Carbohydrates

1. CONDENSATION REACTION

α(1, 4)/α(1, 6)/β(1, 4) glycosidic* bond forms between C1 of one **α/β glucose*** monomer and C4/ C6 of another **α/β glucose*** monomer

[For cellulose] **Alternate** residues are **rotated 180°* with respect to each other**

Condensation* reaction joining two **α/β** glucose residues occurs with the **removal of one water*** molecule and the reaction is catalysed by **enzymes***

2. AMYLOSE V AMYLOPECTIN V GLYCOGEN V CELLULOSE STRUCTURE AND FUNCTION

Point of Comparison	Amylose	Amylopectin	Glycogen	Cellulose
1. Function	**Plant Storage** Polysaccharide		**Animal Storage** Polysaccharide	**Plant Structural** Polysaccharide
2. Location	Stored as granules in **chloroplasts**		Stored in **liver** and **muscle** cells	Forms the **cell wall** of plant cells
3. Monomer	**α-glucose** monomer		**α-glucose** monomer	**β -glucose** monomer
4. Bonds between monomers	**α(1, 4) glycosidic bond** links monomers ⇒ **Enzymes*** (amylase) that **hydrolyse*** these bonds are commonly available. Hence, glucose units can be readily released for respiration to yield **energy* quickly**	**α(1, 4) glycosidic bond** links monomers within a branch and **α(1, 6) glycosidic bond** links monomers at branch points ⇒ **Enzymes*** that **hydrolyse*** these bonds are commonly available. Hence, glucose units can be readily released for respiration to yield **energy* quickly**	**α(1, 4) glycosidic bond** links monomers within a branch and **α(1, 6) glycosidic bond** links monomers at branch points ⇒ **Enzymes*** that **hydrolyse*** these bonds are commonly available. Hence, glucose units can be readily released for respiration to yield **energy* quickly**	**β(1, 4) glycosidic bond** links monomers ⇒ **Enzymes*** that recognise and hydrolyse **β(1-4) glycosidic* bonds** are rare in nature, hence they are likely to remain intact and thus **insoluble*** in water, hence the integrity of the **cell wall*** is maintained in aqueous environment, making cellulose a suitable structural molecule

5. Orientation of Monomer	All glucose units in the chain have the **same orientation**	**Alternate** residues are **rotated 180°* with respect to each other** ⇒ Cellulose is able to lie **parallel* in straight chains** to allow **extensive interchain hydrogen bonding*** to occur between **OH groups*** of **adjacent parallel chains** forming **microfibrils*** with **high tensile strength*** conferring strength to **cell wall***

6. Structure of molecule	**Helical** molecule ⇒ Helical arrangement allows packing of many glucose residues per unit volume so that more glucose can be oxidised to produce energy, making amylose a **compact* energy storage*** molecule	**Helical** and **branched** molecule ⇒ Helical arrangement allows packing of many glucose residues per unit volume so that more glucose can be oxidised to produce energy, making amylopectin a **compact* energy storage*** molecule **Branching*** presents more ends for **increased accessibility to hydrolytic action by amylases** which works from the tip of the branches, thus making making it more efficient to release glucose	**Helical** and **branched** molecule, but **more extensively branched** than amylopectin ⇒ Helical arrangement allows packing of many glucose residues per unit volume so that more glucose can be oxidised to produce energy, making glycogen a **compact* energy storage*** molecule **Branching*** presents more ends for **increased accessibility to hydrolytic action by enzymes** which works from the tip of the branches, thus making making it more efficient to release glucose	**Long, straight** chain ⇒ Straight chains allow **extensive interchain hydrogen bonding*** to occur between **OH groups*** of **adjacent parallel chains** forming **microfibrils*** with **high tensile strength*** conferring strength to **cell wall***

7. Bonds between molecules	No interchain hydrogen bonding		**OH groups** projecting outwards in both directions allow **extensive interchain hydrogen bonding of adjacent parallel chains**, leading to **microfibril** formation. Microfibrils are then bundled up to form **macrofibrils*** ⇒ **Microfibrils*** with **high tensile strength*** confer strength to **cell wall***
8. Solubility	Starch is a large molecule made up of many **α** glucose residues. Moreover, hydrogen bonds form between **OH groups*** that project into core of helices, hence **no OH groups*** are **available** for **hydrogen bonding with water** Hence starch is **insoluble*** in water and so does not affect **water potential*, thus making it a suitable storage molecule**	Glycogen is a large molecule made up of many **α** glucose residues. Moreover, hydrogen bonds form between **OH groups*** that project into core of helices, hence **no OH groups*** are **available** for **hydrogen bonding with water** Hence glycogen is **insoluble*** in water and so does not affect **water potential*, thus making it a suitable storage molecule**	Cellulose is a large molecule made up of many **β** glucose residues. Moreover, many **OH groups** are involved in forming **extensive interchain hydrogen bonds** with **OH groups** of **adjacent parallel*** cellulose molecules, hence **relatively fewer OH groups*** are **available** for **hydrogen bonding with water** Hence cellulose is **insoluble*** in water, thus the integrity of the **cell wall*** is maintained in aqueous environment, making cellulose a suitable structural molecule

3. BENEDICT'S TEST

Carry out **Benedict's test first***: Place sample solution in a test tube and add equal volume of Benedict's reagent. Shake mixture and heat it in a **boiling water*** bath for 3-4 minutes

Next, carry out acid hydrolysis: **boil*** equal volume of a new sample of test solution with **dilute hydrochloric acid*** for 1min. Cool contents of tube and **neutralise*** acidic content with **sodium bicarbonate*** solution

Repeat the Benedict's test on the sample. If only **non-reducing** sugar (e.g. **sucrose**) is **present***, then initial Benedict's test should give a negative result (i.e. solution remains blue). After hydrolysis, Benedict's test should produce **a brick red ppt***

4. WHY PLANT CELLS STORE STARCH AND ANIMAL CELLS STORE LIPIDS

Lipids contain **more energy per unit mass** compared to carbohydrates. Since animals are **more active** than plants, **more energy** is required e.g. migrating birds will have **less load and more energy** by storing lipids as compared to carbohydrates

(Lipids stored in fat cells of animals allow **buoyancy, thermal insulation** and **protection of vital organs**

Lipids provide **more metabolic water compared to carbohydrates** and hence are useful for storage in **desert animals** e.g. camel

Lipids in the form of energy reserves are required in animals to **hibernate** through winter)

5.WHY IS CELLULOSE SYNTHESISED ON THE CELL SURFACE MEMBRANE

Cellulose forms the cell wall on the exterior of the cell membrane, hence it is optimal to **synthesise it on site** rather than inside cell. Moreover, cellulose is **too large** a molecule to **move across cell membrane**. Furthermore, **cellulose synthase is located on cell surface**, therefore cellulose has to be synthesised on the cell surface membrane

Proteins

1. PRIMARY, SECONDARY, TERTIARY AND QUATERNARY STRUCTURE OF PROTEINS

Amino Acid

An amino acid has 1 α-carbon atom, covalently bonded to 1 carboxyl group, 1 amino group, 1 H atom and 1 variable R-group. The R-group can be a polar, non-polar, acidic or a basic side group

Amino acids undergo **condensation*** reactions to form polypeptides with the removal of 1 water molecule with for every 1 peptide bond formed. The OH group from the carboxyl group and H atom from amino group contribute to the formation of water molecule

5. Tertiary structure: single polypeptide chain further extensively folded and bended into specific 3D conformation held in place by hydrophobic interactions, ionic bonds, disulfide bridges and hydrogen bonds formed between R groups;

6. Quaternary structure: association of two or more polypeptide chains which are held together by same four types of interactions involved in the tertiary structure;

7. Globular protein is made up of polypeptide chain(s) folded into spherical shape while and fibrous protein is made up of long polypeptide chains forming long, straight strands;

Primary

specific number and sequence of amino acids in a single polypeptide chain with amino acids linked by peptide bonds

Secondary

α-helix or β-pleated sheet held in place by hydrogen bonding between C=O and N-H groups of the polypeptide backbone. α-helix is single polypeptide wound into a coil with turns linked by **hydrogen bonds*** formed between **C= O*** group of one amino acid residue and **N-H*** group of another amino acid residue, four amino acids away. A β-pleated sheet is a flat sheet with **hydrogen bonds*** formed between **C= O*** group of one amino acid residue and **N-H*** group of another amino acid residue on adjacent regions/segments;

Tertiary

Individual haemoglobin chains are twisted and folded into a compact, globular* shape/ structure;

Quaternary

Example

1. Named protein = Haemoglobin;

2. Primary structure refers to the unique sequence and number of amino acids in a polypeptide linked by peptide bonds*;

3. Secondary, tertiary and quaternary structures are hence direct consequences of primary structure;

4. Secondary structure refers to the regular coiling and folding/pleating of the polypeptide held by hydrogen bonds* between C=O and N-H groups of the polypeptide backbone;

5. α-helix is single polypeptide wound into a coil with turns linked by hydrogen bonds* formed between C=O* group of one amino acid residue and N-H* group of another amino acid residue, four amino acids away;
(since this example here is haemoglobin there is no beta pleated sheets)

6. Tertiary structure refers single polypeptide chain further extensively folded and bended into specific 3D conformation, held by bonds between R-groups within same polypeptide;

7. Tertiary structure is maintained by hydrophobic interaction, hydrogen bonds, ionic bonds; (in case of haemoglobin there is no disulfide bonds)

8. Quaternary structure involves more than 1 polypeptide held together by the same types of interactions/bonds; (in case of haemoglobin there is no disulfide bonds, thus only 3 types instead of 4 types of intermolecular interactions)

9. each Hb has 4 polypeptides: 2 -globin* subunits and 2 -globin* (i.e. each polypeptide has 1 haem group with it);

SECONDARY STRUCTURE

2. α-HELIX V β-PLEATED SHEETS

	α-helix	β-pleated sheet
Shape	**coiled**	**flat/sheet-like structure** **R: pleated sheet**
Location of hydrogen bonding Note: formed between C=O & N-H groups of polypeptide backbone	***Hydrogen bonds**** **is formed between *C=O** group of one amino acid residue and *N- H** group of another amino acid residue, four amino acids away within a single polypeptide backbone	***Hydrogen bonds**** **is formed between *C=O** group of one amino acid residue and *N-H** group of another amino acid residue, on adjacent regions/segments of a single polypeptide chain;

TERTIARY STRUCTURE

3. FIBROUS V GLOBULAR

Pt of comparison	Fibrous protein	Globular protein
Shape	**Made up of long polypeptide chains forming long, straight fibres;**	**Made up of polypeptide chains folded into roughly spherical shape;**
Presence of OH groups Hydrogen bonding with H_2O	**It is large and limited in its ability to form hydrogen bonds* with water. Insoluble in water since extensive hydrogen bonds already formed between residues in different polypeptides;**	**Polar R groups are exposed to water molecules in the aqueous environment. Soluble since these polar groups can form hydrogen bonds* with water molecules.**
Constituent amino acids	**Less variety of amino acids are used to construct the protein; Note: repetitive regular sequence of amino acids. (e.g. tripeptide, gly-X-Y repeats in collagen)**	**More variety of amino acids are used to construct the protein; Note: unique/specific (no repetitive sequence) amino acid sequence**
Length of polypeptide	**Length of polypeptide and sequence of amino acids may vary slightly between two samples of the same protein, yet protein is still functional;**	**Length of polypeptide and sequence of amino acids are always identical between two samples of the same protein, or else protein may not be functional;**

	Note *Secondary structure** most important to its overall conformation;	Note *Tertiary structure** most important to its overall conformation;

4. SIGNIFICANCE OF R-GROUP

1. Types and location of R groups will determine type and location of bonds* formed to maintain tertiary structure*

2. and specific 3D conformation* and hence function* of the protein; [Must mention this idea/concept for full credit]

3. Non-polar* R groups of different amino acids cluster hydrophobic interactions* - causes protein to fold as hydrophobic side groups are shielded from water ;

4. When R groups contain sulphur* (e.g. in amino acid cysteine) covalent disulfide bonds*;

5. O and N are electronegative while H* of -NH or -OH groups are electropositive. Both attracted hydrogen bonds*;

6. Acidic* and basic* R groups are charged* ionic bonds*;

7. Presence of R groups that are too large will hinder the formation of secondary structure*;

QUATENARY STRUCTURE

5. STRUCTURE AND FUNCTION AND COLLAGEN AND HAEMOGLOBIN

Collagen

Collagen: (max 6 marks)

1. fibrous structural protein, in a connective tissue, skin, tendon;

2. has 3 polypeptide* chains wound together to form a tropocollagen* molecule;

3. numerous hydrogen bonds* form between amino acids of adjacent polypeptide chains between NH and CO groups of peptide linkage, as well as between OH groups of hydroxyproline residues;

4. thus contributes high tensile strength;

5. also makes molecule insoluble in water since extensive H bonds already formed between residues in different polypeptides, limiting interaction with water molecules;

6. small glycine* residues allow formation of a very tight/compact triple helical structure;

7. covalent cross-links* form between lysine* residues at the C and N ends of adjacent/ parallel tropocollagen molecules;

8. forming collagen fibrils which lie in parallel bundles to form collagen fibres ;

9. staggered ends to prevent weak 'fault lines' across the fibre;

10. thus contributes high tensile strength;

—

1. Forms long fibres which may run parallel to one another;
2. Insoluble in water due to its large size, relatively weak ability to form hydrogen bonds with water*;
3. Has a primary structure of regular repetitive sequences which results in an ordered helical secondary structure;

—-

1. has 3 polypeptide* chains wound together to form a tropocollagen* molecule;

2. numerous hydrogen bonds* form between amino acids of adjacent polypeptide chains between NH and CO groups of peptide linkage, as well as between OH groups of hydroxyproline residues;

3. small glycine* residues allow formation of a very tight/compact triple helical structure;

4. covalent cross-links* form between lysine* residues at the C and N ends of adjacent/parallel tropocollagen molecules ;

5. forming collagen fibrils which lie in parallel bundles to form collagen fibres ;

6. staggered ends to prevent weak 'fault lines' across the fibre ;

<u>Haemoglobin</u>

1. Haemoglobin;

2. 4 polypeptide subunits: 2 -globin* subunits and 2 -globin* subunits;

3. each subunit is arranged so that most of its hydrophilic amino acid side chains are on external surface while its hydrophobic amino acid side chains are buried in interior;

4. makes it soluble in water/aqueous environment, can be transported and carry O_2 from lungs to tissues vice versa;

5. each subunit is made of globin polypeptide and a prosthetic (non-protein) component called haem group*;

6. each haem group consists of a porphyrin ring* and an iron ion (Fe^{2+})*;

7. Fe^{2+} of haem group binds temporarily to O_2, so 1 Hb molecule can carry up to 4 O_2, at a time forming oxyhaemoglobin; (ref. transport oxygen in blood)

8. 4 subunits held together by intermolecular interactions formed between R groups (hydrogen bonds, ionic bonds and hydrophobic interactions), allows movement that influences affinity for oxygen allowing for cooperative binding* of oxygen;

9. As a result binding of one oxygen molecule to one haemoglobin subunit induces a conformational change in remaining 3 subunits so that their affinity for oxygen increases;

Haemoglobin: (max 6 marks)

1. 4 polypeptide subunits: 2 -globin* subunits and 2 -globin* subunits;

2. each subunit is arranged so that most of its hydrophilic amino acid side chains are on external surface while its hydrophobic amino acid side chains are buried in interior;

3. makes it soluble in water/aqueous environment, can be transported and carry O_2 from lungs to tissues vice versa;

4. each subunit is made of globin polypeptide and a prosthetic (non-protein) component called haem group*;

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8. As a result binding of one oxygen molecule to one haemoglobin subunit induces a conformational change in remaining 3 subunits so that their affinity for oxygen increases;

5. ROLES OF PROTEINS

1. Enzymatic catalysis function (Any 2 points)

. a) polypeptide chain folds in such a way to form active sites which are complementary in shape and charge to the specific substrate;

. b) specific catalytic R-groups in different active sites interact with the specific substrate forming a temporary association;

. c) active site is flexible, being able to adjust its shape to better fit substrate-induced fit;

. d) enzyme may interact with allosteric regulators that causes shape changes that affect binding of substrate – affecting affinity for substrate;

