University of Jordan
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GENETICS & Molecular Biology

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Subject: DNA repair mechanism

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We have discussed types of DNA mutations in the last lecture, in this lecture we will discuss DNA repair mechanisms.
We have to maintain DNA, make sure that it is correct and there is no mutation, in order to have a healthy individual, prevent certain diseases like cancer. So how the cell maintains the DNA??
By **DNA repair mechanisms.**
lets start ;) ,, BESMELLAH

there are different levels of DNA repair systems including :
1- prevention of errors before they happen
2- direct reversal of damage
3- excision – repair pathway
4- postreplication pathway
5- recombinational repair
so, let us go through them in some details >>>>

* Prevention of errors before they happen:

How can cells prevent errors before happening?
In this topic we will talk about two examples about how cells avoid errors:
1- getting rid of reactive oxygen species which produced normally in the body.
Reactive oxygen species are group of molecules that have a single electron in their outer shell, they have the ability to attack any other molecule (like DNA) stealing one electron from it, leaving that molecule damaged.
So, if this happened with DNA molecule, it is a problem, HOW do cells prevent this?
What cells have done is that they have enzymes like superoxide dismutase, this enzyme, particularly in peroxisomes, it converts free radicals into something that is safer, look to the reaction below:

$$2\,O_2^{\cdot-} + 2\,H^+ \xrightleftharpoons{\text{Superoxide dismutase}} O_2 + H_2O_2$$

In this reaction, superoxide dismutase has converted the superoxide into hydrogen peroxide which is another reactive oxygen specie but it is less harmful than superoxide.
But even after this reaction we still have H2O2 which is one of reactive oxygen species, so we have another enzyme called catalase which converts H2O2 into water in a very fast manner as in the reaction below:

So, by these enzymes, reactive oxygen species are removed before they could damage the DNA.

2- Do you remember when we talked about 8-oxodG (GO)! Which is a damaged base …..

**** remember:**
GO molecule is formed as a result of free radical attack on normal G base.
DNA polymerase enzyme once see a GO within the DNA it will read it as T, so it will be paired with A instead of C.

NOW,

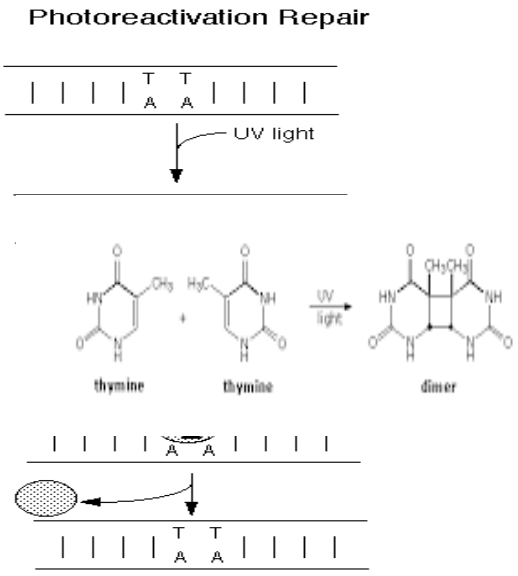
Cells have a protein known as mutT, this protein, which present in E.coli, detect the presence of this molecule and prevent incorporation of this damaged base into DNA.

* Direct reversal of damage:

let's say that damage has occurred, HOW the cells deal with that?! Let us see how ☺

One example on problems that our cells repair after it happened is something called cyclobutane pyrimidine or photodimers.
What is the meaning of photodimers?
it is a condition that appear if DNA molecule has exposed to UV light , lets assume that there are two T located after each other in one strand , as a result of UV light on this DNA strand these two T will make a bond between each other (making cross link) resulting in a photodimer .

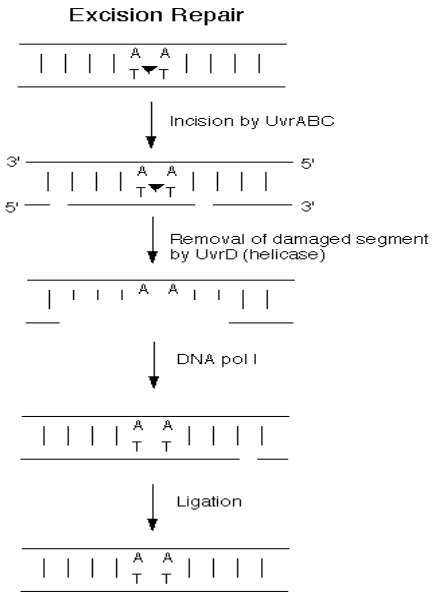
Assume that DNA polymerase in a bacterial cell doing his replication job, and it meets a photodimer, it will stop replication process. HOW bacterial cell will solve this problem??
Bacterial cell has an enzyme called photolyase, what it does is that it is reverse the reaction by breaking the linkage (cross link) between the two T.



