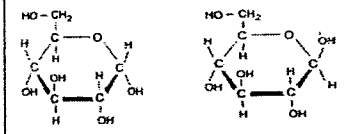
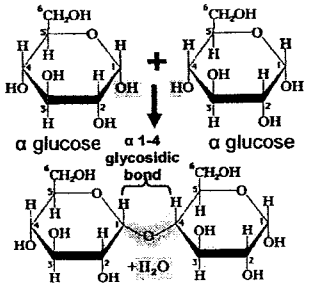
Carbohydrates

Describe the formation and the breakage of a glycosidic bond.

Carbohydrates: $C_n(H_2O)_m$

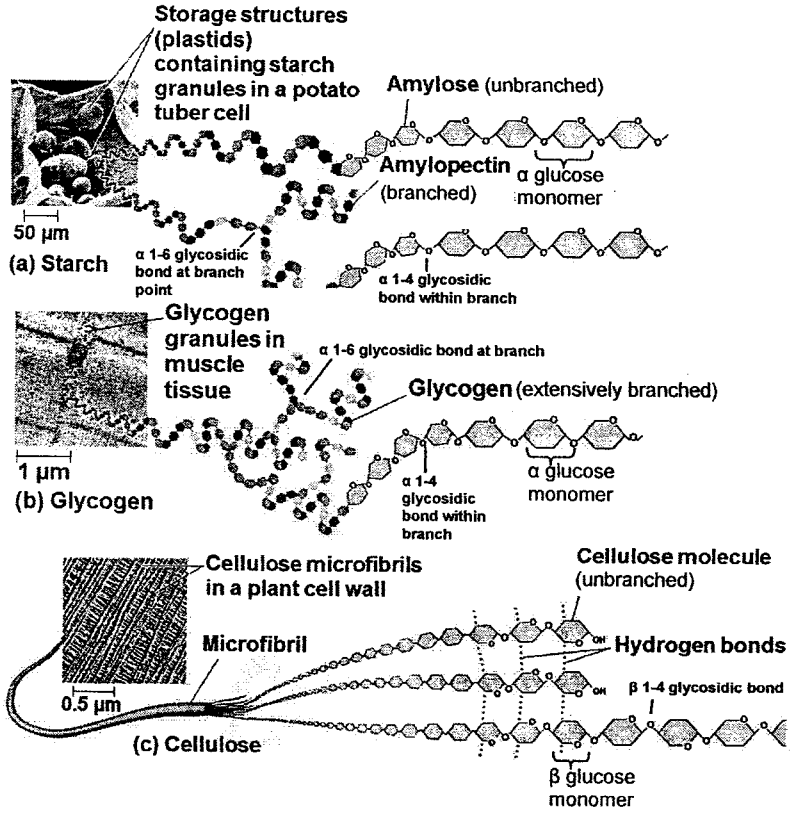
Monosaccharides	Features
 α glucose β glucose (note: You must know how to draw α and β glucose molecules)	1. general formula:$(CH_2O)_n$ 2. have a **carbonyl (C=O)** group 3. **small size** and have multiple **hydroxyl (OH)** groups → forms **H bonds with water** and hence readily **soluble in water** and easily transported in animal and plant transport systems 4. ring structures exhibit **α- and β- isomerism** (α – if OH grp at C1is below ring and on opposite side of C6 and β – if OH grp at C1 is above ring and on same side as C6) 5. are all **reducing sugars**. 6. e.g. glucose, galactose and fructose.
Disaccharides	**Features**
 α glucose α 1-4 glycosidic bond α glucose Maltose	1. are made up of 2 monosaccharides joined by a **glycosidic bond** formed between two monosaccharides *by a **condensation reaction that involves the loss of a water molecule.*** 2. can be split into their component monosaccharides is via **hydrolysis reaction** where, with the **addition of one molecule of water,** the **glycosidic bond** can be broken 3. many hydroxyl groups extending out of the ring → forms H bonds with water and hence readily **soluble in water** and easily transported in animal and plant transport systems 4. **all are reducing sugars except sucrose** 5. e.g. Sucrose = glucose + fructose Maltose = glucose + glucose Lactose = glucose + galactose
Polysaccharides	**Features**
	1. general formula:$(C_6H_{10}O_5)_n$ 2. are made up of many monosaccharides joined by **glycosidic bonds** formed between them by **condensation reactions** which involve the **loss of water molecules.**

Compare the storage and structural forms of starch, glycogen and cellulose and their roles in plants and animals.

Point of Comparison	Starch	Glycogen	Cellulose
Function	**Plant storage** polysaccharide	**Animal storage** polysaccharide	**Plant structural** polysaccharide
Location	Stored as granules in **chloroplasts**	Stored in **liver** and **muscle** cells	**Cell walls** of plant cells
Monomer	Made up of **α-glucose** monomers	Made up of **α-glucose** monomers	Made up of **β-glucose** monomers
Bond between monomers	In **amylose: α(1-4) glycosidic bond** links monomers; In **amylopectin: α(1-4) glycosidic bond** links monomers within a branch and **α(1-6) glycosidic bonds** links monomers at branch points	**α(1-4) glycosidic bond** links monomers within a branch and **α(1-6) glycosidic bonds** links monomers at branch points	**β (1-4) glycosidic bond** links monomers in a molecule
Orientation of monomer	All glucose units in the chain have the **same orientation**	All glucose units in the chain have the **same orientation**	**Alternate** glucose units **rotated 180° with respect to each other**
Structure of each molecule	Amylose is a **helical** molecule while amylopectin is a **helical** and **branched** molecule	**Helical** and **branched** molecule, like amylopectin, but **more extensively branched**	**Long, straight/linear** chain
Bonds between molecules	No interchain hydrogen bonding	No interchain hydrogen bonding	**OH groups** projecting outwards in both directions allow **interchain hydrogen bonding** →leads to **microfibril** formation

How the structures of starch and glycogen make them good **STORAGE** molecules	How the structure of cellulose makes it a good **STRUCTURAL** molecule
1. **Large molecule** made up of **many α-glucose** monomers Hence it is a huge **energy store** as it can be hydrolysed to yield **many glucose** molecules that can be used as a **respiratory substrate to obtain ATP**.	1. **Adjacent glucose units are inverted 180° with respect to each other** and hence form a **linear molecule** with free OH groups projecting out in both directions which can **hydrogen bond with OH groups of other cellulose molecules lying parallel to it** and form **microfibrils.** Thus the microfibrils have **high tensile strength.**
2. Comprise of **helices** a. can **pack many subunits per unit volume** b. in which most OH groups are involved in **intramolecular H bonding, few OH groups available for H bonding with water.** Hence they are **insoluble in water** and the Ψw of cells are unaffected by their presence.	2. As a macromolecule, cellulose has **few OH groups available to hydrogen bond with water** as most are involved in interchain hydrogen bonding. Thus only the surface of the microfibril has OH groups that can hydrogen bond with water. Thus cellulose is **insoluble in water** and the Ψw of cells are unaffected by its presence.
3. Amylopectin and glycogen are **branched** a. they have **multiple branch ends** which hydrolytic enzymes can work on. Thus more glucose molecules can be released and **more ATP can be generated by respiration per unit time.** b. branching **optimizes packing** of many subunits per unit volume and hence **more energy can be stored per unit volume** c. branching **reduces the accessibility of water** and thus **reduces the solubility of the molecule.** Thus the Ψw of cells are unaffected by their presence.	3. The meshwork of microfibrils that form the cell wall have a **porous structure** and hence makes the cell wall a. **freely permeable** to water and solutes and allows movement of substances in and out of cells. b. **strong** and **rigid** and prevent the plant cells from bursting due to osmotic stress.
	4. **Cellulases** that hydrolyse cellulose are **found in very few organisms.** Thus **cellulose cannot be hydrolysed** and used as a respiratory substrate and is a good structural molecule.

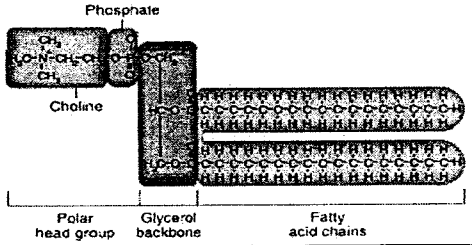
Test	Procedure	Observations and Deduction
Benedict's Test for Reducing Sugars	1. Place 2 cm^3 of test solution in a test tube. 2. Add equal volume of **Benedict's** reagent. 3. Shake the mixture. 4. Heat it by immersing the tube in boiling water bath for 3-4 minutes.	Presence of **reducing sugar** is indicated by formation of **brick-red precipitate**. → colour of final solution depends on amount of reducing sugar present (test is semi-quantitative) → varies from green to yellow to brown to brick red
Test for Non-Reducing Sugars	If a negative result for **Benedict's test** is obtained for the test solution, then 1. boil equal volume of test solution with dilute hydrochloric acid for about 1 minute to hydrolyse disaccharide to monosaccharides 2. cool contents of tube. 3. **neutralise** acidic content with **sodium bicarbonate** solution. 4. carry out Benedict's test for reducing sugar.	Presence of non-reducing sugar indicated by: • A blue solution remains when Benedict's test is first carried out. • After acid hydrolysis, Benedict's test is carried out again → colour of final solution depends on amount of sugar present
Iodine Test for Starch	1. Add a few drops of iodine solution to 1 cm^3 of test solution (or a piece of test specimen). 2. Observe any colour change.	Presence of starch is indicated by blue-black coloration.



Lipids

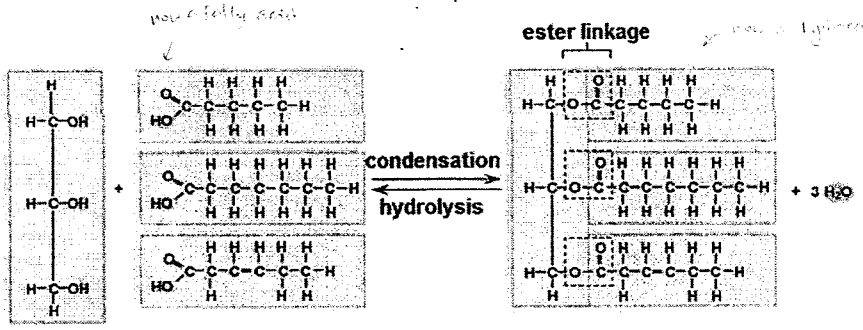
Analyse the molecular structure of a triglyceride and a phospholipid, and relate these structures to their functions in living organisms

- Lipids have a much **smaller proportion of oxygen to hydrogen and carbon** compared to carbohydrates
- They are **insoluble in water**
- Can be classified into: triglycerides, phospholipids and steroids

	Triglyceride	Phospholipid
Structure	Consists of **three** long **non-polar, hydrophobic hydrocarbon tails** associated with a **glycerol backbone**. Formed when 2 kinds of smaller molecules join together: • **glycerol** o alcohol with 3 carbons, each bearing a hydroxyl group • **fatty acids** o **long hydrocarbon chains** with a carboxyl group (-COOH) o **hydrophobic** in nature as they have non-polar C-H bonds in the hydrocarbon chains o may be saturated or unsaturated.	Consists of **two** long **non-polar, hydrophobic hydrocarbon tails** attached to a **glycerol backbone** (not three). The third hydroxyl group of the glycerol is joined to a **negatively charged** (not polar!) **phosphate group**. Additional small molecules, usually charged or polar, can be linked to the phosphate group to form a variety of phospholipids.  (NB: the charged *phosphate head is* not on the same side as the non-polar, hydrophobic hydrocarbon tails)

Condensation reactions take place in the formation of both, though in phospholipids, a **phosphate group** is added in place of a **non-polar, hydrophobic, hydrocarbon chain** of a **fatty acid** molecule.

In triglycerides: **Three non-polar, hydrophobic, hydrocarbon chains** are joined to a **glycerol backbone** by an **ester linkage**. An ester linkage is formed between a hydroxyl group (-OH) and a carboxyl group (-COOH) via a **condensation** reaction. One **water molecule is removed** for each fatty acid joined to the glycerol.



Properties	**Non-polar**. Do not form **hydrogen bonds** with water molecules, ie they are **hydrophobic**, and therefore are **insoluble in water**.	**Amphipathic** as they have **both hydrophilic** and **hydrophobic** regions • hydrophobic hydrocarbon tails are excluded from water while hydrophilic charged phosphate head (and its attachments), will interact with water molecules • In water, they self-assemble into aggregates such as a phospholipid bilayer or micelle which has a **hydrophobic core** that is shielded from water.
Function	1. Excellent **energy store** (38 kJg^{-1}) (a gram of fat stores more than twice as much energy as a gram of glycogen or starch (per unit mass) and hence is a **compact energy store**) • as it has a **high proportion of C-H bonds** in hydrocarbon chains from which energy can be released during **oxidation (main role)** • as it is insoluble, and thus will not affect **water potential** of cell nor be easily transported out of a cell. 2. Production of **metabolic water** • Oxidation of triglycerides produces **metabolic water**. Particularly important for desert animals. 3. **Protect** internal organs 4. **Thermal insulation** – by subcutaneous fat (the layer of fat beneath the skin). 5. Improve **buoyancy** 6. Reservoir for **storage of fat soluble vitamins**	1. Major component of **phospholipid bilayer** of **membranes of cells** and organelles which acts • as a **barrier** to **polar** and **charged molecules/ions** as its **hydrophobic core** has a very low permeability to polar and charged molecules • as a **boundary** between the intracellular & extracellular aqueous environment and allow **compartmentalization** within a cell. 2. Major component of **liposomes** • Artificial vesicles surrounded by a phospholipid bilayer that can be used to carry therapeutic DNA into a target cell.

- Steroids
  - (a) **Properties of steroids e.g. cholesterol**
    - Insoluble in water and soluble in organic solvents.
    - Cholesterol has a characteristic **four fused ring** structure and is largely hydrophobic. Like phospholipids, it is **slightly amphipathic**, having a **hydrophilic** hydroxyl (OH) group and the **hydrophobic** ring structure.
      - The hydroxyl group of cholesterol interacts with phosphate heads of phospholipids while the hydrophobic ring structure interacts with the hydrocarbon tails of the phospholipids via **hydrophobic interactions.**
  - (b) **Function of steroids e.g. cholesterol**
    - Cholesterol **regulates membrane fluidity** i.e. it stabilises membrane.
      - The membrane is prevented from being **overly fluid at higher temperatures** as cholesterol **restricts phospholipid movement**
      - The membrane is prevented from being **overly firm at lower temperatures** as cholesterol **prevents close packing** of phospholipids

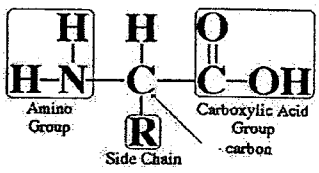
Carry out the emulsion test for fats

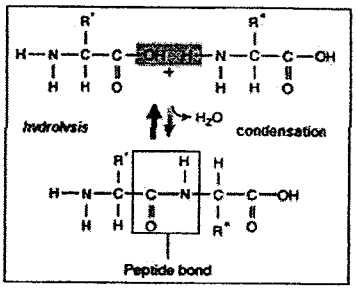
Emulsion Test for Lipids	If the test sample is a solution: 1. Add 2 cm^3 of ethanol to two drops of test sample in a test tube. Mix well and allow to stand for 2 minutes. 2. Decant the ethanol into another test tube containing 2 cm^3 of water. If the test sample is a solid: 1. Add 2 cm^3 of ethanol to ground test sample in a test tube. 2. Mix well to dissolve any lipids if it is present and allow it to stand for 2 minutes. 3. Decant the ethanol into another test tube containing 2 cm^3 of water.	Presence of lipids indicated by: • A homogeneous (or clear) solution is formed with ethanol and an ***emulsion*** was formed when water was added. If lipids are absent, a clear solution remains.

Proteins

Describe the structure of an amino acid and the formation and breakage of a peptide bond

- **Amino Acids – Basic structural unit of proteins**
  - **Structure**
    - consists of an **carbon atom** covalently bonded to 4 groups
      1) **hydrogen atom**, 2) **amino group (-NH_2)**, 3) **carboxyl group (-COOH)**, 4) **variable R group**
  - **Properties of amino acids**
    - Classified according to their R groups as **polar, charged or non-polar**
    - Exist as zwitterions in solution – carry both positive and negative charges
    - Act as buffers
      - Can donate or accept H^+, therefore able to act as an acid or as a base – **amphoteric**
      - Essential in biological systems – sudden change in pH could adversely affect performance of proteins like enzymes





- **Polypeptides**
  - Amino acids are joined by a **peptide bond** via a **condensation reaction** with the **removal of one water molecule**.
  - Further addition of amino acids results in the formation of a **linear** polymer called a **polypeptide**
    - Regularly repeating part, the main chain, is referred to as the **backbone**.
    - Variable part comprises the distinctive **variable R groups**
  - polypeptide folds into a **specific three-dimensional shape / conformation**

- The nucleotide **sequence in DNA** determines **aa sequence** in polypeptide which determines **types** and **locations of R groups** which determines **R group interactions** which determines **3D structure** and **function of protein.**

Explain the meaning of the terms *primary structure, secondary structure, tertiary structure* and *quaternary structure* of proteins, and describe the types of bonding (hydrogen, ionic, disulfide and hydrophobic interactions) which hold the molecule in shape.

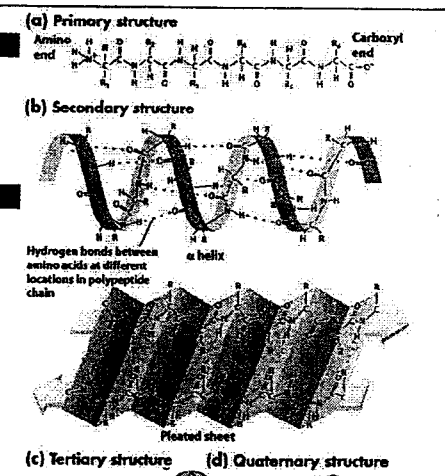
- 4 levels of organization in the structure of proteins

(a) Primary structure

- Refers to the **number and sequence of amino acids** in a single polypeptide chain.
- Linear structure maintained by **peptide bonds**
- The **sequence of amino acids** (and their **R groups**) in a polypeptide chain determines the **type and location of chemical bonds/interactions**, and hence the **3D conformation** and **characteristics** of a particular protein.

(b) Secondary structure

- Refers to the spatial arrangement formed by **regular coiling or pleating** of a **single polypeptide** chain in **one direction.**
- Maintained by **hydrogen bonds** at regular intervals
  - Formed between **C=O and N-H** groups of the **polypeptide backbone.**
  - **R groups are <u>not</u> involved**
- Examples of secondary structures:
  - **α-helix**
    - Made up of a **single polypeptide** chain which is wound into a **coiled/spiral** structure.
    - A **hydrogen bond** forms between the **C=O group** of one amino acid residue and the **N-H group** of another amino acid residue **four amino acids away** along the backbone of a **single polypeptide**
    - There are **3.6** amino acid residues in **every turn** of the helix
  - **β-pleated sheet**
    - **Two or more regions/segment** of a **single polypeptide chain** lying side by side are linked together by **hydrogen bonds**
    - A **hydrogen bond** forms between the **C=O group** of an amino acid residue on **one segment** and a **N-H group** of another amino acid residue on an **adjacent segment** along the backbone of a **single polypeptide**
    - Chains may run **parallel** (same direction) or **anti-parallel** (opposite directions)
    - Forms **flat sheet which** becomes folded

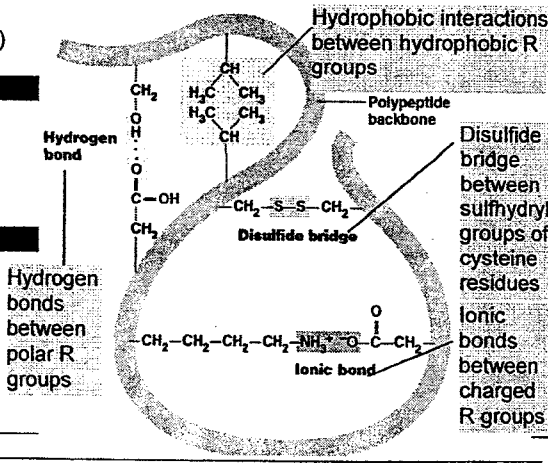


(c) Tertiary structure

- Structure formed by **further extensive folding and bending** of a **single polypeptide chain**, giving rise to the **specific 3D conformation** of a protein
- Maintained by all **4** types of interactions
  - **hydrogen bonds, ionic bonds, hydrophobic interactions** and **disulfide bonds**
  - formed **between R groups** of amino acid residues

(d) Quaternary structure

- Refers to the association of **two or more polypeptide** chains into one functional protein molecule
- Maintained by all **4** types of interactions
  - **hydrogen bonds, ionic bonds, hydrophobic interactions** and **disulfide bonds**
  - formed **between R groups** of amino acid residues of **different** polypeptides
- Constituent chains of a multimeric protein can be identical or different.



Analyse the molecular structure of a protein with a quaternary structure e.g. haemoglobin as an example of a globular protein and collagen as an example of a fibrous protein, and relate these structures to their functions.

Example	Structure	Function
Haemoglobin (**globular** protein) →transports oxygen in the blood **(The binding of successive O_2 molecules facilitates binding of the next. Binding of the 1st O_2 molecule increases the affinity of haemoglobin for oxygen and hence facilitates the binding of the 2nd O_2 molelcule. Binding of the 2nd O_2 molecule facilitates the binding of the 3rd O_2 molecule and so on.)	1. Haemoglobin has a **quaternary** structure made up of 4 **polypeptide subunits**: 2 **α-globin** subunits and 2 **β-globin** subunits. Each subunit is made of globin polypeptide and a prosthetic (non-protein) component called **haem group.** Each haem group consists of a **porphyrin ring** and **an iron ion** (Fe^{2+}) 2. Each subunit is arranged so that most of its **hydrophilic amino acid side chains** are on **external surface** while its **hydrophobic amino acid side chains** are buried in **interior** 3. The 4 subunits held together by intermolecular interactions formed between R groups (hydrogen bonds, ionic bonds and hydrophobic interactions). No disulphide bridges.	→Fe^{2+} **of haem group binds temporarily to O_2**, so **1 Hb molecule can carry up to 4 O_2**, at a time forming oxyhaemoglobin →Thus haemoglobin is **soluble in an aqueous environment**, can be transported and carry O_2 from lungs to tissues vice versa →As a result binding of one oxygen molecule to one haemoglobin subunit induces a **conformational change** in remaining 3 subunits so that their **affinity for oxygen increases.** This is known as the ****cooperative binding of oxygen.**
Collagen (**fibrous** protein) → an essential component of connective tissue in the human body..	1. A **collagen molecule** (aka **tropocollagen molecule**) consists of **three helical polypeptide chains** (not α-helices) wound around each other like a rope. (has quaternary structure) 2. Each chain contains about 1000 amino acids and contain a repeating sequence, usually a repeating tripeptide unit: **glycine-X-Y**, where X is usually **proline,** Y is usually **hydroxyproline.** The tropocollagen molecule can form *a* **compact coil** as almost every third amino acid in each polypeptide chain is a **glycine, the smallest amino acid.** This allows it to fit into the restricted space in the center of the triple helix. 3. Extensive **hydrogen bonds** form **between residues of the polypeptides,** hence interaction with water molecules are limited. 4. Adjacent tropocollagen molecules are arranged in a **staggered** manner 5. **Cross-linking** (involving **covalent bonds** involving **lysine residues**) of adjacent tropocollagen molecules results in the formation of fibrils. 6. Bundles of fibrils together form large and long collagen fibres. 	→**Bulky** and relatively **inflexible** proline and hydroxyproline residues confer **rigidity** on the molecule. →**Increases tensile strength** (ability to resist snapping due to stretching) and **increases tensile strength** and makes the molecule **insoluble in water** →**Staggered/overlapping arrangement minimizes points of weaknesses along fibrils** →Greatly **increases tensile strength.** →Large size of collagen makes it **insoluble**, an important property for a structural molecule

	Fibrous protein	Globular protein
Shape	Made up of long polypeptide chains forming **long, straight fibres.**	Made up of polypeptide chains folded into **roughly spherical** shape.
Solubility in H_2O	It is **large** and limited in its ability to form **hydrogen bonds with water →Insoluble in water** since **extensive hydrogen bonds already formed between residues** in different polypeptides.	**Polar R groups** are exposed to water molecules in the **aqueous** environment→**Soluble in water** since these polar groups can form **hydrogen bonds with water molecules.**
amino acid sequence	**Less variety of amino acids** are used to construct the protein. i.e. consists of repetitive regular sequence of amino acids. (eg tripeptide, gly-X-Y repeats in collagen)	**More variety of amino acids** are used to construct the protein i.e. consists of non-repetitive amino acid sequence
Length of polypeptide	**Length of polypeptide** may **vary slightly** between two samples of the same protein, yet protein is still functional.	**Length of polypeptide** is **always identical** between two samples of the same protein, or else protein may not be functional.
Example	collagen	haemoglobin

Carry out Biuret test for proteins

Test	Procedure	Observations and Deduction
Biuret Test (A test for **peptide bonds**)	1. Place $2cm^3$ of test solution in a test-tube 2. Add equal volume of 5% NaOH solution 3. Shake the mixture well 4. Add two drops of 1% copper sulphate solution, shaking well after each drop.	Presence of protein indicated by a **purple colour** developing slowly.

Denaturation of proteins

3D shape of a protein may be changed by:
(a) Heat (affect hydrogen bonds, hydrophobic interactions)
(b) Acids/Alkalis (affect hydrogen and ionic bonds)
(c) Inorganic ions (affect ionic bonds)
(d) Organic solvents (affect hydrophobic interactions)
(e) Mechanical forces (affect hydrogen bonds)
NB: 1. Peptide bonds not affected. 2. Strength of bonds: **hydrophobic interaction<H bonds<ionic bonds <disulphide bridges <peptide bonds**

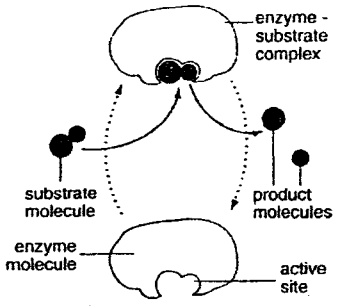
Enzymes

Explain the mode of action of enzymes in terms of enzyme specificity, active site, enzyme-substrate complex and lowering of activation energy.

- Enzymes are **biological catalysts** – they speed up the rate of reactions and are chemically unaltered at the end of the reaction & thus can be reused, and are effective in small amounts
- Most enzymes are **globular proteins**, each with an **active site** with **specific 3D conformation** that is **complementary in shape and charge** to a specific **substrate.**
- The globular structure of enzymes contribute towards their solubility.

- 2 models explain why enzymes are **highly specific**:
  1. ***Lock & key hypothesis***
     → the enzyme's (the lock) active site has a **conformation which is complementary in shape and charge to a specific substrate** (the key)
     → allowing the substrate to bind, hence forming an **enzyme-substrate complex**
     → ***products formed no longer fit the active site and will hence leave the active*** site, making it available for another substrate to bind

  2. ***Induced fit hypothesis***
     → binding of the substrate to the enzyme active site induces a **conformational change in the active site** of the enzyme such that it now provides a **more precise fit for the substrate**
     → thus enzyme can perform its catalytic function more effectively



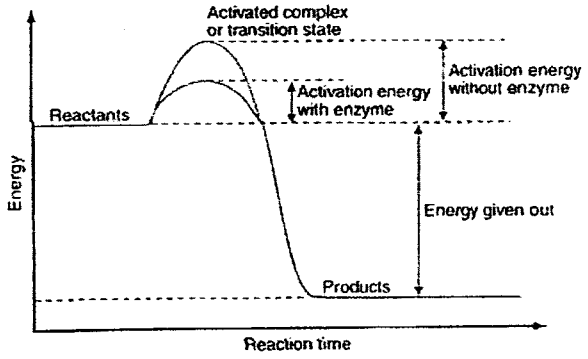
- In an enzyme-catalysed reaction:
  → **Effective collisions** take place between the enzyme and substrate
  → Resulting in the formation of an **enzyme-substrate complex**

- **Amino acids residues** that make up the enzyme have different functions:
  1. **Contact residues** – found in the **active site** – help to position the substrate in the **correct orientation** via weak interactions such as **hydrogen bonds, ionic bonds & hydrophobic interactions.**
  2. **Catalytic residues** – found in the **active site** – act on bonds in the substrate and help to catalyse the conversion of substrate to product
  3. **Structural residues** – interact with each other to maintain the overall **3D conformation** of the protein
  4. Non-essential residues – found on surface of the protein – no specific function

- Enzymes speed up metabolic reactions/allows metabolic reactions to proceed at moderate temperatures by **lowering activation energy (E_A)** of the reaction, through:

  1. **proximity effects** – temporary binding of substrates in **close proximity** in the enzyme active site increases chances of a reaction (vs depending only on random collisions between reactants in the absence of enzymes)
  2. **strain effects** – **slight distortion of substrates** as they bind to enzyme **strains bonds in substrates** that need to be broken for products to form
  3. **orientation effects** – substrates are held by enzymes in their active sites in an the **correct orientation** such that the **bonds in substrates will be exposed to chemical attack**
  4. **microenvironment effects** – enzymes **create the appropriate microenvironment for a reaction** to occur e.g. hydrophobic amino acids in enzymes create a water-free zone that allows non-polar reactants to react more easily
  5. **acid-base catalysis** – **R groups of acidic and basic amino acids** in enzyme active site **facilitate reaction** between substrates

  Hence there will be a **greater proportion of substrate molecules with energy greater than the activation energy**, allowing the reaction to proceed faster than a uncatalysed reaction.



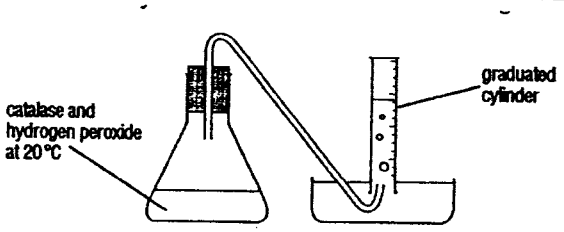
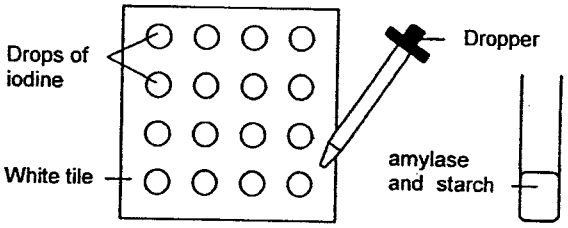
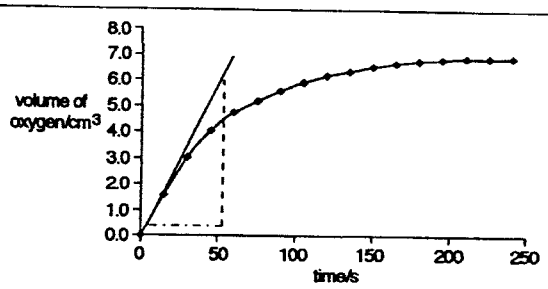
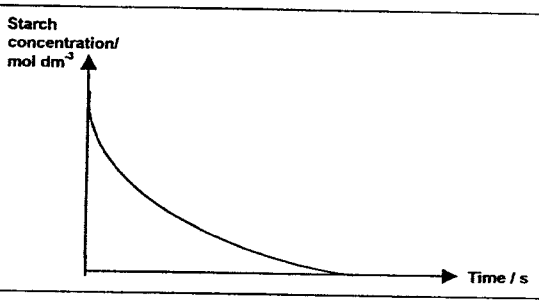
- **Enzyme cofactors**

  1. **inorganic ions** (e.g. magnesium ions in PCR)
     → mould enzyme or substrate and allows enzyme-substrate complex to form more easily

  2. **prosthetic group** (e.g. haem group of cytochrome oxidase in electron transport chain in inner mitochondrial membrane accepts electrons from cytochrome C and transfers them to oxygen to form water)
     → permanently bound to enzyme
     → transfers atoms/chemical groups between active site of enzyme and another substance

  3. **coenzymes** (e.g. NAD transfers electrons in certain redox reactions in respiration)
     → are organic molecules that are required by certain enzymes to carry out catalysis
     → they bind to the active site of the enzyme and participate in catalysis but are not considered substrates of the reaction
     → function as intermediate carriers of electrons or specific atoms that are transferred in the overall reaction

Following the time course of an enzyme-catalysed reaction by measuring the rates of formation of products (eg. using catalase) or rate of disappearance of substrate (eg. using amylase)

Method	Measuring rate of product formation	Measuring rate of disappearance of substrate
Example	$2H_2O_2 \xrightarrow[\text{catalase}]{} 2H_2O + O_2$	Starch $\xrightarrow[\text{amylase}]{}$ Reducing sugars (detected by iodine) 1) blue-black in presence of iodine → incomplete digestion of starch 2) brown in presence of iodine → complete digestion of starch
Dependent variable	Volume of O_2 evolved per unit time / over a fixed period of time	Change in intensity of blue-black colouration of starch-iodine complex per unit time / over a fixed period of time
Fixed variable	Concentration of substrate and enzyme kept constant All other conditions that affect an enzyme-catalysed reaction, eg. pH, temperature kept constant	Concentration of substrate and enzyme kept constant All other conditions that affect an enzyme-catalysed reaction, eg. pH, temperature kept constant
Experimental set-up	 Note: Delivery tube may be attached directly to a gas syringe.	
Procedure	1. Add enzyme (catalase) to H_2O_2 , mix and start the stopwatch. 2. O_2 evolved can be measured by the downward displacement of water in a graduated cylinder (as shown above) or using a frictionless gas syringe. 3. Record volume of O_2 evolved at fixed time intervals	1. Add enzyme (amylase) to starch and start the stopwatch immediately 2. Add fixed time intervals, transferred a drop of the reaction mixture to a white tile with a drop of iodine 3. In the presence of starch, iodine turns from yellowish brown to blue-black 4. Intensity of the blue-black colouration can be measured using a colorimeter 5. Use a standard curve to convert the colorimeter reading to starch concentration
Graph		
Rate	Initial rate of reaction = Gradient of curve at time 0 sec	Initial rate of reaction = Gradient of curve at time 0 sec
Trend	Volume of O_2 evolved increases with time Rate of O_2 production decreases with time	Intensity of blue-black colouration / concentration of starch decreases with time Rate of change in intensity of blue black colouration / Rate of decrease of starch decreases with time

- To investigate how factors like pH, temperature, [enzyme] or [substrate] can affect the rate of reaction:
  - Vary independent variables e.g. pH / temperature / [enzyme] / [substrate]
  - Keep all other variables constant.
  - For each condition, plot a graph of amount of product formed vs time and obtain the initial rate of reaction.
  - Plot a graph of rate of reaction vs the factor investigated (pH / temperature / [enzyme] / [substrate])

Notes:

Investigate and explain the effects of temperature, pH, [enzyme] and [substrate] on the rate of enzyme-catalysed reactions

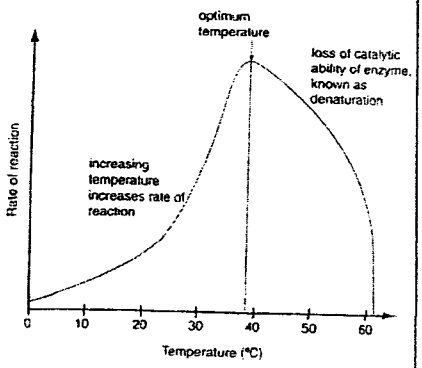
Effect of temperature

As temperature. increases,
→ K.E. of enzyme and substrate molecules increases
→ **Frequency of effective collisions** between enzyme and substrate molecules increasesses
→ **Rate of enzyme-substrate complex formation** increases
→ Number of substrate molecules with **sufficient energy to overcome the activation energy barrier** and form products increases and **rate of reaction** increases.

Temperature coefficient, Q_{10} = $\frac{\text{Rate of reaction at } (x+10)^{\circ}C}{\text{Rate of reaction at } x^{\circ}C}$

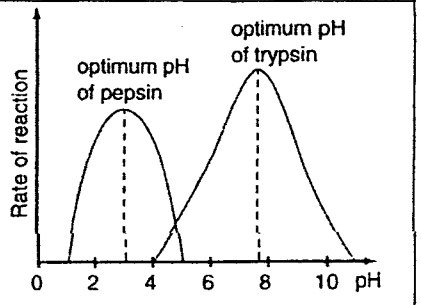
When Q_{10}=2, for **every 10°C rise in temperature**, the rate of reaction/ product formation **doubles**

Beyond the optimum temperature,
→ K.E. of enzyme and substrate molecules continue to increases
→ **Intramolecular vibrations** increases
→ hydrogen bonds, ionic bonds and hydrophobic interactions that maintain the 3D conformation of the enzyme are **disrupted**
(* Covalent disulphide bonds are harder to break and thus can withstand higher temperature. Hence enzymes with higher optimum temperature tend to have a larger proportion of disulfide bridges or more intramolecular interactions.)
→ Specific conformation of active site is lost
→ Substrate **no longer complementary to the shape and charge of active site** and cannot bind to it
→ Rate of **enzyme-substrate complex** formation **decreases** and rate of reaction decreases.



