

DNA Repair Mechanism



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- 4) Double strand break repair systems (*Homologous recombination (HR) and Nonhomologous End-joining (NHE-J)*)

DNA mismatch and damage



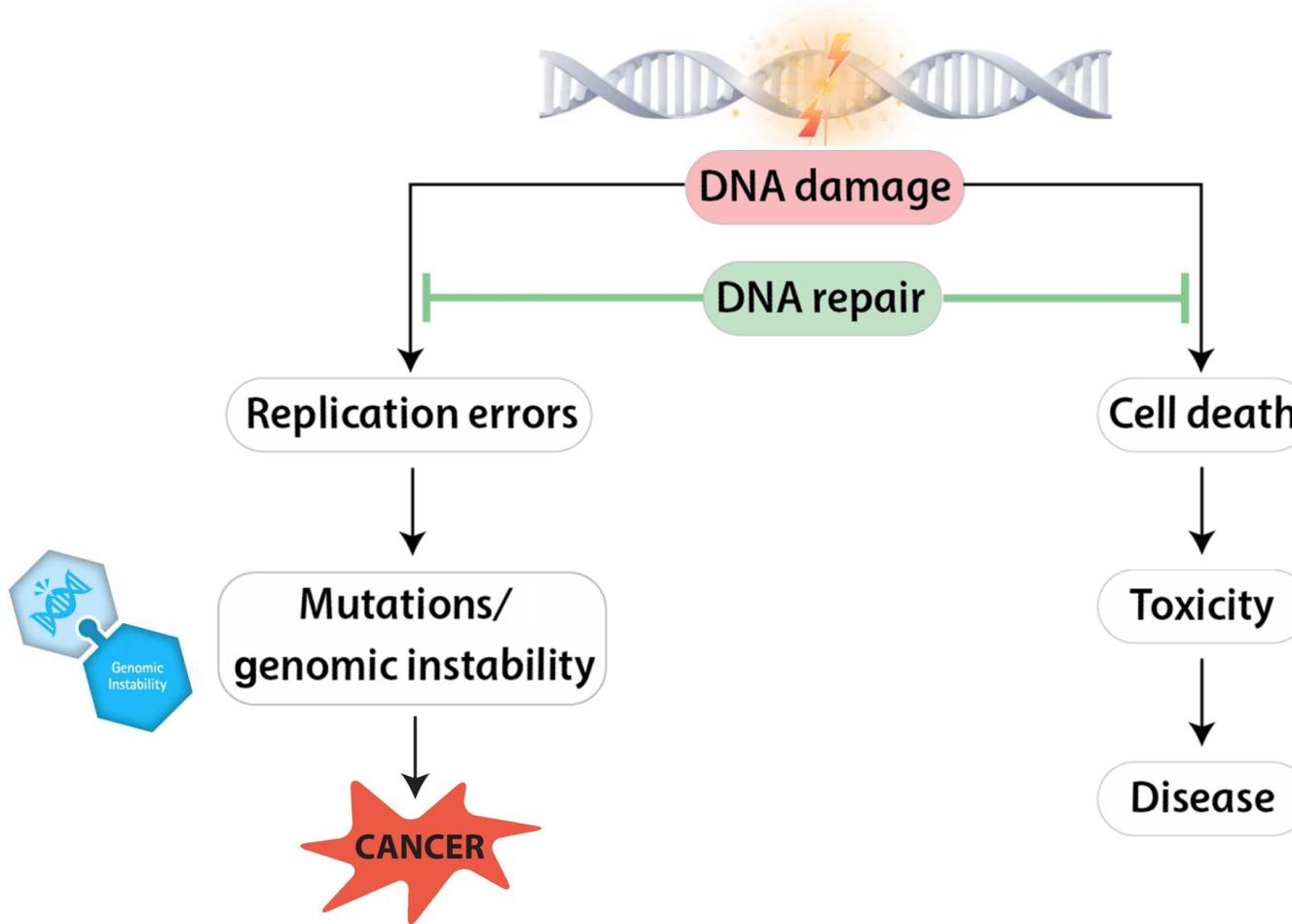
Endogenous
(Cellular metabolic processes)

- Mismatch of DNA bases
- Hydrolysis
- Oxidation
- Alkylation

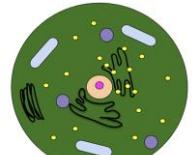
Exogenous
(Environmental factor)

- Ultraviolet (UV) radiation
- Ionizing radiation (IR)
- Chemical agents

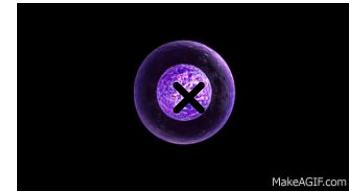
What if there is no DNA repair?



Apoptosis



Senescence



MakeAGIF.com

Cell division

Repair system



Endogenous
(Cellular metabolic processes)

Mismatch repair system (MMR)

- Mismatch of DNA bases
- Hydrolysis
- Oxidation
- Alkylation

Base Excision repair (BER)

Exogenous
(Environmental factor)

Nucleotide Excision repair (NER)

- Ultraviolet (UV) radiation
- Ionizing radiation (IR)
- Chemical agents

Double strand break

Mismatch repair (MMR) system

Mismatch Repair (MMR): corrects replication errors (**base-pair**

mismatches and small insertion-deletions

replication, by removing mismatches and resynthesizing it using the parental strand