2. Transport function (Any 2 points)

. a) different proteins may be associated with different prosthetic groups e.g. haem in each of the 4 subunits of haemoglobin;

. b) Fe^{2+} in each haem is able to reversibly bind oxygen oxygen carrier;

. c) positive co-operativity – binding an oxygen molecule enhances conformation of next subunit to bind another oxygen molecule;

. d) changes in primary structure sickle vs normal haemoglobin – amino acid sequence changes loss of function;

3. Structural function

. a) almost every third amino acid is glycine in primary structure allows close fit of 3 polypeptide chains to form compact coil;

. b) covalent cross-links between lysine residues at the C and N terminus of adjacent/parallel tropocollagen molecules and their staggered arrangement allows the formation of fibrils fibres, thus high tensile strength;

4. Buffering role: charged R-groups on plasma proteins can accept H^+ or OH^- ions in solution to prevent (small) changes in pH in blood;

5. Membrane transport proteins

. a) Protein is folded such that hydrophobic and hydrophilic amino acids are arranged such that hydrophilic amino acids are along the channel to allow charged particles/ions to pass through ion channel

. b) Some transport proteins may have binding site for molecules being transported.
When the molecule being transported binds to the transport proteins, it results in a conformational change that translocate the molecule across the membrane. ATP may be used;

Functions	Example & Description
Enzymatic catalysis;	Lysozyme / sucrose / maltase / amylase with elaboration on the reaction it catalyses;
Transport;	Haemoglobin with elaboration on how it transports oxygen / Proton pumps / ion channel proteins with elaboration on how they transport molecules across the membrane;
Structural support;	Collagen with elaboration on how it serves its structural role;
Control of growth and differentiation;	Insulin / glucagon with elaboration of how it regulates blood glucose concentration;
Receptor;	Insulin receptor with elaboration of how it is involved in signal transduction / neurotransmitter receptor with elaboration of how it is involved in synaptic transmission;
Immune protection;	Antibodies with elaboration of how it is involved in recognition and binding to foreign particles/antigen;
Coordination of motion;	Proteins involved in muscle contraction;

6. DENATURATION

1. Increase in kinetic energy* causes amino acids in polypeptide chain to vibrate violently;

2. breaking hydrogen bonds* (between NH and CO groups of amino acids), causing α-helix to uncoil;

Mitosis and Meiosis

TIPS
- To find number of **chromosomes**, count the number of **centromeres**
- To find **amount of DNA**, count the number of **chromatid threads**

MITOSIS

1. MITOSIS

Stage	Amount of DNA per cell	Number of Chromosomes per cell
Normal	x	2 sets
Interphase	**2x** DNA replication	2 sets
Prophase	2x	2 sets
Metaphase	2x	2 sets
Anaphase	2x	**4 sets** Centromeres divide, sister chromatids now called chromosomes
Telophase	2x	4 sets
Cytokinesis	**x** Nuclear envelope reforms	**2 sets** Cells have completely divided

Interphase

During **interphase***,
1. Organelle synthesis occurs in the G1 and G2 phases
2. **Semi-conservative DNA replication*** during S phase of interphase
3. Centrioles replicate during interphase

Prophase ('Prominent')

During **prophase***,
1. Chromatin threads **condense** to form chromosomes and join at the **centromere**, thus becoming visible
2. Two pairs of centrioles migrate to **opposite poles*** and spindle fibres **extend** from each pole to **kinetochore** and **metaphase plate**
3. Nuclear envelope **disintegrates** and nucleolus **disappears**

Metaphase ('Meet')

During **metaphase***,
1. Kinetochore microtubules align chromosomes align in a **single row** at **equator***/on **metaphase plate***
2. **Kinetochore microtubules/spindle fibres*** from **both** poles **attach** to the centromere of chromosome

Anaphase ('Apart')

During **anaphase***,
1. **Centromeres*** divide (! 'replicate' and 'split' are NOT accepted)
2. Kinetochore microtubules/spindle fibres **shorten** and **pull identical sister chromatids***, now called **daughter chromosomes***, **centromeres first***, hence **resulting in characteristic 'V' shape**, to **opposite poles*** (! 'contract' is NOT accepted)
3. **Non-**kinetochore microtubules/spindle fibres **elongate**, causing the 2 poles to move further apart

Telophase ('The end')
During **telophase***,
1. **Chromosomes* uncoil** into long and thin **chromatin*** threads
2. Spindle fibres **disintegrate**
3. Nuclear envelope **reforms** around the chromosomes at each pole and nucleoli **reform**

Cytokinesis
Animal cells - **Cell membrane invaginates** towards the middle, forming a **cleavage furrow** (by microfilaments in 'drawstring effect'). Cell membranes fuses, thus separating the 2 daughter cells

Plant cells - **Vacuoles** appear in the middle of the cell. They **coalesce** to form a **cell plate**, separating the daughter cells

2 genetically identical diploid daughter cells formed, with nuclei that have the **same number of sets of chromosomes** as parent

2. WHY IS ANAPHASE THE LEAST FREQUENTLY OBSERVED STAGE
Anaphase is a very short-lived stage and quickly proceeds to telophase since **separation** of **sister chromatids*** occurs **very fast**

3. EXPLAIN THE NEED FOR GENETIC STABILITY (GENETICALLY IDENTICAL CELLS)
During **S phase of interphase***, **semi- conservative DNA replication occurs** where DNA unwinds and both parental DNA strands act as **templates*** for the synthesis of a complementary strand by adding of nucleotides by **complementary base pairing**. This ensures that **2 genetically identical sister chromatids*** are formed

During **anaphase***, separation of **genetically identical sister chromatids** occurs when (**centromeres*** divide and kinetochore microtubules/spindle fibres **shorten** and **pull** genetically identical **sister chromatids***, now called **daughter chromosomes***, **centromeres first***, hence **resulting in characteristic 'V' shape**, to **opposite poles***)(from 1. MITOSIS Anaphase)

This ensures **even distribution of DNA in the daughter cells** which will thus contain the **same number of chromosomes** with the same **alleles*** as the parent cell. Hence, the daughter cells produced during mitosis are **genetically identical** (not only to one another but) **to their parents** (as well) as **no genetic variation occurs**. Hence, these cells will have **all the genes necessary for survival of the cell/organism** and hence are **already adapted to the environment that allowed the parents to thrive**. These genes will be **faithfully inherited with every replication cycle** so that the resulting cells can continue to function normally. Thus mitosis allows for **growth, repair and asexual reproduction** to produce **genetically identical daughter cells** which are **clones** of each other

4. IMPORTANCE OF MITOSIS FOR GROWTH, REPAIR AND ASEXUAL REPRODUCTION
Mitosis is a form of **nuclear division** which results in the formation of **two cells** that are **genetically identical** to the parents, hence maintaining **genetic stability**

Mitosis is important in

1. **Growth**, as it allows for an **increase in the number of genetically identical cells** in a multicellular organism and contributes to growth via **increase in its size and mass**

2. **Repair**, as it allows **damaged/worn-ou**t cells to be **replaced** e.g. skin cells and **lost** parts of an organism to be **replaced** e.g. lizard's tail, thus leading to the **repair of tissue**

3. **Asexual reproduction**, as it allows for the production of new organisms from just a single parent e.g. **vegetative propagation** of onion bulbs and hence the fusion of gametes is not necessary. (This ensures **even distribution of DNA in the daughter cells** which will thus contain the **same number of chromosomes** with the same **alleles*** as the parent cell. The

daughter cells produced during mitosis are **genetically identical** (not only to one another but) **to their parents** (as well) as **no genetic variation occurs**. Hence, these cells will have **all the genes necessary for survival of the cell/organism** and hence are **already adapted to the environment that allowed the parents to thrive**)(3. EXPLAIN THE NEED FOR GENETIC STABILITY). This allows for **rapid reproduction** for **colonisation of the habitat**

5. HOW IS DNA CONDENSED
When **negatively charged*** DNA is coiled around **8 positively charged*** proteins called **histones***, they form **nucleosomes*** that come together to form a **solenoid** which is coiled to form **looped domains** which then further **supercoil** to form a **condensed chromosome**

6. FUNCTION OF
Centrioles (Str → Fn)
Centrioles are a **pair*** of **cylindrical, rod-like** structures which are **perpendicular*** to each other. Each consists of **9 triplets*** of **microtubules*** arranged in a ring

After centrioles replicate during interphase, the two pairs of centrioles move to **opposite poles*** during **prophase***. The centrioles become part of the **microtubule-organising centre*** in which the assembly of spindle and asters occur

Nucleolus
Nucleoli are the sites of **rRNA synthesis*** where the transcription of genes from ribosomal DNA occurs. Assembly of **rRNA*** and **ribosomal proteins*** into **ribosomal subunits*** is required for translation

Nuclear Pores
Nuclear pores allow **mRNA** to move out of the nucleus into the cytoplasm for **translation*** to polypeptide at the ribosomes and allow **nucleotides** to move into nucleus for **transcription*** or **DNA replication***. They allow **ribosomal proteins** to move into the nucleus for **ribosome subunit assembly** at the nucleolus. They allow the **large and small ribosomal subunits** to leave the nucleus so that the subunits can come together during **translation***

Centromeres
They are **non-coding tandem repeat sequences*** at one location along the length of a chromosome where **sister chromatids*** adhere to each other and allow proteins called **kinetochores***, and subsequently **kinetochore microtubules/spindle fibres*,** to attach.

(During **metaphase***, kinetochore microtubules align chromosomes in a **single row** at **equator***/ on **metaphase plate***. **Kinetochore microtubules/spindle fibres*** from both poles **attach** to the centromere of chromosome (consisting of 2 sister chromatids. During **anaphase***, **centromeres*** divide and kinetochore microtubules/spindle fibres **shorten** and **pull** genetically identical **sister chromatids***, now called **daughter chromosomes***, **centromeres first***, hence **resulting in characteristic 'V' shape**, to **opposite poles***)(from 1. MITOSIS Metaphase and Anaphase)

7. WHY IS THE NUCLEOLUS NOT PRESENT DURING MITOSIS
No rRNA is synthesised during mitosis so the nucleolus is not needed. Hence, chromatin condenses into chromosomes which separate during mitosis such that the tips of chromosomes pairs now no longer form the distinct nucleolus

MEIOSIS
8. MEIOSIS

Stage	Amount of DNA per cell	Number of Chromosomes per cell
Normal	x	2 sets
Interphase	**2x** DNA replication	2 sets
Prophase I	2x	2 sets
Metaphase I	2x	2 sets
Anaphase I	2x	2 sets Homologous pairs separate; centromeres do not divide (differs from Mitosis Anaphase)
Telophase I	2x	2 sets
Cytokinesis	**x** Nuclear envelope reforms	**1 set** Cells have completely divided
Prophase I	x	1 set
Metaphase I	x	1 set
Anaphase I	x	**2 sets** Centromeres divide, sister chromatids now called chromosomes
Telophase I	x	2 sets
Cytokinesis	**½x** Nuclear envelope reforms	**1 set** Cells have completely divided

Interphase
During **interphase***,
1. Organelle synthesis occurs in the G1 and G2 phases
2. **Semi-conservative DNA replication*** during S phase of interphase
3. Centrioles replicate during interphase

Prophase I ('Prominent')
During **prophase I***,
1. Chromatin threads **condense** to form chromosomes and join at the **centromere**, thus becoming visible
2. **Synapsis*** occurs where homologous chromosomes pair up to form **bivalents** and **chiasmata*** form between **non-sister*** chromatids of homologous chromosomes
3. **Crossing over*** of **corresponding segments** at chiasma leads to an **exchange** of equivalent portions of genetic material to form **non-identical sister chromatids**. The non-sister chromatids* of the **homologous chromosomes*** may have **different alleles***, hence breakage of linkage groups and forming new linkage groups after crossing over creates new combinations of alleles on chromatids
4. Two pairs of centrioles migrate to **opposite poles*** and spindle fibres **extend** from each pole to **kinetochore** and **metaphase plate**
5. Nuclear envelope **disintegrates** and nucleolus **disappears**

Metaphase I ('Meet')
During **metaphase I***,
1. Kinetochore microtubules align chromosomes in **two rows** at **equator***/on **metaphase plate*** with each chromosome on either side of the equator. The orientation of chromosomes of each bivalent is completely independent of the orientation of the other bivalents (Independent Assortment of Homologous Chromosomes)
2. **Kinetochore microtubules/spindle fibres*** from **different** poles **attach** to the centromere of chromosome (consisting of 2 sister chromatids)

Anaphase I ('Apart')
During **anaphase I***,
1. ~~Centromeres* divide~~ (! Centromeres do NOT divide)
2. Kinetochore microtubules/spindle fibres **shorten** and **pull homologous chromosomes***, ~~now called daughter chromosomes*,~~ **centromeres first***, hence **resulting in characteristic 'V' shape**, to **opposite poles*** (! 'contract' is NOT accepted)
3. **Non-**kinetochore microtubules/spindle fibres **elongate**, causing the 2 poles to move further apart

Telophase I ('The end')
During **telophase I***,
1. **Chromosomes* uncoil** into long and thin **chromatin*** threads
2. Spindle fibres **disintegrate**
3. Nuclear envelope **reforms** around the chromosomes at each pole and nucleoli **reform**

Cytokinesis
Animal cells - **Cell membrane invaginates** towards the middle, forming a **cleavage furrow** (by microfilaments in 'drawstring effect'). Cell membranes fuses, thus separating the 2 daughter cells

Plant cells - **Vacuoles** appear in the middle of the cell. They **coalesce** to form a **cell plate**, separating the daughter cells

2 genetically different haploid daughter cells formed, with **half** the **number of sets of chromosomes** as parent due to **reductive division**

Prophase II ('Prominent')
During **prophase II***,
1. Chromatin threads **condense** to form chromosomes and join at the **centromere**, thus becoming visible
2. Centrioles replicate (! since there is no interphase between cytokinesis and prophase II)
3. Two pairs of centrioles migrate to **opposite poles*** and spindle fibres **extend** from each pole to **kinetochore** and **metaphase plate**
4. Nuclear envelope **disintegrates** and nucleolus **disappears**

Metaphase II ('Meet')
During **metaphase II***,
1. Kinetochore microtubules align chromosomes align in a **single row** at **equator***/on **metaphase plate*** with each chromosome on either side of the equator. The orientation of chromosomes is completely independent of the orientation of the other chromosomes (Random Orientation of Non-Identical Sister Chromatids of Each Chromosome)
2. **Kinetochore microtubules/spindle fibres*** from both poles **attach** to the centromere of chromosome (consisting of 2 sister chromatids)

Anaphase II ('Apart')
During **anaphase II***,
1. **Centromeres*** divide (! 'replicate' and 'split' are NOT accepted)
2. Kinetochore microtubules/spindle fibres **shorten** and **pull non-identical sister chromatids***, now called **daughter chromosomes***, **centromeres first***, hence **resulting in characteristic 'V' shape**, to **opposite poles*** (! 'contract' is NOT accepted)
3. **Non-**kinetochore microtubules/spindle fibres **elongate**, causing the 2 poles to move further apart

Telophase II ('The end')
During **telophase II***,
1. **Chromosomes* uncoil** into long and thin **chromatin*** threads
2. Spindle fibres **disintegrate**
3. Nuclear envelope **reforms** around the chromosomes at each pole and nucleoli **reform**

Cytokinesis
Animal cells - **Cell membrane invaginates** towards the middle, forming a **cleavage furrow** (by microfilaments in 'drawstring effect'). Cell membranes fuses, thus separating the 2 daughter cells

Plant cells - **Vacuoles** appear in the middle of the cell. They **coalesce** to form a **cell plate**, separating the daughter cells

4 genetically different haploid daughter cells formed, with **half** the **number of sets of chromosomes** as parent due to **reductive division**

9. NEED FOR REDUCTIVE DIVISION (MEIOSIS) PRIOR TO FERTILISATION
Reduction division refers to the production of 4 **haploid*** gametes, with **half** the **number of sets of chromosomes** as parent, from a **diploid*** cell

1. Maintenance of Chromosome Number in offspring in every generation

During reductive division (meiosis), **diploid** cells (which have 2 complete sets of chromosomes) undergo two nuclear divisions (Meiosis I and II) to give rise to **haploid gametes** (which have only 1 complete set of chromosomes). During fertilisation, 2 haploid games (one male and one female) fuse to give rise to a **diploid zygote**. Hence the **chromosome number in the zygotes is restored** when fertilisation occurs

