CORE IDEA 2: GENETICS AND INHERITANCE
TOPIC 2.6: GENE MUTATION

Learning Outcomes:

(l) Explain what is meant by the terms *gene mutation* and *chromosomal aberration*.

For gene mutation, knowledge of how substitution, addition and deletion could change the amino acid sequence (including frameshift) is required.

(m) Explain how gene mutations can result in diseases (including sickle cell anaemia)

Use the knowledge gained in this section in new situations or to solve related problems.

References:

Reece, J. B. et al. (2018). *Campbell Biology* (11th Ed). Chapter 17: From Gene to Protein, pp407 – 410.

Reece, J. B. et al. (2018). Campbell Biology (11th Ed). Chapter 15: Linkage and Chromosomes, pp356 – 359.

Note: This textbook is available in our library. You may wish to borrow it to supplement your reading when necessary.

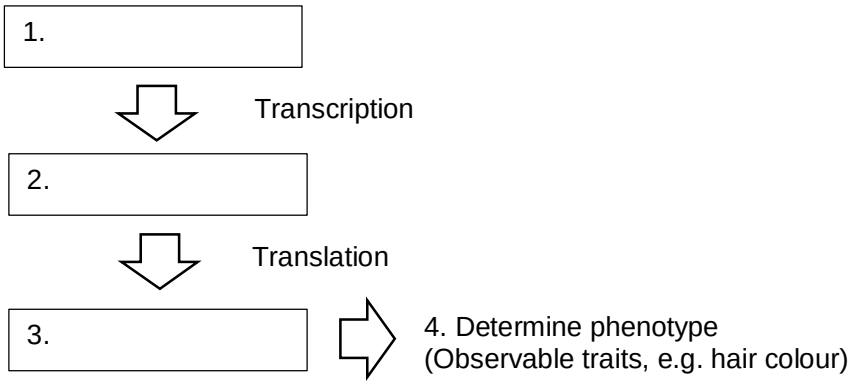
CONTENTS

1. Introduction

Genetic information is stored in linear sequences of base-pairs in DNA. Transcription and translation convert this genetic information into polypeptides, which become functional proteins.

Recall the Central Dogma of Molecular Biology

What is the significance of a change in DNA structure and sequence?



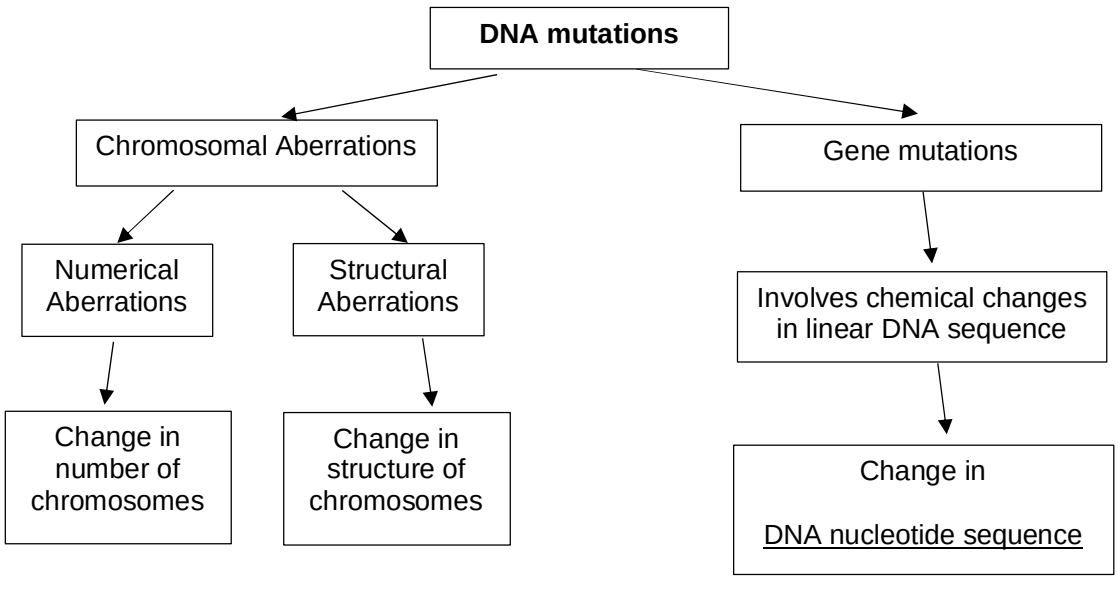
Mutations are **changes** in the **nucleotide sequence of DNA** or **structure/number of the chromosome**. This results in an alteration to an organism's phenotype.

Mutation can be spontaneous (e.g. errors during DNA replication resulting in base pair mismatches) or induced by a mutation-causing agent (e.g. Ethidium Bromide)

DNA mutations can have numerous biological implications

- Disease causing (e.g. mutations to proto-oncogenes contribute to onset of cancer)
- Creation of new gene variants, which contributes to diversity of genes and allows natural selection and evolution to take place

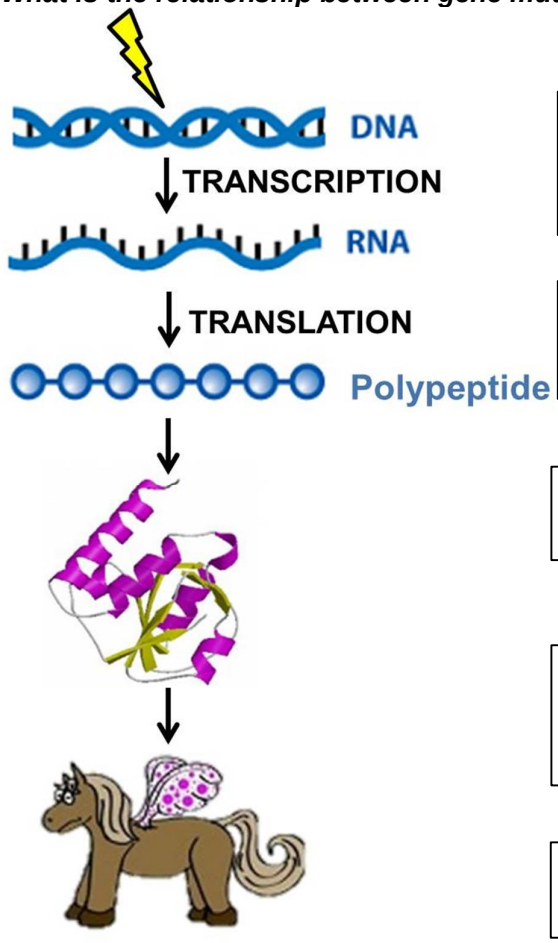
There are two main types of mutation: **gene mutation** and **chromosomal aberration**.



