TEXT

Various classifications of mutations are known, each based on a definite criterion. Some of the common types of mutations are:

1. Mutations based on direction:

   I. Forward mutation
   II. Reverse mutation

(I). Forward mutation: The change from wild-type to mutant-form is called a forward mutation, e.g. The wild-type long pea plants got mutated to dwarf plants. Sometimes, however, the designation of the wild type and mutant is quite arbitrary. They may simply represent two different phenotypes.

(II). Reverse mutation: If a mutant is again mutated at the same site in such a manner that its phenotype returns back to the wild type, the second mutation will be reversion of the first one, or a back mutation. The back mutation can be of two types:

> (i) True back mutation: where the second mutation is at the same genetic site where the first mutation had taken place, and the phenotype as well as the genotype of the organism revert back to wild type.

(ii) Supression mutation: In such a mutation, the second mutation is at a site other than the site of the first mutation. However, the second mutation nullifies the effect of the first mutation and the phenotype of the organism is back to the wild type. But the genotype of the revertant will show mutations at two separate locations.

2. Mutations based on the cause:

I. Spontaneous mutation

II. Induced mutation

(I). Spontaneous mutation: In this type of mutation, there is no apparent reason for the change(s) in the DNA. These take place spontaneously without any known cause. The deamination of bases, especially of cytosine, and the spontaneous depurination (which is very slow) are good examples of this type of mutation (Fig. 1, A & B).
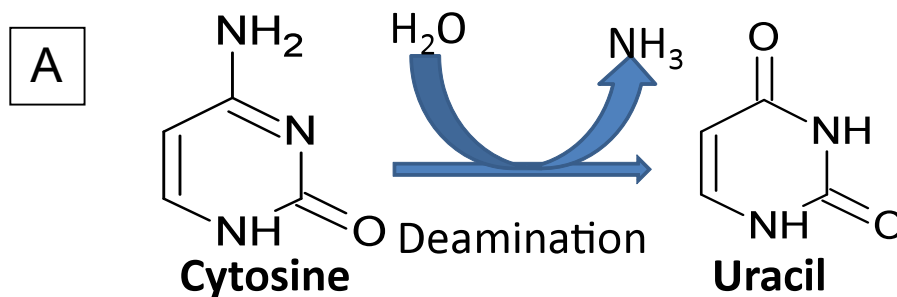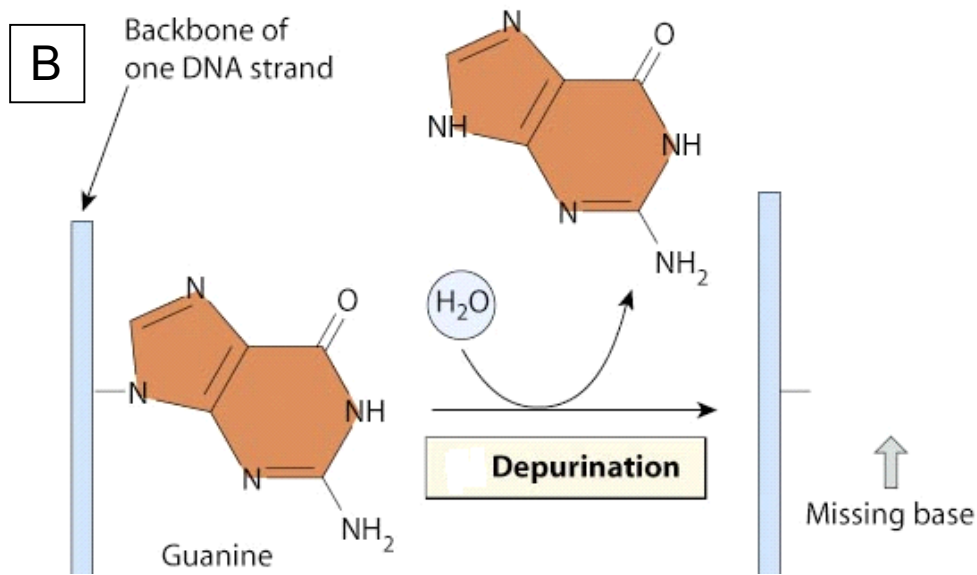
Figure 1. Deamination of cytosine (A) and Depuration of Guanine (B)

(II). Induced mutation: In this type of mutation, the change is caused by certain physical or chemical agents. These agents are known as mutagens. Exposure to mutagens results in mutation. Some of the known mutagens are the ionizing radiations, such as X-rays, γ-rays, cosmic rays and UV light (especially of very low wavelength). The radiation with UV light of the wavelength of less than 100 nm are very strong mutagens. Other mutagens include the chemicals which react with nucleic acids and the nucleotide analogs which can be incorporated in place of a normal nucleotide. These analogs can form a mis-base pairing with a wrong

nucleotide and a mutation will take place during the next cycle of the DNA replication.

