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DNA Mutation and Repair

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What is a mutation?

- Substitution, deletion, or insertion of a base pair.
- Chromosomal deletion, insertion, or rearrangement.

Somatic mutations - occur in somatic cells and only affect the individual in which the mutation arises.

Germ-line mutations - alter gametes and passed to the next generation.

Mutations are quantified in two ways:

1. Mutation rate = probability of a particular type of mutation per unit time (or generation).
2. Mutation frequency = number of times a particular mutation occurs in a population of cells or individuals.

Two types of point mutations:

1. **Base pair substitutions.**

    1. **Transitions**
        - Convert a purine-pyrimidine to the other purine-pyrimidine.
        - 4 types of transitions; A ↔ G and T ↔ C
        - Most transitions results in synonymous substitution because of the degeneracy of the genetic code.

    1. **Transversions**

        - Convert a purine-pyrimidine to a pyrimidine-purine.
        - 8 types of transversions; A ↔ T, G ↔ C, A ↔ C, & G ↔ T
        - Transversions are more likely to result in nonsynonomous substitution.

2. **Base pair deletions and insertions**

Missense mutation :

Base pair substitution results in substitution of a different amino acid.

Nonsense mutation:

Base pair substitution results in a stop codon (and shorter polypeptide).

Neutral mutation:

Base pair substitution results in substitution of an amino acid with similar chemical properties (protein function is not altered).

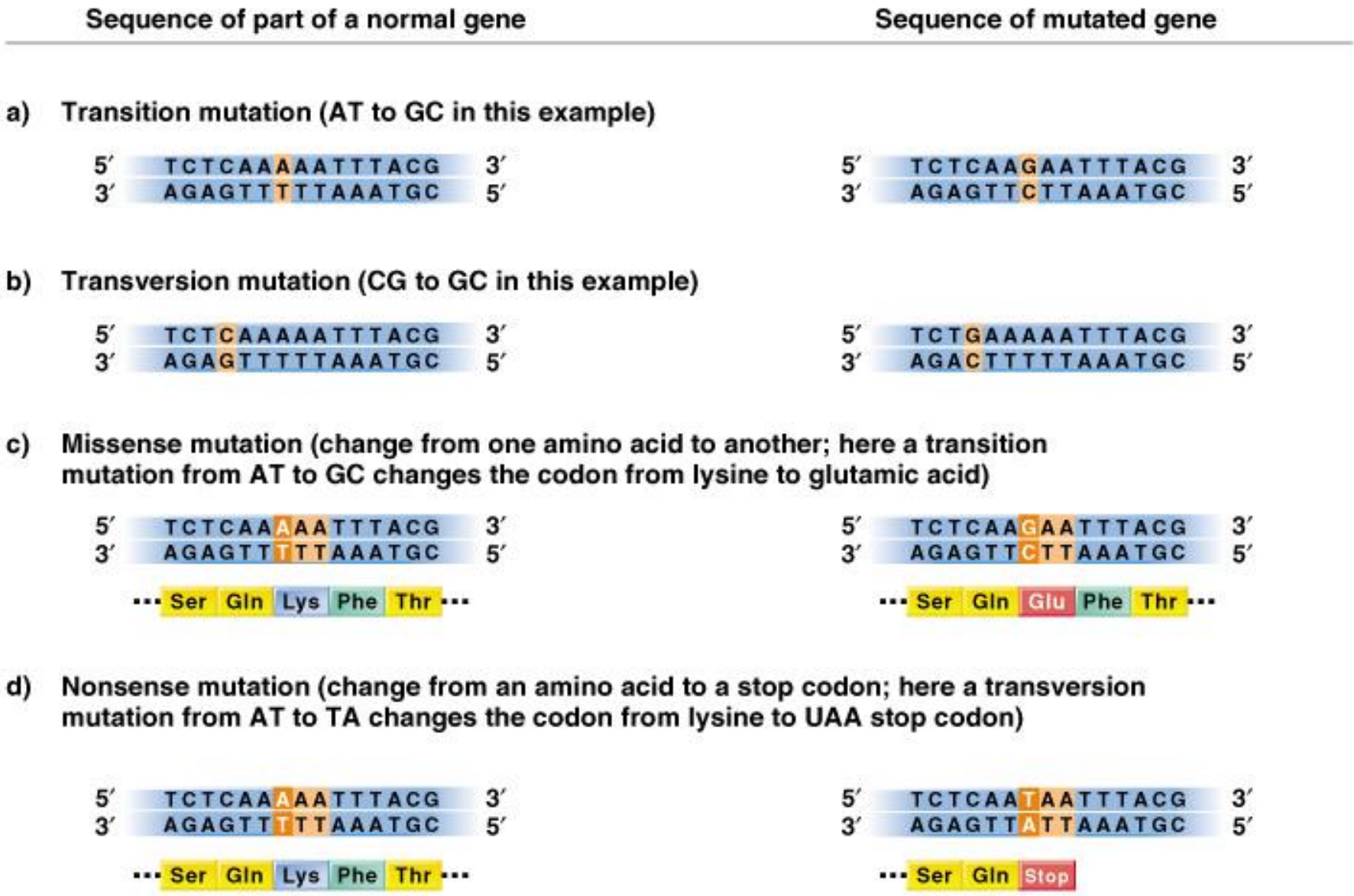
Silent mutation:

Base pair substitution results in the same amino acid.

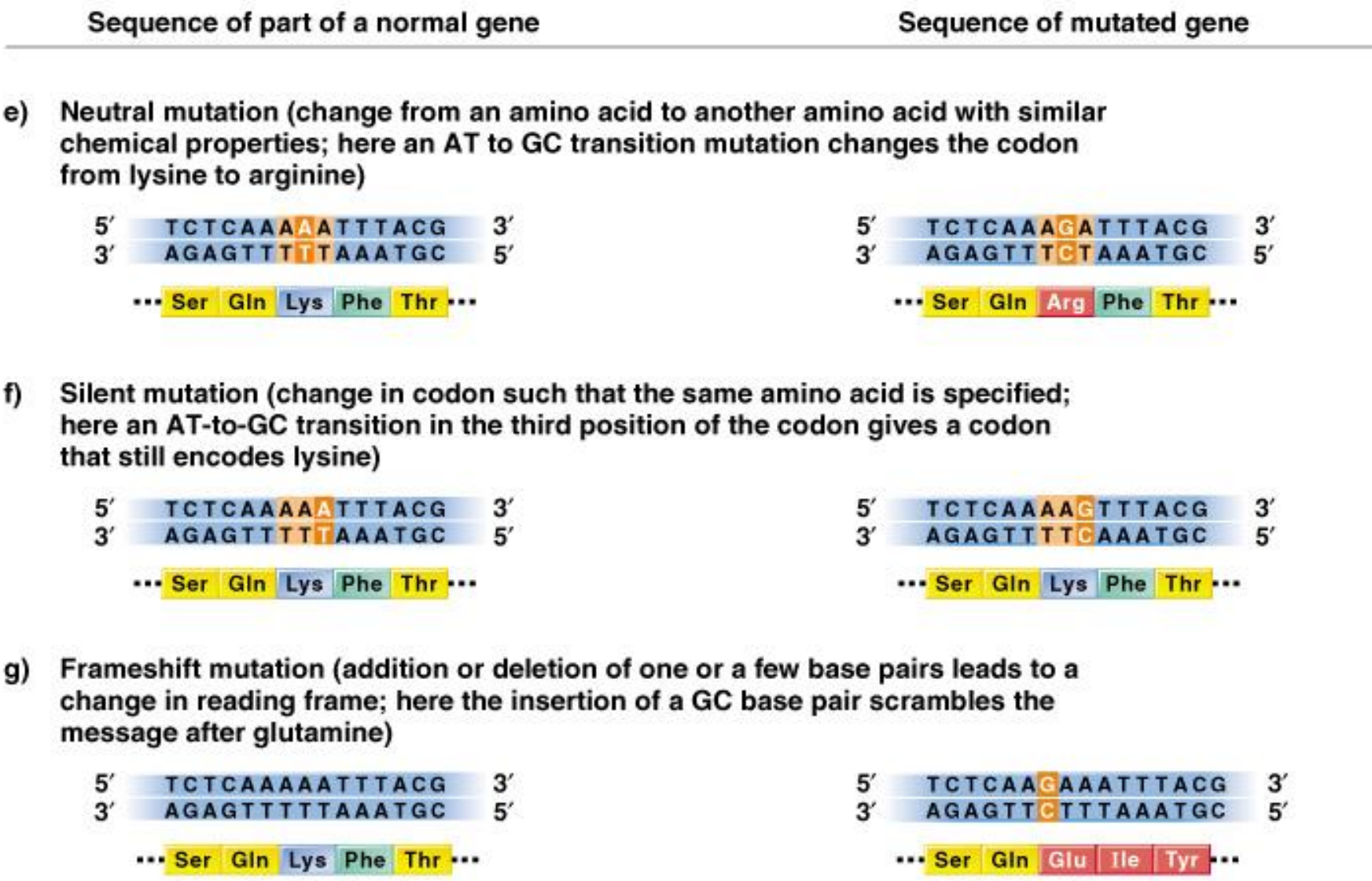
Frameshift mutations:

Deletions or insertions (not divisible by 3) result in translation of incorrect amino acids, stops codons (shorter polypeptides), or read-through of stop codons (longer polypeptides).

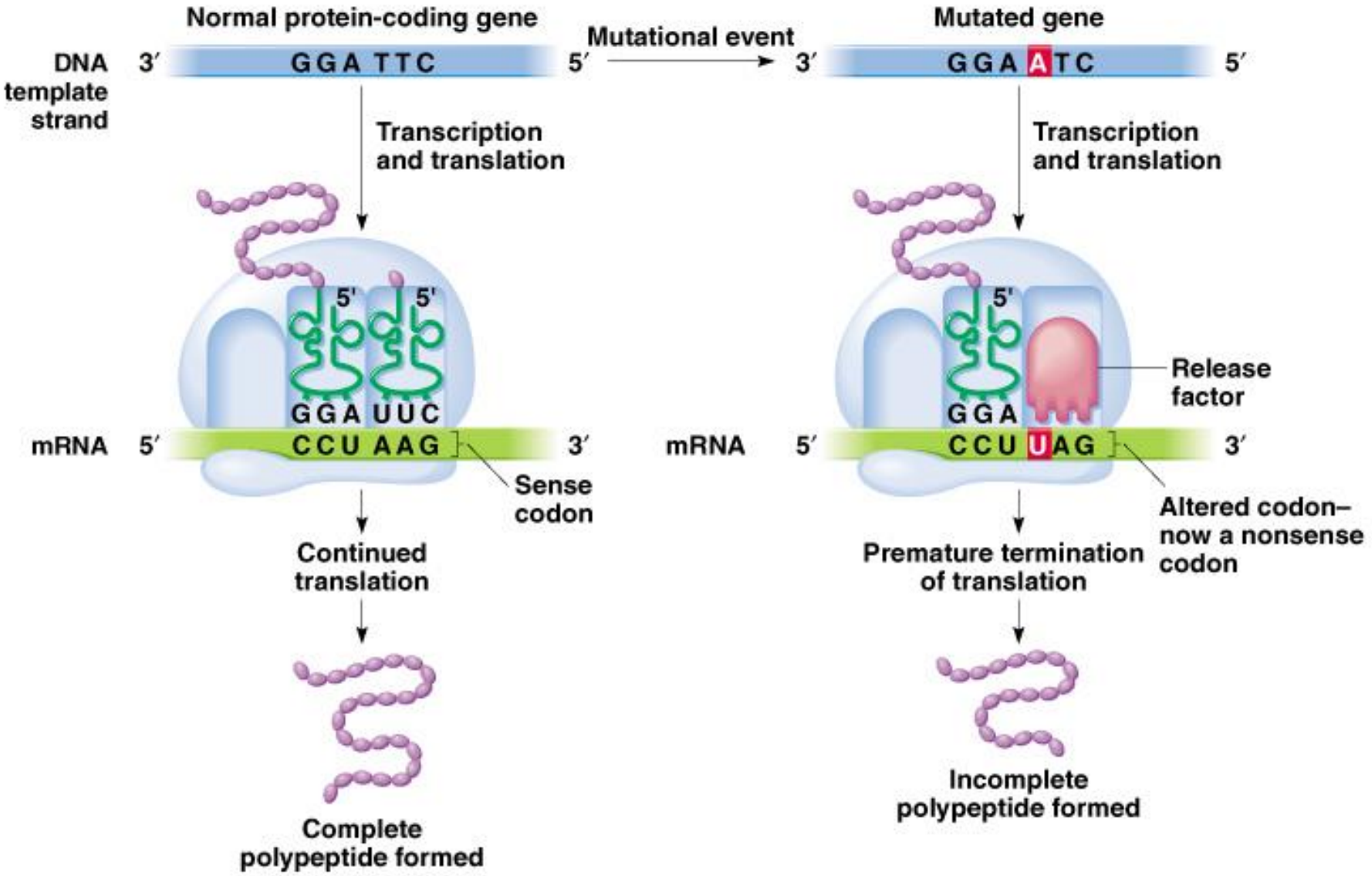
Types of base pair substitutions and mutations



Types of base pair substitutions and mutations



Effect of a nonsense mutation on translation



Reverse mutations and suppressor mutations:

Forward mutation

Mutation changes wild type (ancestral) to mutant (derived).

Reverse mutation (back mutation)

Mutation changes mutant (derived) to wild type (ancestral).

- Reversion to the wild type amino acid restores function.
- Reversion to another amino acid partly or fully restores function.

Suppressor mutation

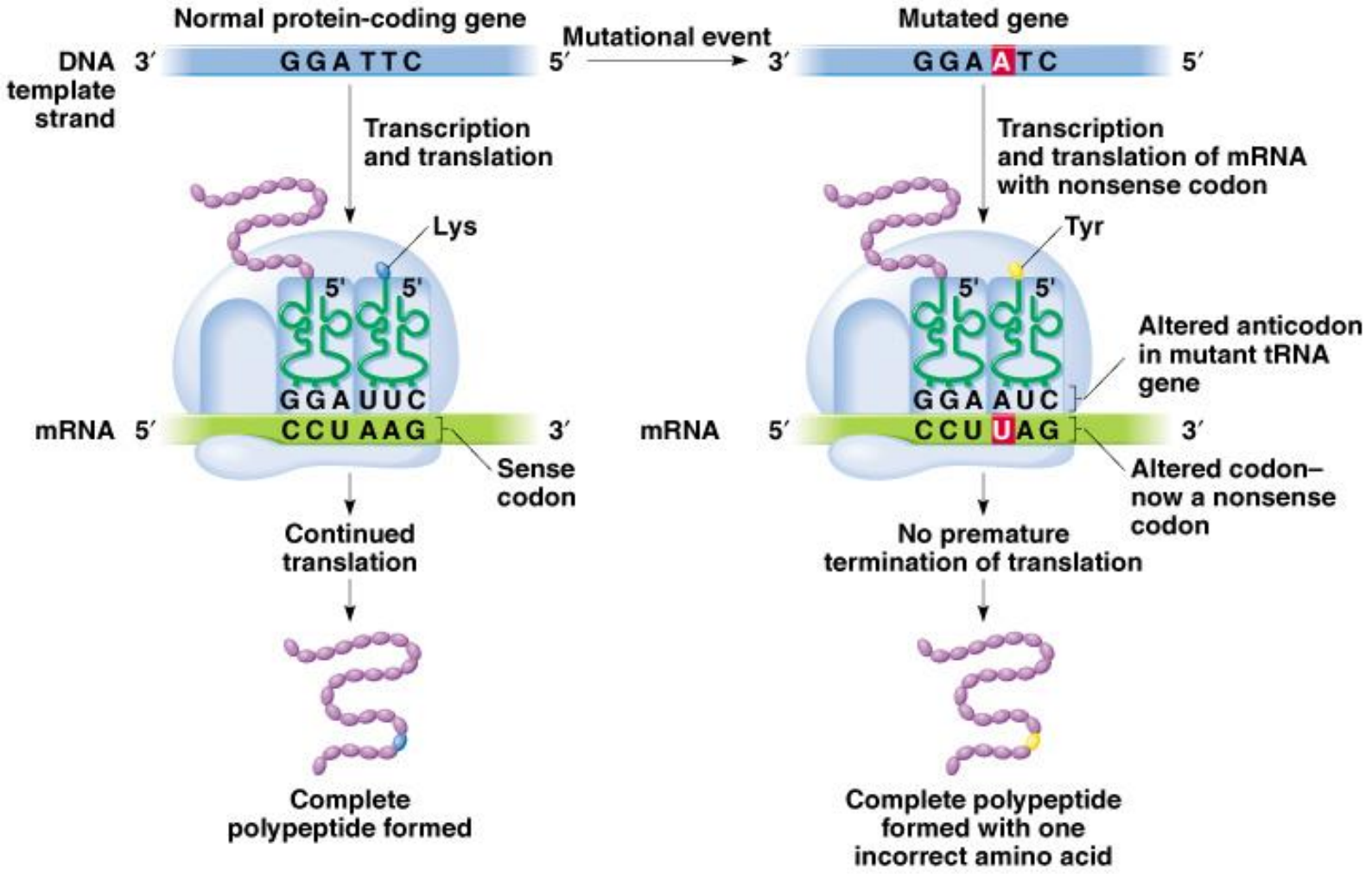
Occur at sites different from the original mutation and mask or compensate for the initial mutation without reversing it.

