MOLECULAR BIOLOGY

SHEET (6)

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Black color: doctor's explanation, Navy color: slides, Blue color: extra information, Red color: Dr. Khaldun's notes, highlighted sentence: modified, purple color: book notes .

-CHAPTER 9: DNA Damage and Repair.

Before starting, it's important to know the difference between DNA repair and proofreading

DNA repair: happens after replication

Proofreading: happens during replication

- its crucial to preform DNA repair to avoid mutations (even though some mutations can be useful)

- DNA could be damaged by many factors and those factors may be internal or external.

DNA damage is a common occurrence, Cells require a restoration process (DNA repair)

DNA damage can come from:

- Exogenous agents **(External factors)** come from the surrounding environment.
  such as hydrogen peroxide from external sources ,or drugs or Exposure to high energy electromagnetic radiation: UV Light, Gamma Rays or X-Rays

- Endgenous agents **(Internal factors)** which are formed inside the cell by normal metabolic pathways, could also damage the DNA,
  such as the spontaneous deamination or what is called tautomerization

All of these could affect DNA and cause damage to the structure of the DNA and thus the DNA will not be able to do its biological activities such as replication, transcription or expression of any gene

-UV light

■types

Firstly, we are going to mention the three bands of the UV light and how they differ from each other :

1) UV-C (wavelengths 100-295 nm):
   Includes the DNA absorption maximum-recall that it has a wavelength of 260 nm and is represented by the notation (lambda 260)-
   very little UV-C reaches the earth's surface because of the ozone layer in the stratosphere which prevents it from doing so.
   It leads to a much greater incidence of skin cancer.

2) UV-B (wavelengths 296-320 nm):
   Is accountable for about 10% of the UV radiation reaching the earth's surface. Furthermore, it is responsible for most of the DNA damage in the skin.

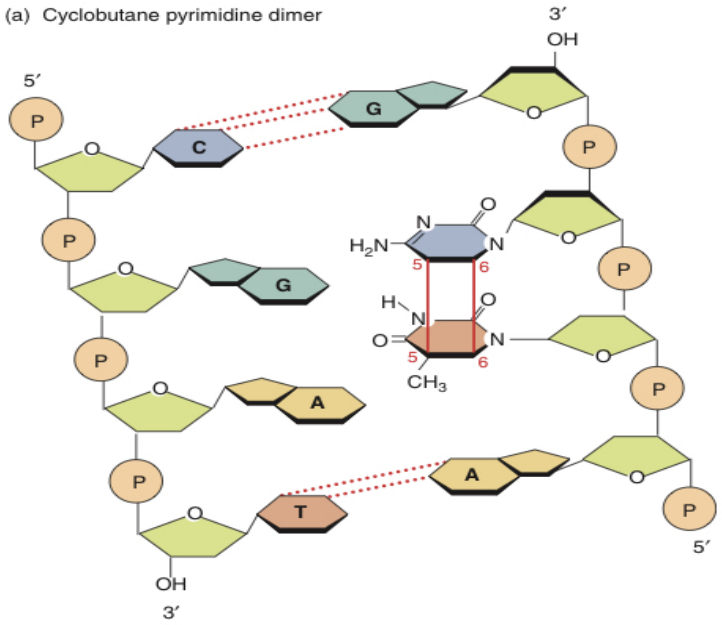
3) UV-A (wavelengths 321-400 nm):
   Is accountable for most UV light reaching earth. Also, it penetrates the skin more deeply than UV-B but is less efficient at causing DNA damage.
   The sunlight's tanning effects are largely due to UV-A. However, constant exposure to UV-A comes at the steep price of skin aging, wrinkling and an increased risk of skin cancer.

■Damage

2 major types of pyrimidine dimers account for nearly all UV-induced damage:

1.Cyclobutane pyrimidine dimers:

- Most common are thymine-thymine dimers.Also, they account for about 75% of UV induced damage. Cytosine-thymine and cytosine-cytosine dimers also form but at slower rates.
- B-DNA can accommodate a single dimer, but the helical axis is forced to bend



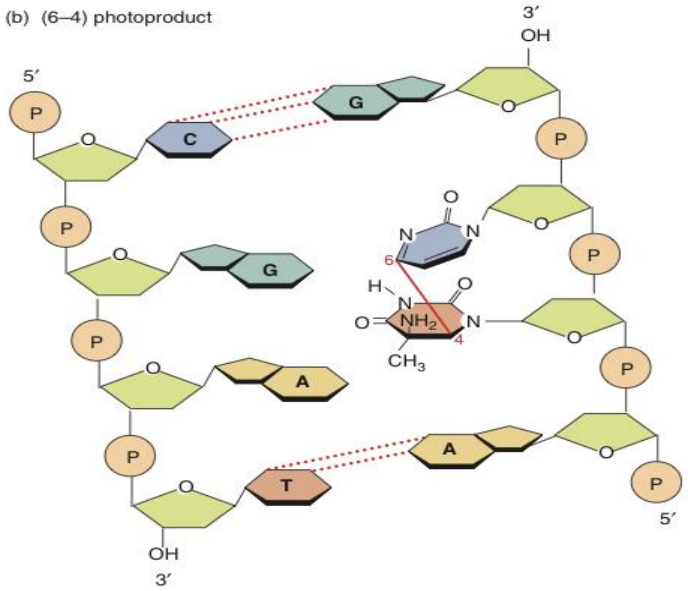
(a) Cyclobutane pyrimidine dimer: two complementary strands of the DNA with two adjacent pyrimidines, they attach covalently to each other by (5-5) and (6-6) carbon atoms and they make what is called cyclobutane pyrimidine dimer.

(This will prevents hydrogen bonding between complimentary bases, lead to bending in sugar phosphate backbone)

Thymidine dimers **(thymine-thymine dimers) (Cyclobutane pyrimidine dimers)** UV exposure will make 2 **covalent bond** between these two neighboring Thymidine bases.

2.The (6-4) photoproduct: causes a major distortion in B-DNA.

Another structure will form.The (6-4) photoproduct which is cyclic structure, there will be a covalent bond between carbon or nitrogen between two thymidine or two pyrimidine at atom C4 and C6 and that will Cause distortion of the DNA and prevent it from its activities.



(b) 6-4 photoproducts:

sometimes covalent bond form between carbon 6’ and carbon 4’ that will form what is called The (6-4) photoproduct (butane product , cyclic product)

Ultraviolet light also promotes the formation of (6-4) photoproducts by introducing a bond between
the C-6 atom of one 3’-pyrimidine (either cytosine or thymine) **and**
the C-4 atom of an adjacent 5’-pyrimidine (usually cytosine) on **the same DNA strand**.

Notes: The (6-4) photoproducts cause a major distortion in B-DNA because the two pyrimidine rings are perpendicular to each other.

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من ذا يراك وليس يذهبُ همّهُ

-X-rays and gamma rays

-X-Rays and Gamma Rays are Ionized Radiation.

