Carbohydrates

Structure & properties of α-glucose and β-glucose

- Reducing sugars: carbohydrates that act as reducing agents due to free aldehyde or ketone groups
- Monosaccharides:
  Small, crystalline
  Dissolve easily in water (due to polar –OH groups → readily form H bonds with water)

- α-glucose: –OH group on Carbon 1 projects below the plane of the ring
- β-glucose: –OH group on Carbon 1 projects above the plane of the ring

Formation & breakage:

1. Type of bond formed
2. Between which molecules
3. Between which functional group
4. Condensation/hydrolysis
5. Addition/removal of water
6. Catalysed by (enzyme)

Formation	Breakage
E.g. α-1, 4 glycosidic bond forms between the –OH group of carbon 1 of one α-glucose monomer and the –OH group of carbon 4 of another α-glucose monomer. This is a condensation rxn with the removal of 1 water molecule and is catalysed by enzymes.	E.g. α-1, 4 glycosidic bond between the –OH group of carbon 1 of one α-glucose monomer and the –OH group of carbon 4 of another α-glucose monomer is broken. This is a hydrolysis rxn with the addition of 1 water molecule and is catalysed by maltase.

Structure and properties of:
Starch (amylose & amylopectin)

Structure:	Function:
Amylose (is an unbranched chain structure that) consists of many α-glucose residues linked by α-1, 4 glycosidic bonds	Glycosidic bonds in amylose can be hydrolysed easily → release of a large number of glucose monomers → oxidised during respiration to produce large amt of ATP. Starch acts as an accessible source of glucose.
The angle of these bonds causes the chain to coil helically into a more compact shape, allowing the packing of many glucose molecules per unit volume	Due to large number of glucose molecules present, starch (amylose) serves as a good energy source in plants → glucose is oxidised during respiration to produce ATP
Most –OH groups are projected into the interior of the amylose helix, hence there are no free –OH groups to form hydrogen bonds with water → insoluble molecule	The insoluble nature of starch (amylose) prevents its diffusion out of the cell → does not change the water potential within the cell. Starch suitable as an energy storage molecule
Amylopectin has a backbone of α-glucose residues held tgt by α-1,4 glycosidic bonds. It is highly branched with side chains formed by α-1,6 glycosidic bonds	Many branched ends allow many hydrolytic enzymes to act on the branched ends at any one time → amylopectin easily broken down into glucose monomers to be used as respiratory substrates. Amylopectin serves as an accessible source of glucose

Cellulose

Structure:	Function:
A cellulose molecule consists of a long chain of β-glucose residues linked by β-1, 4 glycosidic bonds which make the cellulose chain straight Successive β-glucose residues are rotated 180° with respect to its adjacent residue → –OH groups projecting outwards from each cellulose chain in all directions Hydrogen bonds are formed between the –OH groups of neighbouring cellulose chains lying in parallel → extensive cross-linking that binds the chains rigidly together (in bundles of 60-70) form microfibrils and macrofibrils which confer high tensile strength, stability and support	Cellulose is the main structural component of plant cell walls. The high tensile strength of microfibrils → prevents plant cell from lysing when water enters by osmosis As a plant cell inflates with water, pressure develops inside → turgid → help support plants which lack wood Arrangement of fibres around the cell helps to determine the shape of the plant cell as it grows

Most –OH groups involved in formation of H bonds btwn cellulose chains → fewer –OH groups available for H bonding with water. Large molecule → insoluble in water Macrofibrils arranged in several layers running in diff directions	Allows cell wall to remain permeable to water and other solutes
Glycosidic bonds in cellulose are not easily hydrolysed unless specific enzymes are present	Serve as a good structural molecule

Glycogen

Structure:	Function:
Glycogen has a backbone of α-glucose residues held together by α-1, 4 glycosidic bonds, allowing the packing of many glucose molecules per unit volume	Due to the large number of glucose molecules present, glycogen serves as a good energy source in animals, as glucose is oxidised during respiration to produce ATP
Glycogen is highly branched with side chains formed by α-1, 6 glycosidic bonds	Many branched ends allow many hydrolytic enzymes to act on the branched ends at any one time → glycogen can be easily broken down into its glucose monomers (used as respiratory substrates) Glycogen serves as an accessible source of glucose
The glycosidic bonds in glycogen can be hydrolysed easily	Release of a large number of glucose monomers → oxidised during respiration to produce a large amount of ATP. Glycogen acts as an accessible source of glucose
Glycogen is a large polysaccharide, which makes it insoluble in water	Large and insoluble nature of glycogen prevents its diffusion out of the cell → does not change the water potential within the cell → glycogen suitable energy storage molecule

Lipids

Insoluble in water, dissolve readily in organic solvents
Proportion of oxygen is much less in Lipids than Carbs

Structure and properties of glycerol and fatty acids
Glycerol($C_3H_8O_3$):

- 3 carbons bonded to –OH
- Eah –OH can condense with a fatty acid
- Polar, hydrophilic, soluble in water (presence of –OH groups, form H bonds)

Fatty acids (R.COOH):

- R: long hydrocarbon chain (differs)
- Non-polar, hydrophobic, insoluble in water (presence of non-polar hydrophobic hydrocarbon chain, unable to form H bonds with water)
- Saturated:
  Max no. of H atoms
  Each C atom is joined to the next by single covalent bonds
- Unsaturated:
  At least 1 carbon-carbon double covalent bond
  Causes kinks in hydrocarbon chain
  Kinks: unsaturated fatty acids cannot pack closely tgt, resulting in weaker hydrophobic interactions btwn fatty acid molecules. Less thermal energy needed to disrupt these weak hydrophobic interactions to bring abt change in state (solid to liquid). → unsaturated fatty acids hv low b.p. than saturated.
- Longer hydrocarbon chains: more extensive hydrophobic interactions, more heat to disrupt hydrophobic interactions

Formation and breakage of ester bonds

Formation (esterification):	Breakage (saponification: basic/alkaline):
An ester bond forms between one (hydroxyl) –OH group of glycerol and one (carboxyl) –COOH group of a fatty acid by condensation with the removal of 1 water molecule, catalysed by enzymes.	An ester bond between one (hydroxyl) –OH group of glycerol and one (carboxyl) –COOH group of a fatty acid is broken by hydrolysis with the addition of 1 water molecule, catalysed by enzymes/lipase. (addition of acid/base)

Structure and properties of triglycerides and phospholipids

Triglyceride:

Structure	Properties
Higher proportion of hydrogen and almost insignificant proportion of oxygen in triglycerides compared to in carbs. Higher ratio of energy storing C-H bonds compared to carbs	More than twice the amt ATP released from oxidation of a given mass of triglycerides compared to an equal mass of carbohydrates (aerobic respi) Good energy source
Triglycerides contain more energy per gram than carbs/proteins (in birds/insects where small mass is necessary– mass kept to minimum) (seed dispersal makes small mass necessary) (long term food store for animals in cold climates→ hibernation)	Most weight efficient means for animals and plants to store energy Good energy storage molecule
Large, non-polar in nature→ insoluble in water	Do not change water potential within the cell, can be stored in large amts within the cell→ good energy storage molecule
Higher proportion of hydrogen and almost insignificant proportion of oxygen compared to carbs When oxidised, metabolic water is produced Aerobic respi→ complete oxidation of triglycerides produces larger amt of metabolic water per gram of triglyceride than equal mass of carbs	Better source of metabolic water (metabolic water retained in desert animals like camels, helping them survive when no liquid water to drink)
Triglycerides are poor conductors of heat, reducing what loss from body	Good thermal insulators (cold climates, blubber to reduce heat loss from deeper regions of body to surroundings)
Triglycerides less dense than water	Provide buoyancy to aquatic mammals (eg seals/whales)

Phospholipids:

Structure	Function
Each molecule consists of 1 polar hydrophilic phosphate head and 2 non-polar hydrophobic fatty acid tails Aq environments: phosphate head face outwards and interact with aq medium and fatty acid tails face inwards shielded from aq medium Fatty acid tails face inwards, buried inside two layers formed by phosphate heads (phospholipid bilayer)	Compartmentalisation of cell, separate cytoplasm from ecf Allow separate compartments within cell, for specialised metabolic pathways to take place.
Hydrophobic core of csm → selective barrier btwn the aq exterior and aq interior of the cell	Restrict movement of charged ions and polar molecules across the membrane
Non-polar fatty acid tails of phospholipids held tgt by weak hydrophobic interactions. Phospholipids can move about rapidly via diffusion within their own layer Unsaturated fatty acid tails → kinks, cannot pack closely → membrane fluidity	This contributes to membrane fluidity Forms transient pores that allow small non-polar molecules to diffuse in

Membrane structure and transport

Phospholipids:

- In aq environment, polar hydrophilic phosphate head face outwards and interacts with the aq medium while non-polar hydrophobic fatty acid tails face inwards and are shielded from the aq medium (fatty acid tails make up the hydrophobic core)
- Charged ions and polar molecules repelled by hydrophobic core of csm
- Held tgt by weak hydrophobic interactions between non-polar fatty acid tails (can move laterally via diffusion within own layers → transient pores)
  Proteins (including glycoproteins)
- Embedded randomly in phospholipid bilayer
- Non-polar R groups of amino acids in proteins form hydrophobic interactions with non-polar fatty acid tails of phospholipids
- Polar R groups form H bonds with polar phosphate heads of phospholipids
- Intrinsic proteins:
  Hydrophilic ends are exposed to aq solution on either side of membrane
- Extrinsic proteins:
  Not embedded, loosely bound via ionic/H bonds with phosphate heads of phospholipids or exposed parts if integral proteins

Phospholipids	Proteins
1. Aq environments → phospholipid molecules of cell membranes align with one another to form a phospholipid bilayer → compartmentalisation of the cell, separating the cytoplasm from the extracellular fluid, enabling separate compartments to be formed inside the cell (e.g. organelles), allowing specialised metabolic pathways to take place. 2. Hydrophobic core of csm → selective barrier btwn the aq exterior and aq interior of the cell, restrict movement of charged ions and polar molecules across the membrane 3. Non-polar fatty acid tails of phospholipids held tgt by weak hydrophobic interactions. Phospholipids can move about rapidly via diffusion within their own layer. Forms transient pores that allow small non-polar molecules to diffuse in 4. Unsaturated fatty acid tails → kinks, cannot pack closely → membrane fluidity	1. act as enzymes to catalyse reactions 2. act as receptors with a specific binding site with a specific complementary shape to the shape of a chemical messenger 3. serve as transport proteins such as channel proteins or carrier proteins that help regulate movement of polar molecules/charged ions across membranes, via facilitated diffusion or active transport 4. involved in cell-cell recognition where the carbohydrate chain of **(glyco)proteins** serves as markers that distinguishes one cell from another 5. involved in cell-cell adhesion (membrane proteins of adjacent cells may be held tgt in various kinds of junctions), binding cells together into tissues

Glycolipids	Cholesterol
1. involved in cell-cell recognition where the carbohydrate chain functions as a marker that distinguishes one cell from another 2. involved in cell-cell adhesion by binding cells together into tissues 3. act as receptor sites for chemical signals such as hormones	1. acts like a plug by reducing the escape or entry of charged ions and small polar molecules through the membrane. 2. Cholesterol maintains membrane fluidity. Relatively warm temperatures → cholesterol decreases membrane fluidity by restraining the movement of phospholipids Relatively low temperatures → cholesterol hinders the close packing of phospholipids and increases membrane fluidity.

Function of CSM

Function:	Description:
Compartmentalisation	CSM separates the cytoplasm from the extracellular fluid→ keeping desirable substances within the cell and undesirable substances out Membranes surrounding cell organelles enable separate compartments to be formed inside the cell → specialised metabolic pathways take place Membranes also allow transport proteins, enzymes and receptors to be embedded → increase in surface area for metabolic rxns to occur efficiently
Selective barrier	Hydrophobic core of membranes acts as a selective barrier, restricting movement of charged ions/polar molecules across the membrane into the organelles (selectively permeable)
Transport	Transport proteins / channel proteins / carrier proteins embedded in membranes regulate the movement of substances into and out of the organelles
Enzyme activity	Protein embedded may be an enzyme with active site exposed to substances in adjacent aq solution Can be embedded in the membrane and ordered as a team that carries out sequential steps of a metabolic pathway
Cell-cell adhesion	Membrane proteins of adjacent cells may be held tgt in various kinds of junctions, binding cells together into tissues
Cell-cell recognition	Carbohydrate chain in glycolipids and glycoproteins functions as a marker that distinguishes one cell from another
Signal transduction (cell signalling)	Specific signals on the extracellular surface of the cell are detected and relayed to the inside of the cell, triggering a specific cellular response within the cell

Simple diffusion

- Net movement of molecules from a region of higher conc to lower conc, down a conc gradient
- Passive (no ATP), continues until a dynamic equilibrium is reached
- Factors:
    1. Conc gradient
    2. Temp
    3. Distance
    4. Surface area
    5. Molecular size (Smaller molecules→ faster)
    6. Nature of diffusing molecule (fat-soluble molecules diffuse more rapidly thru CSM than water-soluble)

7. Nature of structure (no. of transient pores)
8. State of matter (fastest in gas, slowest in solids)

Osmosis

- Net movement of water molecules from a region of higher water potential to a region of lower water potential, across a selectively permeable membrane
- Passive process (no ATP)
- Plant cells:
  Pure water: Cell wall stretches, develops tension, resist further expansion→ fully turgid
  Lower w.p.: cytoplasm pulls away from cell wall (gap filled with solution) → plasmolysis
  The point where cell membrane just starts to pull away from cell wall→ incipient plasmolysis
  Cell membrane fully withdrawn from cell wall→ full plasmolysis
- Animal cells:
  Higher w.p.: Osmotic lysis→ cell bursts/lyse
  Lower w.p.: Crenated

Facilitated diffusion

- Passive movement of polar molecules/charged ions from a region of higher conc to a region of lower conc, down a conc gradient, across a selectively permeable membrane, with the aid of specific transport proteins
- Passive (no ATP)

1. Channel proteins
   - Forms a water-filled pore in the membrane
   - A.a. residues with polar/charged R groups make up inner lining of the hydrophilic channel, allow water soluble molecules to pass thru relatively easily
   - Transports polar hydrophilic molecules/charged ions in and out of cells
   - Selective channels, allowing only certain molecules/ions but not others
   - Some channels open & close in response to certain signals (e.g. binding of another molecule to the channel)
2. Carrier protein
   - Binding sites specific to a polar molecule/charged ion
   - Shape of molecule/ion must be complementary to shape of binding site (specific)
   - Carrier protein undergoes change in conformation

Active transport

- Active movement of polar molecules/charged ions from a region of lower conc to region of higher conc, against conc gradient, across selectively permeable membrane with the aid of specific carrier proteins
- ATP is used
- Carrier proteins undergo conformational change
- Movement is unidirectional (not reversible)
- Allows cells to take up nutrients even when conc outside cell is low, allows cell to get rid of waste even when conc outside is high
- Cells that carry out active transport have:

Numerous mitochondria
High respiratory rate (factors that increase rate of respi, increases rate of active transport)
High conc of ATP

Exocytosis

- Form of bulk transport (transport of large molecules in/out of cell across membranes by enclosing materials within a vesicle/vacuole) where the cell secretes macromolecules by the fusion of vesicles with the plasma membrane
- Active process, requires ATP

1. Secretory vesicle containing the material to be secreted moves towards the CSM
2. Membrane of the secretory vesicle fuses with CSM
3. Vesicle opens to the exterior and releases contents to the ECF via exocytosis

Endocytosis

- Form of bulk transport where cell takes in macromolecules/ecf by forming new vesicles from the CSM
- Active process, requires ATP
- Provides a means by which macromolecules (too large or hydrophilic, cannot diffuse) in ECF are brought into cells

1. CSM invaginates to form a flask-shaped depression which envelops/engulfs the materials from the ECF
2. Invagination becomes sealed off, enclosing the extracellular material to form an endocytic vesicle which moves into the cell

- Phagocytosis:
  - Process by which the cell can obtain solid particles (e.g. bacteria/food) which are too large to be absorbed via diffusion/active transport
  - Specialised cells like phagocytes (e.g. WBC like macrophages, Amoeba while feeding)
  - Solid particles digested via intracellular digestion
    1. Solid particles taken up by phagocytosis to form phagocytic vesicle
    2. Lysosome fuses with the vesicle and discharges its contents into the vesicle
    3. Lysosome's hydrolytic enzymes digest particles. Digested substances diffuse into cytoplasm from the vesicle and are absorbed by the cytoplasm
    4. Vesicle membrane fuses with the plasma membrane and any indigestible matter is released out of the cell via exocytosis

- Pinocytosis:
  - used for the intake of fluid droplets (pinocytic vesicles smaller than phagocytic vesicles)
  - Unlike phagocytosis which is specific (cell discriminates between different types of particles), pinocytosis is not discriminating and the cell will take in all solutes dissolved in droplets

Proteins

Str and properties of amino acids
Str:

- Central α carbon atom bonded to
- A basic amino group (-NH2)
- An acidic carboxyl group (-COOH)
- A hydrogen atom
- R group

R groups:

- Non polar: no charge, no oxygen atoms (glycine- → smallest a.a. found in collagen)
- Polar: Oxygen (Cysteine → sulfhydryl group to form disulfide bonds)
- Acidic: negatively charged
- Basic: positively charged

Property of R group	Property of R group	Bonds formed
Non-polar	Non-polar	Hydrophobic interactions
Polar	Polar	H bonds
Polar	acidic/basic	H bonds
Acidic	Basic	Ionic bonds
R group containing sulfhydryl group (-SH)	R group containing sulfhydryl group (-SH)	Disulfide bonds Only in Cysteine

Properties of amino acids:

- Colourless, crystalline solids
- High mp, denatures at high temps
- Soluble in water
- Amphoteric
- Amino acid with +ve and -ve charges is called a zwitterion. They have an isoelectronic point, which is the pH at which the zwitterion conc is max

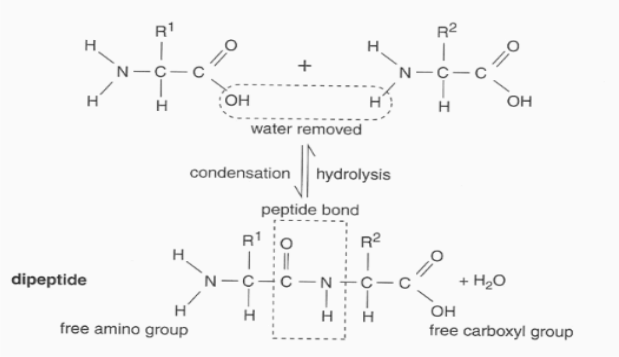
Being amphoteric, amino acids can act as buffers in solutions (resist pH change)

Describe formation and breakage of peptide bonds

Formation	Breakage
A peptide bond is formed between the amino group of 1 amino acid and the carboxyl group of another amino acid in a condensation reaction with the removal of 1 water molecule. This is catalysed by peptidyl transferase	The peptide bond between the two amino acids is broken by hydrolysis with the addition of one water molecule. This is catalysed by protease / enzymes.

Each incoming amino acid is added to the carboxyl end of the chain

- N-terminal (amino end)
- C-terminal (carboxyl end)



Primary str, secondary str, tertiary str, quaternary str (H bonds, ionic bonds, disulfide bonds, hydrophobic interactions)

- Each protein is a functional unit that is made up of one or more polypeptides precisely folded and coiled into a molecule with a specific 3D conformation. Ots function depends on its unique conformation

Primary str:

- Precise number, type and sequence of amino acids held tgt by peptide bonds
- Contains information for the protein's folding into a specific shape & determines the further levels of organisation

Secondary str:

- Primary str coils and folds into geometrically regular repeating structures, mainly α-helices and β-pleated sheets respectively
- They are stabilised by H bonds btwn C=O and -NH groups in the main chain of the polypeptide
- α-helix:
  - Elastic and flexible
  - makes 1 complete turn every 3.6 amino acids
  - Stabilised by intramolecular H bonds btwn the O of C=O group and H of -NH group of every 4th peptide bond
  - fibrous protein made of α-helices is keratin
- β-pleated sheet
  - Flexible but inelastic
  - two or more regions of 1 polypeptide chain lie parallel to each other and are held tgt by intramolecular H bonds btwn the O of C=O group and H of -NH group in the polypeptide backbone
  - the segments are folded in patterns as a result of H bonds at regular intervals
  - found in proteins whose function require strength (e.g. silk fibroin used by silkworms)

Tertiary str:

- Polypeptide chain is further bent, coiled and folded to form a precise 3D conformation
- Specific 3D conformation maintained by intramolecular H bonds, ionic bonds, disulfide bonds, hydrophobic interactions btwn R groups of amino acids
- Diversity of globular proteins is largely due to differences in tertiary str. The specific 3d conformation is responsible for the biological function/role of the protein
- Hydrophobic interactions: polar/hydrophilic R groups face outwards to form H bonds with water, non-polar/hydrophobic R groups are buried and form the core. Hydrophobic interactions help hold them tgt

Quaternary str:

- Quaternary structure consists of an aggregation of 2 or more polypeptide chains
- Subunits are held together by intermolecular hydrogen bonds, ionic bonds, disulfide bonds and hydrophobic interactions between the R groups of amino acids

Effect of temperature & pH on protein str

- Temp: H bonds, hydrophobic interactions
- pH: ionic, H bonds

- **Temp**: increasing temp leads to an increase in KE supplied to the protein. High heat disrupts the H bonds and hydrophobic interactions that maintain the secondary and tertiary str of the protein. This results in a loss of specific 3d conformation of the protein and its active site, leading to denaturation.

- **pH**: a change in pH alters the ionic charge of the COO- (acidic) and NH3+ (basic) R groups of the amino acids. Additional H+ ions in acids can combine with the COO- groups to form COOH while the reduced number of H+ ions can cause NH3 groups to donate a H+ and form NH2. ionic bonds and H bonds that maintain the tertiary str of the protein are disrupted. This results in a loss of specific 3d conformation of the protein and its active site, leading to denaturation.

Fibrous proteins	Globular proteins
Regular sequence of amino acids Consists of long parallel chains of polypeptides, cross-liked at intervals to form fibres or sheets Show little to no tertiary str Insoluble in water and are physically tough and stable structures	Highly irregular sequence of amino acids Consists of polypeptide chains which are tightly folded and coiled to form a spherical shape Complex tertiary str Soluble in water and relatively unstable structures
Functions	Functions
High tensile strength: provide structural support (e.g. keratin, collagen)	Enzymes: speed up rate of rxn Antibodies: produced by B cells Membrane proteins, receptor proteins

	Transport proteins (haemoglobin)

Describe str and function of:

- Haemoglobin (transport)

Structure	Function
Haemoglobin consists of **2 α-chains** and **2 β-chains**. Each chain is **coiled to form α helices** (secondary structure), which is further bent and folded into a globular protein subunit, maintained by intramolecular H bonds, ionic bonds and hydrophobic interactions	This allows for packing of more haemoglobin (Hb) into the red blood cell, thus more oxygen molecules (O2) can be carried and transported
The four protein subunits are packed closely together, held together by H bonds, ionic bonds and hydrophobic interactions, resulting in a compact haemoglobin	
The hydrophilic R groups of amino acids on the surface of the haemoglobin face outwards and interact with the aqueous medium	This maintains the solubility of haemoglobin in aqueous medium and allowing for mobility around the body via the bloodstream as it can bind and transport O2 dissolved in the blood
The four protein subunits are packed such that the hydrophobic R groups of amino acids face inwards into the centre of the molecule and are shielded from the aqueous medium	This allows for haemoglobin to be held in its precise compact 3D conformation, thus more can be packed into the red blood cell and more O2 is carried and transported
Each protein subunit contains a **prosthetic haem group with an Fe2+**	This allows for the reversible binding of O2 (to Fe2+ of haem group), enabling haemoglobin to carry and readily release O2 to respiring tissues
A complete Hb molecule has four protein subunits with four haem groups	Each haemoglobin molecule can carry and transport 4 O2 molecules at a time, enabling more efficient transport of O2 around the body
The haemoglobin molecule is **allosteric in nature** and can undergo changes in conformation. Binding of oxygen to 1 haem group facilitates the binding of oxygen to the other haem groups on the same haemoglobin molecule.	**Cooperative binding** facilitates easier binding and release of subsequent O2

- Collagen (structural)

Structure	Function
Primary: **Glycine-X-Y**, where X is usually proline and Y is hydroxyproline or hydroxylysine Secondary: each polypeptide chain coils into the shape of a loose helix. The high proportion of proline and hydroxyproline residues prevents the formation of intramolecular H bonds for α-helices	
Three helical polypeptide chains wind tightly around each other, and are bound to one another by **inter**molecular H bonds, forming a **tropocollagen**	Tropocollagen is the basic structural unit of collagen, the collective H bonds confers tensile strength to collagen
Almost every third amino acid in each polypeptide chain is glycine. **Glycine's small size** allows the three helical polypeptide chains to lie close together to form a tight coil	
Each tropocollagen interacts with another tropocollagen running parallel to it. The ends of the parallel tropocollagen molecules are staggered and covalent cross-links form between the carboxyl end of 1 tropocollagen and the amino end of another	The out-of-step cross-links lead to the formation of collagen fibrils, leading to greater tensile strength, allowing it to withstand large pulling forces
Collagen fibrils are further assembled to form collagen fibres	Results in structural protein that is flexible but inelastic, resulting in collagen having high tensile strength and can withstand large pulling forces

- G-Protein Linked Receptor (signalling)

Structure	Function
G-protein linked receptor comprises a single polypeptide chain coiled into s**even transmembrane α-helices**	This allows it to be embedded in and span the cell surface membrane so that signals detected outside the cell can be relayed inside the cell
G-protein linked receptor has an extracellular region	serves as the specific binding site for ligands which are unable to pass freely across the membrane
G-protein linked receptor has an intracellular region	This serves as the specific binding site for G proteins

Enzymes

Explain **mode of action of enzymes** in terms of:

1. **Active site**
    - The enzyme active site has a specific 3D conformation and distribution of electrical charge → only substrates of complementary shape and charge will fit into. This gives rise to the specificity of enzyme action
2. **Lowering of activation energy**
    - Enzymes enable reactions to proceed faster by lowering the activation energy
    - They remain chemically unchanged at the end of the reactions → can be reused
    - Enzymes reduce the activation energy by:
      - Holding the substrates close together at the correct angle and orientation at the active site for successful interaction and collision
      - By straining the chemical bonds within the substrates until they break
3. **Enzyme specificity**
    - Enzymes are highly specific in the reactions they catalyse
    - **Spatial fit**: Only substrates of complementary shape will enter and bind to an active site with distinctive conformation
    - **Lock-and-Key hypothesis**: the shape of the substrate must be complementary to that of the enzyme's active site
    - **Induced-fit hypothesis**: as the substrate enters and binds to the active site, it induces a conformational change in the shape of the enzyme → substrate can fit even more snugly into the active site, allowing enhanced interaction between the chemical groups of the substrate and the catalytic amino acid residues at the active site
    - **Chemical fit**: Enzyme and substrate must also be chemically compatible: have charge and hydrophilic/hydrophobic complementarity
4. **Enzyme-substrate complex**
    - Upon successful collisions between enzymes and substrates, enzyme-substrate (E-S) complexes are formed
    - Temporary weak bonds (H bonds, ionic bonds, hydrophobic interactions) between the substrate and active site are formed
    - Eventually leading to the formation of products → leave the active site as the products no longer fit into the active site

Effects of temperature, pH, enzyme/substrate conc

1. **Temperature:**
    - **At low temp**
      Enzyme activity is very low. The enzyme is said to be inactivated as it possesses minimal KE for collision with substrate to form E-S complexes
    - **As temp increases**
      Before optimum temp is reached, the rate of rxn increases. The increase in heat increases the KE of substrate and enzyme, hence increasing molecular motion of substrate and enzyme. The frequency of effective and successful collisions

between the substrate and enzyme increases, thus increasing the rate of formation of E-S complexes

- **At optimum temp**
  Max rate of rxn occurs. There is the highest frequency of effective and successful collisions between the substrate and enzyme.
- **Beyond optimum temp**
  Rate of rxn decreases despite molecules having increased KE. Heat has disrupted the H bonds and hydrophobic interactions within the secondary and tertiary structures of the enzymes, resulting in the loss of its specific 3D conformation of the enzyme and active site. The enzyme is denatured. The substrate can no longer bind to the active site of the enzyme to form E-S complexes, decreasing the rate of formation of E-S complexes.
- Temperature coefficient
  $Q_{10}=\frac{rate\ of\ reaction\ at\ (x+10)\ ^{\circ}C}{rate\ of\ reaction\ at\ x\ ^{\circ}C}$

2. **pH**
   - **At optimum pH**
     Max rate of rxn occurs. Bonds maintaining the secondary and tertiary structures of the enzyme are intact, enabling the highest frequency of effective and successful collisions btwn enzyme and substrate. This increases the rate of formation of E-S complexes
   - **At any other pH other than optimum**
     Rate of rxn decreases. Change in pH alters the ionic charge of the acidic COO- and basic NH3+ R groups on the amino acids at the active site of the enzyme. The ionic bonds and H bonds that help maintain the specific 3D conformation of the active site are disrupted, resulting in loss of specific 3D conformation of the enzyme and its active site. The enzyme is denatured. As a result, the substrate can no longer bind to the active site, rate of formation of E-S complexes decreases.

