) by splitting the dimers.

- They should be exposed to visible light to be photo-activated.



Slide (28): Direct Repair system

2. O6-methylguanine methyltransferase (MGMT) which transfer methyl group from G to a cysteine residue within the enzyme itself.



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

Slide (29): Base Excision Repair

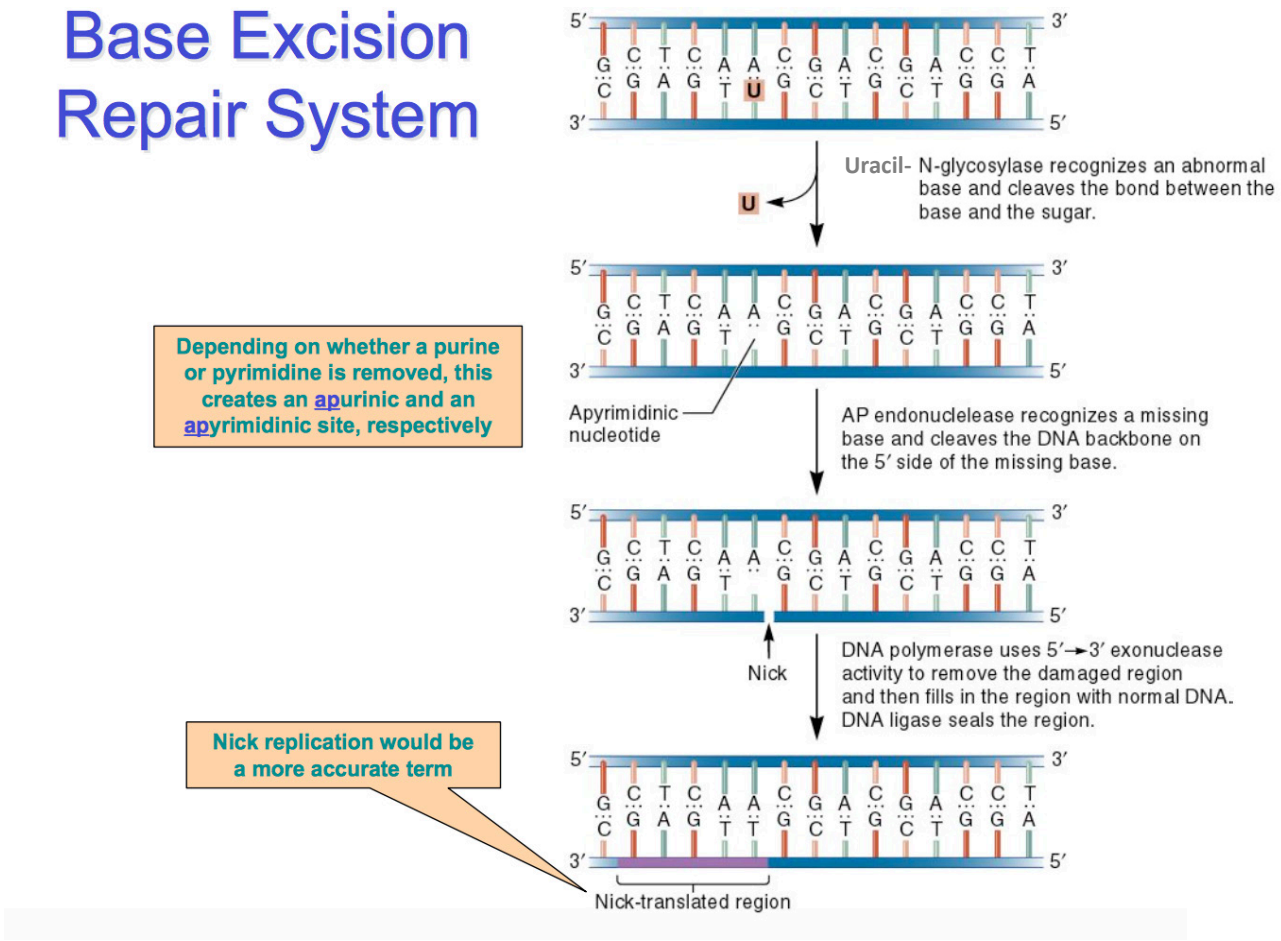
• Base excision repair (BER) involves a category of enzymes known as DNA-N-glycosylases like uracil DNA glycosylase.

• 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.