Induced mutations can be of two types:

        i. Radiation-induced mutations and

        ii. Chemical-induced mutations

(i). Radiation-induced mutation: A number of radiations, especially the electromagnetic waves with 100 nm or smaller wave length cause ionization. The examples are the X-rays, γ-rays and cosmic rays. However, it was found that some mutations can take place even at very low exposure dose occurring for long time, or at high dose for relatively short time. Thus there is no safe level of radiation, and even a very small dose may be unsafe. Among the radiations, UV is relatively a weak mutagen. The normal level of UV in the sun light is not strong enough to cause mutation (Fig. 2). Any damage to the DNA is repaired readily by the cell. UV is absorbed by both the purine and the pyrimidine bases and these are converted in to an excitable state which is more reactive. The UV of 254 nm or lower wavelength is the most damaging. It can cause the formation of pyrimidine dimmers. The relationship between the UV dose and the mutation rate is highly variable.

200 nm	280 nm	320 nm	400 nm	500 nm	600 nm	700 nm	800 nm
UV-C	UV-B	UV-A	BLUE		RED	FAR-RED	

PAR

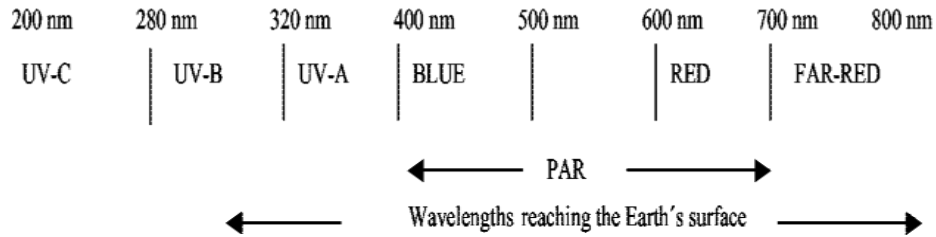Wavelengths reaching the Earth's surface

Figure 2. Wavelengths reaching to earth from sun

(ii). Chemical-induced mutation: A number of chemicals are mutagenic in nature. Some of these chemicals are commonly used by human beings, while the others are not that common. In general, the chemicals can be grouped in to two classes.
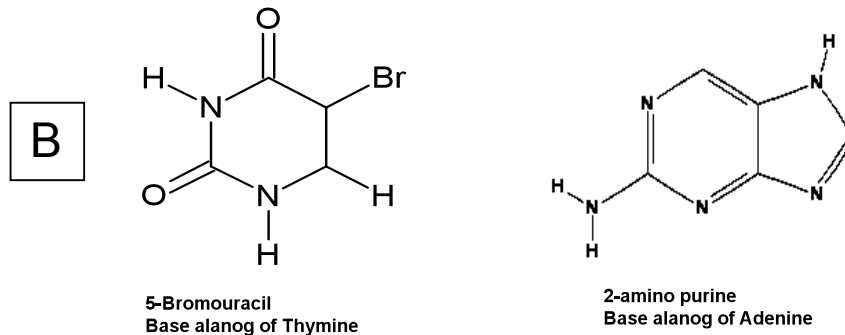
(i). Chemicals which can cause mutation to both the replicating and the non-replicating DNA. Some of the examples of this class of chemicals are nitrous acid (HNO_2), alkylating agents etc.

- Nitrous acid: it causes oxidative deamination of the bases in DNA.
- Alkylating agents and hydroxylating agents: These chemicals transfer a CH_3 or C_2H_5 group to the bases.

(ii). Chemicals which can cause mutation to only replicating DNA, such as acridine dyes and the base analogs.

