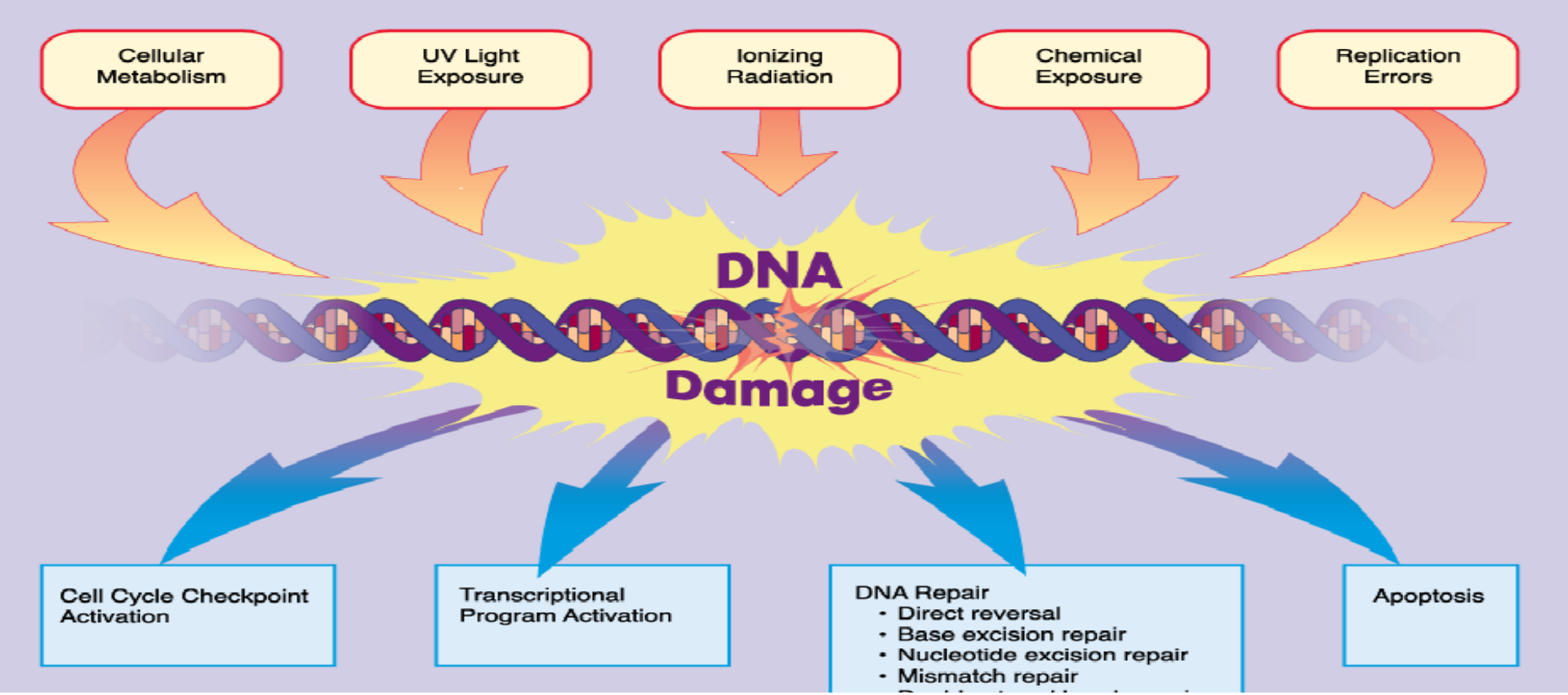


DNA Damage and Repair Mechanism

DNA impairment/damage

- DNA in the living cell is subjected to many chemical alterations.
- Damage to DNA is nothing but effect on primary structure of double helix
- The genetic information encoded in the DNA has to remain uncorrupted.
- Any chemical changes must be corrected.
- A failure to repair DNA produces a mutation.
- It occurs due to environmental factors and normal metabolic processes inside the cell.
- It occurs at rate 10,000 to 10,00,000 molecular lesions per cell per day.

Agents that Damage DNA

DNA damage can occur due to two main agents:

- **Endogenous** cellular processes
- **Exogenous** agents.

