DNA Replication and Repairing System

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Replication Definition

DNA replication is the process by which the genome's DNA is copied in cells. Before a cell divides, it must first copy (or replicate) its entire genome so that each resulting daughter cell ends up with its own complete genome.

- DNA replication is **semiconservative**. Meaning that each strand in the double helix acts as a template for synthesis of a new, complementary strand. This process is beginning with one starting molecule to two "daughter" molecules, with each newly formed double helix containing one new and one old strand.
- Cells need to copy their DNA very quickly, and with very few errors (or risk problems such as cancer). To do so, they use a variety of enzymes and proteins, which work together to make sure DNA replication is performed smoothly and accurately.

- One of the key molecules in DNA replication is the enzyme **DNA polymerase**, is chief enzyme mainly involved in *repair* and deoxy nucleotide *polymerization.*
- Responsible for synthesizing new DNA: by adding nucleotides one by one to the growing DNA chain, using only those that are complementary to the template.
- Requires *a template* and a *primer* (starter) utilizing *deoxyribonucleoside triphosphate* as building block for DNA replication (dATP,dCTP,dGTP and dGTP) to synthesize DNA in the 5' to 3' direction.
- The addition of nucleotides requires energy. This energy comes from the nucleotides themselves, which have three phosphates attached to them (much like the energy-carrying molecule ATP). When the bond between phosphates is broken, the released energy is used to form a bond between the incoming nucleotide and the growing chain.

- DNA replication requires other enzymes in addition to DNA polymerase, including **DNA primase**: Initiate synthesis of RNA primer.
- **DNA helicase**: Unwinding DNA
- **DNA ligase**: Nick sealing enzyme between Okazaki fragments on lagging strand. Requires energy
- and **topoisomerase:** Nick sealing enzyme, relieve torsional strain resulting from helicase induced unwinding. Does not require energy.
- **SSB proteins**: single strand binding proteins ,bind to each strand and stabilize the complex and prevent re-annealing of double strands of DNA.

Stages of DNA replication

DNA replication can be thought of in three stages:

1. **Initiation:** DNA synthesis is initiated at particular points within the DNA strand known as '**origins**', which have specific coding regions(A+T rich region) .

- These origins are targeted by initiator proteins(O protein) leads to local denaturation and unwinding of site origin providing a short region of SS DNA which acts as template for initiation of synthesis.

- This go on to recruit more proteins that help aid the replication process, forming a replication complex around the DNA origin.

- Multiple origin sites exist within the DNA's structure; when replication of DNA begins, these sites are referred to as **replication forks.**

- Within the replication complex is the **DNA helicase**. This enzyme allows for processive unwinding of the double helix producing torsional strain leading to produce a nick on one strand of DNA, and exposes each of the two strands so that they can be used as a template for replication.
- It does this by hydrolyzing the ATP used to form the bonds between the nucleobases, thereby breaking the bond holding the two strands together.
- This nick is quickly resealed by topoisomerase enzyme. The formation of high energy covalent bond, that is why it doesn't require energy.
- Later the **SSB** proteins will be recruited, and do its action.

- **DNA primase** is another enzyme that is important in DNA replication. It synthesizes a small RNA primer, which acts as a ‘kick-starter’ for DNA polymerase enzyme. This enzyme is ultimately responsible for the creation and expansion of new strands of DNA.

2. Elongation : Once DNA Polymerase has attached to the two un wined strands of DNA (the **template** strands), it is able to start synthesizing new strands of DNA to match the templates.

- DNA polymerase is only able to extend the primer by adding free nucleotides to the **3' end** and *reading* the template 3′ to 5′, *synthesizing* in a 5′ to 3′ direction.

- One of the template strands is read in a **3' to 5'** direction, therefore the new strand will be formed in a 5' to 3' direction.

