The University of Jordan

Dentistry 020

Molecular Biology

Title: DNA MUTATION & REPAIR MECHANISM (SHEET 18)

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DNA mutations and repair mechanisms

We now know all process and mechanisms takes place inside cells so we want to see the mutation that can take place as result of these mechanisms and external mechanisms as well and see how cells use the molecular mechanisms inside them to repair them

❖Mutations

First, mutation is: an any change in the genetic material ,or any thing that happens in the DNA nucleotides.

✵We can classify these mutation as:

•somatic mutation→ are mutation occur in somatic cells(any type of cell inside the body except the reproductive cells "sperms and egg") and are not transmitted (not heritable.)

•germline mutation→occurs in gametes (sperm or egg) and are heritable.

✵Classification according to size of these mutation:

•Micromutations → if they involve small region of the DNA like few nucleotides, to observed these mutation we should use PCR sequencing (doctor said we will not n take about these technique "PCR sequencing")

•macromutation → involve chromosome so it large and can be observed under the light microscope

✵Classification according to how they occur:

•spontaneously mutation → naturally occurring and arise in all cells: meaning that they just happen ,they can be result of DNA replication mistakes(lesion) **or** by STH inside cells like reactive oxygen species hiding DNA

•induced → produced when an organism is exposed to external factors that causes mutation and these are called mutagenic agent (or simply mutagen),

And some mutagens are carcinogens meaning that they can cause cancer ,example: ionizing radiation like UV light "sun light" or X-ray machine that we use to look at bones or stuff inside our body

❖Macro mutation

As we said macromutation are large mutation and they occur at the level of chromosome and can be observed under light microscope

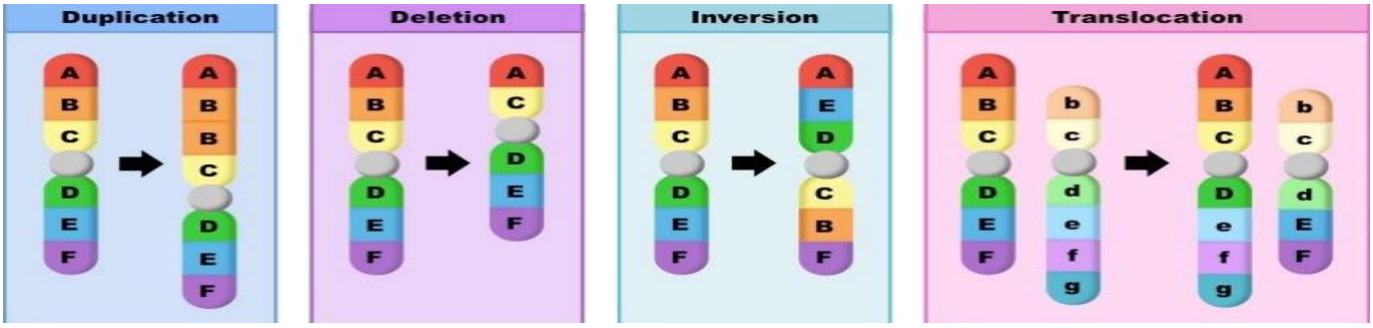
❋Types of macro mutation : (look at the pictures below for clarification).

•duplication → for example you have a certain chromosome with different regions (A,B,C.D,E,F)and we have duplication of region B so it's a large duplication!!

•deletion → deletion of region so B is deleted as whole

•inversion of DNA segments → so we have BC inverted with region DE

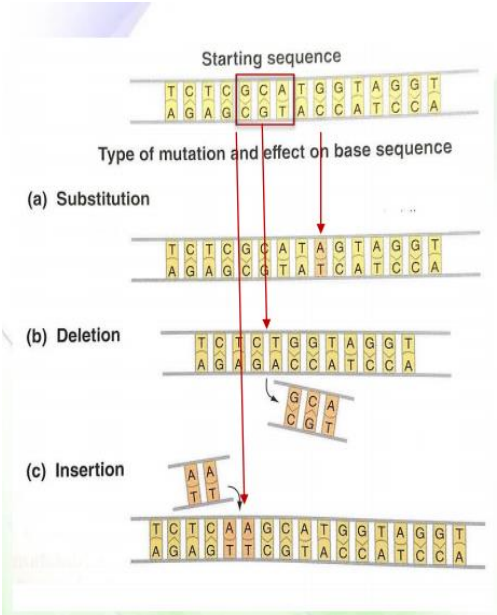
•translocation → you have a removal region from one chromosome and get translocated into another chromosome