-Ionizing radiations : capable of atom ionization (remove electrons and form ions)

-X-rays and gamma rays cause many different types of DNA **damage**(direct and indirect damage)

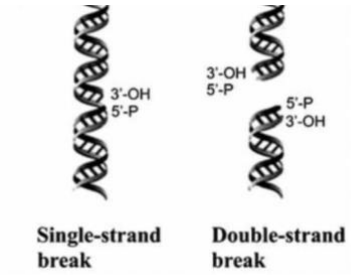
1. **Direct damage**
   – DNA or water tightly bound to it absorb the radiation
   sometimes the DNA itself or the water itself that is bound to the DNA upon absorption, will also produce some damages to the DNA.
2. **Indirect damage**
   – Water molecules surrounding DNA absorb the radiation and generate reactive species (free radicals) water molecules which are surrounding the DNA will be activated and oxygen reactive species will be generated as radiation is absorbed

What will happen when cells are exposed to radiation?
sometimes this radiation won't only lead to single stranded molecules breaking ,but both strands could be broken by exposure to these radiations such as (X-Rays or gamma) that will cause what is called chromosomal aberrations or chromosomal abnormalities, such as:
deletions or duplication, translocations or inversions

-Lesions: regions in which damage has occurred
-What damage?
1) breaking of phosphodiester bond
2) breaking of chromosome itself.

Lesions may be isolated or clustered:

Clustered lesions: Double-stranded breaks can cause a variety of chromosomal aberrations (Translocations, Inversions)



Translocations: (segments breaking away from the chromosome and then reinserting at new sites)

means some parts or part of the chromosome will leave that chromosome and attach to another chromosome for example part of chromosome number 21 will be linked to chromosome number 5 or 6 that will cause damage to the genes and thus could cause serious genetic diseases.

Inversions: (segments breaking away from the chromosome, inverting from end to end, and then reinserting at original breakage site) .

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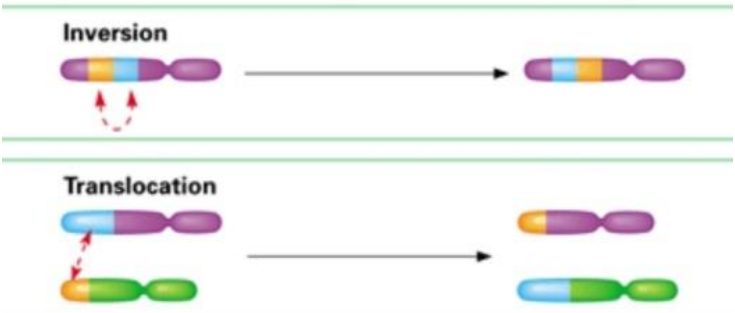


we will talk about these when we take about chromosome karyotyping (to detect chromosomal abnormalities)

≈65% of the DNA damage caused by x-rays and γ-rays is due to indirect effects.
Formation of 3 highly reactive chemical species as they have high energy that could damage the DNA molecule and break it.

*H2O+ (water radical cation)

*OH (hydroxide radical)

*O2 - (superoxide)

Each one attacks and damages biological molecules they encounter. A wide variety of chemical changes take place when that molecule happens to be DNA.

DNA is damaged by *hydrolytic cleavage* reactions

What are the bonds that are affected by these free radicals of water or oxygen after absorption of X-Ray or gamma rays?

1. Phosphodiester bonds: located in the backbone of the DNA (least dangerous)
   Spontaneous phosphodiester bond cleavage occurs very rarely (which introduces a nick) and probably doesn't make a significant contribution to DNA damage.

2. N-glycosyl bonds: between pentose sugar and the nitrogenous base
   Mostly,N-Glycosyl bonds cleavage upon exposure to radiation, when a nitrogen base is cleaved or when N-Glycosyl bond is cleaved then the nitrogenous base will be removed that forms a site that is called abasic site or AP site (from apurinic or apyrimidinic) Furthermore, AP site formation sensitizes the neighboring 3'-phosphodiester bond to cleavage.
   When there is no site of nitrogen base, the information will be lost from the DNA, so this is very serious (no base identifier), (when there is one or more AP sites, the DNA strand will make a poor template because it lacks

the information required to direct accurate replication and transcription).

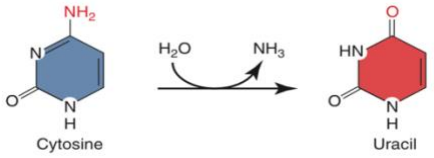
According to current estimates, about 10000 purine and 500 pyrimidine bases are lost from DNA in a mammalian cell each day. These observations are consistent with in vitro experiments showing that purine N-glycosylic bonds are more easily hydrolyzed than pyrimidine N-glycosylic bonds.

Student Q: what is difference between abasic site and bulge: bulge in double stranded DNA OR RNA will happen when alkyl group enter the helix cause adduct (إضافة)that form bulging and it is detected if there is a damage in the DNA OR RNA, bulging contains big groups make distortion of DNA structure that is recognized by repair system.
DNA adducts are covalent modifications of the DNA that result from exposure to specific carcinogens.(cancer causing reagents)

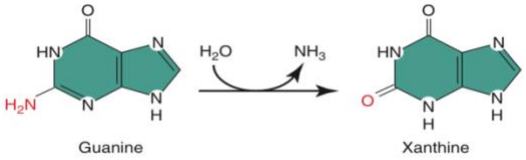
3. Bonds linking amine groups to the rings in C,A and G (deamination:is the process of breaking these bonds): the bond between the amino group and the ring attached to it. .(**covalent bond between that amino group and any atom on the nitrogen base**) .
   -Note: thymine is not mentioned because it lacks an amine group
   -Note: the amino group linked covalently to a nitrogenous base's ring is outside the ring not within it.

Water-mediated deamination leads to conversion of:

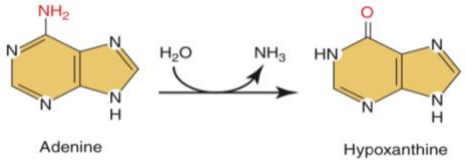
a) Cytosine to Uracil: hydrologic DNA deamination is estimated to take place about 100 to 500 times a day in a mammalian cell, whereas combine guanine and adenine deaminations are estimated to occur 1% or 2% of that value. Also, uncorrected C to U conversion will cause a C-G base pair replaced by T-A (transition mutation).



b) Guanine to Xanthine: Xanthine does not form stable base pairs with either cytosine or thymine, so this conversion may lead to arrested DNA synthesis.

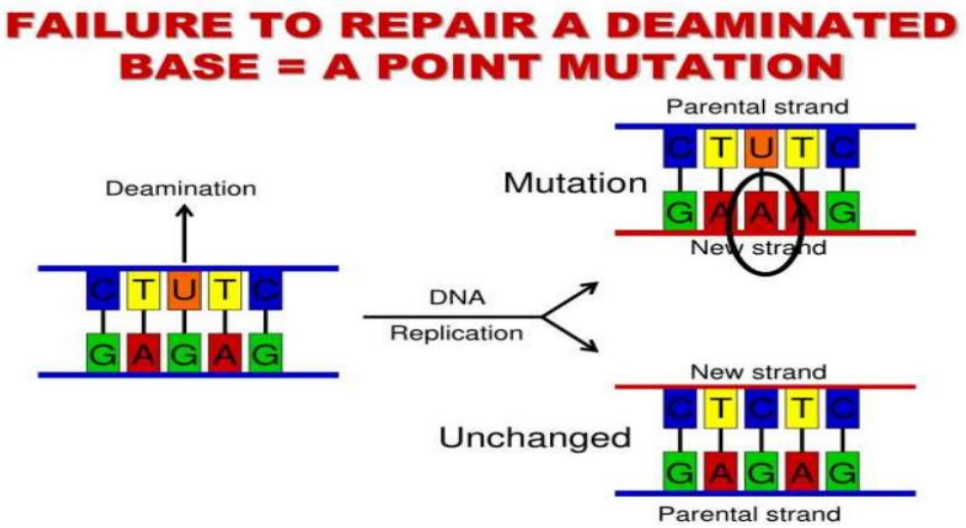


c) Adenine to Hypoxanthine: If not repaired, it will cause a T-A base pair to be replaced by a C-G base pair (transition mutation).



d) 5-methylcytosine (modified base of cytosine) to thymine: Found concentrated in the CpG islands, which are small segments of DNA often present in regulatory elements termed promoters that are located just before the transcription unit that they regulate. The term CpG island is derived from the fact that the CpG sequence is present in these DNA sections at a much higher frequency than in the rest of the DNA. The frequency of spontaneous deamination of 5-methylcytosine bases in CpG islands is even greater than that for cytosine. It is results in the conversion of a C-G base pair into T-A base pair (transition mutation).

