Molecular Biology (6)

DNA repair

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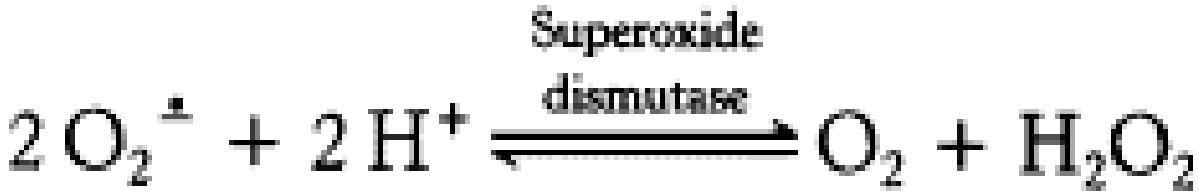
Repair mechanisms

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

Prevention of errors before they happen

Reactive oxygen species

- Enzymes neutralize potentially damaging compounds before they even react with DNA.
  - Example: detoxification of reactive oxygen species and oxygen radicals.



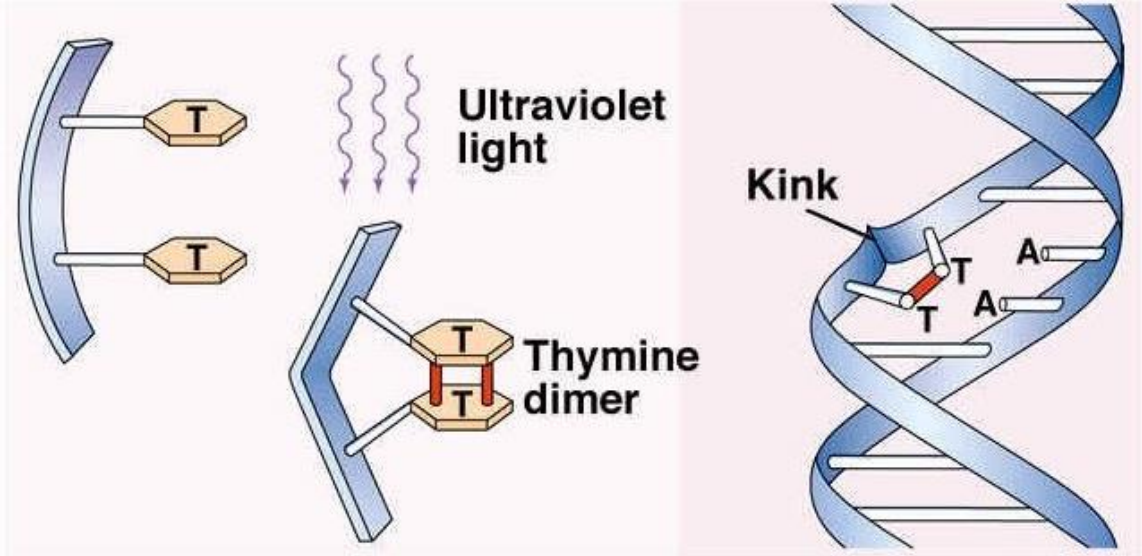
$$2\,O_2^{\bullet-} + 2\,H^+ \rightleftharpoons O_2 + H_2O_2$$

$$2\,H_2O_2 \rightleftharpoons O_2 + 2\,H_2O$$

Direct reversal of damage

Pyrimidine dimers

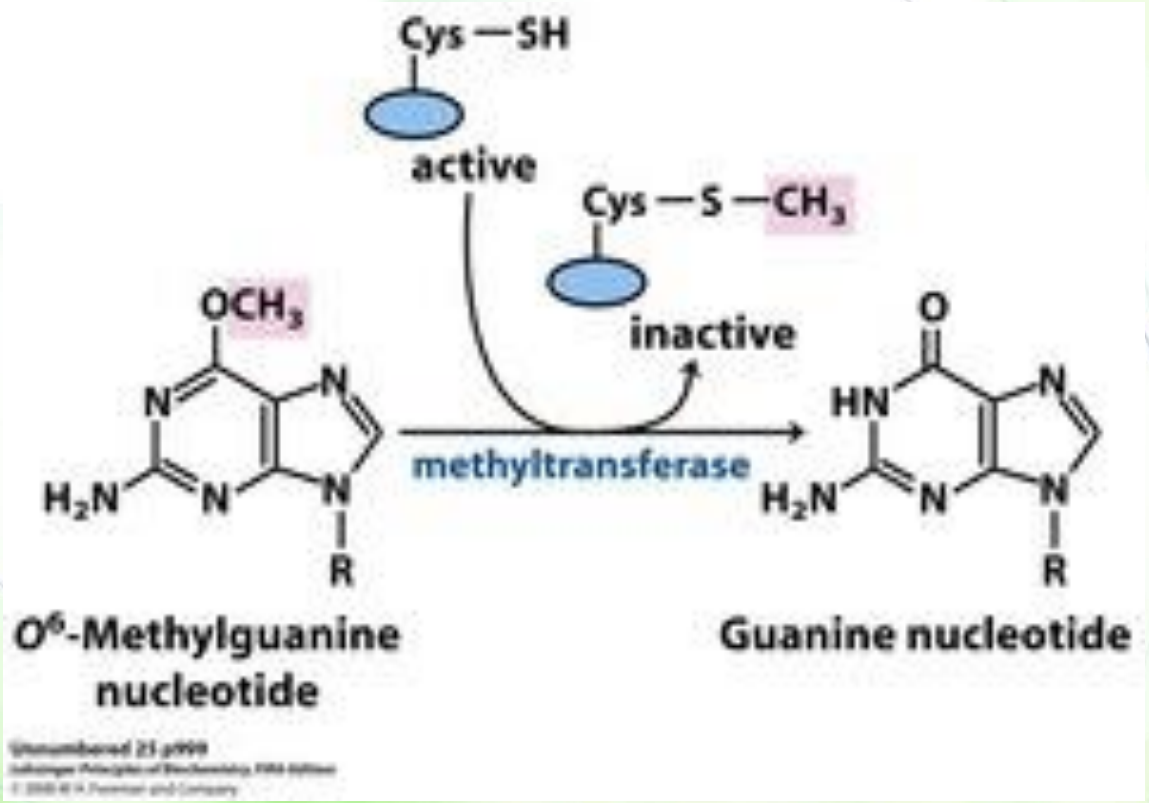
- Some lesions can be repaired by reversal of DNA damage.
- Exposure to sunlight causes UV light to hit DNA results in the formation of a covalent interaction between two adjacent pyrimidine bases (50–100 reactions per second) forming structures known as cyclobutane pyrimidine dimers, commonly between two thymines.
- This product is a mutagenic photodimer.
- Pyrimidine dimers are reversed by enzymes known as photolyases, but they do not exists in humans.