❖Micro mutation

❋Types

•point mutation → you have change of one nucleotide ,for example you have GC changed to AT (LOOK TO PICTURE,FIRST SMALL RED ARROW)
The most common and include substitutions, insertion, and deletion



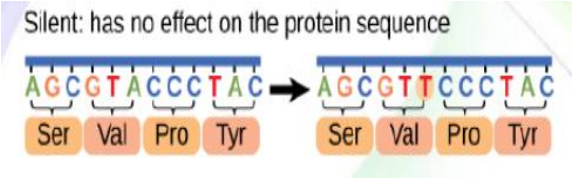
•deletion or insertion of a few nucleotides to long stretches of DNA ,for example(look to square box in the picture) you have GCA can be deleted as a whole or (longest red arrow) insertion between CG for example AA ,So insertion or deletion can be to one or several nucleotide

❖pointmutations

Mutations in one nucleotide, there are a different types:

•silent mutation → no change in amino acid sequence of a polypeptide or protein

For example; you have AGC followed by **GTA** and you have change **of A into a T** so if we go back to genetic code table both of these codon (GTA & GTT) are code for Val so no change in amino acid

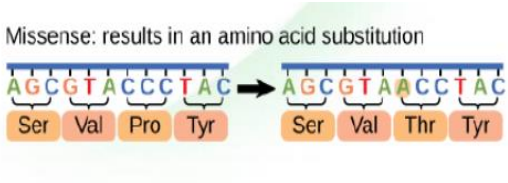


Notice: **we talk about change in third nucleotide and usually change in third nucleotide doesn't change the amino acid that is encoded by a DNA**

Usually, usually silent mutation doesn't have any effect.

•Missense mutation → sense like logic and missense is a wrong logic

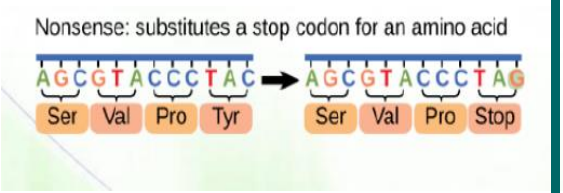
For example ;we have change in CCC to ACC and that result in changing amino acid **from Pro into Thr**



•nonsense mutation → that means there is no logic that means changing of codon that code for amino acid to a stop codon

For example ; changing **TAC to TAG** and that's a stop codon tht result in protein or polypeptide that is shorter than normal so we call this protein:

truncated "cut" protein



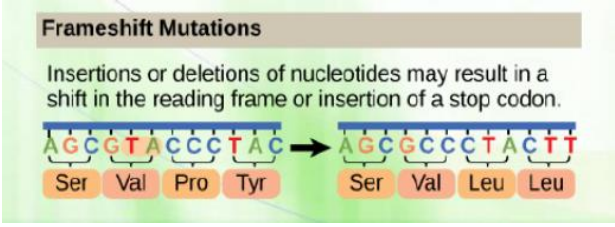
•frame shift mutation → every codon can be imagined that every codon exist in a frame so you have AGC within a frame and you have GTA within another frame and so on....

So what happen here: (see the picture on the next page)if you have deletion of **TA** that result in shifting of alongside **CC** frame to previous frame so instead of having **GTA** we will

have **GCC** so that shift a frame and result in changing every amino acid that occur after frame shifted and result in production of a different polypeptide after word (mutation)

*And we have a different type of frameshift mutation (insertion or deletion)