3. **Enzyme concentration**
   - **Low enzyme concentration**
     Rate of rxn is low. As enzyme conc increases, rate of rxn increases linearly. As enzyme conc increases, more active sites are available for effective and successful collisions with the substrate. There is an increase in the frequency of effective and successful collisions btwn the enzyme and the substrate, increasing rate of formation of E-S complexes. Enzyme conc is the limiting factor.
   - **Very high enzyme concentration**
     As enzyme conc increases, rate of rxn remains constant. An increase in enzyme conc will not result in a further increase in rate of rxn as enzyme conc is no longer the limiting factor, substrate conc is the limiting factor

4. **Substrate concentration**
   - **Low substrate concentration**

Rate of rxn increases with substrate conc. Many enzymes have unoccupied active sites. Substrate conc is the limiting factor

- **As substrate concentration increases**
  Rate of rxn increases linearly. There are more substrates available for successful collisions with the enzymes. There is an increase in the frequency of successful collisions btwn the enzyme and substrate, increasing rate of formation of E-S complexes. Substrate conc is the limiting factor
- **At very high substrate concentration**
  As substrate conc increases, rate of rxn remains constant. All available enzyme active sites are saturated with substrates at any one time. Substrate conc is no longer the limiting factor. Enzyme conc is the limiting factor

Competitive vs non-competitive inhibitors

Competitive	Non-competitive
Structurally similar to substrate	Not structurally similar to substrate
Enters and binds to active site	Binds at allosteric site
Binds temporarily	Binds temporarily/permanently
Competes with substrate for active site and prevents formation of E-S complexes	Does not compete with substrate for active site but changes conformation os active site, prevents formation of E-S complexes
Rate of rxn reaches max level	Rate of rxn reaches lower max level
Effect of inhibition can be overcome by adding more substrate	Effect of inhibition cannot be overcome by adding more substrate
Degree of inhibition depends on relative conc of inhibitor and substrate	Degree of inhibition does not depend on relative conc of inhibitor and substrate

Competitive:

- At low substrate conc, as conc of substrate and inhibitor are similar, competitive inhibitor competes with substrate for binding at active site. Frequency of E-S collisions is similar to frequency of E-I collisions. Number of E-S complexes formed is about the same as E-I complexes formed, leading to low rate of rxn
- At high substrate conc, substrate can out-compete competitive inhibitors for binding to the active site. Frequency of E-S collisions is higher than frequency of E-I collisions. More E-S complexes are formed than E-I complexes, leading to higher rate of rxn
- At very high substrate conc, substrate can out-compete competitive inhibitors for binding to the active site. This allows max rate of rxn to be reached.

Cell Structure and Function
Cell theory:

1. Cells are the smallest unit of life
2. All cells come from pre-existing cells
3. Living organisms are composed of cells

Animal cell	Plant cell
Absence of cell wall	Presence of cell wall
Nucleus anywhere in cell, often central	Nucleus at edge of cell
Cytoplasm present throughout cell	Cytoplasm normally thin layer at edge
Absence of tonoplast	Presence of tonoplast
Presence of centrioles	Absence of centrioles in higher plants
Numerous vacuoles	Large single central vacuole (cell sap)
Absence of pits	Presence of pits in cell wall
Absence of plasmodesmata	Presence of plasmodesmata in cell wall
Absence of middle lamellae (cells are joined by intercellular cement)	Presence of middle lamellae which join cell walls of adjacent cells
Presence of lysosomes	Absence of lysosomes
Absence of plastids	Presence in large numbers (Chloroplasts)
Presence of cilia/flagella	Absence of cilia/flagella in higher plants
Glycogen granules used for storage	Starch grains used for storage
Almost all cells capable of division	Only some capable of division

Double membrane: nucleus, mito, chloroplasts

Nucleus (plural: nuclei)

Structure	Function
Largest organelle Spherical (avg 5 micrometre in diameter) Contains DNA (genes that control activities of the cell)	Contain DNA Direct cellular activities - Protein synthesis - DNA replication during cell division

Nuclear envelope

Structure	Function
Double membrane, outer membrane continuous with RER Perforated by nuclear pores	Separates DNA in nucleus from cytoplasm Regulate entrance and exit of small polar molecules and macromolecules

Nucleolus (plural: nucleoli)

Structure	Function
Largest structure in the nucleus Formed by large loops of DNA from a number of chromosomes containing genes coding for ribosomal RNA (rRNA) Number of nucleoli increases in cells actively involved in protein synthesis	Site of rRNA synthesis - Contains genes coding for rRNA and is the site of transcription Site of ribosomal subunit formation - rRNA combines with proteins to form large and small ribosomal subunits - They leave the nucleus via nuclear pores and enter cytoplasm where the subunits combine to form a ribosome

Nucleoplasm & chromatin

Structure
Nucleoplasm: - Gel-like matrix within nucleus - Contains chromatin and one or more nucleoli and a variety of substances Chromatin: - Uncoiled and uncondensed form of chromosomes (appears in non-dividing cells) - Prepares to undergo mitosis/meiosis: chromatin condenses to form chromosomes

Endomembrane system:

- Includes nuclear envelope, RER, SER, Gb, lysosomes, vacuoles & vesicles, CSM

1. Protein synthesised by RER bound ribosomes enters the lumen of the RER (protein modification may occur here)
2. Transport vesicles containing proteins bud off from the cisternae of the RER and move towards the cis face of the Gb
3. Membrane of transport vesicles fuse with the membrane of the cisternae at the cis face of the Gb
4. Protein is emptied into the cisternal space for further modification, sorting and packaging into secretory vesicles
5. A secretory vesicle containing the proteins buds off from the cisternae of at the trans face of the Gb and are directed to the CSM by microtubules

6. Membrane of the secretory vesicle fuses with the CSM and the protein is secreted out of the cell via exocytosis

RER

Structure	Function
- Network of sheets (flattened cisternae) - Ribosomes present on membrane - Membrane continuous with outer membrane of nuclear envelope - Prominent in protein secreting cells	Site of protein synthesis - Proteins destined for secretion/incorporation into membranes are first synthesised as polypeptides by ribosomes attached to RER - They are then transported into the RER lumen through the pore in the RER membrane Intracellular transport system - Transport proteins synthesised by ribosomes to Gb via transport vesicles budding off from RER membrane - Membrane also replenishes the membrane of the Gb Modification of proteins - Can take place in the RER lumen. May be modified by enzymes which add chemical groups (e.g. glycosylation)

SER

Structure	Function
- Network of tubules - ribosomes absent	Synthesis of lipids - Including phospholipids and steroids Carbohydrate metabolism - Product of glycogen hydrolysis is glucose phosphate, which cannot exit the cell and enter the blood - SER removes phosphate from glucose, which can then leave the cell Detoxification of drugs and poisons (liver) - SER contains detoxifying enzymes that add hydroxyl groups to drugs, making them more soluble and easier to remove (excretion in kidneys) Stores Ca2+ ions - Necessary for muscle contractions - When the muscle is stimulated by a nerve impulse, calcium ions are pumped into and stored within cisternal space of SER. they are released from SER in response to extracellular signals

Gb

Structure	Function
Consists of a stack of flattened membrane-bound sacs called cisternae together with Golgi vesicles	Further modifies, sorts and packages proteins into transport vesicles - Protein modification includes phosphorylation (activates/deactivates the protein) and glycosylation (targets proteins for association with membranes) - Proteins are sorted and packaged to various vesicles at the trans face. They are either transported to other parts of the cell or destined to release contents outside Formation of lysosomes

	- Lysosomes containing hydrolytic enzymes fuse with food vacuoles/autophagic vacuoles to digest Production of plant cell wall components - Secretory vesicles with the polysaccharide (mucus) moves to the cell surface and fuses with the CSM, releasing contents via exocytosis - Vesicles containing cell wall materials (pectins) fuse to form the new cell plate. Membranes of the vesicles become to new CSM of daughter cells, while their contents contributes to the middle lamella and the new cell wall

Lysosomes

Structure	Function
Single membrane bound vesicle Contains hydrolytic enzymes (protease, nuclease, lipase etc) Hydrolytic enzymes: synthesised by RER bound ribosomes, transferred to Gb for further processing (budding vesicles at trans face, remains in cytoplasm) Acidic contents, low optimum pH 5	Intracellular digestion - Digest large, insoluble material (phagocytosis) - Fuse with phagocytic vesicle, release enzymes into vacuole → digestion - Products of digestion absorbed & assimilated by cytoplasm, undigested remain in vacuole → migrate to CSM → exocytosis Autophagy - Digest parts of cell (damaged/worn out organelles), replace old organelles - Membrane of ER encloses worn-out organelle → autophagic vesicle - Autophagic vesicle: fuses with lysosomes → autophagic lysosomes → digest organelles → digested materials absorbed and assimilated for further use Autolysis - Self-digestion of a cell by releasing hydrolytic enzymes into cytoplasm → after cell damage/death

Mitochondrion (plural: mitochondria)

Structure	Function
Rod-shaped (smaller than chloroplasts) Enclosed by double membranes, separated by intermembrane space Outer membrane is smooth, inner is highly-folded, giving rise to infoldings (cristae, singular: crista) - Increase SA, allow more proteins involved in respi to be embedded → increase aerobic repi **efficiency** - Inner membrane: enzymes, electron transport chains, ATP synthase Interior: semi-fluid matrix with 70S ribo, double stranded circular DNA and enzymes	Site of aerobic respiration to synthesise ATP - Energy in the form of ATP is released during oxygen required oxidation of glucose - ATP channelled to other parts Heat production - 55% energy released is given off as heat, maintaining body temp in warm-blooded animals

Chloroplasts

Structure	Function
Chromoplasts that contain chlorophyll (green pigment), carotenoids (yellow/orange pigments) Disc shaped, enclosed by double membrane Inner membrane: fluid filled space (**stroma**), contains enzymes, sugars, ds circular DNA, starch, lipid droplets & 70S ribo Interconnected set of flat, disc-like sacs called **thylakoids** (each disc) Thylakoids arranged in stacks called **grana** (stacks of thylakoids, singular: **granum**) Sacks of grana joined by **intergranal lamellae** (singular: intergranal lamella) Photosynthetic pigments (eg chlorophyll, carotenoids), electron transport chains and ATP synthase embedded in thylakoid membranes Within thylakoid membranes: chlorophyll molecules arranged along with accessory pigment molecules into photosystems	Site of photosynthesis - Process where green plants synthesise simple organic materials (hexose sugars) from inorganic materials (water, CO_2) using light energy and photosynthetic pigments to trap light energy - Light dependent stage: thylakoids - Light independent stage: stroma Involved in synthesis of biomolecules - Eg synthesis of most a.a., fatty acids, purines and pyrimidines

Ribosomes

- Found in both pro & eukayotes
- 70S: prokaryotes, mito, chloroplasts
- 80S: eukaryotes
- Cells with high rate of protein suntehsis have lrge number of ribo, prominent nucleoli (eg pancreatic cells)

Structure	Function
Last to be sedimented in centrifuge Small & large ribosomal subunit Each subunit composed of rRNA and protein Assembly: rRNA and proteins Subunits exported to cytoplasm via nuclear pores where they attach to mRNA to form a functional ribo	SIte of polypeptide synthesis - Translate genetic message carried by mRNA into specific primary structure (a.a. sequence) of a polypeptide chain - Large RS contains peptidyl transferase, catalyses formation of peptide bonds between adjacent a.a.

CSM

Function:	Description:
Compartmentalisation	CSM separates the cytoplasm from the extracellular fluid→ keeping desirable substances within the cell and undesirable substances out Membranes surrounding cell organelles enable separate compartments to be formed inside the cell → specialised metabolic pathways take place Membranes also allow transport proteins, enzymes and receptors to be embedded → increase in surface area for metabolic rxns to occur efficiently
Selective barrier	Hydrophobic core of membranes acts as a selective barrier, restricting movement of charged ions/polar molecules across the membrane into the organelles (selectively permeable)
Transport	Transport proteins / channel proteins / carrier proteins embedded in membranes regulate the movement of substances into and out of the organelles
Enzyme activity	Protein embedded may be an enzyme with active site exposed to substances in adjacent aq solution Can be embedded in the membrane and ordered as a team that carries out sequential steps of a metabolic pathway
Cell-cell adhesion	Membrane proteins of adjacent cells may be held tgt in various kinds of junctions, binding cells together into tissues
Cell-cell recognition	Carbohydrate chain in glycolipids and glycoproteins functions as a marker that distinguishes one cell from another
Signal transduction (cell signalling)	Specific signals on the extracellular surface of the cell are detected and relayed to the inside of the cell, triggering a specific cellular response within the cell

Centrioles

- Found in animal and lower plant cells, absent in higher plant cells
- Found in region called centrosome near nucleus

Structure	Function
Pair of rod-like structures made up of 9 triplet microtubules arranged in a ring Positioned perpendicular to each other	Nuclear division (meiosis and mitosis) - Acts as the microtubule organising centre - Produce spindle fibres radiating towards the metaphase plate during cell division

Structure of nucleic acid & gene expression (I)

Structure of DNA/RNA nucleotides
Purine: Double ring, A,G
Pyrimidine: single ring, CUT

Formation of nucleotides
- Nitrogenous base is linked to C1 of the pentose sugar by a condensation reaction to give a nucleoside
- Another condensation reaction joins the phosphate group to the nucleoside at C5 of the pentose sugar to form the nucleotide

Function of nucleotides
- Monomers of DNA and RNA
- Source of chemical energy (eg adenosine triphosphate ATP)
- Can be chemically modified and used as a signalling molecule inside cells
- Combined with other chemical groups to form coenzymes

Formation of polynucleotides
- A phosphodiester bond forms between the –OH group on C3 of the pentose sugar of one nucleotide and the phosphate group on C5 of the pentose sugar of the adjacent nucleotide, by condensation with the removal of one water molecule, catalysed by DNA polymerase.
- Continued condensation reactions lead to the formation of a polynucleotide
- A polynucleotide strand consists of a sugar-phosphate backbone with the nitrogenous bases projecting outwards
- Each polynucleotide strand has 2 distinct ends, a 5’ end and a 3’ end
- Nucleotides are always added to the 3’ end

Structure and function of DNA:
Structure
- DNA molecule is a double helix made up of **2 complementary polynucleotide strands**
- The two strands coil around each other to **form a right-handed double helix**
- The strands are **antiparallel**. One strand runs in the 5’ to 3’ direction while the complementary strand runs in the 3’ to 5’ direction
- Each (polynucleotide) strand consists of very long chain of DNA nucleotides, with each DNA nucleotide comprises **a deoxyribose sugar, a phosphate group and one of the four nitrogenous bases – Adenine, Thymine, Cytosine or Guanine**
- Each strand contains a **sugar-phosphate backbone** held together by **phosphodiester bonds** between **C3 of the deoxyribose suga**r of one nucleotide and **C5 of the deoxyribose sugar** of the adjacent nucleotide
- The nitrogenous bases are arranged as side groups of the polynucleotide strands
- The **width between the 2 sugar-phosphate backbones** is constant at **2 nm**, which is equal to the **width of 1 purine (AG) + 1 pyrimidine (CUT)**

- **One complete turn** of the double helix measures **3.4 nm i**n length and comprises **10 base pairs**
- The double helical nature of DNA results in the surface of the DNA molecule to have **major grooves and minor grooves**
- The nitrogenous bases of 1 strand pair with nitrogenous bases of the opposite strand via **hydrogen bonds**. There are **2 hydrogen bonds between A and T**, and **3 hydrogen bonds between C and G**
- **Base pairing is specific and complementar**y. Adenine pairs with thymine, guanine pairs with cytosine **(AT, GC)**
- The DNA molecule is **further stabilised by hydrophobic interactions between the stacked nitrogenous bases**

Function

- Storage of genetic information
- Passing down genetic material from one generation to the next
- Direct protein synthesis by coordinating the process of transcription and translation in the cell

Structure of eukaryotic genome

- Histones are +vely charged proteins that interact with the -vely charged DNA. 5 types of histones: H1, H2A, H2B, H3, H4
- Non-histone proteins are -vely charged proteins that interact with the +vely charged histones
- -vely charged DNA wound around a +vely charged histone octamer, consisting of 2 molecules of H2A, H2B, H3 and H4 each, to form a **nucleosome**
- **Individual nucleosomes** are **connected by strands of linker DNA and H1 histones** to give a **10 nm** nucleohistone complex.
- With the aid of **H1 histones**, the nucleohistone complex **coils** to form a **30 nm chromatin fibre**
- The 30 nm chromatin fibre folds to **form looped domains** that are **attached to a base of scaffolding proteins** (non-histones), forming the **300 nm chromatin fibre**.
- The 300 nm chromatin fibre is **further coiled and compacted** to form the **highly-condensed chromosome**.
- Compaction of chromosomes protects the relatively fragile DNA molecules from breaking as they are pulled apart during anaphase
- Eukaryotic genome generally have higher proportion of non-coding DNA to coding DNA
- Non-coding DNA (eg telomere and centromeres) are found in all eukaryotic chromosomes to maintain its structural integrity

Replication of DNA

Semiconservative replication

1. Two parental strands separate due to the breaking of H bonds between complementary bases
2. both strands act as templates for the synthesis of new complementary DNA strands
3. Each new DNA molecule consists of one original DNA strand and one newly synthesised DNA strand

Origin of replication

Prokaryotes:

- Circular bacterial DNA has single origin of replication
- Proteins that initiate DNA replication recognise the origin of replication and attach to the DNA, separating the 2 DNA strands and opening up a replication bubble
- Replication proceeds in both directions until the entire DNA molecule is copied

Eukaryotes:

- Each chromosome has thousands of origins of replication
- Multiple replication bubbles form and fuse, speeding up copying of the very long DNA molecules
- At each end of the replication bubble is a replication fork, a Y-shaped region where the parental strands are being unwound and new strands of DNA are elongating

Replication of DNA:

- Semi-conservative replication begins at origins of replication

Step 1:

- **Helicase** causes the DNA molecule to **unwind and unzip at the origin of replication** and the H bonds between complementary bases break, causing the **2 parental DNA strands to separate**

Step 2:

- **Single-stranded DNA binding proteins** bind to the 2 separated parental DNA strands to **stabilise the ssDNA** formed so each strand at the **unwound region can serve as a template** for the synthesis of a new complementary strand
- (extra pt) Topoisomerase helps to relieve the supercoiling in the DNA by breaking a phosphodiester bond of the strained strand, swivelling the strand and then rejoining the cut strand

Step 3:

- **Primase** catalyses the formation of a **short RNA primer**, the start of a new strand in the 5' to 3' direction

Step 4:

- **DNA polymerase** then **binds to the RNA primer** and **adds free DNA nucleotides** to the **free 3' –OH end** of the **RNA primer**
- DNA polymerase **can only work in one direction from 5' to 3'**, and can only add nucleotides to the **free 3' –OH end of an existing strand**
- Free DNA nucleotides **attach by complementary base pairing via H bonds** with the complementary bases in the DNA template, **A=T, G≡C**
- **DNA polymerase catalyses the formation of phosphodiester bonds** between adjacent DNA nucleotides
- **Synthesis** of newly synthesised strands occurs in the **5' to 3' direction**, thus the DNA **template is read in the 3' to 5' direction**, as DNA strands are **antiparallel**
- The **leading strand** is **synthesised continuously** because the **DNA polymerase is moving in the same direction as the unwinding and unzipping of DNA**
- The **lagging strand** is **synthesised discontinuously**, resulting in Okazaki fragments

Step 5:

- **RNA nucleotides** of all the RNA primers are **replaced with DNA nucleotides** by **another DNA polymerase**

Step 6:

- **DNA ligase** seals the gaps between the DNA fragments by **catalysing the formation of phosphodiester bonds** between adjacent nucleotides to **form a continuous strand**

Step 7:

- At the end of each round of replication, the original DNA strand and newly-synthesised strand **rewind into a double helix**
- Each new DNA molecule consists of one original DNA strand and one newly synthesised DNA strand

End replication problem

Eukaryotic DNA replication:

- During DNA replication, DNA polymerase requires a free 3’ –OH end of an existing strand to add free nucleotides
- An RNA primer is synthesised to provide a free 3’ –OH end for the addition of free nucleotides, and is then replaced by DNA nucleotides
- The RNA primer removed from the 5’ end of the newly synthesised DNA strand cannot be replaced with DNA molecules as there is no free 3’ –OH end
- Since DNA polymerase is unable to complete the replication at the 5’ end of lagging strands, this creased a 3’ overhang at the end of the chromosome
- Due to this, the ends of chromosomes shorten with each round of DNA replication
- Telomeres are found on the chromosomal ends and are non-coding, shortening of chromosomal ends leads to shortening of the telomeres without any deleterious effects
- Genes/genetic info within the chromosomes will thus not be lost

Reason for production of Okazaki fragments

1. The shape of the active site of DNA polymerase and its enzyme specificity allows the enzyme to work in only one direction from 5’ to 3’
2. DNA polymerase can only add DNA nucleotides to the free 3’ –OH end of an existing strand
3. Two parental DNA strands are antiparallel

Structure of nucleic acid & gene expression (II)

Advantage of having mRNA as intermediate

- DNA can be protected, maintain fidelity of genetic information
- Gene expression can be amplified: many copies of RNA synthesised from one gene, each RNA many copies of polypeptide
- Regulation of gene exp: more steps in pathway, more opportunities to control

Central dogma of molecular biology: one gene codes for one polypeptide

Transcription:

- process where DNA is used as template to direct synthesis of RNA molecule with base seq complementary to a section of DNA, takes place in nucleus

Translation:

- Process where mRNA used as template to direct synthesis of polypeptide. Sequence of bases in mRNA converted into specific number, type and sequence of a.a. in the polypeptide, takes place at ribo

	Transcription	Translation	DNA replication
Location	Nucleus	Ribosomes on RER/ free ribosomes in cytoplasm	Nucleus
Begins at	Promoter	Start codon (AUG)	Origin of replication
Ends at	Terminator	Stop codon (UAA, UAG, UGA)	
Template	DNA	mRNA	DNA
Monomer	RNA nucleotides	Amino acids	DNA nucleotides
Enzyme	RNA polymerase	Aminoacyl tRNA synthetase, peptidyl transferase	Helicase, DNA polymerase, primase, DNA ligase, requires RNA primer
Bonds formed in pdt	Phosphodiester bonds	Peptide bonds	Phosphodiester bonds
Template read in	3’ to 5’	5’ to 3’	3’ to 5’
Products	RNA	Polypeptide chain	DNA
Products synthesised in	5’ to 3’	Amino end to carboxyl end	5’ to 3’

Transcription:

Stage 1: initiation

- Specific nucleotide seq (Promoter) instructs RNA polymerase where to start transcription
- Promoter is binding site for RNA polymerase, upstream of gene
- Promoter dictates which strand is template
- RNA polymerase and GTFs R&B to core promoter, forming TIC
- RNA polymerase unwinds and unzips DNA molecule, breaking H bonds between complementary bases and starts transcribing the gene

Stage 2: elongation

- RNA polymerase moves 3' to 5' direction, continues to unwind and unzip DNA molecule
- Free RNA nucleotides align themselves opposite template strand via complementary base-pairing
- U in mRNA pairs with A in the DNA, A with T, C with G, G with C, forming H bonds with complementary DNA bases
- RNA polymerase joins RNA nucleotides together by catalysing formation of phosphodiester bonds between RNA nucleotides → synthesis of RNA
- RNA polymerase catalyses addition of RNA nucleotides to 3' –OH end of growing RNA strand → RNA strand synthesised in 5' to 3' direction
- As RNA synthesis continues, RNA strand peels away from DNA template, allowing 2 separated DNA strands to rewind in region already transcribed
- A single gene can be transcribed simultaneously by several molecules of RNA polymerase, increases number of mRNA synthesised per unit time

Stage 3: Termination

- RNA polymerase reaches a specific seq of bases called terminator, signalling end of gene
- RNA polymerase dissociates from RNA molecule and gene, newly synthesised RNA molecule is released

Post-transcriptional modifications

- **5' capping**
  5' end of pre-mRNA molecule is modified by **addition of a 7-methylguanosine cap**
  Functions:
  1. **Protect** mRNA from degradation by **ribonucleases**
  2. Act as **signal** to **facilitate export** of mature mRNA out of nucleus
  3. **Promotes initiation** of transcription. **Translation initiation factors bind to 5' cap** and initiate translation by **recruiting small ribosomal subunits** (translate 5' to 3' until find AUG) to bind at 5' cap
- **Splicing**
  Process and significance of RNA splicing
  > introns interspersed within pre-mRNA are excised and exons that flank the intron are spliced together to form a **continuous coding seq** and ensure the synthesis of the correct polypeptide

- **3' polyadenylation**
  PolyA polymerase adds 50-250 **adenine** nucleotides to the 3' end of the mRNA forming a polyA tail
  Function:
  1. PolyA tail act as a **signal** to **facilitate export** of mature mRNA out of nucleus
  2. **Recognised** by poly(A)-binding protein. Binding of poly(A)-binding protein **enhances mRNA stability** and **prevents degradation** of the mature mRNA by ribonucleases

Structure and function of mRNA, tRNA, rRNA

a) mRNA

Structure:

- Ss molecules, synthesised in nucleus during transcription
- Transcribed from gene in DNA via CBP, base seq is complementary to DNA template strand
- Limited lifespan

Function

1. Act as template for polypeptide synthesis
   - Mature mRNA exported out of nucleus via nuclear pores and enter the cytoplasm (bind to ribosomes)
   - Ribosomes synthesise polypeptides during translation
   - One codon on mRNA codes for a specific a.a.

b) rRNA

Structure:

- Large complex molecule, segments of single stranded and segments of double stranded helices (due to CBP via H bonds)
- rRNA transcribed from rRNA genes found in nucleolus, synthesised at nucleolus

Function:

1. Combines with proteins in nucleolus to form small and large ribosomal subunits, combine to form ribosomes in cytoplasm
2. rRNA in the small ribosomal subunit CBP to mRNA during translation
   - Contains base seq complementary to regions in the mRNA, determines start codon position
3. rRNA in large ribosomal subunit CBP to tRNA in P site (peptidyl-tRNA site) and A site (aminoacyl-tRNA site)
4. Ribozyme peptidyl transferase in large ribosomal subunit catalyses formation of peptide bonds between adjacent a.a.

c) tRNA

Structure:

- Smallest of the RNAs, single stranded polyribonucleotide, 80 RNA nucleotides that fold back upon itself to form a clover-leaf shape with 3 loops (anticodon loop, activating enzyme site, ribosome recognition site) and a stem

- Held together by H bonds between complementary bases of different regions
- tRNA transcribed from tRNA genes in nucleus

Functions:

1. Act as intermediate molecule between codon of mRNA and a.a. sequence of the polypeptide chain
2. Carry correct a.a. from cytoplasm to polypeptide chain being synthesised at ribosome

Structure	Roles
Anticodon loop	Triplet seq (anticodon) that CBP with a particular codon on mRNA
CCA stem (3' end)	3' end always ends in base seq 5'-CCA-3' , 5' end always ends with G. the 3' end attach covalently to specific a.a. coded for by the anticodon of the tRNA
Activating enzyme site	Site for aminoacyl-tRNA synthetase that catalyses attachment of tRNA with specific a.a.
Ribosome recognition site	Make specific base pairing with rRNA in ribosomes

Different RNA molecules last for different lengths of time depending on the functions they serve ;

mRNA:

- Durability of mRNA is shorter compared to tRNA and rRNA
- [function] mRNA is used as template for translation
- Half-life of mRNA is determined by length of poly-A-tail
- [benefit] varying half-life of mRNA regulates gene expression at translational level
- [benefit] amount of gene products can be regulated temporally and spatially

rRNA

- [function] rRNA associates with ribosomal protein to form ribosomes
- ribosomes are sites of protein synthesis (essential for cellular functions)

tRNA

- [function] tRNA carries amino acid to the ribosome
- [function] tRNA is an intermediate molecule between mRNA codon and a.a. sequence of the polypeptide
- [benefit] long lasting RNA can be reused for gene expression/protein synthesis
- [benefit] conserves the cell resources
- [benefit] allows expression of different gene products to continue

Features of genetic code (PUNDT)

Punctuated

- Includes start and stop signals
- AUG has dual function: start signal and methionine

- UAA, UAG, UGA stop codons: signal for termination, do not code for a.a.

Universal
- Same triplet of nucleotides codes for the same a.a. in all organisms

Non-overlapping
- Genetic code is encoded as a sequence of non-overlapping triplets
- Codons do not overlap

Degenerate
- Due to the degeneracy of the genetic code, more than 1 codon can code for the same a.a.

