4

Molecular biology sheet

Doctors 2022 أثر | medicine | MU

DOCTOR:

Prof. Samir Mahgoub

DONE BY:

Ibrahim Amjad

CORRECTED BY:

Rahaf Mohamed

Mutations and DNA Repair

This lecture is a very important one. In the last lecture we studied that:

***Lac* operon includes genes that encode for 3 enzymes:**

a) ***Lac* Y: encodes lactose permease to transport lactose into the cell for inclusion of energy.**

b) ***Lac Z:* encodes β- galactosidase which cleaves lactose into glucose and galactose to be used for producing energy. However, these metabolic pathways result in catabolites that should be removed by detoxification (detoxication) for removal of toxic compounds. Which is by the way an outdated inaccurate scientific term since not all the products of these pathways are toxic. So, the expression does not really deliver the actual function of enzymes working on this particular function. Accordingly, it was replaced with xenobiotics; which is defined as catabolism (removal) of any foreign compound from the cell regardless its toxicity.**

c) ***Lac* A: encodes galactoside- o- acetyltransferase which is responsible for removing xenobiotics. Why do we have to remove such products? Due to end- product- inhibition concept. To accelerate any interaction there should be availability of reactants, on the same time, to continue the activation of this reaction, catabolites should be removed or they will go back and cause negative feedback inhibition to other products. This negative feedback can be caused by major end products or by- products. For example, ATP (Adenosine Tri Phosphate) is the main objective of all the cell to produce energy to maintain activities. However, Accumulation of ATP inside the cell inhibits more production of it, that's why ATP has to be: 1) exported, 2) consumed by the cell or 3) exchanged for ADP (Adenosine Di Phosphate) to make the ADP higher inside and the ATP higher outside, so that the metabolic pathway is more active and more production of energy (in the form of ATP) is happening for different cellular activities.**

- **We have $3.5 * 10^9$ nucleotides (base pairs, bp) in our genes. If our DNA is laid up end- to- end, the total length will be around 2 meters. We have enough of DNA to make 70 round trips from earth to sun.**
- **During replication of DNA by different types of DNA polymerases, every 500 bps a mistake (mutation) happens. If we took the whole bps ($3.5 *10^9$) / number (#) of bps for each mutation (500) = our bodies almost encounter 70 million mutations during DNA replication.**
- **99% of these mutations are corrected by themselves (spontaneously); as one of the DNA polymerases has a proofreading ability.**

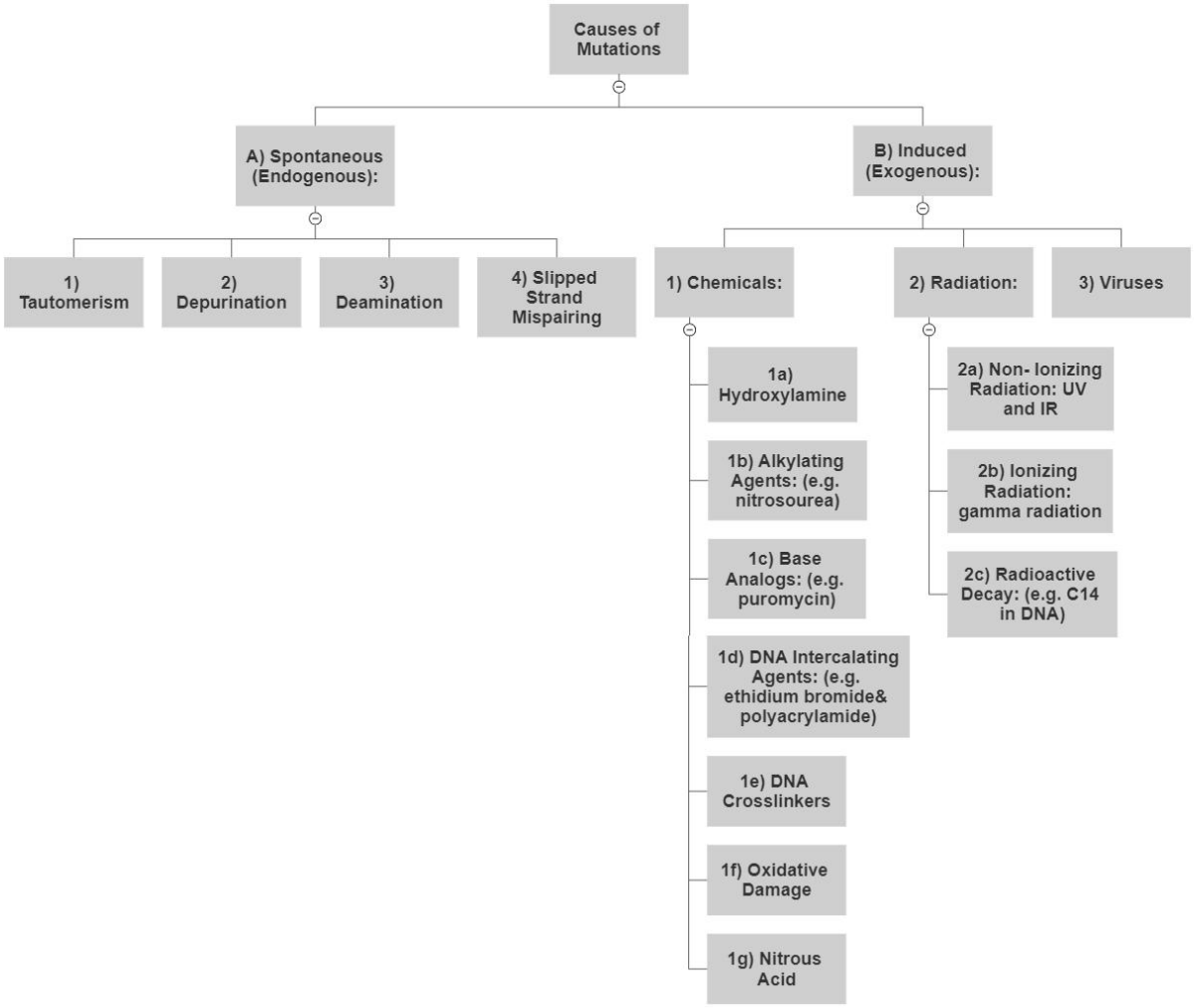
- **Of the remaining 1%:**
  a) **99% happen in the non- coding introns (where they may or may not have effects). Those are regulatory regions.**
  b) **1% happen in the coding regions including: promoter (which is the most dangerous as transcription factors [TFs] won't recognize the binding site and nothing will even start), inside the gene (it depends on the exact site) and 3' end (in this case, mRNA will be longer and consequently less stable as it will be more subjected to degradation, small interfering RNA [siRNA]).**
- **As long as mutations are transient, it'll be repaired and no problems will happen. However, if they are persistent and became permanent, it'll be transmitted from a generation to another and will cause different consequences and most of them tend to have dangerous effects.**
- **In molecular biology and genetics, mutations are changes in a genomic sequence (on different forms: point mutation, deletion or insertion), and can be defined as sudden and spontaneous changes in genomic sequence in the cell. If it's insertion or deletion of a # of bps in a particular region in the DNA frameshift will happen. What if it is of 3 bps? Addition/ deletion of 1 amino acid (aa). This aa could have been added/ removed to/ from a specific region and it will disturb this protein (let it be an enzyme) leading to a loss of its biological activity.**
- **Another example, if an X enzyme has a cysteine aa in its active site with its sulfhydryl group (SH) responsible for the interaction. If this cysteine was deleted; there will be no complete binding between active site of enzyme and binding site of substrate. That being said, insertion/ deletion can have no effect, decrease the activity minimally or inhibit the activity completely.**
- **Mutations are caused by radiation (even if non- ionizing radiations such as UV (ultra- violet) and IR (infra- red) radiations. Due to Ozone (O3) decay and the deliberate tanning on beaches, there was a noticeable increase in # of skin cancer cases in many foreign countries than before due to the amounts of UV radiations affecting them. As per the ionizing radiations; such as gamma rays that are used in some laboratory chemicals it is more dangerous. Sometimes for DNA hybridization or for sequencing of DNA we used sulfur 35 and phosphorus 32 substances. Sulfur 35 omits beta rays (heavy particles and can't penetrate the skin) and is therefore considered generally less dangerous than phosphorus 32 which omits gamma rays (travels on the speed of light [3 *10^8 m/s] and can penetrate the skin), viruses and mutagenic chemicals (for example, anti- insects- sprays [pesticides] with their common**

