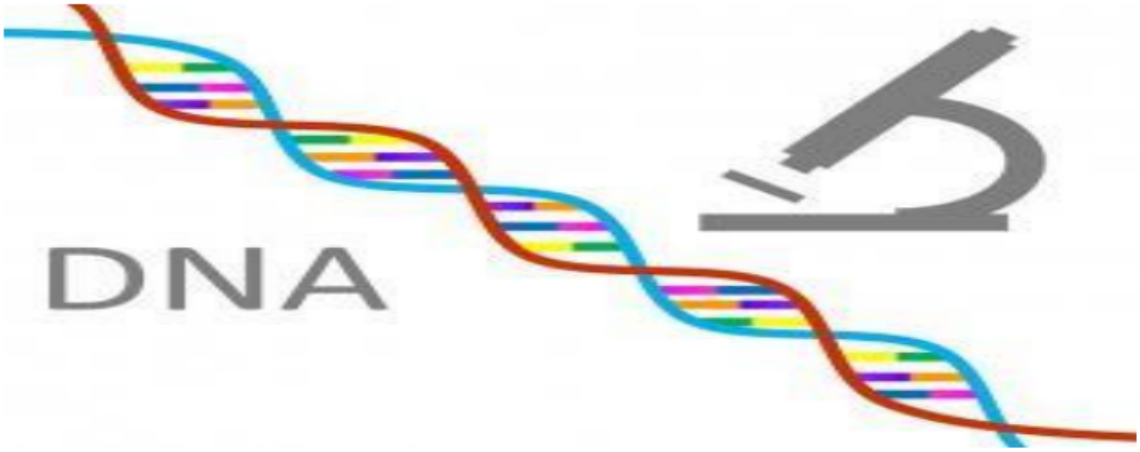
The University of Jordan

Dentistry 020

# MOLECULAR Biology




Tittle : Repair mechanism

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Final correction :Hadeel qafisheh

## Repair mechanism :

- Prevention of errors before they happen
- Direct reversal of damage
- Excision repair pathways
  1-Base excision repair
  2-Nucleotide excision repair
  3-Transcription-coupled repair
- Mismatch repair and post-replication repair
- Translesion DNA synthesis
- Recombinational repair

❖**REPAIR MECHANISM :**It is mechanism that repair the mutated DNA or prevent the mutation.

*The first two mechanisms are discussed in previous sheet*.

## ③Excision Repair Pathways:

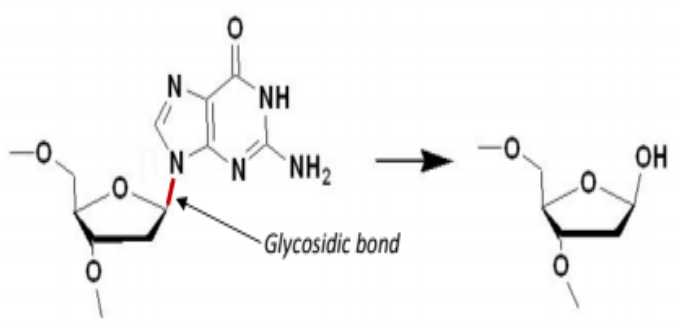
The excision repair pathways are different and complex mechanisms ,excision which mean cut ,so these pathways involve cutting pieces of DNA that are damaged .

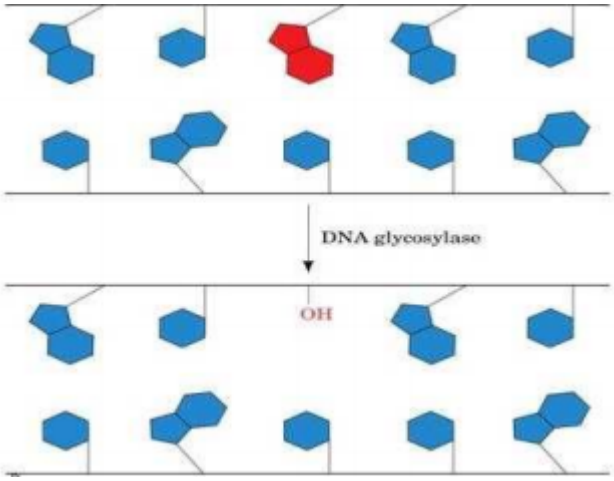
### *Base Excision Repair

→So lets say that the base itself is damaged and it has to be removed ,so there are an enzyme known as **DNA glycosylases** that they cleave N-glycosidic bond "liberating the altered base and generating an apurinic or an apyrimidinic site, both are called **AP sites"** so actually these enzymes remove damaged base cause creation of AP site and then these base will be repaired and replaced by an enzyme

known as **AP endonucleases** (AP endonucleases cleave the phosphodiester bonds at AP sites.)

