BIO-373

Genetics & Genomics

# Mutation and DNA repair

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# Plan

1. Classification of mutations
2. Spontaneous mutations
3. Induced mutations
4. DNA repair systems

# Mutations

- **Mutation = new alteration in DNA sequence**

- Only way to introduce new genetic variation in the gene pool of a species
- May occur anywhere in the genome, but frequency is increased in some regions called “mutational hotspots”

# 1. Classification of mutations

# Classification of mutations

- **Based on molecular change**
  - **Point mutation** or **base substitution**
    - **Missense mutation**: Results in a new triplet coding for a different amino acid
    - **Nonsense mutation**: Results in a new stop codon (translation terminated prematurely) – *TAG, TAA, TGA*
    - **Silent mutation**: Results in a new triplet coding for the same amino acid
  - **Insertions / deletions (indels)**
    - **In-frame indels**: respect the fame of triplet reading during translation
    - **Frameshift indels**: shift the frame → usually results in premature stop due to a new stop codon
  - **Large structural variants**
    - **Deletions, duplications, inversions, translocations**…

# Classification of mutations

- **Based on location**
  - **Somatic mutations** occur in any cell except germ cells; are not heritable
  - **Germline mutations** occur in gametes; can be transmitted to the next generation

  - **Autosomal**
  - **X-linked and Y-linked**
  - **Mitochondrial**

# Classification of mutations

- **Based on detectable impact**
  - Neutral
  - Loss-of-function
  - Gain-of-function
  - Visible (morphological)
  - Nutritional (biochemical)
  - Behavioral
  - Regulatory
  - Lethal
  - Conditional/temperature-sensitive

# Classification of mutations

- **Based on mode of acquisition**
  - **Spontaneous mutations**
    - Arise naturally, from normal biological processes that alter the DNA bases
  - **Induced mutations**
    - Result from influence of extraneous factors, either natural or artificial (radiation, UV light, chemicals, etc.)

# 2. Spontaneous mutations

# Causes of spontaneous mutations

1. **Replication errors**
   - Replication is imperfect
   - DNA polymerase occasionally inserts incorrect nucleotides
   - Repair mechanisms are not 100% effective

Two major types of replication errors:

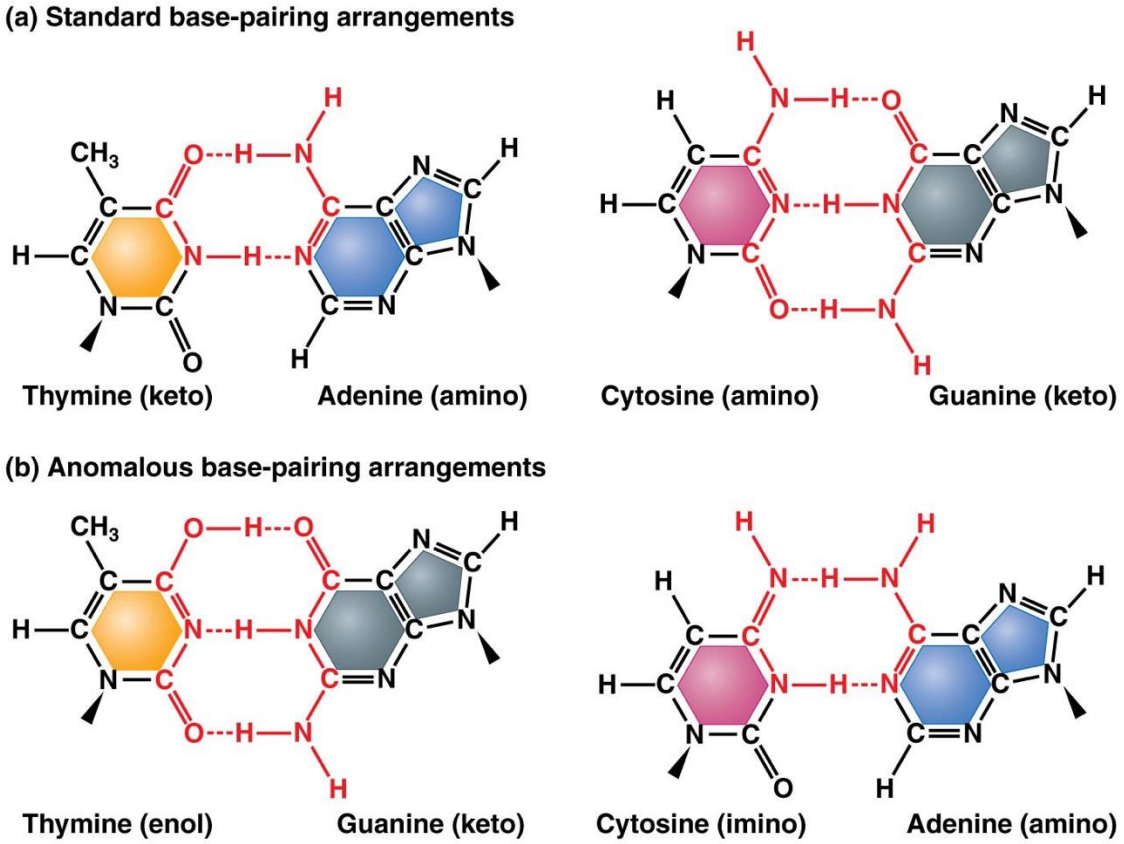
- Replication slippage
- Tautomeric shifts

