8

Molecular Biology

Doctor 2019 | Medicine | JU

Sheet Slides

DONE BY

Mohammad Frejat

CONTRIBUTED IN THE SCIENTIFIC CORRECTION

Hani Shihadeh

CONTRIBUTED IN THE GRAMMATICAL CORRECTION

Hani Shihadeh

DOCTOR

Mamoun Ahram

DNA Repair

What is DNA repair?

If there is a mutation in the DNA of the cell... it can detect these mutations and the cell would activate a number of pathways and mechanisms to repair the DNA.

* It is important to repair DNA because it determines a cell's fate… whether it dies or lives and prospers.

There are several mechanisms to detect DNA mutations and also mechanisms to repair them.

Repair mechanisms

- Prevention of errors before they happen.
- Direct reversal of damage.
- Excision-repair pathways, which include:
  - Base excision repair
  - Nucleotide excision repair
  - Transcription-coupled repair
- Mismatch repair
- Translesion DNA synthesis
- Recombinational repair

Prevention of errors before they happen.

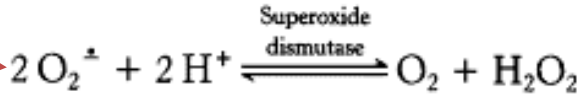
*The cell removes anything that can damage DNA before anything happens to DNA (before it is transcribed, etc…).

Enzymes neutralize potentially damaging compounds before they even react with DNA.

Example: detoxification of reactive oxygen species and oxygen radicals.

- One of the most dangerous factors on DNA are reactive oxygen species, an example is oxygen radicals which are oxygen molecules that are missing an electron (also called: superoxide).
  - ✓ Oxygen radicals are highly unstable and very reactive.
  - ✓ They try to attack other molecules and steal electrons from them.
- These molecules are naturally produced in the cell; when metabolism is taking place in mitochondria, reactive oxygen species and oxygen radicals are produced.
- They can also be generated as a result of external radiation.
  - ➢ When they attack other molecules (lipids, proteins, DNA, RNA… basically anything they find in front of them) they oxidize them (steal an electron from them) and the oxygen radical becomes reduced.
- The Repair process consists of 2 reactions:
  - ➢ The first reaction produces hydrogen peroxide which is also a reactive oxygen species

This is a symbol for an O2 radical (O2 molecule missing an electron)



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

  - ➢ Hydrogen peroxide is removed enzymatically via an enzyme called catalase (this reaction is one of the fastest reactions in the body).

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} O_2 + 2\,H_2O$$

❖ Once this oxygen radical steals an electron from another molecule, this other molecule is damaged.

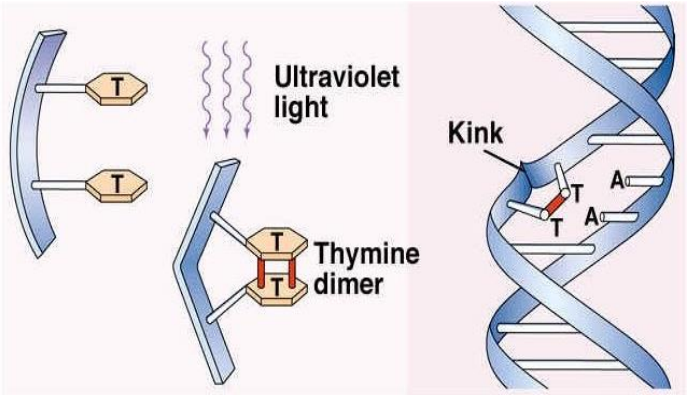
- If the damaged molecule is a protein or a sugar, they can be regenerated and the cell wouldn't be affected.
- If the damaged molecule is a lipid in the plasma membrane, the plasma membrane is damaged and this causes cell death.
- If the damaged molecule is DNA, this causes mutations (Strand breaks, depurination, the formation of apurinic and apyrimidinic sites).

Direct reversal of damage

*If the damage does take place, the cells can reverse it directly.

*Some lesions can be repaired by reversal of DNA damage.

- **Exposure to sunlight** (causes UV light to hit DNA (and this is common)) results in the formation of a covalent interaction between two adjacent pyrimidine bases (50–100 reactions per second).
- This forms structures known as **cyclobutane pyrimidine dimers**, commonly between two adjacent thymines.
- This product is a **mutagenic photodimer**.
    - Pyrimidine dimers cause distortion of DNA structure and this creates a problem in which DNA polymerase and RNA polymerase can't perform their function (replication and transcription, respectively), and as a result, the cell commits suicide.
    - In bacteria (and not in mammals and placental organisms such as humans and animals) there is an enzyme called photolyase.