Sometimes although there will be deamination and that is because of radiation or spontaneous deamination of some nitrogenous bases such as (adenine ,guanine or cytosine) (T hasn't amino group)when they are deaminated the base pairing of these deaminated cells nitrogen bases will be changed and if the DNA replicated while they are deaminated, mutation would be produce and those type of mutation could be of **transition** type or **transversion** type and this is exactly what happened when a specific nitrogen base is deaminated.



Supposedly,we have these double stranded DNA and as you notice G base pair with U how this U comes? It resulted from the deamination of cytosine in which G was base pair to it, so cytosine when it is deaminated the result will be uracil.

If this DNA molecule replicated, every strand will work as template (parental strand) to form new (daughter) molecules in the first molecule with deaminated cytosine the newly synthesized strand will have a mutation (A instead of G), so this molecule does not resemble the parent molecule,it is mutated molecule and will cause genetic diseases, so the deamination is very serious problem in nucleic acid (DNA) that will create mutation.

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واصبرْ وثابرْ فالنجاحُ محققُ

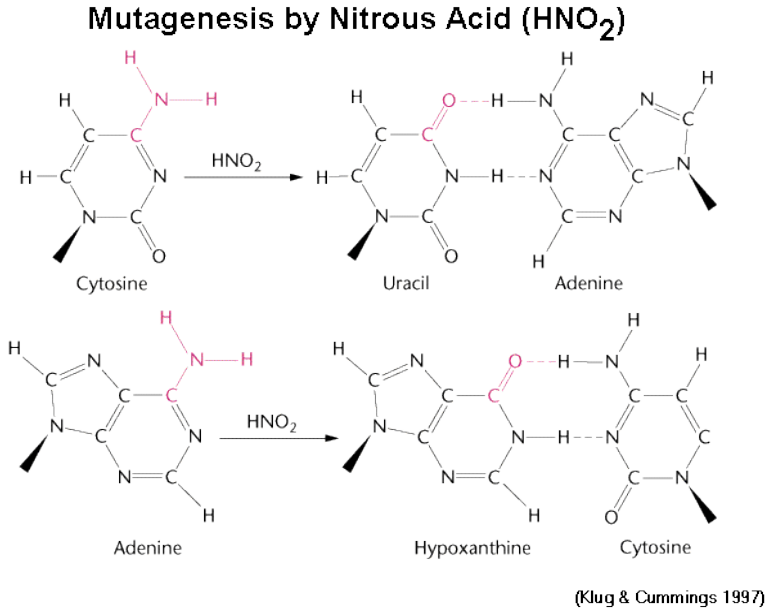
-chemicals, radiations cause of deamination.

Also use a lot of preservative material like **Nitrous acid (HNO2)** and **bisulfite (HSO3-)** will cause deamination of NB in the DNA

Nitrous acid (HNO2): which is formed from nitrites, used as preservatives in processed meats such as bacon, sausage, and hot dogs, reacts with the amine groups attached to the ring structures in cytosine, adenine, and guanine, greatly increasing their rate of deamination.

Bisulfite (HSO3-): an additive that is sometimes present in wine, beer, fruit juices, and dried fruits, also greatly increases the rate of cytosine deamination but does not affect purine (guanine and cytosine) or 5-methylcytosine deamination.

below there is an example of mutation

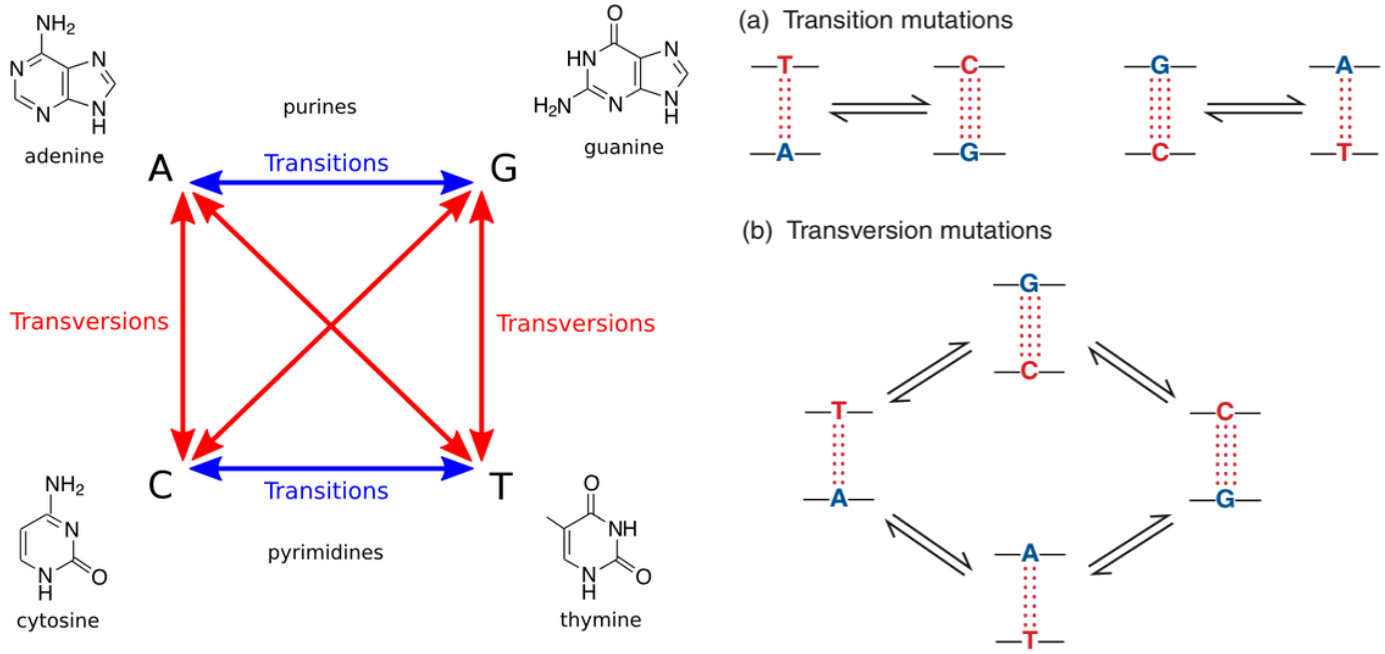


(Klug & Cummings 1997)

The first part: cytosine deaminated by radiation or chemicals like (HNo2(Nitrous acid)) will be uracil, cytosine originally is base pairing with guanine, when it is deaminated and converted to uracil now uracil will base pair to adenine so if this deaminated DNA replicates, the mutation will be produce.

Second part: adenine when it is deaminated will be converted to hypoxanthine and this deaminated adenine (hypoxanthine) it is going to base pair cytosine instead of thymine the mutation will be produced.
**These cause instability in DNA molecule.
When adenine is deaminated and converted to hypoxanthine it will base pair to cytosine, because hypoxanthine is very close to adenine in structure, so hydrogen bond will be formed, but not the conversional one. It will not base pair with the thymine and this base pairing will cause mutation.