DNA structure is distorted and, thus, replication and transcription cannot proceed.

Repair of O^6-methylguanine

- This is done via O^6-methylguanine methyltransferase.



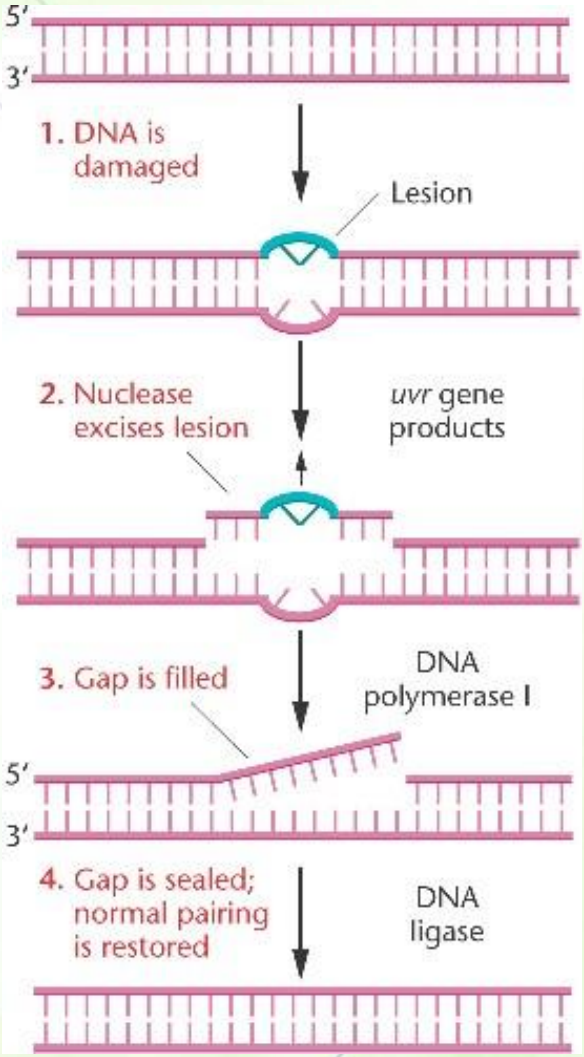
Excision-repair pathways

Types

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

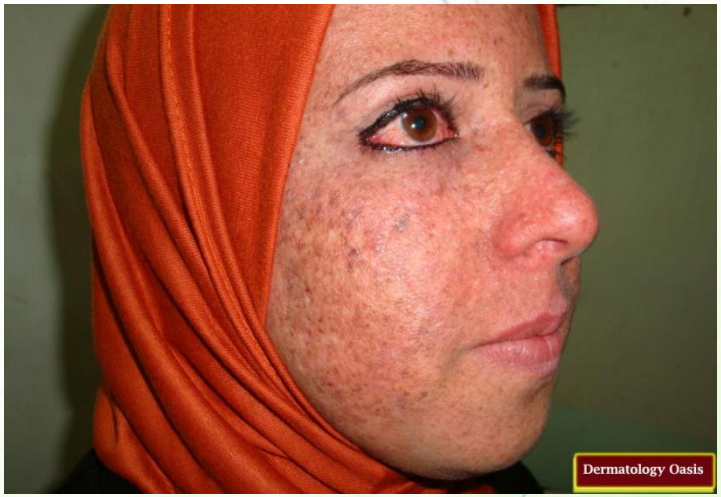
General excision repair (nucleotide excision repair)

- 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 bacteria, the UvrABC protein complex does this work.
- A helicase removes the strand.
- The gap is filled by DNA polymerase I and a ligase seals the breaks.