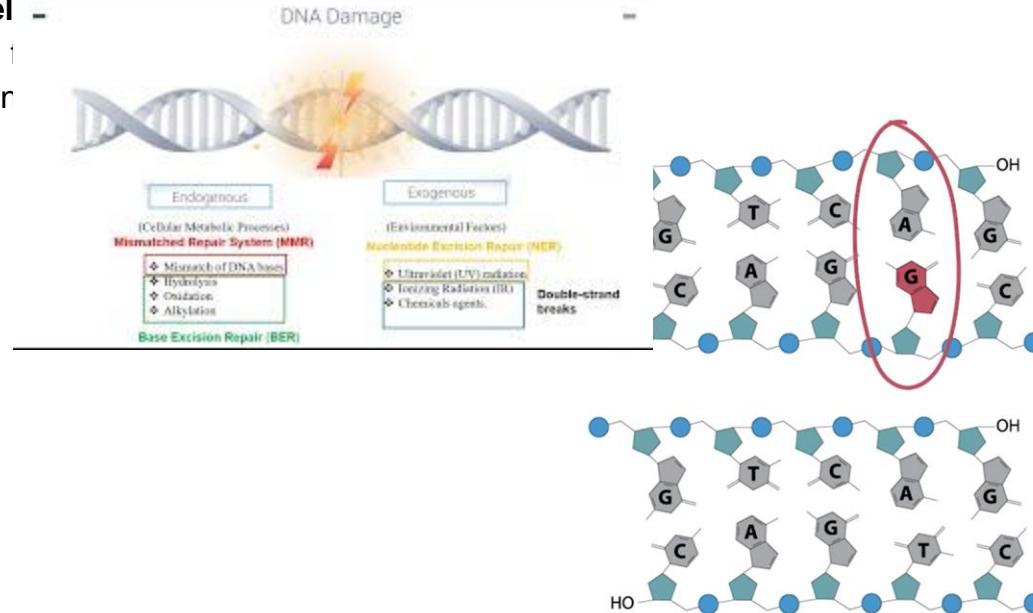
General steps of MMR:

- 1) **Recognize**
- 2) Strand Discrimination
- 3) Recruit key components
- 4) **Incision**
- 5) **Excision**
- 6) **Resynthesize**
- 7) **Ligation**

Types of MMR:

- 1) **MMR in Bacteria:** Methyl-Directed Mismatch Repair

- 2) **MMR in Eukaryotes and Archaea:** Clamp-Directed Mismatch Repair



MMR in bacteria: Methyl-Directed Mismatch Repair

Core mechanism: *Methyl-directed mismatch repair.*

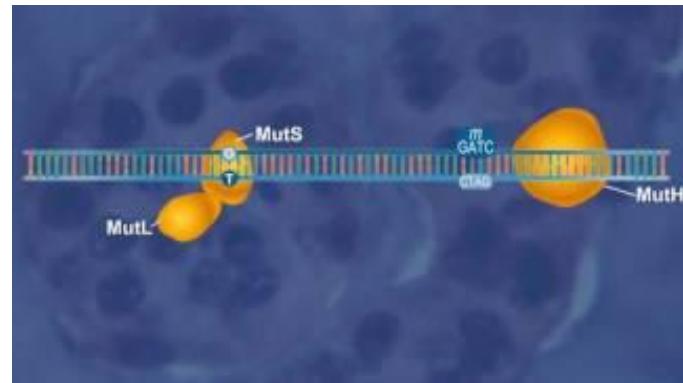
Relies on **DNA adenine methylation at GATC sites** to distinguish the old strand from the newly synthesized strand.

Key Components

- **MutS** → recognizes base-pair mismatches & small insertion–deletion loops → clamps (ATP)
- **MutL** → mediator protein → bridges MutS to MutH
- **MutH** → **endonuclease**, cuts the **unmethylated** strand
- **Exonucleases** → remove DNA containing the error
- **DNA polymerase III** → synthesizes the gap
- **DNA ligase** → seals the nick

Methyl-Directed MMR Types in Bacteria

- **MutH-dependent, methyl-directed system (*E. coli*)**
- **MutH-independent systems** → MutL provides strand incision;

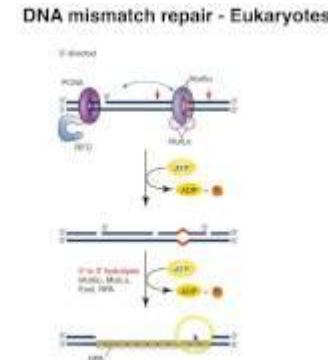


MMR in Eukaryotes and Archaea: Clamp Directed Mismatch Repair

Core mechanism: *Clamp-directed mismatch repair*: Uses the **sliding clamp PCNA** to identify the newly synthesized strand.

Key components:

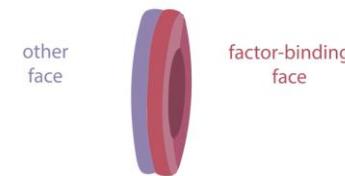
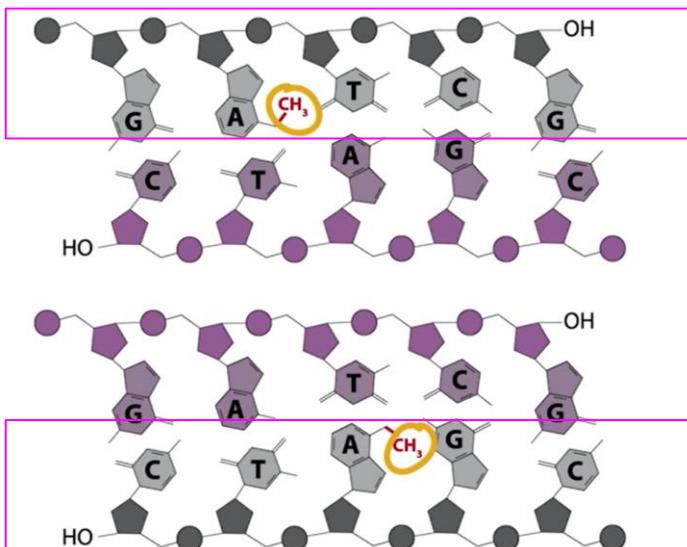
- **MutS homologs** → Bind to distorted DNA after replication.
 - **MSH2–MSH6 (MutS α)** → single-base mismatches
 - **MSH2–MSH3 (MutS β)** → small insertion–deletion loops
- **MutL homologs**:
 - **MLH1–PMS2 (MutL α)** → has **endonuclease** activity
- **PCNA (sliding clamp)** → directs repair to the new strand
- **Exonucleases** → remove DNA past the mismatch
- **DNA polymerase δ / ϵ** → resynthesize
- **DNA ligase** → seal the strand



