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Topic: MUTATION AT PHENOTYPIC, BIOCHEMICAL AND MOLECULAR LEVEL

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MUTATION AT PHENOTYPIC, BIOCHEMICAL AND MOLECULAR LEVEL

REFERENCES:

- ✓ iGenetics, 3rd Edition, Peter. J Russell
- ✓ Friefleder's Essential of Molecular Biology by Geordge M. Malacinski
- ✓ Cell Biology molecular biology genetics evolution and Ecology VERMA AND AGARWAL
- ✓ http://www.botanylibrary.com/plant-breeding-2/mutagens/2-main-types-of-mutagens-mutations-plant-breeding-botany/14299
- ✓ https://geneticeducation.co.in/mutagen-definition-types-and-effect/
- ✓ https://www.genome.gov/genetics-glossary/Frameshift-Mutation
- ✓ https://www.sciencedirect.com/topics/neuroscience/frameshift-mutation

Any change in sequence of DNA, which is reflected in corresponding RNA and Protein can be regarded as mutation. This change can be in a single base pair or a change in more than one base pairs may be involved. Environmental changes can cause mutations anywhere in the genome. A unicellular organism and multicellular organism, both are subjected to such changes. Apart from environmental factors, such changes can be induced to obtain desired results in lab.

Mutation can have various effects which can range from mild diseases to lethal consequences. Mutation has also contributed in evolution of species.

Definition

Mutation can be defined as any sudden heritable change in the DNA.

Mutant refers to any organism or any gene that is different from the Wild type (Normal).

Mutagen refers to any physical or chemical agent, which causes mutation to occur or increase its frequency of occurrence.

MUTATION AT PHENOTYPIC, BIOCHEMICAL AND MOLECULAR LEVEL

Very few mutations create a phenotype change, mutations can be inherited and sometimes passed from one generation to another, if mutation cause a new phenotype it may make an organism to adapt in a better way to a particular environment it may result in such changes which can result in diseases lethal consequences.

Dominant mutation changes in phenotype next generation but a recessive mutation produces phenotypic changes only only when it is present in homozygous condition.

Mutations at the molecular level

Mutations at the molecular level cause permanent changes sequences of bases present in DNA.

Following kind mutation can be produced at molecular level

1. Deletion of bases
2. Insertion of bases
3. Inversion
4. Replacement or substitution of a base pair.

As we have already studied that deletion, insertion and inversion happen due to breakage and re joining

of DNA segments
Base pairs can be substituted by the process of transition and transversion

Biochemical basis of mutation
The chemical and physical properties of each protein determined by its Amino acid sequence so that single amino acid change is capable of in activating a protein. a Single is change in a DNA molecule can lead to an alteration in the I know acid sequence of a protein.
Example
Substitution of methionine which is uncharged, lysine, can destroy the three dimensional structure of a protein because of the newly introduced positively charged amino acid
Exception for this rule are silent and neutral mutations. Silent mutations do not Alter The Amino acid sequence neutral mutations reduce changes which are not significantly effective to change the structure of protein.
Shapes of protein are determined by such a variety of interactions that sometimes amino acid substitution only partially disruptive.
For example, isoleucine mites substitute successfully for leucine, but if we substitute it with a bulky amino acid such as phenyl alanine, then certain stereochemical changes main result but the hydrophobic cluster is maintained.
Such mutations are called Leaky mutations.
Inhumans several heriditary disorders maybe due to Leaky mutations, such as mutation in a gene that codes for essential in enzyme glucose 6 phosphate dehydrogenase, is a point mutation, which produces a defective enzyme with reduced catalytic activity. Search individuals develop hemolytic anaemia when exposed to various substances.
Many types of mutation lead to total elimination of the activity of the protein mutations are base addition, base deletion which come under frameshift mutation discussed below and chain termination mutation or nonsense mutation.
All the different kinds of mutation, their causative agents are discussed in detail below.

