BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Biomedical Science

Course Code: BM35C5
Course Title: Molecular Biology

Unit-II

Replication

Dr. R. POORNIMA
Guest Faculty
Department of Biomedical Science

Unit II:

Replication: Principle and mechanism of replication- Conservative, Semi conservative, Uni- directional and bi-directional mode of replication- Enzymes and accessory proteins involved in replication- Prokaryotic and Eukaryotic DNA replication. DNA damage& repair (Direct repair systems, mismatch repair system, base excision repair and recombination repair)

PRESENTATION: 3

WHAT IS DNA DAMAGE ?

- DNA damage is an alteration in the chemical structure of DNA , such as a break in a strand of DNA, a nucleobase missing from the backbone of DNA, or a chemically changed base such as **8-OHDG.**
- The genetic information encoded in the DNA must remain uncorrupted.
- Any chemical changes must be corrected.
- A failure to repair DNA produces a mutation.

SOURCES OF DAMAGE CELL

ENDOGENOUS DAMAGE

- It includes damage from within the cell.
- It also includes replication errors
- Ex: Mismatch of DNA bases, Hydrolysis, oxidation, alkylation, Free radicals.

EXOGENOUS DAMAGE

- It includes damage caused by external agents
- UV rays, Infrared rays , chemical agents

CONSEQUENCES OF DNA DAMAGE

- Leads to genome instability.
- Increased cancer risk.
- Accelerated aging.
- Neurodegenerative diseases.

DNA REPAIR

❖DNA can be damaged by a variety of processes,

1. some spontaneous

2. Damaged caused by environmental agents

3. Error occurs during replication (introduction of mismatched base pairs such as G paired with T)

❖ The cellular response to this damage includes a wide range of enzymatic systems that catalyze some chemical transformations in DNA metabolism

TYPES OF DNA REPAIR MECHANISMS

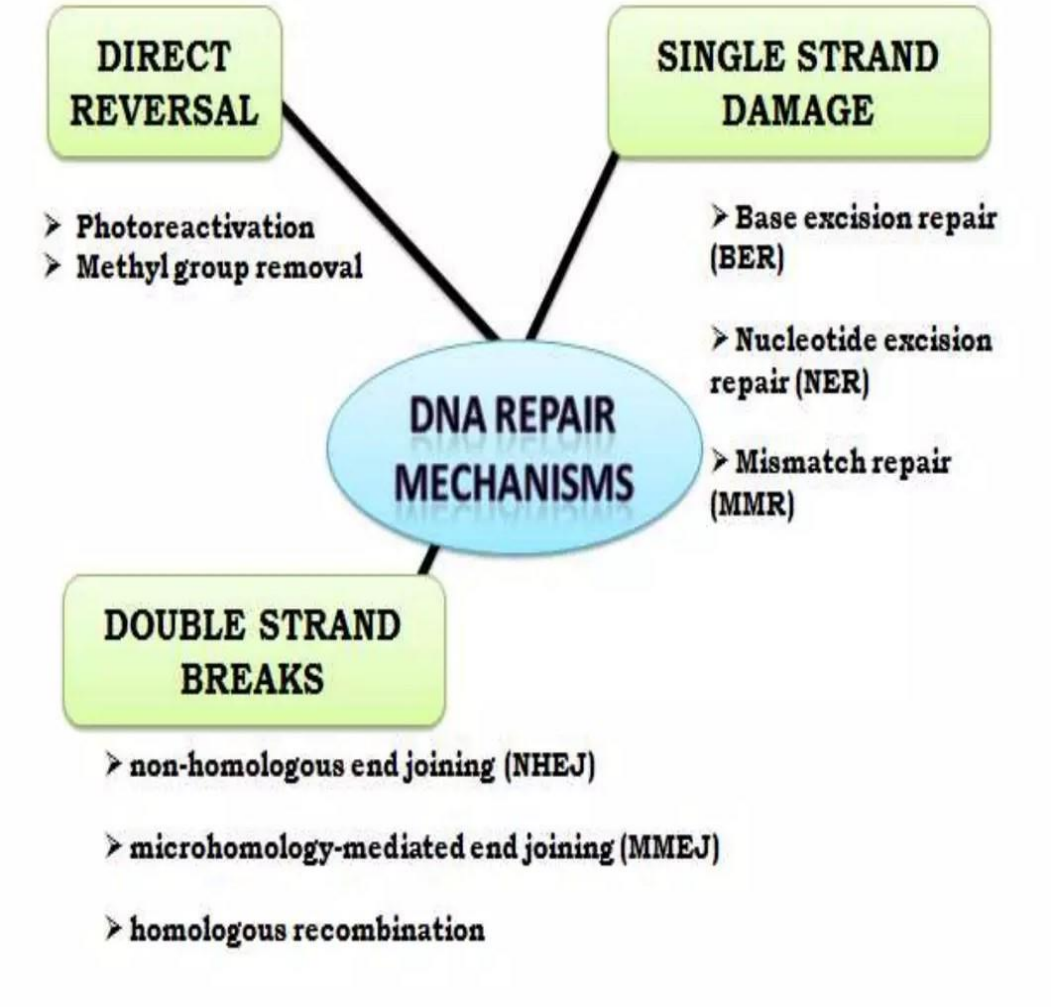
1.Direct repair system.

