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Mutation

Most biological molecules have a limited lifetime. Many proteins, lipids and RNAs are degraded when they are no longer needed or damaged, and smaller molecules such as sugars are metabolized to compounds to make or store energy. In contrast, DNA is the most stable biological molecule known, befitting its role in storage of genetic information. According to the classic definition mutations are sudden heritable changes in the DNA.

The process (change) itself is still called mutation, but due to the fast development of genetics and genomics, two terms related to the variations in the sequence had to be modified. Next to the above mentioned definition the term **mutatio**n is also used to indicate a disease-causing change or sometimes rare change. Similarly, **the term polymorphism** is used both to indicate a non disease-causing change or a change found at a frequency of 1% or higher in the population. In the era of advanced DNA sequencing tools and personal genomics, these earlier definitions of mutation and polymorphism are antiquated. a mutation would be a "DNA variant" acquired over the lifetime of an organism, i.e. a **somatic mutation**. In this sense, mutations are the principal causes of many diseases like cancer but are typically not inherited by their offspring. Alterations in the DNA of germ cells – sperms and eggs – can be inherited by offspring and are currently called **germline mutations**. In this case, the term mutation should be used only if the germline "variant" has been detected using as a reference the germline DNA of the same individual.

1-Mutagens and types

Mutations that are caused by agents that damage the DNA are known as **induced mutations**. Agents that mutate DNA are called **mutagens** and are of three main types: mutagenic chemicals, radiation and heat. Even if there are no dangerous chemicals or radiation around, mutations still occur, though less frequently. These are **spontaneous mutations**. Some of these are due to errors in DNA replication.The enzymes of DNA replication are not perfect and sometimes make mistakes. In addition, DNA undergoes certain spontaneous chemical reactions (alterations) at a low but detectable rate and this rate goes up with increasing temperature.

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A- The most common mutagens are toxic chemicals

That react with DNA and alter the chemical structure of the bases. For example, EMS (ethyl methane sulfonate) is widely used by molecular biologists to mutagenize growing cells. It adds an ethyl group to bases in DNA and so changes their shape and their base-pairing properties. Nitrite converts amino groups to hydroxyl groups and so converts the base cytosine to uracil. Nitrite is used experimentally to mutate purified DNA, such as a cloned gene carried on a plasmid, while the plasmid is in the test tube. The mutagenized DNA is then transferred back into a cell to identify the mutations that were generated. During DNA replication, the DNA polymerase misidentifies these altered bases and puts in the wrong bases in the new complementary strand of DNA it is making (figure 7.1).