Effect of pH

At optimum pH,
→ Conformation of enzyme active site is most ideal for substrate binding and **rate of reaction** is highest
As pH **deviates from the optimum**,
→ Excess H^+ or OH^- ions affects the **ionisation of the R-groups** of the amino acids residues
→ Ionic charges on the basic (eg. NH_2) and acidic (eg. COOH) **R-groups** of the amino acid residues are altered
→ Where excess H^+ results in $-COO^-$ groups becoming -COOH and excess $-OH^-$ results in $-NH_3^+$ becoming $-NH_2$
→ Thus **ionic bonds** and **hydrogen bonds** that maintain the **conformation of the enzyme active site is disrupted**
→ Thus the interaction between substrate and catalytic residues in the active site of enzyme is disrupted
→ Rate of enzyme-substrate complex formation decreases
→ Rate of reaction/product formation decreases
(Note: if the change in pH affects the charges of the R **groups of the**
(1) **catalytic residues** in the active site, the **catalytic activity** of enzyme may be lost
(2) **contact residues** in the active site, this may **affect the temporary binding between the enzyme and substrate** and thus no enzyme-substrate complex forms.
(3) **structural residues,** the **tertiary structure** of the protein and its **active site can be affected** and this would denature the enzyme.)



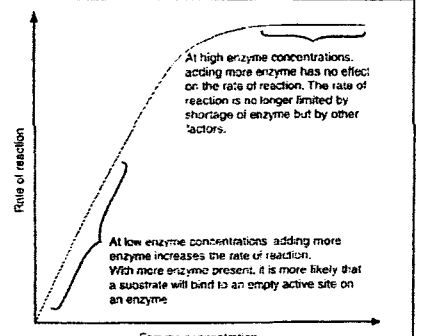
Effect of [enzyme]

Initially, when [enzyme] is low, as [enzyme] increases,
→ **Frequency of effective collisions** between enzyme and substrate molecules increases
→ **Rate of enzyme-substrate complex formation** increases and **rate of reaction** increases

At **linear portion** of graph, **[enzyme] is limiting**
→ Increasing [enzyme] will result in a **proportional increase** in the rate of reaction

At curved portion of graph, [enzyme] is not the only limiting factor. Some other factor is also limiting

At the plateau, **[enzyme] is no longer the limiting factor** (Other factors are limiting)
→ Increasing [enzyme] will not affect the rate of reaction

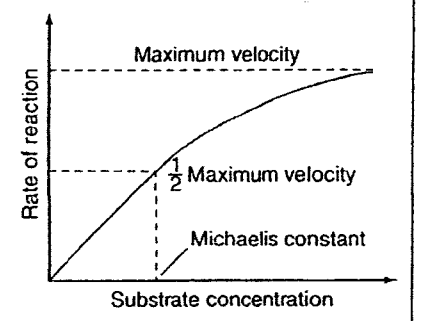


Effect of [substrate]

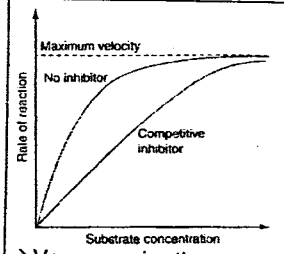
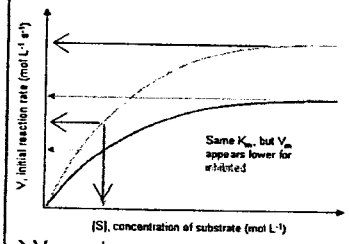
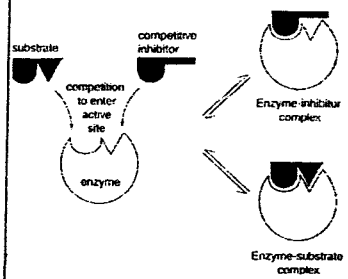
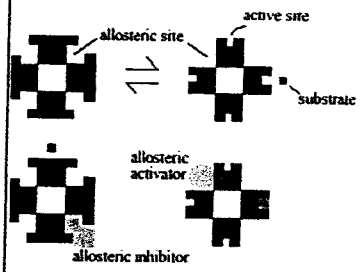
Initially, when [substrate] is low, as [substrate] increases,
→ **Frequency of effective collisions** between enzyme and substrate molecules increases
→ **Rate of enzyme-substrate complex** formation increases and **rate of reaction** increases as **active sites** of enzymes are **readily available** and **substrate concentration is limiting**

Beyond a certain [substrate],
→ All active sites of enzymes are **saturated** with substrate at any one point in time
→ **[substrate] is no longer limiting** and **enzyme concentration is limiting,** the rate of reaction will remain constant(graph plateaus) and reaches maximum velocity (V_{max}).

Michaelis constant (Km): [substrate] at which reaction proceeds at half its max. rate
→ Low Km – high affinity between enzyme & substrate
→ High Km – low affinity between enzyme & substrate



Explain the effects of competitive and non-competitive inhibitors (including allosteric inhibitors) on the rate of enzyme activity

	Competitive Inhibition	Non-competitive Inhibition	Allosteric Inhibition / Activation
Inhibitor / Activator binds to	Active site	Site other than active site	Allosteric site
Shape and charge of inhibitor / activator	Similar in conformation and charge to substrate	Not similar in conformation and charge to substrate	Not similar in conformation and charge to substrate
Structue of enzyme	• Can consist of 1 subunit with 1 active site OR • Can be a multimeric enzyme with multiple subunits, each with an active site	• Usually a 1 subunit with 1 active site	• Multimeric enzyme with multiple active sites o Presence of cooperative binding of substrate to active site • Has multiple allosteric sites o But, binding of 1 inhibitor /activator is sufficient to inhibit /acitivate the activity of the enzyme
Effect of inhibitor / activator on enzyme & the rate of the enzyme-catalysed reaction	• Inhibitor is **structurally similar to substrate** & hence binds **reversibly** to **active site** of enzyme → Hence inhibitor **competes with substrate** for active site of enzyme → This **reduces the availability of active sites for substrate binding** → rate of reaction decreases	• Inhibitor is **not structurally similar substrate** & hence binds to a **site other than the active site** → This results in a **conformational change in the enzyme active site** → Thus substrate cannot bind to active site → rate of reaction ↓ses	• Inhibitor/ Activator is not structurally similar substrate & hence binds to **allosteric site** → This results in conformational change in enzyme → Binding of inhibitor stabilises enzyme in an inactive state Thus the rate of reaction↓ses → Binding of activator stabilises enzyme in an active state Thus the rate of reaction↑ses
Effect of ↑ [substrate] on the inhibition	• At high [substrate], **substrate is more likely to bind active site** than the inhibitor to form enzyme-substrate → Hence the **same Vmax can be reached at high [substrate]** → Thus the **effects of inhibition can be overcome at high [substrate]**	• Inhibitor binds to **site other than active site** and changes the conformation of the active site → Hence the inhibitor **effectively decreases the available [enzyme] as it forms an enzyme-inhibitor complex** → Hence the effects of the inhibition **cannot be overcome by increasing [substrate]**	
Graph demonstrating effect of inhibitor / activator	 → Vmax remains the same → Km increases	 → Vmax decreases → Km remains the same	→ The enzyme freely oscillates between the active form and the inactive form. → When the activator binds to the enzymes, the active form is stabilized. → When the inhibitor binds to the enzyme, the inactive form is stabilized.
			

End-product Inhibition

→ Regulation of a metabolic pathway by the end-product of the pathway

→ **End-product** can function as an **allosteric inhibitor** to an enzyme present **early in the pathway** by binding to its **allosteric site** and prevent further synthesis of the product.

e.g. Synthesis of isoleucine from threonine.

Initial substrate (Threonine) → Intermediate A → Intermediate B ------ End-product (Isoleucine)

Enzyme 1 (threonine deaminase, an allosteric enzyme) — Enzyme 2 — Enzyme 3

Feedback Inhibition

Isoleucine binds to allosteric site of enzyme → active site of enzyme changed, no longer binds to threonine

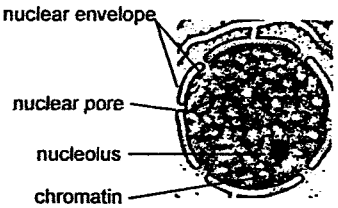
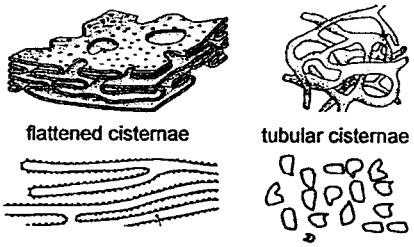
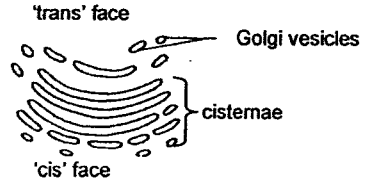
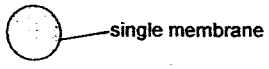
Note: Although enzyme inhibition can both be reversible & irreversible, we are more concerned with **reversible inhibitors** in our syllabus.

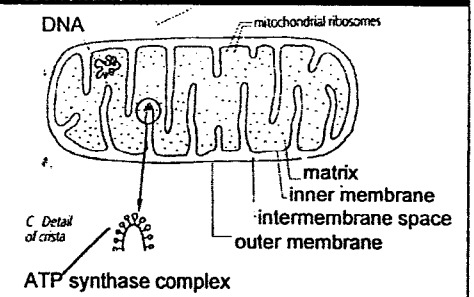
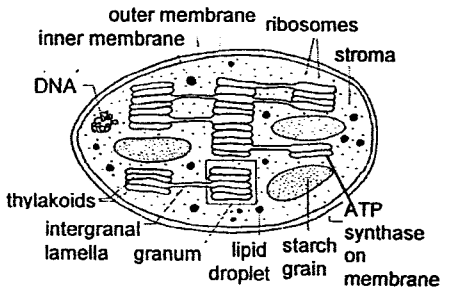
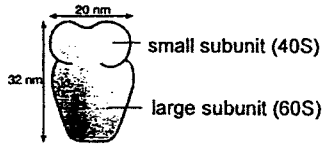
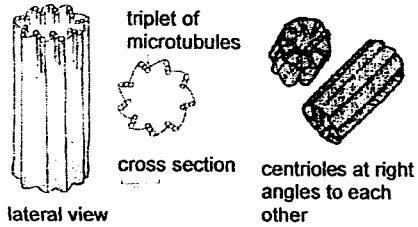
Cell Structure

Use a graticule and stage micrometer to measure cells and be familiar with units (millimetre, micrometre, nanometer) used in cell studies
*** Interconversion of units (mm, μm, nm)**
1 mm = 10^3 micrometers (μm)
1 μm = 10^3 nanometers (nm)

Describe and interpret drawings and photographs of typical animal and plant cells as seen under the electron microscope, recognising the following membrane systems and organelles: rough and smooth endoplasmic reticulum, Golgi body, mitochondria, ribosomes, lysosomes, chloroplasts, cell surface membrane (this is covered under the cell membrane summary), nuclear envelope, centrioles, nucleus & nucleolus.

Membrane-bound organelles

Organelle	Description	Function
Nucleus	**Nucleus**	
	• Prominent, **spherical** organelle in eukaryotic cell • Surrounded by a **nuclear envelope** (a double membrane) which is **perforated with pores & continuous with RER** • Contains the **nucleolus & chromatin**	• To **contain** the **hereditary material** • To **control cell activities** by **synthesising mRNA** which will be translated into proteins which are needed in the cell
	Nucleolus	
	• Non membranous, sphere/s within nucleus • Contains large amounts of DNA,rRNA & protein	• To **synthesise rRNA**, a component of ribosomes • **Assembly of large & small ribosomal subunits** using rRNA synthesised in nucleolus & proteins exported from cytoplasm
[NB: Substances pass between nucleus & cytoplasm via the nuclear pores. They are 1) free nucleotides & enzymes (for DNA replication & transcription), proteins (to make up ribosomal subunits) which enter the nucleus 2) mRNA, tRNA and large and small ribosomal subunits which leave the nucleus]	**Chromatin** • **Hereditary material** of the cell • Are **thin, elongated threads** of **DNA** coiled around **histone proteins** • 2 types of chromatin are present 1. **Euchromatin** (lightly stained, transcriptionally active, exists in a diffused, extended state) 2. **Heterochromatin** (darkly stained, transcriptionally inactive, usually found along the edge of nucleus).	
Endoplasmic Reticulum (ER)	• **Consists of the RER & SER**	
Rough ER Smooth ER 	**Rough ER (RER)** • A network of membranous **flattened sacs** called **cisternae** • Has **ribosomes** attached to the outer surface • **Continuous** with the **outer membrane** of the **nuclear envelope**	• To **transport of proteins** which are synthesised by the ribosomes on its surface to the Golgi apparatus via **transport vesicles** • To allow **proteins to fold** into their **native conformation** in the cisternal space & **glycosylate** them
1.The RER and SER together act as the membrane factory of the cell by adding membrane proteins and phospholipids to its own membrane. 2.Protein channels on the RER surface • Hold the ribosome in position • Allow the entry of polypeptides synthesised on the ribosomes on the surface into the lumen	**Smooth ER (SER)** • A network of membranous **tubular sacs** called **cisternae** • **Lacks ribosomes** on outer surface (Singular: cisterna, Plural:cisternae)	• To **synthesise lipids** and **carbohydrates** • To **detoxifiy drugs & poisons** (Thus SER abundant in liver)
Golgi Apparatus	• Membrane-bound **flattened sacs** called **cisternae** & associated Golgi vesicles • Consists of a **'forming'** or **'cis' face** where new cisternae are being formed by fusion of transport vesicles from ER & a **'maturing'** or **'trans' face** from which Golgi vesicles continuously bud off.	• To **glycosylate** to proteins and lipids to form glycoproteins and glycolipids respectively • **To modify existing glycoproteins** and **glycolipids** by modifying/cleaving the existing sugar chains • **To sort** and **package** proteins into different vesicles and **target** the proteins to different parts of the cell or for **secretion** • To form **lysosomes** • To **synthesises polysaccharides** such as pectin which is transported in vesicles to the cell membrane.
		
Lysosome	• Membranous sac containing **hydrolytic enzymes**	• To **digest material engulfed** by the cell (phagocytosis) • To **release enzymes** from cells • To **digest unwanted or worn-out organelles** (autophagy) • To **self-destruct** a cell after its death (autolysis)
	[NB: The hydrolytic enzymes work best in the acidic environment of the lysosme. Thus if a lysosome bursts, the enzymes are not very active as the cytosol has a neutral pH. However, if many lysosomes burst, then the cell will be destroyed.]	

Organelle	Description	Function
Mitochondrion 	• Spherical or rod shaped structures surrounded by a **double membrane** 1. the **outer membrane** is **smooth** 2. the **inner membrane** is highly **convoluted** with infoldings called **cristae** • Between the membranes is the **intermembrane space** • Cristae project into semi-fluid **matrix** containing **circular DNA, 70S ribosomes, phosphate granules & enzymes** for aerobic respiration • **ATP synthase complex** on inner membrane **projects into matrix**	• Acts as the **site** for certain stages of **aerobic respiration** to generate **energy** in the form of **ATP** 1) Inner mitochondrial membrane is highly folded & hence increases surface area for **oxidative phosphorylation** 2) Mitochondrial matrix is the site of the **link reaction** & the **Krebs cycle**
Chloroplast 	• Lens-shaped structure surrounded by a **double membrane** • Within the chloroplast is an **internal membrane system** which consists of flattened sacs called **thylakoids** (a stack of thylakoids = **granum**) & **intergranal lamella** • Fluid within chloroplast surrounding the grana is called **stroma** (contains **circular DNA, 70S ribosomes, enzymes & starch grains**) • **Chlorophyll molecules** are located on the **thylakoid** membrane. • **ATP synthase complex** on thylakoid membrane **project into stroma**	• Chloroplasts contain chlorophyll which convert solar energy to chemical energy through **photosynthesis** via 1) Site of **light-dependent reactions** (i.e. cyclic & non-cyclic photphosphorylation) which occurs in the **thylakoid membrane** 2) Site of **light-independent reactions** (i.e. Calvin cycle) which occurs in the **stroma** [NB: Like bacteria, both chloroplasts & mitochondria contain circular DNA, 70S ribosomes. This led to the **endosymbiont theory** which proposed that the ancestors of the mitochondria and choloplasts were oxygen using non-photosynthetic prokaryotes and photosynthetic prokaryotes respectively, that were engulfed by an ancestral eukaryotic cell.]
Non membrane-bound Organelles		
Organelle	**Description**	**Function**
Ribosome 	• Consists of a **small & a large subunit** • A complex of **protein & rRNA** • May be found intact **freely floating** in the **cytosol** or **bound to ER** (or outer membrane of nuclear envelope as the ER is continuous with the nuclear envelope) during translation. The small & large subunit only come together during translation.	• Act as the **site for protein synthesis** [NB: Free ribosomes produce proteins that function within the cytosol while bound ribosomes synthesise proteins meant for insertion into membranes, packaging within organelles or secretion out of cell.]
Centrioles 	• A **pair** of hollow cylinders made up **of 9 triplets of microtubules** (hollow tubes made of the protein tubulin) each • The two rod-like cylinders are positioned at **right angles** to each other • Found in a region called the **centrosome** which is the microtubule organising centre (MTOC)	• To act as **microtubule organising centre** (MTOC) during **spindle formation** in cell division [NB: 1) During cell cycle, the centrioles replicate & move to opposite ends of the cell. They play a role in nuclear division in animal cells by helping to organise the spindle fibres (which are made up of microtubules) 2) In higher plants, centrioles are absent.]

The **endomembrane system** consists of the nuclear envelope, rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), Golgi apparatus (GA), lysosomes and various vesicles and the plasma membrane.

DNA is transcribed in the nucleus to mRNA→mRNA leaves the nucleus via the nuclear pores→mRNA is translated into polypeptides on the ribosomes of the RER→polypeptides enter the lumen of the cisterna of the RER where it undergoes modification→transport vesicle buds off from the RER and carry the proteins to the GA →vesicle fuses with the 'cis' face of the GA and the proteins undergoes further modification, sorting and packing →a secretory vesicle containing the protein will bud off from the 'trans' face of the GA and be transported to and fuse with the cell surface membrane, releasing the protein content of the vesicle by exocytosis. **Microtubules direct the movement of the transport vesicle to the GA and the secretory vesicle to the cell surface membrane.**

Explain and distinguish between resolution and magnification

Resolution: The minimum distance whereby two points can be separated and still be distinguished as two points.

Magnification: The degree to which the viewed image is larger than the specimen.

M: Magnification : $\frac{\text{Length of drawing}}{\text{Length of specimen}} = \_\_\_X$ **(NB: 1smallest division of eyepiece graticule under Low Power (X10 objective) = 10μm**
1smallest division of eyepiece graticule under High Power (X40 objective) = 2.5μm)
(Units usually used is micrometers)

Annex: (FYI only)

Draw plan diagrams of tissues and calculate linear magnifications of drawings

* When making drawings remember, **STAMP**

S: Size of drawing must occupy 2/3 of given space

T: Title must include : plane of section, name of specimen, name of tissue / cell,magnification e.g. T.S. of epidermal cell of onion (LP)

A: Annotation e.g. cytoplasm starts to pull away from cell wall (not just cytoplasm)

M: Magnification

P: Proportion: e.g. Cell wall cannot be too thick when drawing a plant cell. It must be proportional to the rest of the cell.

Cell Membranes

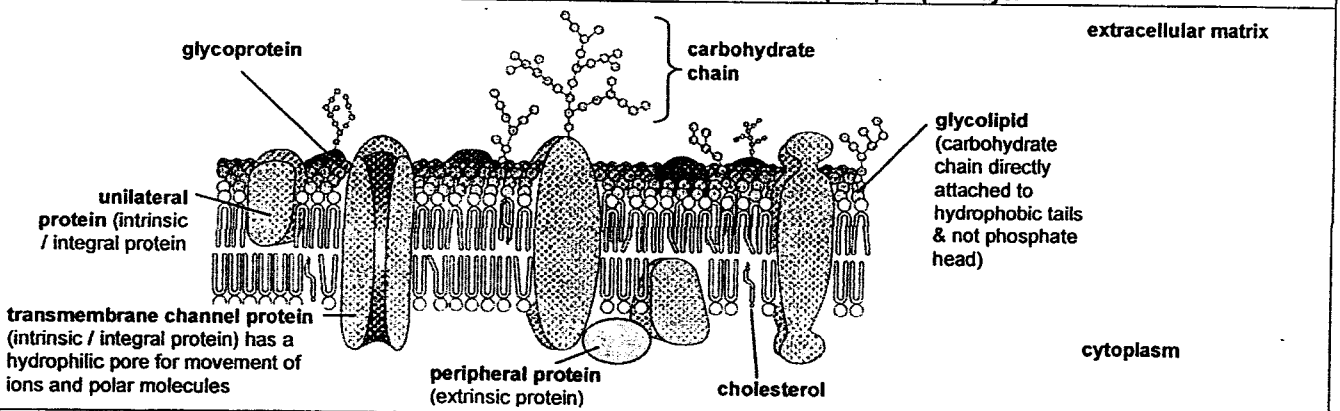
Describe and explain the fluid mosaic model of membrane structure, including an outline of the roles of phospholipids, cholesterol, glycolipids, proteins & glycoproteins

Plasma membrane or Cell surface membrane

➔ **about 7.5nm thick**

➔ **said to have a fluid mosaic model structure**

- 'Fluid' because the membrane is a **dynamic** structure where the phospholipids & proteins are able to **move** (**lipids** can move both **laterally** & **transversely (flip flop)** while **proteins move laterally** due to weak interactions between the biomolecules)
- 'Mosaic' because of the **random** arrangement of **proteins** which are **embedded** in the **phospholipid bilayer**



Components	Characteristics of components	Functions
Phospholipid bilayer	- Each phospholipid molecule is **amphipathic** i.e. has 1 **hydrophilic, negatively charged** phosphate head & 2 **hydrophobic, non-polar hydrocarbon tails** all attached to a **glycerol backbone** - In an **aqueous** environment, they arrange to form a phospholipid bilayer where the **phosphate heads** interact with the **aqueous medium** and the hydrocarbon tails form a **hydrophobic core** in the interior of the bilayer	-As a major **component of cell membranes**, it 1) Acts as a **boundary** between the intracellular & extracellular environment 2) Allows **compartmentalisation** within cell ➔ hence it **regulates the movement** of substances into & out of the cell/cell compartments by acting as a **barrier to movement of ions, polar** and **large molecules** (NB:The **charge, polarity** and **size** of molecule influence their ability to pass through the cell membrane.The presence of **unsaturated hydrocarbon tails with kinks** will affect the fluidity of the membrane.)
Cholesterol	- Found in between phospholipid mlcs in membranes of eukaryotes - Has a characteristic **4 ring** structure - **Slightly amphipathic** as it has a **hydrophilic, polar, hydroxyl group** & a **hydrophobic 4 ring** structure - The hydroxyl group of cholesterol aligns with the charged phosphate heads of the phospholipids while the rest of it is tucked into the **hydrophobic core** of the membrane.	- Cholesterol **regulates membrane fluidity** i.e. it **stabilises** the membrane. ➔ The membrane is prevented from being overly fluid at warmer temperatures as cholesterol restricts phospholipid movement through its interactions with the phospholipids. ➔ The membrane is prevented from being overly firm at lower temperatures as cholesterol prevents the close packing of phospholipids and hence prevents solidification/crystallisation. - Cholesterol **stabilizes** the lipid bilayer due to van der Waals interactions between the rigid fused ring structure and the lipid bilayer
Proteins	- 3 types : unilateral, transmembrane & peripheral - Have domains that are hydrophobic(aas with non-polar R gps) & hydrophilic (aas with polar or charged R gps) Thus are said to be **amphipathic**. [NB: Transport across membranes is vital to a cell, 1) for the excretion of waste such as urea to maintain homeostasis in the cell; 2) for the intake of nutrients such as glucose which acts as a respiratory substrate to provide energy in the form of ATP for the cellular activites; 3) for the maintenance of ionic gradients across nerve cells so that impulses can be transmitted; 4) for the secretion of synthesised products such as hormones like insulin to the bloodstream to maintain blood glucose levels.]	- Function as channels/carriers for facilitated diffusion, osmosis, active transport 1) **Channel Proteins** ➔ are **transmembrane** proteins ➔ **may be gated**, which means they can open to allow the diffusion of solutes or they can be closed to prohibit diffusion. Thus enables cells to regulate the movement of solutes. e.g. voltage gated Na^+ channel in neurons ➔ **may not be gated** i.e. forms an open passageway for the direct diffusion of ions or molecules across the membrane from a high to a low solute concentration. e.g. aquaporins 2) **Carrier Proteins** (have 2 alternative conformations) ➔ bind the solute on **one side** of the membrane and as a result the protein undergoes a **conformational change** that allows access of the solute to the **opposite side** of the membrane. e.g. glucose transporter ➔some are pumps that usually use **ATP** to move solutes **against a concentration gradient** (from a low solute concentration to a high solute concentration). e.g. Na^+ - K^+ pump The above two types of proteins are necessary for the movement of charged particles (e.g. H^+) and polar, uncharged molecules (e.g. glucose, water). In contrast, non-polar mlcs can penetrate the hydrophobic core of the bilayer. - Function as **enzymes** (e.g. acetylcholinesterase which are found on post-synaptic membrane to hydrolyse neurotransmitter acetylcholine) - Function as **receptor proteins** (e.g. insulin receptor) to which a specific ligand will bind to. The formation of the ligand-receptor complex will initiate an intracellular signaling cascade for signal transduction.) - Function to **stabilise membrane structure** as they are non-covalently bound to cytoskeleton (on cytoplasmic side) & extracellular matrix (on extracellular side)
Glycoproteins	Carbohydrate chains associated with membrane proteins	As the sugar component can be very diverse the carbohydrate chains can - Function as **markers/recognition sites** in **cell-cell recognition and adhesion** e.g. allows cells to be attached to one another to form tissues and organs; - Function as **receptors** e.g. for specific chemicals like hormones
Glycolipids	Carbohydrate chains associated directly with hydrophobic tails of membrane (& not the phosphate head)	

Outline the roles & functions of membranes within cells and at the surface of cells

1) Membranes are a **partially permeable barrier** which act as a **boundary** a) between inside and outside of cell, (b) between organelle and cytoplasm (e.g. Golgi apparatus & cytoplasm) & (c) betw. compartments within an organelle (e.g. mitochondrial matrix & intermembrane space).
2) Membranes allows for **compartmentalisation** which allow
   (i) **unique environments** to be formed for highly specialised activities
   (e.g acidic environment in lysosomes for hydrolytic enzymes to work)
   (ii) **spatial separation** of biochemical processes & thus their **sequential operation** within a cell
   (e.g. protein modification in RER and further protein modification, sorting and packaging in the GA)
   (iii) **accumulation** of **ions** to high concentrations
   (e.g.accumulation of a high concentration of H^+ in the intermembrane space of the mitochondria enable a proton gradient to be established for chemiosmosis)
3) Membranes act as a **surface for chemical reactions** to occur in a sequential manner
   → membranes may have functionally-related proteins grouped together so that sequential biochemical processes can occur
   (e.g. the thylakoid membranes of the chloroplast have electron carriers & ATP synthetase for chemiosmosis to occur.)
4) Membranes **increase surface area** for chemical reactions
   (e.g. inner mitochondrial membrane is highly folded to hold more electron transport chains and ATP synthetase)
5) Membranes surface topography enable **communication** of cell with surroundings
   → the unique combination of proteins/glycoproteins/glycolipids on surface of different cells enable
   (a) **cell-cell recognition** and **adhesion** so that tissue formation is possible,
   (b) **viruses** to recognize and infect **host cells** and
   (c) ligands to recognized specific receptors so that **signal transduction** can occur.

(h) (Knowledge of diffusion, osmosis, active transport, endocytosis and exocytosis is required)

Type of transport	ATP reqmt	Trpt protein reqmt	Movement across conc. gradient	Something to note
Diffusion	no	no	down	Definition: Net movement of mlcs. or ions from a region of high concentration to a region of low concentration, down a concentration gradient. e.g. O_2 diffuses from the lungs to the blood
Facilitated diffusion	no	yes	down	- Transport proteins facilitate diffusion of substances that are insoluble in phospholipids bilayer e.g. 1) transmembrane hydrophilic channel proteins (e.g. aquaporins) 2) carrier proteins (e.g. glucose transporters) 3) voltage gated protein channels (e.g voltage-gated Na^+ channels) open & close when an action potential is generated in nerve cells.
Active transport	yes	yes	up	Definition: Energy (ATP) consuming transport of mlcs. or ions across a membrane through a transmembrane transport protein against a concentration gradient. - Requires ATP & transports mlcs. across a membrane - Energy is required because the substance is moving against its natural tendency to diffuse in the opposite direction - e.g. Na^+-K^+ pump (e.g. in maintenance of polarised state of nerve cells) - Movt. of each mlc or ion is in one direction (unlike diffusion which is reversible)
Bulk transport	yes	no	down/up	- Requires ATP but not considered as active transport as it **does not** transport mlcs. across a membrane through a transmembrane transport protein. (However, it is an active process.) 2 types: **1)Exocytosis:** Secretion of macromolecules (e.g. waste materials) to the exterior of the cell by fusion of vesicle with the plasma membrane **2)Endocytosis:** Infolding or extension of cell surface membrane to form a vesicle or vacuole, thus allowing cell to aquire macromolecules and particulate matter respectively. a)**Phagocytosis:** solids taken into cell via a vacuole (cell 'eating')(e.g. white blood cells engulf bacteria) b)**Pinocytosis:** liquids taken into the cell via vesicles (cell 'drinking')(e.g. human egg cell takes up nutrients from surrounding follicles)
Osmosis	no	no	down	Definition: Net movement of water from a region of high water potential to a region of low water potential down a water potential gradient thru' a partially permeable membrane.

Annex:
Investigate the effects on plant cells of immersion in solutions of different water potentials. *(ONLY FOR PRACTICALS)*

- **Water potential** of a solution → tendency for water molecules to leave a solution.
  In plant cells, water potential depends on:
  1) the extent by which dissolved solutes lowers water potential i.e. the solute potential
  2) pressure exerted by the cell wall against the cell surface membrane i.e. pressure potential

Water potential of a plant cell = Solute potential of cell contents + pressure potential of cell wall

Ψ_w = Ψ_s + Ψ_p

The SI unit is Pascal (Pa).

- **Water potential of pure water is zero.** This is the maximum for water potential.

When we add solute,	(1) Ψ_w becomes –ve	(2) concentration of solute increases
When we add more solute,	(1) Ψ_w becomes more –ve	(2) concentration of solute increases more
When we add even more solute,	(1) Ψ_w becomes even more –ve	(2) concentration of solute increases even more

- **At incipient plasmolysis, $\psi_p = 0$.** Thus $\psi_w = \psi_s$ → the plasma membrane just starts to pull away from the cell wall.
- Net water movement occurs from a region of **high water potential** to a region of **low water potential** down a **water potential gradient**.

CELL & NUCLEAR DIVISION

Describe, with the aid of diagrams, the behaviour of chromosomes during mitosis & meiosis & the associated behaviour of the nuclear envelope, cell membrane & centrioles (Names of the main stages are expected, but not the sub-divisions of prophase.)

(1) INTERPHASE
1. Organelle synthesis occurs in the G_1 & G_2 Phases
2. **DNA replication** occurs during **S phase**

(2) PROPHASE I
1. Chromatin threads condense. Each chromosome is visible as two sister chromatids joined at the centromere
2. **Synapsis** occurs i.e. homologous chromosomes pair up to form **bivalents**
3. **Crossing over** occurs between **non-sister chromatids** of homologous chromosome pairs, forming **chiasmata** (sites of crossing over). **Corresponding segments** of non-sister chromatids are **exchanged**
4. Centrioles migrate to opposite poles and spindle fibres extend from each pole to kinetochores & metaphase plate
5. Nuclear envelope disintegrates
6. Nucleolus disappears

(3) METAPHASE I
1. Kinetochore microtubules align homologous pairs at the **equator** in **two rows** with each chromosome on either side of the equator
* The orientation of chromosomes of each bivalent is completely independent of the orientation of the other bivalents.

(4) ANAPHASE I
1. **Kinetochore microtubules shorten** and homologues are led by their **centromeres** to opposite poles. **Non-kinetochore microtubules** elongate causing the 2 poles to move further apart. (Centromeres ~~do not divide~~)

(5) TELOPHASE I
1. Each pole has a haploid set of **chromosomes**
2. Chromosomes **uncoil** into **chromatin**
3. Spindle fibres disintegrate
4. Nuclear envelope reforms around the chromosomes at each pole & nucleoli reappear

(6) CYTOKINESIS (See step 6 of mitosis)
2 HAPLOID daughter cells result

(7) PROPHASE II
1. Chromatin threads condense
Each chromosome is visible as two sister chromatids joined by a centromere
2. Centrioles duplicate & migrate to opposite poles. Spindle fibres extend from each pole to kinetochores & metaphase plate
3. Nuclear envelope disintegrates
4. Nucleolus disappears

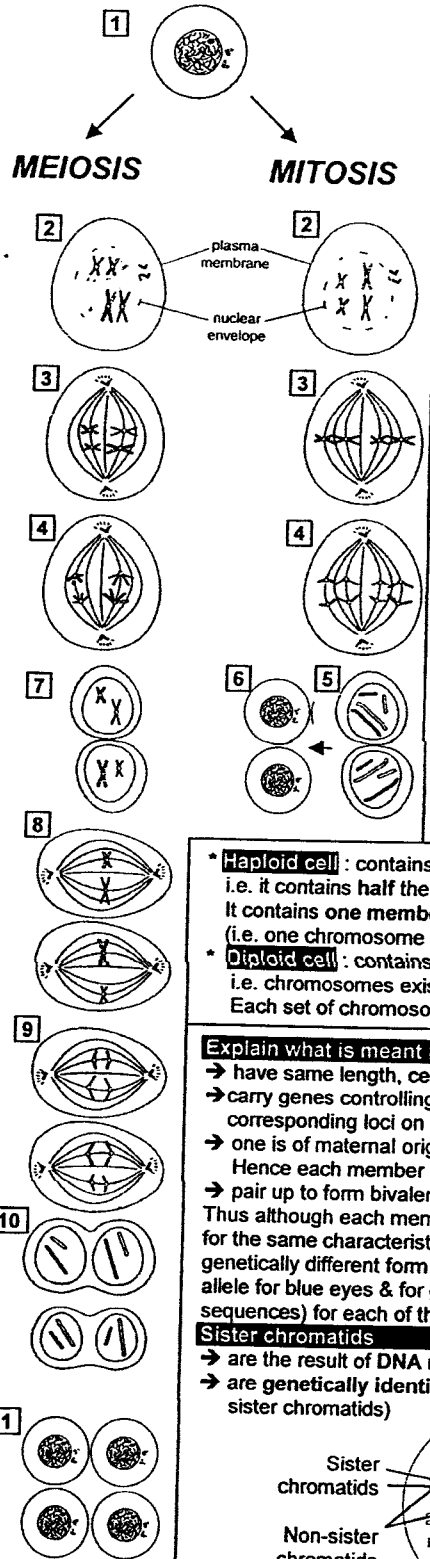
(8) METAPHASE II
1. Kinetochore microtubules align chromosomes at the **equator** in **one row**
The orientation of sister chromatids of each chromosome is completely independent of the orientation of the others

(9) ANAPHASE II
1. **Centromeres** of each chromosome **divides**
2. Kinetochore microtubules shorten & pull the Sister chromatids (now called chromosomes) **centromeres first** to opposite poles. **Non-kinetochore microtubules** elongate causing the 2 poles to move further apart.

(10) TELOPHASE II
1. Chromosomes **uncoil** into **chromatin**
2. Spindle fibres disintegrate
3. Nuclear envelope reforms around the chromosomes at each pole of both cells
4. Nucleoli reappear

(11) CYTOKINESIS (See step 6 of mitosis)
4 HAPLOID daughter cells result

NOTE :(i) Some cells skip telophase I and cytokinesis, entering prophase II directly after anaphase I.There is no interphase II.**(ii)** Centromeres divide NOT SPLIT



(1) INTERPHASE
1. Organelle synthesis occurs in the G_1 & G_2 phases
2. **DNA replication** occurs during **S phase**

(2) PROPHASE "prominent"
1. Chromatin threads condense. Each chromosome is visible as two sister chromatids joined at the centromere
2. Centrioles migrate to opposite poles & spindle fibres extend from each pole to kinetochores & metaphase plate
3. Nuclear envelope disintegrates
4. Nucleolus disappears

(3) METAPHASE "meet"
1. Kinetochore microtubules align chromosomes at the **equator** in **one row**.