A

$N(H_3C)_2$ N $(CH_3)_2N$

ACRIDINE ORANGE

5-Bromouracil
Base alanog of Thymine
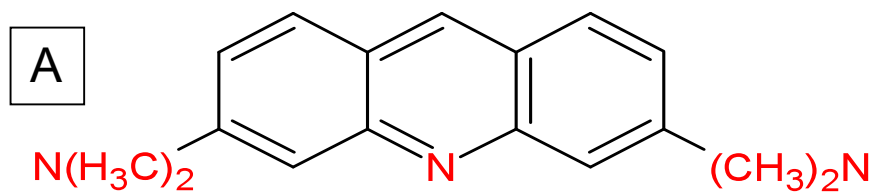
2-amino purine
Base alanog of Adenine

Base analogs

Figure 3. Acridine orange (A) and Base analogs (B)

- Acridines: Acridine dyes cause the frame shift mutation.

- Base analogs: A number of modified bases can be incorporated in place of a normal base during the DNA replication as the DNA polymerase cannot differentiate between the normal base and its analog.

3. Mutations based on the tissue of origin:

I. Somatic mutations

II. Germinal mutations

(I). Somatic mutation: Mutations in somatic cells (all cells other than reproductive cells) are called **somatic mutations**. This type of mutation is not passed on to the next generation as the gametes are not involved. For maintaining somatic mutation, cloning of the organism containing this mutation is

necessary. Navel oranges and red delicious apples are two examples of somatic clones.

Changes that give these two fruits their desirable qualities apparently followed spontaneous mutation in single cells. In each case, the cell carrying the mutant gene reproduced, eventually producing an entire branch that had the characteristic of the mutant type (Gardner, 2008). Plant scientists grafted mutant branches onto the stocks of "normal" trees. After the graft was established, cuttings from that original graft were grafted onto tree stocks. In this way the mutation was maintained and proliferated.

(II). Germinal mutation: If a gene is altered in a germ cell, the mutation is termed a **germinal mutation**. Because the germ cells give rise to gametes, the mutation is passed along to the next generation by sexual means. Typically germinal mutations are not expressed in the individual containing the mutation.

4. Mutations based on type of trait affected:

I. Morphological or visible mutations

II. Biochemical mutations

(I). Morphological or visible mutations: In this type of mutation a change in external features (phenotype) of the

organism takes place, which includes colour, shape, size, etc. For example, albino ascospores in *Neurospora*, kernel colour in corn, curly wings in *Drosophila,* and dwarfism in pea.

*(II). **Biochemical mutations:*** are caused by some deficiency and the defect can be overcome by supplying the nutrient or any other chemical compound, in which the mutant is deficient (Gupta, 2007). Such mutations have been observed in prokaryotes (e.g., bacteria and fungi), as well as in eukaryotes (e.g. *Drosophila* and humans).

For bacteria, biochemical mutants need to be grown on a media supplemented with a specific nutrient. Such mutants are called auxotrophs (Gupta, 2007).

5. Mutations based on cytology:

  I. Chromosomal mutations

  II. Gene or point mutations

  (I). Chromosomal mutations: are of two types

    (i). Change in chromosome number

    (ii). Chromosome aberrations

(i). Change in chromosome number: In this type of mutation, an entire chromosome may either be lost or get duplicated resulting in aneuploidy. If a chromosome is gained, it will

result in the formation of a chromosome which will have three homologues, and is referred to as the trisomic. The loss of one chromosome will result in a chromosome having only a single homologue which is referred to as a monosomic. Sometimes, it may be the loss or gain of a complete pair of chromosomes, resulting in the formation of euploidy (Jain, 2000).

(ii). Chromosome aberrations: Chromosome aberrations are extensive damage in the DNA, which may be due to breakage of chromosomes or due to transposition of the transposons. Such mutations result in major changes in genetic information, and are caused due to some unusual events, such as in the survivors of atomic bombs exploded in Japan in the second World war (Jain, 2000).

(II). Gene or point mutation: It is the substitution of one base pair for another, or the duplication or deletion of a single base pair. Such mutations are referred to as point mutations (Gardner, 2008). If the change is a replacement of some kind, then a new codon is created. In many cases, this new codon, upon translation, results in a new amino acid. One of the outcomes of redundancy in the genetic code is partial protection of the cell from the effects of mutation; common amino acids have the most codons, similar amino

acids have similar codons, and the wobble position of the codon is the least important position in translation. However, when base changes result in new amino acids, new proteins appear. These new proteins can alter the morphology or physiology of the organism and result in phenotypic novelty or lethality.