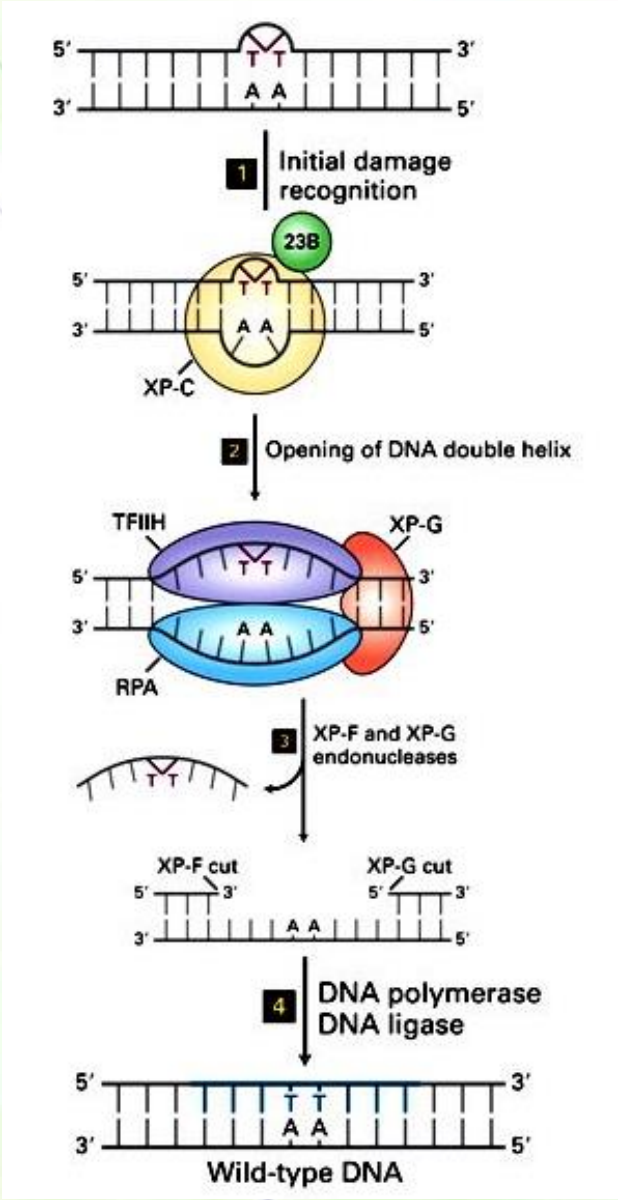
In human...

- 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 causes a condition known as Xeroderma pigmentosum (XP).



XP proteins

- XP is caused by defective genes designated as XPA to XPG.
- These protein have different functions including damage recognition and enzyme activities (endonuclease, helicase)
- A transcription factor, **TFIIH**, functions as a helicase that unwinds the cleaved strand.
- A single-stranded DNA binding protein called **replication protein A (RPA)** protects the undamaged DNA strand.