functional group of benzo(a)pyrene compound that is considered carcinogenic and can cause bronchogenic carcinomas in lungs because of its persistent mutations, as well as errors that occur during meiosis or DNA replication, also can be induced by the organism itself, by cellular processes such as hypermutation.

- Mutations can result in several different types of change in sequences of DNA, which can either of no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Mutations don't all the time cause dangerous consequences. On the contrary, some mutations can cause no effects at all, when? A mutation could be of no effect if it happens in a wobble- position. In wobble- hypothesis, the change of the third position bps in a genetic code (codon) has lower percentages (%) compared to the 1st or 2nd position bps especially if the aa of this genetic code has 4- fold degenerate site (4 different genetic codes [with different bp in the 3rd position] yields the same aa) in this case, no matter how we change the 3rd position bp from A to T to G to C, as the same aa will result. However, that does not mean that mutations only happen in the 3rd poisition. On the contrary, it may happen in the 1st and 2nd positions in a genetic codon, and if they happen they will be real mutations.
- Due to the damaging effects that mutations can have on genes, by Allah's mercy organisms have mechanisms such as DNA repair to remove mutations based on their size and type of mutations. Back to the 99% of the remaining 1% of mutations that happen in the non- coding regions, those mutations might happen in an enhancer (it increases the rate of transcription) or a silencer (it decreases the rate of transcription) sites. Mutations at these sites will cause no regulation of transcription, no stimulation of transcription and will definitely slow down the rate of protein synthesis. However, remember it can happen in an empty site and has no effects at all.
- Mutations can involve large sections of DNA becoming duplicated, usually through genetic recombination.

Causes of Mutations:

- Two classes of mutations: A) Spontaneous (endogenous) mutations (where there is no need for virus, radiation or mutagenic chemicals) and B) induced (exogenous) mutations caused by mutagens.

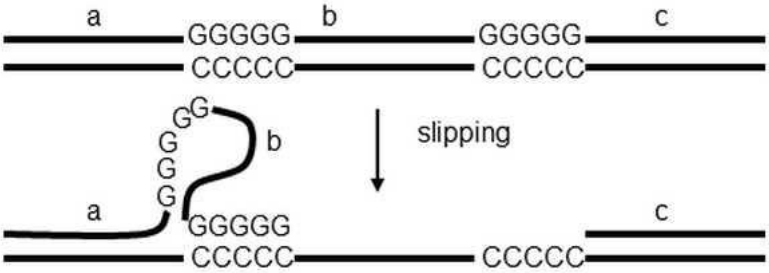


A) Spontaneous mutations can be caused by:

1. **Tautomerism: a base is changed by the repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base resulting in incorrect base pairing during replication. Changing the position of 1 hydrogen atom of one bp (nucleotide) which itself will affect the base pairing (A with T and G with C). Accordingly, hydrogen bonds sites are changed (no base pairing) and DNA (partially or fully) will be separated. Normally, DNA strands should not be separated from each other, the only allowed separation occur in replication under the effect of helicase enzyme. Remember that polymerases (DNA and RNA) can not work on a helical double stranded (DS) DNA, that is why helicase enzyme is important to make those polymerases able to work on single stranded (SS) DNA structures.**
2. **Depurination: De- prefix means loss or removal of something; accordingly, it is defined as**

loss of a purine base (A or G) to form an apurinic site (AP site). It may also happen between pyrimidines (C, T or U) to form a apyrimidinic site. However, it is more common between purines as they are less stable (have weaker bonds among them). So, more susceptible to oxidative stress and chemical modifications.

3. Deamination: changes a normal base to an atypical base containing a keto group in place of the original amine group. It is the removal of amino group from cytosine bp to uracil (keto- group). Remember that DNA does not have uracil and that tRNA (transfer RNA) has 4 unusual ribonucleotides that some of them are responsible for wobble hypothesis, those are: 1) inosine, 2) pseudouridine, 3) dihydrouridine and 4) thymine.
4. Slipped Strand Mispairing: denaturation (breaking down hydrogen bonds between bps) of the new strand from the template during replication, followed by renaturation in a different spot ("slipping"), which can lead to insertions or deletions. Due to 1) denaturation of a segment of DNA (breaking down hydrogen bonds) 2) slipping occur. Then the process will be followed by 3) renaturation (joining the bps back with new hydrogen bonds). The following picture shows slipping process.