# Causes of spontaneous mutations

- **Replication slippage**
  - Small insertions and deletions resulting from loops in the template strand during replication ➔ DNA polymerase misses looped out nucleotides
  - More common in repeat sequences
    - Hot spots for DNA mutation
  - Known to cause genetic diseases
    - Fragile-X syndrome
    - Huntington disease

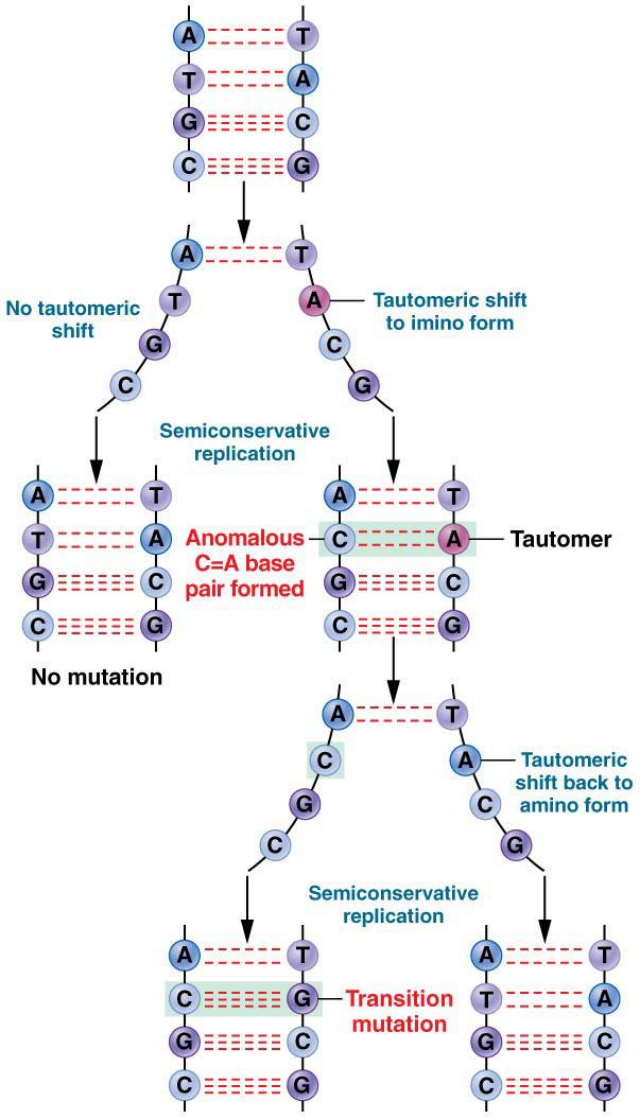
# Causes of spontaneous mutations

- **Tautomeric shifts**
  - **Tautomers:** alternate chemical forms of nucleotides



# Causes of spontaneous mutations

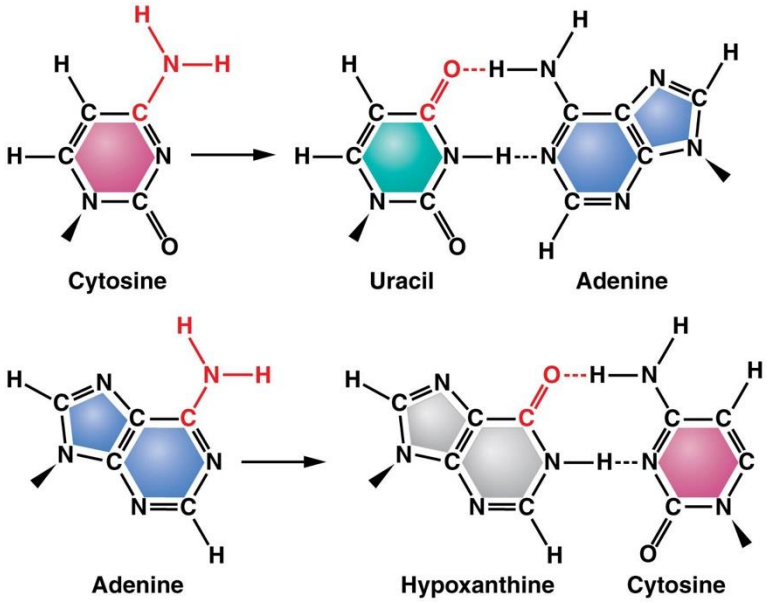
- **Tautomeric shifts**
  - A tautomeric shift changes the bonding structure, resulting in noncomplementary base pairing
  - May lead to permanent base-pair changes and mutations