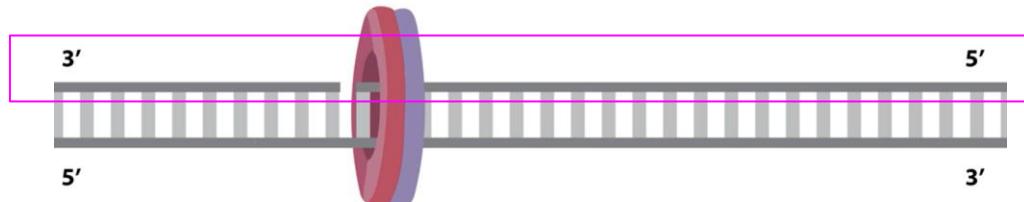
Repair Steps

- MSH complex detects mismatch
- Recruits MLH/PMS
- PCNA directs MutL α to nick the new strand
- Exonuclease excises DNA beyond the mismatch
- Polymerase fills gap → ligase seals

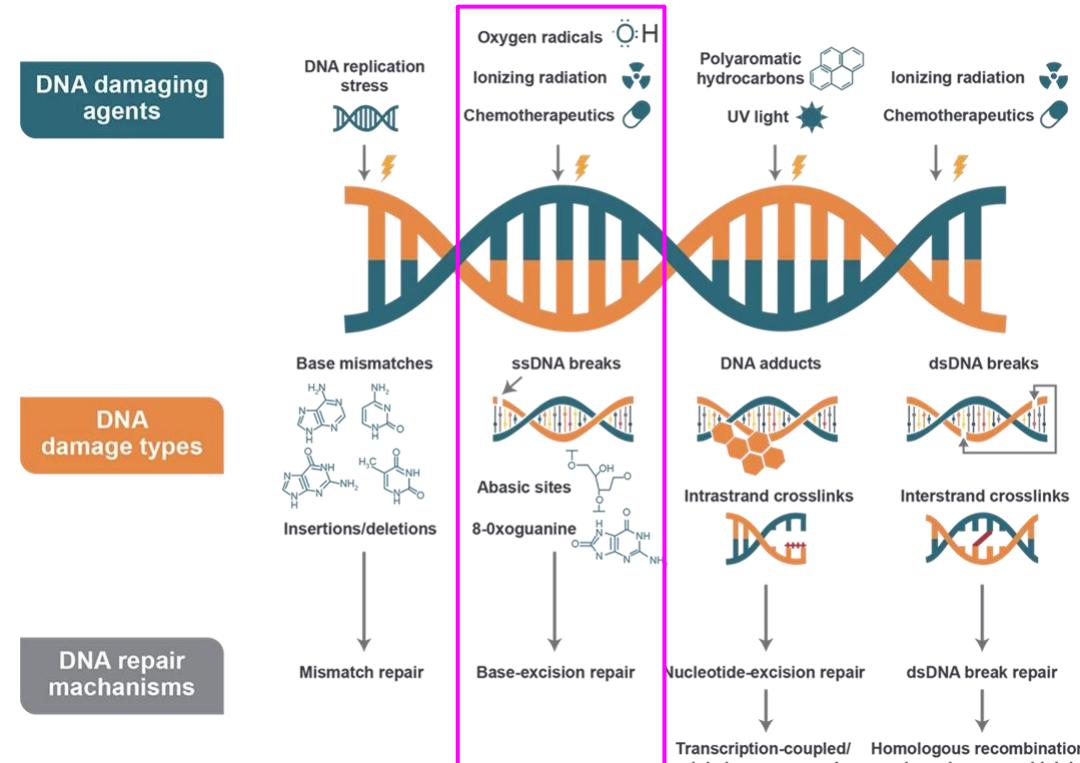
DNA adenine methylation vs Sliding Clamps



Which direction would the sliding clamp load?



DNA repair mechanism



Base damage types

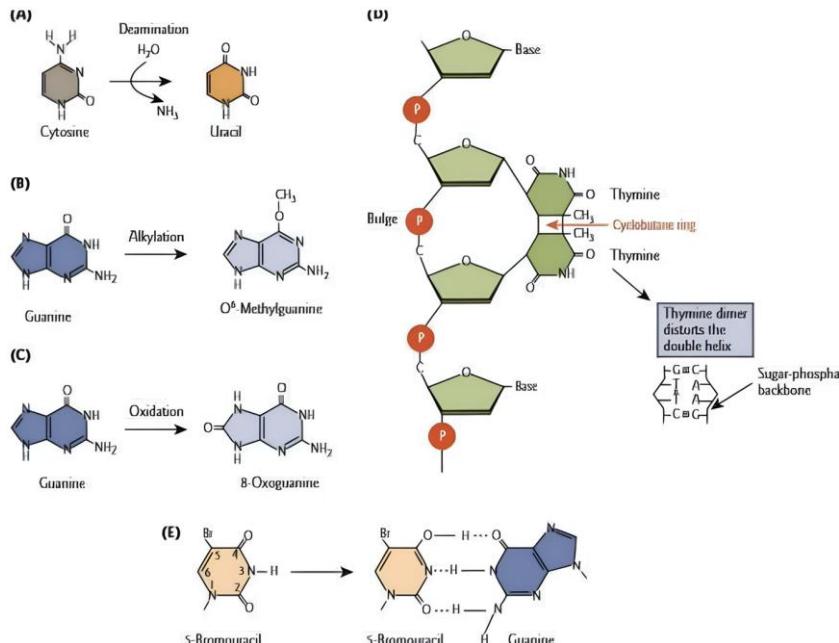


Figure 7.3 Types of DNA damage. (A) Single base change by deamination of cytosine to uracil. (B) Alkylation of the oxygen on carbon atom 6 of guanine generates O^6 -methylguanine. (C) Oxidation of guanine generates 8-oxoguanine. (D) UV radiation induces the formation of a cyclobutane ring between adjacent thymidylates, forming a thymine dimer. This leads to structural distortion of the duplex DNA. (E) 5-Bromouracil, a base analog of thymine, can mispair with guanine.

