Molecular Biology (5)

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
- Transcription-coupled repair
- Mismatch repair
- Translesion DNA synthesis
- Repair of double-strand breaks
- Recombinational repair

Prevention of errors before they happen

Reactive oxygen species

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

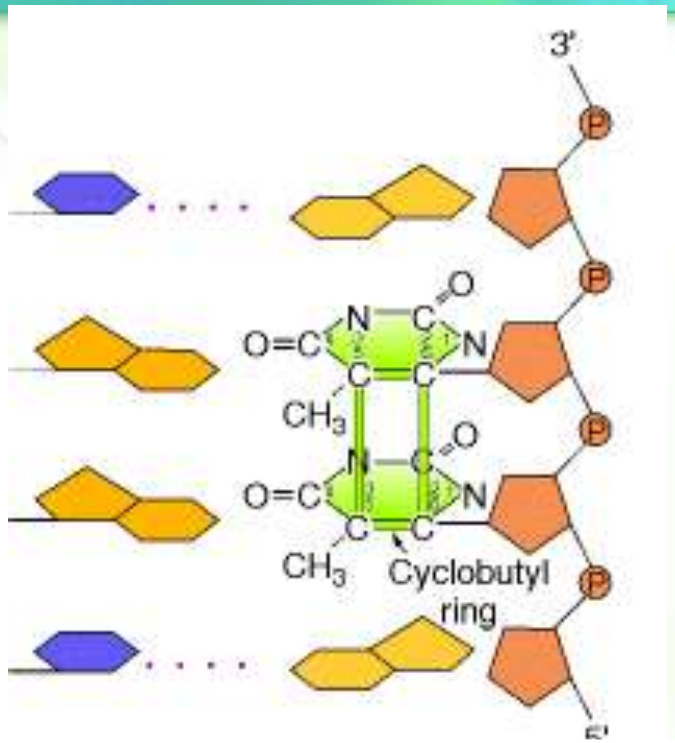
$$2\,O_2^{\bullet-} + 2\,H^+ \xrightleftharpoons{\text{Superoxide dismutase}} O_2 + H_2O_2$$

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

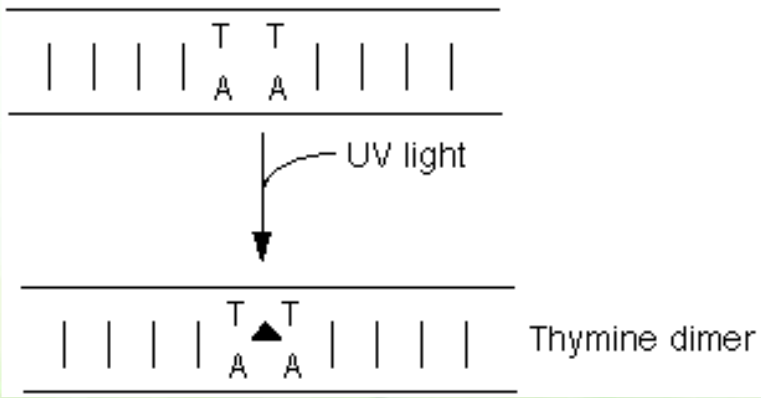
Direct reversal of damage

Cyclobutane pyrimidine

- Some lesions can be repaired by reversal of DNA damage.
- UV light that hits DNA results in the formation of a covalent interaction between two adjacent pyrimidine bases forming structures known as cyclobutane pyrimidine dimers, most frequently between two thymines.
- This product is a mutagenic photodimer.