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:

(i) <u>Silent mutations:</u> are mutations that do not result in a change to the amino acid sequence of a protein. They may occur in a region that does not code for a protein, or they may occur within a codon in a manner that does not alter the final amino acid sequence.

(ii) <u>Missense mutations:</u> where a single nucleotide is changed to cause substitution of a different amino acid. This will cause the resulting protein nonfunctional. In other words, missense mutations code for a different amino acid.

(iii) <u>Nonsense mutations:</u> which code for a stop and can truncate the protein.

6. Mutations based on molecular mechanism:

I. Base substitution mutations

II. Frameshift mutations

(I). Base substitution mutation: A type of mutation involving replacement or substitution of a single nucleotide base with another base. It is of two types:

(i). Transition mutation

(ii). Transversion mutation

(i). <u>Transition mutation:</u> The replacement of a purine in one strand of DNA with the other purine and the replacement of a pyrimidine in the complementary strand with the other pyrimidine is called transition (Fig. 4, A). Transitions are routinely produced by base analogues. Two of the most widely used base analogues are the pyrimidine analogs 5-bromouracil (5BU) and the purine analogs 2-aminopurine (2AP) (Fig. 3, B).
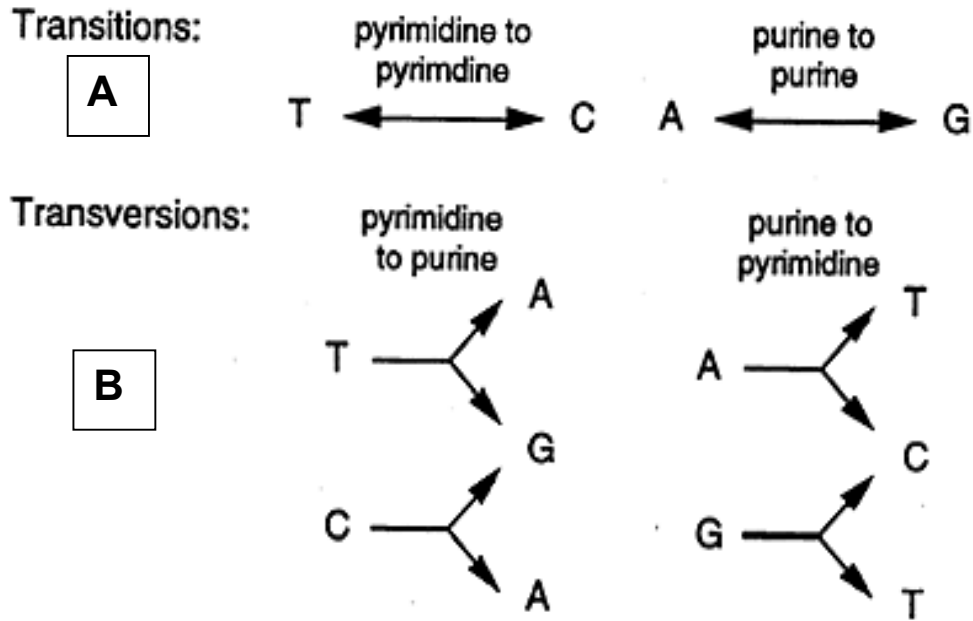
Fig. 4. Transitions (A), and Transversion (B)

The mutagenic mechanisms of the two are similar. The 5-bromouracil is incorporated into DNA in place of thymine; it acts just like thymine in DNA replication and, since it doesn't alter the hydrogen bonding, should induce no mutation. However, it seems that the bromine atom causes 5-bromouracil to tautomerize more readily than thymine does. Thus, 5-bromouracil goes from the keto form (Fig. 5, A) to the enol form (Fig. 5, B) more readily than thymine. Transitions frequently result when the enol form of 5-bromouracil pairs with guanine.