(REMEMBER: *glycosidic bond ;connect the sugar with the base at 1' C*) ,.



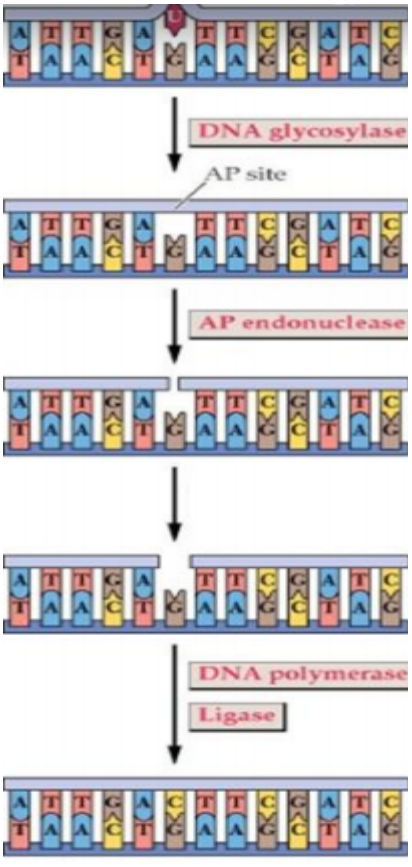

*Each cell in the human body can lose several thousand purine bases daily.

**NOTICE:** DNA glycosylases **do not** cleave phosphodiester bonds

## ◆EXAMPLE OF FUNCTION OF GLYCOSYLASES:

① enzyme known as **uracil-DNA glycosylase**,that work *specifically* on uracil in DNA "its not normal to have DNA with uracil" so what this enzyme does is that removes uracil from DNA → creating an AP site and then → AP endonuclease create a neck "cut" in phosphodiester bond between the AP site and the base right before → so dexoribose is removed → and then DNA polymerase comes and fills in the gap and DNA ligase and re-forms the bond.

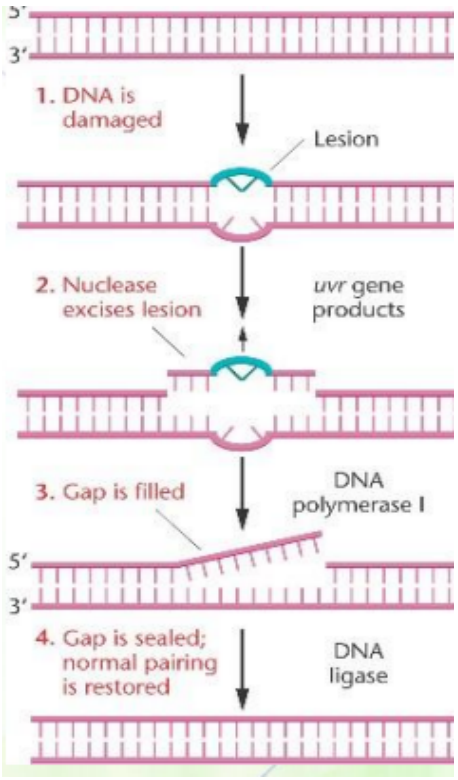
**"REMEMBER**:if uracil stays in DNA then DNA is replicated we will have **UA** that eventually become **TA"**



"**REMEMBER** that uracil (U) shouldn't exist in the DNA ,but these uracil residues result from spontaneous **deamination** of cytosine ©"

## *General(Nucleotide) Excision Repair:

→you have an activation of a complex of a proteins and this happen ❋**IN** **BACTERIA** ,these protein known as :**UvrABC** protein and each one of them have a certain function, but what the collectively do that they: recognize the lesion (pyrimidine dimer) → then one of them cuts the DNA surrounding the dimer (so piece of DNA containing the dimer is removed) → creating a large gap → DNA polymerase 1 fills in the gap by DNA followed by DNA ligase

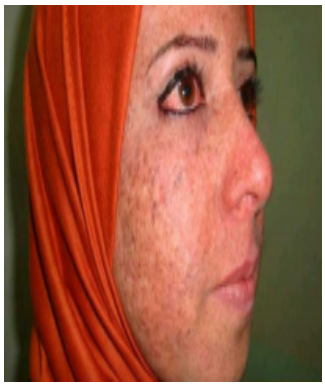



"**REMEMBER**: the **function** of DNA polymerase 1 "lecture 6" ⇨*(removal of RNA primer) of each Okazaki fragment.
*Fills in the gaps between the lagging-strand fragments.
***DNA repair."**

◆ This system includes the breaking of a phosphodiester bond on either side of the lesion, on the same strand, resulting in the excision of an oligonucleotide

## ❋IN Humans :

We have similar mechanism in human cells,and defect of these proteins can cause **zeroderma pigmentosum** ,have these



freckles " نمش" in the skin in face and these are damaged cell and cancer can be resolved if DNA is not repaired.