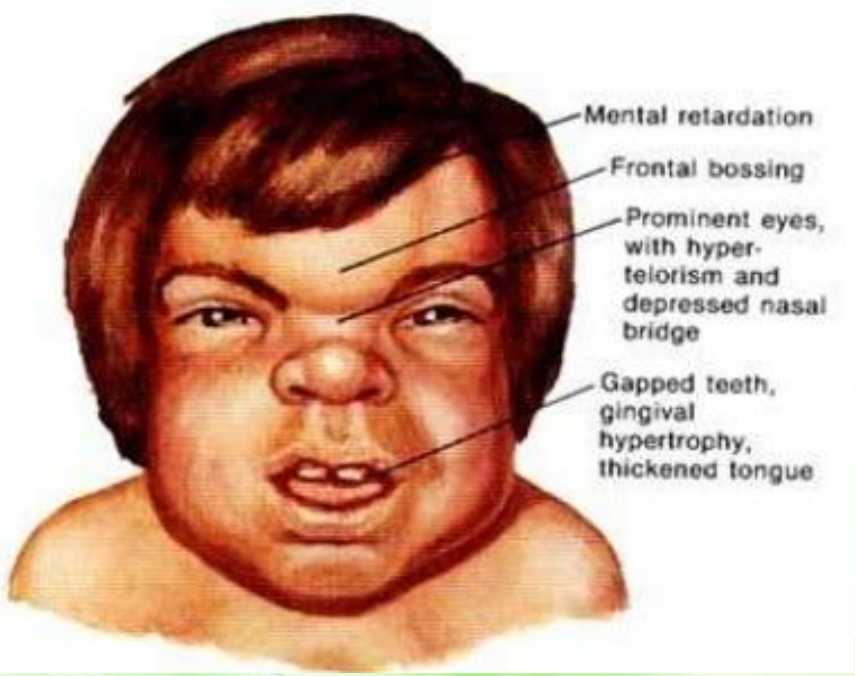


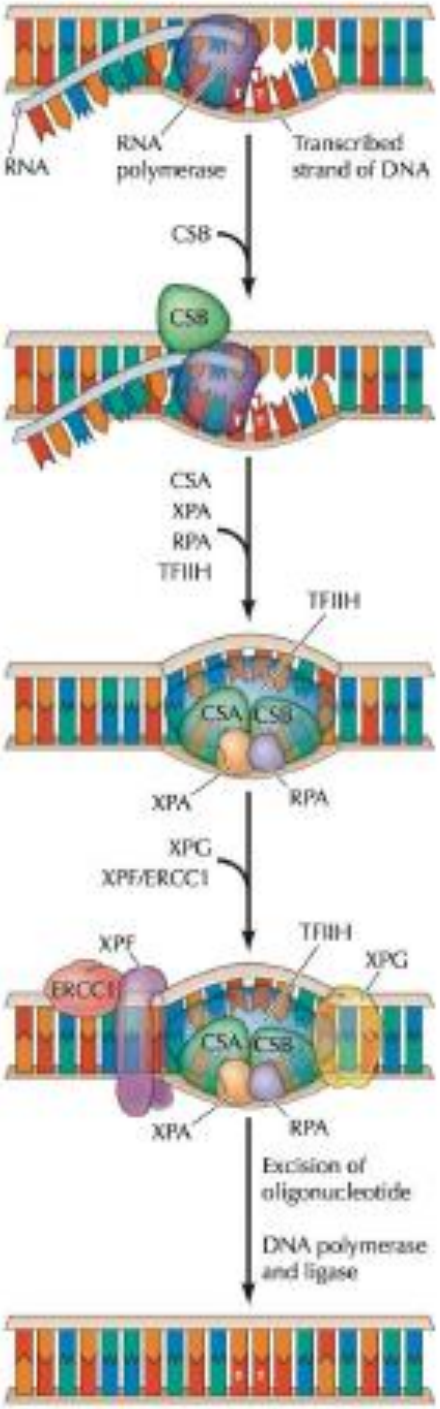
Transcription-coupled repair

- In both eukaryotes and prokaryotes, there is a preferential repair of the transcribed strand of DNA for actively expressed genes.
- RNA polymerase pauses (stalls) when encountering a lesion.
- The general transcription factor TFIIH and other factors carry out the incision, excision, and repair reactions.
- Then, transcription can continue normally.

Cockayne's syndrome

- Cockayne's syndrome: a condition caused by mutation in a CSB protein, which recognizes that the RNA polymerase is stalled due to a mutation.
- It recruits XPA, ,RPA, and TFIIH.

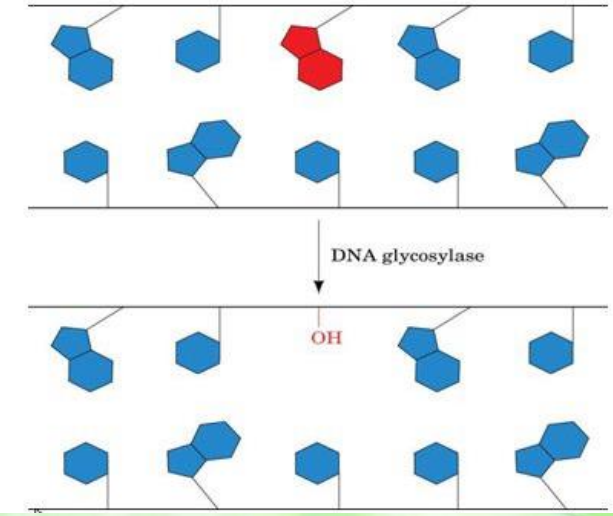


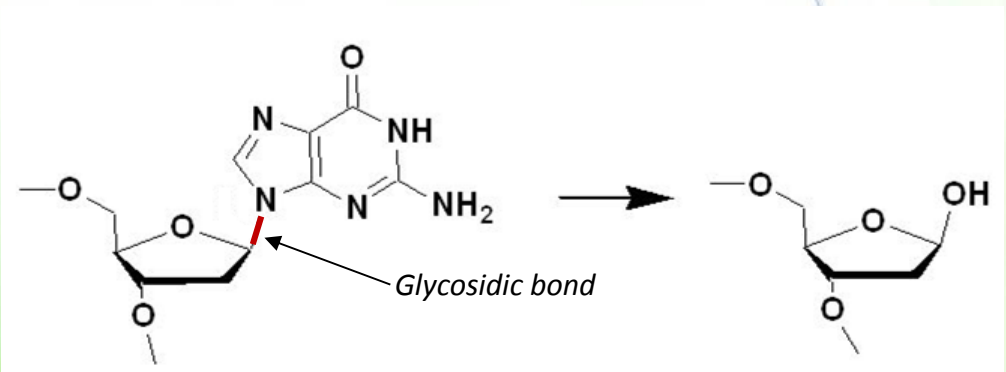


Specific excision pathways

Base excision repair pathway

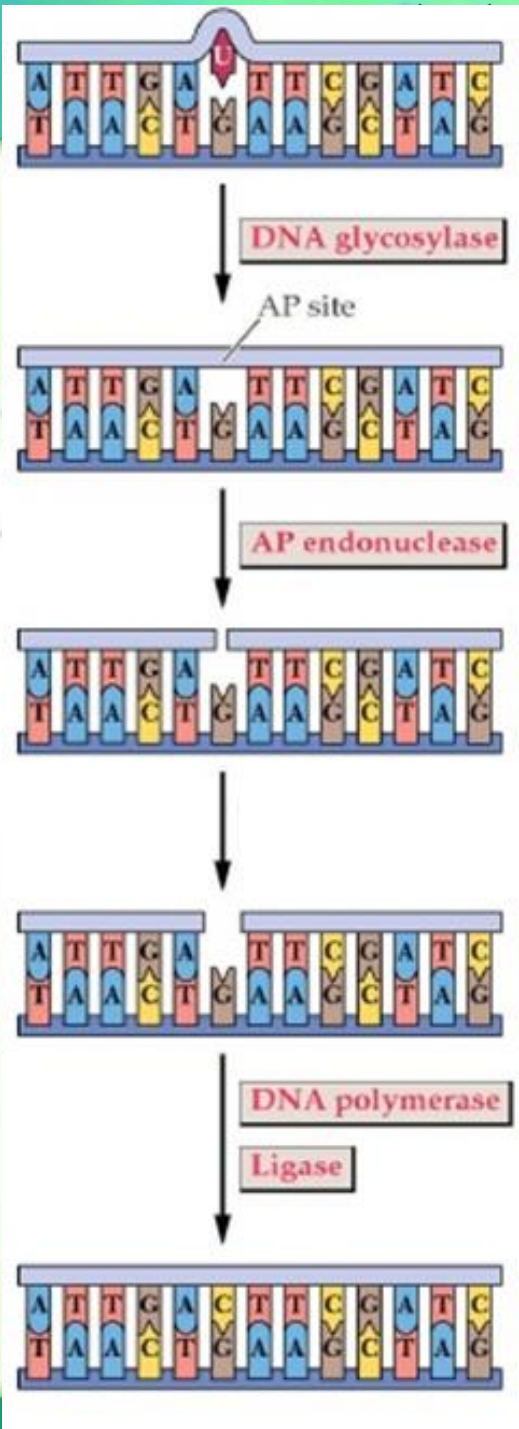
- Each cell in the human body can lose several thousand purine bases daily.
- DNA glycosylases do not cleave phosphodiester bonds, but instead cleave N-glycosidic (base-sugar) bonds of damaged bases, liberating the altered base and generating an apurinic or an apyrimidinic site, both are called AP sites.
- The AP site is repaired by an AP endonuclease repair pathway.