The 2-aminopurine is mutagenic by virtue of the fact that it can, like adenine, form two hydrogen bonds with

thymine. When in the rare state, it can pair with cytosine. Thus, at times it replaces adenine, and at other times guanine. It promotes transition mutations.
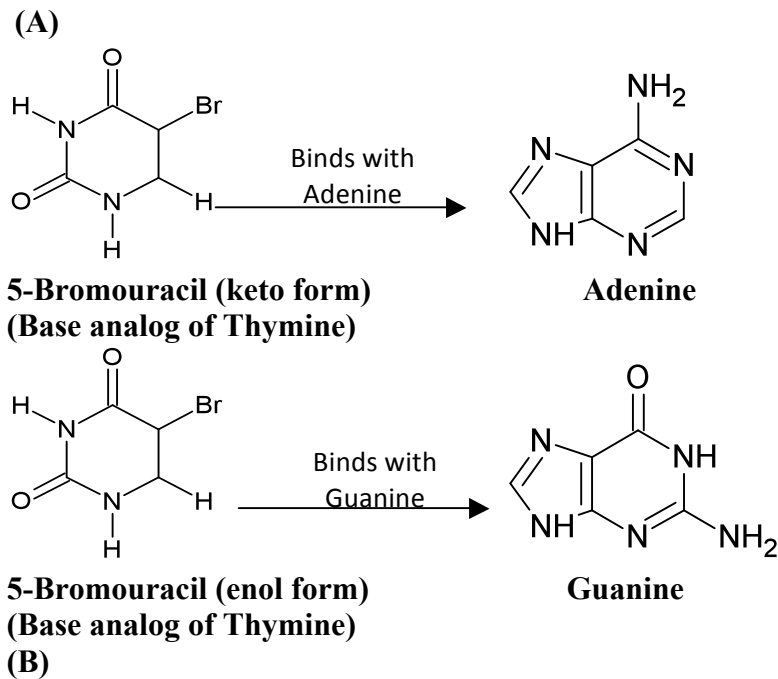

Figure 5: Base pairing of 5-bromouracil with (A) adenine and (B) guanine in its two tautomeric forms.(*Principles of Genetics by Gardner/Simmons/Snustad, Eighth Edition) with some modifications.*

Nitrous acid (HNO_2) also readily produces transitions by replacing amino groups on nucleotides with keto groups (–NH_2 to=O). The result is that cytosine is converted to uracil, adenine to hypoxanthine, and guanine to xanthine. Transition mutation results from two of the changes. Uracil pairs with adenine instead of guanine, thus leading to a UA base pair in place of a CG base pair (Fig. 5, B); hypoxanthine (H) pairs with cytosine instead of thymine, the original base paired with adenine (Fig. 5, A).
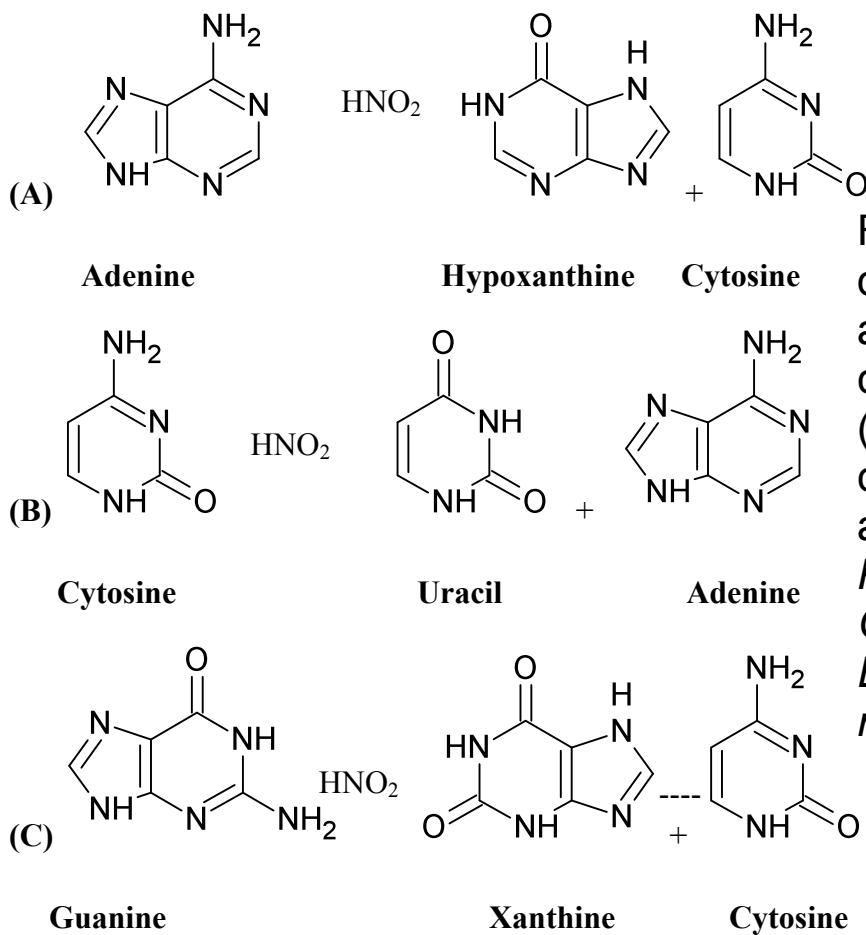