The picture is from an external source

B) Induced mutations can be caused by:

1. Chemicals:
   a. Hydroxylamine
   b. Alkylating agents (e.g. nitrosourea) which can mutate both replicating and non-replicating DNA. It happens in both replicating and resting phases (between two cell cycles).
   c. Base analogs, in contrast to alkylating agents, can only mutate the DNA when the analog is incorporated in replicating the DNA. If X has a Y analog, it means that X and Y are very similar. When of the important base analogs, we mentioned in protein synthesis inhibitors is puromycin, which is an analog to tyrosine aa, but since it is not an aa it cannot form peptide bonds. So, on a ribosomal subunit during protein

elongation process, peptidyl transferase enzyme will transfer the aa found on P site to A site in order to bind it to the aa linked to tRNA on the A site by peptide bond. However, due to puromycin inability to form peptide bonds (not an aa) it won't bind and elongation of protein synthesis will be terminated.

d. DNA intercalating agents: e.g. 1) ethidium bromide (a dye that stains DNA bands, due to its toxicity everyone should wear gloves and masks while using it) and 2) polyacrylamides: this substance is formed under the influence of direct heat and it has been found in more than 170 types of food most commonly Shawarma and grilled food. It is highly carcinogenic especially to the colon.

e. DNA crosslinkers.

f. Oxidative damage.

g. Nitrous acid: it has a very bizzare (weird) way of working; converts amine groups on A and C to diazo groups, altering their hydrogen bonding patterns which leads to incorrect base pairing during replication and consequently no hydrogen bonds.

2. Radiations:

a. Non- ionizing radiation (IR and UV). Ultraviolet radiation (non-ionizing radiation) in which two nucleotide bases in DNA – cytosine and thymine – are most vulnerable to this radiation that can change their properties. UV light can induce adjacent pyrimidine bases in a DNA strand (two bases on the same strand) to become covalently joined as a pyrimidine dimer (most commonly thymine dimer). But why is it even a problem? According to the sequence of central dogma, formation of such dimers will cause a kink on this strand, this will stop the replication of DNA followed by arresting transcription and oxidative damage (oxidative stress) due to the formation of free radicals or Reactive Oxygen Species (ROS). ROS: are compounds of extra unpaired electron on an oxygen atom, so instead of 8 electrons it will have 9 electrons. ROS are of major 4 types: 1) Singlet oxygen ($^1O_2$), 2) Superperoxide (O_2^-), 3) Hydroxyl radical (HO) and 4) Lipid peroxide (hydrogen peroxide, H_2O_2). We naturally

have polyunsaturated fatty acids (FA) in plasma membrane, if it's exposed to oxidative stress by any means those FA will be converted to peroxides. Those peroxides will then go on a chain of reactions until they give us the most dangerous type of free radicals which is Malondialdehyde (MDA). Imagine having free radicals with a free unpaired electron on oxygen. This extra electron has the ability to travel to naïve compounds (DNA, RNA and proteins) causing permanent mutation due to excessive production. That's why after a certain age people tend to use antioxidants. The most important ones are 1) Vitamin E , 2) Vitamin C and 3) Selenium element (it is a cofactor that works with an enzyme called glutathione peroxidase, which is responsible for converting hydrogen peroxides to water molecules. Note that glutathione is an antioxidant peptide not an aa and vitamin D has no antioxidant effects. UV radiation, particularly longer-wave UVA, can also cause oxidative damage to DNA.

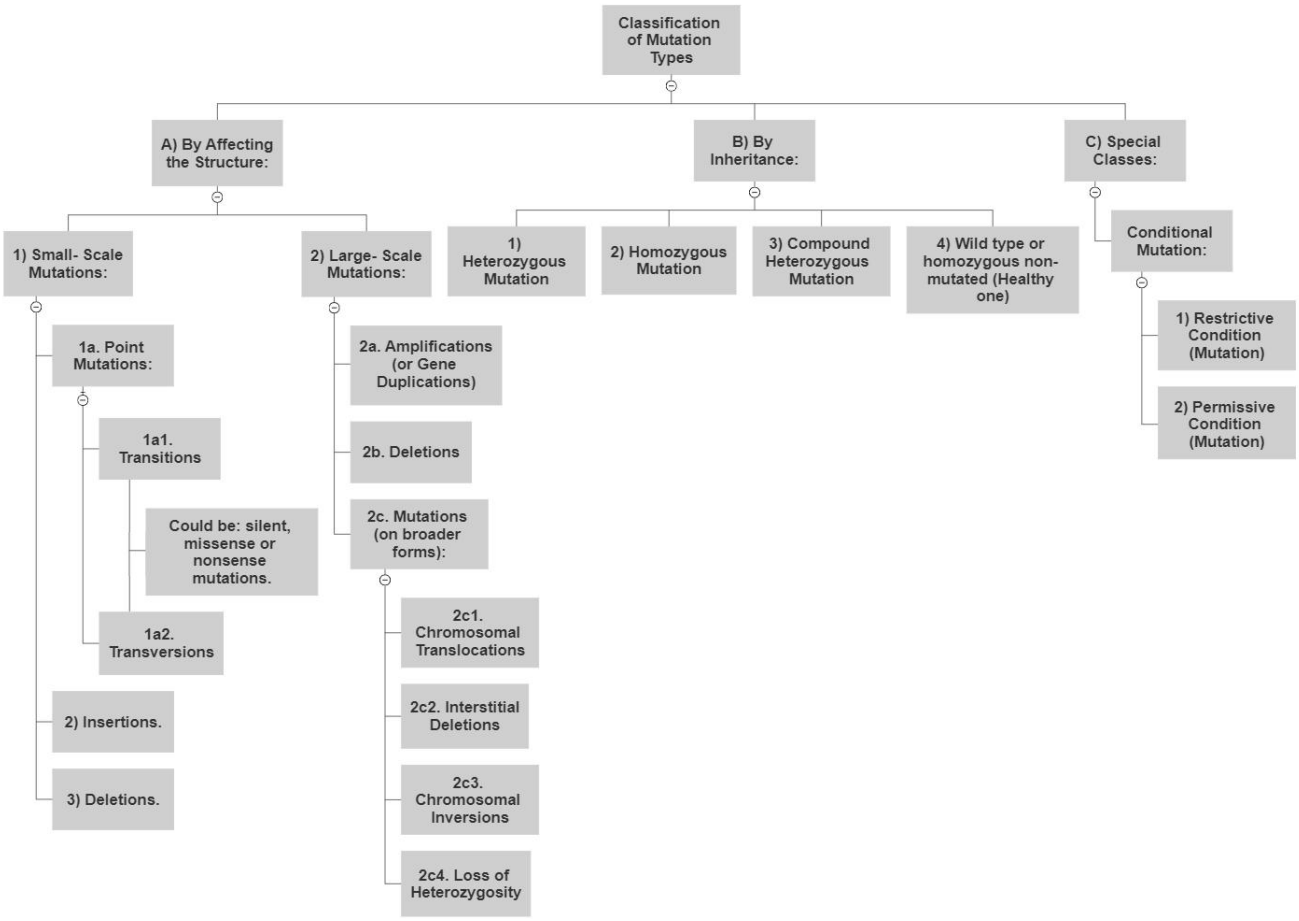
b. **Ionizing radiations**: like beta and gamma rays.
c. **Radioactive decay, such as 14C in DNA**

