Mutations: Nature & types

Microbiology VI

MUTATIONS

- **Mutations** can be defined as heritable changes in the sequence of nucleotides of a cell's DNA.
- Mutant: The organism or the cell, which shows the effects of a mutation, is called a **mutant**.

Significance of mutation:

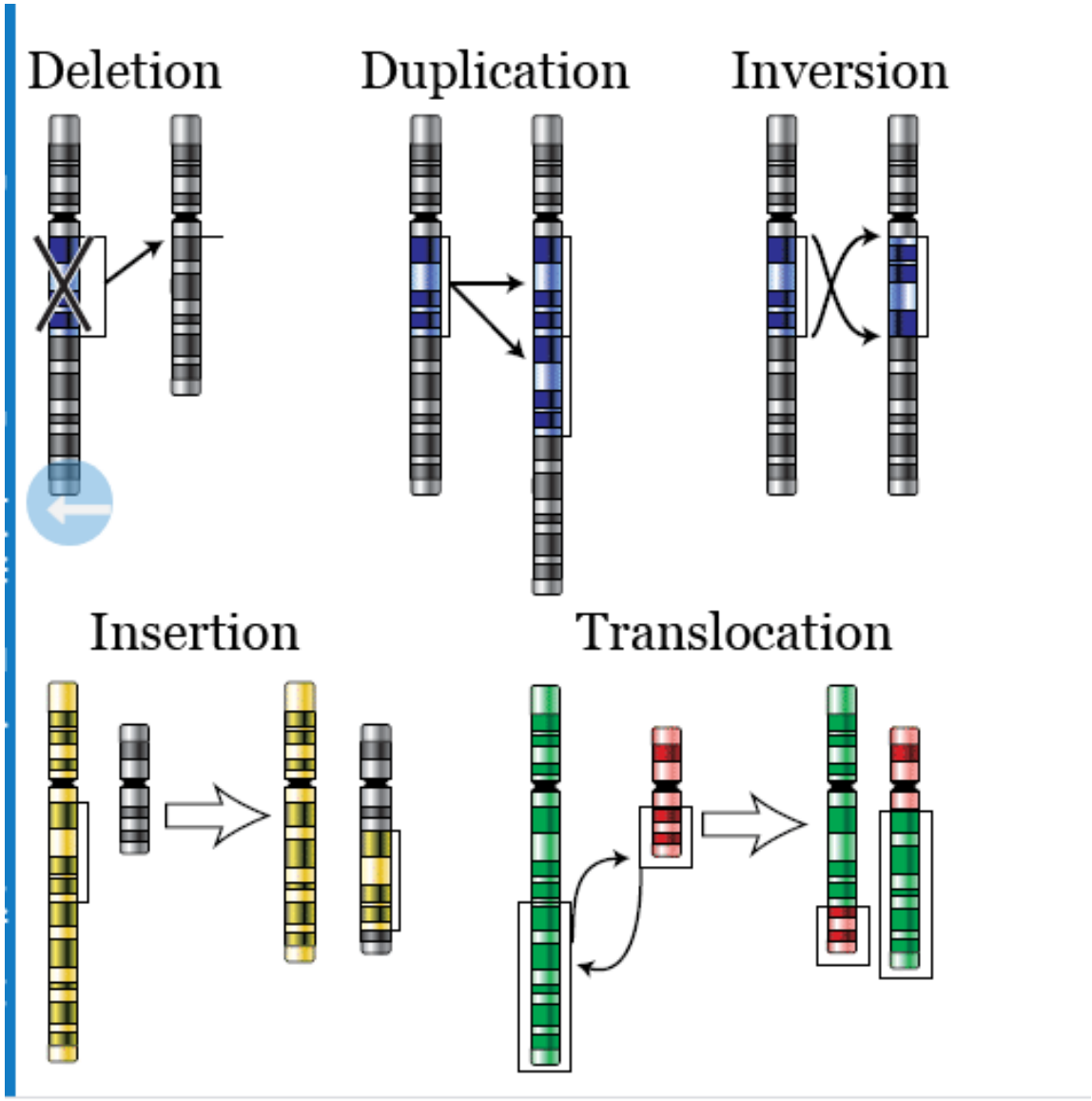
- Mutations give rise to
  - a new genetic trait or a changed genotype
  - characterized by altered phenotypes or phenotypic expressions.
- Mutations always change the genotype
  - it may or may not be expressed in the phenotype
  - depending on the nature of the mutation.

- Mutations account for evolutionary changes in all the organisms, including microorganisms.
- It is also responsible for the various alterations that produce different strains within species.

- Types of mutations:
  - Chromosomal mutations
  - Point mutations
  - Frameshift mutations

Chromosomal Mutations

- Mutations that occur at a macroscopic level.
- Large sections of chromosomes can be altered or shifted, leading to changes in the way genes are expressed.
- Types of chromosomal mutations:
  - Translocations
  - Inversions
  - Deletions
  - Nondisjunction


Deletion
Duplication
Inversion
Insertion
Translocation

Translocations & Inversions

- Translocation
  - The interchange of large segments of DNA between two chromosomes.
  - Can change gene expression if a gene is at the translocation breakpoint or if it is reattached so that it is incorrectly regulated
- Inversion
  - Occurs when a region of DNA flips its orientation with respect to the rest of the chromosome.
  - Rotates, end for end
  - This can lead to the same problems as translocations.

Deletions & Nondisjunction

- Deletion
  - Sometimes large regions of a chromosome are deleted.
  - This can lead to a loss of important genes.
- Nondisjunction
  - Sometimes chromosomes do not divide correctly in cell division
  - When large regions of a chromosome are altered (such as translocation), the chromosome may not segregate properly during cell division
  - One daughter cell will end up with extra genetic material, one will end up with less than its share
  - This is called *nondisjunction.*
  - When there are extra or too few copies of a gene, the cell will have problems

Point mutations:

- It is a base substitution in which one base is substituted for another at a specific location in a gene.

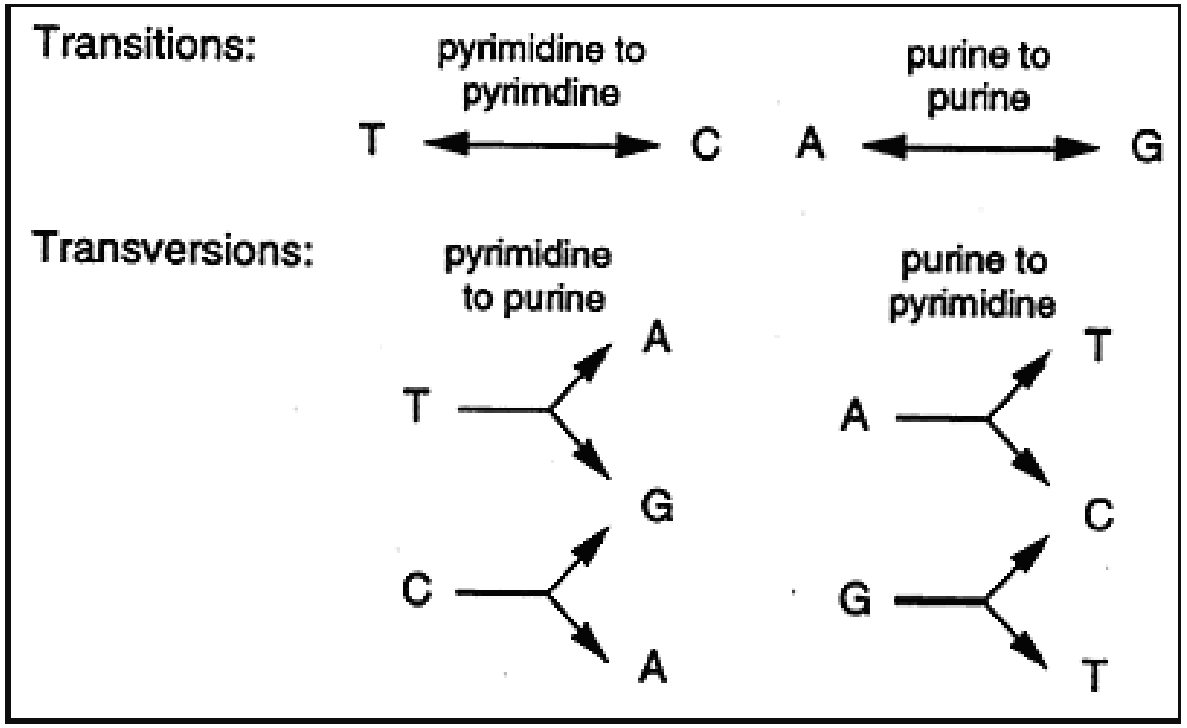
1. Transition:

- The substitution of one purine for another purine
- one pyrimidine for another pyrimidine
- Ex. A-T base pair is replaced by a G-C base pair and *vice versa*.

2. Transversion:

- It is the replacement of a purine by a pyrimidine, or *vice versa*.