If meiosis did not occur, the fusion of gametes during sexual reproduction will result in the **doubling** in the number of chromosomes with **every successive generation**. Hence reduction division (meiosis) ensures that the **maintenance of chromosome number** in a sexually reproducing species in **every generation**

2. Maintenance of Genetic Variation in offspring in every generation

(see 10. HOW MEIOSIS AND RANDOM FERTILISATION LEAD TO VARIATION)

10. HOW MEIOSIS AND RANDOM FERTILISATION LEAD TO VARIATION

1. During **prophase I***, (**synapsis*** occurs where homologous chromosomes pair up to form **bivalents** and **chiasmata*** form between **non-sister*** chromatids of homologous chromosomes **Crossing over*** of **corresponding segments** at chiasmata leads to an **exchange** of equivalent portions of genetic material to form **non-identical sister chromatids**. The **non-sister chromatids*** of the **homologous chromosomes*** may have **different alleles***, hence breakage of linkage groups and forming new linkage groups after crossing over creates new combinations of alleles on chromatids)(from 8. MEIOSIS Prophase I) thus giving rise to a variety of offspring

2. Independent Assortment/Random Orientation

    1. **Independent assortment of homologous chromosomes*** at the metaphase plate during **metaphase I*** and subsequent separation during **anaphase I***. This can result in gametes with $\mathbf{2^n}$ * possible combinations of maternal and paternal chromosomes where n is number of chromosome pairs

    2. **Random orientation of non-identical sister chromatids of each chromosome*** at the metaphase plate at **metaphase II*** and their subsequent separation during **anaphase II*** results in gametes with different combinations of maternal and paternal chromosomes and eventually in a variety of offspring

3. Genetically Different Gametes/Sexual Reproduction

    1. Meiosis produces haploid gametes that are **genetically different** from the parent, contributing to genetic variation

    2. During **fertilisation* in sexual reproduction**, **random fusion* of gametes** results in greater number of genotypic combinations of a zygote ($\mathbf{2^n \times 2^n}$) and hence a variety of offspring with a variety of genotypes and possible phenotypes

11. HOMOLOGOUS CHROMOSOMES

Homologous chromosomes are a pair of chromosomes that have the same **length***, **centromere position*** and **staining pattern***. They carry **genes*** controlling the **same inherited characteristics** but they may have **different alleles*** at the **corresponding loci** on both members of a homologous chromosome pair, one of paternal and one of maternal origin, and pair up to form **bivalents** during **prophase I***. Hence each member of a pair is **genetically different** from each other

12. CANCER

The cell cycle contains **regulatory checkpoints** that ensure **normal cell division and growth**. **Dysregulation** of the regulatory checkpoints in the cell cycle can result in **uncontrolled cell division** via **gain-in-function mutations** in proto-oncogenes and **loss-of-function mutations** in tumour suppressor genes

<Name factor e.g uv light✓/ethidium bromide✓/Human Papilloma Virus (HPV) ✓>
Other factors:
1. Chemical Carcinogens - asbestos/tar in tobacco smoke
2. Ionising Radiation - x-rays/gamma rays
3. Viruses - Human Immunodeficiency Virus (HIV)

<> causes mutations, which results in changes in the nucleotide sequence. **Gain-of-function mutation*** cause **proto-oncogenes*** to be converted to **oncogenes*** or cause the transfer of the **proto-oncongene** gene via translocation to another chromosome with an **enhancer** where it is **excessively** expressed. This results in the production of proteins (growth factors) that **stimulate** cell division or **prevent production** of inhibitors that stop cell growth, hence causing cells to **divide excessively**.

Loss-of-function mutation* result from the mutation of **both tumour suppressor*** alleles that originally prevented tumour formation, hence cells **lose** their ability to **stop dividing**

This causes the cell to:
1. Undergo **uncontrolled cell division*** resulting in excessive cell growth and proliferation that forms a **mass of cells** called a **tumour***
2. **Lose contact inhibition***
3. No longer undergo **programmed cell death* (apoptosis)**
4. Undergo **angiogenesis*** (where blood vessels grow into the tumour and supply it with nutrients and means to metastasise)

When tumour cells become detached and spread to other parts of the body via the blood stream to form new tumours the condition is called **metastasis*** (migration of cells to other locations via blood vessels) and the tumour is said to be malignant and the individual is said to have cancer. Tumours can compress or invade other tissues and organs, thus leading to reduced function and death

13. IMPORTANCE OF CELL CYCLE REGULATORY CHECKPOINTS
1. Cell cycle regulatory checkpoints control the **rate** of cell division and the number of cell cycles a cell undergoes, thus allowing for the **coordination of growth** by **regulating the growth** of one tissue in **proportion** to others

2. Such control also helps to **minimise exposure to mutations** as an **increased number** of DNA replication cycles **increases the chance** of alterations to DNA. This thus prevents tumour formation by preventing uncontrolled cell division, hence avoiding the effect of tumours, which can compress or invade other organs, thus leading to reduced function and death

14. MITOSIS V MEOISIS

Feature	Mitosis	Meiosis
1. Location and Cell Type	Mitosis occurs in **somatic cells** in **all parts** of the body	Meiosis occurs in **precursor sex cells** in **reproductive organs** that give rise to **gametes**
2. Number of Nuclear Divisions	**One**	**Two**
3. Prophase	During mitosis, **synapsis** does not occur, there is no **chiasmata** formation and no **crossing over** of corresponding segments Centrioles **do not replicate**	During prophase I, 1. **Synapsis*** occurs where homologous chromosomes pair up to form **bivalents** and **chiasmata*** form between **non-sister*** chromatids of homologous chromosomes 2. **Crossing over*** of **corresponding segments** at chiasma leads to an **exchange** of equivalent portions of genetic material to form **non-identical sister chromatids**. The **non-sister chromatids*** of the **homologous chromosomes*** may have **different alleles***, hence breakage of linkage groups and forming new linkage groups after crossing over creates new combinations of alleles on chromatids Prophase II, however, is similar to prophase of mitosis, except that centrioles **replicate** in prophase II
4. Metaphase	During mitosis, 1. Kinetochore microtubules align chromosomes align in a **single row** at **equator***/on **metaphase plate*** 2. **Kinetochore microtubules/ spindle fibres*** from **both** poles **attach** to the centromere of chromosome	During metaphase I, 1. Kinetochore microtubules align chromosomes align in **two rows** at **equator***/on **metaphase plate*** with each chromosome on either side of the equator. The orientation of chromosomes of each bivalent is completely independent of the orientation of the other bivalents (Independent Assortment of Homologous Chromosomes) 2. **Kinetochore microtubules/spindle fibres*** from **different** poles **attach** to the centromere of chromosome (consisting of 2 sister chromatids)

5. Anaphase	During anaphase, 1. **Centromeres*** **divide** (! 'replicate' and 'split' are NOT accepted) 2. Kinetochore microtubules/ spindle fibres **shorten** and **pull** **identical sister chromatids***, now called **daughter chromosomes***, **centromeres first***, hence **resulting in characteristic 'V' shape**, to **opposite poles*** (! 'contract' is NOT accepted)	During anaphase I, 1. Centromeres **do NOT divide** 2. Kinetochore microtubules/spindle fibres **shorten** and **pull** **homologous chromosomes*** **centromeres first***, hence **resulting in characteristic 'V' shape**, to **opposite poles*** (! 'contract' is NOT accepted) Anaphase II is similar to metaphase of mitosis, except that **non-sister chromatids** separate
6. Telophase & Cytokinesis	During telophase and cytokinesis, **2 genetically identical diploid** daughter cells formed, with nuclei that have the **same** **number of sets of chromosomes** as parent	During telophase I and cytokinesis, **2 genetically different haploid** daughter cells formed, with **half** the **number of sets of chromosomes** as parent due to **reductive division** During telophase II and cytokinesis, **4 genetically different haploid** daughter cells formed, with **half** the **number of sets of chromosomes** as parent due to **reductive division**
7. Result of nuclear division (Ploidy and Variation)	Mitosis is known as **replicative** division as daughter cells have the **same** **number of sets of chromosomes** as parent	Meiosis is known as **reductive** division as daughter cells have the **half** the **number of sets of chromosomes** as parent

Photosynthesis

LIGHT DEPENDENT REACTIONS (Read 1. PHOTOACTIVATION OF CHLOROPHYLL - 4. PRODUCTS AND BY PRODUCTS OF PHOTOPHOSPHORYLATION in sequence)

1. PHOTOACTIVATION OF CHLOROPHYLL
Photosystems consist of a **reaction centre** surrounded by many **light harvesting complexes**. The reaction centre contains of **two special chlorophyll a molecules** and a **primary electron acceptor**. **Photosynthetic pigments** (bound to proteins) are **arranged in light harvesting complexes**

When a **photon of light*** at 680/700nm is absorbed by an **accessory pigment** molecule in the light harvesting complex of photosystem II/I, one of its electrons is **excited to a higher energy state**. This is photoactivation. As the excited electron drops to its ground state, the energy released is passed on to the next pigment molecule and excites another electron in it. Energy is relayed from pigment to pigment, via resonance transfer of energy, until it reaches **one of the two** specialised chlorophyll a(P680/700) in the reaction center of photosystem II/I.

When the specialised chlorophyll a molecule absorbs the energy, an **electron is excited** (**Chlorophyll a → Cholrophyll a+ + e-**), leaving an **e- hole** in PSII/PSI. This excited electron emitted from chlorophyll a (P680/P700) is captured by the **primary electron acceptor**, in the reaction centre

2. ABSORPTION SPECTRUM

<u>Chlorophyl a and b</u>

The **highest rates** of photosynthesis corresponds to the **absorption peaks** of chlorophylls a and b at about 425nm and 665nm. The **frequencies of lower absorbance**, 480 to 600nm, corresponds to **reduced photosynthetic activity**. This shows that chlorophylls a and b are the **primary** pigments involved in photosynthesis

<u>Carotenoids</u>

At around 440 to 480nm, photosynthetic rate was higher than can be accounted for by the absorption spectrum of chlorophylls a and b alone. Carotenoids absorb strongly at these wavelengths. Carotenoids, an accessory pigment, **broaden the spectrum of wavelength over which photosynthesis can occur by channeling the energy absorbed to the reaction centre**

<u>What precautions must be taken for experiments where light intensity is independent variable?</u>

Supposing other factors such as pH, concentration of CO2, temperature are kept constant

1. Light must be **monochromatic** (one wavelength). Hence, use **prism** to separate white light
2. Experiment must be performed in the **dark** (except for light of that particular wavelength)
3. Light **intensity and duration** must be kept the same for each wavelength
4. Allow the plant to **adjust**/acclimatise to the new wavelength before taking readings

3. PHOSPHORYLATION (*CYCLICAL*)

Excited electrons flow down a series of **increasingly electronegative** electron carriers along the electron transport chain (ETC) which links PS II to PSI, **losing energy** during the transfer. *Electron flow is cyclical passing from PS I (as electron donor) to ETC and going back to PS I.* The **energy lost through redox reactions during this e- flow down the electron transport chain** is used to **actively pump H+ from**

the stroma across the thylakoid membranes into the thylakoid space (**inwards**). This creates a **H+ gradient** across thylakoid membrane which acts as a proton-motive force. Since the membrane is impermeable to ions, **proton/H+ diffuses** down its concentration gradient back into the stroma via **ATP synthase***, which drives ATP synthesis where ADP is phosphorylated to ATP. This process of **coupling the energy released from the flow of protons to the phosphorylation** of ADP to form ATP is known as **chemiosmosis**

What contributes to the high H+ concentration in the thylakoid space? (in order of importance)

1. **Proton pump** (**actively pumps H+** from the stoma across the thylakoid membranes into the thylakoid space. This creates a **H+ gradient** across thylakoid membrane which acts as a proton-motive force. Since the membrane is impermeable to ions, **proton/H+ diffuses** down its concentration gradient back into the stroma via **ATP synthase***, which drives ATP synthesis where ADP is phosphorylated to ATP. This process of **coupling the energy released from the flow of protons to the phosphorylation** of ADP to form ATP is known as **chemiosmosis**)(from 3. PHOSPHORYLATION (CYCLICAL)

2. **Photolysis of water** (which is enzyme-catalysed reaction catalysed by enzymes on **inner thylakoid membrane**) which occurs in the thylakoid space. During the splitting of water, the **H+ released** contributes to the **high H+ concentration in the thylakoid space**, while the O atom combines with another O atom resulting in the release of **molecular oxygen** as a **by-product**)(from 4. PRODUCTS AND BY-PRODUCTS OF PHOTOPHOSPHORYLATION)

3. **Lack of permeability** of thylakoid membrane (since the **hydrophobic core*** of the membrane is **impermeable to protons**)(from 9. FUNCTION OF THYLAKOID MEMBRANE)

4. **Reduction of NADP+ to NADPH** occurs in the **stroma** and hence (**reduces the H+ concentration in the stroma** thereby ensuring the steepness of the H+ gradient across the membrane)(from 4. PRODUCTS AND BY-PRODUCTS OF PHOTOPHOSPHORYLATION)

4. PRODUCTS AND BY-PRODUCTS OF PHOTOPHOSPHORYLATION

Non-cyclical

e- lost from PSII is **replaced** by an e- related from the **splitting of water** (which is enzyme-catalysed reaction catalysed by enzymes on **inner thylakoid membrane**) which occurs in the thylakoid space. During the splitting of water, the **H+ released** contributes to the high H+ concentration in the thylakoid space, while the O atom combines with another O atom resulting in the release of **molecular oxygen** as a **by-product** ($H_2O + 2e^- \rightarrow 2H^+ + \frac{1}{2}O_2$)

When the e- from PSII reaches the end of the 1st ETC, it will then fill the e- hole in PSI

Non-cyclical/Cyclical

The e- from the 2nd ETC is finally accepted by **NADP+** (the final electron acceptor) which is **reduced to NADPH** ($NADP^+ + e^- + H^+ \rightarrow NADPH$) by **NADP reductase** which is found on the thylakoid membrane. This **reduces the H+ concentration in the stroma** thereby ensuring the steepness of the H+ gradient across the membrane

Overall

The ATP and NADPH produced will be used in the Calvin Cycle. ATP is source of energy and is used to drive the Calvin Cycle. ATP also used in the **regeneration of RuBP***. The **high energy e- stored in NADPH provides the reducing power** for the **reduction*** of GP to **glyceraldehyde-3-phosphate*** (G3P)(3C) to synthesise sugar

5. CYCLIC VS NON-CYCLIC PHOTOPHOSPHORYLATION

Features	Non-cyclic photophosphorylation	Cyclic photophosphorylation
1. End product(s)	This process produces **ATP, NADPH and O2**	This process produces **only ATP**
2. Photosystem involved	This process involves **both** PS II and I	This process involves **only PS I**
3. Source of electrons	Involves splitting of water by an **enzyme**. Electron donor is **water** ! Note: direct donor is P680	Electron donor is **P700** and it **does not involve** the splitting of water by an enzyme
4. Pathway of electrons	Electron flow is in **one direction** from water as electron donor through **two ETC to NADP+** via two photosystems	Electron flow is **cyclical** passing from PS I as electron donor to 1st electron transport chain (ETC) and going back to PS I
5. Final electron acceptor	Final electron acceptor is **NADP+**	Final electron acceptor is **P700**
6. Maintenance of high proton/ H+ concentration in thylakoid space	High H+ concentration in the thylakoid space is due to **active transport** of H+ ions by **electron carriers of ETC** from stroma, across the thylakoid membrane into the thylakoid space, and **photolysis of water** ($H_2O + 2e^- \rightarrow 2H^+ + \frac{1}{2}O_2$)	High H+ concentration in the thylakoid space is due to **active transport** of H+ ions by **electron carriers of ETC** from stroma, across the thylakoid membrane into the thylakoid space

