Genetics
& Cell biology

◉ Sheet ○ Slides

Number

6

Done by:

Kinan Obiedat

Corrected by:

Abdelrehman Tayel

Doctor

Mamoun

This lecture will discuss DNA repair mechanisms.

Even though some mutations might be useful as a natural selection , they are harmful in general . That's why we need different levels of DNA repair .

1-Prevention of errors before they happen

2-direct reversal of damage

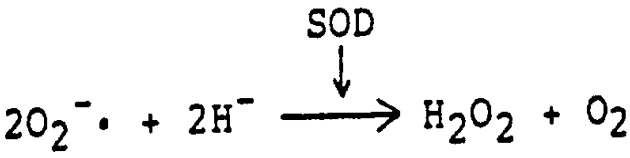
3-Excision-repair pathways

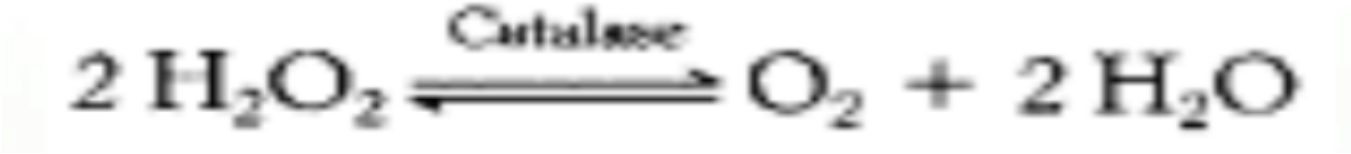
4-post-replication repair

1-Prevention of errors before they happen:

Basically it is the removal of harmful substance that could cause mutations to the DNA by some enzymes such as superoxide dismutase, catalase and peroxide dismutase. **Superoxide dismutase** converts the superoxide free radical into hydrogen peroxide in peroxisome, then this hydrogen peroxide is converted to water by the action of **Catalase** in peroxisome also**.**

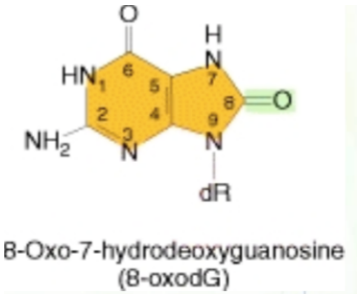
*Hydrogen peroxide is less harmful than superoxide but still dangerous because it is a reactive oxygen species.





Another example is a protein called **mutT** in E.coli that prevents the polymerase to incorporate a guanine base analogue called “8- oxydeoxyguanosine or 8-oxodG" in DNA .

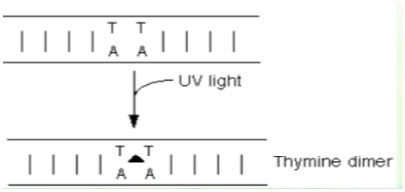
*8-oxodG is formed from free radical attack of DNA and pairs with A rather than C.



8-Oxo-7-hydrodeoxyguanosine
(8-oxodG)

2-Direct reversal of damage: due to interaction between two adjacent pyrimidine bases .

If for example, a strand of DNA has T base followed by another T was exposed to UV light, as a result they may form a dimer through a covalent bond forming **cyclobutane pyrimidine dimers**. This product is **mutagenic photodimer**, which happens usually to thymine bases. Bacteria have systems where they can reverse the dimer formation by breaking the covalent bond between the two T bases.



3-Excision-repair pathways:

-This system is more complex.

-What happens is that we are going to cut a piece of DNA that contains damaged bases

-There are different mechanisms related to this system :

A-general excision repair.

B- coupling of transcription and repair.

C- specific excision pathway.

1-General Excision pathway: is also called the nucleotide excision repair system and is found in both humans and bacteria, but we are going to study the bacterial one since the human one is very complicated.
- Let's say we also have a pyrimidine dimer. So how this pathway deals with this damage??

A-The system first recognizes the mutation.