- **Transition mutation** means that when purines are converted to purines or pyrimidines are converted to pyrimidines.
- **Transversions mutation** Changes of NB from purines to pyrimidines or from pyrimidines to purines



نقل فؤادك حيث شئت من الهوى
ما الحب إلا للحبيب الأول
كم منزل على الأرض يألفه الفتى
وحنينه أبدآ لأول منزل

***Oxidative damage*:**

Reactive oxygen species damage DNA

- OH(hydroxide radical)

Generated by ionizing radiation or cellular hydrogen peroxide H_2O_2 in the presence of a metal ion such as Fe^{+2}

Hydroxyl radicals, are known to cause more than 80 different kinds of base damage.

Relatively little hydrogen peroxide (H_2O_2) is present in cells because of two enzymes that destroy it:

1) Catalase
2) Peroxidase

■Damages

1) **8-oxoguanine:**

oxoG-A —REPLICATION —>T-A transversion mutation

DNA is exposed or damaged by oxidative damaging factors, some components in tobacco(تبغ) will produce **oxidation molecules** that will modify **Nitrogen bases**, and among those is a **guanine**, which will be converted to what is called it **8-Oxoguanine**.

Now when we have oxidative damage because of **this hydroxide radicals (**from radiation through water or oxidation through smoking) the 8-Oxoguanine is formed **(an oxygen will be added at atom number 8 on guanine)** this 8-Oxoguanine will base pair with both adenine (in the syn-conformation) and cytosine (in the anti-conformation).

-Normally guanine base pair with cytosine but oxidize guanine base paired with adenine, If the 8-oxoguanine not repaired and replicated, the guanine will be one of the parental and the adenine will be the next one,

this will produce daughter molecules different from the parental G-C. If that happens and the DNA is **replicated**

It results,

G-A is converted to a T-A,

guanine (purine) to pyrimidine

this is called the **TRANSVERSION** mutation. (طفرة الاستبدال)

2) **Can produce cytotoxic mutations:**

thymine glycol —> inhibit replication.

Polyhydroxide radicals will be attached to thymine, and a **thymine glycol will be formed**, **and it carries many hydroxyl groups and that will inhibit DNA replication.** So, these are some of the things that could happen because of **oxidations** that will cause damage to our DNA.

Alkylation damage by *monoadduct Formation*

The product formed is called an adduct as chemicals or drugs, sometimes that will affect our DNA.

These alkyl groups which come from alkylating agents used for chemotherapy.

Alkylating agents:will donate electrophiles that transfer methyl, ethyl, or larger alkyl groups to electron-rich atoms in the DNA.

DNA is readily attacked by electron seeking chemicals termed electrophiles.

Many different kinds of naturally occurring and synthetic chemical agents are known to transfer alkyl groups to DNA.

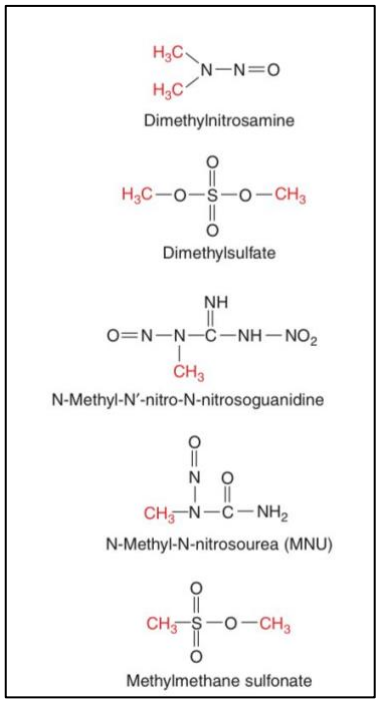
Alkylation takes place at several electron-rich atoms within the DNA, such as:

a) Nitrogen and oxygen atoms external to the base ring systems
b) Nitrogen atoms in the base ring systems except those linked to deoxyribose (through glycosylic linkage)

c) Non-bridging oxygen atoms in the phosphate group

This alkyl group could have one side of attachment to the to 1 strand and in this case, it will form a monoadduct and if it has two sides of attachment between the two strands it will cause the bulky adduct or diadduct.
adduct will disturb the structure of the DNA as well, it will stop its activities like replication and transcription.

Examples of few methylating agents:



The exposure of the DNA to dimethylnitrosamine leads to the production of a monoadduct in which a single methyl group attaches to DNA.
((Environmental agents aren't included))

Chemical Cross-Linking Agents

How we Metabolization alkyl groups??

This alkylating agents (chemicals) mainly aromatic rings fused with each other , they enter the endoplasmic reticulum and they will be modified(hydroxyl groups are attached to it, some other groups also attached) after this

modification by adding or removing some groups, some chemical groups on or from this alkylating agents that will cause an activation of this alkylating agents and will be able to interfere with the DNA and stick together inside the helical structure of the DNA.

Many alkylating agents have 2 reactive sites and can form intrastrand or interstrand cross-links

This is one of the activated **Alkylating agents** and this can bind at this site (it does attack two guanines at their N-7 on opposite strands of the DNA double helix)(p328), in this is strand and able to bind at this site for this strand.

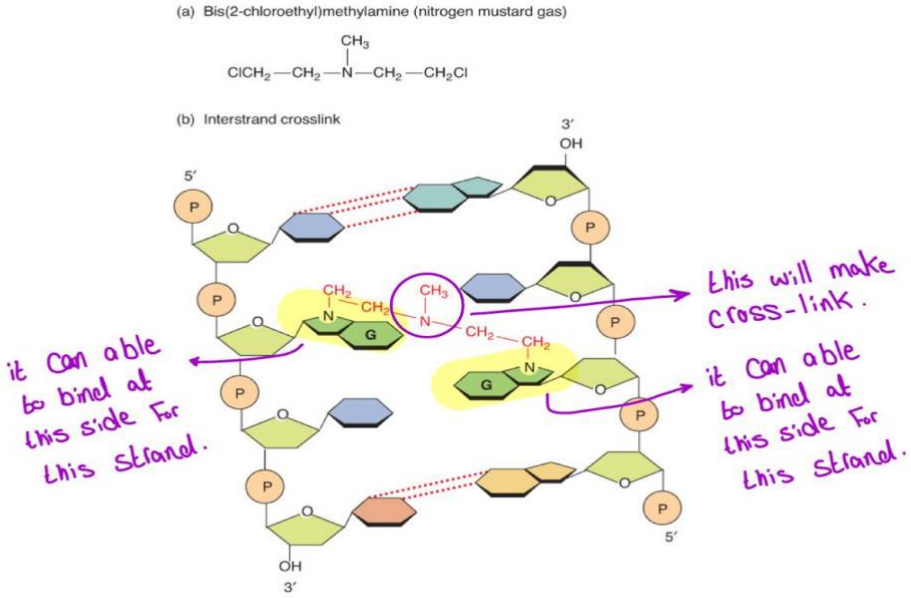


Figure 09.13: Nitrogen mustard gas, an agent that causes crosslink formation.

This will make an inter strand crosslink.

1. Interstrand crosslinks prevent strand separation and are lethal
2. It will stop replication.
3. It will stop transcription.

– Crosslinking agents are often used as chemotherapeutic reagents:

Chemotherapy (that is given in cancer) includes alkylating agents.