(the process is more complex than its bacterial counterpart. However, the basic steps are the same as those in E. coli.)

## ❋XP PROTEIN:

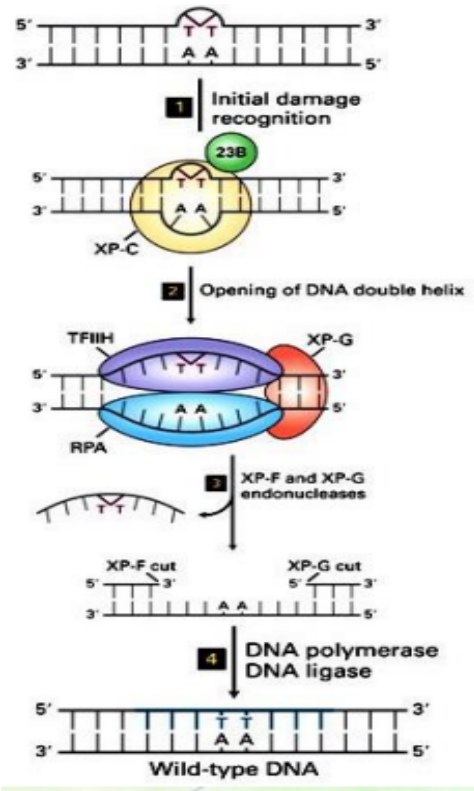
Proteins that are involved in this general excision pathway are known as **XP protein** and there are different protein as; XPA, XPB, XPC , XPD…….XPG, and each one of them is in fact protein with a specific function (one recognizing the lesion, other one cuts in the DNA and third degrades the DNA , and enzyme activities

"endonuclease, helicase"and so on….)

One of these protein known as:①TFIIH "transcription factor 2 H" → function as a helicase ,so when DNA is cleaved it is removed by TFIIH "unwinds

the cleaved strand. "(step number 2)

On the step number 3 we have a single stranded DNA and as we said before cells don't like to see single stranded DNA because "go back to sheet number 6 if ypu want to know", we also talked about protein which is the ②replication protein A that protect the single stranded DNA during replication and it does the same thing here so when the piece of DNA that containing the pyrimidine dimer is removed the RPA coats the undamaged DNA strand protecting it and after that DNA polymerase comes and filling in the gap and DNA ligase forms a phosphodiester bond

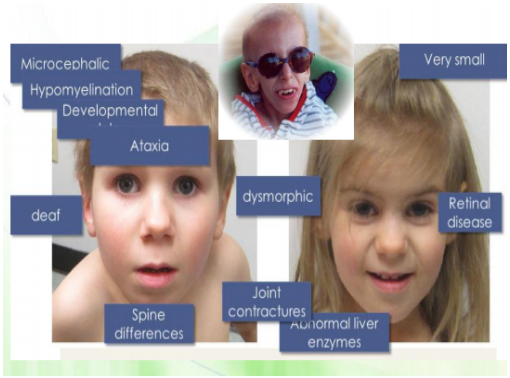


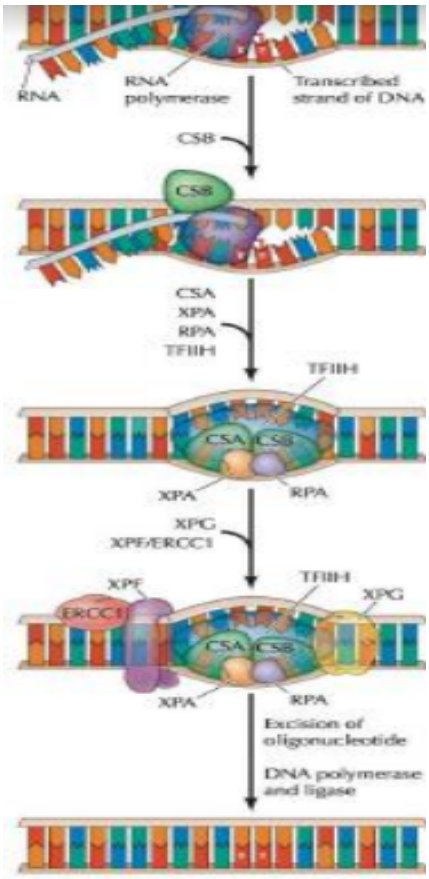
## *transcription-coupled Repair

This mechanism is linked to transcription which is synthesis of RNA by RNA polymerase.

→lets say that we have an active gene needs to be transcribed but its damaged (this damaged also called lesion) so the RNA polymerase which is responsible for RNA synthesis it *stops (pauses,stalls)* ,can not continue because there is damaged DNA ,but there is a proteins that recognizes that RNA polymerase has stopped so this protein gets activated and work with other protein to repair the DNA and then, transcription can continue normally.