- Intragenic suppressors occur on the same codon; e.g., nearby addition restores a deletion
- Intergenic suppressors occur on a different gene.

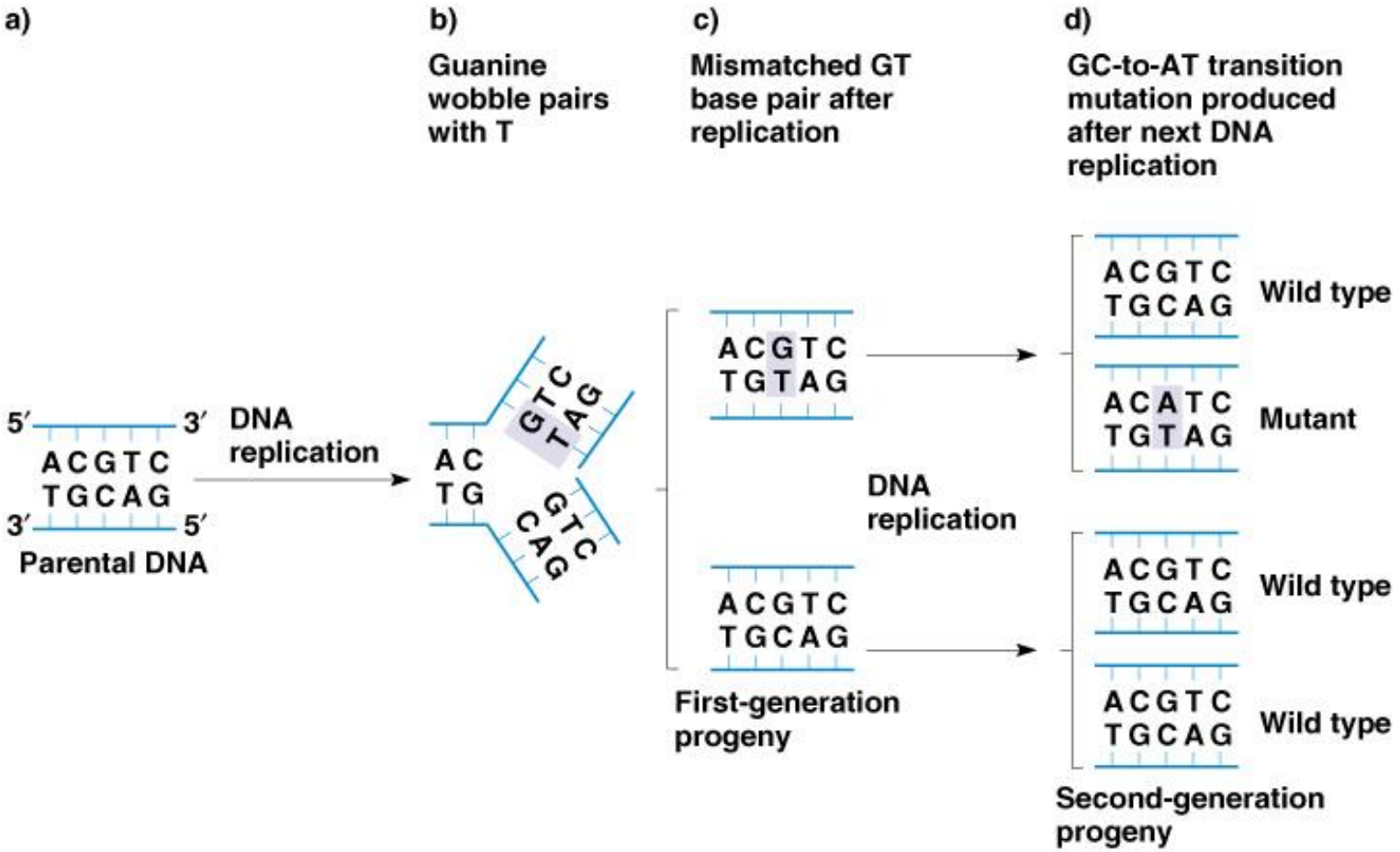
Intergenic suppressor genes:

- Many function in mRNA translation.
- Each suppressor gene works on only one type of nonsense, missense, of frameshift mutation.
- Suppressor genes often encode tRNAs, which possess anti-codons that recognize stop codons and insert an amino acid.
- Three classes of tRNA nonsense suppressors, one for each stop codon (UAG, UAA, UGA).
- tRNA suppressor genes coexist with wild type tRNAs.
- tRNA suppressors compete with release factors, which are important for proper amino acid chain termination.
- Small number of read-through polypeptides are produced; tandem stop codons (UAGUAG) are required to result in correct translation termination.