2.Excision repair

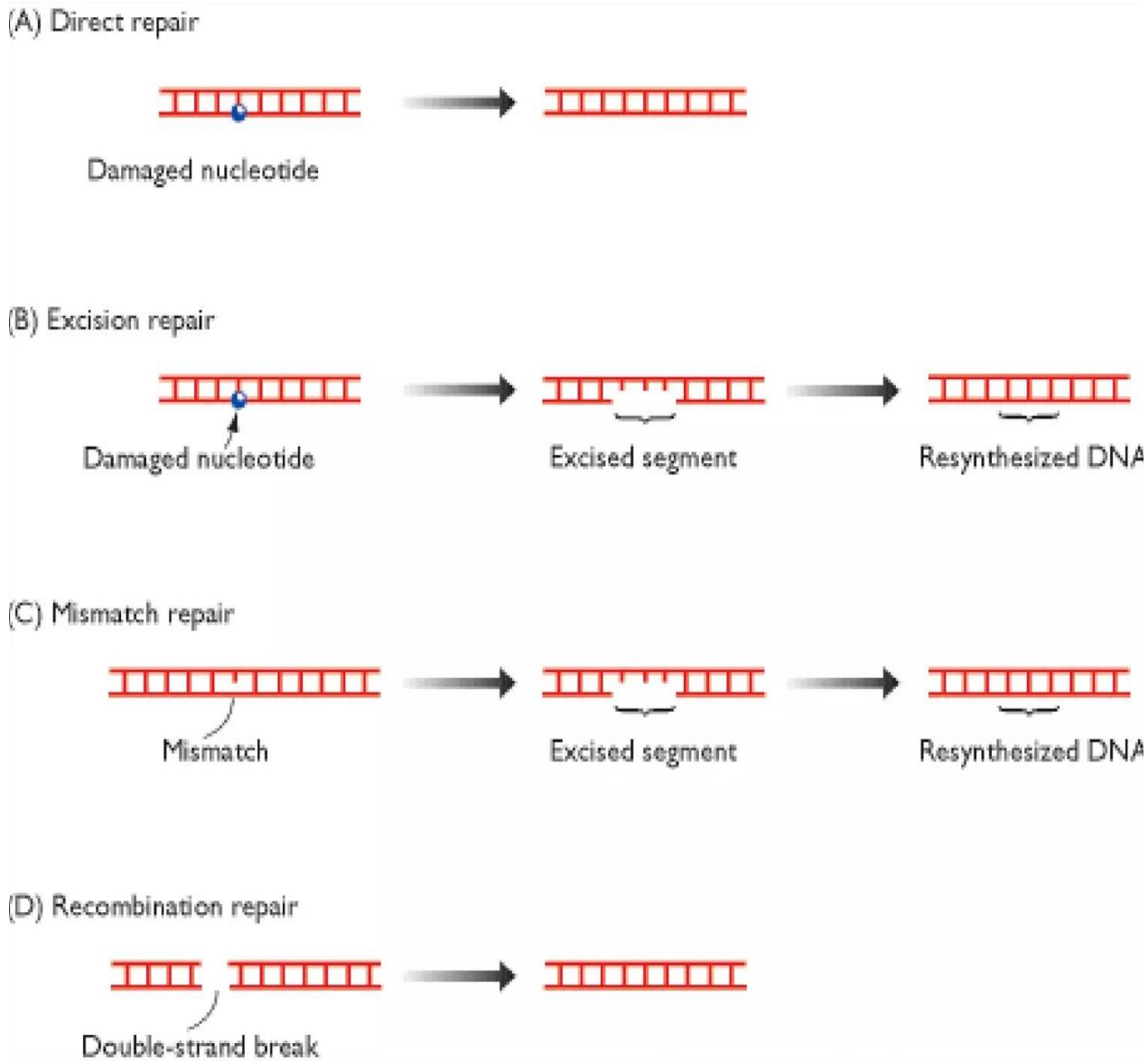
- Base Excision repair system.
- Nucleotide excision repair

3.Mismatch repair system.

4. recombination repair system.