** **Pay attention**: <u>this does not happen in humans</u>, we don't have such photolyase enzyme.

* Excision – repair system:

systems that cut part of DNA in which the mutation is located.



there are three types of excision – repair system :

1- general excision repair:

also known as nucleotide excision repair , let us see what is the mechanism of work for this one ,, assume that we have a mutation(for example : a photodimer which can't be rectified by photolyase since it doesn't exist in our cells) ! , so how can we repair this?

simply , by the general excision repair >>>>

what happens exactly that this mutation detected by protein called Uvr , there are 3 proteins that belong to Uvr family which are Uvr A , B and C, they all together work as a complex in the following mechanism :

1- detect the mutation

2- creating a single cut in one of the strands (at this point they act as endonucleases)

3- followed by removal of part of the strand which contains the mutation and thus creating a gap

** remember :
in bacteria we have 3 types of DNA polymerases :
type 1 :
1- filling the gaps after removal of primers
2- DNA repair

Type 2:

DNA repair

type 3: DNA polymerization (synthesis)

in humans we have 9 or 10 different types of polymerases , but no one know which one of them involved in certain repair mechanism .

4- this gap must be filled ,, * in bacteria it is filled by DNA polymerase 1 *

5- following by DNA ligase forming a phosphodiester bond .

Note:
this process (excision repair) is more complex in human cells than it is in bacteria counterpart, but the basic steps are the same as in bacteria.

-student question :
how the bacteria decide which of the two ways they have to use in repair a photodimer mutation , since they have a photolyase enzyme as well as general excision repair ??

the doctor answered that it depends on :
1- the extend of damage
2- number of photodimers
3- the availability of photolyase
4- depend on the environment

**** pay attention :**
photolyase doesn't make any cut or lesion in the DNA strand , it just reverses the dimerization reaction by break cross linkage between the two T .
A defect in the general excision repair system in our cells will lead to a condition known as Xeroderma pigmentosum , which appear as Freckles on the face due to exposure to sun light making a lot of photodimers which are not fixed , eventually this will cause skin cancer .

2- coupling of transcription and repair :
lets say that RNA polymerase has to transcript certain gene , when it encounter a mutation in DNA strand (a photodimerization for example) it stops transcription then activates a repair system through a protein known as TFIIH (Transcription factor II Human) ,which carries the incision, excision, and repair reactions. So, its stops transcription until the DNA is repaired then transcription continues again.

So this is a mechanism where you have a linkage between transcription and repair system via this transcription factor (TFIIH).

*Xeroderma pigmentosum has more than one type, each type is associated with defect in certain repair system, and actually there is a type that's associated with defective coupling of transcription and repair system.

3- Specific excision pathways

in this topic we will discuss two systems (pathways) which are:

a- DNA glycosylase repair pathway

glycosylases are enzymes that degrade the glycosidic bond between the sugar and the base, so if the base is damaged, they can remove this base.

there are different types of glycosylases (there is a glycosylase for each base A,T,G,C,U) , but they all generate what called AP sites (Apyrimidinic site or Apurinic site) , meaning that we have a site that doesn't have a base neither purine nor pyrimidine .

What is the work mechanism of glycosylases??

> remember :
> deamination of cytosine is one of spontaneous mutations in DNA , when cytosine is deaminated it will give ketone which will converted to uracil .
> DNA polymerase will read the U as T pairing it with A instead of G resulting in transition mutation .

