Phenotypic effects and variations in the genetic material

(part 2)

The composition of DNA molecule:

DNA molecule is composed of two strands coiled around each other like a spiral ladder called double helix where each side consists of both deoxyribose sugar (five-carbon sugar) and phosphate group. Its steps consist of groups of nitrogenous bases. The base may be either ring base (a purine) adenine (A) and guanine (G), or single-ring base (a pyrimidine) cytosine (C) and thymine (T). The adenine pairs with thyamine (A = T), while the guanine pairs with cytosine (G = C). Hydrogen bonds between the base portions of the nucleotides hold the two chains together. Because these two chains are held together by hydrogen bonding between the bases on the different strands, all the bases are on the inside of the double helix, and the sugar-phosphate backbones are on the outside in alternating form. This complementary base-pairing enables the base pairs to be packed in the energetically most favorable arrangement in the interior of the double helix. In this arrangement, each base pair is of similar width, thus holding the sugar-phosphate backbones an equal distance apart along the DNA molecule. The way in which the nucleotide subunits are lined together gives a DNA strand a chemical polarity. If we think of each sugar as a block with a protruding knob (the 5′ phosphate) on one side and a hole (the 3′ hydroxyl) on the other, each completed chain, formed by interlocking knobs with holes, will have all of its subunits lined up in the same orientation. Moreover, the two ends of the chain will be easily distinguishable, as one has a hole (the 3′ hydroxyl, -OH) and the other a knob (the 5′ phosphate, P) at its terminus. This polarity in a DNA chain is indicated by referring to one end as the 3′ end and the other as the 5′ end (i.e. its two ends are
chemically different). The 3’ end carries an unlinked -OH group attached to the 3’ position on the sugar ring; the 5’ end carries a free phosphate group attached to the 5’ position on the sugar ring.

To maximize the efficiency of base-pair packing, the two sugar-phosphate backbones wind around each other to form a double helix, with one complete turn every ten base pairs. Each turn of DNA is made up of 10.4 nucleotide pairs and the center-to-center distance between adjacent nucleotide pairs is 3.4 nm. The coiling of the two strands around each other creates two grooves in the double helix. The wider groove is called the major groove, and the smaller the minor groove.
DNA replication is the process by which DNA makes a copy of itself during cell division:

1. The first step in DNA replication is to ‘unzip’ the double helix structure of the DNA molecule.

2. This is carried out by an enzyme called helicase which breaks the hydrogen bonds holding the complementary bases of DNA together (A with T, C with G).

3. The separation of the two single strands of DNA creates a ‘Y’ shape called a replication ‘fork’. The two separated strands will act as templates for making the new strands of DNA.

4. One of the strands is oriented in the 3’ to 5’ direction (towards the replication fork), this is the leading strand. The other strand is oriented in the 5’ to 3’ direction (away from the replication fork), this is the lagging strand. As a result of their different orientations, the two strands are replicated differently:

![DNA replication fork diagram](image-url)
Leading Strand:
5. A short piece of RNA called a **primer** (produced by an enzyme called primase) comes along and binds to the end of the leading strand. *The primer acts as the starting point for DNA synthesis.*
6. **DNA polymerase** binds to the leading strand and then ‘walks’ along it, adding new **complementary nucleotide** bases (A, C, G and T) to the strand of DNA in the 5’ to 3’ direction.
7. This sort of replication is called *continuous*.

Lagging strand:
5. Numerous RNA primers are made by the primase enzyme and bind at various points along the lagging strand.
6.Chunks of DNA, called Okazaki fragments, are then added to the lagging strand also in the 5’ to 3’ direction.
7. This type of replication is called *discontinuous* as the Okazaki fragments will need to be joined up later.

In both cases:
8. Once all of the bases are matched up (A with T, C with G), an enzyme called **exonuclease** strips away the primer(s). The gaps where the primer(s) were are then filled by yet more complementary nucleotides.
9. The new strand is proofread to make sure there are no mistakes in the new DNA sequence.
10. Finally, an enzyme called DNA ligase seals up the sequence of DNA into two continuous double strands.
11. The result of DNA replication is two DNA molecules consisting of one new and one old chain of nucleotides. This is why DNA replication is described as *semi-conservative*, half of the chain is part of the original DNA molecule, half is brand new.
12. Following replication the new DNA *automatically winds up* into a double helix.
So what are nucleotides for, exactly?