DNA glycosylases

- Numerous DNA glycosylases exist.
  - Example: uracil-DNA glycosylase, removes uracil from DNA.
    - Uracil residues, which result from the spontaneous deamination of cytosine can lead to a C→T transition if unrepaired.
- 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.

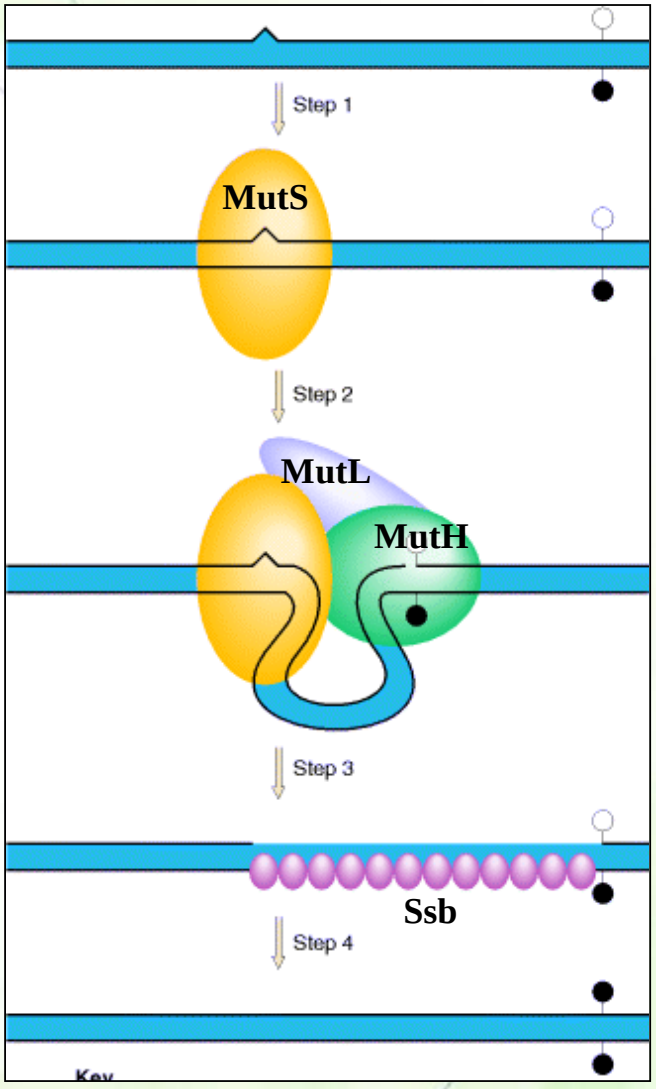


Postreplication repair

Mismatch repair system (prokaryotes)

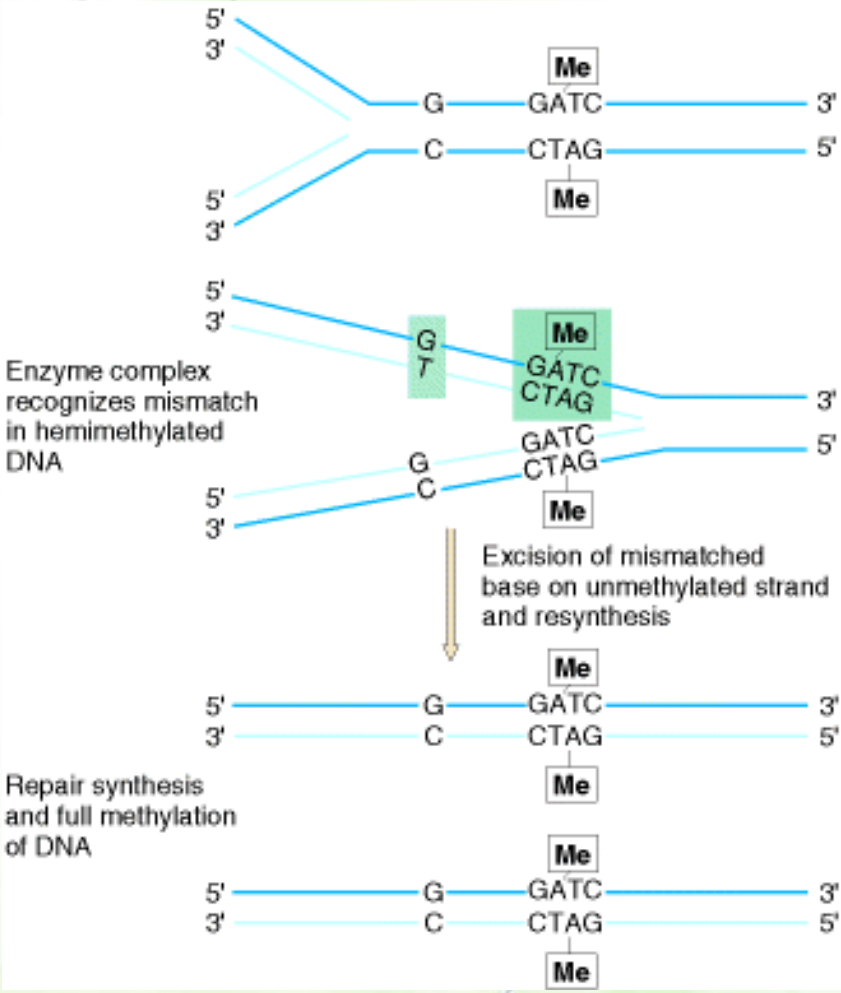
- It recognizes mismatched base pairs.
- It determines which base in the mismatch is the incorrect one.
- It excises the incorrect base and carries out repair synthesis.
- This is mediated by the mut protein system.