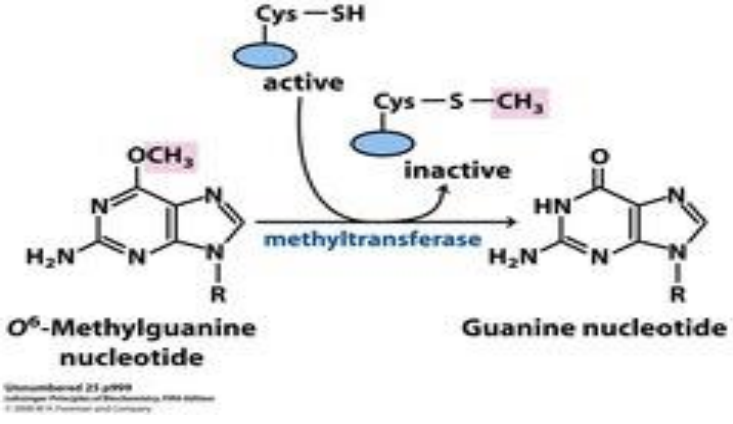
- This enzyme lyses(removes) the dimers (the covalent links between the thymines) reversing the dimer into 2 normal adjacent thymine bases.

➢ There are other mechanisms (in humans) to remove these dimers such as nuclear excision repair (which will be discussed later on).

Repair of O^6-methylguanine

This is a reverse mechanism that contributes to DNA repair.

An enzyme called O^6-methylguanine methyltransferase removes the methyl group transferring it to a protein and the guanine becomes normal again.

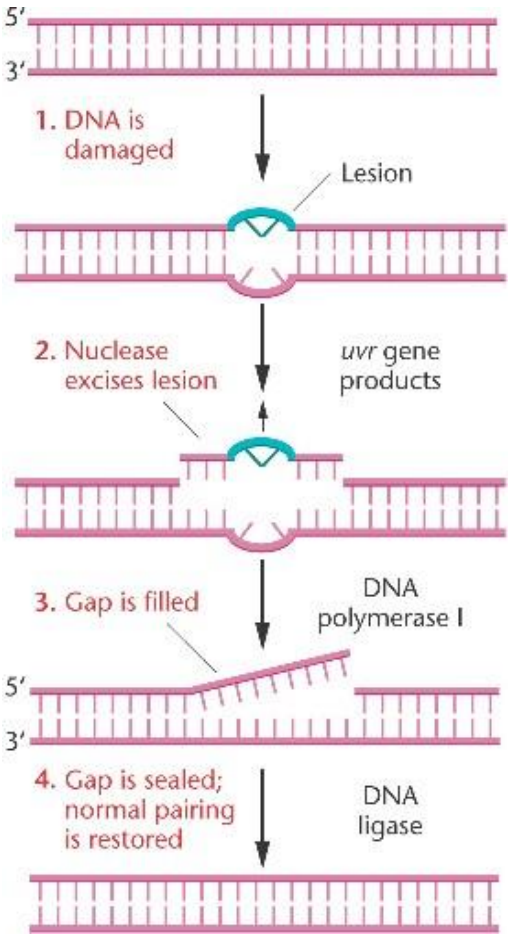


Excision-repair pathways

- General excision repair
- Coupling of transcription and repair
- Specific excision pathways
- Mismatch repair

General excision repair (nucleotide excision repair).

**NOTE: we usually start talking about bacteria because their mechanisms are simpler and we understand them better than eukaryotic cell mechanisms.



❖ What happens here is that we have activation of a complex of proteins which are known as UvrA, UvrB, UvrC.

❖ Each one has a function and is recruited to the lesion sites where it makes nicks (single base cuts of the phosphodiester bonds between two nucleotides) to the left and right of the lesion which produces an oligonucleotide.

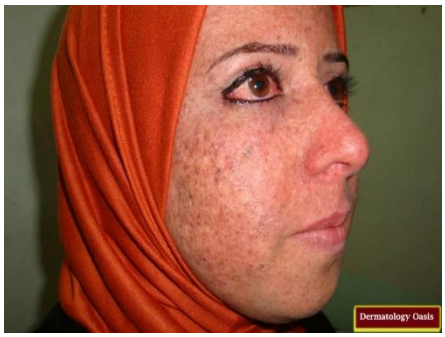
❖ The oligonucleotide that results (which contains the lesion and some adjacent nucleotides to the right and left) is removed by a helicase.

❖ The gap that results from this removal is filled by DNA polymerase 1 (remember that this polymerase synthesizes DNA in DNA repair mechanisms as we said in the DNA replication lecture).