3. Viruses:

- most of viruses rely on reverse transcriptase enzyme (RT), which has 3 activities: 1) RNA-dependent DNA polymerase activity, 2) DNA- dependent DNA polymerase activity and 3) RNase activity. Note that RT does not have proofreading activity. So, the rate of mutations when a virus's genetic material invades that of a host is high especially if it is an RNA virus as seen in many dangerous viruses as HIV and hepatitis. When a virus's genetic material enters a host cell it will start depending on RN for synthesizing DNA. Why do viruses have to make DNA on the first place? To complete the structure of the newly developed viruses. That being said, RT is important as it allows viral DNA to be incorporated (integrated) into host DNA and then to direct host DNA into synthesizing proteins required to complete the structures of the newly formed viruses. One good RT that we have is called telomerase enzyme, it is considered good as it lengthens linear chromosomes.
- **Viruses that use RNA as their genetic material have rapid and high mutation rates to adapt to their surroundings and more effectively move from host to host, which can be an advantage since these viruses will evolve constantly and rapidly, and thus evade the defensive responses of the human immune system, treatments and vaccines.**

- **A mutation can help the virus gain traits that better help it reproduce quickly or adhere better to the surface of human cells.**
- **As a virus replicates, its genes undergo <u>random genetic mutations</u>. Over time, these genetic copying errors can, among other changes to the virus, lead to alterations in the virus' surface proteins or antigen, which will eventually lead to viral mutations that makes it difficult to treat and diagnose such viruses, that was clearly evident in COVID19 pandemic crisis where no one vaccine can work against all the developed mutants.**

Classification of Mutation Types:



Mutations can generally be divided according to different criteria. One of the is A) according to the region of mutation (<u>by affecting the structure</u>), into: small scale (taking a small portion of DNA) and large scale.

- **The sequence of a gene can be altered in a number of ways. Mutations in the structure of**

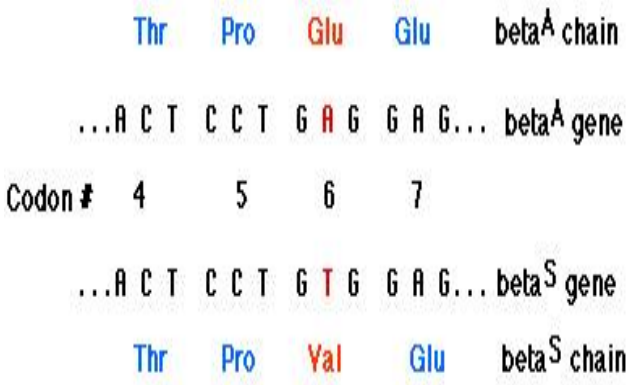
genes can be classified as:

1. Small- scale mutations: such as those affecting a small gene in one or a few nucleotides, including:
   a. Point mutations, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another, in general it happens when one nucleotide (deoxyribonucleotide) is replaced by another. These changes are classified as:
      1) Transitions: more common, during which exchange a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine, (C ↔ T) happens.
      2) Transversions: less common yet more dangerous, during which exchange a purine for a pyrimidine or a pyrimidine for a purine (C/T ↔ A/G) happens. A point mutation can be reversed by another point mutation to be fixed, in which the nucleotide is changed back to its original state (true reversion) or by second-site reversion. If A was replaced with G in a strand X, but G is not agreeing with the bp in the second strand Y because strand Y has T. So, upon replacement of A with G in strand X, T in strand Y should have been replaced with C, what if there's no C in strand Y? in this case we put A back on its place on strand X with another point mutation, this is called true reversion or second-site reversion.

   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 (in other words, what are the effects of point mutations?):

   - Silent mutations: which code for the same amino acid. Like nothing has happened at all. It happens with 4/3/2 folds degenerate sites on wobble position (3rd position bp), which result in genetic codes for the same aa. Note that silent mutations are not exclusive for 3rd positions bp changes, it may also happen if a single aa has different genetic codes with differences in bps on 1st or 2nd positions. However, problems arise with non- degenerate sites; where a certain aa has only one genetic code and any changes in bps in any position yields a different aa.
   - Missense mutations: which code for a different amino acid. Upon having a point mutation, a genetic code for another aa resulted. An example of missense mutations is that responsible for sickle cell disease (a type of anemia); where hemolysis of red blood cells (RBCs) happens and under the microscope those RBCs have a crescent ☾ or a C- shape instead of the normal biconcave disc shape. Here, a

point mutation in the genetic code of glutamic acid happens resulting in changing the aa to valine aa. We all know that glutamic acid is a non- essential aa (our bodies can produce) while valine is an essential aa (our bodies can't produce so we have to eat food that has it). So, is not that a good thing to have our bodies producing essential aa? Nope! That's not the case here. Glutamic acid is considered a polar (hydrophilic) aa while Valine is a nonpolar (hydrophobic) aa. That being said, glutamic acid is found on the exterior surface since it's nonpolar (closer to the nonpolar plasma membrane), when it's replaced with the polar valine, it gets inside the cell resulting in sickling (cutting or lysing) the cells and different properties result consequently.