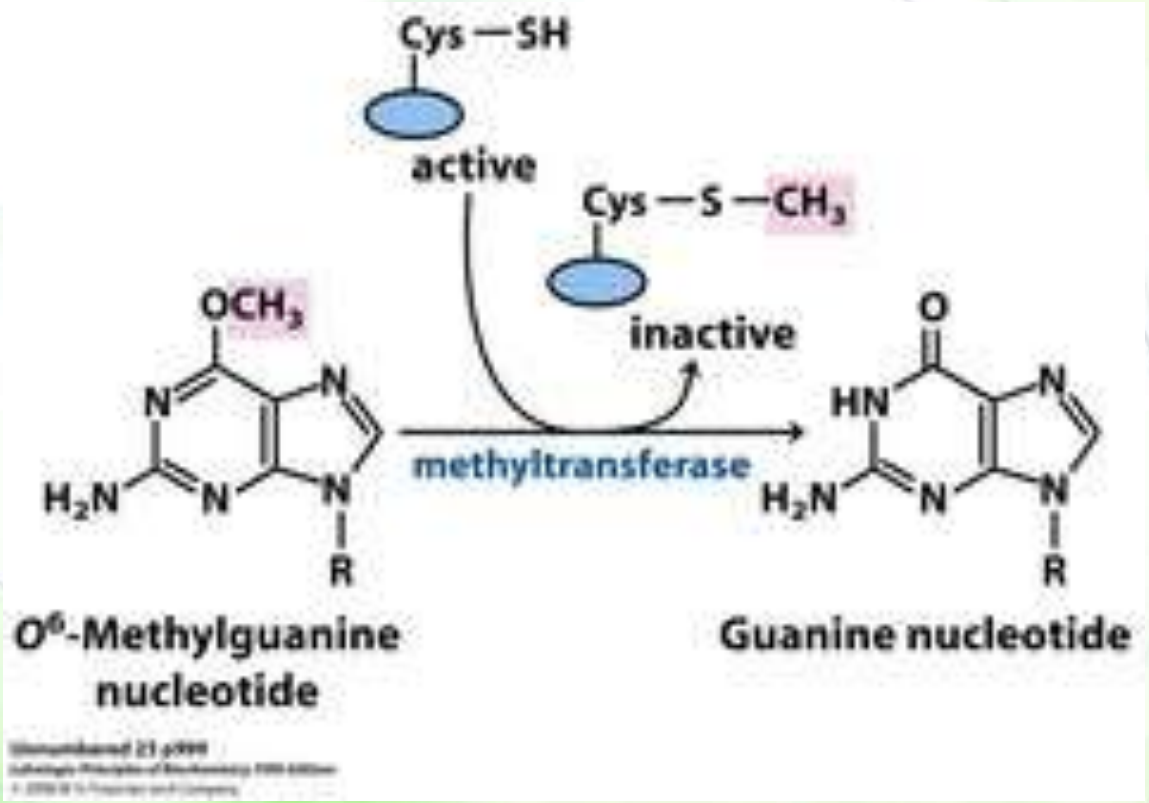


Photoreactivation Repair



Repair of O6-methylguanine

- This is done via O6-methylguanine methyltransferase.



Exision-repair pathways

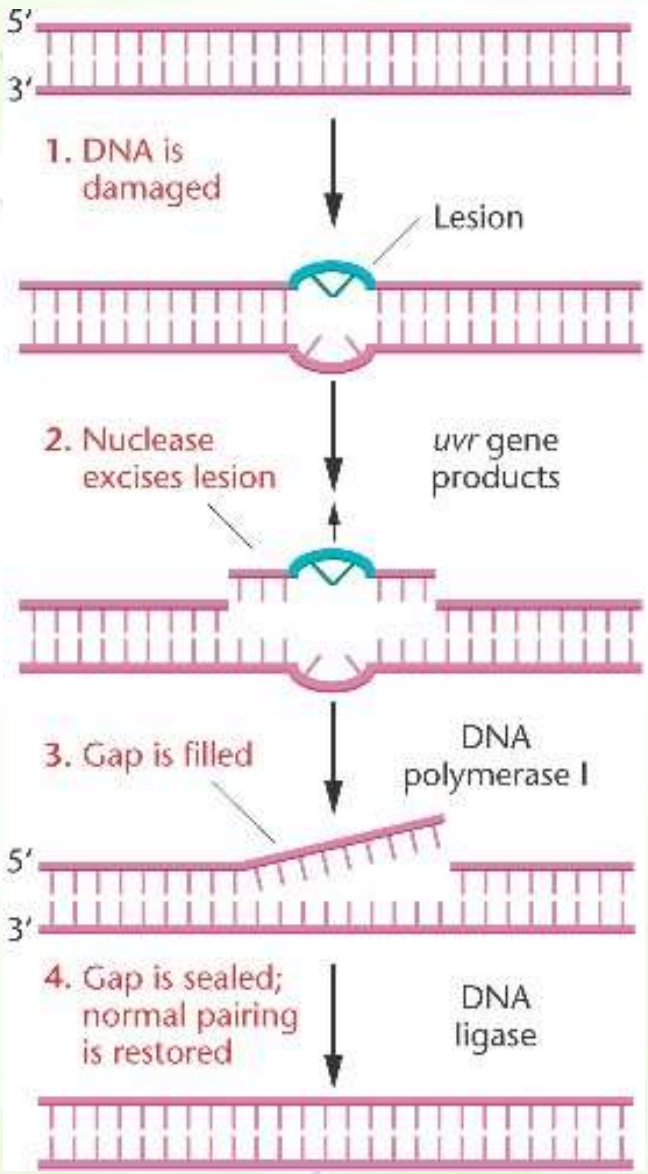
Types

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

General excision repair

AKA: 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 and a ligase seals the breaks.