6. [X-RESPIRATION] PHOTOPHOSPHORYLATION VS OXIDATIVE PHOSPHORYLATION

	Photophosphorylation	Oxidative Phosphorylation
Similarity 1: Proton Pump	Energy lost from the flow of electrons along an electron transport chain is used to **actively pump** protons across membrane to generate **proton gradient**	
Similarity 2: Chemiosmosis	ADP is phosphorylated to form ATP via **ATP synthase*** using energy directly from the flow of protons down its gradient via **chemiosmosis**	
Differences		
3. Location	Process takes place in the **thylakoid membrane** of **chloroplasts**	Process takes place in the **inner membrane** of **mitochondria**
4. Source of energy	Energy for synthesis of ATP comes ultimately from **light**	Energy for the synthesis of ATP comes from the **oxidation of glucose** which **stores chemical energy**;
5. Electron donors	**Water** is the electron donor in the non-cyclic pathway while **Photosystem I** is the electron donor in the cyclic pathway	**NADH** and **FADH2** are the electron donors to the first electron carrier of ETC

6. Electron acceptor	**NADP+** is the final electron acceptor in the non-cyclic pathway while **Photosystem I** is the final electron acceptor in the cyclic pathway	**Oxygen** is the final electron acceptor (and it combines with H^+) and is reduced to water
7. By-product	Splitting of water produces **oxygen** as by-product during non-cyclic pathway	**Water** is produced as by-product when oxygen combines electron and proton at the end of ETC
8. Energy conversion	**Light** energy is converted to **chemical** energy in the process	**Chemical** energy (from glucose) is converted to **chemical** energy (in the form of ATP) in the process
9. Location of proton reservoir	Protons are pumped **inwards** from stroma across the thylakoid membrane, **into the thylakoid space** to establish a proton gradient for ATP synthesis	Protons are pumped **outwards** from mitochondrial matrix across the inner membrane, **into the intermembrane space** to establish a proton gradient for ATP synthesis

LIGHT INDEPENDENT REACTIONS

7. CALVIN CYCLE

Step	Products involved	Molecules involved
1. Carbon Fixation	3 CO2 (3C) → 3 unstable 6C intermediate → 6 GP (3C)	2 ATP → 2 ADP

The Calvin Cycle occurs in the **stroma** of chloroplast

Step 1: Carbon fixation

During carbon fixation stage, CO2 is combined with **ribulose bisphosphate (RuBP)*** (5C) to form an unstable 6 carbon molecule which will immediately split to form 2 molecules of **glycerate phosphate*** (GP)(3C). Carbon fixation is catalysed by enzyme, **RuBP carboxylase*** (Rubisco)

Step 2: Reduction by NADPH

The high energy e- stored in **NADPH** provides the reducing power for the **reduction*** of GP to **glyceraldehyde-3-phosphate*** (G3P or ! triose phosphate)(3C). When GP is reduced to G3P, NADP+ is regenerated to carry out its role as an electron carrier from the light dependent reactions. **ATP** (from light-dependent reaction) is the source of energy required. **6** ATP and 6 NADP+ are required to fix G3P. G3P is the first sugar formed in photosynthesis and the end product of Calvin cycle

Step 3: Regeneration of RuBP

5 molecules of G3P are used to **regenerate* 3** RuBP so that the cycle of carbon dioxide fixation can continue. This requires **3** ATP (from light-dependent reaction)

The net synthesis of **1** molecule of G3P requires **3** CO2 to be fixed

2 molecules of G3P may be used to form **1** molecule of glucose (hexose sugar), hence **6** CO2 is required to fix **2** molecules of G3P using **6** ATP to form **1** molecule of glucose. In total, **12** ATP and **6** NADP are used in the Calvin Cycle

8. [X-RESPIRATION] ROLE OF NADP AND NAD

NADP
NADP+ is a **coenzyme*** which carries both high energy protons and electrons. It is the **final electron acceptor*** in the **non-cyclic light dependent reactions** where it is reduced to NADPH. The e- from the 2nd ETC is finally accepted by **NADP+** (the final electron acceptor) which is **reduced to NADPH** (NADP+ + e- + H+ → NADPH) by **NADP reductase** which is found on the **thylakoid membrane grana**. This **reduces the H+ concentration in the stroma** thereby ensuring the steepness of the H+ gradient across the membrane. It is found on the thylakoid membrane grana. **Protons and high energy electrons** are carried in reduced NADP (NADPH) between **photosystems** in light dependent reactions to be used in the **Calvin cycle in the stroma** of the chloroplast where the high energy e- stored in **NADPH** provides the reducing power for the **reduction*** of GP to **glyceraldehyde-3-phosphate*** (G3P)(3C). When GP is reduced to G3P, NADP is regenerated to carry out its role as an electron carrier from the light dependent reactions.

NAD

Organic molecules are oxidised during glycolysis, link reaction and Krebs cycle and the high energy electrons and protons from the oxidation process are **transferred to the coenzyme NAD+ to form NADH** (reduced NAD+). NAD serves as a **mobile electron (and proton) carrier** to carry the high energy electrons and protons from these organic molecules to the electron transport chain on the cristae of mitochondria. The high energy electrons in NADH are used to **reduce the electron acceptors** of the electron transport chain, while NADH itself gets **re-oxidised**. As electrons pass down the chain, the release of energy in a series of redox reactions is coupled to the oxidative **phosphorylation of ADP to form ATP**. The protons liberated in the oxidation of NADH is used to **establish the proton motive force** necessary for ATP synthesis (the H+ can be either pumped into intermembrane space or combines with oxygen to form water in the matrix). Reoxidation of NADH allows the **regeneration of the coenzyme NAD+**, allowing it to pick up more protons and electrons from Krebs cycle, link reaction and glycolysis, so that these reactions can continue. Each reduced NAD in the matrix yields **3 ATP** through oxidative phosphorylation.

9. FUNCTION OF THYLAKOID MEMBRANE

1. Provides a **large surface area** to **embed** many photosynthetic pigments / chlorophyll molecules for **light absorption***

2. Maintains the **sequential arrangement** of the **photosystems*** and **electron carriers** of **electron transport chain*** for the flow of electrons

3. Maintains **proton gradient** for ATP synthesis since the **hydrophobic core*** of the membrane is **impermeable to protons** and this is essential for chemiosmosis

4. Allows for **many ATP synthase*** to be **embedded** so ATP can be produced as protons flow down their gradient via **chemiosmosis*** from thylakoid space to stroma

FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS

10. LIMITING FACTORS

CO2
CO2 is a raw material for Calvin cycle, used in carbon fixation of RUBP* catalyzed by **Rubisco***. Increased concentration of CO2 will increase rate of photosynthesis. Since atmospheric concentration of CO2 is at a low level of 0.04%, CO2 becomes a limiting factor of photosynthesis;

Light Intensity

Light plays an important role in light dependent stage of photosynthesis as it excites electrons in photosynthetic pigments to higher energy state through photoactivation. Different wavelengths of light stimulate photosynthesis differently. Rate of photosynthesis is high under wavelengths of light about 700nm (red light) & about 470 nm(blue light). Light intensity is rarely limiting during daylight hours;

11. CHANGES TO GP AND RuBP QUANTITIES WHEN

Light → Dark
In the dark, **light dependent reactions*** do not occur and therefore **ATP*** and **NADPH*** is not produced. The amount of RuBP decreases as it is carboxylated and converted to GP since carbon fixation continues as it does not require ATP or NADPH. **RuBP regeneration*** is slowed due to the lack of ATP from the light dependent reactions and hence causing an eventual lack of G3P. GP accumulates in the dark because GP is not reduced into G3P due to lack of ATP and NADPH. Thus, the amount of GP increases. The decrease in RuBP corresponds to a two-fold increase of GP. This is because 2 GP is formed from every 1 RuBP

CO2 REDUCED

RuBP increases

RuBP being the carbon dioxide acceptor, would increase, as less is used in carbon fixation at low CO2 concentration. RuBP regeneration continues as GP continues to be converted to RuBP, giving rise to more RuBP and less GP

GP decreases

GP (in the presence of ATP and NADPH) reduced to **G3P*** and eventually into RuBP, therefore quantity of GP drops. Little RuBP is fixed, due to low CO2 concentration, therefore there is very little GP replacement

12. EFFECT OF LIGHT INTENSITY ON CO2 ASSIMILATION RATE

Highest Light Intensity

At higher light intensity when CO2 assimilation rate levels off, the chloroplasts are **saturated* with light** and photosynthesis is occurring at maximum rate. Light is no longer a limiting factor and other factors such as temperature become the limiting factor

Lowest Light Intensity

At the lowest light intensity there is low or no photosynthesis occurring because there are less photons of light for photoactivation of chlorophyll molecules in the light dependent stage of photosynthesis. Respiration occurs at a higher rate than photosynthesis

13. COMPENSATION POINT

The **light compensation point*** is the point where at that value of light intensity, the rate of respiration equals the rate of photosynthesis. Hence, the amount of CO2 given out during respiration is equivalent to the amount of CO2 fixed during the light independent stage of photosynthesis. Thus, there is no net gain in dry mass and no growth as the products of photosynthesis (e.g. glucose) are used up in respiration. Plants with high light compensation point require higher light intensity for photosynthesis

Respiration
AEROBIC RESPIRATION
GLYCOLYSIS

Step	Products involved	Molecules involved
1. Phosphorylation of Sugars	Glucose (6C) → Fructose 1,6-bisphosphate	2 ATP → 2 ADP
2. Lysis	Fructose 1,6-bisphosphate (6C) → 1 Glyceraldehyde-3-Phosphate (G3P)(3C) + 1 Dihydroxyacetone Phosphate (3C) ! Dihydroxyacetone Phosphate is converted into G3P, hence a total of 2 G3P is produced	-
3. Oxidation by Dehydrogenation	2 G3P → 2 1,3-bisphosphoglycerate (3C)	2 NAD → 2NADH (H removed from G3P and transferred to proton acceptor NAD)
4. Substrate level Phosphorylation (1)(2)	2 1,3-bisphosphoglycerate (3C) → 2 Glycerate-3-Phosphate (GP)(3C) → 2 Pyruvate (3C)	2 ATP → 2 ADP 2 ATP → 2 ADP

1. ATP SYNTHESIS + SIGNIFICANCE
2 **ATP*** are used to phosphorylate glucose to fructose-1,6-bisphosphate. Subsequently 4 **ATP*** are produced via **substrate level phosphorylation** when 2 **glyceraldehyde-3-phosphate (G3P)** molecules are converted to 2 **pyruvate** molecules. Thus a **net*** of 2 **ATP*** are produced. As oxygen is not required for this step, muscle contraction can still take place even when no oxygen available which would result in **oxidative phosphorylation*** being unable occur to meet the ATP demand. **Glycolysis*** can still occur as the **NAD needed for glycolysis is regenerated** from **fermentation reactions** via anaerobic respiration, and thus glycolysis is still able to meet the ATP demand.

ANAEROBIC RESPIRATION
2.
There is a net production of **2 ATP** per **glucose molecule oxidised** during **anaerobic respiration**. This is **19 times lower** compared to ATP production from aerobic respiration which produces **38 ATP** per glucose molecule oxidised

In the absence of oxygen as the f**inal electron acceptor** to **accept electrons** from the electron transport chain (ETC), electron carriers **remain reduced** and so NADH and FADH2 can **no longer donate electrons** to the ETC. Thus, reactions in the mitochondria (oxidative phosphorylation, Krebs cycle and link reaction), which produce the **bulk of ATP**, do not occur. In the absence of oxygen, **glycolysis* alone** can still occur to continue to produce **only 2 ATP** per glucose oxidised. As glycolysis proceeds **NADH accumulates** because it is **unable to donate** its electrons to the ETC and hence **cannot be oxidised**. NAD+ hence **cannot be regenerated** during oxidative phosphorylation. NAD+ needs to be regenerated in order for glycolysis to continue. Therefore, NAD is regenerated from fermentation reactions in aerobic respiration where **pyruvate** acts as the **final electron acceptor** and is first decarboxylated to ethanol then reduced to ethanol/ lactate by NADH. In the process, NAD+ is regenerated so that it may return to take part in glycolysis to continue producing 2 ATP

3. FERMENTATION REACTIONS

Steps	Products involved	Molecules involved
Alcohol Fermentation in Yeast		
	2 Pyruvate → 2 Ethanal + 2 CO2	-
Reduction	2 Ethanal → 2 Ethanol	2 NADH → 2 NAD+ Alcohol Dehydrogenase
Lactate Fermentation in Animals		
Reduction	2 Pyruvate → 2 Lactate	2 NADH → 2 NAD+ Lactate Dehydrogenase

Yeast

In **yeasts**, **Pyruvate** is converted to ethanal and CO2. Ethanal is then reduced by electrons from NADH in the presence of **alcohol dehydrogenase** to **ethanol**

Animals

In **animals**, **Pyruvate** is reduced by electrons from NADH in the presence of **lactate dehydrogenase** to **lactate**

4. ALCOHOL V LACTATE FERMENTATION

Point of Comparison	Alcohol Fermentation in Yeast	Lactate Fermentation in Animals
Similarity	NADH is reoxidised to regenerate NAD^+, which can be used in glycolysis to generate more ATP	
1. End Product	Pyruvate is reduced to **ethanol via ethanal**	Pyruvate is reduced to **lactate**
2. Presence of Decarboxylation Step	Pyruvate first undergoes a decarboxylation step to **produce one molecule of CO_2** before it is reduced	No decarboxylation step takes place
3. Enzymes involved	**Alcohol dehydrogenase** reduces the ethanal to ethanol	**Lactate dehydrogenase** reduces the pyruvate to lactic acid

5. FATE OF PYRUVATE IN AEROBIC V ANAEORBIC RESPIRATION

In the presence of **oxygen***, pyruvate is actively transported into mitochondria and is oxidized to **carbon dioxide*** and **water***. In the absence of **oxygen***; pyruvate remains in the cytosol and is reduced to **lactate***

LINK REACTION

Step	Products involved	Molecules involved
Oxidative Decarboxylation	2 Pyruvate + 2CoA → 2 Acetyl CoA	2 NAD → 2 NADH, 2 CO2 produced

Link reaction takes place in **mitochondrial matrix** when oxygen is present. Pyruvate is a**ctively transported** into the mitochondria matrix via a transport protein. 2 pyruvate (3C) molecules undergo an **oxidative decarboxylation step** to form 2 acetyl CoA (2C) molecules. **2 NADH and 2 CO2** are produced

KREBS CYCLE
6.