◆And the protein that recognizes that RNA polymerase has stopped is known as **CSB** protein the **reason for this name** ➢comes from disease known as cocaine's syndrome→ a condition caused by mutation in a CSB *protein* and people with this condition have very special feature as you can see in the picture below **(you don't have to memorize these)**




### The mechanism of these protein:

When these CSB protein recognizes that the RNA polymerase has stopped it recruits all of the other XP proteins such as XPA ,TFIIH, RPA…. that carry out the incision"cut", excision, and repair the DNA , and once DNA is repaired RNA polymerase can resume RNA synthesis.
*In both eukaryotes and prokaryotes, there is a preferential

repair of the transcribed strand of DNA **for actively expressed genes.**

## ④Mismatch repair and post-replication repair:

Usually these mechanisms are activated after replication ,so if DNA polymerase make mistakes during DNA replication are creating mismatches or mispairing and these mispaired nucleotide are repaired using different mechanisms .

### This mechanism:

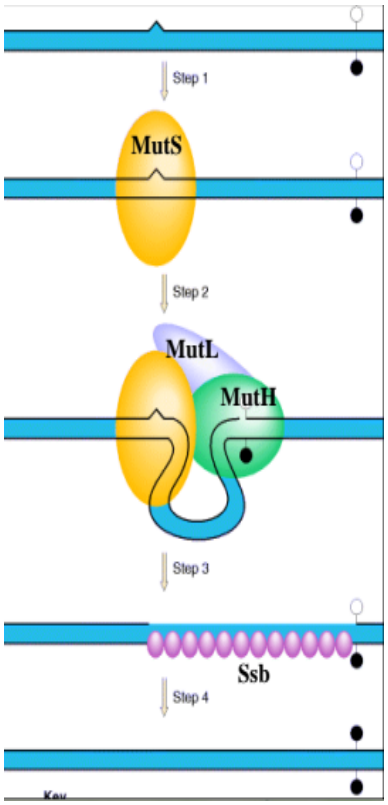
➢It recognizes mismatched basepairs.

➢It determines which base in the mismatch is the incorrect one.

➢It excises the incorrect base and carries out repair synthesis.

### ❋IN PROKARYOTES

→ lets say that DNA polymerase makes a mistake there is a mismatch (instead of having GC we have GT) .in bacteria there is a protein that recognizes this mismatch and these protein known as **MutS (yellow structure in picture)** that work with other proteins known as **MutL & MutH** and they collectively they: recognize the presence of a mismatch → creat a single cut through endonuclease activity → exonuclease that removes this **torsion** DNA (look to picture,the curved area that's will be removed) → so we have a gap & single strand DNA binding protein protecting the single stranded DNA → gap filled in by DNA polymerase 1



So lets say that we have a G here (here refered to triangle area in top of the above picture) and we have a T on the complementary strand

**Since the G & T nucleotide looks normal ,How bacteria will know which one is the original strand ? which one is the correct base? Which one is most be removed?**

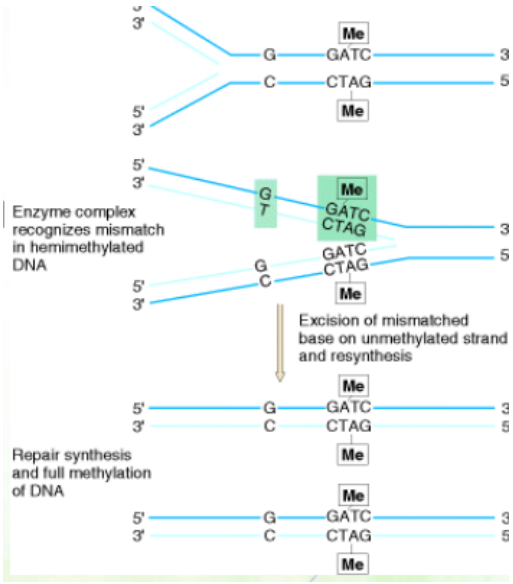
⇨**the answer is:** through DNA methylation

→so the original DNA strand is methylated while newly synthesized strand is not methylated and this methylation of original strand take place at the **adenine**

Since methylation take place at adenine the responsible enzyme known as adenine methylase "that add methyl to adeninie".

So we have here a mismatch (green highlighted area) , so repair mismatch mechanism looks at the unmethylated strand and know that's one new that mismatched should be removed from

So the T is removed and it can be fixed and resynthesis occur, and Once the DNA replication is done ,the DNA methylase methylate the newly synthesized strand as well so it is considered an original strand .