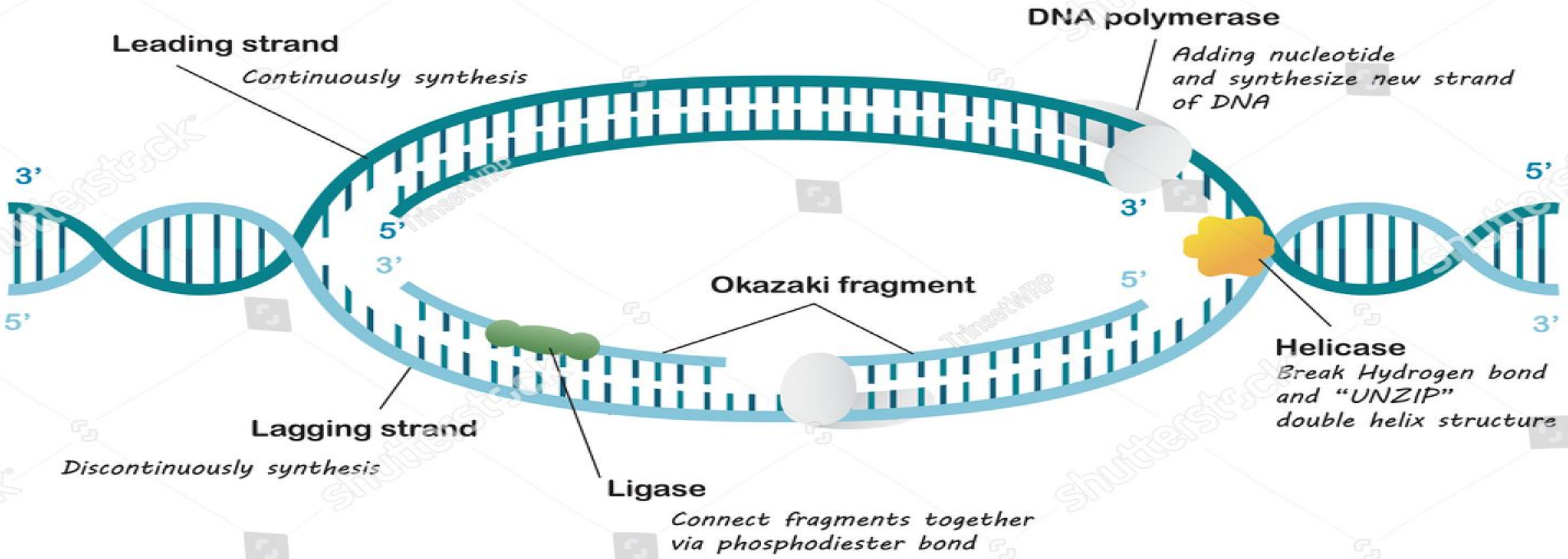
- This newly formed strand is referred to as the **leading strand.** Along the leading strand, **DNA primase** only needs to synthesize an RNA primer **once,** at the beginning, to initiate DNA polymerase in this step.

- However, the other template strand (the **lagging strand**) is antiparallel and is therefore read in a **5’ to 3’** direction.

- Continuous DNA synthesis, as in the **leading strand**, would need to be in the 3′ to 5′ direction, which is impossible as DNA polymerase cannot add bases to the 5′ end. Instead, as the helix unwinds, RNA primers are added by RNA primase to the newly exposed bases on the **lagging strand** and DNA synthesis occurs **in fragments** (discontinuous), but still in the 5′ to 3′ direction as before. These fragments are known as **Okazaki fragments**.

Scientific diagram

DNA Replication



3.Termination

- The process of expanding the new DNA strands continues until there is either no more DNA template strand left to replicate (at the end of the chromosome) or two replication forks meet and subsequently **terminate.**

- Once DNA synthesis has finished, the newly synthesized strands are bound and stabilized.

- For the lagging strand, two enzymes are needed to achieve this stabilization: **RNAase- H** removes the RNA primer at the beginning of each Okazaki fragment, and **DNA ligase** joins these fragments together to create one complete strand.