# Causes of spontaneous mutations

## 2. DNA base damage

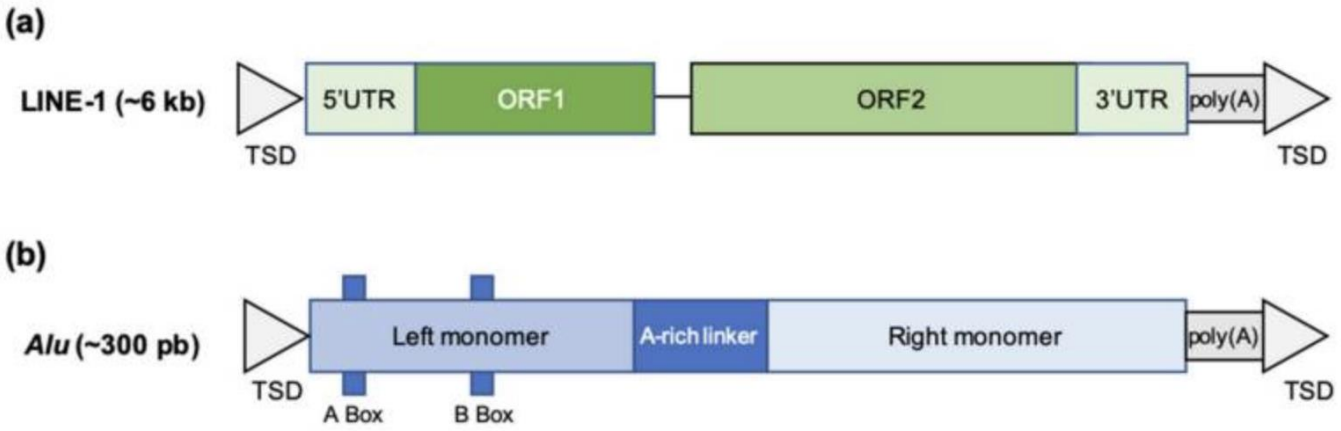
- **Depurination**: loss of a base, most often a purine (guanine or adenine)
- **Deamination**: the amino group in cytosine or adenine is converted to a keto group: cytosine is converted to uracil, and adenine to hypoxanthine



# Causes of spontaneous mutations

## 3. Transposable elements

- Also called transposons or “jumping genes”
- DNA elements that move within or between genomes
- Can act as naturally occurring **mutagens**
- Examples in humans: *Alu* and LINE-1 repeats, which together make close to 30% of the genome (only a very small portion still active, i.e., capable of transposition)



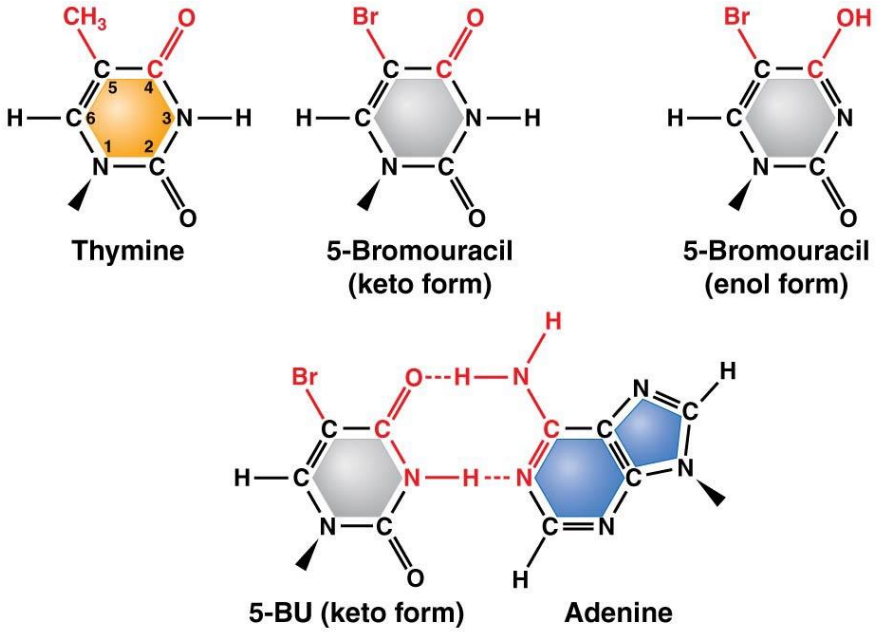
# 3. Induced mutations

# Induced mutations

- Caused by **mutagens** = agents that induce mutations
  - Base analogs
  - Alkylating agents
  - Intercalating agents
  - Adduct-forming agents
  - UV light
  - Ionizing radiation
  - Free radicals
  - Viruses