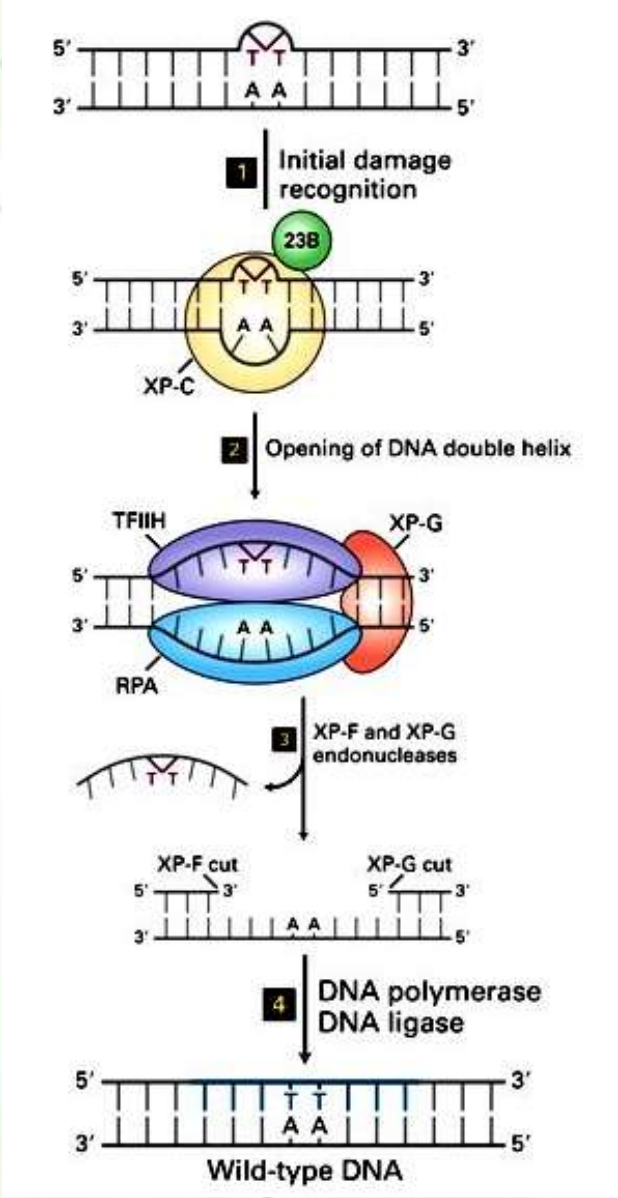


In human…

- 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 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.