Types of mutation

There are various kinds of mutations. We can classify mutation in different ways.

based on type of cells

- Somatic
- Gametic

according to size and quality -

- multiple mutation
- point mutation
- Frameshift Mutations
  - Due to Base addition
  - Due to base Deletion

according to origin

- Spontaneous
- Induced

according to direction

- forward
- reverse

according to magnitude of phenotypic effects

- Dominant
- recessive
- isoalleles
- lethal

according to consequence produced by change in amino acids sequence

- missence
- Ts mutations
- Nonsense or chain termination
- Silent

according to types of chromosomes

- autosomal mutations
- sex chromosonal mutations

Classification based on type of cells involved in mutation

1.**Somatic Mutation**

Mutations which occur in non reproductive or somatic cells of the body are called somatic mutations.

If these changes occur during embryonic stage then many cells of the body might be affected.

Somatic mutations also cause cancers and many fatal diseases (unilateral retinoblastoma) in man.

2. **Germline mutation**

These mutations occur in reproductive cells called as gametes. Such changes can be transmitted from one generation to another.

Eg: eye tumor retinoblastoma and Wilms tumor, a childhood malignancy of the kidney.

Point mutation (Gene mutation)

When changes occur in a single base or single base pair then this type of mutation is called point mutation

It can be of various types

1. **Base substitution**

A nucleotide is replaced with another nucleotide. For example, sickle cell anemia is caused by a substitution in the beta-hemoglobin gene, which alters a single amino acid in the protein produced.

It can be of two types

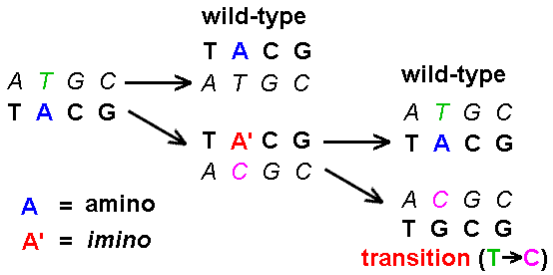
a. **Transition**: One Purine is replaced with another Purine or one Pyrimidines is replaced with another Pyrimidines. This phenomenon occurs due to **Tautomerization.** Altered form of bases are created by single proton shift and such altered bases are called **rare states** or **Tautomers**

Eg: Amino from Adenine is changed to Imino form, keto form of thymine changes to enol form

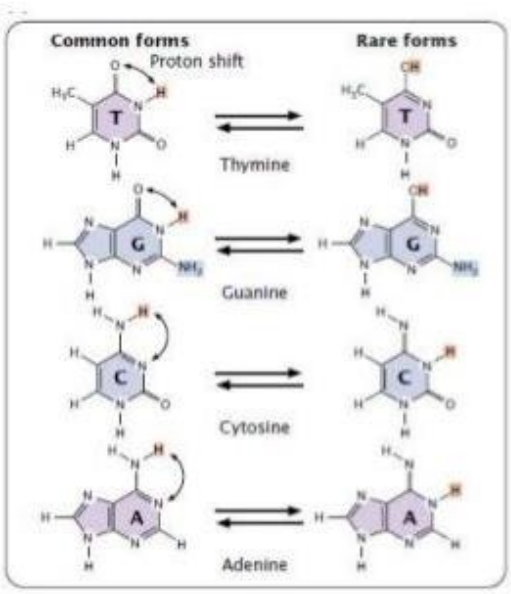
Such altered bases cannot form hydrogen bond to its normal parther (A═T, G≡C)

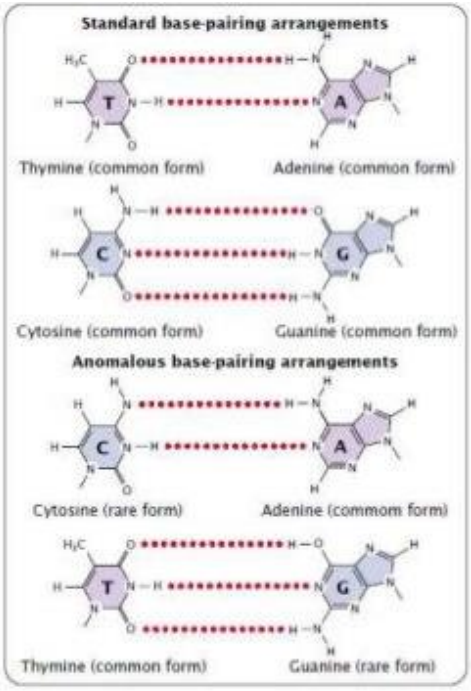
Normal base Pairing: A═T

Altered base pairing A*═C, cytosine in new strand during replication will cause **copy error mutation**