(4) ANAPHASE "apart"
1. **Centromeres** of each chromosome **divide**
2. **Kinetochore microtubules shorten** and pull the sister chromatids (now called chromosomes) **centromeres first** to opposite poles. **Non-kinetochore microtubules** elongate causing the 2 poles to move further apart

(5) TELOPHASE "the end"
1. Chromosomes **uncoil** into **chromatin**
2. Spindle fibres disintegrate
3. Nuclear envelope reforms around the chromosomes at each pole & nucleoli reappear

(6) CYTOKINESIS
Animal cells: Cell membrane invaginates towards the middle, forming a **cleavage furrow**. Cell membranes join up and separate the 2 daughter cells
Plant cells: Vacuoles appear in the middle of the cell. They **coalese** to form a **cell plate**, separating the 2 daughter cells

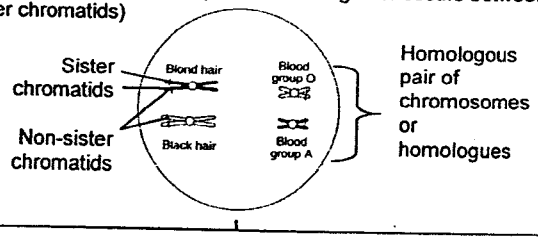
* **Haploid cell** : contains **one complete set** of chromosomes
i.e. it contains **half** the number of chromosomes as a diploid cell.
It contains **one member of each homologous pair** of chromosomes (i.e. one chromosome from either parent)

* **Diploid cell** : contains **two complete sets** of chromosomes
i.e. chromosomes exist as homologous pairs
Each set of chromosomes is from one parent.

Explain what is meant by homologous pairs of chromosomes
- → have same length, centromere position & staining pattern
- →carry genes controlling the same inherited characteristics at the corresponding loci on both members of a homologous pair
- → one is of maternal origin + one is of paternal origin
Hence each member of a pair is genetically different from each other
- → pair up to form bivalents during prophase I of meiosis

Thus although each member of the homologous pair may have genes coding for the same characteristics (e.g. eye colour) at corresponding loci, they are genetically different form each other as they usually have different alleles (e.g. allele for blue eyes & for green eyes, each of which have different nucleotide sequences) for each of the characteristics.

Sister chromatids
- → are the result of **DNA replication** and thus have the **same alleles**
- → are **genetically identical** (before crossing over occurs between non-sister chromatids)



* **Q: What is the difference between chromosomes & chromatin?**
Chromatin: Complex of DNA & protein
Chromosome: 1) Uncondensed form → a mass of long, thin, thread-like fibres of chromatin.
2) Condensed form → chromatin that has been condensed by coiling and folding many times upon itself & appear is short, thick, structures

Explain the need for the production of genetically identical cells & the fine control of replication.

- During **S phase of interphase/before mitosis,**

  ↓

  replication of DNA occurs by semi-conservative replication where both parental DNA strands are used as template for **complementary base pairing**

  ↓

  This ensures that **2 gentically identical DNA molecules** (i.e. **sister chromatids**) form

- During **anaphase, separation of identical sister chromatids** occurs when centromeres separate and the resulting chromosomes are pulled to opposite poles of the cell

  ↓

  This ensure **even distribution of the DNA in the daughter cells** which will thus contain the **same hereditary material/same alleles/ same DNA/ same base sequence / same number of chromosomes**

- Thus daughter cells produced during mitosis are genetically identical to the parents i.e. **no genetic variation occurs.**
  These **cells will have all the genes necessary for survival of the cell/organism** and will be **faithfully inherited with every replication cycle** so that the resulting cells can continue to function normally.

  → Thus **mitosis** allows for **growth, repair & asexual reproduction** which require the production of genetically identical cells. (i.e. cells are clones of each other) .

Explain the importance of mitosis in growth, repair & asexual reproduction.

- Mitosis is a form of **nuclear division** which results in the formation **two nuclei/cells** that are **genetically identical** to the parents i.e. mitosis maintains genetic stability.

- Mitosis is important in

(1) Growth → as it allows for an increase in the number of genetically identical cells in a multicellular organism & contributes to an increase in its size & mass.

(2) Repair → as it allows damaged/worn-out cells to be replaced (e.g. skin cells) & lost parts of an organism to be replaced (e.g. lizard's tail)

(3) Asexual reproduction → as it allows for the production of new individuals from just a single parent (e.g. vegetative propagation of onion bulbs) (The fusion of egg & sperm from two different parents is not necessary)

↓

where offspring produced are genetically identitcal to the parents and hence are already adapted to the environment that allowed the parents to thrive

↓

This allows for rapid reproduction for colonisation of the habitat

Explain the need for reduction division (meiosis) prior to fertilisation in sexual reproduction

(i) for maintenance of <u>chromosome number</u> in offspring in every generation

- During reduction division (meiosis), **diploid cells** (which have 2 complete sets of chromosomes) undergo two nuclear divisions (Meiosis I & II) to give rise to **haploid gametes** (which have only 1 complete set of chromosomes).
- During fertilisation, 2 haploid gametes (one male & one female) fuse to give rise to a diploid zygote. Hence the **chromosome number in the zygote is restored when fertilisation** occurs.
- If meiosis did not occur, the fusion of gametes during sexual reproduction will result in the doubling in the number of chromosomes with each successive generation.
- Hence reduction division(meiosis) ensures that the **maintenance of chromosome number** in a sexually reproducing species in **every generation**

(ii) for maintenance of <u>genetic variation</u> in offspring in every generation

- During meiosis, **independent assortment of chromosomes** and **crossing over** ensures a wide variety of **genetically different gametes** are produced
- This leads to a great **variation in offspring** produced which result from the random fusion of gametes during sexual reproduction.

Explain how meiosis & random fertilisation can lead to variation.

*** During meiosis,**

(a) **Crossing over** between **non-sister chromatids** of homologous chromosomes results in new combinations of alleles on chromatids. (& eventually a variety of offspring)

(b) **Independent assortment of homologous chromosomes at the metaphase plate & their subsequent separation** during metaphase I & anaphase I respectively &
Random orientation of non-identical sister chromatids of each chromosome at the metaphase plate & their subsequent separation during metaphase II and anaphase II respectively
→ results in gametes with different combinations of maternal & paternal chromosomes. (& eventually in a variety of offspring)

*** Random fusion of gametes**
→ **during sexual reproduction/fertilisation** results in offspring with a variety of genotypes & possibly phenotypes (& hence a variety of offspring)

Distinguish Between Mitosis & Meiosis

Feature	Mitosis	Meiosis
Location & cell type	Somatic cells in all parts of the body	**Precursor sex cells** in **reproductive organs** that give rise to **gametes**
No of nuclear divisions	One (Note: DNA replication occurs only once)	Two (Note: DNA replication occurs only once)
Prophase	**No synapsis/** homologues do not pair up; **No chiasma** formation; **No crossing over** of corresponding segments of non-sister chromatids;	**Prophase I** Synapsis occurs/homologues pair up to form **bivalents(tetrads)** **Chiasma** formation & **Crossing over** of corresponding segments of non-sister chromatids of homologous chromosomes (results in **non-identical sister chromatids** with **new combinations of alleles**) **Prophase II** → Similar to prophase of mitosis
Metaphase	Chromosomes, each consisting of a pair of sister chromatids, **align individually** on equator (i.e form a single row) Each centromere attaches to **spindle fibres from both poles**	**Metaphase I** **Homologous chromosomes align in pairs** at the equator (ie. form 2 rows) Centromeres of each chromosome attaches to **spindle fibers from different poles** **Metaphase II** → similar to metaphase of mitosis except that chromosomes consist of non-identical sister chromatids
Anaphase (is the least frequently observed phase as it is the shortest phase.)	**Separation of centromere;** **Separation of identical sister chromatids** to opposite poles; (NB: 1.Once centromeres separate, each sister chromatid is called a chromosome 2. During anaphase, kinetochore microtubules shorten while non-kinetochore microtubules lengthen as they slide pass each other, causing the cell to elongate.)	**Anaphase I** **NO separation of centromere;** **Separation of homologous chromosomes** (i.e. pair of sister chromatids move to same pole); **Anaphase II** → similar to anaphase except that **non-identical sister chromatids separate**
Telophase	**2 daughter nuclei** which are **genetically identical** & have the **same chromosome number as parental cells**	**Telophase I** → **2 daughter nuclei** which are **genetically different** & each has **half the chromosome number as parental cells)** **Telophase II** → **4 daughter nuclei** which are genetically different & each has **half the chromosome number as parental cells)**
Result of nuclear division	**2 genetically identical** daughter cells form; No variation occurs(in the absence of mutation); Daughter cells have the **same number of chromosomes** as parental cells → hence called **replicative** division	**4 genetically different** daughter cells form; Genetic variation has occurred (in the absence of mutation); Daughter cells with **half** the chromosome number as parental cells→ hence called **reductive** division

Explain how uncontrolled cell division can lead to cancer , & identify causative factors (e.g. genetic, chemical carcinogens, radiation, loss of immunity) which can increase the chances of cancerous growth. (Knowledge that dysregulation of checkpoints of cell division can result in uncontrolled cell division and cancer is required, but details of the mechanism is not required.)

Explain how uncontrolled cell division can lead to cancer

- Regulatory checkpoints in the cell cycle that ensure normal cell division & growth
- Dysregulation of the checkpoints in the cell cycle can result in uncontrolled cell division
- Dysregulation of the checkpoints is due to gain-in-function mutations in proto-oncogenes and loss-of-function mutations in tumour suppressor Genes. This can result in

1) excessive cell growth and proliferation (due to mutations in proto-oncogenes and/or tumour suppressor genes)
2) loss of contact inhibition (i.e. cells can grow on top of each other and form a tumour)
3) evasion of apoptosis (i.e. cells no longer undergo programmed cell death)
4) angiogenesis (i.e. blood vessels can grow into the tumour)

- If metastasis (i.e. tumour cells may separate from the primary site and migrate to other parts of the body via the bloodstream and proliferate there) then occurs, the tumour is referred to as being malignant and cancer is said to have occurred.

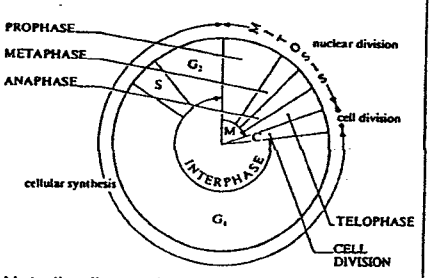
Gain-of-function mutation (dominant mutation) → e.g. when a proto-oncogene is mutated to form an oncogene
→ mutation in just one copy of the gene results in increased cell growth and proliferation due to the increased synthesis/activity of a functional product (which was not produced previously) due to mutation
Thus the mutation is said to be dominant.

Loss-of-function mutation (recessive mutations) → e.g. mutations in tumour suppressor genes
→ mutations in both copies of the gene necessary for loss of tumour suppression
→ even when one copy is mutated, the non-mutant copy still produces a functional gene product which will result in tumour suppression.
Thus the non-mutant copy will mask the effect of the mutant copy and hence the mutation is said to be recessive.

Causative factors which increase the chances of cancerous growth

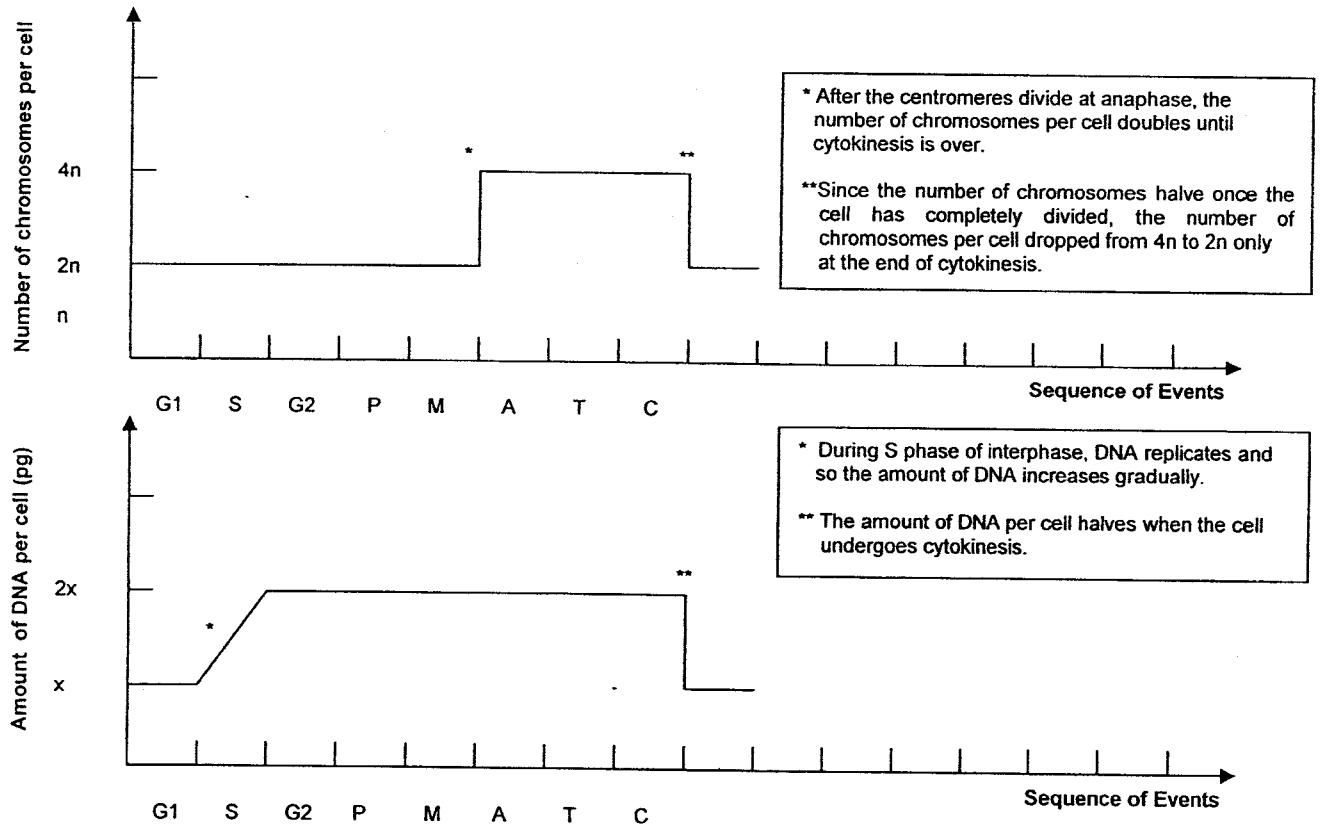
A. **Environmental factors** → exposure to carcinogens (eg. uv light, tar in cigarette smoke, ethidium bromide etc.)
B. **Infection with certain viruses** (e.g. human papilloma virus) or **bacteria** (e.g. *Helicobacter pylori*)
C. **Genetic predisposition** → due to family history
D. **Age** → chances of getting cancer increases with age due to accumulation of mutations over a lifetime.

The Cell Cycle

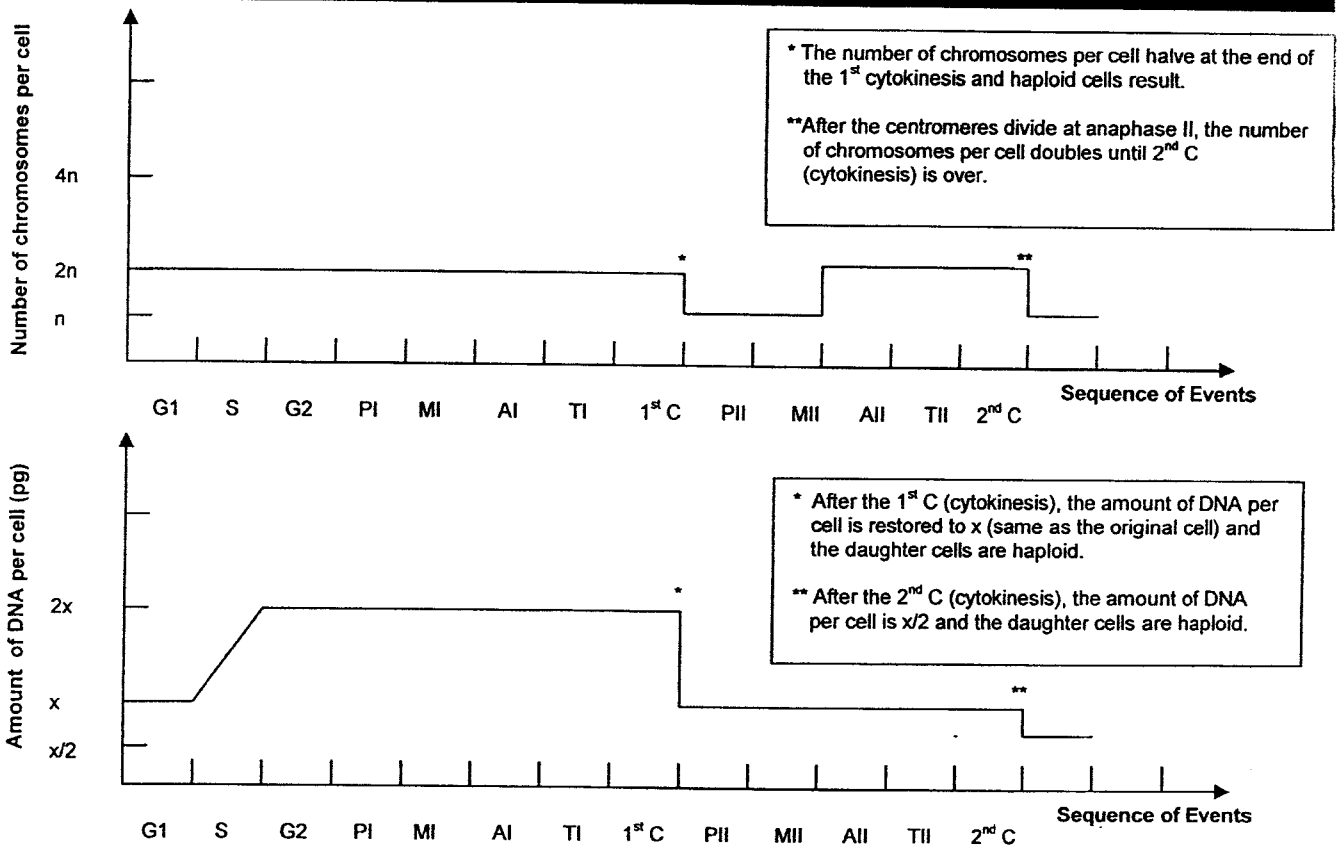


Not all cells need to be replaced at the same rate or at the same time. There are **built-in controls in the cell cycle, (i.e. checkpoints),** that determines how fast a cell divides and how many cycles it will undergo.

MITOSIS



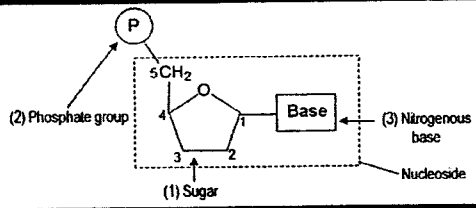
MEIOSIS



Do note that the amount of DNA **per nucleus** (not shown in the above graphs) doubles during S phase of interphase. Then, during mitosis it halves at telophase when the nuclear envelope reforms. During meiosis, the amount of DNA **per nucleus** halves during telophase I and halves again during telophase II.

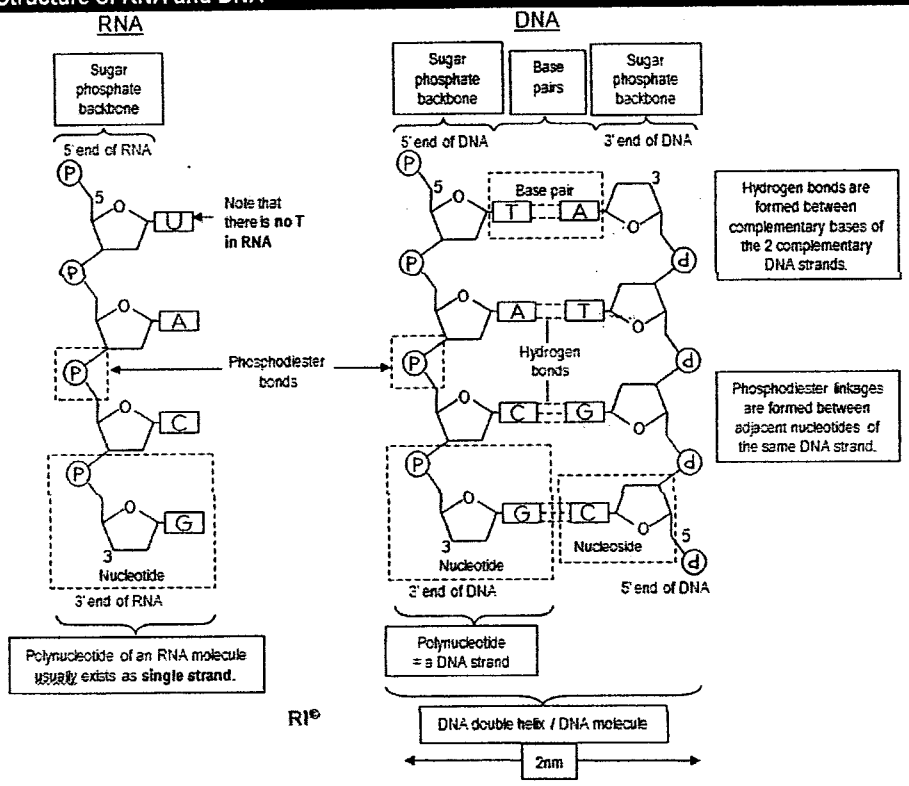
Nucleic acid		RNA (mRNA, tRNA & rRNA)	DNA
Pentose (five carbon) sugar		Ribose	Deoxyribose
Nitrogenous bases	Purines (2 rings)	Adenine & Guanine	Adenine & Guanine
	Pyrimidines (1 ring)	Cytosine & Uracil	Cytosine & Thymine
Complementary base pairing occurs between		Adenine & Uracil (2 H bonds) Cytosine & Guanine (3 H bonds)	Adenine & Thymine (2 H bonds) Cytosine & Guanine (3 H bonds)
Structure		Single Stranded	Double Stranded
Found in (location)		Cytoplasm, Nucleus	Nucleus

Structure of a nucleotide (a nucleoside + phosphate group = nuceloside monophosphate)



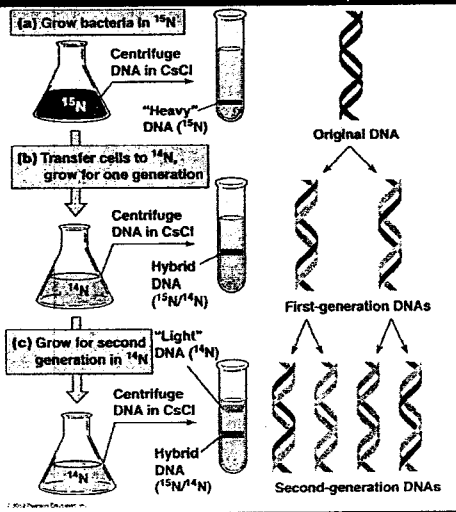
Nitrogenous base: attached to C1
Phosphate group: attached to C5
OH group attached to C3: involved in phosphodiester bond formation
If H is attached to C2 → deoxyribose sugar
If OH is attached to C2 → ribose sugar

Structure of RNA and DNA



In DNA
* A:T = 1:1 and C:G = 1:1
* (A+G) = (C+T)
  i.e. no.of purines = no.of pyrimidines
* Purines: 2 rings (baby's are pure & go googooGAGA)
* Pyrimidines: 1 ring (CUT food on dinner plate)
* Constant width between sugar phosphate backbone = 2nm
* 2 strands are anti-parallel: one strand runs in the 5' to 3' direction, while the other strand runs in the 3' to 5' direction → DNA is said to have directionality
* 1complete turn of the double helix has 10 base pairs and spans a distance of 3.4nm
* 1 DNA molecule is made up of 2 strands of DNA

Evidence for semi-conservative replication



(a) A stock of parental *E. coli* were grown for many generations in ^{15}N **medium as the only source of nitrogen** until ^{15}N was incorporated into the nitrogenous bases of all bacterial DNA.

(b) The *E.coli* containing ^{15}N-^{15}N were then **transferred into a medium containing only ^{14}N.** The transferred *E. coli* were allowed to **divide once and were then collected.** The DNA extracted and centrifuged in CsCl were **all hybrid (^{14}N-^{15}N) DNA.** This excluded conservative replication in which no hybrids form.

(c) Some of these cells were then allowed to **divide once more.** The DNA extracted and centrifuged in CsCl were **half hybrid (^{14}N-^{15}N) DNA and half "light" (^{14}N-^{14}N) DNA.** This excluded dispersive replication in which no pure ^{14}N-^{14}N can be obtained.

3 hypotheses for DNA replication mechanism:

1) Semi-conservative replication
→both strands separate by the **breaking of hydrogen bonds** and each strand acts as a **template** for the synthesis of a new strand through **complementary base pairing.**
Thus each DNA molecule formed is a **hybrid consisting of 1 original strand and 1 newly synthesized strand.**

2) Conservative replication
→2 parental strands re-associate after acting as templates, thus restoring the original double helix. The other DNA molecule consists of 2 newly synthesized strands.

3) Dispersive replication
→Parental DNA molecule is fragmented and dispersed. Daughter molecules are madeup of a mixture of old and newly synthesized parts.

Mutations

A gene mutation is an **alteration in the sequence of nucleotides** which may change the **sequence of amino acids** in a polypeptide chain. This may change the **3D shape of the protein**, affecting the **protein function** and subsequently affect the **characteristics (phenotype)** of the organism.

Type of mutation	Substitution	Inversion	Insertion	Deletion
Description	**Replacement** of one nucleotide	Two or more nucleotides are in the wrong sequence	One or several nucleotides are inserted into a sequence	One or several nucleotides are removed from a sequence
Result of mutation	1 codon changed	1 or more codons changed	Shifts reading frame from point of mutation	Shifts reading frame from point of mutation
Effect on protein	Minor/Major	Minor / Major, depending on whether a frameshift occurs	Usually Major	Usually Major
			If the number of nucleotides inserted or deleted are a **multiple of three**, there will change the primary sequence but a **frame shift will not result.**	

Examples of diseases due to gene mutation:

Name of disease	Sickle-cell anaemia	Cystic Fibrosis
Protein affected	Beta-globin chain of haemoglobin From **HbA** to **HbS**	Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
Description of change	Change in **DNA : CTC** to **CAC** (substitution) Change in **mRNA : GAG** to **GUG** Change in amino acid : **glutamate** to valine	Change in DNA: **Deletion of 3 nucleotides** on exon 10 of **chromosome 7 (deletion).** Loss of amino acid phenylalanine
Effect of the change	Charged and **hydrophilic glutamate** changed to non-polar and **hydrophobic valine** in HbS. **At low oxygen concentrations**, HbS undergoes an **conformation change** which will cause the **hydrophobic patches on different HbS to stick together. This polymerization of HbS** results in the formation of abnormal, rigid, **rod-like fibres.** Shape of **red blood cell distorted – sickle shaped.**	**Missing or defective CFTR** (a channel protein). Cl^- not transported out of the epithelial cells of the tissues, especially lungs. Na^+ and Cl^- retained within the cells cause the water potential within the cell to become **more negative.** Water retained within epithelial cells as a result. Mucus in the lumen becomes thick and undiluted. Bacterial infection may occur.
Effects of disease	Sickle red blood cells are more **fragile** and **break easily.** This results in **shortage of red blood cells** and **poor oxygen transport.** This leads to **anaemia**, lack of **energy and heart failure.** Sickle red blood cells may also **lodge in small blood vessels** and **interfere with blood circulation.** This will lead to **organ damage.**	In the lungs, thick mucus may cause **lung infections and difficulty in breathing.** In the pancreas, thick mucus may block the pancreatic duct → **indigestion.** In the sweat glands, it will prevent reabsorption of NaCl resulting in very **salty and copious sweat** production. **Death** occurs by age 30.

Process	Replication	Transcription	Translation
Location	Nucleus (also in mitochondria and chloroplasts)	Nucleus	Cytoplasm
Begins at	**Origin of replication**	**Promoter**	**Start codon** (AUG) (AUG: Are U Good?)
Ends at	Where 2 adjacent replication bubbles meet / Telomeres	**Termination sequence**	**Stop codon** (UAG,UAA,UGA) (UAG: U Are Good UAA: U Are Awful UGA:U are Good & Awful)
Template	DNA (both strands)	DNA (template / non-coding strand)	mRNA
Monomers	**Deoxyribonucleotides**	**Ribonucleotides**	**Amino acids**
Complementary base-pairing	Adenine & Thymine Cytosine & Guanine	Adenine & Uracil Thymine & Adenine Cytosine & Guanine Guanine & Cytosine	Complementary pairing between codon and anti-codon
Enzymes Involved	**DNA polymerase, Helicase, Primase, DNA Ligase, Topoisomerase**	**RNA polymerase** (Poly A polymerase & endonuclease in eukaryotes)	**Aminoacyl – tRNA synthetase** **Peptidyl transferase (a ribozyme)**
Bonds within molecule formed	**Phosphodiester bonds, Hydrogen bonds**	**Phosphodiester bonds**	**Peptide bonds**
Ribosomes involvement	No	No	Yes
Template strand is read in	3' to 5' direction	3' to 5' direction	5' to 3' direction
Molecule is synthesized in	5' to 3' direction	5' to 3' direction	from the amino end to the carboxyl end
Proof reading	Yes	-	-
Product (s)	2 DNA molecules	mRNA,tRNA rRNA,snRNA etc.	Polypeptide chain
Product destination	Nucleus	Cytoplasm	Cytoplasm/ Cell membrane/Outside cell

Role of DNA

The main role of DNA is to store information and pass it on from one generation to the next.
It is a suitable store of information as:
a) It can be **replicated accurately** → daughter cells have identical copies of DNA as the parent cell
Weak hydrogen bonding between the two strands allow them to separate and act as a template for new strand synthesis
(Adenine forms 2 hydrogen bonds with thymine and cytosine forms 3 hydrogen bonds with guanine through complementary base pairing)

b) It is a **stable** molecule → can be passed on to the next generation without loss of the coded information (Why DNA needs to be stable?)
Collectively, numerous hydrogen bonds hold the two strands of DNA together and adjacent nucleotides in each strand are joined by strong covalent phosphodiester bonds

c)There is a **backup of code**
DNA is double stranded and one strand to serve as a template for the repair of the other if a mutation occurred

d) Coded information can be **readily utilised/accessed**
Weak hydrogen bonding allows the template strand to separate from the non-template strand allowing transcription to take place
Complementary base pairing allows the faithful transfer of info from DNA to RNA in transcription, which will be translated to protein subsequently

Role of mRNA:

1) Messenger RNA (mRNA) serves as a 'messenger' that, in eukaryotes, **takes the information out of the nucleus** via the nuclear pore **to the cytoplasm** where **translation** takes place.
2) mRNA acts as a **template for translation**, as each codon within the coding region of the mRNA represents an amino acid in a polypeptide.

Role of tRNA:

They **bring in specific amino acids in a sequence corresponding to the sequence of codon in mRNA** to the growing polypeptide.
It can facilitate translation due to:
(1)its ability to **bind to a specific single amino acid**
(2)the ability of the **anticodon to base-pair with the mRNA codon**

Role of rRNA:

(1) rRNA **associates with a set of proteins to form ribosomes.**

(2) rRNA is the main constituent of the interface between the large and small subunits of the ribosome
Thus the **small ribosomal subunit can bind to the mRNA** as complementary base pairing can occur between the **rRNA in the mRNA binding site of the small ribosomal subunit and the mRNA.**

(3) rRNA is the main constituent of the P site (peptidyl-tRNA binding site) and A site (amino-acyl tRNA binding site) on the large ribosomal subunit
Hence rRNA **enables the binding of aminoacyl-tRNAs to the P site and A site**

(4)An rRNA molecule (peptidyl transferase) on the large ribosomal subunit also **catalyses the formation of the peptide bond** between the amino group of the new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site.

DNA REPLICATION

1. **Before DNA replication,** free deoxyribonucleoside triphosphates are **manufactured in the cytoplasm** and transported into the nucleoplasm via nuclear pores.
2. DNA replication occurs at **S phase of interphase.**

UNZIPPING OF PARENTAL STRAND

3. Replication begins at specific points of the DNA molecule each of which is known as an **origin of replication (*ori*).**
4. **Helicase** binds to origin of replication. It **disrupts hydrogen bonds** between complementary base pairs, causing parental strands to **unzip and separate.**
5. **Single-strand binding proteins** keep the strands **apart** so that they can **serve as templates** for the synthesis of new strands.
6. **Topoisomerase relieves "overwinding" strain ahead of replication forks** by breaking, swiveling and rejoining DNA strands.

ADDITION OF PRIMER

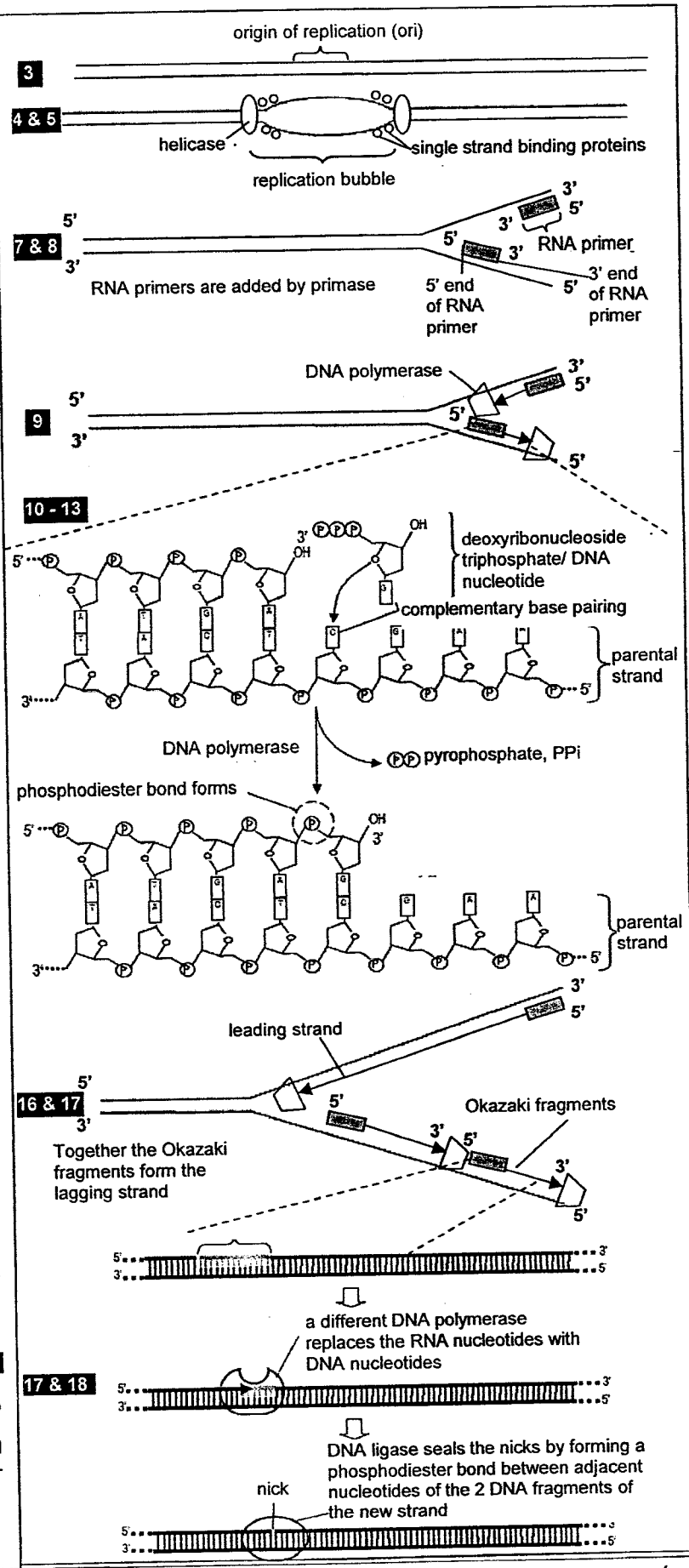
7. **RNA primer** is added to each template (parental) strand by the enzyme **primase.**
8. RNA primer provides a **free 3' OH end** for **DNA polymerase** to recognise and start DNA synthesis of the complementary daughter strand.
9. DNA polymerase can only add **deoxyribonucleotides** (DNA nucleotides) to a **pre-existing 3'OH end** of a nucleotide.

SYNTHESIS OF DAUGHTER STRANDS

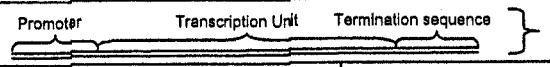
10. DNA **polymerase** uses the parental strand as a template and aligns the **free activated deoxyribonucleoside triphosphates (dNTPs) in a sequence complementary** to that of the **parental strand.**
11. **Adenine** base pairs with **Thymine** and vice versa. **Guanine** base pairs with **Cytosine** and vice versa.
12. DNA polymerase catalyses the formation of **phosphodiester bonds between adjacent daughter DNA nucleotides of the newly synthesised strand.**
13. Removal of the pyrophosphate (PPi) from the deoxyribonucleoside triphosphate (dNTP) and the subsequent hydrolysis of PPi provides the energy to drive the polymerization reaction.
14. As DNA polymerase moves along the template, it **proof reads** the previous region for proper base pairing. Any **incorrect deoxyribonucleotide is removed and replaced** by the correct one.
15. The **leading strand** is synthesized **continuously** in the **5' to 3' direction.**
16. The **lagging strand** is synthesized **discontinuously.** Its synthesis is similar to leading strand, **except that the lagging strand is synthesised in fragments** known as **Okazaki fragments.** Each fragment is initiated by an RNA primer before the addition of DNA nucleotides.
17. A **different DNA polymerase** then **excises** the **RNA primer** and **replaces** it with deoxyribonucleotides.
18. **DNA ligase seals the nicks** by forming **phosphodiester bonds** between adjacent nucleotides of the each of the DNA fragments on the new strand.