Slide (30): Base Excision Repair



- In the very first step "figure" the "A" which is located oppositely to the "U" should be replaced with G.

خطأ مطبعي بالشكل.

- The 3rd activity of DNA Polymerase enzyme is 5'-3' Exonuclease activity.

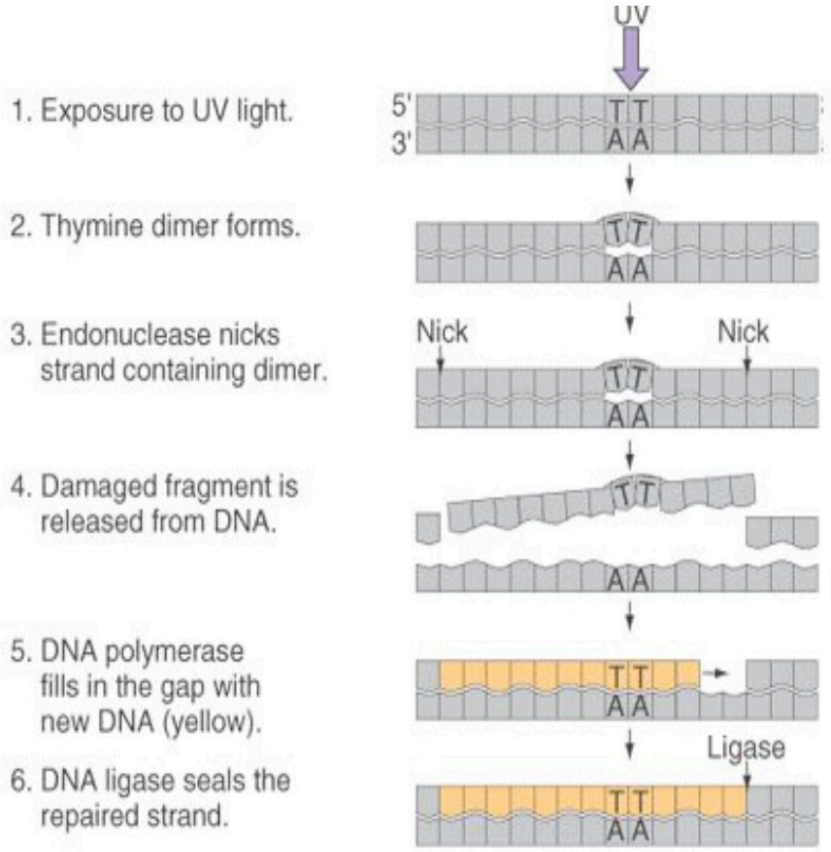
Slide (31): Nucleotide Excision Repair

• 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.

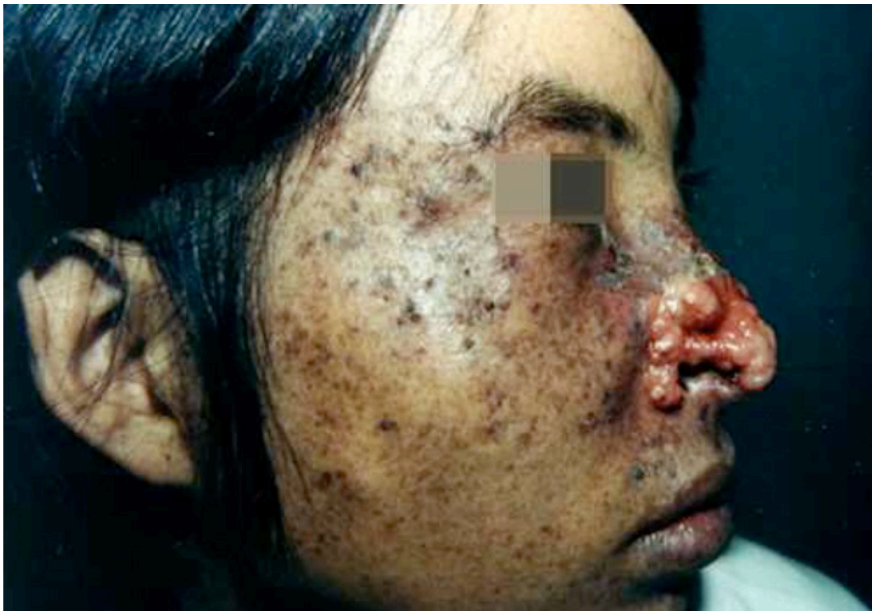
Slide (32): Nucleotide Excision Repair "Reading"