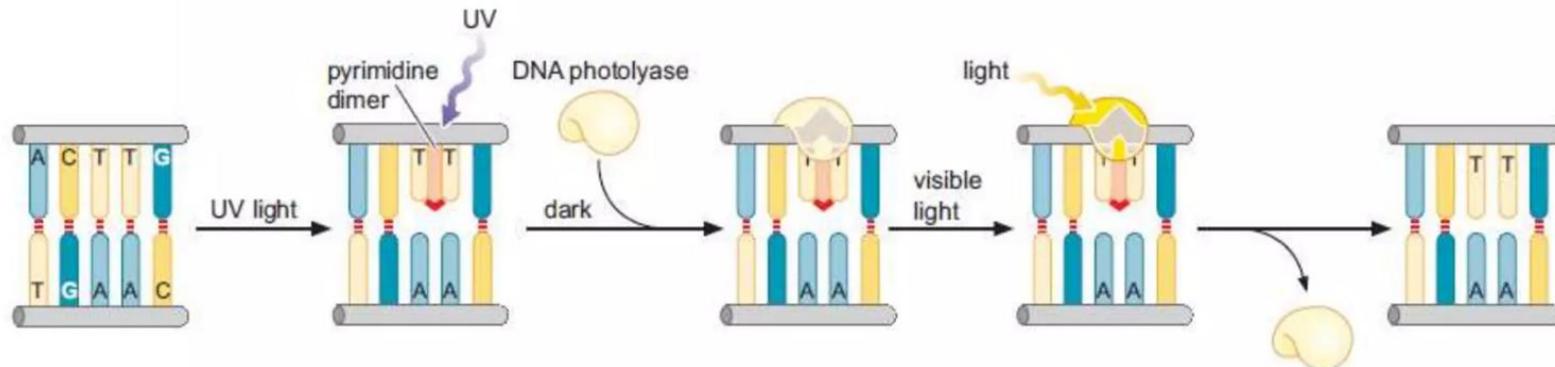
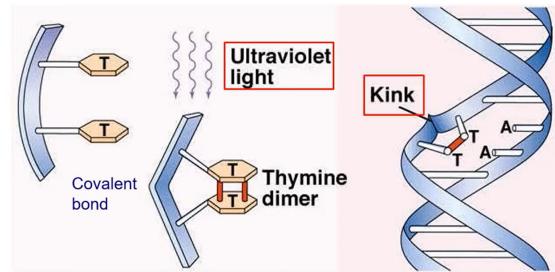
Direct reversal repair

- This system act directly on damaged nucleotides and convert each one back to its original structure.
- But only a few damaged nucleotides can be repaired directly.
- Pyrimidine dimers are repaired by a light-dependent direct system called photoreactivation.
- O6-methylguanine-DNA methyltransferase I and II (MGMT), also called DNA alkyl transferases, remove the modified bases like O6-alkylguanine and O4-alkylthymine.
- The photolyase protein is not found in all living cells. However, the DNA alkyl transferases are widespread in nature.

Direct reversal repair

Example – PHOTOREACTIVATION

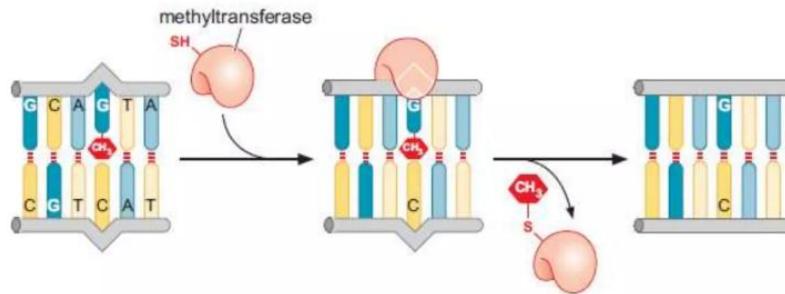
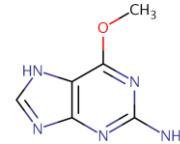
- Photoreactivation directly reverses the formation of pyrimidine dimers that result from ultraviolet irradiation.
- In photoreactivation, the enzyme DNA photolyase captures energy from light and uses it to break the covalent bonds linking adjacent pyrimidines.



Direct reversal repair

Another example:

- removal of the methyl group from the methylated base O⁶-methylguanine.
- In this case, **a methyltransferase removes the methyl group** from the guanine residue by transferring it to one of its own cysteine residues.
- This is costly to the cell because the methyltransferase is **not catalytic**; having once accepted a methyl group, it cannot be used again.



Base Excision Repair

Steps of Base Excision Repair

1. Damage Recognition

DNA glycosylase identifies the abnormal base.

2. Base Removal

Glycosylase cleaves the N-glycosidic bond → creates an **AP site**.

3. Backbone Cleavage

AP endonuclease cuts the sugar-phosphate backbone.

4. End Processing

Residual sugar fragments are removed.

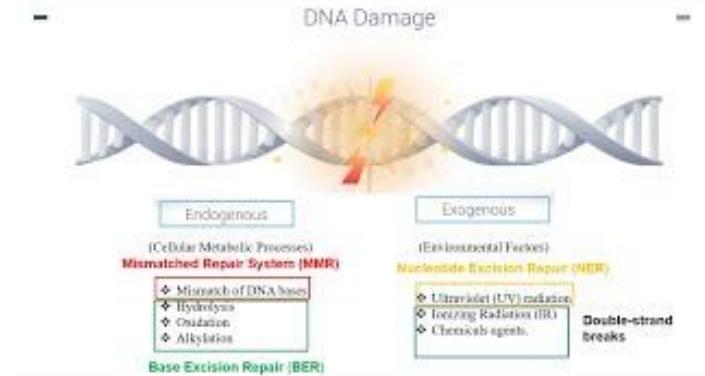
5. DNA Synthesis

DNA polymerase inserts the correct nucleotide(s).