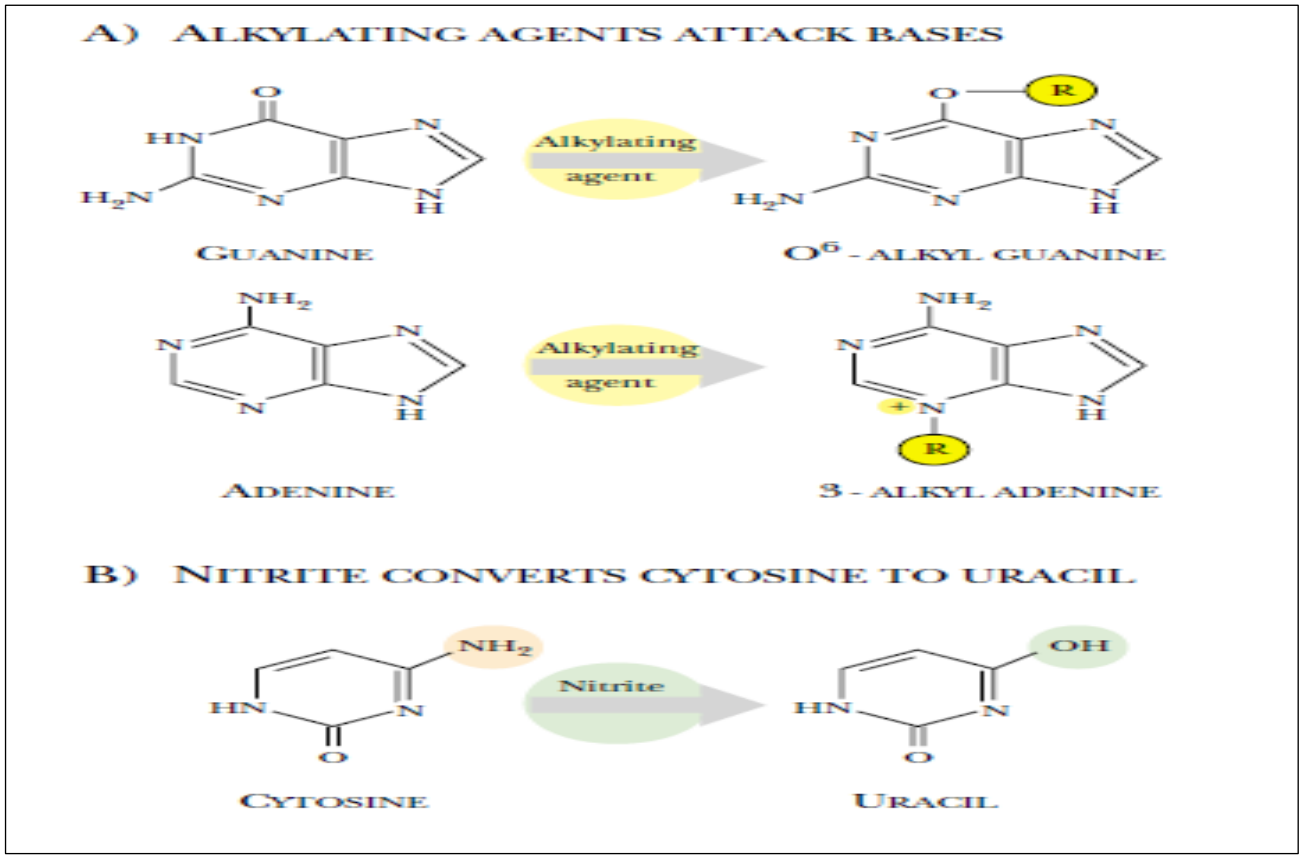

FIGURE 7.1 ***Base Alteration by Chemical Mutagens*** A) Alkylating agents alter the structure of bases by adding alkyl groups. B) Nitrite will convert cytosine to uracil (which pairs with adenine).

Base analogs are chemical mutagens that mimic the bases found in natural DNA. For example, bromouracil resembles thymine in shape. It is converted by the cell to the DNA precursor, bromouridine triphosphate, which DNA polymerase inserts where thymine should go. Unfortunately, bromouracil can flip-flop between two alternative shapes . In its alternate form, bromouracil resembles cytosine and pairs with guanine. If

bromouracil is in its misleading form when DNA polymerase arrives, a G will be put into the new strand opposite the bromouracil instead of A. Some mutagens imitate the structure of a base pair rather than a single base. For example, **acridine orange** has three rings and is about the size and shape of a base pair. Acridine orange is not chemically incorporated into the DNA. Instead, it squeezes in between the base pairs in the DNA a process referred to as **intercalation**. During DNA replication, the DNA polymerase mistakes the intercalating agent for a base pair and puts in an extra base when making the new strand. As discussed above, insertion of an extra base will change the reading frame of the protein encoded by agene. Since this will completely destroy the function of the protein, intercalating agents are highly hazardous mutagens.

A **teratogen** is an agent that causes abnormal development of the embryo, which results in gross structural defects. Teratogens may or may not cause mutations. The most famous example is thalidomide, which resulted in the birth of malformed children with missing limbs. Thalidomide interferes with the development of embryos as opposed to causing mutations. Although the mechanism responsible for the malformations remains uncertain, it is known that thalidomide prevents blood vessels from forming (i.e. it is anti-angiogenic), which may partly explain the drug's ability to cause birth defects.