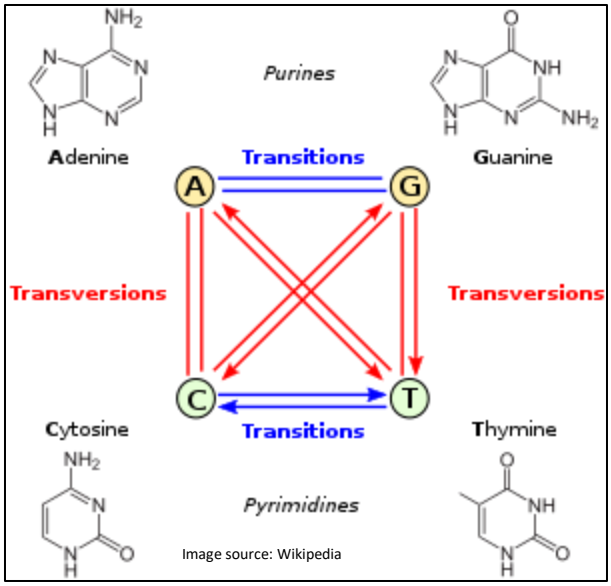
Ionization can cause loss of Hydrogen from N1 of many bases, thus leading to abnormal base pairing. Thymine in ionized state can base pair with guanine.





T' (*enol*) pairs with **G** (keto)
C' (*imino*) pairs with **A** (amino)
G' (*enol*) pairs with **T** (keto)
A' (*imino*) pairs with **C** (amino)

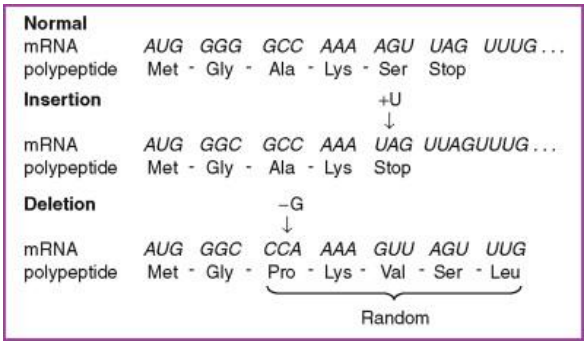
b. **Transversion:** A Purine is replaced by a Pyrimidines or vice versa
A transversion can be spontaneous, or can be caused by ionizing radiation or alkylating agents. It can only be reversed by a spontaneous reversion.

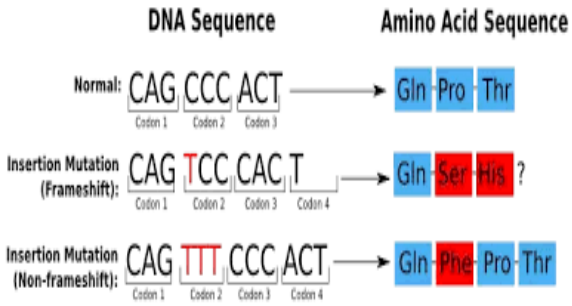


Base substitution mutation can have various consequences; accordingly, the mutations may be of following types

a. **Silent Mutation**: a wild type codon is replaced by an altered codon for the same amino acid
   GAG →GAA
   (Glu) (Glu)
b. **Neutral mutation**: A wild type codon is replaced with an altered codon which codes for a different but functionally similar amino acid. The resultant protein's function might be affected.
   GAG →GAU
   (Glu) (Asp)
c. **Missesnse Mutation**: A wild type codon is replaced with an altered codon which codes for a different and dissimilar amino acid. The resultant protein may become nonfunctional.
   GAG→AAG
   (glu) (Lys)
d. **Nonsense Mutation**: A wild type codon is replaced with an altered codon which is a termination codon. This causes termination of incomplete protein chain which is non functional.

2. **Frameshift mutation:**
   insertion or deletion **(Indel)**of one or more base pairs results in a shift in reading frame of the resulting mRNA molecule and leads to synthesis of non functional protein.
   Each group of three bases (**Codon)** corresponds to one of 20 different amino acids used to build a protein. If a mutation disrupts this reading frame, then the entire DNA sequence following the mutation will be read incorrectly.
   Eg: Tay–Sachs disease





   a. **Insertion or Base addition:** : addition of one or more extra nucleotide to gene
   Some mutagens like acridine dye, proflavin etc can insert between two successive bases of DNA strand thus allowing insertion of an extra nucleotide in the stretched area.
   b. **Base deletion**: loss of a base from a gene.