- *Short-patch BER*: replaces 1 base
- *Long-patch BER*: replaces 2–10 bases

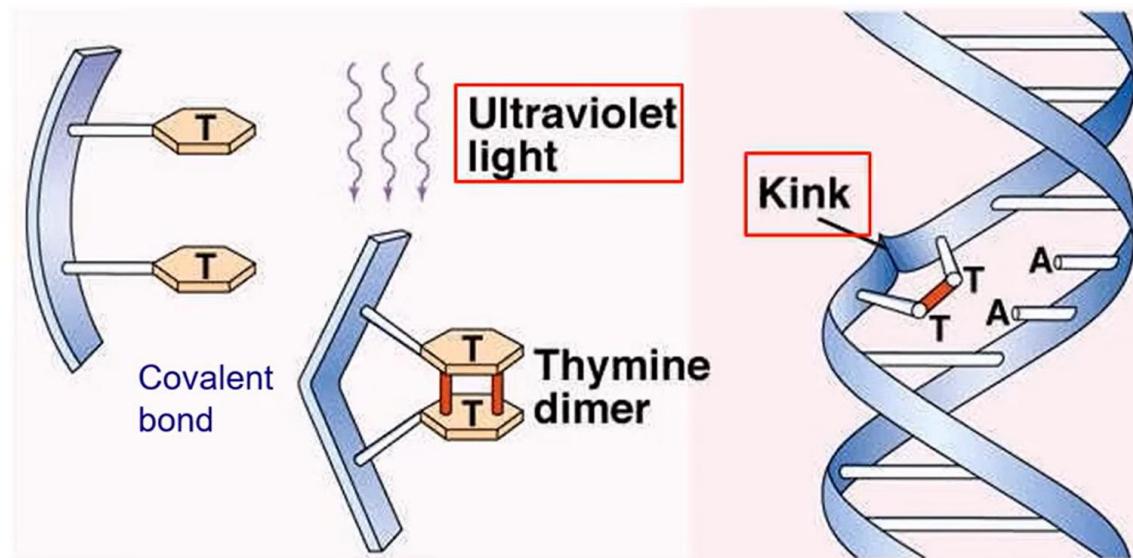
6. Ligation

DNA ligase seals the strand.



Nucleotide excision repair

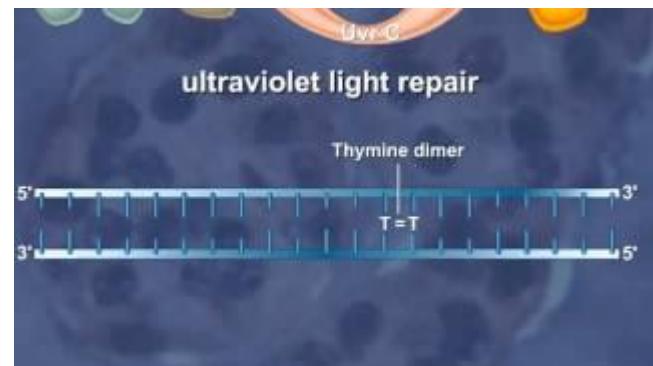
Pyrimidine dimer



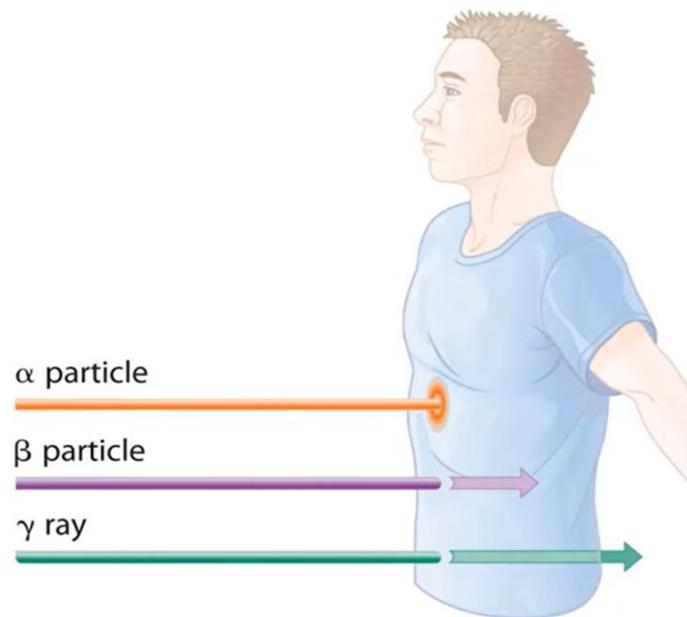
Nucleotide excision repair

NER in *E. coli*: Stepwise Process

- **Detection:**
UvrA₂–UvrB complex binds DNA and scans for helix distortion.
- **Damage Recognition:**
Complex stops at the lesion (e.g., thymine dimer); UvrA dissociates while UvrB remains bound.
- **Incision:**
UvrC binds to UvrB and cuts the damaged strand on both sides of the lesion.
- **Removal:**
UvrD helicase unwinds and releases the excised DNA fragment.
- **DNA Synthesis:**
DNA polymerase fills the gap using the intact strand as a template.
- **Ligation:**
DNA ligase seals the phosphodiester backbone.