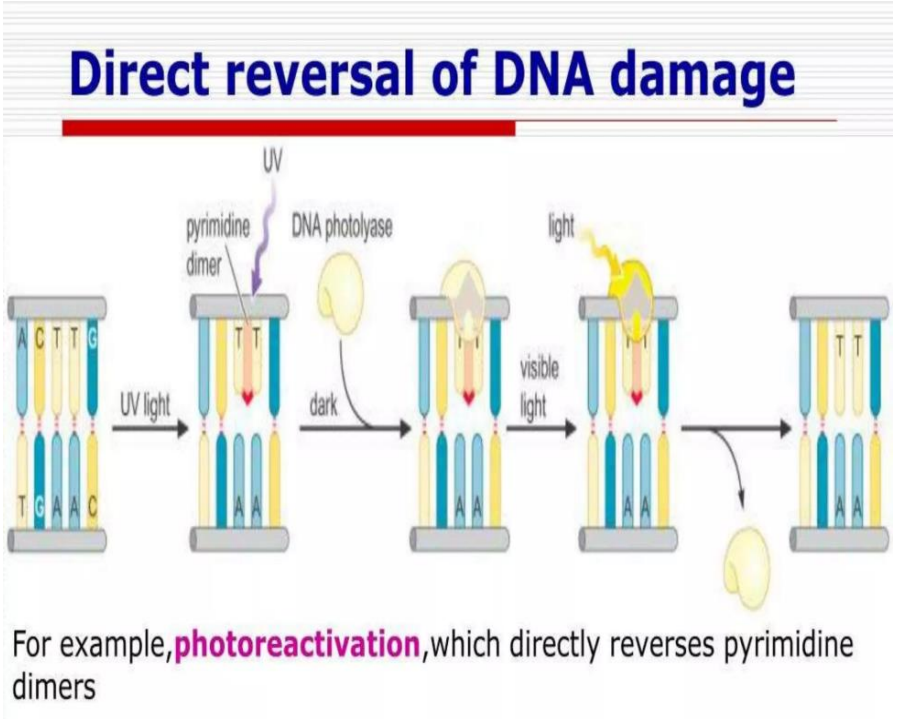


Types of DNA Repair Mechanisms

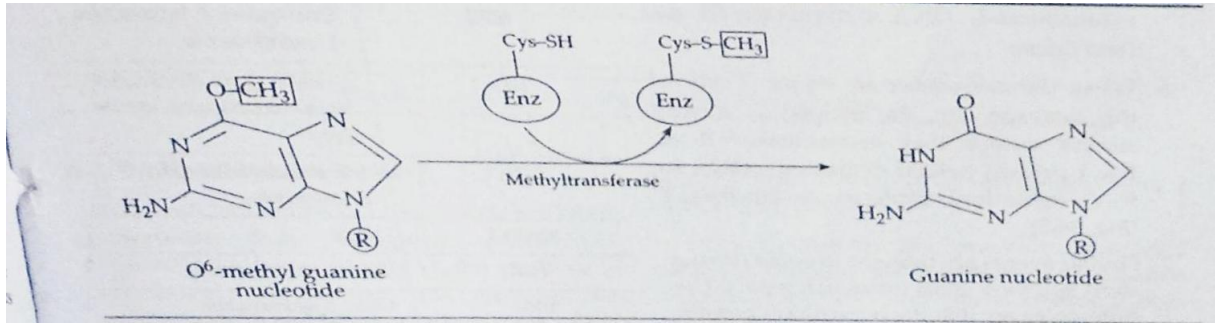


DIRECT REPAIR SYSTEM

- Direct reversal repair system is a DNA repair mechanism that directly fixes specific types of DNA damage without the need for excision or replacement
- Pyrimidine dimers resulted from the UV light induced reaction can be reversed by the enzymes photolyase that uses that uses the energy derived from absorbed light. .This process is called as photoreactivation .
- When stimulated by light with a wavelength between 300 and 500 nm, the enzyme binds to Pyrimidine dimers and converts them back to the original monomeric nucleotides.



- o6-methyl guanine tends to pair with thymidine during replication and therefore causes G=C to G= T mutations .
- Direct repair of o-methyl guanine is carried out by 06-methylguanine-DNA –methyl transferase, which catalyses the transfer of –CH3 group to specific cys residue of the same protein (Enzyme). This enzyme then become inactivated so it is not truly an enzyme.