Triplet
- The code is a triplet code

Translation
- tRNA, ribosomes, enzymes (aminoacyl-tRNA synthetase, peptidyl transferase), protein factors (e.g. initiation factors, protein release factor), source of chemical energy (ATP, GTP)

Step 1: Amino acid activation
- A specific a.a. is attached to the 3' end of a specific tRNA, forming an aminoacyl-tRNA, catalysed by a specific aminoacyl-tRNA synthetase
- Active site of each type of aminoacyl-tRNA synthetase fits only a specific combination of a.a. and the anticodon on the tRNA
- The a.a. that the tRNA attaches to is determined by the specific anticodon on the tRNA

Step 2: Initiation
- A special initiator tRNA carrying a.a. methionine (Met), with anticodon UAC, binds to the small ribosomal subunit with the aid of initiation factors
- Small ribosomal subunit (containing initiator tRNA) R&B to 5' end of mRNA and moves along the mRNA until it reaches first AUG codon → serve as start codon
- Anticodon UAC of initiator tRNA CBP via H bonds with the start codon AUG on the mRNA
- The union of the initiator tRNA, small ribosomal subunit and mRNA is followed by the attachment of the large ribosomal subunit, completing the TIC
- GTP is also required to bring all the components together
- At completion of the initiation process, initiator tRNA fits into the P site of the large ribosomal subunit and the vacant A site is ready for the next aminoacyl-tRNA

Step 3: Elongation
- Anticodon of the next incoming aminoacyl-tRNA, carrying its specific a.a., undergoes CBP and forms H bonds with the mRNA codon in the A site of the ribosome
- A peptide bond is formed between the amino end of the a.a. in the A site and the carboxyl end of the growing polypeptide chain in the P site, catalysed by peptidyl transferase
- After the peptide bond has been formed, the ribosome translocates one codon downstream along the mRNA in a 5' to 3' direction

- This moves the tRNA carrying the growing polypeptide in the A site to the P site, the tRNA in the P site now moves to the E site and leaves the ribosome. Vacant A site is now free to receive the next incoming aminoacyl-tRNA

Step 4: Termination

- Elongation continues until a stop codon, UAA, UAG or UGA, reaches the A site of the ribosome
- Protein release factor R&B to the stop codon on the mRNA, causing the addition of a water molecule to the polypeptide
- This reaction hydrolyses the completed polypeptide from the tRNA that is in the P site, releasing the polypeptide from the tRNA and the ribosome
- The remainder of the translational complex then disassembles

Polyribosomes:

- One a ribosome moves past AUG, a second ribosome can attach to mRNA, cluster of ribosomes called polyribosomes

Protein synthesis in prokaryotes

- Absence of nuclear envelope, translation of mRNA can begin as soon as the 5' end of mRNA peels away from DNA. No post-transcriptional modifications are carried out after transcription, hence translation can begin
- RNA polymerase involved in transcription and ribosomes involved in translation are both found in the cytoplasm of bacterium

Gene Mutation

Gene mutation: change in the sequence of DNA nucleotides in the gene

- Involves just 1 base pair → point mutation
- Most likely when DNA is replicated during S phase of interphase

Base pair substitution:

- Replacement of one nucleotide base pair with another base pair in a gene
- Result in **missense** mutation and results in significant change in the encoded protein
  > **codes for a different a.a.**, a.a. may have different properties (charge/size) due to different R group, change the polypeptide sequence, and hence the protein's specific 3D conformation, lead to production of non-functional protein
  > occurs in region crucial for protein's str/function: non functional protein

- Result in **nonsense** mutation and results in significant change in the encoded protein
  > codon coding for a.a. **changes to stop codon**
  > translation terminated prematurely, truncated/shortened polypeptide produced instead → non-functional protein

- Result in **silent** mutation with no/little effect on the encoded protein
  > mRNA codon changed, but codes for the **same a.a.**
  > due to degeneracy of genetic code, more than 1 codon can code for the same a.a.
  >OR different a.a. Coded for, causes little effect on polypeptide
  > **similar size and properties**/ located in region of polypeptide **not essential** for protein str/function (e.g. structural residues of an enzyme that maintains overall globular shape of enzyme, not in active site)

Base pair addition/deletion

- gain or loss of one or more nucleotide base pairs in a gene
- Result in **frameshift** mutation where reading frame is altered
  > all nucleotides downstream from the deletion will be **improperly grouped** into incorrect codons and read in different sets of three
  > **a.a. sequence** from point of addition/deletion will be changed, resulting in non-functional protein

- Result in **nonsense** mutation
  > there could be a **stop codon downstream** of mutation, resulting in translation being terminated prematurely, a **truncated**/shortened polypeptide would be produced instead → non-functional protein

- Result in **silent** mutation, little/no effect on encoded protein
  > bases added/removed in **multiples of 3** and a.a. replaced is located in non-crucial regions of protein

Sickle cell anaemia:

- Haemoglobin S (HbS) instead of Haemoglobin A (HbA)
- **Description**:
  Due to a single base pair substitution of the gene coding for ß chain, adenine replaces thymine in the template strand (DNA triplet changed from CTC to CAC in the gene). In the mRNA formed, GUG is coded for instead of GAG, resulting in a missense mutation, Hydrophobic valine replacing hydrophilic glutamic acid at the 6th amino acid position of β chain. This results in a change in the 3D conformation of haemoglobin to produce HbS instead of HbA

1. **Type** of mutation: single base pair substitution
2. Change in **DNA**: adenine replaces thymine in the template strand
3. Change in **mRNA**: GUG is coded for instead of GAG
4. **Impact** of mutation: missense mutation
5. **A.a.** change: Hydrophobic valine replacing hydrophilic glutamic acid
6. **Polypeptide** change
7. **Protein** change: 3D conformation of haemoglobin to produce HbS instead of HbA

Effect on **phenotype**: (Hb/RBC, organs etc)

- Decreases the solubility of deoxygenated HbS, at low oxygen concentration, hydrophobic areas of different HbS would stick together
- HbS molecules polymerise and precipitate out of solution to form rigid fibres
- Changes to the shape of RBC from circular biconcave shape to become sickle shape

Consequences:

- Sickle shaped RBCs ineffective in transporting oxygen
- Due to shape, RBCs clump together and block capillaries, impeding blood flow → deprives multiple organs of oxygen resulting in organ damage (bone & kidneys)
- Sickle shaped RBCs have shorter lifespan and haemolyse readily, resulting in anaemia
- Sickle shaped RBCs also accumulate in the spleen for destruction, leading to enlargement of the spleen

Mutations:

- Induced or spontaneous

Types of mutagen	Mutagen	Effect
Chemical	Nitrous acids	Deaminates adenine in DNA such that it behaves like guanine (affects CBP)
	Mustard gas	Replaces guanine in DNA with other bases
Physical	X-ray	Gene and chromosomal aberrations
	UV rays	Distorts structure of DNA

Significance to evolution:

- Increase genetic variation within populations/ gene pool for natural selection

- Mutations in immunoglobulin genes have potential to generate antibodies of high affinity towards antigens

Molecular techniques

Polymerase Chain Reaction (PCR)

- **Selectively amplify** a specific target DNA sequence
- Reagents:
    1. **Template DNA** (DNA containing target DNA sequence to be amplified)
    2. **Primers**
        - Synthetic ssDNA fragments
        - **Forward and reverse primers** complementary to sequences at **3' ends** of target DNA sequence to be amplified
        - Functions:
            1. Mark out section of DNA to be amplified
            2. Provide a **free 3' –OH end** for Taq polymerase to add dNTPs
    3. **Deoxyribonucleoside triphosphates (dNTPs)**
        - DNA **nucleotides** used for PCR, such as dATP, dCTP, dGTP, dTTP
    4. **Taq polymerase**
        - type of heat stable DNA polymerase
        - **Thermostable**, will not denature under high temperatures, optimum temp: **72** deg C
        - Functions:
            1. R&B to **3' –OH end of primer** and **add free dNTPs** to the primer
            2. Free dNTPs CBP via H bonds with exposed bases in template DNA
            3. Taq polymerase catalyses the formation of a phosphodiester bond between adjacent dNTPs, synthesising **new DNA strands in the 5' to 3' direction**
- Procedures:

  - **Thermal cycler**, automatically heat and cool the tubes in a very short time
  - Reaction mixture includes: **DNA fragment** containing the target sequence to be amplified, **<u>excess</u> forward and reverse primers**, **Taq polymerase**, **free dNTPs**, **buffer solution** at **optimum pH**
  - 30-40 cycles

  Step 1: **Denaturation**
  - Heated to **95** deg C
  - **H bonds** between complementary bases of the dsDNA fragment break
  - **dsDNA separates into ssDNA**

  Step 2: **annealing** of DNA primers
  - Cooled to **65** deg C
  - **DNA primers CBP** via H bonds with complementary sequences at the **3' end** of target sequences in the ssDNA

  Step 3: Primer extension
  - Heating to **72** deg C

- Taq polymerase synthesises the rest of the **new strand** of DNA by adding dNTPs to the 3' –OH ends of both primers by catalysing the formation of phosphodiester bonds between adjacent dNTPs
- Taq polymerase start to denature after 30 cycles, when exposed to high temps for a longer duration, more enzymes will be denatured due to H bonds being disrupted
- Number of cycles=n
- Number of ds DNA molecules = 2^n
- Number of DNA strands = 2^{n+1}

Advantages of PCR

1. High **sensitivity**
   Capable of amplifying sequences from minute amounts of target DNA
2. **Speed**
   Millions of copies of target DNA can be obtained in a relatively short amount of time
3. **Specificity**
   Specific process which amplifies only target sequences, primers used are specific
4. **Automation**
   Taq polymerase (withstand high temps), allows the technique to be fully automated, no need to replace Taq polymerase after every cycle
5. **Robustness**
   Can amplify specific DNA sequences from material where DNA is badly degraded or embedded in medium
6. **Ease of use**
   Easy to set up and use thermal cycler to carry out
   Computer software can design specific primers is readily available

Disadvantages of PCR

1. Too **sensitive**
   Minute amt of **contaminant** DNA which contains a seq complementary to primers can be amplified along with target DNA → misleading result
2. Requires **prior information** of the target DNA sequence
   Prior info of target DNA seq required in order to design specific primers for selective amplification of target DNA seq
3. **Limit** to size of DNA fragment to be amplified
   Size range of DNA fragments to be amplified is **0.1-5kb**
4. There is **infidelity** of DNA replication
   Taq polymerase has **no proofreading function**

Gel electrophoresis

Explain how gel electrophoresis is used to separate fragments of DNA

1. DNA is **-vely charged** due to phosphate groups of the sugar phosphate backbone
2. When placed in an electric field, -vely charged DNA molecules move towards the **anode**, away from the cathode
3. DNA fragments of different molecular sizes in a given sample can be separated based on their **different rates of migration** through the gel.
4. **Larger** DNA fragments move **slower** through the pores, thus move a **shorter distance** and can be found **nearer to the well**
5. **Rate of migration** is **inversely proportional** to the **molecular size** of the DNA fragments.

Process:

Step 1: **casting** of agarose gel

- Molten agarose poured into casting try and allowed to solidify
- With the help of a comb, small indentations (wells) are formed at one end of the gel where DNA samples can be loaded
- Each well correspond to one lane
- After the gel solidifies, it is placed in a buffer solution

  Function of buffer solution:

  1. Buffer solution contains **ions** that will **conduct electricity** to allow -vely charged DNA to move through gel towards the anode when the electric current is turned on
  2. **Maintain pH** at a relatively constant value

Step 2: loading of samples

- Prior to loading, DNA samples are mixed with a loading dye (glycerol and coloured dye, e.g. bromophenol blue)
- **Function of loading dye:**
  1. **Glycerol <u>increases density</u>** of DNA sample → sinks to bottom of the well
  2. DNA is invisible to the naked eye, glycerol is colourless → colour of loading dye enables one to **locate** DNA sample
  3. Loading dye moves towards anode, allowing **visual tracking** of DNA sample migration
- DNA ladder: consists of DNA fragments of known sizes → **<u>estimate</u> approximate size** of molecule on a gel after electrophoresis by comparing positions

Step 3: running of gel

- Current on: DNA fragments migrate out of well and travel through gel towards anode
- DNA fragments of different molecular sizes separate based on different rates of migration
- Larger fragments → moves slowly → found nearer to well

Step 4: visualisation

- **Loading dye reaches end** of gel, current turned off
- Gel stained with **DNA binding dye**, usually **ethidium bromide**, placed under **UV light**
- Bands visible, ethidium bromide bound to DNA fluoresces under UV light
- Higher number of DNA fragments → thicker bands

Southern blotting and nucleic acid hybridisation

- Too many bands from gel electrophoresis → smear
- Southern blotting: transferring DNA from gel onto nitrocellulose membrane
- Nucleic acid hybridisation: use radioactive probe to detect specific nucleotide sequences via hybridisation

- Procedure:
    1. DNA sample is cut by **restriction enzymes**, dsDNA fragments separated by gel electrophoresis
    2. Gel from gel electrophoresis is placed on top of a sponge which is soaked in **alkali** solution,
    3. A sheet of **nitrocellulose membrane** followed by a stack of paper towels are placed on top of the gel
    4. Alkaline solution is drawn upwards through the gel, **denaturing** the dsDNA, transferring ssDNA fragments to the nitrocellulose membrane → **adhere firmly** in the same positions as they were in the gel
    5. Nitrocellulose membrane is placed in a sealed plastic bag containing **radioactively-labelled ssDNA probes** (complementary to the DNA sequence of interest) → **hybridise** with DNA fragments (containing sequence of interest) on the nitrocellulose membrane via **CBP**
    6. Nitrocellulose membrane is removed from the bag and washed thoroughly → remove unhybridised probes
    7. **Autoradiography** → placing **X-ray film** over the nitrocellulose membrane. Radioactivity in the bound probes → **expose** the film → form an image corresponding to the bands that have hybridised with the probe, allowing the pattern for the particular gene to be **visualised**

Outline the process of **genetic fingerprinting**:

- DNA sample is **isolated** from ___ and **digested by restriction enzymes**
- Carry out **gel electrophoresis**. DNA fragments are separated through a gel in an electrical field where rate of migration is inversely proportional to their molecular size
- Transfer the DNA fragments onto a **nitrocellulose membrane** using alkaline solution (via capillary action) → **denature** the dsDNA fragments into ss fragments
- Carry out **nucleic acid hybridisation** using **ss radioactive DNA probes** → hybridise by CBP to DNA fragments of complementary sequence)
- Autoradiography by **exposing X-ray film** to obtain the genetic fingerprint

Explain how the genetic fingerprints could be used to confirm that it originated from:

- Obtain the genetic fingerprint of ___ via the process of genetic fingerprinting
- Compare the genetic fingerprints of ___ and that of ___
- If ___ are from ___, they will have **similar genetic fingerprints**
- If there is a **match in banding pattern**, it confirms that ___ is from ___

Organisation of Eukaryotic Genome'

End replication problem

Eukaryotic DNA replication:

- During DNA replication, DNA polymerase requires a free 3' –OH end of an existing strand to add free nucleotides
- An RNA primer is synthesised to provide a free 3' –OH end for the addition of free nucleotides, and is then replaced by DNA nucleotides
- The RNA primer removed from the 5' end of the newly synthesised DNA strand cannot be replaced with DNA molecules as there is no free 3' –OH end
- Since DNA polymerase is unable to complete the replication at the 5' end of lagging strands, this creased a 3' overhang at the end of the chromosome
- Due to this, the ends of chromosomes shorten with each round of DNA replication
- Telomeres are found on the chromosomal ends and are non-coding, shortening of chromosomal ends leads to shortening of the telomeres without any deleterious effects. Genes/genetic info within the chromosomes will thus not be lost
- Cells with critically short telomeres trigger apoptosis/cannot undergo further cell division. Hence, end replication problem limits the number of cell divisions

Structure and function of non-coding DNA

Intron: non-coding DNA seq that interrupt a gene-coding seq

- **Structure**:
  - interspersed between exons
  - pre-mRNA undergoes RNA splicing: introns excised, exons spliced tgt to form mature mRNA with continuous coding seq. Hence, DNA of eukaryotic gene longer than mature mRNA
  - Pre-mRNA can also undergo alt RNA splicing, such that a single pre-mRNA can produce more than 1 type of mature mRNA, depending on the combination of exons spliced. One gene can code for more than one polypeptide.
- **Function**:
  1. Enable alternative RNA splicing and the subsequent production of multiple polypeptides from one gene

Centromeres: constricted region on a chromosome where 2 sister chromatids are joined. Spindle fibres attach during nuclear div

- **Structure**:
  - DNA in centromeric region includes non-coding DNA made up of a series of tandem repeats, heavily methylated
  - Consists of specific DNA seq to which a number of centromere-associated proteins bind, forming a structure called the kinetochore
- **Function**:
  1. Ensure proper nuclear division

2. Hold sister chromatids together (until anaphase of mitosis and anaphase II of meiosis II)
3. Site on the chromosome where the kinetochore assembles and spindle fibres from the centrioles attach during nuclear division
4. Ensure proper alignment and segregation of homologous chromosomes / sister chromatids to opposite poles of the cell

Telomeres: nucleoprotein complexes composed of telomeric DNA bound by telomere-specific binding proteins found at both ends of linear eukaryotic chromosomes

- **Structure:**
  - Non-coding DNA, series of tandem repeats synthesised by telomerase
  - ss region of DNA at 3' ends (3' overhang). The 3' end of the telomeric DNA, aided by telomere-specific binding proteins, bend back on itself to form a physical loop (t-loops) at the telomere (protects the ends of chromosomes)
  - Packaged into heterochromatin (highly condensed DNA)
- **Functions:**
  1. Ensure genes are not lost or eroded due to end replication problem with each round of DNA replication, preventing loss of important genetic information.
  2. **Protect** and **stabilise** terminal ends of chromosomes by forming a physical loop → confers stability to linear chromosomes by <u>preventing accidental fusion</u> of the single-stranded end of one chromosome to the single stranded end of another chromosome via complementary base pairing. Telomeric DNA is bound by <u>specific telomere-specific binding proteins</u> → protect the chromosomal ends from <u>degradation by exonucleases</u>
  3. Telomeres that are critically short trigger apoptosis (programmed cell death)
  4. Telomeres allow their own extension, by providing an attachment point for correct positioning of telomerase

Telomerase: enzyme that adds telomere repeat sequences to 3' end of DNA strands

- Ribonucleoprotein
- **RNA**:
  - Nucleotide sequence 3'-AAUCCC-5' that acts as template for new telomere segments at the 3' end of the telomere
  - Telomerase RNA CBP with 3' end of DNA, provide template for CBP of new DNA nucleotides
  - Telomerase translocates 5' to 3' direction, allowing telomere seq (5'-TTAGGG-3') to be repeated, extending telomeric DNA
- **Protein**:
  - Telomerase reverse transcriptase (TERT)
  - Active site determines the specific region of DNA to be bound and hence the region of DNA to be elongated
  - New DNA nucleotides undergo CBP with telomerase RNA as template (3'-AAUCCC-5')

- TERT catalyses the formation of PDiester bonds between adj DNA nucleotides, elongating DNA by adding telomere repeat seq (5'-TTAGGG-3') to the 3' end of the DNA strand

Control elements: non-coding DNA segments that transcription factors R&B to in order to regulate transcription of genes

Promoters: specific segment of non-coding DNA located upstream of the transcription start site

- **Structure**:
  - Recognition site for binding of GTFs and RNA polymerase
  - specific promoter of each gene dictates which of the 2 DNA strands is to be transcribed, and which is used as template
  - **Core promoter:** located within 40bp upstream of transcription start site
  > found in all protein-coding genes
  > upstream of the gene containing transcription start site and a TATA box
  > TATA box is a TA rich region, seq 5'-TATAAA-3'
  > GTFs (TFIID, TFIIB, TFIIA, TFIIH) R&B to TATA box
- **Function**:
  1. binding site for GTFs and RNA polymerase
  2. GTFs (e.g. TFIID) R&B to the TATA box and recruit RNA polymerase to R&B to core promoter. This forms the transcription initiation complex (TIC) and initiates transcription at the start site. This allows for basal level of transcription of the gene

  - Upstream promoter (could exist): located 200bp upstream
  > binding site for other specific transcription factors (e.g. activators)
  > improves efficiency of promoter by recruiting GTFs and RNA polymerase to core promoter

Enhancers: positive regulatory elements, located far away from promoter (distal control element), upstream/downstream of start site, or within an intron/near the gene they control

- **Functions**:
  1. binding site for specific transcription factors called activators
  2. binding of activators to enhancers accelerates the assembly of a TIC at the promoter, increasing rate of transcription of the gene
  3. activator protein R&B to enhancer, bends DNA, bring activator in contact with GTFs and RNA polymerase, forming a stable TIC

Silencers: negative regulatory elements, located much further upstream/downstream of promoter (distal control element)

- **Function**:
  1. binding site for specific transcription factors called repressors
  2. Repressors R&B to silencers, preventing assembly of TIC, inhibits/decreases rate of transcription of the gene

Terminators: extra reading: specific segment of non–coding DNA at the end of the gene which signals end of transcription

- RNA polymerase transcribes the polyadenylation signal seq (3'-TTATTT-5') found before the terminator. It dissociates 10-30bp after the seq
- Polyadenylation signal seq 3'-TTATTT-5' is usually transcribed into the mRNA polyA seq 5'-AAUAAA-3', which can function as stop codon (UAA, UGA, UAG)

Gene: specific seq of nucleotides in DNA which codes for a polypeptide or RNA

- **Structure**:
  - consist of both coding and non-coding seq
  - coding region made up of exons interspersed with introns
  - non-coding region made up of regulatory seq (e.g. enhancers, promoter, terminator)

- Each gene occupies a specific gene locus
- Classification: products
  Protein coding genes/ RNA coding genes
- Classification: function of products
  Housekeeping genes: products involved in routine metabolic functions
  Specialised genes: products synthesised only in particular cell types

Regulation of gene expression in eukaryotes

Importance/significance of regulation:

- Ensures that the appropriate genes are expressed at the appropriate time to synthesise the **right type** and **amount** of protein/RNA products for proper functioning of the cell

Differential (spatial, temporal) gene exp can be regulated at 5 levels

1. Chromatin level: modify DNA from euchromatin (loosely packed) to heterochromatin (tightly packed) or vice versa
    a) Chromatin remodelling complex (proteins alter structure of nucleosomes temporarily, can be either gene exp on or off)

b)	Histone acetylation	Histone deacetylation
c)	DNA demethylation	DNA methylation
	Gene exp ON	Gene exp OFF

2. Transcriptional level: control transcription, require interaction of control elements (enhancer, silencer) with GTFs and specific transcription factors (activator, repressor proteins)
    a) Binding of GTFs to TATA box to facilitate recruitment of RNA polymerase to R&B to core promoter (basal rate of transcription)
    b) Binding of activator proteins to enhancers (increase rate of transcription)
    c) Binding of repressor proteins to silencers (decrease rate of transcription)

3. Post-transcriptional level: control/regulate pre-mRNA after it has been transcribed to form a mature mRNA and can leave the nucleus, enter cytosol to be used at template for translation
    a) 5’ capping (addition of 7-methylguanosine cap to the 5’ end of the pre-mRNA)
    b) 3’ polyadenylation (addition of polyA tail to the 3’ end of the pre-mRNA)
    c) RNA splicing (may involve alternative RNA splicing)

4. Translational level: controls translation. Determine whether translation can be initiated or the amount of proteins that could be translated from the mature mRNA
    a) Regulating initiation of translation
    b) Regulating half-life of mature mRNA

5. Post-translational level: control functionality of the proteins and life-span of proteins synthesised
    a) Biochemical modifications (glycosylation → targets proteins for association with CSM, phosphorylation → activates protein)
    b) Protein degradation

1. Chromatin level

Chromatin alternates between heterochromatin and euchromatin
Heterochromatin

- Highly condensed
- DNA associates more tightly around histones (promoter region not exposed), limiting access of RNA polymerase and transcription factors to promoters of genes
- Genes not expressed, gene exp OFF

Euchromatin

- Loosely packed
- DNA associates less tightly around histones (promoter region exposed), promoting access of RNA polymerase and transcription factors to promoters of genes
- Genes expressed, gene exp ON

- Gene expression controlled by modifying chromatin str
- Chromatin modification alters chromosome compaction, determines ease of transcription initiation

a) Chromatin remodelling complex:

- protein complexes that alter the str of nucleosomes temporarily
- **DNA less tightly coiled around histones**, allow RNA polymerase and GTFs to access promoter, R&B and initiate transcription
- **DNA more tightly coiled around histones**, prevents RNA polymerase and GTFs from accessing the promoter, inhibiting transcription

b) Histone acetylation (promote)

- Addition of acetyl groups (-COCH3) to lysine residues on histone tails
- **Process**:
  > **Histones acetyltransferase (HATs)** adds acetyl groups to **lysine** on histone tails
  > **Non-acetylated lysine residues** on histone tails are **+vely** charged, **interact strongly** with **-vely** charged **phosphate groups on DNA** backbone, **increasing affinity** of DNA for nucleosome surface (DNA more tightly wound around nucleosome)
  > **Acetyl groups remove +ve charge** on histones, **decreasing electrostatic interactions** between -vely charged DNA and histones. Affinity of histones for DNA reduced
- **Consequence**:
  > DNA **coils less tightly** around nucleosomes, chromatin decondenses. Promoter of gene more accessible to GTFs and RNA polymerase, promotes assembly of TIC, transcription easier to be initiates, gene exp ON

b) Histone deacetylation (inhibit)

- **Process**:
  > **Histones deacetylase (HDACs)** removes acetyl groups from histones

> Removal of acetyl groups **restores +ve charge** on histones, resulting in **strong electrostatic interactions** between -vely charged DNA and histones. This increases the affinity of DNA for the nucleosome surface

- **Consequence**:
  > DNA coils more tightly around nucleosomes, chromatin condenses. Promoter of gene less accessible to GTFs and RNA polymerase, inhibit assembly of TIC, inhibits transcription, gene exp OFF

c) **DNA methylation (inhibits)**

- Addition of methyl groups (-CH3) to selected **cytosine residues at the CpG islands**
- Highly methylated DNA found in heterochromatin (e.g. DNA at centromeric region, non-coding, not expressed)
- **Process**
  > Catalysed by **DNA methyltransferase**
  > addition of methyl groups **physically blocks** binding of GTFs and RNA polymerase to promoter
  Methylated DNA **recruits additional DNA-binding proteins** (e.g. transcriptional repressors → R&B to silencers, histone deacetylase → remove acetyl groups, repressive chromatin remodelling complexes)
- **Consequence**:
  > forming a more compact, inactive **heterochromatin**, tighter nucleosomes. Promoter is inaccessible to GTFs and RNA polymerase, preventing formation of TIC, preventing transcription, gene exp OFF

b) DNA demethylation (promotes)

- Process
  > Catalysed by **DNA demethylase**
  > **DNA that is actively transcribed** has **methyl groups removed** from cytosine residues at CpG islands
- Consequence:
  > form a less tightly coiled **euchromatin** str, promote transcription. Removal of methyl groups enables GTFs and RNA polymerase to R&B to promoter, forming TIC to initiate transcription, gene exp ON

2. Transcription level

a) **GTFs** (R&B to promoter)

- GTFs (e.g. TFIID, TFIIB, TFIIA, TFIIH) **facilitate recruitment of RNA polymerase** to R&B to core promoter, forming the stable TIC and initiates transcription at the start site (TFIID contains TATA binding protein TBP that R&B to TATA box)
- GTFs **stabilise TIC** (allows RNA polymerase to bind more securely) and the progression of RNA polymerase to elongation stage. This allows for the **basal level of transcription** of the gene

b) Specific Transcription Factors (**STFs**, R&B to enhancer/silencer)

- STFs include transcriptional **activators** and transcriptional **repressors**
- Activator proteins R&B to **enhancers**, repressor proteins R&B to **silencers**
- Upon R&B, the rate of transcription of a gene increases/decreases
- Different gene expression: STFs work in a tissue/cell specific or time-dependent manner

i) Transcriptional activators (R&B to enhancer seq)

- Details:
  Effects: **Bending of DNA**, brings **activator in contact with GTFs and RNA polymerase** via **protein-protein interactions** to facilitate binding of GTFs and RNA polymerase to promoter
- **Recruits, positions and modifies** the **transcription machinery** at the promoter to form a **stable TIC** → **increase rate** of **initiation** of transcription
- Assist in **recruiting HATs and chromatin remodelling complex** to promoter region and **increase accessibility** of the transcription machinery to the promoter
- **Accelerates the assembly** of a **stable TIC** at the promoter and increases the rate of transcription of the gene.

ii) Transcriptional repressors (R&B to silencer reg)

- Details:
- **Change in looping patterns** of DNA so **activators** bound to enhancers are **prevented from binding to GTFs** at promoter
- **Inhibit assembly** of TIC at promoter
- **Repress** chromatin remodelling complexes, **prevent changes** to **chromatin str** that **facilitates** transcription
- **Recruit histone deacetylases** to make promoter **inaccessible** to GTFs and RNA polymerase
- Binding of repressors to silencers **inhibit** transcription/**decrease rate** of transcription

	Enhancer	Silencer
STFs involved	Activator R&B to enhancer	Repressor R&B to silencer
Effect of binding STFs on rate of transcription	Accelerates assembly of TIC, increases rate of transcription	Decreases rate of transcription
Effects on DNA and transcription machinery	Leads to the bending of DNA	Change the looping patterns of the DNA → regulatory proteins bound onto the enhancer and promoter regions are not brought into contact
	Brings activator in contact with GTFs and RNA pol via protein-protein interactions to facilitate the binding of GTFs and RNA pol to promoter	Prevent activators from binding to GTFs

	Recruits, positions and modifies the transcription machinery (GTFs and RNA pol) at promoter to form a stable TIC → increase rate of initiation of transcription	Inhibit assembly of the TIC at promoter
	Assist in recruiting HATs and ATP-dependent chromatin remodelling complex to promoter region and increase accessibility of GTFs and RNA pol to promoter	Repress the chromatin remodelling complexes to prevent changes to chromatin structure that facilitates transcription Recruit histone deacetylases to make promoter inaccessible to GTFs and RNA pol

3. Post-transcriptional level (5' capping, splicing, polyA)

a) 5' capping

- When RNA polymerase transcribes abt 25 nucleotides of pre-mRNA, **5' end** of pre-mRNA molecule is modified by **addition of a 7-methylguanosine cap**
- **Functions**:
    4. **Protect** mRNA from degradation by **ribonucleases**
    5. Act as **signal** to **facilitate export** of mature mRNA out of nucleus
    6. **Promotes initiation** of translation. **Translation initiation factors bind to 5' cap** and initiate translation by **recruiting small ribosomal subunits** (translate 5' to 3' until find AUG) to bind at 5' cap

b) 3' polyadenylation

- Completely transcribed pre-mRNA contains a **polyadenylation signal seq (AAUAA) at 3'** end
- **Ribonucleases** recognises polyadenylation signal seq and **cleaves the mRNA** at the **3'** end. **PolyA polymerase** adds 50-250 **adenine** nucleotides to the 3' end of the mRNA forming a polyA tail
- **Function**:
    3. PolyA tail act as a **signal** to **facilitate export** of mature mRNA out of nucleus
    4. **Recognised** by poly(A)-binding protein. Binding of poly(A)-binding protein **enhances mRNA stability** and **prevents degradation** of the mature mRNA by ribonucleases

c) RNA splicing

- Process
    1) i) **Small nuclear ribonucleoprotein particles (snRNPs)** consist of small nuclear RNAs associated with proteins. Several snRNPs join with additional proteins to form a larger assembly called a **spliceosome**
    2) snRNPs **recognise splice sites** at each end of an intron (5' site = GU and 3' site = AG)