- Nitrogen Mustard Gas for treating certain forms of lymphoma and leukemia.
- Cisplatin

This compound is called **nitrogen mustard gas**, it is highly lethal, damaging the DNA and killing the person this compound previously was used in the chemical wars and it was affecting **the neuromuscular junctions** and CNS(Page 327), but later also it was discovered that **it crosslinks the double strand DNA and damage it.**

This compound is called **nitrogen mustard gas**, it is highly lethal, damaging the DNA and killing the person this compound previously was used in the chemical wars and it was affecting **the neuromuscular junctions** and CNS(Page327), but later also it was discovered that **it crosslinks the double strand DNA and damage it.**

Recap:Type of DNA damages:

1- Exposure to radiations such as a UV light (form thymidine dimers)
2- X-ray and gamma radiation (breaking the chromosomes, hydrolysis water and produce a free radical with high energy that could change the DNA structure or do some other breakage of other bonds, some bases are lost (form abase site)).
3- Alkylating agents such as drugs, preservatives (مواد الحافظة)that are able to insert some alkyl groups between the strands or within a strand.

اليوم عيد الاستقلال يا جماعة، طبعا حتشوفها مع الفاينل بس خذوا بيت شعر

كل الشقائق في بطحاء أردنا

إكليل غار وحب حين نلقاك

REPAIRING

Now, what is the mechanism to repair or fix these damages?

Some of them **are direct mechanisms and some of them are indirect mechanisms.**

Direct Reversal of Damage

1-photo reactivation **2-the alkyltransferases** (wasn't discussed)

photo reactivation:

- Alber Kelner's experiment: (page 333)
  The first clue to the existence of an enzyme that catalyzes the direct reversal of DNA research was motivated by the desire to isolate antibiotic-resistant bacterial mutants.
  Firstly, Kelner irradiated the bacteria with UV light which killed most of the bacteria. The next step was to test the survivors to isolate the desired mutants he noticed that the cells that were placed in the dark after UV irradiation had a much lower survival rate than those exposed to the light through laboratory window.
  Conclusion of Kelner's experiment: exposure to visible light reversed UV light's bactericidal effects.

- Renato Dulbecco's experiment: (page 333)
  Renato Dulbecco observed a similar phenomenon when studying Phage T2 (a bacteriophage which is a virus that attacks bacteria).
  Dulbecco explained this observation by proposing that the plate on the top of the pile (stack of plates) is more exposed to the fluorescent bulb used to illuminate the laboratory than the plates in the bottom.
  Dulbecco called this light-dependent phenomenon "photoreactivation".

- Claud Rupert's experiment: (page 333+334)
  Claud S. Rupert and coworkers devised an in-vitro photoreactivation system. There straightforward approach was to isolate DNA from the gram-negative bacteria "Haemophilus influenzae", irradiate the isolated bacterial DNA with UV light to inactivate it's transforming ability and then demonstrate that a cell-free "Escherichia coli" extract, acting in the presence of visible light, restores its transforming ability.

  Conclusions

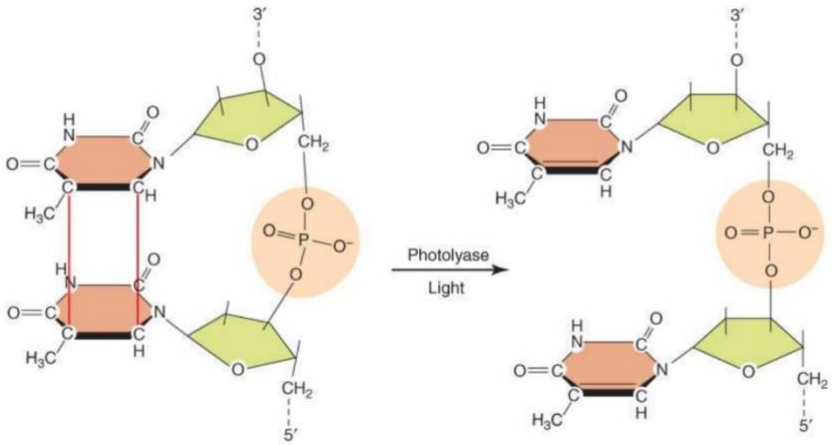
  - Photolyases use and utilize energy provided by blue light (350-450nm) to reverse DNA UV-induced damage.
  - Because we know that the photoreactivation enzyme catalyzes the disruption of C-C bonds(covalent bond), the enzyme was given the name Cyclobutane pyrimidine dimer photolyase.
  - Bacterial cells that lack this photolyase cannot repair cyclobutane pyrimidine dimer lesions by photoreactivation.
  - This enzymes is present in a wide variety of organisms including bacteria, the archaea, plants, and animals but not in humans or other placental mammals.
  - The photolyases have two light absorbing pigments,
    - The first one, which contains the active site and is required to repair Cyclobutane pyrimidine dimer damage.
    - The second one, which is not required for repair, captures light with wavelengths that would not otherwise be available for the first pigment to absorb and transfers the energy to the first pigment. It thereby permits the photolyase to use available light energy much more efficiently than would otherwise be possible.

– UV irradiation induces cyclobutane dimer formation
– Photoreactivation reverses this

Early observations suggested that UV damage in bacterial DNA could be repaired by exposure to visible light, that is called photoreactivation., there are Photolyase enzymes are activated by visible light and break down the covalent bonds between the pyrimidines and convert Cyclobutane pyrimidine dimer (for example: thymidine dimer) into normal bases (thymine in this case), OR the (4-6) photoproducts into normal nitrogenous bases.

Photolyase reverses the damage caused by cyclobutane pyrimidine dimer formation Photolyase is specific to break covalent bond between two thymines (pyrimidines, generally speaking) when it is activated by visible light.

Look here(in the image below), Although this was formed by the UV light exposure, this is thymidine dimer in one strand which are neighbors to each other (left part).And by photolyase enzyme which will be activated by visible light will be able by specific mechanism to break these covalent bonds and convert the thymidine dimer to normal thymidine(right part).



This is a direct damage reversal. (The visible light does not include X-ray nor gamma rays, nor UV light. The visible light is safe).

6-4 photolyase catalyzes the reaction in FIG 9.15.

(6-4) **photolyase** catalyzes the reaction shown here

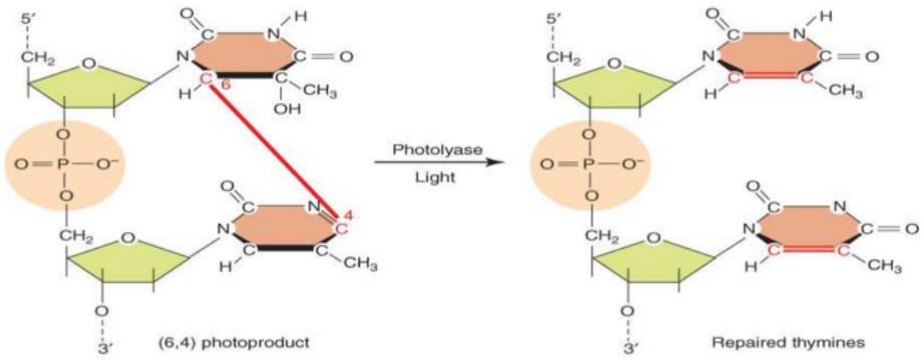


Figure 09.15: Reaction catalyzed by the (6-4) photolyase.

