بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 21

DNA mutation & repair pt.1

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Reviewed by : NST

Molecular Biology (13)

DNA mutations and repair mechanisms
Gene editing by CRISPR-Cas9

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Mutations:

What are mutations?

- A genetic mutation is a change in the genetic material (Specifically in DNA).

  In viruses, it depends on whether the genetic material is DNA OR RNA.
  - Somatic mutations occur in somatic cells and are not transmitted.
  - Germline mutations occur in gametes and are heritable.

- The damaging effect of mutations has variable *sizes*.
  - **Micromutations** involve **small** regions of the DNA. (change in one/few nucleotide), can't be detected by microscope, so we use DNA Sequencing or PCR methods to identify micro mutations.
  - **Macromutations** involve chromosomes, (Extra or missed chromosomes, Q arm missed)

1. **Mutation**: A rare change in DNA (<1% of the population) that may cause disease or alter phenotype.
2. **Polymorphism (SNP)**: A genetic variation (>1% of the population) that does not typically cause disease but may affect traits under specific conditions.

Causes of DNA mutations

- DNA mutations can arise **spontaneously** or can be **induced**.

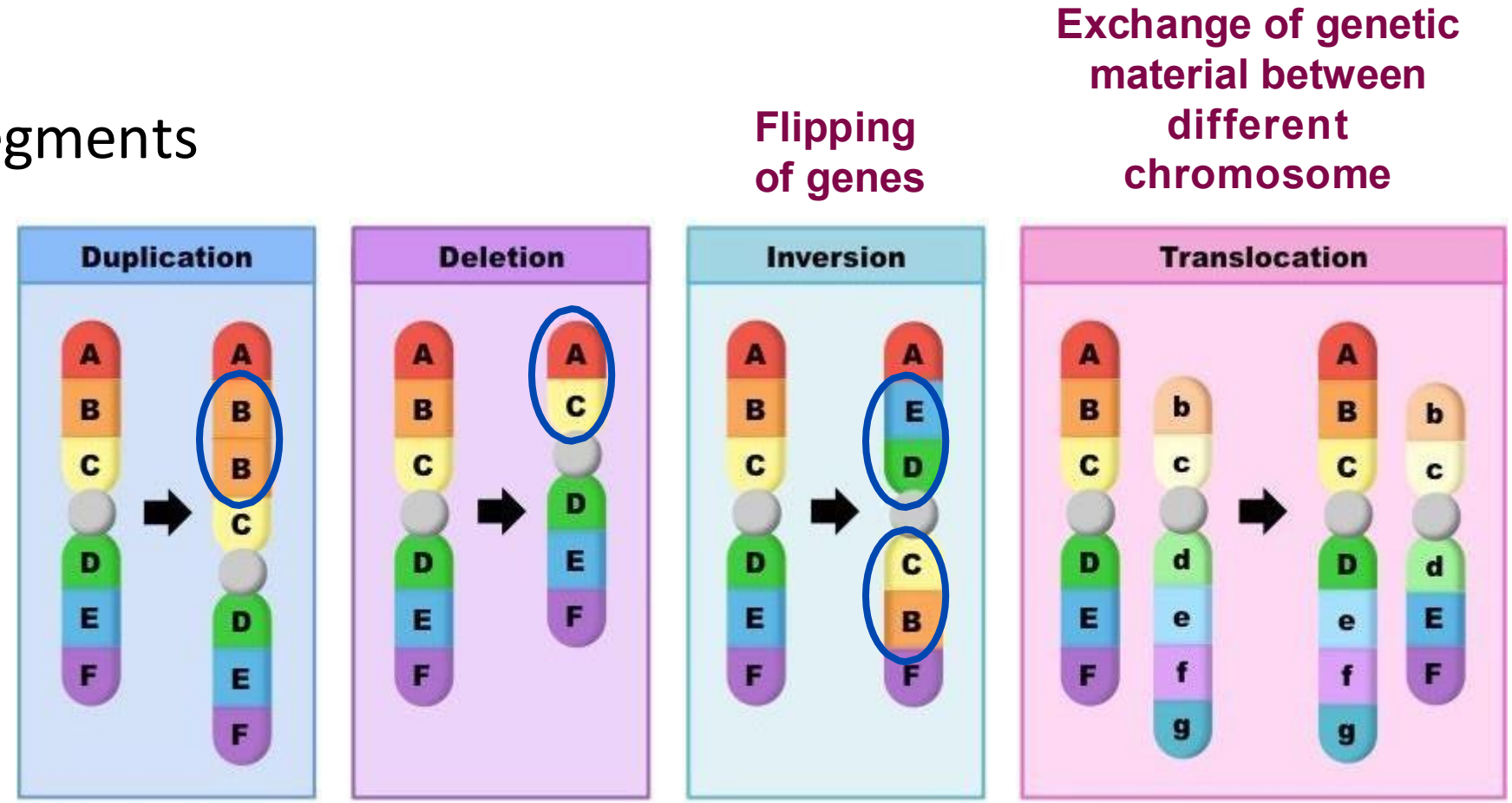
- Spontaneous mutations are naturally occurring and arise in all cells.
  - They arise from a variety of sources, including errors in DNA replication and spontaneous lesions, without any interference. (Oxidative stress)
- Induced mutations are produced when an organism is exposed to a mutagenic agent (or mutagen), external factor.
  - Some mutagens are carcinogens (cancer-causing)
    - Ionizing radiation UV Light