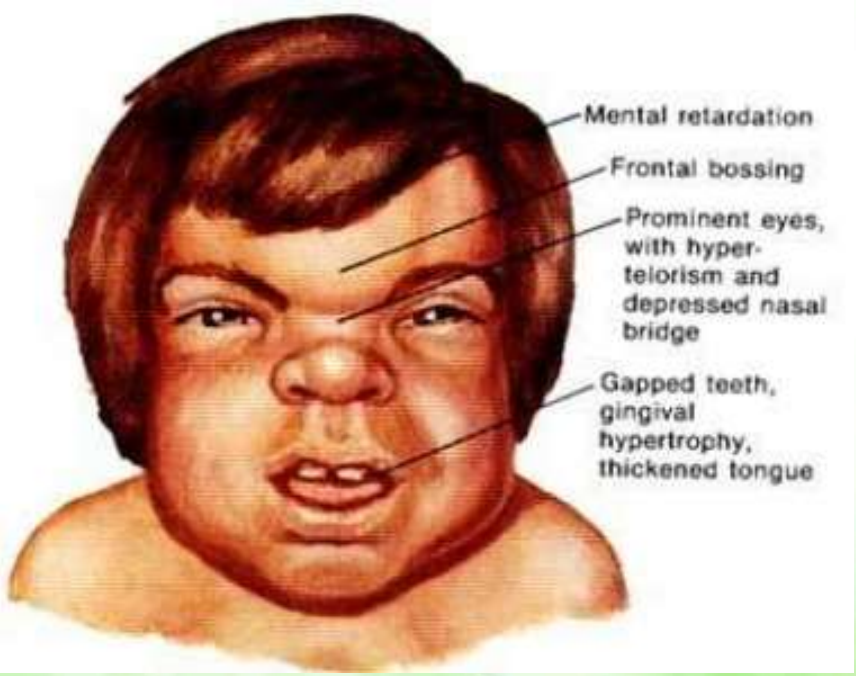


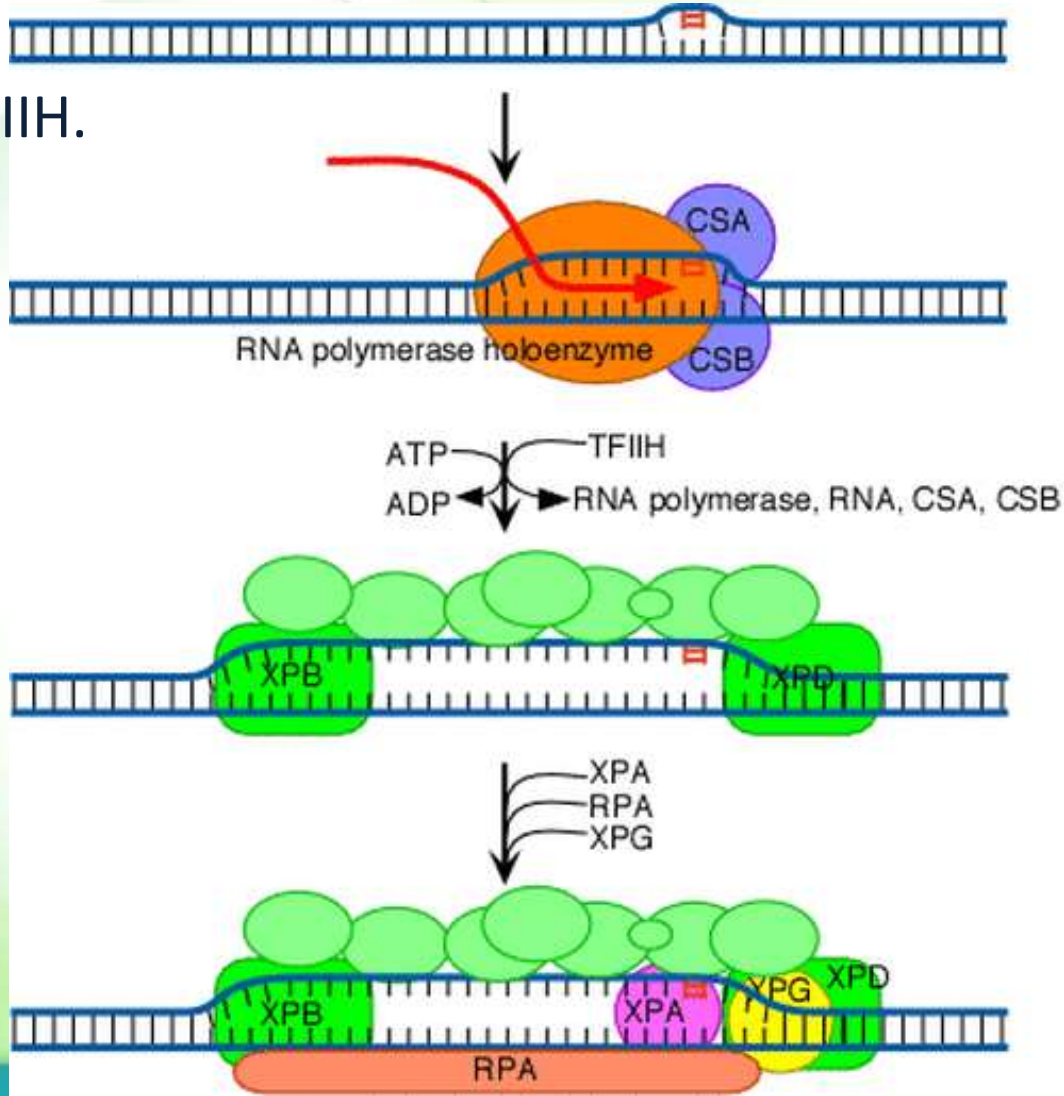
Transcription and repair

- In both eukaryotes and prokaryotes, there is a preferential repair of the transcribed strand of DNA for actively expressed genes.
- RNA polymerase pauses 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.





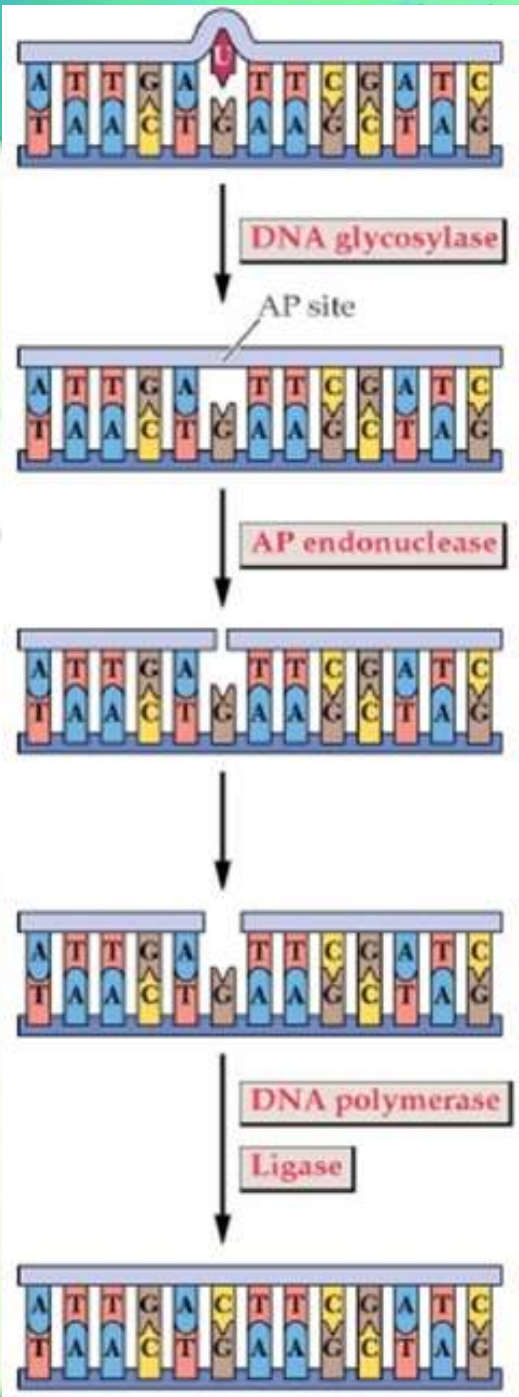
Specific exision pathways

DNA glycosylase repair pathway

- 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 resulting AP site is then 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.
- The AP endonucleases cleaves the phosphodiester bonds at AP sites.
- The deoxyribose is removed. DNA polymerase fills in the gap, and DNA ligase and re-forms the bond.