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

Slide (33): Nucleotide Excision Repair

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

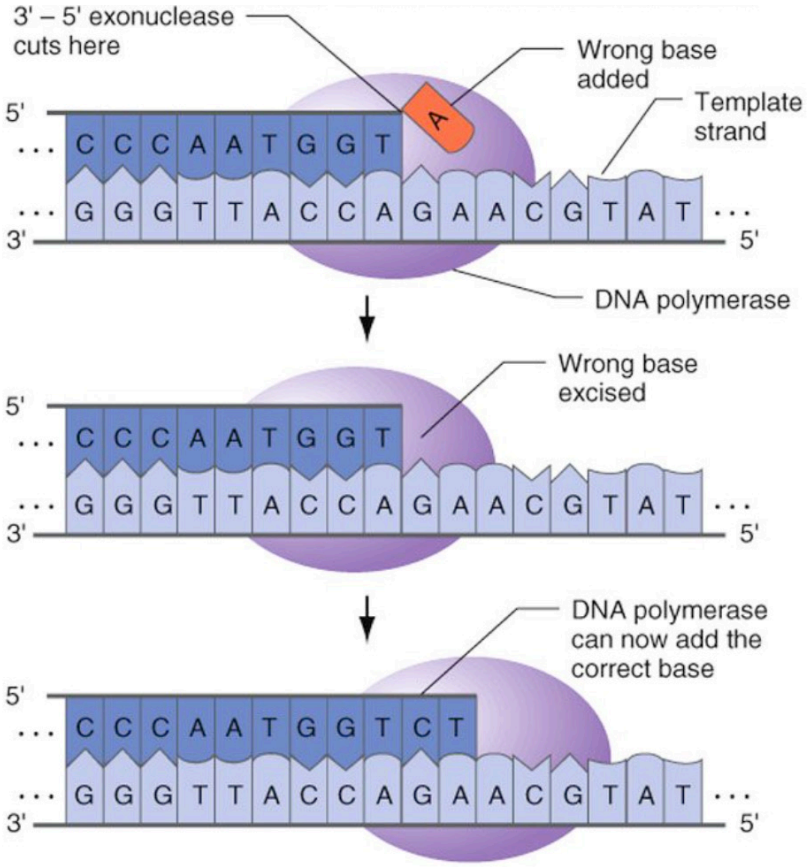


Slide (34): Strand-directed Mismatch Repair

• 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.



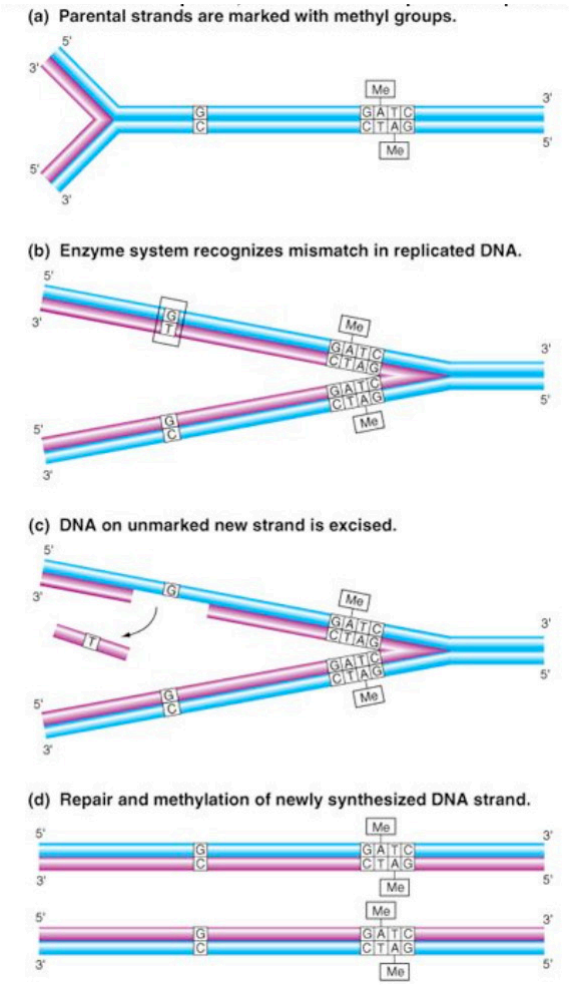
Slide (35): Strand-directed Mismatch Repair

• 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.