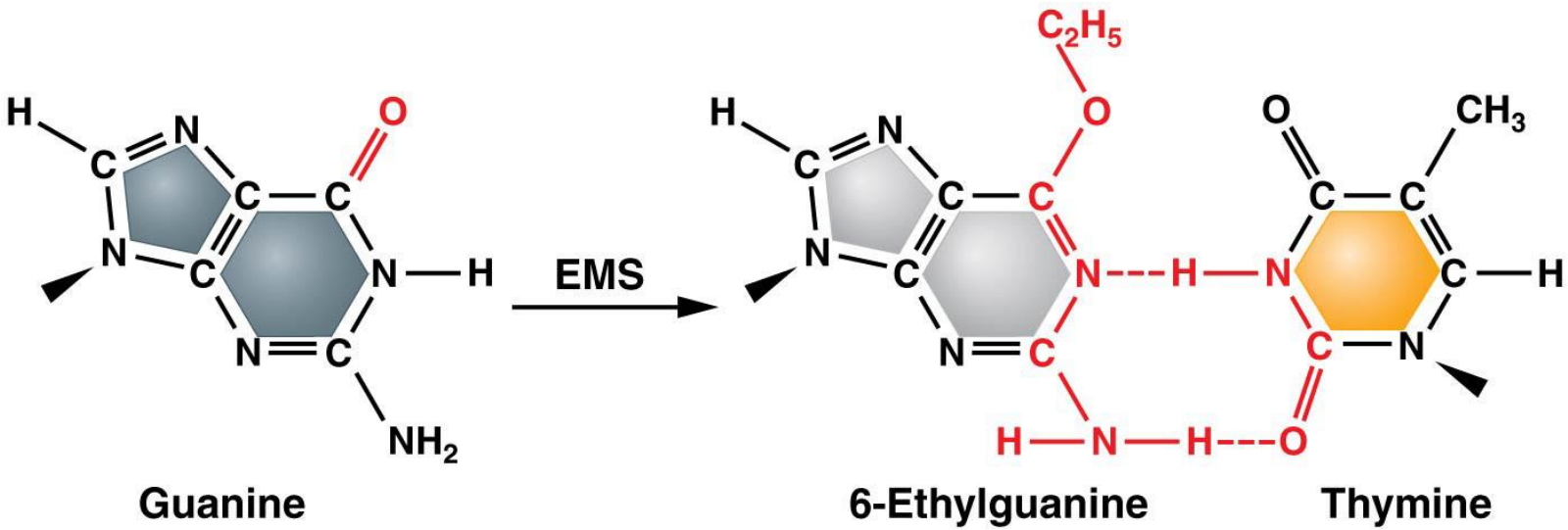
# Base analogs

- Can substitute for purines or pyrimidines during nucleic acid biosynthesis
- Example: 5-Bromouracil behaves as thymine analog



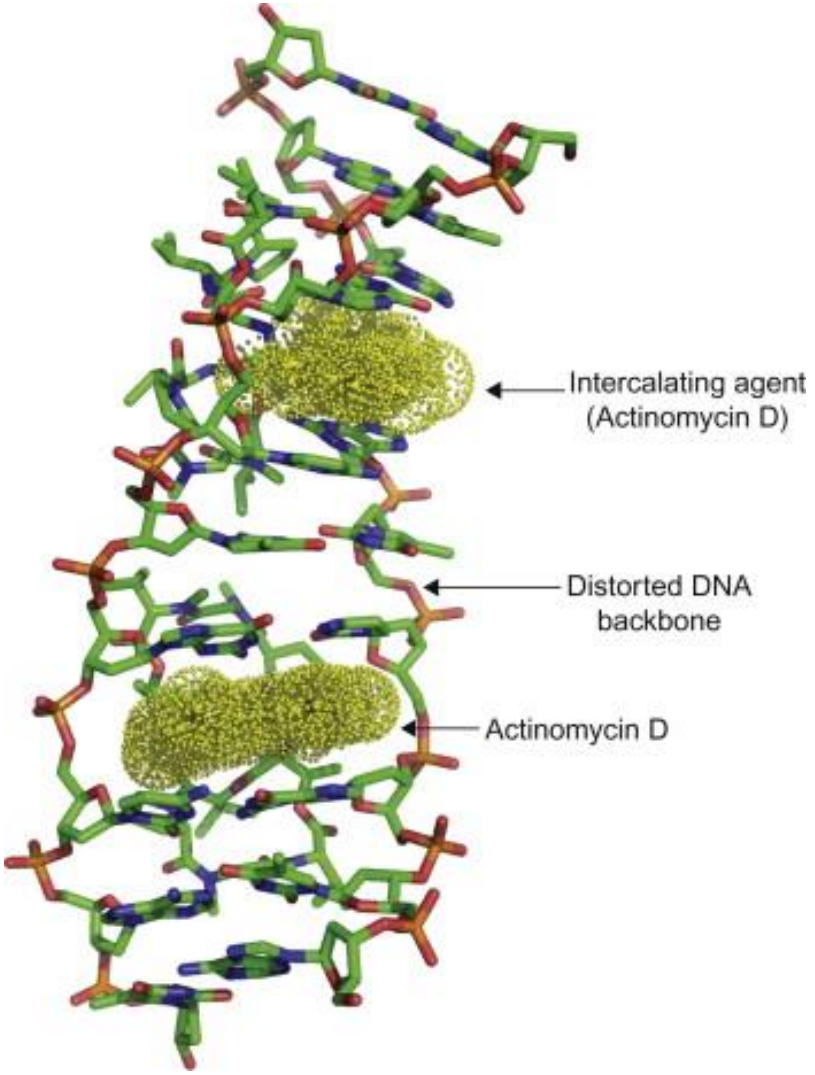
# Alkylating agents

- Donate alkyl group ($CH_3$ or $CH_3CH_3$) to amino or keto groups in nucleotides
- Alter base-pairing affinity
- Examples: Ethyl Methyl Sulfonate, mustard gas



# Intercalating agents

- Chemicals with dimensions and shapes that wedge between DNA base pairs
- This causes base-pair distortions and DNA unwinding
- Example: Actinomycin D, Ethidium bromide