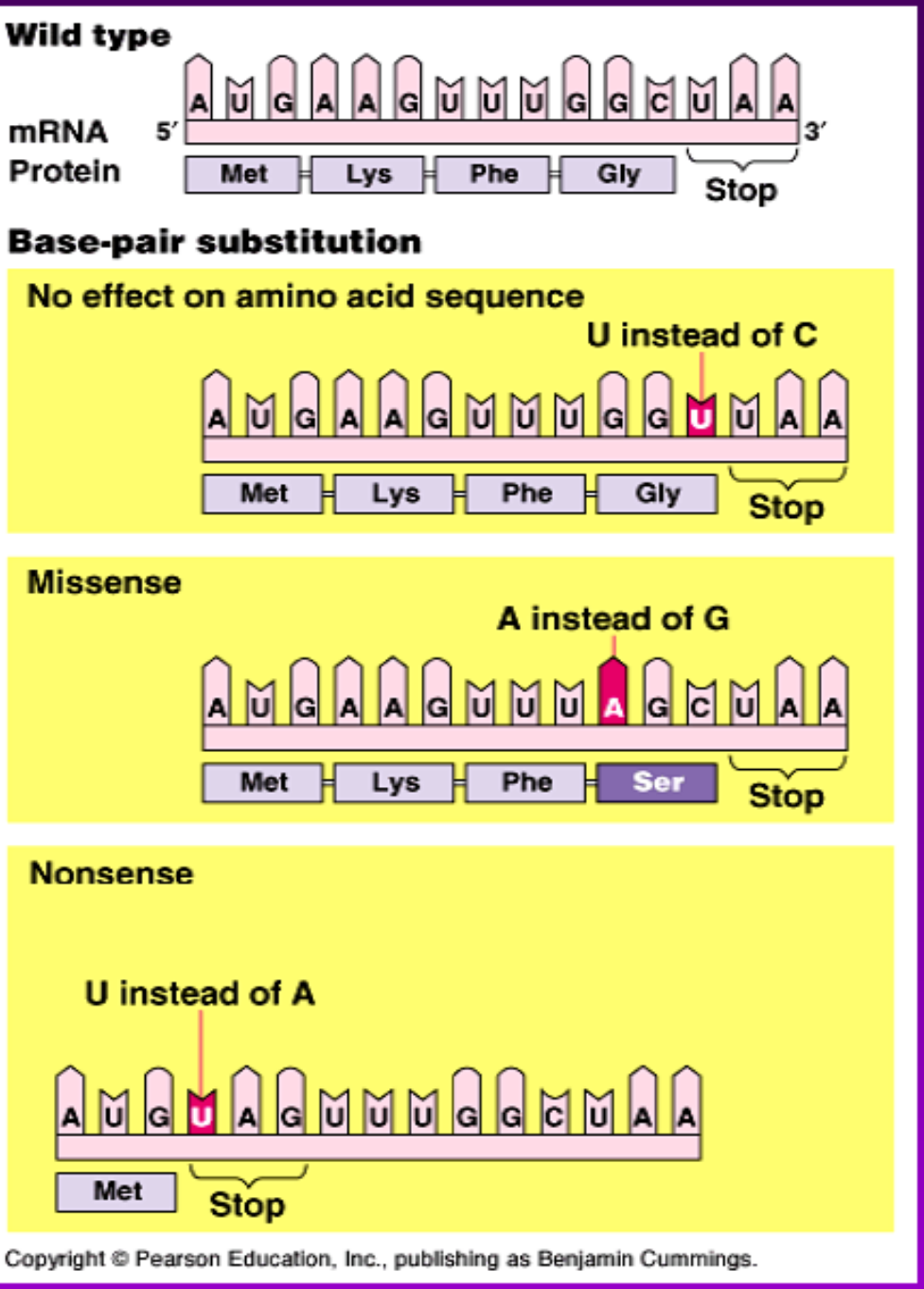
Point mutation



Purine – adenine, guanine;
Pyrimidine – cytosine, thymine

Point Mutations

- *Point mutations* are single base pair changes.
- Three possible outcomes:
- *Nonsense mutation*
  - Creates a stop codon where none previously existed.
  - This shortens the resulting protein, possibly removing essential regions.
- *Missense mutation*
  - Changes the code of the mRNA.
  - Which changes the resulting amino acid
  - This may alter the shape and properties of the protein.
- *Silent mutation*
  - Has no effect on protein sequence.
  - Because the genetic code is redundant, some changes have no effect



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Effects of point mutations

Type	Description	Example	Effect
Silent	mutated codon codes for the same amino acid	CAA (glutamine) → CAG (glutamine)	none
Missense	mutated codon codes for a different amino acid	CAA (glutamine) → CCA (proline)	variable
Nonsense	mutated codon is a premature stop codon	CAA (glutamine) → UAA (stop)	usually serious

Frameshift Mutations

- Insertions or deletions have a disastrous effect
- mRNA is "read" as a series of three letter words
- Insertions or deletions that are not multiples of three, shift the reading frame

Frameshift Example

- Given the coding sequence:

  AGA UCG ACG UUA AGC

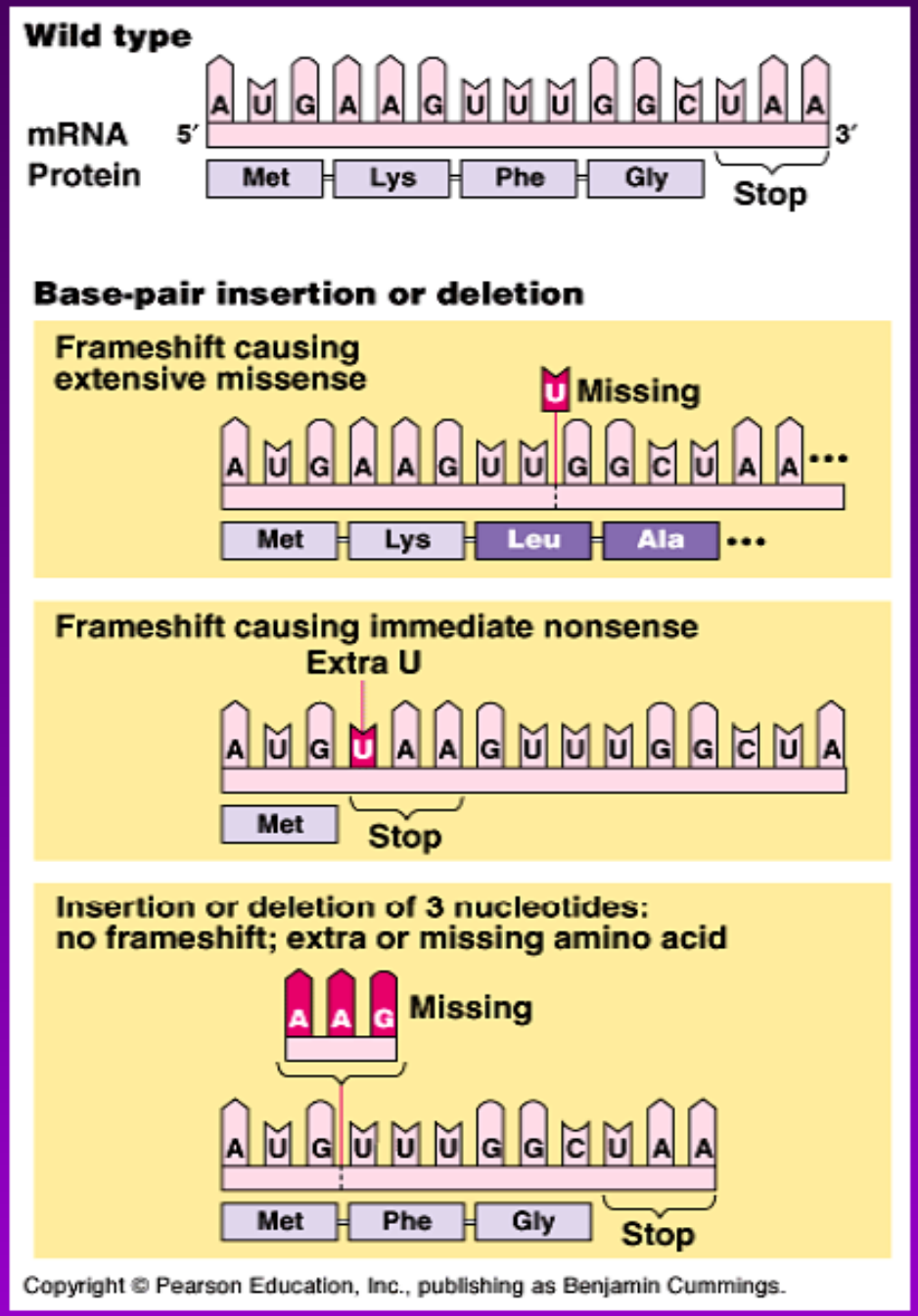
- corresponding to the protein:

  arginine - serine - threonine - leucine - serine

- The insertion of a C-G base pair between bases 6 and 7 would result in the following new code:

  AGA UCG CAC GUU AAG C

- which would result in a non-functional protein: arginine - serine - histidine - valine - lysine
- Every amino acid after the insertion will be wrong.
- The frame shift might even generate a stop codon which would prematurely end the protein.



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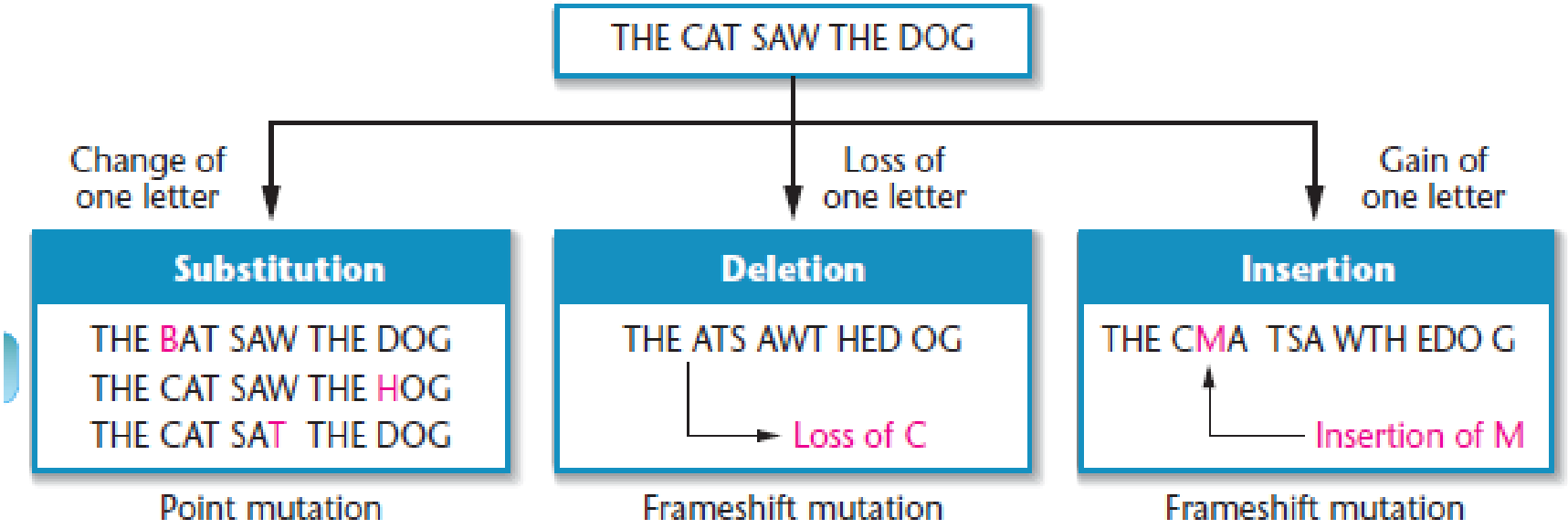
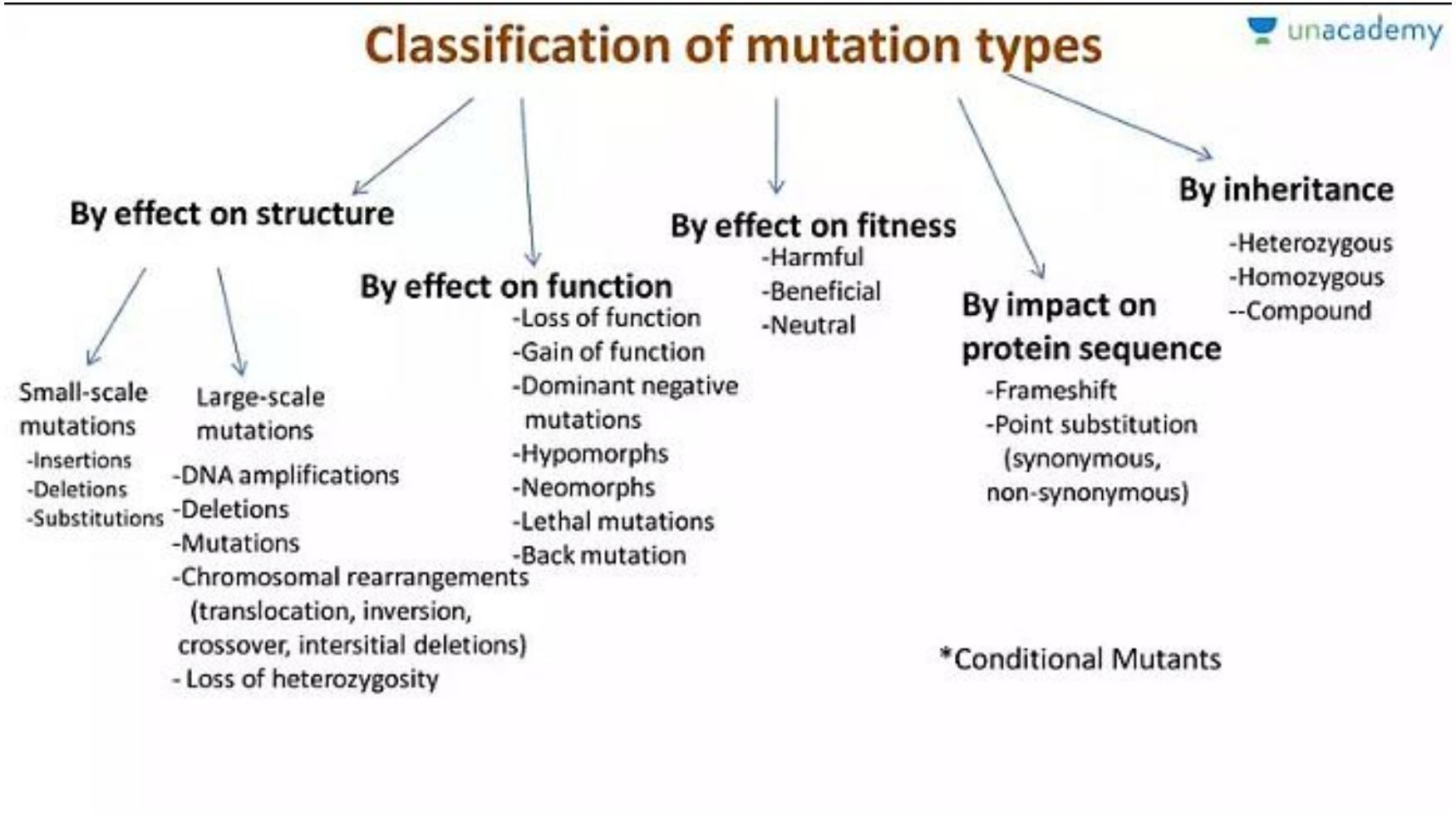