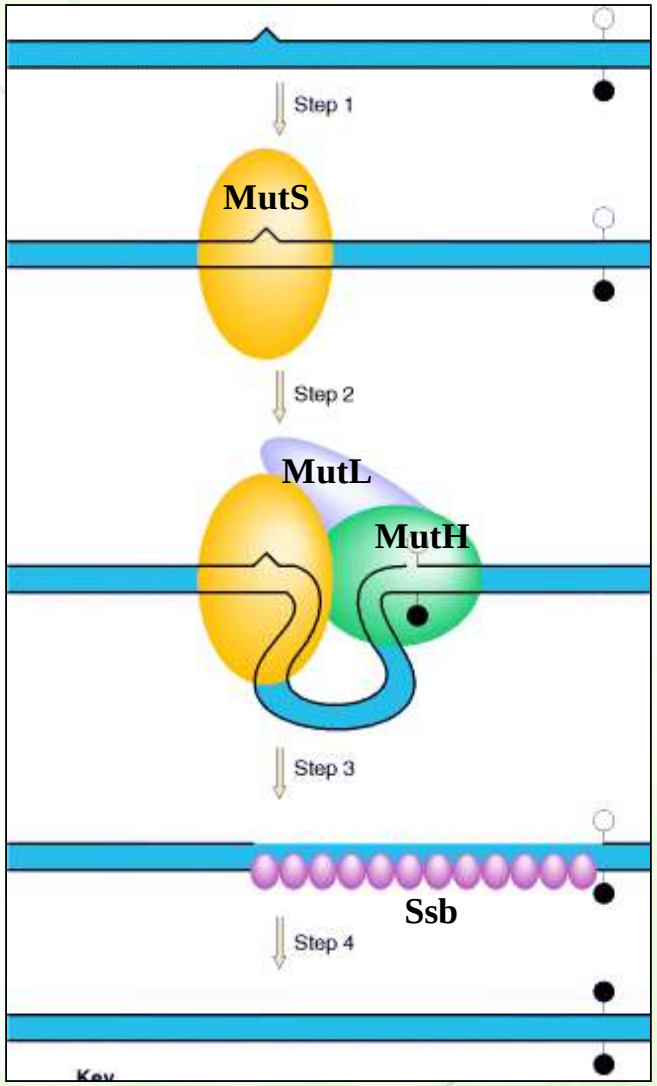


Postreplication repair

Mismatch repair system (prokaryotes)

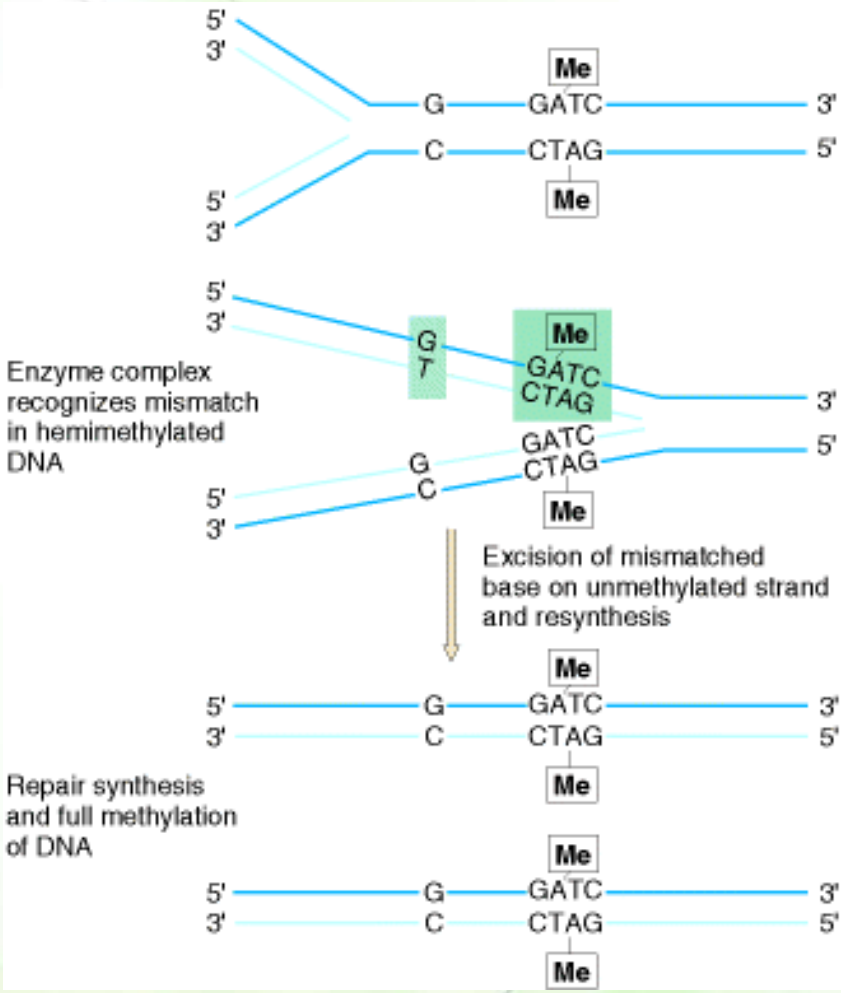
- Recognize mismatched base pairs.
- Determine which base in the mismatch is the incorrect one.
- Excise the incorrect base and carry out repair synthesis
- This is mediated by the mut protein system.