EXCISION REPAIR

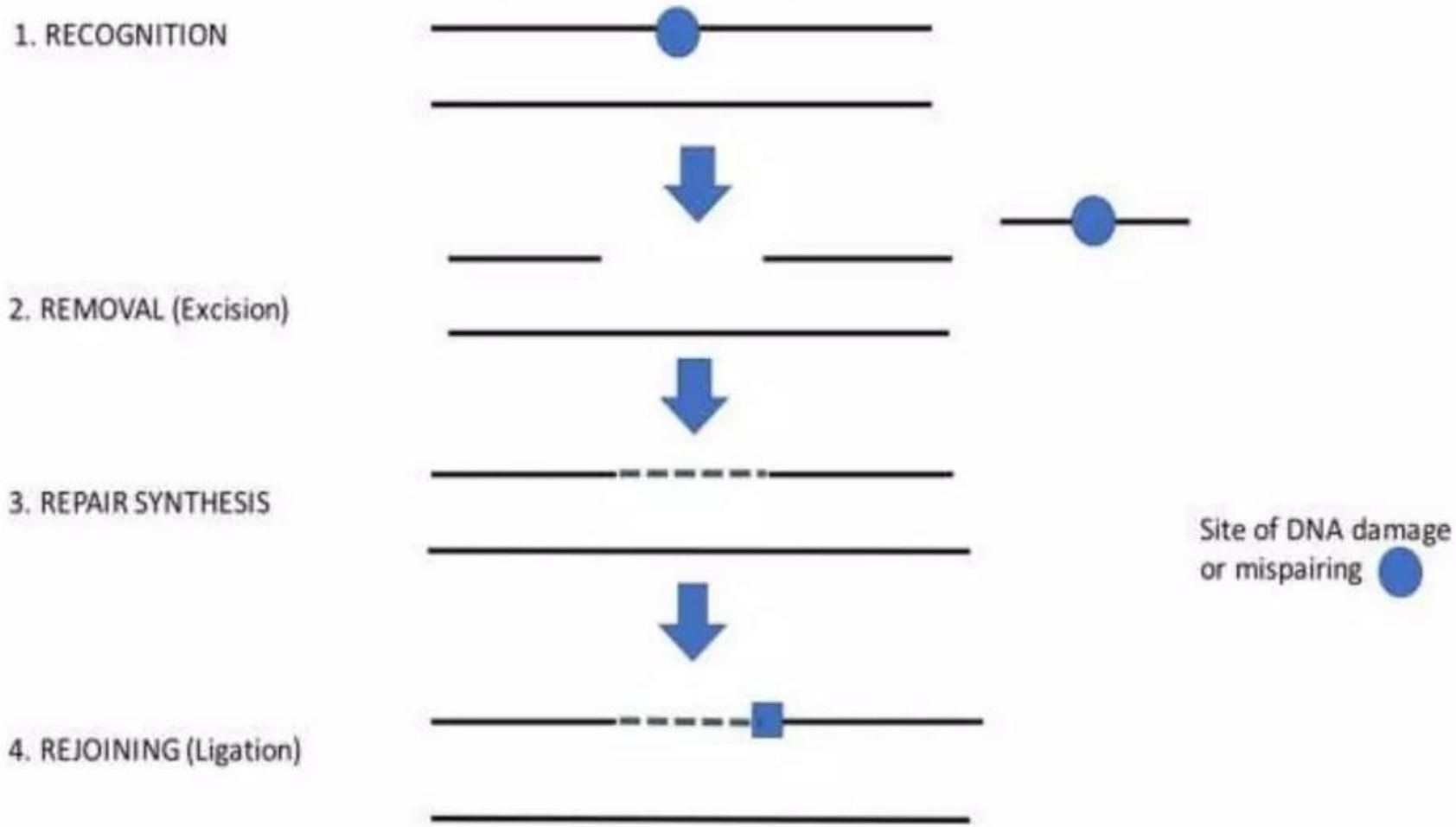
- It involves the excision of a segment of the polynucleotide containing a damaged site , followed by the resynthesis of the correct nucleotide sequence by a DNA polymerase
- Excision → Resynthesis → Ligation

Excision repair is of two types :