❖ DNA ligase links the fragment (that is synthesized by DNA polymerase 1) with adjacent DNA by forming phosphodiester bonds between them.

- Forming protein complexes is a method which organisms use a lot as it is efficient.
- All proteins that work on a certain mechanism form a complex to work together at the same time on the same region rather than being separate.

- In human cells, the process is more complex than its bacterial counterpart. However, the basic steps are the same as those in E. coli.
- Defect in this mechanism (DNA repair by nuclear excision) causes a condition known as Xeroderma pigmentosum (XP).
  *derma=skin *pigmentosum=pigment
- This is a result of cell death (skin cells) because of DNA damage that can't be repaired



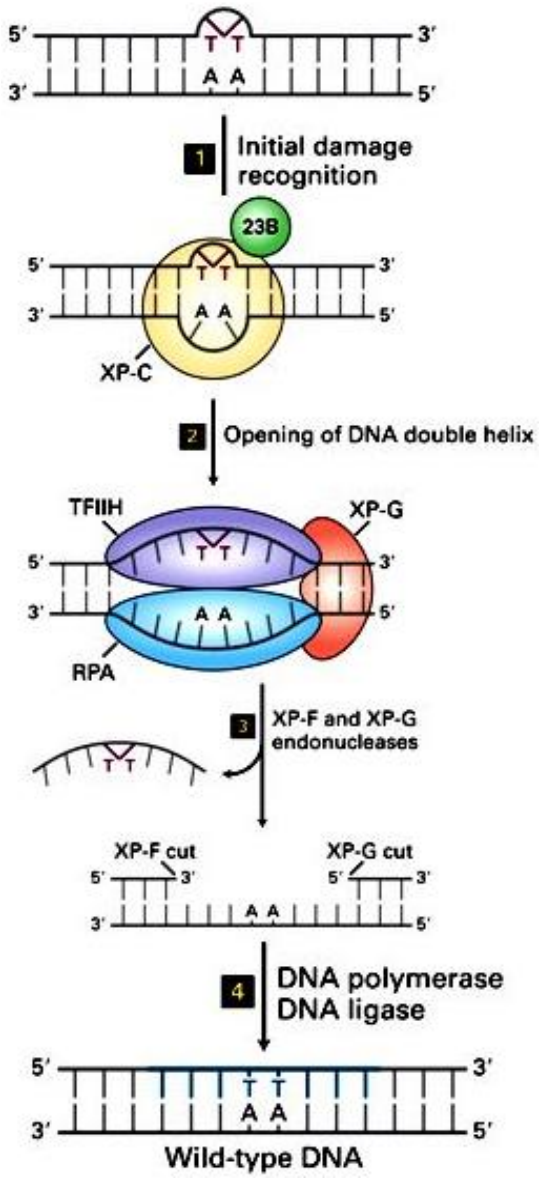
XP proteins

A group of proteins that work together.

- ✓ XP is caused by defective genes designated as XPA, XPB, XPC ……. XPG.
- ✓ These proteins have different functions including damage recognition and enzyme activities (endonuclease, helicase).

❖ A **transcription factor**, **TFIIH**, functions as a helicase that unwinds the cleaved strand and it forms a complex and helps in removing the nucleotide that contains pyrimidine dimers.

❖ **A single-stranded DNA binding protein** called **replication protein A** (**RPA**) protects the undamaged DNA strand and coats the single strand DNA and protects it from degradation (because cells tend to degrade any single strand they detect but here we need the strands to be separated to synthesize the new fragment that replaces the damaged one→to allow the DNA polymerase and DNA ligase to perform their function).



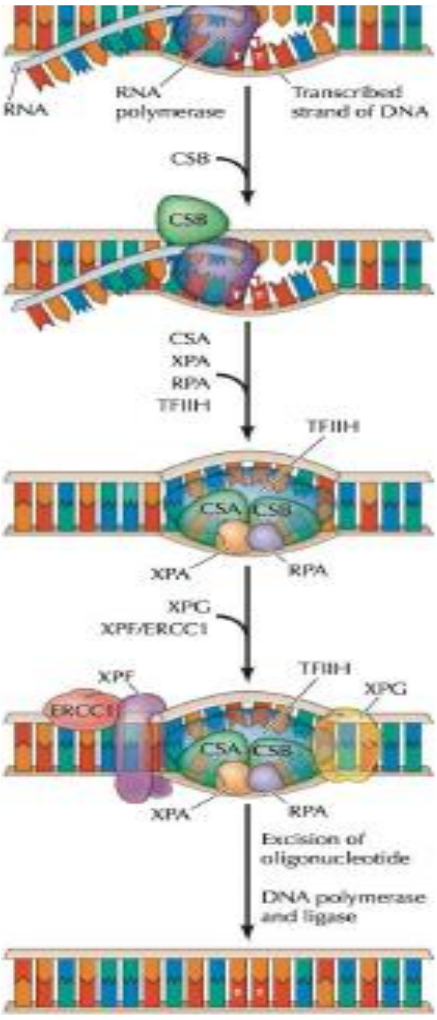
Transcription-coupled repair.