The nucleotides act as a special language, one used to 'write the recipes' for chemicals the organism makes, specifically **proteins**. Most stretches of nucleotides are called **junk DNA** because they don't code (contain the recipe) for anything. A small fraction, however, is critical to your survival and to making the organism that it is. This 2% of nucleotides code for every protein in the body makes and are on stretches of DNA called **genes**. Each gene codes for a chain of **amino acids** which results in a specific protein.

**Protein synthesis** is the series of steps taken by cells in order to create a functional protein, used for building muscle, repairing tissue, and functioning as enzymes. These steps involve varying parts, but one vital component exists within them. This vital component is called **messenger RNA**, or **mRNA**, which is the link between your DNA and creation of an actual protein.
Protein Synthesis

There is an organelle called a ribosome (rRNA) that exists outside the nucleus, which is responsible for reading the message contained in DNA and then synthesizing proteins. mRNA carries the message coded by our DNA outside the nucleus to the ribosome. The ribosome can then read this message and produce protein in a process called translation.

In protein synthesis, a succession of transfer RNA molecules (tRNA) charged with appropriate amino acids are brought together with an mRNA molecule and matched up by base-pairing through the anticodons (three letters complementary to codon) of the tRNA with successive codons (three letters) of the mRNA. The amino acids are then linked together to extend the growing protein chain, and the tRNAs, no longer carrying amino acids, are released after facing a stop codon (UAA, UAG, or UGA).
II. Mutation (or point mutation)

It is a change in the nature of the hereditary factors that control the traits of a living organism which results in a change in the living organism’s traits. This change in the hereditary trait is mainly changes in the chemical composition of one or more genes i.e. changes at the DNA level (nucleotide sequence) so, it is may be a permanent and not repaired according to its strength.

Mutations in genes can have either with no effect (neutral), or beneficial (alter the product of a gene), or detrimental (prevent the gene from functioning properly or completely).

Mutations can be classified according to (1) the inheritance or (2) origin of mutation.

(1) according to inheritance

Germinal Mutations: They occur in reproductive cells (germ cells/eggs and sperms) so, transmit to the offspring of the mutant individual (heritable) but not the individual itself.

Somatic Mutations: They occur in somatic cells so, affect only the individual and are not transmissible to future generation (non-heritable).

(2) according to origin of mutation

Spontaneous Mutations: Its percentage is very low in nature. It occurs due to the influence of the surrounding environment and no mutagens are involved in it (natural processes).

It causes natural variations between genera and species and are inherited across the successive generations.

Induced Mutations: It is controlled by human to obtain desirable traits.

Mutations can be induced by either physical or chemical ways.
Irradiation is an example of physical mutagen, such as X-rays, gamma-rays and Ultraviolet light (UV) are the common examples used. Irradiation causes the breakage of chromosomes, which may result in chromosomal rearrangements or other events that may be lethal to the cell.

Ethyl methanesulfonate (EMS), N-methyl-N’-nitro-N-nitrosoguanidine (MNNG), N-methyl-N-nitrosoguanidine (NTG), ethidium bromide (EtBr) and acridine orange (AO) are example of chemical mutagens. They can act in a variety of ways depending on the properties of the chemical and its reactions with the bases of the DNA.

**What are they mode of actions and applications?**

Throughout the life of an organism, its cells are exposed to number of agents that have the potential to damage the DNA and so, induced mutations. Accumulated damage to the DNA over a period of time is considered to be a case of transformation of cells to an abnormal state. For the cell to overcome this damage, a variety of repair mechanisms have evolved that serve to reverse the effects of some spontaneous and induced mutations as pyrimidine dimers and nucleotide excision repair.

**I. UV radiation:**

It is less energetic, and therefore non-ionizing, but its wavelengths (as photons) are preferentially absorbed by bases of DNA (purines and pyrimidine) and by aromatic amino acids of proteins, so it has important biological and genetic effects.

When cells (eukaryotic or prokaryotic) are subjected to UV light, the reaction of UV photons with cellular DNA occurs. As photons are absorbed by DNA molecules (mainly bases), an excited state is produced which allows for the rearrangement of electrons resulting in the formation of photoproducts. The photoproducts interfere with DNA replication (if not repaired) and cause specific mutations in DNA. DNA sequences of
the exposed cells form pyrimidine dimers by which adjacent pyrimidine molecules (thymine or cytosine bases) link together by covalent attachment (C≡C or T=T double bonds) (figure below).