A) Base excision repair

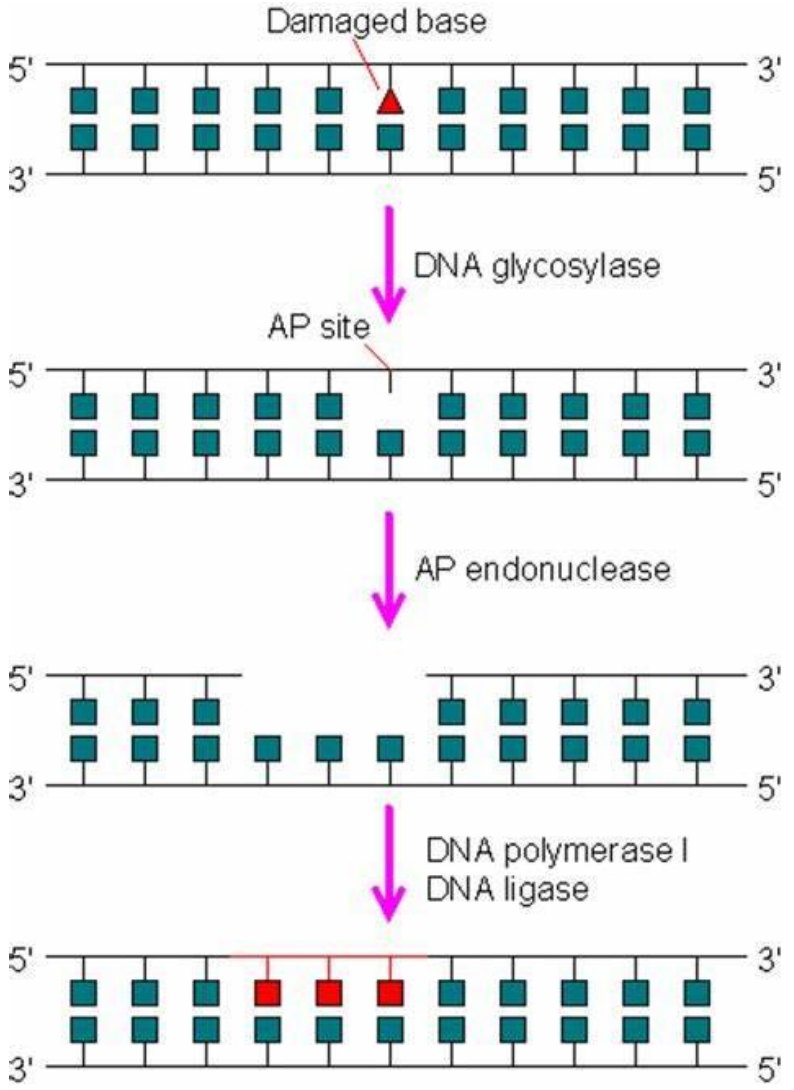
B) Nucleotide excision repair

Common Steps in Excision Repair Mechanisms



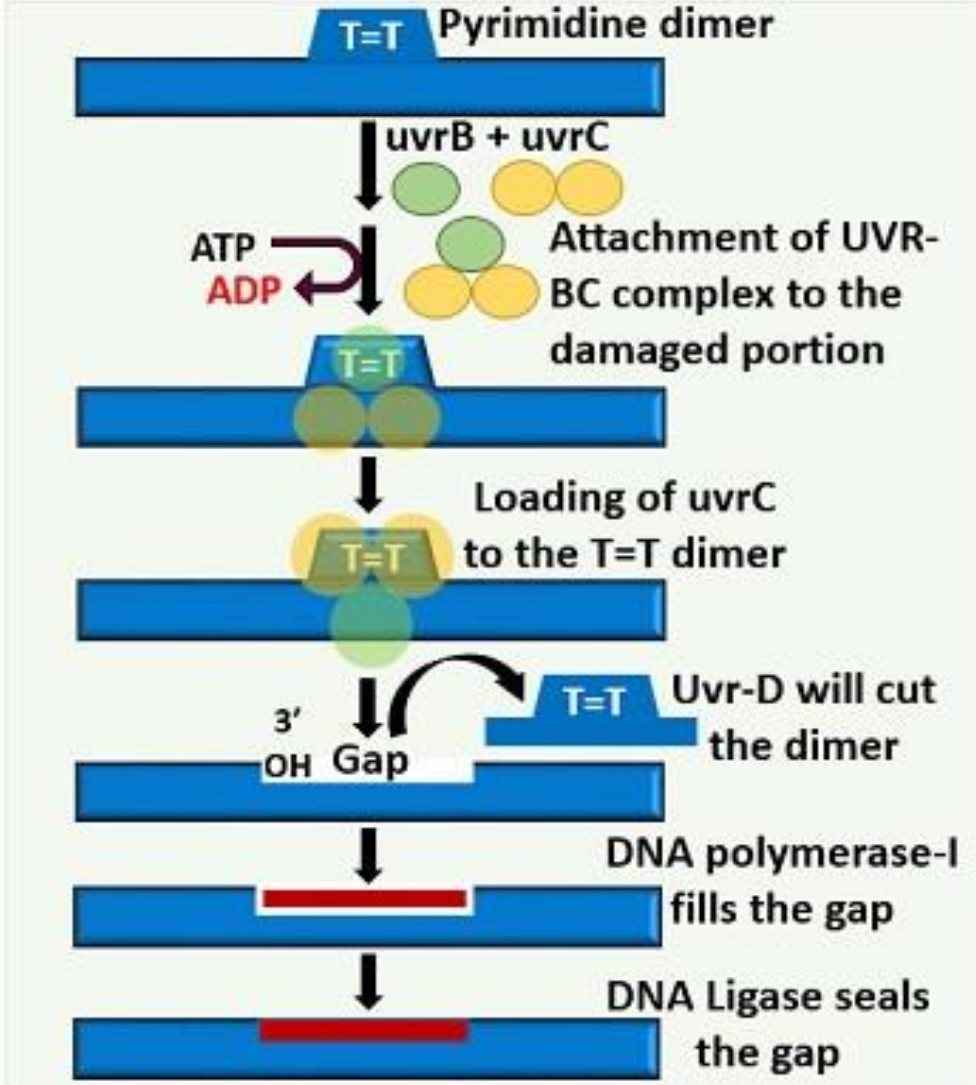
BASE EXCISION REPAIR

- Base excision repair (BER) is a DNA repair mechanism that removes and replaces damaged bases.
- The defective bases is cut out and replaced with correct base occurs due to deamination, depurination, alkylating agents.
- One example of BER is the repair of uracil-containing DNA. In this process, a DNA glycosylase recognizes and removes the uracil base, creating a gap in the DNA called the AP site. The gap is then cleaved by an enzyme called AP endonuclease.
- After that, the remaining sugar is removed, and the gap is filled using DNA polymerase and sealed with ligase.



NUCLEOTIDE EXCISION REPAIR

- In the first stage , a trimer comprising two Uvr A and one copy of Uvr B attaches to the damaged site of DNA possibly recognizing the area of distortion of the double helix.
- After recognition of DNA , the UvrA subunit leaves the complex and it allows Uvr C to bind forming Uvr BC-complex.
- This Uvr-BC complex thus cuts the polynucleotide either sides of the damaged sites. At first Uvr B cuts the DNA at 5^{th} phosphodiester bond down stream of the damaged nucleotide.