*So if we have for example insertion of one nucleotide that result in complete sequence of amino acid after word (after that mutation) and same thing if we have deletion or insertion



of 2 nucleotide

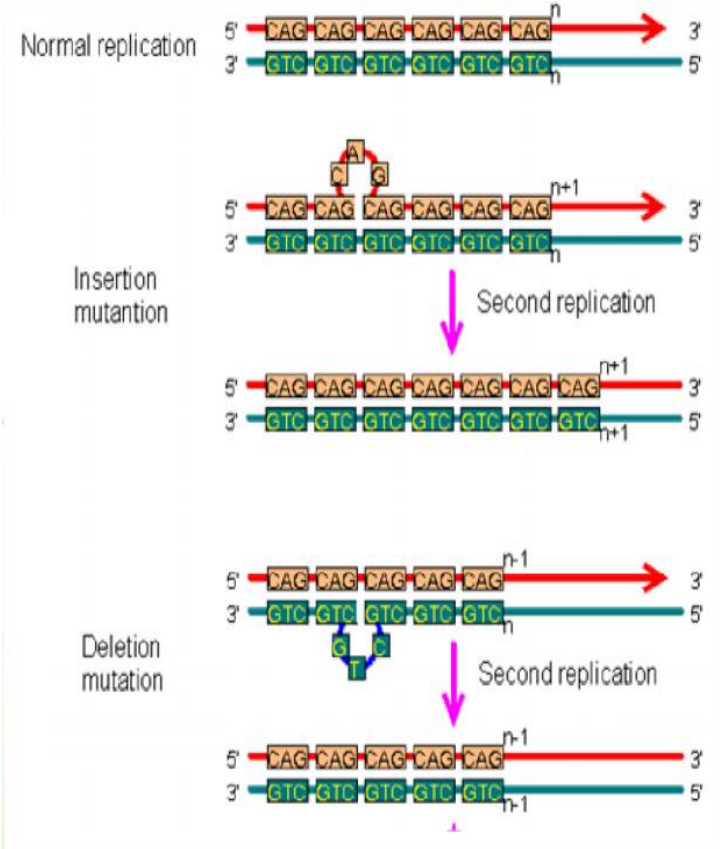
*but what if we have insertion or deletion of 3 nucleotide : that depends, if these nucleotides are inserted <u>between</u> 2 frame then there is <u>insertion</u> of amino acid,

or if you have deletion for example of CCC all deleted what happenes we will have deletion of Proline so we will have Valine then Tyrosine .....

or if we have insertion of 3 nucleotide <u>within</u> a frame that result in production of a different polypeptide

❖repeated sequences and DNA replication

DNA replication is quite accurate but there are mutation can take place (insertion or deletion) of bases depend on the DNA sequence so usually insertion and deletion can take place if we have repeat for example, we have this FIRST GREEN strand in picture that read by the DNA polymerase so it read :GTC GTC GTC ..... so DNA polymeraseوهون ببلش would stop and يتحزر ويحكي 9 لا لا 10خلص خليني اضيف كمان او يلا اقلل فهو بتخربط وبينسى قديش ضاف (بيحكيش say mmmm how many هو طبعا بس مشوها) GTCs I had added ,10 no no 9 and he say lets add more or lets add less so it get confused basically ,so you have CAG CAG CAG that are synthesized so you can have



insertion or deletion of CAGs so these replict can result in mistakes during DNA replication

So the cell can have **extra or less** number of repeat and that result in number of diseases

Because if we have increase number of repeat more than normal that results in different type of mutation (these mutation will discussed later on)

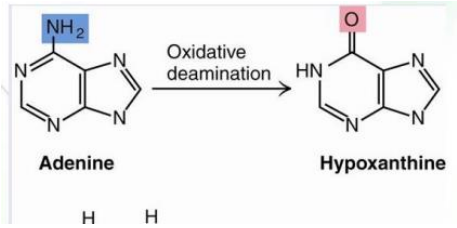
<u>Bach to lecture to understand this concept correctly (10:13 until 13:16 minute)</u>

❖deamination (an example of a certain mutation)(spontaneous)

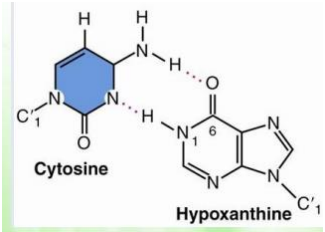
•Spontaneous mutation so they naturally occur within cells normally without any external effect

•so we have deamination reaction and deamination mean removal of amino group

For example;* you have an adenine can be deaminated so resulting in a change in this amino group into ketone group and that result in different molecule named hypoxanthine so that's mean that would be differences in base pairing because A base with T