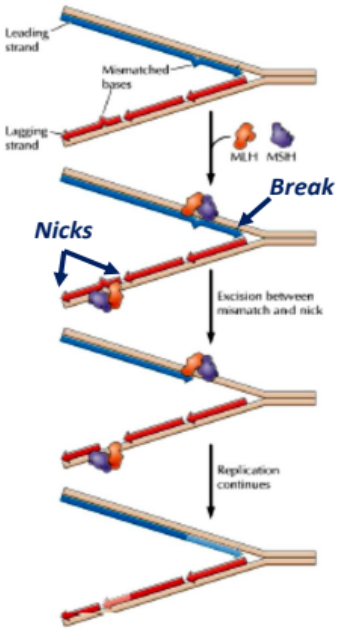



***NOTE:** However, it takes the adenine methylase several minutes to methylate the newly synthesized DNA, The mismatch repair system in bacteria takes advantage of this delay to repair mismatches in the newly synthesized strand.

## ❋IN HUMAN

The mechanism here is little bit different but what is important it **is catalyzed by** 2 protein known as **MLH1 & MSH2** and these protein are very similar to the bacterial MutS & MutL proteins except they don't function after replication is done its not

post replication repair rather it is as called replication mechanism so as replication mechanism take place mismatches are repaired .



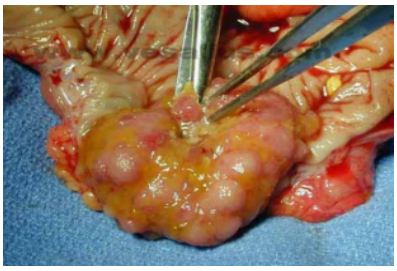

→lets say we have a mismatch in the leading strand so these protein will recognize the mismatch and they create a cut (remove part of the leading strand) and then they start all over again

→let say we have a mismatch in the okazaki fragment ,again these proteins will recognize the mismatch and they remove part of the okazaki fragment that contain mismatch and its replaced by okazaki fragment that has start right after .

*NOTE: The newly synthesized lagging strand could be identified by nicks at either end of Okazaki fragments, whereas the leading strand might be identified by its growing 3' end."this note is written on the slide but doctor don't talk about it"

these 2 proteins are really important because defect on these 2 protein can lead to a type of colon cancer known as **HEREDITARY NONPOLYPOSIS COLON CANCER ( HNPCC)** and is constitute 15% of colon cancer

cancer is a result of accumulation of genetic mutations



It is mainly caused by mutations in MSH followed by mutated MLH.

عن أبو هريرة رضي الله عنه قال: قال الرسول صلى الله عليه وسلم:"ما من رجل رأى مبتلى فقال: الحمدلله الدي عافاني مما ابتلاك ,وفضلني على كثير ممن خلق تفضيلا، لم يصبه \الك البلاء"

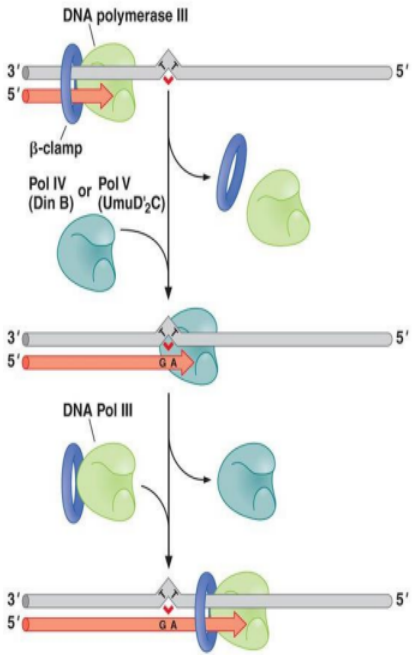
الحمدلله الدي عافانا مما ابتلى به كثيرا من خلقه

## ⑤Translesion DNA synthesis

◆Translesion means jumping over a lesion,

(NOTICE that there are different ways by which pyramidine ddimer is repaired, try to recall them!)

→ lets say there is a pyramidine dimer and we have DNA polymerase 3 “in bacteria” synthesizing DNA and during that it see a pyrimidine dimer so its can not complete synthesizing of DNA ,so it gets released from DNA and DNA polymerase **4 or 5** coming in and it jumps over the lesion and it adds nucleotides of its own ,




The whole purpose is that to resume (continue) to finish and complete DNA synthesis, as we said before cells don’t like to die and they prefer to create mutation rather than die ,so these polymerases “ 4 or 5” add any nucleotide in hope of repairing DNA later on, then this polymerase is released and then DNA polymerase 3 coming over again continuing DNA synthesis .

“Stay hopefully as these polymerses”

◆so this repair mechanism has low fidelity (because it creates mutations), and these polymerases (4 & 5) lack proofreading mechanism, and, hence, are error-prone.

◆But these polymerases can assume that it is a pyrimidine dimer so they add As in place of these dimer, its not always the case but they more often add As guessing that it’s a pyramidine dimer result in reducing the rate of mutation .” so that TT dimers are often replicated correctly.”