- The second cut is made by Uvr C at the 8^{th} phosphodiester bond upstream , resulting 12-nucleotide excision.
- The excised site is removed. DNA helicase-II presumably detaches the segment breaking the bases pairs holding it to the second strand.
- Thus Uvr C detaches from the complex, only Uvr B remains in place and prevents pairing of single stranded region.
- The gap is now filled by DNA polymerase-I and the phosphodiester bond is formed by DNA ligase to close the nick.

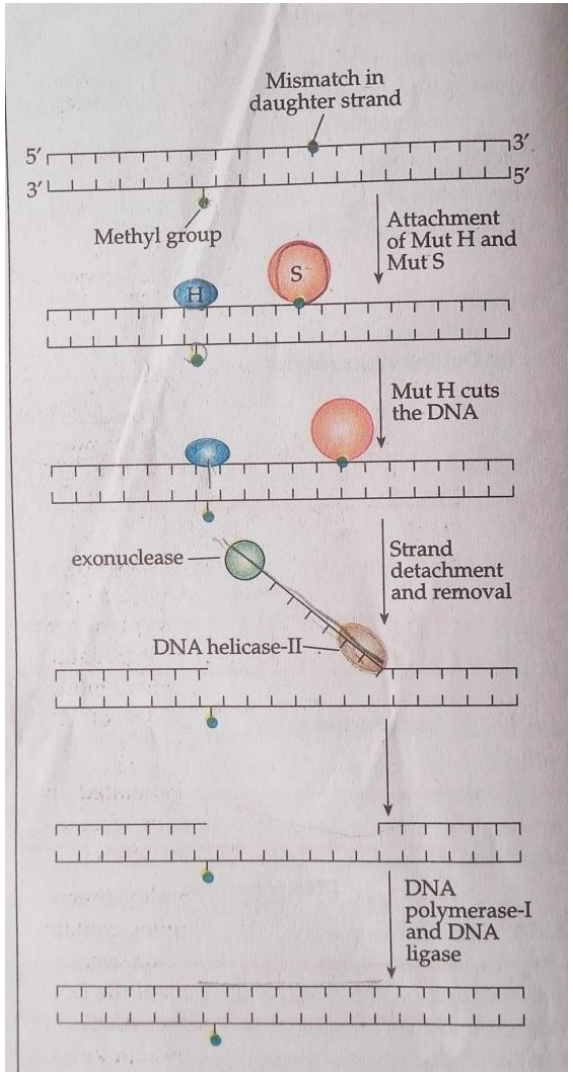


NUCLEOTIDE EXCISION REPAIR

MISMATCH REPAIR SYSTEM

- MutS protein recognizes the mismatch.
- MutH distinguishes the two strands by binding with an unmethylated 5’GATC3’ sequence.
- MutL may be an interfering protein linking MutS and MutH.
- MutH acts a site – specific endonuclease ,cleaving the unmethylated strand on the 5’side of the G in GATC.
- When the mismatch is on the 5’ side of the cleavage site, the unmethylated strand is unwound and degraded in the 3’ 5’ direction from the cleavage site through the mismatch and this is then replaced with new DNA.
- This process requires combined action of DNA Helicase-II , SSB exonuclease-I , DNA polymerase – III and then ligase.