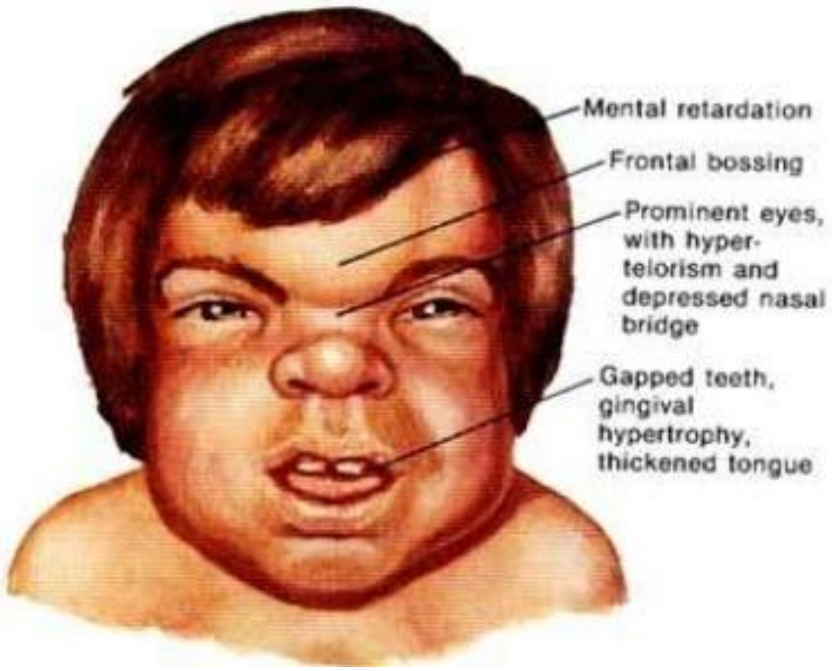
- In both eukaryotes and prokaryotes, there is a preferential repair of the transcribed strand of DNA for actively expressed genes (The cell prefers to repair active genes.
- RNA polymerase pauses (stalls) when encountering a lesion (such as a pyrimidine dimer).
- The **general transcription factor TFIIH** and **other factors** carry out the incision (make a cut), excision (removing the fragment that is produced from the cut), and repair reactions (synthesize DNA to fill the gap).
- Then, transcription can continue normally (the RNA polymerase binds again and continues synthesis of DNA)

Cockayne's syndrome:

A condition caused by a mutation in a CSB protein

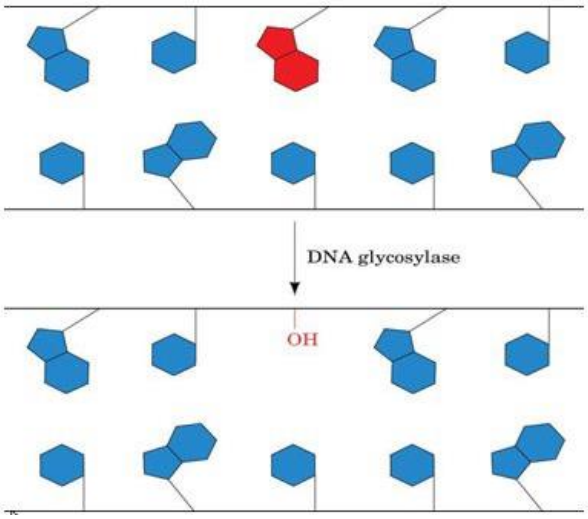
- These proteins recognize that the RNA polymerase is stalled (paused) due to a mutation (for example a pyrimidine dimer).
- It recruits XPA, RPA, and TFIIH which perform the previous mechanism (incision, excision, and repair).