SO if hypoxanthine is exist in the DNA and this DNA is replicated the DNA polymerase will read this hypoxanthine as a guanine and place cytosine complementary to it (instead of having AT pairing we would have a GC pairing) so that result in mutation

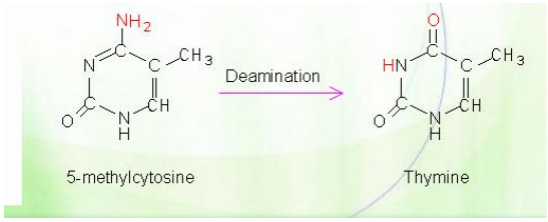


_BUT How can DNA polymerase do that (normally read a G) ,why would not add something else rather than G or why wouldn't it stop DNA replication ? the reason is cells don't like to die (because if the cell stop DNA replication would die) so since hypoxanithine look like a G so it pairs it with C and continuous in the DNA replication.

•Also, we can have deamination of cytosine ,and this deamination converted cytosine to uracil (uracil in DNA!!!) SO when the DNA polymerase read DNA and synthesizes a new DNA during replication it see a U and would pair it with A !SO we would have UA pairs instead of CG pairs and then this U can be removed and replaced by a T (FINAL RESULT :having TA pairing)

•REMEMBER: when we are talking DNA modification and that cytosine can be methylated in promoter region

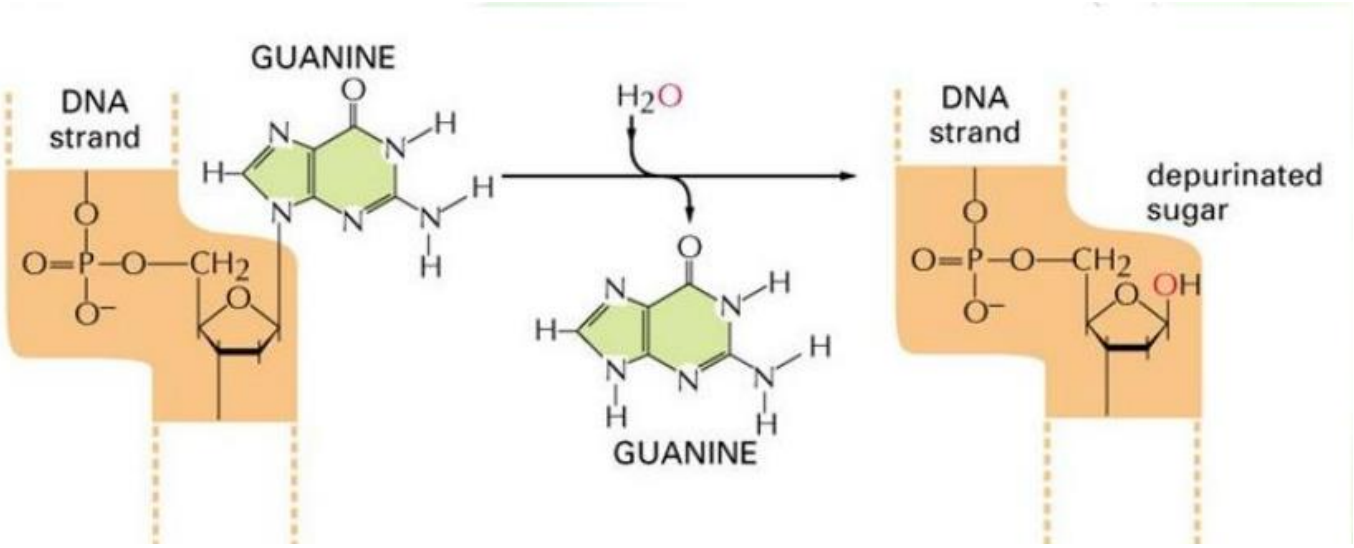
So if methylcytosine deamminated that would result converted it to T and again when DNA polymerase read a T that would no problem with it and simply put an A opposite to it (instead of CG we would have TA)



❖Depurination "SPONTANEOUS"

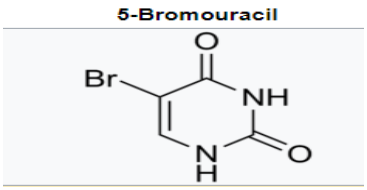
•depurination mean; removal of a purine

•cleavage of the glycosidic bond between base (guanine) and dexoribose (sugar)creating apurinic sites (AP sites) and then during replication as DNA read that site and see that has no base so what it does !? it will insert anything (random base pair can be inserted) ,hopping that DNA can be repaired later on .