MISMATCH REPAIR SYSTEM

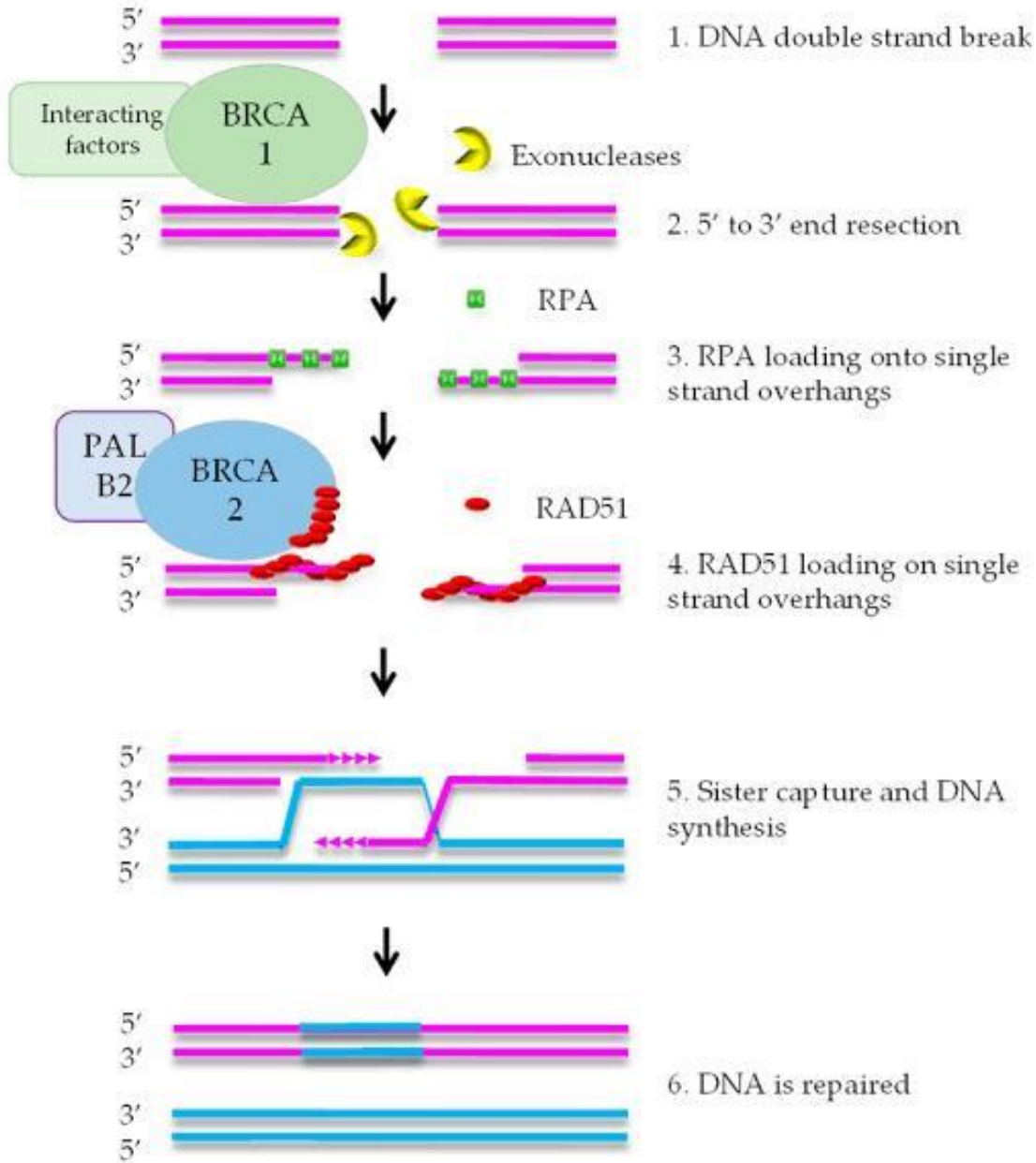


DOUBLE-STRAND BREAKS

- Double-strand breaks (DSBs) in DNA can be repaired through two pathways:
- Homologous recombination (HR) and non-homologous end joining (NHEJ).