END OF REPLICATION

19. Complementary parental and daughter strands **rewind** into a double helix.
20. Each resultant helix consists of **one parental strand** and **one daughter strand.** Hence this is called **semi-conservative DNA replication.**



TRANSCRIPTION :the synthesis of RNA using a DNA template
(occurs in nucleus)



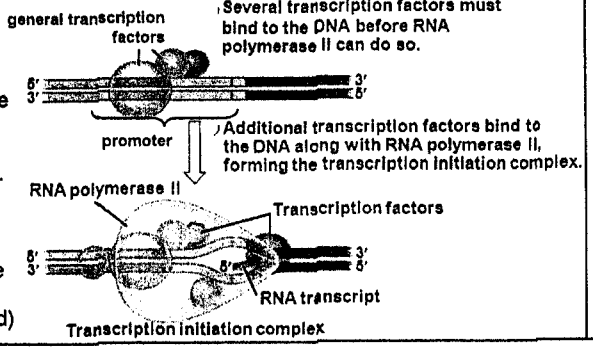
3 main components of a gene

IN EUKARYOTES

INITIATION

1. RNA polymerase is a **multimeric** complex.
2. **General transcription factors** first **assemble along the promoter.**
3. General transcription factors **recruit RNA polymerase & position it** correctly on the promoter.
4. The complex of RNA polymerase and transcription factors is called the **transcription initiation complex.**
5. RNA polymerase **unzips** the 2 strands of the DNA double helix at promoter by breaking **hydrogen bonds** between base pairs.
6. Only one strand is used as the **template** to synthesise mRNA.

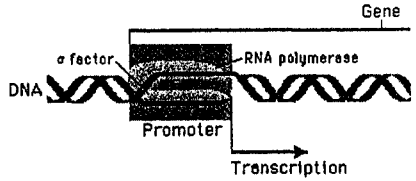
(N.B. The **template** strand is also the **non-coding** strand)



IN PROKARYOTES

INITIATION

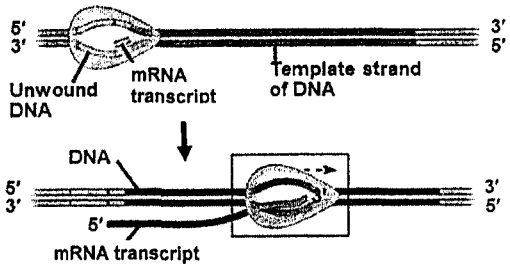
1. RNA polymerase is a **multimeric** (made up of many subunits) complex.
2. **Sigma factor** associates with **core RNA polymerase** forming the RNA polymerase **holoenzyme**
3. As the holoenzyme scans along the DNA, its sigma factor recognizes and binds to the promoter.
4. RNA polymerase **unzips** the 2 strands of the DNA double helix at promoter by breaking **hydrogen bonds** between the base pairs.
5. Only one strand is used as a **template** to synthesise mRNA.



IN EUKARYOTES AND PROKARYOTES

ELONGATION

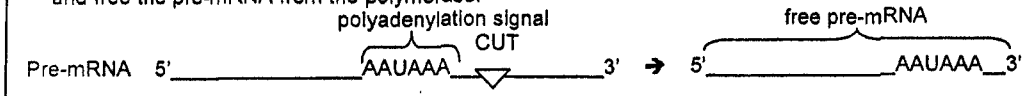
1. Free ribonucleotides match up with the template DNA strand by **complementary base pairing.**
2. Cytosine base pairs with guanine, guanine base pairs with cytosine, thymine base pairs with adenine & adenine base pairs with uracil.
3. RNA polymerase catalyses the formation of covalent **phosphodiester bonds** between adjacent ribonucleotides.
4. As each **ribonucleoside triphosphate** is brought in, 2 of its terminal phosphate groups are removed and the 5' end of the resulting **ribonucleotide** is added to the **3' end** of the growing RNA strand via the formation of a phosphodiester bond.
5. Thus the **mRNA strand is synthesized & elongated in the 5' to 3' direction** & the **template DNA strand is read in the 3' to 5'direction.**
6. As the transcription complex continues to move down the DNA double helix, unzipping the 2 strands, and synthesizing mRNA, the **region of DNA that has just been transcribed, reanneals.**



IN EUKARYOTES

TERMINATION

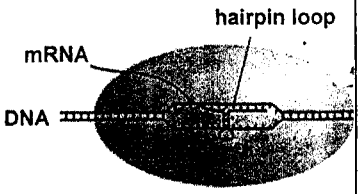
1. RNA polymerase transcribes a sequence on the DNA, which codes for a **polyadenylation signal (AAUAAA)** in the **pre-mRNA.**
2. Proteins (endonucleases) bind at a point (10 to 35 nucleotides) **downstream** of the polyadenylation signal to cut and free the pre-mRNA from the polymerase.



IN PROKARYOTES

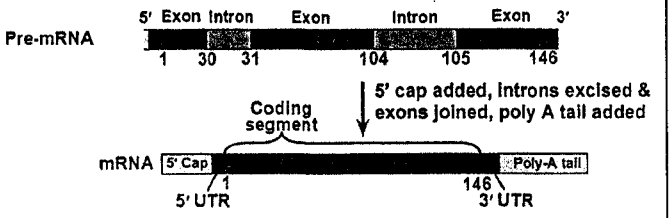
TERMINATION

1. After transcribing through a **termination sequence** (in DNA), the **transcribed terminator** (an mRNA sequence) folds back to form a **hairpin loop.**
2. The loop acts as a termination signal that causes the mRNA and RNA polymerase to be released.



POST-TRANSCRIPTIONAL MODIFICATION (Only in EUKARYOTES)

1. **Addition of methylguanosine cap to 5' end of pre-mRNA**
   → the 5' cap protects mRNA from degradation by ribonucleases that degrade RNA form the 5'end, serves as a recognition signal for the small ribosomal subunit to assemble & begin translation & facilitates the export of mature mRNA form the nucleus)
2. RNA **splicing** (which requires ATP) by spliceosomes which **excise introns**(non-coding seq.) & **join exons**(coding seq).
3. **Synthesis of poly A tail** (polyadenylation) by the enzyme poly A polymerase which adds adenine nucleotides downstream of polyadenylation sequence, AAUAAA.
   →protects mRNA from degradation by ribonucleases, making it a more stable template for translation and directs the export of mRNA through nuclear pores into the cytoplasm.



TRANSLATION :the synthesis of a polpeptide using genetic information encoded in an mRNA molecule. There is a change in "language" from nucleotides to amino acids

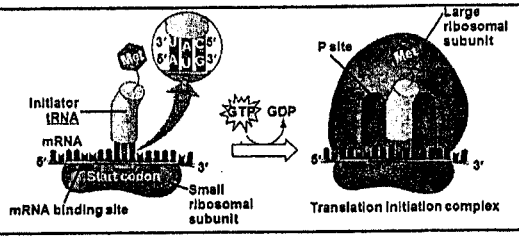
Amino acid activation: amino acid + tRNA $\xrightarrow[\text{ATP}]{\text{aminoacyl-tRNA synthetase,}}$ aminoacyl tRNA

Amino acid activation is NOT part of translation but essential for translation to occur.

Each amino acid is covalently attached to the 3' CCA stem of a specific tRNA by a specific aminoacyl-tRNA synthetase.
There are 20 different aminoacyl tRNA synthetases. Each enzyme recognizes a specific amino acid & the unique identity sites at the 3'CCA stem & the anticodon loop of a specific tRNA.
Thus. 20 different aminoacyl tRNAs can be formed.

INITIATION
(in both EUKARYOTES & PROKARYOTES) (GTP is required)

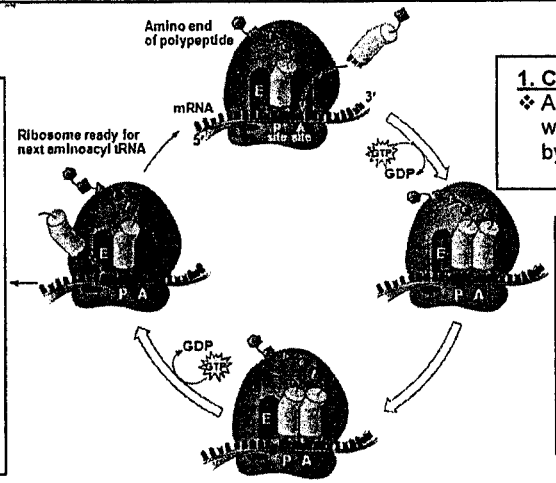
1. Initiation factors facilitate the binding of the small ribosomal subunit to both mRNA and initiator tRNA.
2. The large ribosomal subunit binds, completing the ribosome, resulting in the formation of the **translation initiation complex**.
3. The initiator tRNA will be positioned at the **P site (peptidyl-tRNA binding site).**
4. The **A site (aminoacyl-tRNA binding site)** will be vacant for the addition of the next aminoacyl tRNA molecule.
5. GTP is required for the initiation stage.



IN EUKARYOTES	IN PROKARYOTES
• **Initiation factors** and **initiator tRNA** (carrying methionine) bind to **small ribosomal subunit.** • Small ribosomal subunit then recognizes & binds to the **5' 7 methylguanosine cap** of the mRNA.& **moves in the 5' to 3' direction** along the mRNA in search of the **start codon, AUG.** • The initiator tRNA associates with the start codon by complementary base pairing (This is followed by points 2-5 under initiation.)	• **Initiation factors** bind to the **small ribosomal subunit** and facilitate the binding of the small ribosomal subunit to **Shine-Dalgarno sequence** so that the **start codon can be correctly positioned** before the initator tRNA and large ribosomal subunit bind. (This is followed by points 2-5 under initiation.)

ELONGATION
(in both EUKARYOTES & PROKARYOTES) (GTP is required)

<u>3. Translocation</u>
- Ribosome shifts one codon down mRNA in **5' to 3' direction.**
- The tRNA from the **A site** is shifted to the **E site (exit site)** and released into cytosol.
- The aminoacyl-tRNA with growing polypeptide is translocated from A site to P site.
- Empty A site is ready to receive the next incoming aminoacyl tRNA, with anticodon complementary to mRNA codon exposed at A site.
- The process is repeated until a stop codon is reached.

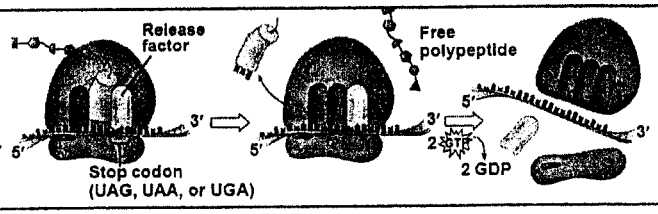


<u>1. Codon recognition</u>
- Anticodon of incoming aminoacyl tRNA base pairs with complementary mRNA codon in **A site** by forming hydrogen bonds.

<u>2. Peptide bond formation</u>
- **Peptidyl transferase** in large ribosomal subunit **catalyses peptide bond formation** between amino acid in A site and methionine/ amino acid at carboxyl end of growing polypeptide chain in the P site.
- The methionine/amino acid dissociates from the initiator tRNA it was bound to.

TERMINATION
(in both EUKARYOTES & PROKARYOTES) (GTP is required)

1. When the **stop codon (UAG, UAA, UGA)** reaches the A site, **release factors** enter the A site.
2. Binding of the release factors causes the **hydrolysis of the bond** between the **polypeptide chain** & the **tRNA** at the P site.
3. The polypeptide is released from the ribosome as it completes its folding into it secondary & tertiary structure.
4. The ribosome disassembles into its subunits.

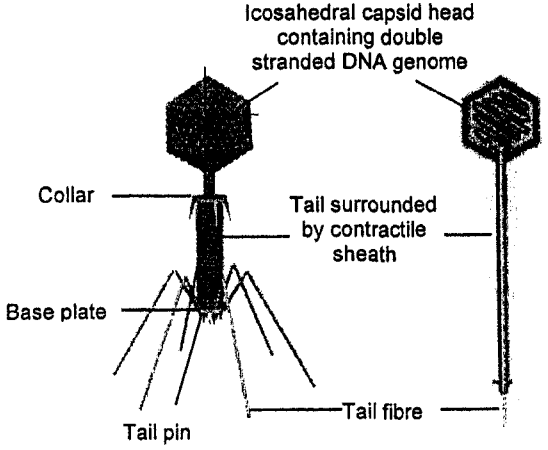


- **Post-translational modification** of proteins **occurs in eukaryotes** (i.e. cleaving of proteins into smaller functional peptides or modification of proteins) and **prokaryotes.**
- In eukaryotes, mRNA undergoes post-transcriptional modification within nucleus before being transported to the cytoplasm for translation.
  In **prokaryotes, mRNA can be translated while it is being transcribed.**

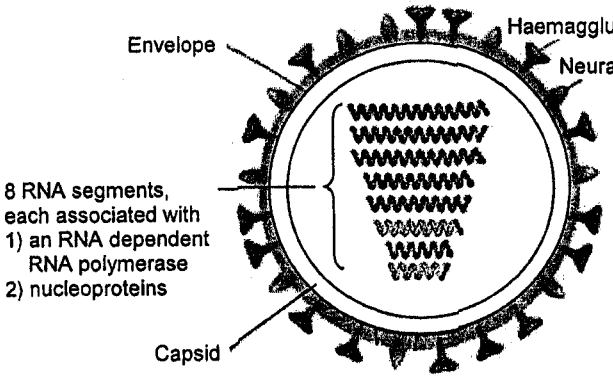
- **Are viruses are considered living or non-living?** Living as they contain **genetic material**. However, non-living because they have **no cellular organization** and only show characteristics of living things when in host cell.
  Characteristics of living things include1) metabolic activity 2) cellular organization 3) ability to reproduce and grow in numbers 4) ability to respond to stimuli and adapt to environment
- **Why are viruses obligate parasites?** This is because viruses, like obligate parasites, **depend on host cells to complete their life cycle.**

Structure of Viruses

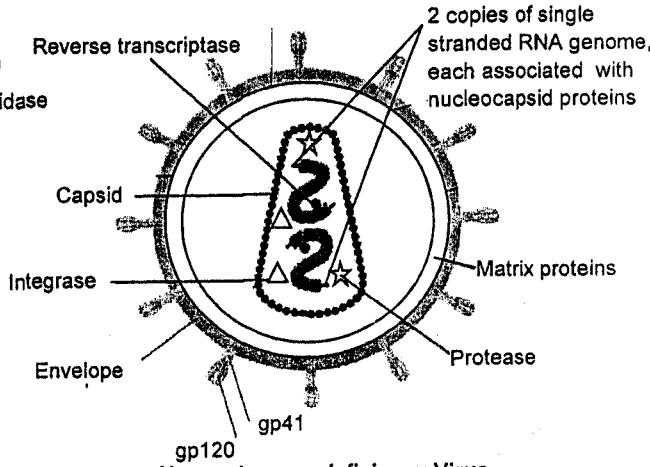
Size: 10-300nm	Bacteriophages		Animal Viruses	
			Enveloped	
	T4 phage	Lambda phage	Influenza	Human Immunodeficiency Virus (HIV)
Genome ▪ Nucleic acid that codes for synthesis of viral components and enzymes for viral replication & assembly ▪ Can be either DNA/RNA, single/double-stranded	▪ Double-stranded DNA		▪ **(-) strand RNA** →viral genome is **complementary to viral mRNA** ▪ **8 different segments of single stranded RNA** associated with **nucleoproteins** ▪ Each RNA segment is packed with 3 polymerase proteins which come together to form an **RNA-dependent RNA polymerase** enzyme complex which replicates and transcribes the viral genome in the host cell	▪ **(+) strand RNA** →viral genome has the **same sequence as viral mRNA** ▪ **2 identical copies of single stranded RNA** bound to **nucleocapsid proteins**
Capsid ▪ Protein coat that surrounds and protects viral genome ▪ Comprise subunits called capsomeres	▪ Icosahedral capsid head		▪ Present.	▪ Present, conical shaped Enzymes reverse transcriptase, integrase and protease found in capsid
Envelope ▪ Phospholipid bilayer surrounding the nucleocapsid ▪ Derived from host cell membrane ▪ Embedded with viral glycoproteins involved in host cell recognition	▪ Absent		▪ Glycoproteins embedded in envelope: haemagglutinin (80%) & neuraminidase (20%)	▪ Glycoprotein embedded in envelope: gp41 gp120 is attached to gp41



T4 phage **Lambda phage**



Influenza Virus



Human Immunodeficiency Virus

Antigenic Drift and Antigenic Shift

Antigenic Drift : When the influenza virus replicates in its host cell, mutations frequently occur due to the **poor proofreading mechanism** of the **viral RNA-dependent DNA polymerase**. Over time, there is an **accumulation of mutations in the viral genome.** Sometimes, these mutations produce viruses with modified antigens (e.g. glycoproteins such as haemagglutinin or neuraminidase). If these viruses infect a host that does not have the antibodies that recognise these modified antigens, the host becomes susceptibleto the virus.

Antigenic Shift: When a **bird strain** of influenza A and **human strain** of influenza A infect a **single cell** of an **intermediate host** (e.g.a **pig**), **genetic reassortment** can occur. Thus when new viruses are assembled in the host cell, they can have **new combinations of RNA segments**. Sometimes, genetic reassortment produces viruses with new antigens (e.g. glycoproteins such as haemagglutinin or neuraminidase). If these viruses infect a **human host** that does not have the antibodies that recognise these modified antigens, the host becomes susceptibleto the virus.

Virus Life Cycle

Stages	Bacteriophage		Enveloped animal viruses	
	T4 phage (Lytic phage)	Lambda phage (Temperate phage)	Influenza	HIV
1. **Attachment** Virus recognises and attaches to host cell	▪ **Attachment sites** on tail fibres **adsorbs** to **complementary receptor sites** on bacterial surface (e.g. *E.coli*)		Enveloped viruses use viral glycoproteins to bind to specific receptor molecules on host cell. ▪ **Hemagglutinin binds to complementary sialic acid receptor** on host cell (e.g. **epithelial cells in respiratory tract)** membrane	▪ **gp120 binds to complementary CD4 receptors** on T **helper cells or (macrophages)** with the help of a co-receptor.
2. **Penetration** Viral genome introduced into host cell	▪ Bacteriophage releases **lysozyme** which **digests bacterial cell wall** ▪ This allows the **release of molecules** from the bacterium which triggers a change in shape of the proteins in the base plate which causes the **contraction of tail sheath** which will drive the **hollow core tube** through **cell wall** ▪ When the tip of the hollow core tube **reaches the plasma membrane, phage DNA is injected** into the bacterial cell ▪ The empty capsid remains outside		Release of capsid into host cell cytosol ▪ Virus enters host cell by **endocytosis** (the process involves ***invagination*** of membrane) ▪ Endocytic vesicle fuses with **lysosome** → which lowers the pH → causes viral envelope to fuse with lipid bilayer of vesicle → nucleocapsid is released into cytosol Degradation of capsid to release viral genome (uncoating) Capsid degraded by cellular enzymes and the 8 viral RNA segments that are released into cytosol **enter the nucleus**	▪ With the help of **gp41, the viral envelope fuses with host cell membrane** → nucleocapsid is released into cytosol (NB: HIV can also enter by endocytosis) Degradation of capsid to release viral genome (uncoating) Capsid degraded by cellular enzymes→ the 2 viral RNA strands and enzymes are released into the **cytosol**
3. **Replication** Synthesis of viral components & viral genome replication	▪ **Host cell macromolecular synthesizing machinery** is used to synthesise phage proteins ▪ **Early phage proteins:** degrade host DNA ▪ Phage DNA synthesized using host cell nucleotides and early proteins ▪ **Late phage proteins:** are phage enzymes and structural components	▪ **Linear** phage **DNA circularizes** and **inserted** into **host cell genome** by enzyme **integrase** ▪ The integrated phage DNA is known as a **prophage** ▪ Expression of phage genes is **repressed** by phage **repressor proteins**. Hence new phages are not synthesized ▪ Prophage replicates along with bacterial chromosome ▪ During **spontaneous induction, cellular proteases** are activated. They destroy the repressor proteins ▪ The prophage is then excised from the bacterial genome ▪ The replication phase of lytic cycle then occurs.(on left)	▪ Viral **RNA-dependent RNA polymerase** uses viral genome as a template to synthesise mRNA ▪ mRNA 1. **enters cytosol** →translated into **viral structural components** (Capsid proteins are made in the cytosol. Envelope glycoproteins are made in the RER & eventually are embedded in host cell membrane) 2. can also **act as template** for synthesis of **new viral RNA genome** in the nucleus. Viral RNA genome then exits nucleus.	▪ **Reverse transcriptase** makes DNA strand using viral RNA as template to form a DNA-RNA hybrid. The RNA is then degraded and the 2nd DNA strand is made → double-stranded DNA molecule produced ▪ Viral DNA **enters nucleus** → inserted into host cell genome by **integrase** → Viral DNA known as **provirus** →can remain **latent** for a long time ▪ Upon **activation**, viral DNA transcribed to viral RNA which **enters cytosol** ▪ Viral RNA can either act as mRNA and be translated into proteins or become part of the genome of the new virions ▪ mRNA 1. is translated to **viral polyproteins** 2. is translated into envelope glycoproteins gp120 and gp 41 in the RER and eventually are embedded in the host cell surface membrane.
3. **Maturation** Assembly of complete viruses	▪ Phage DNA and capsid assemble into a DNA-filled head ▪ Head, tail and tail fibers assembled independently & join in a specific sequence. ▪ First, tail fibers join the tail, then DNA-filled head attaches to the tail		▪ Capsid proteins associate with host cell membrane where viral glycoproteins are inserted. ▪ Nucleoproteins associate with the RNA genome and then interact with capsid proteins that have associated with the glycoproteins embedded on the plasma membrane. ▪ This initiates the budding process.	**For HIV, maturation is completed only after release of virus.** ▪ The viral RNA genome and polyprotein assembles at the cell surface membrane where viral glycoproteins have been inserted.
4. Release	▪ Phage **lysozyme** synthesised within the cell breaks down the bacterial cell wall ▪ Bacterial cell membrane lyses and release the newly formed virions		▪ Newly formed viruses bud off by ***evagination***, acquiring host cell membrane with embedded viral glycoproteins ▪ **Neuraminidase facilitates the release** of the new virions from the host cell membrane by cleaving sialic acid from the host cell receptor.	▪ Newly formed viruses bud off by ***evagination***, acquiring host cell membrane with embedded viral glycoproteins ▪ Viral **protease** cleaves polyproteins, forming viral enzymes and proteins. ▪ The viral RNA genome and enzymes are then encapsulated by a protein coat to form a **capsid** ▪ The mature HIV virus (virion) is now able to infect neighbouring cells.

Influenza Life Cycle

(1) **Hemagglutinin** recognizes & binds to **sialic acid** receptor on host membrane

(2a) Virus enters host cell by **endocytosis** (which involves ***invagination*** of membrane)

(2b) Endocytic vesicle fuses with **lysosome** → which lowers the pH → causes viral envelope to fuse with lipid bilayer of vesicle → nucleocapsid is released into cytosol

(2c) Capsid degraded by cellular enzymes and viral RNA **enters nucleus**

Note:
Only 2 out of the 8 RNA segments are shown due to space constraints in the diagram)

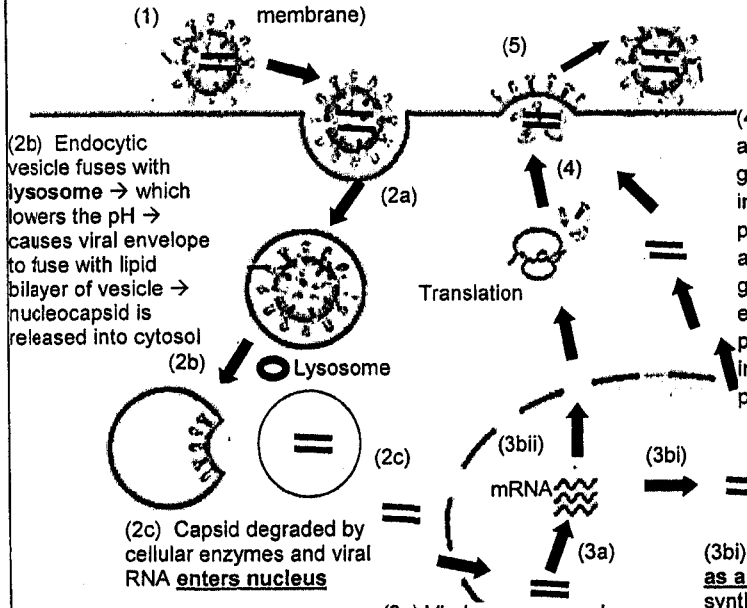
(3a) Viral genome **used as a template** for **mRNA** synthesis by **RNA-dependent RNA polymerase.**

(3bii)mRNA then **enters cytosol** → translated into **viral structural components**
(Capsid proteins are made in the cytosol. Envelope glycoproteins are made in the RER & are eventually embedded in host cell membrane)

(3bi) **mRNA** can **used as a template** for synthesis of **new viral RNA genome** in the nucleus

(4) Nucleoproteins associate with the RNA genome and then interact with capsid proteins that have associated with the glycoproteins embedded on the plasma membrane. This initiates the budding process.

(5) Newly formed viruses bud off by ***evagination***, acquiring host cell membrane with embedded viral glycoproteins. **Neuraminidase facilitates the release of the new virions from the host cell** membrane. Host cell may or may not be lysed.



HIV Life Cycle

(1) **gp120 binds to CD4 receptors** on T lymphocytes (or macrophages) with the help of a co-receptor.

(2a) With the help of **gp41, the viral envelope fuses with host cell membrane** → nucleocapsid is released into cytosol

(2b) Capsid degraded → viral RNA and enzymes released into **cytosol**

(3a) **Reverse transcriptase** makes DNA strand using vira RNA as template → RNA degraded → 2nd DNA strand made → double-stranded DNA molecule (ds DNA) produced

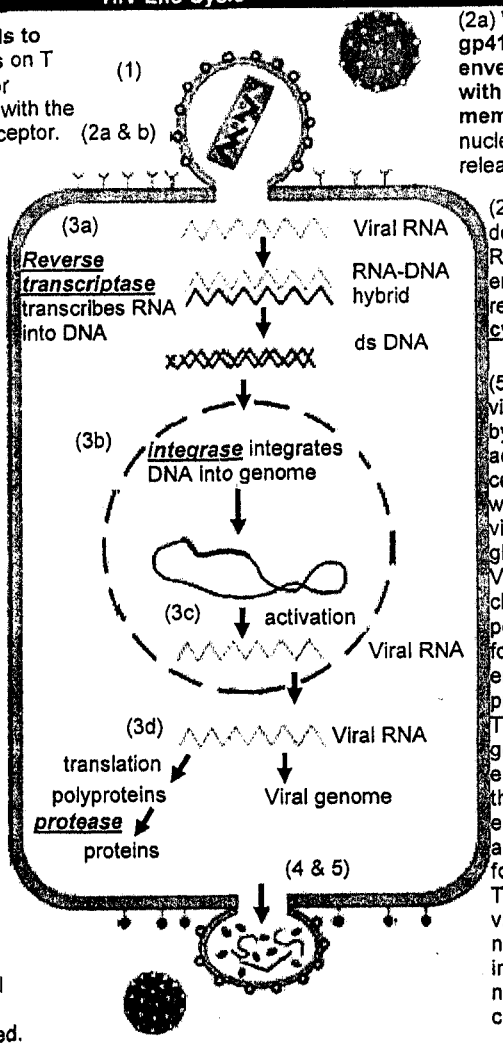
(3b) Viral DNA **enters nucleus** → inserted into host cell genome by **integrase** → Viral DNA known as **provirus** →can remain **latent** for a long time

(3c) Upon **activation**, viral DNA is transcribed to viral RNA which **enters cytosol** Viral RNA can either act as mRNA and be translated into proteins or become part of the genome of the new virions.

(3d) mRNA is translated to
1. **viral polyproteins** which are cleaved into functional proteins by **protease**
2. envelope glycoproteins gp120 and gp 41 in the RER which eventually are embedded in the host cell surface

(4) Viral RNA genome and polyprotein assemble at the cell surface membrane where viral glycoproteins have been inserted.

(5) Newly formed viruses bud off by ***evagination***, acquiring host cell membrane with embedded viral glycoproteins. **Viral protease** cleaves polyproteins, forming viral enzymes and proteins. The viral RNA genome and enzymes are then encapsulated by a protein coat to form a **capsid.** The mature HIV virus (virion) is now able to infect neighbouring cells.



Pathogenecity of Influenza

When influenza will bind to sialic acid receptors on epithelial cells of respiratory tract
↓
Influenza replicates within it and then buds off. Infected epithelial cells eventually lyse
↓
The build up of dead epithelial cells results in inflammation and symptoms of influenza appear
→runny nose & scratchy throat
↓
The epithelial layer weakens and the individual is more susceptible to bacterial infections like pneumonia

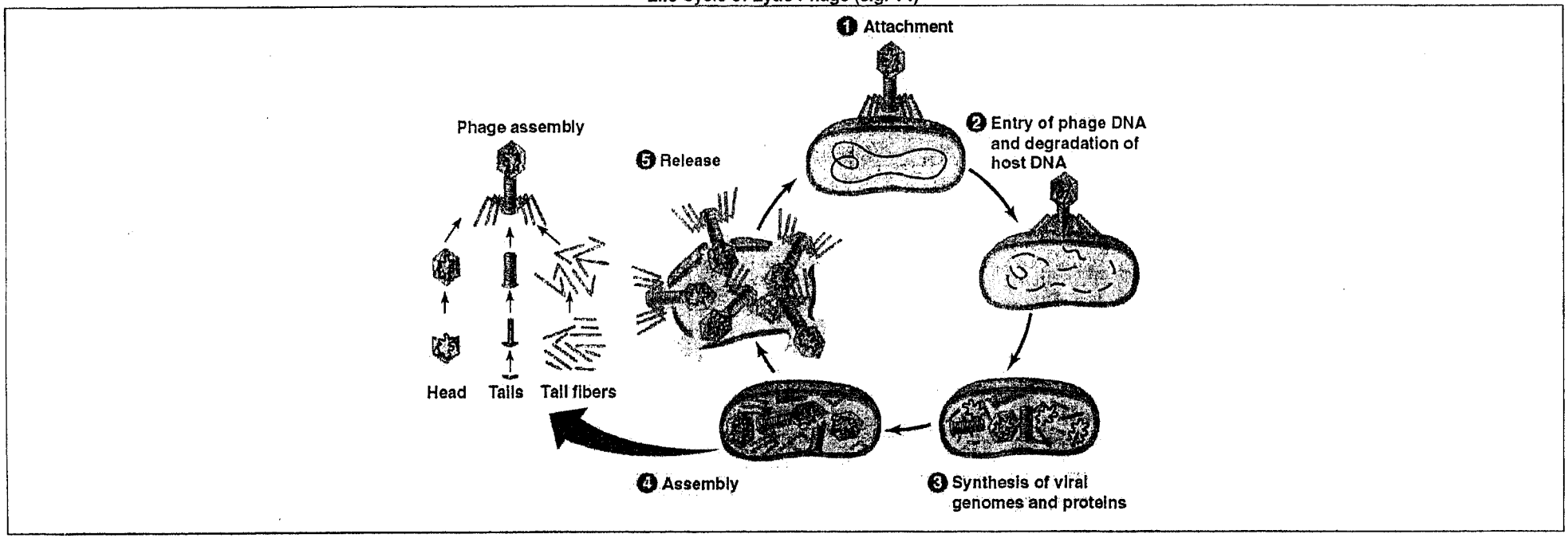
- Treatment:1) antibiotics for bacterial infections
  2) antiviral drugs which target viral enzymes i.e enzyme inhibitors
  e.g:Tamiflu for some strains of influenza

Pathogenecity of HIV

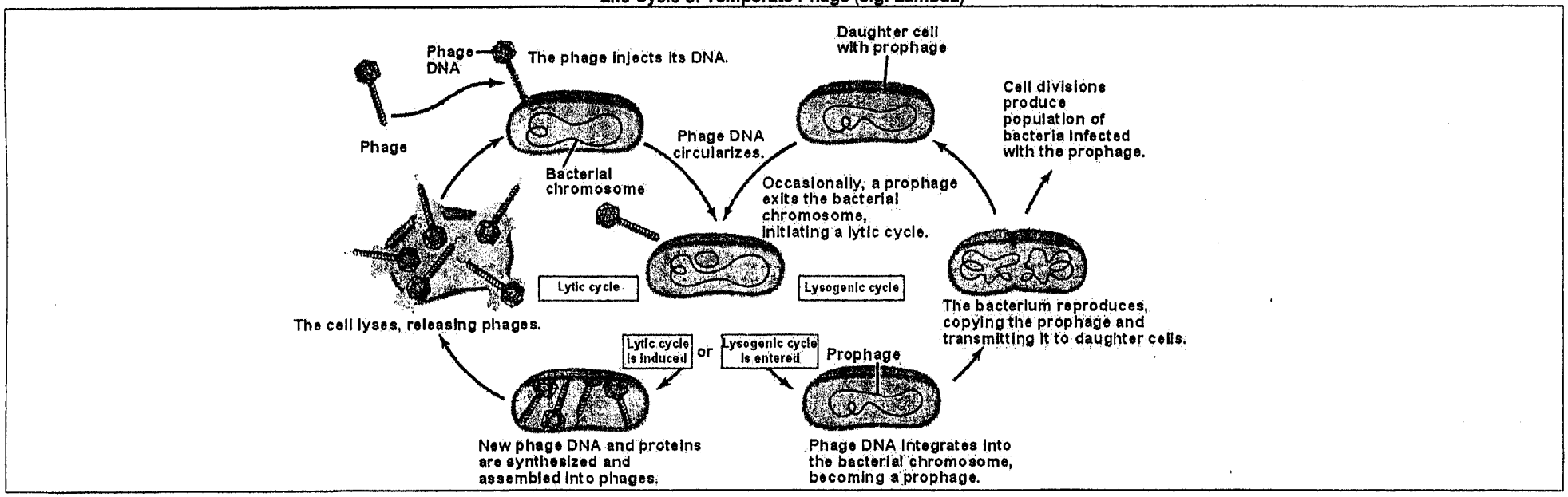
When HIV binds to CD4 receptor on a T helper cell, a type of T lymphocyte
↓
HIV replicates within it and then buds off. Infected T helper cells eventually lyse.
↓
With fewer T helper cells, the immune system is depressed & individuals are more susceptible to opportunistic infections. When infections become unmanageable→AIDS → death

- Virus able to avoid detection by immune system as it mutates at a high rate during replication →surface proteins altered →prevent recognition & elimination by immune system
- Treatment: drug cocktail that targets (1) enzymes(RIP) i.e. enzyme inhibitors
  (2) glycoproteins (gp120) i.e. entry inhibitors

Life Cycle of Lytic Phage (e.g. T4)



Life Cycle of Temperate Phage (e.g. Lambda)



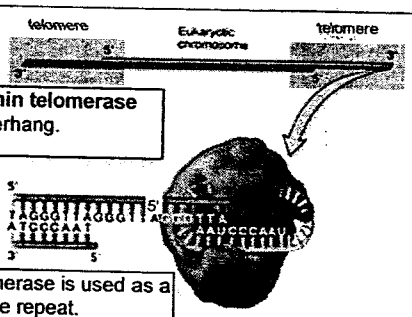
General structure of a eukaryotic and prokaryotic cell:

Feature	Eukaryotic cell	Prokaryotic cell (bacteria)
Cell size	Larger: 10-100μm in diameter	Smaller: 0.5 - 5μm in diameter
Nucleus	Nucleus with nuclear envelope present;	No true nucleus / No nuclear envelope
Genetic material	Linear DNA associated with many proteins; Plasmids absent	Circular DNA associated with few histone-like proteins; Plasmids present
Ribosome	80S;Ribosomes may be attached to ER or free in cytoplasm	70S;No ER present. Ribosomes free in cytoplasm.
Organelles	Many membrane bound organelles present;	No membrane bound organelles;
Cell walls	Composed of cellulose in plants & chitin in fungi	Composed of peptidoglycan or murein
Photosynthesis	In chloroplast	At plasma membrane
Respiration	In mitochondrion and cytoplasm	At plasma membrane

Structure and organization of the genome of a prokaryotic and eukaryotic cell:

Feature	Eukaryotic Genome	Prokaryotic Genome
Size	Larger	Smaller
Appearance	Multiple, linear molecules	Generally a single, circular molecule
Molecule	Double helix DNA	Double helix DNA
Association with proteins	Yes – large amounts of it e.g. histones, scaffold proteins	Yes – relatively less histone-like proteins
Level of DNA packing/coiling	High: DNA double helix → 10nm fibre or nucleosomes (DNA+many histones) → 30nm fibre or solenoid (present at interphase)→ looped domain (300nm fibre) → chromosome (at metaphase) (See * below)	Relatively low: DNA double helix → domains (DNA+few proteins) → supercoiling (See * below)
Location	Nucleus	Nucleoid region – not membrane-bound
Extrachromosomal DNA	Yes – if you consider mitochondria and chloroplast circular DNA	Yes – plasmids (much smaller rings of DNA)
Number of genes	25,000	4,500
Non-coding regions	Common – about 98%	Not common – typically less than 15%
i. presence of introns	many	None (rare)
ii. presence of promoters	yes	yes
iii. presence of repeated sequences	many	few
iv. presence of enhancers/ silencers	many	None (rare)
Presence of operons	very few	many

How telomerase works



1. A short 3-nucleotide segment of **RNA within telomerase** binds to part of a **DNA repeat** in the 3'overhang.

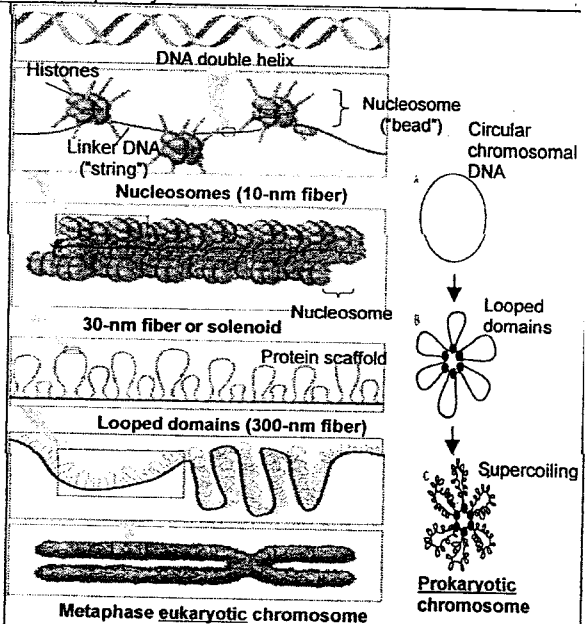
2. The **adjacent part** of the RNA within telomerase is used as a **template** to synthesise a short 6-nucleotide repeat.