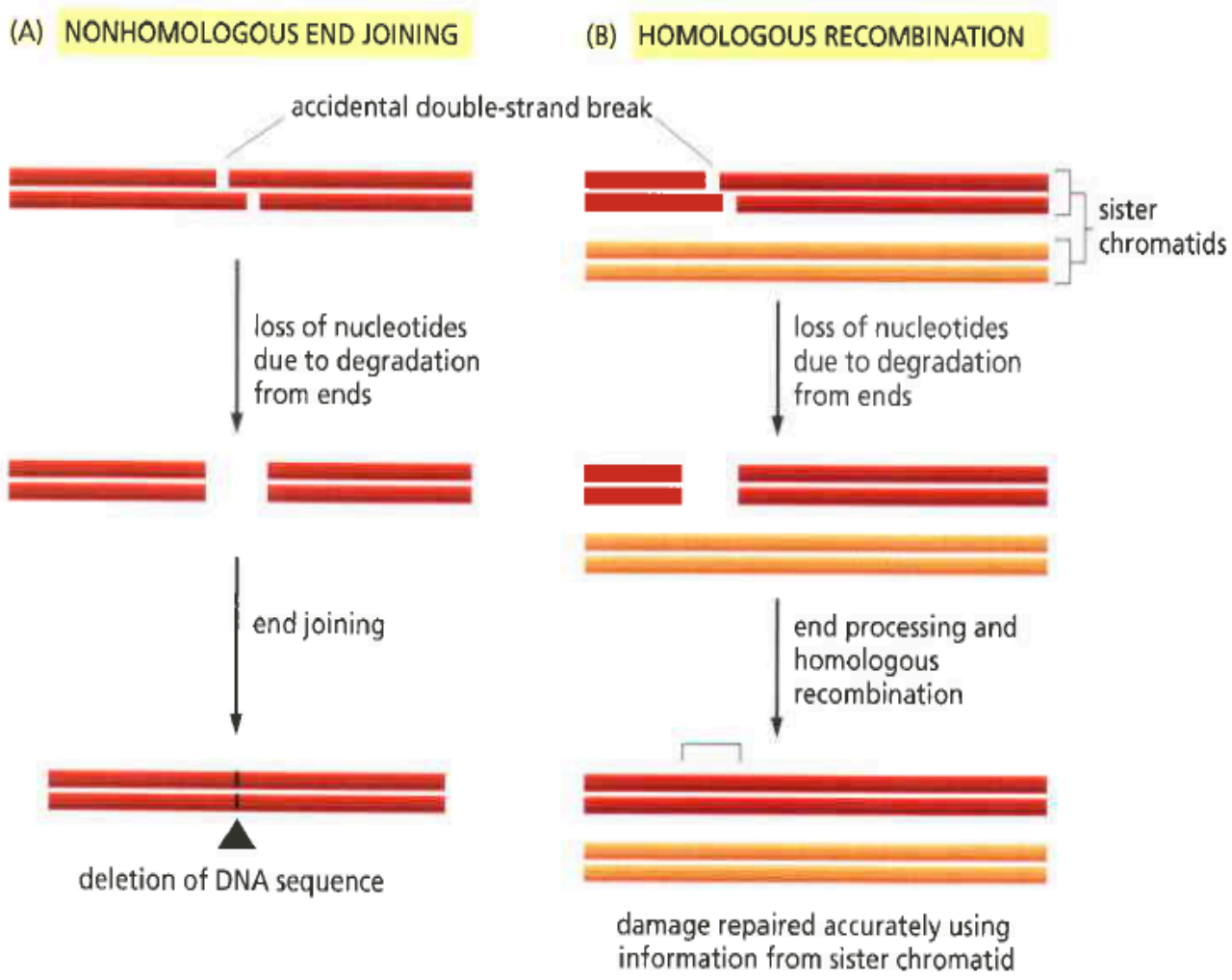
Slide (36): Double strand breaks repair (DSB)

• 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 (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.