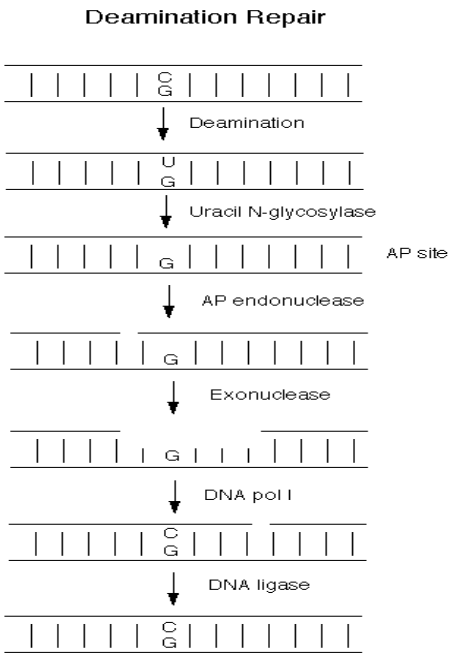
Before DNA replication starts, what the repair systems do is that they scan the whole DNA structure to make sure that it is free of any mutations.

there is a specific glycosylase known as uracil-DNA glycosylase that checks if there is any U in the DNA , if it find U within DNA the following will occure :

1- this uracil-DNA glycosylase will remove just the U (removing the base only) leaving the whole nucleotide structure (pentose and phosphate stay as they are)

2- then an endonuclease known as AP endonuclease will make a cut (cleaves the phosphodiester bond at AP site)

3- then an exonuclease removing chunk of the

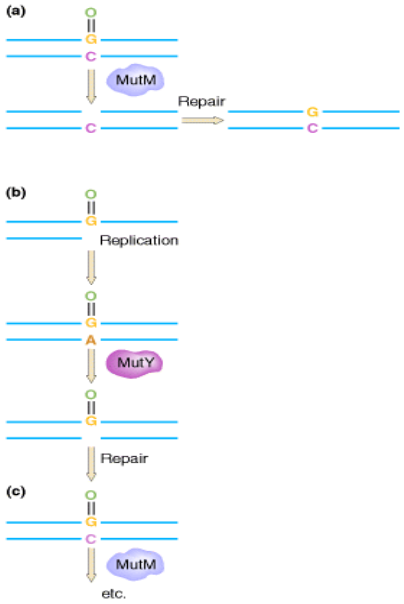


strand
4- DNA polymerase 1 fills in the gap in bacteria
5- and finally , DNA ligase re-forms the phosphodiester bond within the strand .
Note: glycosylase are part of specific excision repair system but depurination is a type of spontaneous lesion mutation.

the second specific pathway we will discuss is :

b- GO (8-oxodG) system :

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.
   There is another protein called mutM, which will remove GO from DNA, then DNA polymerase adds a normal G instead.

****to sum up:**

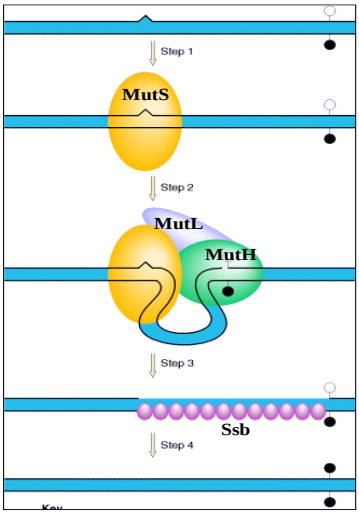
we have 3 types of mut protein:
1- mutT which is responsible for preventing incorporation of GO in DNA.
(preventing errors before they happen)
2- mutM which is responsible for removing GO from DNA (the error has happened but here we prevent the incorporation of it

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.

* postreplication repair :

This system works on DNA after replication has already occurred, it's one of the most important repair systems known as Mismatch repair.
If there is a mismatch in DNA strand either A with C or G with T, we have to fix this mismatch BUT how??

the repair mechanism of mismatching involve a different set of proteins known also as mut proteins but they have different symbols which are mutS, mutL , mutH ,, these proteins detect if there is a mismatch .



the mechanism of function that these mut proteins will form a complex which will recognise the mismatch, makes a cut (just like what happens in the general excision repair) and degradation the part of the strand that has mismatch , then filled in by polymerase 1 in bacteria followed by ligase connecting the strand again .

Note:

Mismatch repair system has the same exact mechanism as general excision repair system except that we ae dealing with mismatches not mutations.