3. After the repeat is made, telomerase **translocates** 6 nucleotides to the right in the **5' to 3'direction of the template strand** and begins to **make another repeat.**

4. Then **primase** makes an RNA primer **near the end of the telomere. DNA polymerase** adds nucleotides to the 3'OH end of the primer and hence **synthesizes a complementary strand** . The nick is then sealed by DNA ligase. The RNA **primer is eventually removed.**

Newly synthesized strand — RNA primer to be removed

NB: Germ cells (cells that give rise to gametes), **cancer** cells and **embryonic stem** cells have telomerase activity which will ensure that vital coding DNA is not lost as they divide continuously.



Packing of DNA in eukaryotic and prokaryotic DNA

NB:
1) DNA + histones = chromatin
2) Euchromatin: transcriptionally active
3) Heterochromatin: transcriptionally inactive
4) During differentiation of any one cell type, **non-coding regions** and **genes that are not expressed** in the differentiated cell form part of the tightly packed **heterochromatin.**

Non-coding DNA (does not code for proteins or RNA)

Regulatory Sequences

Introns

- **non-coding** DNA located **within a gene,** specifically **between exons** in a specific segment of **DNA** (also present in **pre- mRNA)**
- found in eukaryotes

Role

- enables a process called **'alternative RNA splicing'** to occur where different exons of a single pre-mRNA can be spliced such that **many different mature mRNAs** are produced eg. mature mRNA 1 → exons 1, 2, 3; mature mRNA 2 → exons 1, 3 etc.
- **one gene** can now code for **more than one polypeptide**

Promoter

- **non-coding** DNA located **just upstream** of the transcription start site of a gene
- called **proximal control element**
- **has critical elements/short sequences** (a) eukaryotes (TATA box, CAAT box and GC box) and (b) prokaryotes (-10 and -35 sequences)

Role

- **recognition & binding site** for RNA polymerase
- **transcription initiation site**

Enhancer

- **non-coding** DNA usually located **far away** from the promoter (usually much further upstream or downstream)
- called **distal control element**
- found in eukaryotes

Role

- **activators** (which are **specific transcription factors**) bind to it
- enhance / increase the frequency of transcription by **promoting the assembly of the transcription initiation complex** (with the help of **DNA bending proteins** that bend **spacer DNA**)

Activators and repressors are not enzymes & do not have an active site. Each has a DNA binding domain that is complementary in shape & charge to a specific sequence of DNA.

Silencer

- **non-coding** DNA usually located **far away** from the promoter (usually much further upstream or downstream)
- called **distal control element**
- found in eukaryotes

Role

- **repressors** (which are **specific transcription factors**) bind to it
- silence/repress the frequency of transcription by **preventing the assembly of the transcription initiation complex** (e.g. repressor can interact with general transcription factors and prevent assembly of transcription initiation complex)

Repetitive DNA

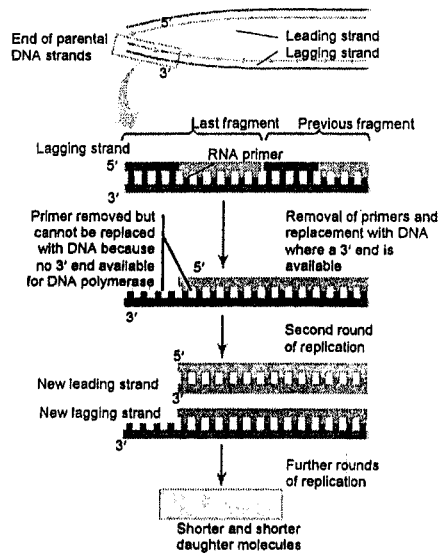
Telomere

1- **non-coding** DNA made up of a **series of tandem repeat** sequences (a specific sequence of nucleotides occurring many times in a row)

2- found at **both ends** of **linear**, eukaryotic chromosomes

3- in humans, each repeat has the sequence 5' TTAGGG 3'

4- have a **single stranded region at their 3' ends known as the 3' overhang** (due to a **limitation of DNA polymerase**, this region of DNA does not have a complementary strand)



Role

1-Telomeres ensure that vital genetic information is not lost due to the shortening of DNA with each round of DNA replication due to the end replication problem.

Due to limitation of DNA polymerase (i.e. it requires a free 3'OH of a pre-existing strand to add nucleotides and hence **cannot replace the last primer on the lagging strand with DNA**), the DNA molecule **shortens with each round of replication**. Hence, telomeres, which are **non-coding** sequences at the **ends of linear** chromosomes will be lost before any vital genetic information is.

2-Telomeres protect and stabilize the terminal ends of chromosomes by forming a loop with the 3'overhang.

The 3' single stranded end of a chromosome can loop back and displace the same sequence in the upstream telomeric region while binding to the complementary sequence of the other strand.

This prevents single-stranded terminal end of one chromosome from annealing to a complementary single-stranded terminal end of another chromosome. If two chromosomes join this way, the cell's DNA repair machinery may detect it as **damaged DNA** due to double stranded breaks and **trigger apoptosis**.

3-Telomeres allow their own extension, as they have a 3' overhang which provides an attachment point for the correct positioning of the enzyme telomerase. Although telomeres shorten with every round of DNA replication, telomerase activity in germ cells, embryonic stem cells and cancer cells can maintain telomere length. (See *** on pg1 on how telomerase works)

Centromere

- **non-coding** DNA made up of a **series of tandem repeat sequences**
- found at a constricted region of a condensed chromosomes

Role

1- allow **sister chromatids to adhere** to each other

2- allow kinetochore **proteins** (which recognize specific sequences in centromeric region)to bind, and **subsequently spindle fibres to attach** so that **sister chromatids / homologous chromosomes can be separated** to opposite poles.

Ultimately, allows proper alignment and segregation of chromosomes.

Regulation of gene expression at various stages in eukaryotes and prokaryotes

Genomic/DNA	Eukaryotes (only)
Amplification of specific genes (occurs only during a specific stage of development of the organism)	**Gene amplification** to increase the **copy number** of **specific** genes (as some gene products/proteins are needed in higher amounts) 1) **rRNA gene clusters in oocytes (mature eggs) of South African clawed toad (*Xenopus laevis*)** →chromosome gives rise to an **extrachromosomal circular ring** of DNA carrying the rRNA gene cluster. From this ring, many **more copies** of circular DNA are synthesized by **rolling circle mechanism**, giving rise to **millions of rRNA genes**. The rRNA genes will be transcribed to rRNA which are an important component of ribosomes. With more **ribosomes, massive protein synthesis** can occur **after fertilization** of the oocyte for **rapid growth of the embryo**. 2) **Chorion (eggshell) gene cluster in follicle cells in ovary of *Drosophila melanogaster*** →**multiple replications of a small region of the chromosome** containing the **chorion gene cluster** occurs. Replication is initiated within the chorion gene cluster and is terminated randomly, giving rise to a **replication bubbles nested within larger replication bubbles**. This results in many copies of the chorion gene cluster which meets the demand for **high levels of chorion mRNA and hence chorion protein** which **envelops and protects the zygote**.
Histone acetylation & deacetylation	**Histone acetylation** → addition of acetyl group to certain amino acids (e.g. lysine) in the histone by enzyme histone acetylase →removes positive charge on histones → loosens electrostatic interaction between the negatively charge DNA and the positively charged histones→chromatin decondenses → promotes transcription **Histone deacetylation** →removal of acetyl groups from histones by histone deacetylase →restores tighter interaction between DNA & histones →chromatin condenses→ inhibits transcription
Chromatin remodeling complex	**Chromatin remodeling complex** are protein complexes which →**alter structure of nucleosomes temporarily** and changes **accessibility of RNA polymerase and transcription factors to promoter** 1) can cause DNA to be more tightly coiled around histones →inhibits transcription 2) can cause DNA to be less tightly coiled around histones →promotes transcription
DNA methylation & demethylation	**DNA methylation** (Once DNA methylation occurs in certain genes in a cell, it is usually permanent for the entire lifetime of the cell.) →addition of a **methyl group** to selected **cytosine** residues inhibits transcription by 1) blocking the binding of transcription factors at the promoter and hence preventing the formation of the transcription initiation complex 2) recruiting DNA-binding proteins (e.g. transcriptional repressors, histone deacetylases and repressive chromatin remodeling complexes) **DNA demethylation** →removal of methyl group from cytosine residues promotes transcription

Transcription	Eukaryotes	Prokaryotes
Promoter strength	**Critical elements in promoter are the** 1) **TATA box at -25 sequence** (i.e. located 25bp upstream of transcription start site) is important in determining the **precise location** of the **transcription start site** 2) **CAAT** and **GC** boxes improve efficiency of promoter by helping to **recruit general transcription factors** and **RNA polymerase** **Strength of promoter**: the degree of similarity of critical elements to consensus sequence is not critical in controlling gene expression.	**Critical elements in promoter are** 1) **sequences at -10 (Pribnow box) region determines precise location of transcription start site.** 2) and sequences at -35 region **Strength of promoter**: determined by degree of similarity between critical elements and **consensus sequence**→ the greater the similarity, the greater the frequency of transcription
Promoter recognition	**Promoter recognized by general transcription factors** General transcription factors assemble at promoter region and then recruit RNA polymerase to form a transcription initiation complex and **start transcription**. Eukaryotic **transcriptional regulation depends on the presence of specific transcription** factors, activators and repressors that bind to enhancers and silencers respectively. (See below)	**Promoter recognized by sigma factor** Sigma factor binds to core RNA polymerase to form RNA polymerase holoenzyme. As the holoenzyme scans along the DNA, its **sigma factor recognizes and binds to the promoter** elements at both the -10 and -35 sequences. **Different sigma factors recognize different promoters**. Hence, controlling the **availability of different sigma factors** will determine which genes are transcribed.
Regulatory proteins	**Specific transcription factors (activators and repressors)** **Activators bind to enhancers** → promote assembly of transcription initiation complex → transcription frequency increases (Bound activator may recruit histone acetylase and chromatin remodeling complex to increase accessibility of promoter) **Repressors bind to silencers** → prevent assembly of transcription initiation complex → transcription frequency decreases (Bound repressor may recruit histone deacetylase and/or chromatin remodeling complex to decrease accessibility of promoter.)	**CAP and repressors** **Positive gene regulation** → binding of activated Catabolite Activator Protein (CAP) to the CAP binding site at the promoter of the *lac* operon , increases the affinity of RNA polymerase to the promoter → transcription frequency increases. **Negative gene regulation** → binding of repressor to the operator (in both *lac* and *trp* operon) prevents RNA polymerase from binding to the promoter site → transcription frequency decreases

Post-transcriptional	Eukaryotes (only)
Addition of 5'cap	1) **Addition of 5'cap** - a **7-methylguanosine** nucleotide is added to the 5' end of the pre-mRNA - added shortly after transcription begins i.e. it occurs **co-transcriptionally** - helps the cell to **recognize** mRNA (amongst other RNAs) so that subsequent steps such as splicing and polyadenylation can occur - The 5' cap a) acts as a **signal** to **export mRNA out of nucleus** b) **protects** the growing pre-mRNA chain **from degradation** by ribonucleases→more proteins can be made c) is **recognized by translation initiation factors** which have bound to **small ribosomal subunit** so that initiation of translation can occur.
Splicing	2)**Splicing** - the process where **introns** (noncoding regions within a gene) are **excised** and **exons** (coding regions within a gene) are **joined** together. →functional proteins can be produced - carried out by a complex of **proteins** and **snRNA** called **spliceosomes** - a spliceosome recognises the points of excision, which is determined by the sequence of nucleotides at **intron-exon boundaries** **Alternative splicing** -where different exons of a single pre- mRNA can be spliced such that different mature mRNAs are produced eg. mature mRNA 1→exons 1, 2, 3; mature mRNA 2 → exons 1, 3 - **one gene can now code for more than one polypeptide**
Addition of poly A tail	3) **Addition of poly A tail at 3'end of pre-mRNA** - the 3'end of pre-mRNA is cleaved enzymatically at a site of about 10-35 nucleotides downstream of a polyadenylation signal, AAUAAA - immediately after the cleavage, **poly-A polymerase** adds a long sequence of adenosine monophosphates (ribonucleotides) which forms a poly(A) tail - this process is called **polyadenylation** and occurs immediately **after transcription** - The poly A tail a) acts as a **signal** to **export mature mRNA** out of nucleus b) **protects** mature mRNA **from degradation** by ribonucleases c) **works with 5'cap** to regulate **translational efficiency** during initiation of translation

Translation	Eukaryotes	Prokaryotes
mRNA half-life/stability	Eukaryotic mRNA half-life/stability is determined by the **length of its poly-A tail. The longer the poly-A tail, the longer the mRNA can be used as a template to make proteins** The poly-A tail is removed by ribonucleases in the 3' to 5' direction until a critical length is reached. This will trigger removal of the 5'cap and degradation of the mRNA from the 5'end as well.	a) Prokaryotic mRNA have relatively **short half-life** as they are rapidly **degraded** by RNases soon after they are synthesised. This enables bacteria to **rapidly adjust synthesis of proteins** (regulate gene expression) in response to **environmental changes**. b) Under certain conditions, **anti-sense RNA** which is **complementary** to part of the **mRNA to be degraded** will be synthesised. It will complementary base pair with mRNA to form double stranded RNA. This double-stranded RNA 1) is then targeted for degradation by ribonucleases. 2) will block translation of the mRNA
Binding of small ribosomal subunit	a) During translation initiation, **small ribosomal subunit binds to 5'cap of mRNA**. This can be prevented by binding of **translational repressor** to 1) 5'cap 2) 3' untranslated region which intereferes with the interaction between the 3'poly-A tail , the 5'cap and the small ribosomal subunit which is needed for translation b) During translat ion initiation, **initiation factors bind to small ribosomal subunit.** These initiation factors **facilitate the binding of the small ribosomal subunit to the 5'cap.** The **availability of translation initiation factors** is determined by whether or not they are phosphorylated. Some initiation factors are activated by phosphorylation while others are inactivated by phosphorylation. Without activated translations initiation factors, translation cannot begin.	During translation initiation, **initiation factors bind to the small ribosomal subunit** and facilitate the binding of the small ribosomal subunit to **Shine-Dalgarno sequence** so that the **start codon can be correctly positioned** before the initator tRNA and large ribosomal subunit bind. Translation initiation can be blocked by 1) binding of **translational repressor protein** at/near Shine-Dalgarno sequence which prevents small ribosomal subunit from binding 2) binding of **anti-sense RNA** complementary to the mRNA **near the Shine-Dalgarno sequence** which prevents small ribosomal subunit from binding 3) regulating **availability of translation initiation factors** which facilitate binding of small ribosomal subunit to Shine-Dalgarno sequence

Post-translational	Eukaryotes and Prokaryotes
	1) **Covalent modification/cleavage** (eg. attachment of prosthetic groups, glycosylation, disulphide bond formation) of polypeptides make them functional proteins 2) **Phosphorylation /dephosphorylation** can up or down regulate of protein activity 3) **Protein degradation** by proteasome determines long a protein remains in a cell (Proteins targetted for degradation are tagged with ubiqutin and then recognised and degraded by the proteasome.)

Proto-oncogenes

1. code for proteins that stimulate **normal cell growth and proliferation** e.g. growth factor
2. when mutated, they are known as **oncogenes** which
   - (a) increase the **amount** of proto-oncogene's protein product
     - (i) by a **point mutation** in base sequences of **regulatory elements** (e.g. promoters of proto-oncogenes)
       This can lead to increased transcription, and excess production of the proto-oncogene protein product (e.g. growth factor) and hence → excessive cell growth and proliferation
     - (ii) **gene amplification**, where the **number of proto-oncogenes in a cell is increased** (due to a mistake made during DNA replication)
       This can lead to excessive production of proto-oncogene protein product (e.g. growth factor) and hence → excessive cell growth and proliferation.
     - (iii) **chromosomal translocation** such that the **proto-oncogene ends up under the control of a enhancer**
       This can lead to excessive production of proto-oncogene protein product (e.g. growth factor) and hence→ excessive cell growth and proliferation.
     - (iv) **retroviral integration** which can
       1) **inactivate a silencer** of a proto-oncogene →which can upregulate the transcription of the proto-oncogene
       2) **insert an enhancer** → that can upregulate the transcription of the proto-oncogene

     Both can lead to excessive production of proto-oncogene protein product (e.g. growth factor) and hence→ excessive cell growth and proliferation.
   - (b) increase the **intrinsic activity** of the proto-oncogene protein product
     - (i) by a **point mutation within** the **proto-oncogene**
       This **changes the amino acid** sequence of the proto-oncogene protein (e.g. growth factor) which can then become **hyperactive or more resistant to degradation.**
       This can lead to excessive cell growth and proliferation.
3. e.g. **ras gene**: transduces signals from growth factors to downstream signaling process and **increases cell division**

Tumour suppressor genes

1. codes for protein products that **inhibit cell division**
2. when mutated, they are inactivated. Mutations could be due to (a) point mutations, (b)chromosomal translocation or (c) retroviral integration
3. e.g. **p53 gene**: codes for a **specific transcription factor** (an **activator**) that can activate genes involved in
   - (a) **cell cycle arrest**
     → gives the cell enough time to repair damaged DNA and prevent formation of mutant daughter cells
   - (b) **DNA repair**
     → prevents mutations that may lead to the formation of oncogenes or inactivated tumour suppressor genes
   - (c) **initiating apoptosis when DNA damage is beyond repair**
     → which will thus remove cells with damaged DNA with the potential to cause cancer.

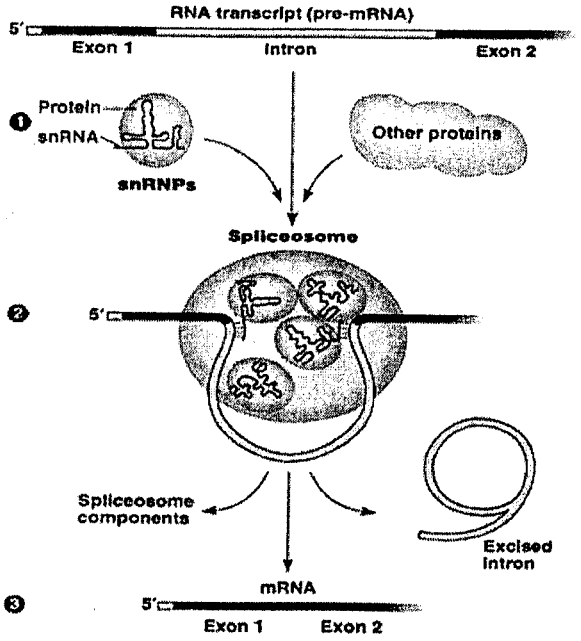
Gain-in-function and loss-of-function mutations

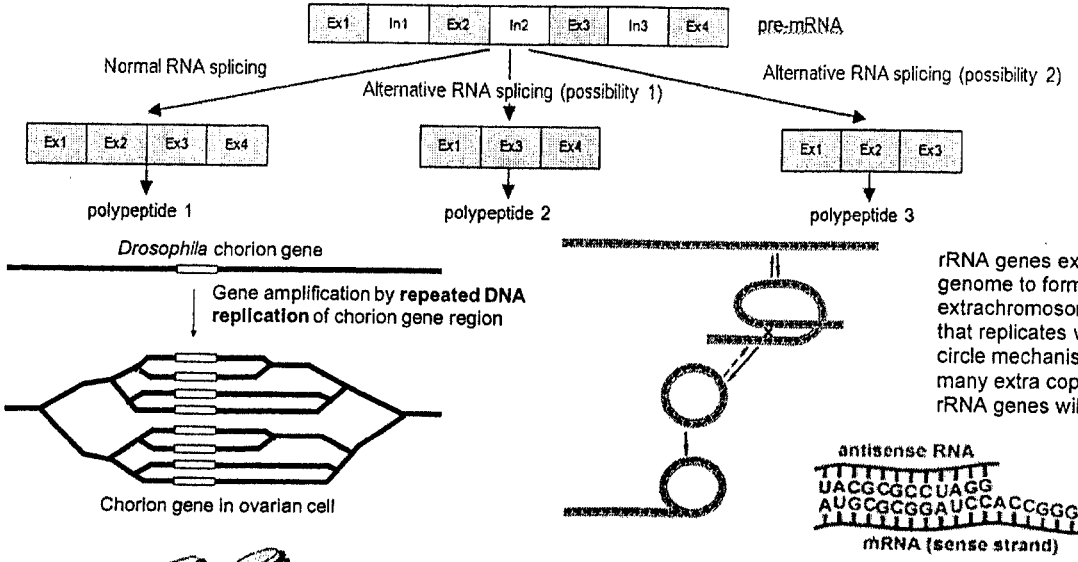
Gain-in-function mutation → e.g. when a proto-oncogene (e.g.ras) is mutated to form an oncogene
(dominant mutation) → mutation in just one copy of the gene results in increased cell growth and proliferation due to the increased synthesis/activity of a functional product due to mutation.
Thus the mutation is said to be dominant.

Loss-of-function mutation → e.g. mutations in tumour suppressor genes(e.g.p53)
(recessive mutations) → mutations in both copies of the gene necessary for loss of tumour suppression
→ even when one copy is mutated, the non-mutant copy still produces a functional gene product which will result in tumour suppression.
Thus the non-mutant copy will mask the effect of the mutant copy and hence the mutation is said to be recessive.

Why is development of cancer a multi-step process?

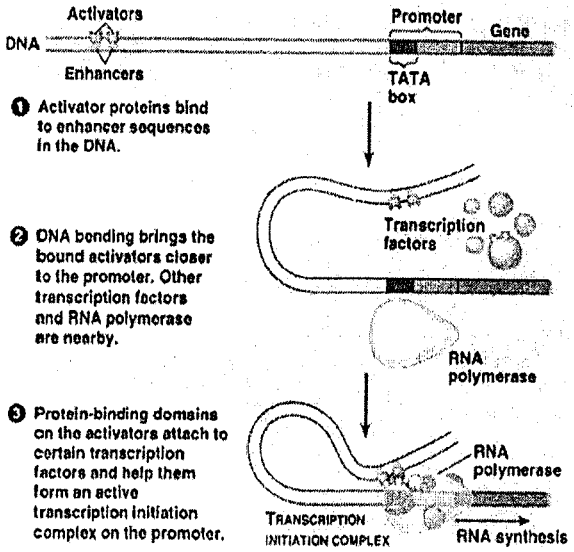
- The development of cancer requires the **accumulation of mutations** in the genes which **control regulatory checkpoints** of the cell cycle in a **single cell**
- This will **disrupt** the **normal cell cycle**, thus causing the cell to undergo **excessive cell growth and proliferation**
- A **gain-in-function** mutation is a dominant mutation where mutation in just **one copy/allele of a proto-oncogene** will result in its **overexpression** which will result in the production of excessive **amounts of hyperactive/degradation resistant growth factors** leading to cell proliferation
- **Loss-of- function** mutations is a recessive mutation where mutations in **both copies/alleles of a tumour suppressor gene** will **disrupt their ability to inhibit cell cycle, enable DNA repair and promote apoptosis**
- **Upregulation/activation** of the genes coding for **telomerase** result in telomeres being lengthened and the cell can thus **dividing indefinitely** as the chromosomes are prevented from shortening with DNA replication cycle.
- **Loss of contact inhibition*** will enable the cells to **grow into a tumour/mass of cells.**
- **Angiogenesis** must occur within the tumour so that the **blood vessels** formed can **transport oxygen and nutrients for its growth.**
- Finally the cells must **metastasise.** i.e **leave the primary site** and **spread to other tissues in different parts of the body** via the **blood stream** and **form tumours there.**
- Since the above steps should occur in order for cancer to develop. As it takes years to accumulate these mutations, the chances of developing cancer increases with age.

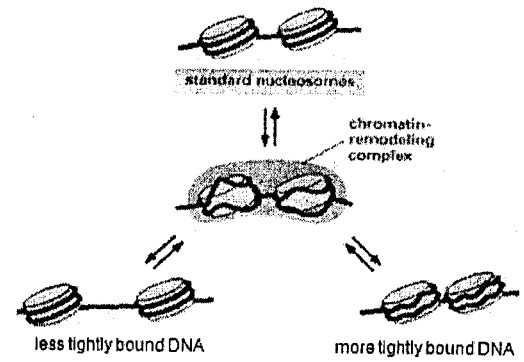


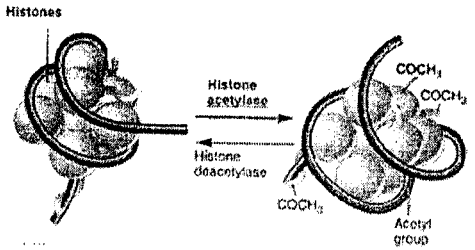


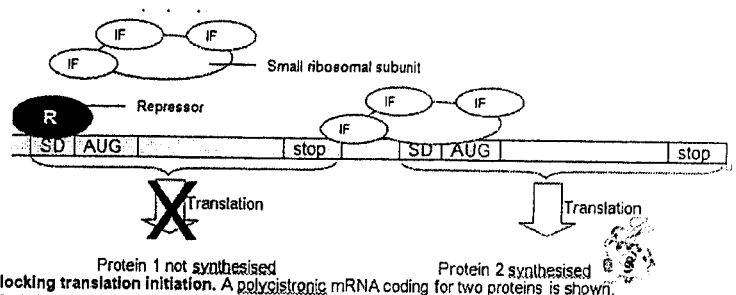
rRNA genes excised from genome to form circular extrachromosomal DNA that replicates via rolling circle mechanism.Hence many extra copies of the rRNA genes will result

Anti-sense RNA forms a double-stranded and reduces half-life of mRNA.

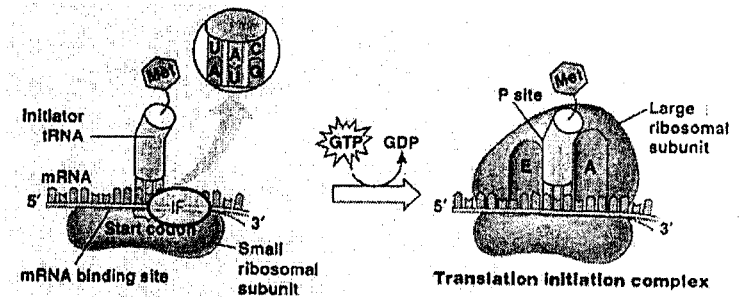








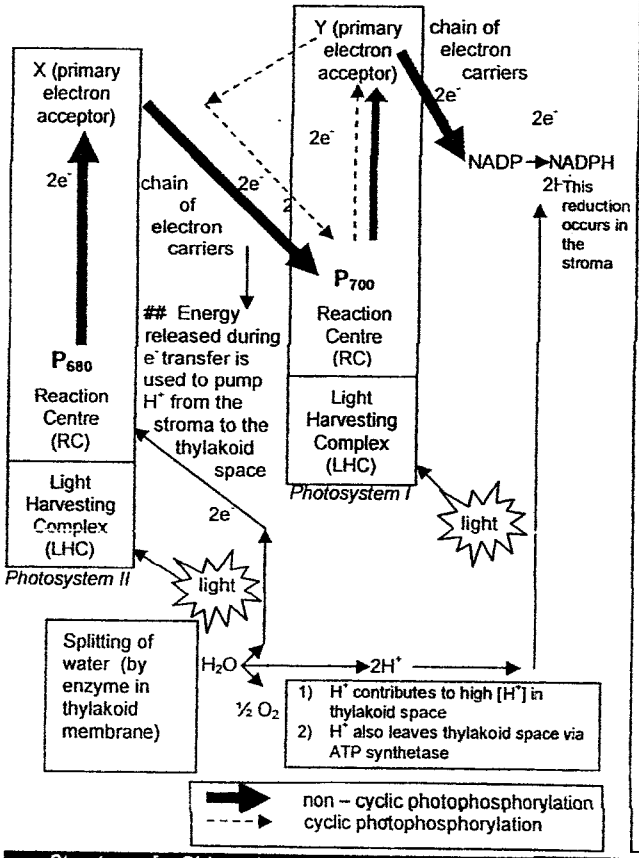
. **Blocking translation initiation.** A polycistronic mRNA coding for two proteins is shown.
SD: Shine-Dalgarno sequences. IF: Initiation factors



Initiation factors ('IF' in diagram) are needed for translation initiation.

Photosynthesis ($6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$)

Light Dependent Reactions (on thylakoid membrane)



* **Primary** pigment :**special chl a** mlc (P680 in PSII and P700 in PS I)
Accessory pigments :other chl a & chl b mlcs & carotenoids (fd in LHC)

Non-cyclic photophosphorylation

* When a photon of **light is absorbed** by an **accessory pigment** molecule in the LHC of PS II, one of its electrons is excited to a higher energy level. As the excited electron drops to its ground state, the energy released is passed on to the next pigment molecule. This **resonance transfer** of energy continues until the **special chlorophyll a** mlc in the **RC** is reached.

* When the special chlorophyll a molecule absorbs the energy, an **electron is displaced**, Chl a→chl $a^+ + e^-$, leaving an **e^- hole** in PS II. The displaced e^- is accepted by a **primary e^- acceptor (X)** & then passed down a series of increasingly electronegative electron carriers (of the ETC) losing energy during the transfer. The **energy lost during this e^- flow is** used to **actively pump H^+** from the stoma to the thylakoid space. (See **##** below)

* The e^- lost from PSII is replaced by an e^- released ***from the splitting of water*** (which is an enzyme-catalyzed reaction) which occurs in the thylakoid space. During the splitting of water, the **H^+ released,** contributes to the high $[H^+]$ in the thylakoid space, while the O atom combines with another O atom resulting in the release of ***molecular* oxygen as a by-product**.

* Meanwhile, PSI loses an e^- in a manner similar to PSII leaving an **e^- hole** in PS I. The displaced e^- is accepted by a **primary e^- acceptor (Y)** & then passed down a series of electron carriers of a 2nd ETC. (Energy is not released during e^- transfer down this 2nd ETC.). The e^- is finally accepted by **NADP** (the final electron acceptor) which is **reduced** to **NADPH** ($NADP+e^-+H^+ \rightarrow NADPH$) by **NADP reductase** which is found on the thylakoid membrane.

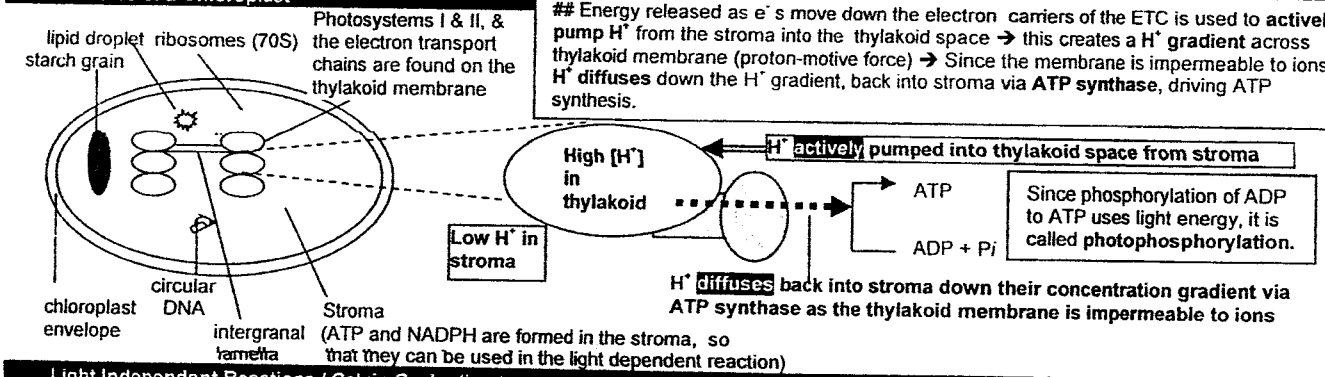
* When the e^- from PSII reaches the end of the 1st ETC, it will then fill the e^- hole in PSI.

* The **ATP** & **NADPH** produced will be used in the Calvin cycle.(The high energy e^-s stored in NADPH provide the reducing power for the synthesis of sugar)

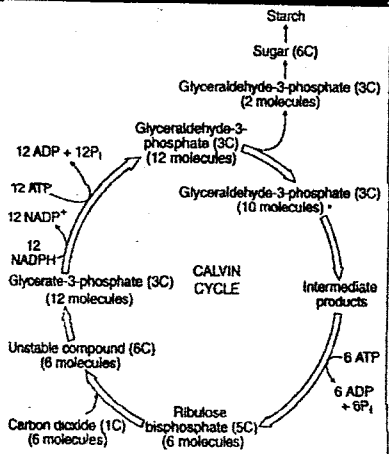
Cyclic photophosphorylation

* In **cyclic photophosphorylation**, electrons raised to a higher energy level from RC of PSI are recycled back to PSI through the electron transport chain. Only PSI is involved and only ATP is produced. (NADPH is not produced during cyclic photophosphorylation.)

Structure of a Chloroplast



Light Independent Reactions / Calvin Cycle (in stroma)



* Substances required from light reaction: **NADPH & ATP**

* **Carbon fixation** : CO_2 combines with RuBP (5C) in the presence of the enzyme ribulose bisphosphate carboxylase **(Rubisco)** to form an unstable 6C compound which breaks down into 2 molecules of GP/PGA (3C).

* **Reduction** and sugar formation : GP is reduced to G3P/TP/PGAL(3C). **ATP** and **NADPH** are needed for the reaction.

* **Regeration of RUBP**: G3P molecules can either be converted to sugars and then polymerized to starch or enter a series of reactions driven by **ATP** to **regenerate RuBP** to allow CO_2 fixation to continue.

* C & O atoms of sugar ($C_6H_{12}O_6$) come from CO_2 & H atoms come from NADPH (or indirectly from H_2O)

* Products of light independent reaction : 1) **G3P** (a triose sugar)
2) **NADP & ADP** (which are recycled to the light reactions)

(Note: GP: Glycerate-3-phosphate/ Glycerate phosphate; PGA: Phosphoglyceric acid
G3P: Glyceraldehyde-3-phosphate; TP: Triose phosphate; PGAL: Phosphoglyceraldehyde)

Energy released as e^-s move down the electron carriers of the ETC is used to **actively pump H^+** from the stroma into the thylakoid space → this creates a **H^+ gradient** across thylakoid membrane (proton-motive force) → Since the membrane is impermeable to ions, **H^+ diffuses** down the H^+ gradient, back into stroma via **ATP synthase**, driving ATP synthesis.