Classification of Mutation according to origin

1. **Spontaneous mutation**

These mutations occur suddenly in nature and their origin is unknown. They are also called background mutation.

2. **Induced mutation**

   Some mutations can be artificially induced in organism by exposing them to certain environmental condition which contains mutagens.

   Site specific mutatgenesis is a type of induced mutation where genetic engineering techniques are used to construct mutatnt DNA molecules containing mutations at specific site

Classification of Mutation according to diection

1. **FORWARD MUTATION**

when a mutation created an organism which is different from its wild type, then it is known as forward mutation

2. **BACKWARD MUTATION OR REVERSE MUTATION**

When mutant can be reversed or an abnormal phenotype can be converted back to wild type then it is known as backward or reversed mutation.

They are of following types.

a. True Reversion

   If the mutation occurs in the same site as the original mutation and restores back the gene to wild form.

b. **SINGLE SITE MUTATION**

some mutations can change only one single neucleotide. For example due to a single mutation Adenine changes to Guanine and due to reverse mutation Guanine changes back to adenine.

c. **MUTATION SUPPRESSOR**

When mutation occurs in a different site from where the mutation has already occurred then mutation in this second site might reverse the mutation in the primary site. Then such secondary mutation is called mutation suppressor. Such even is called **pseudoreversion**

i. **EXTRAGENIC SUPPRESSOR**

Secondary mutation occurs in a different gene than that of the already mutant gene.

ii. **INTRAGENIC SUPPRESSOR**

Secondary mutation occurs in a different nucleotide of the same gene in which a primary mutation has already occurred and shifts back the reading frame.

iii. **PHOTOREACTIVATION**

thymine dimmers which are formed due to Ultraviolet light, are reversed using specific enzymes (photolyase) in presence of visible light. The thymine dimer breaks into monomers and restores original form

iv. **EXCISION REPAIR OR DARK REACTIVATION**

Reversal of UV induced Mutation takes place in absence of light. Various enzymes such as exonuclease, endonulclease, DNA polymerase and Ligases are involved.

Classification of Mutation according to chromosomal type

1. Autosomal mutations

It occurs due in autosomal chromosomes

2. Sex chromosomal mutation

   It occurs only in sex chromosomes

Classification of mutation based on Magnitude of Phenotype effect

1. **DOMINANT MUTATION**

these mutations are due to a dominant gene which is mutant, eg : aniridia disease in humans

2. **RECESSIVE MUTATION**

when a recessive gene is mutant, then the phenotypic effect can be seen only if it is in homozygous condition.

3. **ISOALLELES**

some mutations causes very little change in phenotype such that it can be detected only by some special technnique.

4. **LETHAL MUTATIONS**

these mutations causes death of an organism

5. **SUBVITAL MUTATIONS**

these mutations reduces the chance of survival of an organism

6. **SUPERVITAL MUTATIONS**

these mutation improve biological fitness of an organism

Mutation rate

The frequency with which mutation occurs or henes mutate is called mutation rate. The regions of DNA where mutation occurs at a higher frequency is called **HOT SPOTS.**

Rate of mutation is influenced by certain factors

1. **Genetic control**

   Certain genes called **MUTATOR GENES** can increase the rate of mutation in drosophila.

   Eg: A mutant methylating enzyme (Dam enzyme), methylates those sequence of DNA which the mismatch repair system uses to discriminate parental strand from daughter strand

   Certain **SUPPRESSOR GENE** may suppress the rate of mutation

   TRANSPOSONS are certain genetic regions which cause spontaneous mutation

2. **Viral control**

   Virus may cause mutation or increase the rate of mutation in certain organisms

3. Environmental control

   Temperature, chemicals and certain radiations have been linked to many mutations

Mutagens

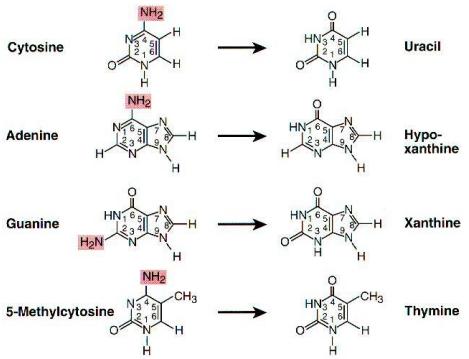
Mutagen refers to any physical, chemical or Biological agent, which causes mutation to occur or increase its frequency of occurrence.