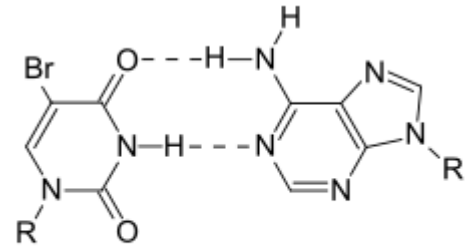
❖Incorporation of base analogs "INDUCED"

•**Base analogs** have similar structure to normal nucleotides and are incorporated into DNA during replication.



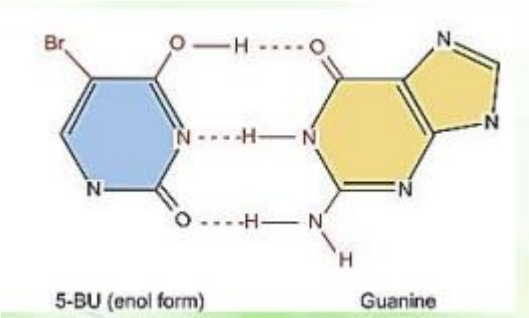
• 5-bromouracil (5-BU), an analog of thymine, pairs with adenine, but, when ionized, it pairs with guanine (because it looks like cytosine)

•5-bromouracil (5BU) →IF inserted into DNA by mistake by (DNA polymerase for example), so if you have an AT and during a DNA replication 5BU inserted into DNA ,since is look like a T , what happenes is that A it get place in the DNA and the 5BU would pair with A



5-BrU (keto) Adenine

If **this 5BU ionized** and be in <u>his ion form</u> which is <u>complementary to guanine</u> →if we have a round of replication the upper strand (A) would be normally. But the other (lower) strand would pairs with G because ion form of 5BU look like a C (please notice to difference between these 2 picture look to carbon alongside brome carefully)



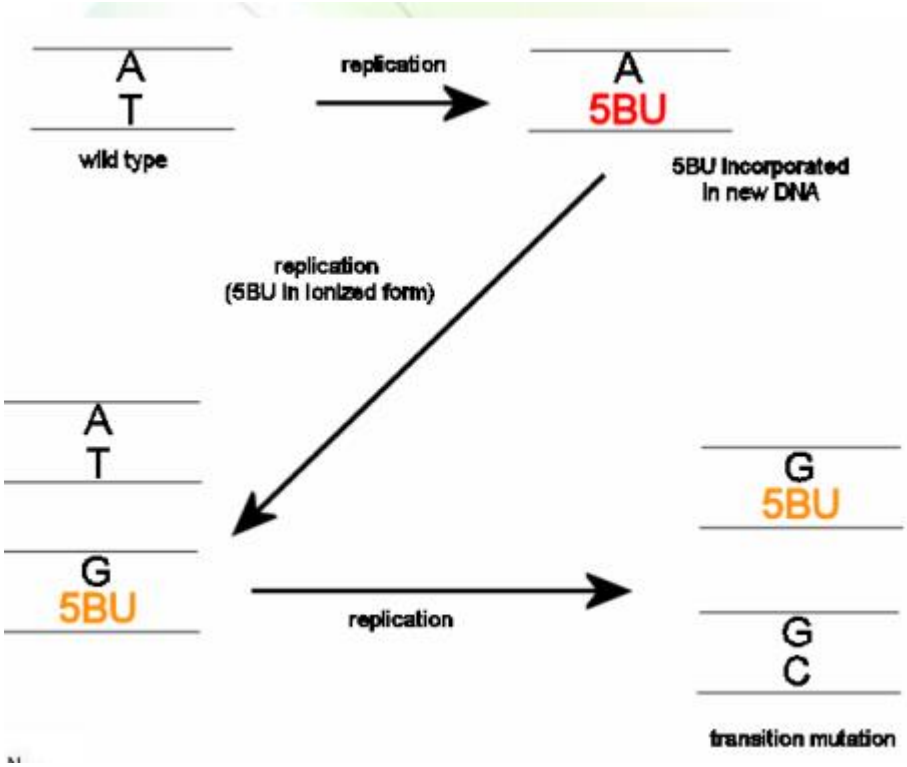
And if we have again another round of replication what hapenes that G would read normally ,but 5BU would pair with G so finally ! we have a change from AT into GC