UV irradiation also induces the formation of (6-4) photoproduct. Although it's name is derived from the chemical bond linking carbon-6 and carbon-4 of adjacent pyrimidines on the same strand, but additional chemical changes also take place during dimer formation. Due to this fact, **investigators thought it would be quite unlikely for a single enzyme to catalyze direct reversal of (6-4) photoproduct lesion.**

Takeshi Todo, Taisei Nomura, and coworkers reported in 1993 that "Drosophila melanogaster" has a photolyase that reverses (6-4) photoproduct lesions. **This photolyase was designated as (6-4) photolyase to distinguish it from Cyclobutane pyrimidine dimer photolyase.**

Although (6-4) photolyase is widely distributed in plants and animals, **this enzyme has not been detected in bacteria or mammals that have been tested to date.**

Organisms can also repair dimer lesions introduced by UV irradiation by excising damaged nucleotides and replacing them with normal nucleotides. **This type of excision repair is the major pathway for repairing UV-induced damage to DNA in humans and other organisms that lack both photolyases**

Another mechanism of direct damage reversal is dealkylation and IT **IS NOT INCLUDED**

ألا ليت أيامَ الصيفِ ثلاثة

وسائرَ أيامِ السّنينِ.. شتاءُ

Indirect Reversal of Damage

Now we will talk about three indirect mechanisms to repair damages in DNA:
(Base excision, Nucleotide excision repair, Mismatch repair)

1. Base excision repair.

-This pathway removes and replaces the damaged or inappropriate bases

- Damage to DNA bases caused by deamination, oxidation, and alkylation that cannot be repaired by a single reversal enzyme must rely on a multistep pathway - base excision repair-
Enzymes in the base excision repair pathway also participate in single-strand break repair.
(you must know the role of each enzyme)

-Base excision derives its name from the 1st step of N-glycosyl bond cleavage that forms an abasic site

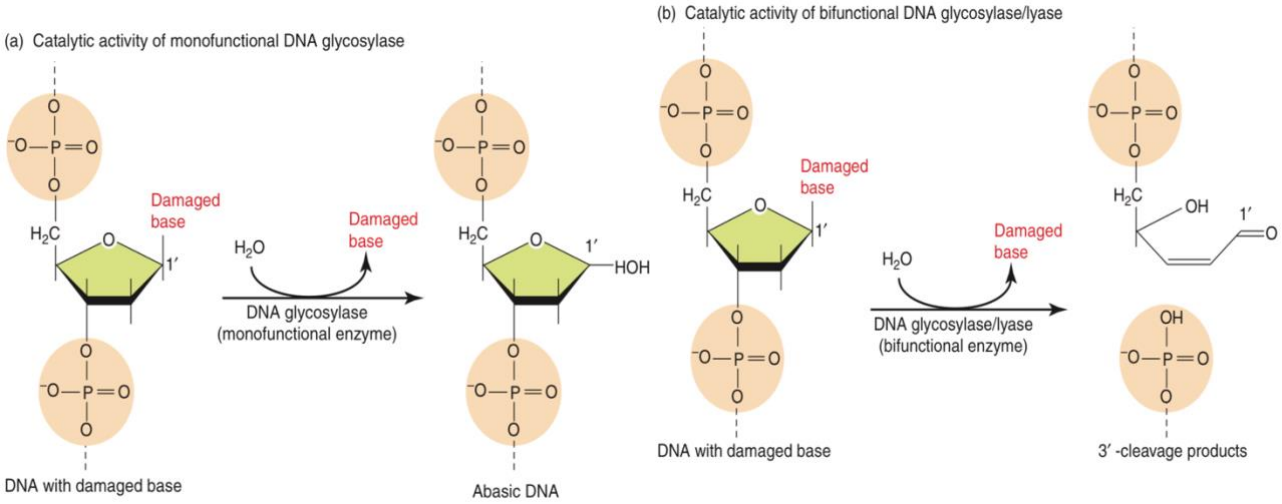
• Cells must use different enzymes

1. Some N-glycosylases are **monofunctional** as they have the ability to **only** excise a damaged base (cleaving N glycosyl bond)
===> is designated as DNA glycosylase

2. **multifunctional**, others have additional AP lyase function that cleaves the bond between the sugar and the phosphate 3' to the damaged site
so its capable of :
1) cleaving N glycosyl bond
2) cleaving phosphodiester bond,
===> is designated as DNA glycosylase/lyase

In the base excision repair it will cleave the base by breaking N-glycosidic bond. So if we have a damaged base, the excision repair system will remove that base by breaking Nglycosidic bond and using other enzymes in order to remove that base and replace it by the proper one.

Because no single enzyme can distinguish the four bases normally present in the DNA from the wide variety of altered bases due to oxidation, alkylation and deamination, cells must use many different enzymes to perform this function



(a) In this case we have a damaged base here, by using an enzyme called glycosylase it will break the glycosidic bond to remove the damaged base and as a result, we will end up with a sugar without any base attached to it. This is called abasic (without base strand DNA) site or AP site (apurinic or apyrimidinic)

(b) Sometimes the enzyme glycosylase has another function in addition to breaking glycosidic bond, it can hydrolyze the 3º end of the phosphodiester bond and leave the DNA(AP lyase activity) (as we have seen above)

Note:

1) H2O replaces damaged nitrogenous base on 1’ which is excised by glycosylase

2) AP-endonuclease breaks phosphodiester bond (now we only have sugar with phosphate on one side of the nick and free 3’-hydroxyl group on the other side).

The base excision repair pathway

The pathway can be divided into **two stages** in both bacteria and eukaryotes: (the enzymes involved are found in the eukaryotes not bacteria)

this figure summarizes the two stages of base excision repair process.

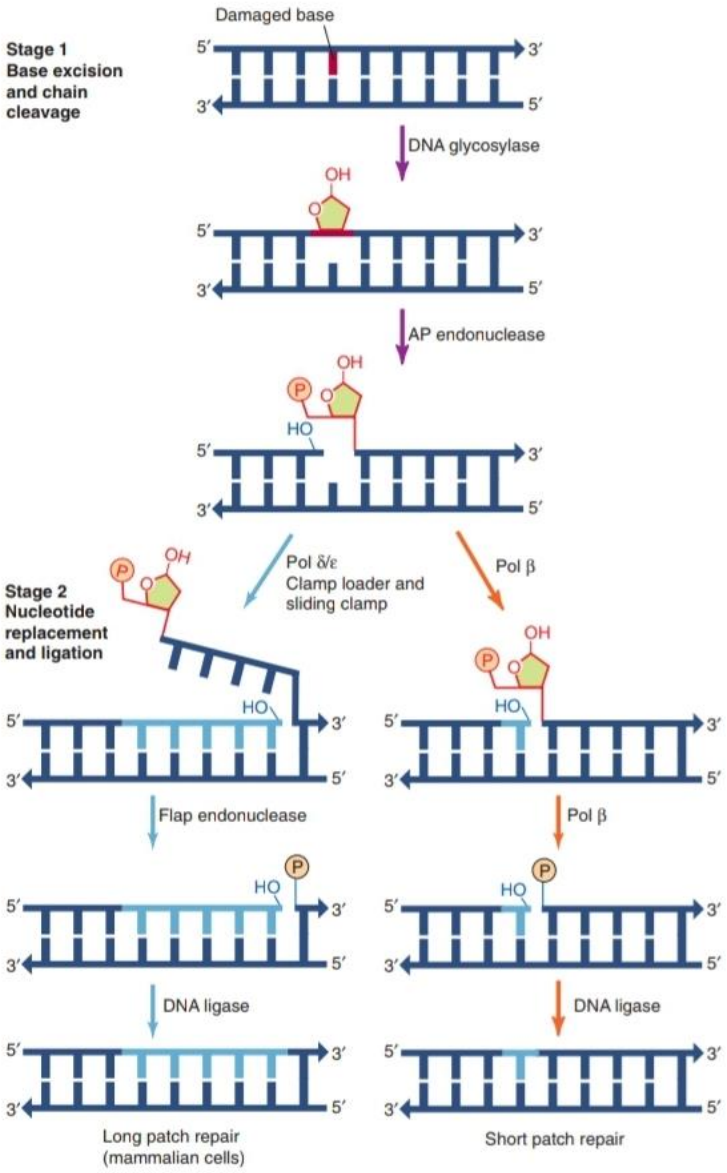


Figure 9.18

Stage1: Base excision and chain cleavage:

(this happens in the beginning of both pathways short or long)

- DNA glycosylase excises the damaged base.