There are three types of Mutagens

1. Physical mutagens
2. Chemical Mutagens
3. Biological Mutagens

Effect of chemical mutagen on DNA

1. **Deamination**:
   Chemicals like nitrous acid causes oxidative deamination of DNA bases. During this process, the amino group of DNA is replaced by Hydroxyl group.
   Adenine → Hypoxanthine
   By tautomeric shift, Hypoxanthine pairs with cytosine
   Cytosine →Uracil, now uracil can pair with adenine.



2. **Hydroxyl amine and Hydrazine**
   Cytosine is converted to hydroxylcytosine on treatment with Hydroxyl amine. Hydroxylcytosine can pair with adenine. Thus a GC pair coverts to AT pair subsequently.
3. **Alkylating agent**
   Eg: Diethyl Sulphate (DES), Dimethyl Sulphate (DMA), Methyl methane sulphonate (MMS), ethyl ethane sulphonate(EES), Ethyl methane Sulphonate (EMS)
   Alkylating agents carry reactive alkyl groups and can form following changes
   d. They can add ethyl or methyl group to guanine, which can base pair with adenine.
   e. They can remove alkylated guanine (depurination), if the gap itself or if filled with wrong base, it may cause mutation.
4. **Base analogues**
   Base analogs have structures similar to bases of DNA and can be incorporated into a replicating strand.
   Eg: 5 Bromouracil or %- Bromodeoxyuridine (in its keto form) can substitute Thymine in a DNA strand. This change a AT base pair GC base pair
5. **Amino purines (2-AP)**

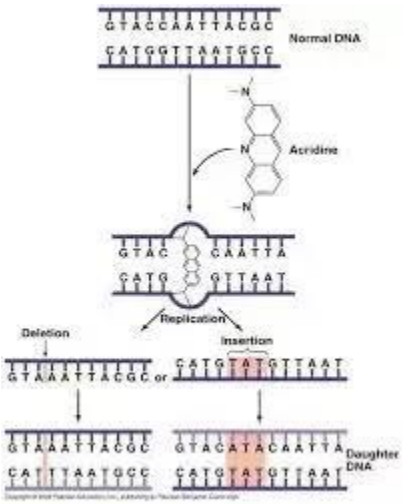
It is a base analogue which can form a base pair both with C and T. 2-AP acts by switching Pyrimidines. It is incorporated opposite thymine during one round of replication and in another round it pairs with cytosine, thus producing an AT →GC transition.

6. **Inhibition of precursor of nucleic acid**

Azaserine, a potent Alkylating agent inhibits the synthesis of Purine

Urethrane inhibits Pyrimidines synthesis.

7. **Intercalating agents**: These are planar three ringed molecules whose dimensions are roughly the same as those of a Purine – Pyrimidines base pairs. They get inserted between two adjacent base pairs in a DNA molecule causing insertion or deletion. EtBr- ethidium bromide used during the Agarose gel electrophoresis is one of the intercalating agents. Other intercalating agents like proflavine, acridine orange or daunorubicin operated by the same mechanism alike the EtBr. The molecules intercalate between the bases of DNA and disrupt its structure. If it is incorporated during the replication, it can cause frameshift mutation. It may also block transcription.



8. **Metal ions:**

Metal ions also dangerous to our DNA as it acts in varieties of different ways. Nickel, chromium, cobalt, cadmium, arsenic, chromium and iron are some of the common metal ions cause mutations.

The metal ions work by producing ROS, hindering DNA repair pathway, cause DNA hypermethylation or may directly damages the DNA.

9. **Other chemical mutagens:**

ROS- reactive oxygen species, benzene, synthetic rubber and rubber products, sodium azide, aromatic amines, alkaloids, deaminating agents and PAH are other mutagens creates different mutations.