![DNA damage diagram](image)

Linked pyrimidine molecules are unable to encode purines on messenger RNA molecules during the process of protein synthesis. Moreover, replication of the chromosome in binary fission is impaired. The damaged organism can no longer produce critical proteins or reproduce, and it quickly dies. Ultraviolet light is especially effective in inactivating viruses. However, it kills far fewer bacteria than one might expect because of DNA repair mechanisms. Once DNA is repaired, new molecules of RNA and protein can be synthesized to replace the damaged molecules.

**Application:**

Ultraviolet light used as surface and air disinfectant effectively reduces the microbial population only when direct exposure takes place as it is not very penetrating. It is used to limit airborne or surface
contamination in a labs hospital room, morgue, pharmacy, toilet facility, or food service operation.

Ultraviolet (UV) light consists of light of wavelengths between 40 to 390 nm, but wavelength in the 200 nm range are most effective in killing the vegetative cells of microorganisms. But according to some books, wavelength between 260 – 265 nm is most effective. It is noteworthy that UV light may not be that effective against all bacterial spores.

**Note:** UV light can cause permanent damage human eyes, and prolonged exposure can cause burns and skin cancer in humans. So to help sanitize the air without irradiation humans, these lights can be turned on when there rooms are not in use.

### II. Ionizing radiation:

Both, X rays and Gamma rays have wavelength shorter than the wavelength of ultraviolet light. X rays have wavelength of 0.1 to 40 nm, and gamma rays, which have even shorter wavelength, are forms of ionizing radiation, so named because it can dislodge electrons from atoms, creating ions. Ionizing radiation damages DNA and produces peroxides, which act as powerful oxidizing agents in cells. This radiation can also kill or cause mutations in human cells if it reaches them.

**Production of Gamma rays:**

Gamma rays are emitted by certain radioactive elements such as cobalt, and electron beams are produced by accelerating electrons to high energies in special machines. Gamma rays penetrate deeply but may require hours to sterilize large masses. In comparison with other physical modification methods, such as microwave, UV, ultrahigh hydrostatic pressure and hydrothermal treatment, irradiation treatment is rapid, convenient and more extensive because ionizing energy penetrates through the polysaccharide granule rapidly.
Production of X rays:

X rays, which are produced by machines in a manner similar to the production of electron beams, are similar in nature to gamma rays. High energy electron beams have much lower penetration power but usually require only a few seconds of exposure.

Mode of action:

Radiation effects on living organisms are mainly associated with the chemical changes but are also dependent on physical (dose rate, dose distribution, radiation quality) and physiological/environmental (temperature, moisture content and oxygen concentration) factors. The action of radiation on living organisms can be divided into direct and indirect effects. Normally, the indirect effects occur as an important part of the total action of radiation on it. One, or a few, hits of the ionized particles may only cause nonlethal mutations, some of them conceivably useful. More hits are likely to cause sufficient mutations to kill the microbe: there is considerable variability in radiation resistance between microbial species; in general, viruses are more radiation resistant than bacterial spores, which in turn are more resistant than vegetative organisms, yeasts and moulds.

Much progress has been made towards identification of the mechanism of inactivation. Many hypotheses have been proposed and tested regarding the mechanism of cell damage by radiation (radiotoxins). Nowadays, it seems certain that lethality is primarily the consequence of genetic damage, directly damaging the cellular membranes, enzymes or energy metabolism.

The principal effect of ionizing radiation is the ionization of water, which forms highly reactive radicals (OH\(^-\), e\(^-\)aq and H\(^+\)).
The action of the hydroxyl radical (OH\textsuperscript{-}) is responsible for an important part of the indirect effects as it targets the organic cellular components as DNA in the chromosomes. Ionizing radiation can affect DNA either directly, by energy deposition in this critical target, or indirectly, by the interaction of radiation with other atoms or molecules in the cell or generate metabolites that can modify important cell components or surrounding the cell like water.