FIGURE 16–1 Analogy showing the effects of substitution, deletion, and insertion of one letter in a sentence composed of three-letter words to demonstrate point and frameshift mutations.

Classification of Mutations


Classification of mutation types
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By effect on structure
Small-scale mutations
-Insertions
-Deletions
-Substitutions
Large-scale mutations
-DNA amplifications
-Deletions
-Mutations
-Chromosomal rearrangements (translocation, inversion, crossover, intersitial deletions)
- Loss of heterozygosity
By effect on function
-Loss of function
-Gain of function
-Dominant negative mutations
-Hypomorphs
-Neomorphs
-Lethal mutations
-Back mutation
By effect on fitness
-Harmful
-Beneficial
-Neutral
By impact on protein sequence
-Frameshift
-Point substitution (synonymous, non-synonymous)
*Conditional Mutants
By inheritance
-Heterozygous
-Homozygous
--Compound

Mutations based on phenotypic effects:

- Based on type and location of mutations show phenotypic effects such as:
  - loss-of-function mutation
  - null mutations
  - gain-of-function
  - neutral mutations
  - visible mutations
  - nutritional mutation
  - biochemical mutations
  - regulatory mutations
  - lethal mutation
  - conditional mutations
  - temperature-sensitive mutations

- **Loss-of-function mutation:**
- Reduces or eliminates the function of the gene product.
- Any type of mutation
  - from a point mutation
  - deletion of the entire gene
- may lead to a loss of function.

- **Null mutations:**
- Mutations that result in complete loss of function
- Can be either dominant or recessive

- Dominant loss-of-function mutation:
- Presence of a defective protein product binds / inhibits the action of the normal gene product which is also present in the same organism

- **Gain-of-function:**
- Results in a gene product with enhanced or new functions
  - due to a change in the amino acid sequence of the protein that confers a new activity.

- Mutation in a regulatory region of the gene
  - leads to expression of the gene at higher levels
  - synthesis of the gene product at abnormal times or places

- **Neutral mutations**:
- Mutations that occur at non coding regions in genome of eukaryotes, do not affect gene products or gene expression.

- **Visible mutations:**
- Mutations affecting a morphological trait that are easily observed
- It is recognized by their ability to alter a normal or wild-type visible phenotype.
- Ex. *Drosophila*

- **Nutritional mutation:**
- Mutations exhibit nutritional effects
  - results in a loss of ability to synthesize an amino acid or vitamin.
  - Auxotrophs: mutant, phototrophs: wild type
  - Ex. bacteria and fungi
- **Biochemical mutations**:
- Biochemical effects that results in diseases
- Ex. In humans – sickle cell anemia and hemophilia
  - Such mutations effect the wellbeing and survival of the affected individual
  - morphological characters not affected

- **Regulatory mutations**:
- Mutations may affect the regulation of gene expression.
  - regulatory gene can produce a product that controls the transcription of other genes.

- Mutation in regulatory gene results in:
  - disrupts normal regulatory processes
  - inappropriately activate or inactivate
  - expression of a gene
- Ex. ***lac* operon**

- **Lethal mutation**:
- Mutation that interrupt a process that is essential to the survival of the organism.

- Ex. Bacteria mutant:
  - lost the ability to synthesize an essential amino acid
  - cease to grow and eventually will die
  - when placed in a medium lacking that amino acid.

- In humans: Tay–Sachs disease and Huntington disease (destruction of nerve cells in brain).

- **Conditional mutations**:
- Expression depends on the environment of the organism
- Mutation detected only in certain conditions

- **Ex. Temperature-sensitive mutations:**
- At a "permissive" temperature - the mutant gene product functions normally
- Loses its function at a different -"restrictive" temperature.

- **Based on the survival of an individual**

1. **Lethal mutation** – when mutation causes death of all individuals undergoing mutation are known as lethal

2. **Sub lethal mutation** - causes death of 90% individuals

3. **Sub vital mutation**– such mutation kills less than 90% individuals

4. **Vital mutation** -when mutation don't affect the survival of an individual are known as vital

5. **Supervital mutation** – This kind of mutation enhances the survival of individual

- **Based on tissue of origin**

1. Somatic mutation-

A mutation occurring in somatic cell is called somatic mutation.

In asexually reproducing species somatic mutations transmits from one progeny to the next progeny

2. Germinal Mutation-

When mutation occur in gametic cells or reproductive cells are known as germinal mutation.

In sexually reproductive species only germinal mutation are transmitted to the next generation

- **Based on direction of mutation**

1. Forward mutation- When mutation occurs from the normal/wild type allele to mutant allele are known as forward mutation

2. Reverse mutation- When mutation occurs in reverse direction that is from mutant allele to the normal/wild type allele are known as reverse mutation

Based on causes of mutations

❑ Spontaneous Mutation: occurs naturally without any cause.

- They arise most commonly during replication of DNA due to errors base pairing of nucleotides in the old and new strands of DNA.
  - **Tautomerism**
  - **Depurination**
  - **Deamination**
  - **Slipped strand mispairing (replication slippage)**
- The rate of spontaneous mutation is very slow.
- Rate of spontaneous mutation is higher in prokaryotes than in eukaryotes.

Based on causes of mutations

- Induced Mutation: mutations produced due to treatment with either a physical or chemical agents.
- The agents capable of causing such mutations are called mutagens.

Physical agents:
UV radiation
X-rays
gamma rays

Chemical agents:
Base analogs: 5-bromouracil (5-BU), 2-amino purine
Alkylating agents: methyl-nitrosoguanidine, ethyl methane sulphonate (EMS),
Deaminating agents: nitrous acid
Intercalating agents: acridine orange and proflavin
Carcinogens: aflatoxin B1