Step	Products involved	Molecules involved
-	2 Acetyl CoA (2C) + 2 Oxaloacetate (4C) → 2 Citrate (6C)	-
Oxidative Decarboxylation	2 Citrate (6C) → 2 α-ketoglutarate (5C)	2 NAD → 2 NADH, 2 CO2 produced
Oxidative Decarboxylation	2 α-ketoglutarate (5C) → 2 Oxaloacetate (4C)	2 NAD → 2 NADH, 2 CO2 produced
Substrate level Phosphorylation		2 ATP → 2 ADP
Reduction		2 FAD → 2 FADH2 2 NAD → 2 NADH

Krebs Cycle: 6 NADH produced, 2 FADH2 produced, 2 ATP produced, (4 CO2 produced)
Glycolysis: 2 NADH produced, 2 ATP produced
Link Reaction: 2 NADH produced, (2 CO2 produced)
Total: 10 NADH produced, 2 FADH2 produced, 4 ATP produced

1 NADH yields 3 ATP, 1 FADH2 yields 2 ATP

10x3 + 2x2 + 2 + 2 = 38 ATP

Krebs cycle takes place in the **mitochondrial matrix** when oxygen is present. Acetyl CoA (2C) formed through link reaction combines with oxaloacetate (4C) to form citrate (6C);
Citrate is decarboxylated and dehydrogenated to form α-ketoglutarate (5C) and NADH.
Each decarboxylation step results in a loss of carbon in the form of a carbon dioxide.
Regeneration of oxaloacetate (4C) involves one decarboxylation step and three dehydrogenation steps to yield 2 NADH, 1 $FADH_2$ and 1 CO_2. High energy electrons originally from the glucose molecule have now been transferred to electron carriers NAD+ and FAD

$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$ (or reduced NAD)
$FAD + 2H^+ + 2e^- \rightarrow FADH_2$ (or reduced FAD)

1 ATP is also produced through substrate-level phosphorylation during this regeneration process. All the carbon in glucose is lost as carbon dioxide. Altogether 1 molecule of glucose will yield 6 NADH, 2 $FADH_2$ & 2 ATP through the Krebs cycle. The coenzymes with their reducing power will next be transported to the electron transport chain where the bulk of ATP is generated

7. ROLE OF NAD

As different substrates of the Krebs cycles are oxidized by dehydrogenation**,** the electrons and protons are transferred to the **coenzyme NAD$^+$**. NAD^+ is reduced to NADH. NADH will eventually transfer these electrons to the electron transport chain on the cristae of the mitochondrion, resulting in regeneration of NAD^+ for the synthesis of ATP via **oxidative phosphorylation***

8. [X-PHOTOSYNTHESIS] CALVIN X KREBS CYCLE

	Calvin Cycle	**Krebs Cycle**
Site	Occurs in the **stroma*** in the chloroplast	Occurs in the **matrix*** in the mitochondrion
Electron and Proton carrier(s)	**NADP+** accepts electrons and proton to be reduced to **NADPH**	**NAD+/ FAD** accepts the electrons and proton to be reduced to **NADH/$FADH_2$**
Carbon Dioxide	**Fixed** by ribulose bisphosphate carboxylase (Rubisco)	**Released** by **oxidative decarboxylation**
ATP	It is **used** during the **reduction of GP to G3P** and **regeneration of RuBP**	It is **synthesised** during **substrate level phosphorylation**

OXIDATIVE PHOSPHORYLATION
9.
Reduced NAD and FAD donate electrons to the electron transport chain (ETC). As electrons are passed down electron carriers of increasing electronegativity, energy release is coupled to the pumping of H+ into the intermembrane space to generate a proton motive force. As H^+ ions diffuse through the **ATP synthase*** back into the matrix, ATP is produced from ADP and inorganic phosphate via **chemiosmosis***. **Oxygen*** is the **final electron acceptor*** at the end of the ETC, which will combine with H^+ to form water

10. WHY DOES FADH2 YIELD LESS ATP THAN NADH
The electrons from FADH2 are also transferred down the ETC. However, FADH2 releases the electrons lower in the ETC as compared to NADH. Hence, less energy is released from FADH2 during electron transfer. The regenerated FAD then can pick up electrons and protons from the Krebs cycle

11. ROLE OF OXYGEN
Oxygen is the **final electron acceptor*** at the end of the **electron transport chain*** (ETC), where $2e^- + 2H^+ + \frac{1}{2}O_2 \rightarrow H_2O$. This reaction is catalysed by **cytochrome oxidase**. By removing electrons, oxygen re-oxidises the ETC so that NADH and $FADH_2$ can continue to donate electrons to the chain, thereby allowing **oxidative phosphorylation*** to continue to produce ATP. This in turn allows the regeneration of **NAD^+*** and **FAD*** allowing them to pick up more protons and electrons from the link reaction, Krebs cycle and glycolysis

12. HOW IS MITOCHONDRIA ADAPTED FOR AEROBIC RESPIRATION

1. Through **compartmentalization**, the **double membrane** of the mitochondrion **isolates** the Krebs cycle and oxidative phosphorylation from reactions occurring in the cytoplasm, such as glycolysis. This improves the efficiency of the different reactions by **concentrating the enzymes needed in a fixed compartment** and providing **optimal conditions** required for each reaction to take place i.e. Krebs cycle in the matrix and oxidative phosphorylation across the cristae

2. The inner membrane is **highly folded into cristae**, which **increases the surface area** for **attachment** of many electron carriers involved in the electron transport chain and enzymes such as ATP synthase. By being **membrane bound**, these enzymes involved in oxidative phosphorylation can be attached in an **ordered sequence** that facilitates the transport of electrons. This improves efficiency

3. In between the double membranes is the intermembrane space. The **phospholipid bilayer** making up the membranes that enclose this region are **impermeable to charged ions**. The enclosed intermembrane space therefore allows the **build up of protons** here by the proton pumps found on the inner membrane, enabling the **establishment of a proton gradient across the inner membrane**. This **proton motive force** is then coupled to the production of ATP from ADP by ATP synthase. Therefore, this compartmentalisation allows **chemiosmosis** to take place;

4. The **selective permeability** of the mitochondrial double membrane to oxygen and pyruvate enables a constant supply of these substrates to enter the mitochondrion, and also for CO_2 to leave as a by-product.

13. ARE THERE NET CHANGES TO [NAD/NAD+] IN THE WHOLE PROCESS OF RESPIRATION?

No **net*** change as **NAD+*** is reduced to **NADH*** when it accepts electrons during **glycolysis***, **link reaction*** and **Krebs cycle***. During **oxidative phosphorylation*, NADH*** is oxidised to regenerate **NAD+*** as it donates electrons to the **electron transport chain***

Mendelian Genetics

1. STANDARD RATIOS

Ratio	Feature
Complete Dominance | Monohybrid	
3:1	Heterozygote x Heterozygote
2:1	Heterozygote x Heterozygote ! Lethal Alleles (Homozygous Dominant is lethal)
1:1	Heterozygote x Homozygous Recessive ! Test Cross ? x Homozygous Recessive
Complete Dominance | Dihybrid	
9:3:3:1	Heterozygote (both traits) x Heterozygote (both traits)
1:1:1:1	Heterozygote (both traits) x Homozygous Recessive (both traits)
Codominance | Monohybrid	
1:2:1	Heterozygote x Heterozygote e.g. I^AI^B x I^AI^B ($I^AI^BI^O$ blood type system)

2. EPISTATIC RATIOS

Ratio	Feature (Case Study)	Deviation from...
9:3:3:1 R_P_: R_p'p': rrP_: rrp'p' Walnut:Rose:Pea:Single	Recessive Epistasis (Comb Type of Chicken)	9:3:3:1 Complete Dominance | Dihybrid
Explanation: rr and p'p' genotypes are epistatic over the walnut comb phenotype		
9:7 C_P_:C_p'p':c'c'P_:c'c'p'p' Purple:White	Recessive Epistasis (Flower Colour in Sweet Pea)	9:3:3:1 Complete Dominance | Dihybrid
Explanation: c'c' genotype is epistatic over P/p' locus Gene C codes for enzyme C which converts colourless precursor into a colourless intermediate		

HOW DOES EVIDENCE SUPPORT POLYGENIC/CONTINUOUS VARIATION

There is a range of values observed for <>. Although each gene only has a small effect, these genes act in an additive manner leaving to continuous variation

WHY IS THERE A DIFFERENCE IN VARIATION BETWEEN F1 AND F2 FOR CONTINUOUS VARIATION
F2 shows a **wider range of variation** as compared to F1. In F1, there is only 1 genotype, hence any variation in **heterozygous** individuals is due **only to the environment**. The wide range of **variation in F2** is due to **meiosis in the formation of gametes** from the heterozygous F1 due to processes such as: 1. **independent assortment** of **homologous** chromosomes, 2. **crossing over** which forms new linkage groups and 3. **random fusion of gametes** during fertilisation. The additive effect of different allelic combinations of multiple gene result in a wider range of phenotypes than F1

!
1. Diploid + Trait controlled by one gene with two alleles showing codominance = Discontinuous variation

Evolution

1. WHY POPULATION IS THE SMALLEST UNIT TO EVOLVE + DEFINITION OF EVOLUTION

A population is a group of **interbreeding individuals** of the **same species**. Variation exists in a population. **Natural selection acts on the individuals** in a population and results in the **perpetuation of favourable characteristics (determined by favourable alleles)** in **successive generations**. Thus, over many generations, a **higher proportion** of the population will bear the **favourable characteristics/alleles**. Since evolution is a measure of **changes in allele frequencies in a population over time**, only a population can involve, not individuals

5 AGENTS OF EVOLUTION

1. Mutation
2. Natural Selection
3. Disruption of Gene Flow
4. Genetic Drift

Genetic drift is random change in allele frequencies due to chance events. There are 3 types of effects/events:

1. **Founder Effect** - A small group of individuals separate from a larger population and establish a colony in a new location. Because the founder individuals of the new colony are only a small sample of the original population, certain alleles are overrepresented or underrepresented. Thus, genetic variation is usually reduced
2. **Bottleneck effect** - A population size is dramatically reduced due to catastrophe and then rebounds (in size after a few generations). The initial reduction leads to certain alleles being overrepresented or underrepresented among the survivors and even though the population numbers may rebound to the original, genetic variation is usually reduced
3. **Neutral mutations** that do not manifest in the phenotype and are thus selectively neutral will experience genetic drift. Their allele frequencies change due to chance and not natural selection

5. Nonrandom Mating/Sexual Selection: Individuals choose mates on basis of favourable phenotypes. Favourable genotypes thus propagate at higher allelic frequency

MUTATION

3. HOW GENETIC VARIATION ARISES

Mutation

DNA sequence changes when there is a **mutation***. The DNA may be damaged by the reactive oxygen radicals generated in the mitochondrion and mitochondria DNA repair mechanisms may not be as robust, leading to DNA mutation

Feature	Gene Mutation	Chromosomal mutation
1. Definition	A gene mutation is a change in the sequence of bases in the DNA of one gene	A chromosomal mutation is a change in chromosomal structure usually involving several gene loci or number of chromosome
2. Mechanism	A gene mutation can be brought about by **substitution**, **deletion** or **insertion** of a nucleotide that changes the **triplet code** and hence the **amino acid**	A change in chromosomal structure can be brought about by **deletion** (segment of chromosome is missing e.g. cri-du-chat disease, **inversion** (chromosome segment is detached, inverted by 180° and reattached to chromosome), **translocation** (segment of chromosome is detached are reattached to different non-homologous chromosome) and **duplication** (an extra segment of chromosome is present) of a chromosomal segment Moreover, a change in number of chromosomes can result from **non-disjunction*** during **anaphase/anaphase I and anaphase II** of **mitosis/meiosis** respectively
3. Consequence of mutation with example	A gene mutation may change the **triplet code** and hence the **sequence of amino acids** in a polypeptide chain, alter the **3D conformation of protein** and thus affect **protein function** and subsequently affect the **phenotype** of the organism Mutations in non-coding regions e.g. promoter and enhancer may result in phenotypic variation as well E.g. A single base **substitution** of a **T with and A** in gene coding for **β-globin chain** results in new codon that codes for non-polar hydrophobic amino acid **valine** instead of polar hydrophilic **glutamate** resulting in the disease sickle cell anaemia	A change in chromosomal structural may result from e.g. translocation where segment of chromosome is detached are reattached to different non-homologous chromosome E.g. Burkitt's lymphoma (cancer) results from translocation of a segment of chromosome containing a protooncogene to another chromosome where the region has active gene transcription. This, upregulates gene expression i.e. gene becomes oncogenic **Aneuploidy*** is a condition where the cell does not have a chromosome number that is a multiple of the haploid number e.g. (2n + 1) or (2n – 1) as one or more chromosomes are overrepresented or underrepresented E.g. Down syndrome results from an extra chromosome 21; **Polyploidy*** is a condition where there are three or more times the haploid number of chromosomes e.g. 3n (triploidy) as more than 3 homologous sets of chromosomes are present

Meiosis

Independent assortment and segregation of homologous chromosomes during metaphase I and anaphase I respectively as well as **independent assortment and segregation of sister chromatids** during metaphase II and anaphase II respectively results in gametes with numerous combinations of maternal and paternal chromosomes

Crossing over between **non-sister chromatids** of **homologous chromosomes** during prophase I results in more allelic combinations

Sexual Reproduction

Random fusion of gametes add to the variety of genotypes. Different genotypes will result in different phenotypes and these will act as raw materials for natural selection

4. HOW RECESSIVE ALLELES ARE PRESERVED

1. **Diploidy/Heterozygote protection**
   A gene can be dominant or recessive. Dominant alleles mask the effect of recessive alleles. Thus even if recessive alleles may be less favourable in the current environment, they persist because they are propagated in heterozygous individuals where the disadvantageous trait does not manifest and hence is not selected against
2. **Heterozygote advantage**
   This occurs when heterozygotes have greater fitness than both kinds of homozygotes

   e.g. In a region where malaria is prevalent, heterozygous individuals with the HbAHbS genotype do not develop sickle cell anaemia and at the same time have **less chance of contracting malaria**. They are able to survive and reproduce in malaria-infected regions. Therefore **both** the HbA and HbS alleles of these people remain in the population. Thus the HbS allele confers a survival advantage on people who have one copy of the allele and is therefore maintained in the population at a relatively high frequency

3. **Frequency-dependent selection**
   In frequency dependent selection, selective advantage of the phenotype depends on how common it is. The more common phenotype is selected against and the less common phenotype is selected for

   e.g. Scale eating fish in Lake Tanganyika are either “left-mouthed” or “right-mouthed”. The “left-mouthed” fish attacks its prey’s right while the “right-mouthed” fish attacks its prey’s left. The prey guards itself against attack from whatever phenotype of scale-eating fish is most common in the lake. So from year to year, selection favours whichever mouth phenotype is least common. As a result, the frequency of “left-mouthed”fish and “right-mouthed” fish oscillates over time and frequency-dependent selection keeps the frequency of each phenotype close to 50%

5. WHY VARIATION IS IMPORTANT IN NATURAL SELECTION

While most mutations are disadvantageous, and will be selected against and the allele frequency decreases, others may confer a selective advantage, and organisms possessing these alleles will be selected for and the allele frequency in a population will increases; thus resulting in evolution

When mutations take place during gamete formation, they are inherited by offspring and can have an impact on evolution as these mutations are a source of new alleles in an existing gene pool. Thus these genotypic changes may result in phenotypic differences which contributes to an increase in genetic variation in a population. Variation is the **phenotypic differences** between individuals in a population due to genotypic differences. Hence, it is the **raw material for natural selection to act on**

When **environmental changes** occur, the variation amongst individuals results in some individuals surviving better and reproducing more successfully than others, as organisms possessing alleles that are disadvantageous will be selected against thus decreasing their allele frequency in a population over time, whereas organisms possessing alleles that confer a selective advantage are **best adapted** to the particular environmental condition and these

alleles will be **selected for** through natural selection, increasing the allele frequency in a population over time, thus resulting in evolution

If not for variation, there would be **no differential selection** as natural selection would either **favour all organisms or select against all of them**

NATURAL SELECTION
6. NATURAL SELECTION
1. Overproduction of offspring

Natural populations overproduce offspring

2. Constancy of numbers

However the **size** of most populations stay **relatively constant** as many offspring **die** before they reach **reproductive age**

3. Struggle for existence

This is because individuals of a species are constantly **competing** with each other for **limited resources** e.g. food, mates, shelter. Other **selection pressures** such as **disease** and **predators** also impose a limit on their numbers

4. Variation within a population

Individuals in a population show **variation*** (genetic difference due to the presence of different alleles, thus leading to phenotypic difference) for **natural selection*** to act on

5. Survival of the fittest by natural selection

Among the variety of individuals, some individuals with **phenotypes conferring a selective advantage that makes them better adapted to the environment** are **selected for by the environment** while individuals with disadvantageous phenotypes that are less well adapted to the environment will be selected against

6. Like produces like

Individuals with advantageous phenotypes are more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **advantageous phenotypes** (and hence **alleles**) **are passed on** to the offspring, leading to an increase in proportion of individuals with **advantageous phenotypes** (and hence **alleles**), leading to an **increase in advantageous allele frequency** in the population

7. Formation of a new species

Over **hundreds and thousands of successive generations**, with genetic isolation (i.e. no gene flow), evolutionary changes occur and may form new species if **reproductive isolation*** occurs which is necessary for speciation

7. ENVIRONMENTAL FACTORS AS FORCES OF NATURAL SELECTION
Industrial Revolution and the Peppered Moth

Before the Industrial Revolution in 1848, there **were** 2 forms of peppered moths (**Biston betularia**), the lighter form and the melanic form. There was a greater proportion of the higher form than the melanic form. The melanic form arose by **spontaneous mutation**.