The endogenous cellular processes include:

- oxidation of nitrogen bases
- generation of DNA strand interruptions from reactive oxygen species
- alkylation of bases,
- hydrolysis such as deamination, depurination, depyrimidation
- bulky adducts formation
- mismatch of bases due to error in the replication process of DNA
- monoadduct damage due to change in mononitrogen base
- diadduct damage

Agents that Damage DNA

Exogenous agents include:

- UV radiations such as UV-B light which cause direct DNA damage by cross-linking between C and T bases creating pyrimidine dimers and indirect damage by creating radicals
- Ionizing radiations damage DNA by radioactive decay or cosmic ray causing strand breaks .
- Depurination and single strand breaks caused by thermal disruption at elevated temperature can affect the DNA helix structure.
- Industrial chemicals such as vinyl chloride, hydrogen peroxide and polycyclic aromatic hydrocarbons present in smoke, soot and tar create huge diversity of DNA adducts such as ethenobases, oxidized bases, alkylated bases, phosphotriesters and cross linking of DNA.

Agents that Damage DNA

- Chemicals in the environment
- Aromatic hydrocarbons, including some found in cigarette smoke .
- Plant and microbial products, e.g. the Aflatoxin produced in moldy peanuts.
- Chemicals used in chemotherapy, especially chemotherapy of cancers.
- DNA damage is totally different from mutation. It occurs physically and can be repaired but mutation is change in base sequence and can’t be repaired.

DNA repair mechanisms

S.No.	Damage	Damaging Agent	Example
1	BER	Reactive oxygen species, X-Rays, alkylating agents, Spontaneous reactions	Oxidation (8OxoG) Uracil, Single strand Break
2	MMR	Replication error	A-G mismatch, T-C mismatch, Insertion, Deletion
3	NER	UV lights and polycyclic aromatic hydrocarbons	Bulky adducts, intrastrand cross link
4	DSBR	X-Rays, Ionozing radiations, antitumor agent	Double strand break, Interstrand crosslink

Common Pathway of DNA repair mechanisms

a. Lesion detection: Proteins are bound to DNA lesion

b. Damaged DNA removal: Nucleases, glycosylases etc remove damaged part

c. Repair/Resynthesis: DNA ligase, DNA polymerase

d. Effects on other cellular processes: Replication and/or cell division to allow more time for repair to occur

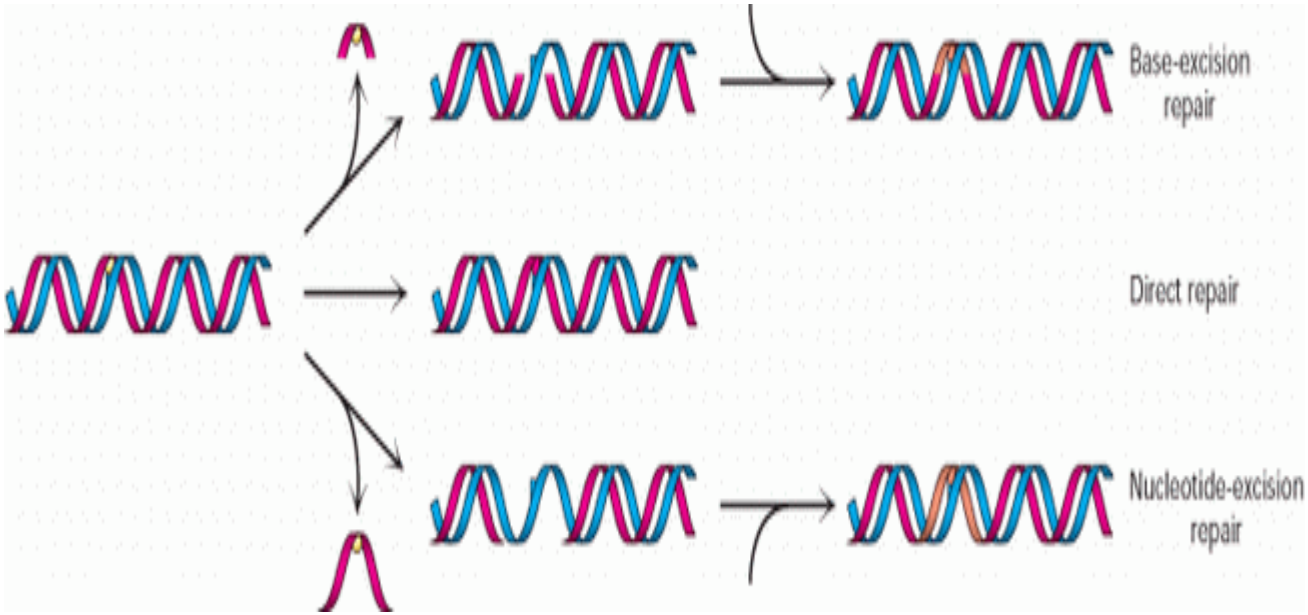
e. Consequence: Accurate repair-Survival, Inability to Repair-Cell death, Misrepair-Genome Instability