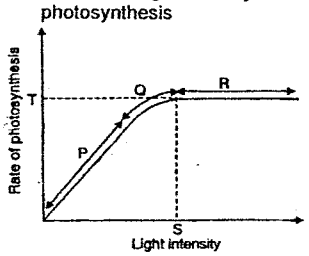
Phosphorylation = addition of a phosphate group to a molecule [eg: ADP + P_i (inorganic phosphate)→ ATP]
Photophosphorylation = formation of ATP from ADP + P_i using light energy in photosynthesis
Non-cyclic photophosphorylation = Electrons obtained from PS II → Primary electron acceptor (X)→ electron transport chain → PSI → Primary electron acceptor (Y) → electron transport chain →NADP.
Electron from the photolysis of water replaces the electron lost form PSII.
Cyclic photophosphorylation = Electrons that are raised to a higher energy level are lost from PSI, but are recycled back to PSI through the 1st electron transport chain.
Together cyclic & non-cyclic photophosphorylation produce sufficient ATP & NADPH to drive the Calvin cycle.

Chemiosmosis: an energy coupling mechanism that uses energy stored in the form of hydrogen ion gradient across a membrane to synthesise ATP.
Photoactivation: When a chlorophyll molecule absorbs light, the energy from this light raises one of its electrons to a higher energy level. That chlorophyll molecule is said to be photoactivated.

Resonance transfer: When a chlorophyll molecule absorbs light, the energy from this light raises one of its electrons to a higher energy level. When the excited electron returns to its ground state, the energy released is transferred to another pigment molecule. This is called resonance transfer.

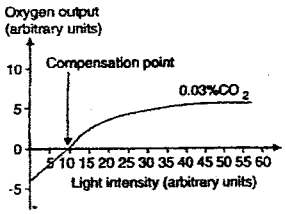
Limiting factor: Any environmental factor that - by its decrease or increase, absence or presence - alters the growth, metabolic processes or distribution of organisms and populations most significantly.
If you increase a particular variable and there continues to be a **proportional relationship** between the values on the x & y axes, it is referred to as the only limiting factor.
At P: light intensity is a limiting factor (note linear relationship between x and y values)
At Q: light intensity is not the only limiting factor. Some other factor is also limiting. (eg: CO_2 concentration)
At R: light intensity is no longer limiting. (How do you know this? Even when light intensity is increased, there is no increase in the rate of reaction.) Some other factor is limiting.

S: **Light saturation point**: Light intensity beyond which an increase in light intensity will not increase the rate of photosynthesis

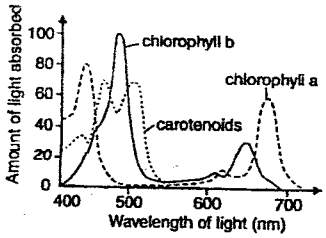


Compensation point: is the light intensity at which the rate of photosynthesis is equal to the rate of respiration.

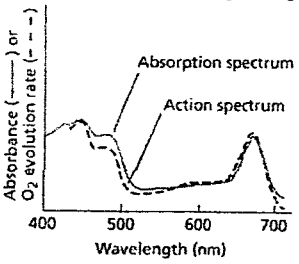
At compensation point, all the CO_2 produced during respiration is used in photosynthesis and all the oxygen produced in photosynthesis is used in respiration. Hence there is no net gain / loss of CO_2.



Absorption spectrum: a record of the amount of light absorbed at each wavelength.

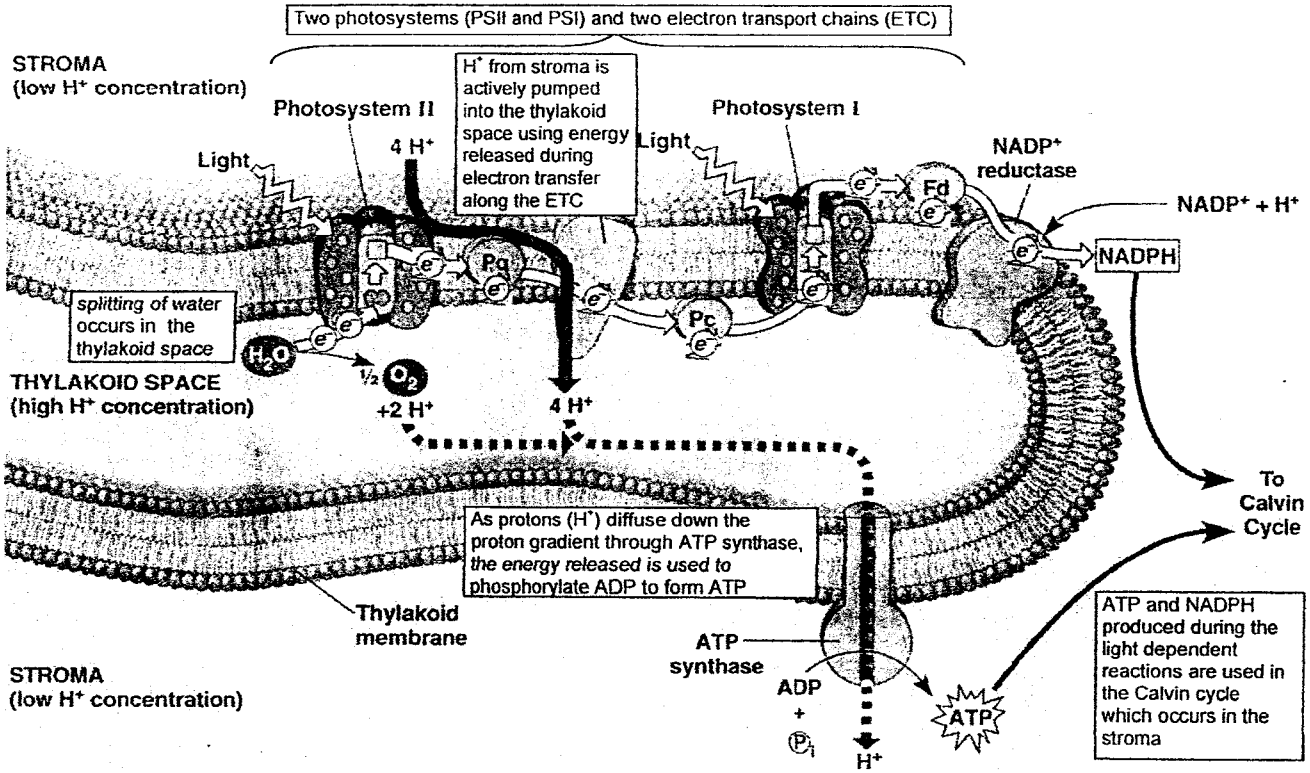


Action spectrum: a record of the amount of photosynthesis occurring at each wavelength of light



NB:
Q: What contributes to high H^+ concentration in the thylakoid space? (in order of importance)
1) proton pump (which actively pumps H^+ into the thylakoid space)
2) photolysis of water (catalysed by enzymes on inner thylakoid membrane)
3) lack of permeability of thylakoid membrane to H^+. (due to its hydrophobic core)
4) reduction of NADP to NADPH occurs in the stroma & hence reduces the H^+ concentration in the stroma therby ensuring the steepness of the H^+ gradient across the membrane

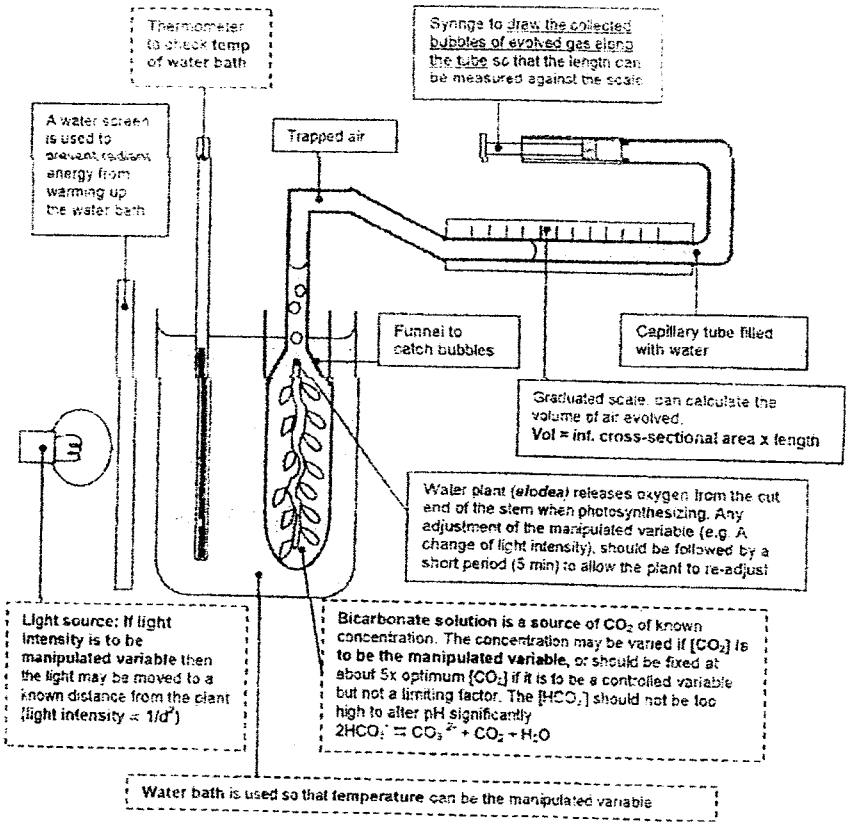
Q: Describe the function of the thylakoid membrane in photophosphorylation.
1) Provides a large surface area to embed many photosynthetic pigments / chlorophyll molecules for light absorption
2) Maintains the sequential arrangement of the photosystems and electron carriers of electron transport chain for the flow of electrons
3) Maintains proton gradient for ATP synthesis since the hydrophobic core of the membrane is impermeable to protons and this is essential for chemiosmosis
4) Allows of many ATP synthase to be embedded so ATP can be produced as protons flow down their gradient via chemiosmosis from thylakoid space to stroma



3 KEY Factors Affecting The Rate Of Photosynthesis

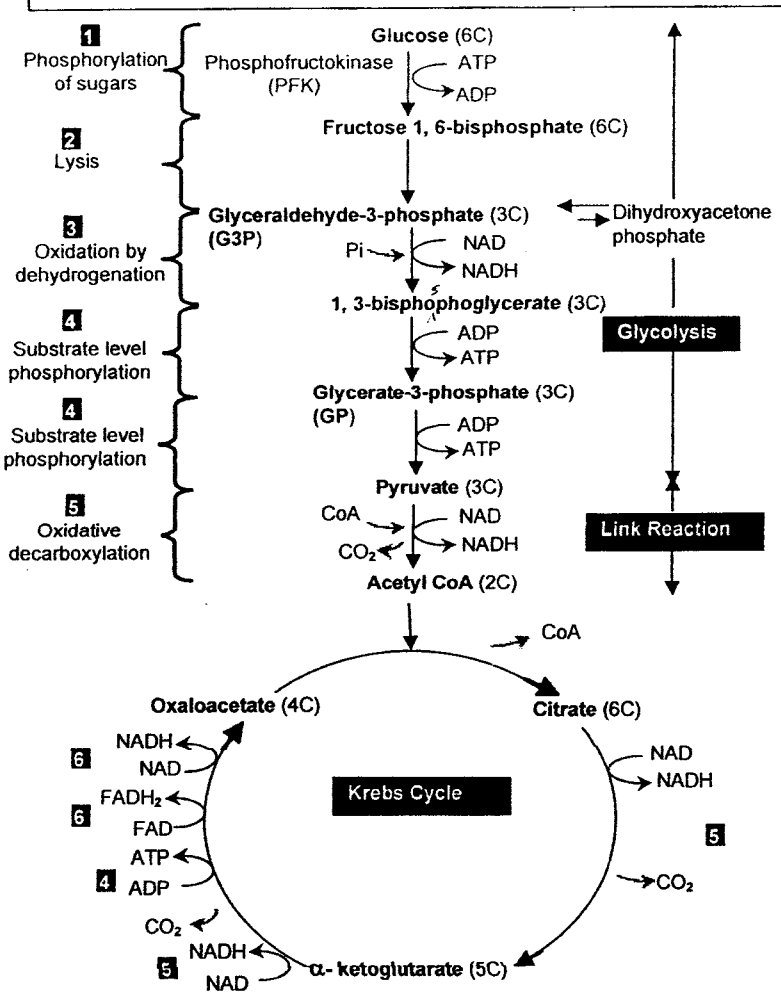
1) light intensity 2) CO_2 concentration 3) temperature
(other factors which may limit rate of photosynthesis include chlorophyll or oxygen concentration, specific inhibitors like herbicides, water, pollution etc.)
Below is the setup to measure **rate of oxygen evolution by a water plant during photosynthesis**. Only 1 limiting factor should be tested at a time.

Rate of Photosynthesis is proportional to the volume of gas evolved. Since bubbles of evolved gas are collected over a fixed duration of time,
Rate of Photosynthesis = $\frac{\text{collected volume } (mm^3)}{\text{time (minutes)}}$ = ___mm^3 of evolved O_2/min (at a known temperature, t°C)



Respiration: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

Aerobic respiration: (1) involves oxidation of glucose (2) produces 38 ATP mlcs per glucose mlc oxidised, CO_2 & water (3) requires oxygen



GLYCOLYSIS (in cytosol)

* 1 glucose mlc → 2 pyruvate mlcs
* 2 NADH + net 2 ATP are produced
* O_2 is **not required** for this step.

1 Phosphorylation of sugar

→ addition of phosphate group from ATP activates the sugars & commits it to the glycolytic pathway

2 Lysis

→ **1 mlc** of fructose-1,6- bisphosphate **(6C) lyses** to form **2 mlcs** of glyceraldehyde-3-phosphate (G3P/TP)(3C)

→ Hence the number of products formed (eg: ATP, NADH, $FADH_2$, CO_2 etc.) in **all subsequent rxns** *(i.e. Link, Krebs, OP.) including glycolysis is* ***doubled***.

3 Oxidation by dehydrogenation

→ glyceraldehyde-3-phosphate (G3P/TP) undergoes oxidation/dehydrogenation and phosphorylation

→ NAD is reduced to NADH

4 Substrate-level phosphorylation

→ enzyme-mediated ATP synthesis

→ involves transfer of a phosphate group (P_i) from a **substrate mlc** in a metabolic pathway to **ADP**.

→ occurs during glycolysis and Krebs cycle

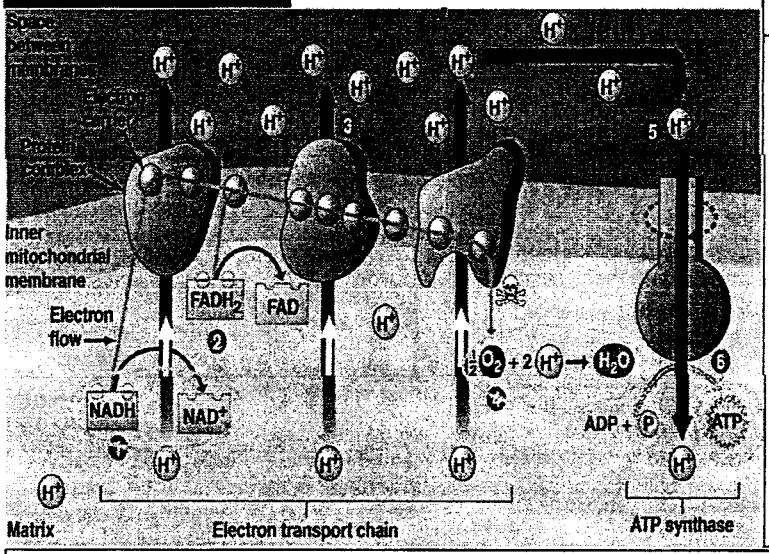
LINK REACTION (in mitochondrial matrix)

* If O_2 is present, pyruvate is **actively transported** into the mitochondrial matrix via **a transport protein**
* 2 pyruvate mlcs (3C) undergo **5 oxidative decarboxylation** to form 2 acetyl CoA mlcs (2C)
* 2 NADH + 2 CO_2 are produced

KREBS CYCLE (in mitochondrial matrix)

* When **1 glucose molecule** is oxidised, 2 Acetyl CoA mlcs form and enter the Krebs cycle. Thus **2 turns** of the Krebs cycle is necessary to oxidise 1 mlc of glucose.
* Acetyl CoA (2C) combines with oxaloacetate (4C) to form citrate (6C)
* Citrate (6C) is converted to α-ketoglutarate (5C) by **5 oxidative decarboxylation**
* α-ketoglutarate (5C) then goes through a series of enzymatic reactions (i.e **5 oxidative decarboxylation**, **4 substrate level phosphorylation** & **6 oxidation**) and is converted to oxaloacetate (4C).
* When oxaloacetate (4C) is **regenerated** ATP, NADH & $FADH_2$ are also produced.
* 6 NADH + 2 $FADH_2$ + 2 ATP + 4 CO_2 are produced

Oxidative Phosphorylation



OXIDATIVE PHOSPHORYLATION (on inner mitochondrial membrane)

* When **molecular oxygen (O_2) is available, NADH** from glycolysis, the link reaction & the Krebs cycle, donates high energy e^-(s) to the first electron carrier of the electron transport chain (ETC) on the inner mitochondrial membrane.
* The e^- first carrier is thus reduced & the **NAD** which is **regenerated** can pick up e^-(s) and protons from **glycolysis**, the **link reaction** or the **Krebs cycle.** The first reduced e^- carrier then transfers the e^- to the next e^- carrier & reduces it while the first carrier itself becomes reoxidised.
* The transfer of **electrons** continues in this manner until they combine with H^+ & O_2 **(the final e^- acceptor)**, to form metabolic H_2O in the matrix. This reaction is catalysed by **cytochrome oxidase.** ($2e^- + 2H^+ + \frac{1}{2}O_2 \rightarrow H_2O$)

* As e^- **are transferred** down the increasingly electronegative **electron carriers** in the **ETC, energy is released.** This energy is used to **actively pump H^+** from the **mitochondrial matrix to intermembrane space.**

* This creates a **proton gradient** across the inner mitochondrial membrane. The **energy stored** in the form of a H^+ gradient across a membrane is known as a **proton-motive force.**

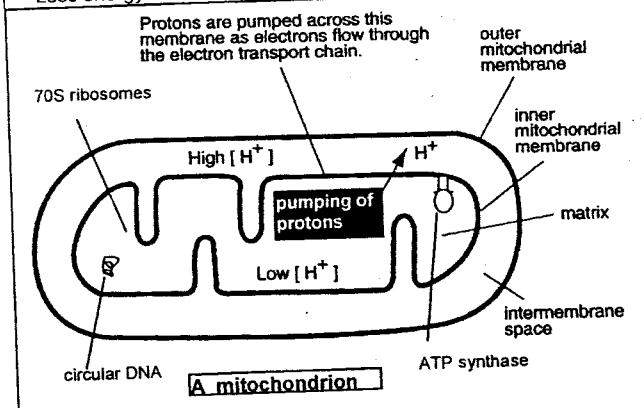
* As **protons diffuse through ATP synthase** (which projectsinto the matrix) down the H^+ concentration gradient into the mitochondrial matrix, **ATP synthase is activated** and it **phosphorylates ADP to ATP in the matrix.**

	CO_2	ATP	NADH	$FADH_2$
Glycolysis	-	2 (net)	2	-
Link reaction	2	-	1 x 2 = 2	-
Krebs cycle	4	2	3 x 2 = 6	2
Sub-total	$6CO_2$	4ATP	10 NADH	2 $FADH_2$

Oxidative phosphorylation
* From **1NADH** → **3 ATP** (or **2.5 ATP**) form
* From **1$FADH_2$** → **2 ATP** (or **1.5 ATP**) form
Thus from **10 NADH** and **2 $FADH_2$** → (10 X 3) + (2 X 2) = **34 ATP** form

Total ATP from the oxidation of 1 glucose molecule: 34 + 4 = 38 ATP

* The e^-(s)from $FADH_2$ are also transferred down the ETC. However, $FADH_2$ releases the e^-(s) lower in the ETC compared to NADH. Hence, Less energy is released from $FADH_2$ during e^- transfer. The **regenerated FAD** then can pick up e^-(s) and protons from the **Krebs cycle**.



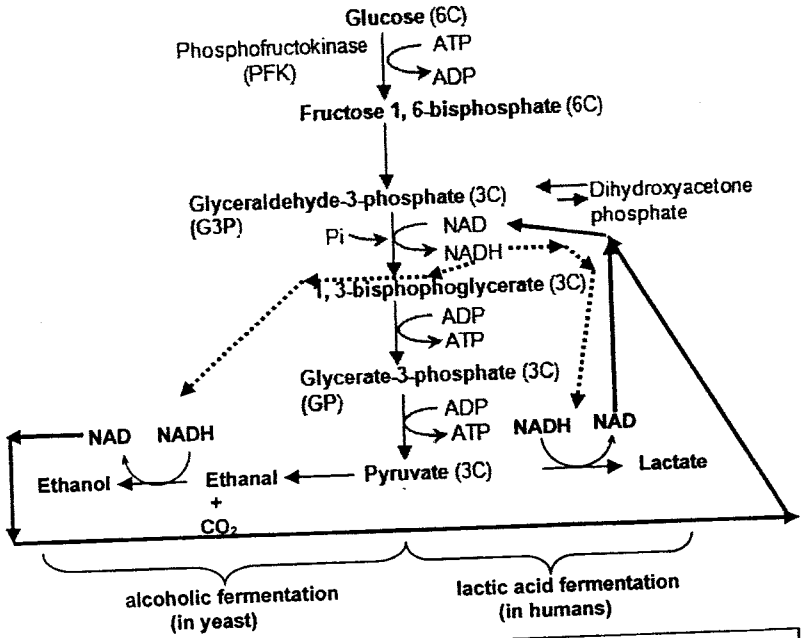
A mitochondrion

* NAD : nicotinamide adenine dinucleotide (a coenzyme)
* FAD : flavine adenine dinucleotide (a coenzyme)
* NAD
  → a coenzyme and a mobile electron carrier
  → carries electrons & protons (in its reduced form, NADH) from organic molecules to electron carriers in ETC & reduces them while, NADH itself is reoxidised to NAD^+

* The number of ATP molecules produced per glucose molecule can vary between 36 to 38. This is because the mitochondrial membrane is impermeable to the NADH generated by glycolysis. The H^+ and electrons of the NADH are passed to either NAD or FAD inside the mitochondrion via a **shuttle system**, and if passed to FAD, only 2 ATP will be produced instead of 3.

* **Chemiosmosis**: an energy coupling mechanism that uses energy stored in the form of hydrogen ion gradient across a membrane to synthesise ATP.

Anaerobic respiration: (1) involves oxidation of glucose in absence of oxygen (2) produces 2 ATP mlcs per glucose molecule



Lactate is transported in the blood to the liver where it is converted back to pyruvate which can then enter the link reaction again during aerobic respiration.

* In the **absence of oxygen (O_2)**, there is no **final e^- acceptor** to accept electrons from the electron transport chain (ETC).

* Electron carriers remain reduced and so NADH and $FADH_2$ can no longer donate electrons to the ETC. Hence **oxidative phosphorylation (OP) cannot occur .**

* In absence of oxidative phosphorylation, there there is **no regeneration of NAD & FAD** and thus the **link reaction & Krebs cycle cannot occur** (because there are no e^- acceptors i.e. NAD & FAD).

* In the absence of oxygen, **glycolysis can still occur as the NAD needed for glycolysis is regenerated from fermentation reactions.**

* **Alcoholic fermentation** occurs in **yeasts** while **lactate fermentation** occurs in **muscles of animals.**

* Both fermentation reactions **regenerate NAD from NADH** in order to keep **glycolysis going. ATP** is only **produced from glycolysis.**

* In **animals** → **pyruvate** is reduced by e^- (s) from NADH in the presence of **lactate dehydrogenase** to **lactate.**
In **yeasts** → pyruvate converted to **ethanal** and CO_2 . Ethanal is then reduced by e^- (s) from NADH in the presence of **alcohol dehydrogenase to ethanol.**

* Thus **pyruvate or ethanal** are the **final e^- acceptors** during **anaerobic respiration.**

* Only **2 ATP mlcs.** are produced **per glucose mlc.** during **anaerobic respiration.** This is **19 times** lower compared to aerobic respiration which produces **38 ATP** mlcs. per glucose mlc.

* Fermentation reactions occur in the **cytosol.**

ATP : adenosine triphosphate

* ATP: universal energy currency
* Energy released from glucose oxidation during respiration is used to make ATP from ADP + P_i.
* ATP made can then be hydrolysed to ADP + P_i, releasing energy in the process.
* Removal of terminal phosphate group from ATP yields 30.6 kJ/mol of free energy.
  → This energy is useful for cellular work such as muscle contraction, maintenance of constant body temperature and active transport.
* When ATP is hydrolysed, it is incorrect to say that a "high energy bond" is broken. Instead modification occurs to the molecule as a whole and there is a net release of energy when the phosphate group is removed.
* ATP is actually **carrier of energy, does not store energy** (compare: fats and glycogen which store energy).
* ATP is the same nucleoside triphosphate used to form RNA.

Differences between Photosynthesis and Aerobic Respiration

Features	Photosynthesis	Aerobic Respiration
Anabolic / Catabolic processes	An anabolic process which results in the synthesis of carbohydrate molecules from simple inorganic molecules and light energy. $6CO_2 + 6\ H_2O$ → $C_6H_{12}O_6 + 6O_2$	A catabolic process which results in the breakdown of carbohydrate molecules to simple inorganic molecules. $C_6H_{12}O_6 + 6\ O_2$ → $6CO_2 + 6\ H_2O$
Storage of energy	*Energy from light is accumulated and stored in* carbohydrates. (Making food using energy from light.)	*Energy is incorporated into ATP* for use in energy requiring processes. (Using food to make energy/ATP)
Oxygen	Oxygen is released.	Oxygen is used.
Carbon dioxide and water	Carbon dioxide and water are used.	Carbon dioxide and water are produced.
Change in dry mass	Process results in an increase in dry mass.	Process results in a decrease in dry mass.
Organelle involved	Chloroplast	Mitochondrion (and cytosol)
Occurence	Process occurs only in cells possessing chlorophyll and only in the presence of light.	Process occurs in all living cells throughout their lifetime.
Electron carrier	NADP	NAD, FAD
Major reactions	Light dependent (cyclic and non-cyclic photophosphorylation) and light independent (Calvin cycle) reactions	Glycolysis, link reaction, Krebs cycle and oxidative phosphorylation
High H^+ concentration in	Thylakoid space	Intermembrane space

Differences between Photophosphorylation and Oxidative Phosphorylation

Features	Photophosphorylation	Oxidative Phosphorylation
Location	Thylakoid membrane of chloroplast	Inner membrane of mitochondrion
Involvement of light energy	Light energy is required for splitting of water.	Light energy is not required.
Source of energy for synthesis of ATP	Energy for synthesis of ATP comes ultimately from light.	Energy for the synthesis of ATP comes from the oxidation of glucose which stores chemical energy.
Electron donors	Water is the electron donor in the non-cyclic pathway while Photosystem I is the electron donor in the cyclic pathway.	NADH and $FADH_2$
Electron acceptors	NADP is the final electron acceptor in the non-cyclic pathway while Photosystem I is the final electron acceptor in the cyclic pathway.	Oxygen is the final electron acceptor (and it combines with H^+) and is reduced to water.
Establishing proton gradient for ATP synthesis	Protons are pumped inwards, from stroma, across the thylakoid membrane, into the thylakoid space.	Protons are pumped outwards, from the matrix, across the inner membrane, into the intermembrane space.

Distinguish between Calvin and Krebs cycle

Features	Calvin cycle	Krebs Cycle
Site	Stroma of chloroplast	Matrix of mitochondria
Electron or hydrogen carriers involved	NADP	NAD, FAD
Carbon dioxide	Fixed by ribulose bisphosphate carboxylase (Rubisco)	Released by oxidative decarboxylation
ATP	Used in reduction of GP to G3P and regeneration of RuBP	Synthesised by substrate level phosphorylation

Distinguish between Non-cyclic and Cyclic Photophosphorylation

Features	Non-cyclic Photophosphorylation	Cyclic Photophosphorylation
Conditions under which process occurs	When plants require ATP and NADPH	When plants require ATP only
Pathway of electron	Non-cyclic (Z-scheme)	Cyclic
Photosystems involved	I and II	I only
First electron donor (source of electron)	Water	Photosystem I
Last electron acceptor (destination of electrons)	NADP	Photosystem I
Establishing proton gradient for the synthesis of ATP	High H^+ concentration in the thylakoid space is due to the photolysis of water and active transport of H^+ from stroma, across the thylakoid membrane, into the thylakoid space.	High H^+ concentration in the thylakoid space is due to the active transport of H^+ from the stroma, across the thylakoid membrane, into the thylakoid space.
Products	ATP, NADPH and oxygen	Only ATP

Homeostasis and Cell Signalling

* **Homeostasis** refers to the **maintenance of a stable internal environment independent of fluctuations in the external environment** by **self-regulating** & **negative feedback** mechanisms so that the **organism can function optimally**.
  Self-regulation: where a **corrective mechanism is triggered by the very entity which is to be regulated**
  (e.g. control of blood glucose levels is triggered by changes in blood glucose levels)
  Negative feedback: a mechanism which brings about **increasing stability of a system** i.e. it **removes any deviations from the set point**
  i.e. a change in a variable triggers a response that counteracts the initial change.
  (e.g. when blood glucose level goes higher than set point, insulin is secreted to return glucose levels to set point).
* **Hormones:**
  → secreted by **endocrine** glands (ductless glands) **directly** into the **bloodstream**
  → effective in **small quantities** (as signal amplification, that occurs during signal transduction, will lead to the production of a strong cellular response)
  → act on specific **target cells** which have **specific cell surface receptors**
  → each type elicits **different cellular responses** & after having served their function, are rapidly broken down
  → can be classified into 3 main classes: 1) proteins / peptides (insulin & glucagon), 2) amines (adrenaline & thyroxine), 3) steroids (oestrogen)
  → may be **hydrophobic** (e.g. oestrogen) & hence readily transverse the **hydrophobic core of the phospholipid bilayer** of cell membrane and bind to **specific receptors within the cell**
  → may be polar/charged & **hydrophilic** (e.g. insulin & glucagon) & hence bind to the **specific receptors on the cell membrane** (e.g. RTK & GPCR)

* **Pancreas:**
  → is an organ that is both an **endocrine (islets of Langerhans)** gland & an exocrine (acinar cells) gland
  → the **islets of Langerhans** contain **alpha** cells which secrete **glucagon**; and **beta** cells which secrete **insulin** into the bloodstream
  (insulin and glucagon are secreted constantly and work in an **antagonistic** fashion; it is their relative concentrations and not their actual levels that are critical to maintain normal blood glucose levels at the set point which is **90mg/dL**)

* **Glucose:**
  → key **respiratory substrate**
  → **only energy source** that the **brain** can utilise
* **Glycogen:**
  → stored in **liver** and **muscles**
* Insulin **triggers** the conversion of glucose to glycogen
* Glucagon **triggers** the conversion of glycogen to glucose
  (It is incorrect to say that insulin converts glucose to glycogen as insulin binds to the insulin receptor which triggers a signal transduction pathway that eventually leads to the conversion of glucose to glycogen in the cell. Likewise, it is incorrect to say that glucagon converts glycogen to glucose.)

* A **deviation from the set point i.e. stimulus (e.g. high blood glucose levels)**
  → is detected by **detectors (e.g. beta cells in islets of Langerhans)** (N.B. Sometimes detectors are referred to as receptors)
  → which secretes an appropriate **signal (e.g. insulin)**
  → which binds to the **cell surface receptors** of the cell** (e.g. **cell surface insulin receptor, RTK**) of the **effector** (e.g. **liver/muscle cells**)
  → which brings about an appropriate **response** that **restores the condition to the set point** (e.g. **blood sugar levels return to set point**)
  → this serves as **negative feedback** to detectors (e.g. beta cells) to decrease secretion of signal (e.g. insulin)

Cell signaling (3 stages):

1) Ligand-receptor interaction:
→ **ligand/signal** binds to a specific, **ligand-binding site** (which is **complementary in shape and charge** to the ligand) on the **extracellular domain** of the **cell-surface receptor** to form a **ligand-receptor complex.**

2) Signal transduction & amplification:
→ where binding of the signal to the protein receptor causes a **conformational change** in the **intracellular domain** of the protein receptor which **initiates** the signal transduction. i.e. the signal is converted to a form that can bring about a specific cellular response.
→ transduction usually occurs in a series of multiple steps in a **signal transduction pathway**.
→ the signal transduction pathway is mediated by intracellular signaling proteins (e.g. kinases^^) or small molecules(e.g. cAMP) or ions.
→ the presence of multiple **catalytic** steps in a signal transduction pathway allows **amplification** of the signal, where the number of activated molecules increases with each subsequent step.(Hence signal amplification occurs during signal transduction.)

3) Cellular response:
→ where the transduced signal triggers specific changes in cellular function, metabolism, or development (e.g. gene expression) by **targeting proteins** such as gene regulatory proteins, ion channels, components of a metabolic pathway etc.
(^^ kinases phosphorylate proteins while phosphatases dephosphorylate proteins)

Advantages of a cell signaling pathway:

1) Facilitates **amplification** of signal
   → **small number of signal molecules** binding to the receptors can produce a **large cellular response** as the **number of activated molecules increases with each catalytic step** in the pathway
2) One signal molecule may elicit **many cellular responses** via many pathways in a cell
   → **2nd messengers** or **relay proteins** may activate multiple proteins involved in different signal transduction pathways to produce **multiple cellular responses**
3) Provides **many checkpoints for regulation** as cellular responses can be **terminated/regulated** at
   (i) At Reception:
   → extracellular first messenger can be degraded by enzymes in the extracellular space
   → endocytosis of cell surface receptors to prevent ligand-receptor interaction can prevent signal transduction
   → endocytosis of the entire ligand-receptor complex can prevent signal transduction
   (ii) During Signal Transduction Pathway
   → e.g. phosphatases dephosphorylate & inactive the relay proteins → inhibit further signal transduction
   → production of inhibitors that bind to the intracellular domain of the ligand-receptor complex and /or any of the intracellular signal proteins in the signal transduction pathway to prevent transduction of the signal.
4) One signal can allow the **coordinated activation of many cells** simultaneously
5) Ensures **specific reactions** are triggered as a **specific signal** will bind to a **specific receptor** and will elicit specific reactions in specific cell types.
6) A signal molecule can **activate genes in nucleus** upon binding to cell surface receptor **without the need to move into nucleus.**

Advantages of signal transduction...

Control of Blood Glucose Levels: An Overview

	INSULIN	GLUCAGON
A **stimulus**	An **increase in blood sugar level above 90 mg/dL**	A **decrease in blood sugar level below 90 mg/dL**
↓	↓	↓
is detected by a **detector/receptor** (which is a cell that detects the change)	is detected by the **beta cells** of islets of Langerhans of pancreas	is detected by the **alpha cells** of islets of Langerhans of pancreas
↓	↓	↓
which releases a **ligand/signal** (e.g. insulin/glucagon)	which secretes **insulin (1st messenger)**	which secretes **glucagon (1st messenger)**
↓	↓	↓
which recognises and binds to **cell surface receptors** (e.g. RTK / GPCR)	which recognises and binds to cell surface receptors known as **insulin receptors (a receptor tyrosine kinase, RTK) which exist as linked dimers**	which recognises and binds to cell surface receptors known as **glucagon receptors (a G-protein coupled receptor, GPCR)**
↓	↓	↓
on the **effector** (which is a cell where the cellular response occurs).	on the **liver** (or muscle) cell	on the **liver** cell
↓	↓	↓
Ligand-binding causes a **conformational change** in the **intracellular,cytoplasmic domain** of the receptor, activating it This **initiates signal transduction.**	Ligand-binding causes RTK to undergo **changes in conformation** in its **intracellular, cytoplasmic domain**	Ligand-binding causes GPCR to undergo **changes in conformation** in its **intracellular, cytoplasmic domain**
↓	↓	↓
The signal is **further transduced** and **amplified.**	This conformational change exposes the active sites of the kinases in each of the subunits of the receptor and triggers **autophosphorylation** i.e. where **kinases on 1 subunit cross-phosphorylates tyrosine residues on the other subunit**	This conformational change causes an **inactive G protein to bind to the GPCR**, and **release** its bound **GDP** and allow **GTP to bind** in its place. GTP binding causes a **conformational change in the G protein, activating it.**
↓	↓	↓
	Phosphorylated tyrosine residues serve as **docking sites** for other relay proteins	Activated G protein dissociates from the receptor and translocates along the **cytoplasmic side** of the cell membrane to bind to and activate an enzyme, **adenylyl cyclase**
	↓	↓
	Relay proteins activated by binding or via phosphorylation by RTK	Adenylyl cyclase converts ATP to **cAMP** (2nd messenger) which activates **Protein Kinase A**
	↓	↓
	Relay proteins may be kinases which can go on to phosphorylate other proteins when activated	Activated Protein Kinase A can phosphorylate other proteins
	↓	↓
	a phosphorylation cascade	
	↓	↓
which results in the following **cellular responses**	Phosphorylation activates protein kinases in a cascade which eventually activates **glycogen synthase** which catalyses **glycogen synthesis from glucose.** (i.e. increase **glycogenesis** in liver & muscle) Other cellular responses: e.g. 1) **Translocation of glucose transporters from the membrane of cytoplasmic vesicles to the cell membrane.** This **increases glucose intake** into cells e.g. 2) Increased **rate of glycolysis** e.g. 3) Increased lipid & protein synthesis	Protein kinase A activates phosphorylase kinase which activates **glycogen phosphorylase** which catalyses the **breakdown of glycogen to glucose** (i.e. **glycogenolysis** in liver & muscle cells) Other cellular responses: e.g. 1) Increased **gluconeogenesis** (synthesis of glucose from non-carbohydrate sources)
↓	↓	↓
which eliminates the stimulus,	Blood glucose levels **decreases**	Blood glucose level **increases**
↓	↓	↓
resulting in a diminished response.	This is detected by the receptor (detector, i.e. beta cells) which then **decreases insulin production**	This is detected by the receptor (detector, i.e. alpha cells) which then **decreases glucagon production**

Note:

* **First messenger:**
  → extracellular ligand/signal molecule that binds to membrane receptor e.g. insulin, glucagon etc.

