Mutations and DNA repair

- In molecular biology and genetics, **mutations** are **changes in a genomic sequence**, and can be defined as **sudden and spontaneous changes** in the cell.

- Mutations are caused by **radiation**, **viruses** and **mutagenic chemicals**, as well as **errors** that occur during meiosis or **DNA replication**, also can be induced by the organism itself, by cellular processes such as hypermutation.

- Mutations can result in several different types of change in sequences of **DNA, which** can either of **no effect**, **alter the product** of **a gene**, or **prevent the gene from functioning properly or completely**.

- Due to the damaging effects that mutations can have on genes, organisms have mechanisms such as **DNA repair** to remove mutations.

- Mutations can involve **large sections of DNA** becoming duplicated, usually through genetic **recombination**.

Causes of mutations

- Two classes of mutations:
- **Spontaneous mutations** and **induced mutations** caused by mutagens

Spontaneous mutations can be caused by:

1- **Tautomerism**: a base is changed by the repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base resulting in incorrect base pairing during replication.

2- **Depurination**: loss of a purine base (A or G) to form an apurinic site (**AP site**).

3- **Deamination**: changes a normal base to an atypical base containing a keto group in place of the original amine group.

4- **Slipped strand mispairing**: denaturation of the new strand from the template during replication, followed by renaturation in a different spot ("slipping"), which can lead to **insertions** or **deletions**.

Induced mutations can be caused by:

A- Chemicals

- Hydroxylamine

- Alkylating agents (e.g. nitrosourea) which can mutate both **replicating and non-replicating DNA**.

- In contrast, a **base analog** can only mutate the DNA when the analog is incorporated in **replicating the DNA**.

- DNA intercalating agents (e.g. **ethidium bromide**)

- DNA crosslinkers

- Oxidative damage

- Nitrous acid converts amine groups on A and C to diazo groups, altering their hydrogen bonding patterns which leads to incorrect base pairing during replication.

B- Radiation

- Ultraviolet radiation (non-ionizing radiation).

- Two nucleotide bases in DNA – cytosine and thymine – are most vulnerable to radiation that can change their properties.

- UV light can induce adjacent pyrimidine bases in a DNA strand to become covalently joined as a pyrimidine dimer.

- UV radiation, particularly longer-wave UVA, can also cause oxidative damage to DNA.

- Ionizing radiation

- Radioactive decay, such as ^{14}C in DNA

C- Viruses

- Viruses that use **RNA** as their genetic material have **rapid and high mutation** rates to adapt to their surroundings and more effectively move from host to host, which can be an advantage since these viruses will evolve **constantly** and **rapidly**, and thus **evade** the **defensive responses** of the human immune system, treatments and vaccines.

- A mutation can help the virus gain traits that better help it reproduce quickly or adhere better to the surface of human cells.

- As a virus replicates, its genes undergo **random genetic mutations**. Over time, these genetic copying errors can, among other changes to the virus, lead to alterations in the virus' surface proteins or antigen

Classification of mutation types

By affecting the structure

- The sequence of a gene can be altered in a number of ways.
- Mutations in the structure of genes can be classified as:

A- Small-scale mutations, such as those affecting a small gene in one or a few nucleotides, including:

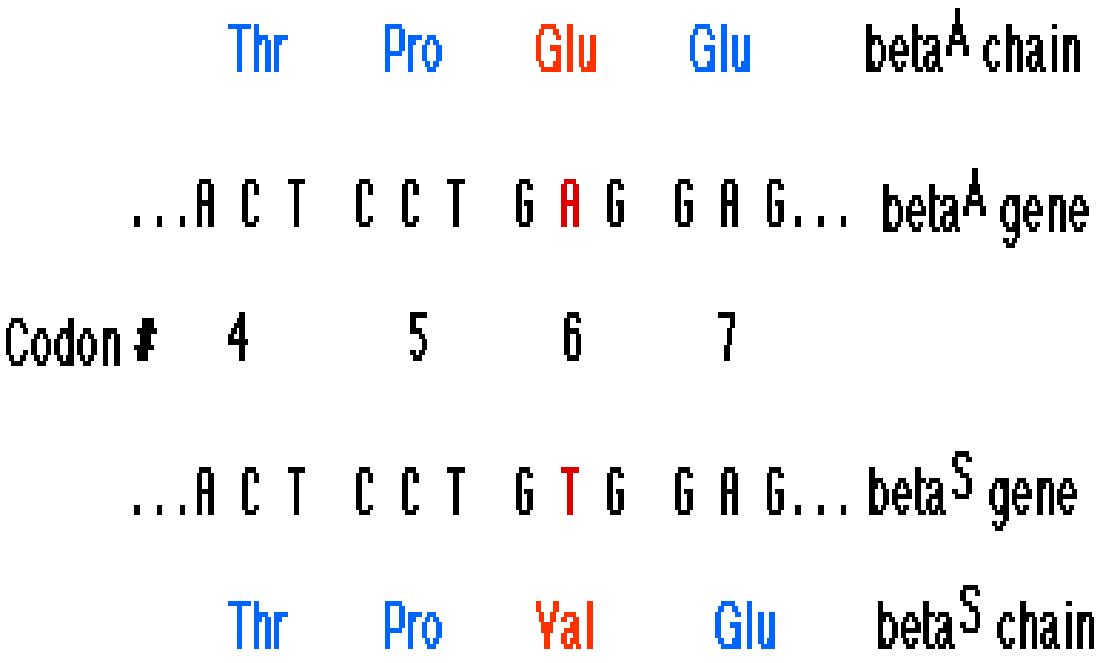
1- Point mutations, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another.

- These changes are classified as **transitions** or **transversions**.
- Most common is the **transition** that exchanges a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine, (C ↔ T).
- Less common is the **transversion**, which exchanges a purine for a pyrimidine or a pyrimidine for a purine (C/T ↔ A/G).

- A point mutation can be reversed by another point mutation, in which the nucleotide is changed back to its original state (true reversion) or by second-site reversion.

- Point mutations that occur within the protein coding region of a gene may be classified into three kinds, depending upon what the erroneous codon codes for:

1- Silent mutations: which code for the same amino acid.
2- Missense mutations: which code for a different amino acid.
3- Nonsense mutations: which code for a stop and can truncate the protein.



2- <u>Insertions</u> add one or more extra nucleotides into the DNA.

- They are usually caused by transposable elements, or errors during replication of repeating elements (e.g. AT repeats).
- Insertions in the coding region of a gene may alter splicing of the mRNA (splice site mutation), or cause a shift in the reading frame (**frameshift**), both of which can significantly alter the gene product.
- Insertions can be reverted by excision of the transposable element.