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



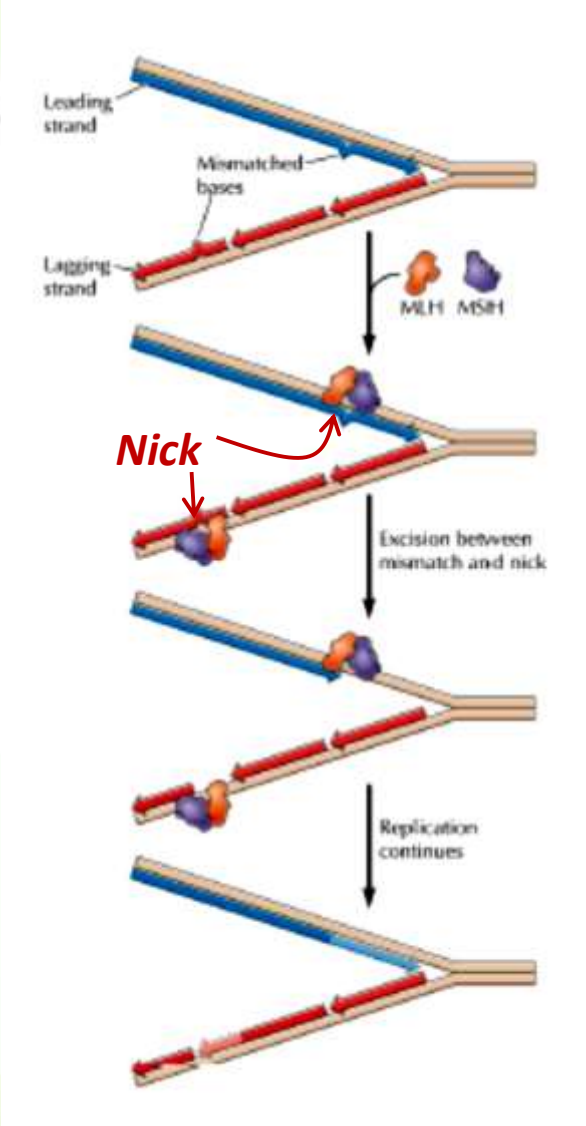
DNA methylation

- 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.



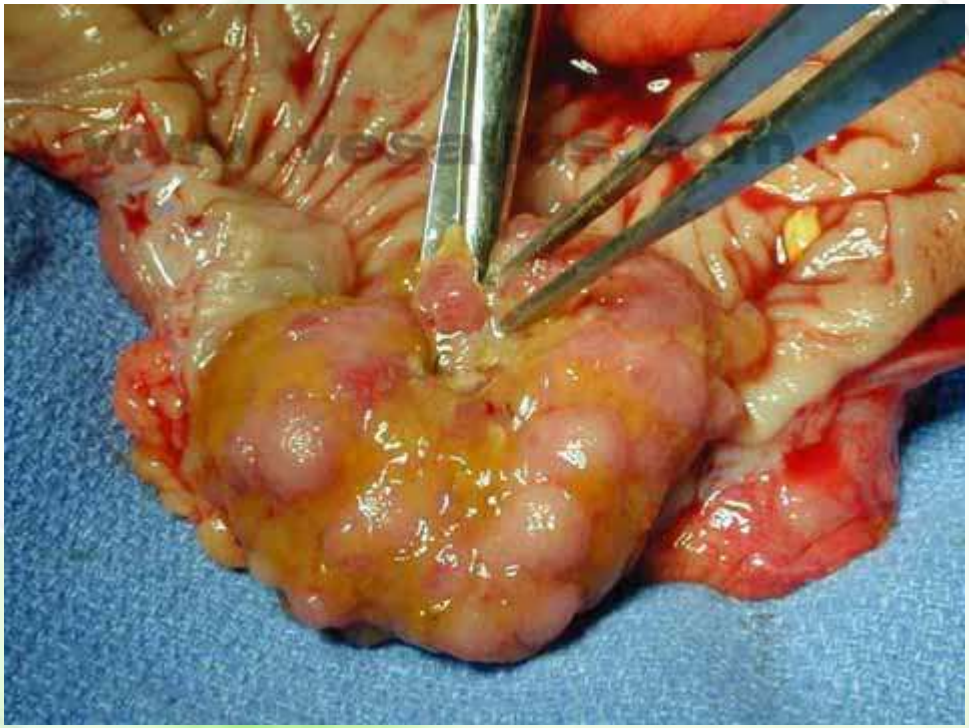
In humans

- The mismatch repair system has also been characterized in humans.
- Two of the proteins, hMSH2 and hMLH1, are very similar to their bacterial counterparts, MutS and MutL, respectively.