B- Radiation Causes Mutations

Some types of radiation cause mutations. High frequency electromagnetic radiation, ultraviolet radiation (UV light), X-rays and gamma rays (g-rays), directly damage DNA. X-rays and g-rays are **ionizing radiation**; that is, they react with water and other molecules to generate ions and free radicals, notably hydroxyl radicals. Ionizing radiation is responsible for about 70 percent of the radiation damage to DNA. The other 30 percent of the radiation damage is due to direct interaction of X-rays and g-rays with DNA itself. In the early days of molecular biology, X-rays were often used to generate mutations in the laboratory. X-rays tend to produce multiple mutations and often yield rearrangements of the DNA, such as deletions, inversions and translocations. Ultraviolet radiation is electromagnetic radiation with wavelengths from 100 to 400 nm. It is nonionizing and acts directly on the DNA. The bases of DNA show an absorption peak at around 254 nm and UV close to this wavelength is absorbed very efficiently by DNA. In particular, UV causes two neighboring pyrimidine bases to cross-react with each other to give dimers. Thymine dimers are especially frequent . Although DNA polymerase can proceed by skipping over thymine dimers, this leaves a single-stranded region that needs repairing. The repair process in turn causes the insertion of incorrect bases in the newly synthesized strand. Ultraviolet radiation is emitted by the sun. Most of it is absorbed by the ozone layer in the upper atmosphere, so it does not reach the surface of the earth. Damage to the ozone layer by the chlorinated hydrocarbons used in aerosol sprays and refrigerants has allowed more UV radiation to reach the surface of this planet, especially in certain

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areas. This has probably contributed to the increased frequency of skin cancer noted in recent years. **In addition to electromagnetic radiation**, there are other forms of radiation, such as the a-particles and b-particles emitted by radioactive materials along with g-rays. Most a-particles are too weak even to penetrate skin but b-particles may cause significant damage to DNA and other biological molecules. However, a-emitters can be mutagenic if they have entered the body, for example by being breathed in or swallowed (figure 7.2).

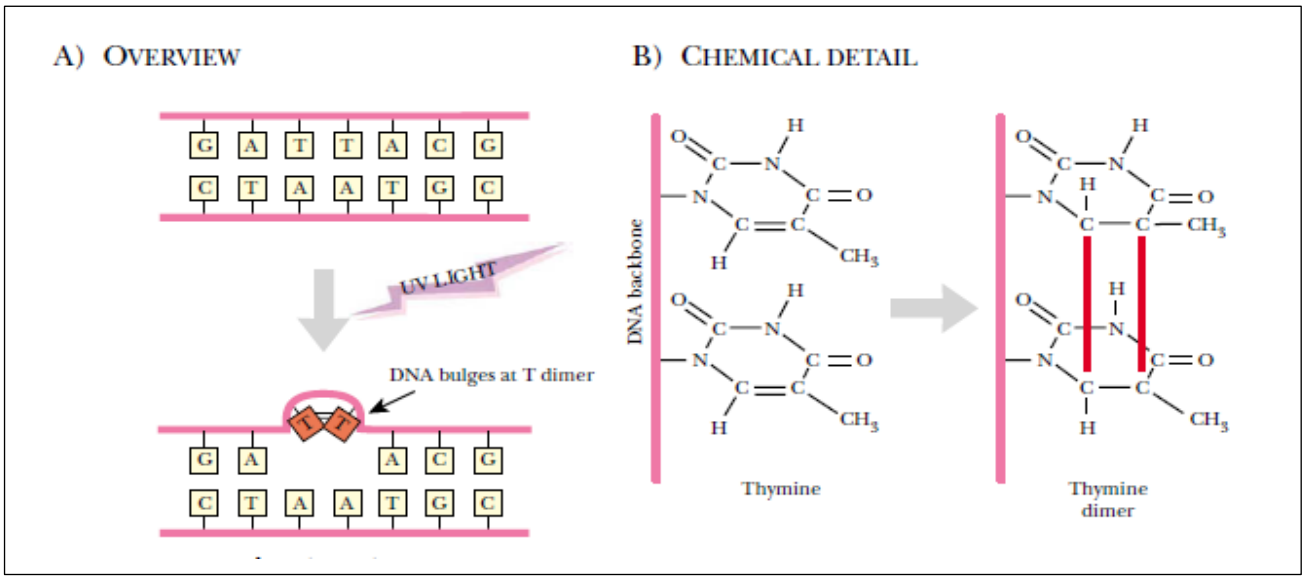

FIGURE 7.2 ***Thymine Dimers*** A) Ultraviolet light (UV) sometimes results in the formation of a thymine dimer (red). B) The detailed chemical structure of the thymine dimer is shown.