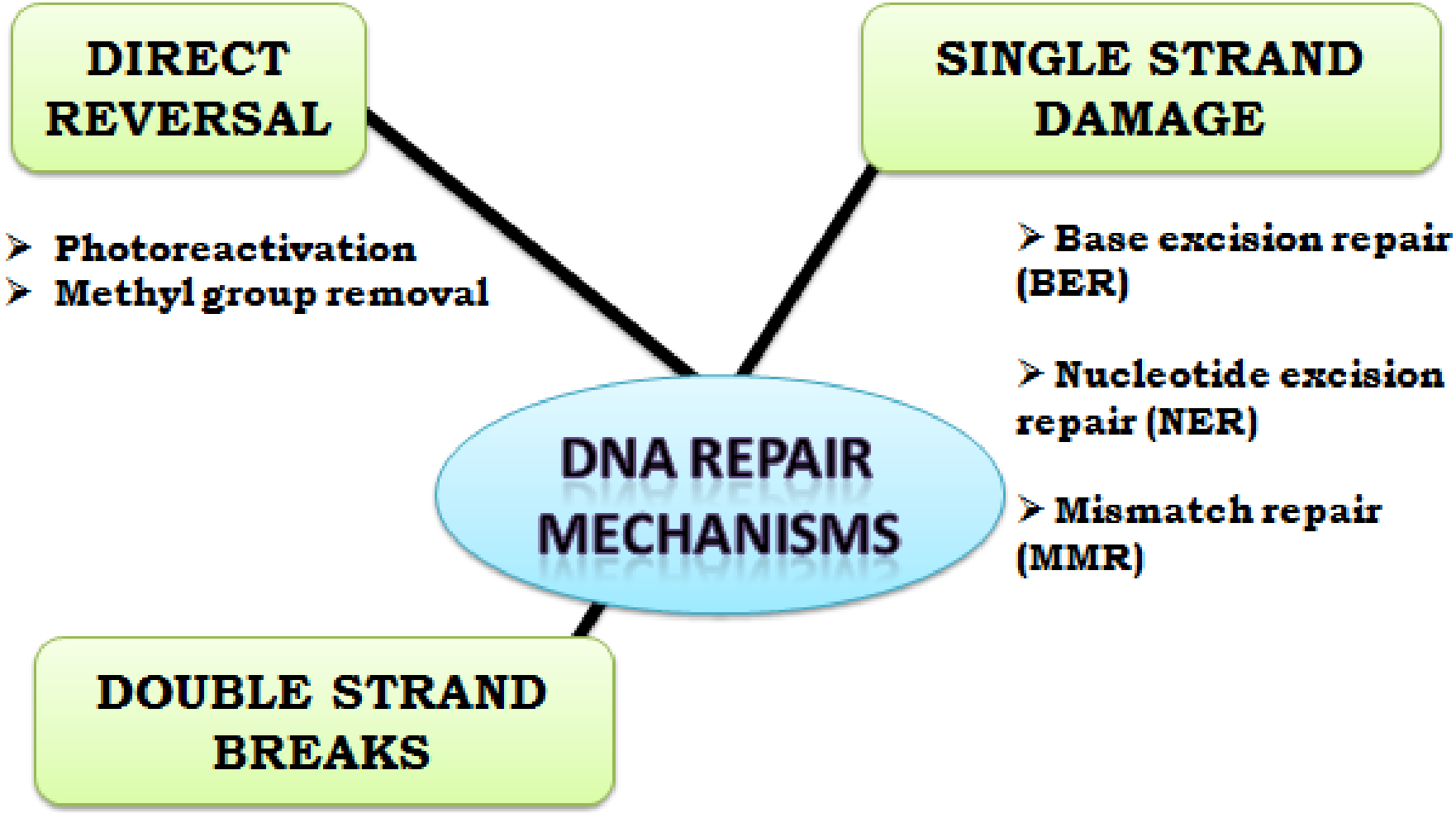
DNA Repair

- DNA repair can be grouped into two major functional categories:

A) Direct Damage reversal

B) Excision of DNA damage




DIRECT REVERSAL
SINGLE STRAND DAMAGE
Photoreactivation
Methyl group removal
Base excision repair (BER)
Nucleotide excision repair (NER)
Mismatch repair (MMR)
DNA REPAIR MECHANISMS
DOUBLE STRAND BREAKS
non-homologous end joining (NHEJ)
microhomology-mediated end joining (MMEJ)
homologous recombination

A) Direct Damage Reversal

The direct reversal of DNA damage is by far the simplest repair mechanism that involves a single polypeptide chain, with enzymatic properties which binds to the damage and restores the DNA genome to its normal state in a single-reaction step. Direct reversal repair eliminates some DNA and RNA modifications without using excision, resynthesis, and ligation. Therefore, because direct reversal repair does not require breaking of the phosphodiester backbone, it is error-free and preserves genetic information.