More will be elaborated on in Topic 2.7

Focus of this topic (Topic 2.6)

What is the relationship between gene mutation and protein synthesis process?



Mutation in DNA resulting in **change** in ……

↓

Change in ……..

↓

Change in ……..

↓

Affect ……. of polypeptide into specific ……..

↓

Change in ……..

↓

Change in ……..

Learning Outcome 2(l) modified:

Explain what is meant by the term's ***gene mutation***. For gene mutation, knowledge of how substitution, addition and deletion could change the amino acid sequence (including frameshift) is required.

2. Point Mutations

Gene mutation is a **change in the sequence of nucleotide bases** of DNA in a particular gene.

A **point mutation** usually refers to **a single nucleotide base change in a gene**.

There are 3 different ways that DNA sequence can be changed.

1. Substitution
2. Insertion
3. Deletion

2.1 Base-pair Substitution

Substitution mutation **replaces one base** with another.

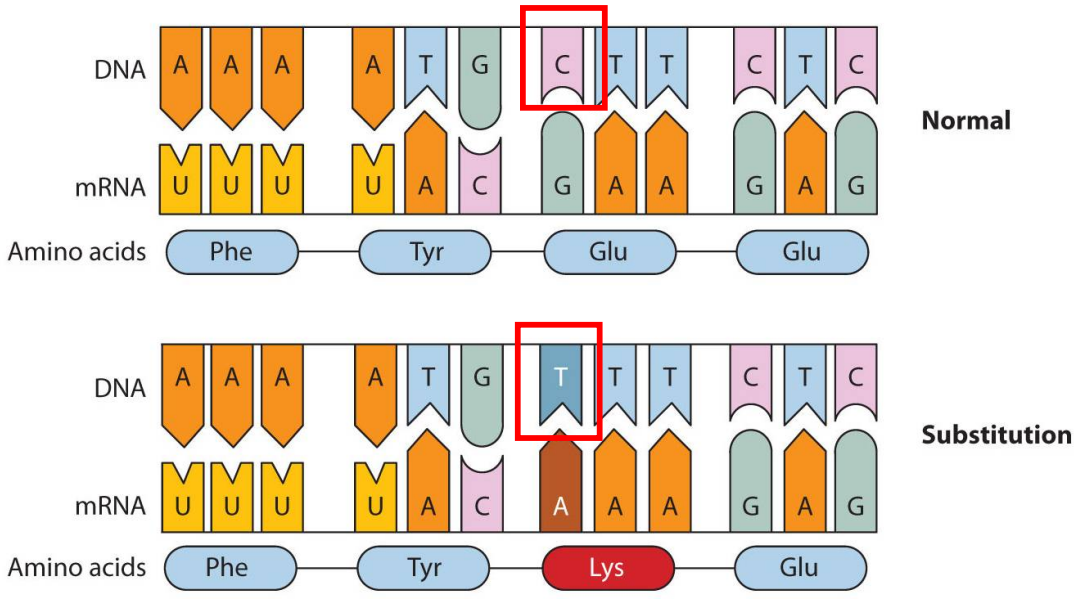


Fig 2.1.1: Substitution mutation, where Cytosine in DNA is substituted with a Thymine

Base-pair substitution mutation can result in 3 possible consequences:

a) Consequence 1: Missense mutation

Missense mutation is when the substitution causes a change in DNA sequence and subsequent change in mRNA codon, eventually resulting in a **single amino acid change** in the primary structure of the protein.

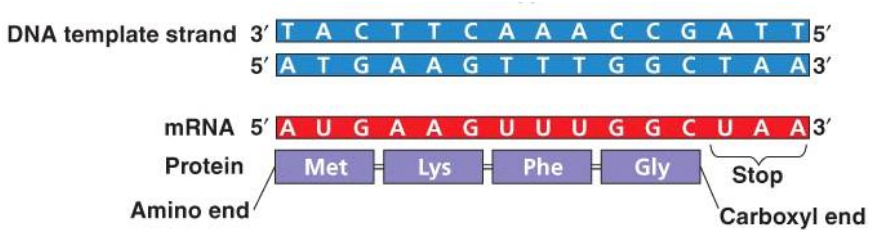


Fig 2.1.2: No DNA mutation present.

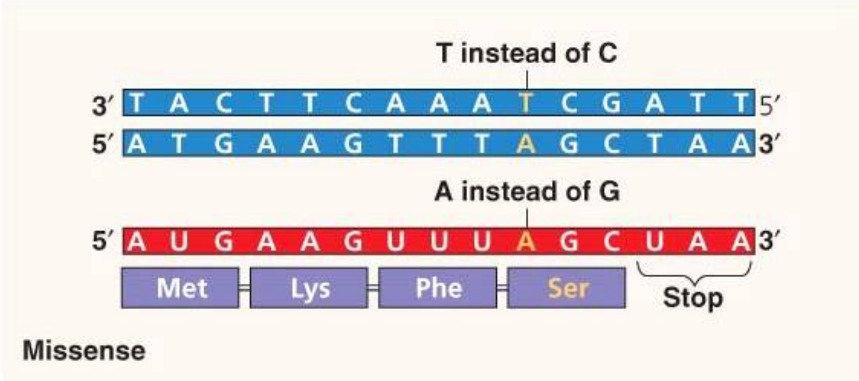


Fig 2.1.3: Missense mutation as a result of base-pair substitution (T replaces C in DNA template strand), leading to a change in mRNA sequence (A replaces G) and a change in amino acid sequence (from Gly to Ser).