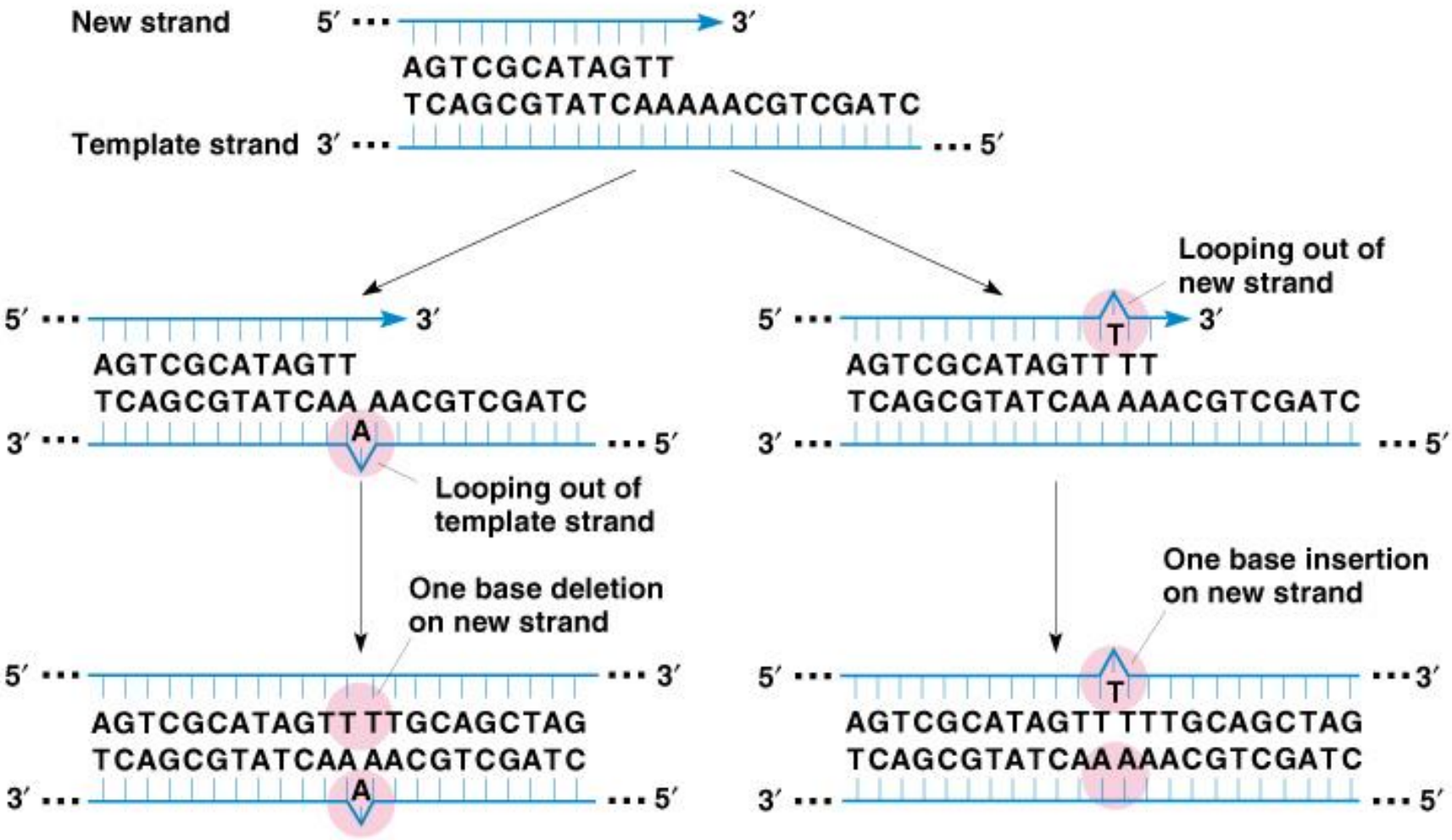
tRNA suppressor gene mechanism for nonsense mutation



Mutation caused by mismatch wobble base pairing



Addition and deletion by DNA looping-out.



Spontaneous mutations:

Depurination

Common; A or G are removed and replaced with a random base.

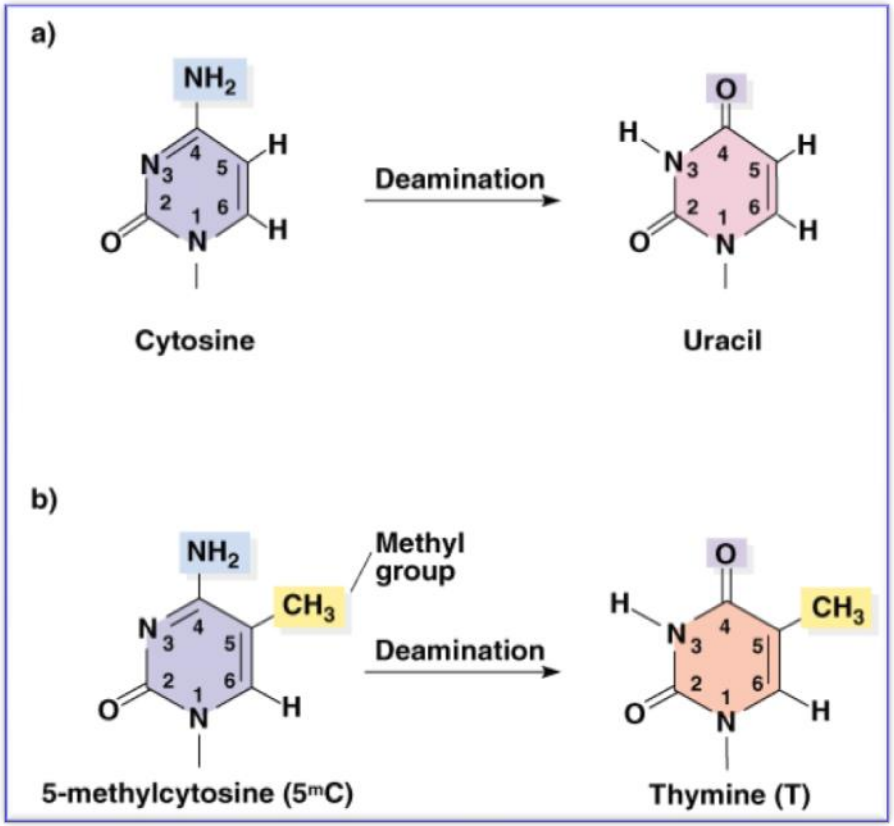
Deamination

Amino group is removed from a base (C → U); if not replaced U pairs with A in next round of replication (CG → TA).

Prokaryote DNA contains small amounts of $5^{M}C$; deamination of $5^{M}C$ produces T (CG → TA).

Regions with high levels of $5^{M}C$ are mutation hot spots.

Deamination



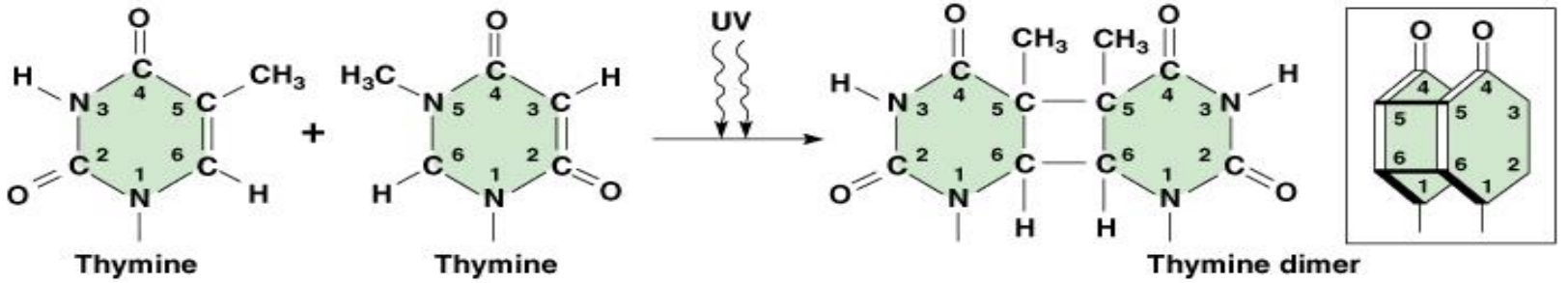
Induced mutations

Radiation (e.g., X-rays, UV)

Ionizing radiation breaks covalent bonds including those in DNA and is the leading cause of chromosome mutations.

Ionizing radiation has a cumulative effect and kills cells at high doses.

UV (254-260 nm) causes purines and pyrimidines to form abnormal dimer bonds and bulges in the DNA strands.



Thymine dimers induced by UV light.