Direct repair

- Photoreactivation reverses UV damage (bacterial cell) →photolyase.

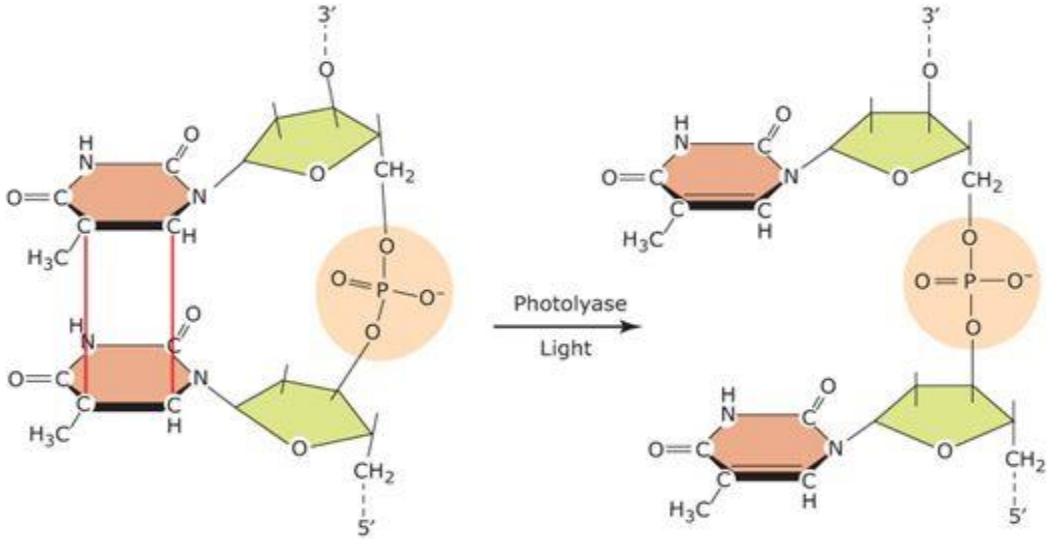


Figure 3.38: Photolyase reverses UV-induced pryimidine dimers

- Alkyltransferases remove alkyl groups from DNA.
  - Suicide enzyme
  - Cys alkylation

B) Excision of DNA damage

- ❑ Base excision repair (BER)
- ❑ Nucleotide excision repair (NER)
- ❑ Mismatch repair (MMR)
- ❑ Strand break repairs

❖ In these reactions a nucleotide segment containing base damage, double-helix distortion or mispaired bases is replaced by the normal nucleotide sequence in a new DNA polymerase synthesis process.

❖ All of these pathways have been characterized in both bacterial and eukaryotic organisms.

Base Excision Repair (BER)

- The name itself implies that it is the predominant mechanism responsible for the repair of damaged DNA bases.
- In this repair mechanism the DNA helix or the backbone of DNA is not cut out, only the base is cut from the target site. It is opposite to NER.
- It contains enzymatic reactions pathway. It only removes the damaged bases by cleaving the N-glycoside linkage.
- BER is initiated by DNA glycosylases, which catalyze the hydrolysis of the N-glycosidic bonds, linking particular types of chemically altered bases to the deoxyribose-phosphate backbone.
- DNA damage is excised as free bases, generating sites of base loss called apurinic or apyrimidinic (AP) sites.