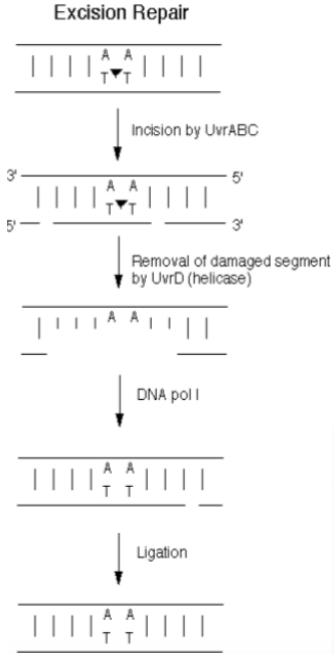
B- After that UVrABC enzyme makes 2 nicks in the phosphodiester bond on either side of the lesion that contains the damaged bases.

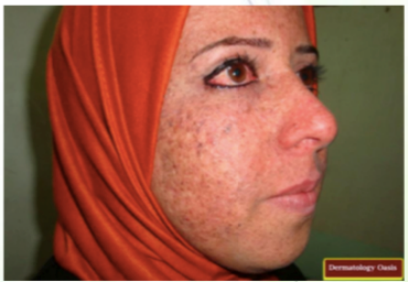
-Note: Nick means a break of the phosphodiester bond.

C- Then helicase enzyme (or **UVrD enzyme**) removes a piece of DNA from the damaged strand, which contains the damaged pairing of the two bases.

D-Finally DNA **polymerase I** fills the gap with the correct bases again and ligase connects them with phosphodiester bond.

- In humans it's much more complicated with other complexes of proteins named **XP** A-B-C and so on. They were named so because they have noticed that problems in such system would result in a condition known as **xeroderma pigmentosum**. In which UV light hits the DNA causing pyrimidine dimers, the XP system fails to repair the DNA so this conditions happens.





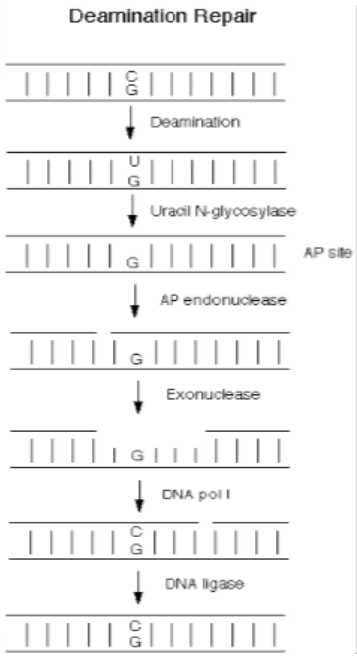
2-During transcription: enzymes called RNA polymerase are associated with transcription factors (TF2 A-B-C…etc.) the most important one is **TF2H** that has many functions. One of them is that during synthesis of RNA " transcription ", TF2H recognizes damages or mistakes to form a complex with XP proteins to repair through the same mechanism mentioned in the general system (recognition, nicking. …etc.). Then transcription can continue normally.

3-In the specific pathway: we are talking about more specific damages in the DNA. We have enzymes known as DNA glycosylases . They recognize the incorrect bases to cleave the glycosidic bond between the sugar and the base . This will result in AP sites which means Apurinic sites or Apyrimidinic sites (sites without purin nor pyrimidin).Glycosylases have specified functions , some of them recognize the uracil only for example . So it breaks the glycosidic bond between the uracil and its sugar keeping a complex of phosphate and sugar without the purine base (AP site). After that, AP endonucleases cleave phosphodiester bond forming a gap (makes **one nick**), then exonucleases can take over to get rid of a larger fragment. A polymerase I fills the gap inserting cytosine instead of uracil and a ligase re-forms the bond.

-Note: - Exonuclease does the same function of helicase found in the general system , in which both remove a piece of the DNA that contains the damage .

***Other glycosylases work on other bases such as T or C.**

* Why would we find uracil in the DNA ??! Because uracil actually results from spontaneous deamination of cytosine, so that we have uracil glycoslase to fix this base with the correct one.



We have another specific system in bacteria, which is the **GO system** referring to **8-oxodG** (guanine base analogue). Assume that mutT protein, which is responsible for preventing incorporation of GO in DNA, fails to do its job, and we have a GO within DNA strand, how can the cell now fix this problem??