Note: All carcinogens are mutagens, but not all mutagens are carcinogens

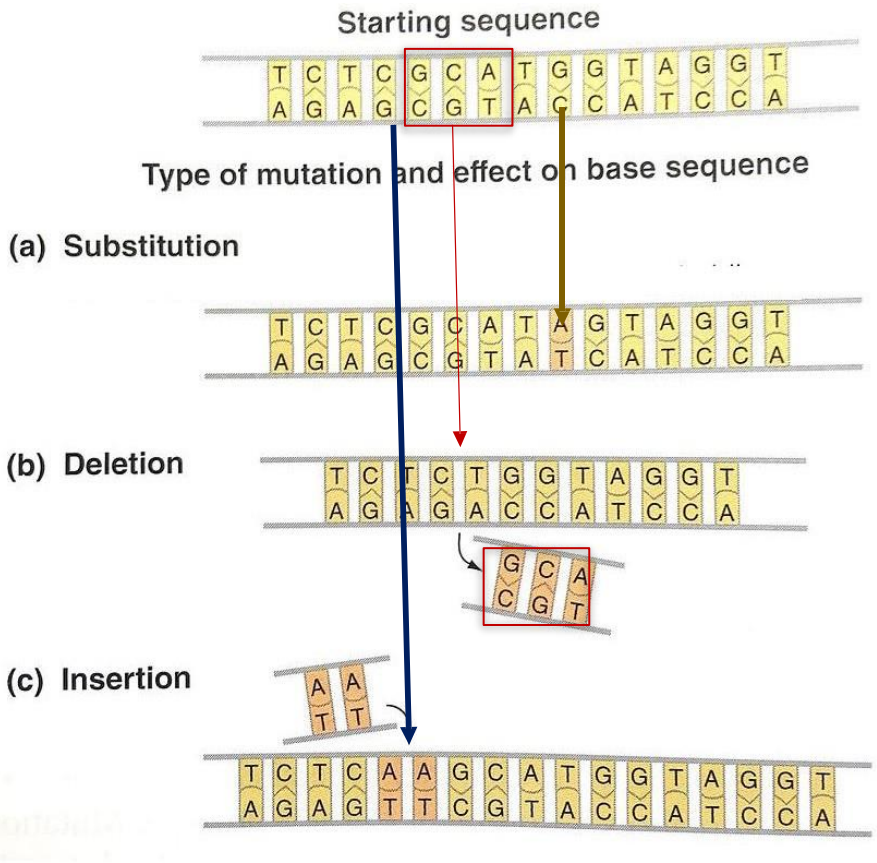
Macromutations

at the chromosomal level

- Translocations
- Inversion of DNA segments
- Duplications
- Deletions

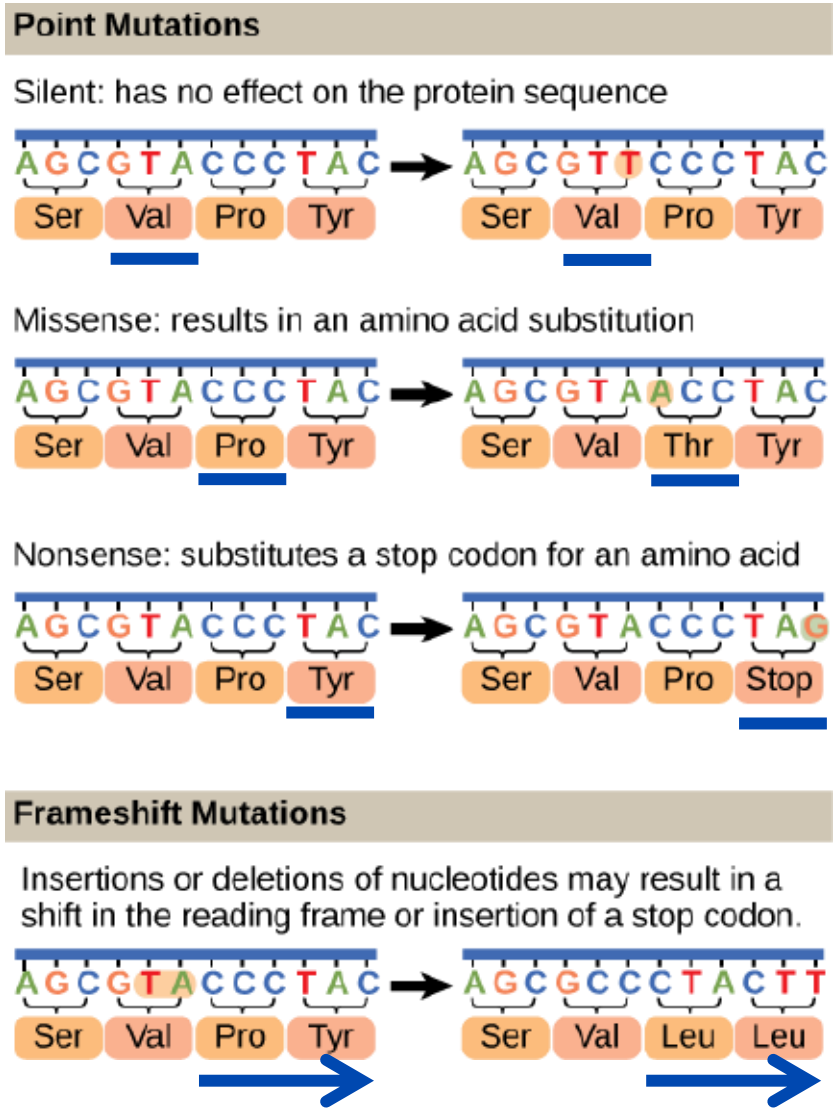


Types of micromutations



- Point mutations (single/few nucleotide)
  - The **most common** and include substitutions, insertion, and deletion.
- Deletions or insertions of a few nucleotides to long stretches of DNA (bad effect, but unrecognized on chromosomes level).

Point mutations



- A point mutation occurs in a genome when a single base pair is added, deleted, or changed.
- *Trillions of mutations happen in our DNA **daily**.*

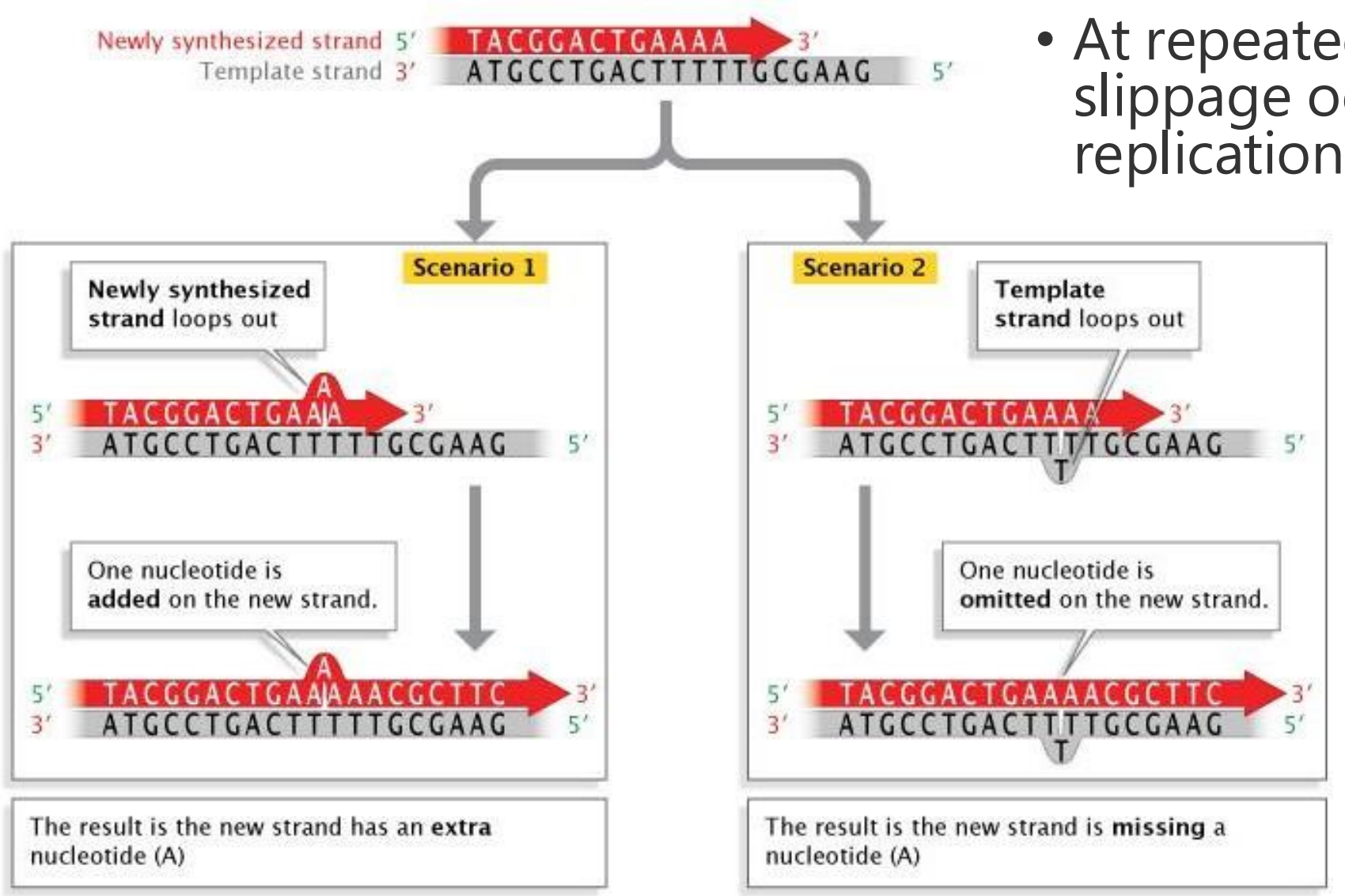
➢ Point mutations are classified based on their effects on the amino acid sequence:

1. **Silent mutation**: No change in the amino acid sequence, (same amino acid sequence)
2. **Missense mutation**: Substitution of one amino acid for another.
3. **Nonsense mutation**: Conversion of a codon into a stop codon, leading to premature termination of translation, forming a shorter polypeptide, ex: Becker dystrophy.
4. **Frameshift mutation**: Insertion or deletion of nucleotides that shifts the reading frame, altering the entire downstream amino acid sequence, ex: duchenne muscle dystrophy.

Comparison Between Duchenne and Becker Muscular Dystrophy

Condition	Type of Mutation	Effect on Protein Synthesis
Duchenne muscular dystrophy	Frameshift (disrupts the reading frame of the dystrophin gene)	Complete loss of protein function; more severe form.
Becker muscular dystrophy	Frameshift (in less critical regions of the dystrophin gene)	Partial protein function retained; less severe but still significant.

Repeated sequences, DNA replication, and strand slippage



- At repeated sequences, strand slippage occurs during DNA replication.
- This results in adding or deleting a nucleotide on the newly synthesized strand.

Further Clarification:

➢ **Mutation Occurrence**:

- Mutations commonly occur in **repeated sequences** of the genome, either within template or newly synthesized strand.

➢ **DNA Polymerase Errors**:

- Although **DNA polymerase** is highly accurate, it can make mistakes in repetitive sequences due to the **dynamic nature of DNA**.

➢ **Strand Slippage**:

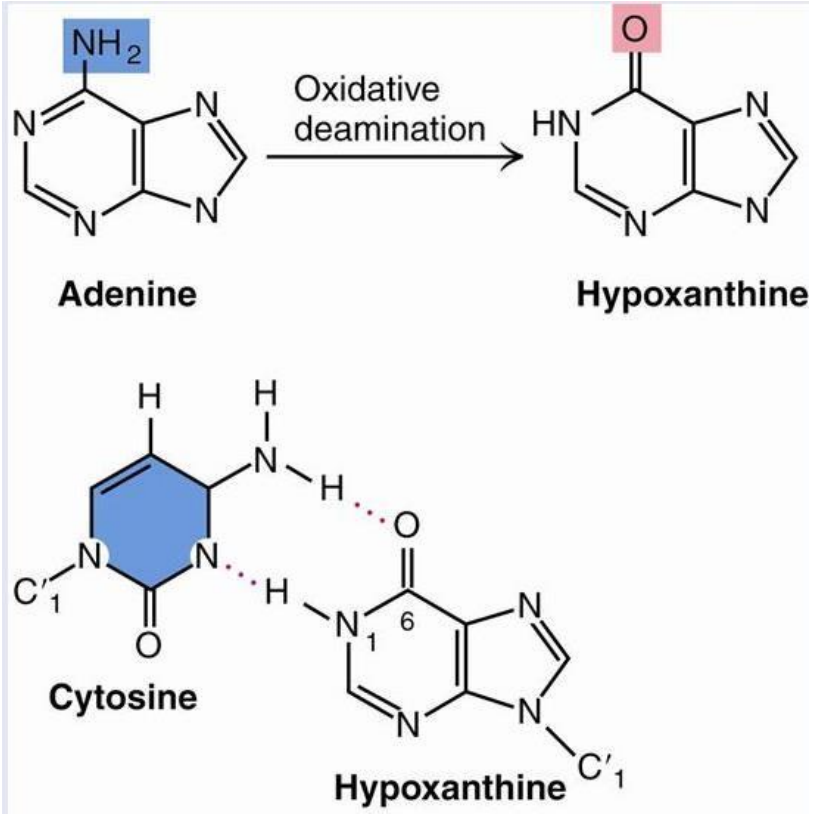
- happens during replication, causing misalignment between the template and the new strand.
- This can lead to **deletions** or **insertions (mainly)** in the DNA sequence, such as Huntington disease in which many repeated sequences accumulate.

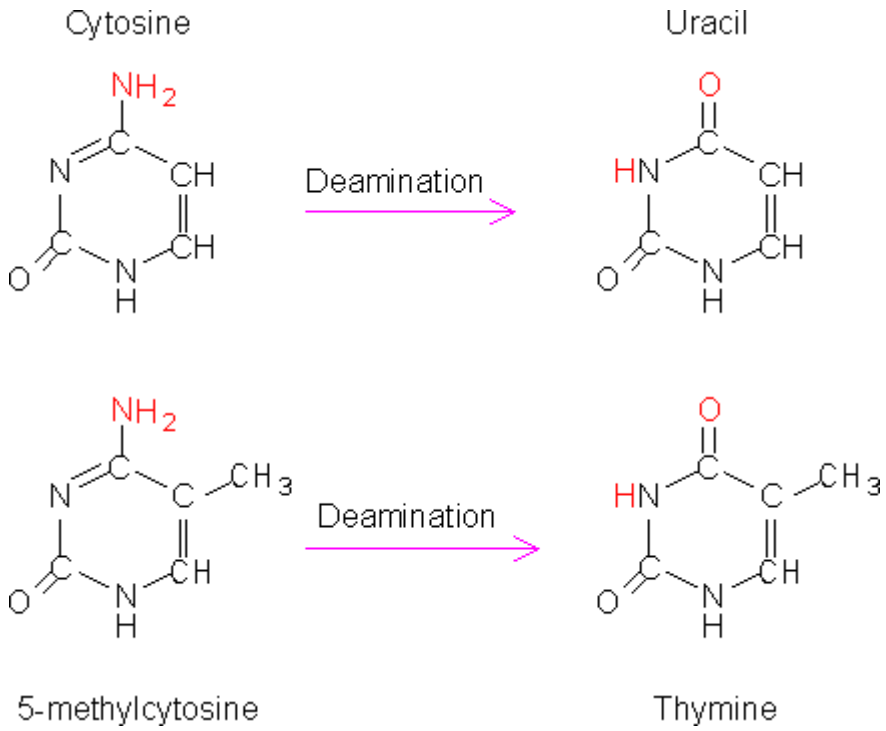
➢ **DNA Looping**:

- In strand slippage, **DNA looping** occurs when part of the strand misaligns, resulting in replication errors.

Deamination (spontaneous) Common mutation

- The deamination of **cytosine yields uracil**.
- The deamination of methylated cytosine yields thymine.
- The deamination of adenine yields hypoxanthine.





Examples of Deamination: (you must differentiate between nitrogen bases structures to clearly understand it.

➢ **Adenine to Hypoxanthine**:

- Deamination converts **adenine** into **hypoxanthine**.
- During DNA replication, **DNA polymerase** misreads hypoxanthine as **guanine** due to **shared structural features (carbonyl group)**.
- As a result, hypoxanthine pairs with **cytosine, (It looks like changing (A) to (G)).**

➢ **Cytosine to Uracil**:

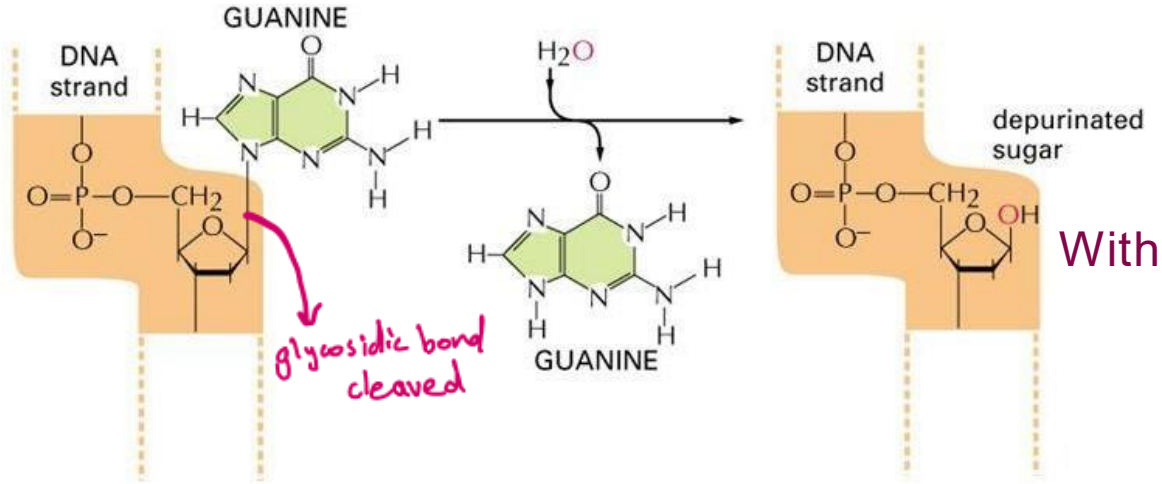
- Deamination of **cytosine** produces **uracil**, which is not a normal DNA Base, because uracil is only present in RNA not DNA.
- If not repaired, uracil pairs with **adenine**, causing mutations during replication.

➢ **5-methylcytocine to Thymine**:

- It's acceptable and doesn't need repair because Thymine is normally present in DNA.

Depurination (spontaneous)

- Cleavage of the glycosidic bond between the base (A or G) and deoxyribose creates an apyrimidinic or apurinic site (AP site).
- During replication, a **random base** can be inserted across from an AP site resulting in a mutation, with a 25% chance of being correctly replaced.



Without nitrogenous base

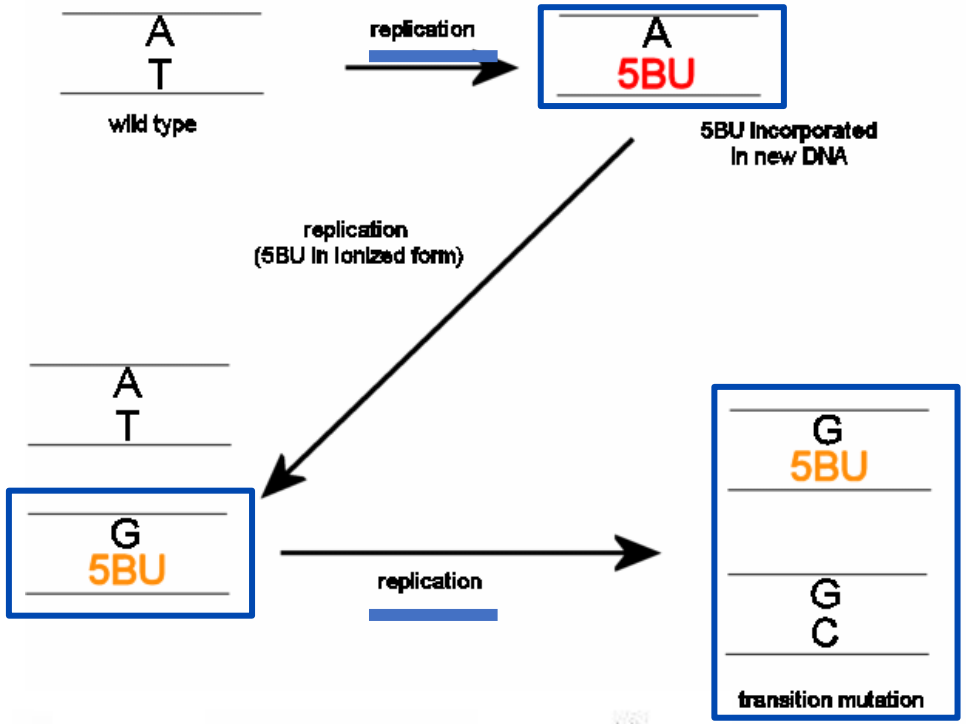
- Release of adenine or guanine bases

The Cell has two options:

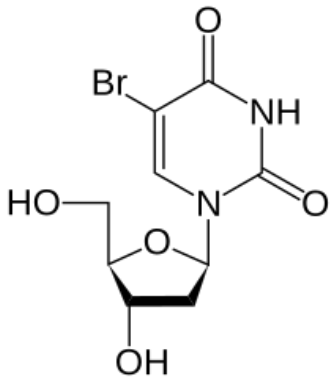
1. Stop replicating
2. Use a random base (commonly occur)