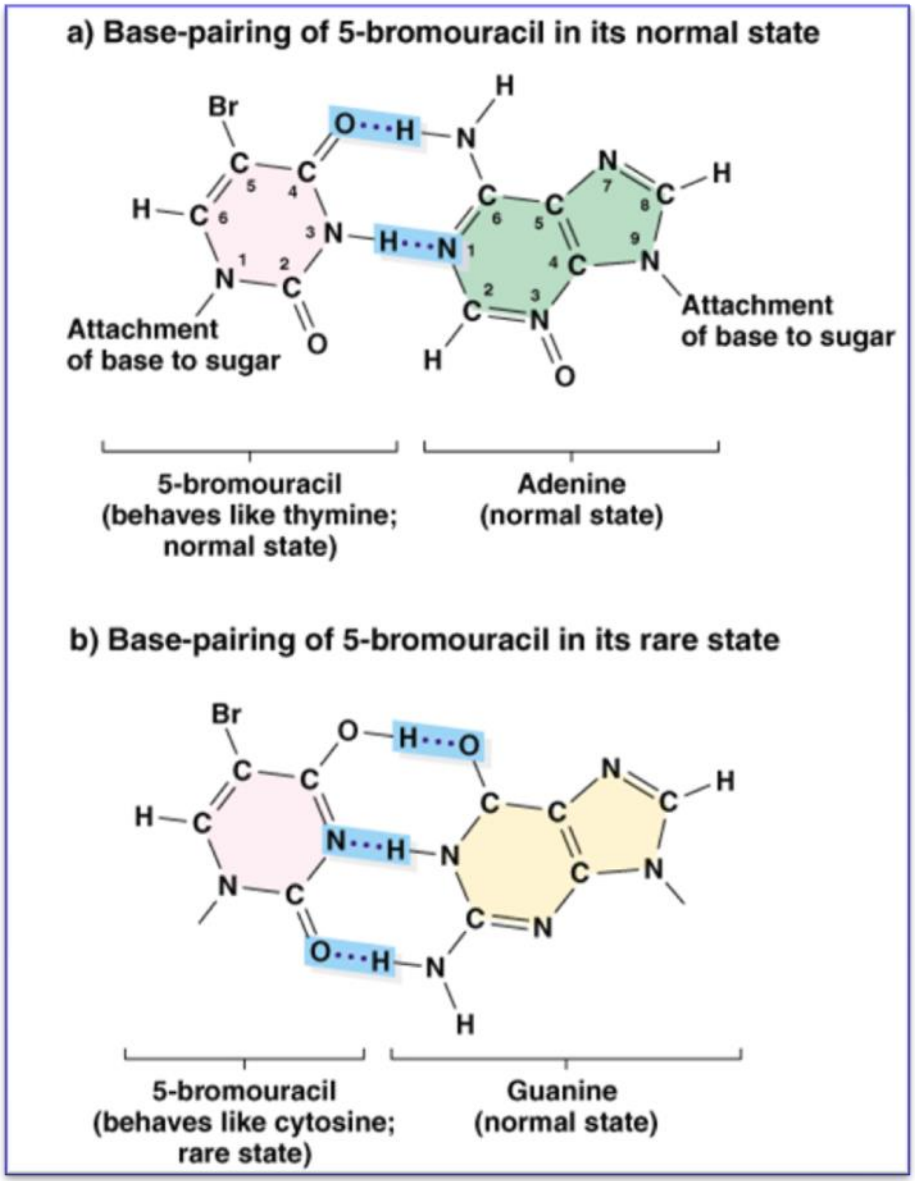
Induced mutations:

Chemical mutagens

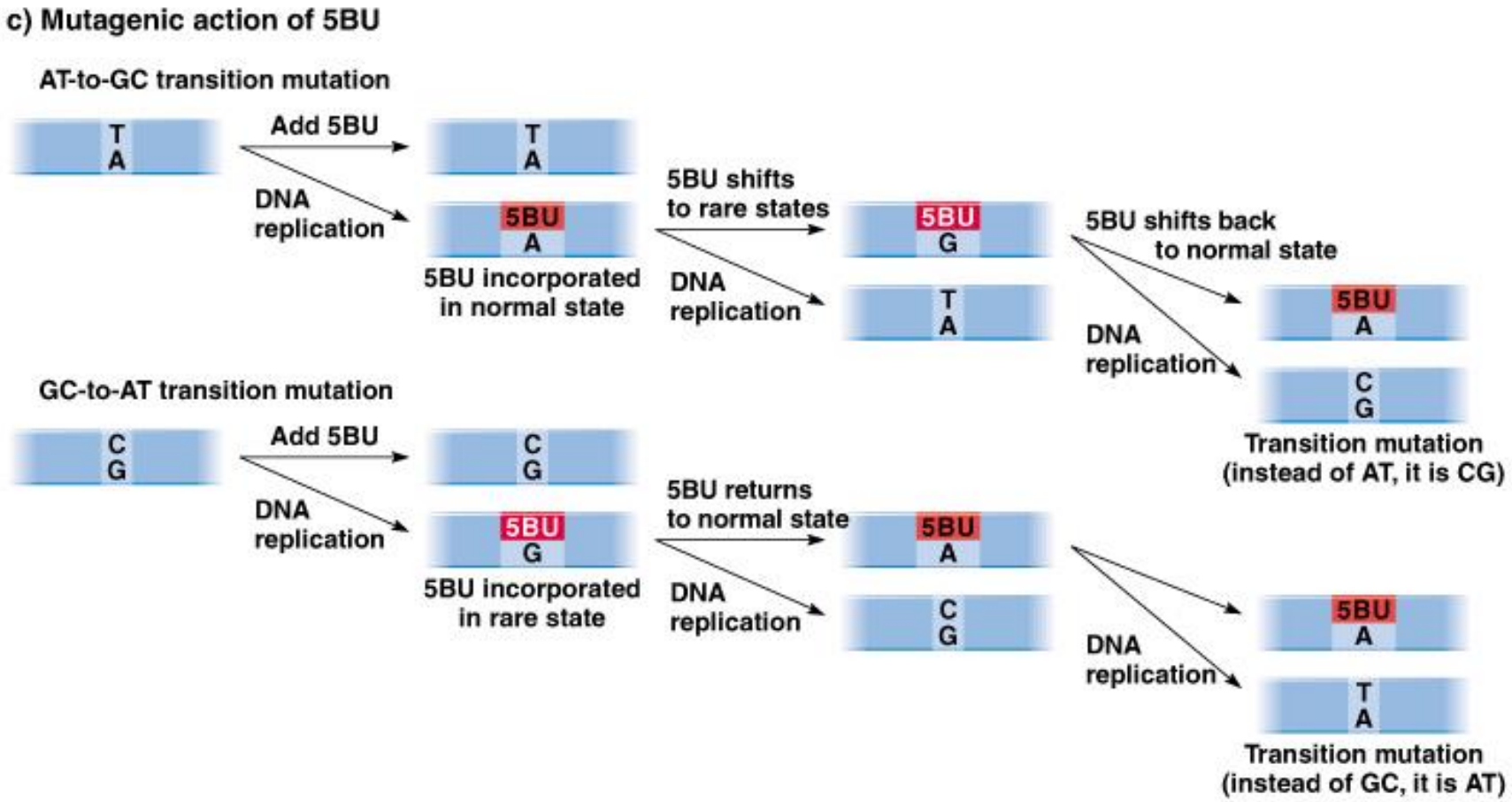
Base analogs

- Similar to normal bases, incorporated into DNA during replication.
- Some cause mis-pairing (e.g., 5-bromouracil).
- Not all are mutagenic.