IMPORTANT: What happens when the amino acid changed is chemically similar or chemically dissimilar to the original amino acid ?

Chemically Similar	Chemically Dissimilar
Change in amino acid in 1˚ protein structure → new amino acid has same R grp property → No change in folding of polypeptide → No change in specific 3D configuration of protein → No change in function of protein → No change phenotype	Change in amino acid in 1˚ protein structure → new amino acid has different R grp property → Change folding of polypeptide → Change specific 3D configuration of protein → Change function of protein → Change in phenotype

b) Consequence 2: Nonsense mutation

Nonsense mutation is when the substitution results in a **premature stop codon** (UAG/UAA/UGA) in the resultant mRNA. During translation, the synthesis of the **polypeptide is terminated prematurely** resulting in a **truncated** polypeptide chain (a shorter polypeptide chain than usual).

Most nonsense mutation frequently results in **non - functional** protein especially if the mutation occurs at the beginning of the protein. This results in a **phenotypic change** in the individual.

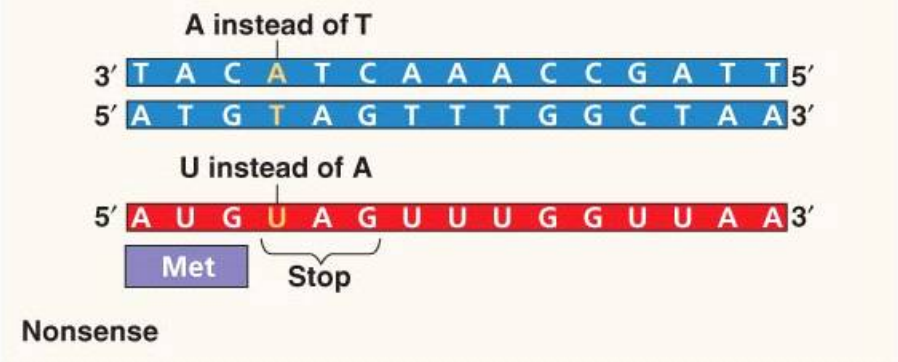


Fig 2.1.4: Nonsense mutation as a result of base-pair substitution (A replaces T in DNA template strand), leading to a change in mRNA sequence (U replaces A). This results in a stop codon (UAG) and polypeptide is truncated.

c) Consequence 3: Silent mutation

Silent mutations is when the substitution results in change in the DNA sequence and mRNA codon but **no change in amino acid sequence**. Hence **no phenotypic change** is observed in the individual. This is due to degeneracy of genetic code.

Silent mutations usually occur when the mutation affects the **third base** of the codon resulting in a degenerate code coding for the same amino acid.

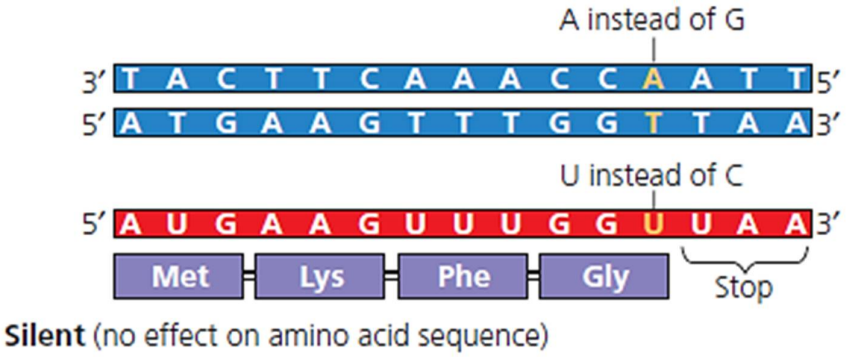


Fig 2.1.5: Silent mutation as a result of base-pair substitution (A replaces G in DNA template strand), leading to a change in mRNA sequence (U replaces C) but no change in amino acid sequence. Refer to Fig 3.1.2, both GGC and GGU code for Gly. This is due to degeneracy of genetic code.

2.2 Base-pair Insertion and Deletion

Base-pair insertion or deletion is the **addition** or **removal** of **one** nucleotide in the DNA sequence.