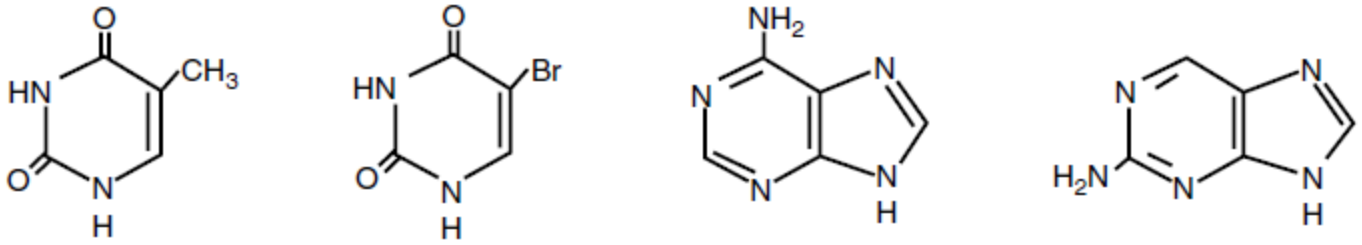
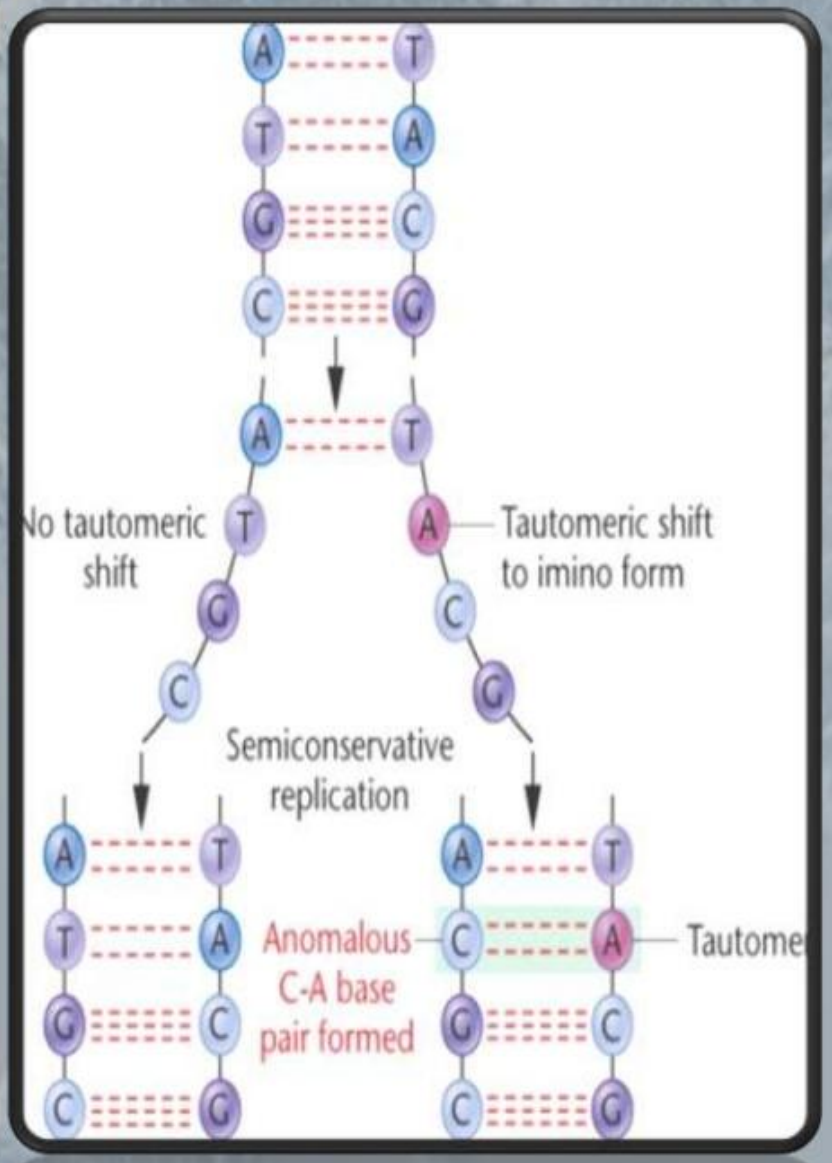


Figure 14.13 Structures of nitrogenous bases and their mutation-causing analogs

Spontaneous Mutations

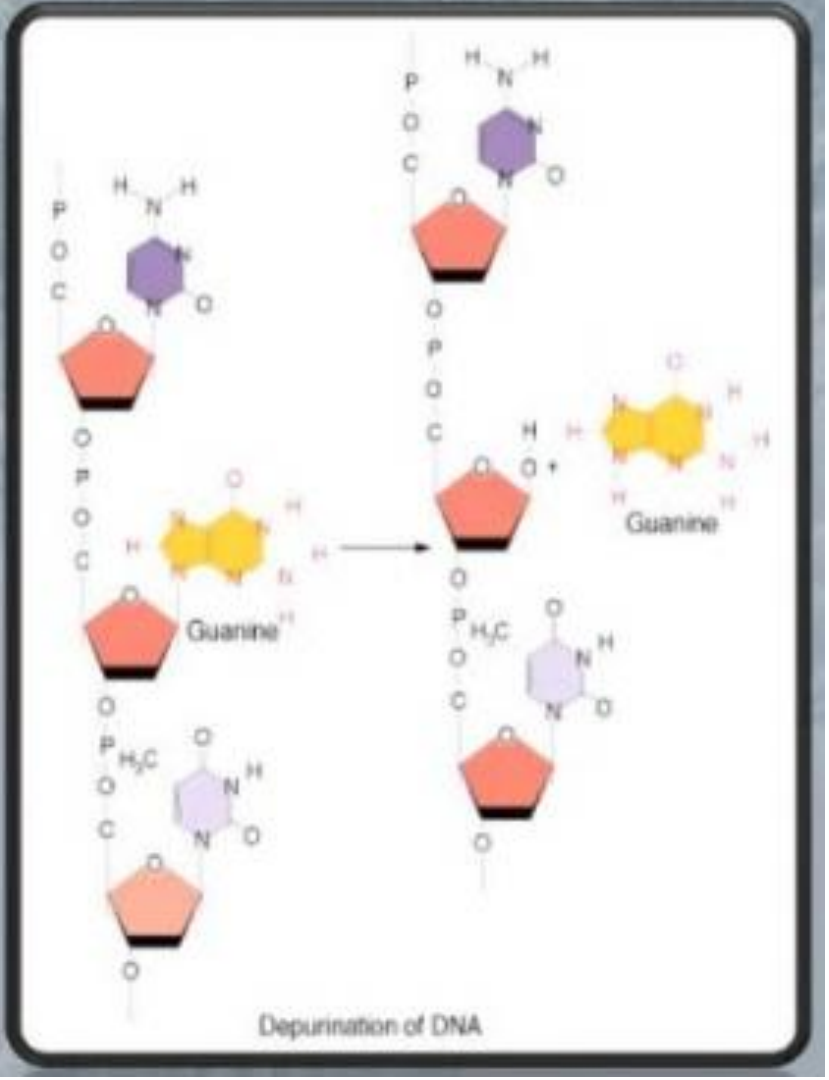
These mutations can be caused by:

- **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.
- The ability of a molecule to exist in more than one chemical form is called tautomerism .
- All the four common bases of DNA (A, G, C and T) have unusual tautomeric forms, which are rare.



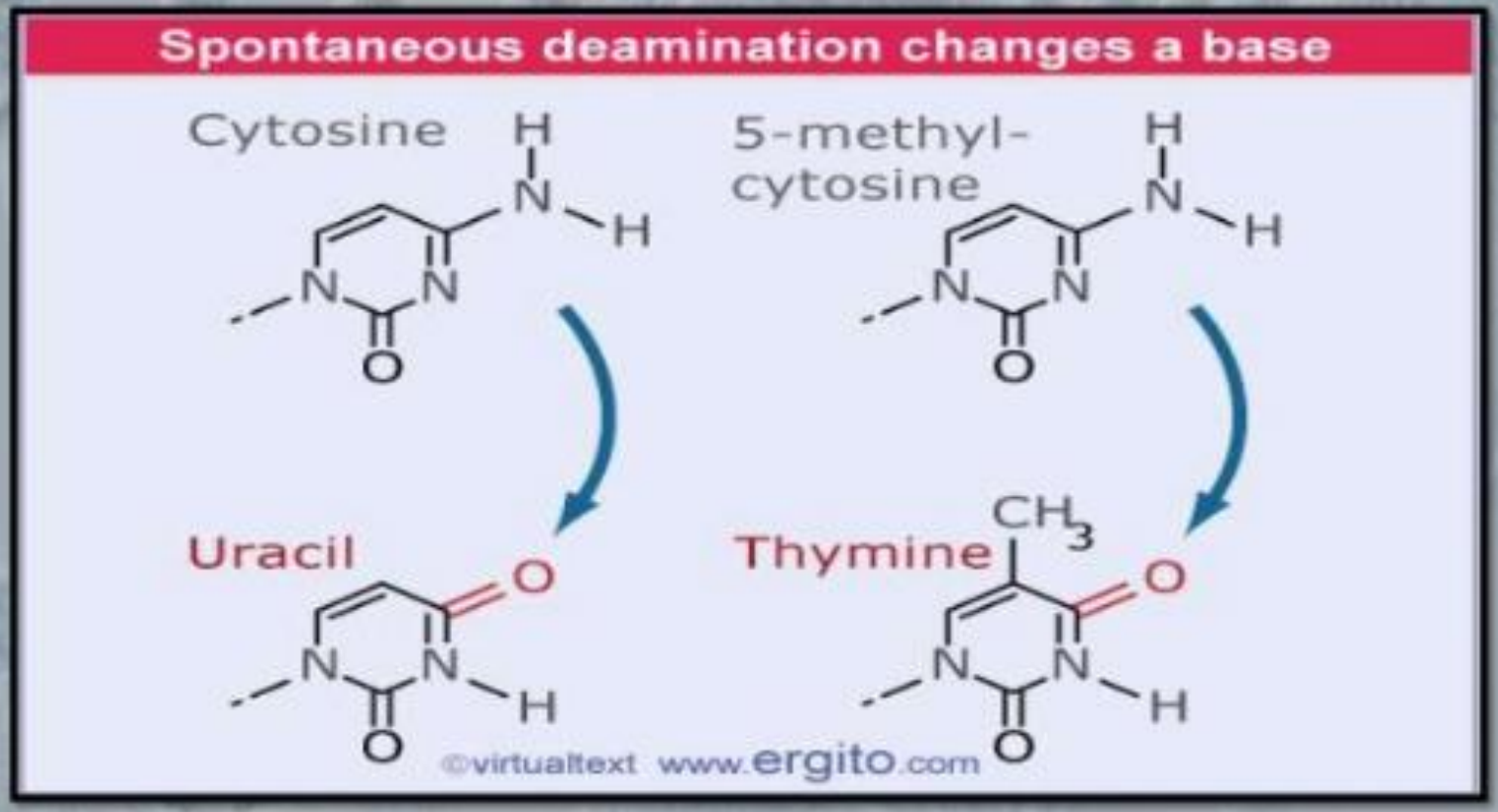
Spontaneous Mutations

- **Depurination** – In molecular genetics, **depurination** is an alteration of DNA in which the purine base (adenine or guanine) is removed from the deoxyribose sugar by hydrolysis of the beta-N-glycosidic link between them.
- Loss of a purine base form an apurinic site (AP site). where the sugar phosphate backbone remains and the sugar ring has a hydroxyl (-OH) group in the place of the purine.