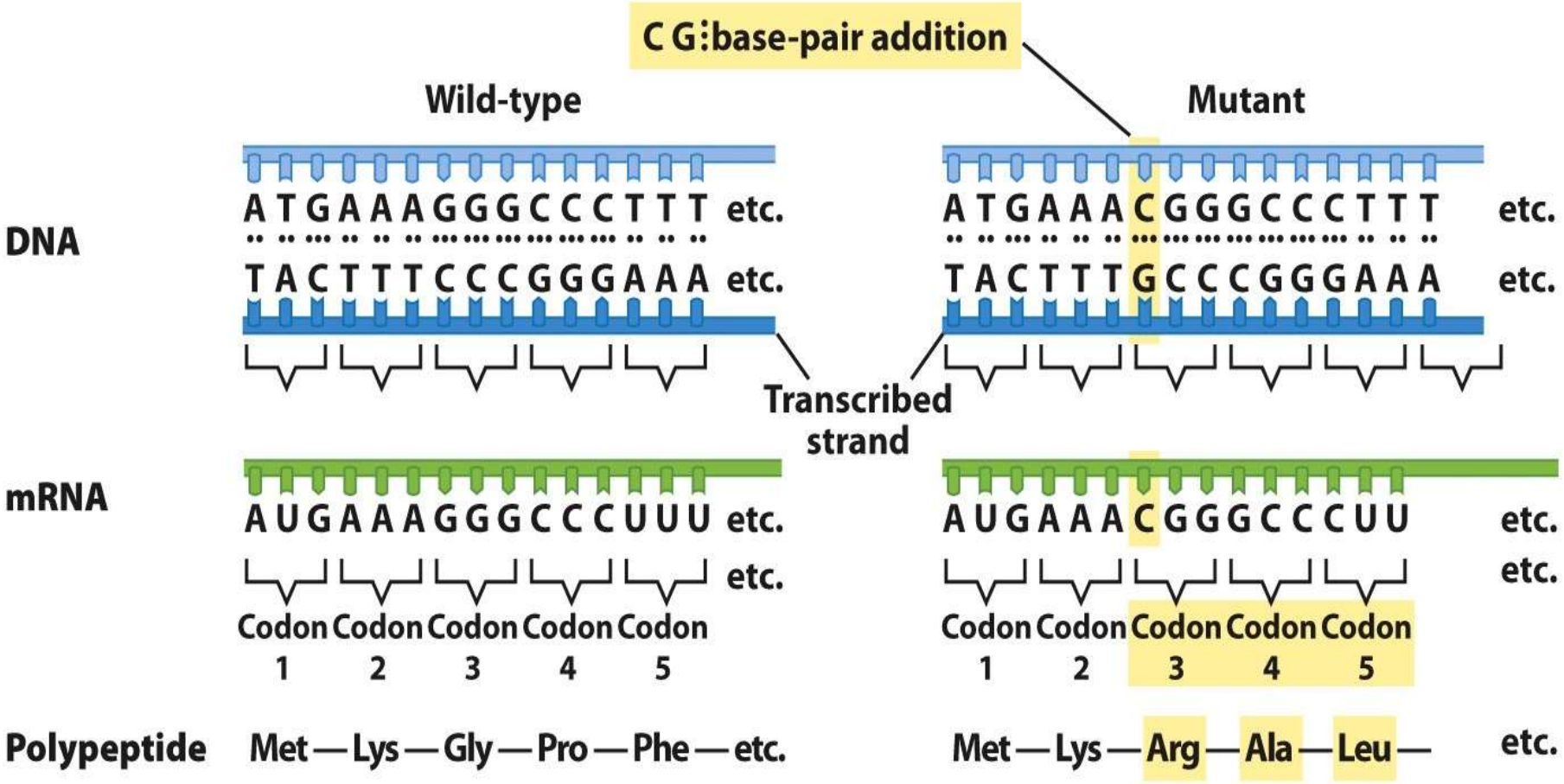
3- <u>Deletions</u> remove one or more nucleotides from the DNA.

- Like insertions, these mutations can alter the reading frame of the gene.
- They are generally irreversible: though exactly the same sequence might theoretically be restored by an insertion, transposable elements able to revert a very short deletion (say 1–2 bases) in any location are either highly unlikely to exist or do not exist at all.

- **<u>Note</u>** that a deletion is not the exact opposite of an insertion: the former is quite random while the latter consists of a specific sequence inserting at locations that are not entirely random or even quite narrowly defined.

Frameshift Mutations

Insertions or deletions of one or two base pairs alter the reading frame of the gene distal to the site of the mutation.



B- Large-scale mutations in chromosomal structure, including:

1- Amplifications (or gene duplications) leading to multiple copies of all chromosomal regions.

2- Deletions of large chromosomal regions, leading to loss of the genes within those regions.

3- Mutations potentially bringing together separate genes to form functionally distinct fusion genes (e.g. bcr-abl). These include:

- **Chromosomal translocations**: interchange of genetic parts from non-homologous chromosomes.
- **Interstitial deletions**: an intra-chromosomal deletion that removes a segment of DNA from a single chromosome, thereby apposing previously distant genes.
- **Chromosomal inversions**: reversing the orientation of a chromosomal segment.
- **Loss of heterozygosity**: loss of one allele, either by a deletion or recombination event, in an organism that previously had two different alleles.