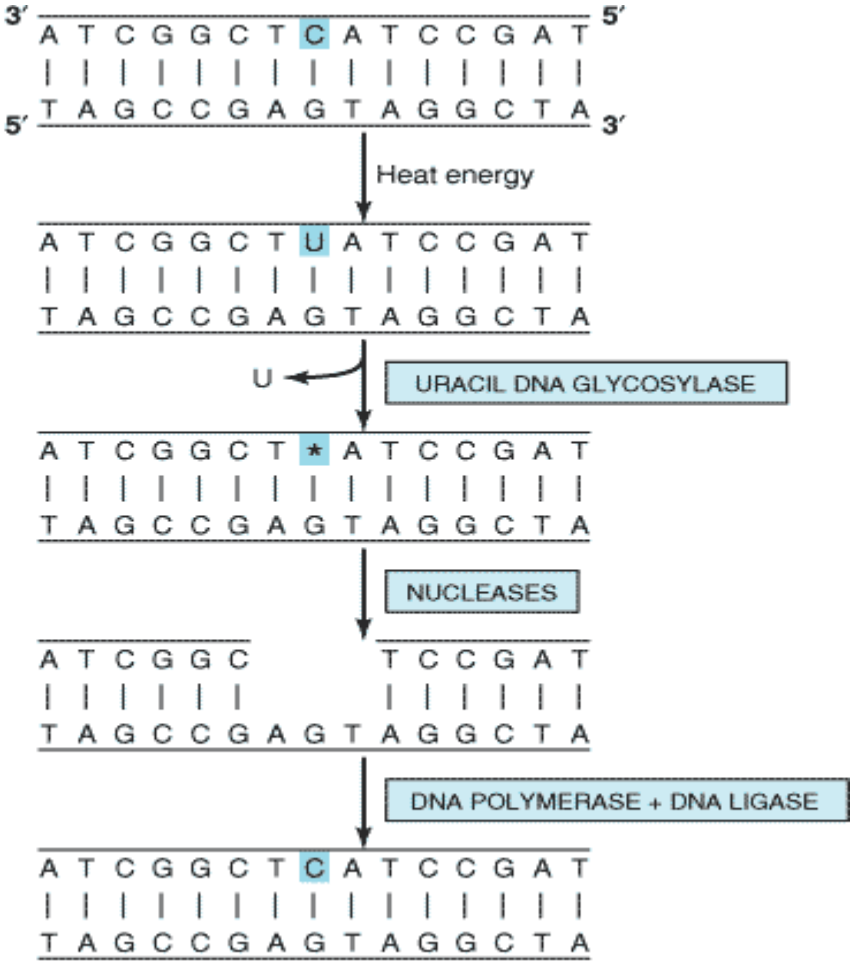
Base Excision Repair (BER)

- The AP sites are substrates for AP endonucleases.
- These enzymes produce incisions in duplex DNA as a result of the hydrolysis of a phosphodiester bond immediately 5' or 3' to each AP site.
- The ribose-phosphate backbone is then removed from the DNA through the action of a specific exonuclease called deoxy ribophosphodiesterase or dRpase.
- Finally, the DNA polymerase and a ligase catalyze the incorporation of a specific deoxyribonucleotide into the repaired site, enabling correct base pairing.

Base Excision Repair (BER)

Base excision-repair of DNA

- The enzyme uracil DNA glycosylase removes the uracil created by spontaneous deamination of cytosine in the DNA.
- An endonuclease cuts the backbone near the defect.
- An endonuclease removes a few bases.
- The defect is filled in by the action of a DNA polymerase
- The strand is rejoined by a ligase.



Nucleotide excision repair (NER)

- This mechanism is used to replace regions of damaged DNA up to 30 bases in length.
- Common causes of such DNA damage include ultraviolet (UV) light, which induces the formation of cyclobutane pyrimidine-pyrimidine dimers, and smoking, which causes formation of benzo[*a]pyrene-guanine adducts.*
- Ionizing radiation, cancer chemotherapeutic agents, and a variety of chemicals found in the environment cause base modification, strand breaks, cross-linkage between bases on opposite strands or between DNA and protein, and numerous other defects.
- These are repaired by a process called nucleotide excision repair.

Nucleotide excision repair (NER)