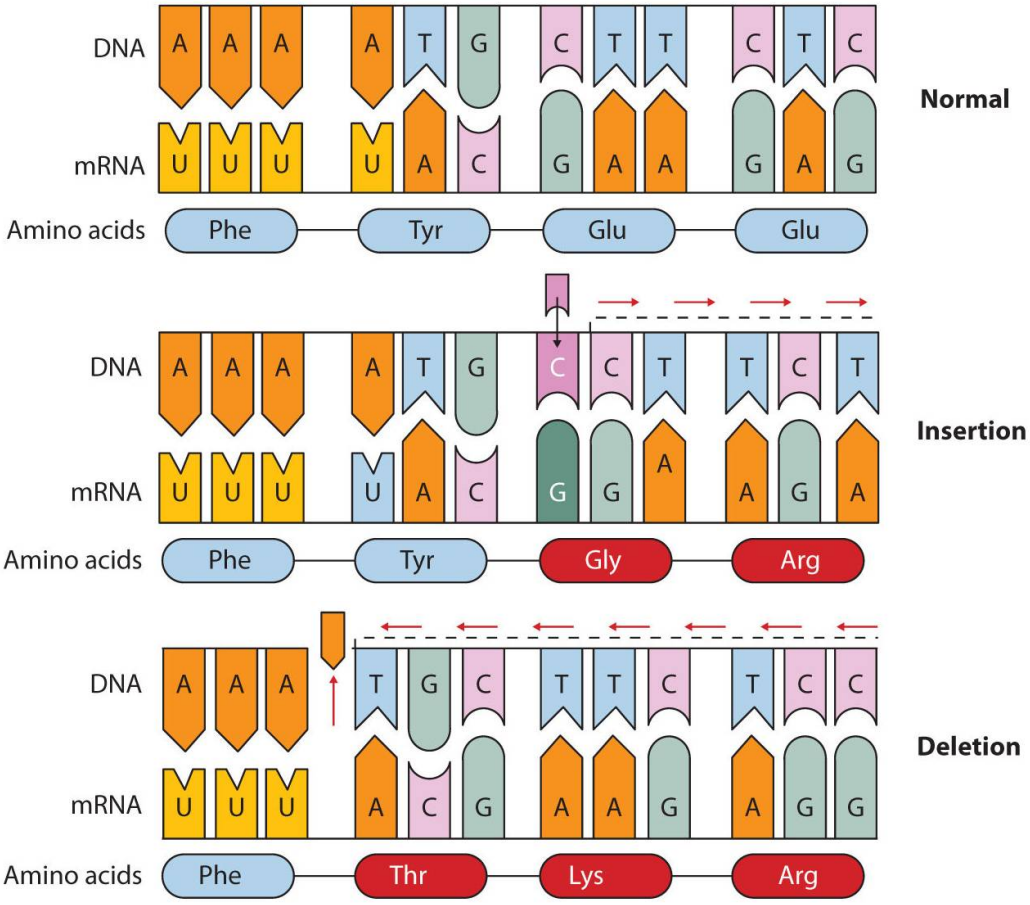


Fig 2.2.1: Insertion and deletion mutation

If the base-pair addition or base-pair deletion does not occur in multiples of 3 nucleotides, a **frameshift mutation** can occur. Frameshift mutations cause a **change in the reading frame of the codons**, which leads to severe consequences.

Frameshift mutation have severe consequences:

- **nonsense mutation** (Fig 2.2.2) or
- **extensive missense mutation,** extensive change of sequence of amino acids downstream of the mutation (Fig 2.2.3).

This is usually more severe than substitution mutation as a larger part of the polypeptide will be changed due to the change in reading frame resulting in the change of multiple amino acids downstream of the mutation site.

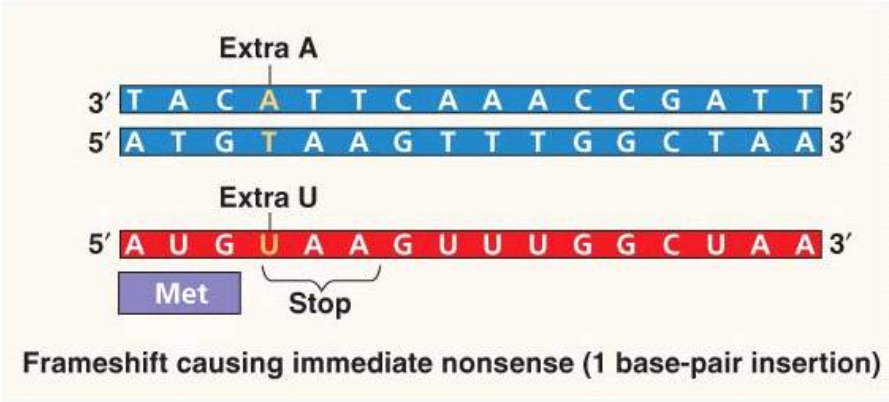


Fig 2.2.2: Frameshift mutation as a result of base-pair insertion (extra A inserted in between 3rd and 4th base in DNA template strand), leading to a nonsense mutation.

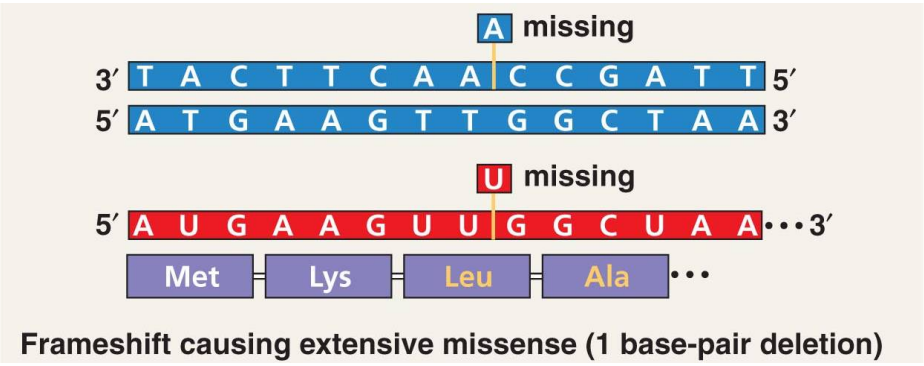
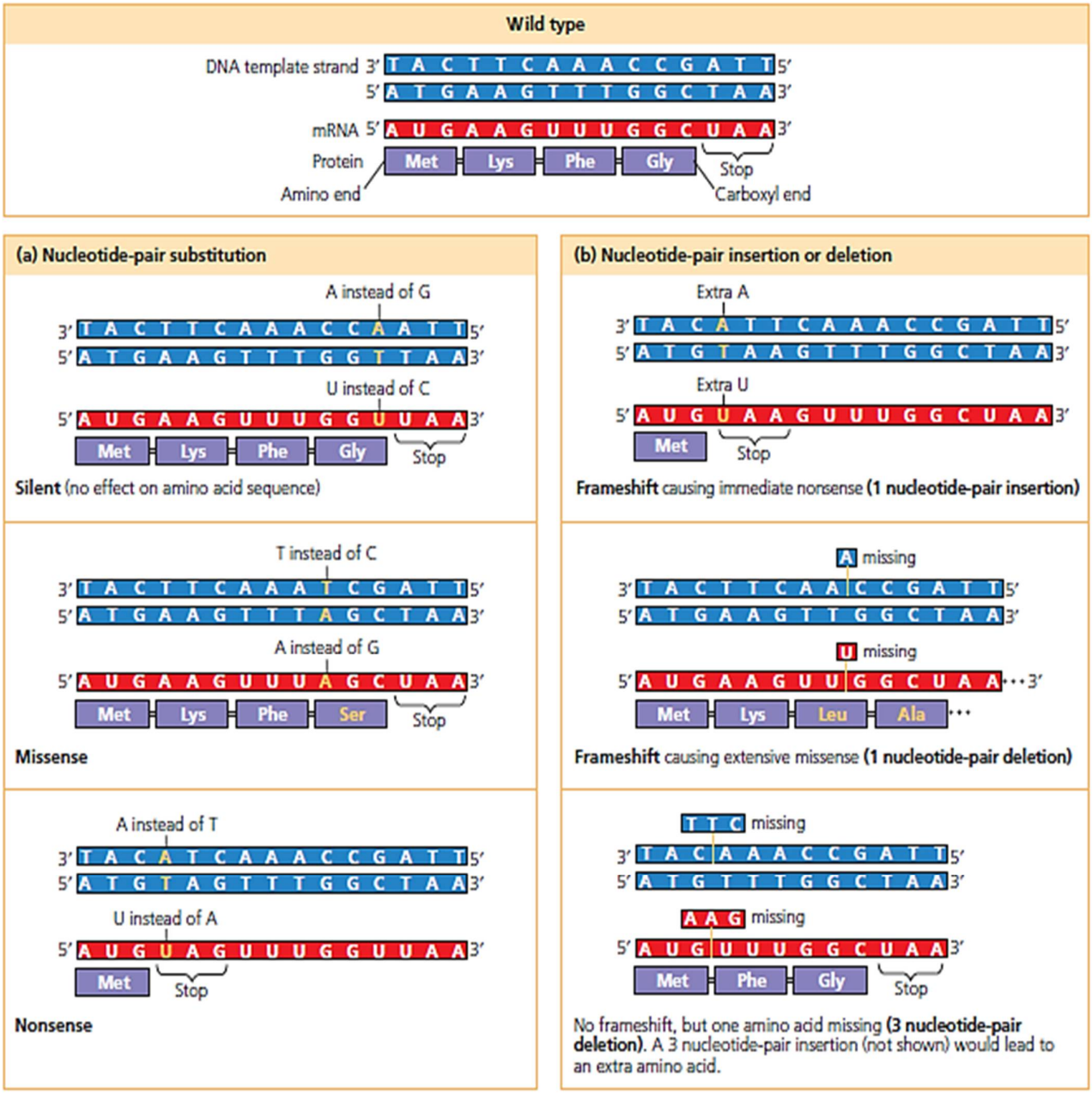