3) Spliceosome interacts with splice sites at the ends of an intron. It cuts at specific points to release the intron, then immediately joins together the 2 exons that flanked the intron

- **Process and significance of RNA splicing**
  > introns interspersed within pre-mRNA are excised and exons that flank the intron are spliced together to form a **continuous coding seq** and ensure the synthesis of the correct polypeptide

Alternative RNA splicing

- Involves **specific regulatory proteins** which control intron-exon choices by binding to regulatory seq within pre-mRNA
- Different/more than 1 mature mRNA molecules are produced from the same pre-mRNA, depending on which RNA segments are treated as exons and which as introns
- Importance:
  - enables a **single gene** to code for **more than 1 polypeptide**, depending on which exons are spliced together to form a continuous coding seq

4. Translational level

a) Regulation of **initiation** of translation

(i) binding of **translation initiation factors** to **small ribosomal subunit**

- Translation initiation factors **recruit and facilitate binding of small ribosomal subunits** to the **5' cap of mature mRNA**, so that initiation of translation can occur
- translation initiation factors are involved in **locating** the **5' cap** of the mature mRNA, **scanning** the mature mRNA for the start codon **AUG**, and locating the **binding site of initiator tRNA to AUG**
- Phosphorylation (activates proteins) and dephosphorylation (deactivate) of translation initiation factors (proteins) is found to activate/repress translation

(ii) binding of **translational repressors** to **mature mRNA**

- **Regulatory proteins** known as **translational repressors** R&B to specific seq, usually the **5' untranslated region** (5' UTR) of **mature mRNA** and **block** binding of ribosomes

b) **Half-life** of mature mRNA

- **Length of polyA tail** determines **stability** of mRNA. Longer tail → more stable → mRNA can be used as template for translation longer
- mRNA **degradation**: **polyA tail (3'end) steadily removed** by **ribonucleases** in **3' to 5'** direction until **critical length** is reached → trigger **removal of 5' cap** and degradation of mRNA from 5' end as well
- All mature mRNA subjected to polyA tail shortening, decapping and degradation, but **rate differs**
- Controlling **lifespan and stability** of mature mRNA → determines **duration** for which translation can occur → control over number of protein molecules translated
- E.g. mRNA stability can be under hormonal control → mRNA that codes for vitellogenin (protein synthesised in liver of female frogs, transported to oviduct, used in forming egg yolk proteins). Under influence of estradiol (hormone), half life of vitellogenin mRNA synthesis can be as high as 500 hours compared to 165 hours in its absence

5. Post-translational level

a) protein processing

- Polypeptides must be processed through **addition/removal of functional groups** to yield functional protein

(i) **biochemical modification** (e.g. glycosylation, phosphorylation/dephosphorylation)

- **Glycosylation** targets protein for association with membranes (at RER or Gb, addition of carbohydrate chain)
- **Phosphorylation** reversibly alters activity of protein (e.g. **activates** the protein/enzyme) or yields a functional protein (catalysed by **kinases**)

(ii) **structural** modification

- **Removal of a.a.** from precursor protein may occur to produce mature protein

b) Protein degradation

- Proteins can be marked for degradation
- **Ubiquitin ligase** adds **ubiquitin** to protein
- Ubiquitin-tagged protein recognised by **proteasome**, which **cleaves** the protein into smaller peptides → further degraded by enzymes in cytoplasm

Structure of virus

	T4 phage	Lambda phage	influenza	HIV
genome	dsDNA	dsDNA	8 segments of linear ssRNA	2 copies of ssRNA
envelope	absent	absent	present	present
Host cell	bacteria	bacteria	Animal epithelial cells of respiratory tract	Animal immune cells (T cells, macrophages)
entry			Receptor mediated endocytosis	fusion
exit	lytic	lysogenic	budding	budding
			Haemagglutinin (entry), Neuraminidase (exit)	gp120, gp41

Viral genome:

- Genes code for synthesis of coral components
- Either RNA or DNA
- May comprise of since nucleic acid molecule or segmented
- Circular or linear
- Ss or ds
- May be associated directly with nucleoproteins
- Nucleoprotein + genetic material = nucleocapsid

Capsid:

- Outer protein coat, made of indiv protein subunits (capsomeres)
- Function: protect, attach and introduce genome into host cells

Envelope

- Function: additional protective coat
- Glycoprotein spikes: attach virus to specific surface receptor molecules on membranes of susceptible hosts

Reproductive cycle of virus

Cell theory:

4. Cells are the smallest unit of life
5. All cells come from pre-existing cells
6. Living organisms are composed of cells

Living characteristics of viruses

- Contain nucleic acids (either DNA or RNA, never both)
- Able to reproduce (only in suitable host cell)

Non-living characteristics of viruses

- Acellular (no cytoplasm/cellular organelles), do not hv cell str
- Outside of host cells, viruses do not carry out any metabolism, move, grow or divide
- Replication of DNA or RNA can only occur within host cell
- Viruses depend on host cells for reproduction because they lack most of the metabolic machinery and cellular organelles (e.g. enzymes for metabolism, ribosomes for protein synthesis)
- Obligate parasites

Explain why viruses are described as obligate parasites.

- Viruses contain either DNA or RNA and thus their replication can only occur within host cells
- Viruses lack resources e.g. amino acids and ATP
- Viruses lack most of the reproductive / metabolic machinery or structures e.g. enzymes for metabolism and ribosomes for protein synthesis

Influenza

- enveloped
- 8 segments of linear ssRNA and RNA dependent RNA polymerase within capsid
- RDRP catalyse synthesis of a complementary RNA (cRNA) strand
- Haemagglutinin HA (entry) and Neuraminidase NA (exit)
- HA is a glycoprotein that R&B to the specific sialic acid containing receptors on the CSM of host cells
- NA is an enzymes that facilitates the exit and release of newly replicated viruses from the infected host cell via budding
- 13H, 9N

Reproductive cycle of influenza

Step 1: adsorption

- The haemagglutinin glycoproteins (on the viral envelope) recognise and bind to specific sialic acid containing receptors on the host cell CSM

Step 2: penetration and uncoating

- Virus enters the host cell via receptor-mediated endocytosis

- host cell CSM invaginates and pinches off, engulfing the virus in an endocytic vesicle which enters the cytoplasm
- Acidification causes the viral envelope to fuse with the endocytic vesicle membrane, releasing the nucleocapsid
- Uncoating, whereby the capsid is then degraded by cellular enzymes, releasing the viral nucleic acid and RDRP

Step 3: replication

- Viral nucleic acid serves as the template for the synthesis of cRNA using the viral RNA dependent RNA pol (RDRP)
- cRNA serves 2 functions:
    1. Act as template for the synthesis of new copies of viral RNA
    2. Function as mRNA and undergo translation to produce viral proteins (capsid proteins, glycoproteins)

Step 4: maturation

- Glycoproteins (HA, NA) synthesised by ribosomes on the RER are transported to the CSM via vesicles
- Incorporated into host cell's CSM via fusion and are clustered in patches that serve as 'exit points'
- Capsid (made by free ribosomes) assembled around the viral RNA and RDRP

Step 5: release

- The new influenza viruses bud off from the host cell's plasma membrane at 'exit points' via budding
- At this point, the new viruses would still adhere to the host cell receptors through haemagglutinin
- Neuraminidase catalyses the cleavage of sialic acid residues from HA, facilitates the release of the newly replicated viruses

HIV

- Retrovirus with 2 copies of ssRNA
  Replicate via reverse transcription of viral RNA to DNA
- Inside capsid: reverse transcriptase and integrase
  Outside capsid: HIV protease
- Reverse transcriptase: synthesises cDNA from viral RNA template. Newly synthesised cDNA strand is used as template for synthesis of a second DNA strand to form dsDNA
- Integrase: enzymes responsible for integrating dsDNA into host cell's DNA
- HIV protease cleaves immature HIV polyprotein into individual functional viral proteins
- Gp120: specific conformation that allows the virus to R&B to specific receptors on CSM of host
- Association between gp120 and gp41 with host cell receptors leads to fusion of HIV envelope and CSM

Why is HIV described as a retrovirus?

- HIV is a retrovirus as it replicates by means of reverse transcription of its ssRNA genome into dsDNA by reverse transcriptase
- Followed by integrating the dsDNA into the host cell genome by integrase

Step 1: adsorption

- gp120 on the HIV envelope recognises and binds to a CD4 molecule on the CSM of the host cell, inducing a conformational change in gp120
- gp120 binds to the host cell's chemokine receptor (CCR5 for T cells, macrophages or CXCR4 only T cells), bringing about a conformational change in gp41 on HIV virion

Step 2: penetration and uncoating

- Fusion of the viral envelope with the host cell's cell surface membrane allows the genetic material and capsid to enter the target cell
- Capsid is later removed by cellular enzymes, releasing the viral RNA and various enzymes (RT, integrase) during uncoating

Step 3: replication

- RT catalyses the synthesis of cDNA complementary to viral RNA. a RNA: DNA hybrid is formed
- RNA strand is degraded. Second DNA strand is synthesised, which is complementary to the cDNA, forming dsDNA
- dsDNA incorporated into host cell DNA as a provirus by integrase
- Host RNA pol transcribes provirus genes into viral RNA molecules that function as:
    1. mRNA for translation of viral proteins
    2. Genetic material for new virus particles

Step 4: maturation and release

- Translation of HIV mRNA leads to synthesis of gp120 and qp41 (synthesised by ribo on RER) and immature viral polyprotein
  > gp120 and gp41 are transported to CSM of host cell via transport vesicles from RER and embedded in CSM
  > immature viral polyproteins are transported to CSM, where viral progeny begins assembling
- New viral RNA assembles with newly synthesised proteins to form new viral particles
- Eventually, the virus particles buds off at exit points, acquiring host CSM with viral glycoproteins embedded
- HIV protease cleaves immature HIV polyproteins into individual functional proteins (capsid proteins, RT, integrase) giving rise to a mature infectious virus

Antigenic drift (gradual)

- Point mutation in the gene that codes for viral protein can occur during the replication of viral RNA. Accumulation of mutations results in slight changes to the shape of viral protein, leading to antigenic drift
- Host immune system no longer recognises the antigenic sites on the HA on new strain of influenza virus

- Change in shape in new HA may enable it to be complementary in shape to other membrane receptors found on new types of host cells

Antigenic shift (sudden)

- Different strains of a virus infect the same host cell simultaneously.
- Random assembly / genetic reassortment of RNA segments from these different strains of the virus leads to novel combinations of viral genes coding for viral proteins to form a new strain, resulting in antigenic shift
- 3 methods
    1. Direct transmission from another species to humans
    2. Transmission of virus to an intermediate animal host and later from intermediate animal host to humans
    3. 2 or more existing strains infect the same host cell simultaneously, random assembly of RNA segments from these different strains (genetic reassortment) produces new subtypes containing novel combinations of the viral genes
- Characterised by major changes in subtypes of HA and NA

Bacteriophages:

- Viruses that only infect bacteria
- Virulent: lytic cycle, T4 bacteriophage
- Temperate bacteriophage: Lytic and lysogenic cycle, lambda bacteriophage

Lytic cycle of T4 phage

1. Adsorption
    - The T4 phage uses the attachment sites on its tail fibres to recognise and adsorb to specific receptor sites on the host bacterial cell wall (host cell wall lipopolysaccharides/proteins serve as receptors)
2. Penetration
    - The T4 phage tail fibres anchor the base plate pins to the cell surface, while the tail sheath contracts to drive a hollow tube through the bacterial cell
    - The (double-stranded) phage DNA is injected into the bacterial cytoplasm
3. Replication
    - Phage genes are expressed using the host's cell metabolic machinery (RNA pol, ribo) and resources. Phage genes code for:
    - Enzymes which disrupt normal cellular functions in the host bacterium by shutting down the bacterium's macromolecular synthesis (DNA, RNA,protein)
    - Phage nucleases hydrolyse the bacterial chromosome so that DNA nucleotides released from the degraded DNA are used in the synthesis of many copies of new phage DNA
    - Phage uses host cell's metabolic machinery to synthesise more phage enzymes and phage str components (capsid, tail fibres)
4. Maturation

- Phage components (capsid, tail, tail fibres) which assemble and enclose the newly replicated phage DNA, forming new phages

5. Release
    - Phage directs production of lysozymes → breaks down peptidoglycan cell wall of host bacterium, resulting in osmotic lysis of the bacterium to release newly assembled bacteriophages

Lysogenic cycle of lambda phage

1. Adsorption
    - The lambda phage uses the attachment site on its tail fibre to recognise and adsorb to specific receptor site on the host bacterial cell wall.
2. Penetration
    - Tail sheath contracts to drive a hollow tube through the bacterial cell well
    - dsDNA is injected into the bacterial cytoplasm
    - Lambda phage DNA circularises inside the bacterium so as to prevent host exonucleases from degrading it
3. Replication
    - In the lysogenic cycle, lambda phage DNA is incorporated into specific sites of the host bacterium's chromosome. At this stage, the inserted phage DNA is called a prophage
    - One of the prophage genes that codes for a repressor protein is expressed. The repressor protein represses the expression of the bacteriophage genes controlling phage replication. Hence, phage replication is blocked
    - Phage is dormant
4. Induction
    - Environmental factors could induce the lambda phage to enter lytic cycle
    - Repressor proteins are no longer made and the lambda phage DNA is excised from the bacterial chromosome
    - The phage switches from lysogenic to lytic mode of replication and ends with lysis of host cell

Structure of bacteria

Asexual repro & genetic variation in prokaryotes

F plasmid differs from the bacterial chromosome:

1. [Number of genes] Plasmid has fewer genes whereas bacterial chromosome has more genes
2. [Presence of AbR genes / xenobiotic resistance genes] Plasmid has antibiotic resistance genes / xenobiotic resistance genes but bacterial chromosome does not have antibiotic resistance genes / xenobiotic gene (confer selective advantages)
3. [Gene product for cell metabolism] Absence of genes required for cell metabolism on plasmid but presence of genes required for cell metabolism are found on bacterial chromosome (e.g. production of enzymes)

Binary fission:

- Division of a single parent bacterial cell into 2 genetically identical daughter cells
- Example of vertical gene transfer (transfer of genetic material from parent to daughter cells)

What happens to the genome of the bacterial cell during binary fission?

1. DNA replication
    - Circular DNA molecule undergoes DNA replication which begins at the origin of replication (ori) resulting in the formation of a replication bubble
    - The DNA double helix separates into 2 strands and each strand acts as a template for the synthesis of a daughter strand by semi-conservative replication
    - From a single origin of replication, DNA synthesis progresses in both directions around the circular chromosome until the entire chromosome has been replicated
    - Supercoiling ahead of the replication fork is relieved by topoisomerase, an enzyme which breaks a phosphodiester bond of the strained strand, swiveling the strand and rejoining the cut strand
2. Chromosome segregation
    - As the chromosome replicates, the newly formed ori and the original ori move to opposite poles of the cell (and becomes attached to the CSM at opposite poles of the cell)
    - The cell also elongates by producing more cytoplasmic, CSM and cell wall components to prepare for division into two
3. Cytokinesis
    - When DNA replication is complete, and the bacterium has reached twice its initial size, the CSM starts to invaginate
    - Invagination of the CSM along with the deposition of new peptidoglycan cell wall (septum), eventually divides the parent cell into 2 genetically identical daughter cells

Genetic recombination

- DNA is integrated into the recipient chromosome
- Result of horizontal gene transfer in which genetic material from a donor bacterium cell is transferred into a recipient cell
- Result in new allele combinations, which may confer antibiotic and xenobiotic resistance or the ability to use new metabolites

Transformation

- Transformation is the uptake of naked, foreign DNA by competent cells from the surrounding environment, resulting in the alteration of a bacterial cell's genotype and phenotype
- Naked dsDNA from surrounding environment binds to the competence factors on the CSM of a competent cell. One strand is degraded by exonucleases, while the other strand enters the bacterium
- The ssDNA taken up by the bacterium can be incorporated into its chromosome via homologous recombination by crossing over where there is sufficient homology between the DNA fragments and bacterial chromosome
- The resultant cell is a recombinant cell. If different alleles for a gene were exchanged, there will be a change in the organism's phenotype

Transduction

- process by which bacterial DNA/genes is transferred from one bacterium (host cell) to another (recipient cell) via a bacteriophage

Generalised transduction:

- Infection if bacterium by a virulent bacteriophage (T4)
- When a virulent bacteriophage undergoes lytic cycle, a small piece of the host bacterial cell's degraded DNA is mistakenly packaged within the capsid of a defective phage
- The defective phage infects another bacterial cell (recipient). No new phages can be synthesised
- Foreign bacterial DNA can then be incorporated into the recipient cell's chromosome through homologous recombination
- Recipient cell now becomes a recombinant cell
- As any random portion of the donor bacterial DNA may be transferred, this process if called generalised transduction

Specialised transduction

- Infection of a bacterium by a temperate bacteriophage (lambda)
- When a temperate bacteriophage enters into lytic cycle from lysogenic cycle, a small region of the host bacterial DNA adjacent to the prophage is excised and the phage-host hybrid DNA is packaged within the capsid of a defective phage
- The defective phage infects another bacterial cell and inject the piece of phage-host hybrid DNA into the newly infected bacterial cell cytoplasm

- The phage-host hybrid DNA is incorporated into recipient bacterium's DNA via homologous recombination OR integration of phage-host hybrid DNA as the phage enters the lysogenic cycle
- As only bacterial genes adjacent to the integrated prophage may be transferred, this process is called specialised transduction

Features	Generalised	Specialised
Type of bacteriophages involved	Virulent	Temperate
Example	T4	Lambda
Genetic material in defective phage	Any random fragment of bacteria's degraded DNA instead of the phage genome is packaged within the capsid	A small region of the bacterial DNA that was adjacent to the prophage (Phage-host hybrid DNA) is packaged within the capsid
Incorporation of bacterial DNA into recipient cell's chromosome	Foreign bacterial DNA is incorporated into the recipient bacterial cell's chromosome through homologous recombination	Phage-host hybrid DNA is integrated into the recipient bacterial cell's chromosome as the phage enters the lysogenic cycle (OR through homologous recombination)

Conjugation

- Conjugation is the direct transfer of genetic material from one bacterial cell to another, through a temporary link between two cells (requires cell to cell contact)
- The F+ donor cell produces the sex pilus to attach to specific receptors on the F- recipient cell. Upon making contact, the sex pilus retracts, pulling the two cells closer
- A single strand of F plasmid breaks at a specific point (origin of transfer) and the F plasmid is transferred as a single strand from the F+ donor cell to the F- recipient cell via the cytoplasmic mating bridge
- Each ssDNA remaining in its respective cell now acts as a template for the synthesis of a complementary daughter strand
- The complementary strand to the ssDNA remaining in the F+ donor cell is synthesised via rolling circle mechanism
- Once semi-conservative replication is complete, transferred DNA circularises resulting in an F plasmid

Role of F plasmid

- F plasmid contains the F factor which carries genes to produce sex pilus
- presence of the F plasmid with the F factor enables a bacteria to become a donor cell

- donor cell can produce sex pilus to attach to a recipient cell, pulls it closer and a temporary cytoplasmic bridge is formed
- F plasmid carry genes for antibiotic-resistance

Regulation of gene expression in prokaryotes

Operon

- Segment of DNA containing a cluster of structural genes that make up a single transcription unit and controlled by the same promoter and operator.
- The structural genes codes for enzymes / functionally related proteins involved in a single metabolic pathway.

Advantage of grouping structural genes into operons
E.g. why operons are necessary in prok/ advantages of operons in prokaryotes

1. Allow for the simultaneous regulation of related genes with related functions / which are involved in the same metabolic activity
2. Genes coding for related proteins in a single biochemical pathway / of related functions are grouped into an operon for easier control
3. Bacteria able to utilise a variety of carbohydrates as respiratory substrate (for their energy requirement).
4. Allow the bacteria to adapt and respond to environmental changes.
5. Genes on the operons are only expressed when required, thus preventing wastage of energy and resources
6. This confers selective advantage to the bacteria.

Structural gene

- Region of DNA that codes for a protein or RNA molecule that forms part of a structure or has an enzymatic function.

Polycistronic mRNA:

- Single mRNA that codes for many proteins
- Punctuated with start and stop codons
- Separate polypeptides produced during translation

Promoter

- Specific non-coding DNA sequence that RNA polymerase R&B to prior to transcription
- Located upstream from the coding region

Regulatory gene

- Region of DNA that codes for a specific protein product that regulates (positively or negatively) the expression of the structural genes
- Constitutively expressed (always expressed)
- Regulatory proteins have a DNA binding site and an allosteric site

Operator

- Segment of DNA situated between the promoter and the first structural gene, that controls access of the RNA polymerase to the structural genes
- Active repressor protein R&B to the operator, physically blocks the RNA polymerase from binding to the promoter region

Regulatory proteins:

- Activator: R&B to activator binding sites upstream of the promoter and enhances recruitment of RNA polymerase to the promoter/enhances the ability of RNA polymerase to unwind DNA at the promoter region, increasing rate of transcription
- Repressor: R&B to operator and physically blocks the RNA polymerase from binding to the promoter/prevents RNA polymerase from moving downstream, decreasing rate of transcription

Regulation of gene expression
- Prevent waste of resources
- Adapt and respond to constant environmental changes

Constitutive genes
- Continually expressed in all cells
- Essential components

Inducible genes (lac operon)
- Genes turn on in response to a substance (inducer)
- Inducers taken up from the environment inactivates active repressors, turning ON gene expression

Repressible genes (trp operon)
- Genes turn off in response to a substance (co-repressor)
- Co-repressors taken up from the environment activates inactive repressors, turning OFF gene expression

1. Scenario
2. Repressor
3. Operon (ON/OFF)
4. RNA polymerase, promoter, transcription occur/not
5. Protein product (enzyme/polypeptide etc)

Repressible system (trp operon)
- Promoter: binding site of RNA polymerase
- Operator: binding site of active trp repressor
- trpE, trpD, trpC, trpB, trpA: structural genes
- Terminator: termination sequence that marks the end of the operon
- Tryptophan: co-repressor
- Polycistronic mRNA that codes for 5 polypeptides: 5’ - trpE-trpD-trpC-trpB-trpA - 3’

1. Tryptophan absent
2. Repressor is in its inactive conformation and is unable to R&B to operator
3. Operon is turned ON
4. RNA polymerase is able to R&B to the promoter and is able to transcribe the structural genes of the trp operon. Polycistronic mRNA is synthesised and translated into 5 polypeptides
5. Enzymes that synthesise tryptophan are produced and thus tryptophan is synthesised

1. Tryptophan is present and binds to the trp repressor at its allosteric site
2. Repressor protein changes from inactive to active conformation and can R&B to the operator
3. Operon is turned OFF
4. RNA polymerase is physically blocked from binding to the promoter and is unable to move downstream to transcribe the structural genes of the trp operon. mRNA is not synthesised
5. Enzymes that synthesise tryptophan are not produced. Tryptophan is not synthesised

Inducible system (lac operon)

- Promoter: binding site of RNA polymerase
- Operator: binding site of lac trp repressor protein
- CAP binding site: binding site of catabolite activator protein (CAP)
- lacZ: codes for β-galactosidase, hydrolyses lactose into glucose and galactose, catalyses the intramolecular isomerisation of lactose to allolactose (inducer)
- lacY: codes for lac permease → membrane protein for transport of lactose into the cell
- lacA: codes for lactose transacetylase / β-galactoside transacetylase
- Terminator: termination sequence that marks the end of the operon
- Allolactose: inducer
- Polycistronic mRNA that codes for 3 polypeptides: 5' - lacZ-lacY-lacA - 3'

Negative control

1. Absence of lactose → absence of inducer molecule allolactose
2. Lac repressor synthesised in its active conformation and R&B to the operator
3. Operon is turned OFF
4. RNA polymerase is physically blocked from binding to the promoter and is unable to move downstream to transcribe the genes of the lac operon. mRNA is not synthesised
5. β-galactosidase, lac permease and β-galactoside transacetylase are not produced

1. Presence of lactose (due to small amount of lac permease present in the plasma membrane, some of the lactose outside the cell gets transported into the cell)
2. Lactose is converted to allolactose by β-galactosidase (transcription of lac operon is induced by inducer allolactose)
3. Allolactose binds to the allosteric site of lac repressor. The lac repressor changes its 3D conformation and becomes inactive. Repressor protein is unable to bind to/dissociates from the operator
4. The operator site is exposed, and the lac operon is turned ON
5. RNA polymerase is able to R&B to the promoter, and move downstream to transcribe the structural genes of the lac operon. mRNA is synthesised
6. The enzymes – β-galactosidase, lac permease and β-galactoside transacetylase, are synthesised

Positive control of lac operon

- Catabolite repression
- Catabolite activator protein (CAP)
- Cyclic AMP (cAMP) → low glucose conc, increase cAMP conc
- CAP is an activator of transcription, synthesised in inactive conformation. Binding of cAMP to allosteric site of CAP activates CAP

1. Lactose present, low levels of glucose (low glucose conc, increase cAMP conc)
2. When glucose is scarce, there is an increase in concentration of cAMP. cAMP binds to the allosteric site of CAP. CAP assumes its active conformation and can recognise and bind to the CAP binding site (upstream of lac promoter)
3. The attachment of CAP bends the DNA and recruits RNA polymerase to the promoter
4. RNA polymerase R&B to the promoter at high affinity
5. Transcription of the lac operon proceeds at a high level (transcription rate increase) EFFECT: cell will use lactose as a respiratory substrate

1. Lactose present, high level of glucose
2. When the amount of glucose in the cell increases, cAMP concentration falls. Without cAMP, CAP assumes its inactive conformation and disengages from the CAP binding site
3. CAP is inactive, RNA polymerase R&B to the promoter at very low affinity
4. Transcription of lac operon proceeds at only a low level (transcription rate decrease) EFFECT: cell will use glucose first. When glucose is depleted, cell will start to use lactose

	Feature	trp operon	lac operon
1	Default state of repressor	trp repressor is synthesized in the inactive form	lac repressor is synthesized in the active form
2	effector molecule	tryptophan acts as a co-repressor	allolactose acts as an inducer
3	effect of effector molecule on operon	In the presence of co-repressor (tryptophan), operon is turned OFF	In the presence of inducer (allolactose), operon is turned ON
4	type of operon	lac operon is an inducible operon	trp operon is a repressible operon
5	products + metabolic pathway	protein products of trp operon is involved in the synthesis of tryptophan	protein products of lac operon is involved in the breakdown of lactose
6	Str + metabolic pathway	cluster of 5 structural genes coding for enzymes involved in synthesis of tryptophan	cluster of 3 structural genes coding for enzymes and proteins involved in breakdown of lactose
7	When does the repressor bind to the operator	when co-repressor binds to allosteric site of the repressor, trp repressor in its active form R&B to the operator	lac repressor in its active form R&B to the operator

Mitosis

Centromere

1. hold sister chromatids together until anaphase of mitosis and divide to allow separation of sister chromatids
2. DNA at the centromere is essential for proper alignment and separation of sister chromatids to opposite poles of the cell during nuclear division
3. ensure proper segregation of chromosomes by being the site on the chromosome where the kinetochore assembles and spindle fibres from the centrioles attaches during nuclear division
4. consist of specific non-coding DNA made up of a series of tandem repeat sequences, to which a number of centromere-associated proteins bind, forming the kinetochore

homologous chromosomes

- Chromosome pairs with the same structure and the same genes are found at the same positions (loci)

Why does the chromosome appear as a double arm structure?

- Semi-conservative DNA replication occurs during S phase of interphase, producing two identical DNA molecules
- which coil and condense during prophase of mitosis to form a chromosome consisting of two identical sister chromatids held together at the centromere
- Thus the chromosome appears as a double arm structure.

Centrioles:

1. 2 pairs of centrioles move to the opposite poles of the cell and determine the polarity of the cell
2. Act as the microtubule-organising centre (MTOC) – the centrioles produce spindle fibres radiating from the centrioles at the poles towards the equator of the cell
3. Centrioles organise the synthesis of spindle fibres which lead to the separation of chromatids during cell division

Spindle fibres:

1. Guide movement of chromosomes during nuclear division
    - Align chromosomes at metaphase plate
    - Move chromatids/chromosomes to opposite poles of the cell during anaphase
2. Ensure each daughter cell receives an exact number of chromosomes during cell division

Kinetochore spindle fibres:

1. Fibres attach to kinetochore of the centromere of the chromosome and pull the sister chromatids to the opposite poles of the cell during anaphase

Non-kinetochore spindle fibres (extend from pole to pole)

1. Push the two spindle poles apart, resulting in cell elongation and pushes the two poles further apart
2. Aids ultimately in cytokinesis

Interphase:

Phase	Events
G1	- Period of high metabolic activity resulting from cell growth - Intensive cellular synthesis resulting in cellular growth. Cell increases in size. New proteins (eg histones) are synthesised. tRNA, mRNA, rRNA produced. All cytoplasmic cell organelles (eg mito, ribo)
S	- Semi-conservative DNA replication occurs. DNA content is doubled. 2 sister chromatids are held together at the centromere
G2	- Cell continues to grow and undergo intensive cellular synthesis. - Pair of centrioles replicate and mitotic spindle of microtubules is formed

Mitosis:

- Process by which a cell nucleus divides to produce two genetically identical daughter nuclei containing the same chromosome number and the same genetic makeup as the parent cell

Prophase

- Chromatin condense and become distinct structures called chromosomes
- The two pairs of centrioles move to opposite poles of the cell and determine the polarity of the cell and determine the polarity of the cell
- Short microtubules radiate from the centrioles, forming spindle fibres (kinetochore microtubules attach to centromere, non-kinetochore microtubules just extend)
- Nucleolus disappears
- Nuclear envelope disintegrates and fragments into nuclear membrane vesicles

Metaphase

- 2 pairs of centrioles are positioned at opposite poles of the cell
- Kinetochore spindle fibres pull on centromeres, arranging the chromosomes in a single row on the metaphase plate
- Tension created by the spindle fibres from opposite poles pulling on the chromosomes may actually temporarily shorten the distance between the poles of the cell

Anaphase

- Spindle fibres attached to the centromeres shorten and contract, pulling the sister chromatids to opposite poles of the cell
- Centromeres divide and sister chromatids separate at the centromere
- Each sister chromatid becomes a daughter chromosome

Telophase

- Daughter chromosomes pulled by spindle fibres attached to centromere reach opposite poles of the cell
- Chromosomes uncoil, lengthen and become indistinct to form chromatin again
- Spindle fibres disintegrate
- Nuclear membrane vesicles bind to the chromosomes and fuse together. Nuclear envelope reforms around the chromatin in each daughter cell
- Nucleolus reappears in each daughter cell

Cytokinesis

Animals

- Rings of microfilaments assemble at the metaphase plate of the cell beneath the CSM
- Contraction of filaments causes the invagination of the CSM and leads to the cleavage furrow forming across the metaphase plate of the cell
- Continual contractions ultimately draws the furrow inwards until the cell membranes in that region join up, fuse together to form 2 separate daughter cells

Plants

- Due to the rigid cell wall, the cell cannot constrict
- Growth of a cell plate during telophase across the metaphase plate of the parent cell from inside out
- Plate formed by fusion of carbohydrate-filled vesicles produced by Golgi body
- As more vesicles are added, cell plate grows until it stretches across the cell and its membranes fuse with the cell membrane
- Contents of the vesicles contribute to the new middle lamella, resulting in the formation of 2 daughter cells

Plant cell	Animal cell
Centrioles absent	Centrioles present
No asters form	Asters form
Occurs mainly at shoot and root meristems	Occurs in tissues throughout body
Involves formation of cell plate across the metaphase plate of parent cell	No cell plate forms
No furrowing and cleavage of cytoplasm at cytokinesis	Involves furrowing and cleavage of cytoplasm at cytokinesis

Explain the **significance of the mitotic cell cycle** in animal tissues.