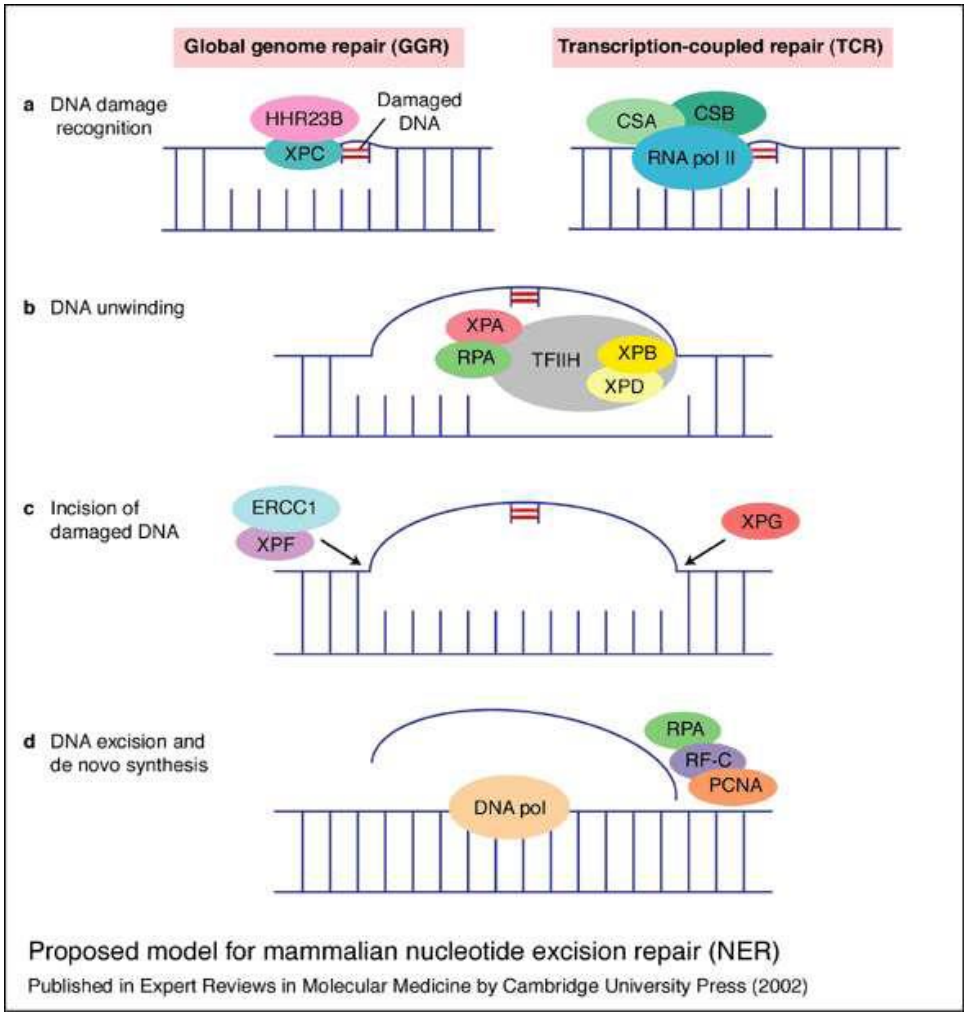
- NER is a much more complex biochemical process than BER, especially in eukaryotic cells.
- Several gene products are required in a multiple step process, during which the ordered assembly of DNA proteins provides an enzymatic complex that discriminates damaged from undamaged DNA.

Nucleotide excision repair (NER)

• In eukaryotic cells the enzymes cut between the third to fifth phosphodiester bond 3' from the lesion, and on the 5' side the cut is somewhere between the twenty-first and twenty fifth Bonds.

• Thus, a fragment of DNA 27–29 nucleotides long is excised.

• After the strand is removed it is replaced, again by exact base pairing, through the action of yet another polymerase, and the ends are joined to the existing strands by DNA ligase.



Proposed model for mammalian nucleotide excision repair (NER)

Published in Expert Reviews in Molecular Medicine by Cambridge University Press (2002)

Mismatch repair (MMR)