1.If the GO was detected before DNA replication it will be rectified and removed by a protein called mutM. After GO removal a guanine will be added by DNA polymerase1.

2.If the GO was detected after or during DNA replication we will end up having a GO paired with adenine instead of guanine paired with cytosine. In order to repair this mutation a protein called mutY will remove the mispaired adenine from the opposite strand and cytosine will be placed instead by DNA polymerase1. Then mutM will remove GO and a guanine will be added by DNA polymerase1.

- This mechanism found in bacteria.
- Don't forget that when GO pairs with A the mutation here is called **transversion mutation**.

-SO basically mutM is responsible for the removal of GO, mutY is responsible for the removal of the wrong base after replication.

****To sum up:**

We have 3 types of mut protein:
1- mutT which is responsible for preventing incorporation of GO in DNA.(preventing errors before they happen)
2- mutM, which is responsible for removing GO from DNA (the error has happened but here we prevent the incorporation of it before DNA replication so that the daughter cells won't house it).
3- mutY which act on the complementary strand after replication; removing the mispaired base (adenine) which will be replaced by cytosine and then mutM will remove GO which will be replaced by guanine.

-Refer to this figure, which summarizes GO system mechanisms.

4-Post replication mechanism

1-Mismatch repair system: when there is mismatch pairs between DNA bases after finishing the replication.

*This mechanism is found in both bacteria and humans, but we will study the bacterial one since the human system is very complex.

-This system works after replication when for example guanine pairs with thymine instead of cytosine.

- Note this system works after S phase after the replication.
Complexes of mut proteins **recognize** the damage such as mutY that we have mentioned above. **It causes then one nick in** the DNA, then exonucleases take over. Finally the space if filled out by DNA polymerase I. (its much more complicated in humans).

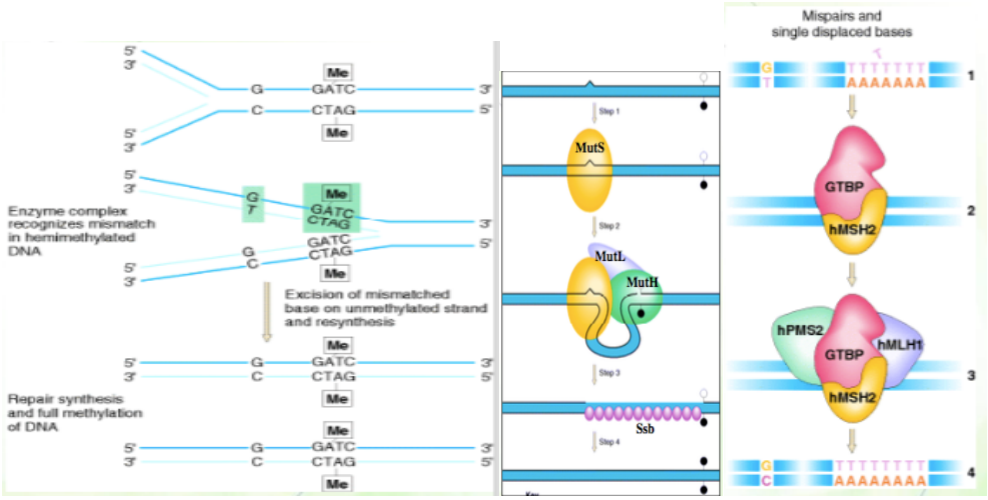
*Actually, what is happening that a piece of the strand, which has the incorrect base, is removed then is corrected by DNA polymerase 1.

Now the question is, will these systems remove the guanine to insert an adenine or they will remove the thymine to insert a cytosine? What happens is that after the replication there is a period where DNA repair systems work to check if there are any mistakes, if not, methylation of **adenine by adenine methylase** in the new strand happens. But if we have a mismatch mistake, the original strand will be already methylated while the newly formed one won't. So they recognize the non methylated strand to repair the damage within it.In humans , the mechanism is the same but using different proteins such as **hMSH2** and **hMLH1**.

* Note that bacteria have **mutS** and **mutL** instead.

*Adenine methylation is what helps in determining the old strand from the new strand (it help in recognizing the incorrect base).

- Please refer to the figures below for better understanding.