Slide (37): Double strand breaks repair (DSB)



Slide (41): Frameshift Mutation

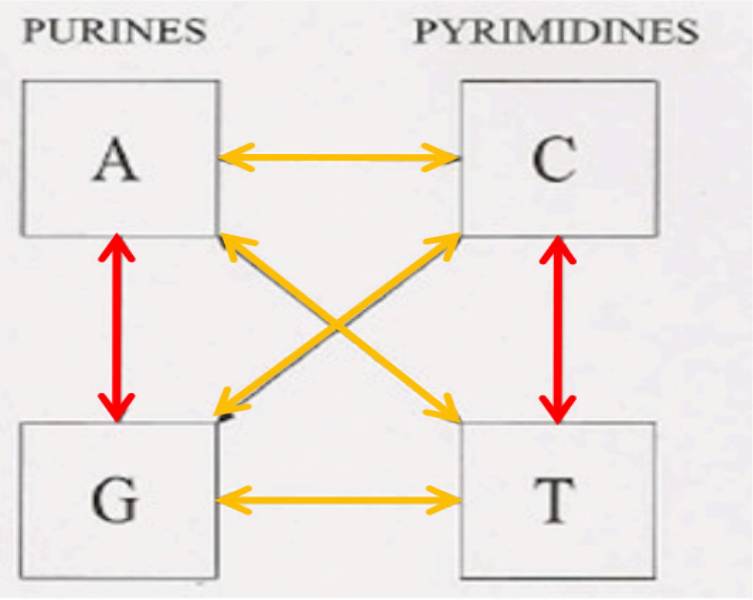
• Frameshift mutation: any addition (insertion) or deletion which alters the reading frame (i.e. not in a multiple of three).

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 ···; A

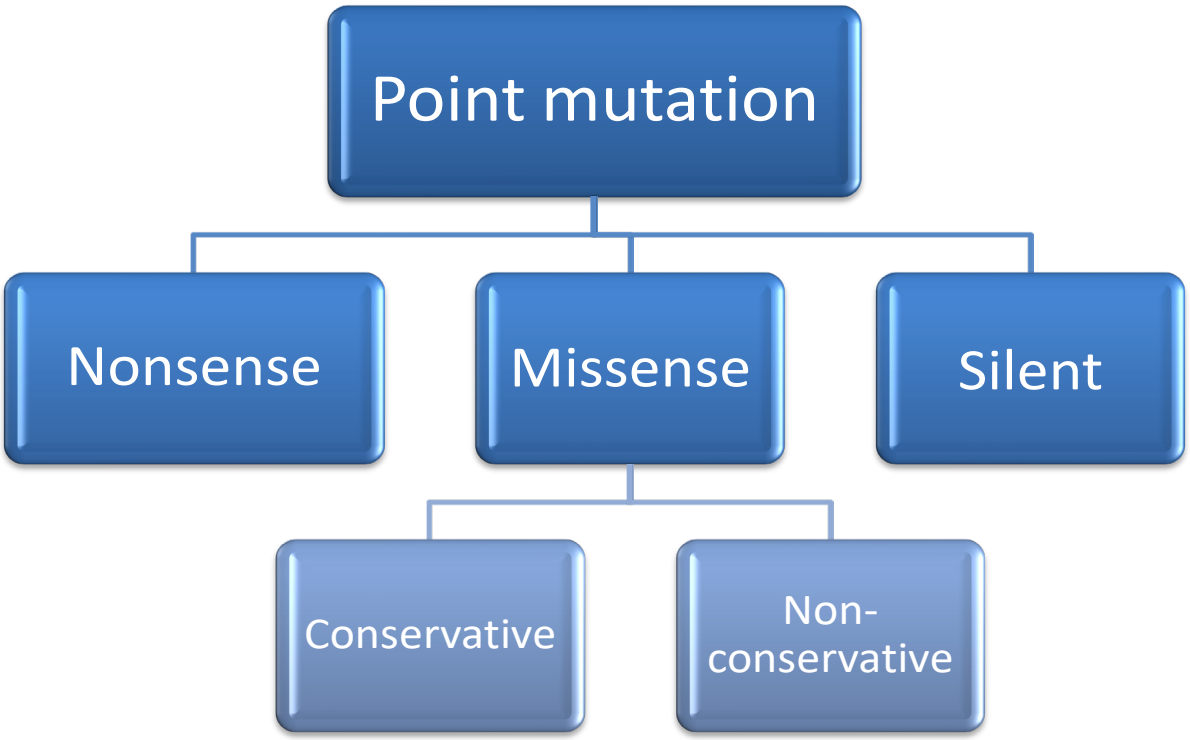
Slide (42): Point Mutation

• 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.