Mutagenic efffects of 5-bromouracil



Mutagenic effects of 5-bromouracil



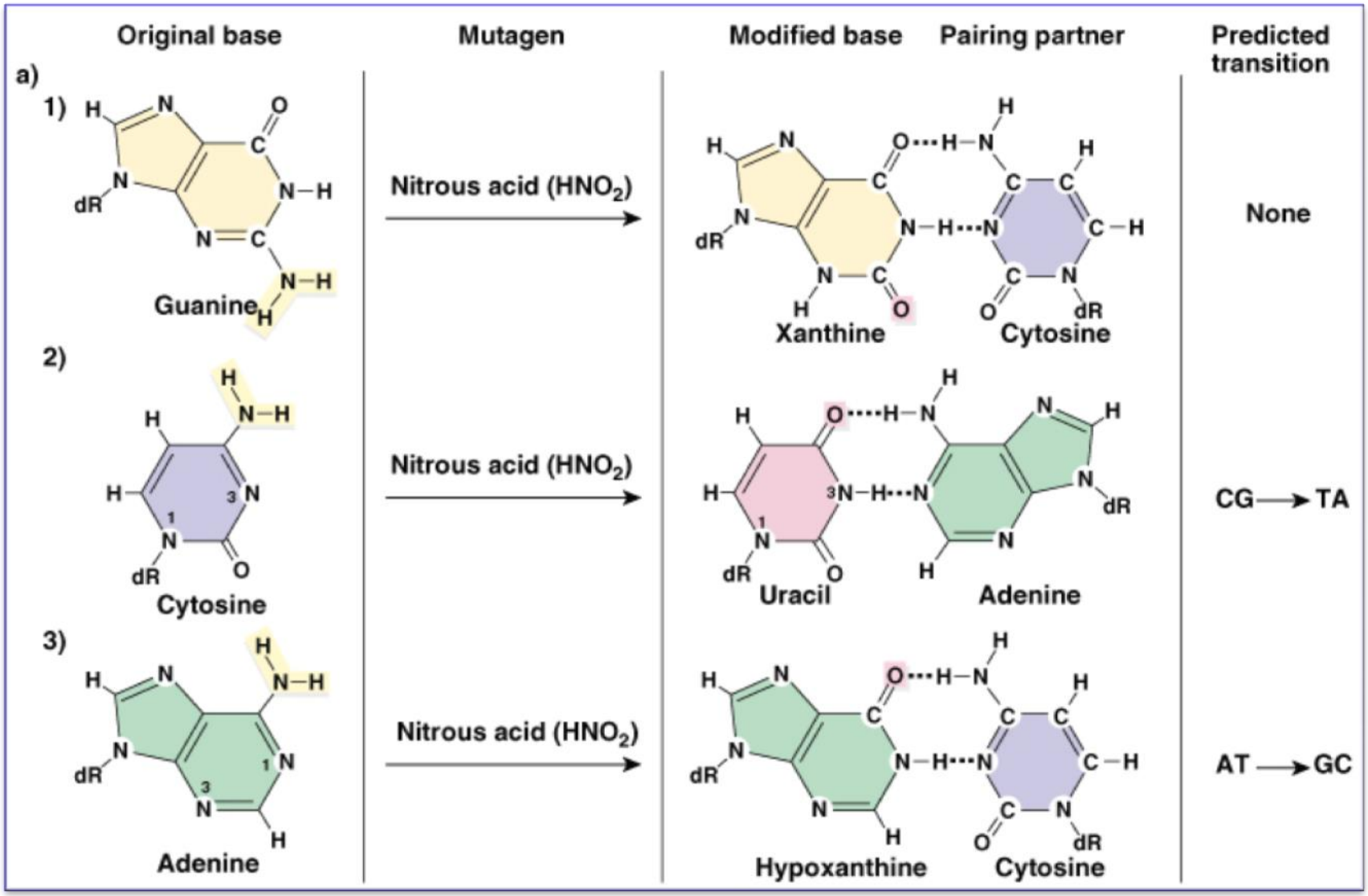
Induced mutations:

Chemical mutagens

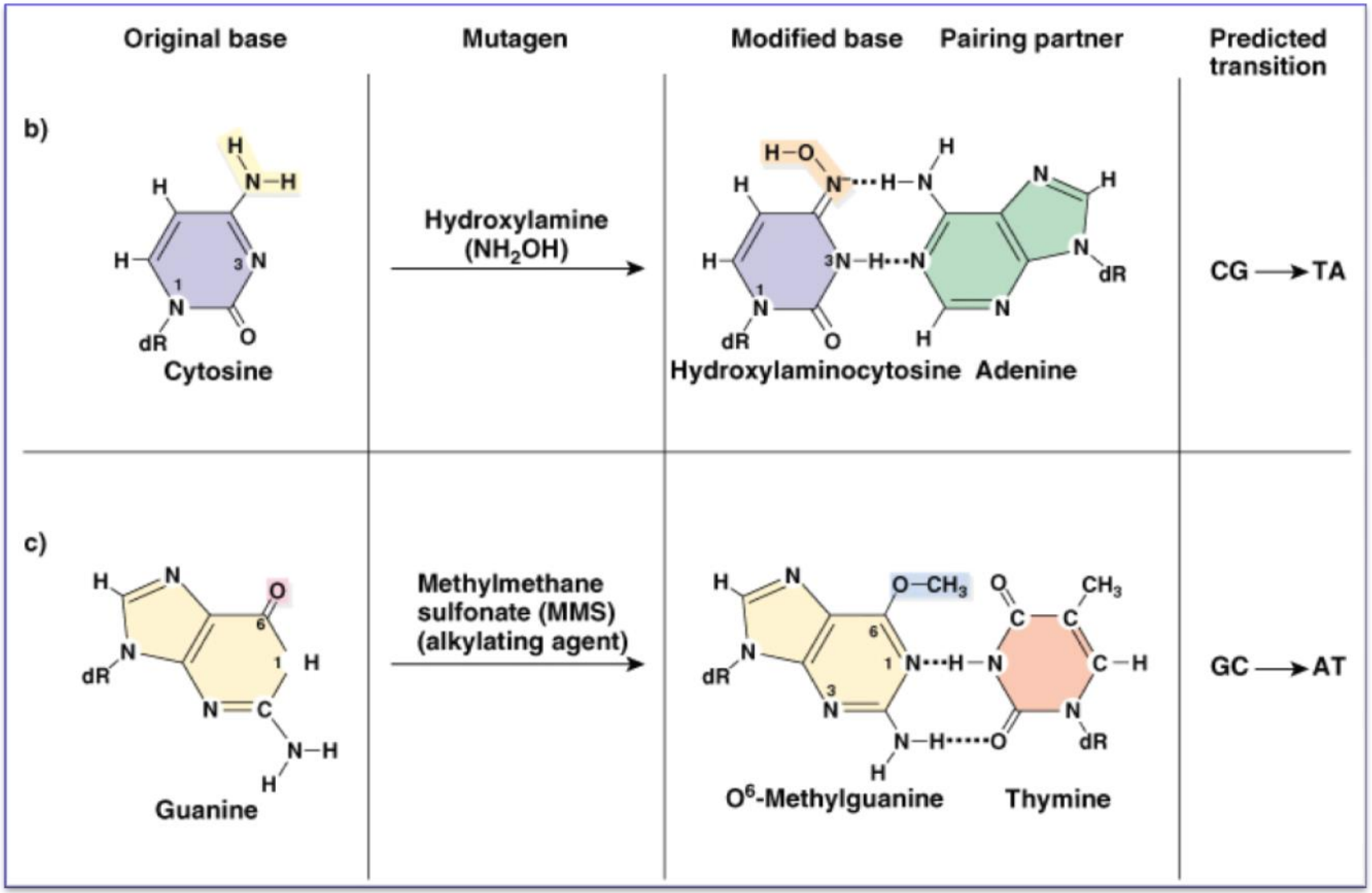
Base modifying agents, act at any stage of the cell cycle:

- Deaminating agents
- Hydroxylating agents
- Alkylating agents

Base-modifying agents



Base-modifying agents

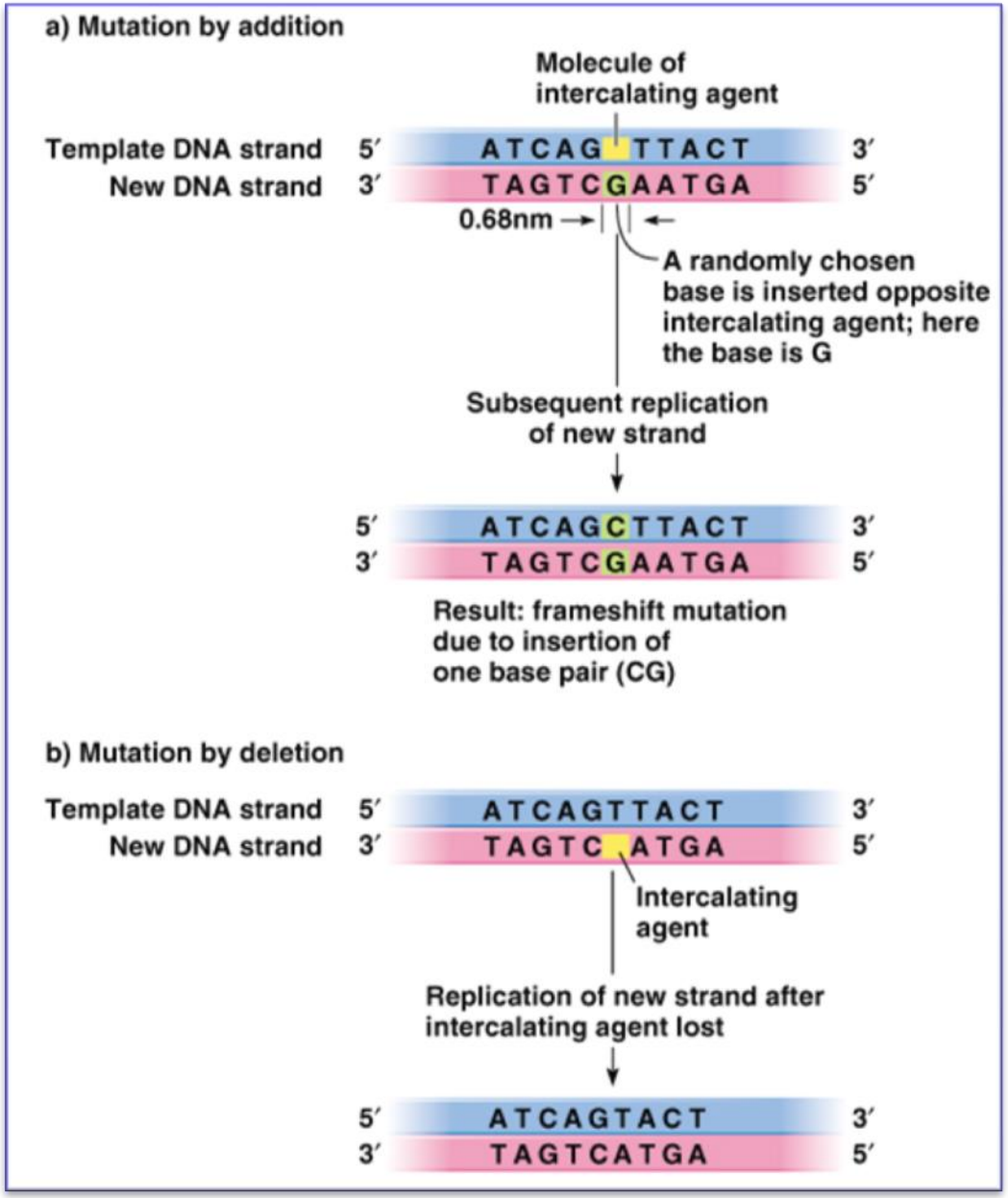


Induced mutations:

Chemical mutagens

Intercalating agents:

- Thin, plate-like hydrophobic molecules insert themselves between adjacent base-pairs,
- Mutagenic intercalating agents cause insertions during DNA replication.
- Loss of intercalating agent can result in deletion.
- Examples: Proflavin, Ethidium bromide


a) Mutation by addition
Molecule of intercalating agent
Template DNA strand
New DNA strand
5′ ATCAG TTACT 3′
3′ TAGTCGAATGA 5′
0.68nm
A randomly chosen base is inserted opposite intercalating agent; here the base is G
Subsequent replication of new strand
5′ ATCAGCTTACT 3′
3′ TAGTCGAATGA 5′
Result: frameshift mutation due to insertion of one base pair (CG)
b) Mutation by deletion
Template DNA strand
New DNA strand
5′ ATCAGTTACT 3′
3′ TAGTC ATGA 5′
Intercalating agent
Replication of new strand after intercalating agent lost
5′ ATCAGTACT 3′
3′ TAGTCATGA 5′

Detecting environmental mutations:

Ames Test (after Bruce Ames)

Ames Test is an inexpensive method used to screen possible carcinogens and mutagens.

- Histidine auxotroph *Salmonella typhimurium* (requires Histidine to grow) are mixed with rat liver enzymes and plated on media lacking histidine.

- Liver enzymes are required to detect mutagens that are converted to carcinogenic forms by the liver (e.g., procarcinogens).

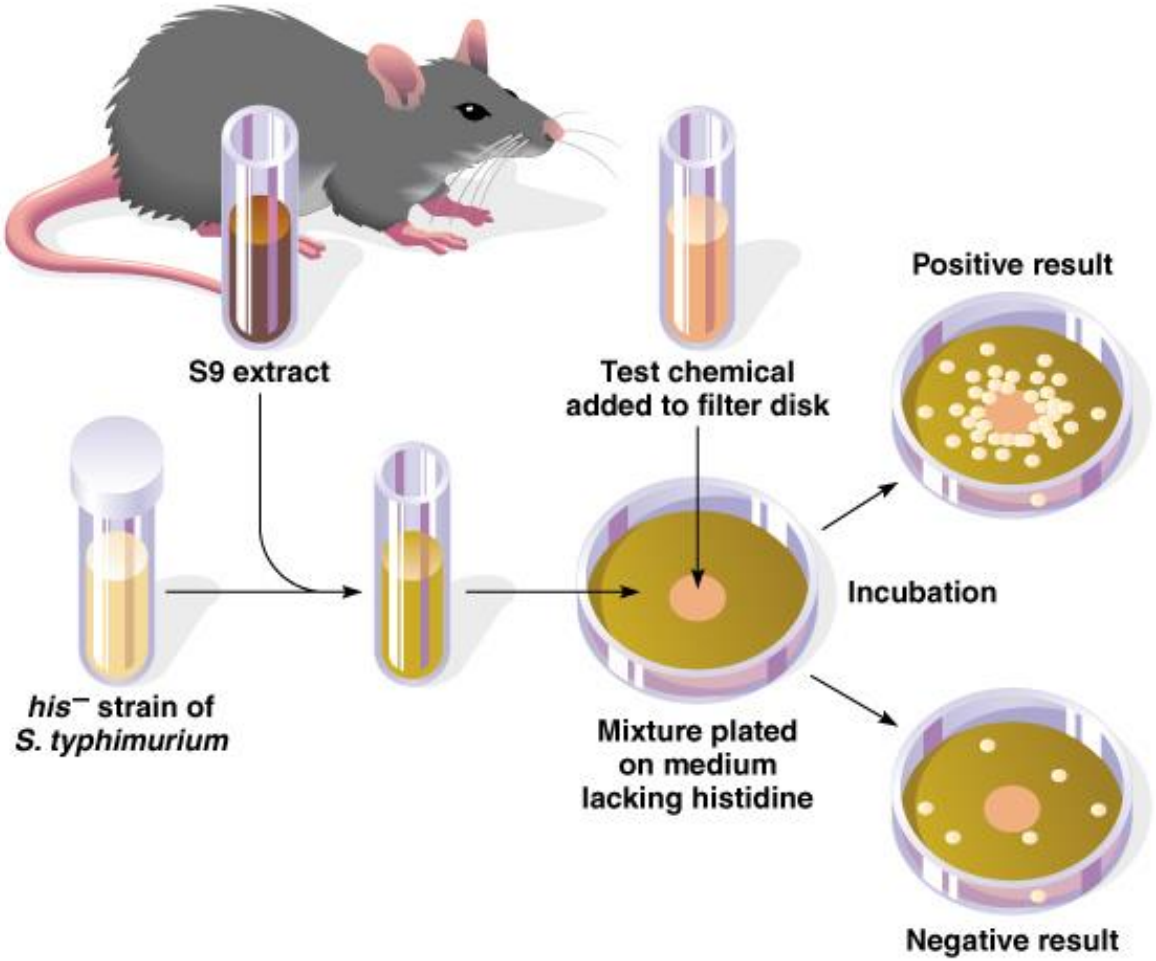
- Test chemical is then added to medium.

- Control plates show only a small of revertants (bacteria cells growing without histidine).

- Plates innoculated with mutagens or procarcinogens show a larger of revertants.

- Auxotroph will not grow without Histidine unless a mutation has occurred.