C- Biological mutagens:- such as virus and transposons that can isert themselves with in a gene and destroy its function

2-Phenotypical classification of mutation

Although all mutations represent biochemical changes ,they have various manifestations such as

1- **Colour mutation ;-** these change in the colour e.g. red eyes to white eyes in *Drosophila* , red flowers to whit flowers in peas plant
2- **Morphological mutation**;- these change the morphology of the mutant e.g normal wings to curly wings in *Drosophila* , normal coloniesto fluffy colonies in the fungus *Aspergillus* .
3- **Resistant mutation** ;- these make mutant capable of growth on chemical or antibiotic that are toxic to the wild type e.g drug resistant mutations in microorganisms .

4- **Auxtrophic (nutritional) mutations** ;- these make mutant unable to grow on the minimal medium (MM) unless a certain nutrient is added to the medium.the wild type can grow on MM and is called prototrophic .

3- The Major Types of Mutation

A single mutation is a single event and a multiple mutation is the result of several events. A single mutational event, however large or complex its effect, is regarded as a single mutation. A mutation that involves only a single base is known as a **point mutation**. A **null mutation** totally inactivates a gene; the expression "null mutation" is a genotypic term. Complete absence of a gene product may or may not cause a detectable phenotype. A **tight mutation** is one whose phenotype is clear-cut. The complete loss of a particular enzyme may result in no product in a particular biochemical pathway. For example, the complete inability of a bacterium to grow if provided with a certain sugar is an example of a tight mutation. A **leaky mutation** is one where partial activity remains. For example, 10 percent residual enzyme activity might allow a bacterium to still grow, albeit very slowly.
The major types of sequence alteration are as follows, and will be discussed separately below:

1. **Base substitution:** one base is replaced by another base.If one base is replaced by another, a base substitution mutation has occurred. These may be subdivided into **transitions** and **transversions**. In a transition a pyrimidine is replaced by another pyrimidine (i.e., T is replaced by C or vice versa) or a purine is replaced by another purine (i.e., A is replaced by G or vice versa). A transversion occurs when one base is replaced by another of a different type; for example, a pyrimidine is replaced by a purine or vice versa.
2. **Insertion:** one or more bases are inserted into the DNA sequence. Genes may also be inactivated by insertions of DNA. If a foreign segment of DNA is inserted into the coding region, then the gene is said to be disrupted. (Fig. 7.3).The cause of insertion mutations may be divided into two distinct categories. Some of these mutations are the result of **mobile genetic elements**, usually thousands of bases long, inserting themselves into a gene. Other insertion mutations, usually only one or a few bases long, are caused by mutagenic chemicals or by mistakes made by DNA polymerase.