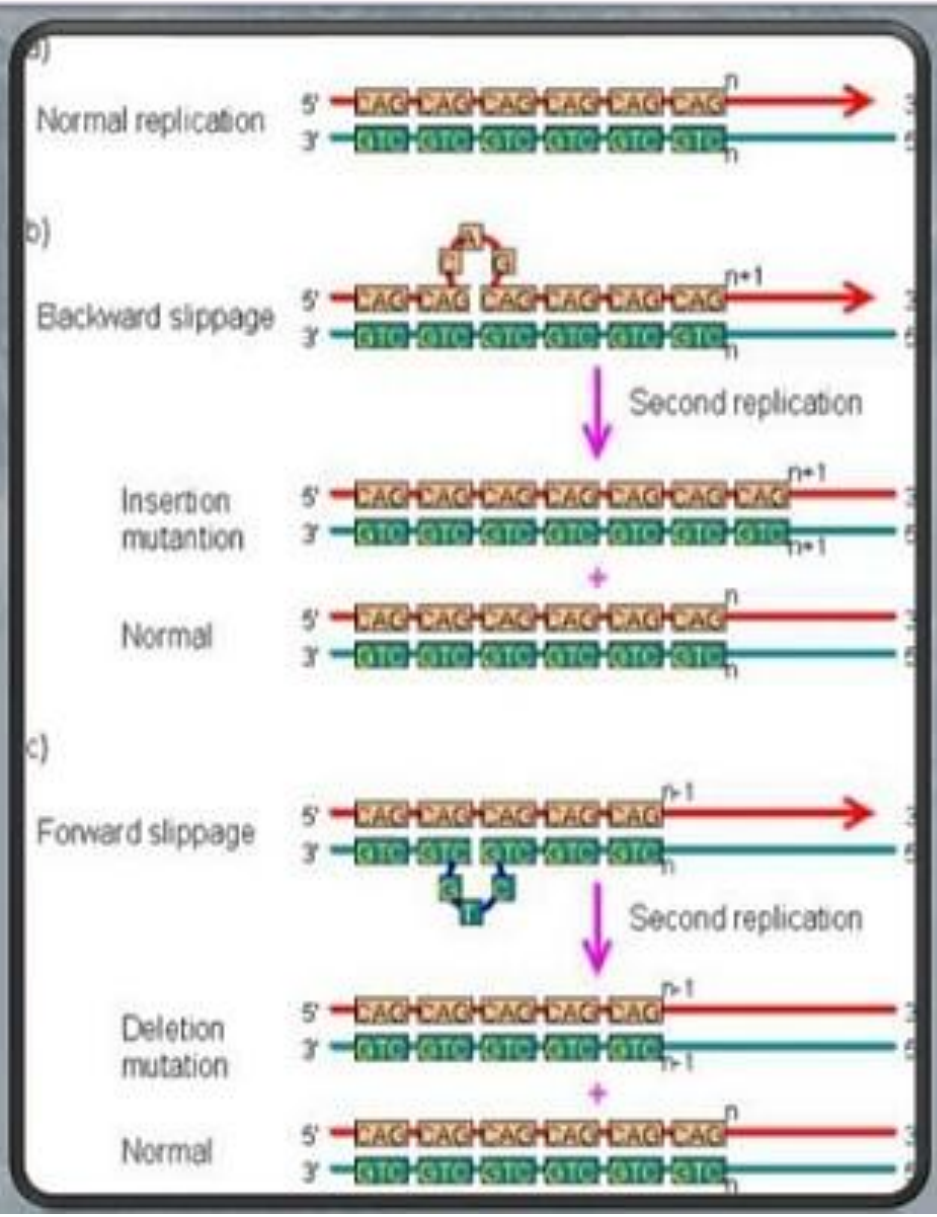
Spontaneous Mutations

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



Spontaneous Mutations

- **Slipped strand mispairing** - Denaturation of the new strand from the template during replication, followed by renaturation in a different spot lead to insertions or deletions.
- (SSM) is a process that produces mispairing of short repeat sequences between the mother and daughter strand during DNA synthesis

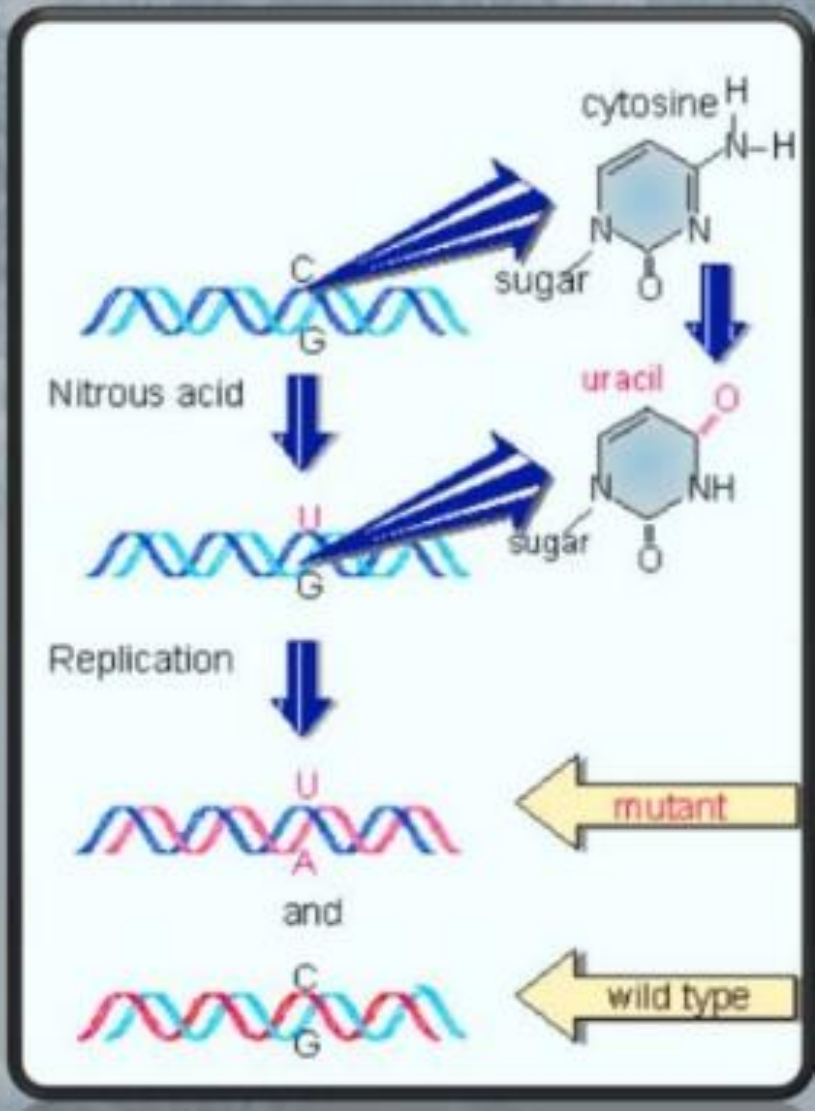


Induced Mutations

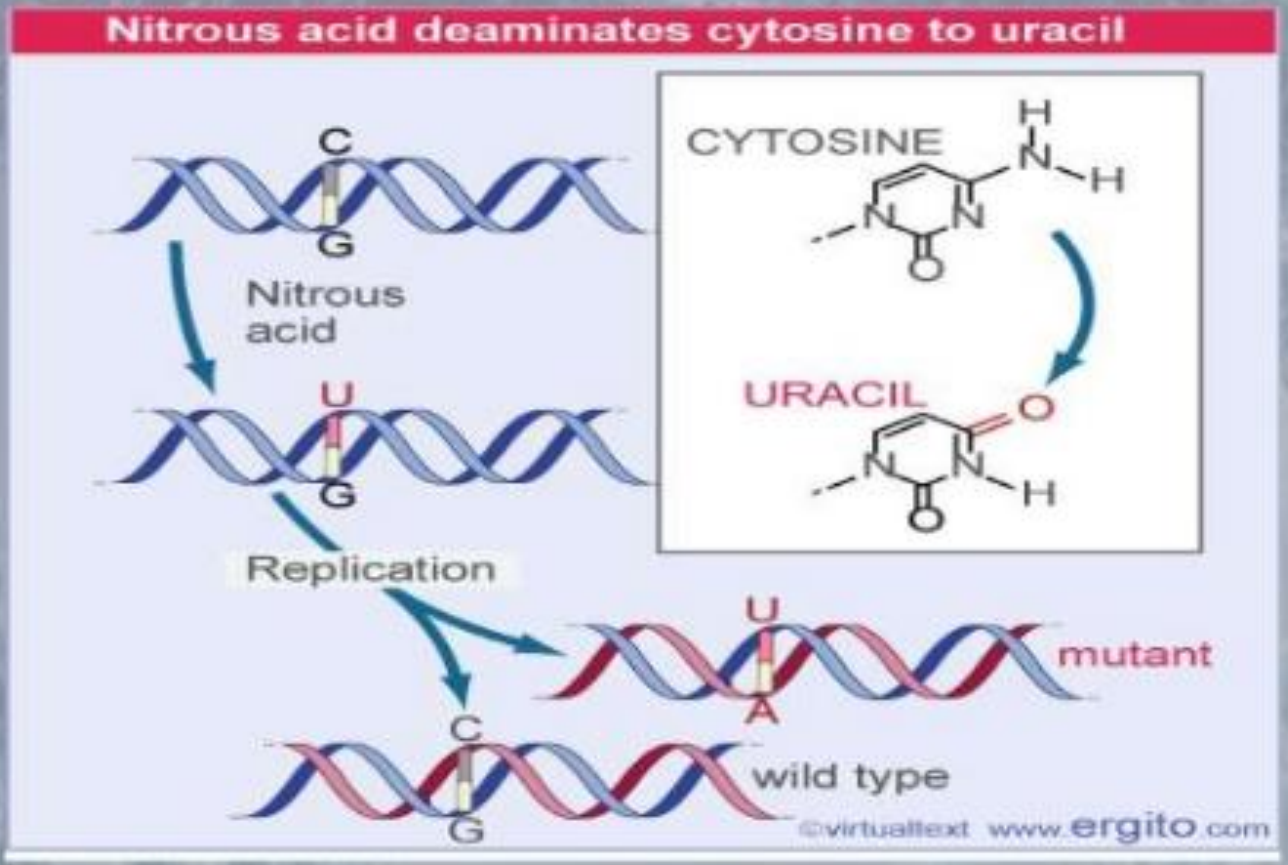
- Ingredients that cause mutations are called mutagens. Mutagen is divided into three, namely:

1- Mutagenic chemicals like;

- Hydroxylamine NH2OH
- Base analogs (e.g. BrdU)
- Alkylating agents
- Agents that form DNA adducts
- DNA intercalating agents
- DNA crosslinkers
- Oxidative damage



Induced Mutations



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.