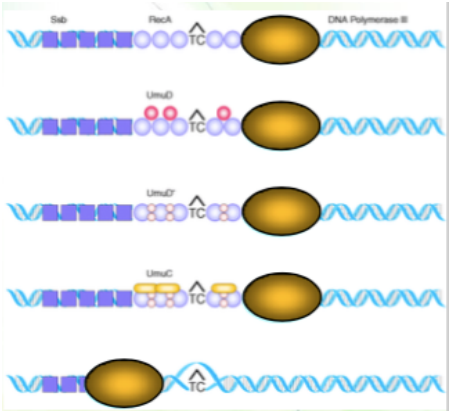


***What's really important about our mismatch repair system is that they are associated with a type of colorectal cancer known as hereditary nonpolyposis colon cancer (HNPCC) . Since we are not able to repair the mismatch mistakes because hMSH2 and hMLH1 are defective , so accumulation of mutations occur which leads to caner .**

2-SOS: proteins known as recA and in addition to UmuC and UmuD will take over with the help of DNA polymerase 3 here . What happens here isn't really repairing . The cell loves to complete the replication as fast as possible and stay alive.So if some kind of mutations such as TC photodimer causes arrest in the replication , the SOS system will not wait for the DNA repair systems to repair the damage . So ssb (single-strand-binding protein) and recA bind to the dimer and signal the cell to synthesize UmuC and UmuD . This complex will allow the polymerase to pass by the damage and add random bases to proceed with the replication (actually most of the bases which will be inserted are incorrect). **The problem is that it might cause accumulation of mutations** as well, unless post-replication systems repaired the damage after .

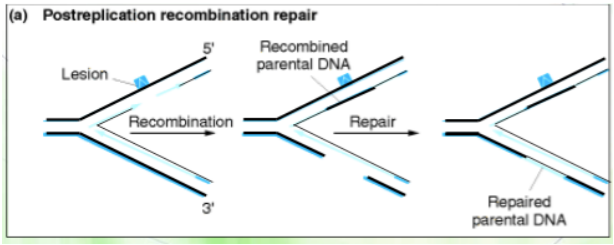
* This system was found in the bacteria and we are not sure if this system present in human or not.

- Summary>>> cell can not wait to repair the damages so it proceeds by inserting random bases in order to finish its replication and that done by the previously mentioned proteins.



3-Recombinational repair system: if there is a lesion in part of one original strands of the DNA , DNA polymerase cant continue the replication because it cant read bases on the original strand in lesion containing region , so what is the solution ?? We all know that the other original strand is complementary to the one which has lesion, so by logic this undamaged original strand (the other original strand that doesn't have a lesion) must have the same sequence of nucleotides as the new/complementary strand that complement the original lesion containing one.
So this recombinational repair system will make a cut in the old strand putting it in the new complementary strand (to be specific putting it in the area on the complementary strand that facing the lesion on the original one). but now we have a gap in the undamaged original strand since it donate apart from it to the complementary strand , so how can we fill the gap ??
Since the complementary strand of the undamaged original strand is correct and doesn't have any mutations we can use it as a template to fill the gap in the original undamaged strand.

-Summary >>>> DNA polymerase leaves a gap where the lesion found >>>then this gap is filled by a cut of the other similar copy of DNA by the action of **recA** enzyme >>>> the new gap from the sectioned copy filled with the correct bases by certain mechanism >>>>> the first lesion is then repaired by nucleotide excision-repair system.



-This system occurs in our systems and are associated with some types of breast cancers .**BRCA1 and BRCA2 are important for the recombinational** repair . So if we have DNA breaks , ATM will recognize the damage to phosphorylate BRCA1 and BRCA2 . They get activated and activate the **recombinational repair**.

- **BRCA1 is also responsible for transcription- repair** coupling . Thus, mutations in BRCA1 will increase the accumulation of mutations causing **breast cancer** in females as well as **ovarian cancer**.

-BRCA2 some of its activity is resembles BRCA1 but it is not the same.

THE END

((A MAN WHO VIEWS THE WORLD THE SAME AT FIFTY AS HE DID AT TWENTY HAS WASTED THIRTY YEARS OF HIS LIFE))

" MUHAMMAD ALI KLAY"