1. Mitosis produces genetically identical cells and thus enables a multicellular organism to grow and develop from one single cell.
2. Mitosis produces genetically identical cells to replace damaged and worn out cells, allowing renewal and repair of tissues.
3. Mitosis produces genetically identical cells for asexual reproduction so that the species can colonise a particular stable and well-adapted habitat within the shortest period of time
4. Mitosis produces genetically identical cells thus contributing to genetic stability within populations of cells derived from the same parental cell.

How does mitosis **contribute to genetic stability**

1. Semi-conservative DNA replication occurs during S phase of interphase to form sister chromatids which are genetically identical to parent DNA
2. During metaphase, each chromosome is arranged on the metaphase plate such that each sister chromatid of a chromosome faces the opposite pole of the cell
3. Separation of sister chromatids during anaphase leads to formation of genetically identical daughter chromosomes → ensures an even distribution of daughter chromosomes into daughter nuclei → forming two genetically identical nuclei in daughter cells
4. There is no crossing over between non-sister chromatids of homologous chromosomes and thus there is no genetic recombination

Explain the **need for the tight control** of the mitotic cell cycle.

1. The mitotic cell cycle is controlled by 3 checkpoints - G1 checkpoint, G2 checkpoint and M checkpoint, which determine whether or not the cell cycle can proceed
2. This ensures that DNA replication is accurate and bring about DNA repair mechanisms if required. Requirements of each checkpoint must be met before cells move on to next stage
3. This can prevent dysregulation of checkpoints of cell division, prevent excessive cell cycle progression (rapid cell cycle) and uncontrolled cell division which can lead to tumour formation and possibly cancer

Cell signalling and communication

Outline the main stages of cell signalling

Ligand-receptor interaction

1. Ligand recognises and binds to the specific binding site of the receptor on CSM or within the target cell
2. Ligands are complementary in shape to the specific binding site of the specific receptor protein

Signal transduction

3. Binding of the ligands induces the receptor to undergo conformational change
4. Relay molecules and various proteins amplify and transduce the signal into a specific cellular response
5. Signals could be transmitted through second messengers such as cyclic AMP, or phosphorylation cascades via protein kinases
6. Signal transduction occurs in multiple steps known as the signal transduction pathway. Presence of multiple steps allows amplification of the signal, where the number of activated molecules increases with each subsequent step

Cellular response

7. The transduced signal triggers a specific cellular response, which may be a change in gene expression / catalysis by an enzyme / rearrangement of the cytoskeleton

G-protein linked receptor str and function

1. GPLR is made up of a single polypeptide chain with 7 transmembrane α-helices , allowing it to be embedded in and span the CSM, allowing signals detected outside the cell to be transduced inside the cell
2. Transmembrane region of the α-helices composed mainly of non-polar amino acids. This allows hydrophobic interactions to form between the amino acids and the hydrophobic core of the CSM
3. GPLR has an extracellular region which may be glycosylated to serve as a specific binding site for ligand
4. GPLR has an intracellular region to serve as a specific binding site for G-protein
5. The specific binding sites composed mainly of charged/polar amino acids, allowing hydrogen bonds / ionic bonds to form between the binding sites and the ligands and G-proteins

General GPLR action

1. A ligand R&B to the specific binding site of the GPLR on the target CSM and induces a conformational change in the receptor
2. The receptor now binds to G protein and activates it
3. A molecule of GTP replaces the GDP on the G protein
4. Activated G protein dissociates from the receptor and binds to and activates enzymes
5. Activated enzymes triggers the next step in the pathway, leading to a cellular response

6. When the ligand is no longer present, the G protein system returns to its inactive form when the GTP attached to the G protein is hydrolysed by its own GTPase to GDP. inactive G protein dissociates from the enzyme and becomes available for reuse

Enzyme linked receptors (tyrosine kinase receptors)

- Two individual polypeptides, each with:
    1. An extracellular ligand binding site
    2. An α-helice spanning the CSM
    3. An intracellular tail containing multiple tyrosines

General RTK action

- When ligand R&B to the pacific binding site of the receptor on the target CSM, the two receptor polypeptides change conformation and dimerise
- Dimerisation activates the tyrosine kinase region of each polypeptide with each tyrosine kinase phosphorylating the tyrosines of the other polypeptide
- Activated tyrosine kinase receptor is recognised by specific relay proteins in the cell. Inactive relay proteins R&B to specific phosphorylated tyrosines, change shape and become activated
- Each activated relay protein can trigger a signal transduction pathway, leading to a specific cellular response. Thus tyrosine kinase receptors can trigger multiple signal transduction pathways at once

Signal transduction

- Second messengers (eg cAMP)
  - small, hydrophilic molecules, usually non-protein
  - cAMP binds to and activates another protein, protein kinase A
  - Activated protein kinase A triggers downstream signalling events, which involves sequential phosphorylation of various other proteins, leading to cellular response
- Phosphorylation cascade
  - carried out by protein kinases
  - proteins undergo sequential phosphorylation, and the addition of phosphates causes proteins to activate, which in turn activates other proteins, triggering numerous reactions in the cell at once

Kinase (activates):

- An enzymes that catalyses the transfer of phosphate groups from phosphate donating molecules to specific substrates in a process known as phosphorylation
- Activated protein kinase (A) triggers downstream signaling events which involves the sequential phosphorylation of various other proteins / signal is transmitted through a phosphorylation cascade
- The number of protein molecules being phosphorylated increases with each level of the cascade Relay molecules and various proteins amplify and transduce the signal into a specific cellular response

Phosphatase (deactivates):

- Catalyse the removal of phosphate groups from the proteins by hydrolysis, reversing the action of protein kinases

Signal amplification

- Small number of ligands produce large cellular response
- Number of activated molecules at each consecutive step of the transduction pathway increases at each level of the phosphorylation cascade

Glucagon, GPLR

1. Glucagon R&B to the specific binding site of GPLR on the target CSM and induces a conformational change in the receptor
2. The receptor now binds to G protein and activates it. A molecule of GTP replaces the GDP on the G protein. The activated G-protein dissociates from the receptor and activates inactive adenyl cyclase to active adenyl cyclase
3. Adenyl cyclase catalyses the conversion of ATP to cAMP
4. cAMP acts as a second messenger and triggers downstream signalling events such that glycogen phosphorylase is activated
5. Glycogen phosphorylase will catalyse the breakdown of glycogen to glucose (glycogenolysis)
6. so that blood glucose concentration increases and returns back to normal levels

Glucagon also stimulates:

- an increase in the rate of conversion of amino acids and glycerol to glucose (gluconeogenesis)

Insulin, RTK

1. Insulin recognises and binds to the specific binding site of RTK on the target CSM, causing the 2 receptor polypeptides to undergo a conformational change and dimerise
2. Dimerisation activates the tyrosine kinase region of each polypeptide. Each tyrosine kinase phosphorylates the tyrosines of the other polypeptide
3. Activated RTK is recognised by specific relay proteins which pass the signal on and lead to the activation of glycogen synthase
4. Glycogen synthase catalyses the conversion of glucose to glycogen (glycogenesis)
5. For storage in liver and muscle cells so that blood glucose concentration decreases and returns back to normal levels

Insulin also stimulates:

- an increase in the rate of respiration using glucose as a respiratory substrate, which will be broken down and oxidised to form CO_2 and water
- an increase in the permeability of the CSM of target cells to glucose, to increase the rate of uptake of glucose from the blood by almost all body cells (especially muscle cells)
- Conversion of excess glucose to fats for storage in adipose cells/tissues

Advantages of cell signalling systems

1. A ligand reaching the cell membrane can activate genes in the nucleus without the need to move into the nucleus
2. A single ligand can trigger a large cellular response, resulting in signal amplification
3. Provide different points at which a cell's response can be regulated
4. Specificity of cellular responses due to the collection of receptor proteins, relay proteins and enzymes
5. Many cells can be activated simultaneously, as long as the cells have the specific receptors for the ligand to recognise and bind to

Infectious diseases

Feature	Innate immune system	Adaptive immune system
Specificity to pathogen	Non specific	High specificity to pathogen
Immunological memory	absent	Possession
Clonal expansion	absent	Upon activation

Adaptive immune response

Primary	Secondary
Result of primary contact with Ag	Result of second and subsequent exposure to same Ag
Responding cell is naive B and T cell	Responding cell is memory cell
Longer lag phase (4-7 days)	Shorter lag phase (3-5 days)
Ab peak in 7-10 days	Ab peak in 3-5 days
Longer time to establish immunity	Shorter time to establish immunity
Ab level declines rapidly	Ab level remains high for longer period
Ab lower affinity for Ag	Ab higher affinity for Ag

Feature	Artificially acquired active immunity	Naturally acquired passive immunity
Intention of introducing Ag/Ab	Deliberate introduction (vaccine)	non-deliberate introduction from mother in breast milk/across placenta
source	vaccine/foreign Ag in injection	Ab pass on
production of Ab and memory cell	Ab and memory cell produced	Ab and memory cell not produced
Durability	Long lasting	Short lived
Lead time for protection	Protection not immediate	Protection immediate

Innate immune system
Features:

- **Non-specificity**
  Different types of cells participate in innate immunity, receptors by which they recognise pathogens are limited in their specificity. Each cell can recognise and respond to many different types of pathogens. Many cells can respond to the same pathogen too.

- **No immunological memory**
  reacts in a similar manner upon repeated exposures
- **No clonal expansion**

Physical barriers
- Epithelial surfaces

Chemical barriers
- Kill pathogens or weaken its effects
- Mucus, tears, saliva trap pathogens
- Acidic pH of stomach mucus prevents growth of microorganisms
- Antimicrobial enzymes → lysozyme (found in mucus, tears and saliva) cleaves glycosidic bonds of peptidoglycan → bacterial cell lysis
- Antimicrobial peptides → defensins act within minutes to disrupt the cell membranes of bacteria, fungi, and the membrane envelopes of some viruses → involve insertion of hydrophobic region of the peptides into the membrane bilayer & formation of a pore that makes the membrane leaky

Cellular components - phagocytic cells

Inflammation → protective response
- Function: eliminate pathogens by ensuring that immune cells needed are delivered to the right place at the right time (specialised phagocytes e.g. macrophages, neutrophils, dendritic cells, other lymphocytes, Ab)
- Release of histamines and cytokines by mast cells and macrophages respectively → increase blood flow to the site of infection → recruiting specialised phagocytes
  Leakage of fluid & proteins from the blood into the tissue space to produce redness and swelling
- Capillary permeability: dilation of blood vessels (redness), increase temp due to increased blood flow (heat), increased fluid in tissue space (swelling)

Innate immune system:
1. Recognition of pathogen
   - Phagocytic cells are activated when their specific receptors recognise structures unique to the pathogen (e.g. bacterial lipopolysaccharides found in bacterial cell walls) leading to several mechanisms to eliminate the infection

2. Activation of immune cells
   - An infection triggers the secretion of histamine by mast cells
   - Histamine is a signaling molecule → triggers dilation of blood vessels → increased blood flow → recruiting more phagocytic cells (macrophages, neutrophils, dendritic cells) to the site of infection
   - Also increases permeability of blood vessels near site of infection to antimicrobial peptides

- Macrophages secrete other signaling proteins (cytokines) → dilate blood vessels, increase blood flow → further recruit and increase the movement of more phagocytic cells to the site of infection
- Binding of pathogens to receptors embedded on CSM of phagocytic cells activates the phagocytic cells. If successful, the pathogen will be eliminated at this stage.
- Macrophages and dendritic cells also act as APCs to present Ag from peptide fragments of pathogen proteins to B and T cells to activate the adaptive immune response → further resolve the infection (pathogens may outnumber macrophages, not all pathogens are ingested and eliminated)

Antigen Ag
- toxin or any foreign substance (carbohydrate, lipid, nucleic acid, protein) that is recognised by the host immune system which induces an immune response, stimulating production of Ab
- Epitopes: parts on a single Ag that bind to the specific Ag-binding site of an Ab or TcR

APC
- Role: Link innate immune system to adaptive immune system when Ag from pathogens are presented by APCs to B and T cells to activate adaptive immune response

Ag presentation
1. The binding of pathogen to receptor on CSM of the phagocyte activates the phagocyte
2. The phagocyte engulf the pathogen by phagocytosis and form phagosome containing the pathogen
3. Fusion of phagosome containing pathogen with lysosome
4. digestion with lysosomal/hydrolytic enzymes
5. Ag from surface of the pathogen are digested and processed into short peptides
6. Peptides bind to MHC proteins to form MHC-Ag complexes which are transported to the CSM of APC via vesicles
7. Vacuole containing undigested substances moves to the CSM and undigested substances are released out of the APC via exocytosis
8. MHC-Ag complex on the membrane of APC are ready for presentation to B cells and T cells to activate the adaptive immune response

Adaptive immune system
Features:
- **High specificity** → each specific Ag receptor (on B and T cell) or Ag binding site (on Ab) binds to a specific Ag through 3D complementarity
- **Possession of immunological memory** → responds faster to the same pathogen that was previously encountered
  Advantage → optimises ability of the immune system to combat persistent and recurrent infections. Each encounter with a pathogen generates more memory cells and activates previously generated memory cells

- **Clonal expansion upon activation** → a lymphocyte (B or T cell) will be stimulated to undergo clonal expansion to produce large numbers of genetically identical daughter cells → undergo differentiation to form effector cells sharing same Ag specificity

Development of B and T cells

T cells
- CD4+ T cells develop into helper T cells (Th) → produce cytokines that activate B cells and macrophages
- CD8+ cells develop into cytotoxic T cells (Tc) → kill cells infected with viruses and other pathogens

Role of T cells
- APCs display Ag fragments bound to MHCs to TcRs on naive T cells. Naive T cells whose Tcrs can R&B to the MHC-Ag complexes are activated (clonal selection)
- Activated T cells secrete cytokines and undergo clonal expansion and differentiation to develop into effector T cells (Th, Tc) and memory T cells

Helper T cells secrete cytokines which:
1. Activates B cells to undergo clonal expansion and differentiation into plasma cells and memory B cells
2. Stimulate CD8+ T cells to undergo clonal expansion and differentiation into Tc cells
3. Promote recruitment of more monocytes, macrophages and Th cells from blood to area of damage

Cytotoxic T cells
1. Kill virally infected cells
    - Secrete proteins that kill and infected cell by
    - Perforins: forming pores in the CSM
    - Granzymes: breaking down the cell contents and the virus it carries
    - Eventually lysing the cell

Memory T cells
1. Persist long after an infection has resolved. They quickly divide by mitosis, proliferate and undergo differentiation to develop into large numbers of effector T cells upon re-exposure to the same Ag, providing the system with immunological memory against past infections
- Increased number of T cells increases chances of coming across APCs
- Hence, there is a faster and stronger response for the second and subsequent exposure to the same infectious agent → high efficient clearance of the infectious agents

Role of B cells
- First activation signal occurs upon BcR R&B to Ag (antigen stimulation)

- Once the naive B cell is activated, BcR and bound Ag are endocytosed into the B cell. Ag is processed into short peptides and attached to MHC proteins to form MHC-Ag complexes
- MHC-Ag complexes are transported to the CSM → B cell now acts as APC to Th cells
- TcR on Th cells R&B to MHC-Ag complex on B cell CSM
- Th cell provides a second activation signal to B cell
  Th secrete cytokines which activates B cell to undergo clonal expansion and differentiation into plasma cells and memory B cells.

Plasma cells
- secrete Ab for Ab mediated response

Memory B cells
- Persist long after an infection has resolved. They quickly divide by mitosis, proliferate and undergo differentiation to develop into large numbers of plasma cells upon re-exposure to the same Ag, providing the system with immunological memory against past infections
- Memory cells can be rapidly reactivated → secondary response much larger and more rapid, usually leading to high efficient clearance of the infectious agent

Antibodies
- Globular proteins
- Soluble form secreted from plasma cells
- Membrane bound form attached to surface of B cell → referred to as BcR

Str and function of IgG
- General: globular protein with quaternary structure held together by intermolecular H bonds, ionic bonds, disulfide bonds and hydrophobic interactions between R groups of amino acids
- Consists of 4 polypeptide chains (2 identical heavy chains and 2 identical light chains
- Each light chain pairs with a heavy chain, held tgt by disulfide bonds

1. Fab contains specific Ag-binding site to bind to specific Ag (of pathogens) to prevent pathogen from attaching to host cell receptor (neutralisation of pathogen)
   OR
   Ag-binding site bind to specific Ag to prevent pathogen from attaching to host cell receptor (neutralisation of pathogen)
2. Fc region binds to the Fc receptor on phagocytes/macrophages and activate the phagocytes/macrophages so that phagocytosis of the pathogen occurs and the pathogen is ingested and killed (opsonisation)
3. Constant region determines the different class of Ab and the different biological function, thus determining the mechanism used to destroy antigens
   OR
   determines if the Ab is secreted or membrane bound

4. Each antibody has 2 identical Ag-binding sites, allowing it to bind to 2 of the same Ag and concentrates the Ag together (agglutination) causing a reduction in the number of infectious units to be dealt with
5. hinge region allows the independent movement of/confers flexibility to the 2 Ag binding sites, allowing both Ag binding sites to bind to Ag that are spaced at variable distances apart
6. disulfide bonds holds each light chain with a heavy chain and holds the two heavy chains together to maintain the Ab as a globular protein with a quaternary structure

Roles of Ab

1. Neutralisation of pathogens or toxins
    - Ab R&B to Ag on pathogens and block penetration of pathogen through epithelial barrier or
    - Attachment of pathogens to specific host cell receptors, preventing infection of the host cell, thus neutralising the pathogens
2. Opsonisation to facilitate phagocytosis
    - Process of R&B of Ab to Ag on the pathogen to mark the pathogen for uptake by phagocytic cells
    - Ag binding site at Fab region of Ab R&B to specific Ag followed by binding of Fc region of Ab to Fc receptor on phagocyte to activate the phagocytic cell
    - Phagocytosis of pathogen occurs and pathogen in ingested and killed

Generation of diverse range of Ab

- Genes coding for immunoglobulin are found at 3 gene loci and each locus is made up of multiple gene segments
- Heavy chain (VDJ gene segments and C segments)
- Light chain (VJ gene segments and C segments)
- Light chain: Kappa (chromosome 2) or lambda chain (chromosome 22)

Somatic recombination (before clonal selection)

- form of DNA rearrangement where various gene segments are joined together randomly, and some intervening segments are enzymatically removed followed by rejoining of remaining sequences. SOmatic recombination occurs prior to Ag contact during B cell development in the bone marrow
- Multiple copies of the variable (V), diversity (D), and joining (J) segments (within each gene locus) can be joined together randomly in different combinations to form novel amino acid sequences in the antigen-binding regions of the antibodies. This results in the highly diverse repertoire of antibodies found on B cells (BcR).
- Somatic recombination occurs prior to antigen contact in the bone marrow → production of naive B cells which expresses BcR that are specific to a particular Ag

Somatic hypermutation (during clonal expansion)

- Somatic hypermutation is a random point mutation in the rearranged VDJ/VJ regions in activated B cells.

- occurs only in Th cell-activated B cells (takes place outside the bone marrow)
- As a result, each of these B cells with a mutation will acquire slight amino acid difference in the variable regions of the Ig chains, possessing slightly different BcRs and varying specificity and affinity for the particular Ag, resulting in further diversification of this Ab
- If the altered Ag-binding sites bind Ag better than the original (higher affinity), the B cells will then be selected to differentiate into plasma cells and memory B cells
- With repeated exposures to the same Ag, activated B cells will produce Ab of successively greater affinities to the antigens (affinity maturation), leading to faster eradication of pathogens
- Secondary response can elicit Ab with several fold greater affinity to the same Ag than in a primary response

Class switching

- Form of DNA rearrangement at the constant gene segment of the heavy chain locus in Th cell-activated B cells.
  (Portions of the Ab heavy chain locus are removed from the chromosome. Gene segments surrounding the deleted portions are rejoined to retain a functional Ab gene that produces Ab of a different isotype)
- Fc region of the Ab heavy chain is changed, but the variable region (Fab) of the heavy chain remains unchanged
- Occurs only on Th cell-activated B cells (2 activation signals)
- Changes a B cell's production of antibody from one isotype to another
  (IgG, IgM, IgA, IgD and IgE. Different classes of Ab contain different constant region of the Ab heavy chain)
- Since the variable region does not change, class switching does not affect antigen specificity
- Ab retains Ag-binding specificity and affinity for the same Ag, but can **interact with different effector molecules, increasing functional diversity**

Vaccination

- Vaccination is a process whereby vaccine is administered to an individual to stimulate a long lasting, active immunity against diseases
- Stimulates immunity without causing the disease, generate memory B and T cells, providing faster and stronger secondary immune response
- Attenuated (less virulent pathogen or dead pathogen)
- Non disease causing pathogen containing same AG found on disease causing pathogen
- Subunit vaccine: fragments of pathogen

Smallpox

- Cowpox virus is non virulent to humans, but have the same Ag as smallpox virus → vaccinated individuals produce memory B and T cells with receptors with Ag binding sites complementary to Ag on smallpox virus
- Vaccination of high enough proportion of the population can break the disease transmission cycle

Factors contributing to success

1. Virus did not mutate frequently
    - The same vaccine can be used for the whole programme and did not need to be changed
2. Cowpox virus are harmless
    - This allows the use of a 'live' virus vaccine which results in a stronger immune response
3. Development of vaccine technology
    - Vaccine is heat stable and suitable for hot countries / isolated areas / rural areas.
    - Ease of administration → 1 dose enough to give life-long immunity, no boosters
    - Cheap and easy to produce
4. Easy identification of infected individuals
    - Few/no symptomless carriers and no animal reservoir required as the virus infects humans only → infected people are easy to identify and isolated to prevent the spread of the disease.
5. Mass vaccination
    - Mandatory for each country to run their own mass vaccination campaigns and eradicate the disease within their own borders. When a high enough proportion of individuals gets vaccinated (95%), it confers herd immunity to unvaccinated individuals. The disease gets contained and unvaccinated individuals (those not eligible for vaccination e.g. pregnant women) obtains a certain degree of protection
6. Vaccination of a high enough proportion of the population can thus break the disease transmission cycle

Benefits of vaccination

1. Protect the individual against disease by conferring immunity to the individual without prior exposure to a specific pathogen
2. Upon encounter with the pathogen, memory B and T cells can rapidly divide and differentiate to form effector B and T cells to provide secondary immune response against that pathogen
3. Confer lifelong immunity to the individual because memory B and T cells are long-lived and contribute to immunological memory to the vaccinated individual
4. Confer herd immunity to unvaccinated individuals in a community e.g. pregnant women and immunocompromised people
5. When infected, the immunised individual will not spread the pathogen as readily to another individual as compared to a susceptible individual
6. Herd immunity reduces possibility of transmission between individuals and thus, unvaccinated individuals have a very low risk of becoming infected
7. Contribute to the eradication of infectious diseases within human population if pathogen relies only on human as host
8. If herd immunity has been established and maintained in a population for a sufficient period of time, there will be no disease transmissions within that population. If this is

done to the whole human population within an endemic region, the virus can be eradicated when every individual is immune

Risks

1. Adverse reactions after vaccination have been observed in small numbers of individuals e.g. life-threatening allergic reaction, fainting and rashes etc
2. Some individuals are more susceptible to vaccination risks than others e.g. individuals with weakened immune system
3. For live attenuated vaccines, pathogens used are modified to be less virulent. However, there is a possibility of the pathogen regaining its virulence and causing disease
4. Some pathogens mutate very quickly. A new vaccine is needed every year

Name of pathogen	Type of pathogen	Name of disease
Influenza virus	virus	influenza
Human immunodeficiency virus (HIV)	virus	Acquired immunodeficiency syndrome (AIDS)
Mycobacterium tuberculosis	bacterium	tuberculosis

HIV and AIDS

- HIV targets cells with CD4 proteins on their CSM (Th cells, macrophages, dendritic cells)
- When HIV infect Th cells, some viral DNA integrate into chromosome of Th cells (provirus) and remain dormant, evading the adaptive immune system
- Over a long period of time, untreated HIV will lead to the replication of new virus that triggers the destruction of Th cells. Excessive budding of new virions from Th cells result in disruption of CSM permeability and function.
- When the Th cell count declines below a critical level in an affected person, cell mediated immunity is lost and the adaptive immune system becomes impaired.
- The immune system thus becomes so weak that one becomes susceptible to opportunistic infections (e.g. TB and pneumonia)
- AIDS occurs when the opportunistic infections become uncontrollable when associated with immunodeficiency caused by HIV (Th cells falls below 200 cells/cubic mm)

Influenza virus and influenza

- Affects the epithelial cells lining the respiratory tract
- Inhaled, infects and damages the epithelial cells → excessive budding of newly synthesised virions depletes CSM of epithelial cells, causing the epithelial lining of the upper and lower respiratory tract to be lost
- In response, these cells produce excessive mucus and secrete cytokines which cause inflammation to activate the immune system → typical flu symptoms
- If the influenza virus gets into the lower respiratory tract, it can lead to more serious complications such as viral pneumonia, which is severe inflammation of the lungs

Mycobacterium tuberculosis causes TB

Target organ:

- Lungs causing pulmonary tuberculosis

Mode of transmission:

- Transmitted from person to person by fine, aerosol droplets, in which bacterial cells are carried within, when an infected person with the active/infectious TB disease sneezes or coughs. The aerosol droplets are inhaled by an uninfected person

Mode of infection:

- Once inside the lungs, alveolar macrophages phagocytose the bacteria and form phagosomes containing M. tuberculosis
- Inside the phagosome, M. tuberculosis inhibits the fusion of the phagosome with lysosomes. No phagolysosome is formed and no lysosomal enzymes are available to kill the bacteria
- M. tuberculosis survives and continues to multiply inside the macrophages.
- A tubercle is formed which consists of macrophages in a tight ball-like formation. At the center of the tubercle, cell death by necrosis occurs resulting in the rupture of CSM of macrophage and release of cell contents to the surroundings. At this stage, the tubercle remains intact
- The disease may be arrested at this stage and remains latent for several years. People with this latent infection do not spread the disease to others

Active TB

- The tubercle ruptures and releases M. tuberculosis into the bronchiole and infection spreads throughout the lungs. This results in development of a productive cough that facilitates aerosol spread of infectious bacteria
- The lungs will be progressively destroyed by the formation of cavities due to the rupturing of tubercles.