By inheritance

- By pattern of inheritance The human genome contains two copies of each gene – a paternal and a maternal allele.

1- A heterozygous mutation is a mutation of only one allele.

2- A homozygous mutation is an identical mutation of both the paternal and maternal alleles.

3- Compound heterozygous mutations or a **genetic compound** comprises two different mutations in the paternal and maternal alleles.

4- A wild type or homozygous non-mutated organism is one in which neither allele is mutated. (Just not a mutation).

Special classes

Conditional mutation

- For example, **a temperature-sensitive mutation** can cause **cell death** at high temperature (**restrictive condition**), but might have no deleterious consequences at a lower temperature (**permissive condition**).

DNA repair systems

-Repair mechanisms are divided into 2 categories:

1- Damage reversal simplest; enzymatic action restores normal structure without breaking backbone

2- Damage removal involves cutting out and replacing a damaged or inappropriate base or section of nucleotides.

1- Damage reversal

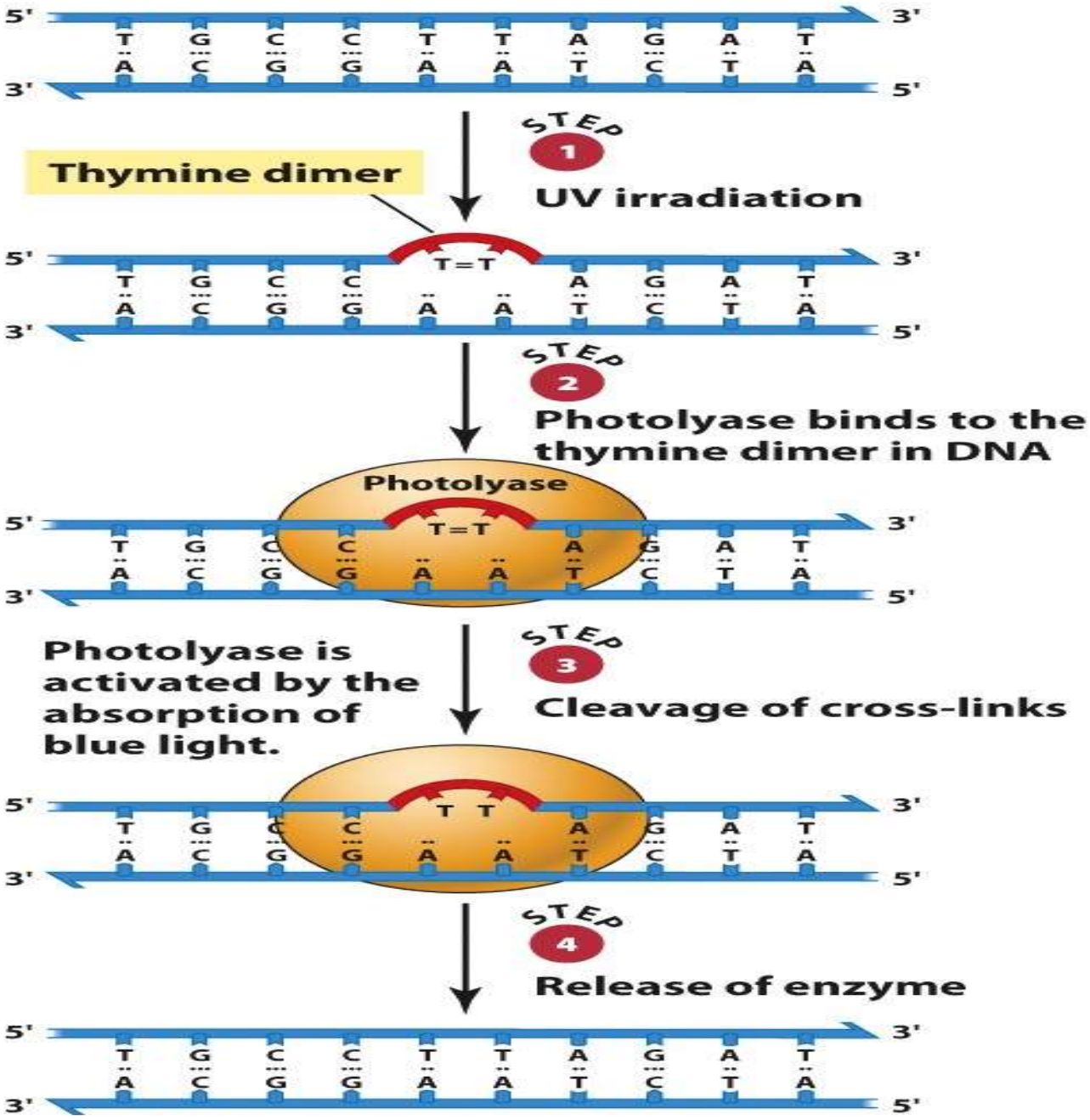