Induced Mutations

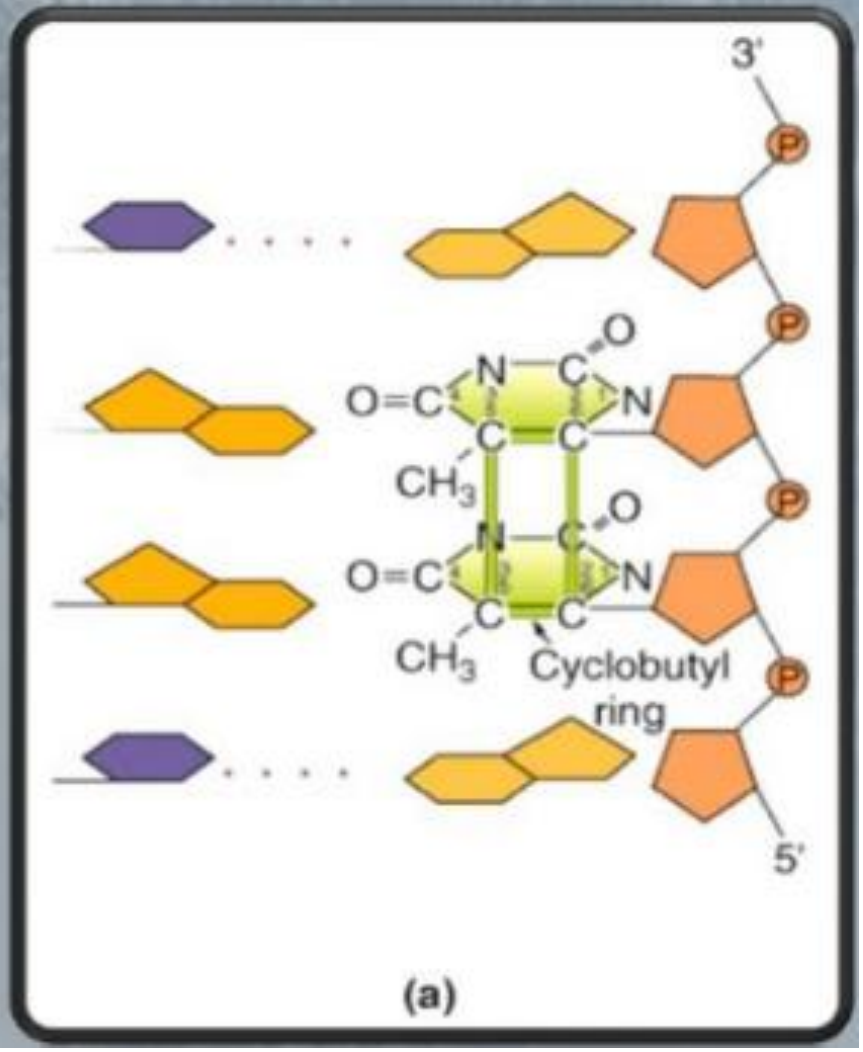
Mutagens:

Physical agents

- Ultraviolet rays. (can cause skin cancer).
- Radioactive rays.
- Gamma rays.

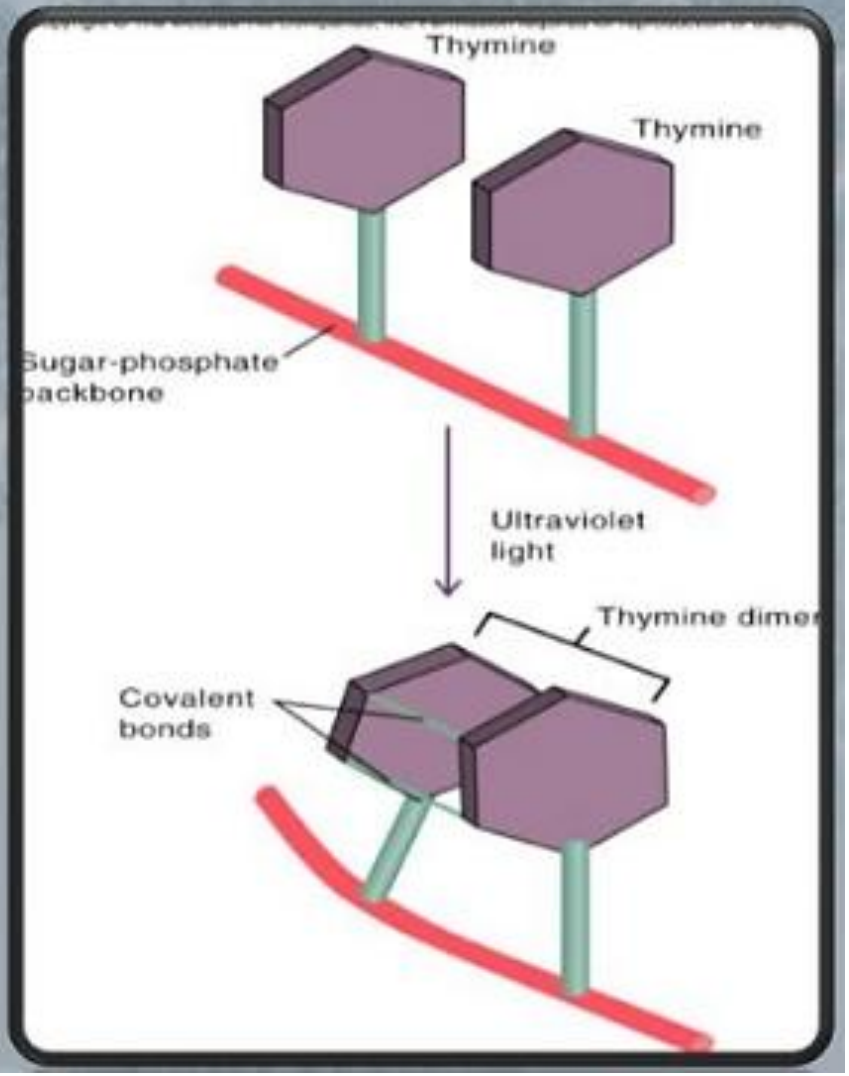
Induced Mutations

- Ultraviolet light is absorbed by the nucleic acid bases, and the resulting influx of energy can induce chemical changes.

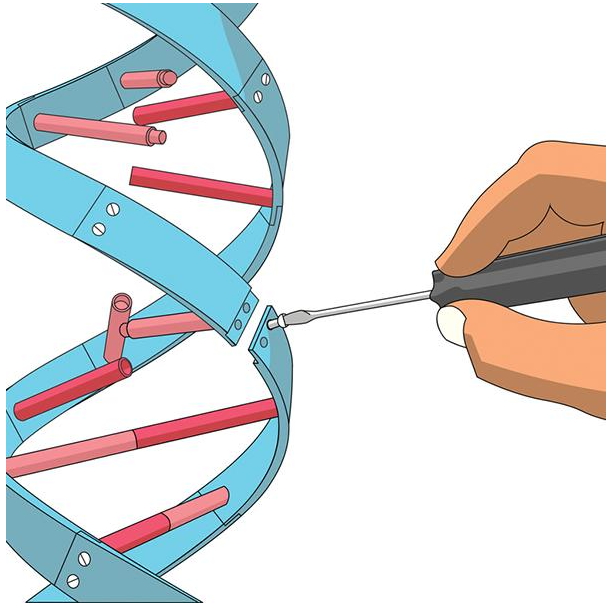


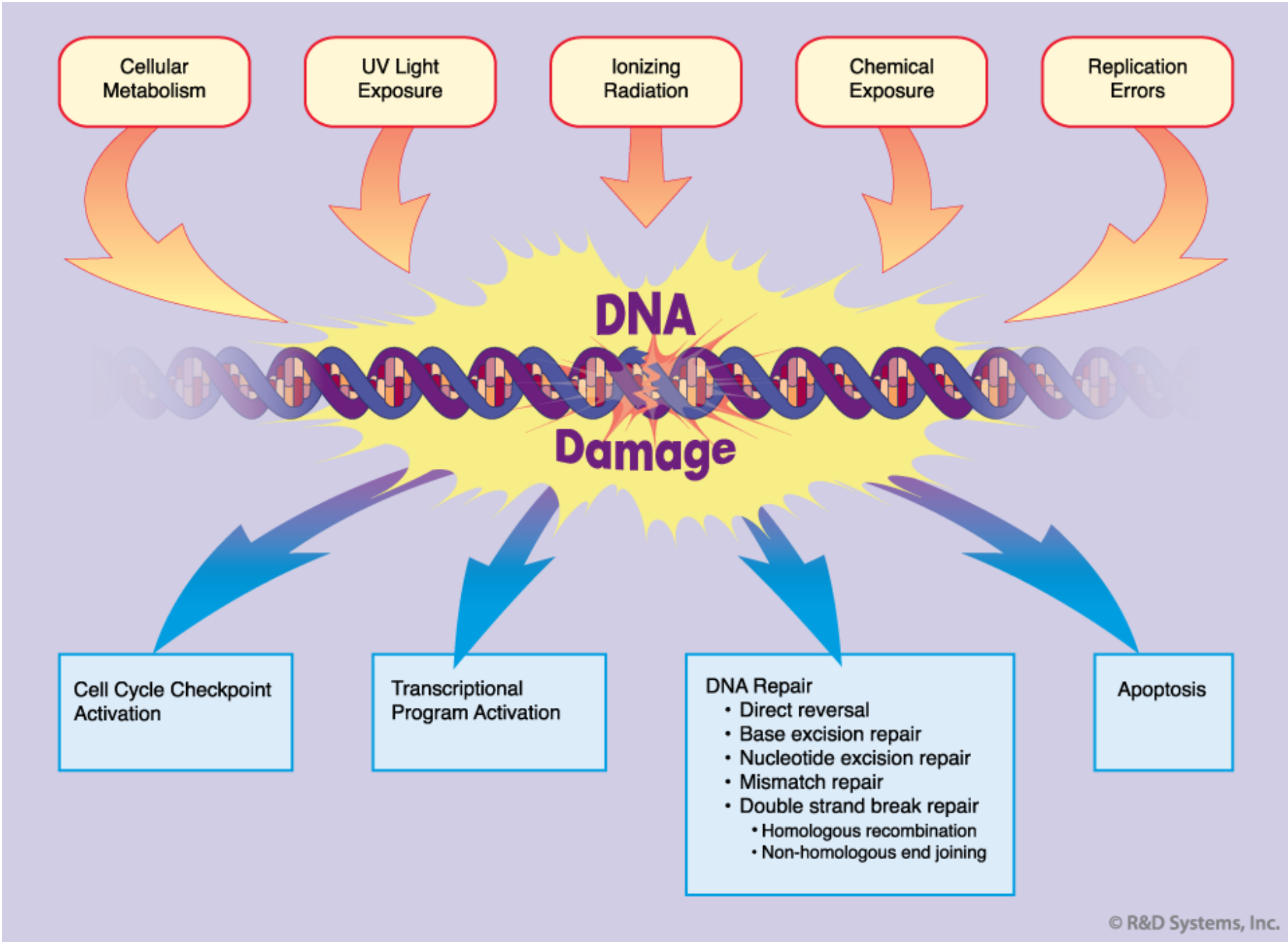
Induced Mutations

- The most frequent photoproducts are the consequences of bond formation between **adjacent pyrimidines** within **one strand**, and, of these, the most frequent are cyclobutane pyrimidine dimers (CPDs).
- T CPDs are formed most readily, followed by T-C or C-T; C-C dimers are least abundant.