Treatment:

- Antibiotic treatment, antibiotics are required to kill/inhibit bacterial growth and replication, given at least 2 different types to minimize risk of bacteria developing resistance thus ensuring that all the bacterial cells are killed

Mode of action of antibiotics

- Bactericidal (kill bacteria)
- Bacteriostatic (inhibit growth and replication)
- Do not eradicate bacteria, but serves to limit growth/infectection of bacteria to a level where the patient's immune system can inactivate/eliminate the pathogen
- Prevent deaths caused by bacterial infections and the transmission of disease
- Antibiotics are effective towards bacteria because bacteria usually build up antibiotics within their cytoplasm

Cell wall synthesis

1. Penicillin inhibits the synthesis of peptidoglycan, a component of the bacterial cell wall essential for maintaining the structural integrity of the bacterial cell

2. Penicillin inhibits the enzyme transpeptidase and prevents the formation of cross-links between adjacent chains of peptidoglycans
3. The individual peptidoglycan chains remained separated as cross-links cannot be formed
4. Bacterial cell walls of dividing bacterial cells are weakened and osmotic lysis occurs due to high osmotic pressure within the bacterial cells

Protein synthesis (translation)

1. Streptomycin binds to the small ribosomal subunit (30S) of the bacterial ribosome so that initiator tRNA cannot bind to the small ribosomal subunit
2. An unstable mRNA-ribosome complex is formed such that N-Formylmethionine-tRNA (fMet-tRNA) cannot bind to the small ribosomal subunit
3. This leads to codon misreading and eventual inhibition of protein synthesis

1. Tetracycline inhibits protein synthesis by blocking the entry of aminoacyl-tRNA to the A site of bacterial ribosome ;

Nucleic acid synthesis

1. Rifampin inhibits RNA synthesis by binding to bacterial RNA polymerase thus preventing transcription
2. Quinolones inhibit bacterial DNA gyrase or topoisomerase, thereby inhibiting DNA replication and transcription

Stem Cells

Applications of stem cells

1. Lead to the discovery of new medical treatments that would alleviate the suffering of patients suffering from presently incurable diseases
2. Regenerate damaged tissues, making them valuable in treating diseases and injuries such as heart disease
3. May be used to grow new organs, reducing the dependency on organ donors and the risks of transplant rejection
4. Mimic human tissues for testing new drugs, reducing reliance on animal testing and improving drug safety

- Stem cells used have to be treated with chemicals to stimulate proliferation as they are typically inactive and require molecular signals to effect cell proliferation in vitro, such as growth factors

Unique features of stem cells

1. Can remain undifferentiated and unspecialised for a long time
2. Capable of dividing indefinitely and self-renewal by mitosis
    - Have activated telomerase genes and can produce high levels of telomerase → lengthening of telomeres, will not reach critically short length → no apoptosis triggered
    - Symmetrical division: 2 identical daughter stem cells, same developmental and differentiation potential. This maintains a pool of stem cells for further cell division
    - Asymmetrical division: produce one daughter stem cell that is identical to parent stem cell and one progenitor cell. Progenitor cell can differentiate into more specialised cells whose differentiation potential is more restricted than the parent cell
3. Can undergo differentiation, giving rise to specialised cell types upon receiving appropriate molecular signals (eg hormones, growth factors)
    - Differential gene expression

Developmental potential

- Cell potency: totipotent, pluripotent, multipotent, unipotent

a) Zygotic stem cells
    - Totipotent: can differentiate into all cell types that make up an entire organism including extra-embryonic tissue (e.g. placenta)

b) Embryonic stem cells
    - Pluripotent: can differentiate into any cell type that make up an organism except the extra-embryonic tissue such as the placenta
    - Inner cell mass in blastocysts gives rise to embryonic stem cells
    - Functions:
      > ability to differentiate into any cell type except extra-embryonic membranes for the formation of a foetus/adult organism

> Inner cell mass can give rise to the derivatives of the three germ layers, endoderm, mesoderm and ectoderm, which eventually form all the highly specialised cells needed to produce an adult organism

c) Blood stem cells (haematopoietic stem cells)
   - Multipotent: can differentiate into a limited and related range of stem cells and tissues in an organism
   - Functions:
     > undergo differentiation to form the different types of blood cells to maintain the specific tissues where they reside by replacing worn-out or damages rbcs and wbcs

Contrasting feature	Myeloid stem cells	Lymphoid stem cells
Type of immunity	Differentiate to form cells which are mainly involved in innate immunity	Differentiate to form cells which are mainly involved in adaptive immunity
Role	form cells which generate inflammatory responses/act as phagocytes	form cells involved in cell-mediated immunity/ Ab production / providing rapid response against virally-infected cells
Effector cells	Macrophage, neutrophil, rbc, platelets	T lymphocytes, B lymphocytes, Natural killer cells

Embryonic stem cells vs bscs

Contrasting feature	embryonic	bsc
potency	pluripotent	multipotent
role	differentiate into the three germ layers to give rise to cell types required for the formation of a foetus / adult organism	differentiate to form the different types of blood cells to maintain the specific tissues where they reside by replacing worn-out or damaged rbcs and wbcs
sources	Unused IVF embryos Embryos created for research from donor eggs and sperms	Bone marrow of adults Umbilical cord blood Placenta Peripheral blood

Induced pluripotent stem cells (iPSCs)
- Adult cells genetically reprogrammed to return to a pluripotent state
- Characteristics of iPSCs:
  1. similar to embryonic stem cells in terms of cell surface markers, morphology, long telomere length, ability to differentiate along a given lineage because embryonic stem cell-specific genes are expressed
  2. can differentiate in vitro into derivatives of all 3 primary germ layers

Ethical implications of application of stem cells in research and medical applications

1. Destruction of embryos to obtain ESCs poses a moral dilemma of destroying a potential human life to save another
2. Creation of embryos for research and as a source of stem cells is too controversial → embryos are treated as a source of spare parts to benefit others
3. Oocyte retrieval is an invasive procedure and the risks of medical complications are high → ovarian hyper-stimulation syndrome, bleeding, infection, death
4. Concerns that female donors could be exploited for their oocytes, especially in poorer countries/countries with fewer legal restrictions
5. Harvesting of embryos for therapeutic purposes goes against many religious and cultural beliefs → some believe that the human race does not have the authority to tamper with nature
6. Issues pertaining to individual informed consent and privacy:
    a) individuals involved in donating oocytes & embryos must be informed about possible risks and potential future uses of the donated embryos
    b) patients involved in stem cell therapies → concerns about privacy and confidentiality of medical information, e.g. whether such information should be released to third parties (insurance companies)
    c) Breaches of confidentiality might subject patients to unwanted attention /harassment from opponents of human ESC research
7. May not be available and affordable for all. High cost of treatment could become a luxury available only to the rich and powerful, with the poor failing to gain access to the benefits of stem cell therapy

How human iPSCs overcome some of the ethical implications

1. Avoidance of the destruction of embryos in order to obtain pluripotent stem cells/ESC as it poses a moral dilemma of destroying a potential human life to save another human life
2. Provides an alternative to the controversial creation of embryos specially for research purposes as pluripotent stem cells should not be treated as a source of spare parts
3. Avoids possible exploitation of female donors for their eggs, especially those in poorer countries if embryos are created for research purposes
4. Bypasses the risks involved in oocyte retrieval as it is an invasive procedure and the medical complications from oocyte donation are high

Cancer

Need for regulation of mitosis:

1. The cell cycle is tightly regulated as it is important for normal cell division and development.
2. The mitotic cell cycle is controlled by three checkpoints - G1 checkpoint, G2 checkpoint and M checkpoint which determine whether or not the cell cycle can proceed.
3. These checkpoints ensure that key processes such as DNA replication is accurate / bring about DNA repair mechanisms if required, before cells move on to the next stage.
4. This can prevent dysregulation of checkpoints of cell division, prevent rapid cell cycle progression and uncontrolled cell division which can lead to tumour formation and possibly cancer.

Differences between normal and cancerous cells

	Normal	Cancer
Contact inhibition	Show contact inhibition. Cells do not divide further when in contact	Do not show contact inhibition. Cells divide even when they make contact with neighbouring cells, resulting in cells growing into a tumour
Anchorage dependence	Exhibit anchorage dependence. For cells to divide, must be attached to a physical surface	Lack of anchorage dependence. Divide even when not attached to a physical surface
Differentiation	Differentiate to become specialised cells	Fail to differentiate
Cell adhesion/metastasis	Exhibit cell adhesion	Lak cell adhesion, can detach and metastasise
Nucleo-cytoplasmic ratio	Low nucleo-cytoplasmic ratio	High nucleo-cytoplasmic ratio

Molecular differences

	Normal cells	Cancer cells
POG	Have POG	Have oncogenes
TSG	Presence of TSG	TSG absent/mutated
Cell division	Controlled cell division	Uncontrolled cell division
Apoptosis	Show apoptosis	Do not show apoptosis
Telomerase activation	Absence of telomerase activity. Cells cannot divide indefinitely	Presence of telomerase activity. Cells can divide indefinitely
Angiogenesis	Do not stimulate new blood vessels	Stimulates growth of new blood vessels within tumours

Ability to differentiate properly	Differentiate to become specialised cells	Fail to differentiate. Cells remain in immature and non-functional state

Tumour:

- Interfere with the activity of the cells and organs that surround them
- Compress tissues, preventing normal blood flow or nerve function
- Invade surrounding tissues, killing normal cells

Development of cancer

1. The development of cancer is a multi-step process which requires the accumulation of mutations in a single cell, angiogenesis and metastasis.
2. Gain in function mutation in (one copy of) a proto-oncogene will result in excessive amounts of / hyperactive / degradation resistant protein products, leading to uncontrolled cell division
3. Loss of function mutation in (both copies of) tumour suppressor genes results in non-functional / missing protein, which will disrupt the ability of the cell to arrest cell cycle / activate DNA repair / trigger apoptosis, leading to uncontrolled cell division
4. Mutation in the genes involved in apoptosis causes cells to evade programmed cell death (even when the DNA of the cells are irreparably damaged).
5. Activation of genes coding for telomerase prevents the progressive shortening of chromosomes / telomeres with each round of DNA replication, thus the cancer cells can divide indefinitely
6. These mutations in the same somatic cell results in the rate of cell division to be greater than the rate of cell death, hence leading to uncontrolled cell division and tumour formation.
7. Angiogenesis, the growth of new blood vessels, occurs within the tumour to transport oxygen and nutrients to support the growth of the tumour.
8. When cells of the tumour invade surrounding tissue and metastasise to other sites, the tumour is considered to be malignant and cancer has developed.

Proto-oncogenes: ras (gain-of function mutation/dominant mutation)

- A proto-oncogene is a class of gene which codes for proteins involved in the regulation of normal cell growth and proliferation/division.
- When proto-oncogenes are mutated, they are referred to as oncogenes.
- Oncogene expression leads to an increase in the:
    1. Amount of proto-oncogene protein
    2. Intrinsic activity of protein such that the protein is hyperactive / more resistant to degradation than the normal protein

Genetic mutations:

1. Point mutation
    (a) Point mutation in POG
        - Base pair substitution within POG, change the a.a. sequence of the POG protein. This changes the POG protein to one that is hyperactive or more degradation resistant than normal protein

(b) Point mutation in control elements (eg enhancer, promoter) of POG
    - Increased rate of transcription, excessive production of POG protein (eg growth hormones)

2. Amplification of proto-oncogene
    - Mistakes made during DNA replication can lead to increase in number of copies of POG located on a selected region of the chromosome in the cell
    - Lead to excessive production of POG proteins that stimulate excessive cell proliferation
3. Chromosomal translocation
    - Translocation of the POG within/across a chromosome occurs
    - Result in POG to be under the control of an enhancer / strong promoter
    - Result in increased rate of transcription of POG and hence excessive production of the POG proteins that stimulate excessive cell division

ras gene → Ras protein (G protein)
Normal role of Ras protein:

- Binding of growth factor to growth factor receptor triggers a series of reactions, resulting in the binding of GTP to inactive Ras protein, activating the Ras protein. Activated Ras protein relays signals to a cascade of protein kinases, leading to cell division. Last kinase activates a transcription factor to turn on genes for proteins that stimulate cell cycle
- Cellular response: synthesis of protein that stimulates cell cycle

Mutation in ras gene:

- Result in constitutively active/hyperactive Ras protein without intrinsic GTPase activity and GTP remains bound to Ras protein
- Constitutively active Ras protein can increase cell division even in the absence of growth factors

Tumour suppressor genes: p53 (TSG: loss of function mutation, recessive mutation)

- Class of genes which code for proteins that inhibit cell division and help to prevent uncontrolled cell growth and cell division
- Protein products activate cell cycle arrest, DNA repair, trigger apoptosis

Normal role of p53 gene

- Codes for a specific transcription factor, p53 protein (activator) that R&B to enhancer to promote synthesis of cell cycle-inhibiting proteins
- When there is DNA damage: p53 gene is activated to produce p53 protein, which R&B to DNA and induces the transcription of several genes, whose protein products are involved in various growth-inhibiting pathways
    (a) Cell cycle arrest
    (b) DNA repair
    (c) Initiate apoptosis when DNA damage is beyond repair

Mutation in p53 gene

- Point mutation, chromosomal translocation or retroviral integration
- Cell cycle and proliferation will not be inhibited in cells with defective p53 proteins

- Cells with damaged DNA proceed through cell cycle without DNA repair
- Cells with irreparable DNA damage do not enter apoptosis
- Cells continue to proliferate with defective genome

	Gain of function mutation	Loss of function mutation
Definition	causes a gene product to be expressed in excessive amt/ hyperactive / resistant to degradation Gene products of POG such as Ras protein	causes a gene product to be non-functional Gene products of TSG such as p53 protein
Nature of mutant allele	Dominant	Recessive
Number of alleles that have to be mutated for effect to take place	Only one copy	Both copies
Effect on cell cycle	Gain of function mutation of POG result in overstimulation of the cell cycle	Loss of function mutation of TSG results in no DNA repair as the cell cycle cannot be arrested. Cells with accumulated mutations keep dividing.

Factors that increase risk

1. Genetic predisposition
    - An individual inheriting an oncogene or mutant allele of TSG will be at higher risk of developing cancer
2. Exposure to chemical carcinogens such as tobacco/tar in cigarettes
    - Chemical carcinogen: agent that causes cancer/accelerate development
    - Damages DNA, causes gene mutation, leads to cancer
3. Exposure to ionising radiation such as X rays, UV radiation
    - Carries energy high enough to cause ionisation
    - Damage DNA and result in development of cancer
4. Age and loss of immunity
    - Ability to repair damaged DNA or destruction of abnormal cells by immune system may become less efficient with age
    - Poor immune system (AIDS)
5. Lifestyle factors
6. Infection by viruses and bacteria
    - Viruses carries oncogenes that may be introduced into the cell
    - Viral DNA inserted into genome, leading to conversion of POG into oncogene/disruption of TSG
    - Virus directs expression of viral proteins that inactivate TSG to render host cell more susceptible to cancer
    - Eg human papilloma virus (cervical cancer), Helicobacter pylori (stomach cancer)

Meiosis

Interphase I
Meiosis I: reduction division
- Synapsis (pairing of homologous chromosomes), followed by separation of homologous chromosomes
- Each daughter cell is haploid in nature

Meiosis II
- Chromatids within each daughter cell is separated, resulting in 4 haploid daughter cells

Prophase I
- Chromatin condenses to form chromosomes made up of two identical sister chromatids held together by the centromere
- Homologous chromosomes pair up to form a bivalent/tetrad during synapsis
- Crossing over may occur between non-sister chromatids of homologous chromosomes
- Sections of chromatids with the same genes break and rejoin, giving new combinations of alleles and forming recombinant chromatids
- Nucleolus disappears. Nuclear envelope disintegrates and fragments into nuclear membrane vesicles
- Spindle fibres form. Spindle fibres from one pole of the cell attach to one chromosome of each bivalent, which spindle fibres from the opposite pole attach to the other homologue at its centromere

Metaphase I
- Chromosomes are arranged along the metaphase plate in their homologous pairs/bivalents
- Independent assortment of homologous chromosomes occurs, whereby the arrangement of chromosomes of each bivalent is completely independent of the orientation of other bivalents

Anaphase I
- Centromeres do not divide
- Homologous chromosomes are pulled towards opposite poles of the cell, when spindle fibres attached to the centromere shorten and contract

Telophase I
- Homologous chromosomes reach opposite poles of the cell and uncoil, lengthen and become indistinct to form chromatin again
- spindle fibres disintegrate
- Nuclear membrane vesicles bind to the chromosomes and fuse together. Nuclear envelope reforms around each set of chromosomes in each daughter cell. Nucleolus reappears

Prophase II

- Chromatin condenses to form chromosomes made up of two (recombinant) chromatids held together by the centromere
- 2 pairs of centrioles move to opposite poles of the cells
- Spindle fibres formed at right angles to the original spindle of meiosis I

Metaphase II

- Spindle fibres pull on centromeres, chromosomes are arranged in a single row on the metaphase plate
- Centromeres are at right angles to the spindle axis
- Independent assortment of chromatids occurs, whereby the arrangement of (recombinant) chromatids of each chromosome is completely independent of the orientation of other chromosomes

Anaphase II

- Spindle fibres attached to the centromeres shorten and contract, pulling daughter chromosomes to opposite poles of the cell
- Centromeres divide and (recombinant) chromatids separate at the centromere
- Each (recombinant) chromatid is now a daughter chromosome and daughter chromosomes are pulled to opposite poles of the cell

Telophase II

- Daughter chromosomes reach opposite poles of the cell and uncoil, lengthen and become indistinct to form chromatin
- Spindle fibres disintegrate
- Nuclear membrane vesicles bind to the chromosomes and fuse together. Nuclear envelope reforms around each set of chromosomes in each daughter cell. Nucleolus reappears
- Each nucleus now possesses half the number of chromosomes as the original parent cell. Each daughter nucleus is haploid

Stage	In a cell		Stage	In a cell	
	No. of chromosomes	Amt of DNA		No. of chromosomes	Amt of DNA
G1	2n	X	Prophase I	n	X
Prophase I	2n	2X	Meta I	n	X
Meta I	2n	2X	Ana I	2n	X
Ana I	2n	2X	Telo I	2n	X
Telo I	2n	2X	Cytokinesis	n	½ X
Cytokinesis	n	X			

Significance of meiotic cell cycle

1. Meiosis allows the maintenance of the constant chromosome number in every generation of a species.
2. Meiosis allows genetic variation in the genotype and phenotype of offspring produced.

Need for reduction division

1. Reduction division in meiosis I allows for **maintenance of the constant chromosome number** in every generation of a species
2. This results in the production of haploid gametes with half the chromosome number as the diploid parent cell
3. During fertilisation, the fusion of haploid gametes restores the diploid chromosome number for each species
4. Crossing over between non-sister chromatids of homologous chromosomes during prophase I of meiosis I / Independent assortment of homologous chromosomes in metaphase I of meiosis I / Independent assortment of recombinant chromatids in metaphase II of meiosis II
5. leads to **new combination of alleles in the gametes formed** and results in genetic variation in the genotype and phenotype of offspring

How does meiosis lead to genetic variation

1. Crossing over between non-sister chromatids of homologous chromosomes during prophase I of meiosis I.
2. Sections of non-sister chromatids with the same genes will break and rejoin to give new combination of alleles in the gametes formed.
3. Independent assortment of homologous chromosomes in metaphase I of meiosis I / independent assortment of recombinant chromatids in metaphase II of meiosis II.
4. Results in gametes with different combinations of maternal & paternal chromosomes leading to new combination of alleles in the gametes formed.

Factors that lead to genetic variation

1. independent assortment of chromosomes (M1 of M1 and M2 of M2)
    - Independent assortment of homologous chromosomes during metaphase I of meiosis I and independent assortment of recombinant chromatids during metaphase II of meiosis II
    - Results in independent assortment of paternal and maternal chromosomes between the nuclei of daughter cells producing new combinations of alleles in gametes
2. Crossing over between non-sister chromatids of homologous chromosomes
    - During prophase I
    - Sections of non-sister chromatids with the same genes will break and rejoin to give new combinations of alleles (on chromosomes of the gametes)
3. Random fertilization of haploid gametes

- Random fertilisation of gametes during sexual reproduction → new combinations of alleles in the zygote & adds to genetic variation in the genotype and phenotype of offspring

Homologous chromosomes

1. Homologous chromosomes are chromosome pairs with the same structure, same length same shape, same centromere position, and the same genes are found at the same gene loci
2. (diploid cells) Each homologue of a homologous pair is derived from the maternal parent while the other homologue is derived from the paternal parent
3. Each homologue of a homologous pair may carry different alleles (for the same gene) at a particular locus
4. At prophase I of meiosis I, homologous chromosomes pair up to form a bivalent during synapsis
5. Each chromosome appears as a double structure made up of two identical sister chromatids held together by the centromere

Meiosis	Mitosis
During prophase I, synapsis occurs - homologous chromosomes pair up (to form bivalents)	No synapsis, homologous chromosomes remain separated / No pairing of homologous chromosomes
During prophase I, crossing over between non-sister chromatids of homologous chromosomes may occur	No crossing over between non-sister chromatids of homologous chromosomes
During metaphase I, homologous chromosomes line up in their homologous pairs on the metaphase plate	During metaphase, single chromosomes line up in a single row on the metaphase plate
During anaphase I, homologous chromosomes separate Centromeres do not divide	During anaphase, sister chromatids separate Centromeres divide
Results in the haploid number of chromosomes in each daughter cell	Maintains the diploid number of chromosomes in each daughter cell
Involves two rounds of nuclear division to give rise to 4 daughter cells	Involves one round of nuclear division to give rise to 2 daughter cells

Chromosomal aberration

- defined as numerical aberration (the change in chromosome number) or structural aberration (alteration to chromosome structure)

(a) **Aneuploidy** is a condition in the nucleus where there are <u>one or several chromosomes more than or less than the diploid number of chromosomes.</u>

- Result from non-disjunction during anaphase I or anaphase II, and when such a gamete carrying n-2, n-1, n+1 or n+2 chromosomes fuses with a normal haploid gamete

(b) **Polyploidy** is a condition of the nucleus where there are three or more times the haploid number of chromosomes, e.g. 3n, 4n and 5n.
    - Result from non-disjunction, the fusion of a diploid gamete with a normal haploid gamete giving a triploid nucleus.

Example of aneuploidy:
- Down's syndrome
- Extra copy of chromosome 21 is found (Trisomy 21, 3 copies of chromosome number 21)
- Desc: mentally disabled / broad head / rounded face, almond-shaped eyes / flattened bridge of the nose / short stature

Structural aberrations
- During meiosis, a chromosome may break into one or more segments, causing changes to the structure of chromosome
- Can be caused by damaging agents such as ionising radiation (UV rays, X Rays)

(a) Translocation
    - section of a chromosome breaks off from the chromosome and becomes attached to another chromosome.

(b) Duplication - addition of genes
    - a section of a chromosome replicates such that a set of gene loci is repeated.

(c) Deletion - loss of genes
    - a chromosome breaking at two points with the middle portion of the chromosome displaced and the two ends joining together.

(d) Inversion
    - a chromosome breaking at two locations and the middle portion flips through 180 deg before rejoining.

Gene mutation
- Defined as a change in the sequence of DNA nucleotides in the gene
- Base pair substitution – replacement of one nucleotide bp with another in a gene, resulting in missense/nonsense mutation/ significant change in the encoded protein/silent mutation on the encoded protein
- Base pair addition – gain of a nucleotide bp in a gene, resulting in frameshift mutation, whereby the reading frame is altered / nucleotides downstream from the mutation are improperly grouped into incorrect codons and read in different sets of threes
- Base pair deletion – loss of a nucleotide bp in a gene, resulting in frameshift mutation, whereby the reading frame is altered / nucleotides downstream from the mutation are improperly grouped into incorrect codons and read in different sets of threes

Inheritance

Definitions:

1. Gene
    - Specific sequence of nucleotides in the DNA which codes for a polypeptide or RNA. each gene occupies a specific gene locus on a chromosome and determines a specific characteristic/trait in the organism
2. Locus
    - position of a gene on a chromosome or within a DNA molecule
3. Allele
    - an alternative form of a gene (responsible for determining the contrasting characteristics of a gene), which occupy the same locus of a pair of homologous chromosomes. May be dominant, recessive or codominant
4. Dominant allele
    - Allele that shows its phenotype even in the presence of an alternative allele
    - (heterozygote) expression of gene product of dominant allele masks the effect of the gene product expressed by the other recessive allele
5. Recessive allele
    - Allele that shows its phenotype only in the presence of another identical recessive allele (homozygous)
6. Codominance
    - 2 different dominant alleles of the same gene are expressed independently of one another and influence the phenotype in the heterozygote
    - Occurs when the heterozygote has a phenotype that is different from the phenotypes of either homozygotes → more than 2 phenotypes
7. Incomplete dominance
    - Form of intermediate inheritance in which 1 allele for a specific trait is not completely expressed over an alternative allele
    - Occurs when heterozygote has a phenotype that is distinct from and intermediate to the phenotypes of the homozygotes
8. Homozygous
    - Diploid condition in which the alleles at a given locus on a pair of homologous chromosomes are identical (homo dom or homo rec)
9. Heterozygous
    - Diploid condition in which the alleles at a given locus on a pair of homologous chromosomes are different
    - Expression of the dominant allele masks the effect of the recessive allele
10. Genotype
    - Combination of alleles situated at corresponding loci on homologous chromosomes
    - Determine a specific phenotype observed
11. Phenotype
    - Observable characteristics/trait of an organism that is due to the expression of its genotype

- May arise from interaction between the genotype and environment

12. Test cross
    - Cross between an individual of unknown genotype to a homozygous recessive individual. Offspring ratios are used to deduce the genotype of the unknown, and/or linkage relationships of its heterozygous loci
13. Linkage (sex-linked or autosomal linkage)
    - 2 or more genes on the same chromosome that do not assort independently in meiosis and are likely inherited together
    - Closer the gene, less likely crossing over will occur

Inheriting genes from one generation to the next via gametes

- Haploid gametes produced for reproduction (half the number of chromosomes as the parent cell, each gamete contains only one copy of each gene)
- Fertilization: gametes fuse to form diploid zygote → diploid cell with 2 sets of chromosomes → 2 copies of each gene, same position on the 2 homologous chromosomes

Linkage of genotype to phenotype:

- Expression of genes give rise to functional products
- Central dogma: DNA via transcription to RNA via translation to protein

Mendel's first law of segregation

- Characteristics of a diploid organism are determined by alleles which occur in pairs. The 2 alleles for each gene segregate during ana1 of meio1 so that each gamete carries only one allele of each gene

Second law of independent assortment

- During gamete formation, 2 alleles of a gene segregate into different gametes independently of the segregation of the 2 alleles of another gene

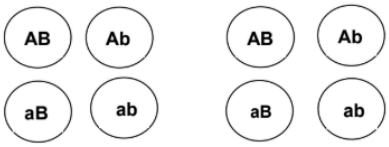
Monohybrid inheritance:
RR x rr → all Rr
Rr x Rr → Rr:rr → 3:1 ratio
Rr x rr → Rr:rr → 1:1

Dihybrid:
AaBb x AaBb →
A_B_ : A_bb : aaB_ : aabb
9 :3 :3 :1
AaBb x aabb →
A_B_ : A_bb : aaB_ : aabb
1 :1 :1 :1

Let A represent the dominant allele for smooth grains.
Let a represent the recessive allele for wrinkled grains.
Let B represent the dominant allele for purple grains.
Let b represent the recessive allele for yellow grains. mp1;

F1 phenotype: smooth, purple grains x smooth, purple grains
F1 genotype: AaBb x AaBb mp2;

Gametes:



mp3;

Fertilisation

	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

F2 genotype : AABB : AAbb : aaBB : aabb
AABb Aabb aaBb
AaBB
AaBb mp4;

F2 phenotype : smooth, purple grains : smooth, yellow grains : wrinkled, purple grains : wrinkled, yellow grains

F2 phenotypic ratio : 9 : 3 : 3 : 1 mp5;

Codominance:
Let W represent the allele for the production of white feathers
Let B represent the allele for the production of black feathers
W and B exhibit codominance

Incomplete dominance
Let R represent the allele for red colour
Let W represent the allele for white colour
R and W exhibit incomplete dominance

	Codominance	Incomplete dominance
Alleles	A, B	A, B
Expected ratio	Hetero x hetero AA : AB : BB 1 : 2 :1	Hetero x hetero AA : AB : BB 1 : 2 :1

Multiple alleles
I^A → type A antigen on CSM of RBC, anti-B antibody in blood
I^B → type B antigen on CSM of RBC, anti-A antibody in blood
i → neither, both anti-A and anti-B antibody in blood
I^A and I^B exhibit codominance
I^A and I^B are dominant to i

Linkage:
Sex linkage:

E.g. haemophilia

Let X^H represent the X chromosome with the dominant allele for normal blood clotting.

Let X^h represent the X chromosome with the recessive allele for haemophilia.

Let Y represent the Y chromosome with neither allele.