◆ **In prokaryotes and eukaryotes**, specialized DNA polymerases can bypass DNA mutations by the ability of DNA polymerases to synthesize DNA over the lesions.

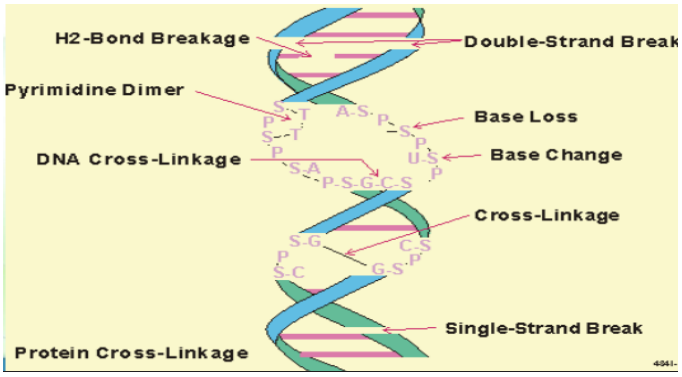
## ⑥Recombinational repair

usually these mechanisms are really complex and they involve in large mutations in the DNA ,and one of the largest inducers of mutations DNA is **ionizing radiation** that can cause different type of mutations by braking up DNA .

◆Ionizing radiation results in the formation of ionized and excited molecules that can cause damage to DNA

## The type of mutation that these radiation can create:

- Creation of AP sites (apurinic or apyrimidinic sites)
- Base damage
- Strand breaks "phosphodiester bonds are broken up"

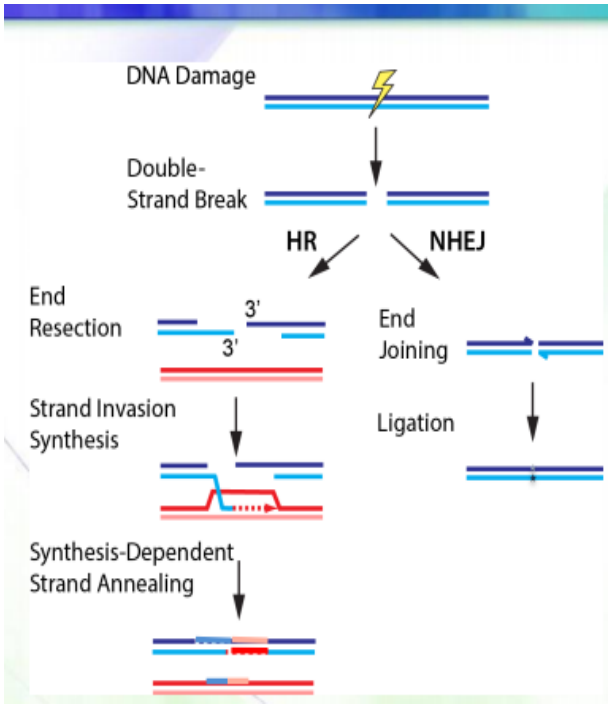



◆There are 2 repair mechanisms that can fix this problem that are known as recombinational repair mechanisms :

**FIRST** type know as; non-homologous end joining (NHEJ) → If there is double strand breaked these protein glued them together ,except that when they are glued, mutations can happen such as insertion, deletion…….

So it's a good system because it fixes the large damage in DNA but mutations take place .

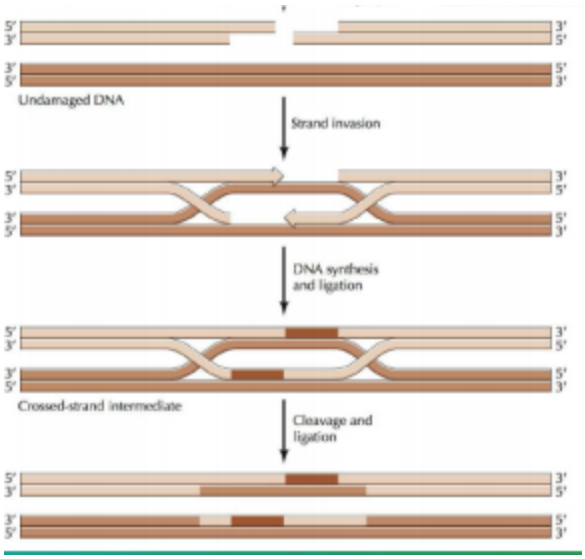
الفكرة هون زي مبدأ المزهرية الملزقة الي كنا نشوفها ببرامج الأطفال ،بتتلزق بس الكسر بضل مبين



**Second :** homologous repair (HR) → This involves Rad51 protein

→Cells take advantage of having a homologous chromosome , that they utilize the second copy of chromosome by taking pieces of it placing it in the damaged DNA so this damaged DNA is repaired and the homologous chromosome with the pieces taken out can be repaired because pieces are taken from one strand only