DNA Replication

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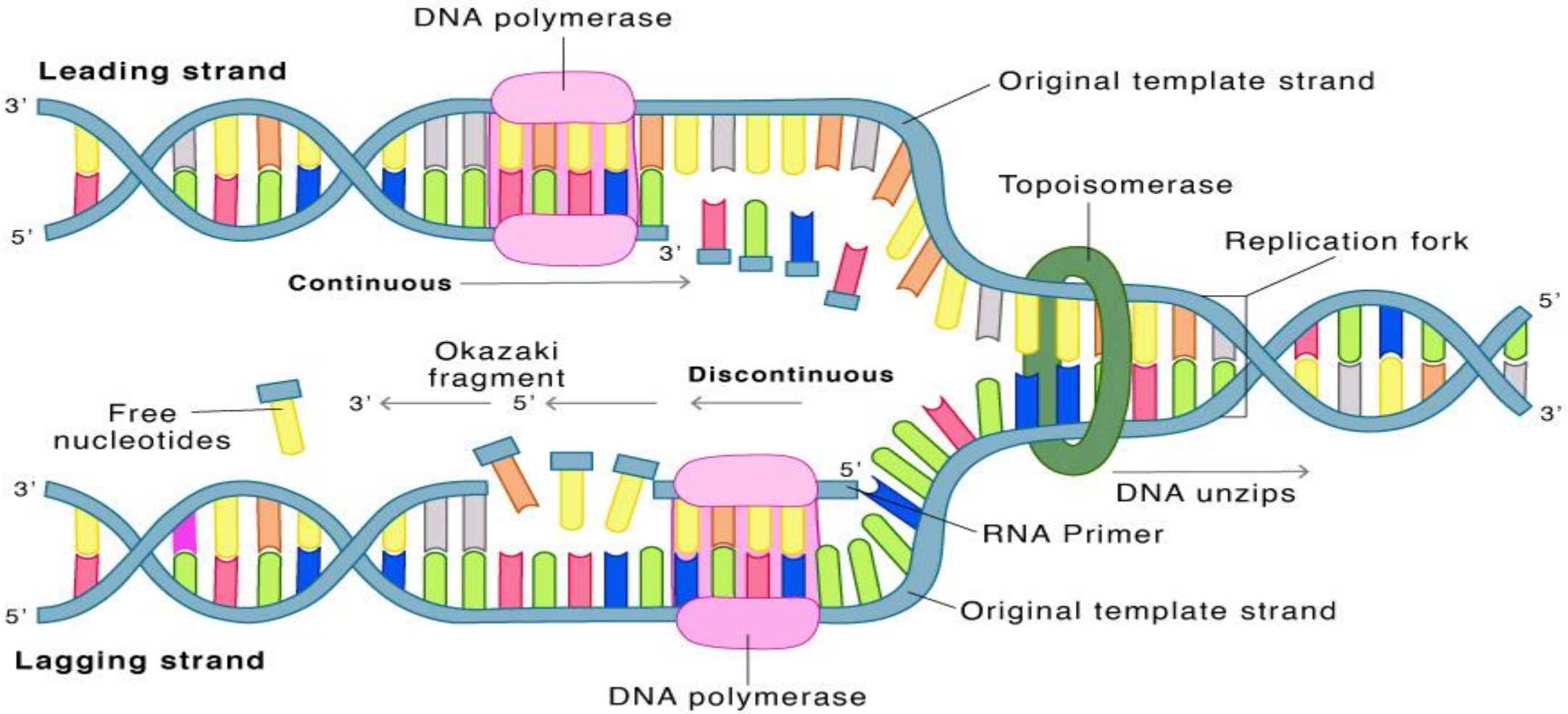


DNA Repair Definition

- DNA repair, any of several mechanisms by which a cell maintains the integrity of its genetic code.
- DNA repair ensures the survival of a species by enabling parental DNA to be inherited as possible by offspring.
- It also preserves the health of an individual.
- Replication errors are minimized when the DNA replication machinery “proofreads” its own synthesis, but sometimes mismatched base pairs escape proofreading.

Sources of DNA Damage

DNAs are constantly being attacked by genotoxic agents which can cause different types of DNA damage. DNA lesions have the capacity to block genomic replication and transcription which can cause mutations and double-strand breaks (DSBs). Sources of DNA damage are broadly classified into the below two categories:

- **Endogenous Sources of DNA Damage** - Mutations acquired over a certain time span can cause genomic instability and endogenous sources contribute a lot to these mutations. Endogenous sources of DNA damage *are reactive oxygen species* which are produced by the usual metabolic activities and *DNA replication errors.*

- **Exogenous Sources of DNA Damage**

- The UV (ultraviolet) radiations from the sun, ionizing radiation from space, radioisotopes on earth which occur naturally, certain industrial chemicals like hydrogen peroxide and vinyl chloride, and many hydrocarbons, including some found in cigarette smoke are all exogenous sources of DNA damage. Chemicals used in chemotherapy, especially chemotherapy of cancers.

Classification of DNA Damage

Based on the source of DNA damage, DNA damages can be classified to :

❑ ***Damages Due to Endogenous Sources***

1-Base oxidation : there could be DNA strand interruptions caused by reactive oxygen species.

2-Alkylation of bases can happen (mostly methylation) for example formation of 1-methyladenine.

3-Hydrolysis of bases - Examples of this are depyrimidination, deamination, and depurination.

4-Mismatch of bases - This happens because of DNA replication errors where a wrong DNA base gets stitched into a place in a newly forming DNA strand. It could also occur when a DNA base is either skipped or inserted by mistake.

Damages Due to Exogenous Sources

1- Crosslinking of adjacent bases due to UV-B light rays. This results in pyrimidine dimers and is called direct DNA damage.

