

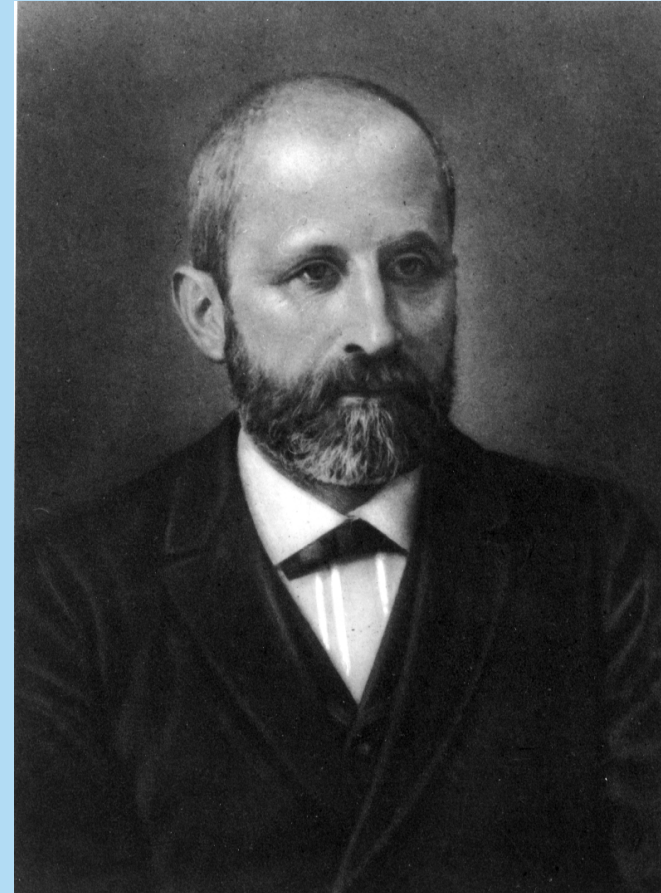
DNA Replication, Damage, & Repair

By: Sai Voleti

DNA Replication

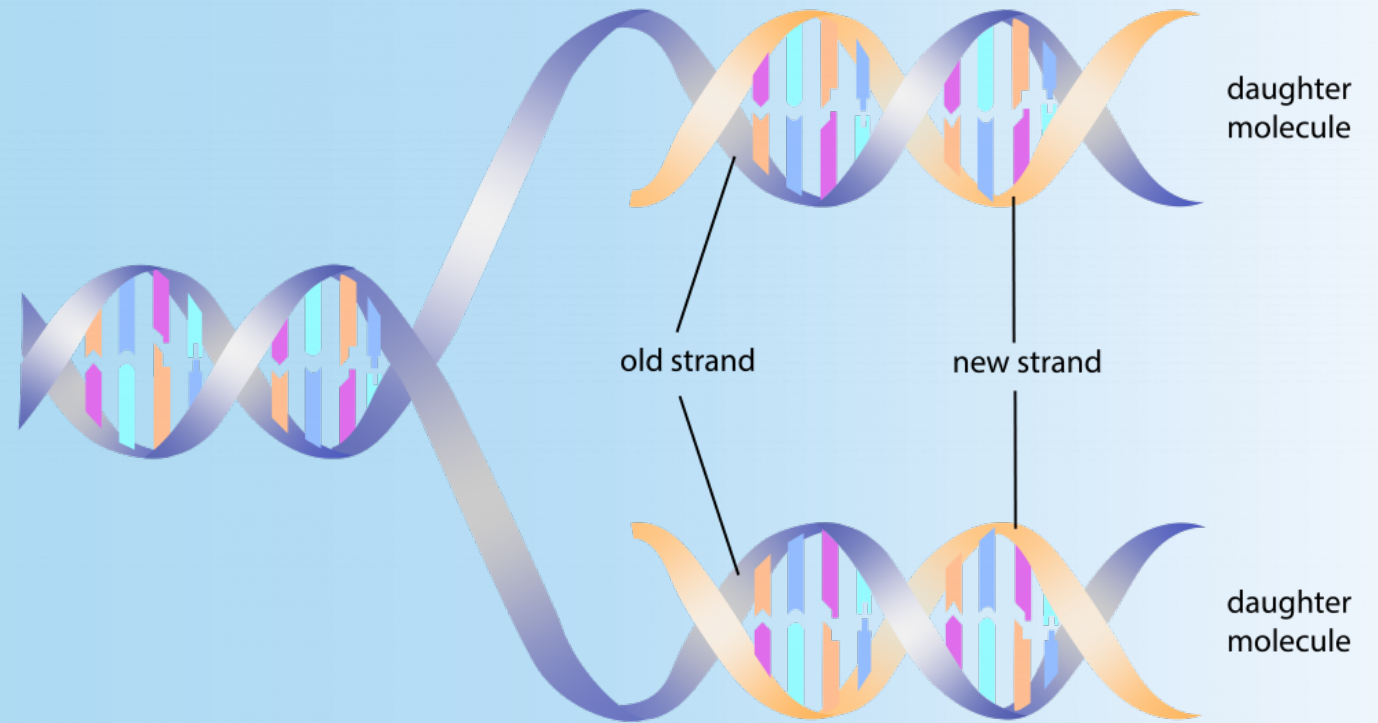
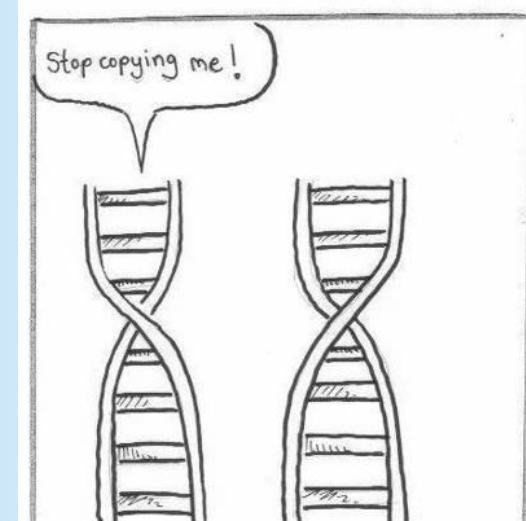
DNA history

- Frederich Miescher
 - First person to isolate DNA in 1869
- DNA looked too simple to hold the genetic code
 - Only four bases, less combinations
- Only almost 90 years later would we find the truth

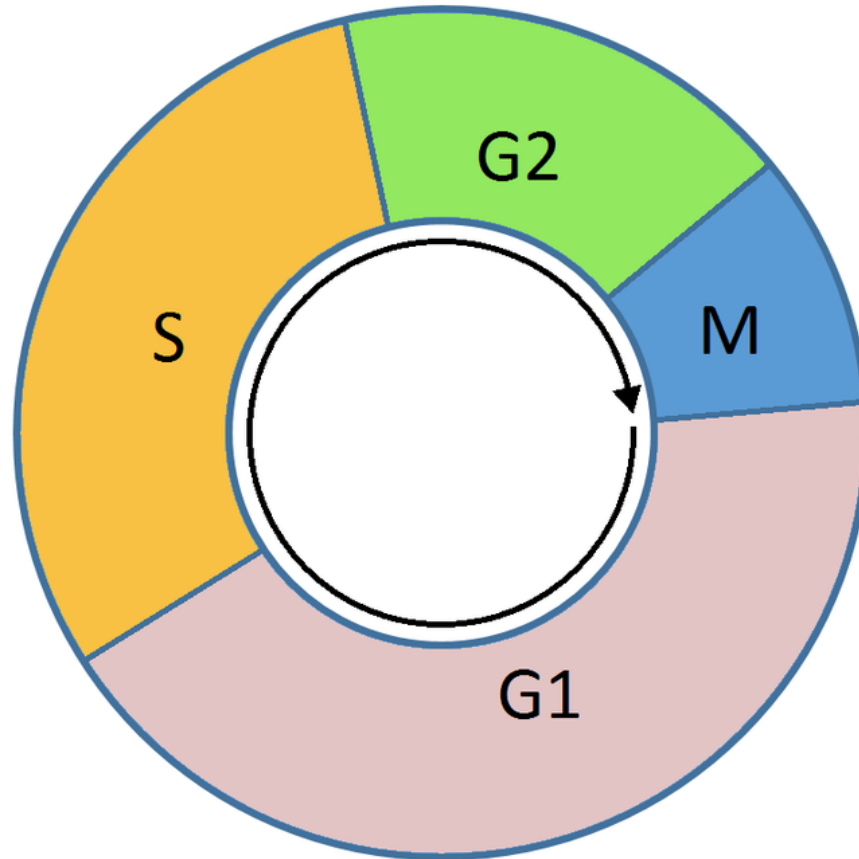


Purpose of DNA Replication

Transmission of genetic information from parent cell to the daughter cell.



S Phase = DNA Synthesis



G1 - Growth

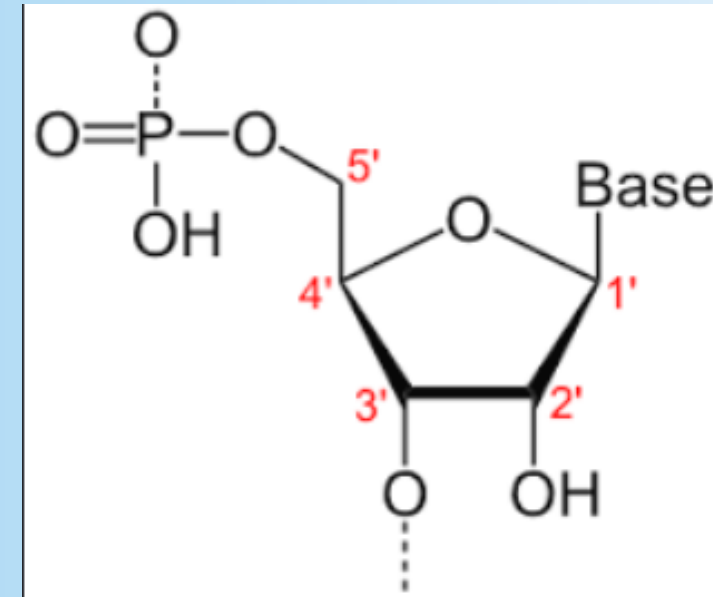
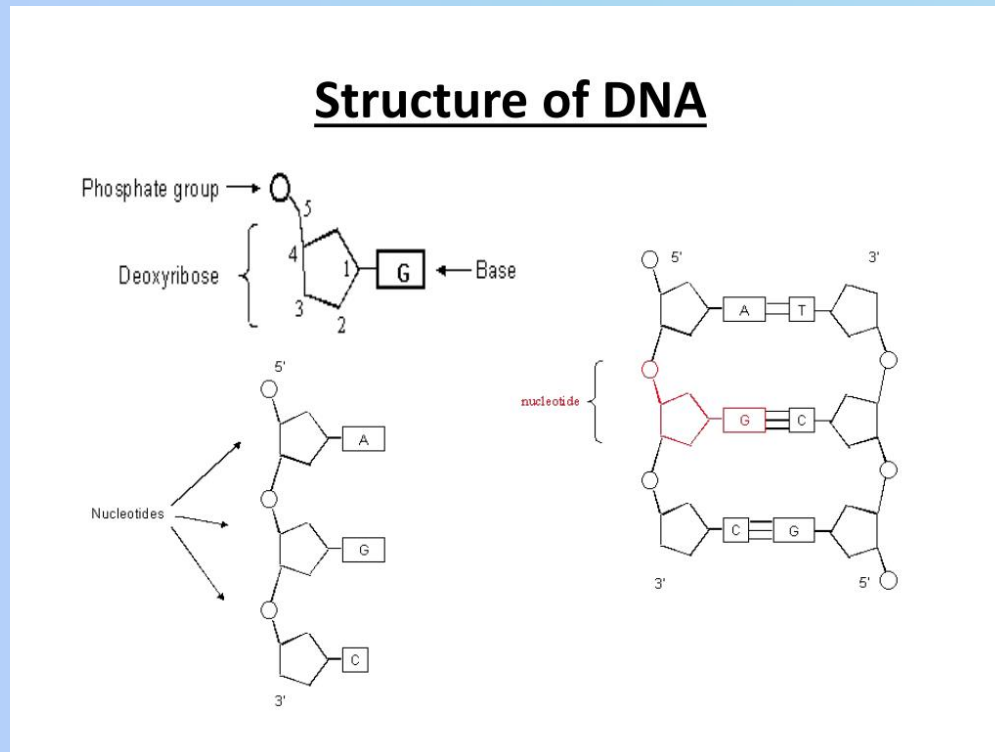
S - DNA synthesis

G2 - Growth and
preparation for
mitosis

M - Mitosis
(cell division)