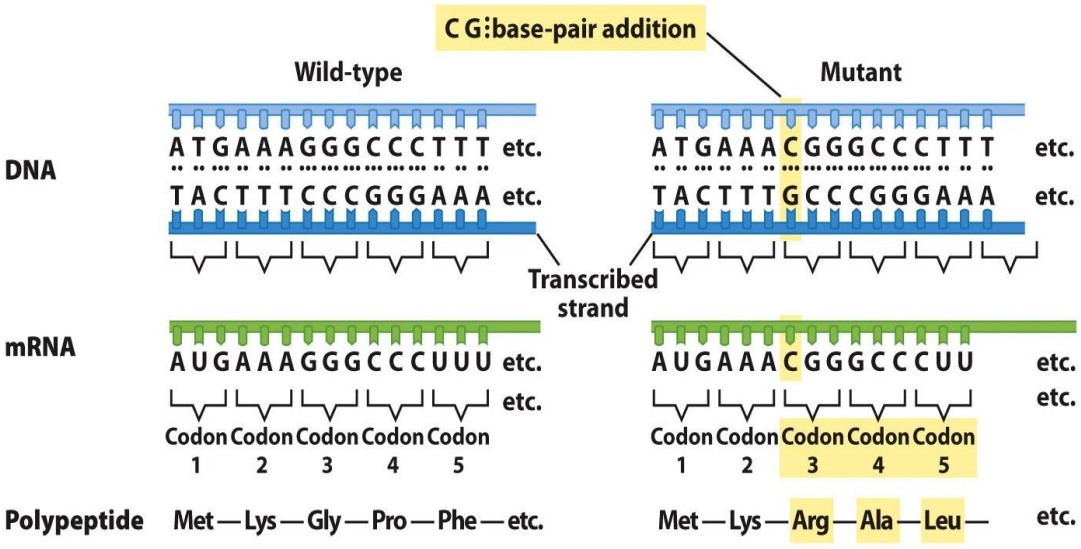


- **Nonsense mutations:** which code for a stop and can truncate (shortens) the protein. The point mutation here changed the genetic code of an aa to a stop codon (UAA, UAG or UGA), resulting in a premature termination of protein synthesis, as the protein synthesizing machinery (specifically ribosomes) will synthesize proteins till it reaches the stop codon. So, no completion of protein synthesis of this particular mRNA happen which result in truncated (shorter) protein. This will definitely affect the function (biological activity) of this protein to either be of: no function, little function or even opposing the original function. In general, that could be a very serious problem because it might not be able to be catabolized.

b. **Insertions:** is another small- scale mutation that is well- organized or well- defined. Where addition of 3 ribonucleotides, less (1,2) or more (4,5,6,7 …etc.) happens. Note that it is not always true that addition of 3 bps is less dangerous, it depends on the genetic code itself and what aa it is coding for. Add one or more extra nucleotides into the DNA. They are

usually caused by transposable elements (DNA sequences that have the ability to change their positions within a genome; "jumping genes"), or errors during replication of repeating elements (e.g. in the very common Huntington's disease where multiple AT repeats are added, affecting the CNS, movements and whole body, it will be explained in pediatrics rotations).

Insertions in the coding region of a gene may alter splicing of the mRNA (splice site mutation), or cause a shift in the reading frame (frameshift), both of which can significantly alter the gene product. Insertions can be reverted by excision of the transposable element. The following figure shows an example of frameshift mutation resulting from the addition of 1 bp (C): Upon the addition of C in the first strand, it will be followed by the addition of the complimentary bp (G) in the transcribed strand (based on Chargaff's rule). Resulting in changing the whole reading frame and the resultant AAs upon translation (compare the 3rd, 4th and 5th AAs in both polypeptides).



c. **Deletions:** another small- scale mutation that is usually random and entails the removal of bp(s) and consequently AA(s). One more time, removal of 3 bps, less or more and all of them can affect the protein produced. Remove one or more nucleotides from the DNA. Like insertions, these mutations can alter the reading frame of the gene. - They are generally irreversible: though exactly the same sequence might theoretically be restored by an insertion, transposable elements able to revert a very short deletion (say 1–2 bases) in any location are either highly unlikely to exist or do not exist at all.

Notes: 1) that a deletion is not the exact opposite of an insertion: the former (deletion) is quite random while the latter (insertion) consists of a specific sequence inserting at locations that are not entirely random or even quite narrowly defined, 2) transposable elements could be inserted or deleted, 3) wild type is the normal healthy DNA and 4) insertions or deletions of one or two base pairs alter the reading frame of the gene distal to the site of the mutation resulting in what is called frameshift mutations.

We said that mutations can be classified by how they are A) affecting the structure into 1) small scale mutations and:

2. Large- scale Mutations: in chromosomal structure, including:
   a. Amplifications (or gene duplications) leading to multiple copies of all chromosomal regions. One extra chromosome, the total number of chromosomes will be 47 as seen in: Down syndrome, Klinefelter syndrome, Patau syndrome and Edwards syndrome.
   b. Deletions of large chromosomal regions, leading to loss of the genes within those regions. Loss of a chromosome, the total number of chromosomes will be 45 as seen in Turner syndrome.
   c. Mutations (on broader forms) potentially bringing together separate genes to form functionally distinct fusion genes (e.g. bcr-abl). These include:
      1. Chromosomal translocations: interchange of genetic parts from non-homologous chromosomes. Normally, each chromosome is a duplicate; one of a paternal origin

(inherited from the father, let it be Y) and one of a maternal origin (inherited from the mother, let it be X). On son's chromosome #7 (for example) X will come from chromosome #7 from his mother and Y will come from chromosome #7 from his father, now, #7 chromosomes XY of the son are called homologous chromosomes; they both contain the same gene type and in the same loci on a genetic map (Loci: particular place or location, singular is locus). What happens in chromosomal translocations is, a part of chromosome #7 will go with chromosome #14 instead of going with another #7. So, what is the problem? As each chromosome has almost 1000 genes (encoding for many different structural characteristics), there will be interactions between different loci which is very dangerous due to: 1) loss of organization and 2) loss of arrangement of genes on a particular chromosome will result in losing the function of those genes as regulatory proteins will also be disorganized. Suppose 5 genes are replaced, we may lose the function of 10 genes in total.

Remember that there are particular sciences that help biochemistry, those are: 1) cell biology, 2) molecular biology and 3) molecular genetics. Molecular genetics is very important as it detects the localization of each gene on a particular chromosome. So, it is never found haphazardly but in a well- organized and well- arranged manner.

2. Interstitial deletions: an intra-chromosomal deletion that removes a segment of DNA from a single chromosome, thereby apposing previously distant genes. One segment of DNA will be removed. Suppose we 20, 30, 50 or 100 genes, it will result in losing the function of 10 lost genes and the function of most genes that depend on them.
3. Chromosomal inversions: reversing the orientation of a chromosomal segment. A part of the chromosome is inverted.
4. Loss of heterozygosity: loss of one allele (one chromosome)?, either by a deletion or recombination event, in an organism that previously had two different alleles. This itself will result in losing the organization and arrangement of genes on that chromosome.

Another classification of mutations types is:

B) By inheritance:

By pattern of inheritance The human genome contains two copies of each gene – a paternal and a maternal allele.