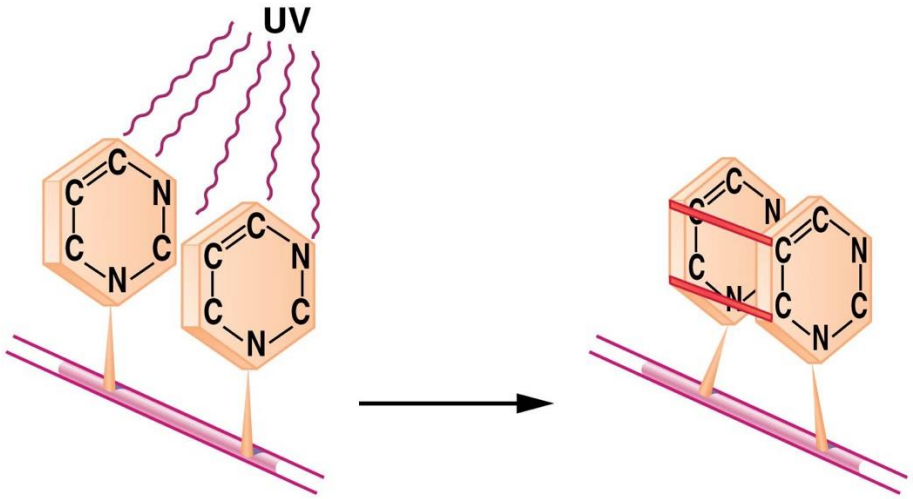


# Adduct-forming agents

- Chemicals that covalently binds to DNA, altering conformation and interfering with replication and repair
- A **DNA adduct** is a segment of DNA bound to a carcinogen
- Examples:
  - Acetaldehyde (component of cigarette smoke)
  - Nitrosamines (used as meat preservatives)
  - Aflatoxin (produced by some *Aspergillus*)
  - Cisplatin (anti-cancer drug)

# UV light

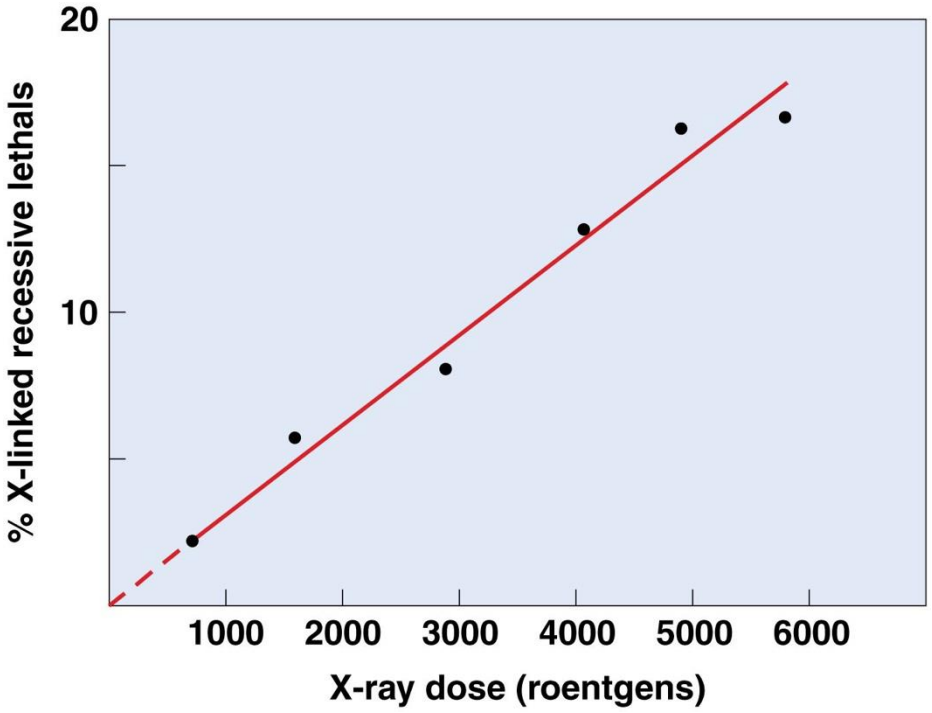
- UV light creates **pyrimidine dimers**
  - Two identical, adjacent pyrimidines create a dimer
  - Lead to distortion of DNA conformation
  - Errors are then introduced during DNA replication




Dimer formed between adjacent thymidine residues along a DNA strand

# Ionizing radiation

- X rays, gamma rays, cosmic rays
- Penetrates deeply into tissues
- Causes ionization of molecules



# Free radicals

- By-products of biochemical reactions in the body, including some metabolic processes and immune system responses
- Highly reactive because they contain one or more unpaired electrons
- Free radicals can affect DNA directly or indirectly:
  - Alter purines and pyrimidines
  - Break phosphodiester bonds
  - Produce deletions, translocations, and DNA fragmentation