In particular, radiation interacts with water, leading to the formation of free radicals that can diffuse far enough to reach and damage DNA. Mainly OH\textsuperscript{-} radicals are in action forming the hydration layer around the DNA molecule, which are responsible for 90% of DNA damage. Consequently, in a living cell, the indirect effect is especially significant. In a general sense, the death of a microorganism is a consequence of the ionizing action of the high energy radiation. It is estimated that the irradiation of a living cell at one gray induces 1000 single strand breaks, 40 double strand breaks, 150 cross-links between DNA and proteins and 250 oxidations of thymine.

**Application:**

1) **Food sterilization (food preservation)** by gamma irradiation is the process of exposing food to ionizing radiation to destroy microorganisms, bacteria, viruses, or insects that might be present in the food. Irradiated
food does not become radioactive, but in some cases there may be some chemical changes. The use of ionizing radiation depends on a country as food irradiation is illegal in some countries. The food industry use radiation to prevent spoilage in seafood by doses of 100 to 250 kilorads, in meats and poultry by doses of 50 to 100 kilorads, and in fruits by doses of 200 to 300 kilorads. Such foods are quite safe and free of both pathogens and radiation. Note: Ionizing radiation not only kills microorganisms in food but also affects material properties of the vessels. Medical devices are made of many different materials, some of which are metals, but most are non-metals, such as ceramics. Radiation itself does not directly affect metals since sterilization energies are safely below any activation thresholds.

2) Several types of microorganism, mainly bacteria and, less frequently, moulds and yeasts, have been found on many pharmaceuticals, disposable dental and medical supplies such as plastic syringes, surgical gloves, suturing materials, and catheters. So high energy electron beams, is used for their sterilization. Note: The sterilization process must be validated to verify that it effectively and reliably kills any microorganisms that may be present on the presterilized product. Radiation sterilization, as a physical cold process, has been widely used in many developed and developing countries for the sterilization of health care products. Earlier, a minimum dose of 25 kGy was routinely applied for many medical devices, pharmaceutical products and biological tissues. Now, as recommended by the International Organization for Standardization (ISO), the sterilization dose must be set for each type of product depending on its bioburden. Generally, the determination of sterilization dose is the responsibility of the principal manufacturer of the medical product, who must have access to a well-qualified microbiology laboratory.
3) As a protection against bioterrorism, the postal service often uses electron beam radiation to sterilize certain classes of mail.

4) These radiations can be used to differentiate between Gram positive and negative bacteria. Gram-positive bacteria are more sensitive to ionizing radiations than gram-negative bacteria.
5) Ionizing radiations are currently used to sterilize such heat sensitive pharmaceuticals as vitamins, hormones, and antibiotics, as well as certain plastics and suture materials.

6) Ionizing radiation is the basic tool of nuclear technology for crop improvement as it causes induced mutations in plants. These mutations might be beneficial and have higher economical values by improving many useful traits in plants as size, flowering time and fruit ripening, fruit color and resistance to pathogens. In wheat, research efforts were to develop plant types with reduced height, which would enable them to tolerate gusty wind and control losses due to lodging and subsequently grain yield.
THE GENETIC EFFECTS OF CHRONIC GAMMA IRRADIATION IN BARLEY

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I. INTRODUCTION

Until recently, mutations in crop plants have been primarily induced by acute irradiations of seeds, pollen grains or dormant buds. There appear to be distinct differences as to the modes of action and the relative efficiencies of y-rays, X-rays, and gamma radiations. This fact, as well as differences in rates and types of mutations produced, has been illustrated in the volume "Mutations in Plants" (Acta Agric. Scand., Vol. IV, 1934). As a means of completing and improving the methods of irradiating for the breeding of new mutations, a co-operative project was established in 1955 at the Harpenden Research Institute, and this project has an average of 100 mutations per year. Although planned for the induction of bud sports in fruit-trees, the Harvard source at Harpenden has become useful for the artificial production of mutations in crop plants, too, as well as for studies on the influence of continuous irradiation on biochemical processes and on plants in general. In this connection we want to refer to similar American studies by Weisenburger and Mattoni (1913), and Weeden et al. (1918).
III. Chemical mutagens:

Chemical mutagens are defined as those compounds that increase the frequency of some types of mutations. They vary in their potency since this term reflects their ability to enter the cell, their reactivity with DNA, their general toxicity, and the likelihood that the type of chemical change they introduce into the DNA will be corrected by a repair system. Most of the following mutagens are used in vivo treatments, but some of them can also be used in vitro.