Fig 2.2.3: Frameshift mutation as a result of base-pair deletion (A at 9th position removed in DNA template strand), leading to an extensive missense mutation.

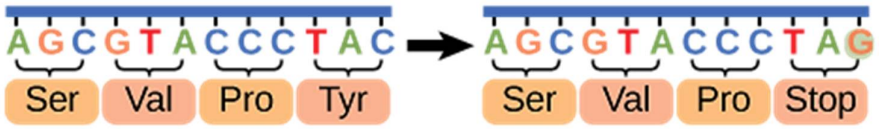
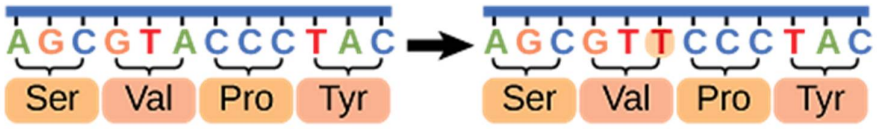
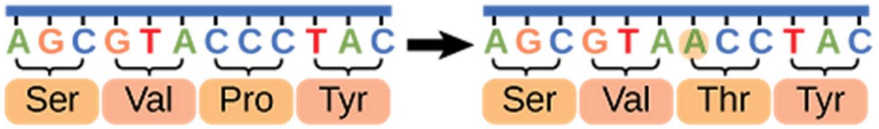
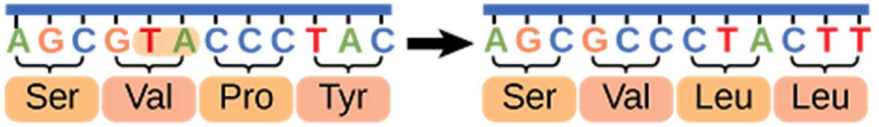
In Summary,

Normal DNA sequence (non-mutated)



Checkpoint 1:

a) Identify the <u>consequence</u> of mutation shown in each point mutation.

Point Mutation	Consequence of mutation
	.....
	.....
	.....
	.....

b) Describe the consequences of a missense mutation.

……

……

……

c) Describe the consequences of a nonsense mutation.

……

……

……

*Answers will only be given in lecture only, so please pay attention.

Checkpoint 2:

Below is a template strand of DNA that is read by RNA polymerase in transcription.

3' **TACTAACATCCCCTCATT** 5'

(a) State the base sequence of the mRNA transcribed from the template strand shown above.

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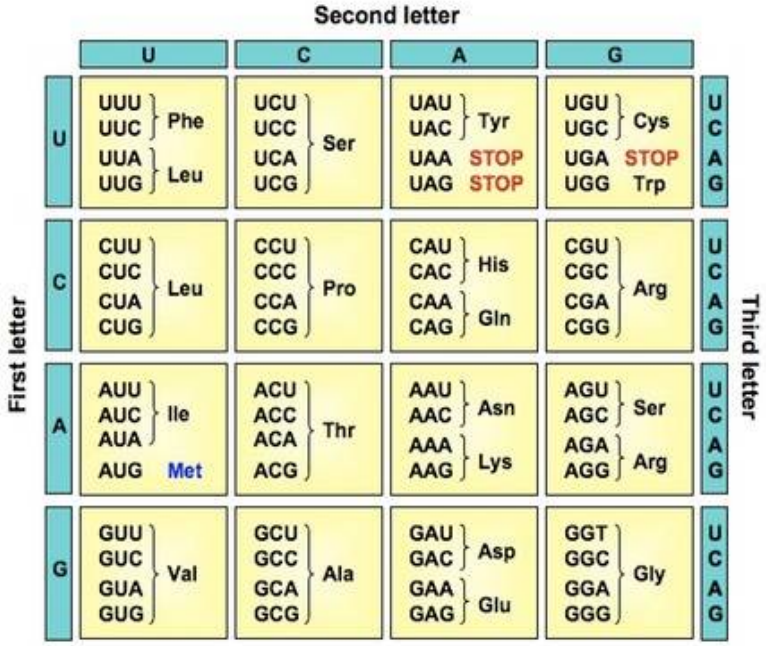
(b) Using the mRNA Genetic Dictionary below, write down the amino acid sequence translated from the mRNA sequence in **(a)**.