# 4. DNA repair systems

# DNA Repair

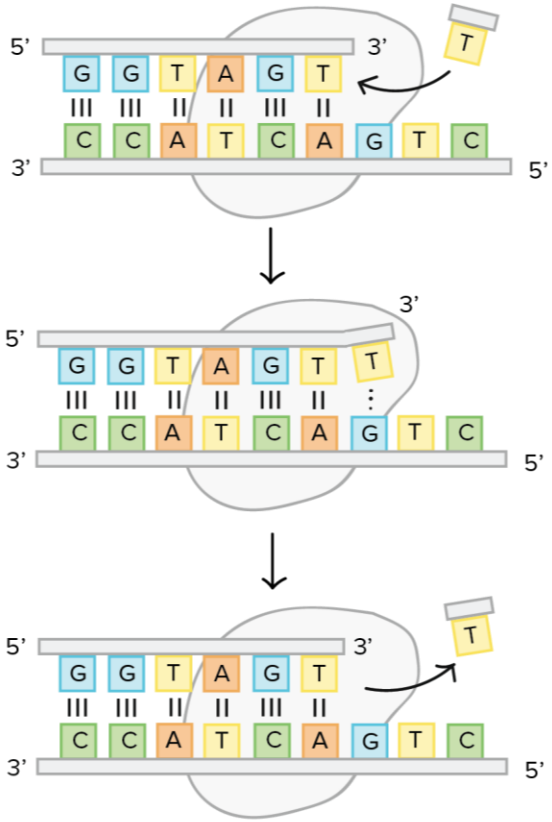
- **Repair systems counteract spontaneous and induced DNA damage**
  - Maintains integrity of genetic material
  - Counteract genetic damage that would result in genetic diseases and cancer
- The balance between mutation and repair results in the **observed mutation rates** of individual genes and organisms.

# Systems of DNA repair

1. During DNA synthesis, most DNA polymerases "check their work," fixing the majority of mispaired bases in a process called **proofreading**
2. Immediately after DNA synthesis, any remaining mispaired bases can be detected and replaced in a process called **mismatch repair**
3. Outside of synthesis, if DNA gets damaged, it can be repaired by various mechanisms, including **chemical reversal**, **excision repair**, and **double-stranded break repair**