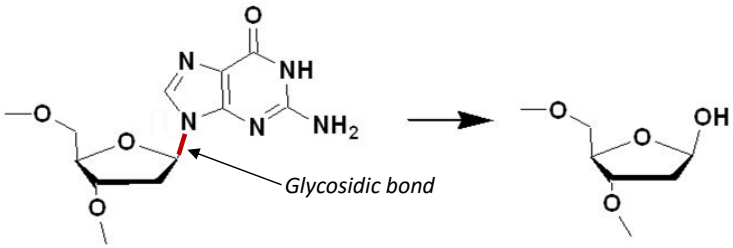




Specific excision pathways

- Each cell in the human body can lose several thousand purine bases daily (spontaneously or via induced mechanisms→external effects) creating AP sites (nucleotides that have a sugar and a phosphate group but no base) because the glycosidic bond is cleaved.
  - ❖ DNA glycosylases do not cleave phosphodiester bonds, but instead cleave N-glycosidic (base-sugar) bonds of damaged bases, liberating the altered base and generating an apurinic or an apyrimidinic site, both are called AP sites.
  - ❖ The AP site is recognized and repaired by an AP endonuclease repair pathway which removes the damaged base and inserts a new base.

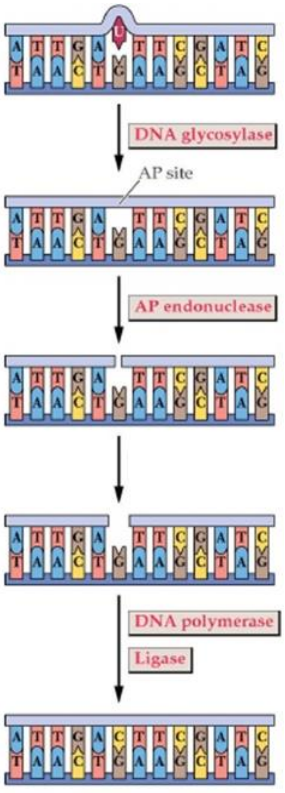




DNA glycosylases

Numerous DNA glycosylases exist.

- ❖ Example: uracil-DNA glycosylase, which removes uracil from DNA (because uracil should only be found in RNA).
- ❖ Uracil residues which results from the spontaneous deamination of cytosine can lead to a C→T transition(instead of having C-G, we would have T-A) if unrepaired(if they aren't removed by glycosylase).
- ❖ AP endonucleases cleave the phosphodiester bonds at AP sites.
- ❖ The deoxyribose is removed.
- ❖ A DNA polymerase fills in the gap and DNA ligase and re-forms the bond.



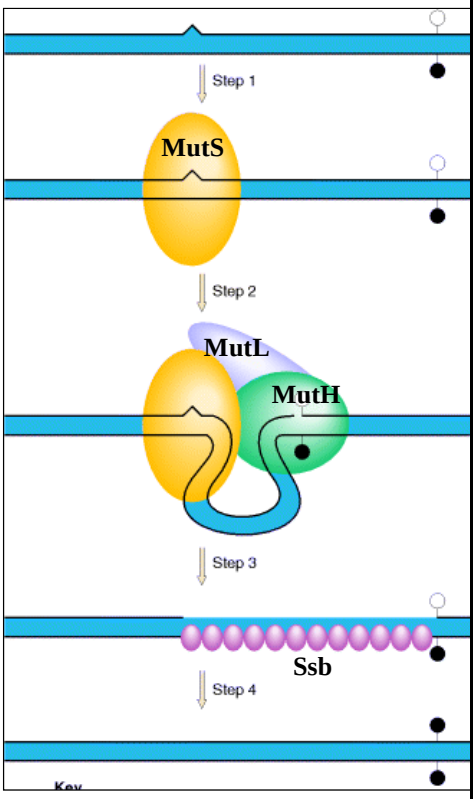
Postreplication repair

Mismatch repair system

**As the name suggests, this mechanism happens when the DNA has a mutation that hasn't been repaired and then the DNA was replicated.

(in prokaryotes)

STEP1: **It recognizes mismatched base pairs** (for example, G-T instead of G-C).



- When this base pair mismatch is detected a group of proteins are activated, known as **mut proteins**, (MutS, MutL, MutH).

STEP2:(The doctor didn't mention what is step2 in the video, but it is probably **recruitment of other mut proteins by MutS**)

STEP 3: **It excises the incorrect base:**

- These proteins determine which base in the mismatch is the incorrect one and makes a nick (single base cuts) on a point way a little ahead the mismatch point (the mismatch point itself is NOT cut in this stage) and this is followed by an exonuclease activity starting from that end (because the single cut made a gap in the strand).
- The exonuclease activity starts from the gap and continues removing nucleotides towards the mismatch point until reaching it, removing it and removing nucleotides a bit further away from it.