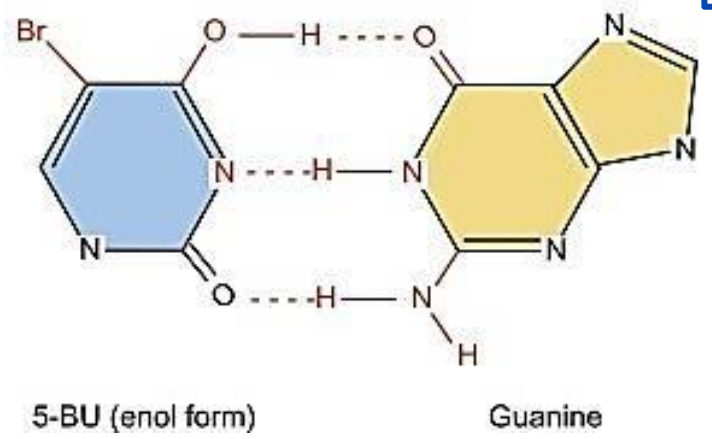
Incorporation of base analogs (induced)

- Base analogs have a **similar** structure to normal nucleotides and are incorporated into DNA during replication.
- 5-bromouracil (5-BU), an analog of **thymine**, **pairs with adenine**, but, when ionized, it pairs with **guanine**.
- Its deoxyriboside derivative (5-bromo-2-deoxy-uridine) is used to treat neoplasms.



- **Used in cancer chemotherapy, this treatment work by halting cell replication to prevent tumor growth.**
- **Non-ionized (5-BU), Thymine, which will link to Adenine (normal base pairing).**
- **Ionized (5-BU), Cytosine, which will link to guanine, (GC instead of AT).**





5-BU (enol form) Guanine

Repair mechanisms

- Prevention of errors before they happen
- Direct reversal of damage
- Excision repair pathways (cutting)
  - Base excision repair
  - Nucleotide excision repair
  - Transcription-coupled repair
- Mismatch repair and post-replication repair
- Translesion DNA synthesis
- Recombinational repair

Prevention of errors before they happen

Reactive oxygen species

- Enzymes neutralize potentially damaging compounds before they even react with DNA.
  - Example: detoxification of reactive oxygen species and oxygen radicals.

See next slide:

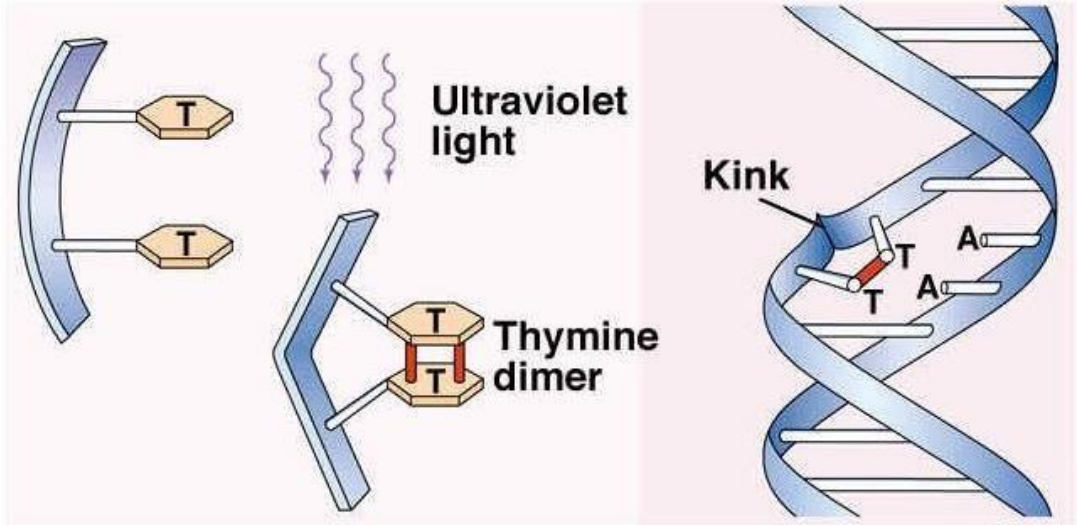
Reactive Oxygen Species (ROS) and Radicals:

- **Increased Oxygen (O_2):** Higher levels make DNA more susceptible to mutations.
- **Superoxide (O_2):** This is a radical with an **unpaired electron**. Because of this, it oxidizes and attacks other molecules.
- **Targets of Attack:** ROS attack proteins, sugars, and lipids (fatty acids).
- **Consequences:** Damage to fatty acids leads to **membrane damage**, which can result in **severe mutations**.
- While proteins and sugars can often be **repaired**, damaged components must be **removed**.
- **Hydrogen Peroxide (H_2O_2):** This is **not a radical**, but it is still highly **reactive**.

Direct reversal of damage

Pyrimidine dimers

- The ultraviolet (UV) wavelength of sunlight causes the formation of **covalent interactions** (50–100 reactions per second) between two adjacent pyrimidine bases, commonly between **two thymine** (on the same DNA strand), structures known as pyrimidine dimers (abnormal) unrecognized by DNA polymerase, which leads to repairing with a random nucleotide causing a mutation.
- This product is mutagenic (skin cancer)
- Pyrimidine dimers are reversed in **bacteria** by enzymes known as photolyases, **which do not exist in humans.**



DNA structure is distorted and, thus, replication and **transcription cannot proceed**.

- **Photolyase** recognizes the covalent linkage in the dimer and reverses it by breaking the bond, restoring the original DNA structure.