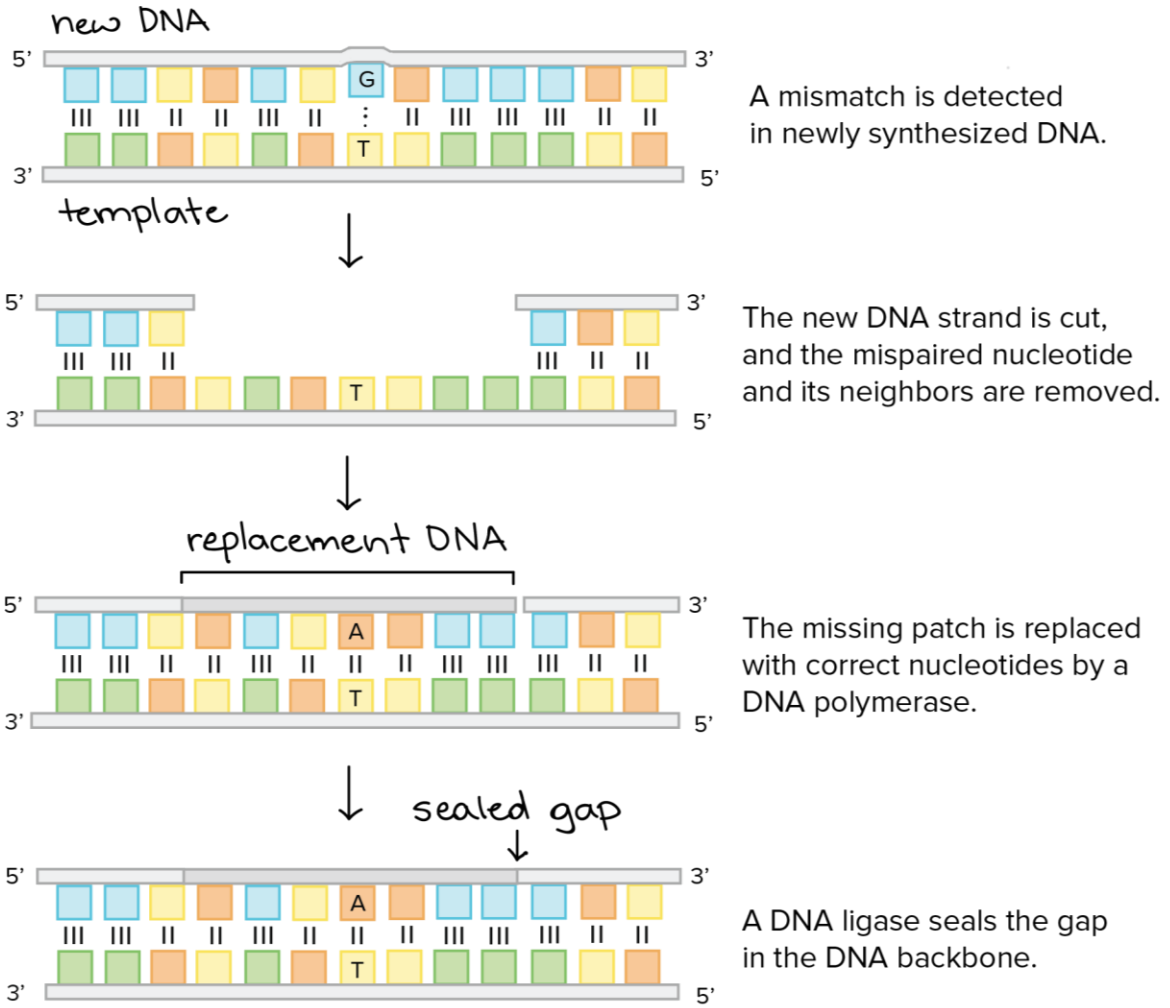
# Proofreading

- Happens during DNA synthesis
- When an incorrect base pair is recognized, the **DNA polymerase** reverses its direction and excises the mismatched base
- This implies that the polymerase has a **3’ → 5’ exonuclease activity**



# Mismatch repair

- Happens right after DNA synthesis
- Removes mismatches that were missed by proofreading
- Needs **nuclease**, **DNA polymerase** and **DNA ligase**



# DNA damage repair

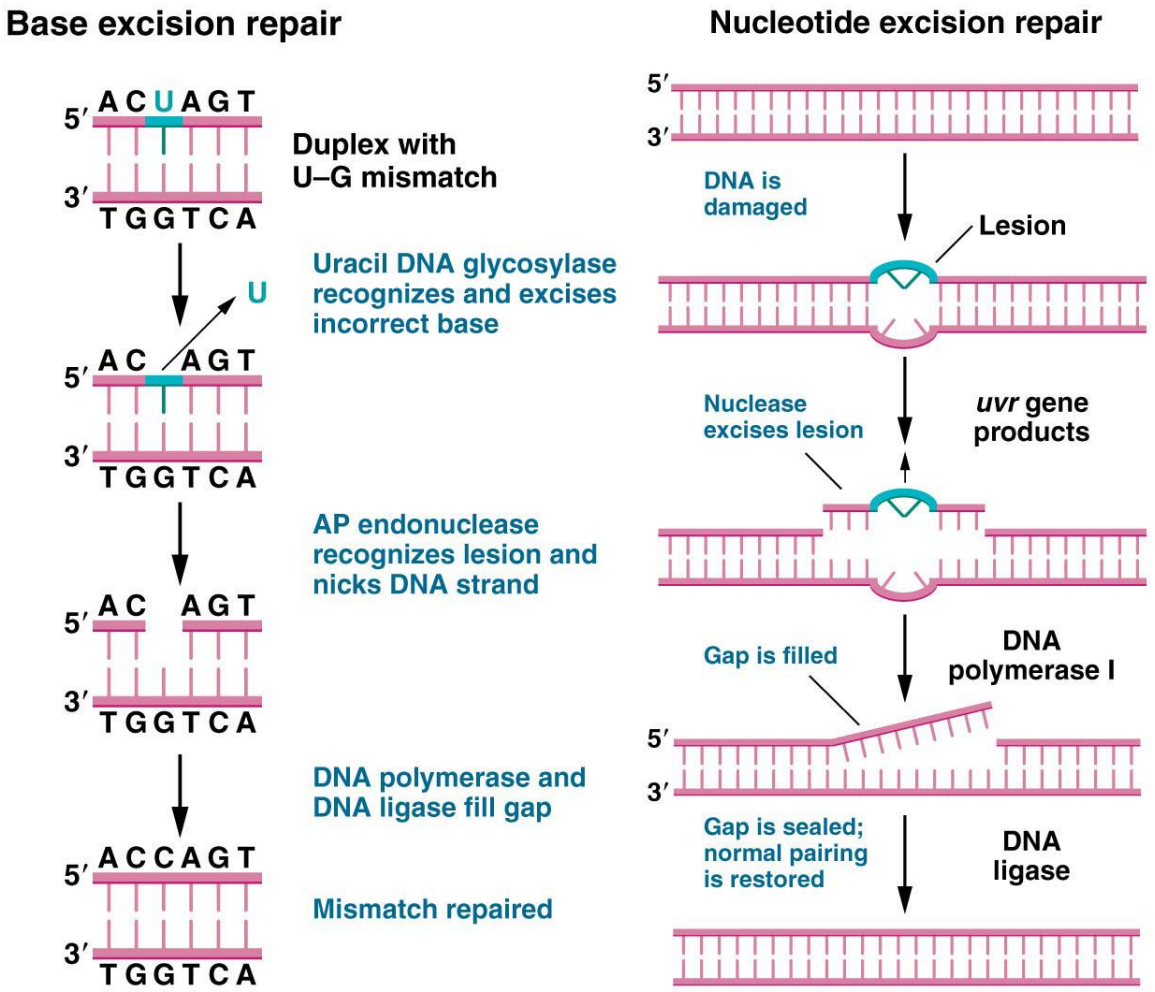
## 1. Chemical reversal

- DNA damage is fixed by reversing the chemical reaction that caused it
- Example: **photoreactivation** in bacteria: DNA photolyase (a light-driven enzyme) can destroy the abnormal covalent bond present in pyrimidine dimers induced by UV light