* **Second messenger:**
  → small, non-protein, water-soluble molecules or ions.
  → can readily spread throughout the cell by diffusion
  → can participate in pathways initiated by **both GPCR and RTK.**
  e.g. cAMP → synthesised from ATP by adenylyl cyclase
  →activates kinases which phosphorylate other proteins

* G protein has **intrinsic GTPase activity** which can hydrolyse GTP to GDP and inactivate the G protein.

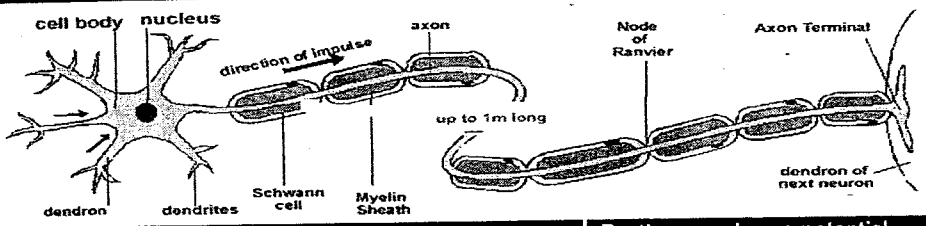
Nervous system

Function of communication systems in our body

Transmit information between different body parts so that bodily functions can be well-coordinated and carried out efficiently

Point of comparison	Endocrine system	Nervous system
Type of stimulus	Internal stimulus	External and internal stimulus
Nature of information	*Chemical* signals ie, hormones	*Electrical* signals within neuron and *chemical* signals across synapses ie, neurotransmitters
Mode of transmission	Endocrine gland secretes hormones directly into the *bloodstream* where they are carried to target organ	Via a system of *neurons* that branch throughout the body
Speed	Slow	Fast
Duration of response	Meant for *long term* effects eg. growth	Meant for *short term* effects
Nature of response	Widespread	Localized
Specificity of pathway	General (entire bloodstream) *BUT* target is specific	Specific pathway of neurons

Structure of a neuron



Neuron structure	Neuron function
Numerous dendrites	Communicate with many other neurons
Axon is long Axon has wide diameter	Transmission of impulse over long distances Increase speed of conduction
Permeability of plasma membrane can be altered by a stimulus	Voltage gated Na^+ channels open → depolarization Voltage gated K^+ channels open → repolarization As a result, action potential is produced
Myelin sheath along axon	Saltatory conduction increases speed
Membranes at nodes of Ranvier have numerous 1)voltage gated ion channels 2) Na^+/K^+ pumps 3) Leak channels	1) Regeneration & transmission of action potential 2) Actively pump Na^+ & K^+ against concentration gradient to restore unequal ion concentration → so another action potential can be generated
Synaptic vesicles	Contain neurotransmitters for synaptic transmission
Numerous mitochondria at pre-synaptic knob	Produce ATP needed for 1) exocytosis of acetylcholine at pre-synaptic knob 2) active transport of choline back into pre-synaptic knob after acetylcholinesterase in synaptic cleft degrades acetylcholine into acetic acid & choline 3) actively pump Ca^+ out of pre-synaptic knob

Resting membrane potential

When neuron is not stimulated, the resting membrane potential is -70mv (intracellular environment **relative** to extracellular environment)

Why the resting membrane potential is negative:

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

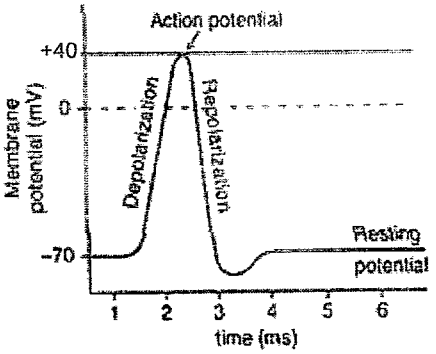
2) **Na^+- K^+ pump** uses ATP to pump **$3Na^+$ out and $2K^+$ in**
Hence there is a net loss of positive ions.

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

Action potential

How it is *generated*

1. Resting state (-70mv): all voltage-gated Na^+ & K^+ channels are closed
2. Depolarization (+40mv): Stimulus results in opening of **some** voltage-gated Na^+ channels → **influx of Na^+. If depolarization reaches threshold potential of -55mv, action potential will be generated.** More voltage-gated Na^+ channels open → further **influx of Na^+** → further depolarization to +40mv. Voltage-gated K^+ channels remain **closed**.
3. Repolarization: At +40mV, all voltage-gated Na^+ channels **close,** influx of Na^+ stops; All voltage-gated K^+ channels **open, efflux of K^+**
4. Hyperpolarization (-90mv): Although resting membrane potential (-70mv) is reached, K^+ continues to leave as voltage-gated K^+ channels are **slow to close**
5. Restoration of membrane potential: All voltage-gated K^+ channels close. Membrane returns to resting potential.

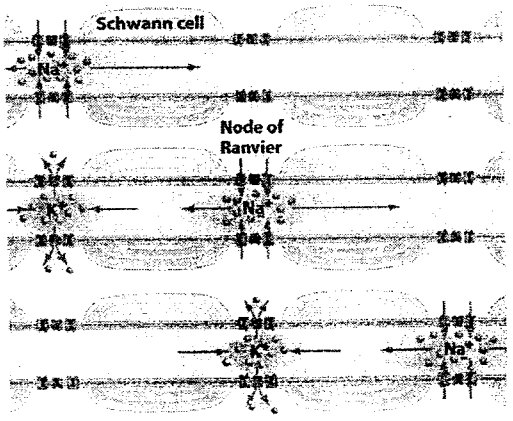


How it is *propagated*

6. Influx of Na^+ can create a **local current** which diffuses sideways
7. Although it can diffuse back to the previous node, the previous node will still be in **refractory** period and will not generate another action potential
8. Therefore impulse is only propagated in one direction down the axon
9. When Na^+ diffuses **downstream** to the **next node**, it causes membrane to depolarize to **threshold potential** so another action potential is generated

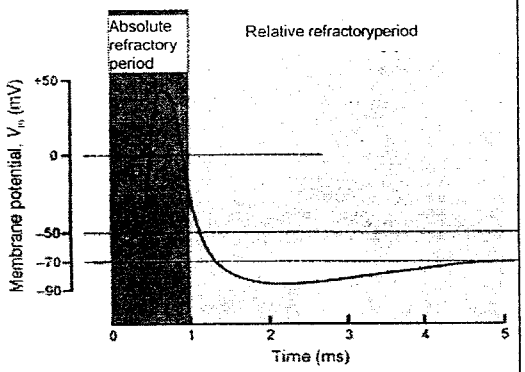
*(Pts 10-12 for **myelinated** neurons only)*

10. Action potential is only generated when it reaches the **next node of Ranvier**
11. As myelin sheath is an electrical insulator due to its high lipid content , it prevents leakage of Na^+ and K^+. Hence action potentials appear to "jump" from node to node
12. This is known as **saltatory propagation** which results in faster transmission of impulse compared to non-myelinated neurons.



Characteristics of action potentials

- Identical in **magnitude & duration**, independent of strength of stimulus
  Reason: magnitude of action potential determined by [Na^+]. Greater the [Na^+] difference, higher the depolarization peak. Since ion concentration remain constant due to homeostasis, peak size is the same.
  NOTE: stronger stimuli = higher **FREQUENCY of action potentials generated*



- Are propagated in **1 direction**
  *Reason (along axon): **Refractory period** (time during an action potential where a normal stimulation will not generate another action potential as voltage-gated Na^+ channels need time to reset.)*

  Reason (across synapse): Only axon terminals have synaptic vesicles and only post-synaptic membranes have neurotransmitter receptor

- Only generated at **nodes of Ranvier** of myelinated neurons
- Are an **all-or-none** event –only when threshold potential is reached is an action potential generated

Speed at which axons transmit action potential depends on:

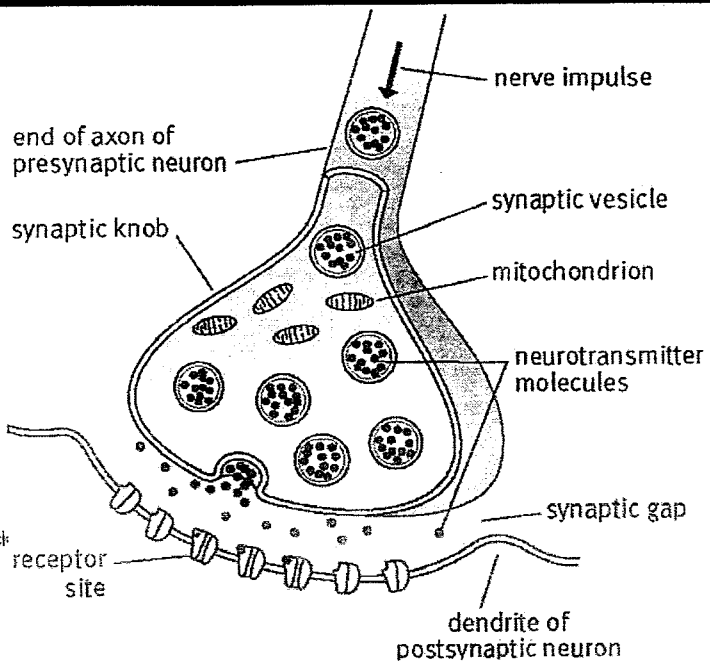
- **Diameter of axon**
  Larger diameter → reduced resistance → faster
- **Presence/absence of myelin sheath**

  Myelinated → faster
  - action potential generated over smaller area of plasma membrane
  - insulation causes local current to spread further along axon interior

Synapses

Transmission

1. Arrival of action potential and **depolarization** of **pre-synaptic knob**
2. causes opening of **voltage-gated Ca^+ channels**
3. **Influx of Ca^+** due to lower [Ca^+] inside relative to outside
4. Stimulates migration of **vesicles containing acetylcholine** to **fuse** with the presynaptic membrane
5. releasing acetylcholine into the synaptic cleft via **exocytosis**
6. Acetylcholine **diffuses** across synaptic cleft
7. And binds reversibly to **specific receptor site** on **ligand-gated Na^+ channel** on **post-synaptic membrane**
8. Causes **conformational change** in **ligand-gated Na^+ channel causing it to open**
9. **Influx of Na^+** results in **depolarization** of **postsynaptic membrane**
10. **If threshold potential is reached, action potential will be generated.**
11. Acetylcholine is **degraded** by **enzyme acetylcholinesterase** into acetic acid and choline. **Choline** is **actively transported** back into pre-synaptic knob where it combines with **acetyl-CoA** to **reform acetylcholine**
12. **Ca^{2+} rapidly removed** by active transport out of pre- synaptic knob via **Ca^{2+} pump**.



Synaptic Delay

- delay in the depolarization of the post-synaptic membrane after the arrival of the impulse at the presynaptic membrane

Reasons for synaptic delay

1. **Calcium influx into pre-synaptic knob**
2. **Movement of synaptic vesicles** towards pre-synaptic membrane to release neurotransmitters by exocytosis
3. **Diffusion** of **neurotransmitter** across **synaptic cleft** form pre-synaptic membrane to post-synaptic membrane.

(a) Explain the binomial nomenclature of a species and hierarchical classification.

❖ The **binomial system of nomenclature** classifies every organism with a genus name & a species name/specific epithet (in modern Latin).

The **genus name** always begins with a **capital letter** & the **species name** always begins with a **lower case letter.**

Both names must be **italicised** when typed and **underlined separately** when written.
e.g. *Homo sapiens* **OR** Homo sapiens
[NB: Nomenclature: the giving of names to biological groups; Binomial: consisting of two parts.]

When asked for the species/ scientific name/binomial name of man, we use "Homo sapiens" and not "sapiens" alone.
Hierarchical classification involves the **grouping of organisms into increasingly inclusive groups**.
Thus related species are grouped into the same genus, related genera are grouped into the same families, related families are grouped into the same order, related orders are grouped into the same class, related classes are grouped into the same phyla, related phyla are grouped into the same kingdom & related kingdoms are grouped into the same domain.

(b) Describe the classification of species into taxonomic groups (genus, family, order, class, phylum, kingdom) and explain the various concepts of the species. Knowledge of biological, ecological, morphological, phylogenetic concepts of species is required.)

❖ Taxon: a recognisable group of organisms at any particular level of classification. For e.g., *Homo sapiens* and Chordata are both taxa.
Organisms are broadly categorised into a series of eight nested groups referred to as a taxonomic hierarchy. They are

Domain,	Kingdom,	Phylum,	Class,	Order,	Family,	Genus,	Species
(Eukarya)	(Animalia)	(Chordata)	(Mammalia)	(Primates)	(Hominidae)	(*Homo*)	(*sapiens*)
(Dumb	King	Phillip	Came	Over	From	Greece	Singing)

❖ **Explain the various concepts of the term species** (see table).

Species concepts	Definitions	Appreciating The Significance Of The Various Concepts
Biological (***)	A species is group of organisms capable of **interbreeding** and producing **fertile, viable offspring.**	According to this concept, members of the same species are reproductively isolated from other species and thus they share a common gene pool and have the same chromosome number. **Advantage:** Organisms being studied can be interbred to see if they produce fertile, viable offspring. **Limitation:** This definition cannot be applied to asexually reproducing organisms and extinct species whose breeding behavior cannot be observed.
Ecological	A species is a group of organisms sharing the same ecological niche*.	According to this concept, the differences between species are due to the differences in the ecological resources that they depend on. This means that if a species can no longer occupy a particular niche, it would be considered a new species. **Advantage:** This definition applies to organisms that reproduce both **sexually** and **asexually**. **Limitation:** This definition cannot be applied to **unrelated species that occupy similar niche**. e.g. the striped possum, a marsupial mammal from Australia, and the aye-aye, a placental mammal from Madagascar, are not related but occupy a similar niche in different parts of the world. They both eat worms/insects in the bark of trees. **Niche - 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 species is a group of organisms sharing similar **body shape, size** and other **structural features.**	When using this concept, researchers may disagree on which structural features distinguish a species as this definition relies on superficial & subjective criteria, & it is known from genetic data that genetically distinct populations may look very similar, & contrarily, large morphological differences can exist between closely related species. **Advantage:** This definition can be applied to all organisms (i.e. sexually and asexually.reproducing organisms) **Limitations:** 1) This definition makes it difficult to determine the **degree of difference** that is required to indicate **separate species** as well as **what structural features** should be used to **distinguish the differences**. 2) Some organisms may be **superficially similar** but have **different evolutionary origins** e.g. the marsupial mole from Australia and the golden placental mole from South Africa.
Phylogenetic	A species is the **smallest group of organisms** that **share a most recent common ancestor** and can be distinguished from other such groups.	The phylogenetic history of a species can be obtained by comparing **homologous morphological structures and/or homologous molecular sequences**, with those of other organisms. Thus this concept takes into account anatomical, molecular & embryological homologies to recreate an evolutionary history to connect a common ancestor to a group of organisms. **Advantage:** Using this definition to classify species will **avoid mistakenly classifying organisms based on superficial morphological similarities** as the characteristics that are compared are based on **common ancestry/homology** **Limitations:** It is difficult to determine the most recent common ancestor of a species.

(c) Explain how species are formed with reference to geographical isolation, physiological isolation and behavioural isolation

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

- **Speciation** is a process by which **one or more new species arise** from a previously **existing species.**
  - Speciation that occurs when **geographical isolation** occurs as a result of the presence of a **physical barrier** is called **allopatric speciation**
  - Speciation that occurs when populations are not separated geographically but gene flow is disrupted by **physiological or behavioural isolating mechanisms** is called **sympatric speciation**

- For speciation to occur, ***gene flow must be disrupted*** between two populations of the existing species. Then **evolutionary changes** (e.g. mutations, natural selection, genetic drift) could occur **independently in each sub population.** If this continues over a **long period of time,** then **new species** can form.
- Gene flow may be disrupted in three ways:
  - **Geographical isolation** – is due to a **physical barrier** (e.g. a body of water) between two sub- populations which blocks migration of individuals and disrupts gene flow
  - **Physiological isolation** – is due to the **unique physiology** (e.g. difference in flowering time due to physiological response to different soil conditions) of different individuals in the same area which disrupts gene flow
  - **Behavioural isolation** – is due to the **unique mating rituals** (e.g. different bird song) and **preferences** of individuals in the same area such that a group of mating individuals isolate themselves from the main population, disrupting gene flow

Geographical isolation

e.g.:Caribbean porkfish in the Caribbean sea, and the **Panamic porkfish** in the Pacific ocean

How speciation occurred

* An **ancestral fish population** was **split into two** by the formation of the Isthmus of Panama about 3.5 million years ago.
* This **geographic isolation disrupted gene flow** between the two sub-populations.
* The divided populations were exposed to **different environments** and thus **different selection pressures.**
* Since there was **variation within the populations, favourable** characteristics were **selected for** and **unfavourable** characteristics were **selected against**
* Thus **evolutionary changes** occured **independently** in each subpopulation i.e. **different genetic changes** occurred in each sub-population (due to mutations, natural selection and genetic drift).
* **Over hundreds and thousands of successive generations** each subpopulation became **reproductively isolated genetically distinct species.**

e.g.: Darwin's finches on the **Galápagos Islands**

How speciation occurred

* An **ancestral population** of Darwin's finches strayed from the **South American mainland** of Ecuador to one of the **Galápagos Islands.** They then **colonized other islands/parts of the islands.**
* The sub-populations were **geographically isolated** in the various islands/parts of the islands and so did not interbreed. Hence **gene flow was disrupted.**
* The divided populations were exposed to **different environments** in the different islands and thus were under **different selection pressures.** Since there was **variation within the populations, favourable** characteristics were **selected for** and **unfavourable** characteristics were **selected against.**
* Thus **evolutionary changes** occured **independently** in each subpopulation i.e. **different genetic changes** occurred in each sub-population (due to mutations, natural selection and genetic drift).
* **Over hundreds and thousands of successive generations,** each subpopulation became **reproductively isolated genetically distinct species.**
* There are now at least **13 species of finches** on the Galapagos Islands, each filling a different niche on different islands/parts of the islands.
* This kind of evolutionary pattern in which there is a **rapid increase in the number of species** produced from a common ancestor upon introduction into new environments is known as **adaptive radiation.**

Physiological isolation

e.g.: two species of **palms** in **Lord Howe island,** *Howea forsteriana* and *Howea belmoreana.*

How the 2 species are now:

* There are also **two soil types** on the island - the older volcanic soil and the younger calcareous soils.
* The two species of palms have **different soil preferences.** One palm species grows in **calcareous soil** while the other grows in **volcanic soil.** Since these **soil types are in close proximity** to each other in many areas, the two species of **palm can coexist in close proximity.**
* However, the two species **flower at different times** due to the **difference in soil conditions** and hence **cannot interbreed** i.e. the two species are **reproductively isolated.**

How speciation occurred

* When the palms that normally grew on volcanic soil started to grow on calcareous soil, a conspicuous **flowering time difference** may have arisen as a **physiological response** to a new substrate.
* This **prevented the interbreeding** between the two palms growing in the two types of soil although they were in close proximity.
* This **disruption of gene flow** resulted in evolutionary changes occurring independently in each sub- population. Since there was **variation within the population, favourable** characteristics were **selected for** and **unfavourable** characteristics were **selected against.**
* Thus **evolutionary changes** occured **independently** in each subpopulation i.e. **different genetic changes** occurred in each sub-population (due to mutations, natural selection and genetic drift).
* **Over hundreds and thousands of successive generations** each subpopulation became **reproductively isolated genetically distinct species.**

Behavioural isolation

e.g.: eastern meadowlark and the **western meadowlark**

How the 2 species are now:

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

How speciation occurred

* In a population of a particular bird species **some members** of the population **developed a new call.**
* Over many generations, the new bird **call became more distinct.**
* Birds began to distinguish between the two calls and tended to **mate preferentially with the members with the same call.**
* This **disruption of gene flow** resulted in evolutionary changes occurring independently in each sub- population. Since there was **variation within the population, favourable** charact eristics were **selected for** and **unfavourable** characteristics were **selected against.**
* Thus **evolutionary changes** occured **independently** in each subpopulation i.e. **different genetic changes** occurred in each sub-population (due to mutations, natural selection and genetic drift).
* **Over hundreds and thousands of successive generations** each subpopulation became **reproductively isolated genetically distinct species.**

(d) Explain the relationship between classification and phylogeny.

- **Classification** uses a **binomial naming system**. It groups of organisms in a **hierarchical manner** into **increasingly inclusive categories** such as species, genus, family, order, class, phylum, kingdom and domain based **mainly** on their **morphological characteristics.** (not evolutionary history)
- **Phylogeny** is the grouping of organisms based on their **evolutionary history** (i.e. **ancestor-descendent relationships**)
  These evolutionary relationships are based on **homologous characters** which are passed down from ancestor to descendants

	Classification	Phylogeny
basis of grouping organisms	organisms grouped based on **overall morphological similarities** and without considering their evolutionary history	organisms grouped based on their **evolutionary history** of organisms (i.e. based on ancestor-descendent relationships)
system of organizing organisms	is a **naming system** where each organism is given a binomial name and grouped into a domain, kingdom, phylum, class, order, family, genus and species using a **hierarchical classification system**	is a system which assigns each organism a position on a phylogenetic tree based on its **evolutionary relationship with other organisms** on the tree
how species are presented	uses **bionomial nomenclature**	uses a **phylogenetic tree** where more closely related organisms are grouped closer together in the different branches of the tree
nature of characteristics	organisms are grouped using **only morphological characteristics** and does not distinguish between analogous and homologous characteristics	organisms are grouped using **homologous characteristics** that are derived from a common ancestor
types characteristics used	uses **morphological characteristics** only	uses (1)**morphological**, (2)**anatomical**, (3)**embryological** as well as (4)**molecular** characteristics (such as DNA, RNA, amino acid sequences) and (5)**fossil record**
strengths and weaknesses	**can easily place** an organism into its well defined group →may wrongly classify organisms that are not related but look similar due to ***convergent evolution****	**cannot immediately place** an organism into the phylogenetic tree as evolutionary history need to be established from multiple sources →rarely classifies wrongly as convergent evolution will be placed in separate branches
inference of speciation events	does not allow inference of historical speciation events	indicates **speciation events** as the node of the phylogenetic tree;
inference of common ancestors	does not allow inference of common ancestors	allows inference of **common ancestors**. Descendants of a common ancestor are represented in the same branch
inference of relationships	cannot infer of how closely related 2 species are especially since they are grouped together in the same hierarchy, "species"	allows accurate inference of **how closely related 2 species are** by looking at how recently they diverged from their common ancestor
application of molecular clock	not possible to use molecular clock to date speciation events	using molecular evidence, can apply the molecular clock to **infer time of speciation**

NB: Systematics is phylogenetic classification. It uses evolutionary relationships to organize classification systems

(e) Explain why variation is important in selection.

- Variation:→ **phenotypic differences** between individuals in a population due to genotypic differences.
  → is the **raw material for natural selection to act on.**
- When **environmental changes** occur, the variation amongst individuals results in some individuals surviving better & reproducing more successfully than others. (i.e. organisms that are **best adapted** to the particular environmental condition will have a selective advantage and will be **selected for** through natural selection).
- **If not for variation**, selection would either **favour all organisms or select against them**. (i.e. there will be no differential selection).

(f) Explain, with examples, how environmental factors act as forces of natural selection.

Example 1:

Before 1848 i.e. before the industrial revolution
→ there were 2 forms of peppered moths (***Biston betularia***), the lighter form and the melanic form
→ there was a greater proportion of the lighter form than the melanic form
→ the melanic form arose by **spontaneous mutation**
By 1895
→ 98% of all peppered moths in industrial areas were the melanic form

[NB: It is incorrect to imply that industrialisation caused the melanic form of peppered moth to appear. The melanic form arose by spontaneous mutations which existed before the industrial revolution.]

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

Selection pressure
↓
With industrialisation, lichens on bark of trees were killed. The darker coloured barks which were once covered with white lichen were now exposed.
Thus lighter forms of moth became easy **prey to birds** & their numbers declined.
The melanic form of moth were camouflaged & thus proliferated.

Differential reproductive success
Thus the melanic form of moths had a selective advantage in polluted areas & increased in frequency there.
and the lighter form of moths had a selective advantage in non-polluted areas & increased in frequency there.

Example 2:

1940s: **antibiotics** first used to kill **bacteria**.

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

[NB: It is incorrect to imply that the antibiotics cause the bacteria to mutate into resistant strains. The resistant strains arose by spontaneous mutations. The antibiotics merely provided the selection pressure that increased the frequency of the resistance allele.]

Selection pressure
↓
Antibiotics consumed

Differential reproductive success
Antibiotics kill most of non-resistant bacteria
Selection pressure now favours resistant mutant strains which have a selective advantage.
These survive and pass on the allele for antibiotic resistance to offspring. Antibiotic-resistant allele frequency increases.

(g) Explain how natural selection may bring about evolution.

- Natural selection is a process in which individuals that have certain inherited traits tend to survive and reproduce at higher rates than other individuals, because of those traits. The theory of natural selection was put forward by Charles Darwin.

(1) **Overproduction of offspring**: All organisms produce a large number of offspring. This can lead to an **exponential increase** in population size **if all** of the **offspring survive.**

(2) **Constancy of numbers**: However the **size** of most populations stay **relatively constant** as many offspring **die** before they reach **reproductive age**

(3) **Struggle for existence** : This is because individuals of a species are constantly **competing** with each other for **limited resources** (eg:food, mates & shelter). Other factors such as **disease** and **predators** also impose a limit on the numbers.

(4) **Variation within a population** : Individuals in a population differ from each other genetically(due to the presence of different alleles) and hence phenotypically. These variations, are a pre-requisite for evolution by natural selection.

(5) **Survival of the fittest by natural selection** : Among the variety of individuals, some individuals will have **characteristics/phenotypes that are better adapted to the environment** & hence are able to **survive** to reproductive age & produce viable, fertile offspring. These individuals (with favourable characteristics, due to favourable alleles) are **selected for by** the environment while those that are less well adapted (with unfavorable characteristics) will be eliminated (i.e. selected against).

(6) **Like produces like** : Individuals with advantageous characteristics are likely to breed successfully and produce **offspring similar to themselves.** Thus **advantageous characteristics** (and **hence alleles) are passed on** to the **offspring** and will **increase in frequency** in the population.

(7) **Formation of a new species:** Over **hundreds and thousands of generations**, with genetic isolation (i.e. no gene flow), **reproductive isolation** will occur and a **new species can form.**

(h) Explain why the population is the smallest unit that can evolve.

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

(i) Explain how homology (anatomical, embryological and molecular) supports Darwin's theory of natural selection (with emphasis on descent with modification).

- **Homology:**
  →refers to similar anatomical, molecular & embryological characteristics found in different species due to **common ancestry.**
  These characteristics present in an ancestral organism developed into different forms as the result of natural selection, as they faced different environmental conditions **(descent with modification)**

- **Homologies suggest common ancestry**: e.g. The pentadactyl limb is a homologous feature of all tetrapods, with different forms in different species. This suggests that they **descended from a common ancestor** which had a basic form of the pentadactyl limb.
  Hence, homologies show "descent with modification": Comparisons of homologies (anatomical, embryological and molecular) between species show how an ancestral homology in a population may have been modified in descendent species through natural selection.
  Homologies provide the basis of comparison: hence homologous traits are what are compared between populations or species as they are derived from a common ancestor which shows the modification process from a basic ancestral form.
  [NB: Non-homologous (analogous) structures, (such as the wing of a bird & the wing of insects) are a result of convergent evolution. Such structures are absent in the common ancestral species as they are derived from different ancestors, although they may have a similar function.]

- **How homologies support Darwin's theory of natural selection:**

	Anatomical Homology	Embryological Homology	Molecular Homology
Definition	Organisms with **anatomical** homology have **morphological structures** such as bones, organs and gross structural features that they share with a **common ancestor.**	Organisms with **embryological** homology share **similar structures during embryonic development** as they shared a **common ancestor** with the same developmental stages.	Organisms with **molecular** homology have **similar DNA, RNA & amino acid sequences** as they share a **common ancestor** that had these molecules.
Named e.g.	**Pentadactyl limb** structure in **forelimbs** of all **tetrapods.** e.g. humans, cats and whales.	**Gill slits** in the **embryo** of fish and man	**Cytochrome C** and **p53** are homologous genes
How they are homologous	The forelimbs have the same **arrangement of bones** but have **different functions** and **superficially look different.** (e.g. walking in cats and swimming in whales)	All vertebrates, including fish and man, have **gill slits during early embryological development** which later develop into gills in fish and part of the ear and throat in humans.	Homologous genes share significant **sequence homology** and when expressed produce **proteins** that have the **same function** in all organisms that possess them.
Descent with modification	The **5 digit pentadactyl limb structure** in the common ancestor was altered by natural selection in the different organisms to **suit their specialised functions/environments**, resulting in **variations of the pentadactyl limb structure**	The **basic embryological body plan** in the ancestral species was altered by natural selection in the different descendant species due to **different selection pressures in the different environments.**	**Nucleotide sequences** in the ancestral genes were modified **due to accumulation of mutations** that occurred over many generations and selection pressures that favoured some mutations over others.
Degree of relatedness	Relying on anatomical appearance is not a reliable indicator of relatedness	The **longer the embryological development remains similar, the more closely related** the 2 species are.	The **greater the sequence similarity between homologous genes, the more closely related** the 2 species are.

[NB: Anatomically homologous structures that are greatly reduced in size or have little to no function are called **vestigial structures.**
Organisms with vestigial structures share common ancestry with organisms in which the structure is still functional.
e.g.1: The hind limbs in whales are reduced to small bones (i.e. vestigial structures) as they are no longer beneficial to whales which swim. However, their presence in whales suggest common ancestry with tetrapods.
e.g.2: The appendix in humans is also a vestigial structure as it is reduced from the cecum of its primate ancestors which was involved in digestion of plant material. Thus, the presence of the appendix in humans, suggests common ancestry with primates.]

(i) Explain how biogeography and the fossil record support the evolutionary deductions based on homologies.

❖ **Biogeography** → is the study of the **past** and **present geographical distribution** of organisms.

If Darwin's theory of descent from a common ancestor were to be supported, then biogeographical evidence should indicate that **closely related species** and their **common ancestors** should be **present in the same geographical region.** This was indeed the case. Typically, the common ancestor establishes itself in one location and as speciation occurs, the new species disperse out of the center of origin. The pattern of species distribution across space (biogeography) points towards Darwin's concept of descent with modification from a common ancestor.

Example:

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

❖ **Continental Drift**

→ When **related species are not distributed in the same geographical region** (when it should), **continental drift can explain this discrepancy.**
→ Drawing on the concept of plate tectonics, we observe that the **continents on Earth drift.**
→So **fossilized remains** that **once might have been found on a supercontinent** may have **split up** into a multitude of **smaller continents distributed over a wide area** after millions of years.

Example:

- In the case of the **extinct mesosaurs**, their fossils are distributed across Africa and South America which are separated by the vast Atlantic ocean. Based on continental drift, the 2 continents were once joined together and that was the time when the ancestor of mesosaur originated.
- This ancestral species gave rise to a variety of mesosaur species that radiated out in this ancient supercontinent.
- When the continent broke up and separated, their fossilized remains were transported to where they are today.
- The concept of descent with modification from a common ancestor still holds true but time and continental drift has separated the descendants.
- The living equivalent used to explain the same concept is the lungfish – a living fossil which is found today only in Africa, Australia and South America.

❖ **Island Biogeography**

Example:

- The Galapagos islands are a group of small islands that are found off the coast of Ecuador, South America.
- **Finches** in the on the **Galapagos islands** bear **similarity** to the finches found on the coast of the **South American mainland.**
- This suggests that they **share a common ancestry.**
- However, there are now **13 different island species.**
- This is because the finches from the mainland migrated to the islands and those that were **best adapted to a variety of different niches in the different islands survived, reproduced there.**
- Since the finches were geographically isolated (which disrupted gene flow) from the mainland & each other, evolutionary changes (e.g. natural selection, genetic drift, mutations) occurred independently in each sub-population and over time they evolved into different species.
- Thus, the **biogeographic distribution of the finches** supports the evolutionary deduction of descent with modification from a common ancestor because the finch species didn't emerge from the Galapagos islands but **came from an ancestral species from the mainland,** which then evolved into different species through **adaptive radiation.**

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

❖ **Fossils**

→relics or impressions of organisms that lived in the past that are preserved in rock.
(The deeper the strata the organism is found in, the earlier it existed.)
→We can tell how old they a fossil is by radioactive /carbon dating techniques.
→When compared across strata, they show an ordered sequence of succession of organisms & how homologous structures have been modified through time (descent with modification).

Example:

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

Example:

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

Thus, the **study of fossils allows us to see the evolution of species through the modification of homologous structures from a common ancestor to the present descendant through a series of transitional forms.**

(k) Explain the importance of the use of genome sequences in reconstructing phylogenetic relationships and state the advantages of molecular (nucleotide and amino acid sequences) methods in classifying organisms.

Importance of using genome sequences in *reconstructing phylogenetic relationships*. [This part is about how important molecular phylogenies are].

- **All living organisms contain nucleic acids in which evolutionary changes** are **captured. Thus more closely related species will have a greater number of similar nucleotides sequences** in their nucleic acids and a **greater number of similar amino acid sequences** in their proteins than less closely related species.
- Comparison of nucleotide and amino acid sequences has revolutionized our **understanding of the evolutionary relationships** for many groups of organisms and has led to **extensive revisions** of the classification of all life-forms.

Advantages of molecular (nucleotide and amino acid sequences) *methods* in classifying organisms. [This part focuses on the advantages of methods].

1. They can be used to compare **all organisms** which **share common genes.**
2. They can be used to compare organisms that are **morphologically indistinguishable** due to **convergent evolution** or because they are **closely related.**
3. Molecular methods are **objective** as molecular character states are unambiguous (e.g. A, C, G & T) whereas some morphological characters, such as those based on the shape of a structure or colour, can be less easy to distinguish objectively.
4. They are **quantitative** as molecular data can be **converted into numerical form** and **statistical analysis** performed to determine **degree of relatedness** by calculating nucleotide differences between organisms.
5. **Changes in nucleotide sequences accumulate over time with clockwork regularity** and this forms the basis of the molecular clock. We can thus **estimate the time of speciation** of modern to ancient species.
6. Some molecular differences may not be reflected as a morphological difference while small genetic differences may not result in a major phenotypic difference. This means that molecular data **does not underestimate nor exaggerate differences** unlike morphological analysis.
7. Molecular methods offer a **large set of characters to be studied quickly**. Each nucleotide position can be considered a character to distinguish between species.
8. Nucleotide sequences for a rapidly increasing number of genomes & amino acid sequences for many proteins can be **accessed from electronic databases for comparative study & classification of all life.**
9. **Specimens need not be complete or alive for comparative analysis** so long as the molecules survive degradation.

(l) Explain how genetic variation (including recessive alleles) may be preserved in a natural population.

How genetic variation *arises* in natural populations

(1) Mutations (Gene mutations, chromosomal mutations)

(a) Gene mutations

These include **substitution, deletion** or **insertion** of a nucleotide that **changes** the **triplet code** & hence the **amino acid**. Mutations in non-coding regions such as the promoter & enhancer can result in phenotypic variation as well.

(b) Chromosomal mutations (may involve changes in chromosome structure and number)

Number:

Polyploidy - when more than 2 homologous sets of chromosomes are present e.g., triploids: 3n, tetraploid: 4n.

Aneuploidy - when one or more chromosomes are over-represented or under-representated e.g Trisomy 21

Structure:

Deletion - when a segment of a chromosome is missing e.g. cri-du-chat disease where there is a deletion of chromosome

Duplication - when an extra segment of a chromosome is present.

Inversion - when a chromosome segment is detached, flipped around 180 degrees & reattached to the rest of the chromosome

Translocation - when a segment from one chromosome is detached & reattached to a different chromosome.

(2) Meiosis

(a) Independent assortment & segregation of homologous chromosomes during metaphase I & anaphase I respectively
Independent arrangement & separation of sister chromatids during metaphase II and anaphase II respectively
→ results in gametes with numerous combinations of maternal & paternal chromosomes.

(b) Crossing over between **non-sister chromatids of homologous chromosomes** results in more allelic combinations.