***NOTE: This mens that 5BU can be present in DNA either opposite A OR G**

5BU normal form -→ looks like T

5BU Ion form -→ looks like C

The result of this is that during a subsequent round of replication a different base is aligned opposite the 5-BrU residue. Further rounds of replication 'fix' the change by incorporating a normal nitrogen base into the complementary strand.



*Thus 5-BrU induces point mutation via base substitution. This base pair will change from an A-T to a G-C or from a G-C to an A-T after a number of replication cycles, depending on whether 5-BrU is within the DNA molecule or is an incoming base when it is ionized.

*WILD TYPE: means normal because when the scientist first studied the genetic basically in fruit fly and they looked wild (the normal type was known as wild type) so the normal sequence of DNA was called a wild type

❖Repair mechanism

Types of repair mechanism:

① Preventing of errors before they happen:

There is a protective mechanism within our cell that preventing of errors before they happen

-the first anything harmful inside cell these things are removed right away ,and an example of these thing:

•Reactive oxygen species → molecules that are oxygen but they are hyper active ,example; radicals "free oxygen radical" ,these free oxygen radical are missing an electron

Like① O_2° which is known as superoxide and this superoxide is very hyper active it means an electron and it can attack any molecule inside a cell and stealing an electron from these molecules so it get relaxed (stable) but at the same time the other molecule that get oxidized is damaged.

So imagine that this superoxide attack DNA so this DNA will be damaged .

Also these radical can attack lipids in membrane and the membrane will damage and that cell death by the way.

②H_2O_2 (HYDROGEN PEROXIDE) ,also reactive molecule and can removed enzymatically by an enzyme known as catalase

Whereas superoxide can be removed by superoxide dismutase

⇨Enzymes neutralize potentially damaging compounds before they even react with DNA (detoxification of reactive oxygen species and oxygen radicals).

②Direct reversal of damage:

•Lets say that thing happen and damages takes place inside the cell

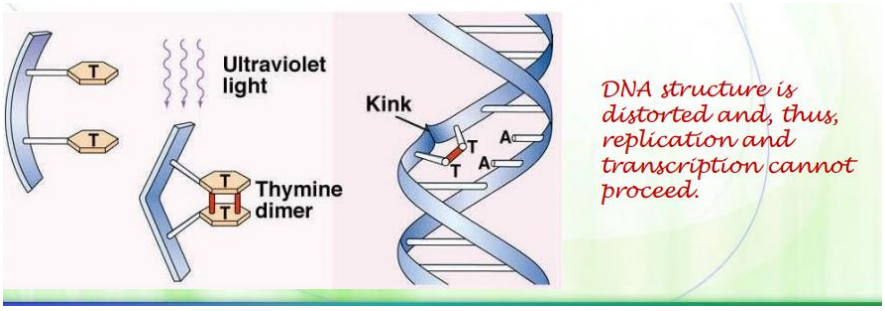
So **How do cells handle such damage? How do they repaired?**

•A type of a common mutation that takes place inside cells is : pyrimidine dimer ⇨ since all exposed to sunlight and once sunlight is basically UV light and its really damaging to DNA and what happenes actually that when UV light hit DNA result in the formation of a covalent interaction between two adjacent pyrimidine dimers and commonly between two thymine (50-100 reactions per second), a lot of mutation takes place but these mutations are repaired and they are repaired in different mechanism in human as in bacteria "we well talk about human later on"