Mode of action:

1- Base analog mutagens

Base analog mutagens are chemicals that structurally resemble normal bases so, fool the DNA replication system. Their essential property is that they base-pair with two different bases thus making mutations because of their lack of consistency in base-pairing as bromouracil and aminopurine. To be mutagens they must be incorporated into the DNA and therefore they need to be present during active DNA synthesis.

5-bromo-deoxyuridine (5-BU), which can exist in two tautomeric forms: typically it exists in a keto form (T mimic) that pairs with A, but it can also exist in an enol form (C mimic) that pairs with G. Aminopurine -adenine analog which can pair with T or (less well) with C.
Each of these chemicals will continue to mutagenize with time because of their constant likelihood of mispairing. By the same argument, it requires subsequent rounds of replication for any mutation to be generated since this requires "mispairing" during replication. Further, it takes another round of replication before the mutation is stabilized, that is, before both strands of DNA have the "mutant information". Until that occurs, the mismatch repair system can still recognize and remove the inappropriate base.

2- Alkylators

Alkylation is defined as replacement of hydrogen on an atom by an alkyl group. Alkylating agents produce highly reactive carbonium ion intermediates or related transition complex which transfer alkyl group to cellular macromolecules by covalent bonds. Alkylating agents cross-link (mispairing) with carboxyl, hydroxyl, amino, sulphydryl and phosphate groups of biomacromolecules results abnormal base paring/ scission of DNA Stand.
These chemicals and thus do not require active DNA synthesis in order to act but still do require DNA synthesis in order to be "fixed". This interferes in the separation of strands and prevents mitosis or arrest cell replication. They are very commonly used because they are powerful mutagens in nearly every biological system. Examples of alkylators include ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), diethylsulfate (DES), and nitrosoguanidine (NTG, NG, MNNG). These mutagens react directly with certain bases but tend to prefer Guanine rich regions, reacting to form a variety of modified G residues, the result often being depurination.
3- Other chemical mutagens

Nitrous acid is another chemical mutagen that causes *oxidative deamination of particular bases*. It converts adenine to hypoxanthine (which now pairs with C), cytosine to uracil (which now pairs with A) and finally guanine to xanthine (which still continues to pair with C). Unlike the above mutagens, nitrous acid alters a base directly to a "miscoding" form (baseless site) and thus does not require subsequent DNA synthesis for its effect.

![Chemical structures](image)
4- Intercalating agents

Intercalating agents (acridine orange, ethidium bromide, proflavine) (figures below) are planar, polycyclic compounds, which can slip between the stacked bases in the major groove of the DNA. Presence of these mutagens stretches the DNA strand thus fooling the replication machinery into inserting extra nucleotide/nucleotides in the opposite strand (insertion mutation).
5- Mutators

Mutations that deleteriously affect the fidelity of DNA replication or repair cause an increase in the basic mutation frequency and are called mutators. There are a number of sorts of these mutations with different levels and types of effects. *Transversion mutations* are those base substitution mutations where a purine replaces a pyrimidine or vice versa (mutT mutations which increase mutation frequency 103-104 times and are specific for A/T to C/G). *Transition mutations* are those where a purine replaces a different purine or where a pyrimidine replaces a
different pyrimidine (mutD generate a range of error types). mutS mutations induced both Transversion and transition mutations.

![Diagrams of Transitions and Transversions](image)

**Note:** Many metals, such as arsenic, cadmium, chromium, nickel and their compounds may be mutagenic. They act in different number of ways like the production of ROS, alteration of the fidelity of DNA replication, DNA hypermethylation and histone deacetylation. Metals such as cobalt, arsenic, nickel and cadmium may also affect DNA repair processes such as DNA mismatch repair, and base and nucleotide excision repair.

**Types of point mutations**

Point mutations can cause changes to an organism. A mutation in DNA alters the mRNA code, which in turn can change what kind of protein is produced.

Point mutations within a gene may be classified either as:

1) base-pair substitutions, or 2) as base-pair insertions or deletions.