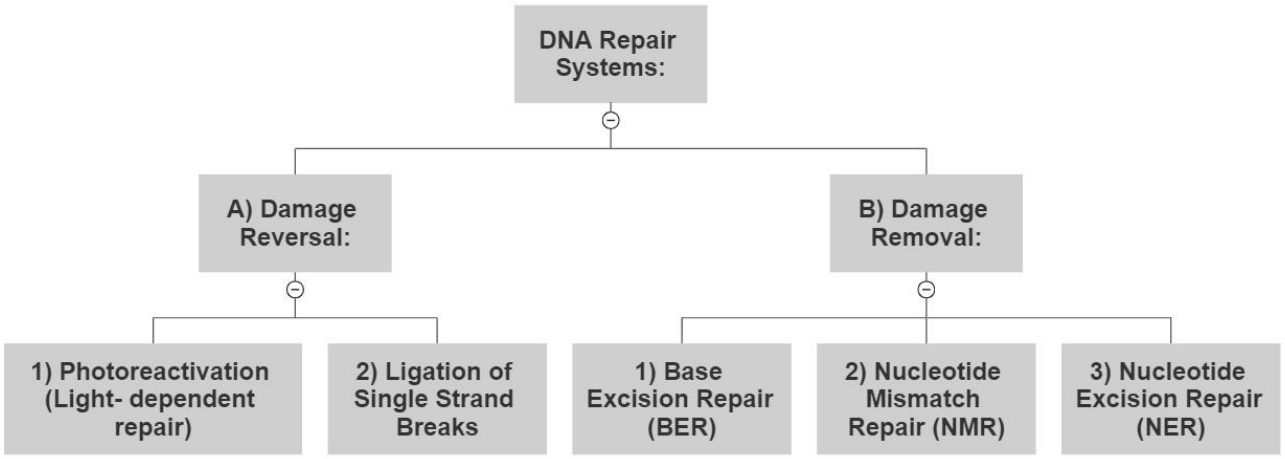
1. **A heterozygous mutation is a mutation of only one allele. One chromosome is removed?**
2. **A homozygous mutation is an identical mutation of both the paternal and maternal alleles. Both the alleles are lost, chromosomes # is now 44 or 22 pairs?**
3. **Compound heterozygous mutations or a genetic compound comprises two different mutations in the paternal and maternal alleles. Let's say gene #4 of the mother and gene #11 of the father each cause a disease; a compound heterozygous mutation. If gene #4 of the mother and also gene #4 of the father cause the disease it's called a compound homozygous mutation.**
4. **A wild type or homozygous non-mutated organism is one in which neither allele is mutated. (Just not a mutation). By this we mean the correct chromosome (the one that is supposed to be found in healthy cells under effects of replication, transcription and protein synthesis).**

The last classification of mutations types is:

C) **Special Classes: Conditional mutation (in special situations; usually when temperature is very high or very low):**

- **For example, a temperature-sensitive mutation (conditional lethal mutation) can cause cell death at high temperature (1. restrictive condition (mutation)), but might have no deleterious consequences (no bad effects) at a lower temperature (2. permissive condition (mutation), this is another mutation yet a good one, or at least a harmless one). So, both restrictive and permissive conditions are in response to changes in temperature.**

DNA Repair Systems:

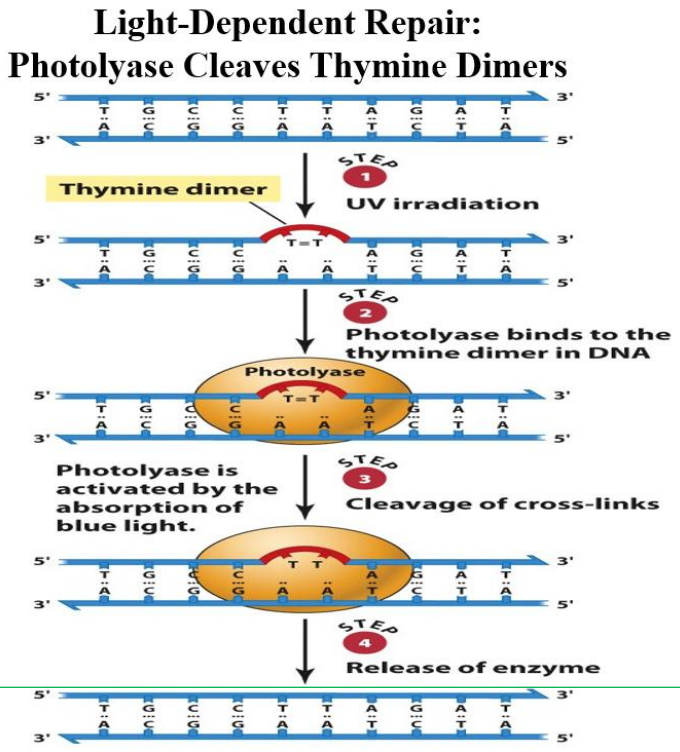


- **Repair mechanisms are divided into 2 categories: a system can do either of:**
  - **A. <u>Damage reversal</u> simplest; enzymatic action (needs enzymes, not spontaneous) restores normal structure without breaking backbone (no breakage of DNA's phosphate backbone, only repair). Instead of deletion of certain nucleotide, it inserts the same needed one and instead of inserting certain nucleotides, it deletes the inserted nucleotides.**
  - **B. <u>Damage removal</u> involves cutting out and replacing a damaged or inappropriate base or section of nucleotides. It's also enzymatic and not spontaneous. However, here we have removal of a DNA segment (the one with the mutation) then DNA polymerase and DNA ligase will work together to fill up this missed region of DNA.**

A. <u>Damage Reversal:</u>

1. <u>Photoreactivation (Light- dependent Repair)</u>:

- **This is one of the simplest and perhaps oldest repair systems: it consists of a single enzyme which can split <u>pyrimidine dimers</u> (break the covalent bond) in presence of light. It usually happens with <u>thymine dimers</u>; where two adjacent thymine molecules on the same strand join together**



covalently forming a kink on this strand which will itself affect replication, transcription and stop the whole central dogma as this strand can't proceed in any of these processes. So, to regain (restore) the normal structure of DNA molecule, this covalent bond kink has to be removed.

- The main enzyme responsible here is photolyase (called photo- as this mutation and repair happen under the effect of UV rays (photons) enzyme which catalyzes this reaction (it fixes the situation by cleaving the thymine dimer kink); it is found in many bacteria, lower eukaryotes, insects, and plants. It seems to be absent in mammals (including humans).
- Note that this repair is reversal not permanent (as there was no cutting of DNA molecule). Only breakage of the covalent bond between the two thymine molecules happened. Once removed, the DNA regained its function.

2. Ligation of single strand

- X-rays and some mutagenic chemicals like peroxides can cause breaks in (cut. So, DNA is not losing any portion of it) backbone of DNA. Accordingly, there is no need for mutation damage removal here, only sealing of this cut will suffice (be enough) using DNA ligase enzyme.
- Simple breaks in one strand are rapidly repaired by DNA ligase.
- Microbial mutants lacking ligase if they ever have single strand breaks, they tend to have high levels of recombination (it seals by itself without the need of ligase enzymes) since DNA ends are recombinogenic (can connect with each other spontaneously without the need of ligase enzyme) (very reactive).