DNA double strand breaks (DSB)



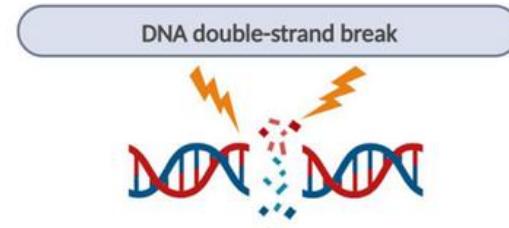
DNA double strand breaks (DSB)



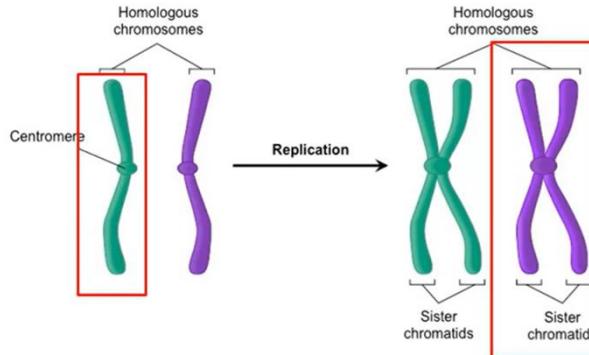
DSBs can cause:

- Cancers
- Genetic disorders
- Developmental abnormalities
- Immunodeficiency
- Neurodegeneration
- Premature aging
- Cell death (apoptosis)

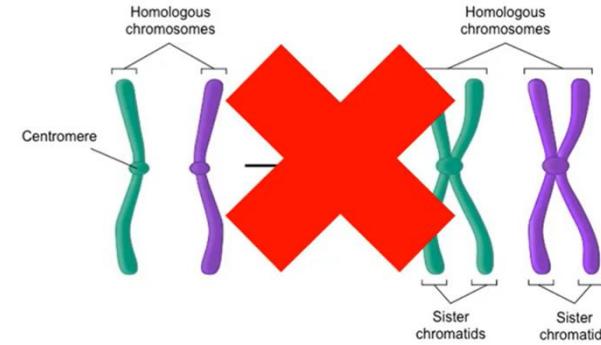
DNA double strand breaks repair



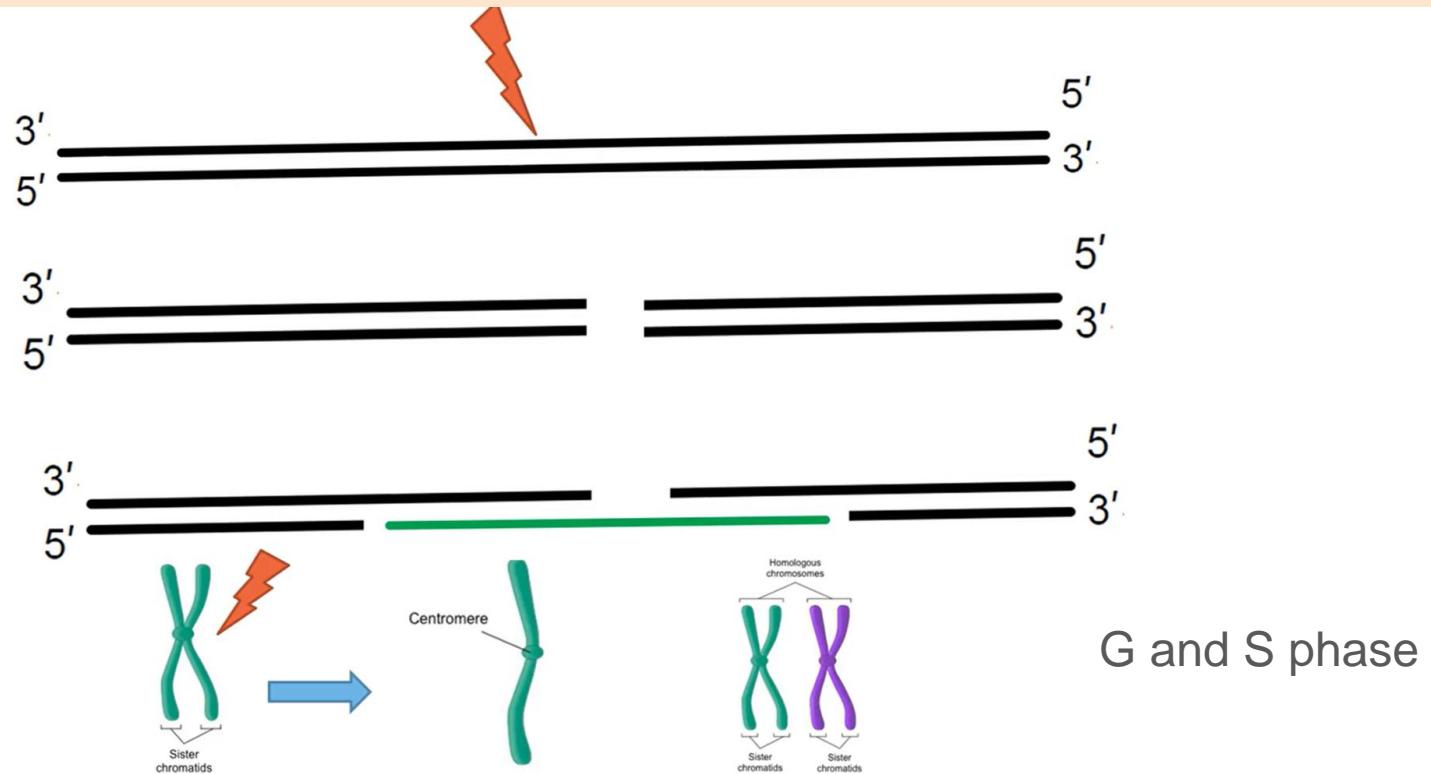
Homologous Recombination (HR)



Nonhomologous End-Joining (NHEJ)