**I. Base-pair substitution mutations:**

It is resulted from qualitative changes in nucleotide composition of a codon i.e. changes of a single nucleotide pair may switch an amino acid. A **missense mutation** makes a slight change to a protein, a **nonsense mutation** blocks a protein's production, a **silent mutation** does not affect
the protein at all and a **readthrough mutation**. These three different effects are all caused by **base substitutions**. They all result from the switching of one base for another.

1) **Silent Mutation**: happen when the new amino acid may have properties similar to those of the amino acid it replaces or the new amino acid is in a region of the protein not essential to its function.

2) **Missense Mutation**: It has a detectable change in a protein. This happen when the alteration of a single amino acid in a crucial area of a protein as in the active site of an enzyme.

Example of missense mutation: **In sickle-cell anemia**. This disease is caused by a slight change in the primary structure of hemoglobin protein of the red blood cells. The normal allele on DNA has the sequence CTT,
which give the mRNA codon, GAA, which codes for the amino acid, glutamic acid. If the allele on DNA has the sequence CAT so, the new mRNA codon will be GUA i.e. one A (adenine) is substituted by U (uracil), new mRNA codon will then codes for the amino acid valine instead of glutamic acid. This change in amino acids leads to abnormal hemoglobin molecules in form of sickle shape.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Missense Mutation</th>
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<tbody>
<tr>
<td>Partial DNA Sequence of Beta Globin Gene: CCT GAG GAG</td>
<td>CCT GTG GAG</td>
</tr>
<tr>
<td>Partial RNA Sequence: GGA CTC CTC</td>
<td>GGA CAC CTC</td>
</tr>
<tr>
<td>Partial Amino Acid Sequence for Beta Globin: CCU GAG GAG</td>
<td>CCU GUG GAG</td>
</tr>
<tr>
<td>Pro — Glu — Glu</td>
<td>Pro — Val — Glu</td>
</tr>
<tr>
<td>Hemoglobin Molecule:</td>
<td></td>
</tr>
<tr>
<td>Red Blood Cell:</td>
<td></td>
</tr>
</tbody>
</table>

**Application:**
- Improvement of myrosinase activity of *Aspergillus* sp. NR4617 by chemical mutagenesis, performed by Nuansri Rakariyatham and his coworkers, published in Electronic Journal of Biotechnology Vol. 9 No. 4, 2006.

3) **Non sense Mutation:** It has an inhibitory effect on the protein. This happen when the mutation is detrimental and creating a useless or less active protein that impairs cellular function. Readthrough Mutation
(non-stop) is a type of non-sense mutation in which the continuation of transcription of DNA happen beyond a normal stop signal, or terminator sequence (do not recognize the stop signal). Readthrough occur in translation, when a mutation has converted a normal stop codon into one encoding an amino acid. This result in extension of the polypeptide chain until the next stop codon is reached, producing a so-called readthrough protein.

II. Base-pair insertions or deletions (indels):
Those are caused by additions (insertions) or losses (deletions) of one or more nucleotide pairs in a gene. Insertions and deletions actually change the length of the DNA strand because they add or subtract one base pair from the code.

**Insertions:**
Insertion mutations occur when extra nucleotides are put into a DNA sequence, making it longer than it should be.

Eg: a DNA sequence reads CAGC.
If a T was accidentally inserted between the G and C when this sequence was being copied, it would now read CAGTC. An insertion mutation has occurred. If this mutation was in a **gene**, or the part of a DNA sequence that codes for a protein, it could be detrimental and result in the production of a nonfunctional protein.

Insertion mutations can be small, like in the above example in which only one nucleotide was inserted, or they can be large, with many nucleotides being added.

**Deletions:**

One nucleotide has been deleted from the sequence. For example, if the original sequence is ATG-AGT-CGT-ATA-TAA, it will code for methionine, serine, arginine, isoleucine, and finally the STOP codon (telling the cell to stop protein production).

After a point deletion, the new sequence might be ATG-AGC-GTA-TAT-AA. In this case, a T has been deleted. The new amino acid sequence is methionine, serine, valine, tyrosine, and then the final AA doesn't code for anything.

Indels mutations have disastrous effect on the DNA codon and the resulting protein more than substitutions do. Due to the triplet nature of gene expression by codons, the mRNA read amplified nucleotides
during translation, which may alter the reading frame of the genetic message resulting in a completely different translation from the original. All these nucleotides will be improperly grouped into codons. A **frameshift mutation** is a genetic mutation caused by indels of a number of nucleotides in a DNA sequence that is not a multiple of three, which causes a shift in the translational reading frame.