  The N-glycosidic bond that attaches this damaged base (in red, check figure 9.18)with the sugar will be broken, and as a result the damaged base will be removed at this stage and an abasic site is formed.

- AP endonucleases hydrolyzes the phosphodiester bond to the 5' side of the abasic site (check figure 9.18). The AP endonuclease generates a nick that has 5'-deoxyribosephosphate (5'-dRP) on one side and a 3'-Hydroxyl group on the other.

Stage2: Nucleotide replacement and ligation:

Can continue by either of **two subtypes**:

a) Short patch repair (non mammalian cells):

   (replaces one nucleotide only in the place of damage)

   Now firstly, DNA polymerase beta (pol β) adds the proper deoxyribonucleotide to the 3'-OH end and removes the 5'-deoxyribose phosphate from the 5'-end on the other side of the nick.

   Then, DNA ligase will ligate and complete the repair process by joining two adjacent ends which will be linked by phosphodiester bond and we will have DNA with repaired proper base.

b) Long patch repair (mammalian cells):

   (replaces two or more nucleotides)

   Note: there is a replacement of more than one nucleotide even if they are not damaged but are near the damaged area

   مثال لربط المعلومة

   A good analogy of this is if doctors wanted to remove a cancer tumor; they remove the tumor and the surrounding cells.

   DNA polymerase delta or epsilon catalyzes chain extension with the assistance of the clamp loader and the sliding clamp

All of them will remove the damaged area and start building or polymerizing and replacing the damaged and the normal ones. As the polymerase adds nucleotides to the 3'-OH end on one side of the nick it displaces the 5'-deoxyribose phosphate (undamaged ones) on the other side of the nick (study figure 9.18 carefully to understand what's going on).

Flap endonuclease (FEN1) cleaves the displaced strands. Then DNA ligase will make phosphodiester bond (seals the remaining nick)and now the DNA is repaired.

*flap:Number of nucleotides don't form hydrogen bonds with the complementary strand and they are attached or glued at single end and cleaved from the other one

The regulatory mechanism by which short or long patch repair pathways are selected is still not well understood. However, according to one hypothesis selection depends on the 5'-deoxyribose phosphate formed by the AP endonuclease. If this group:

Can be removed by DNA pol Beta ==> short patch repair

Cannot be removed by DNA pol Beta ==> long patch repair

(Page 340)

2. nucleotide excision repair

which is used to remove bulky adducts damages from DNA, correcting many different types of structurally unrelated DNA damage, for example: nucleotide excision repair excises UV-induced Cyclobutane pyrimidine dimer, (6-4) photoproducts and damaged bases formed by alkylating agents such as psoralen and aflatoxin B.
(Page 340)

UV-irradiated E.Coli can repair their DNA in the dark, although more slowly (because photoreactivation could not take place) than when incubated in the light. This observation suggests that bacteria has another process in addition to photoreactivation repair UV-induced DNA damage.

Richard Setlow and William Carrier and -working independently- Richard Boyce and Paul Howard-Flanders used a similar approach to investigate this alternative process in 1964. (Page 340_341)

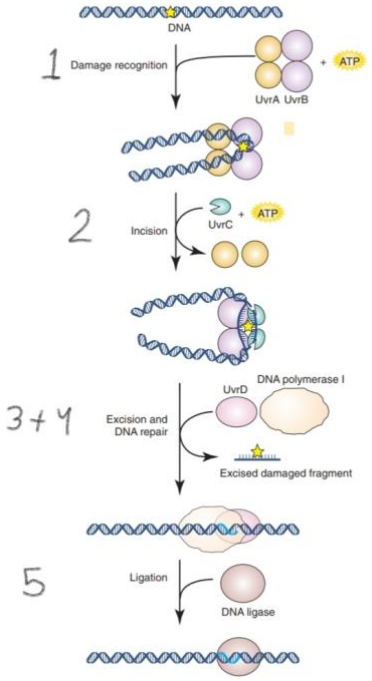
Further studies showed that eukaryotes and the archaea also have nucleotide excision repair pathways.

three E.coli genes-uvrA, uvrB and uvrC- (derived from UV radiation) encode for essential proteins (UvrA, UvrB and UvrC) for damage recognition, incision and excision.

Although the three proteins do not combine to form a stable ternary complex, the polypeptides nevertheless are said to be part of a UvrABC damage-specific endonuclease, or UvrABC endonuclease for short. Some investigators prefer the term UvrABC excinuclease to indicate the proteins participate in excision and incision reactions.
(Page 341)

nucleotide excision repair is involved in the removal of an oligonucleotide that surrounds the damage.

we have **five stages**, which are the same in all organisms:



1.Damage recognition

UvrA & UvrB (UvrB is a helicase) proteins that recognize the damage

UvrB combines with UvrA to form a UvrA2 · UvrB2 complex, which usually binds to DNA at some distance from the damaged site)

-We load this complex onto DNA and it can also be loaded in undamaged areas

-The bulky adducts: they are a bit alkyl groups that make cross linking in one strand or two strands of the DNA (in two sites of the same DNA).

then **UvrB** helicase catalyzes ATP-dependent **movement** of the UvrA2 · UvrB2 complex along the DNA until the protein complex encounters a bulky adduc(yellow star)or helical distortion,

UvrA scans to see if there's damage

then the two UvrA subunits bind on either side of the damaged site without making direct contact with the lesion. Causing the double helix to bend and unwind.

After this local unwinding, the UvrA subunits will be release in an ATP-dependent reaction to produce a stable UvrB2 · DNA complex.

2.cutting DNA on each side of the lesion (incision)

Then two **UvrC** subunits bind to the UvrB2 · DNA complex and make a cut on each side of the lesion (incision)

(The first cut of the damaged strand is four nucleotides toward the 3'-end from the lesion and the second is seven nucleotides toward the 5'-end from the lesion).

3.excision of the oligonucleotide

(removal of the oligonucleotide that has the damage)

UvrD, a helicase, unwinds the damaged oligonucleotide.

UvrD (a helicase) preforms unzipping (unwinding) of damaged region

3.synthesis of a new DNA to replace the excised segment by using the undamaged strand as a template to fill in the gap,

4.ligation of the remaining Nick

To sum up:

In the nucleotide excision mechanism:

Specific proteins → recognition

Specific nuclease → Incision (cut) at both sides of the damage

DNA polymerase 1 → building new oligonucleotide

DNA ligase → ligating

NOTES:

- UvrB, a helicase, interacts with both UvrA and UvrC, although not at the same time
- eukaryotes have a similar repair system (but not the same enzymes involved)
- individuals with the defects in the pathways suffer from autosomal recessive disease called xeroderma pigmentosum (XP).

Extra photos of kids suffering from XP

3. Mismatch repair (The last mechanism used in repairing)

Mismatch repair corrects rare errors that appear in the DNA after replication, such as: base pair mismatches and short deletions or insertions.

the DNA polymerase during DNA replication makes one mismatched pair per 10^5 nucleotides),

then the proofreading by 3′ to 5′ exonuclease increases the DNA fidelity and decreases the rate of error by 100-fold by removing mismatched base pairs before further extension of the growing DNA strand resulting in one error per 10^7 nucleotides (10^{-7} error probability).
it means that one error per 10 million nucleotide polymerized.