Effects of Physical agents on DNA

Physical Mutagens:

Physical mutagens include Heat and various types of radiation, viz. X-rays, gamma rays, alpha particles, beta particles, fast and thermal (slow) neutrons and ultra violet rays

1. **Heat:**

Heat is another mutagen that causes mutations in our DNA. when we heat the DNA, over a certain degree (>95°C), the DNA becomes denatured- two single-stranded DNA generated from the dsDNA. Also, extreme heat also damages DNA and breaks the phosphodiester bonds too.

2. **Radiation:**

Radiations are the first mutagenic agent reported in 1920. UV rays, X-rays, alpha rays, neutrons and other ionizing and non-ionizing radiations are mutagenic.

Usually, radiations directly damage the DNA or nucleotide structure which might be either lethal or sub-lethal. The electromagnetic radiation is also one of the known mutagens that cause lethal or sub-lethal mutations.

A brief description of these mutagens is presented below:

(1) X-Rays:
The wavelengths of X- rays vary from 10^{-11} to 10^{-7}. They are sparsely ionizing and highly penetrating and are generated in X-rays machines. X-rays can break chromosomes and produce all types of mutations in nucleotides, viz. addition, deletion, inversion, transposition, transitions and transversions.
These changes are brought out by adding oxygen to deoxyribose, removing amino or hydroxyl group and forming peroxides. X-rays induce mutations by forming free radicals and ions.

(2) Gamma Rays:
Gamma rays are identical to X-rays in most of the physical properties and biological effects. But gamma rays have shorter wave length than X-rays and are more penetrating than X-rays. They are generated from radioactive decay of some elements like 14C, 60C, radium etc. Of these, cobalt 60 is commonly used for the production of Gamma rays.
Gamma rays cause chromosomal and gene mutations like X-rays by ejecting electrons from the atoms of tissues through which they pass. Now-a-days, gamma rays are also widely used for induction of mutations in various crop plants.

(3) Alpha Particles:
Alpha rays are composed of alpha particles. They are made of two protons and two neutrons and thus have double positive charge. They are densely ionizing, but lesser penetrating than beta rays and neutrons. Alpha particles are emitted by the isotopes of heavier elements. They have positive charge and hence they are slowed down by negative charge of tissues resulting in low penetrating power.
Alpha particles lead to both ionization and excitation resulting in chromosomal mutations.

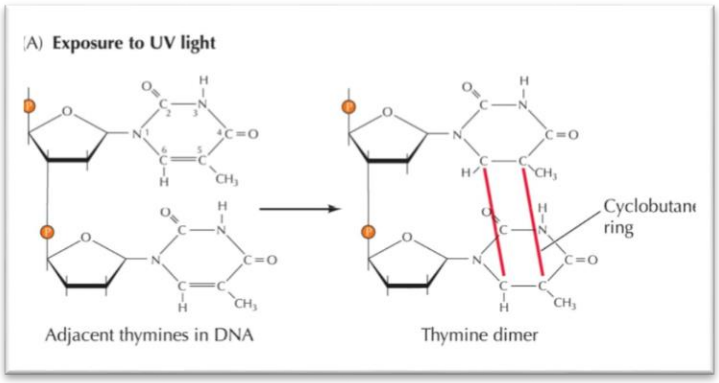
(4) Beta Particles:
Beta rays are composed of beta particles. They are sparsely ionizing but more penetrating than alpha rays. Beta particles are generated from radioactive decay of heavier elements such as 3H, 32P, 35S etc. They are negatively charged, therefore, their action is reduced by positive charge of tissues. Beta particles also act by way of ionization and excitation like alpha particles and result in both chromosomal and gene mutations.

(5) Fast and Thermal Neutrons:
These are densely ionizing and highly penetrating particles. Since they are electrically neutral particles, their action is not slowed down by charged (negative or positive) particles of tissues. They

are generated from radioactive decay of heavier elements in atomic reactors or cyclotrons. Because of high velocity, these particles are called as fast neutrons.
Their velocity can be reduced by the use of graphite or heavy water to produce slow neutrons or thermal neutrons.
Fast and thermal neutrons result in both chromosomal breakage and gene mutation. Since they are heavy particles, they more in straight line. Fast and thermal neutrons are effectively used for induction of mutations especially in asexually reproducing crop species.