2- Free radicals get created by UV-A light. This type of damage is called *indirect DNA damage.*

3- Cosmic rays or radioactive decay can cause ionization radiation which can *break DNA strands.*

4-An increase in the rate of *depurination* (this is loss of purine bases from the backbone of DNA) can occur due to thermal disruption at high temperatures. It also causes *single-strand breaks.*

5-Bulky adduct formation - This occurs due to the covalent bonding of large-sized chemical carcinogens with piece of DNA.

Types of DNA Damage

1.All four of the bases in DNA (**A, T, C, G**) can be covalently modified at various positions.

- One of the most frequent is the loss of an amino group ("deamination") - resulting, for example, in a **C** being converted to a **U**.

2.Mismatches of the normal bases because of a failure of proofreading during DNA replication.

- Common example: incorporation of the pyrimidine **U** (normally found only in RNA) instead of **T**.

3.Breaks in the backbone.

- Can be limited to one of the two strands (a single-stranded break, **SSB**) or on **both strands** (a **d**ouble-**s**tranded **b**reak (**DSB**). Ionizing radiation is a frequent cause, but some chemicals produce breaks as well.

4.Crosslinks Covalent linkages can be formed between bases

1. on the same DNA strand ("intrastrand") or
2. on the opposite strand ("interstrand").

- Several chemotherapeutic drugs used against cancers crosslinked DNA of cancerous cells.

Repairing Damaged Bases mechanisms

Damaged or inappropriate bases can be repaired by several mechanisms:

1- Direct chemical reversal (photo reactivation) of the damage.

- It only repairs the pyrimidine dimers which are present on the DNA's double helix structure. It is also called light induced repair which the repaired enzyme is activated by the visible light (300-600 nanometers) to induce an enzymatic cleavage of thymidine dimers leading to restoration of the monomeric condition.
- Direct reversal of the base lesion rather than excision is the one simplest step error-free and most economical DNA repair mechanism to have evolved.
- Most of these changes are repaired by enzymes, called ***glycosylases***, that remove the mismatched T restoring the correct C.
- This is done without the need to break the DNA backbone (in contrast to the mechanisms of excision repair).

2-Recombinational repair system also called as **Sister-strand exchange** .

- In this process, the un mutated single strand segment from homologous DNA is excised from the ‘good’ strand and inserted into the ‘gap’ opposite the dimer.
- It occur in the first round of DNA replication.

3- Excision Repair The main targets of excision repair are nucleotides and bases, in which the damaged bases are removed and then replaced with the correct ones in a localized burst of DNA synthesis. This is present in all types of biological cells and the primary DNA repair mechanism in human cells.

- There are three modes of excision repair, each of which employs specialized sets of enzymes.
- A- Base Excision Repair (BER)
- B- Nucleotide Excision Repair (NER)
- C- Mismatch Repair (MMR)

Base Excision Repair (BER)

BER refers to the removal of damaged bases. This repair is done by *glycosylase* by hydrolyzing the glycosidic bonds.

The steps and some key players:

1.removal of the damaged base (estimated to occur some 20,000 times a day in each cell in our body!) by a DNA glycosylase. We have at least 8 genes encoding different DNA glycosylases each enzyme responsible for identifying and removing a specific kind of base damage.

2.removal of its deoxyribose phosphate in the backbone, producing a gap. We have two genes encoding enzymes with this function.

3.replacement with the correct nucleotide. This relies on **DNA polymerase beta**, one of at least (11) DNA polymerases encoded by our genes.

4.ligation of the break in the strand. Two enzymes are known that can do this; both require ATP to provide the needed energy.

Nucleotide Excision Repair (NER)

NER targets the distortions in the DNA double helix (such as thymine dimer) or the distortions that happen due to the addition of large chemicals to the bases.

➢ NER differs from BER in several ways.

•It uses different enzymes.

•Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, NER removes a large "patch" around the damage.

➢ ***The steps and some key players****:*

1.The damage is recognized by one or more protein factors that assemble at the location.

2.The DNA is unwound producing a "bubble". The enzyme system that does this is **Transcription Factor IIH** (**TFIIH)**, (which also functions in normal transcription).

3.Cuts are made on both the 3' side and the 5' side of the damaged area so the tract containing the damage can be removed.

4. A fresh burst of DNA synthesis using the intact (opposite) strand as a template fills in the correct nucleotides.

The DNA polymerases responsible are designated polymerase **delta** and **epsilon**.

5.A **DNA ligase** covalently inserts the fresh piece into the backbone.