(A)

| Adenine | $\xrightarrow{HNO_2}$ | Hypoxanthine | Cytosine |

(B)

| Cytosine | $\xrightarrow{HNO_2}$ | Uracil | + | Adenine |

(C)

| Guanine | $\xrightarrow{HNO_2}$ | Xanthine | + | Cytosine |

Figure 6: Oxidativ deamination converts (A adenine to hypoxanthin causing AT→GC transitio (B) cytosine to urac causing GC→ AT tranitio and (C) guanine to xanthin *Principles of Genetics l Gardner/Simmons/Snustac Eighth Edition) with som modifications.*

Thus, in this case, an HC base pair replaces an AT base pair. Both of these base pairs (UA and HC) are transition mutations. Xanthine, however, pairs with cytosine just as guanine does (Fig. 5, C). Thus, the replacement of guanine with xanthine does not cause changes in base pairing.

(ii). <u>Transversion mutations:</u> Base pairing substitution involving the substitution of a purine for a pyrimidine and vice versa is known as Transversion (Fig. 4, B). Ethyl methane sulfonate (EMS) ($CH_3SO_3CH_2CH_3$) and ethyl ethane sulfonate (EES) ($CH_3CH_2SO_3CH_2CH_3$) are agents that cause the removal of purine rings from DNA. The multistep process begins with the ethylation of a purine ring and ends with the hydrolysis of the glycosidic (purine-deoxyribose) bond, causing the loss of the base. These sites where this happens are referred to as AP (apurinic-apyrimidinic) sites. If the AP site is not repaired, any of the four DNA bases could be inserted into the new strand opposite the gap. If thymine is placed in the newly formed strand, then the original base pair is restored; insertion of cytosine results in a transition mutation; insertion of either adenine or guanine results in a transversion mutation. Of

course, the gap is still there, and it continues to generate new mutations each generation until it is repaired. During DNA replication in *E. coli,* the polymerase tends to place adenine opposite the gap more frequently than it places other bases.
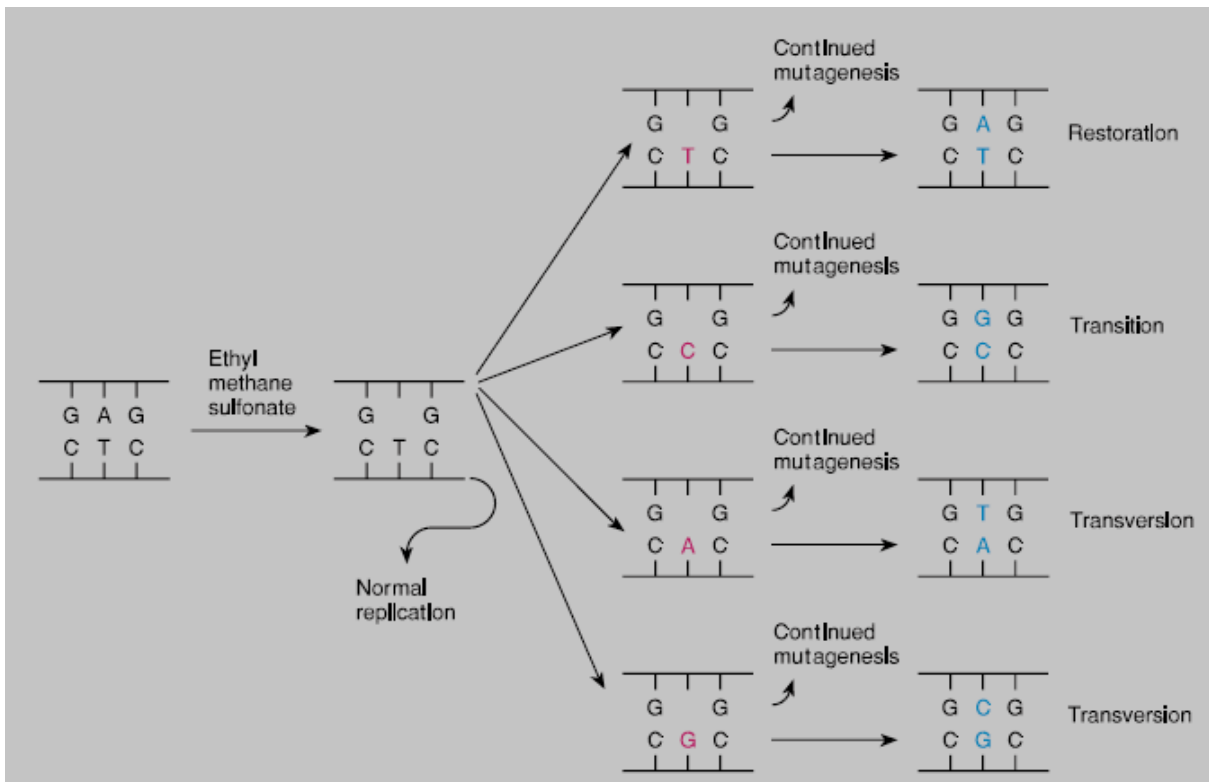