Frameshift mutations have a more dramatic effect on the polypeptide than missense or nonsense mutations. Instead of just changing one amino acid, frameshifts cause a change in all the amino acids in the rest of the gene. The earlier in the sequence the deletion or insertion occurs, the more altered the protein. A frameshift mutation will in general cause the reading of the codons after the mutation to code for different amino acids. The frameshift mutation will also alter the first stop codon ("UAA", "UGA" or "UAG") encountered in the sequence. The polypeptide being created could be abnormally short or abnormally long, and will most likely not be functional.
Eg: Frameshift mutations have been proposed as a source of biological novelty, as with the creation of nylonase (discovered in 1975). **Nylon-eating bacteria** are a strain of *Flavobacterium* that is capable of digesting certain byproducts of nylon 6 manufacture, such as the linear dimer of 6-aminohexanoate. This strain of *Flavobacterium* sp. KI72, became popularly known as nylon-eating bacteria, and the enzymes used to digest the man-made molecules known as nylonase. This discovery led the geneticist Susumu Ohno (1984) to speculate that the gene for one of the enzymes, 6-aminohexanoic acid hydrolase, had come about from the combination of a gene duplication event with a frameshift mutation.

In addition, Frameshift mutations are apparent in Human severe genetical diseases such as Tay-Sachs disease and Cystic Fibrosis.

**How the cell repair or tolerate mutations?**

**DNA repair systems**

Because DNA damage occurs spontaneously and as a result to ubiquitous environmental agents, most organisms possess some capacity to repair their DNA and DNA is the only macromolecule which IS repaired by cells. We can divide "repair" mechanisms into 3 categories: damage reversal, damage removal or damage tolerance.

- **damage reversal**: simplest; enzymatic action restores normal structure without breaking backbone.
- **damage removal**: involves cutting out and replacing a damaged or inappropriate base or section of nucleotides.
- **damage tolerance**: not truly repair but a way of coping with damage so that life can go on.
A. Damage reversal through Photoreactivation

Both prokaryotes (bacteria) and eukaryotes (moulds and yeasts) are capable of repairing many of the different DNA breaks (fractures). Living organisms have developed different strategies to recover from losses of genetic information caused by DNA damages. Photoreactivation is one of the simplest and perhaps oldest repair systems.

The type of DNA damage caused by radiation and some chemicals like peroxides is repaired via the nucleotide excision repair (NER) pathway. This pathway consists of five steps including the following:

1) recognition of DNA lesion (damages to DNA alter its spatial configuration so, that they can be detected by the cell),

2) incision of the damaged strand on both sides of the lesion: it consists of enzyme (photolyase/endonuclease) which can split pyrimidine dimers (break the covalent bond) in presence of light. The photolyase enzyme catalyzes is found in many bacteria, lower eukaryotes, insects, and plants. It seems to be absent in mammals (including humans).

3) removal of the damaged oligonucleotide by exonuclease

4) synthesis of a patch by DNA polymerase (its complementary strand is used to restore it), and

5) ligation of the patch by DNA ligase. Efficient and accurate repair of the damages can take place as long as the integrity of the complementary strand is maintained. Sensitivity is highly influenced by the capability of the strain to repair single-strand breaks as strains that lack this ability are far more sensitive than the others.
B. Damage removal

1. Base excision repair

Base excision repair is a mechanism used to detect, remove certain types of damaged bases from its sugar linkage and replaced them correctly. A group of enzymes called glycosylases play a key role in base excision repair as they cut the base-sugar bond. Each glycosylase detects and removes a specific kind of damaged base. Example: uracil glycosylase--enzyme which removes uracil from DNA. Uracil is not supposed to be in DNA--can occur if cytosine is deaminated. The enzyme recognizes uracil and cuts the glycosyl linkage to deoxyribose. The sugar is then cleaved and a new base put in by DNA polymerase using the other strand as a template.
2. Mismatch Repair

This process occurs after DNA replication as a last "spellcheck" on its accuracy. It is carried out by a group of proteins which can scan DNA and look for incorrectly paired bases (or unpaired bases) which will have aberrant dimensions in the double helix. The incorrect nucleotide is removed as part of a short stretch and then the DNA polymerase gets a second try to get the right sequence.