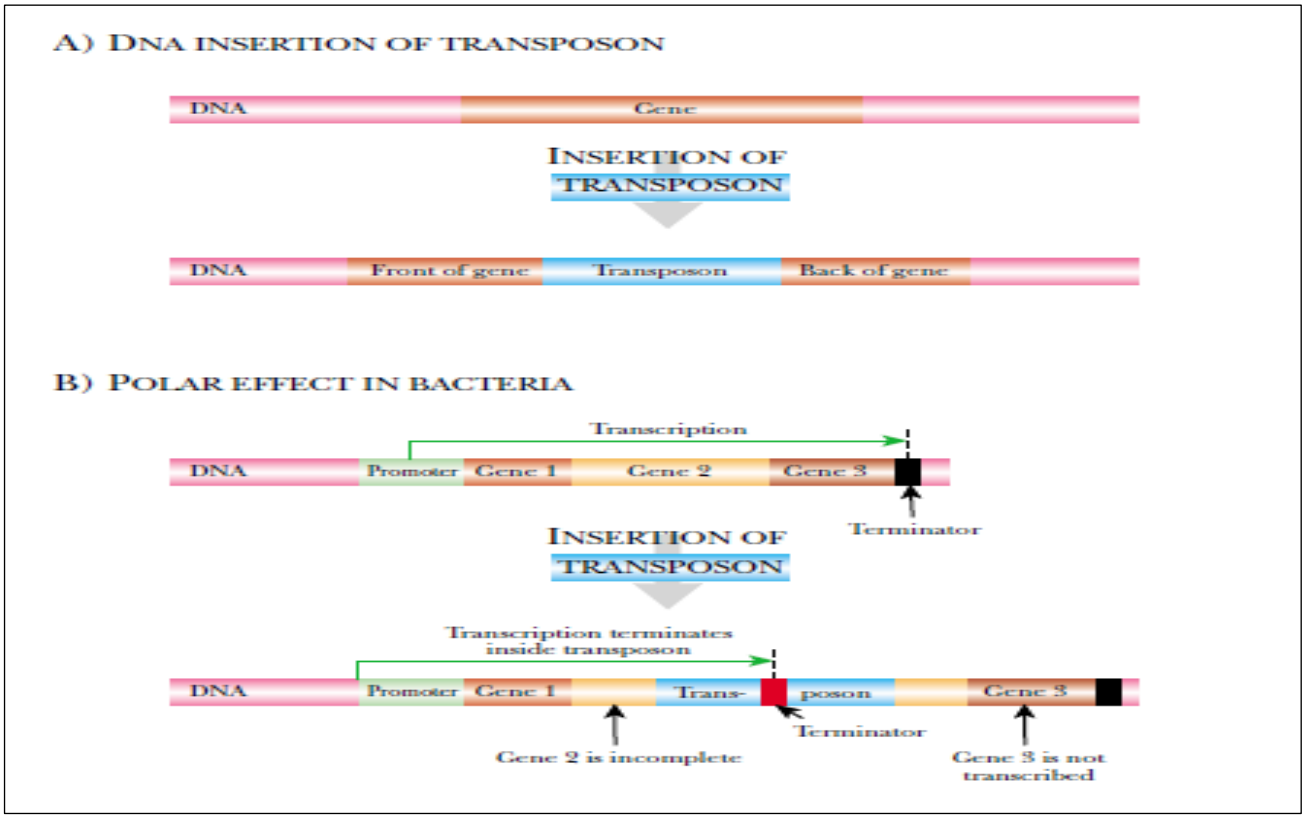

FIGURE 7.3 ***Effects of Insertion Mutations*** A) Insertion of a transposon into the middle of a gene interrupts the coding sequence. B) Insertion of a transposon into the second gene of a bacterial operon with three genes. Gene 1 is the only gene correctly transcribed since the transposon disrupts gene 2 and causes premature termination. Gene 3 will not be transcribed, although its coding sequence is still intact

Occasionally, insertions may activate genes. If an insertion occurs in the recognitionsite for a repressor, binding of the repressor will be prevented and activation of the gene may result.

3. **Deletion:** one or more bases are deleted from the DNA sequence. In particular, we should distinguish between point mutations where one (or a very few) bases are affected, and gross deletions and insertions that affect long segments of DNA. Point deletions and insertions may have major effects due to disruption of the reading frame—see below. Here we will consider the effects of larger deletions. Large deletions may remove part of a gene, an entire gene or several genes. Deletions may also remove part or all of the regulatory region for a gene. Depending on the precise region removed, gene expression may be decreased or increased. For example, a deletion that removes the binding site for a repressor may result in a large increase in activity of the gene in question. Thus loss of DNA may result in elevated activity (fig. 7.4).