Now, there is an **important point**
lets assume that there is a mismatch mutation in a double stranded DNA, HOW does the mut protein complex know which strand from the two that has the false base!! (In other words, how the protein complex distinguish which of the two strands is the original/parent one and which of them is the complementary one??)
the answer is through methylation , there is an enzyme called adenine

methylase , so before replication the original DNA is methylated on adenine , when this DNA replicate we will have one of the strands methylated (which is the original / parent one) , and the other is not methylated yet (the complementary / new one) . now , if there is a mismatching mistake the mut protein complex comes looking for the methylated strand , once it find the methylated one it says : this is the parent strand , that's mean that the mistake is in the other strand , then it will go and do its job .

Conclusion:
mut protein complex in mismatch repair system fixes the unmethylated DNA strand, then after all mismatches been repaired, adenine methylase comes and methylate the complementary / new strand. So, now it can act as original copy of DNA.

All the things we discussed above were about the bacterial mismatch repair system ☺

now, the same mismatch repair system also exist in our cells (human cells), but we have hMSH2 and hMLH1 proteins, which are very similar to their bacterial counterparts, MutS and MutL, respectively.
These two proteins (hMSH2 and hMLH1) are found mutated in colorectal cancer patients who have specific type of colorectal cancer known as hereditary nonpolyposis colon cancer (HNPCC).

* SOS system (save our souls system) :

bacteria have system known as SOS system , this system actually based on the cells' decision , should they commit suicide if they cant repair their DNA OR should they survive although the replication with mutated DNA will accumulate more and more mutations !!!

now in SOS system what happens is that while DNA polymerase III is replicating the DNA, it hits a photodimer, so it cannot continue any more, this region where DNA polymerase 3 has stopped attracts single strand binding protein (Ssb) and recombination A protein (Rec A) which

signal the cell to synthesise UmuC and UmuD proteins which will allow the DNA polymerase to add random nucleotides just for the sake of finishing DNA replication. Now, the problem with this system (SOS) is that it creates a lot of mutations because a number of bases are inserted to DNA randomly which could be fatal.

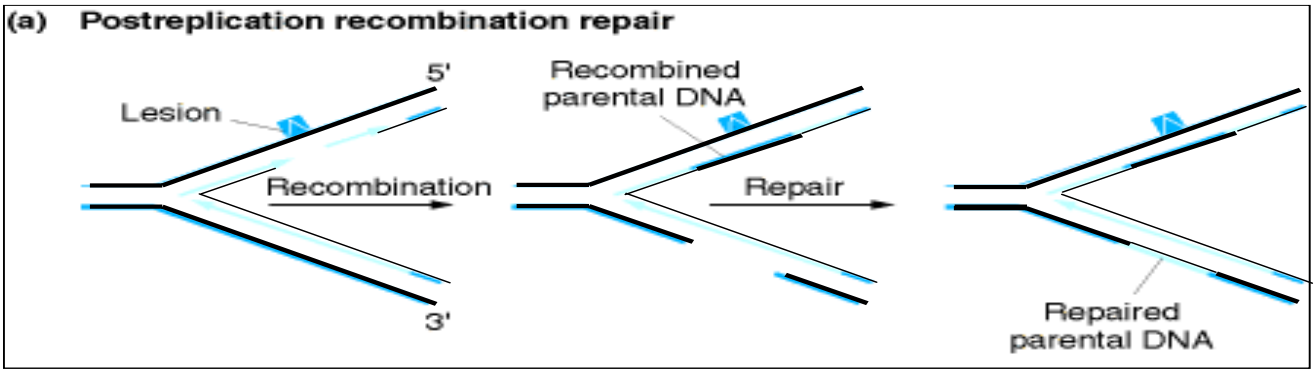
Note: DNA polymerase 2 is used in the SOS system but its role remains unknown.

* 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.

I have done my best to explain this system, but if the idea is not clear yet, look at the pic below:



Note: the original strand still has the mutation but replication was resumed via recombination repair system although it didn't rectify the original strand. This mechanism is commonly used in human cells.

*** breast cancer**

there are two genes that commonly found mutated in familial breast cancer as well as ovarian cancer which are BRCA1 and BRCA2 (BRCA1 and BRCA2) are involved in recombinational repair system , so families with mutated BRCA1 or BRCA2 are at higher risk of developing breast cancer than other individuals .

always keep smiling whatever the condition is, your smile may give hope to someone who needs it

Best wishes