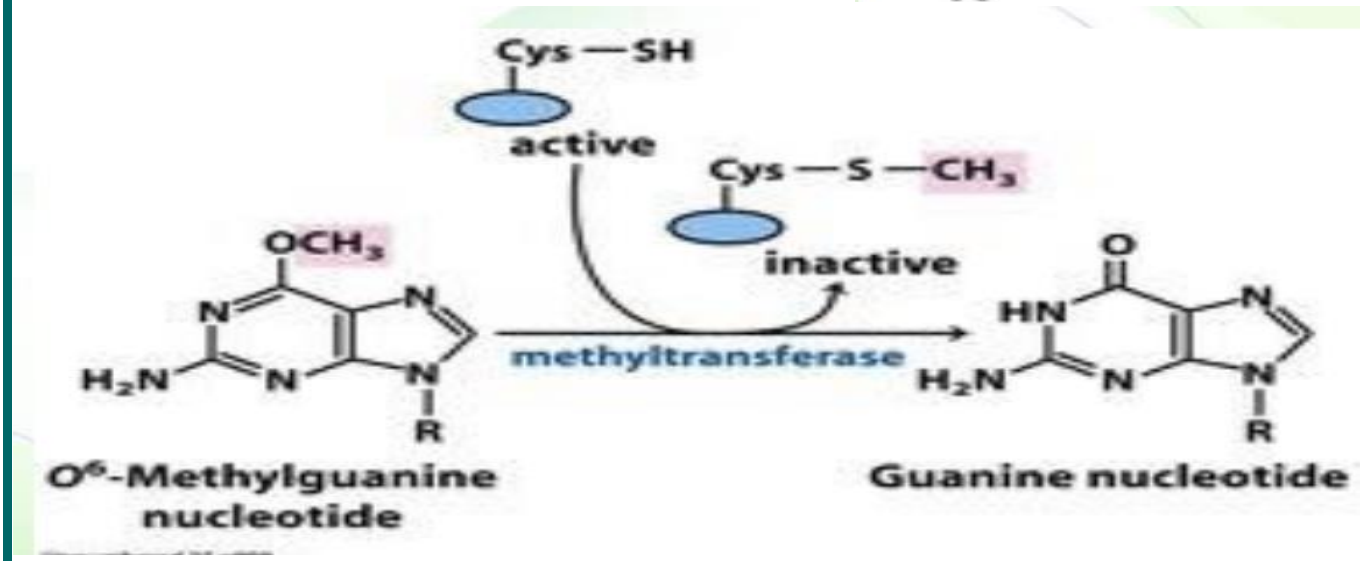
-but in bacteria we have an enzymes known as photolyase (photolyase doesn't exist in human) :is simple reversion of pyrimidine dimers or thymine dimer so these covalent interactions are removed .

•This product is a mutagenic photodimer →so during DNA replication and during transcription enzyme can not proceed they can not synthesize DNA or RNA and that result in cell killing or result in production of mutations

-so within one strand if we have two adjacent TT and UV light hit DNA these Ts would have formation of covalent interactions between these Ts resulting in formation of cyclobutane pyrimidine dimer or thymine dimer

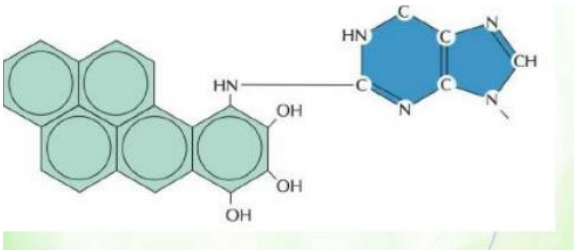


• we can have modification of nucleotides and that causing mispairing during DNA replication so you can have◆ addition of an alkyl group (like methyl) to carbon number 6 in guanine forming 6-methylguanine (6-meG) which mispairs with thymine so instead of having GC we would have meG pairing with T and if we have replication that result in formation of one strand pair of AT so at result we will have a change of GC to AT



*can be repaired enzymatically by an enzyme called methylguanine methyltransferase
→this enzyme will remove the additional alkyl group which is in this case methyl
NOTE:few types of damaged DNA are repaired in this way

Or◆ addition of large objects (large group of molecule as you can see in picture below) to DNA and this is considered mutagenic and it can be carcinogenic as well



Other repaired mechanisms will be discussed in the next sheet

Sorry for any mistake , I do all my effort to mention all point motioned on the lecture

Be the best

version of you,

do the best you can

good luck