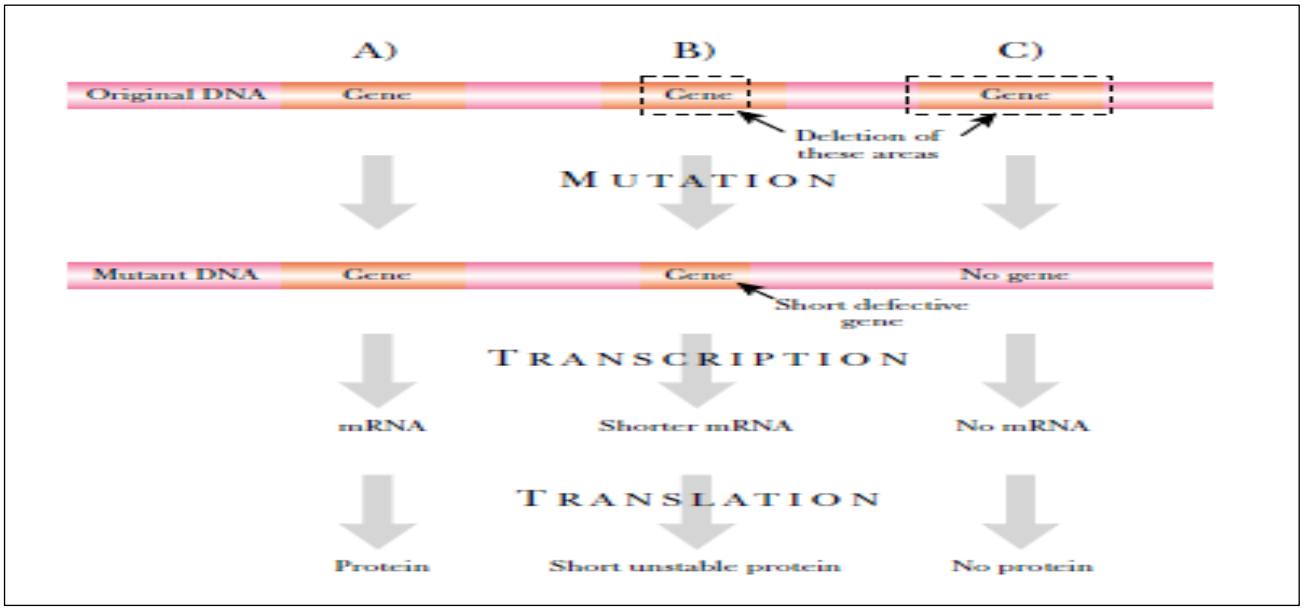

FIGURE 7.4 ***Effects of Deletion Mutations*** A) The wild-type gene produces a normal mRNA and a normal protein. B) A large deletion causes a shorter mRNA and a short unstable protein. C) Deletion of an entire gene results in no mRNA and no protein.

4. **Inversion:** a segment of DNA is inverted, but remains at the same overall location.
5. **Duplication:** a segment of DNA is duplicated; the second copy usually remains at the same location as the original.
6. **Translocation:** a segment of DNA is transferred from its original location to another position either on the same DNA molecule or on a different DNA molecule (Fig. 7.5)

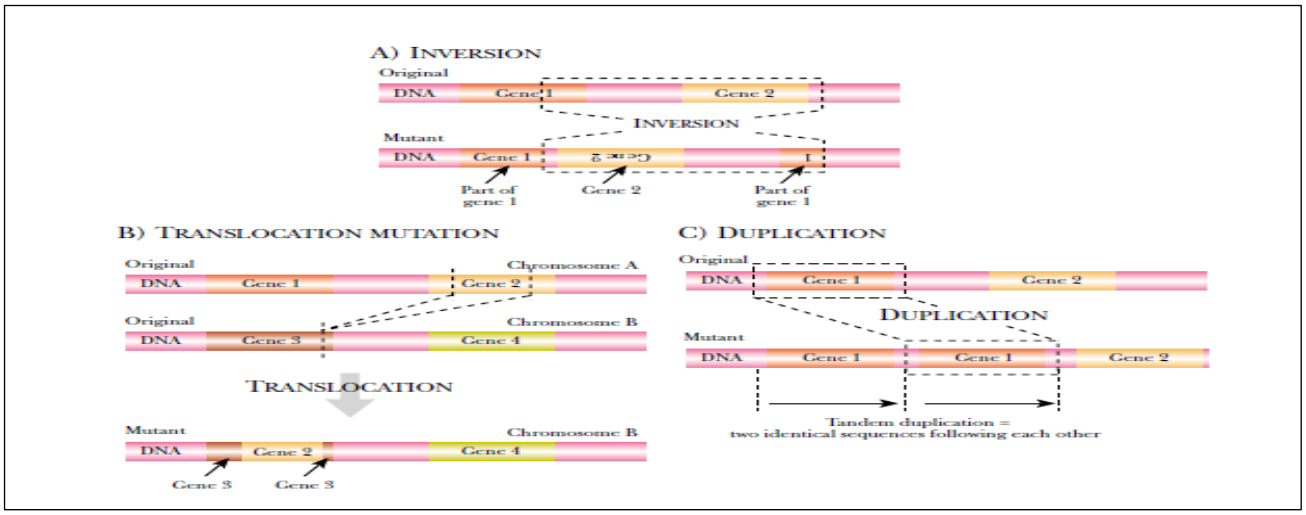

FIGURE 7.5 ***Inversions, Translocations and Duplications***