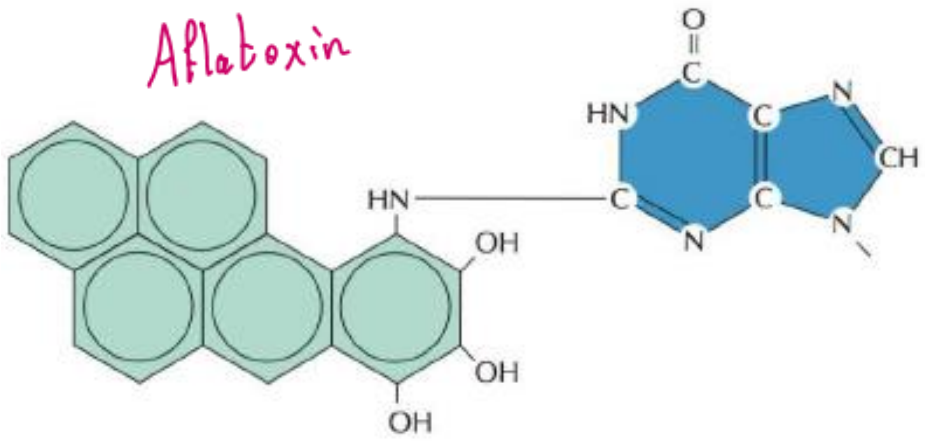
Specific mispairing

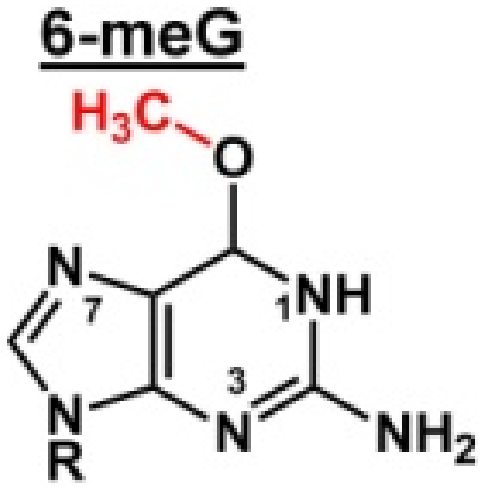
Bases existing in DNA can be altered causing mispairing.

Alkylating agents found in tobacco and car exaust can transfer methyl group to guanine forming 6-methylguanine, which pairs with thymine (6-meG links T, instead of G linking C).

Addition of large chemical adducts by carcinogens such as A polycyclic aromatic hydrocarbon (PAH) and aflatoxins (mold) , **Adding aflatoxins to G may cause cancers).**

These distort DNA structure leading to defective DNA replication and repair.





Specific mispairing:

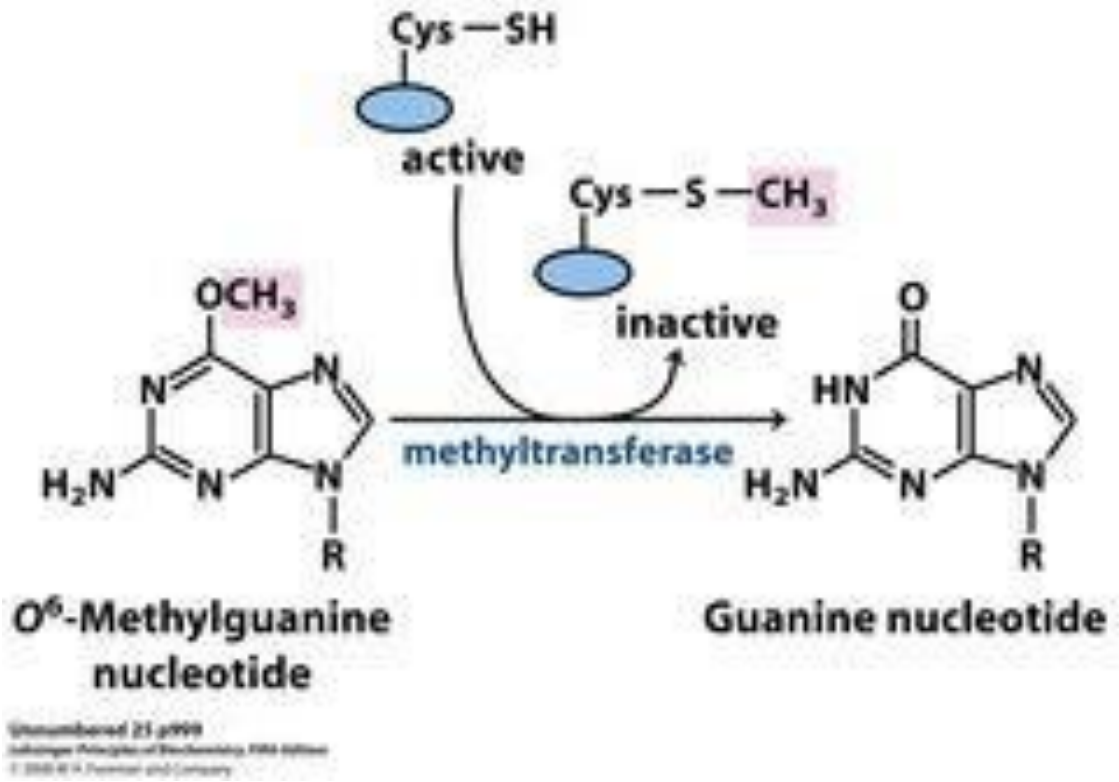
- If the DNA undergoes another round of replication, the thymine, which replaced the cytosine, will appear as a normal base to the replication machinery.
- As a result, the original guanine-to-cytosine pairing is permanently altered to an adenine-to-thymine pairing, completing the mutation.

➢ **Effect of Large Chemicals on DNA Function (able to be reversed):**

- When a **large chemical** is added to a nitrogenous base, it forms a **covalent bond** with the base.
- These chemicals can be found in nature, for example, in **contaminated water**, and when they bind to the base, they disrupt its normal function of DNA polymerase, it will be unable to recognize the base, then random base is used.

Repair of O6-methylguanine

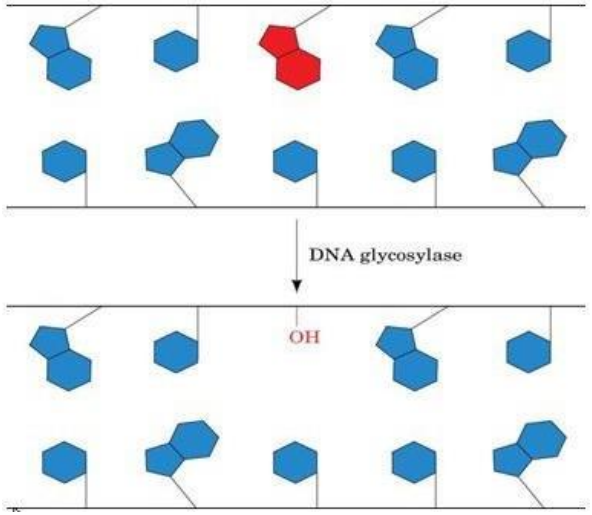
- This is done via O6-methylguanine methyltransferase.
- **Direct reversal** repairs mutations by enzymatically removing the chemical modification (methyl group).