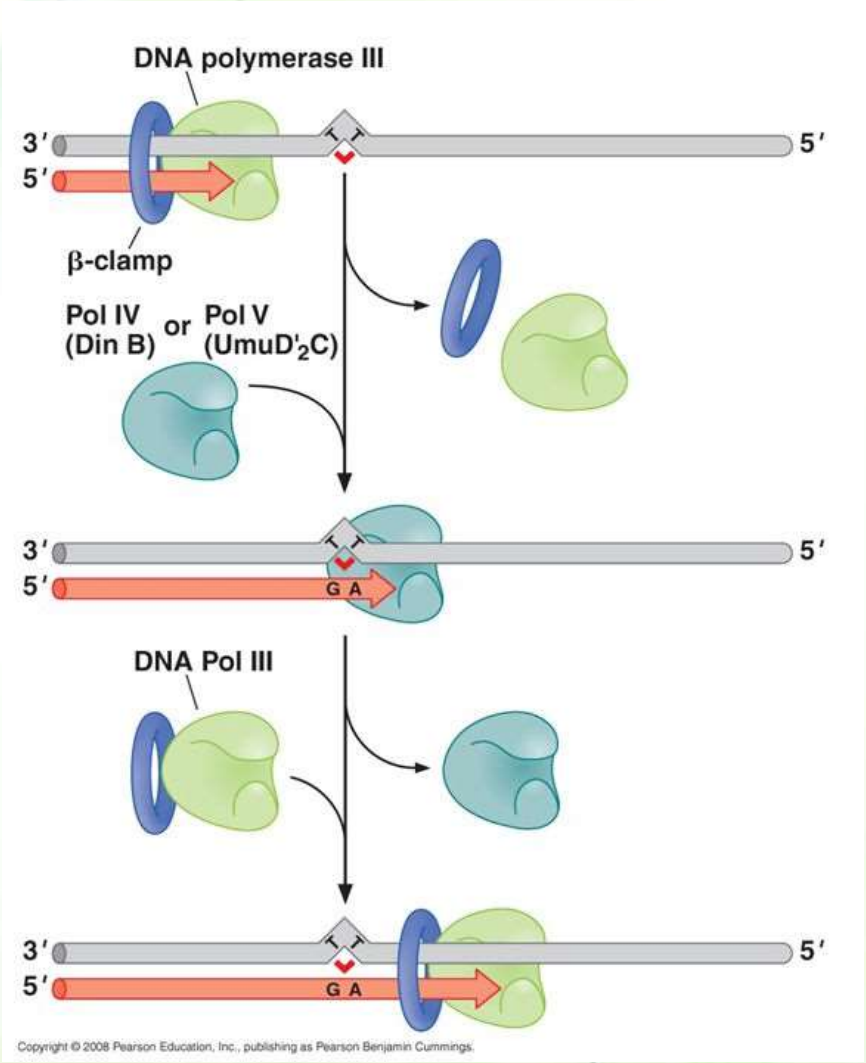
Hereditary nonpolyposis colon cancer (HNPCC)

- 15% of colon cancer cases.
- 50% caused by mutation in MSH and most of the remaining is caused by mutated MLH.