Figure 7. Four possible outcomes after treatment of DNA with an alkylating agent, which removes the purine—adenine in this example. (Source: *Tamarin: Principles of Genetics, Seventh Edition, The McGraw−Hill Companies, 2001*).

(II). Frameshift mutation: A point mutation may consist of replacement, addition, or deletion of a base (Fig. 8). Point mutations that add or subtract a base are, potentially, the

most devastating in their effects on the cell or organism because they change the reading frame of a gene from the site of mutation onward. A frameshift mutation causes two problems. First, all the codons from the frameshift will be different and thus yield (most probably) a useless protein. Second, stop-signal information will be misread. One of the new codons may be a nonsense codon, which causes translation to stop prematurely. Or, if the translation apparatus reaches the original nonsense codon, it is no longer recognized as such because it is in a different reading frame and, therefore, the translation process continues beyond the end of the gene.

Transitions, transversions and frameshift mutation are point mutations and are present among spontaneously occurring mutations.
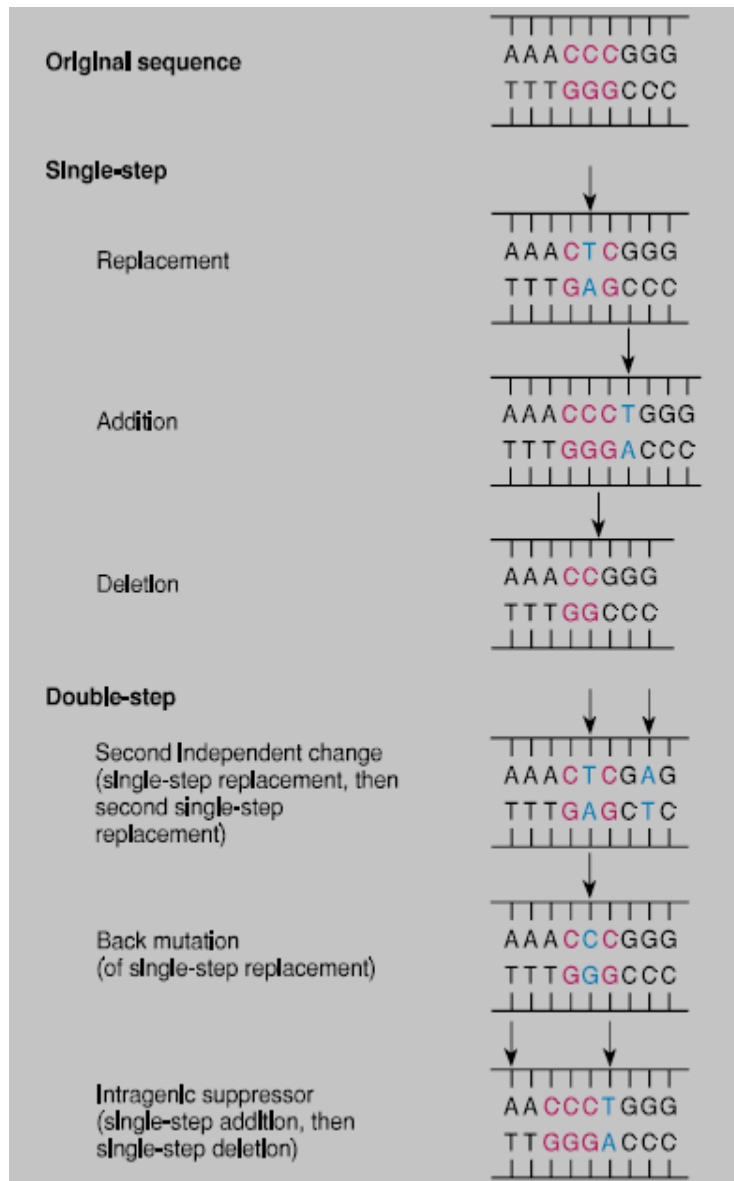
Figure. 8. Types of DNA point mutations. Single-step changes are replacements, additions, or deletions. A second point mutation in the same gene can result either in a double mutation, reversion to the original, or intragenic suppression. In this case, intragenic suppression is illustrated by the addition of one base followed by the nearby deletion of a different base. (Source: *Tamarin: Principles of Genetics, Seventh Edition, The McGraw−Hill Companies, 2001*).