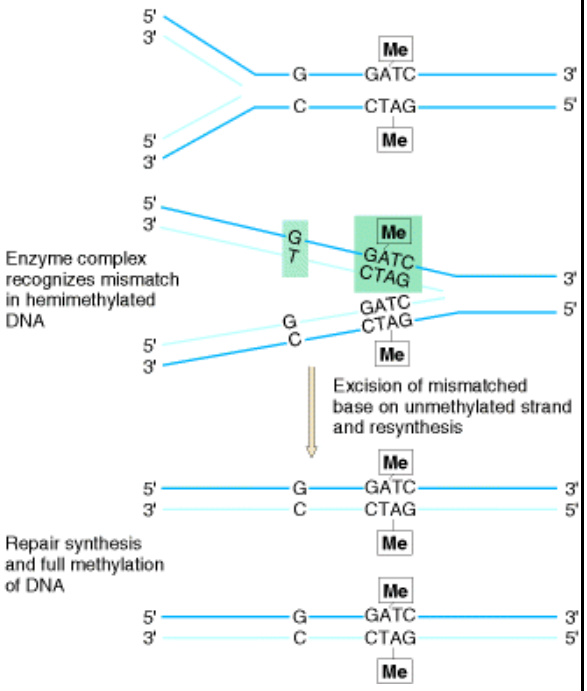
STEP 4: **It carries out repair synthesis:**

- DNA binding proteins bind to the other strand and DNA polymerase 1 begins synthesis of DNA (to fill the gap created by the exonuclease activity) then DNA ligase closes the gap.

BUT…How can the mismatch repair system determine whether G or T is incorrect?

**Dna is methylated (Addition of a methyl group to a DNA) and this happens after replication is completed BUT not immediately.

- The newly synthesized DNA strand is not methylated straight after it is synthesized, so you have a strand that is methylated (the template strand) and a strand that isn't (the newly synthesized strand).
- DNA is methylated following replication by the enzyme, **adenine methylase**.
- However, it takes the adenine methylase several minutes to methylate the newly synthesized DNA.
- Mut proteins know which strand is the template strand and which is the newly synthesized (because one is methylated and one isn't), and thus they repair mismatched BP in the newly synthesized DNA.
- The mismatch repair system in bacteria takes advantage of this delay to repair mismatches in the newly synthesized strand.

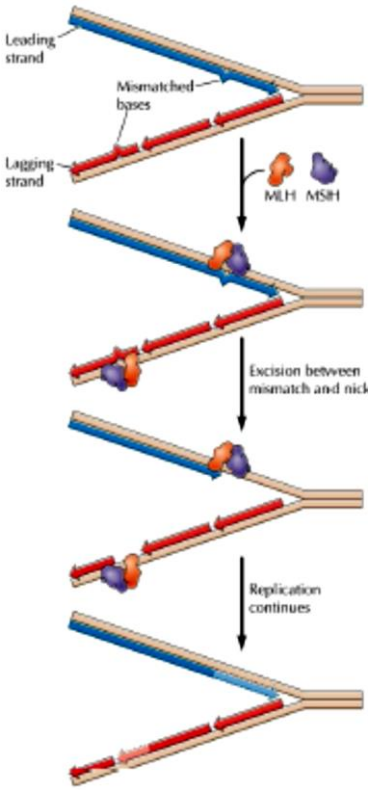


Mismatch repair in humans

- Two proteins, hMSH2 and hMLH1, are very similar to their bacterial counterparts, MutS and MutL, respectively.
  - ✓ Except that we don't have the adenine methylase enzyme in our system.

**SO… How do these proteins know which strand is the newly synthesized one?

- The newly synthesized lagging strand could be identified by nicks at <u>either end of Okazaki fragments</u>, whereas the leading strand might be identified by its growing 3' end (because this is an open end that is being synthesized).
  - ✓ NOTE: **the identification happens <u>during replication</u>.**



Hereditary nonpolyposis colon cancer (HNPCC)

- Colon cancer is a hereditary cancer
- HNPCC constitutes 15% of colon cancer cases.
- It is mainly caused by mutations in **MSH** followed by mutated MLH.
- People who have mutated repair systems have a higher chance of developing cancer.

*What is cancer?

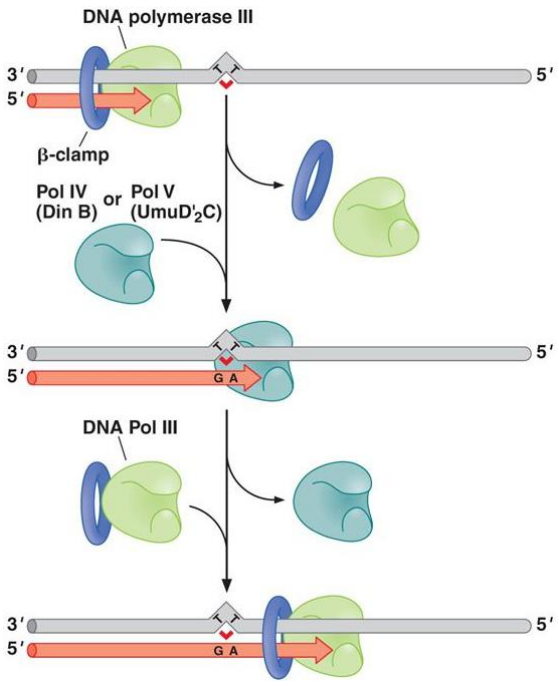
Cancer is uncontrolled cell proliferation

Cancer is generated as a result of accumulation of genetic mutations, and if you have a repair system that is defective, mutations will accumulate and some of these mutations can cause cancer.