B. Damage Removal:

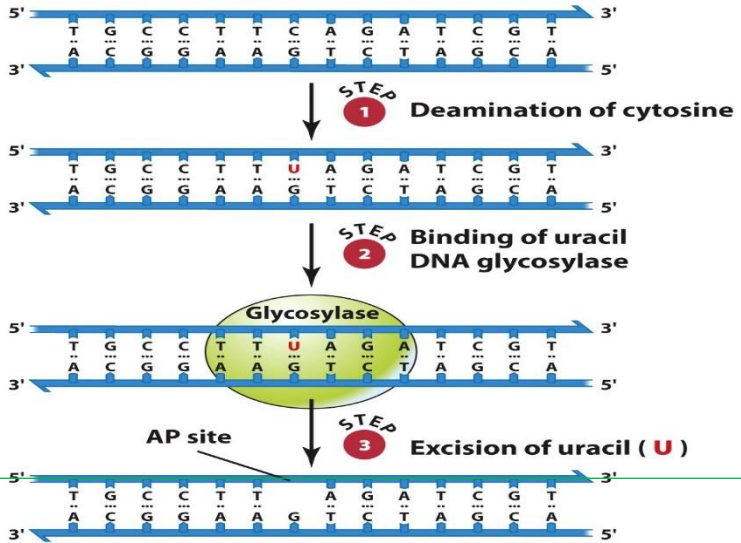
- It is generally of 3 types:

1. Base Excision Repair (BER):

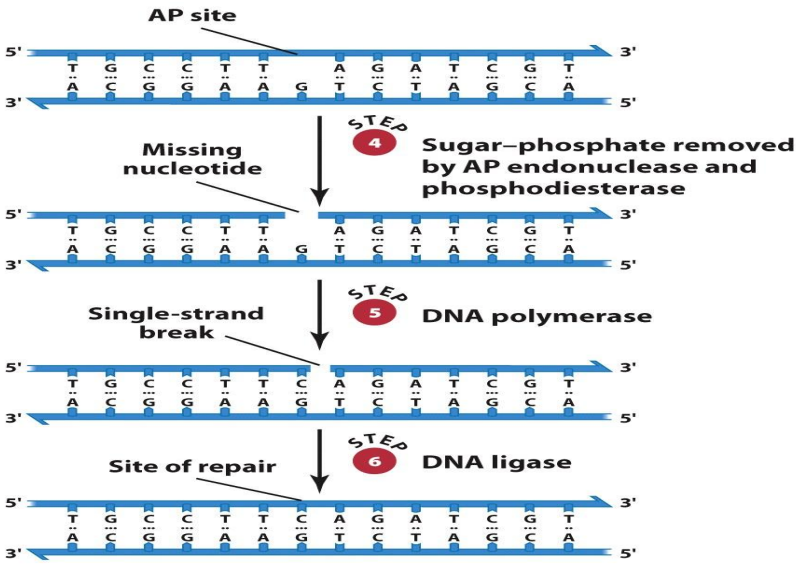
- The damaged or inappropriate base is removed from its sugar linkage and replaced.
- Deamination of cytosine gives uracil and since uracil is not supposed to be in DNA, it should be removed (repaired).
- These are glycosylase enzymes which cut the base-sugar bond as uracil glycosylase

enzyme which removes uracil from DNA to provide AP site (apurinic site; lacking purines).

- It can occur if RNA primers not removed in DNA replication or (more likely) if cytosine is deaminated (this is potentially mutagenic).
- The enzyme recognizes uracil and cuts the glyscosyl linkage to deoxyribose.
- Then, the AP endonuclease removes the AP site and neighboring nucleotides.
- The gap is filled by DNA polymerase I and DNA ligase using the other strand as a template.
- In other words, the three major steps needed in this repair are:
  a. Recognition of the mutation site by uracil glycosylase enzyme.
  b. Excision of the wrong nucleotide (which is uracil in this case).
  c. By DNA polymerase I filling of the gap with a correct nucleotide happens followed by sealing the ends of the DNA molecule together by DNA ligase.
- Notes regarding the next two figures:
  - Uracil DNA glycosylase enzyme has two functions: 1) recognition of mutation site (where U shouldn't exist for example) and binding to it and 2) Cutting the U from this site (note that this enzyme can not anneal U back to C (anneal means replacement with/ addition of the correct complimentary bp). So, the easiest way is to cut it, remove it, add a new one and finally seal it.
  - Removal of the uracil happened in two stages: 1) removal of the nitrogenous base itself (uracil) forming an AP site, followed by 2) removal of sugar-phosphate backbone that will cause a gap in that strand (missing nucleotide).
  - DNA polymerase I will read the deoxyribonucleotides on the complimentary (un- harmed) strand and then adds the correct nucleotide in the missing site (since it's G in the complimentary strand, it will add a C).
  - Finally, the two ends of the two fragments (of the single strand break) that are



left after DNA polymerase I has added the missing nucleotide will be sealed by DNA ligase.

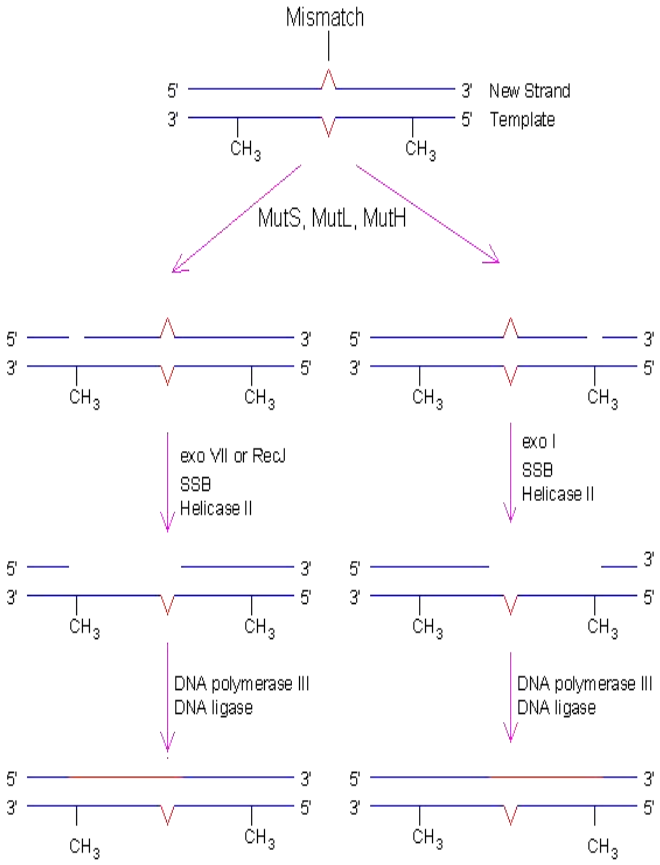


2) Nucleotide Mismatch Repair (NMR):

- This damage removal system consists of a group of proteins.
- The repairing process begins with the 1)protein MutS which binds to mismatched base pairs (recognizes and determines the location of the mutation, where it binds to the bps who lost the complementarity, for example, A is connected with G instead of T)
- Then, 2) MutL is recruited to the complex and activates 3) MutH which binds to GATC sequences on the strand having the mutation.