DNA “5ynth3sis”

DNA synthesis occurs in the 5' → 3' direction, and the template is read in the 3' → 5' direction

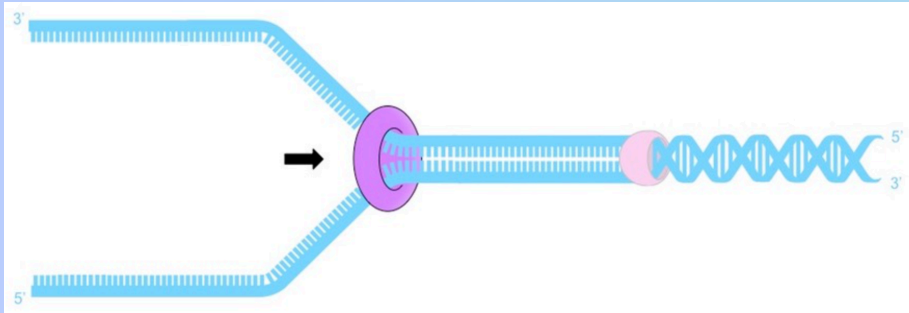


Helicase

Function: Unwinds local segments of DNA double helix at replication fork (ATP-dependent)

Enzyme in Prokaryotes: DnaB

Enzyme in Eukaryotes: MCM

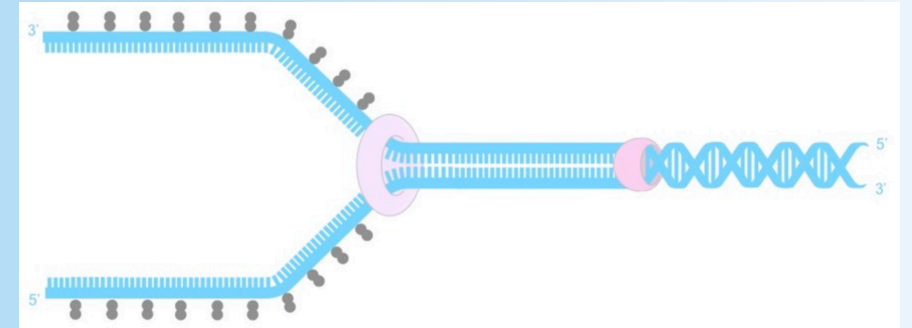


Single stranded binding proteins

Function: Prevents reannealing of separated strands

Enzyme in Prokaryotes: SSB

Enzyme in Eukaryotes: RPA (replication protein A)



Helicase, imagine you are opening a suit**C**ase,
and helicase is your zipper

Topoisomerase

Both Topoisomerase I and Topoisomerase II function to remove supercoils that develop during DNA elongation as parent DNA is separated by helicase



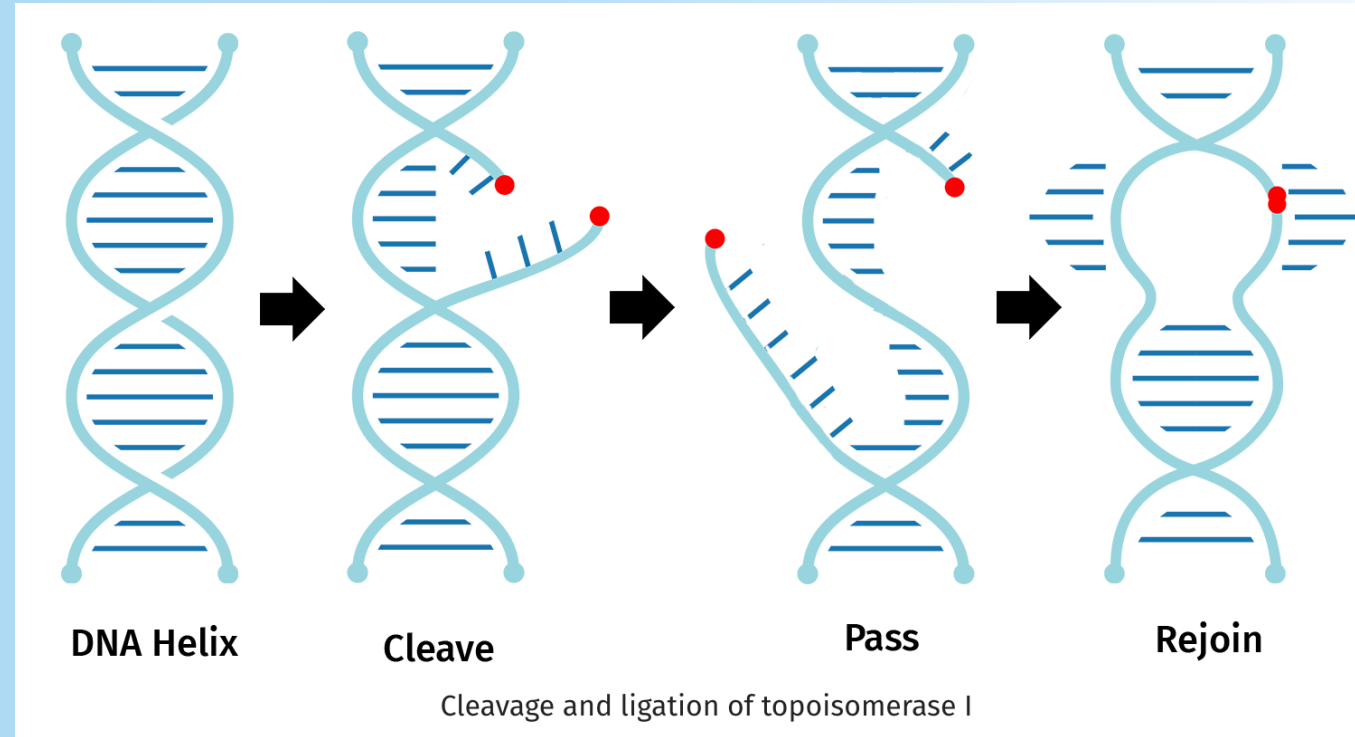
Topoisomerase I

Function:

- Cleaves only one of both DNA strands
- Has nuclease (nuclear bomb, i.e destroys DNA) activity to cut DNA strands
- Also has ligase (glue-gase/gluing enzyme) activity to reseal the ligated strand
- Does not require ATP

Enzyme in Prokaryotes: Topoisomerase I
(Topoisomerase IA)

Enzyme in Eukaryotes: Topoisomerase I
(Topoisomerase IB)



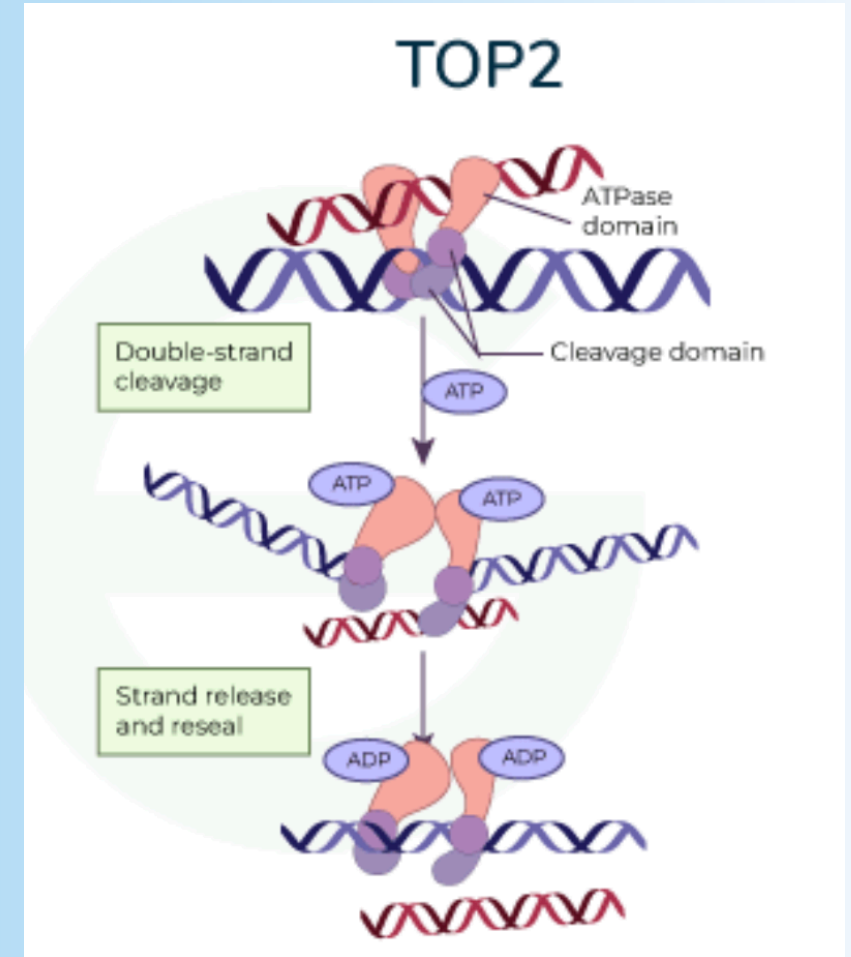
Topoisomerase II

Function: Cleaves both DNA strands for larger structural alterations of DNA (requires ATP)

Enzyme in Prokaryotes: DNA gyrase, (clinically relevant) & Topoisomerase IV

Enzyme in Eukaryotes: Topoisomerase II

[Topoisomerase 1 and 2 - YouTube](#)

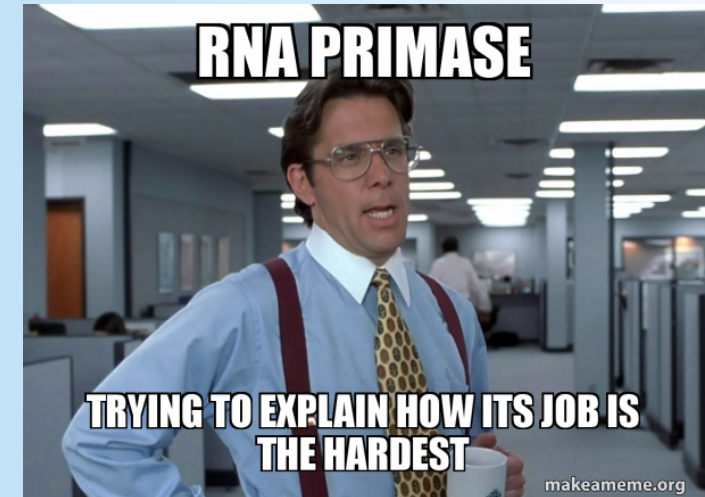
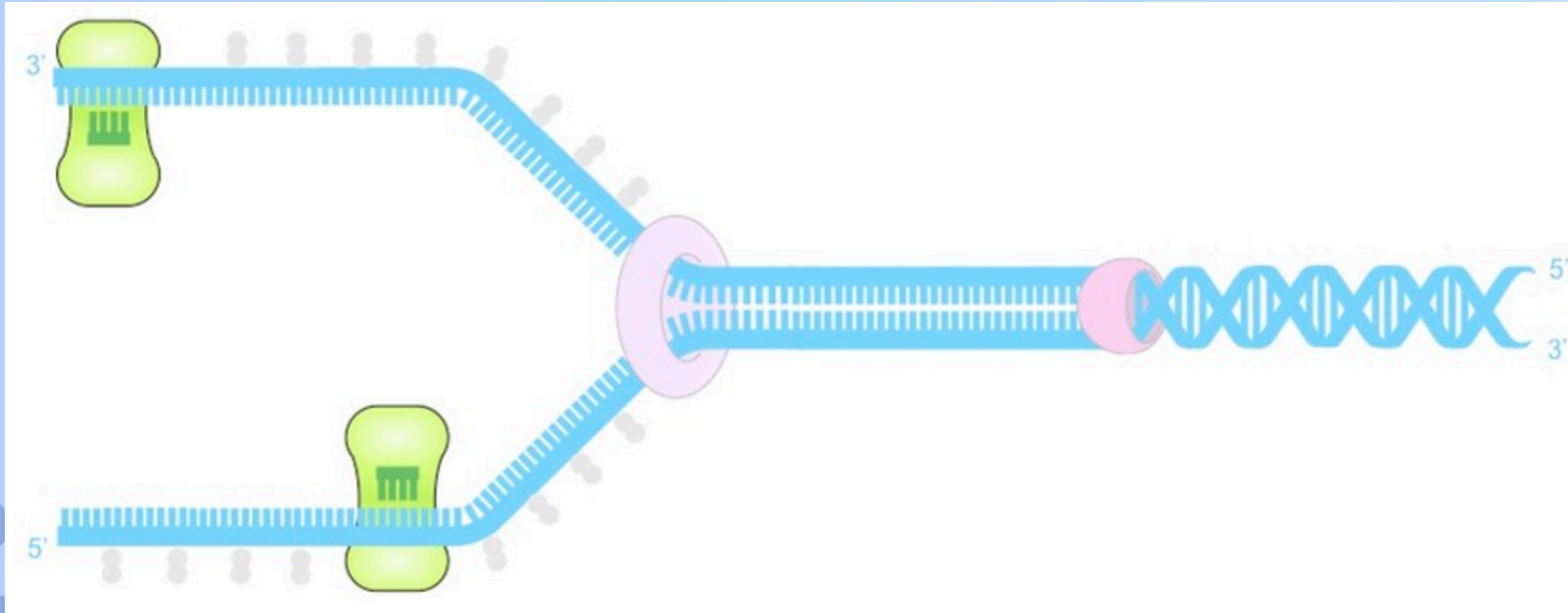