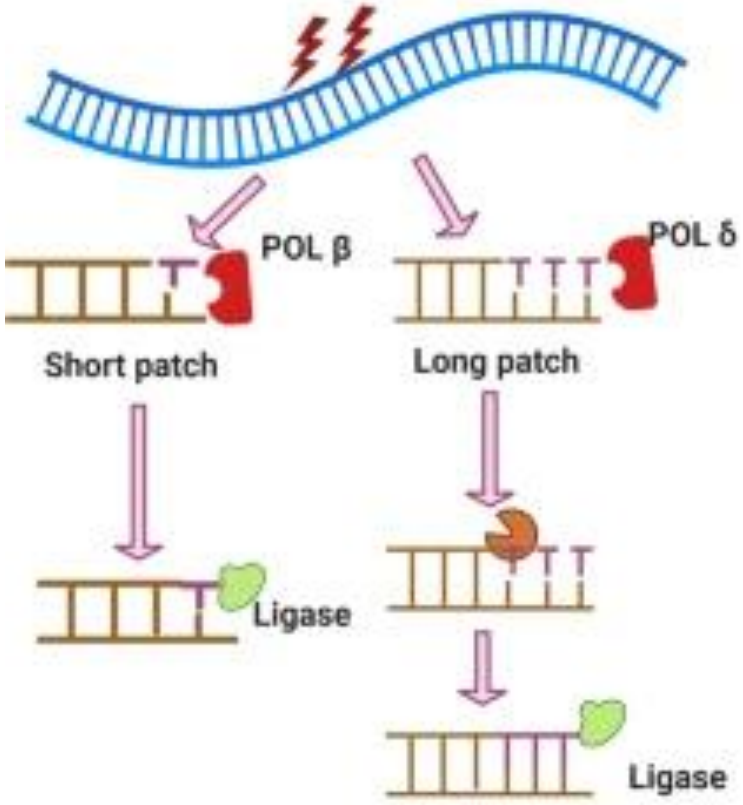
Mismatch Repair (MMR)

Mismatch repair deals with correcting mismatches of the **normal bases**; that is, failures to maintain normal Watson-Crick base pairing (A•T, C•G) due to insertions and deletions during the replication process.

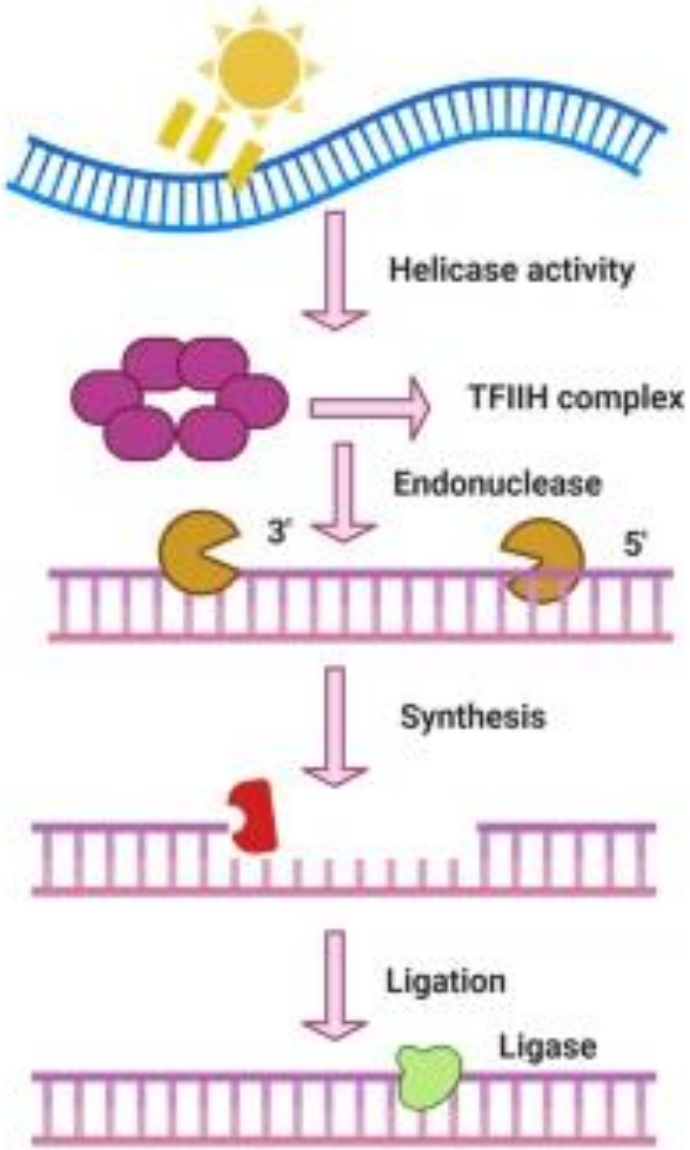
- It can enlist the aid of enzymes involved in both base-excision repair (BER) and nucleotide-excision repair (NER) as well as using enzymes specialized for this function.