That actually results in high frequency of mutations and errors for human genome to have one error per ten million base pairs as we have 6 billion base pairs so there will be a lot of errors per cell division in this case (results in high mutation rate).

……

- Slippage can also occur in repeat sequences

The second type of error that arises during replication, short insertions and deletions, occurs because repeated-sequence motifs such as [CA]n or [A]n in microsatellites sometimes dissociate and then re-anneal incorrectly. As a result of this slippage the newly synthesized strand will have a different number of repeats than the template strand.

……

Sometimes after the DNA replication finishes and some mistakes bypass the proofreading, there is still a chance after DNA replication to repair these mistakes.

We begin our examination of mismatch repair by considering the E. coli mismatch repair system because this system has been the most extensively studied.

The E. coli mismatch repair system can **distinguish** a newly synthesized strand from a parental strand because only the parental strand has **methyl groups** attached to sites with the sequence GATC.

E. coli has a **deoxyadenosine methylase that transfers methyl groups from S-adenosylmethionine molecules to deoxyadenosines in GATC sequences**, but the timing of **methylation lags behind** the time of nucleotide addition at the replication fork by about **2 minutes**, so the newly synthesized strand is transiently unmethylated.

The E. coli mismatch **repair system exploits this period** of transient unmethylation to identify and cut GATC sites in a newly synthesized strand with a mismatch (error).

Three essential E.coli **proteins that are dedicated to mismatch repair** :
MutS, MutL and MutH.

However, they are not sufficient as several **additional enzymes and protein factors make important contributions**, among them are:

a) UvrD (helicase)
b) Single-stranded DNA binding protein (SSB)
c) 5′→3′ exonucleases
d) 3′→5′ exonucleases
e) DNA polymerase III holoenzyme
f) DNA ligase
g) Deoxyadenosine methylase

Recall:

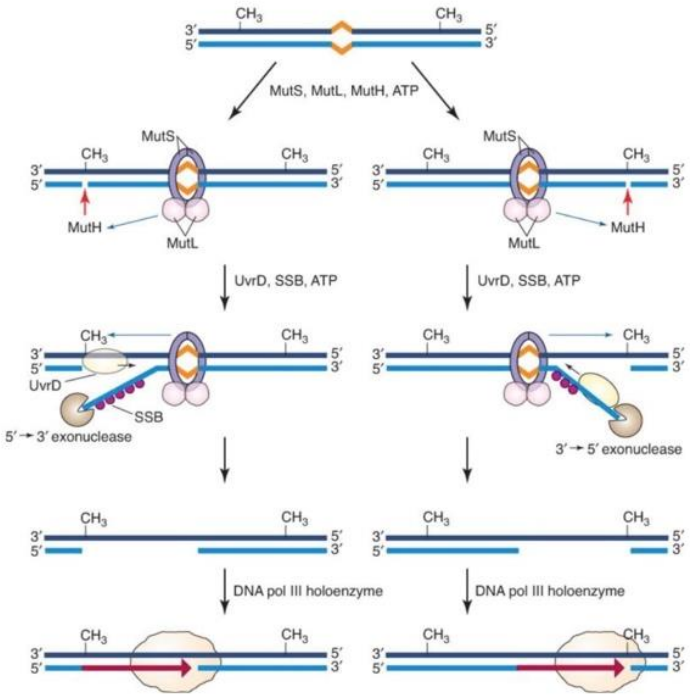
Q) How will the cell know which strand (after the DNA replication) is the daughter strand or the parental strand?

→The newly synthesized strand doesn't have any methyl group but the parental strand has methyl groups.

→It's a benefit to the daughter strand not to be methylated because the system in the cell will be able to tell that this is the strand that has the damage not the parental one because the parental is methylated, so the system will recognize and take the daughter strand, work on it and start fixing the damage.

→ The system uses methyl groups as markers or labels for the parental strand and it's enzymes will not deal with it, and the non-methylated GATC as a marker for the daughter to deal with it and repair damages.

(Meaning that the probability for the system to know the daughter strand when it's not methylated is 100%, while it's 50% when the two strands are methylated)



The mechanism:

1.And after recognition, the protein MutS will recognize the damage and will bind to the mismatch.

2.Then, MutS will recruit another protein (MutL) in an ATP-dependent fashion. The MutS · MutL complex activates MutH endonuclease, which make an incision in the nearest unmethylated GATC sequence (MutH does not bind or cleave fully methylated GATC sites).

*To make it easy SLH =صلح

3.Therefore, MutH generates a nick either on the 5' side to the mismatched pair , or it may from a nick on the 3' side to the mismatched pair, and in both cases there will be a gap (either on the 3' or 5' side to the mismatched repair) (as we have see above)

- When the incision is 5' to the mismatch, a 5'→3' exonuclease hydrolyzes the nicked strand in a 5'→3' direction
- When the incision is 3' to the mismatch, a 3'→5' exonuclease hydrolyzes the nicked strand in a 3'→5' direction

4.UvrD (helicase) unwinds the DNA, and SSB binds to the resulting single strands so the DNA won't rewind.

5.This gab will be filled by the DNA polymerase 3 and then the DNA ligase will come and do the ligation (DNA ligase seals the remaining nick)

……

This happens in prokaryotes, the same system nearly with different names of enzymes and proteins and different labeling of the strands is present in a eukaryotic system.

Eukaryotes:

Eukaryotes have proteins that are similar to the bacterial MutS and MutL proteins but lack a homolog to MutH..
The eukaryotic MutL homolog has endonuclease activity
– Lagging strand is recognized because of Okazaki fragments
– Leading strand recognition is not understood yet

Further details from the book:
All organisms that have mismatch repair system have MutS and MutL homologs (that is, proteins that are like MutS and MutL).
MutH, on the other hand, is present only in E.Coli and other gram-negative bacteria.
Organisms which lack MutH require some means other than recognizing unmethylated GATC in the newly synthesized strand to distinguish it from the parental strand (which makes sense because how is distinguishing the daughter strand from the parental strand beneficial if you couldn't cut or modify it), they need an endonuclease to make a gap (a nick).

The E. coli mismatch repair system suggests a possible explanation: MutH is not required if the DNA molecule already has a nick on either the 5' or 3' side of the mismatch.

Humans:

Humans possess three MutS homologs which participate in mismatch repair: MSH2, MSH3 and MSH6.

⇨ **MSH2 and MSH6** combine to form a heterodimer called MutS(**alpha**): MutS(alpha) initiates mismatch repair at single mismatches and **small** insertion/deletion loops.

⇨ **MSH2 and MSH3** combine to form a second heterodimer called MutS(**beta**): MutS(beta) only initiates mismatch repair at insertion/deletion loops of **various sizes.**

Now how can the human mismatch repair distinguish the daughter strand form the parental strand?

The human mismatch repair system can recognize newly synthesized lagging strand DNA because the mismatched base is on an Okazaki fragment.

What about the leading strand?

Further work is required to determine how eukaryotes distinguish between the newly synthesized leading strand and its template parental strand.

Individuals with a nonfunctional mismatch repair system due to a defective MutS or MutL suffer from **nonpolyposis colon cancer,** an autosomal recessive syndrome that greatly increases their predisposition to develop intestinal cancer.

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V2

Extra photos has been added To page 7and 31
No extra information had been added, the sheet has been reorganised only