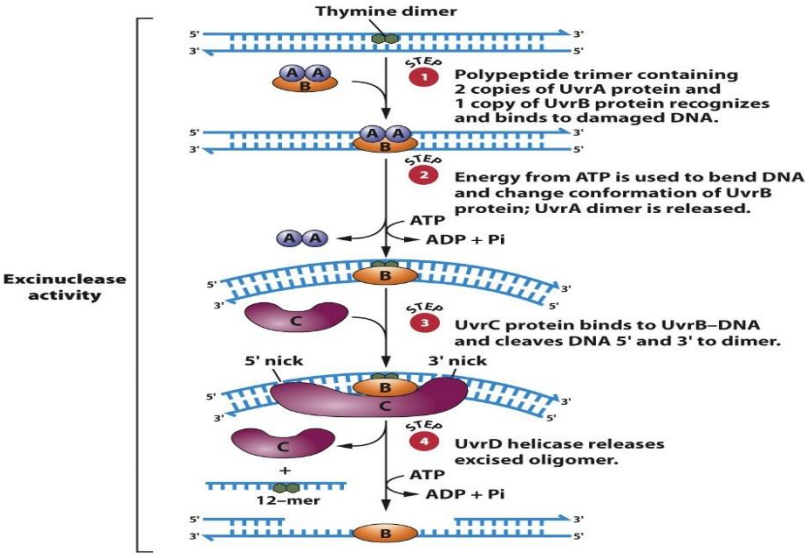
- **Activated MutH cleaves the unmethylated strand at the GATC site. Subsequently, the segment from the cleavage site to the mismatch is removed by exonuclease (with assistance from helicase II (it causes activation) and SSB proteins (single strand binding proteins)). Helicase II and SSB proteins work on both sides 3' and 5'.**
- **If the cleavage occurs on the 3' side of the mismatch, this step is carried out by exonuclease I (which degrades a single strand only in the 3' to 5' direction).**
- **If the cleavage occurs on the 5' side of the mismatch, exonuclease VII.**
- **Then in both cases, the gap is filled by DNA polymerase III and then sealing of the two ends of DNA is done by DNA ligase.**
- **The distance between the GATC site and the mismatch could be as long as 1,000 base pairs, not necessarily close, therefore, mismatch repair is very expensive and inefficient.**
- **NMR is more complicated than BER and it generally happens in bacteria or unicellular organisms with the aforementioned proteins. Where MutS, MutL and MutH do: recognition, binding GATC sequence to give the chance for exonuclease 1 to act on 3' end and exonuclease 7 to act on 5' end for the removal of the mismatch and finally DNA polymerase III and DNA ligase will fill and seal the gap. What about eukaryotes?**
- **Mismatch repair in eukaryotes may be similar to that in E. coli.**
- **Homologs of MutS and MutL have been identified in yeast, mammals, and other eukaryotes.**
- **The proteins responsible for NMR in eukaryotes generally and humans specifically are accordingly: MSH1 to MSH5 are homologous to MutS; MLH1, PMS1 and PMS2 are homologous to MutL.**
- **Mutations of MSH2, PMS1 and PMS2 are related to colon cancer. As these proteins are responsible for repairing mismatches; mutations in genes coding for them result in losing DNA repair system and colon cancer develops easily.**

- **In eukaryotes, the mechanism to distinguish the template strand from the new strand is still unclear.**

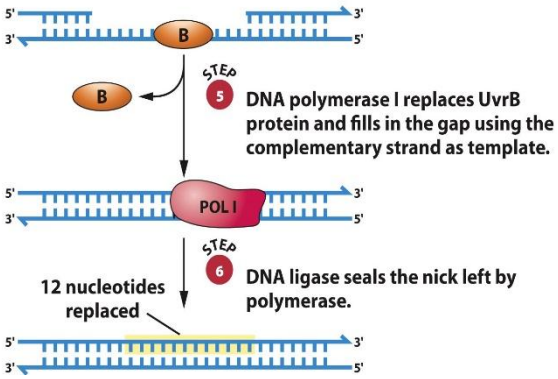


3. **<u>Nucleotide Excision Repair (NER):</u>**
   - **This system works on DNA damage which is "bulky" and creates a block to DNA replication and transcription (so, UV-induced dimers (causing UV- induced mutations) and some kinds of chemical adducts). This system has Ultraviolet Ray X (UvrX) proteins or Radiation xx (RADxx) proteins for repair.**
   - **In <u>E. coli</u>, proteins <u>UvrA</u>, <u>UvrB</u>, and <u>UvrC</u> are involved in removing the damaged nucleotides and the gap is then filled by <u>DNA polymerase I and DNA ligase</u>. UvrA, UvrB and UvrC have functions of 1) recognition and determining the location of a mutation resulted from UV rays 2) they cut and 3) recruitment of needed enzymes (DNA polymerase I and DNA ligse). Upon determining the location, cutting will happen then DNA polymerase will fill the gap with the correct nucleotide and DNA ligase will finally seal the gap.**
   - **In yeast (multicellular organism), the proteins are similar to Uvr's but with different names in the form of <u>RADxx</u> ("RAD" stands for "radiation" and xx are numbers), such as <u>RAD3, RAD10</u>.**

- **Any mutation in NER system (any problems in RAD3, RAD10, DNA polymerase III, DNA ligase.. etc will result in failure of NER system and mutations). One of those mutations that happens in NER system results in a very dangerous condition called Xeroderma Pigmentosum. Humans with the hereditary disease <u>Xeroderma pigmentosum</u> are sunlight-sensitive, they have very high risks of skin cancers on sun- exposed areas of the body and have defects in genes homologous to those required for <u>NER</u> in simple eukaryotes.**



- **<u>NER</u> mutants in lower organisms**



are UV-sensitive.

آخر شيت لمادة الأحياء الجزيئية ✓

فالحمد لله الذي بنعمته تتم الصالحات...

بالتوفيق #أثر

#لجنة_الطب_والجراحة

لا تنسونا ووالدينا من صالح دعائكم. ولكم بالمثل.