- **Recognition** of a mismatch requires several different proteins including one encoded by ***MSH2 gene(tumor suppressor gene)***.
- **Cutting** the mismatch out also requires several proteins, including one encoded by ***MLH1 gene***.
- **Synthesis of the repair patch** is done by **DNA polymerase delta**.

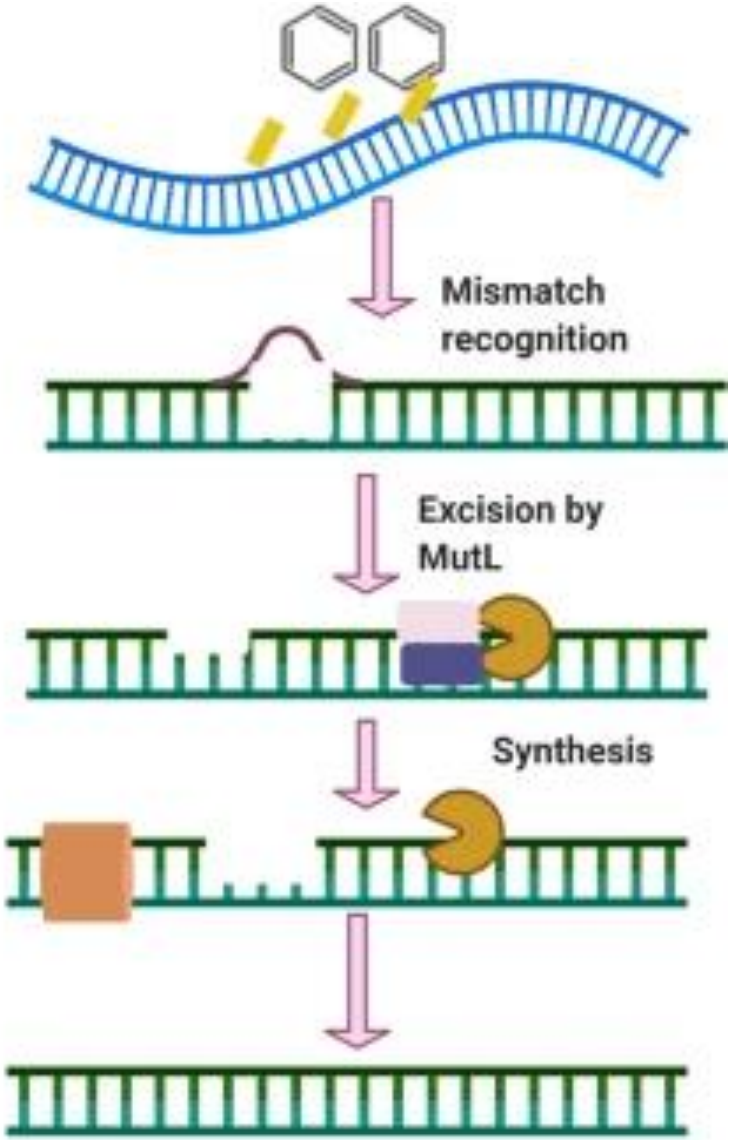
- In *E. coli*, certain adenines become methylated shortly after the new strand of DNA has been synthesized. The MMR system works more rapidly, and if it detects a mismatch, it assumes that the nucleotide on the already-methylated (parental) strand is the correct one and removes the nucleotide on the freshly-synthesized daughter strand. How such recognition occurs in mammals is not yet known.



Base Excision Repair



Nucleotide Excision Repair



Mismatch Repair

Repairing Strand Breaks (Recombinational repair)

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

- Lindahl et al. reported that, each day, our cells may be subject to around 70,000 instances of DNA damage. Most of these lesions are single-strand breaks, and only a few are DSBs, which are less frequent.

1-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).