Primase (DNA-Dependent RNA Polymerase)

Function: Synthesis of short RNA primers

Enzyme in Prokaryotes: Primase (DnaG)

Enzyme in Eukaryotes: Primase activity as a component of DNA polymerase α



DNA-Dependent DNA Polymerase

Polymerase	Function	Exonuclease Activity
α	Synthesizes the RNA primer, initiations DNA synthesis and the lagging strand	None
β	Repair DNA	None
γ	Replicate mitochondrial DNA	3' to 5'
δ	Synthesizes the leading strand, filling DNA gaps after removal of primer	3' to 5'
ϵ	Repair DNA	3' to 5'

<https://youtu.be/TNkWgcFPHqw?si=sVFahs58lxN2DIZ2&t=80>

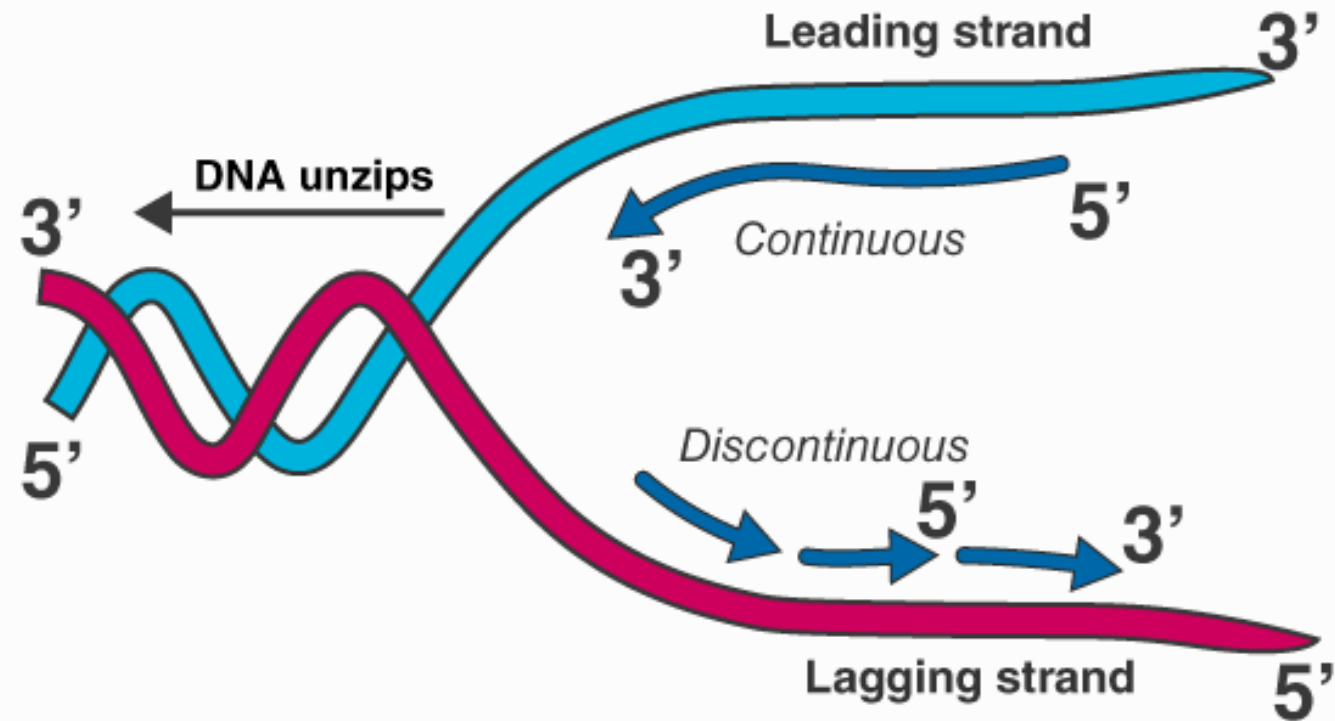
HIGH YIELD

DNA replication be like:



DNA REPLICATION FORK

BYJU'S
The Learning App

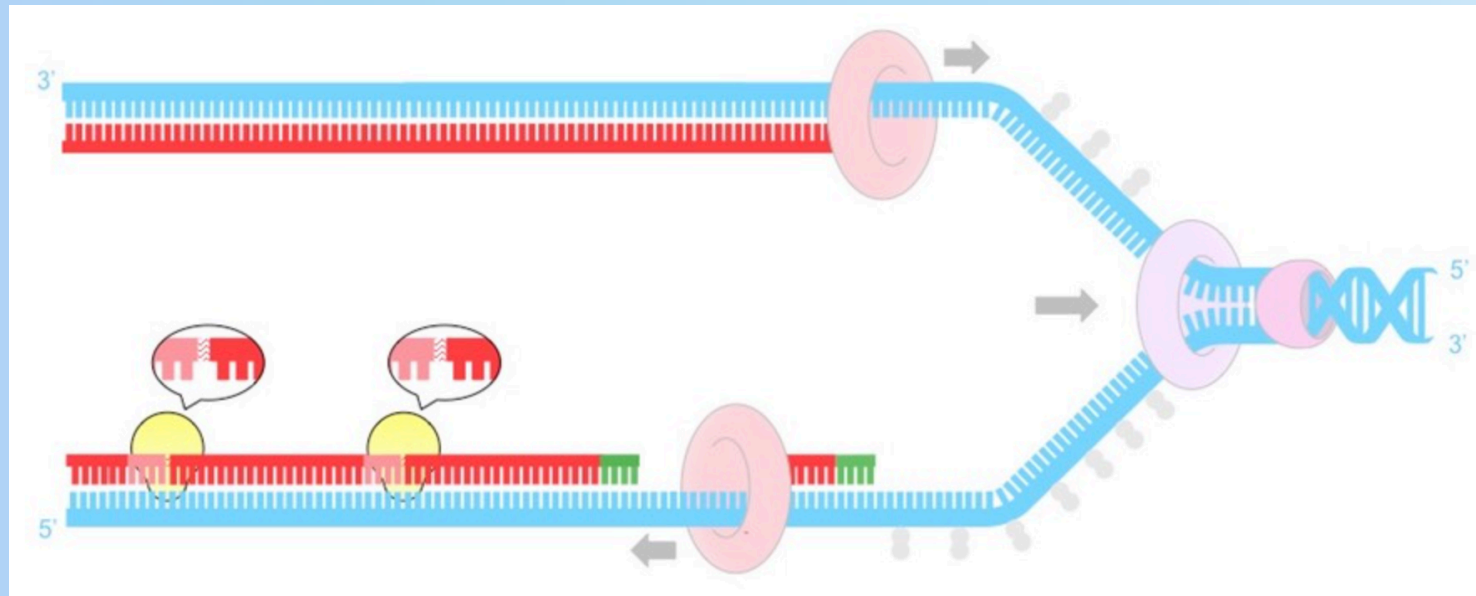


Ligase

Function: Links newly synthesized DNA fragments (Okazaki fragments HIGH YIELD) by catalyzing the formation of phosphodiester bonds (ATP or NAD⁺ dependent)

Enzyme in Prokaryotes: DNA ligase

Enzyme in Eukaryotes: DNA ligase



Ligase Ligates the Okazaki fragments !

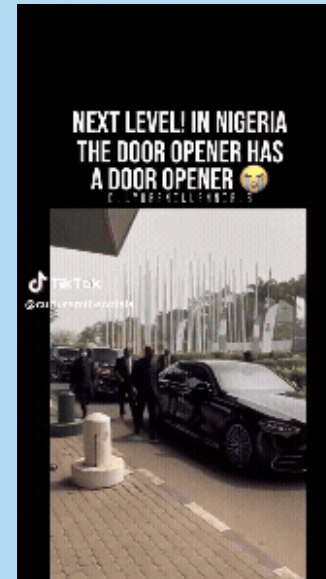
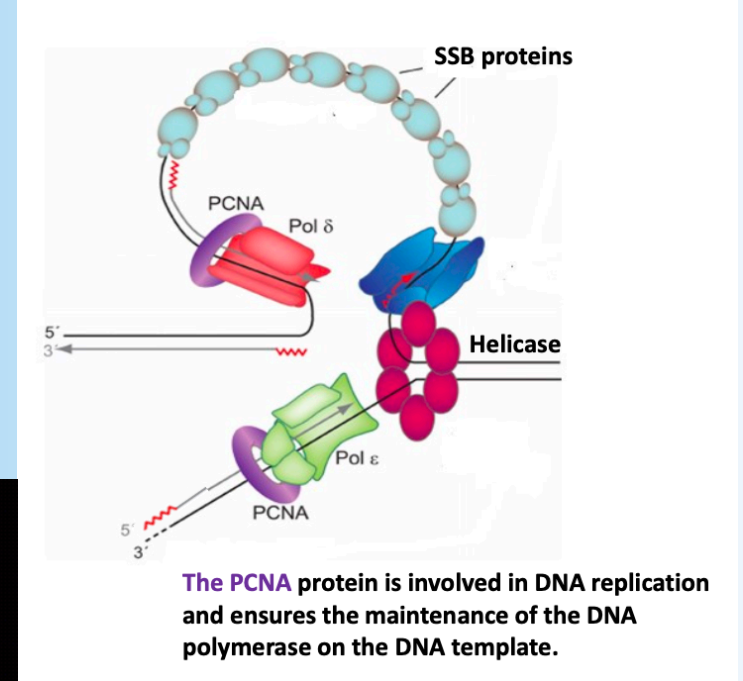
Steps of DNA Replication



Elongation: Clamp and Processivity

Sliding clamp (PCNA): increases the processivity of DNA polymerases, ensuring efficient replication

The clamp loader (RFC) helps load PCNA onto the DNA



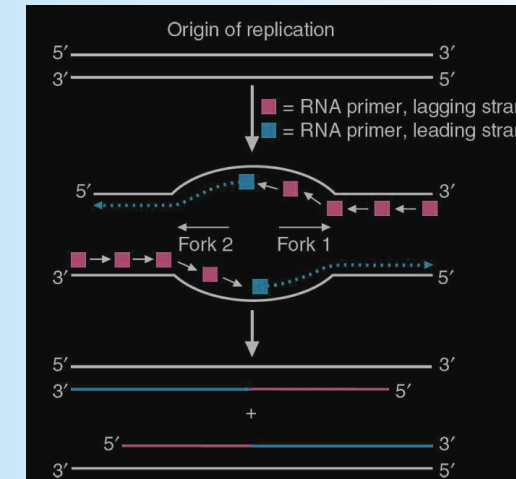
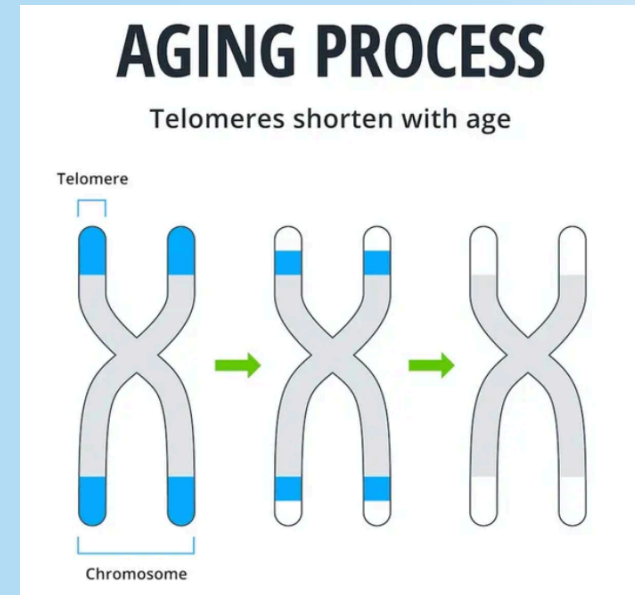
Telomeres

Structure: a noncoding DNA fragment of several thousand base pairs (composed of tandem repeats of TTAGGG) at the 3' end of chromosomes

Function:

- Prevention of structural gene loss during replication of linear DNA double-strands (telomerases lose their function after 30-50 cell divisions)
- Telomeres prevent the continuous loss of nucleotides from the 3' end of DNA during replication and raise the number of possible replication cycles that a given cell can undergo

Maintenance by telomeres (HIGH YIELD): a type of reverse transcriptase that carries its own RNA template (ribonucleoprotein) and can elongate telomeres



To remember the **TTAGGG** sequence added by the **telomerase**: **T**ell **T**hem **A**ll: **G**enes **G**otta **G**o!