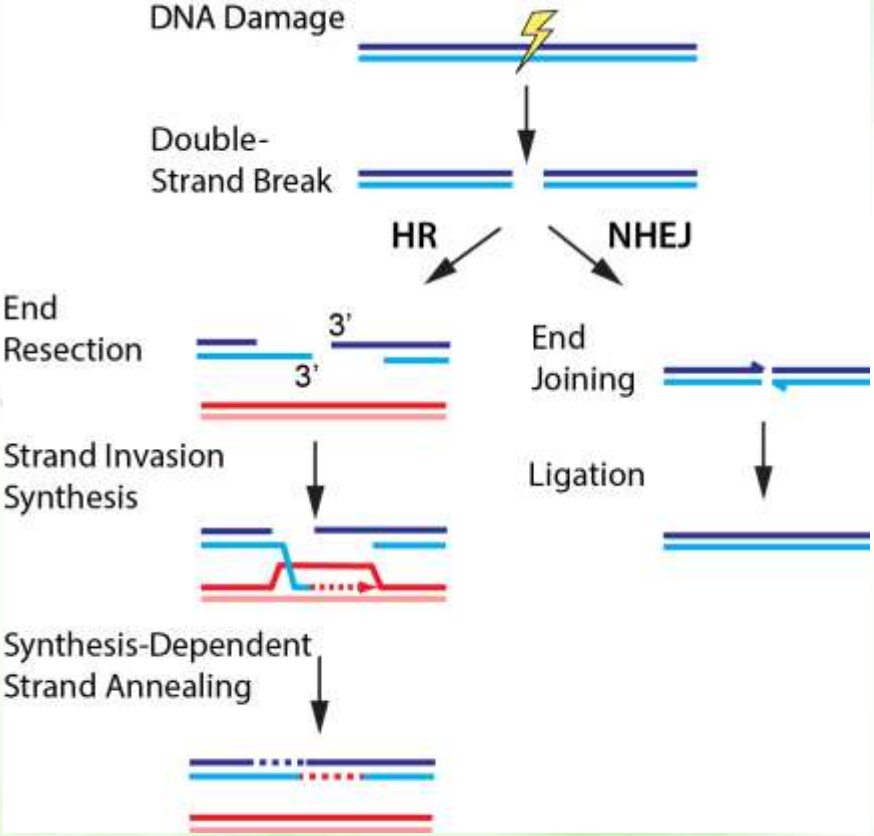


Translesion DNA synthesis

- In prokaryotes and eukaryotes, specialized DNA polymerases can bypass DNA mutations by the ability of DNA polymerases to synthesize DNA over the lesions.
- These DNA polymerases replace the normal replicating enzyme temporarily.
- Although they display some selectivity in base insertion, they have **low fidelity and are error-prone**.



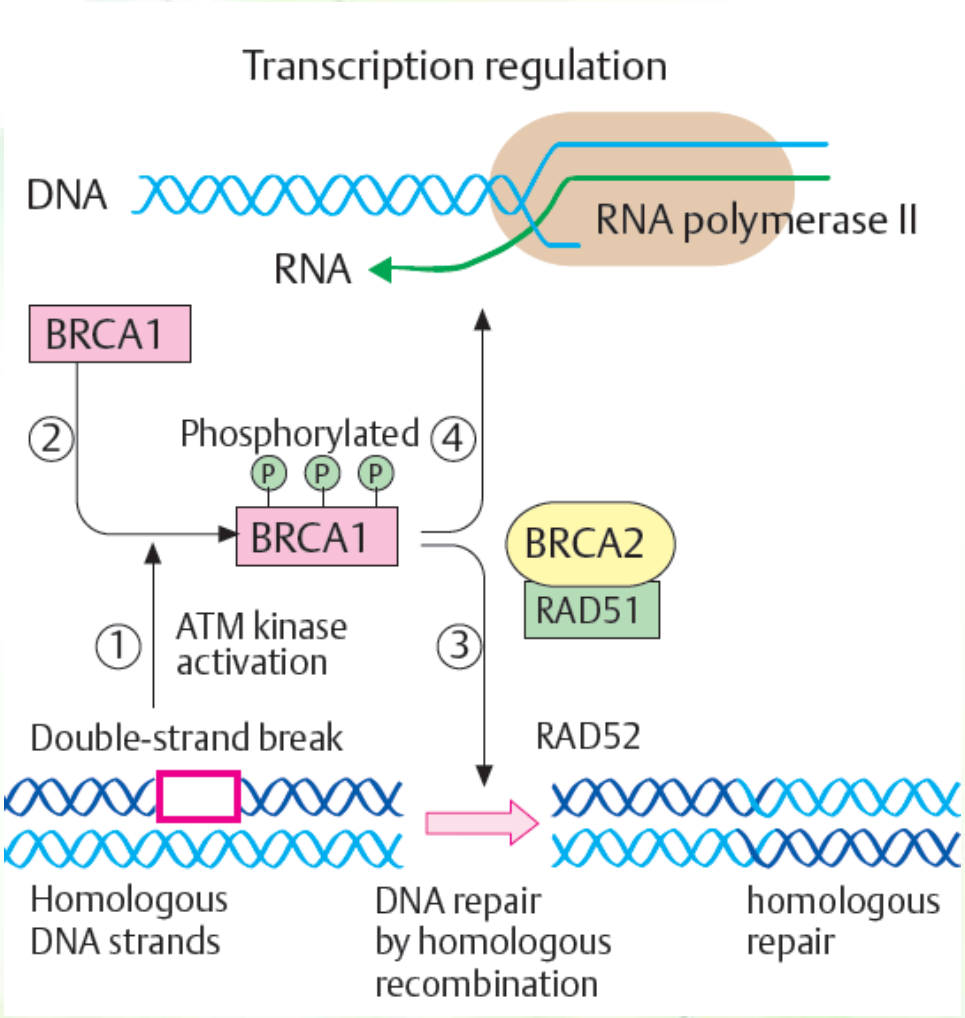
Recombinational repair



- 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 a protein called Rad51.

Breast cancer

- Mutations in BRCA1 account for 2% of all breast cancers and, at most, 5% of ovarian cancer.
- BRCA1 activates homologous recombination repair of DNA double-stranded breaks by recruiting Rad51 to the ssDNA.
- BRCA1 is also involved in transcription and **transcription-coupled DNA repair**.



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