DNA damage and repair





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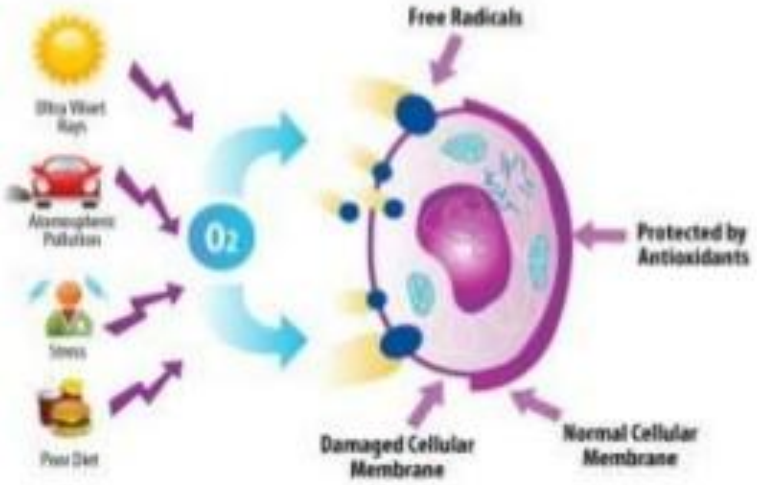
DNA repair

- **DNA repair** is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome.

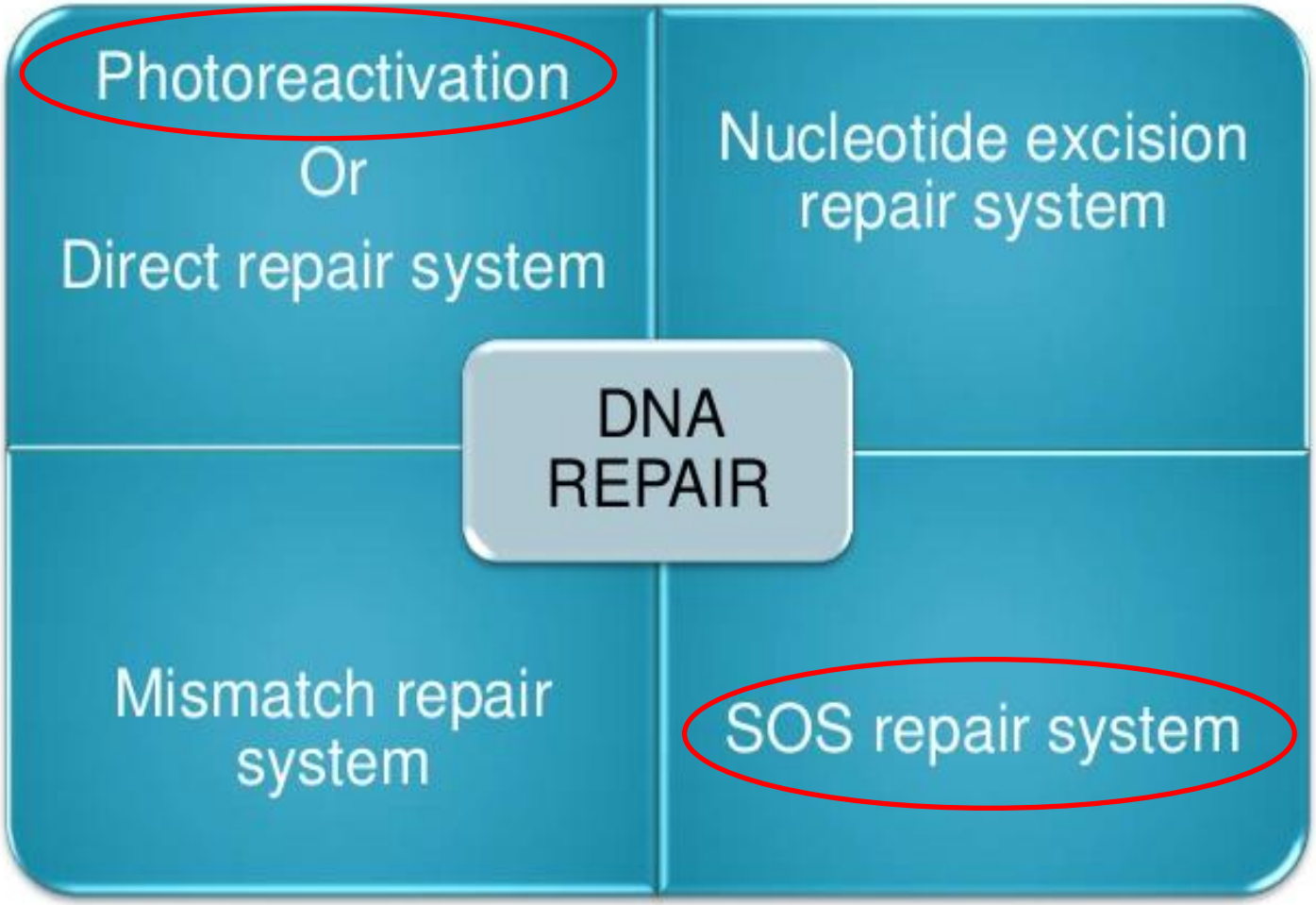
- In human cells, both normal metabolic activities and environmental factors such as UV light and radiation can cause DNA damage, resulting in as many as 1 million individual molecular lesions per day.

- Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell's ability to transcribe the gene that the affected DNA encodes.

- As a consequence, the DNA repair process is constantly active as it responds to damage in the DNA structure. When normal repair processes fail, and when cellular apoptosis does not occur, irreparable DNA damage may occur, including double-strand breaks and DNA cross-linkages.



DNA repair types



Overview - DNA repair types

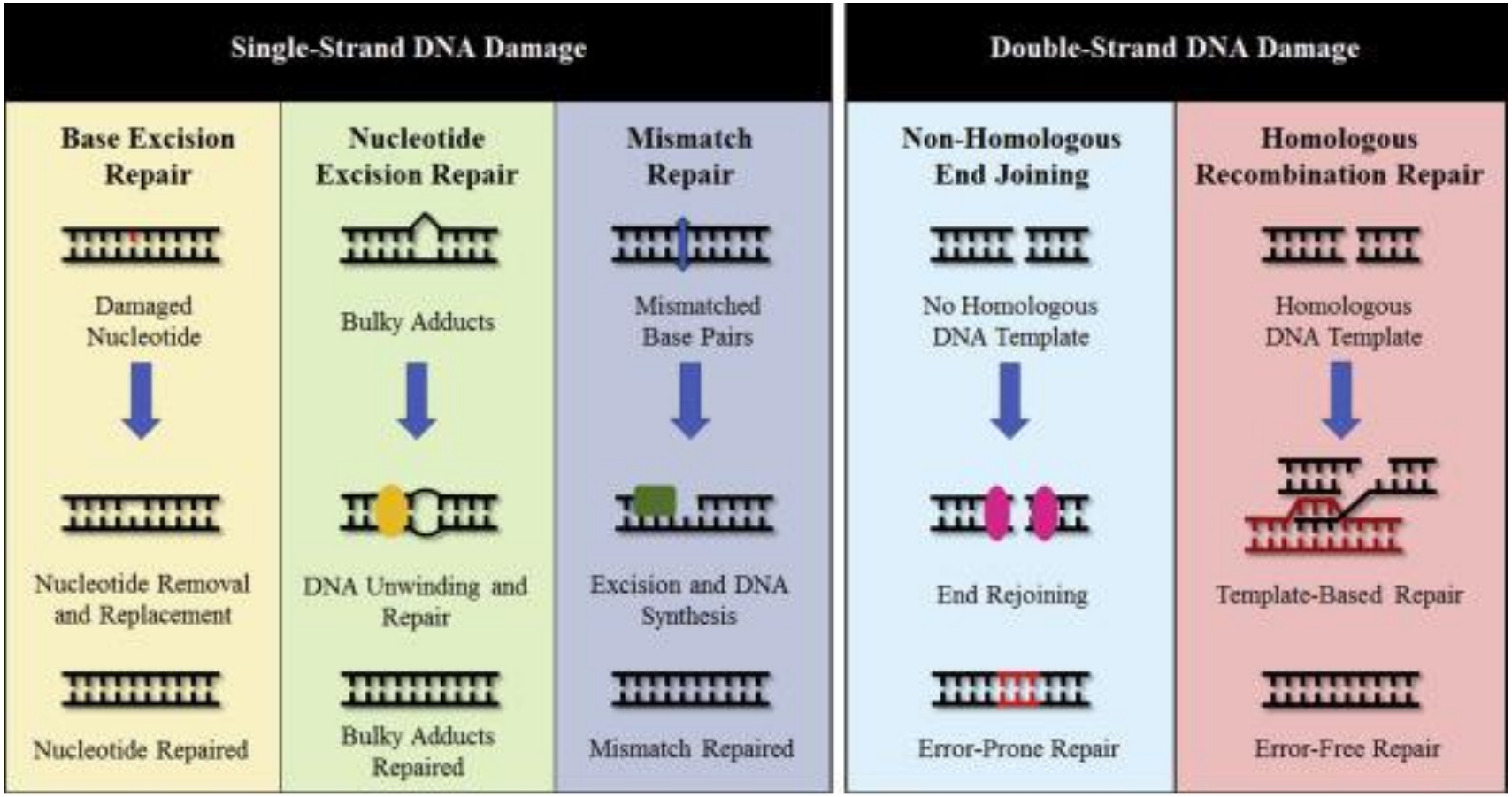
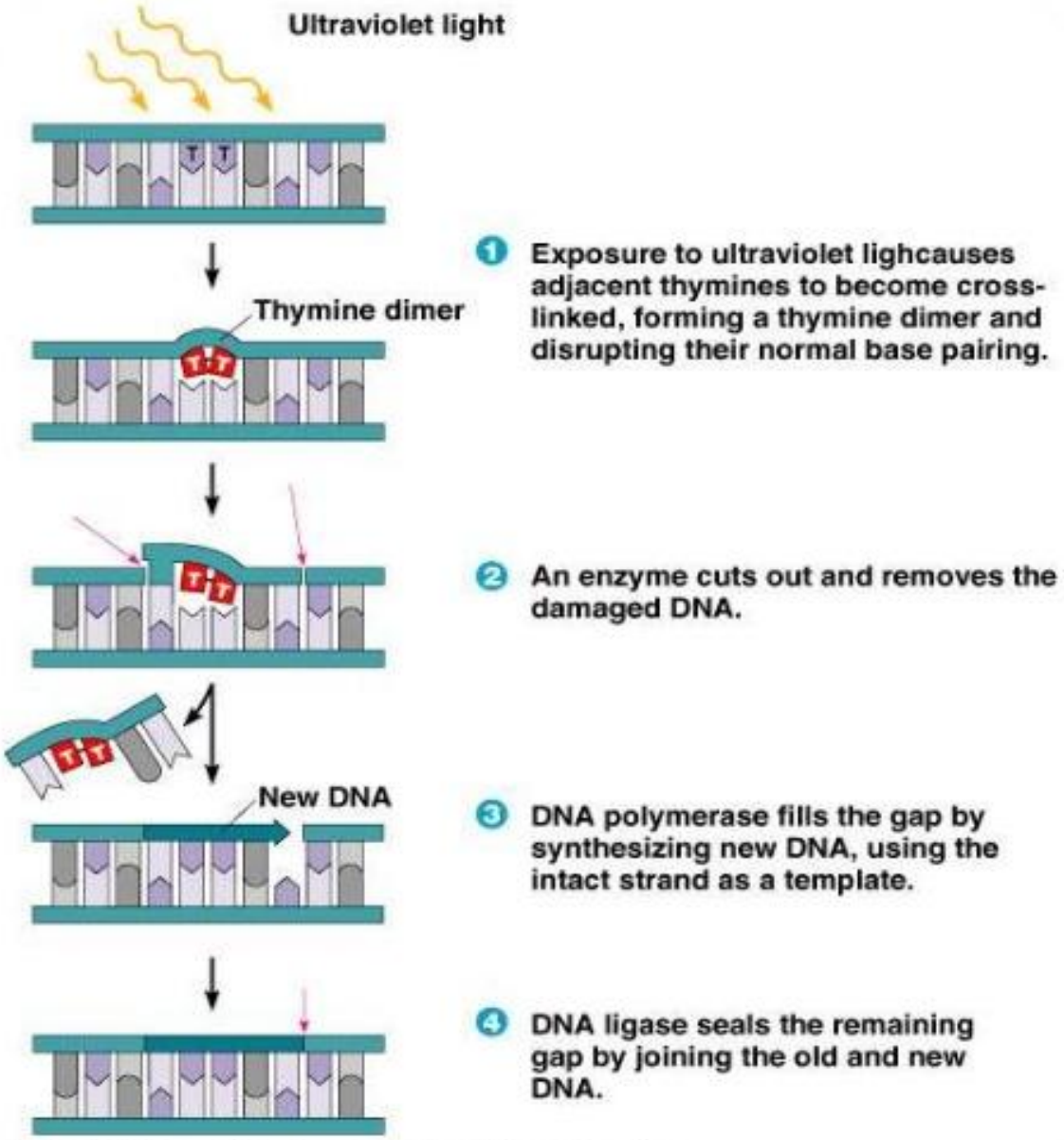