Translesion DNA synthesis

Let's presume that DNA polymerase 3 is synthesizing DNA and it reaches a pyrimidine dimer, what would happen?

- The DNA polymerase 3 stops and is released and the association of other DNA polymerases (DNA polymerase 4 or 5), and they allow for continuation of DNA synthesis even in the presence of the lesion (they synthesize a temporary nucleotide in the lesion site hoping that it will be repaired later on).
- These proteins are then released and DNA polymerase 3 binds and continues synthesis after the lesion site.
- In prokaryotes and eukaryotes, specialized DNA polymerases can bypass DNA mutations by the ability of DNA polymerases to synthesize DNA over the lesions.
- Although DNA polymerase 4 and 5 display some selectivity in base insertion, they have low fidelity (اخلاص) and lack proofreading mechanisms and, hence, are error-prone (associated with a high rate of DNA mutations).



- ✓ It's good that we continued DNA synthesis even though there is a mutation but what's bad is that we created even more mutations.
- ✓ However, there is a bias towards introduction of A's, so that TT dimers are often replicated correctly.

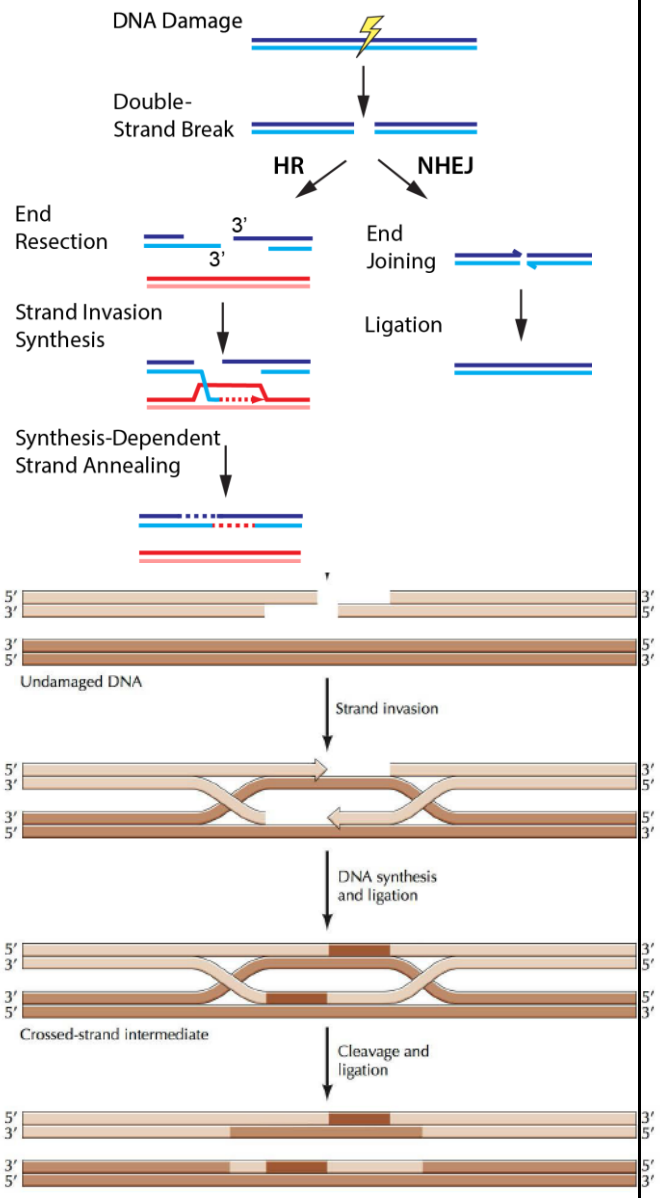
**Any C is likely to be deaminated, inducing a C to T transition (This means that if the pyrimidine dimer was made of two C bases and since there is a bias to introducing A, this is great!).