Ames test

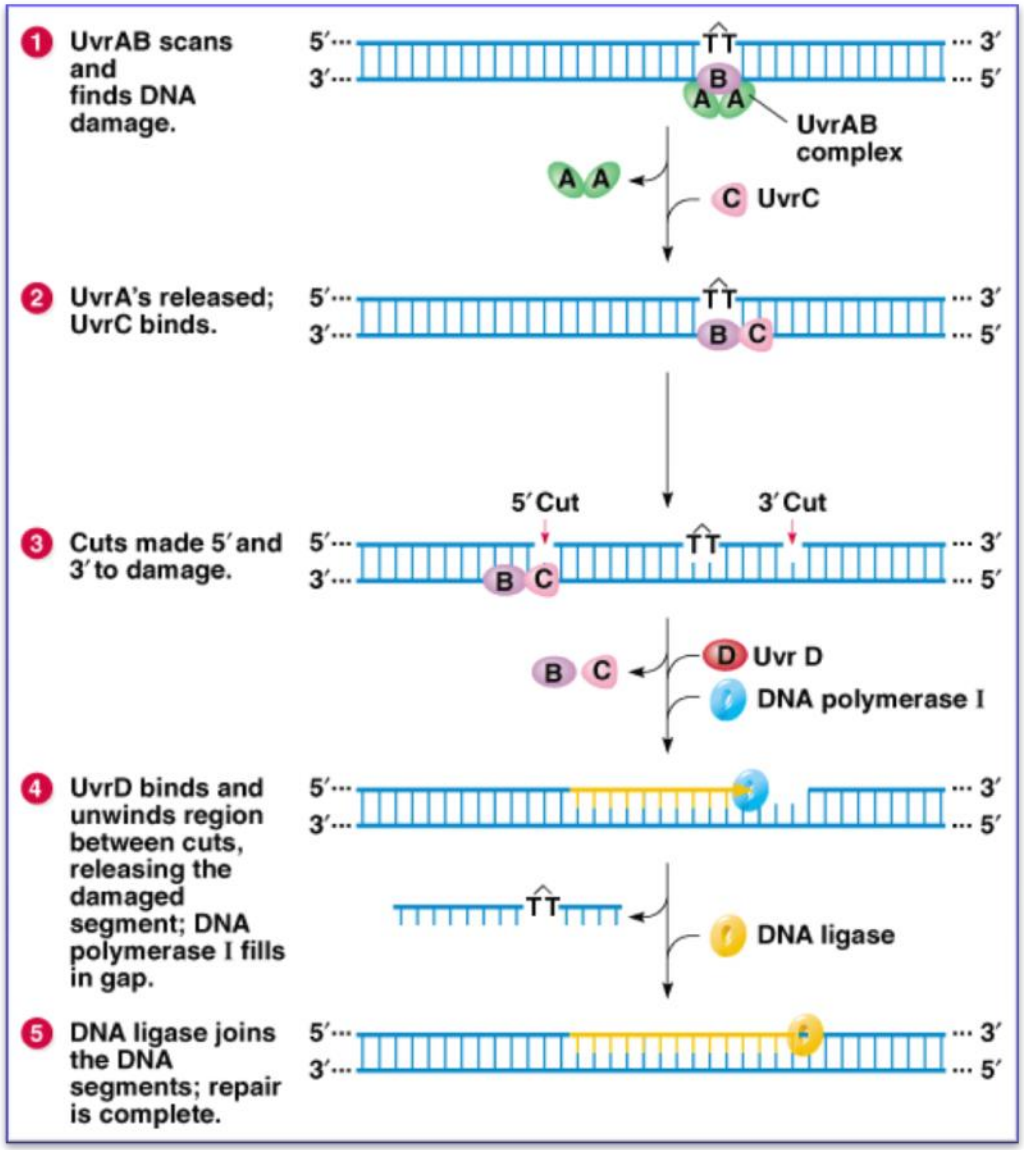

S9 extract
Test chemical added to filter disk
Positive result
his⁻ strain of S. typhimurium
Incubation
Mixture plated on medium lacking histidine
Negative result

DNA repair mechanisms:

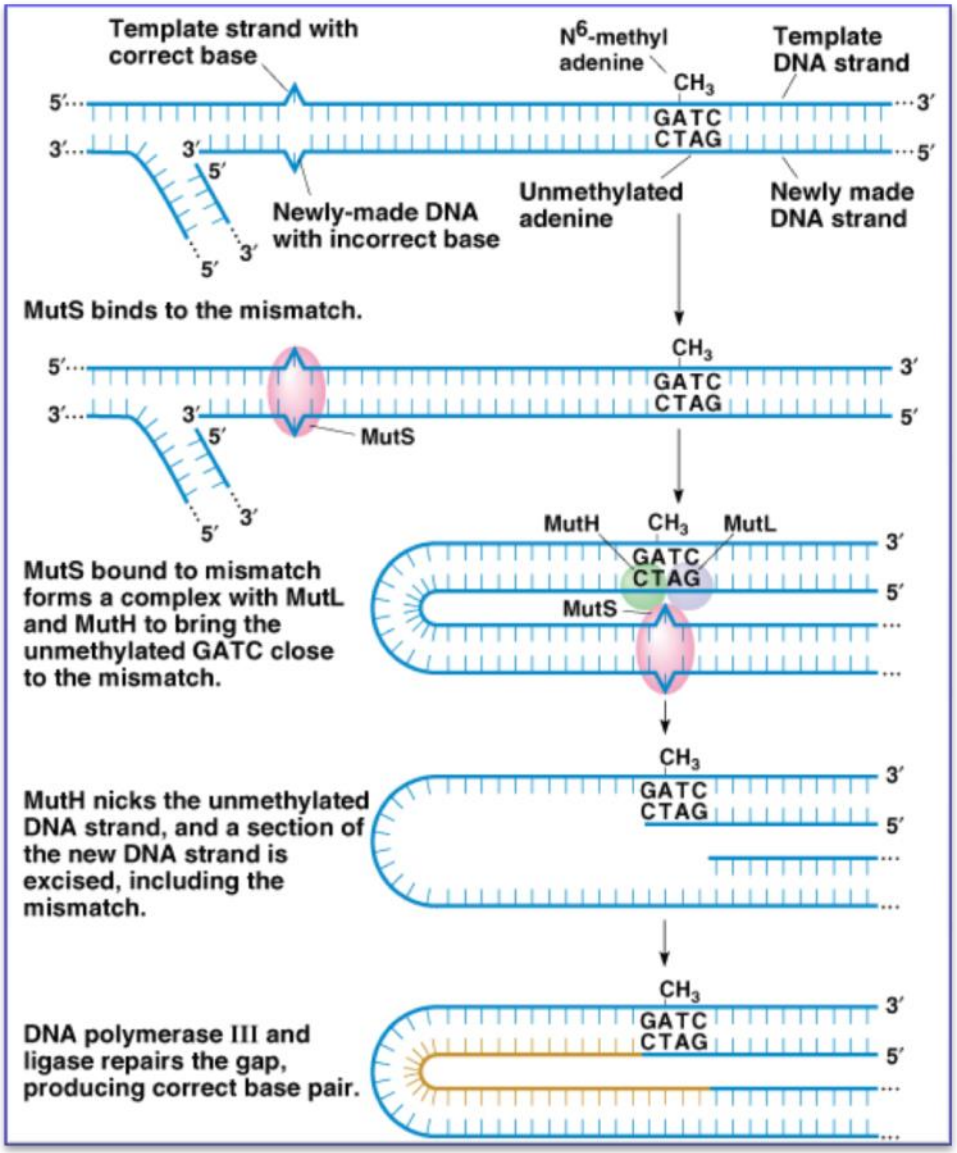
Types of mechanisms

- **DNA polymerase proofreading** - 3’-5’ exonuclease activity corrects errors during the process of replication.
- **Photoreactivation (also called light repair) -** photolyase enzyme is activated by UV light (320-370 nm) and splits abnormal base dimers apart.
- **Demethylating DNA repair enzymes -** repair DNAs damaged by alkylation.
- **Nucleotide excision repair (NER) -** Damaged regions of DNA unwind and are removed by specialized proteins; new DNA is synthesized by DNA polymerase.
- **Methyl-directed mismatch repair** - removes mismatched base regions not corrected by DNA polymerase proofreading. Sites targeted for repair are indicated in *E. coli* by the addition of a methyl (CH_3) group at a GATC sequence**.**

Nucleotide excision repair (NER) of pyrimidine dimmer and other damage-induced distortions of DNA



Mechanism of mismatch correction repair



Thank you…..