Homologous recombination (HR) repair system



Homologous recombination in eukaryotes

1. DSB recognition

- MRN complex = MRE11 + RAD50 + NBS1

2. End resection

- 5' strand is degraded → creates 3' ssDNA overhang

3. Protection of ssDNA

- RPA coats the single-stranded DNA

4. RPA replacement

- RAD51 + BRCA2 form nucleoprotein filament

5. Homology search

- RAD51 locates matching sequence

6. Strand invasion

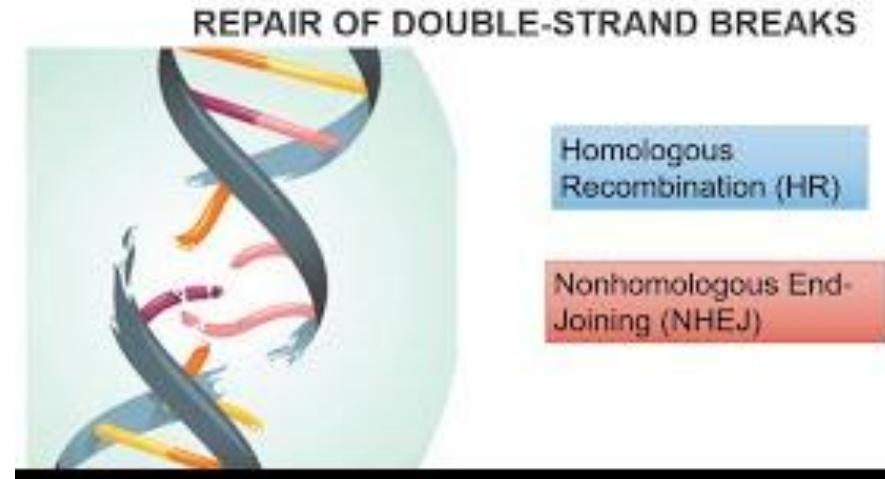
- Formation of a D-loop

7. DNA synthesis

- DNA polymerase extends the 3' end

8. Resolution & ligation

- Intermediates removed → DNA restored

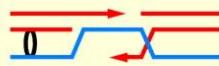


Homologous recombination (HR)

(a) DSB



5' to 3' Resection



5' to 3' Resection

Strand invasion



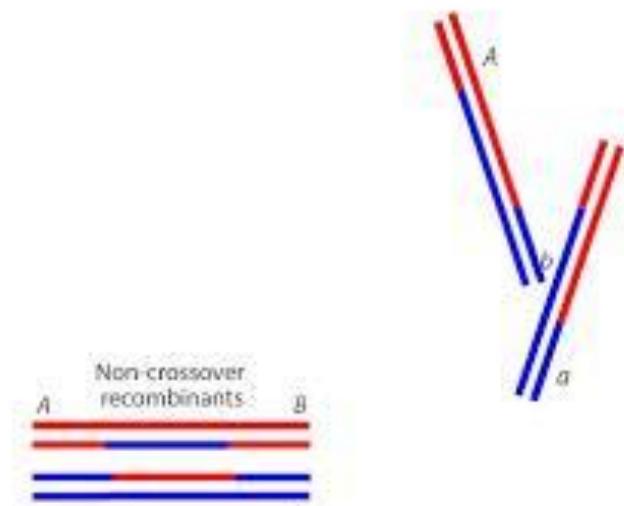
Branch migration and
new DNA synthesis



Holliday junction resolution



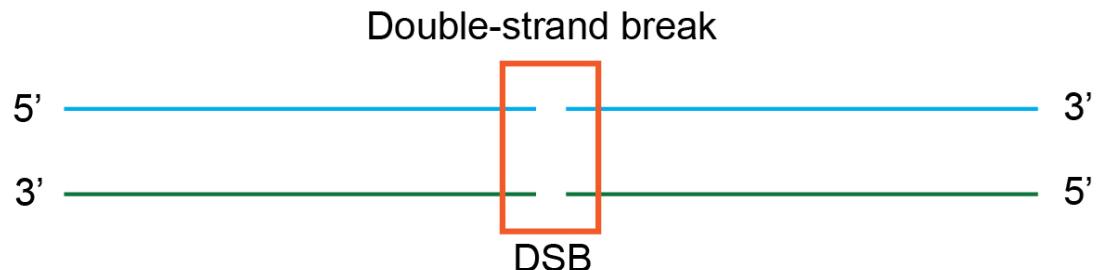
No crossing over Crossing-over of
flanking markers



Homologous recombination in E.coli (bacteria)

1. Double-Strand Break Occurs

DNA is broken by radiation, replication stress, or chemicals.

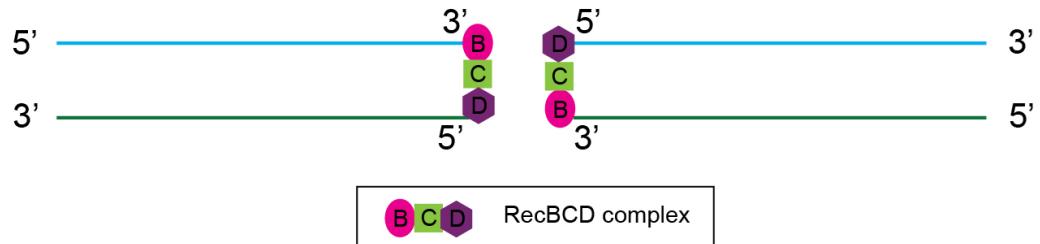


2. Strand resection

RecBCD binds the Break

- unwinds DNA
- trims 5' ends
- creates 3' single-stranded overhang

RecBCD complex loaded at each double-strand break

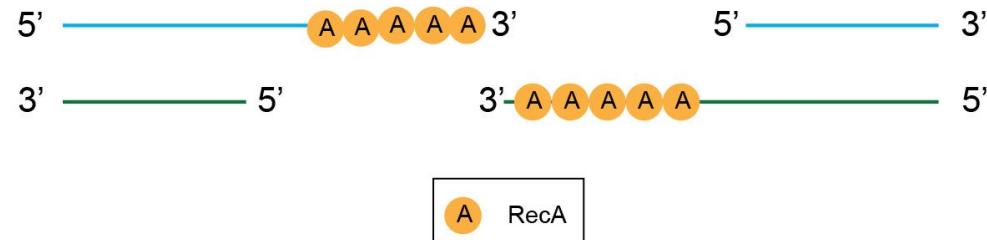


3. Search for homologous DNA

- RecA Loads onto 3' ssDNA
- RecA coats the single strand → forms a nucleoprotein filament.