Reciprocal cross:
2 separate crosses using the same characteristics but reversed sex

Autosomal linkage (4 phenotypes, no longer 16 parts)

- Closer the gene loci, less likely crossing over will occur, higher the chance that alleles of the 2 genes will be inherited together as one linkage group to form gametes with parental types
- Complete linkage: no crossing over. Linked genes inherited together as one linkage group, resulting in only gametes with parental types and hence, only offspring of parental combinations
- incomplete linkage: crossing over occurs between the 2 linked genes on non-sister chromatids of homologous chromosomes
- Crossing over is a chance event, resulting in higher proportion of gametes carrying parental types and lower proportions of recombinant gametes. This results in majority of offspring produced to be of parental combinations and minority to be of recombinant combinations

How to determine position of genes

- Recombinant frequency/Crossover value can be calculated using the formula:

$$\frac{\text{Number of individuals showing recombination}}{\text{Number of offspring}} \times 100\%$$

- One percent COV is equivalent to one map unit
- Low recombinant frequency/ Crossover value indicates that the genes are close together
- If the expected phenotype ratio of 1:1:1:1 is obtained then there is no linkage

Explain the deviation of observed test cross results from expected

- State expected ratio vs observed ratio
    1. Expected test cross ratio is 1:1:1:1 while observed test cross ratio is ____ which deviates from expected ratio

- Account for gametes carrying parental type (identify 2 characteristics, state the 2 genes are linked, discuss their inheritance, and chances of getting gametes carrying parental type)
    2. The 2 genes for wing colour and shape of wing are linked
    3. And the alleles of the 2 genes will be inherited together as one linkage group
    4. Resulting in a higher proportion of gametes carrying parental types

- Account for recombinant gametes (discuss crossing over, chances of getting recombinant gametes)
    5. Crossing over between the 2 linked genes on non-sister chromatids of homologous chromosomes may occur
    6. As crossing over is a chance event, resulting in a lower proportion of recombinant gametes

- Conclusion on offspring
    7. Observed offspring of test cross are majority of parental combinations and minority of recombinant combinations

Epistasis:
- Less than 4 phenotypes, but phenotypic ratio still adds up to 16 parts
- 2 genes 1 trait

Recessive epistasis (9:3:4)
- Recessive epistatic allele hides the phenotypic effects of alleles of the hypostatic gene when the recessive allele is present in the homozygous condition
- A_B_ : A_bb : aaB_ + aabb
  9 :3 : 3+1=4

Let C represent the dominant epistatic allele for pigment production
Let c represent the recessive epistatic allele for no pigment production
Let A represent the dominant hypostatic allele for agouti fur
Let a represent the recessive hypostatic allele for black fur

C_A_ : C_aa : cc_ _
Agouti : Black : albino
9 :3 :4

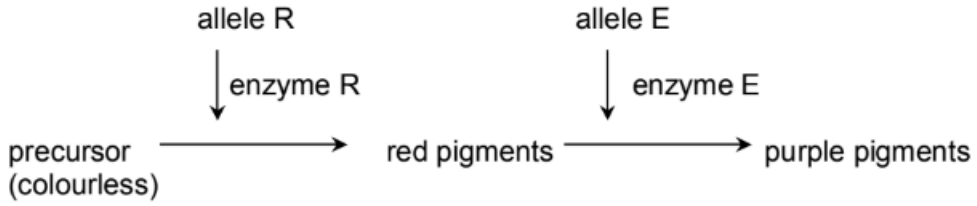
Explain the term epistasis in this context
- Explain the interaction
    1. Fur colour is controlled by 2 genes (gene C/c and gene A/a) occupying different loci
    2. When gene C/c is homozygous recessive at the gene loci, it hides the effect of A/a
- Identify genotypes that led to phenotypes

3. cc is epistatic over the A/a locus, homozygous for the recessive allele at gene C/c results in albino
4. A copy of dominant allele C results in black fur, a copy of dominant allele A results in agouti fur

(d) Explain how different genotypes give rise to a white phenotype. [3]

1. **The grain colour is controlled by two genes (gene R/r and gene E/e) occupying different loci ; (extra point)**
2. **when gene R/r is homozygous recessive at the gene loci, it hides the effect of the gene E/e, rr is epistatic over the E/e locus ;**
3. **homozygous for the recessive allele at gene R/r (rr _ _) results in white grains ;**
4. **Without allele R to code for enzyme R which converts precursor to red pigments, which is then converted to purple pigments by enzyme E coded for by allele E, a white phenotype resulted ;**

For teaching purpose, pathway is not required by question



Dominant epistasis (12:3:1)
A_B_ + A_bb : aaB_ : aabb
9+3=12 : 3 : 4
A locus exhibit dominant epistasis over B locus

1. **The fruit colour is controlled by two genes (gene A/a and gene B/b) occupying different loci ;**
2. **when gene A/a contains a copy of allele A at the gene loci, it hides the effect of gene B/b, A_ is epistatic over the B/b locus ;**
   OR
3. **when a copy of dominant allele A is at the gene loci, it hides the effect of gene B/b, A_ is epistatic over the B/b locus ;**
4. **A copy of dominant allele A results in white fruit (A _ _ _), a copy of dominant allele B (aa B _) results in yellow fruit, homozygous for the recessive allele at gene B/b (aa bb) results in green fruit ;**

Let A represent the dominant epistatic allele for inhibiting pigment production
Let a represent the recessive epistatic allele for pigment production
Let B represent the dominant hypostatic allele for yellow pigment
Let b represent the recessive hypostatic allele for green pigment

F1 phenotypes: white fruit X white fruit

F1 genotypes: AaBb X AaBb ;

Gametes (AB) (Ab) (aB) (ab) X (AB) (Ab) (aB) (ab) ;

Fertilisation:

	(AB)	(aB)	(Ab)	(ab)
(AB)	AABB	AaBB	AABb	AaBb
(aB)	AaBB	aaBB	AaBb	aaBb
(Ab)	AABb	AaBb	AAbb	Aabb
(ab)	AaBb	aaBb	Aabb	aabb

F2 genotype : AABB, AABb, AaBB, AaBb, AAbb, Aabb : aaBB, aaBb : aabb

F2 phenotype : white fruit : yellow fruit : green fruit

F2 phenotypic ratio : 12 : 3 : 1

Duplicate recessive epistasis (9:7)
A_B_ : A_bb + aaB_ + aabb
9 : 3+3+1=7
Dominant allele codes for an enzyme that controls a step in the synthesis of pigment from a precursor. If a dominant allele is absent, its step in the biosynthesis pathway is blocked and the pigment is not produced

Chi squared test

- Purpose:
- to determine if the observed results are significantly different from the expected results
- to estimate the probability that differences between observed and expected results were due to chance

Observed (O)	Expected (E)	Deviation (O-E)	$(O-E)^2/E$ (4sf)

Chi sq value = sum of $(O-E)^2/E$ = ___
Degrees of freedom = no. of phenotypic classes (n) - 1

Null hypothesis

1. The calculated χ^2 value is __, less than the critical χ^2 value of ___ at p= 0.05
2. Value of p is between __ and __, more than p= 0.05 ;
3. Do not reject null hypothesis, the observed and expected results is not significantly different
4. The phenotypic ratio does not differ significantly from the expected ratio of ___, any deviation from the expected is due to chance

Not null hypothesis, not due to chance

1. The calculated χ^2 value is ___, more than the critical χ^2 value of ___ at p= 0.05
2. Value of p is between __ and __, less than p= 0.05 ;
3. Reject null hypothesis, the observed and expected results is significantly different
4. Results obtained does not conform to the expected phenotypic ratio. Any deviation from the expected ratio is not due to chance, but due to other factors such as linkage

t-test

- Compare mean and standard deviation of the 2 groups
- Degrees of freedom = no. of A + no. of B - 2 =

Variation

- Continuous vs discontinuous

Continuous variation:

- Complete gradation from one extreme to the other without any break
- Produced by the effects of many genes (polygenes)
- Genes have similar and additive effects on the same characteristic
- Each of these genes has little effect on the phenotype individually, but combined effect is significant
- Significantly affected by environmental factors

Discontinuous variation

- Clear cut differences with no intermediates
- Controlled by one or few genes
- Relatively unaffected by environmental conditions

Continuous	Discontinuous
Continuous variation giving a range of phenotypes	Discontinuous variation giving discrete phenotypic classes
Characteristic controlled by the combined effect of many genes (polygenes)	Characteristic controlled by 1 or few genes. Each gene may have 2 or more alleles
Effect of individual gene cannot be observed, effect of polygenes are additive	Effect of individual gene can be observed
Environment has large effect on phenotype	Environment has small/no effect on the phenotype

Effect of diet on differentiation of honey bees

1. Diet of the bees will determine whether the eggs develop into queen bees or worker bees
2. Larvae which are fed on royal jelly will develop into queen bees while larvae which are fed a mixture of royal jelly, pollen and honey will develop into worker bees
3. The high protein (royalactin) content of royal jelly stimulates expression of genes for the formation and maturation of the female reproductive system, development of ovaries and a larger abdomen for egg laying

Effect of temperature on fur colour of Himalayan rabbits

- The rabbit has white body with black ears, nose, feet and tail
- Black fur only grows on extremities (only in cool parts of body)
- If fur on the back is shaved and an ice pack is fixed on its back, left in position for weeks and kept cold, black hair begins to develop beneath the ice pack
- They carry the gene required for development of black pigments, but the enzyme encoded by the gene is active only at low temperatures (temperature sensitive)
- Enzyme inactive → no pigment produced, white fur

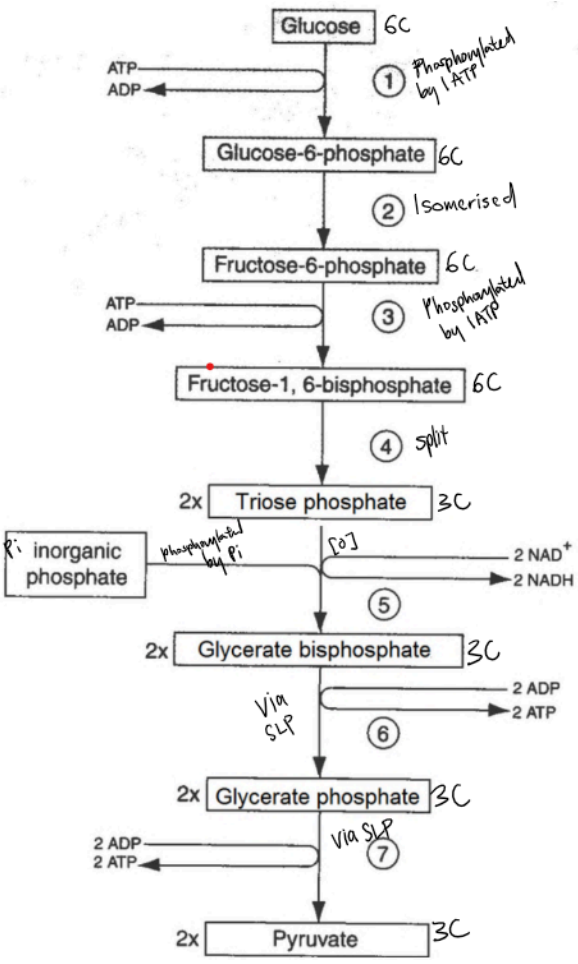
Respiration

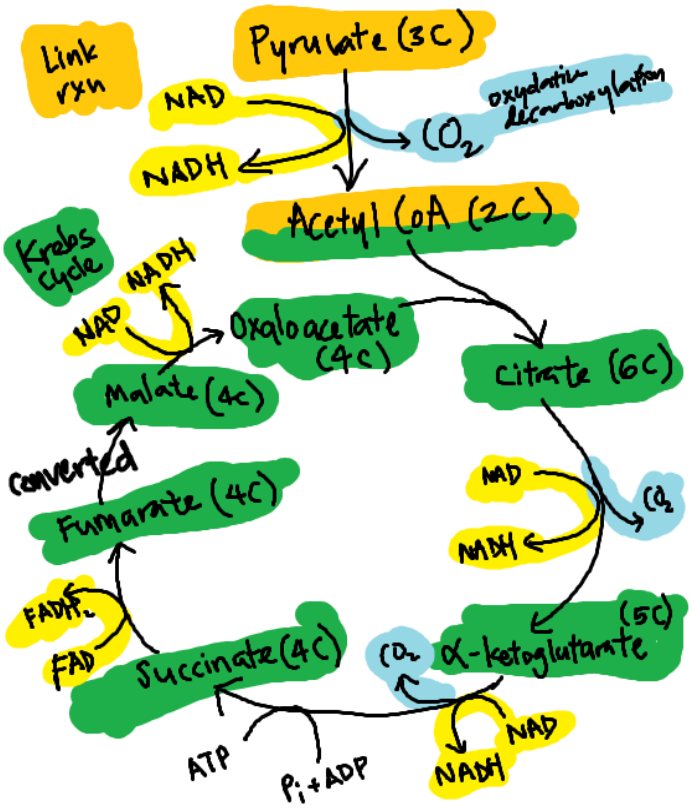
Function of mitochondrion

1. Site of aerobic respi to release ATP through OP
    - ATP is synthesised during oxidation of organic materials such as glucose in the presence of O2
2. Heat production

Functions of mitochondrial membrane

1. Inner mito memb provides large surface area which holds the ATP synthases, enzymes and electron carriers in the ETC needed for aerobic respiration
2. Outer and inner mito memb impermeable to H+. Accumulation of H+ occurs in the intermemb space (formation of electrochemical and proton gradient across the inner mito memb
3. Site of ATP synthesis by chemiosmosis when H+ diffuse from intermemb space down the concentration gradient through ATP synthase back to mito matrix
4. Allows for compartmentalisation within the mitochondria so that specialised metabolic pathways can take place in different regions





Aerobic respiration

- Involves four stages:
  Glycolysis (cytosol)
  Link reaction (mito matrix)
  Krebs cycle (mito matrix)
  Oxidative phosphorylation (inner mito memb)

Glycolysis

- Location: cytoplasm
- Sequence of reactions → 1 molecule of glucose → broken down/oxidised → 2 molecules of pyruvate
- Per glucose: net gain 2 ATP, 2 NADH formed, 2 pyruvate formed

1. Glucose (6C) is phosphorylated by 1 ATP to form glucose-6-phosphate (6C)
2. Glucose-6-phosphate (6C) is isomerised to form fructose-6-phosphate (6C)
3. Fructose-6-phosphate (6C) is phosphorylated by 1 ATP to form fructose-1,6-bisphosphate (6C)
4. Fructose-1,6-bisphosphate (6C) is split to form 2 molecules of triose phosphate (3C)
5. 2 molecules of TP (3C) is phosphorylated by 2 molecules of inorganic phosphate (Pi), undergo [O]/dehydrogenation by 2 NAD to form 2 molecules of glycerate bisphosphate (GBP) (3C)
6. 2 NAD → reduced to form 2 NADH
7. 2 molecules of GBP (3C) are converted to form 2 glycerate phosphate (GP) (3C)
8. 2 ATP formed by substrate level phosphorylation (SLP)
9. 2 molecules of GP (3C) converted to form 2 molecules of pyruvate (3C)
10. 2 ATP formed by SLP

Link Reaction

- Location: mitochondrial matrix
- 2 pyruvate translocated into mito matrix from cytosol via transport protein → undergo oxidative decarboxylation → form 2 molecules of acetyl CoA
- Per glucose: 0 ATP, 2 NADH, 2 CO_2, 2 acetyl CoA

1. 2 CO_2 released
2. 2 NAD are reduced to form 2 NADH
3. 2 molecules of coenzyme A are attached to 2 molecules of oxidised 2C fragments to form 2 molecules of acetyl CoA

Krebs cycle

- Location: mitochondrial matrix
- Completes the oxidative breakdown of glucose to CO_2 and H_2O
- Release H (used in OP)
- 2 molecules of acetyl CoA → undergo oxidative decarboxylation
- Per glucose: 4 CO_2, 6 NADH, 2 $FADH_2$, 2 ATP → formed by SLP

- At the end of the cycle: 2 molecules of oxaloacetate are regenerated (to receive more acetyl groups and the cycle continues)

1. Acetyl CoA (2C) and oxaloacetate (4C) are condensed to form citrate (6C)
2. Citrate (6C) undergoes oxidative decarboxylation to form α-ketoglutarate (5C)
3. 1 CO2 released, 1 NAD is reduced to NADH
4. α-ketoglutarate (5C) undergoes oxidative decarboxylation to form succinate (4C)
5. 1 CO2 released, 1 NAD is reduced to NADH, 1 ATP is formed by SLP
6. Succinate (4C) undergoes oxidation to form fumarate (4C), 1 FAD is reduced to FADH2
7. Fumarate (4C) is converted to form malate (4C)
8. Malate (4C) undergoes oxidation to regenerate oxaloacetate (4C), 1 NAD is reduced to NADH

Oxidative phosphorylation

- Location: inner membrane of mitochondrion

  15. H atoms dissociate from NADH and FADH2 and are split to form H+ and electrons in the mitochondrial matrix, regenerating FAD and NAD

  16. The electrons are transferred down the electron transport chain (ETC) comprising of a series of electron carriers of progressively lower energy levels (via redox reactions) to the final electron acceptor, oxygen, at the end of the ETC

  17. Oxygen combines with H+ and electrons to form water, catalysed by cytochrome oxidase

  18. Electrons are accepted by oxygen, thus electron carriers along the ETC will be oxidised, enabling continued NAD and FAD regeneration

  19. Energy released from the electron transfer is used to pump H+ via active transport from the mito matrix, (across the inner mito memb) into the intermemb space

  20. As the inner mito memb is impermeable to H+, this leads to an accumulation of H+ in the intermemb space which set up a proton gradient (btwn the intermemb space and mitochondrial matrix) for ATP synthesis

  21. H+ diffuse down their proton gradient through the ATP synthase from the intermemb space back to the mito matrix and releases electrical potential energy, which drives the phosphorylation of ADP to form ATP catalysed by ATP synthase

Importance of OP:

- Regenerate NAD and FAD so they can be reused for glycolysis, link rxn and Krebs cycle, allowing aerobic respi to continue
- Produce large amounts of ATP
- Per glucose: 34 ATP (NADH → 3 ATP, FADH2 → 2 ATP)

Anaerobic respi:

- Glyolysis followed by fermentation takes place in the cytosol
- Main purpose: regenerate NAD from NADH in order for glycolysis to continue

Alcoholic fermentation (plants and yeast)

- Per glucose: 2 ATP, regen 2 NAD, 2 ethanol, 2 CO_2
- Following glycolysis:
- Pyruvate (3C) undergoes decarboxylation to form ethanal (2C), catalysed by pyruvate decarboxylase. 1 CO_2 released
- Ethanal (2C) reduced to ethanol (2C) by NADH in the presence of alcohol dehydrogenase. 1 NAD is regenerated

Lactate fermentation

- Per glucose: 2 ATP, regen 2 NAD, 2 lactate
- Following glycolysis
- Pyruvate (3C) is reduced to lactate (3C) by NADH, catalysed by lactate dehydrogenase. NAD is regenerated
- Lactate is reconverted to pyruvate in the liver when aerobic conditions resume

Feature	Yeast cells	Mammalian cells
Process	pyruvate undergoes alcoholic fermentation	pyruvate undergoes lactate fermentation
Decarboxylation	pyruvate is decarboxylated to ethanal	no decarboxylation
Reduction	ethanal is reduced by NADH to ethanol	pyruvate is reduced by NADH to lactate
Enzymes	pyruvate decarboxylase, alcohol dehydrogenase	lactate dehydrogenase
Pdt	ethanol, CO_2, NAD	lactate, NAD

Why smaller yield of ATP per glucose during fermentation

1. During aerobic respiration, for every glucose molecule broken down and oxidised, there is a net gain of 2 ATP from glycolysis, 2 ATP formed during Krebs cycle via SLP and 34 ATP formed during OP, giving a total of 38 ATP
2. During anaerobic respiration, only glycolysis followed by alcoholic/lactic fermentation occurs in the cytosol
3. Net gain of 2 ATP per glucose molecule only in glycolysis formed via SLP
4. Incomplete breakdown and oxidation of glucose meant that a lot of energy remains locked up in lactate or ethanol
5. Hence, there is a small yield of ATP per glucose molecule from anaerobic respiration

Lower rate of utilisation of glucose:

1. During aerobic respiration, for every glucose molecule broken down and oxidised, there is a net gain of 2 ATP from glycolysis, 2 ATP formed during Krebs cycle via SLP and 34 ATP formed during OP, giving a total of 38 ATP
2. During anaerobic respiration, for every glucose molecule broken down and oxidised, there is only a net gain of 2 ATP per glucose molecule from glycolysis formed via SLP
3. This means 19 times less glucose is required in aerobic condition than anaerobic condition to produce the same number of ATP for cell metabolism

Describe the role of oxygen in aerobic respiration.

1. Oxygen is the final electron acceptor at the end of the ETC, combined with electrons + protons → form water, catalysed by cytochrome oxidase.
2. Electrons accepted by oxygen, NADH, FADH2 and electron carriers along ETC are oxidised, regenerating NAD and FAD.
3. This maintains the electron flow along the ETC + built up of proton gradient across the inner mitochondrial membrane for ATP synthesis by chemiosmosis.

Role of NAD and FAD

1. NAD and FAD act as a coenzyme for dehydrogenase and H atom carrier
2. H atom transferred to NAD, reduced NAD to NADH during glycolysis, link reaction and Krebs cycle in aerobic respiration
3. H atom transferred to FAD, reduced FAD to FADH2 during Krebs cycle in aerobic respiration
4. NADH and FADH2 carry the hydrogen atoms to the ETC at the inner mito memb to be used in OP
5. where NADH and FADH2 is oxidised to NAD and FAD respectively, regenerating NAD and FAD for subsequent glycolysis, link reaction and Krebs cycle to proceed

Summary of the by-products of the different stages of aerobic respiration (per glucose molecule)

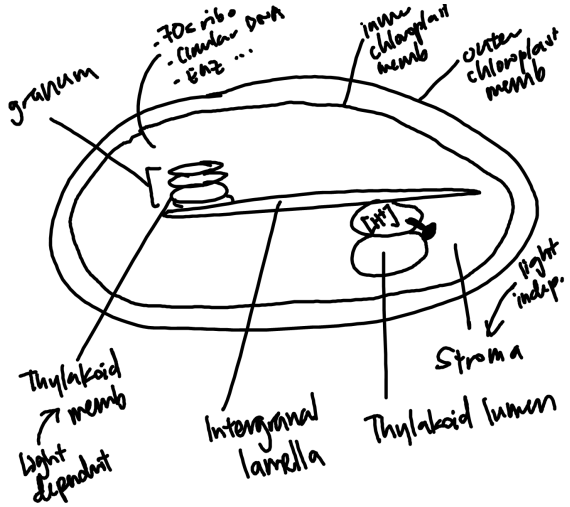
	CO_2	ATP	$NADH + H^+$	$FADH_2$
Glycolysis	0	2	2	0
Link Reaction	2	0	2	0
Krebs Cycle	4	2	6	2
Total	6	4	10	2

ATP yield from the different stages of aerobic respiration (per glucose molecules)

Stage	Substrate-Level Phosphorylation	Oxidative Phosphorylation	Total ATP
Glycolysis	2	6	8
Link Reaction	0	6	6
Krebs Cycle	2	22	24
Total	4	34	38

Photosynthesis

Chloroplasts



Function of thylakoid memb:

1. Provides a large surface area which holds the photosynthetic pigments, ATP synthases, enzymes and electron carriers of the ETC needed for the light dependent reactions ;
2. Holds the photosynthetic pigments in a suitable position for absorbing maximum amount of light energy
3. Impermeable to H+ and accumulation of H+ occurs in the thylakoid lumen, resulting in a proton gradient
4. Site of ATP synthesis by chemiosmosis when H+ diffuse from thylakoid lumen down their electrochemical gradient through ATP synthase back to stroma
5. Allows for compartmentalisation and formation of thylakoid lumen, allows the accumulation of H+ which set up proton gradient for ATP synthesis
6. Allows for compartmentalisation within the chloroplast so that specialised metabolic pathways can take place in different regions e.g. light-dependent reactions at the thylakoids

Outer and inner membranes

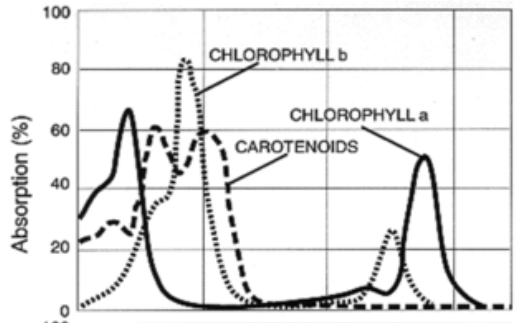
7. Allow for compartmentalisation – the membranes enable separate compartments to be formed, thereby allowing specialised metabolic pathways to take place e.g. light-independent reactions in the stroma
8. Act as selective barriers – hydrophobic core of the membranes restrict movement of charged ions and polar molecules across the membranes, regulating the movement of such molecules into and out of the chloroplast

Photosynthetic pigments:

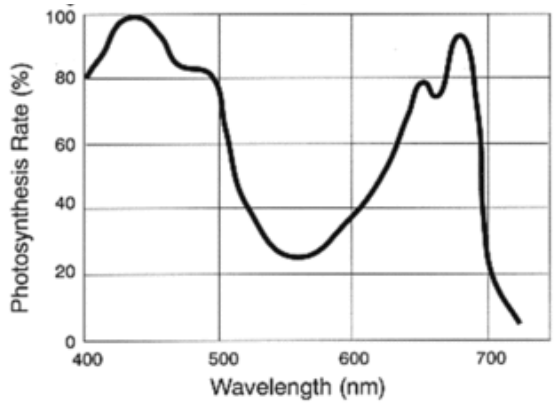
- Primary pigments (special chlorophyll a – P680, P700)
- Accessory pigments

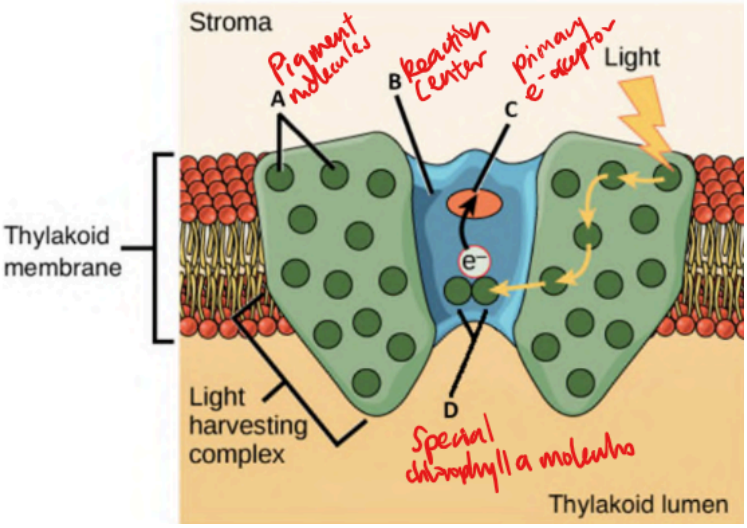
Absorption and action spectra

- Absorption spectrum shows degree of absorption at each wavelength of light by a particular photosynthetic pigment

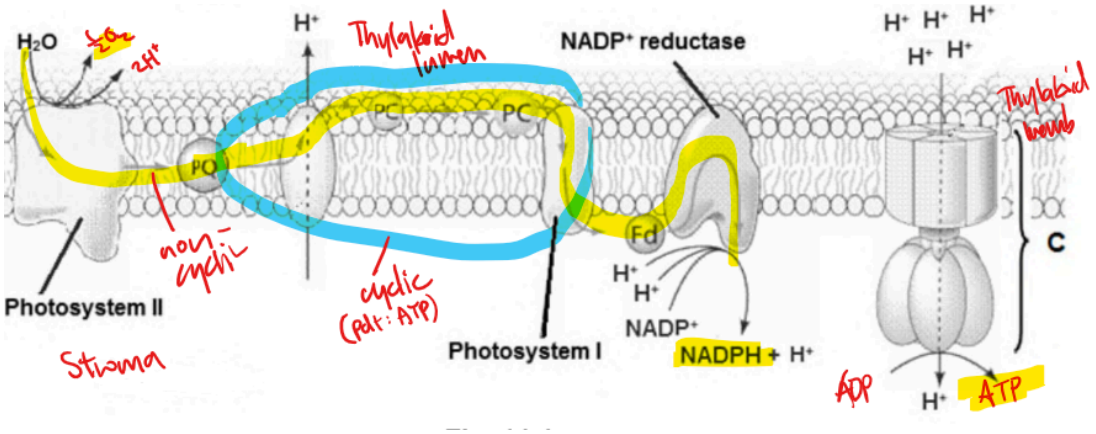


- Action spectrum shows effectiveness of diff wavelengths of light on photosynthesis





PSII (P680)
PSI (P700)



Light dependent reactions
Non-cyclic photophosphorylation (PSII, PSI)
Cyclic photophosphorylation (PSI)

Non-cyclic photophosphorylation
Photoactivation:

1. Photosynthetic pigments such as chlorophyll a, chlorophyll b and carotenoids are embedded on the thylakoid membranes and arranged in photosystems
2. Photons of light strike photosynthetic pigments in PS I and PS II. Energy from the photons of light is passed on from pigment molecules to other pigment molecules via resonance energy transfer until it reaches the special chlorophyll a (P680 and P700) in the reaction centres
3. Electrons in special chlorophyll a are excited and boosted to higher energy levels. These excited electrons are accepted by primary electron acceptors

Flow of electrons down the 1st ETC

4. The primary electron acceptors pass the electrons down the ETC comprising of a series of electron carriers of progressively lower energy levels from PS II to PS I
5. Energy is released and used to pump H+ via active transport from the stroma into the thylakoid lumen

Photolysis of water

6. In PS II, photolysis of water is catalysed by the water-splitting enzyme which splits water (into 2 H+, 2 electrons and ½ oxygen)

Synthesis of ATP

7. Accumulation of H+ in the thylakoid lumen sets up a proton gradient between the thylakoid lumen and stroma of the chloroplast
8. H+ diffuses down their proton gradient through ATP synthase, from the thylakoid lumen back into the stroma, releasing electrical potential energy which drives the phosphorylation of ADP to form ATP, catalysed by ATP synthase

Synthesis of NADPH

9. Electrons released from special chlorophyll a of PS II replaces electrons lost by special chlorophyll a of PS I. Electrons from photolysis of water replaces the electrons lost from special chlorophyll a of PS II

10. Electrons released from special chlorophyll a of PS I combines with H+ from water to form H atoms, which are then used to reduce NADP to NADPH (catalysed by NADP reductase)

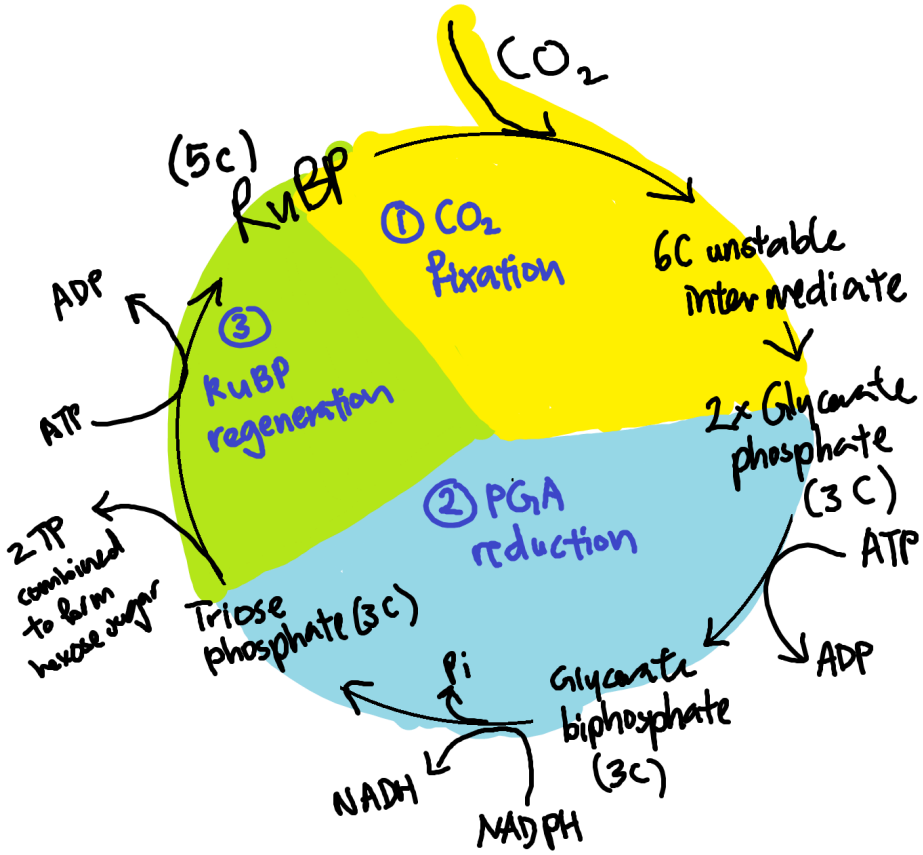
Cyclic photophosphorylation

11. In cyclic photophosphorylation, excited electrons from special chlorophyll a of PS I are passed to the ETC between PS II and PS I before returning to the same special chlorophyll a of PS I

Light independent reaction

Calvin cycle

12. During CO2 fixation, CO2 is fixed by combining with ribulose bisphosphate (RuBP) to form a 6C unstable intermediate, catalysed by rubisco
13. The 6C unstable intermediate breaks down immediately to form 2 molecules of glycerate phosphate (GP) (3C)
14. 2 molecules of GP are phosphorylated by ATP and reduced by NADPH to form triose phosphate (TP) (3C) (PGA reduction)
15. 10 molecules of TP and 6 molecules of ATP are used to regenerate 5 molecules of RuBP so that Calvin cycle can continue (RuBP regeneration)
16. The rest of TP exits the Calvin cycle to produce hexose sugar



To form 1 6C sugar and to regenerate 6 RuBP, CO_2 fixation must occur 6 times (18 ATP and 12 NADPH used)
Every 6 CO_2, 6 RuBP (30C) are carboxylated. 6 RuBP must be regenerated
Every 12 TP (3C x 12 =36C) formed, 10 TP (30C) are used to regenerate 6 RuBP (30C)
1 ATP needed to regenerate 1 RuBP → 6 RuBP need 6 ATP

Limiting factors

- Temperature
  > Calvin cycle are enzyme controlled and thus temperature sensitive
  > T increased, enz denature, stomata close to prevent excessive transpiration
- Light intensity
  > light intensity increases, more photons of light fall on a unit area on the leaves, increase rate of photoactivation, greater number of special chlorophyll a molecules ionised, increase movement of electron down both ETC, result in higher rate of photosynthesis with more ATP and NADPH generated for light independent reaction
  > light saturation occurs
  > rate of respiration=rate of photosynthesis → light compensation point
- CO_2 concentration
  > CO_2 concentration increases, rate of photosynthesis increases proportionally

Describe the role of NADP in photosynthesis.