(REMEMBER:somatic cells are diploid ⇨there are two copies of every chromosome)

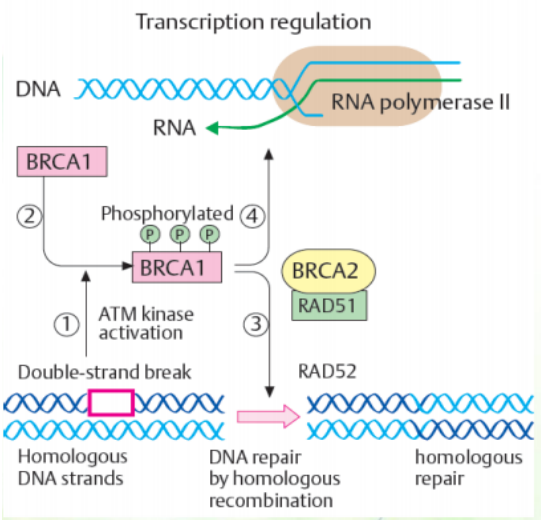



There are 2 proteins that are important in homologous recombination repair in human cells :**BRCA1 & BRCA2** (BRCA:comes from ; breast cancer ) because these protein have been found to be associated with the breast cancer, so defect in these proteins leads to accumulation of mutations that can lead to breast cancer .

" Mutations in BRCA1 and BRCA2 genes are responsible for a portion of hereditary breast and ovarian cancers."

what happens → ***BRCA1*** recognizes the presence of double strand breaks and activated of these protein then they can be involved either in the HR or in transcription coupled DNA repair

***BRCA2*** function with RAD51 protein




Be patient, little remains

😊 قربت أخلص استحملوني شوي .

# wrap-up

| Type of DNA repair | Mechanism | Genes/proteins |
|---|---|---|
| Base excision repair | Removal of abnormal bases | DNA glycosylases |
| Nucleotide excision repair | Removal of thymine dimers and large chemical adducts | XP proteins, CSB |
| Mismatch repair | Correction of mismatched bases caused of DNA replication | MLH1, MSH2 |
| Post-replication repair | Removal of double-strand breaks by HR or NHEJ | BRCA1, BRCA2 |

## This is molecular biology in a nutshell

so in this cource doctor mamoun summarized what molecular biology talk about and the future lies head of us its exciting time for molecular biology,so the few paper are remained we will talk about the future of medicine 7 future of human rise

## Controversial issue:

**we will talk about number of new and this is separated ( not part of the lecture) just to spice up the lecture.**

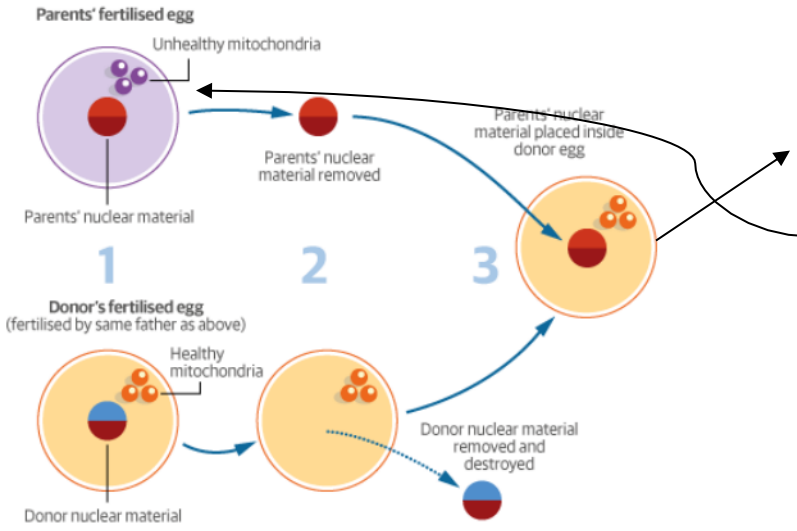
*there is a new way of fixing diseases in the mitochondria ,as we know mitochondria has its own DNA  and if this DNA is mutated that can cause babies born with a certain mitochondrial disease ,and this mitochondria come from mothers specifically because they are replaced in the egg and the contribution of the male is only nuclear DNA (there is hardly any contribution of male mitochondria into generation)

What happens → lets say we have a couples wanting to have a baby except that the mitochondrial DNA of the mother is defective (consist of mutation) this has been fixed by having 3-parent babies.