Variation (black and white peppered moths) existed naturally. Lighter form of moths were well-camouflaged from predators on light coloured, lichen-covered tree barks. Lighter form of moths had a **selective advantage** in non-polled areas and in these areas their frequency became higher

With industrialisation, lichens on the bark of trees were killed. The darker coloured barks which once covered with white lichen were now exposed. The lighter forms of moth were exposed to selection pressure in the form of predators as they became **easy prey to birds** and their number declined. The melanic form of moths were camouflaged and thus proliferated

Hence, there was differential reproductive success as the melanic form of moths had a selective advantage in polluted areas and were selected for, and were (more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **melanic form** (and its **alleles**) **were passed on to their** offspring, leading to an increase in proportion of melanic forms of moths (and hence **alleles for melanic form**), leading to an **increase in frequency of alleles for melanic form** in the population)(6. NATURAL SELECTION), while the lighter forms of moths were selected against, thus decreasing in frequency

Thus, by 1895, 98% of all peppered moths in industrial areas were the melanic form

Antibiotics and Bacteria

In 1940s, antibiotics were first used to kill bacteria

Variation (resistant and non-resistant bacterial strains) existed naturally. Resistant strains **arose by spontaneous mutations** or by gaining a plasmid with an antibiotic resistance gene through conjugation

Selection pressure was in the form of antibiotics consumed by patients

This led to differential reproductive success as antibiotics killed most non-resistant bacteria. Hence the selection pressure now favours resistant mutant strains which have a selective advantage and are selected for, thus (resistant bacteria are more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **antibiotic resistance** (and **hence antibiotic-resistance alleles**) **are passed on to their** offspring, leading to an increase in proportion of resistant bacteria (and hence **antibiotic-resistance alleles**), leading to an **increase in antibiotic-resistance allele frequency** in the population

Adaptive Radiation of Finches

Selection pressure in the form of limited type and availability of food results in competition to exploit varied food sources as different types of food are available in the environment e.g. seeds, cactus flowers, cactus fruits. Finches with beaks suited to feed on a particular type of food available in the environment are selected for and individuals with advantageous phenotypes are more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **advantageous phenotypes** (and **hence alleles**) **are passed on to the** offspring, leading to an increase in proportion of individuals with **advantageous phenotypes** (and hence **alleles**), leading to an **increase in advantageous allele frequency** in the population. Finches with beaks that are not suited to feed on a particular type of food available in the environment will be selected against e.g. If the seeds available in the environment are hard or large, finches with low beak depth will not be able to crush these seeds and hence will be selected against

Descent with modification of pentadactyl limb to form wing structure in Bats

Selection pressure* in the form of availability and type of food and/or a need for escaping ground-based predators resulted in the selection of a forearm that facilitated flight

8. HOW HOMOLOGY SUPPORTS DARWIN'S THEORY OF NATURAL SELECTION (DESCENT WITH MODIFICATION)

Homology refers to the similar anatomical, embryological and molecular characteristics found in different species due to common ancestry. These characteristics present in an ancestral organism developed into different forms as the result of natural selection, as they faced different environmental conditions (**descent with modification**), and hence provide a basis for comparison

Anatomical homology

Organisms with **anatomical** homology have **morphological structures** that they share with a common ancestor

An example is the **pentadactyl (five-digit) limb*** structure in **forelimbs** of all tetrapods, 4-limbed animals e.g. humans, cats, whales and bats, with different forms in different species. This suggests that they **descended from a common ancestor** which had a basic form of the pentadactyl limb. The forelimbs of humans, cats, whales and bats show the same **arrangement of bones** from the shoulder to the tips of the digits, but the appendages have **different functions**: lifting/grasping, walking, swimming and flying and **superficially do not resemble each other**. The **pentadactyl (five-digit) limb*** in the common ancestor was altered by natural selection in the different organisms to **suit their specialised functions/environment**, resulting in **variations of the pentadactyl (five-digit) limb* structure**. Hence, this shows **decent with modification**

Embryological homology

Organisms e.g. fishes, turtles, cats and humans with **embryological** homology share **similar structures during embryonic development** as they shared a **common ancestor** with the same developmental stages.The **longer the embryological development remains similar, the more closely related** the organisms are.

At the **early stage in their embryonic development**, all vertebrates have **gill slits, long bony tail, two chambered heart and a notochord**. The **basic embryological body plan** in the ancestral species was altered by natural selection in the different descendant species due to **different selection pressures in the different environments** e.g. gill slits ultimately develop into structures with very different functions, such as gills in fishes and parts of the ears and throat in humans and other mammals

Molecular homology

Organisms with **molecular** homology have similar **DNA, RNA and amino acid sequences** as they share a **common ancestor** that had these molecules. The **greater the sequence similarity between homologous genes, the more closely related** the 2 species are

Examples are **cytochrome c** gene and the **p53** protein encoding gene, which are homologous genes. Homologous genes share significant **sequence homology** and are typically important enough that every organism possesses them. When expressed, they produce **proteins** that have the **same function** in all organisms that possess them. **Nucleotide sequences** in the ancestral genes are modified **due to accumulation of mutations** that occurred over many generations and selection pressures that favoured some mutations over others

9. HOW BIOGEOGRAPHY AND FOSSIL RECORD SUPPORTS HOMOLOGICAL SUPPORT OF DESCENT WITH MODIFICATION

Biogeography

Biogeography is the study of the **past** and **present geographical distribution** of organisms. If Darwin's theory of descent from a common ancestor is to be supported, then biogeographical evidence should indicate that **closely related species** and their **common ancestors** should be

present in the same geographical region. Biogeography allow us to see how the **distribution pattern of species** suggests the existence of **a common ancestor**, whose descendants dispersed out from the center of origin and were distributed **within a restricted geographical region** and **not throughout the whole world**. Thus, modification amongst species was shaped by **natural selection due to differences in the local environment**

e.g. The great Apes e.g. Chimpanzees, Gorillas, Orang Utans are closely related to one another. They can be only be found in the forests of Africa and Asia. The fossils of the common ancestor of the great apes are also found in the Africa and Asia. This indicates that both the extant ape species and common ancestral apes, from which they descended from, share the same biogeographic regions. This lends support to Darwin's theory of species descending with modification from ancestral ones. If species were to appear spontaneously, we should see great apes being present in similar ecological habitats in other parts of the world where ancestral species were not present e.g. firsts in Australia, or North and South America. However this is to the case

Island Biogeography

e.g. **Finches** in the **Galapagos islands** bear **similarity** to the finches found on the coast of the **South American mainland**. This suggests that they **share a common ancestry**. However, there are now **13 different island species**.

(An **ancestral population** of Darwin's finches strayed from the **South American mainland** of Ecuador to one of the **Galapagos Islands**. They then **colonised other islands**. The subpopulations were **geographically isolated** in the various islands surrounded by bodies of water that acts as a physical barrier preventing interbreeding. Hence **gene flow was disrupted**

The islands due to their differing habitats / environments, present many niches for the species to fill. Hence the divided populations were exposed to **different environments** and thus **different selection pressures**

(Since individuals in a population show **variation*** (genetic difference due to the presence of different alleles, thus leading to phenotypic difference) for **natural selection*** to act on. Individuals with **advantageous phenotypes** and were *best adapted to a variety of different niches in the different islands* were more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **advantageous phenotypes** (and hence **alleles**) **are passed on** to the offspring, leading to an increase in proportion of individuals with **advantageous phenotypes** (and hence **alleles**), leading to an **increase in advantageous allele frequency** in the population) (from 6. NATURAL SELECTION)

Thus **evolutionary changes** e.g. mutations, natural selection, genetic drift occurred **independently** in each subpopulation. Over **hundreds and thousands of successive generations** each subpopulation became **reproductively isolated genetically distinct species**)

There are now at least **13 different island species** of finches on the Galapagos islands, each filling a different niche on different islands

This kind of evolutionary pattern in which there is a **rapid increase in the number of species** produced from a common ancestor upon introduction into new environments is known as **adaptive radiation**)(from 11. SPECIATION Geographical Isolation)

Thus the **biogeographic distribution of the finches** supports the evolutionary deduction of descent with modification from a common ancestor because the finch species did not emerge from the Galapagos islands but **came from an ancestral species from the mainland**, which then evolved into different species through **adaptive radiation**

Fossils

Fossils are relics or impressions of organisms that lived in the past that are preserved in rock. The deeper the strata the organism is found in, the earlier it existed. We can determine the age of a fossil by carbon dating techniques. When compared across strata, fossils show an ordered

sequence of succession of organisms and how homologous structures have been modified through time (descent with modification). Fossils allow us to see the **evolution** of a species through the **modification of homologous structures** from a **common ancestor** to the present descendent through a **series of transitional forms**

e.g. When **horse fossils** are studied, we see an **ordered sequence of progression** in terms of **lengthening of limbs, toe reduction and increase in tooth size** over time that **coincided** with the change in environment from **dense forest to open grasslands**. Through natural selection, these adaptations transformed the ancestral horse into the present-day horse best suited for open grasslands (descent with modification)

e.g. **Transitional fossils** are referred to as the 'missing link' as they share characters of their modern descendants and a prehistoric ancestor and hence support evolutionary deductions best as they **illustrate an evolutionary transition** between the two forms. **Tiktaalik** is an example of a transitional fossil animal between fishes and tetrapods. It provides strong evidence that fish are the ancestors to modern tetrapods as it was similar to its fish ancestors by having fish gills and scales and also similar to its tetrapod descendants by having tetrapod leg bones, lungs, upward positioned eyes and a mobile neck

Continental Drift
When **related species are not distributed in the same geographical region**, **continental drift can explain the discrepancy**. Plate tectonics states that the **continents on Earth drift**. Hence, **fossilised remains** that **once might have been found on a supercontinent** may have **split up** into a multitude of **smaller continents distributed over a wide area** after millions of years

e.g. In the case of the extinct **Mesosaurs**, their fossils are distributed across Africa and South America which are separated by the Atlantic ocean. Based on continental drift, the 2 continents were once joined together at the time of the origin of the Mesosaur. This ancestral species gave rise to a variety of Mesosaur species that radiated out in this ancient supercontinent. When the continent broke up and separated, their fossils remained on the respective separated continents

10. [X-ICS] HOW GEL ELECTROPHORESIS CAN BE USED TO SUPPORT MOLECULAR HOMOLOGY

DNA* from **various organisms*** being compared are extracted using a buffer and purified
Using specific forward and reverse primers that hybridise to the 3' flanking regions of a potential
homologous gene, we carry out polymerase chain reaction/PCR*;

(Stage 1

Heating to 95 C separates two strands of the DNA double helix by breaking the hydrogen bond between complementary bases through increased molecular vibrations, thus denaturing the DNA

Stage 2
Cool to around 64 C, for *specific forward and reverse primers* to anneal. Primers anneal to complementary 3' *flanking regions* of each template/single strand of a *potential homologous gene*

Stage 3
At 72 C , Taq polymerase* synthesises complementary DNA strand. Chain extension occurs from 3' OH end of primer) (16. METHOD)

(Dense loading buffer is mixed with DNA sample to help it sink to the bottom of the well

Loading dyes also added to DNA sample to allow visualisation of the separation process

The fragments of DNA are pipetted into the wells at the top of the gel in a position furthest from the positive electrode. Negatively-charged DNA migrates out of well towards direction of positive electrode when subjected to an electric current. Fragments migrate through agarose gel matrix, made up of a meshwork of polysaccharides. The meshwork of agarose fibres impedes movement of longer fragments more than shorter fragments thus longer fragments migrate slower compared to shorter fragments, resulting in smallest fragments moving furthest/largest fragments least far from well

To **visualise the bands**, the gel can be treated with a staining dye that binds DNA (e.g. **ethidium bromide, a carcinogen**) and **fluoresces under UV light**. Thus the fragment size can be estimated (based on **position** of the band relative to bands in the molecular weight marker) and the **amount of DNA** can possibly be estimated (based on **intensity** and **thickness** of the band))(from 18. GEL ELECTROPHORESIS)

The PCR primers would hybridise to the DNA of the homologous gene it was targeting and amplify the gene. If there was indeed molecular homology amongst the organisms being compared, the gel would reveal the same banding pattern for all the organisms compared. Once it is established that the different organisms show molecular homology, their DNA can then be sequenced to determine the degree of nucleotide similarity with the DNA from closely related organisms being the most similar;

OR

The PCR primers would not amplify the gene not if there was no complementary sequences it can bind to. There is no molecular homology if any one or more of the lanes did not have any bands;

DISRUPTION OF GENE FLOW
10. DEFINITION OF SPECIES
✓ Biological Definition
A species is a group of organisms capable of **interbreeding*** and producing **fertile, viable offspring*** and are reproductively isolated from other species. They have a **common gene pool** and **same chromosome number** and usually have **similar morphological, physiological and behavioural features**

Advantage: Organisms being studied can be interbred to see if they produce fertile, viable offspring

Disadvantage: Definition cannot be applied to **asexually** reproducing organisms and **extinct** species where breeding behaviour cannot be observed

Ecological Definition + Niche Definition
A species is a group of organisms sharing the same **ecological niche***, the habitat where an organism lives and the roles of an organism in its interactions with other organisms in its habitat and environment e.g. predator-prey relationship, decomposer. (Hence differences between species are due to the differences in the ecological resources they depend on. If a species can no longer occupy a particular niche, it is considered a new species)

Advantage: Definition applies to organisms that reproduce sexually and asexually

Disadvantage: Definition cannot be applied to unrelated species that occupy similar niche e.g. the striped possum, a marsupial mammal from Australia, and the aye-aye, a placental mammal from Madagascar, are not related but occupy a similar niche (both eat worms in the bark of trees) in different parts of the world

Morphological Definition
A species is a group of organisms sharing similar **body shape, size** and other structural features

Advantage: Definition can be applied to all organisms (organisms that reproduce sexually, asexually as well as extinct species)

Disadvantage:
1. Definition makes it difficult to determine the **degree of difference** that is required to indicate **separate species** as well as **what structural features** should be used to **distinguish the differences**
2. Some organisms may be **superficially similar** but have **different evolutionary origins** e.g. the marsupial mole from Australia and the golden placental mole from South America

Phylogenetic Definition
A species is the **smallest group of organisms** that **share a most recent common ancestor** and can be distinguished from other such groups (The phylogenetic history of a species can be obtained by comparing **homologous morphological structures and/or homologous molecular sequences** with those of other organisms)

Advantage: **Avoids mistakenly classifying organisms based on superficial morphological similarities** as the characteristics that are compared are based on **common ancestry/homology**

11. SPECIATION
Gene flow is the **transfer of alleles from one population to another**, due to the **movement** of fertile individuals or their gametes. If members of a population migrate and **interbreed** with members of another population, **gene flow has occurred**. **Speciation** occurs when **gene flow is disrupted**

Speciation is a process by which **one or more new species arise** from a previously **existing species**. For speciation to occur, **gene flow must be disrupted** between two populations of the existing species. Then **evolutionary changes** e.g. mutations, natural selection, genetic drift could occur **independently in each subpopulation**. If this continues over a **long period of time**, then **new species** can form

Gene flow may be disrupted in 3 ways

Geographical Isolation

Speciation that occurs when **geographical isolation** occurs as a result of the presence of a **physical barrier** is called **allopatric speciation**

Geographical isolation is due to a **physical barrier** e.g. a body of water between two subpopulations which blocks migration of individuals and disrupts gene flow

e.g. **Caribbean Porkfish** in the Caribbean Sea and the **Panamic Porkfish** in the Pacific Ocean
An **ancestral fish population** was **split into two** by the formation of the Isthmus of Panama that acts as a physical barrier about 3.5million years ago. This **geographic isolation disrupted gene flow** between the two subpopulations.