# DNA damage repair

## 2. Excision repair

- **Base excision repair (BER)**
  - Corrects DNA containing a damaged DNA base
  - A DNA glycosylase recognizes an altered base
- **Nucleotide excision repair (NER)**
  - Repairs bulky lesions that alter/distort the DNA double helix



# DNA damage repair

## 3. Double-stranded break repair

- Double strand breaks are extremely dangerous
- Results in chromosomal rearrangements, cancer, cell death
- Two pathways involved in DSB repair
  - **Nonhomologous end joining**
  - **Homologous recombination**

# Double-stranded break repair

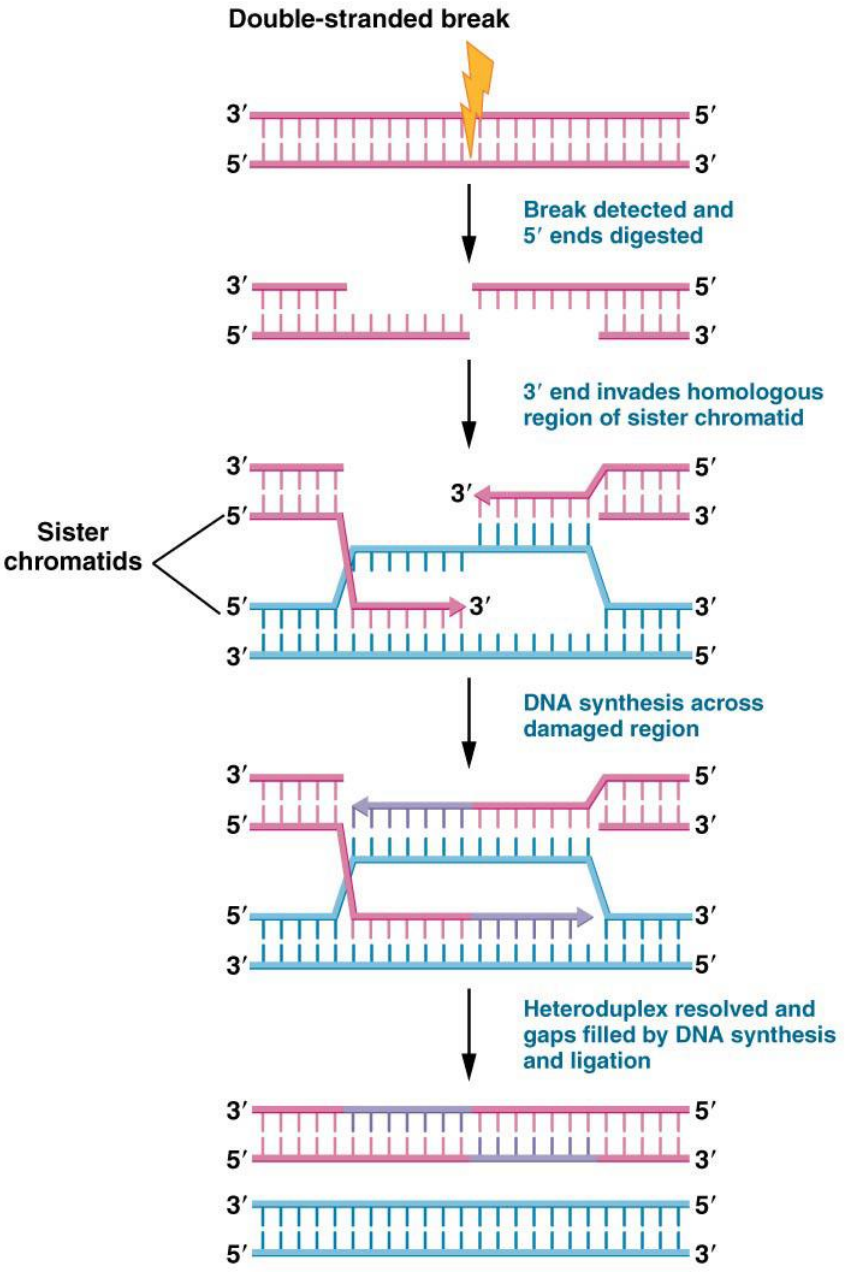
## Nonhomologous end joining

- A complex of proteins binds the 2 free ends and ligate them back together
- Error-prone: typically involves the loss of a few nucleotides at the cut site ➔ non-homologous end joining tends to produce mutations, but this is better than the alternative (loss of an entire chromosome arm)
- Occurs during G1 phase of the cell cycle

# Double-stranded break repair

## Homologous Recombination

- Recognizes break, digests 5′ end, and leaves 3′ overhang
- 3′ end aligns with sequence complementary on sister chromatid (homologous chromosome)
- Occurs during late S or early G2 phase of cell cycle



# Defects in DNA repair

**Consequences of defective DNA repair mechanism:**

➔ **Accumulation of mutations**

➔ **Premature aging and/or cancer**

Example of disease: **Xeroderma pigmentosum**

- Autosomal recessive, can be caused by mutations in 9 different genes (*XPA*, *XPB*, *XPC*, etc.)
- The nucleotide excision repair mechanism doesn't work
- Accumulation of DNA damage due to UV light in skin cells ➔ severe sunburn, keratosis, skin cancers…