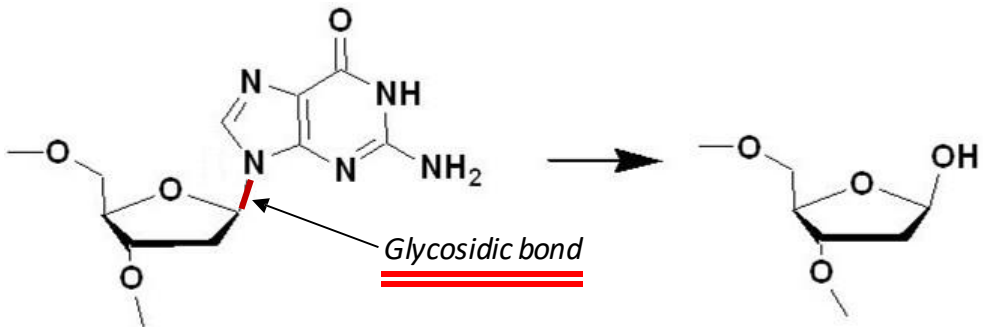


Excision repair pathways

Base excision repair pathway

- Each cell in the human body can **lose several thousand purine bases daily. (depurination).**
- DNA glycosylases do not cleave phosphodiester bonds, but instead **cleave N-glycosidic (base-sugar) bonds of damaged bases**, liberating the altered base and generating an apurinic or an apyrimidinic site, both are called AP sites.
- The AP site is repaired by an AP endonuclease repair pathway.

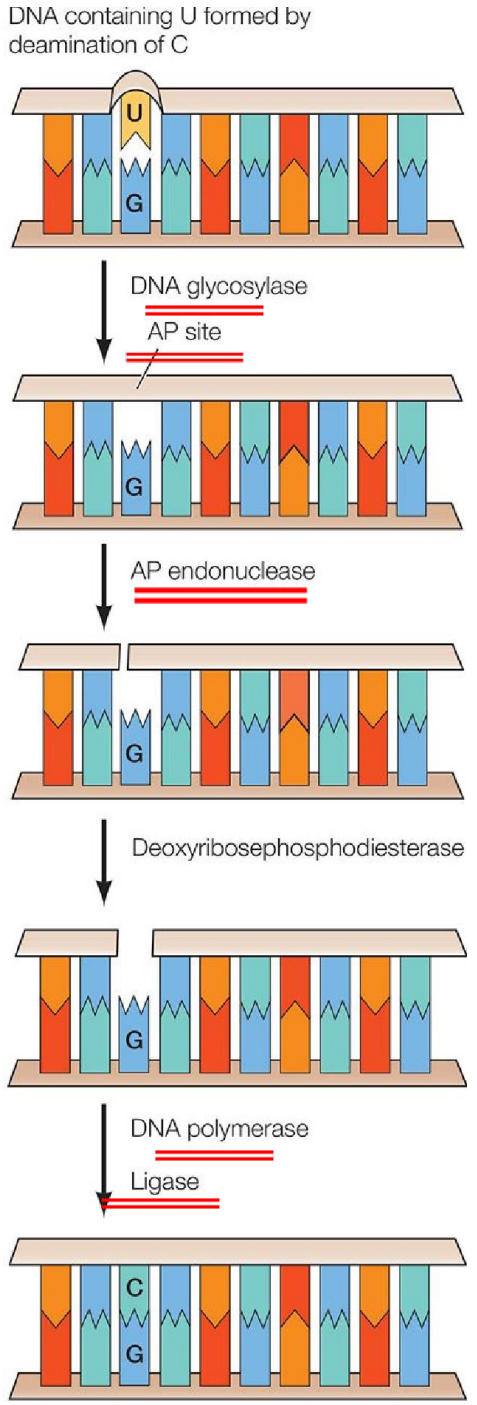




DNA glycosylases

See next slide:

- Numerous DNA glycosylases exist.
  - Example: uracil-DNA glycosylase, removes uracil from DNA.
    - Uracil residues, which result from the spontaneous deamination of cytosine or incorporation of dUTP can lead to a C→T transition, if unrepaired.
- AP endonucleases cleave the phosphodiester bonds at AP sites.
- The deoxyribose is removed.
- A DNA polymerase fills in the gap and DNA ligase and re-forms the bond.

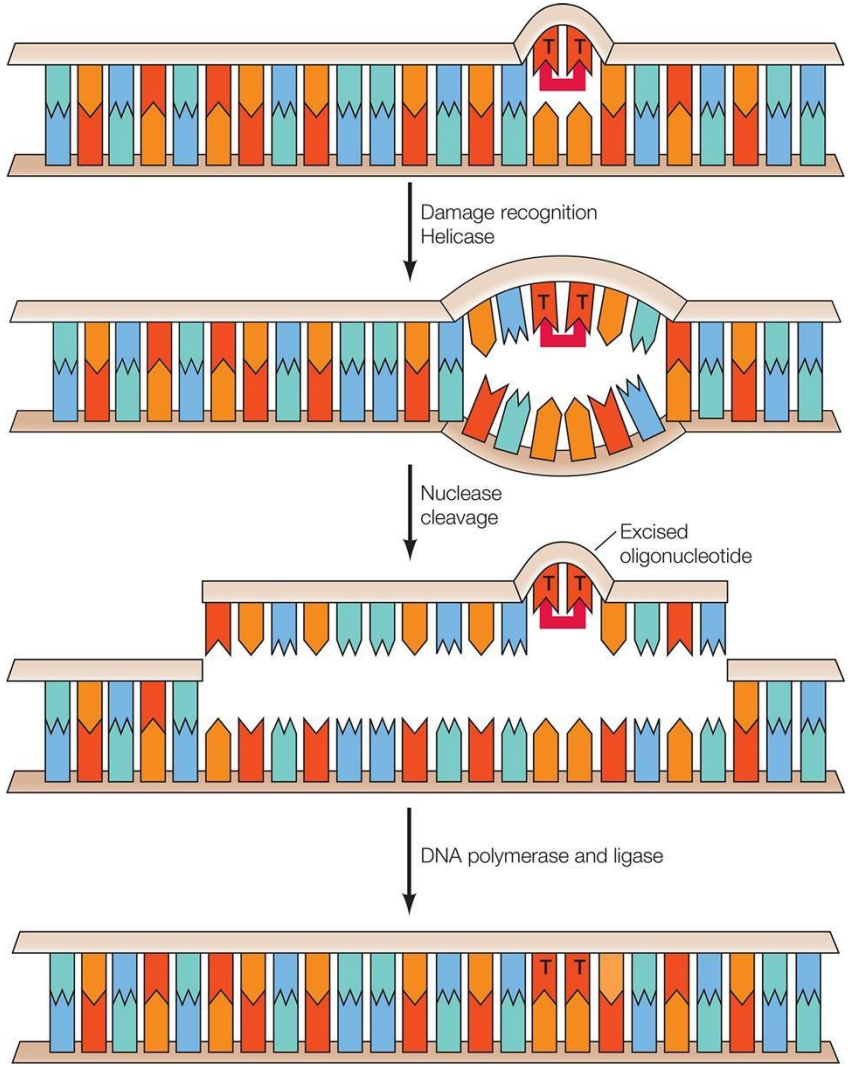


Steps:

1. **Damage Recognition**: A DNA glycosylase, such as **uracil DNA glycosylase** (which cleave the glycosidic bond), identifies and removes the damaged or inappropriate base (uracil).
2. **AP Site Formation**: The removal of the base creates an **apurinic/apyrimidinic (AP) site**.
3. **Cleavage of the AP Site**: **AP endonuclease** cleaves the **phosphodiester bond** at the AP site, leaving a single-strand break.
4. **Removal of Deoxyribose**: The damaged **deoxyribose sugar** is removed from the backbone by additional enzymes.
5. **Filling the Gap: DNA polymerase** inserts the correct base into the gap.
6. **Sealing the Strand**: **DNA ligase** seals the nick in the sugar-phosphate backbone, completing the repair process.