Slide (43): Point Mutation



• At the protein level, point mutation is classified:

1. Silent mutation.
2. Nonsense mutation.
3. Missense mutation (conservative and non-conservative).

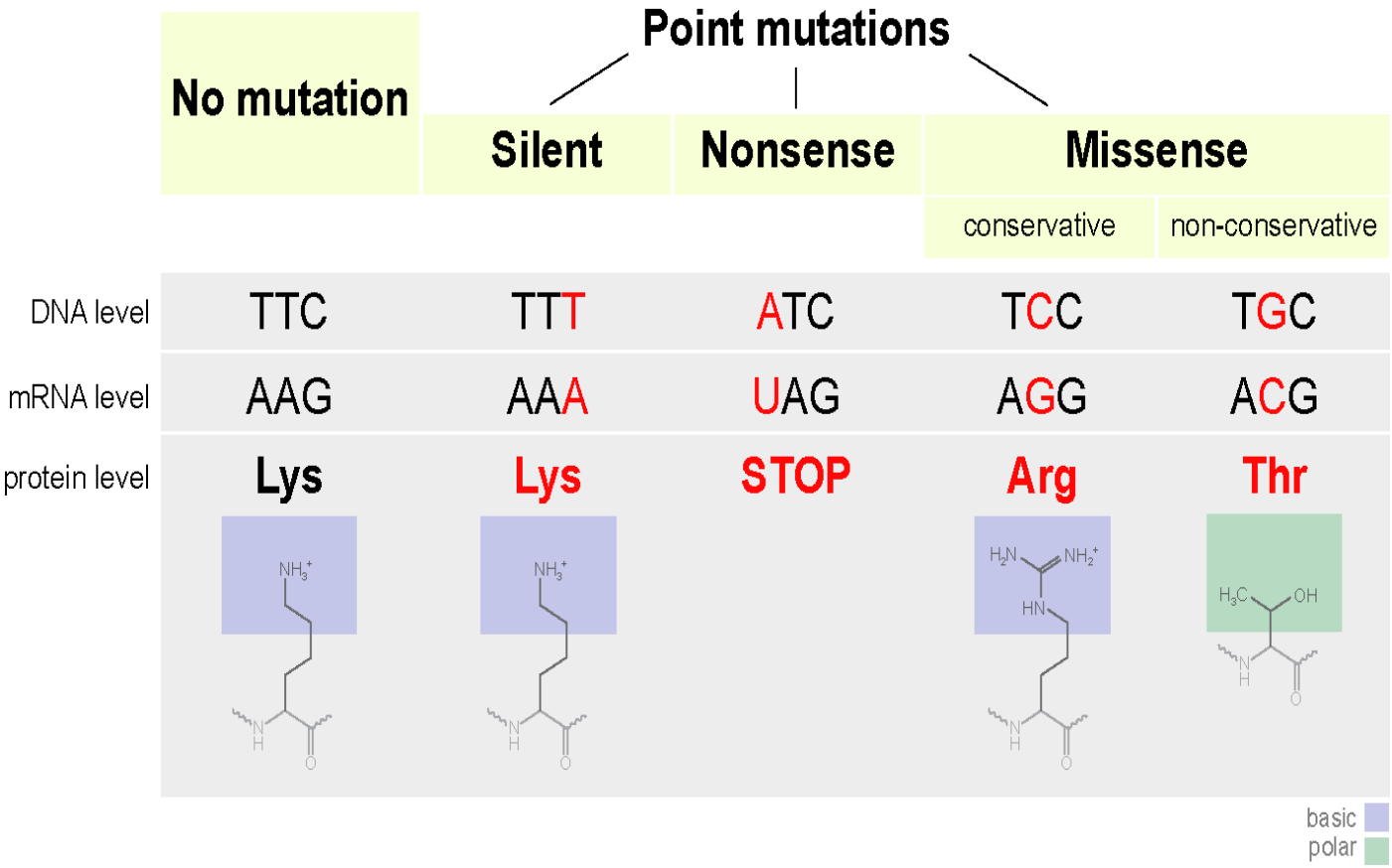
Slide (44): Point Mutation

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.

Slide (45): Point Mutation



Slide (45): Point Mutation

- You should memorize the first figure below, and the last figure is only for reading...

يعطيك العافية، وبالتوفيق جدًا