→ search for homologous DNA

Strand resection and RecA loading

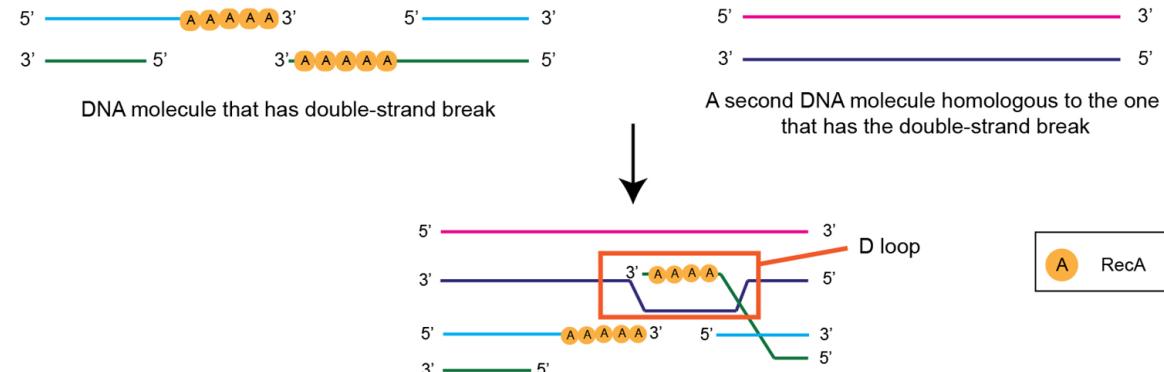


4. Strand Invasion & D-Loop Formation

The RecA-coated strand:

- invades a homologous duplex
- displaces one strand
- forms a **D-loop**.

Strand invasion and displacement



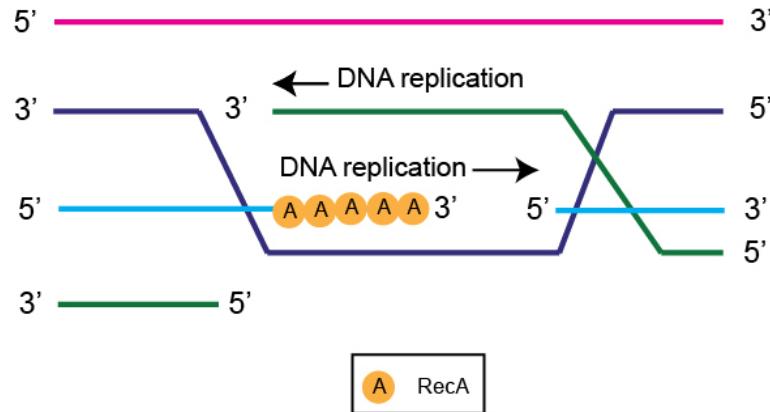
5. DNA Synthesis

- DNA polymerase extends the invading 3' end.
- The second broken end anneals and is also extended.

Strand extension and branch migration

6. Branch Migration

- RuvA & RuvB move the crossover region along DNA.
- This forms **Holliday junctions** between the two molecules.

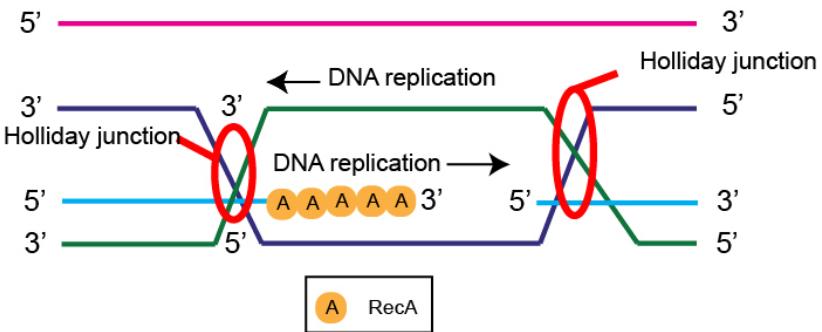


Second cross-over and formation of double Holliday junction

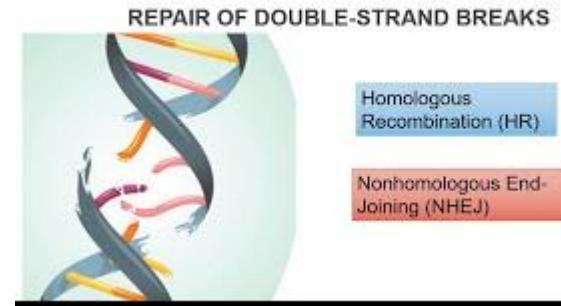
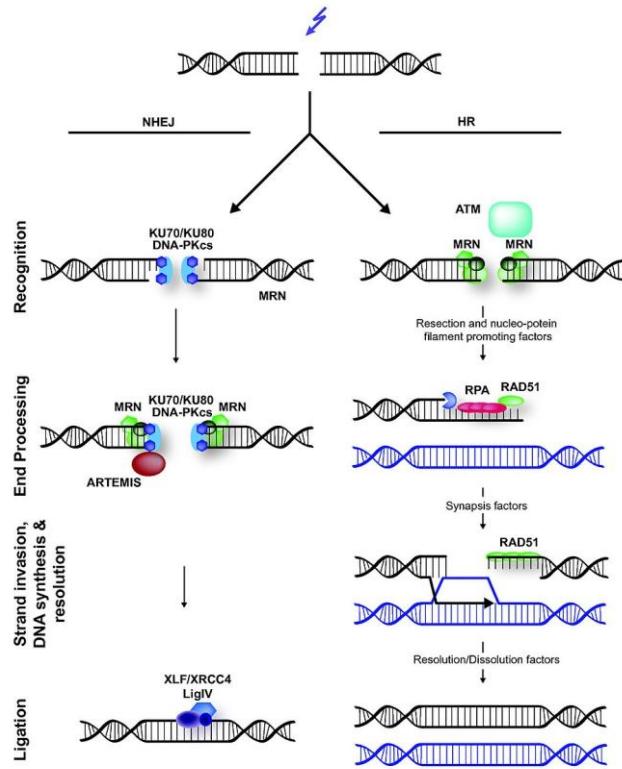
7. Resolution

- RuvC cuts the junctions.
- DNA ligase seals strands.

→ Two repaired DNA molecules are produced.



Nonhomologous End-joining (NHE-J)



Thank you for your
Attention!

DNA structure