The divided populations were exposed to **different environments** and thus **different selection pressures**

(Since individuals in a population show **variation*** (genetic difference due to the presence of different alleles, thus leading to phenotypic difference) for **natural selection*** to act on. Individuals with advantageous phenotypes are more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **advantageous phenotypes** (and hence **alleles**) **are passed on** to the offspring, leading to an increase in proportion of individuals with **advantageous phenotypes** (and hence **alleles**), leading to an **increase in advantageous allele frequency** in the population)(from 6. NATURAL SELECTION)

Thus **evolutionary changes** e.g. mutations, natural selection, genetic drift occurred **independently** in each subpopulation. Over **hundreds and thousands of successive generations** each subpopulation became **reproductively isolated genetically distinct species**)(from 11. SPECIATION)

e.g. **Darwin's Finches** on the **Galapagos Islands**
An **ancestral population** of Darwin's finches strayed from the **South American mainland** of Ecuador to one of the **Galapagos Islands**. They then **colonised other islands**. The subpopulations were **geographically isolated** in the various islands surrounded by bodies of water that acts as a physical barrier preventing interbreeding. Hence **gene flow was disrupted**

The islands due to their differing habitats/environments, present many niches for the species to fill. Hence the divided populations were exposed to **different environments** and thus **different selection pressures**

(Since individuals in a population show **variation*** (genetic difference due to the presence of different alleles, thus leading to phenotypic difference) for **natural selection*** to act on. Individuals with advantageous phenotypes are more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **advantageous phenotypes** (and hence **alleles**) **are passed on** to the offspring, leading to an increase in proportion of individuals with **advantageous phenotypes** (and hence **alleles**), leading to an **increase in advantageous allele frequency** in the population)(from 6. NATURAL SELECTION)

(Thus **evolutionary changes** e.g. mutations, natural selection, genetic drift occurred **independently** in each subpopulation. Over **hundreds and thousands of successive generations** each subpopulation became **reproductively isolated genetically distinct species**)(from 11. SPECIATION)

There are now at least **13 different island species** of finches on the Galapagos islands, each filling a different niche on different islands

This kind of evolutionary pattern in which there is a **rapid increase in the number of species** produced from a common ancestor upon introduction into new environments is known as **adaptive radiation**

Physiological Isolation

Speciation that occurs when populations are not separated geographically but gene flow is disrupted by **physiological or behavioural isolating mechanisms** is called **sympatric speciation**

Physiological isolation is due to the **unique physiology** e.g. difference in **flowering time** due to physiological response to different **soil conditions** of different individuals in the same area which disrupts gene flow

e.g. 2 species of **palms** on **Lord Howe Island**, *Howea forsteriana* and *Howea belmoreana*
There are **two soil types** on the island - the older volcanic soil and the younger calcareous soils. The two species of palms have **different soil preferences**. One palm species grows in **calcareous soil** while the other grows in **volcanic soil**. Since these **soil types are in close proximity** to each other in many areas, the two species of **palm can coexist in close proximity**. However, the two species **flower at different times** due to the **difference in soil conditions** and hence **cannot** and hence the two species are **reproductively isolated**. When the palms that normally grew on volcanic soil started to grow on calcareous soil, a conspicuous **flowering time difference** may have arisen as a **physiological response** to a new substrate. This **prevented interbreeding** between the two palms growing in the two types of soil although they were in close proximity

(Since individuals in a population show **variation*** (genetic difference due to the presence of different alleles, thus leading to phenotypic difference) for **natural selection*** to act on. Individuals with advantageous phenotypes are more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **advantageous phenotypes** (and

hence **alleles**) **are passed on** to the offspring, leading to an increase in proportion of individuals with **advantageous phenotypes** (and hence **alleles**), leading to an **increase in advantageous allele frequency** in the population)(from 6. NATURAL SELECTION)

Thus **evolutionary changes** e.g. mutations, natural selection, genetic drift occured **independently** in each subpopulation. Over **hundreds and thousands of successive generations** each subpopulation became **reproductively isolated genetically distinct species**)(from 11. SPECIATION)

Behavioural Isolation

Behavioural isolation is due to **unique mating rituals** e.g. different **bird song** and **preferences** of individuals in the same area such that a group of mating individuals isolate themselves from the main population, thus disrupting gene flow

e.g. **Eastern Meadowlark** and the **Western Meadowlark**
Both species are **nearly identical in shape, colouration and habitat** and their **ranges overlap** in the central United States. **Mating does not take place** between the eastern and western meadowlarks, largely due to the **difference in their songs**. These differences in songs enable meadowlarks to recognise potential mates as members of their own species. Thus the eastern and western meadowlark are **reproductively isolated**

In a population of a particular bird species, **some members** of the population **developed a new call**. Over many generations, the new **bird call became more distinct**. Birds began to distinguish between the two calls and tended to **mate preferentially with the members of the same cell**.
(Since individuals in a population show **variation*** (genetic difference due to the presence of different alleles, thus leading to phenotypic difference) for **natural selection*** to act on. Individuals with advantageous phenotypes are more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **advantageous phenotypes** (and hence **alleles**) **are passed on** to the offspring, leading to an increase in proportion of individuals with **advantageous phenotypes** (and hence **alleles**), leading to an **increase in advantageous allele frequency** in the population)(from 6. NATURAL SELECTION)

Thus **evolutionary changes** e.g. mutations, natural selection, genetic drift occurred **independently** in each subpopulation. Over **hundreds and thousands of successive generations** each subpopulation became **reproductively isolated genetically distinct species**)(from 11. SPECIATION)

Subspeciation

(Since individuals in a population show **variation*** (genetic difference due to the presence of different alleles, thus leading to phenotypic difference) for **natural selection*** to act on. Individuals with advantageous phenotypes are more likely to **survive** to reproductive age and produce **fertile, viable offspring*** similar to themselves. Thus **advantageous phenotypes** (and hence **alleles**) **are passed on** to the offspring, leading to an increase in proportion of individuals with **advantageous phenotypes** (and hence **alleles**), leading to an **increase in advantageous allele frequency** in the population)(from 6. NATURAL SELECTION)

Thus **evolutionary changes** e.g. mutations, natural selection, genetic drift occurred **independently** in each subpopulation. Over **hundreds and thousands of successive generations** each subpopulation became ~~**reproductively isolated**~~ **genetically distinct species**)(from 11. SPECIATION) R: reproductive isolation because they are still a species

CLASSIFICATION
13. BINOMIAL NOMENCLATURE

14. HIERARCHICAL CLASSIFICATION V PHYLOGENY

Point of comparison	**Classification**	**Phylogeny**
1. (Basis of) grouping of organisms	**Overall morphological similarities** and without consideration of evolutionary history of organisms	**Evolutionary history** of organisms based on **ancestor-descendent relationships**
2. System of organizing organisms	It is a **naming system**. It involves grouping organisms into a kingdom, phylum, class, order, family, genus and species using a **hierarchical classification*** system	Organisms are arranged based on their **evolutionary relationship with other organisms** with each organism assigned a position on a branching tree relative to other organisms
3. How species are presented	Uses **binomial nomenclature***	Uses a **phylogenetic tree*** where more closely related organisms are grouped closer together in the different branches of the phylogenetic tree;
4. Nature of characteristics	Uses **morphological characteristics only** and does not discriminate between analogous or homologous characters	Considers **morphological, anatomical, embryological and molecular characteristics** including DNA, RNA and amino acid sequences and **fossil record** to group organisms
5. Types of characters used	Uses **morphological characteristics only**	Uses **homologous characteristics*** that are derived from a **common ancestor**
6. Strengths and weaknesses	<u>Strength</u> **Easily place** an organism into its well defined group <u>Weakness</u> May wrongly classify organisms that are not related but look similar due to **convergent evolution***	<u>Weakness</u> **Cannot immediately place** an organism into the phylogenetic tree as evolutionary history need to be established from multiple sources; <u>Strength</u> Rarely classifies wrongly as convergent evolution will be placed in separate branches
7. Inference of speciation events	Does not allow inference of historical speciation events	Indicates **speciation events** as the node of the phylogenetic tree;
8. Inference of relationships	Cannot infer of how closely related 2 species are especially since they are grouped together in the same hierarchy, "species"	Allows accurate inference of how **closely related 2 species are** by looking at how recently they diverged from their common ancestor
9. Inference of common ancestors	Does not allow inference of common ancestors	Allows inference of **common ancestors**. Descendants of a common ancestor are represented in the same branch
10. Application of molecular clock	Not possible to apply molecular clock to date speciation events	If molecular evidence is used, can apply the molecular clock to infer **time of speciation**

?! 15. IMPORTANCE OF GENOME SEQUENCES IN RECONSTRUCTING PHYLOGENETIC RELATIONSHIPS (in-progress)

?! 16. ADVANTAGES OF MOLECULAR METHODS OF CLASSIFICATION (in-progress)

1. They are objective. Molecular character states are unambiguous as A, C, G and T are easily recognisable and cannot be confused

2. Data is quantitative and easily converted to numerical form for statistical analysis. The degree of relatedness can be inferred and quantified by calculating the nucleotide differences between species

3. mtDNA does not undergo recombination thus any changes to DNA is due solely to the accumulation of mutations over time making it the ideal candidate for a molecular clock. We can thus estimate the time of speciation

4. Even dead tissue may be used so long as the DNA or protein remains intact and you do not even need an entire specimen;

?! 17. NEUTRAL THEORY OF MOLECULAR EVOLUTION (in-progress)
?! 18. MITOCHONDRIAL DNA AS BASIS FOR COMPARISON (in-progress)
The gene encoding for cytochrome b is a **homologous gene***. (Homologous genes share significant **sequence homology** and are typically important enough that every organism possesses them. When expressed, they produce **proteins** that have the **same function** in all organisms that possess them)(from 8. HOW HOMOLOGY SUPPORTS DARWIN'S THEORY OF NATURAL SELECTION (DESCENT WITH MODIFICATION). This forms the basis of comparison

Yet (**nucleotide sequences** in the ancestral genes are modified **due to accumulation of mutations** that occured over many generations and selection pressures that favoured some mutations over others)(from 8. HOW HOMOLOGY SUPPORTS DARWIN'S THEORY OF NATURAL SELECTION (DESCENT WITH MODIFICATION), hence there are sufficient differences in the DNA of cytochrome b for scientists to distinguish the 3 closely related species

As it is found in the mitochondria, it **does not undergo recombination** and **any mutation accumulates at a regular rate in the maternal line**. Hence, it can be used for the molecular clock

19. ORIGIN OF VIRUSES
Viruses **did not come from a common ancestor**. Viruses are very **diverse**, having RNA or DNA genomes, being double stranded or single stranded, having circular or linear nucleic acid molecules, contain one or many nucleic acid molecules pointing to a very different evolutionary origin/paraphyletic nature for each one of them. Their **replication strategies** are also very diverse, some using reverse transcriptase while others use RNA dependent RNA polymerases and yet others requiring DNA polymerase for its replication. The polyphyletic nature of the diverse groups of viruses therefore suggest that they **do not share a common ancestor***;
The possible evolutionary origins of virus are

<u>The Progressive Hypothesis</u>

They are genetic material that can move within a genome called retrotransposons. Their life cycle resembles that of HIV and other retroviruses and they encode reverse transcriptase as well. We can speculate that they acquired a few structural proteins that would allow them to exit a cell and enter a new cell

<u>The Regressive Hypothesis</u>

The existing viruses may have evolved from more complex, possibly free-living organisms that lost genetic information over time, as they adopted a parasitic approach to replication. e.g. large DNA viruses with large genomes like smallpox and mimivirus;

<u>The Virus-first Hypothesis</u>

The first two hypothesis suggest that cells exists first before viruses. Here viruses evolved first in a pre-cellular world. The very first replicating molecules are RNA, not DNA. We know that

some RNA molecules, ribozymes exhibit enzymatic properties. Viruses could be the first replicating entities. Eventually enzymes for the synthesis of membranes and cell walls evolved, resulting in the formation of cells

Ultimately, viruses can still evolve as they are all parasites that multiply only within a host cell and it is also within the host that they can accumulate mutations and change/evolve through a process called antigenic drift. Moreover viruses can undergo antigenic shift where 2 different viruses may have infected a single host cell and recombined into a new virus. E.g H7N9 a recombination of viruses from wild migratory birds and chicken avian influenza virus with ducks as a host

20. WHY IT MIGHT NOT BE POSSIBLE TO DETERMINE HOMOLOGY FROM FOSSIL RECORD
Fossils may be incomplete or damaged. Furthermore, fossils may have few morphological characters in common that can form a basis of comparison hence evolutionary relationships cannot be drawn from them

21. WHY THERE ARE NO FLIGHTLESS BIRDS ON HAWAII

Flightless birds will have difficulty crossing the sea to reach Hawaii from the older islands. Also, flightless birds might have became extinct before the emergence of Hawaii

Homeostasis and Cell Signalling
1. GLYCOGEN VS GLUCAGON

	Glycogen (Glucose)	Glucagon
1. Type of macro-molecule	It is a **helical** and **extensively branched** polysaccharide	It is a **globular protein**
2. Monomer	**α-glucose** monomer	**Amino acid** residues with different **R-groups**
3. Bonds between monomers	**α(1, 4) glycosidic bond** links monomers within a branch and **α(1, 6) glycosidic bond** links monomers at branch points	**Peptide bonds**
4. Number of monomers per molecule	**Variable** number of glucose per molecule	**Fixed number** of amino acid per molecule
5. Structure	**α glucose*** monomers are joined by α(1-4) glycosidic bonds with α(1-6) glycosidic bonds at branch points;	Formed by a chain of **amino acids*** joined by peptide bonds;
6. Solubility	Insoluble molecule as it is large , being made up of many α glucose residues, hence will not affect water potential of cells. Thus, glycogen functions as good storage molecule	Soluble molecule as it is globular in structure; allows it to be transported in blood plasma to be circulated to its target site
7. Synthesis	**Liver** and **muscle cells** when blood glucose is high	**α cells of islets of Langerhans** of pancreas when blood glucose level decreases below **90mg/dL**

8. Function	Made up of **α** glucose residues, thus serves as **large energy store*** when produced in muscle and liver during glycogenesis;	Hormone that is produced by alpha cells in islets of Langerhans of pancreas. Glucagon is secreted when blood glucose concentration is detected as below set point and it will help regulate blood glucose concentration by stimulating responses that help **raise blood glucose level back to set point*;**

2. CELL SIGNALLING (GENERAL)
3 main steps:

1. ligand-receptor interaction (reception)
2. signal transduction (inclusive of phosphorylation and signal amplification)
3. cellular responses

Ligand-receptor interaction (reception)

A chemical signal is "detected" when signal molecule (ligand) that is structurally **complementary in conformation*** binds to the ligand binding site of a **specific*** receptor protein located at cell surface (or inside cell);

Signal transduction

Binding of a signal molecule (ligand) causes a change in receptor protein conformation. In the case of a transmembrane protein, binding of ligand in extracellular domain of receptor will result in a **conformational change in intracellular domain*** of receptor. Signal transduction is initiated when signal is converted to a form that can bring about a specific response;

Phosphorylation cascade

Signal cascade is often mediated by kinases and phosphatases: Transduction of signal occurs when kinase is activated and will go on to activate a relay protein (usually another kinase) in the next step by phosphorylating it; Phosphatase will inactivate kinase in preceding step to prevent the constitutive transduction of signal;

Signal amplification

This occurs via a signaling cascade; At each catalytic step of cascade, number of activated products is much greater than at preceding step; Hence during transduction, signal amplification occurs too.

Cellular responses

Transduced signal finally triggers a specific cellular response; (e.g. catalysis by an enzyme e.g. glycogen phosphorylase, physical response e.g. rearrangement of cytoskeleton or activation of specific genes in nucleus). Cell signalling ensures activities like these occur in right cells, at right time, in proper coordination with other cells of the organism

3. ACTION OF GLUCAGON (+ CELL SIGNALLING OF GPCR)

A **decrease** in blood sugar concentration below **90mg/dL** (stimulus) is detected by the **α cells of islets of Langerhans** of pancreas (detector/control centre) which secretes **glucagon (1st messenger)** into the bloodstream. Glucagon is transported via the bloodstream and being complementary in shape to the ligand binding site of the receptor;

Recognises and binds to specific cell surface receptors known as glucagon receptors (a G-protein coupled receptor, GPCR) on the **liver** cells (effector) to form ligand-receptor complex. Ligand-binging causes GPCR to undergo **conformational changes** in its **intracellular, cytoplasmic domain**. This conformational change causes an **inactive G-protein to bind to the GPCR**, and **release** its bound **GDP** and allow **GTP to bind** in its place. GTP binding causes a **conformational change in the G protein, activating it**. Activated G-protein dissociates from the receptor and translocates along the **cytoplasmic side** of the cell membrane to bind to an enzyme, **adenylyl cyclase**, and phosphorylate it, thus activating it. Adenylyl cycles catalyses conversion of ATP to **cAMP (2nd messenger)** which binds to and activates **Protein Kinase A (PKA)**. Activation of PKA will initiate a sequential activation of kinases resulting in a phosphorylation cascade to eventually activate glycogen phosphorylase;

(Signaling cascade beginning from cAMP is also amplified where number of activated product is always greater than those in preceding step as one moves down the cascade; adenyl cyclase converts many ATP to cAMP. Numerous cAMPs which are small, non-protein water-soluble molecules are able to diffuse quickly throughout the cytosol to activate many other relay molecules/PKA thus leading to the activation of a large number of glycogen phosphorylases involved in the breakdown of glycogen to generate a high yield of glucose in the liver cells)

Which catalyses the **breakdown of glycogen to glucose** (i.e. **glycogenolysis** in liver and muscle cells). Other cellular responses include increased **gluconeogenesis** (synthesis of glucose from non-carbohydrate sources). Glucose molecules are then released into the bloodstream causing the blood glucose concentration to increase.