Recombinational repair

❖ When double-strand breaks of DNA occur, Recombinational repair takes place by:

1. **Non-homologous end joining (NHEJ):**
   It's like grabbing both ends and gluing them together which fixes DNA, but creates mutations (It's error prone).
2. **Homologous repair**:
   - Remember we are diploid and let's say that one of the parental DNA has a double strand break.
   - Cells utilize the other homologous chromosome to fill the gap by Recombination as well as DNA polymerase filling in the gap using information taken from the other chromosome.

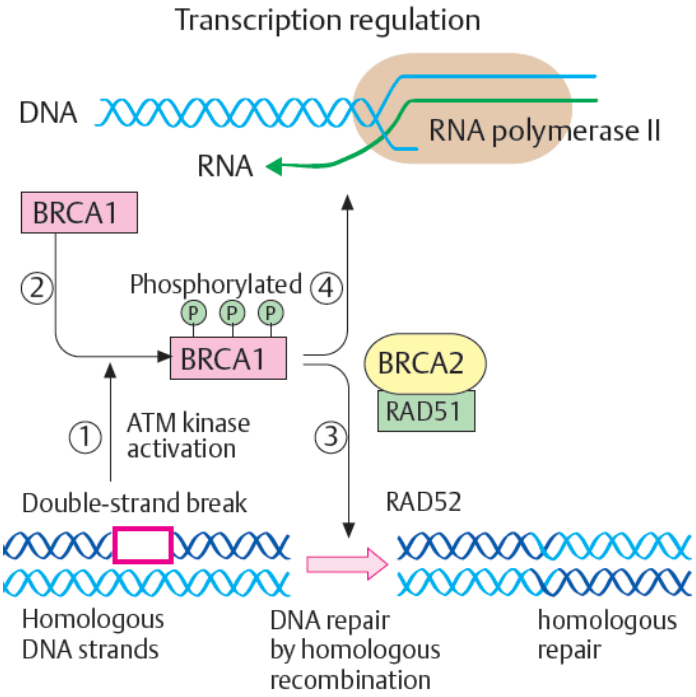
This involves Rad51 protein (they are called rad51 because they were found to be activated when exposed to radiation)



So let's talk about something interesting…

There was a scientist, her last name was king, she was working in the university of Utah and she was trying to look for genes that are associated with hereditary breast cancer and she was able to identify a gene associated with breast cancer known as BRCA1 (BRCA stands for breast cancer) and later on another protein was identified known as BRCA 2.

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



Later on, it was found that both are involved in DNA repair.

- ✓ BRCA1 activates **homologous recombination repair** of DNA double-stranded breaks
- ✓ BRCA2 can recruit Rad51 to the ssDNA.
- ✓ BRCA1 is also involved in transcription and **transcription-coupled DNA repair**.

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

Notes on the table:

- ✓ **Large chemical adducts such as conjugation to benzo-A-pyrene.**
- ✓ **Mismatch repair also includes mut proteins.**

Controversial issue:

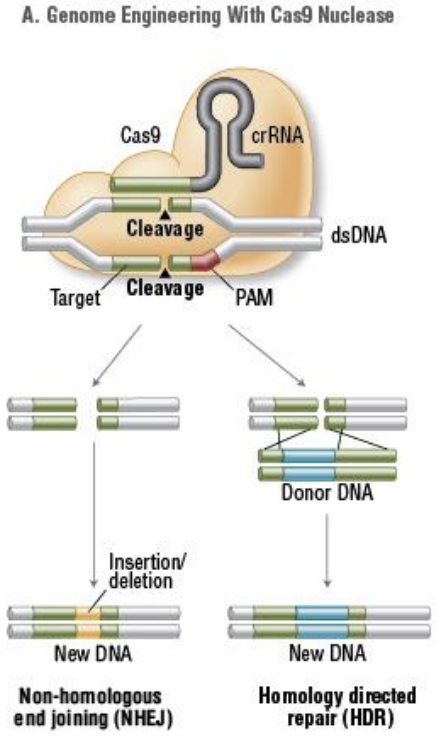
*(this isn't included in the exam, it's just to enrich your minds to think about unusual topics) *

A scientist in the UK decided to study genes important in embryonic development and she wanted to work on fertilized eggs (few days old) and she wanted to change certain genes to learn the functions of these genes and this is useful and can help us improve in-vitro fertilization, as well as repair genes that are damaged and have healthy babies.

She was the first doctor in the UK to work on such a topic and it's really controversial to work with fertilized eggs.

What do you think about manipulating embryonic genes so that the baby doesn't have diseases when born, do you think its ethical or unethical?

**Something similar happened last year in china when a chinese scientist manipulated the genes of twins so that when they are born they would be immune to the HIV virus



and they were born and healthy.

This created heated arguments and the scientist was imprisoned because he didn't get approval for genetic modification.