General excision repair (nucleotide excision repair)

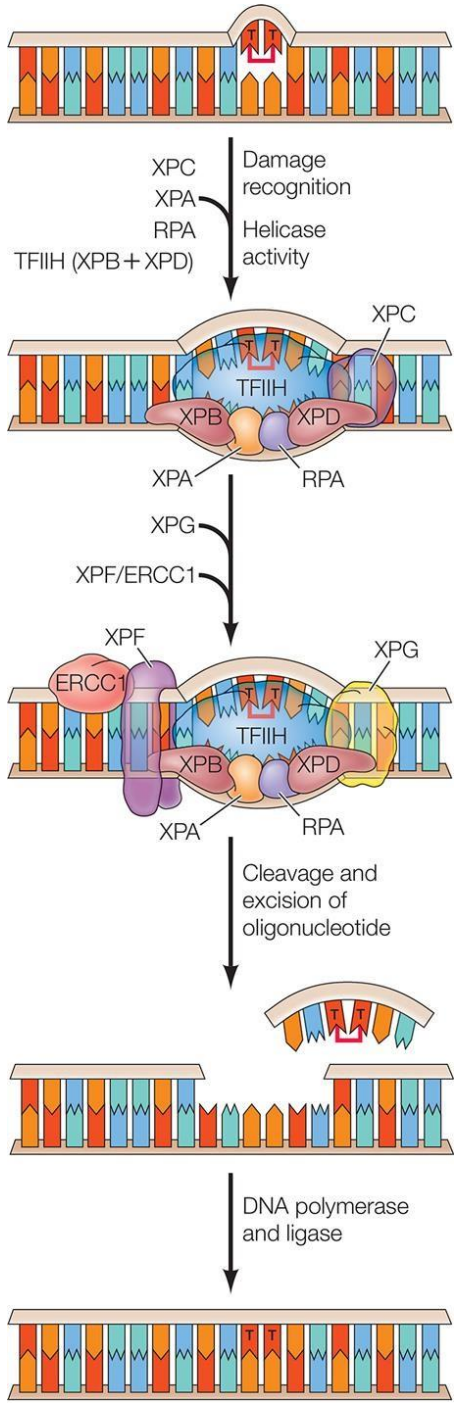
- This pathway corrects pyrimidine dimers and is crucial for maintaining DNA integrity after UV-induced damage.
- Damaged DNA is recognized (by Recognition proteins) and then unwound around the site of damage by a **helicase**.
- The DNA is then cleaved on both sides of a thymine dimer, resulting in the **excision of an oligonucleotide containing the damaged bases.**
- The gap is then filled by DNA polymerase and sealed by ligase.



XP proteins

See next slide:

- DNA damage (e.g., a thymine dimer) is **recognized** by **XPC protein**.
- XPA, Replication protein A (RPA), which binds the single-stranded DNA during DNA replication, and TFIIH form a complex with XPC.
  - TFIIH contains the subunits, XPB and XPD helicases.
- DNA is unwound by TFIIH (XPB and XPD) and XPG.
- XPF/ERCC1 **endonucleases** are recruited, and the DNA is cleaved, excising the damaged oligonucleotide.
- The resulting gap is filled by DNA polymerase and sealed by ligase.



Steps:

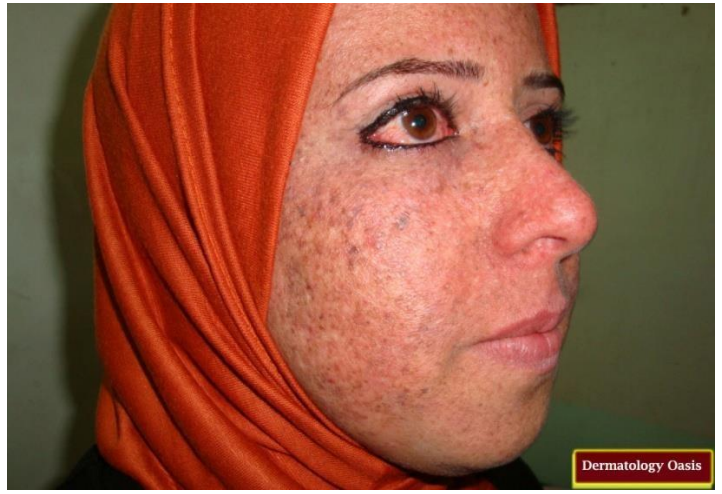
1. **Recognition**: **XP-C** recognizes the **thymine dimer**.
2. **Strand separation**: **XP-A** works with **Replication Protein A (RPA)** and **TFIIH**, where **TFIIH** unwinds the DNA (T Dimer) using its **helicase** activity, and its **kinase** activity to phosphorylation RNA polymerase's tail to start transcription. **Note:** RPA is the protein that prevents hairpin forming during replication.
3. **Cutting**: **Endonucleases** make cuts on both sides of the thymine dimer.
4. **Removal**: The damaged DNA segment containing the dimer is removed.
5. **Repair**: **DNA polymerase** fills in the gap, and **DNA ligase** seals the strand.

Comparison of Base Excision Repair (BER) and Nucleotide Excision Repair (NER)

Base Excision Repair (BER)	Nucleotide Excision Repair (NER)
Repairs single base damage using DNA glycosylases	Repairs larger lesions (e.g., pyrimidine dimers) by removing a short oligonucleotide
Creates an AP site after base removal	Creates a gap by removing the damaged segment
AP endonuclease cleaves the backbone	Helicase unwinds the DNA, endonucleases cut on both sides of the damage
DNA polymerase fills the gap, and ligase seals it	DNA polymerase fills the gap, and ligase seals it

In human…

- Defects in nucleotide excision repair cause a condition known as **Xeroderma pigmentosum (XP), thiamin dimer accumulation (can't be repaired) ,** and **Cockayne's syndrome**.
- Individuals with this disease are **extremely sensitive to UV light** and develop multiple **skin cancers** on the regions of their bodies that are exposed to sunlight.



Additional Resources:

رسالة من الفريق العلمي:

تفكَّر في قوله تعالى: "يَوْمَ تَشْهَدُ عَلَيْهِمْ أَلْسِنَتُهُمْ وَأَيْدِيهِمْ وَأَرْجُلُهُم بِمَا كَانُوا يَعْمَلُونَ" (سورة النور).

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			