Many errors are corrected by proofreading (spellcheck), but a few slip through. Mismatch repair happens right after new DNA has been made, and its job is to remove and replace mis-paired bases (ones that were not fixed during proofreading). Mismatch repair can also detect and correct small insertions and deletions that happen when the polymerases "slips," losing its footing on the template.

C. DNA Damage Tolerance

Despite the existence of multiple mechanisms that remove lesions from DNA, repair is usually not fully efficient, and leaves in the DNA unrepaired lesions. These lesions can interrupt DNA replication, leading to the formation of collapsed forks and single-stranded gaps, which if left
untreated prevent completion of replication and can cause highly deleterious (lethal type of DNA damage) double strand breaks (DSB). DNA damage tolerance converts these structures to double-stranded DNA, without removing the lesion, by DNA repair pathways in eukaryotes: Trans-lesion DNA synthesis (TLS; also termed lesion bypass, or error-prone repair), or by homology-directed repair (HDR; also termed homologous recombination repair, template switch or post-replication repair) or by non-homologous end-joining (NHEJ).

**Trans-lesion DNA synthesis:**

If cells cannot repair some lesions, there is a fail-safe mechanism that allows the replication machinery to bypass these sites of damage. This mechanism is known as trans-lesion synthesis. But because of its high error rate, trans-lesion synthesis can be considered a system of last resort. Trans-lesion synthesis is catalyzed by a specialized class of DNA polymerases that synthesize DNA directly across the site of the damage.

**Translesion DNA Synthesis**

![Diagram of Translesion DNA Synthesis](image-url)

- Replicative polymerase encounters DNA damage on template strand
- Catalytic site of replicative polymerases is intolerant of misalignment between template and incoming nucleotide
- Replicative polymerase is replaced by TLS polymerase which inserts a base opposite lesion
- Base pairing is restored beyond the lesion and replicative polymerase replaces TLS polymerase
- TLS can occur in S or G2

A double-strand break (DSB) is the most lethal type of DNA damage and must be repaired before DNA replication, which has led to the evolution of two major DNA repair pathways in Plants and some eukaryotes: Non-homologous end joining (NHEJ) and homology directed repair (HDR) are two main repair pathways. Classical NHEJ may lead to insertions or deletion, while Micro-homology based alternative NHEJ always results in deletions. HDR is less efficient, but can result in precise integration of a donor DNA template into the genome.

**Homology-directed repair:** HDR relies on template DNA. Homologous recombination is an important process that occurs in somatic cells to repair DSBs and in meiotically dividing cells to exchange genetic material between parental chromosomes. The most common conservative HDR mechanism in plants, which repairs almost all DSBs in somatic cells, is the synthesis-dependent strand annealing (SDSA) pathway (Fig. c). As a DSB occurs, 3’ overhangs are extended from the break site. A 5’ end invades the homologous strand forming a D-loop. Synthesis fills in the gaps using homologous DNA as a template, and the 3’ end reanneals with the second 3’ end without crossover. The result is a precisely integrated template or “donor” DNA strand. In nature, template DNA in the form of a sister chromatid or homologous chromosome is not always available, which may hinder HDR. However, synthetic template DNA can be provided exogenously and used for gene insertion, or replacement. There are many exciting applications in basic and applied science using HDR. For example, HDR was used to engineer an herbicide resistant trait in tobacco plants.

**Non-homologous end-joining:** NHEJ is an error-prone repair pathway. When a DSB occurs, NHEJ can quickly and imprecisely, be used in two ways to repair the break:

In classical NHEJ (Fig. a), several different proteins bind to broken DNA ends and are joined together by a ligase that can result in the insertion
or deletion (indel) of nucleotides. NHEJ often leads to frameshift mutations which can result in premature stop codons, rendering genes non-functional (Fig. a, b). This is helpful for creating knockout plants useful for reverse genetic studies, but can also create desirable agricultural traits. For example, a powdery mildew resistant wheat line was created by knocking out three redundant *MLO* genes.

In micro-homology-based alternative NHEJ (Fig. b), 5′ ends are cut until 3′ overhangs with homology are created. DNA strands then bind at their complementary sequence, and flaps of non-homologous DNA are excised. This typically results in deletions as DNA between homologous sections is removed.