2-Double-Strand Breaks (DSBs)

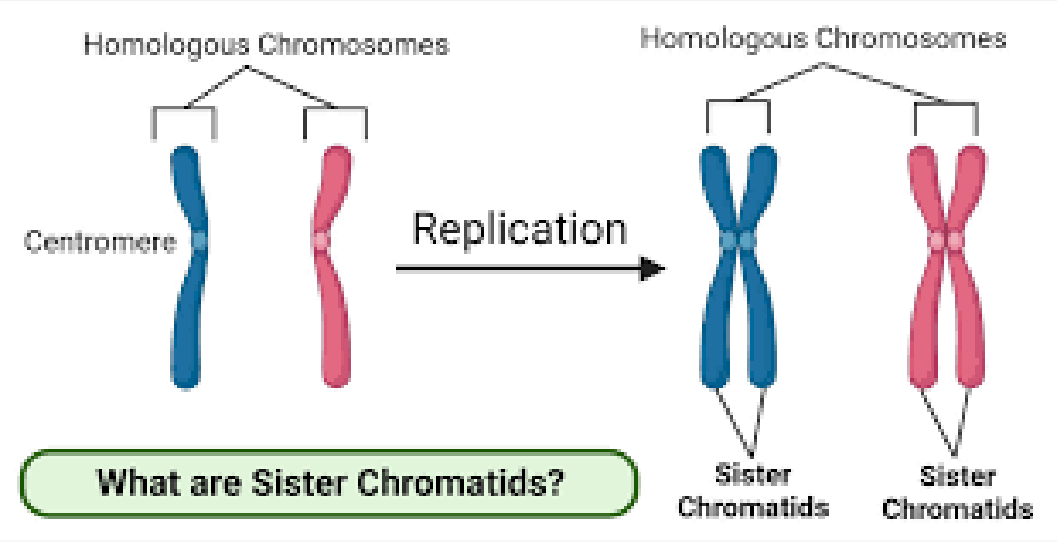
There are two mechanisms by which the cell attempts to repair a complete break in a DNA molecule:

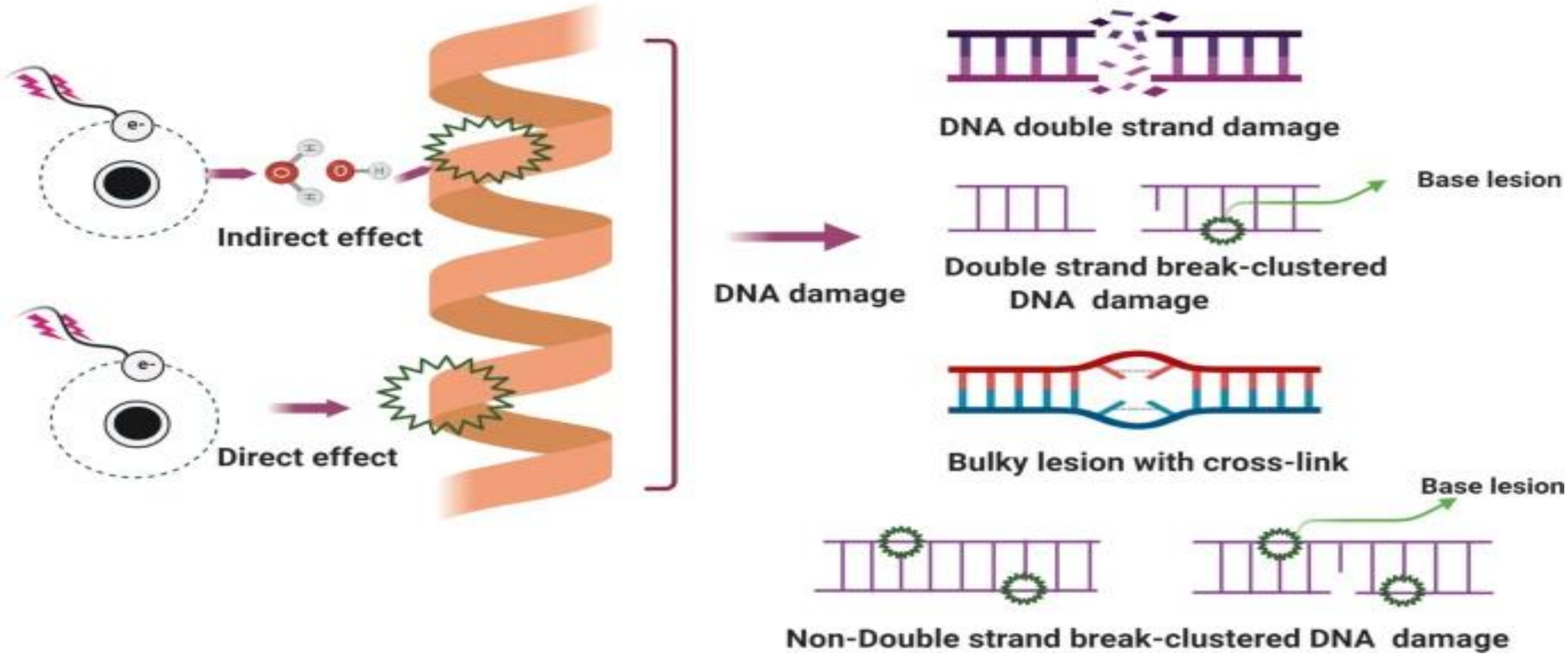
A-Direct joining of the broken ends. This requires proteins that recognize and bind to the exposed ends and bring them together for ligating. They would prefer to see some complementary nucleotides but can proceed without them so this type of joining is also called **Non homologous End-Joining** (**NHEJ**).