All the proteins that were needed NOTE: pic

Helicase

Single-Stranded
DNA-Binding
Proteins (SSBs)

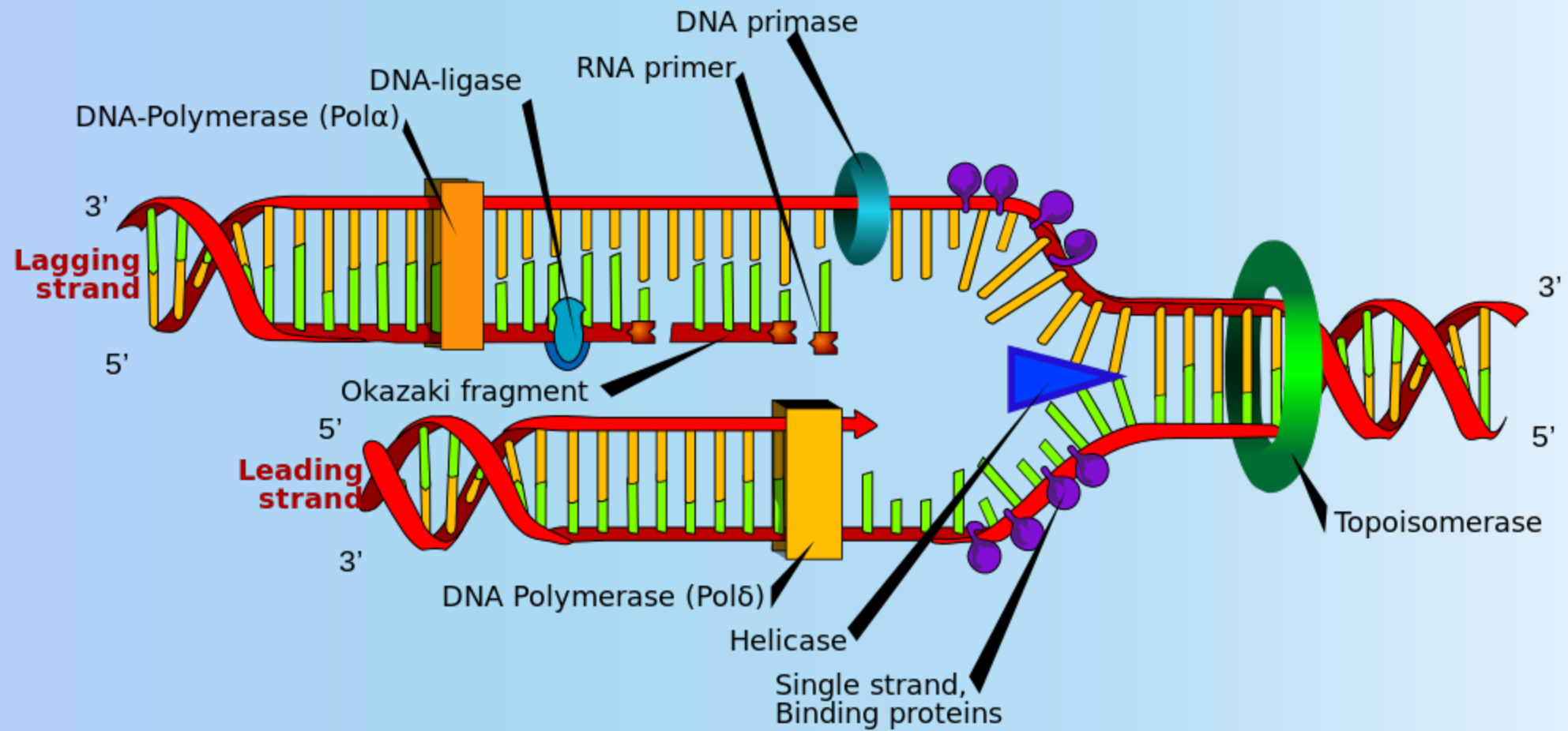
Topoisomerase

Primase (DNA-
Dependent RNA
Polymerase)

DNA-
Dependent DNA
Polymerase

Ligase

Telomerase



DNA Damage

Endogenous vs. Exogenous

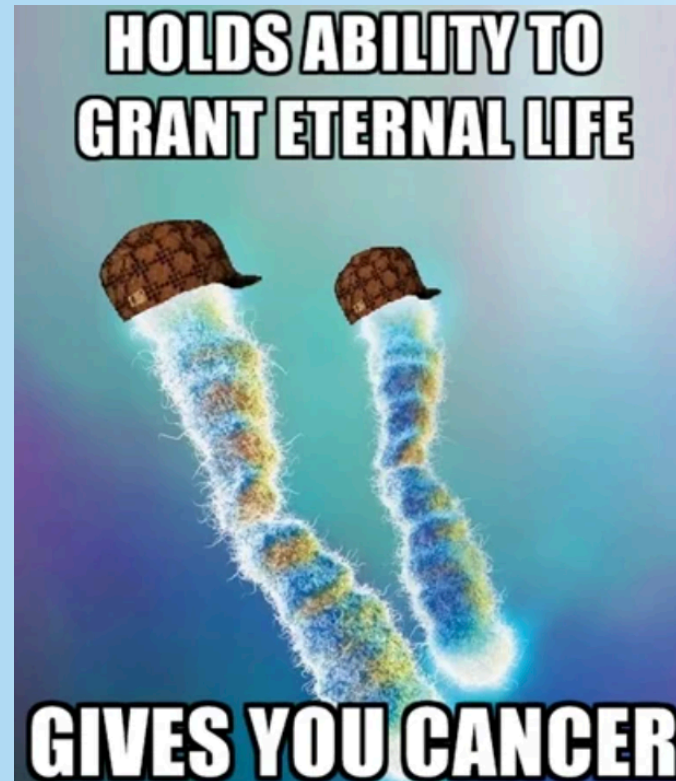
There are both endogenous and exogenous sources of DNA damage, which may result in mutations and/or cell death if not repaired

Endogenous:

- Replication Errors
- Chemical Instability

Exogenous:

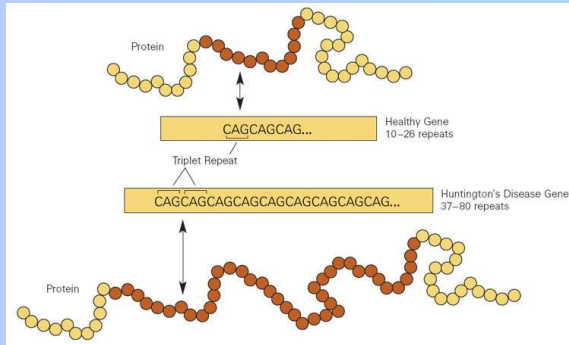
- Toxic substances
- Radiation



Endogenous sources

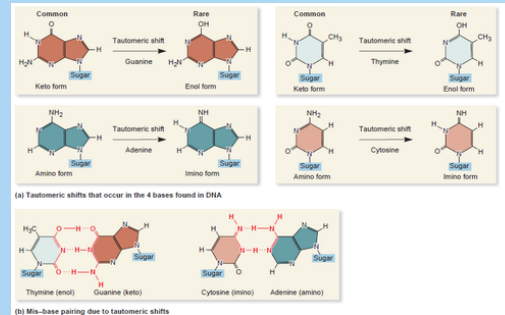
Repetitive sequences:

- DNA polymerase is error-prone in reading repetitive DNA sequences



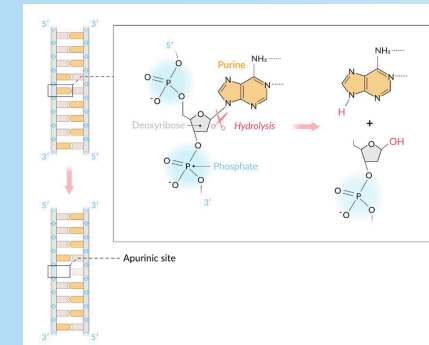
Keto-enol Tautomerism:

- A transient tautomeric shift of nucleotide bases from the usual keto-configuration to the enol-configuration.
- **Most common cause of replication error!**



Depurination:

- Thermal cleavage of the N-glycosidic bond between deoxyribose and a purine base (guanine or adenine) at body temperature

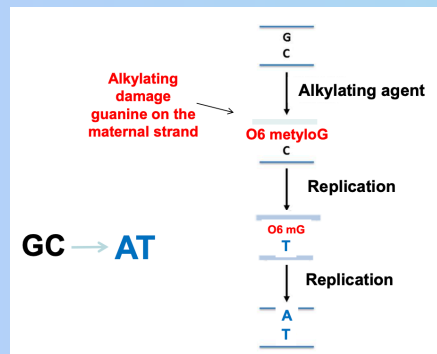


Free Radical Damage:

- Highly reactive oxygen or hydroxyl radicals that cause **oxidative stress** and can chemically alter bases
- Example: **guanosine to 8-oxoguanosine**

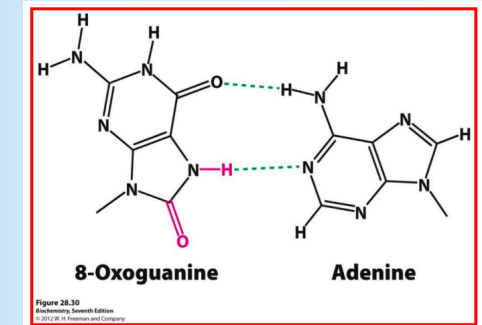
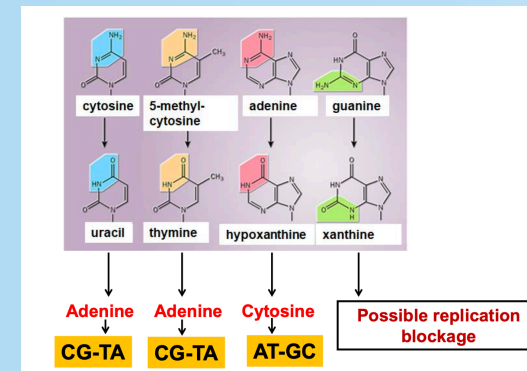
Alkylating Substances:

- Mechanism of Action: methylate or ethylate bases cause incorrect base pairing



Spontaneous Deamination:

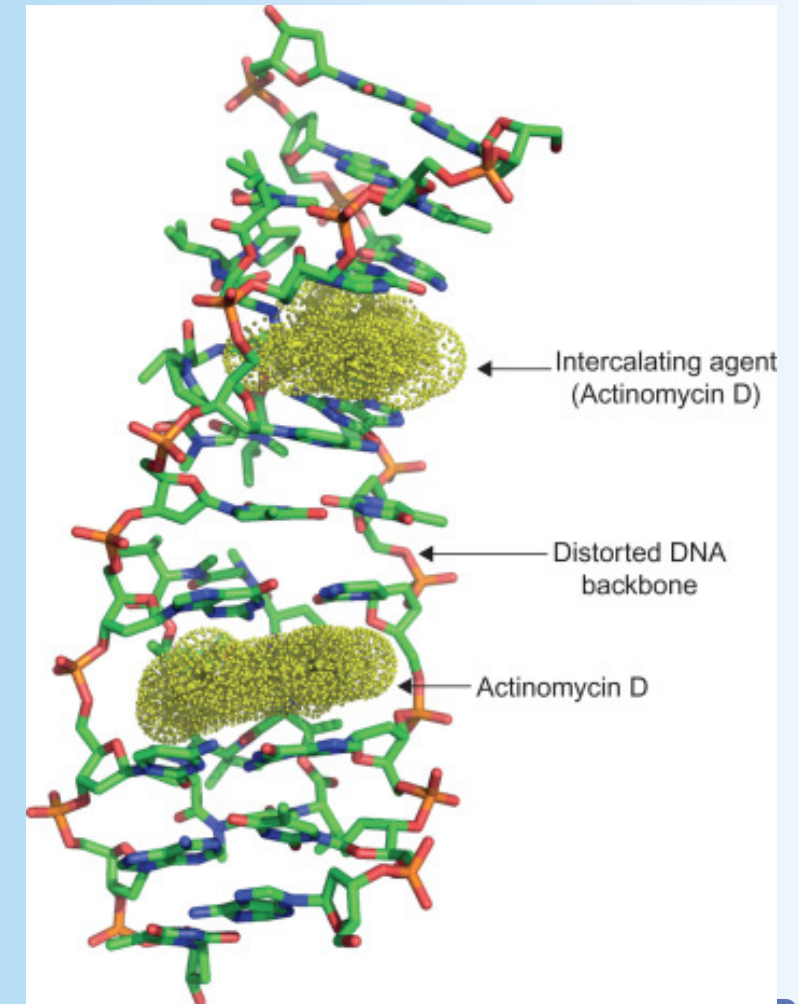
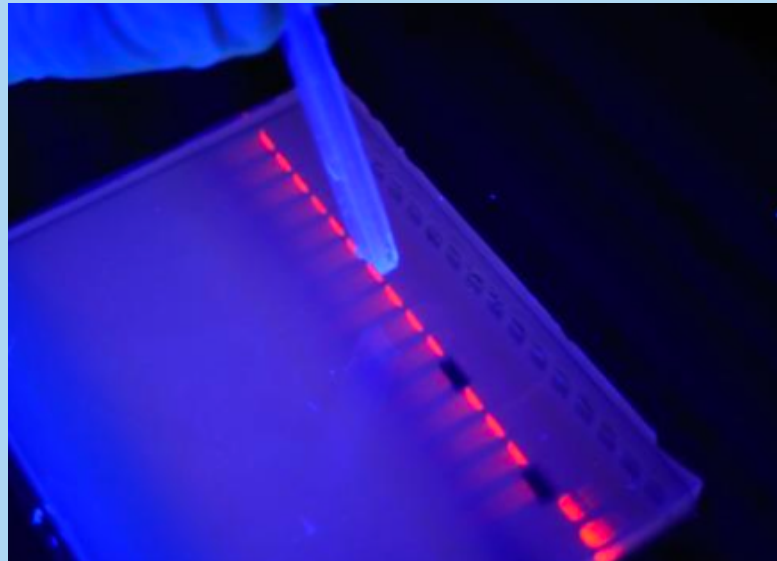
- Deamination of DNA bases causes incorrect base pairing (mismatching)



Exogenous: Toxic Substances

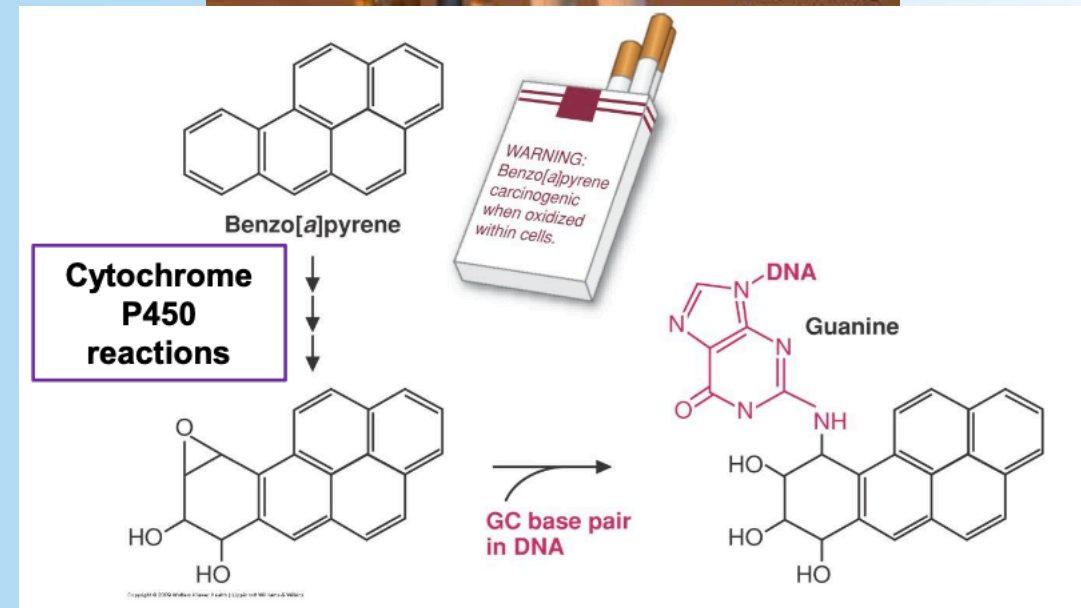
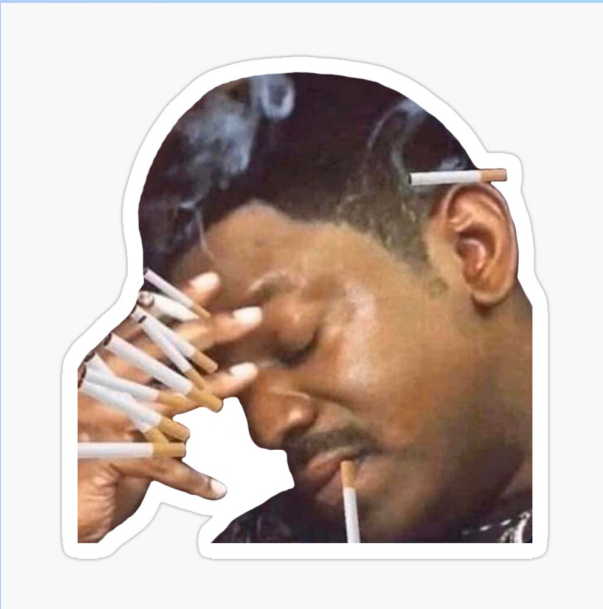
Intercalating Substances:(clinically relevant)

- Mechanism of action: embedding between the stacked DNA base pairs, causing replication to stop and increasing the risk of strand breaks
- Examples: ethidium bromide, acridine dye, dactinomycin



Exogenous: Toxic Substances

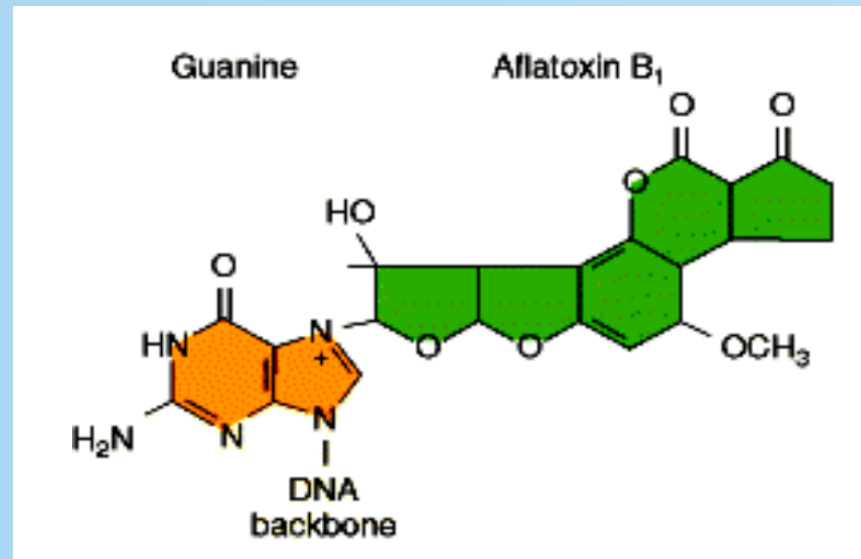
- Polycyclic aromatic hydrocarbons (PAHs) are organic compounds made of multiple fused aromatic rings.
- They are produced by incomplete combustion of organic materials such as coal, oil, wood
- In the liver, PAHs are metabolized by cytochrome P450 enzymes (high yield) (e.g., CYP1A1) into reactive intermediates like diol epoxides.



Exogenous: Toxic Substances

Aflatoxin B₁:

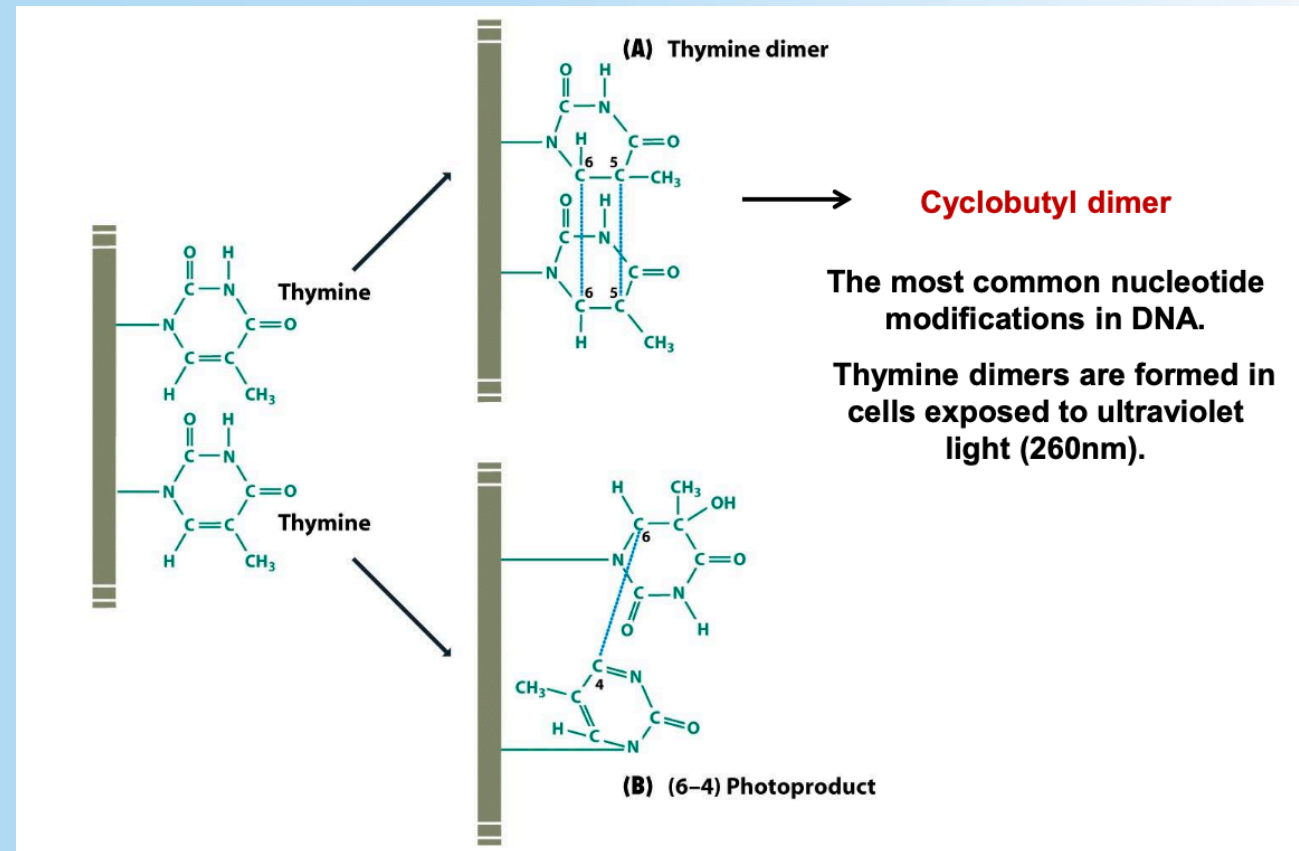
- Aflatoxin B₁ (AFB₁) is a highly toxic and carcinogenic mycotoxin produced by molds, primarily *Aspergillus flavus* and *Aspergillus parasiticus*
- Found in contaminated food products, especially **grains**, **nuts** (e.g., peanuts), and **spices**, stored in warm, humid conditions
- AFB₁ is metabolized in the liver by **cytochrome P450 enzymes (high yield)**(e.g., CYP1A2 and CYP3A4) to form the reactive intermediate **AFB₁-8,9-epoxide**



Exogenous: Radiation

UV Radiation:

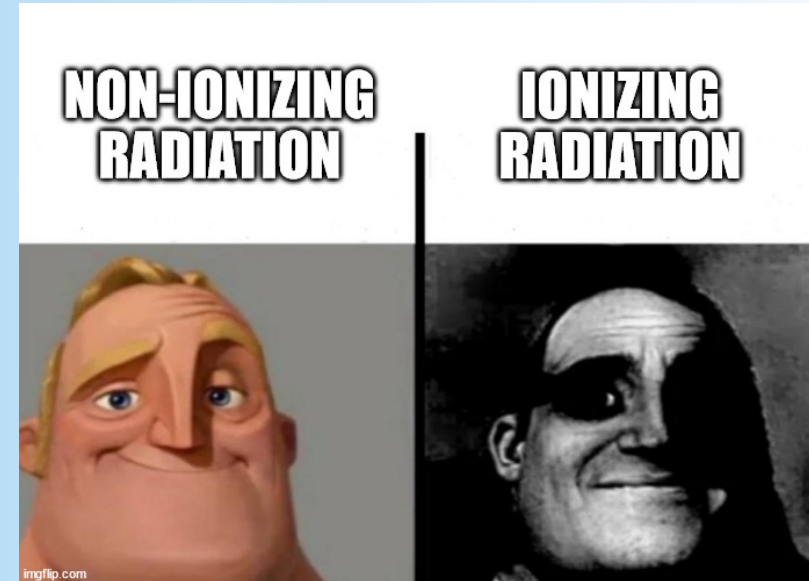
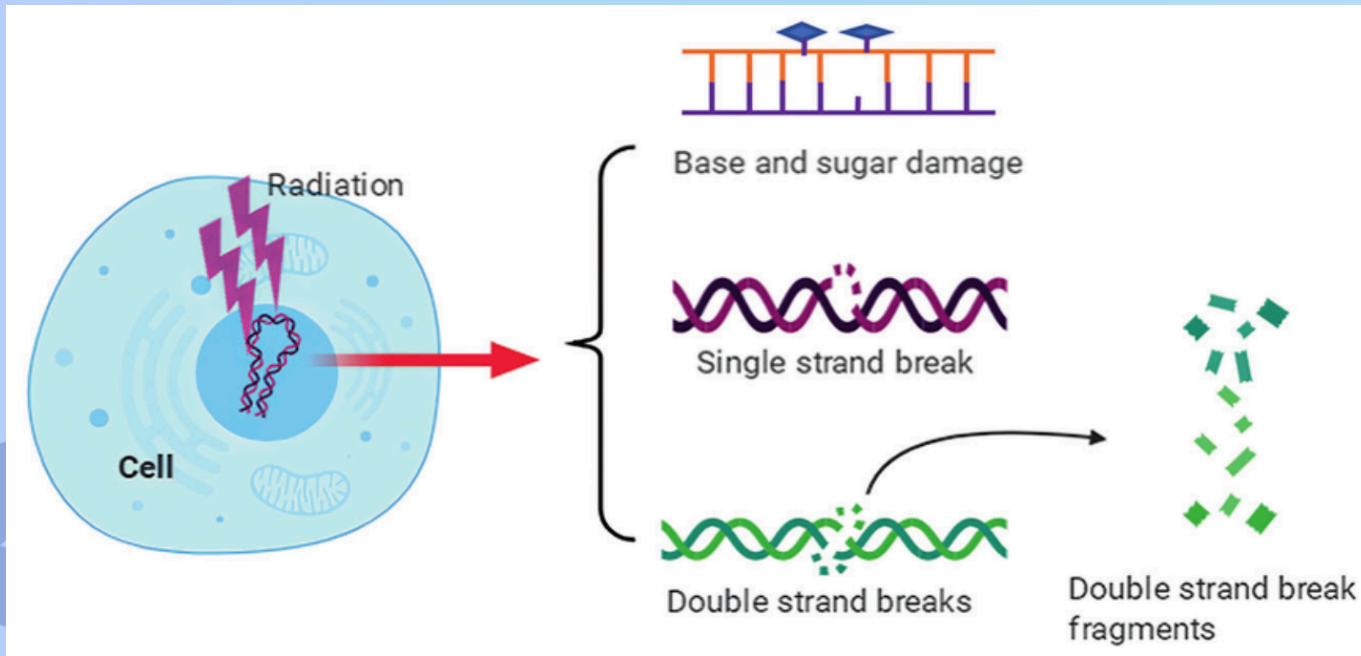
- Both UVA and UVB can result in dimer formation of neighboring pyrimidine bases (**pyrimidine dimers**) (clinically relevant)



Exogenous: Radiation

Ionizing Radiation:

- X-ray and γ -ray radiation as well as α - and β -particles from radioactive decay
- Mainly cause dsDNA breaks, but can also cause ssDNA breaks
- Also result in increased formation of free radicals



DNA Repair

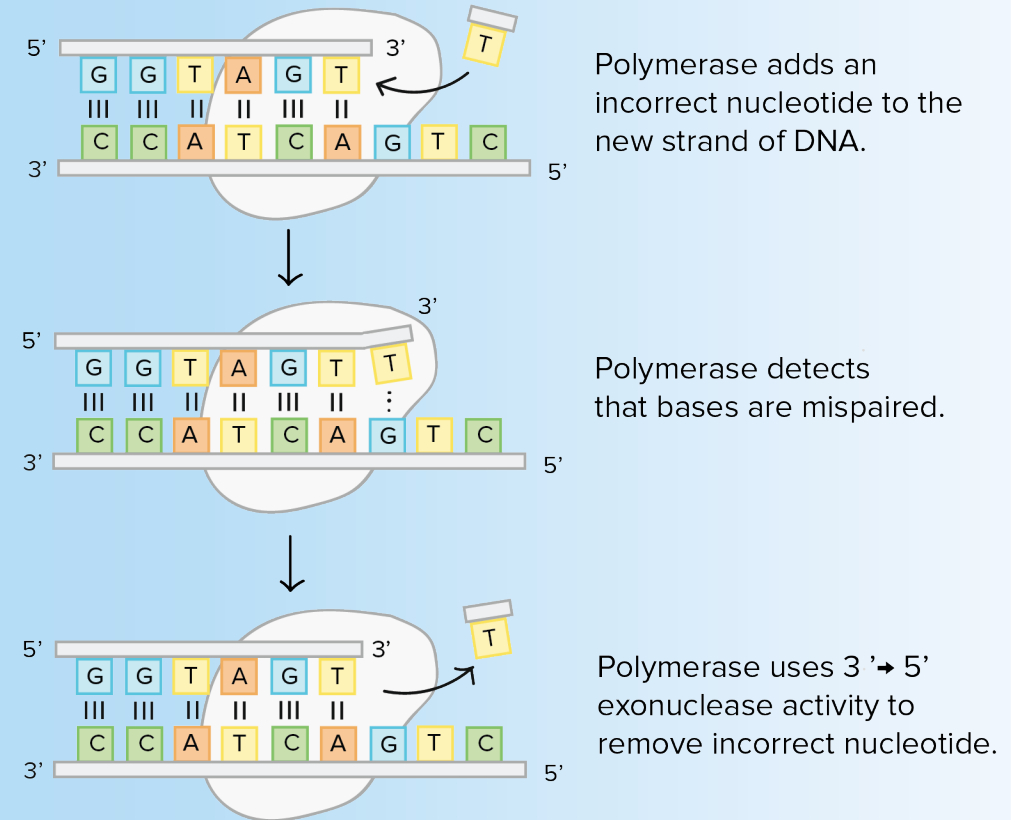
Proofreading and Error Correction

DNA polymerase proofreading:

- DNA polymerase δ and ϵ has $3' \rightarrow 5'$ exonuclease activity to correct misincorporated bases during replication

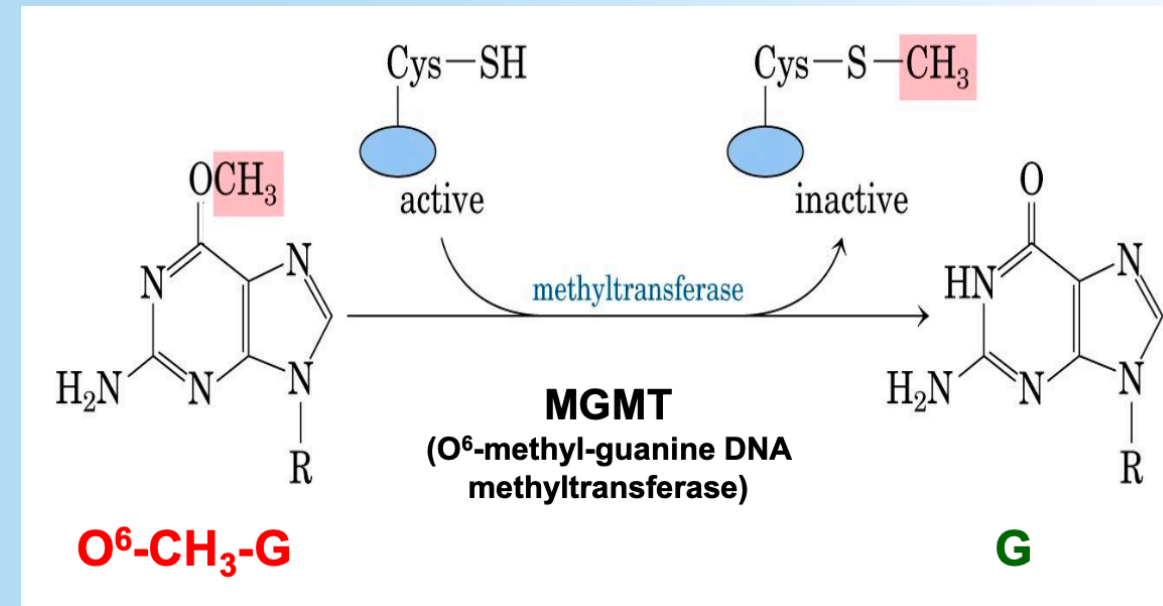
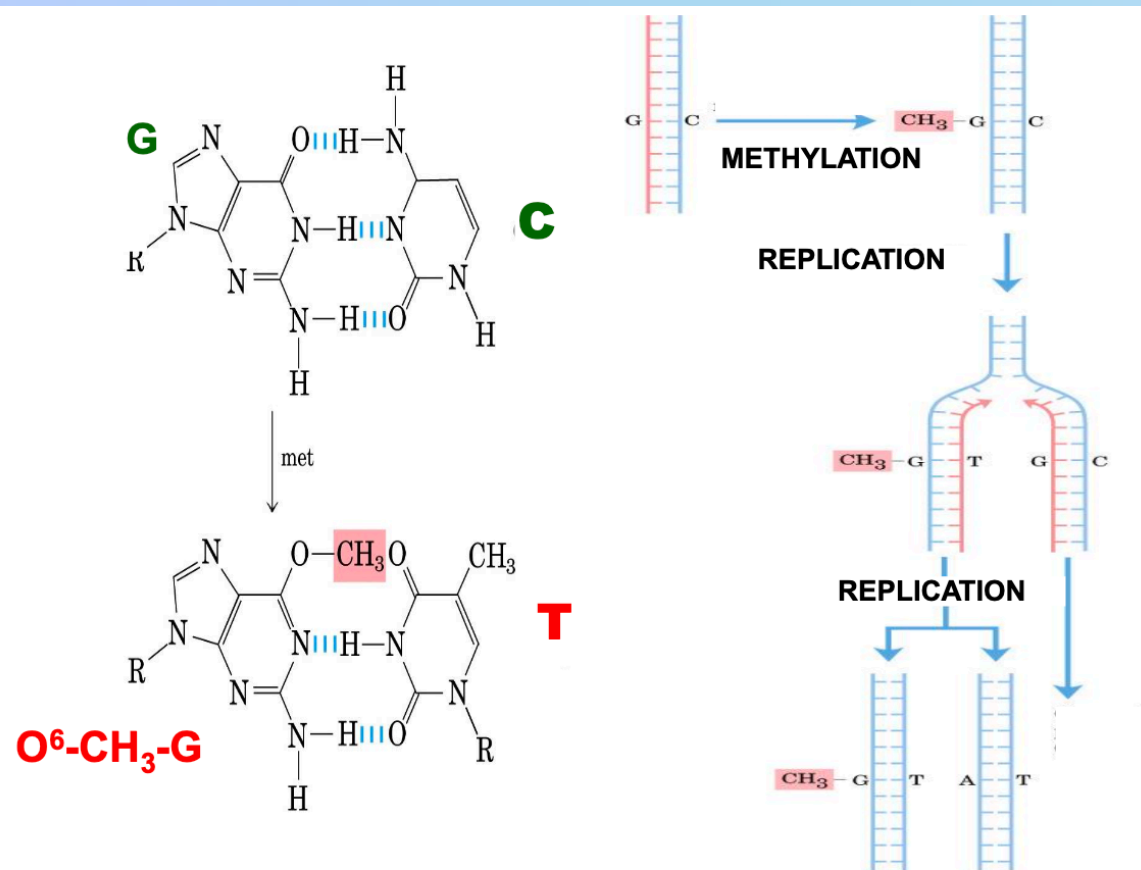
Post-replication repair:

- Additional repair pathways (e.g., mismatch repair) address errors missed during replication