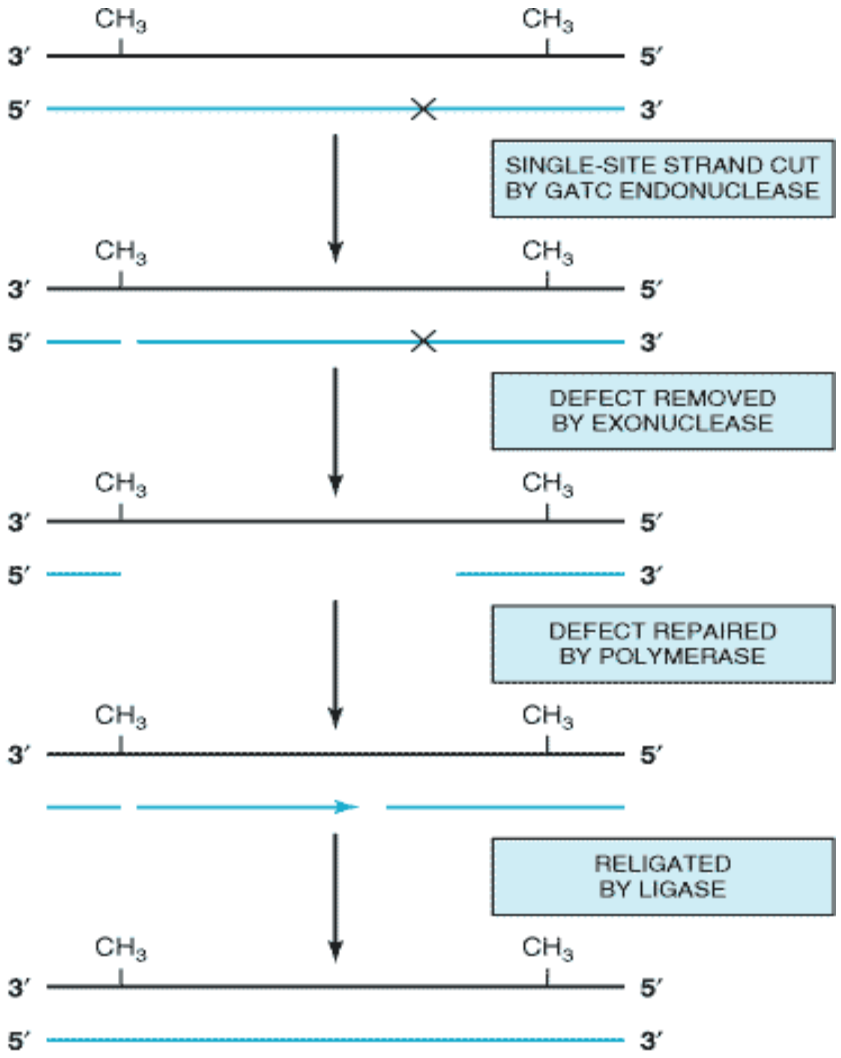
- Mismatch repair corrects errors made when DNA is copied.
- For example, a C could be inserted opposite an A, or the polymerase could slip or stutter and insert two to five extra unpaired bases.
- Specific proteins scan the newly synthesized DNA, using adenine methylation within a GATC sequence as the point of reference
- The template strand is methylated, and the newly synthesized strand is not.

Mismatch repair (MMR)

- This difference allows the repair enzymes to identify the strand that contains the errant nucleotide which requires replacement.
- If a mismatch or small loop is found, a GATC endonuclease cuts the strand bearing the mutation at a site corresponding to the GATC.
- An exonuclease then digests this strand from the GATC through the mutation, thus removing the faulty DNA. This can occur from either end if the defect is bracketed by two GATC sites.
- This defect is then filled in by normal cellular enzymes according to base pairing rules.

Mismatch repair (MMR)

- This mechanism corrects a single mismatch base pair (eg, C to A rather than T to A) or a short region of unpaired DNA.
- The defective region is recognized by an endonuclease that makes a single-strand cut at an adjacent methylated GATC sequence.
- The DNA strand is removed through the mutation, replaced, and religated.



Repairing Strand Breaks

Ionizing radiation and certain chemicals can produce both single-strand breaks (**SSBs) and** double-strand breaks (**DSBs) in the DNA** backbone.

i) Single-Strand Breaks (SSBs):

Breaks in a single strand of the DNA molecule are repaired using the same enzyme systems that are used in Base-Excision Repair (BER).

Repairing Strand Breaks

ii) Double-Strand Break Repair:

There are two mechanisms by which the cell attempts to repair a complete break in a DNA molecule:

1) Direct joining of the broken ends. This requires proteins that recognize and bind to the exposed ends and bring them together for ligating. This type of joining is also called **Nonhomologous End-Joining (NHEJ). A protein called Ku is** essential for NHEJ.

Repairing Strand Breaks

- Errors in direct joining may be a cause of the various **translocations that are associated** with cancers. Examples:
- Burkitt's lymphoma
- Philadelphia chromosome in chronic myelogenous leukemia (CML)
- B-cell leukemia

Repairing Strand Breaks

2) Homologous Recombination:

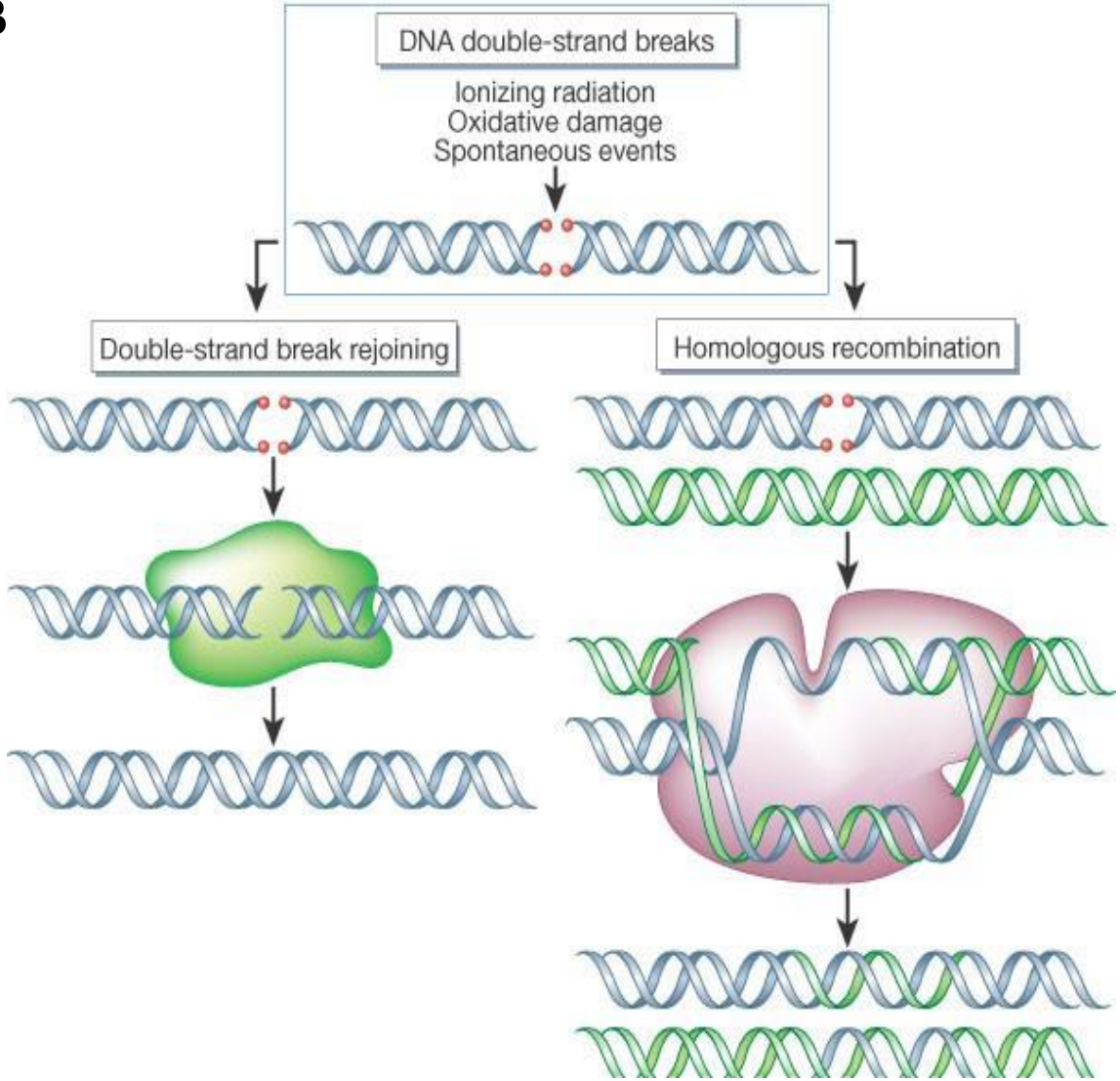
Here the broken ends are repaired using the information on the intact

- **sister chromatid,** or on the
- **homologous chromosome**
- **same chromosome** if there are duplicate copies of the gene on the chromosome oriented in opposite directions (head-to-head or back-to-back).
- Two of the proteins used in homologous recombination are encoded by the genes ***BRCA1 and BRCA2.***
- Inherited mutations in these genes predispose women to breast and ovarian cancers.

Repairing Strand Breaks

Meiosis also involves DSB

- Recombination between homologous chromosomes in meiosis I also involves the formation of DSBs and their repair.
- Meiosis I with the alignment of homologous sequences provides a mechanism for repairing damaged DNA.



Diseases associated with defective DNA repair system

- Ataxia telangiectasia
- Bloom syndrome
- Cockayne's syndrome
- Progeria (Hutchinson-Gilford Progeria syndrome)
- Rothmund-Thomson syndrome
- Trichothiodystrophy
- Werner syndrome
- Xeroderma pigmentosum
- Hereditary non polyposis colon cancer.