***B-Homologous Recombination* (also known as Homology-Directed Repair — HDR).**

- Here the broken ends are repaired using the information on the intact **sister chromatids** (available in G_2 after chromosome duplication), **or** on the **homologous chromosome** (in G_1; that is, before each chromosome has been duplicated), **or in the 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.

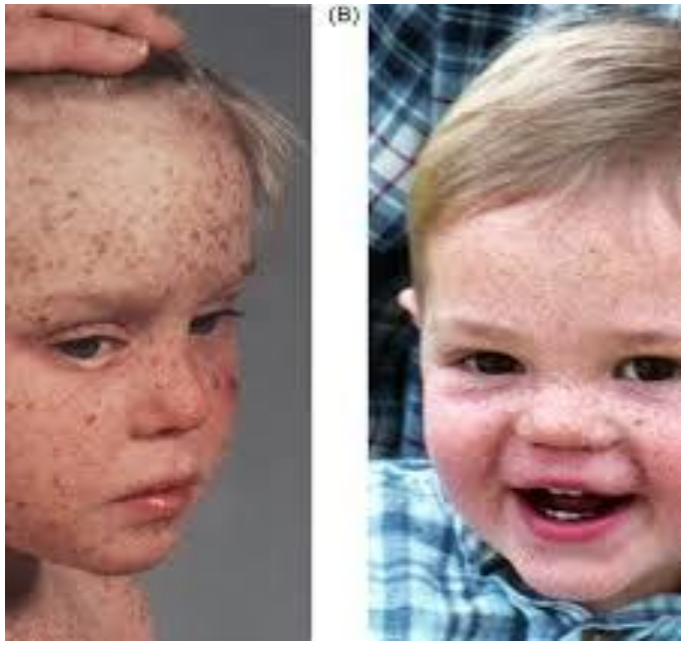



DNA single strand damage
DNA double strand damage
Base lesion
Indirect effect
DNA damage
Double strand break-clustered
DNA damage
Direct effect
Bulky lesion with cross-link
Base lesion
Non-Double strand break-clustered DNA damage

CLINICAL ASPECT OF DNA REPAIR SYSTEM DISORDERS

1- Xeroderma Pigmentosum (XP)

XP, the first DNA repair disorder described in 1874 by Hebra and Kaposi ,is an autosomal recessive syndrome with dermatological, ocular, and neurological manifestations with skin cancer predisposition . XP patients are unable to repair UV radiation-induced DNA damage due to mutations in the NER pathway.



2-Ataxia-Telangiectasia (AT)

AT is an autosomal recessive disorder (familial disorder), is a multi-systemic disease characterized by ataxia secondary to cerebellar degeneration, immunodeficiency with recurrent pulmonary infections, premature aging, ionizing radiation sensitivity, and a high risk of developing cancers of lymphoid origin .
AT is a result of faulty DSB repair pathway.

3-Fanconi Anemia (FA)

- DSB repair syndrome associated with cancer. Autosomal recessive anemia.
- A rare inherited disease characterized by multiple physical abnormalities, bone marrow failure, and a higher-than-normal risk of frequency of cancer.
- FA pathway is defective in recognizes and repairs DNA inter-strand crosslinks that induce a replication block followed by defective repair of DSBs and chromosomal instability.

Cancer Chemotherapy as repair application

•The hallmark of all cancers is continuous cell division.

•Each division requires both

- the replication of the cell's DNA (in S phase) and
- transcription and translation of many genes needed for continued growth.

•So, any chemical that damages DNA has the potential to inhibit the spread of a cancer (carcinogenic). Many (but not all) drugs used for cancer therapy do their work by damaging DNA . *Examples* 6-mercaptopurine (Purinethol®) purine analog. One effect: substitutes for G, inducing strand breaks.

- The cancer patient has many other cell types that are also proliferating rapidly, e.g., cells of the intestinal endothelium, bone marrow and hair follicles.

Anticancer drugs also damage these — producing many of the unpleasant side effects of "chemo“ and poses a significant risk of creating a new cancer, often a leukemia (secondary cancer).