……

(c) Describe the consequence of a single base deletion at position 7 of the DNA sequence.

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Learning Outcome 2(m) modified:

Explain how gene mutations can result in diseases (including sickle cell anaemia).

3. SICKLE CELL ANAEMIA: DISEASE CAUSED BY GENE MUTATION

Normal adult haemoglobin (HbA) is a protein with a quaternary structure, consisting of two α-globin and two β-globin chains.

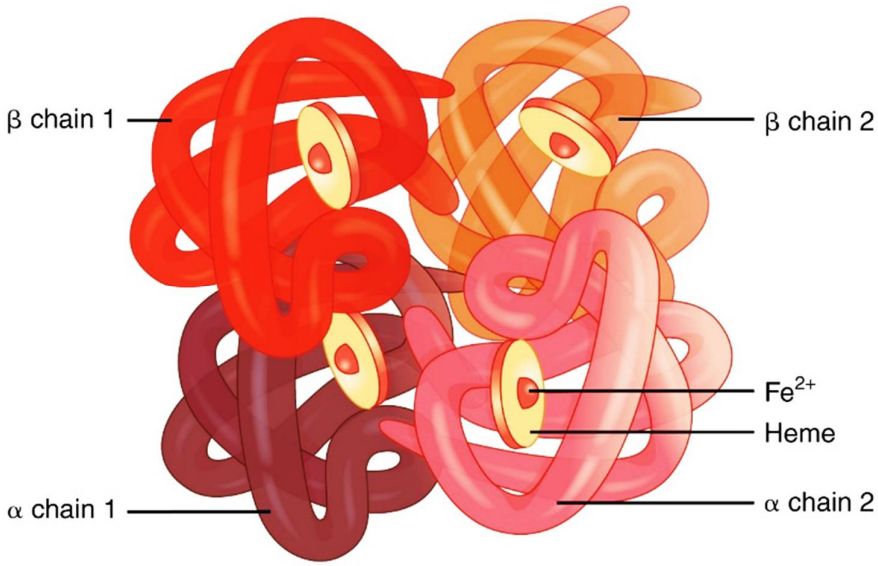


Fig. 3.1.1 Normal haemoglobin molecule (HbA)

In sickle-cell anaemia,

1. A variant haemoglobin protein, **HbS**, is produced instead of HbA.
2. A single base substitution where **T is replaced by A** in the β-globin gene of the template strand.
3. This results in the sixth codon of the mRNA being changed from **GAA to GUA**.
4. Glutamate (**glu**) is **replaced by** valine (**val**) at the sixth amino acid position in the mutated β-globin chain.

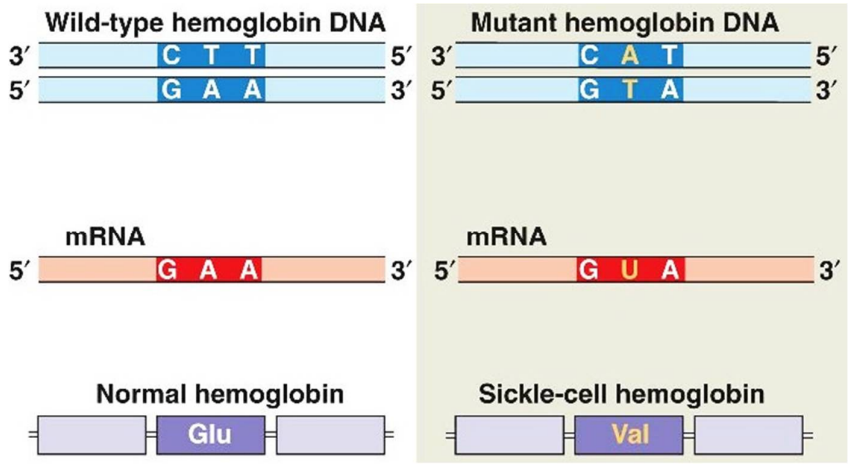


Fig 3.1.2: Comparison of DNA, mRNA and amino acid sequence between normal β-globin chain of haemoglobin A and defective β-globin chain of haemoglobin S.

5. **Glu with a hydrophilic (negatively-charged) R group** is **replaced** by **Val with a hydrophobic R group**. Glu has a hydrophilic R group, which forms ionic bonds with R groups of other amino acids. However, after the mutation to Val, hydrophobic interactions replace these ionic bonds instead, affecting the folding of the polypeptide chain.

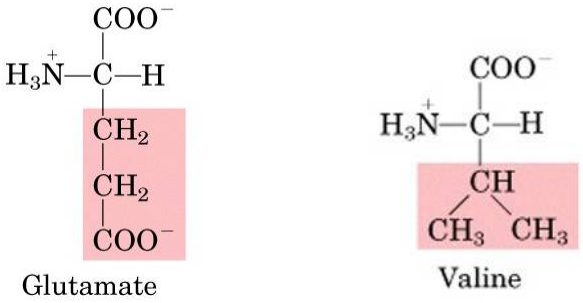


Fig 3.1.3: Structure of amino acids, Glutamate and Valine, with their respective R-groups

6. This results in a **hydrophobic protrusion** in the β-globin chain of oxyhaemoglobin S (oxy-HbS).

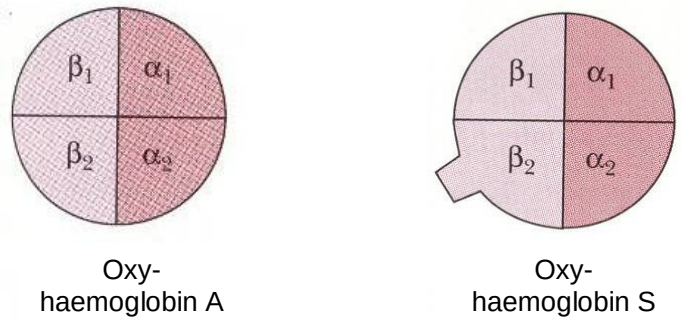


Fig 3.1.4: Presence of hydrophobic protrusion in β-globin chain of oxy-haemoglobin S but not in oxy-haemoglobin A.