7. Mutations based on their effect on survival of organism

     (I). Lethal mutations

     (II). Subvital mutations

     (III). Supervital mutations

(I). Lethal mutations: These involves genotypic changes leading to death of an individual. These are perhaps the easiest to score for a study of mutation frequencies.

(II). Subvital mutations: reduce the chances of survival of the organism in which they occur.

(III). Supervital mutations: cause the improvement of biological fitness under certain conditions.

8. Mutations based on the effect on function

     (I). Loss of function mutations

     (II). Gain-of-function mutations

     (III). Dominant negative mutations

(I). Loss of function mutations: Wild type alleles typically encode a product necessary for a specific biological function. If a mutation occurs in that allele, the function for which it encodes is also lost. The general term for these mutations is loss-of-function mutations. The degree to which the function is lost can vary. If the function is entirely lost, the mutation is called a null mutation. It is also possible that some function

may remain, but not at the level of the wild type allele. These are called leaky mutations.

Loss of function mutations are typically recessive. When a heterozygote consists of the wild-type allele and the loss-of-function allele, the level of expression of the wild type allele is often sufficient to produce the wild type phenotype. Genetically this would define the loss-of-function mutation as recessive. Alternatively, the wild type allele may not compensate for the loss-of-function allele. In those cases, the phenotype of the heterozygote will be equal to that of the loss-of-function mutant, and the mutant allele will act as a dominant.

(II). Gain-of-function mutations: It leads to change in the gene product and it gains a new and abnormal function. These mutations usually have dominant phenotypes. This type of mutation is often called a neomorphic mutation.

(III). Dominant negative mutations: This type of mutation leads to an altered gene product that acts antagonistically to the wild-type allele. These mutations usually result in an altered molecular function (often inactive) and are

characterised by a dominant or semi-dominant phenotype. It is also called antimorphic mutations.

9. Mutations based on the effect of fitness

(I). Beneficial mutations

(II). Harmful mutations

(I). Beneficial mutations: Most of the spontaneous mutations are harmful to the organism as these usually render an organism less efficient. However, the entire process of evolution has been possible only because of the slow mutation. Many mutations allow an organism to be more suitable to survive in an unfriendly environment. These mutations permit the development of new characters, while also give the adoptability to the surrounding.

The induced mutations can be used for providing new characters. The application of such mutations has been more in plant sciences. It has been possible to provide an organism with useful characters, such as pest resistance, stress resistance or the high yielding varieties, etc. For example, it has been possible to develop a variety of *Pennicilium* which has higher yields of penicillin. Such over producers have been very valuable commercially.

(II). Harmful mutations: A harmful mutation is a mutation that decreases the fitness of the organism.

10. Special mutations/ *Conditional mutations*

Conditional mutations: are those which allow the mutant phenotype (including lethality) to be expressed only under certain conditions (e.g. high temperature) called restrictive conditions. Under other or normal conditions described as permissive condition, the mutant expresses normal phenotype.