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



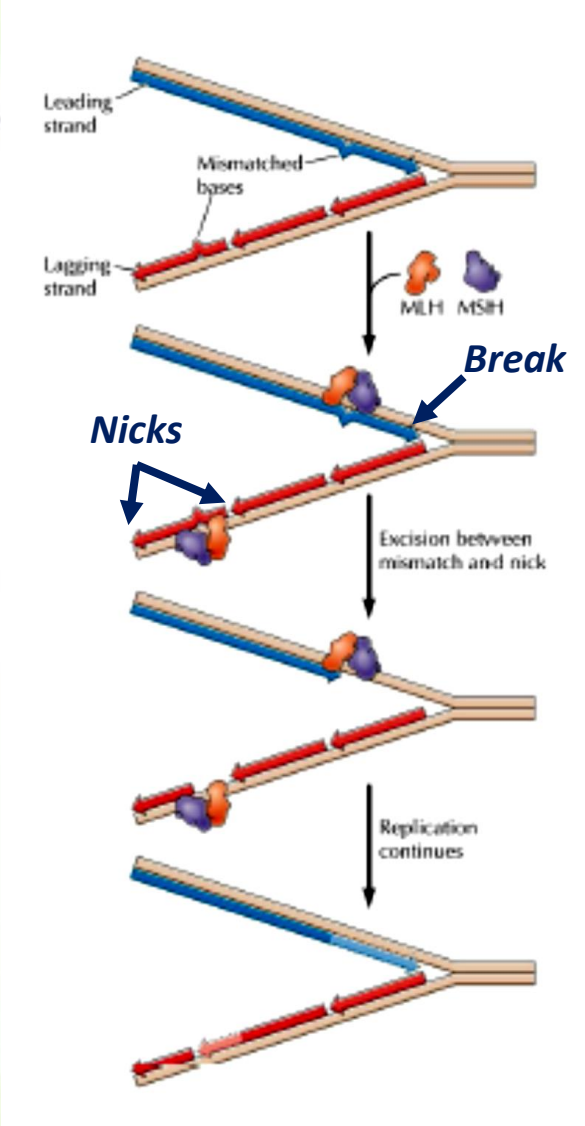
DNA methylation

- DNA is methylated following replication by the enzyme, adenine methylase.
- 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.



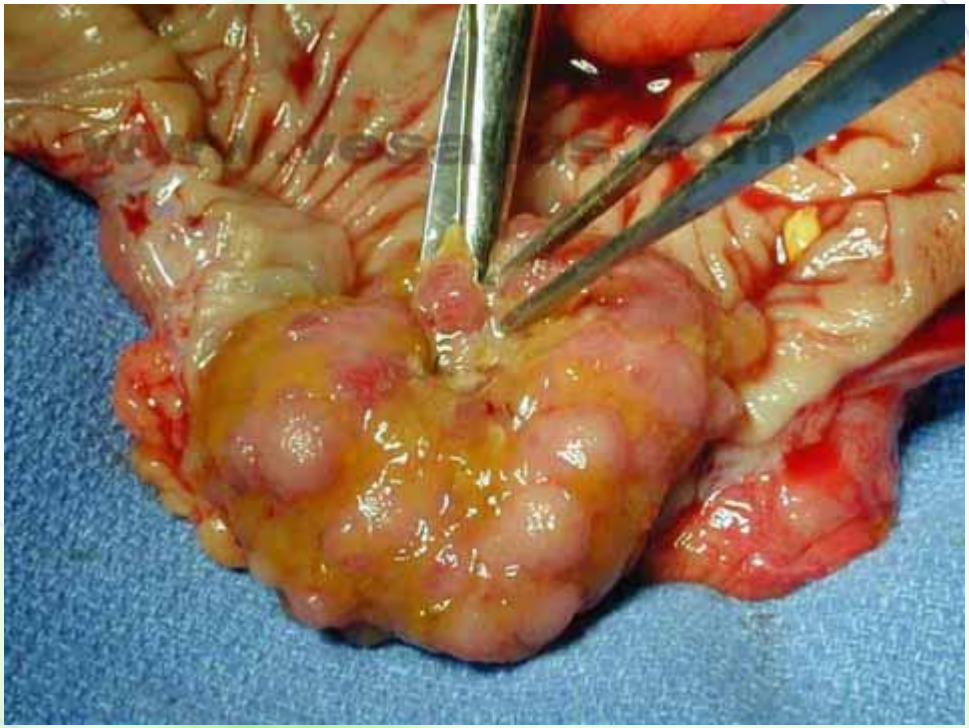
Mismatch repair in humans

- Two proteins, hMSH2 and hMLH1, are very similar to their bacterial counterparts, MutS and MutL, respectively.
- 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.