The **increase** in the blood glucose levels serve as **negative feedback*** in the form of a diminished stimulus to the **α cells of the islets of Langerhans of the pancreas*** and serve to **decrease glucagon secretion**; **until blood glucose level return to set point**.

4. ACTION OF INSULIN (+ CELL SIGNALLING OF RTK)

An increase in blood sugar concentration above **90mg/dL** (stimulus) is detected by the **β cells of islets of Langerhans** of pancreas (detector/control centre) which secretes **insulin (1st messenger)** into the bloodstream. Insulin is transported via the bloodstream and being complementary in shape to the ligand binding site of the receptor;

Recognises and binds to specific cell surface receptors known as **insulin receptors (a receptor tyrosine kinase, RTK)** which

(A) causes the two receptor subunits to dimerise OR

(B) exist as **linked dimers**

on the **liver** and muscle cells (effector) to form ligand-receptor complex. Ligand-binging causes RTK to undergo **conformational changes** in its **intracellular, cytoplasmic domain**. This conformational change exposes the active sites of the kinases in each of the subunits of the receptor and triggers **autophosphorylation** by intrinsic kinase activity of receptor i.e. where **kinases on 1 subunit cross-phosphorylates tyrosine residues on the other subunit**, thus activating them. (Activated tyrosine residues will in turn **phosphorylate tyrosine residues*** on **intracellular domain*** of the RTK). Phosphorylated tyrosine residues serve as **docking sites** for other relay proteins. Relay proteins are activated by binding or via phosphorylation by RTK. Relay proteins may be kinases which, when activated, can go on to phosphorylate other proteins. Phosphorylation activates protein kinases which will initiate a sequential activation of kinases resulting in a phosphorylation cascade to eventually activate **glycogen synthase**;

Which catalyses **glycogen synthesis from glucose** (i.e. increase **glycogenesis** in liver and muscle cells). Other cellular responses include: 1. **Translocation of glucose transporters from the membrane of cytoplasmic vesicles to the cell membrane**, (increasing the amount of glucose transporters on the cell membrane. This increases the permeability of cell membrane to glucose, thus increasing uptake glucose form the blood by cells and therefore, decreases blood glucose concentration). This increases glucose intake into cells. 2. Increased **rate of glycolysis** and 3. Increased lipid and protein synthesis.

The **decrease** in the blood glucose levels serve as **negative feedback*** in the form of a diminished stimulus to the **β cells of the islets of Langerhans of the pancreas*** and serve to **decrease insulin secretion**; **until blood glucose level return to set point.**

5. ADVANTAGES OF CELL SIGNALLING

1. Facilitates **signal amplification** as only a **small number of signal molecules (ligands)** binding to receptors is needed to solicit a **large cellular response** as the **number of activated molecules increases with each catalytic step** in the pathway

2. Provides **multiple checkpoints for regulation** so that cellular responses can be **regulated/ terminated** at:

    1. **Reception**: extracellular 1st messenger can be degraded by enzymes in the extracellular space/endocytosis of cell surface receptors to prevent ligand-receptor interaction, thus preventing signal transduction/endocytosis of the entire ligand-receptor complex to prevent signal transduction

    2. **During Signal Transduction Pathway**: phosphatases dephosphorylate and inactivate relay proteins, thus inhibiting further signal transduction/production of inhibitors that bind to the intracellular domain of the ligand-receptor complex and/or any of the intracellular signal proteins in the signal transduction pathway to prevent signal transduction

3. One signal molecule (ligand) may elicit **multiple cellular responses** via different pathways in a cell. **2nd messengers** or **relay proteins** may activate multiple proteins involved in different signal transduction pathways to produce **multiple cellular responses**

4. Ensures **specific reactions** are triggered as a **specific signal molecule (ligand)** binds to a **specific receptor** as ligands are complementary in shape to the ligand binding site of the receptor and elicit specific reactions via specific pathway in each cell type

5. A signal molecule can **activate genes in nucleus** upon binding to cell surface receptor **without the need to move into nucleus**

6. One signal molecule (ligand) can allow the coordinated activation of many different cells simultaneously

6. LIGANDS CANNOT ACT DIRECTLY ON DNA

Interferon is too large and cannot pass through any transient pores form within cell surface membrane; it is also polar and hydrophilic and cannot pass through hydrophobic core of cell surface membrane because it will be repelled.

7. SIGNIFICANCE OF BLOOD SUPPLY FOR α,β CELLS

Alpha and beta cells produce and secrete hormones glucagon and insulin respectively; extensive blood supply ensures that hormones produced by these cells can be secreted directly into the bloodstream. The hormones will be able to travel quickly to their target organs where they will perform their function.

8. [STR>FN] GPCR

1. GPCR is a transmembrane protein with a ligand binding site on the extracellular domain that binds to ligand and an intracellular domain to bind to inactive G-protein.

Nervous System

RESTING MEMBRANE POTENTIAL

1. HOW IS RESTING MEMBRANE POTENTIAL MAINTAINED/WHY IS IT -VE

When neutron is not stimulated, the resting membrane potential is -70mV (intracellular relative to extracellular environment).

1. [Na+] is higher outside the neuron than inside, [K+] is higher inside the neuron than outside. Since there are **more K+ leak channels than Na+ leak channels**, more K+ leave the neuron than Na+ enter it. Hence there is a net loss of positive ions

2. **Na+-K+ pump** uses ATP to pump **3Na+ out and 2K+ in**. Hence there is a net loss of positive ions

3. Presence of **large, negatively charged organic anions** in the intracellular environment

2. HOW CHARGED IONS NA+/K+ PASS MEMBRANE OF NERVE CELL + IMPORTANCE AND MECHANISM OF PUMPS

Ion Channels

Charged ions move through specific ion channels (e.g. leak, voltage-gated and ligand-gated ion channels) embedded in the membrane of the nerve cells by **facilitated diffusion* down electrical chemical gradient***;

Ion Pump: Importance

Since Na+/K+/Ca2+ ions are charged, they cannot pass readily through the hydrophobic core of phospholipid bilayer of the cell membrane. Using a Na+/K+/Ca2+ pump, Na+/K+/Ca2+ is **actively transported* against their concentration gradient*** across the cell membrane against a concentration gradient* using **ATP***

Ion Pump: Mechanism

Na+/K+/Ca2+ will bind to its binding site found within the hydrophilic pore of the protein pump at one side of the membrane; resulting in a conformation change in the structure of the pump that results in the releases the Na+/K+/Ca2+ ion to the outside of the cell, so that a Na+/K+/Ca2+ concentration gradient is established, high <> relative to <>, (to ensure the synaptic transmission is not continuous)(for Ca2+)

ACTION POTENTIAL

3. MAIN CHARACTERISTICS AND PROPERTIES OF ACTION POTENTIAL

An action potential consists of these phases:

Depolarisation

When a stimulus arriving at a 1st node of Ranvier is **above the threshold potential***; this triggers the opening of the **voltage-gated Na+ channels*** at the node; **Influx of Na+** * down a concentration gradient into the neuron leads to a rapid **depolarisation** of the membrane for the generation of an action potential at the node;

Repolarization

At the **peak of the action potential at +40mV***, voltage-gated Na+ channels will close while the voltage-gated K+ channels will open; efflux of K+ down a concentration gradient leads to the **repolarization*** and restoration of the resting membrane potential so that the membrane can respond to a new stimulus

Hyperpolarization

When the cell's resting membrane potential of -70mV is reached, K+ continue to leave the axon as the voltage-gated K+ channels are slow to close causing **hyperpolarization*** of membrane (i.e. more negative than -70mV, e.g. -90mV).

Other Properties

- An action potential is an **all-or-none** event - only when the threshold potential is reached will an action potential be triggered
- All action potentials generated are **identical in magnitude and duration** (i.e. independent of the strength of the stimulus) and action potentials are generated at a higher frequency if there is a stronger stimulus
- Intensity of stimulus is passed as a **frequency*** of action potentials/impulses;
- Action Potentials are propagated in **one direction**:
  - Action potential undergoes a period called **refractory period*** during which another action potential cannot be initiated as voltage-gated Na+ channels need time to be reset
  - Calcium channels and synaptic vesicles containing neurotransmitters are **only*** found on the presynaptic knob therefore neurotransmitters can only be released from presynaptic neuron to the postsynaptic cell. In addition, receptors that bind with the neurotransmitter are **only*** found on the **post synaptic membrane*,** ensuring the movement of neurotransmitters in one direction from presynaptic membrane across synaptic cleft to the postsynaptic membrane (from 8. WHY IS SYNAPTIC TRANSMISSION UNIDIRECTIONAL)
- Action potentials are **individual** events and cannot add or interfere with one another due to the refractory period
- An action potential **does not diminish in strength** as they are propagated down the axon.

4. TRANSMISSION OF ACTION POTENTIAL ALONG MYELINATED NEURON

The neuron has a **resting membrane potential of ~70mV*** because (1. [Na+] is higher outside the neuron than inside, [K+] is higher inside the neuron than outside. Since there are more K+ leak channels than Na+ leak channels, more K+ leave the neuron than Na+ enter it. Hence there is a net loss of positive ions 2. Na+-K+ pump uses ATP to pump 3Na+ out and 2K+ in. Hence there is a net loss of positive ions 3. Presence of large, negatively charged organic anions in the intracellular environment)(from 1. HOW IS RESTING MEMBRANE POTENTIAL MAINTAINED/WHY IS IT -VE)

(Depolarisation

When a stimulus arriving at a 1st node of Ranvier is **above the threshold potential***; this triggers the opening of the **voltage-gated Na+ channels*** at the node; **Influx of Na+** * down a concentration gradient into the neuron leads to a rapid **depolarisation** of the membrane for the generation of an action potential at the node;

Repolarization

At the **peak of the action potential at +40mV***, voltage-gated Na+ channels will close while the voltage-gated K+ channels will open; efflux of K+ down a concentration gradient leads to the **repolarization*** and restoration of the resting membrane potential so that the membrane can respond to a new stimulus)(from 2. MAIN CHARACTERISTICS AND PROPERTIES OF ACTION POTENTIAL)

Refractory Period

The **Na+ influx also creates local currents/circuits*** of Na+ which diffuse sideways (in both directions) within the axoplasm, driven by its electrochemical gradient;

The diffusion of Na+ down the axon causes depolarisation that will be above the threshold potential when it reaches the next node of Ranvier to cause the voltage-gated Na+ channels in the next node to open and generate an action potential; This results in **propagation of action potentials*** along the myelinated axon of the neuron where the action potential "jumps" from node to node, and enabling impulses to spread by **saltatory conduction*** along the axon. Hence The presence of myelin sheath speeds up the transmission of impulse by **saltatory conduction***

Though the local currents of Na+ ions can travel upstream, the voltage-gated Na+ channel in the previous node of Ranvier would have been in the **refractory period*** and will not respond to any stimulus; Thus the impulse travels in one direction only, down the axon

5. IMPORTANCE/ROLE OF NA+ AND K+ IN IMPULSE TRANSMISSION

NA+

When the membrane is stimulated and depolarises to threshold potential of +40mV, the voltage-gated Na+ channels on the neuron open to allowing influx of Na+ down a concentration gradient into the neuron, causing the membrane to depolarise from -70mV to +40mV. This initiatives an action potential. Na+ in the axon spreads as local currents/circuits to adjacent node of Ranvier to initiate another action potential at the next region.

K+

Contributes to the resting potential of -70mV, where there is a higher concentration of K+ ions in the axon and a higher concentration of Na+ ions outside the axon maintained by Na+/K+ pump

Along an axon:

Upon stimulation, during **depolarisation***, the voltage-gated K+ channels are closed thus no diffusion of K+, while voltage-gated Na+ channels are open thus only allowing influx Na+ ion;

When the membrane is stimulated and depolarises to threshold potential of +40mV, voltage-gated K+ channels will slowly open, efflux of K+ down a concentration gradient leads to the **repolarization*** and restoration of the resting membrane potential so that the membrane can respond to a new stimulus

Due to slow closure of voltage-gated K+ channels, efflux of too much K+ ions leads to **hyperpolarization*** of membrane; which corresponds to the relative **refractory period*** where a larger than normal stimulus is needed to generate a new action potential; so action potentials can only be propagated in one direction down the axon; also limiting the frequency of action potentials production to prevent the axon from being
over stimulated;

At post synaptic neurone:

At post synaptic membrane, when ligand gated K+ channels open, efflux of K+ ions leads to **hyperpolarization*** of membrane resulting in; inhibitory post synaptic potential preventing depolarisation at the postsynaptic membrane/generation of action potential at axon hillock;

6. EFFECT OF MYELINATION OF NEURONS

Myelin sheath contains high level of lipids that wrap around the axon to act as an electrical insulator against the movement of Na+ and K+ ions across it; thus action potentials can only be generated at the **nodes of Ranvier*** where there are high density of voltage-gated Na+ and K+ channels and are exposed to the extracellular fluid;

(Depolarisation

When a stimulus arriving at a 1st node of Ranvier is **above the threshold potential***; this triggers the opening of the **voltage-gated Na+ channels*** at the node; **Influx of Na+** * down a concentration gradient into the neuron leads to a rapid **depolarisation** of the membrane for the generation of an action potential at the node;

Repolarization

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SYNAPTIC TRANSMISSION

7. SYNAPTIC TRANSMISSION + GENERATION OF AP AT POSTSYNAPTIC MEMBRANE

When an action potential reaches presynaptic membrane; depolarisation of the membrane causes **voltage-gated Ca2+ channels open*** allowing an **influx of Ca2+** into the pre-synaptic **knob** down its concentration gradient. The sudden rise in Ca2+ concentration stimulates synaptic vesicles containing neurotransmitters such as acetylcholine to move towards the presynaptic membrane and **fuse with the presynaptic membrane***. This results in the release of **neurotransmitters*** such as acetylcholine (Ach) into the synaptic cleft **via exocytosis***. Ach **diffuses*** across the synaptic cleft and binds to the receptors on **ligand-gated Na+ channels*** on the postsynaptic **membrane**; causing opening of **ligand-gated Na+ channels***. The influx of **Na+*** across the postsynaptic **membrane** causes it to **depolarise***; if the depolarisation exceeds **threshold potential***, an action potential will be generated. The depolarisation needed to reach threshold potential can be due to the (temporal & spatial) summation of several localised depolarisations because the postsynaptic neuron can be receiving several impulses from different synapses. Ca2+ concentration gradient (high outside relative to inside of synaptic knob) is reestablished by calcium ion pump, to ensure the synaptic transmission is not continuous

8. WHY IS SYNAPTIC TRANSMISSION UNIDIRECTIONAL

Calcium channels and synaptic vesicles containing neurotransmitters are **only*** found on the presynaptic knob therefore neurotransmitters can only be released from presynaptic neuron to the postsynaptic cell. In addition, receptors that bind with the neurotransmitter are **only*** found on the **post synaptic membrane*,** ensuring the movement of neurotransmitters in one direction from presynaptic membrane across synaptic cleft to the postsynaptic membrane

9. SYNAPTIC DELAY

Time is required for:

1. The calcium influx into pre-synaptic knob
2. The **movement of synaptic vesicles** towards the pre-synaptic membrane to release neurotransmitter via **exocytosis***;
3. **Diffusion** of the **neurotransmitter*** across the **synaptic cleft** to bind to the receptor on the post-synaptic membrane;

10. WHY IMPULSE TRANSMISSION > SYNAPTIC TRANSMISSION (SPEED)

Synaptic transmission involves the diffusion of neurotransmitters across the synaptic cleft which is slow compared to the rapid saltatory conduction because the diffusion of neurotransmitters is driven by their concentration gradient only while the movement of Na+ during electric transmission is driven by its concentration gradients as well as electrostatic attraction within the axoplasm (electrochemical gradient)

11. EFFECT OF ACETYLCHOLINESTERASE INHIBITOR

The **inhibitor of acetylcholinesterase*** blocks its active site, preventing acetylcholine from binding to it. Thus acetylcholine cannot be broken down; acetylcholine will accumulate and

remain bound to receptor on the post synaptic membrane; Causing Na+ ligand-gated channels to stay open leading to continuous influx of Na+, resulting in repeated depolarisation and production of action potential;