A- Photoreactivation

- This is one of the simplest and perhaps oldest repair systems: it consists of a single enzyme which can split **pyrimidine dimers** (break the covalent bond) in presence of light.
-The **photolyase** enzyme catalyzes this reaction; it is found in many bacteria, lower eukaryotes, insects, and plants.
- It seems to be absent in mammals (including humans).

B- Ligation of single strand breaks

- X-rays and some chemicals like peroxides can cause breaks in backbone of DNA.
- Simple breaks in one strand are rapidly repaired by DNA ligase.
- Microbial mutants lacking ligase tend to have high levels of recombination since DNA ends are recombinogenic (very reactive).

Light-Dependent Repair: Photolyase Cleaves Thymine Dimers



2- Damage removal

A- Base excision repair

-The damaged or inappropriate base is removed from its sugar linkage and replaced.

- These are **glycosylase** enzymes which cut the base-sugar bond as **uracil glycosylase** enzyme which removes uracil from DNA to provide **AP site**.

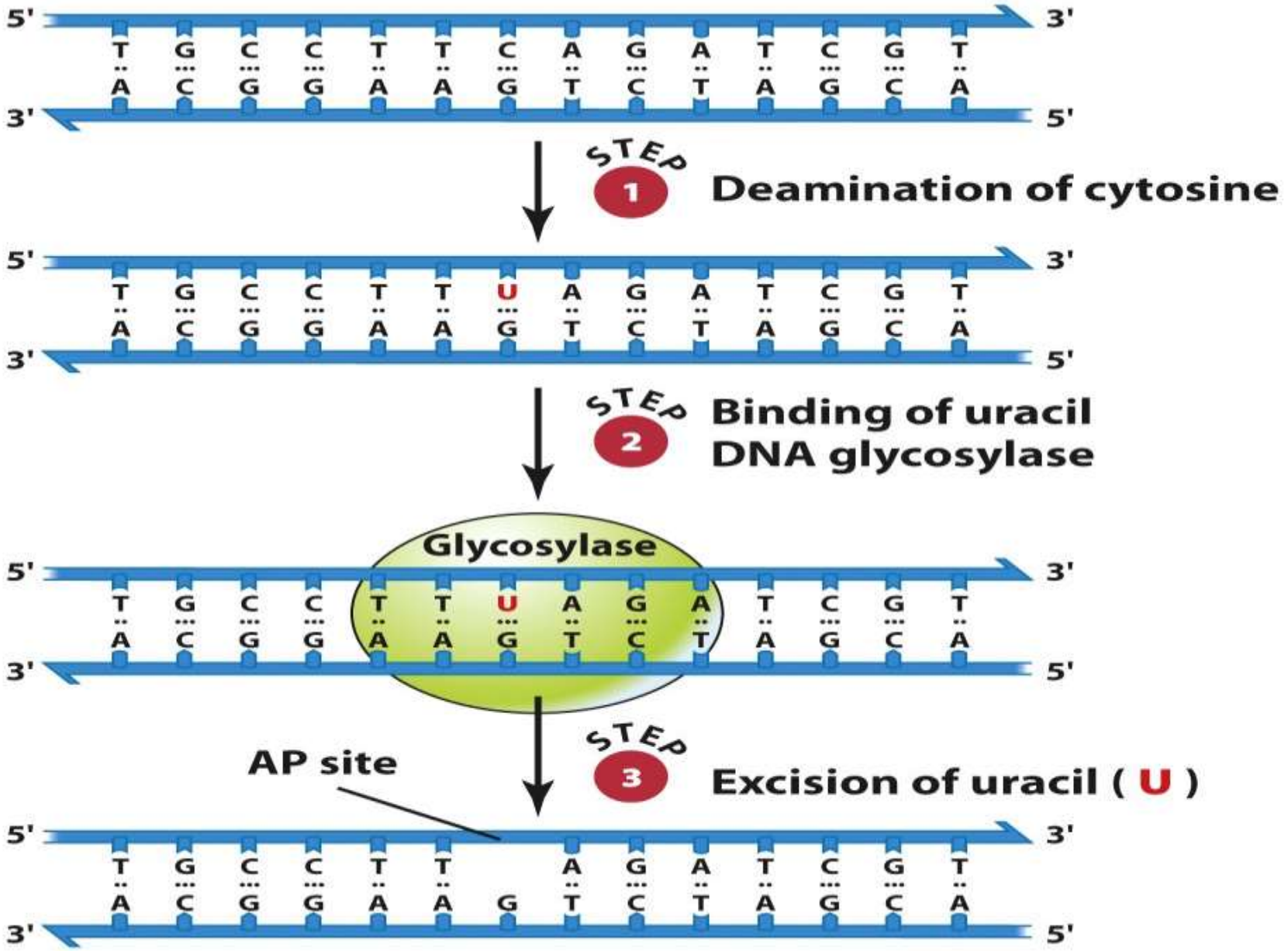
- Uracil is not supposed to be in DNA.