Photo reactivation/direct repair :

- **Photolyases** are DNA repair enzymes that repair damage caused by exposure to ultraviolet light. This enzyme mechanism requires visible light(300-600 nm), preferentially from the violet/blue end of the spectrum, and is known as **photo reactivation**.
- Photolyase is a phylogenetically old enzyme which is present and functional in many species, from the bacteria to the fungi to plants and to the animals.
- Photolyase is particularly important in repairing UV induced damage in plants.
- The photolyase mechanism is no longer working in humans and other placental mammals who instead rely on the less efficient nucleotide excision repair mechanism

- Photolyases bind complementary DNA strands and break certain types of pyrimidine dimers that arise when a pair of thymine or cytosine bases on the same strand of DNA become covalently linked.
- These dimers result in a 'bulge' of the DNA structure, referred to as a lesion.
- Photolyases have a high affinity for these lesions and reversibly bind and convert them back to the original bases.



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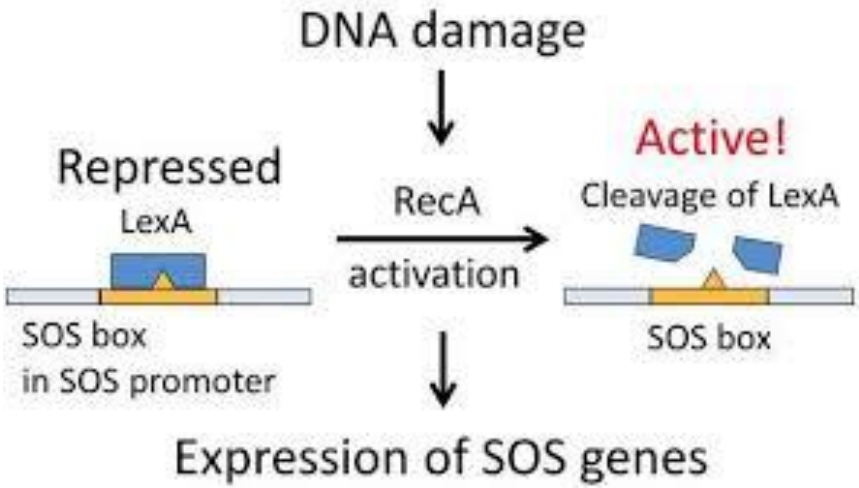
SOS REPAIR / ERROR–PRONE REPAIR :

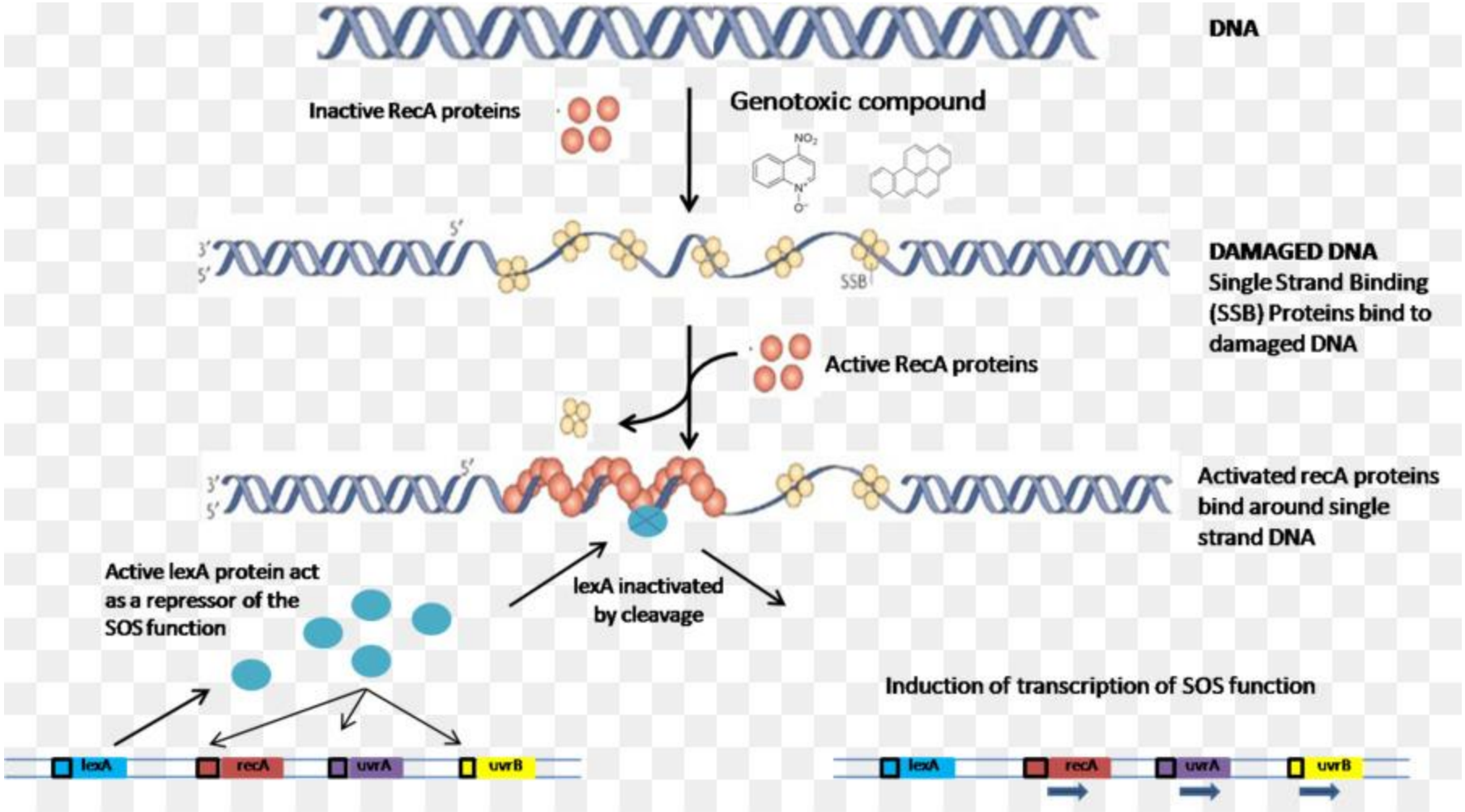
- The **SOS response** is a global response to DNA damage in which the cell cycle is arrested and DNA repair ,mutagenesis are induced.
- The system involves the Rec-A protein (Rad51 in eukaryotes). The RecA protein, stimulated by single-stranded DNA, is involved in the inactivation of the Lex-A repressor thereby inducing the response.
- It is an error-prone repair system that is attributed to mutagenesis.
- The SOS response was discovered and named by Miroslav Radman in 1975.

SOS repair Mechanism:

- **Normal growth**: SOS genes are negatively regulated by LexA repressor protein dimers.
- LexA binds to a 20-bp consensus sequence (SOS box) in the operator region for those genes.
- Some SOS genes are expressed at certain levels even in the repressed state, according to the affinity of LexA for their SOS box.
- **DNA damage:** Activation of the SOS genes occurs after DNA damage by the accumulation of single stranded (ssDNA) regions generated at replication forks, where DNA polymerase is blocked.



SOS Activation



SOS repair contd.

- The damaged DNA cause RecA to trigger the response & results in the auto cleavage of protein called LexA protein .
- RecA is activated on binding on a single-stranded DNA.
- Lex A is a repressor that participate in DNA repair . RecA forms a filament around these ssDNA regions in an ATP-dependent fashion, and becomes activated.
- The activated form of RecA interacts with the LexA repressor to facilitate the LexA repressor's self-cleavage from the operator.

SOS repair contd.

- Once the pool of LexA decreases, repression of the SOS genes goes down according to the level of LexA affinity for the SOS boxes.
- Operators that bind LexA weakly are the first to be fully expressed.
- In this way LexA can sequentially activate different mechanisms of repair.
- Genes having a weak SOS box (such as uvrA, uvrB, and uvrD) are fully induced in response to even weak SOS-inducing treatments.

SOS repair contd.

- Thus the first SOS repair mechanism to be induced is nucleotide excision repair(NER), whose aim is to fix DNA damage without commitment to a full-fledged SOS response.
- This causes filamentation, and the induction of UmuDC-dependent mutagenic repair.