7. In its deoxygenated state (low partial pressure of O_2 / low O_2 concentration), both the normal β-globin chain and the mutated β-globin chain have a **hydrophobic pocket** each.
   *This hydrophobic pocket would have existed with or without the mutation as the mutation occurs in β2 chain, not β1.

8. Hence, at low partial pressure of O_2, the protruding hydrophobic side chain of the abnormal β-globin chain of a deoxy-HbS fits into the hydrophobic pocket of a β-globin chain of a neighbouring Hb molecule.

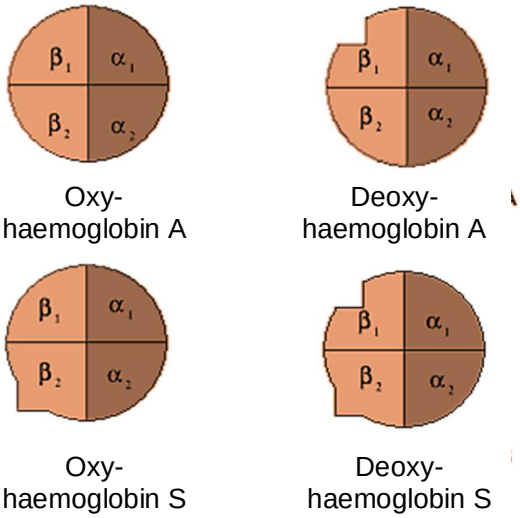


Fig 3.1.5: Structures of oxy-, deoxy-haemoglobin A and oxy-, deoxy-haemoglobin S

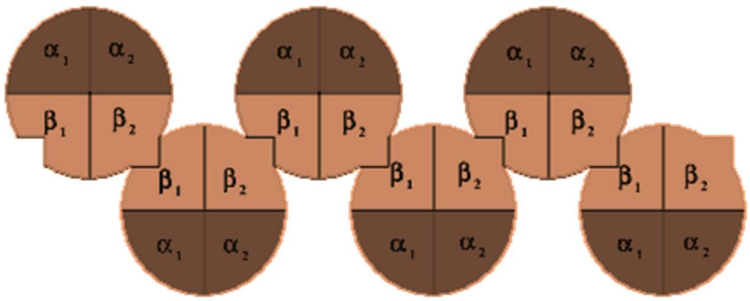


Fig 3.1.6: Hydrophobic protrusion of 1 deoxy-haemoglobin S fits into hydrophobic pocket of another, causing polymerization of haemoglobin S proteins into filaments

9. This results in **aggregation and polymerization** of HbS, thus reducing the solubility of HbS which leads to crystallization into rod-like fibres within the red blood cells.

10. This causes the red blood cell to become **sickle shaped**.
    *RBC becomes sickle shaped, NOT haemoglobin!

11. Normal RBCs are elastic, hence able to change its shape to squeeze through narrow capillaries. However, sickle-shaped RBCs are less elastic due to the damaged red blood cell membrane caused by the crystallisation of HbS.

12. These rigid sickle-shaped red blood cells will block blood capillaries, interfering with blood circulation to organs.

13. Restricted blood flow to organs deprives the cells of oxygen and glucose needed for cellular metabolism, leading to tissue death (e.g. in kidneys).

14. Shorter lifespan thus higher turnover of red blood cells in the liver may also cause liver damage.

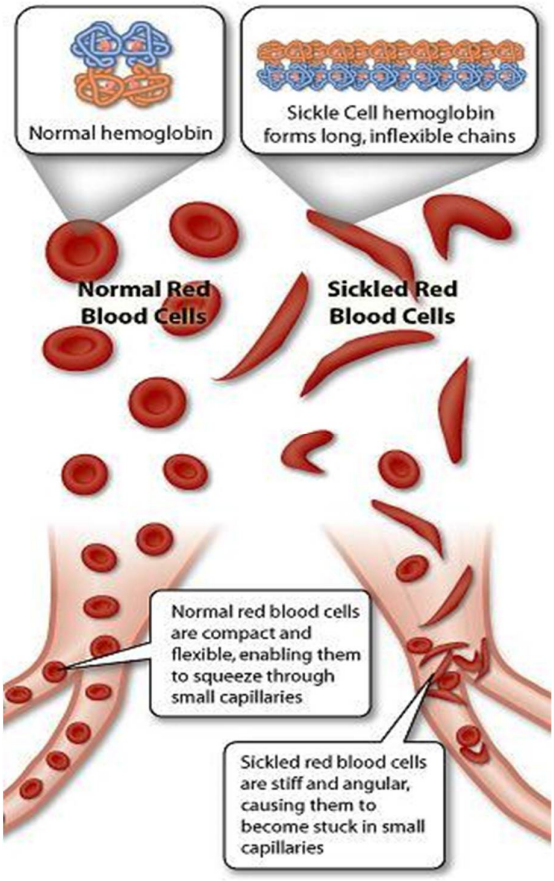


Fig 3.1.7: Normal red blood cells vs sickled cells

Checkpoint 3:

Circle **'T'** or **'F'** in the following statements on DNA replication process.

1	People with sickle cell anaemia are born with it.	**T / F**
2	A single base substitution that changes the 2nd base in the DNA triplet code results in sickle cell anaemia.	**T / F**
3	Valine is replaced with glutamic acid in Haemoglobin S.	**T / F**
4	The hydrophobic pocket is only found in Haemoglobin S.	**T / F**
5	Haemoglobin S changes to sickle-shaped under low partial pressure of oxygen.	**T / F**
6	Red blood cells in people with sickle-cell anaemia crystallise under low partial pressure of oxygen.	**T / F**
7	People with sickle cell anaemia dies of suffocation.	**T / F**

Annex: Causes of mutations

Mutation can be

i. **spontaneous**

E.g. errors during DNA replication resulting in base pair mismatches in the DNA molecule. Such errors could have arisen due to proofreading errors by DNA polymerase.

ii. **induced**

Induced mutations are due to a reaction between a mutagen (mutation-causing agent) with DNA, causing a structural change that affects the base-pairing properties of the affected nucleotide.