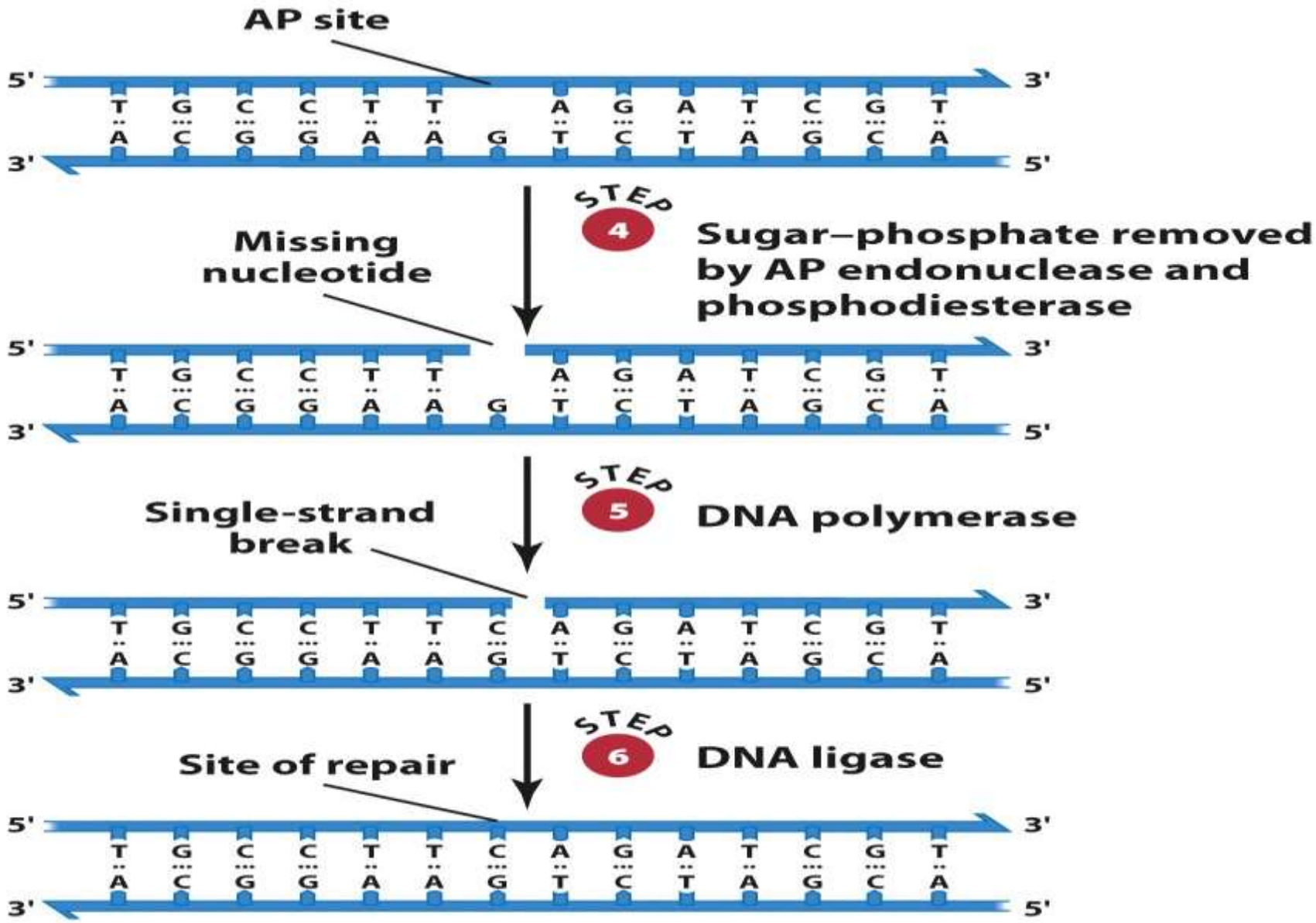
- **It can occur if RNA primers not removed in DNA replication or (more likely) if cytosine is deaminated (this is potentially mutagenic).**