1. Acts as a coenzyme (for dehydrogenase) and as H atom carrier
2. Electron acceptor in non-cyclic photophosphorylation of the light dependent reactions
3. Electrons combine with H+ to form H atoms and reduce NADP to NADPH in non-cyclic photophosphorylation
4. Carries the H atom from the light dependent reaction to the Calvin cycle for the reduction of GBP to TP (stroma)
5. NADPH is oxidised to NADP, leading to the regeneration of NADP for subsequent light dependent reactions

Explain the importance of ATP and NADPH to photosynthesis.

1. ATP serves as the energy source for the light-independent reaction.
2. ATP is needed to phosphorylate GP to form GBP
3. ATP is needed for the regeneration of RuBP.
4. NADPH provides the reducing power for the light-independent reaction
5. NADPH is needed for reducing GBP to form TP which is required for the synthesis of sugars and regeneration of RuBP

Compare the role of reduced NAD in aerobic respiration with the role of reduced NADP in photosynthesis
Similarities:

1. Both function as coenzymes for dehydrogenase
2. Both act as H atom carriers

3. Both NADH and NADPH oxidised to regenerate NAD during OP and NADP during Calvin cycle respectively

Differences:

4. NADH delivers electrons to the mitochondrial ETC during OP whereas NADPH directly donates electrons and H+ to the Calvin cycle in the chloroplast stroma
5. NADH contributes to the production of ATP during OP but NADPH contributes to the reduction of GBP to TP during the Calvin cycle
6. NAD is regenerated (in the mitochondrial matrix) for glycolysis, link reaction, and Krebs cycle whereas NADP is regenerated (in the chloroplast stroma) for light dependent reactions

feature of comparison	light-dependent reactions	light-independent reactions
location	thylakoids	stroma
requirement for light	light required for photoactivation of a pair of special chlorophyll a molecules	light not required
reactants	H_2O, ADP (and P_i), NADP	CO_2, ATP, NADPH
products	ATP, NADPH, O_2 (as a by-product)	triose phosphate (NADP, ADP regenerated)

	feature	Krebs cycle	Calvin cycle
1.	site	matrix of the mitochondrion ;	stroma of the chloroplast ;
2.	role of carbon dioxide	released by oxidative decarboxylation (4 CO_2 lost per glucose molecule) ;	used for carboxylation (6 CO_2 fixed by combining with 6 RuBP) ;
3.	type of reaction	involves oxidation by dehydrogenation, e.g. citrate undergoes oxidative decarboxylation to form α-ketoglutarate ;	involves reduction, e.g. GBP is reduced to triose phosphate ;
4.	fate of coenzyme / hydrogen atom carrier	NADH / $FADH_2$ is formed, NAD is reduced to NADH / FAD is reduced to $FADH_2$;	NADPH is used, NADPH is oxidised to NADP ;
5.	ATP	ATP is synthesised by substrate level phosphorylation ;	ATP is used in the phosphorylation of GP to GBP and in regeneration of RuBP ;
6.	starting point	oxaloacetate is regenerated ;	ribulose bisphosphate is regenerated ;
7.	nature of reaction	catabolic reaction (breakdown of pyruvate) ;	anabolic reaction (formation of triose phosphate) ;

	Feature of comparison	photophosphorylation	oxidative phosphorylation
1.	location	occurs at thylakoid membrane of chloroplast;	occurs at the inner mitochondrial membrane;
2.	involvement of light energy	light energy required to energise the electrons in special chl a;	light energy not required;
3.	involvement of oxygen	oxygen released as a by-product;	oxygen is used as the final electron acceptor;
4.	involvement of water	water molecules are split to form H^+, oxygen and provide replacement electrons for PSII;	water releaseld as a by-product;
5.	electron donor	non-cyclic: water cyclic: special chl a in PSI;	NADH, $FADH_2$;
6.	electron acceptor	non-cyclic: NADP cyclic: special chl a in PSI;	oxygen;
7.	source of energy for ATP synthesis	energy for ATP synthesis comes from light;	energy for ATP synthesis comes from glucose oxidation processes;
8.	establishment of proton gradient	H^+ pumped (inwards) from stroma to thylakoid lumen;	H^+ pumped (outwards) from mitochondrial matrix to intermembrane space;
9.	accumulation of protons in	thylakoid lumen/space;	intermembrane space;
10	energy conversion	light energy → chemical energy;	chemical energy → chemical energy;

Feature of comparison	non-cyclic photophosphorylation	cyclic photophosphorylation
photosystems involved	PS II and PS I	only PS I
pathway of electron flow	non-cyclic (electron from water passes through 2 ETCs to NADP via 2 photosystems)	cyclic (PS I to ETC and back to PS I)
number of ETCs involved	2	1
first electron donor	water	special chlorophyll a / P700 of PS I
final electron acceptor in photophosphorylation	NADP	special chlorophyll a / P700 of PS I
products	ATP, NADPH, oxygen	only ATP
photolysis of water	photolysis of water by water splitting enzyme in PSII	no photolysis of water due to absence of water splitting enzyme in PSI
generation of proton gradient	high H^+ concentration in the thylakoid lumen due to: • photolysis of water by water splitting enzyme in PSII • active transport of H^+ from stroma (across thylakoid membrane) into thylakoid lumen	high H^+ concentration in the thylakoid lumen due to: • active transport of H^+ from stroma (across thylakoid membrane) into thylakoid lumen

Biological Evolution

Importance of variation

1. Genetic variation results in different alleles in the individuals of the same species and hence differences in characteristics
2. Variations in characteristics/different phenotypes are subjected to selection pressure from the environment
3. Natural selection will take place in the population and individuals who are better adapted to the environment will be at a selective advantage.
4. Individuals with favourable characteristics will survive to maturity, reproduce and pass down their favourable alleles to their offspring.

Role of natural selection in evolution

1. Natural selection is differential success in reproduction
2. Spontaneous mutation results in genetic variation in individuals within a population
3. When there is a change in environment, selection pressure will change
4. Since there was variation within the population, individuals who are better adapted to the environment with favourable characteristics will be at a selective advantage
5. They will survive to maturity, reproduce and pass on their favourable alleles to their offspring
6. With each succeeding generation, the proportion of individuals who are at selective advantage increases while those at selective disadvantage decrease
7. Over time, there is a change in allele frequency in the population and this is how natural selection leads to micro evolution

Examples of how environmental factors act as forces of natural selection

Peppered moths:

- 2 types of peppered moth. Black arose from spontaneous mutation. After industrial revolution, lichen on trees killed, bark is darker with soot. Lighter moths now more visible to birds and at selective disadvantage. Darker moths at a selective advantage, survived to maturity and reproduced, passing on favourable alleles to their offspring. Over time, there is an increase in the frequency of melanic allele in the population, leading to evolution

Antibiotic resistant bacteria

- Resistant and non-resistant strains (variation, arise by spontaneous mutation)
- Antibiotics kill non-resistant bacteria. Non-resistant bacteria are selected against. Antibiotic resistant bacteria are at a selective advantage. Selected for and reproduce to pass on favourable allele for antibiotic resistance. Frequency of antibiotic resistance allele in the population increased over time

Population is the smallest unit

- A population is a group of interbreeding individuals sharing the same gene pool
- Individual organisms cannot evolve as each individual has the same genetic makeup all its life. The individuals in a population provide the genetic variation for natural selection

- Individuals in a population live in the same location and are subjected to the same selection pressures

Types of selection:
- Directional
- Disruptive
- Stabilising

	Directional	Disruptive	Stabilising
Selected against	Phenotype at one extreme is selected against	Intermediate phenotypes are selected against	Extreme phenotypes are selected against
Selected for	Favours individuals on one extreme of a phenotypic range	Favours individuals on both extremes of a phenotypic range	Favours more common intermediate variants in a population
Outcomes	Once the new mean phenotype coincides with the new optimum environmental conditions, stabilising selection take over	Can result in polymorphism, where 2 or more distinct forms are found in one species within a population	Does not promote evolutionary change but maintain phenotypic stability
Examples	Peppered moth colours	Snail shell patterns	Human birth weight

How genetic variation is preserved
1. Diploidy - heterozygote protection
    - Recessive apple present in heterozygote not shown in phenotype and will not be exposed to natural selection and possible elimination
    - Recessive alleles that are less favourable or harmful can persist in a population through propagation by heterozygous individuals
2. Heterozygote advantage
    - If individuals who are heterozygous at a particular locus have greater fitness and reproductive success than homozygous individuals, then 2 or more alleles will be maintained at that locus by natural selection
    - E.g. sickle cell anemia. Recessive allele which causes sickle cell anaemia is preserved in malaria zones

Mechanism of evolution
- Natural selection, gene flow, genetic drift, non-random mating, mutation

1. Natural selection
    - Individuals in a population exhibit variations in their heritable characteristics. This results in differential reproductive success as these individuals with favourable characteristics are at a selective advantage and survive to maturity, reproduce and pass on their favourable alleles to their offspring
    - Over time, frequency of favourable alleles increases in the population
2. Gene flow
    - Transfer of alleles from one population to another due to migration of fertile individuals
    - Depends on geographical proximity, mobility of individuals
    - Genetic variability within the recipient population will increase
    - Reduce differences in allele frequencies between neighbouring populations
    - Disruption to gene flow can cause 2 populations to evolve into different species due to differences in allele frequencies over time, such that individuals can no longer interbreed to produce fertile viable offspring
3. Genetic drift (small population size)
    - Change in allele frequencies due to chance
    - Reduce genetic variation in populations through loss of alleles

    (a) Bottleneck
        - Disasters may reduce the size of a population drastically
        - Allele frequencies of surviving population may not be representative of the original population's allele frequencies
        - By chance, certain alleles will be over-represented. Others will be under-represented. Some alleles may be eliminated
        - Results in reduction in genetic variation of a species

    (b) Founder effect
        - A few individuals from a larger population establish a new colony or population, they bring with them only a small fraction of the genetic variation present in the original population
        - Results in reduction in genetic variation in the newly found population
        - Allele frequencies in the newly founded population are different from those of the parent population
4. Non-random mating
    - Individuals select mates on the basis of phenotype. Random fusion of gametes in natural populations therefore does not occur
    - E.g. presence of one or more inherited phenotypes improves the chances of successful mating. This is known as sexual selection. Allele that resulted in a favourable phenotype for mating will increase in frequency over time, leading to evolution
    - Artificial selection: humans select the breeding pair of animals
5. Mutation
    - Generates new alleles and changes the gene pool of a population
    - For mutations to have an impact on evolution, it must occur in gametes
    - Only mechanism that results in formation of new alleles

Biological evolution:

- Descent with modification from a common ancestor

Micro evolution

- small-scale evolutionary changes below species level which is the change in allele frequency of a population over time.

Macro evolution

- large-scale evolutionary changes (may span millions of years) at or above species level which will result in the creation of new species.

Micro-evolution:

1. 630 000 years ago, when the sea level was much lower, there were only 7 islands and the sub-populations of finches on the different islands were exposed to different environments and subjected to different selection pressures, e.g. food resources
2. Since there was variation (e.g. beak shape) within the sub-populations, individuals with favourable characteristics were at a selective advantage and changes in allele frequency occurred independently in each sub-population

Macro-evolution:

3. Over the years, with the sea level rising and the large island separated into several different islands, geographical isolation leading to allopatric speciation occurred
4. The sub-populations of finches did not interbreed and thus gene flow was disrupted, resulting in different species
5. Over successive generations, evolution of different species of finches on the islands occurred, leading to adaptive radiation

Evidence based on homology

- Similarity in characteristics resulting from common ancestry and developed as a result of natural selection and changes in allele frequency is known as homology
- Molecular and anatomical homology support Darwin
- Species with common ancestor should display underlying similarities, even in features that no longer match in function
- Species with higher level of similarity are assumed to have diverged from a common ancestor more recently than species with lower level of similarity, and are thus more closely related

1. Biochemical data (molecular homology)
    - Comparing DNA and amino acid sequences
    - Similar DNA nucleotide/a.a. sequences of homologous genes (haemoglobin genes, cytochrome oxidase genes) most likely acquired the gene from a common ancestor
    - Fewer the differences of homologous genes between species, the more closely related the species will be
2. Anatomical homology
    - Organisms with anatomical homology have similar (anatomically homologous) structures such as bones, organs or any other physical structures derived from a common ancestor

- Structures with different appearances and functions that are all derived from the same body part in a common ancestor are termed homologous features
- Vestigial organs

3. Fossil record (anatomical homology)
    - Study structures of extinct species in order to infer possible evolutionary relationships between past and present species based on homology
    - Transitional fossils
    - Support concept of descent with modification
    - Challenges:
        - lack of continuous fossil record
        - decompose rapidly
        - soft bodied organisms do not fossilise easily
        - only a small fraction of organisms have fossilised
        - only a fraction have been unearthed
4. Biogeography
    - Study of past present and geographic distribution of species

    (a) Descent with modification
        - Closely related species tend to be found in the same geographic region
        - Most island species are closely related to species from the nearest mainland or neighbouring island due to descent with modification from a common mainland ancestor (divergent evolution)

    (b) Environmental pressures driving natural selection
        - Similar ecological niches in distant regions can be occupied by unrelated but similar looking species
        - Convergent evolution

Adaptive radiation:
- Single species evolves into several species occupying different ecological niches due to natural selection

Divergent evolution
- Differentiation of a homologous structure to perform a variety of functions

Species concept

Biological
- A group of organisms <u>capable of interbreeding and producing fertile, viable offspring</u>
- Only considered different if there is <u>no gene flow</u> between the respective populations of different species since their gene pools are so different that breeding between two different species is not possible or prevented due to presence of reproductive barriers
- Advantage
  Organisms can be studied to see if they can interbreed and produce fertile, viable offspring
- Limitations
  Cannot be applied to asexually reproducing organisms and to extinct species whose breeding behaviour cannot be observed

Difficult to determine if populations which are geographically isolated are capable of interbreeding

Ecological

- A group of organisms sharing the same ecological niche
- Niche refers to both the place where an organism lives and its interactions with the environment (i.e. the roles that an organism plays in its habitat - predator, prey, primary producer or consumer, decomposer etc)

Morphological

- A group of organisms sharing similar body shape and other structural features
- Usually used for preserved specimens or fossils

Genetic

- A group of genetically compatible interbreeding organisms that is genetically isolated from other such groups

Phylogenetic

- Smallest group of organisms that share a most recent common ancestor and can be distinguished from other such groups

Biological classification

- Organisation of a species according to shared characteristics

Phylogeny

- Organisation of species to show evolutionary relationships

Classification vs phylogeny

1. Classification is the organisation of species according to shared characteristics and classification may not take into consideration evolutionary relationships.
2. However, phylogeny is the organisation of species to show their evolutionary relationships.
3. Each group possesses unique features (classification)
4. A binomial (two-part) naming system is used (classification)
5. A limitation is that characteristics may be analogous and not homologous (classification)

Speciation:

- Process by which one or more new species arise from a previously existing species
- Over successive generations, each population became a genetically distinct species which cannot interbreed to produce fertile, viable offspring

How new species are formed

1. Geographical isolation (allopatric speciation)
2. Behavioral isolation (sympatric speciation)
    - Unique mating rituals and preferences (e.g. bird song)
3. Physiological isolation (sympatric speciation)
    - Unique physiology (internal functions)
    - E.g. flowering time difference arisen as a physiological response to a new substrate

Long speciation question:

Explain the ways in which islands favour the formation of new species. [6]

Dispersal

1. Founder effect occurs when some members of an ancestral population colonized other islands / other parts of the island to form sub-populations

Selection

2. The sub-populations were exposed to different environments and were thus subjected to different selection pressures e.g. food availability / temperature / different predators
3. Since there was variation within the sub-populations, individuals with favourable characteristics were at a selective advantage and changes in allele frequency occurred independently in each subpopulation

Isolation

4. The sub-populations become geographically isolated leading to allopatric speciation and are also physiologically / behaviourally isolated leading to sympatric speciation
5. The sub-populations did not interbreed (reproductive isolation) and thus gene flow was disrupted

Evolution

6. Over successive generations, evolution of different species on each island occurred, leading to adaptive radiation

Why larger islands have more species

1. More different environments / ecological niches for larger islands
2. More selection pressures e.g. temperature / different predators, etc. on bigger islands
3. More reproductive isolating mechanisms e.g. geographical + suitable example / increased distance between populations to disrupt the gene flow between populations

Why older islands have more species

1. For older islands, there were more time for more successive generations of ____, which provide more chances for changes in allele frequency to occur independently in each subpopulation of ___
2. Hence more chances for reproductive isolating mechanisms to take place e.g. behavioural / physiological + suitable example / flowering times differ

Advantages of using molecular methods to classify organisms

1. Unambiguous and objective. A, T, G, C are easily recognised and hence not dependent on subjective judgements or observations involving qualitative differences
2. Quantifiable and can be converted to numerical form and open to statistical analysis
3. Homologous regions of DNA from different species provides many points of comparison as each nucleotide position is a point of comparison

4. Molecular evidence will avoid the pitfalls of convergent evolution when morphological evidence could be due to convergence / some nucleotide differences are not expressed morphologically (e.g. silent mutations → no phenotypic differences)

5. Nucleotide data can be used to trace evolutionary relationships of species that are so different that there is little morphological homology
6. Nucleotide data can be used to assess phylogenetic relationships that cannot be measured by comparative anatomy

Advantage of mitochondrial DNA

1. No recombination or crossing over takes place in the mitochondrial genome, the differences reflect only mutations
2. There are multiple mitochondria and hence multiple copies of mtDNA in each cell as compared to one copy of nuclear DNA in each cell, making it easier to obtain mtDNA
3. Mitochondrial chromosomes are circular and subjected to less degradation by exonucleases
4. Non-coding mitochondrial control region of mtDNA has a rapid mutation rate to allow for differences in DNA sequence to accumulate and allow better differentiation between closely related species (for use in phylogenetic study)

Climate change

identify and explain the human activities over the last few centuries that have contributed to climate change through accumulation of greenhouse gases (limited to CO2 and methane) including

- burning of fossil fuels linked to increasing energy usage
- clearing of forests/deforestation for agriculture or urban development
- food choices (increasing consumption of meat)

Outline how human activities contribute to climate change. [4]

1. Human activities such as burning of fossil fuels due to increasing energy usage / deforestation for agriculture or urban development / food choices linked to increased meat consumption
2. increases GHG emissions such as CO2 and methane into the atmosphere
3. Increased GHG emissions has resulted in enhanced greenhouse effect, trapping heat in the atmosphere and resulting in global warming and increase in global average temperature
4. This causes melting of polar ice caps / rising sea levels / stress on fresh water supplies / heat waves / heavy rains / death of coral reefs / migration of fishes and insects / release of greenhouse gases in frozen organic matter

explain the effects of climate change as a result of greenhouse gas emissions, including

- melting of polar ice caps
- rising sea levels
- stress on fresh water supplies
- Extreme weather events such as heat waves, heavy rains
- death of coral reefs
- migration of fishes and insects
- release of greenhouse gases in frozen organic matter

explain how climate change brings about environmental stresses which affects plant distribution (vertical and latitude) and plant adaptations, including morphology and physiology

Adaptations:

1. Stomatal closure
    - Increase rate of transpiration, reduce water loss
    - CO2 assimilation and rate of calvin cycle decrease. Net PS decrease
2. Decrease in number of stomata per leaf
    - Reduce water loss via transpiration
3. More well developed roots
    - Take in more water to counter loss of water
4. Decrease in number of leaves and smaller leaf area
    - Reduce water loss by increasing ratio of roots to shoots

discuss the consequences to the global food supply of increased environmental stress resulting from climate change, including the effects on plants and animals of increased temperature and more extreme weather conditions

- Increased environmental stress (increased temp, more extreme weather conditions)
- Decline in global food supply and increased risk of food insecurity

1. Plants (crops)
    - Higher temp, decreased crop yields, decrease in global food supply leading to food shortage
    - Mid latitude regions: warming may benefit crops, longer growing seasons, more precipitation, less frost, increased crop yields
    - Low latitude (tropics and sub tropics): crops are often near maximum temperature tolerance and even moderate temperature increases are likely to reduce water availability, disrupt plant growth and development, decreased crop yields
    - Rise in sea levels, loss of arable land for agriculture, decrease in production of food crops
    - Migration of weeds fungi and insects, emergence of pests and diseases which damage crops and reduce yield
    - Heat waves cause heat stress in crops
    - Decreased water availability disrupt germination and fruit development
2. Animals (fisheries/livestock)
    - Poleward migration of fishes
    - Affect timing of repro and migration, frequent marine and disease outbreaks by parasites and algae bloom
    - Ocean acidification
    - Heat stress on cows, parasites and diseases, reduce food availability of livestock

explain how temperature changes impact insects, including increased temperature leading to increased metabolism and the narrow temperature tolerance of insects

- Narrow temperature tolerance
- Explain how temperature changes impact insects:
    1. Increasing temperature to insects' thermal optimal level leads to higher rate of enzymatic reactions and consequently, increased metabolism of insects
    2. Hence insects develop more quickly under high temperatures not exceeding their optimal levels
    3. Higher temperatures tend to increase the speed of an insect's life cycle and insects mature, mate and reproduce in a shorter span of time

outline the life-cycle of Aedes aegypti as an example of a typical mosquito vector

1. Female Aedes aegypti lay their eggs above the water line in areas likely to temporarily flood such as tree holes and containers
2. Mosquito larvae hatch from the submerged eggs 2 days after the containers are filled with water and feed on microorganisms found in the water

3. Larvae go through developmental stages in which they moult four times over 5 days and these larval stages are called the first to fourth instars
4. When a larva is a fully grown fourth instar, it undergoes metamorphosis into a new form called a pupa
5. After two days, the fully developed adult mosquito forms and breaks through the pupal case

outline the development of viral dengue disease in humans, including host-pathogen interactions, human susceptibility to the virus, pathogen virulence, transmission and drug resistance

- Describe how the dengue virus is transmitted
    1. The dengue virus is transmitted via the bite of an infected female Aedes aegypti mosquito (which acts as a vector)
    2. When the mosquito feeds on the blood of an infected person with the dengue virus during viraemia, the dengue virus enters the mosquito's system in the blood meal
    3. The dengue virus replicates and spreads through the mosquito's body to reach the salivary glands
    4. The infected mosquito can transmit the dengue virus through their saliva to another uninfected person when feeding on their blood

Symptoms of dengue:

- High fever that lasts 2-7 days, severe pain in muscles and joints, decrease in WBC, low platelet, bleeding from nose/gums, easy bruising

Symptoms of severe dengue

- Leakage of blood plasma out of the capillaries
- Stomach and intestinal bleeding

Host pathogen interaction

- Infects nearby skin cells (keratinocytes), infects and replicates inside skin dendritic cells (Langerhans cell)
- Enter via receptor mediated endocytosis
- Innate response activated and infected dendritic cells produce cytokines and recruit macrophages, but WBCs get infected by virus
- Infected dendritic cells and macrophages travel to lymph nodes through the lymphatic system and spread through body to infect more cells
- Spread and increase of the virus results in varaemia
- Cells display viral Ag on surface to activate adaptive immune response. B cells produce Ab (IgM and IgG) that specifically recognise and neutralise dengue viral articles by preventing virus from binding to macrophages and gaining entry
- Tc cells kill virally infected cells

Human susceptibility

- Infected from 4 serotypes

- Antibody-dependent enhancement of infection
- Infected with a second type of dengue serotype will activate body to attack as if the first serotype, but cannot eradicate, aiding the virus in infecting host cell more efficiently

explain how global warming affects the spread of mosquito-borne infectious diseases, including malaria and dengue, beyond the tropics

- Increased in temperature
    1. Results in increase in number of mosquito vectors as increased temperatures lead to increased metabolism of mosquitoes and accelerates their development
    2. Increases activity of female mosquitoes and reduces the incubation time for them to become infective / transmit DENV
    3. Rate of viral replication within vector will increase and extrinsic incubation period will shorten
- Increased in rainfall
    1. More stagnant water

Outline how global warming can affect the spread of dengue. [2]

1. Global warming results in an increase in geographical/distribution range of Ae. aegypti as they have increased survival at higher latitudes
2. Global warming also results in increase in number of mosquito vectors as increased temperatures lead to increased metabolism of mosquitoes and accelerates their development
   OR
3. Increases activity of female mosquitoes and reduces the incubation time for them to become infective
   OR
4. Rate of viral replication within vector will increase and extrinsic incubation period (before DENV becomes transmissible to another host) will shorten

discuss the effects of increased environmental stress (including increased temperatures and more extreme weather conditions) as a result of global climate change, on habitats, organisms, food chains and niche occupation

Discuss the effects of global climate change on the survival and distribution of plants and animals.

- Effects of global climate change

1. Increased GHG emissions has resulted in enhanced greenhouse effect, trapping heat in the atmosphere and resulting in global warming and increase in global average temperature
2. Results in extreme weather conditions such as heat waves, heavy rains, drought
3. Results in earlier arrival of spring in temperate countries with milder winters and longer summers
4. Results in habitat loss, e.g. due to forest fires or melting of ice sheets

- Effects on survival

5. Migratory birds and insects are now arriving at their summer feeding and nesting grounds earlier than they did in the 20th century
6. Phenology – timing mismatch of biological events of different species results in increased vulnerability of species and affect their survival
7. Sea level rising due to the melting of land-based ice sheets and thermal expansion of water
8. Results in increased coastal erosion and flooding in coastal regions
9. Saltwater intrusion into surface water/freshwater spring and ground water increases salinity, not suitable for growth of plants and freshwater fishes
10. Reduction in the extent of ice sheet in the Arctic has threatened the survival of the polar bears
11. Due to reduction in number of seals / increased competition for food / increased distance to travel to find food / loss of breeding sites
12. Increased temperatures lead to ocean warming and shallow waters where corals are found heats up more rapidly
13. At higher water temperature, corals bleach, expelling zooxanthellae from their tissues
14. Bleached corals are unhealthy and may die from starvation or disease, leading to loss of vital nurseries for fishes and habitats for marine species

- Effects on distribution

15. As global average temperature rise, both plant and animal populations are projected to shift upwards to the higher latitude and towards higher altitudes to stay within their ideal range of environmental conditions
16. e.g. Boreal forests gradually shifts into the tundra region while being displaced by temperate forests and grasslands
17. e.g. shift of life zones to higher altitudes up the mountains, results in increased competition among high-altitude plant species and may lead to their extinction
18. e.g. distribution ranges of aquatic species may change as they shift to colder areas of streams or lakes or to oceans at higher latitude, putting them into competition with other species and cause ecological disruptions/separation of prey from predators
19. As plants are more sedentary than animals, certain plant species may not be able to migrate fast enough to escape the consequences of climate change and may result in extinction of that particular species
20. Some species may find themselves in unsuitable habitats/locations or face increased competition, and be unable to adapt to the change in conditions and become extinct

discuss how climate change affects the rich biodiversity of the tropics, including the potential loss of this rich reservoir for biomedicines and genetic diversity for food

- Many prescription drugs derived from plants
- Crop wild relatives (CWR): cousins of well known food crops contain genes that can be used to improve crop qualities via crossbreeding or genetic engineering
- Combat disease outbreaks in food crops