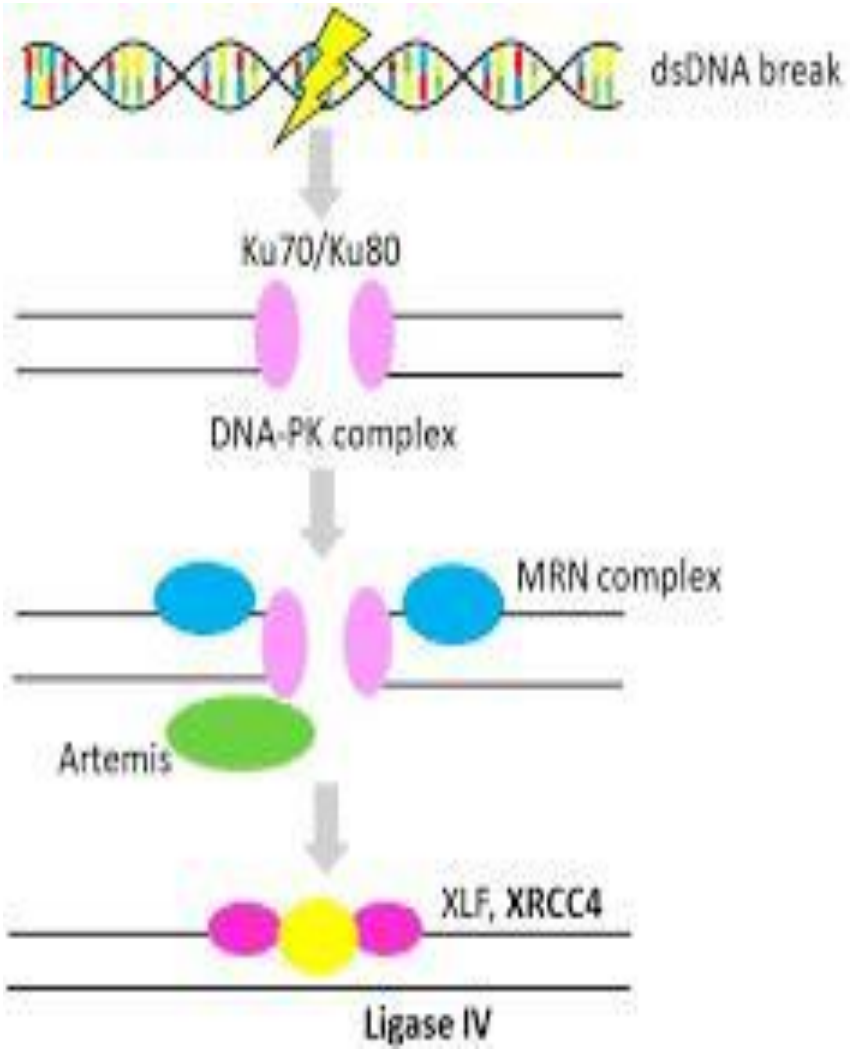
HOMOLOGOUS RECOMBINATION:

- HR is a precise repair pathway that requires a matching DNA sequence as a template.
- It primarily uses the sister chromatid, a copy of the damaged DNA, for repair.
- HR is most active during the S, G2, and M phases of the cell cycle when sister chromatids are present.
- The HR process involves creating single-stranded DNA (ssDNA) by degrading one strand of the DNA break and coating it with proteins like RPA. Rad51 replaces RPA and pairs the ssDNA with a homologous DNA template for repair.


5′
3′
1. DNA double strand break
Interacting factors
BRCA 1
Exonucleases
2. 5′ to 3′ end resection
RPA
3. RPA loading onto single strand overhangs
PAL B2
BRCA 2
RAD51
4. RAD51 loading on single strand overhangs
5. Sister capture and DNA synthesis
6. DNA is repaired

NON-HOMOLOGUS RECOMBINATION

- Double strand breaks are generated by exposure to ionizing radiations or chemical mutagens or during recombinations events .
- The complex contains a protein called ku, having two non-identical subunits, binds to two broken ends of the DNA
- The binding of ku is accomplished by the DNA-PKcs protein kinase.
- It activates a third protein XRCC4, which interacts with the mammalian DNA ligase-IV directing this repair protein to join the double strand broken ends .



DISEASES ASSOCIATED WITH DEFECTIVE DNA REPAIR SYSTEM

- Ataxia telangiectasia.
- Bloom syndrome.
- Cockayne’s syndrome.
- Rothmund –thomson syndrome.
- Werner syndrome.
- Xeroderma pigmentosum.
- Herditary non polyposis colon cancer.

Acknowledgement

- The presentation is being used for educational and non-commercial purposes.
- Thanks are due to all the original contributors and entities whose pictures were used to create this presentation.