-The enzyme recognizes uracil and cuts the glyscosyl linkage to deoxyribose.

-Then, the **AP endonuclease** removes the AP site and neighboring nucleotides.

-The gap is filled by **DNA polymerase I and DNA ligase** using the other strand as a template.



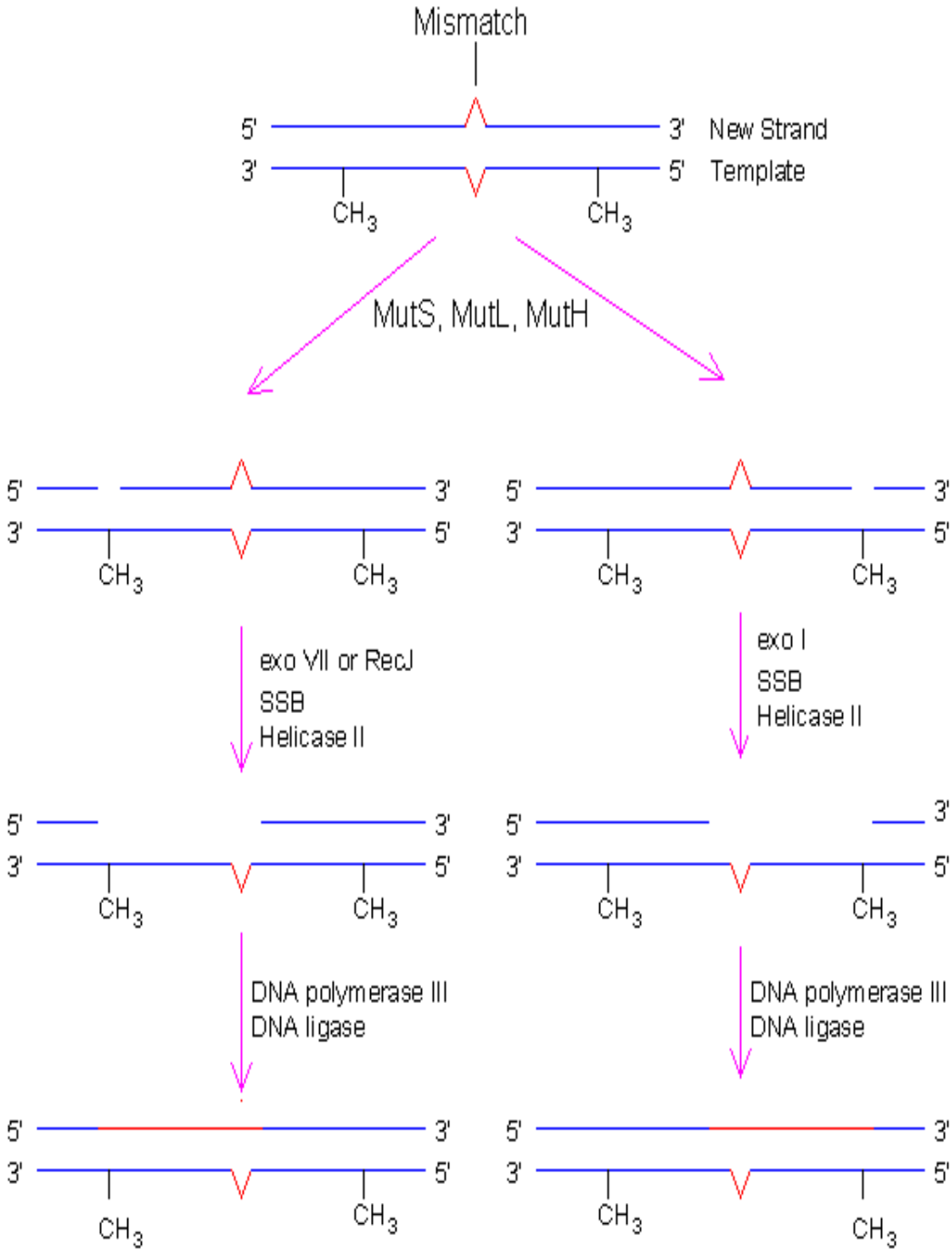


B- Mismatch repair

-The repairing process begins with the **protein MutS** which binds to mismatched base pairs.

- Then, **MutL** is recruited to the complex and activates **MutH** which binds to **GATC** sequences.
- **Activated MutH** cleaves the unmethylated strand at the GATC site.
- Subsequently, the segment from the cleavage site to the mismatch is removed by **exonuclease** (with assistance from **helicase II** and **SSB proteins**).
- If the cleavage occurs on the **3' side** of the mismatch, this step is carried out by **exonuclease I** (which degrades a single strand only in the 3' to 5' direction).
- If the cleavage occurs on the **5' side** of the mismatch, **exonuclease VII**.
- The gap is filled by **DNA polymerase III and DNA ligase**.
- The distance between the GATC site and the mismatch could be as long as 1,000 base pairs. Therefore, mismatch repair is **very expensive** and **inefficient**.