Direct Repair

Some forms of DNA damage can be reversed directly

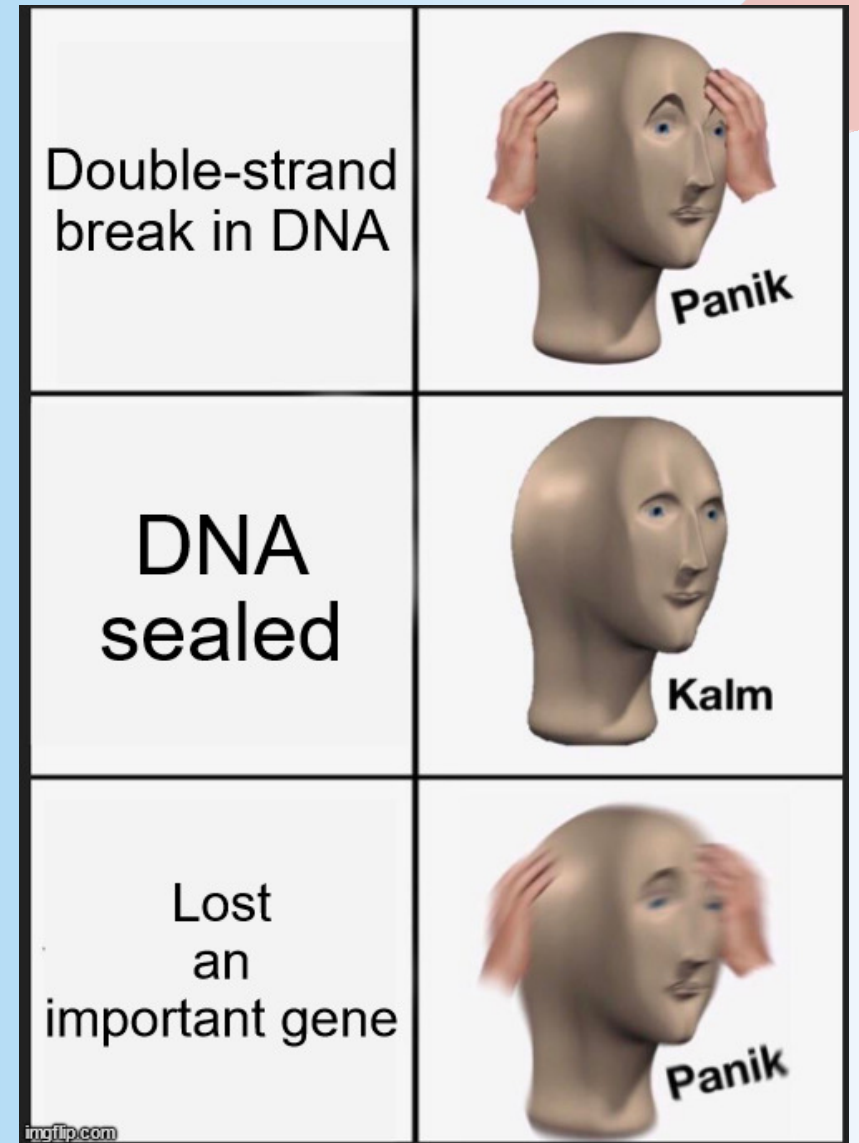


ssDNA Repair:

- Base excision repair
- Nucleotide excision repair
- DNA mismatch repair

dsDNA Repair:

- Nonhomologous end joining
- Homologous recombination repair



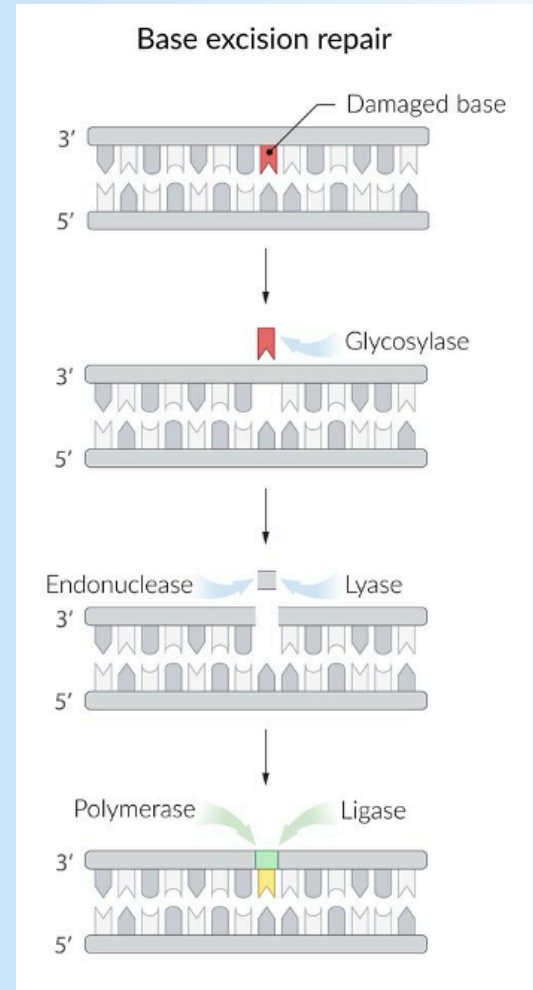
ssDNA Repair: Base Excision Repair

Mechanism:

- Base-specific glycosylase (**DNA glycosylase**) recognizes and removes damaged bases (e.g., deaminated or oxidated), creating an **AP site** (apurinic/apyrimidinic)
- **AP endonuclease** cuts the DNA backbone on the 5' end of the AP site.
- **AP lyase** cuts the DNA backbone on the 3' end of the AP site.
- **DNA polymerase-β** refills the gap
- **Ligase** reseals the strand

Associations with defective repair:

- Repair of spontaneous or toxic deamination
- Certain cancers, e.g., colorectal cancer (MBD4), gastric cancer (NEIL1)



Enzymes of base excision repair: **GEL PLease** = **G**lycosylase, AP-**E**ndonuclease, **L**yase, DNA **P**olymerase, DNA **L**igase

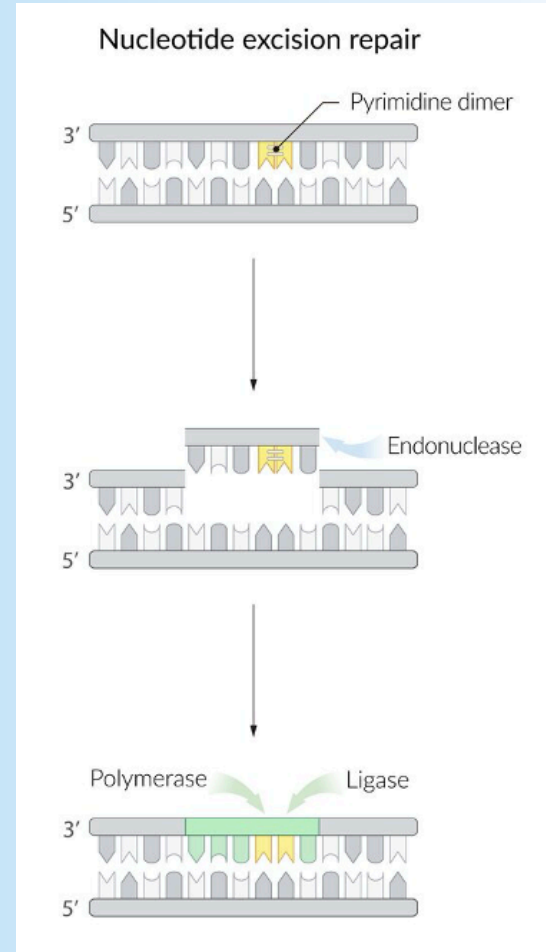
ssDNA Repair: Nucleotide Excision Repair

Mechanism:

- Specific endonucleases recognize the damaged area (e.g., **pyrimidine dimers** that distort the DNA helix) or nucleotides (typically a 12-24 bp section)
- Oligonucleotide containing the damaged region is excised by **endonuclease**
- **DNA polymerase** refills the resulting gap
- **Ligase** reseals the strand

Associations with defective repair:

- **Xeroderma pigmentosum** (UV radiation causes formation of pyrimidine dimers)



Xeroderma Pigmentosum

- Xeroderma Pigmentosum (XP) is a rare, genetic disorder.
- XP results from mutations in genes involved in the repair of DNA damage caused by UV light.



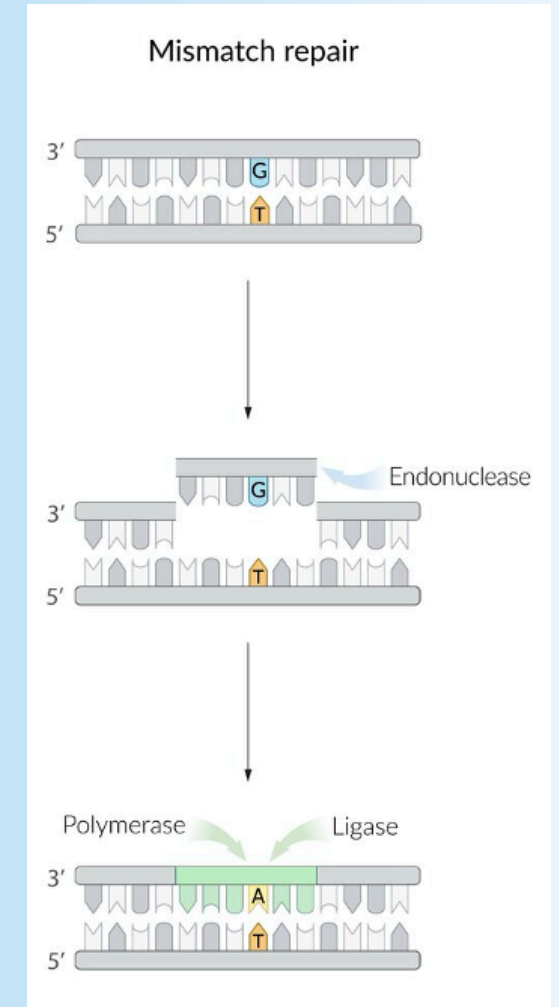
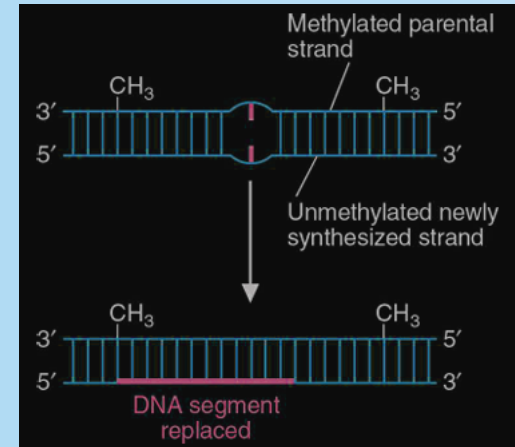
SSDNA Repair: DNA Mismatch Repair

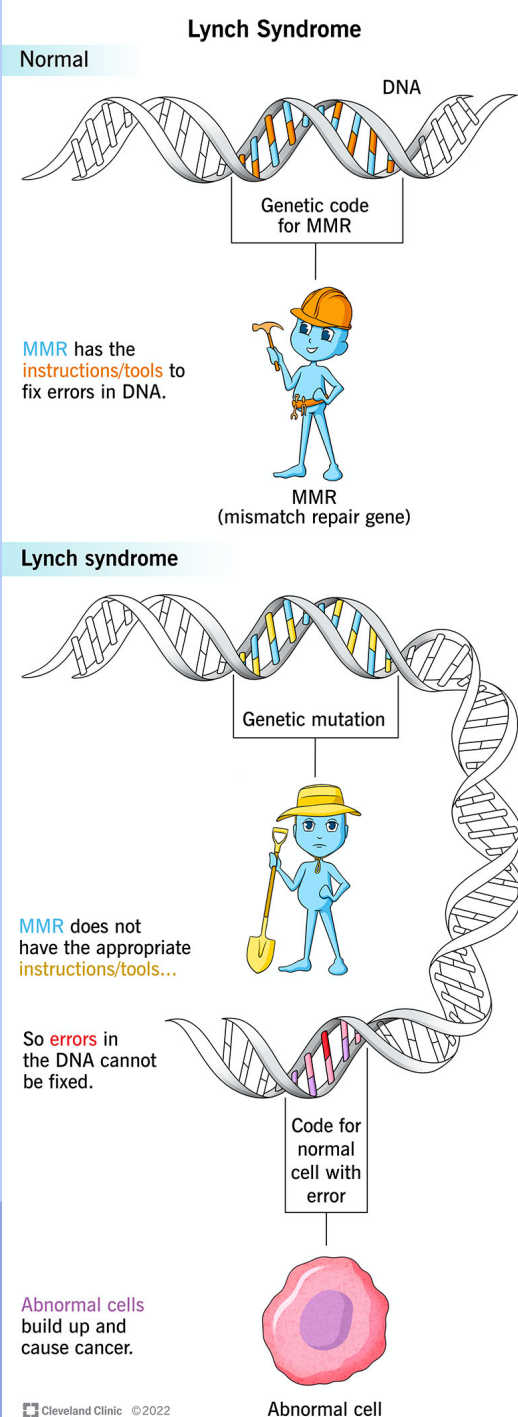
Mechanism:

- Mismatch repair distinguishes the unmethylated new strand from the methylated parent strand
- Recognizes errors via MSH proteins, excises the faulty section with endo- and exonucleases, and restores DNA integrity using DNA polymerase and ligase.