E.g. Ethidium bromide (EtBr) is a well-known chemical mutagen that acts as an intercalating agent. Ethidium bromide and other intercalating agents are flat molecules that can slip between base pairs in the double helix, slightly unwinding the helix and hence increasing the distance between adjacent base pairs.

Radiation like ultraviolet rays and X-rays are examples of physical mutagens. UV radiation can result in pyrimidine dimer formation while ionising radiation like X ray and gamma ray may result in various kinds of mutations that may prevent subsequent replication of the genome.

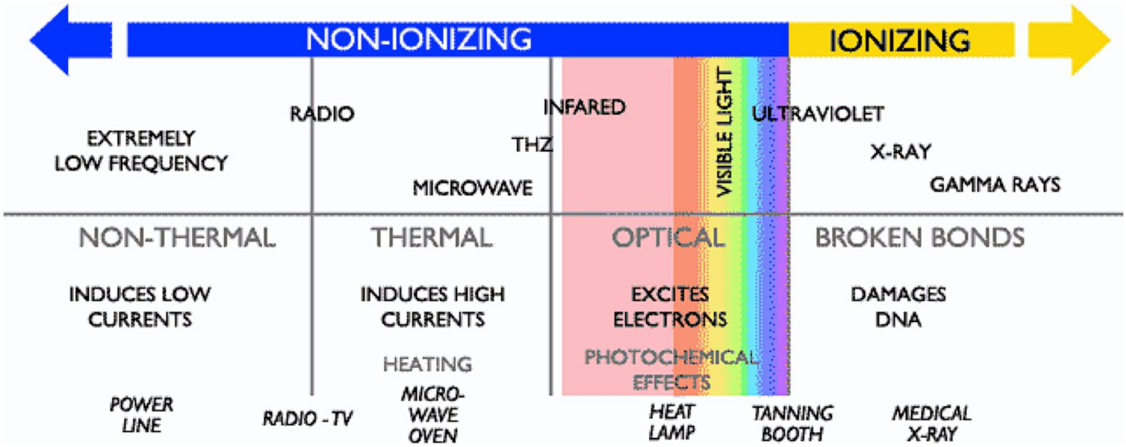


Fig 2.1: Various wavelength of radiation

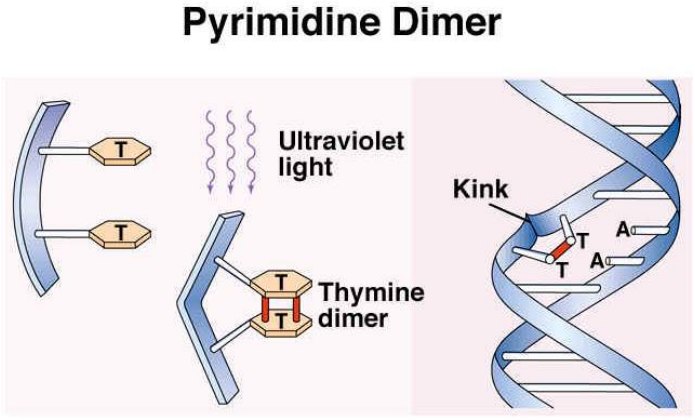


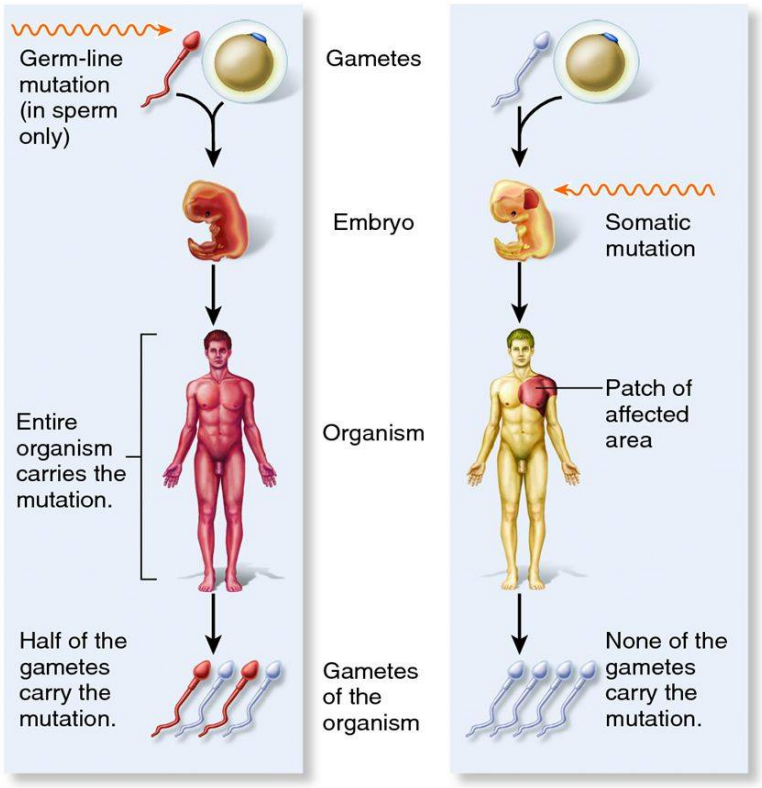
Fig 2.2: Thymine dimer in DNA caused by UV light

Annex: Are all mutations inherited?

Mutations can occur in the DNA somatic cells and / or gametes.

Mutations that occur in gametes or cells that give rise to **gametes** are known as **germline mutations**. These mutations can be **inherited** by the offspring. When a germline mutation is inherited by a person from his parent, the mutation is present throughout the person's life in virtually every cell in the body.

On the other hand, mutations that occur in **somatic** cells are known as **somatic mutations**. Somatic mutations are **not inherited** by the offspring. Such a mutation is only present in daughter cells produced via mitosis from the affected cell (i.e. cell in which the somatic mutation occurred).



(a) Germ-line mutation **(b) Somatic cell mutation**