This is done by taking the mother nucleus and taking donors egg and removes its nucleus so it becomes without any DNA , and the nucleus of the mother that want to have a baby is placed into the nucleus free egg(3), this nucleus contain the mother nucleus DNA as well as mitochondria donor cell and then this egg can be fertilized ,resulting of having 3 types of DNA molecule( father , mother & donors !) and the first baby was born healthy

And this was controversial in the UK because it was first time in the UK

In islam the scholars have decided that is not permissible (haram) should not be done




This egg contain nuclear maternal and healthy mitochondria from donor egg . this egg can be fertilized by the husband of this mother .

https://www.theguardian.com/science/2015/feb/02/three-parent-babies-explained

Not secure | nationalpost.com/news/world/jordanian-couple-has-baby-using-three-parent-genetic-engineering-but-its-actually-abou

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*The **British**-developed technique was performed in **Mexico** by a **Chinese-American** physician who worked in New York*

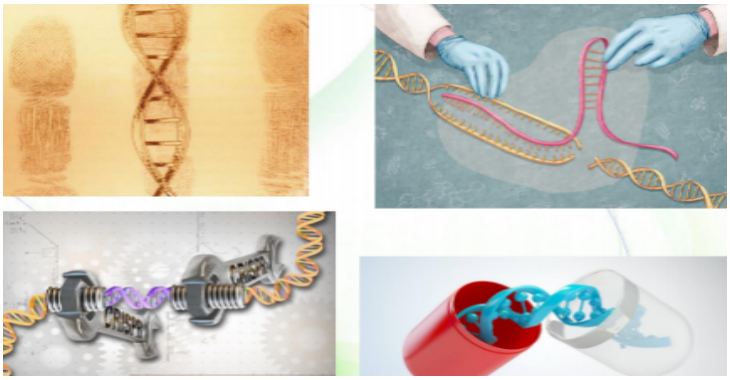
# Jordanian couple has baby using 'three parent' genetic engineering — but it's actually about 2.001 parents

*The Jordanian newborn represents the first successful birth in a new wave of "three parent" techniques, although the procedure is illegal in most countries*

This Jordanian newborn represents the first successful birth in a new wave of "three parent" techniques — ones that are more sophisticated, and that will likely stick around much longer.

We have a jordanian couple they went to Mexico to have a healthy new born without mitochondrial damaged or mutated DNA !!

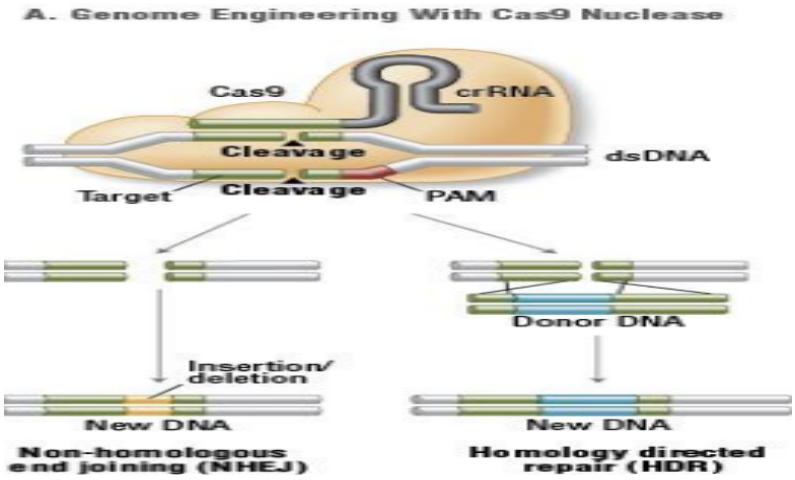
مش رح غير تبا لهم !!



So many things can happen into cells, there is different way to manipulate DNA,

https://www.theguardian.com/world/2019/dec/30/gene-editing-chinese-scientist-he-jiankui-jailed-three-years

It is thought that can be placed in a drug capsule and introduced in cells


Other thing that is controversial as well the scientist who applied for a grant so they get money to do a research on fertilize egg using the Cas9 system they work to mutate DNA in the fertilizer egg to see what the important gene that are necessary for fertilization to understand genetic factor involve in fertilization in hope to improve vitro fertilization or to discover diseases .

The idea of using fertilized egg is always controversial but it allowed by UK system .https://www.healthline.com/health-news/crispr-study-is-first-to-change-dna-in-participants

## The dark side of science


In China, a few years ago scientists did a study on a twins while they were embryos and made them Protect from HIV virus by using CRISPR-Cas9 system to damage a gene known as CCR5 that is an membrane protein that allows the HIV virus to get into human cell

The defective of This gene also responsible to increase intelligence so these babies probably have brains enhanced

***write the article to know***

[The bright side of science]

***[https://www.healthline.com/health-news/crispr-study-isfirst-to-change-dna-in] participants***


**Finally**

**this is the last sheet in this course .**

يعطيكم العافية و فالكم العلامة الكاملة ،

بنعتذر إذا طولنا عليكم بس حاولنا نوجز

كلشي حكاه الدكتور ارعولنا دعوة صادقة من قلبكم

ALWAYS BE THE BEST PERSON YOU CAN BE