Associations with defective repair:

- **Hereditary nonpolyposis colon cancer**





Lynch Syndrome - HNPCC

Lynch syndrome, also known as **hereditary nonpolyposis colorectal cancer (HNPCC)**, is a genetic condition that increases the risk of **colorectal cancer** and other types of cancer due to inherited mutations in **mismatch repair genes**

Lynch syndrome is caused by mutations in **MMR genes**, which are responsible for repairing **mismatched nucleotides** that occur during DNA replication

Autosomal dominant inheritance

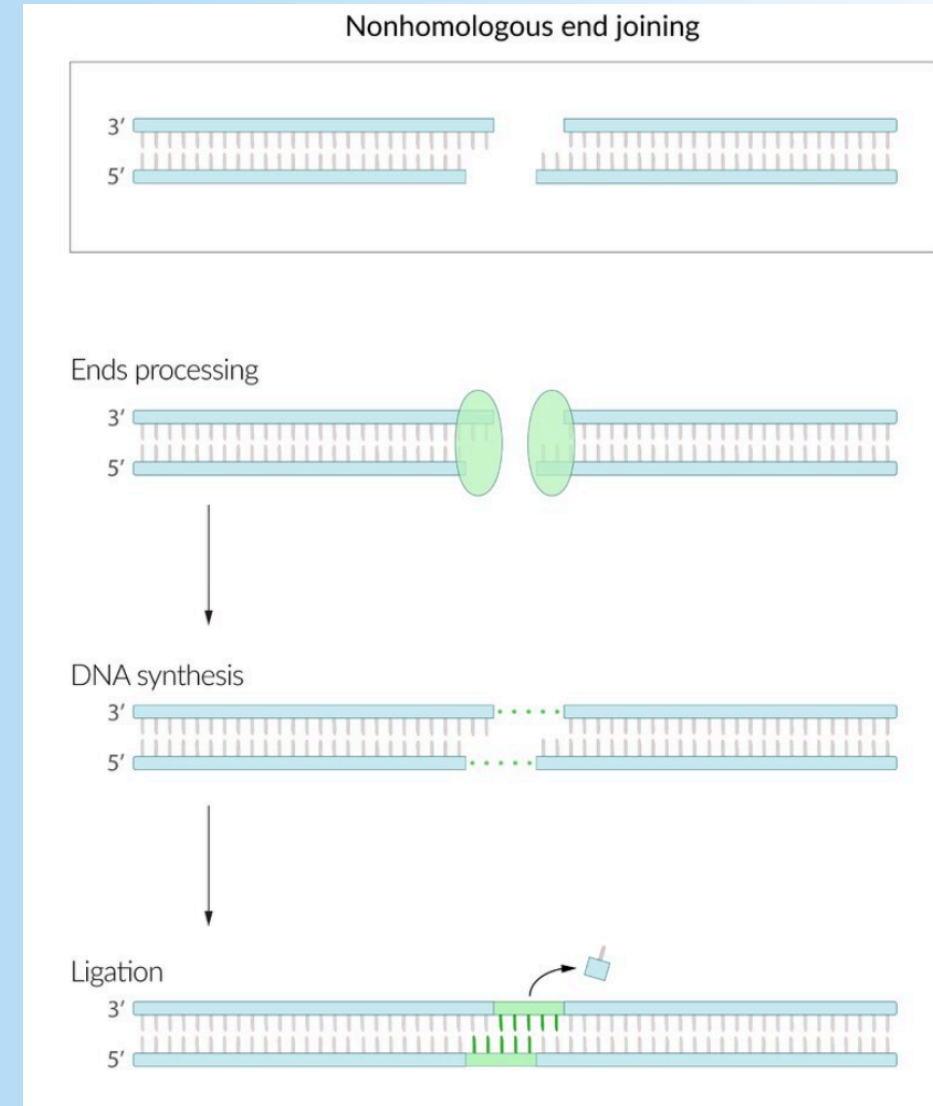
dsDNA Repair: Nonhomologous End Joining

Mechanism:

- DNA that has undergone a double-stranded break is repaired via **DNA ligase**
- Short homologous sequences called microhomologies comprise the single-stranded tails of the DNA ends to be joined.
 - Repair is usually accurate if microhomologies are compatible
 - Nonhomologous end joining is itself prone to errors and mutagenic defects because no error-free template is available
 - Some DNA may be lost or translocated in the process

Associations with defective repair:

- Ataxia-telangiectasia



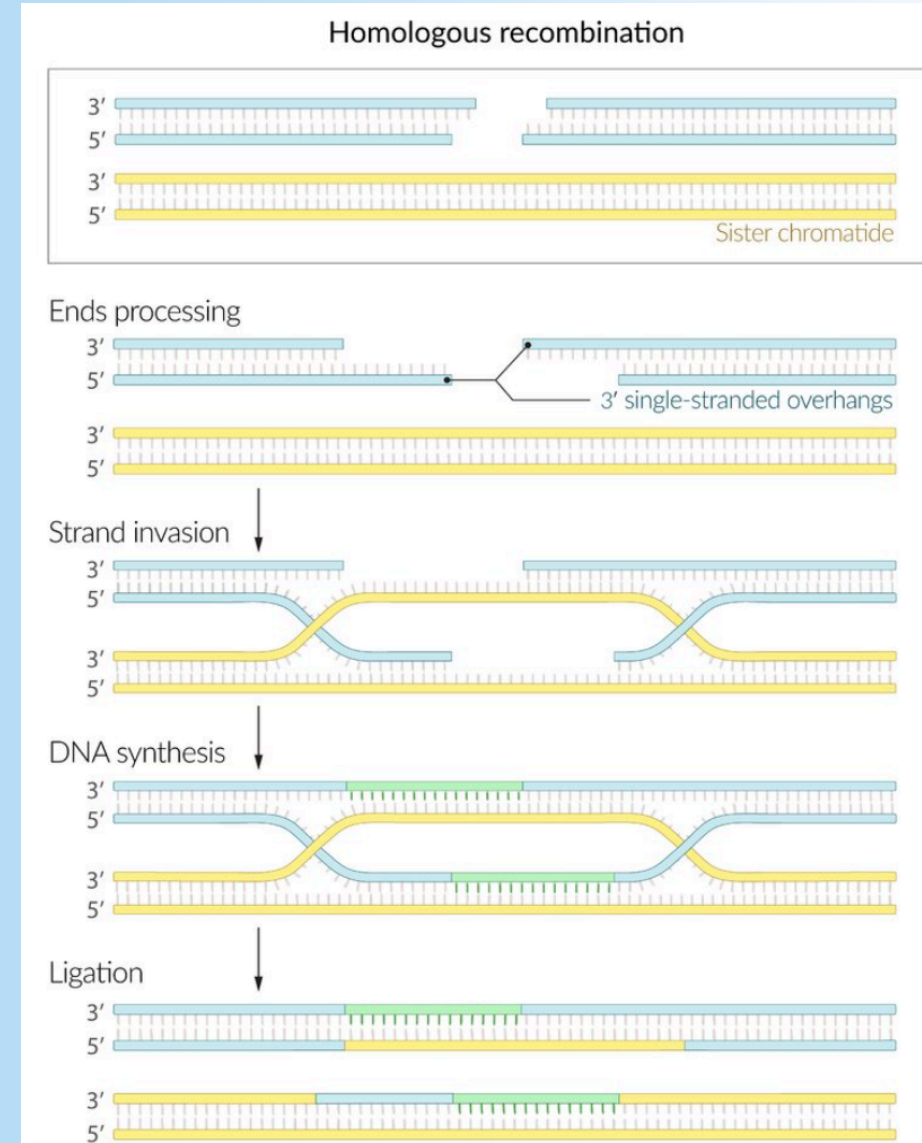
dsDNA Repair: Homologous Recombination

Mechanism:

- Repair by exchanging homologous segments between two DNA molecules (sister chromatids)
- The error can be repaired using the **complementary strand** from the intact sister chromatid
- Requires a (nearly) identical sequence (such as the complementary strand) to serve as a template for repair
- **No DNA is lost in the process**

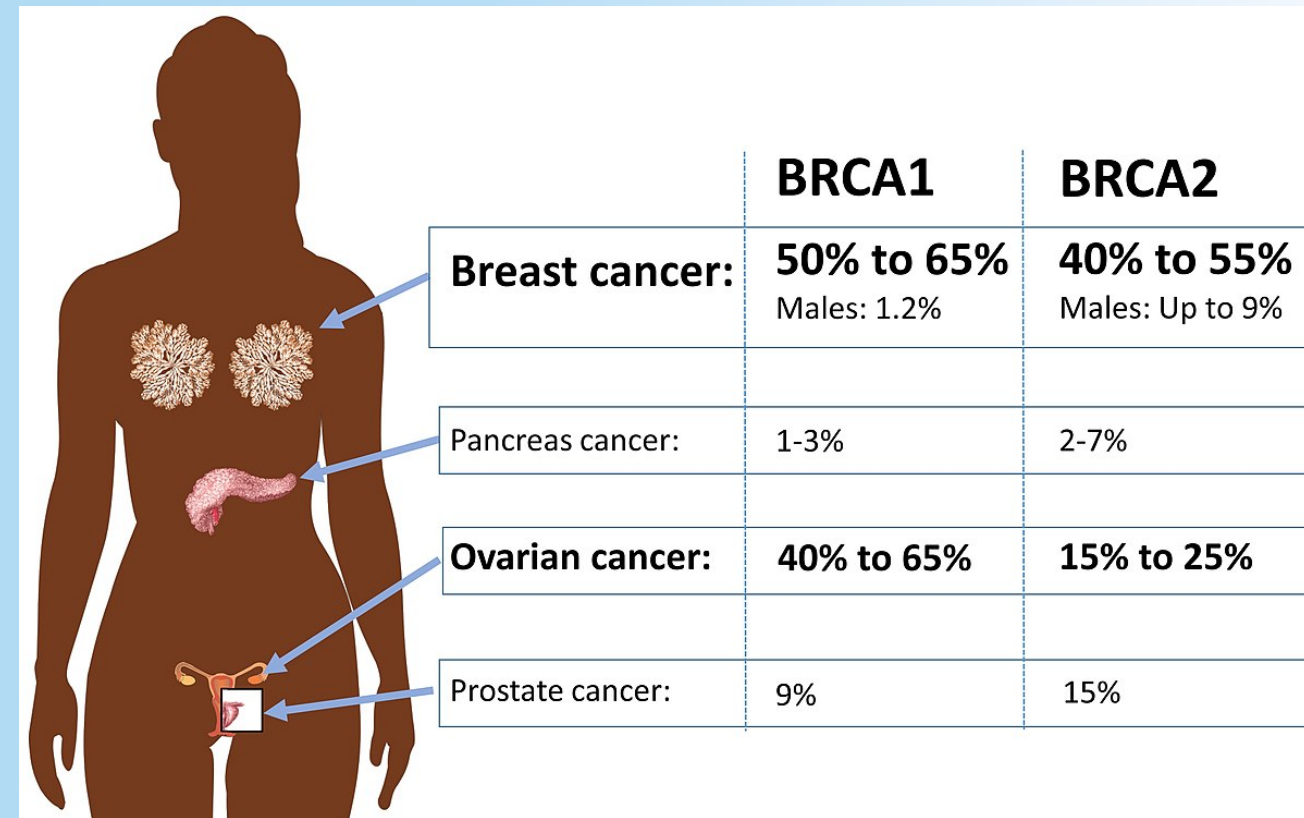
Associations with defective repair:

- **Breast/ovarian cancer** (due to BRCA1 and BRCA2 mutations)
- Fanconi anemia



Breast/Ovarian Cancer

BRCA1 and BRCA2 are key components in the **homologous recombination repair** pathway, which accurately repairs **DNA double-strand breaks** using an undamaged sister chromatid as a template. When this pathway is compromised, cells rely on error-prone repair mechanisms, increasing the risk of mutations



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