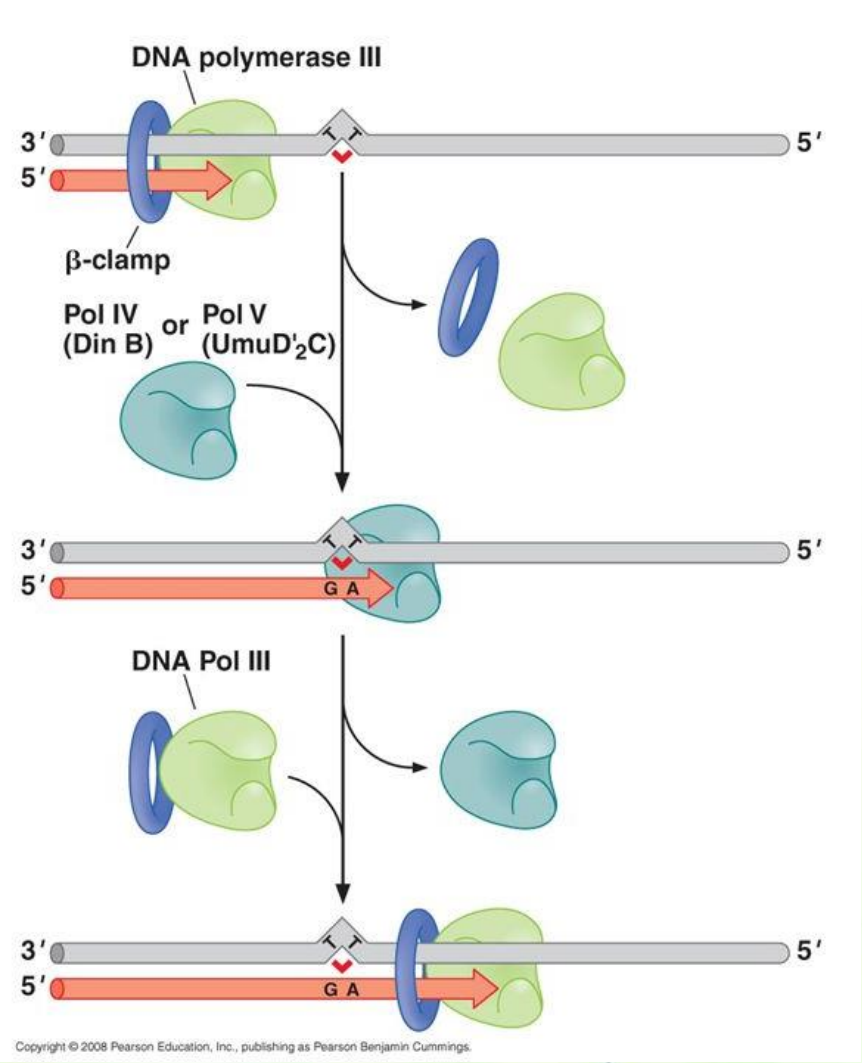
Hereditary nonpolyposis colon cancer (HNPCC)

- 15% of colon cancer cases.
- It is mainly caused by mutations in MSH followed by mutated MLH.