(6) Ultra Violet Rays:

UV rays are non-ionizing radiations, which are produced from mercury vapor lamps or tubes. They are also present in solar radiation. UV rays can penetrate one or two cell layers. Because of low penetrating capacity, they are commonly used for radiation of micro-organisms like bacteria and viruses. In higher organisms, their use is generally limited to irradiation of pollens in plants and eggs in Drosophila. UV rays can also break chromosomes. They have two main chemical effects on Pyrimidines.
The first effect is the addition of a water molecule which weakens the H bonding with its Purine complement and permits localized separation of DNA strands.
The second effect is to join Pyrimidines to make a Pyrimidines dimer. This **dimerization** can produce TT, CC, UU and mixed Pyrimidines dimers like CT.



Dimerization interferes with DNA and RNA synthesis. Interstrand dimers cross link nucleic acid chains, inhibiting strand separation and distribution.

Commonly used physical mutagens (radiations), their properties and mode of action

Type of radiation	*Main properties*	*Mode of action or changes caused*
1. X-rays	S.I., penetrating and non-particulate.	Induce mutations by forming free radicals and ions.Cause addition, deletion, transitions and transversions.
2. Gamma rays	S.I., very penetrating and non-particulate.	Induce mutations by ejecting atoms from the tissues. Cause all types of changes as above.
3. Alpha Particles	D.I., Particulate, less penetrating and positively charged.	Act by ionization and excitation. Cause chromosomal and gene mutations.
4. Beta Rays Particles	S.I., particulate, more penetrating than alpha particles and negatively charged.	Act by ionization and excitation. Cause chromosomal and gene mutations.
5. Fast and Thermal Neutrons	D.I., particulate, neutral particles, highly penetrating.	Cause chromosomal breakage and gene mutations.
6. Ultra Violet Rays	Non-ionizing, low penetrating.	Cause chromosomal breakage and gene mutations.

Note : Particulate refers to particle emitting property DI = Densely ionizing, SI = Sparsely ionizing.

Effect of Biological mutagens on DNA

Viruses, bacteria and transposons (non-coding DNA sequence) are biological mutagens.

Virus: Viruses are common mutagens that causes lethal health issues.

Viruses insert their DNA into our genome and disrupt the normal function of DNA or a gene. Once it inserts DNA, the DNA is replicated, transcribed and translated viral protein instead of our own protein.

Bacteria: Some bacteria are also dangerous to for our DNA- cause inflammation. It provokes DNA damage and DNA breakage.

Transposons: The transposons are non-coding DNA sequences, jumps from one place to another place in a genome and influence the function of genes.

Effect of mutagens:

The mutagens are genotoxic- harmful to our DNA in many ways, some directly affect the DNA some indirectly. And therefore, the exact effect of each mutagen is still unknown to us.

At the chromosomal level, the mutagens can alter the structure or number of chromosomes. As deletion, duplication, insertion, translocation, monosomy and nondisjunction are some of the chromosomal abnormalities caused by mutagens.

The mutagens also affect or dysregulates the molecular central dogma process- replication, transcription and translation.

At the molecular level, the mutagens create different gene mutation results in loss of function, altered function or non-functional protein.

It also alters the codon, deletes bases, alters bases, breaks hydrogen bonds or phosphodiester bonds or changes gene expression.

Some mutagens dysregulate cell proliferation and cell death process and thus cause cancer, those are called carcinogens.

Biological mutagens slower down the DNA repair or DNA synthesis process.
Some of the common types of mutagens based on their effect are enlisted here:
Teratogens: teratogens are the class of the mutagens which causes congenital malformations. X-rays, valproate and toxoplasma are common physical, chemical and biological teratogens, respectively.
Carcinogens: The carcinogens are the class of mutagens induces tumor formation and thus cause cancer. Wide varieties of agents are categorized as carcinogens. X-rays/ UV-rays, Aflatoxins and retroviruses are common physical, chemical and biological carcinogens, respectively.
Clastogens: Clastogens are the class of mutagens responsible for chromosomal- breakage, deletion, duplication and rearrangements. UV-rays, Bleomycins and HIV virus are a common type of physical, chemical and biological clastogens, respectively.
Other non-specific mutagens: other unclassified mutagens are responsible for DNA damage and non-functioning of the DNA repair pathway. X-rays/heat, innumerable and toxoplasma are several non-specific mutagens.