- Mismatch repair in eukaryotes may be similar to that in *E. coli*.
- Homologs of MutS and MutL have been identified in yeast, mammals, and other eukaryotes.
- **MSH1** to **MSH5** are homologous to **MutS**; **MLH1**, **PMS1** and **PMS2** are homologous to **MutL**.
- Mutations of **MSH2**, **PMS1** and **PMS2** are related to **colon cancer**.
- In eukaryotes, the mechanism to distinguish the template strand from the new strand is still unclear.



C- Nucleotide excision repair (NER)

-This system works on DNA damage which is "bulky" and creates a block to DNA replication and transcription (so, UV-induced dimers and some kinds of chemical adducts).

- In **E. coli**, proteins **UvrA**, **UvrB**, and **UvrC** are involved in removing the damaged nucleotides and the gap is then filled by **DNA polymerase I and DNA ligase**.

- In yeast, the proteins similar to Uvr's are named **RADxx** ("RAD" stands for "radiation"), such as **RAD3**, **RAD10**.

- Humans with the hereditary disease **Xeroderma pigmentosum** are sunlight-sensitive, they have very high risks of skin cancers on sun-exposed areas of the body and have defects in genes homologous to those required for **NER** in simple eukaryotes.
- **NER** mutants in lower organisms are UV-sensitive.