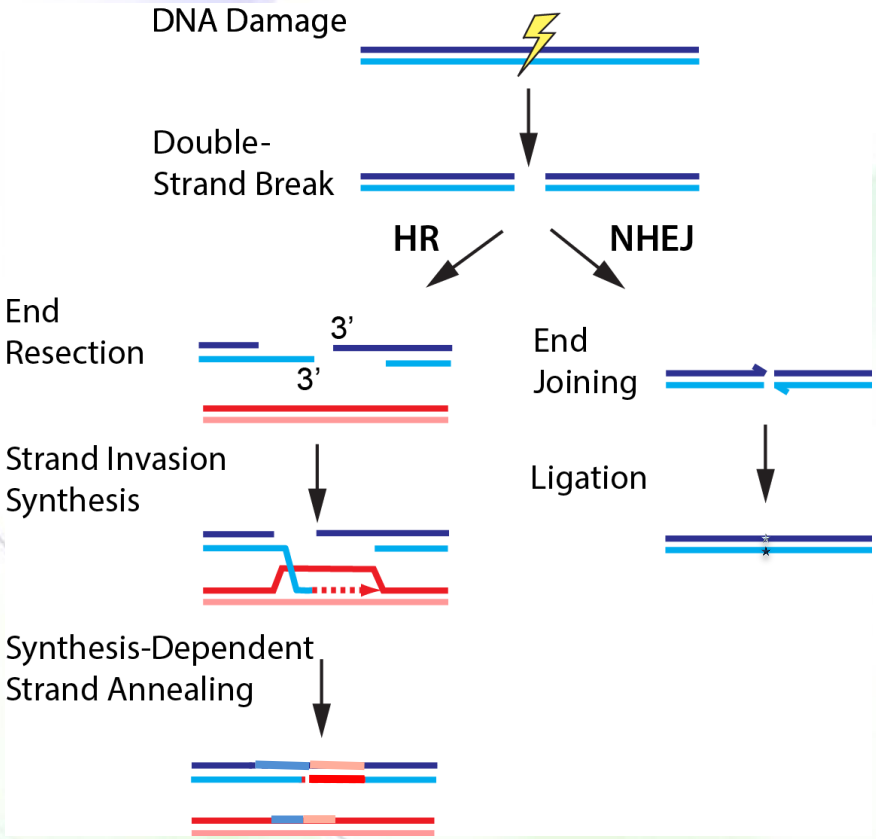


Translesion DNA synthesis

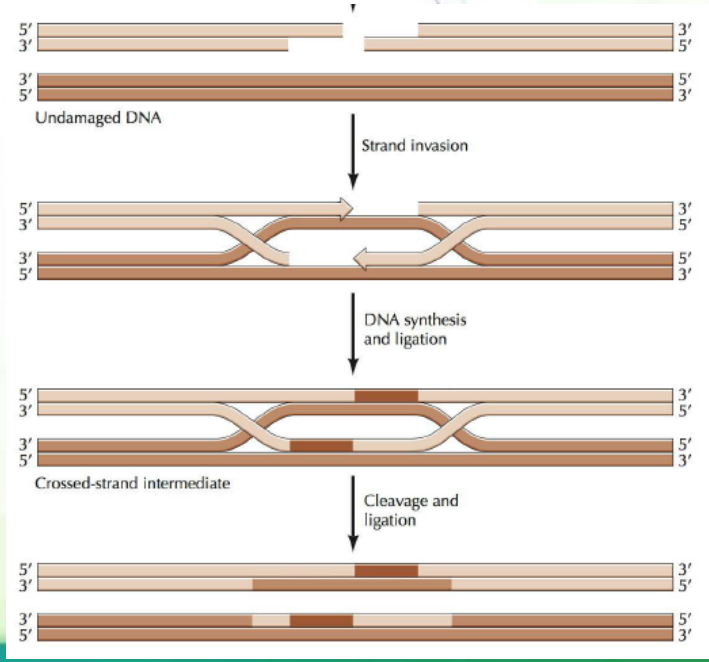
- In prokaryotes and eukaryotes, specialized DNA polymerases can bypass DNA mutations by the ability of DNA polymerases to synthesize DNA over the lesions.
- Although they display some selectivity in base insertion, they have **low fidelity and lack proofreading mechanism and, hence, are error-prone**.
  - Any C is likely to be deaminated, inducing a C to T transition.
- However, they are biased toward introduction of As, so that TT dimers are often replicated correctly.



Recombinational repair

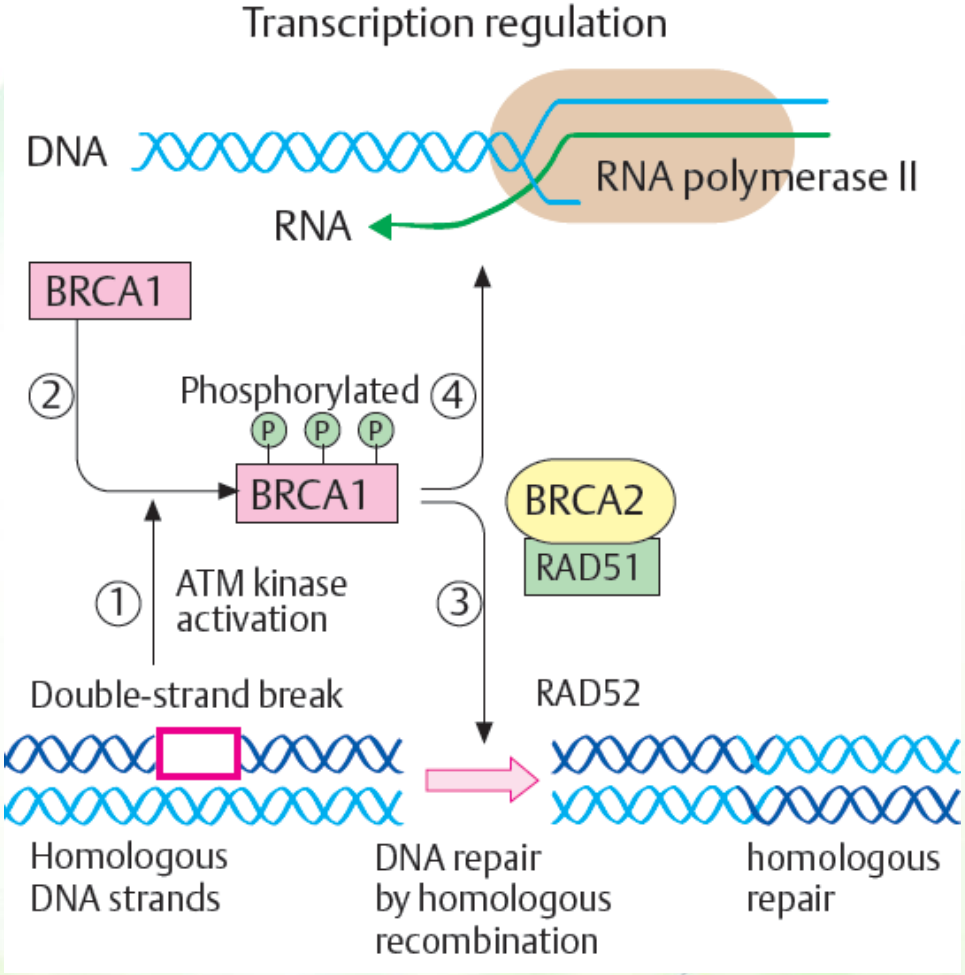


- When double-strand breaks of DNA occur, recombinational repair taakes place by:
  - Non-homologous end joining (NHEJ), which fixes DNA, but **creates mutations**.
  - Homologous repair with the undamaged chromosome.
    - This involves Rad51 protein.



Breast cancer

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

Controversial issue

Gene repair

UK scientists ready to genetically modify human embryos

Researchers awaiting approval to use gene editing in embryos, which they hope will help them understand early stage life and improve fertility treatment

https://www.theguardian.com/science/2016/jan/13/uk-scientists-ready-to-genetically-modify-human-embryos