(3) Sexual Reproduction

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

How recessive alleles are *preserved* in a natural population

(1) Diploidy/Heterozygote protection

A gene can be dominant/recessive. Dominant alleles mask the effect of recessive alleles. Thus even if recessive alleles may be less favorable in the current environment, they persist because they are propagated in heterozygous individuals where the disadvantageous trait does not manifest and hence is not selected against.

(2) Heterozygote advantage

This occurs when heterozygotes have greater fitness than both kinds of homozygotes..

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

(3) Frequency-dependent selection

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

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

(m) Briefly describe the neutral theory of molecular evolution.

(1) The neutral theory states that most of the mutations/variations at the **molecular level** that exist in populations are **selectively neutral**. Examples of selectively neutral mutations are **silent mutations** (mutation may still result in the same amino acid), **conservative mutations** (mutation results in a chemically similar amino acid), mutations in **non-regulatory sequences in non-coding regions** (hence have no effect on the fitness of the organism)
(2) Other mutations which are in lesser proportions are those that that are **disadvantageous** which are quickly **removed** by natural selection anyway; and **advantageous** mutations that are quickly **brought to fixation** (i.e. don't mutate anymore) and remain in the population.
(4) Since most mutations are **selectively neutral**, most evolutionary change we see in populations at the molecular level is driven by **genetic drift** (random change in allele frequencies in a population due to chance events) **rather than natural selection**.
(5) Mutations will **accumulate** at a **constant rate** since they are selectively neutral & this forms the **basis for the molecular clock**.

Annex:

❖ Unrelated species which occupy similar ecological niches may look similar. Why?

(e.g.1) dophins and sharks (e.g.2) sugar gliders (marsupials) in Australia are not related to the flying squirrels (mammals) in North America but look alike
→ evolved independently from different ancestors (i.e.have different evolutionary lineages) in different parts of the world
→ look similar (i.e. have analogous structures) as they have adapted to the same way of life as they live in similar environments with similar selection pressures.
Thus **convergent evolution** has occurred. They have similarities that are shaped by the **environment** rather that through **shared ancestry]**

❖ There are **5 agents that can cause evolutionary change** (i.e. cause change in allele frequency):

(a) natural selection
(b) disruption of gene flow
(c) mutation = source of **new alleles** due to random changes in genes and chromosomes
(d) nonrandom mating = individuals choose mates on basis of favourable phenotypes. Favourable genotypes thus propagated at higher allelic frequency. also called sexual selection
(e) genetic drift = random change in allele frequencies due to chance events
i. **Founder effect** – A small group of individuals separate from a larger population and establish a colony in a new location. Because the founder individuals of the new colony are just a few, certain alleles are overrepresented or underrepresented. Thus, genetic variation is usually reduced.

ii. **Bottleneck effect** – A population size is dramatically reduced due to catastrophe and then rebounds (in size after a few generations). The initial reduction leads to certain alleles being overrepresented or underrepresented among the survivors and even though the population numbers may rebound to the original, genetic variation is usually reduced.

iii. **Neutral mutations,** that do not manifest in the phenotype and thus selectively neutral, experience genetic drift. Their allele frequencies change due to chance and not natural selection.

❖ **Mitochondrial DNA**

→ **does not undergo recombination** thus any changes to DNA is **solely due** to the **accumulation of neutral mutations over time** making it the ideal candidate for a molecular clock as these neutral mutations occur at a constant rate.
(mt DNA may be damaged by the reactive oxygen radicals generated in the mitochondrion)
→ undergoes a **high rate of mutation (relative to nuclear DNA)** as it has **no proof reading mechanism.** As a result the differences in mt DNA are sufficient to distinguish between different species and individuals of the same species as well.
→ contains many different genes e.g. cytochrome B

Isolating, cloning & sequencing DNA (Part 1)

How to clone human growth hormone (HGH) gene

cDNA preparation

* Isolate **total mRNA** from cells of the **anterior pituitary gland** where the HGH gene is expressed (i.e. transcribed & translated) → mRNAs
* Use **reverse transcriptase** to synthesise **cDNA** (complementary DNA) using the **mRNA** molecules as **templates** to form **RNA-DNA hybrids** → RNA-DNA hybrids
* Treat double stranded RNA-DNA hybrids with **RNase** to **break down the mRNAs** → ss cDNAs
* **DNA polymerase** the cDNA strands as templates to synthesize the 2nd cDNA strand. Thus double stranded cDNA molecules are produced. → ds cDNAs

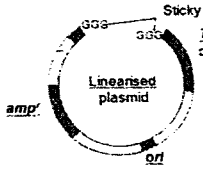
[Do note that many different cDNAs (e.g. HGH cDNA, PFK cDNA, RNA polymerase cDNA etc,) are produced from the total mRNA)]

Digestion, Recombination & Ligation

* Add dCTPS & **terminal transferase** to cDNA mixture (from above)

* cDNA fragments with **sticky ends** that that **do not self-anneal** from

Add dGTPS & **terminal transferase** to plasmids **linearised** with a restriction enzyme which leaves **blunt ends.** (The blunt cut is within the **multiple cloning site** *of the tetracycline* resistance gene.)



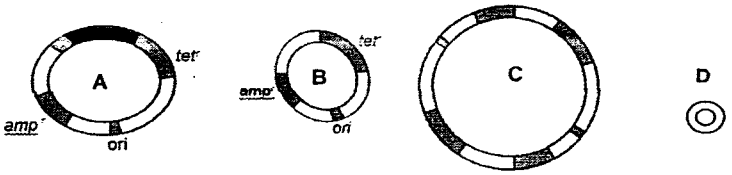
* Vectors with **sticky ends** that **do not self-anneal** but can anneal with **sticky ends of the cDNA fragments**

Mix & incubate to allow **annealing of complementary sticky ends**

Add **ligase** to allow **covalent phosphodiester bonds** to form between **adjacent nucleotides of both** DNA strands

4 possible products in ligation mixture

Why are there 4 possible products? This is because not all of the cDNA & plasmid molecules will have the sticky ends created with the help of terminal transferases.



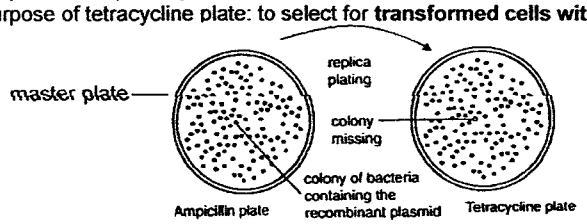
recombinant vector | **vector** | **concatamer** | **circularized insert**

Transformation

* Addition of Ca^{2+} (in the form of $CaCl_2$) to bacterial cells and **brief heat shock** makes them **competent** i.e. capable of taking up DNA from solution
* The ligation mixture is mixed with the bacterial cells and $CaCl_2$.
* This mixture is given a **brief heat shock** to **create transient pores** on the bacterial cell membranes. This allows the above DNA molecules to enter the bacterial cells.
* Only about **1%** of the above bacterial cells would be **transformed.**

Screening

* After transformation, there is a need to screen for bacterial cells containing, the **recombinant plasmids.** Recombinant plasmids would each contain a cDNA fragment which has disrupted the tetracycline resistance gene. i.e. the tetracycline resistance gene would have undergone **insertional inactivation.**
* The bacterial cells are grown on **ampicillin** medium & then **replica plated** on **tetracyclin** medium.
  Purpose of ampicillin plate: to select for **transformed cells**
  Purpose of tetracycline plate: to select for **transformed cells with recombinant plasmid**



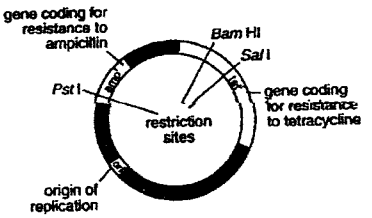
* Bacterial cells with
  1) **cDNA alone (D)** : susceptible to ampicillin & tetracycline → die in both media
  2) **vector alone (B & C):** resistant to both ampicillin & tetracycline →survive in both media (as amp^r and tet^r genes are intact)
  3) **recombinant plasmid (A)** : resistant to ampicillin & susceptible to tetracycline→survive in ampicillin medium but die in tetracycline medium (as amp^r gene is intact) (as tet^r gene is disrupted)
* **Compare** the tetracycline plate with ampicillin plate
  → colonies that **survive in ampicillin medium & die in tetracycline medium** are the colonies that contain the **recombinant plasmid.**

---

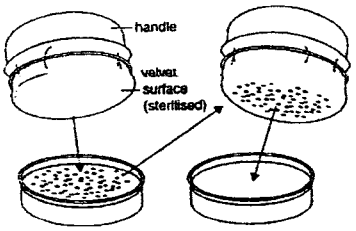
* **Restriction enzymes** (molecular scissors)
  1) are isolated from **bacterial** cells
  2) recognize & bind to specific **palindromic** base sequences (restriction sites) on DNA.
  3) create either **sticky ends/blunt ends** by cleaving of **phophodiester bonds** between **nucleotides** in **both** strands of DNA

e.g. **Sma I**	e.g. **Eco RI**
CCCGGG	GAATTC
GGGCCC	CTTAAG
↓	↓
CCC GGG	G AATTC
GGG CCC	CTTAA G
blunt ends	**sticky** ends

  4) **protect** bacterial cells from **invading viruses** by **degrading the foreign DNA** that enter them
  5) do not cleave the bacterial DNA in the bacteria they are found in as the restriction sites in the bacterial DNA are methylated. **Methylation** protects bacterial DNA from degradation.
* ***DNA ligase*** (molecular glue)
  → catalyses the formation of **phosphodiester bonds** between **adjacent nucleotides** in **both** DNA strands
* **Plasmid** (molecular carrier) e.g.: **pBR322**
  → can function as a **vector** and introduce a gene of interest (which has been inserted into it) into a host cell
  1) single, **circular, self-replicating** piece of double-stranded DNA derived from bacteria
  2) contains at least 2 **selectable markers**
     → eg: antibiotic resistance genes, *lac Z* gene
     → one to allow for selection of **transformed** cells
     → the other to distinguish between **transformed cells with and without recombinant plasmid**
  3) contains an **origin of replication (ori)**
     → to allow replication of plasmid **independent of host chromosome**
     → this creates **multiple copies** of the plasmid and gene of interest **within one bacterium.**
  4) contain **multiple cloning site**
     → has **several different restriction sites**
     → is found **within a selectable marker** which can be disrupted to allow **insertion of a range of different genes** (which are flanked by different restriction sites). **Insertional inactivation** of this selectable marker will allow for **selection of transformed cells with recombinant plasmids.**
  5) contains a **bacterial promoter** so that the bacterial transcriptional machinery can transcribe the cDNA insert.



* **Transformation**: process of **introducing foreign DNA** into host cells resulting in a change in **genotype & phenotype**
* **Host cells**: where the replication & expression of genes in recombinant plasmid occurs
* **Replica plating**: bacterial colonies from one plate are picked up (by the sterile velvet surface of the block) & an imprint of those colonies is made on another plate.



After the imprint is made on the tetracycline plate , the plate must be incubated at 37°C overnight to allow the bacterial colonies to grow.

Probing

* Next **probing** needs to be done to **identify** bacterial colonies with **recombinant plasmid containing the *HGH cDNA***.

* A **replica** of the colonies on the ampicillin master plate is made on a **nitrocellulose filter**. This is done by placing the filter on the master plate over 1-2 days to allow the bacteria to grow on it.

* The nitrocellulose filter with bacterial colonies is treated with **NaOH** to
  1) **lyse** the **bacterial cells** &
  2) **denature** the **DNA** (i.e. 1 double stranded DNA molecule → 2 single strands of DNA)

* **Wash** the filter with saline to **remove cell debris**.

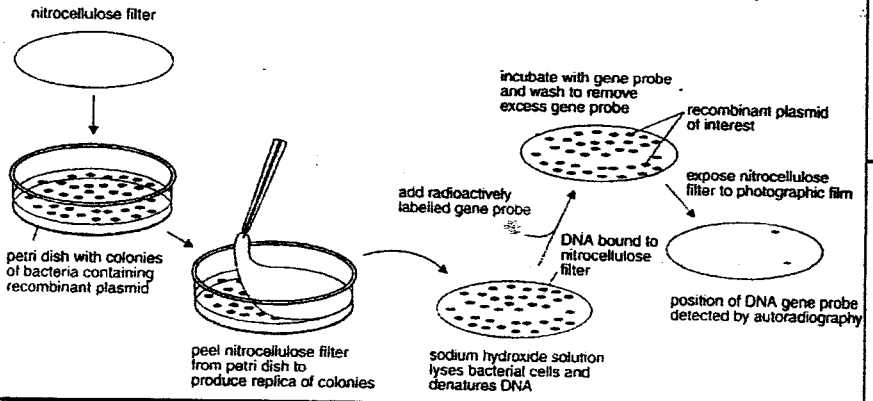
* Bake the filter for few minutes at **80°C** → This will **fix DNA** to the filter.

* **Incubate** the filter in a solution of **specific radioactive probe** whose sequences are complementary to part of the **HGH cDNA**.
  → Thus the probe can **anneal** to **complementary DNA sequences** of the **HGH cDNA**. This specific binding of the probe is called **hybridisation**.
  (Radioactive probe: a **short radioactively labelled single stranded DNA** which consists of sequence that is **complementary to part of the fragment of interest**)

* **Wash** the membrane to **remove any unhybridised probes**.

* Place nitrocellulose membrane to **photographic film**. **Radioactive areas** (i.e. where gene probe binds) will **expose** the film.
  (Autoradiography is a technique used to detect radioactivity.)

* By comparing the exposed photographic film with the ampicillin master plate, colonies which carry the HGH cDNA can be **identified & picked off from the master plate.**



Large-scale production

* With proper nutrients & optimum conditions, colonies containing the HGH cDNA can be grown in **large quantities** in **bioreactors**.

* The bacteria can be **induced** to produce **large amounts** of HGH.

* The HGH can then be **extracted**, **purified** & **packaged** for use.

* **Linkers**
  → synthetic double stranded oligonucleotides with 1 or more restriction sites
  → can be added to ends of cDNA & linearised plasmids by **DNA ligase**. Then they can both be cut by the **same** restriction enzyme so as to produce complementary sticky ends. This way the cDNA and plasmid can anneal. DNA ligase can be added seal the nicks and thus a recombinant plasmid is created.

* ***Lac Z***
  → **is a selectable marker**. It is a gene that codes for the enzyme **β-galactosidase** which breaks down colourless **X-gal into a blue compound**. Bacterial colonies containing **intact *lac Z* gene** (in their plasmids) appear **blue** while bacterial colonies with **disrupted *lac Z* gene** appear **white** (not colourless) when grown in X-gal.

* **Insulin**
  → When synthesising insulin, a cDNA copy of the functional mRNA is used. The cDNA is inserted into a plasmid. This recombinant plasmid is introduced into bacterial cells. The bacteria with the recombinant plasmid will produce **pro-insulin** (which is made up of the **A, B and C chains**). The pro-insulin is extracted and the **C chain is enzymatically cleaved**. The **A and B chains** are then **chemically combined** to form **functional insulin.**

Human insulin is synthesised artificially
1) to meet the demands of an increasing number of diabetic patients
2) some patients are allergic to insulin from animal sources

* Bacteria do not have the enzymes for splicing out the introns. By using cDNA for cloning, the eventual transcription of the gene will produce mRNA without the intron sequences. This will ensure that the polypeptide produced contains the correct amino acids sequence that gives rise to the functional protein.

* Yeast can be used in place of bacteria when using genomic DNA for cloning. It is a eukaryote and thus has the enzymes needed for post-transcriptional splicing of the pre-mRNA to remove the introns.

How to make a genomic library	How to make a cDNA library
Extract genomic DNA from **any somatic cell**	Extract total **mRNA** from a **specific cell type**
↓	↓
Digest the DNA using restriction enzymes	Use **reverse transcriptase** to synthesise a DNA strand complementary to the mRNA template → RNA-DNA hybrid results
↓	↓
Incorporate DNA fragments into vector e.g. plasmid pBR322	Treat RNA-DNA hybrids with **RNase** to **break down mRNA**
↓	↓
Transform host cell with recombinant vector	**DNA polymerase** synthesizes complementary DNA strand
↓	↓
Select for recombinant host cells by carrying out replica plating on antibiotic media	Double stranded cDNA molecules are produced
	↓
	Incorporate DNA fragments into vector e.g. plasmid pBR322
	↓
	Transform host cell with recombinant vector
	↓
	Select for recombinant host cells by carrying out replica plating on antibiotic media

Comparison between genomic and cDNA library

Point of comparison	Genomic library	cDNA library
1. Content	It contains **entire DNA content of an organism** including all coding & non-coding sequences.	It contains entire **protein-encoding DNA content** of the source tissue.
2. Starting genetic material to be isolated	It requires **chromosomal DNA isolation.**	It requires **total mRNA isolation.**
3. Starting material	The starting material can be **from any cell/tissue**.	(Total) mRNA should be isolated **from a single cell/tissue** where the **particular protein** is likely to be produced in **large quantities.**
4. Key enzyme(s) involved before cloning into vector	**Genomic DNA is cleaved with restriction enzymes** before inserting into a vector.	**Reverse transcriptase** catalyzes the **reverse-transcription of mRNA into cDNA** before inserting into a vector (Restriction enzyme needed for cleaving vector).
5. Size of library	There are **larger numbers of different types of clones** in the library (fragments represent the whole genome).	There are **smaller numbers of different types of clones** in the library (≈1% of genome).
6. Intactness of genes	**A gene may be cut in between** when there is a restriction site within the sequence.	**Intact genes** are obtained as cDNAs are derived from mRNAs.
7. Functional use of library (which sequences may be studied)	It is used for studying **introns or regulatory sequences** associated with a gene.	Cannot be used for studying introns or regulatory sequences associated with a gene. It is used for studying the **exact coding sequence** of the gene.
8. Functional use of library (to study patterns of gene expression)	It cannot be used for studying physiological/developmental-based changes in gene expression.	It is used for tracing **changes in patterns of gene expression under different developmental/physiological conditions.**
9. Functional use of library (screening of a gene)	It can be used for the screening, isolation and characterisation **of a gene**, when the **cell type** in which it is expressed is **currently unknown.**	It is used only when the cell type in which the gene is expressed in is known
10. Frequency of fragment inserted into vector / type of clones	Generally, there is equal representation of fragments.	It shows **unequal proportions** of different fragments because different genes are represented in different amounts as there were unequal levels of different mRNAs isolated.

Advantages of using a cDNA library	contains only **coding sequences** → cDNA library has a **smaller number of clones** compared to genomic library and thus is easier to screen for desired gene
	lack introns in mRNA (unlike genomic DNA that contains introns) → eukaryotic genes **can be expressed in prokaryotes that lack enzymes for splicing**
	cDNA obtained from **reverse transcription of mRNA** → coding regions of **gene is intact** (unlike genomic DNA which may be fragmented)
Limitations of using a cDNA library	only **genes that are expressed** in a particular tissue at a **particular developmental stage** can be harnessed. The other genes cannot be harnessed. Thus the cDNA library does not represent the entire genome. (unlike the genomic library)

Advantages of using a genomic DNA library	contains **all the genes** of an organism
	allows for the **study of introns** or **regulatory sequences** associated with a gene
	differences in nucleotide sequences in **diseased and healthy** people can be **compared** by studying the genomic libraries of healthy and diseased individuals
Limitations of using a genomic library	eukaryotic **genomes are very large** → the library will contain many fragments and so it will take a **long time to screen** the library
	use of **restriction enzymes to cut the DNA** → the gene of interest may be cut internally by the restriction enzyme. Hence the **gene of interest will not be intact.**
	eukaryotic genome **contains introns** → DNA fragments will contain introns and the **correct polypeptide cannot be expressed** in bacteria as they **lack splicing mechanisms**

What are the advantages of using bacteria for large scale production of the protein?

1. Bacteria have small genomes and are relatively easy to manipulate genetically.
2. Bacteria have high replication/growth rate, thus giving rise to comparatively higher productivity.
3. Production using bacteria takes up relatively small space.
4. Bacteria can be cultured easily and in any part of the world
5. Avoids risks of infection or disease and problems of allergic effects that proteins (e.g. insulin) extracted from pigs can cause
6. Overcome ethical or religious objections

Isolating, cloning & sequencing DNA (Part 2)

Describe the polymerase chain reaction (PCR) and explain the advantages and limitations of this procedure

Polymerase Chain Reaction

→ Amplifies DNA from a **limited source** of DNA so that there is **sufficient amount** for **analysis**.

*** Process: (3 steps)**

1. **Denaturation:** Double stranded DNA **denatures** into **single stranded DNA** by heating to **95°C** as weak **hydrogen bonds** between bases of each strand is **broken** due to increased molecular vibrations

2. **Primer annealing:** Each **primer anneals specifically** to the end of a **single stranded target DNA sequence** via **complementary base pairing** when the temperature is lowered to **64°C**

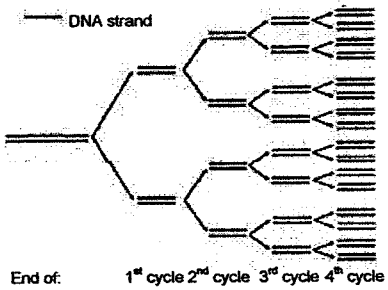
3. **Extension:** **Taq polymerase** synthesizes the **complementary DNA strand** from the **free 3OH' end** of the **DNA primer** when the temperature is increased to **72°C**

*** Advantages:**

1. Only a **minute amount of DNA** is required to carry out PCR as with **each round of PCR**, the number. of copies of **target DNA is doubled**. Thus the number of desired sequence **increases exponentially**.
2. Use of **thermostable** (i.e.**resistant to denaturation at high temperatures**) Taq polymerase allows PCR to be **automated** so DNA can be amplified very **quickly**.

*** Limitations:**

1. **Taq polymerase lacks 3' to 5' proofreading ability**. Hence **errors** occurring early in the PCR reaction will get **compounded** with each subsequent replication cycle.
2. **Knowledge of sequences flanking (i.e. at the 3' ends of) the target sequence** is required in order to **design** appropriate **primers**.
3. **Taq polymerase tends to 'fall off' the DNA template** before chain extension is complete if the strand is too long. Hence there is a **limit to the size** of DNA fragment (~3kb) to be amplified.
4. **Minute amounts of contaminant DNA** can be exponentially **amplified along with target DNA** and affect the **reliability** of the results.



* Number of copies of double stranded DNA = 2^n
(where n is the number of cycles)
* Number of (single) strands of DNA = 2^{n+1}
(where n is the number of cycles)

End of n cycle where n is	1	2	3	4
Copies of ds DNA	2	4	8	16
Strands of ss DNA	4	8	16	32

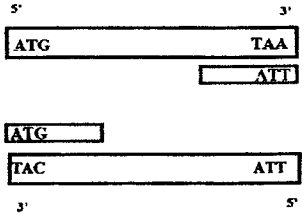
PCR Components

*** Template DNA**

→DNA containing the target sequence to be amplified

*** Primers**

→synthetic single-stranded DNA fragment (20-30 nucleotides long)
→needed to **initiate DNA synthesis** by providing a free 3'OH group for Taq polymerase to bind to and extend
→ **2 different primers** are required. Each is complementary to the sequence at **3'end of each single stranded target DNA sequence.**



→ are required in **large excess** to increase the likelihood of them binding to target DNA sequences (relative to template strands binding each other)

*** Taq polymerase**

→ **thermostable** DNA polymerase which is resistant to denaturation at high temperature

*** Deoxyribonucleotides (dNTPs)**

→ **substrates** for DNA replication made up of dATP, dTTP, dCTP and dGTP

*** Buffer**

→ contains cofactor, **Mg^{2+}**, for proper polymerase function

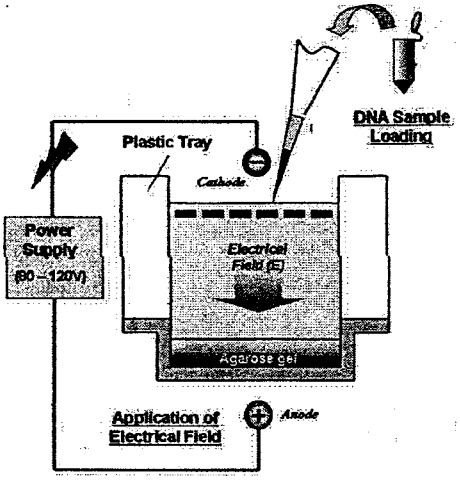
Explain how gel electrophoresis is used to analyse DNA

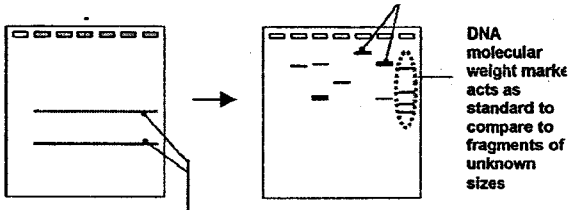
Agarose gel electrophoresis

→ separates DNA based on **fragment size**

*** Steps:**

1. A slab of agarose gel is placed in a **buffer solution** contains **ions** which **allows the conduction of electricity** when the current is turned on.
2. The DNA sample is mixed with a **loading dye** containing **glycerol** & **2 coloured dyes**.
3. Glycerol makes the **DNA sample denser** than the buffer so that the DNA sample can **sink to the bottom of the well.**
4. Since **DNA is invisible**, the dyes **colour the DNA sample** and will indicate if the DNA has been loaded correctly into the well. (NB: Loading dyes do not bind to the DNA.)
5. One dye (corresponds to a smaller fragment and) runs ahead of the DNA sample and gives an indication of **when electrophoresis must be stopped so that the samples do not run out of the gel.** The other dye moves at a speed that corresponds to a larger fragment and gives an indication of the **position of the larger fragments** on the gel.
6. The **2 coloured dyes** thus act as **visual markers** which show the progress of the migration of the DNA fragments in the gel during electrophoresis.
7. DNA samples are pipetted into the **wells** in the gel near the **negative electrode.**
8. **A DNA ladder** (i.e. DNA molecular weight markers) which contains **DNA fragments of known sizes**, is run in one of the lanes and acts as a standard for which to compare fragments of unknown size in the sample.
9. **Negatively charged DNA** is attracted towards the **positive electrode (anode)** when subjected to an **electric current.**
10. The **agarose gel** matrix made of a **meshwork of polymer fibres** which **impedes movement** of longer fragments more than shorter fragments. The **longer fragments** thus migrate **more slowly** compared to **shorter fragments**, leading to a banding pattern observed on the gel.
11. Before the loading dye reaches the end of the gel, the current is turned off.
12. To **visualize the bands**, the gel can be treated with a staining **dye that binds DNA** (e.g. **ethidium bromide, a carcinogen**) and **fluoresces under uv light**.
13. Thus a) the **fragment size** can be estimated (based on **position** of the band relative to bands in the molecular weight marker) and
    b) the **amount of DNA** can possibly be estimated (based on **intensity & thickness** of the band).





The top line corresponds to fragments that are 1100bp and the bottom line corresponds to 100bp fragments.

Note: Bands are only visible after treating with staining dye such as ethidium bromide (visible under UV light).

NB: The higher the concentration of agarose, the finer the pores in the meshwork.
→Thus smaller fragments can be effectively separated.

Outline the process of nucleic acid hybridisation and explain how it can be used to detect and analyse restriction fragment length polymorphisms.

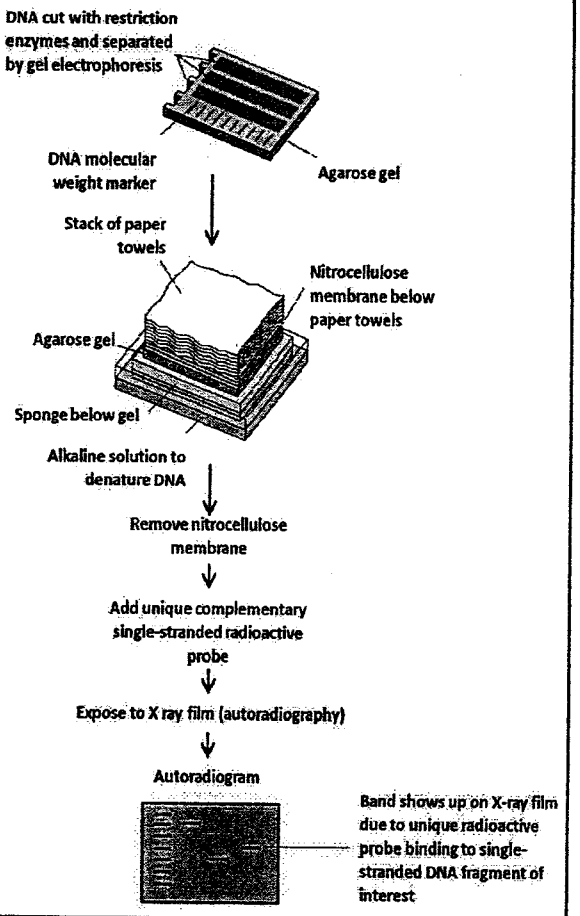
Southern blotting: a nucleic acid hybridisation technique used in RFLP analysis

Tool to **detect specific nucleotide sequences** within a sample of DNA

Steps:
(Continued from Gel electrophoresis)

1. Gel slab is placed under **nitrocellulose membrane**. A stack of paper towels placed on top of nitrocellulose membrane. These are placed in a tray of **alkaline solution**.
2. Absorbent paper towels draw the solution towards themselves and the **alkaline solution denatures double-stranded DNA** into **single-stranded DNA**.
3. Single stranded DNA on the gel is then **drawn upwards onto the nitrocellulose membrane** and **binds to the membrane** (in exactly the same position as they were in the gel).
4. Nitrocellulose membrane is removed and incubated with single-stranded **radioactive DNA probe** which **hybridises** via **complementary base pairing** to the target sequence.
5. The excess unhybridised probes are washed off.
6. **Autoradiography** is performed placing X-ray film over membrane. Radioactive regions **exposes the film** forming an image that correspond to the bands that have base-paired with probe.

[Note: RFLP analysis is a principle/ way of analyzing data. PCR, gel electrophoresis and nucleic acid hybridization are tools used in RFLP analysis.]



Explain how RFLP analysis facilitated the process of i) disease detection e.g. sickle cell anaemia, ii) DNA fingerprinting and iii) genomic mapping in terms of linkage mapping.

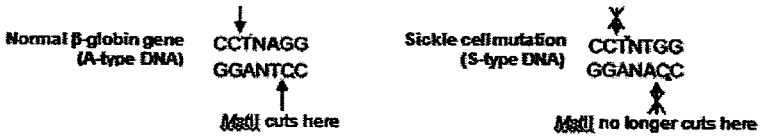
What are RFLPs?

- RFLP = Restriction Fragment Length Polymorphisms
- RFLPs are restriction patterns that arise from restriction digestion using a specific restriction enzyme.
- RFLPs arise due to **DNA polymorphisms** which are **small nucleotide differences** in **different individuals** at **specific locus**.
- Thus due to the **polymorphic** nature of DNA in different individuals, there will be **variations** in the **number/location of restriction sites** or **number of tandemly repeated nucleotide sequences**. This will result in a **unique banding pattern** amongst individuals.

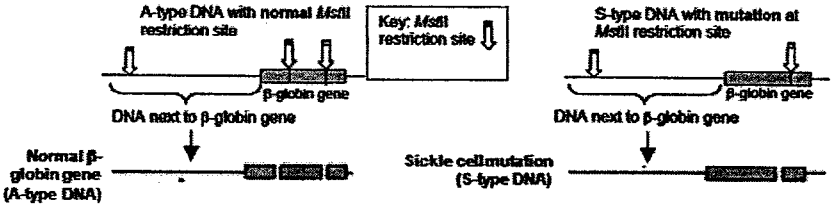
- The DNA polymorphism in sickle cell anaemia is a **single nucleotide polymorphism (SNP)**. There is a difference in a **single base pair** due to a **point mutation**.
- In **sickle cell anaemia** this SNP is within the **coding region**. However the **majority of SNPs** used for RFLP analysis are found in **non-coding regions**.

i) Use of RFLP analysis in disease detection (direct method) e.g. sickle cell anaemia

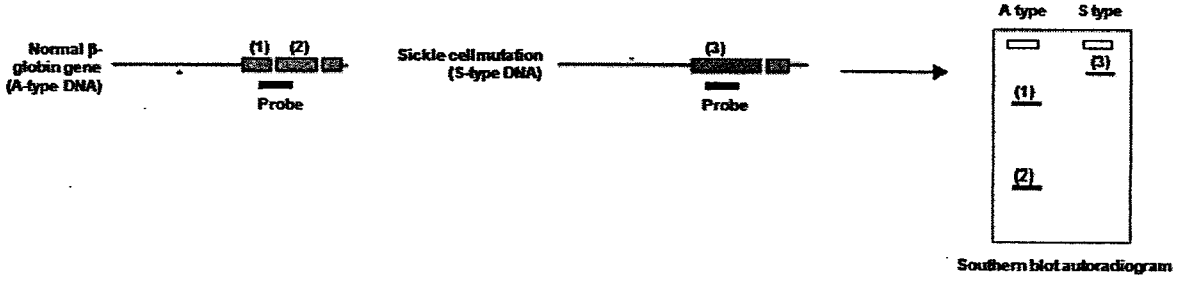
- A **difference in single nucleotide** can result in a **gain OR loss of a restriction site** of an enzyme. Thus when a particular section of DNA in individuals with the mutation and without the mutation is digested with the same restriction enzyme, **DNA fragments of different lengths** will result. **Analysis of the banding pattern** that arises, will allow determination of the **presence of disease-causing allele** or the **normal allele**.
- e.g. in **sickle-cell anaemia**, the disease-causing mutation occurs at restriction site for ***Mst* II** within the **β-globin** gene.
  1. In the **disease causing** allele, nucleotide is **thymine**, thus ***Mst* II** restriction site is **eliminated**.
  2. In the **normal** allele, nucleotide is **adenine**, thus ***Mst* II** restriction site is **retained**.



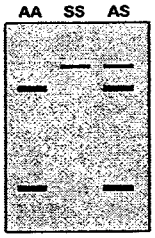
- How to determine the genotype of a person for **β-globin** gene?
  1. Digest **genomic** DNA with *Mst*II to obtain different restriction fragments will arise.



  2. Perform **gel electrophoresis** following by **southern blotting** (use of nitrocellulose membrane, radioactive probes and autoradiography). The **same single-stranded radioactive probe** (which is complementary to part of the target sequence) will be used to detect both the presence of A-type and S-type DNA. Hence the site that the probe binds on the DNA fragments should give different banding patterns on the autoradiogram.



i) Use of RFLP analysis in disease detection (direct method) e.g. sickle cell anaemia(cont'd)



A type DNA will indicate presence of normal β globin.
S type DNA will indicate presence of abnormal β globin (sickle cell).
Note that normal β globin is dominant over abnormal β globin (sickle cell).

AA indicates that individual is **homozygous dominant** who is phenotypically normal.
SS indicates that individual **is homozygous recessive** who is suffering from sickle cell anaemia.
AS indicates that individual is **heterozygous** and is phenotypically normal.

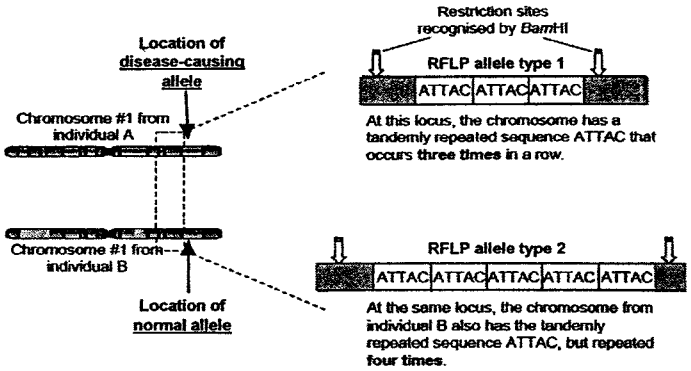
ii) Use of RFLP analysis in detection of other diseases (indirect method)

Using Single Nucleotide Polymorphisms (SNPs)

- In **sickle cell anaemia** the SNP is within the **coding region**. (see above). However the **majority of SNPs** used for RFLP analysis are found in **non-coding regions**. Hence the SNP can be found at **regions flanking the gene responsible for the disease**. The SNP is **tightly linked** to the disease-causing gene thus **inheritance of a particular SNP** will indicate the inheritance of a **corresponding allele of the disease-causing gene.**

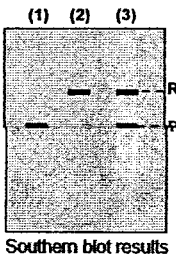
Using Short Tandem Repeats (STRs)

- STRs are repeating sequences of 2 to 6 base pairs of DNA.
- The **number of repeats** can differ in different individuals.
- In some cases, the **STR locus** can be **tightly linked with a disease-causing gene locus**. Hence, a particular number of repeats at the STR locus (hence the length of the fragment) could be linked with the presence of the disease-causing mutation.



- Perform a restriction digest followed by gel electrophoresis and southern blotting (use of nitrocellulose membrane, radioactive probes and autoradiography)

- If RFLP allele type 1 is tightly linked to the disease-causing allele and RFLP allele type 2 is tightly linked to the normal allele, then

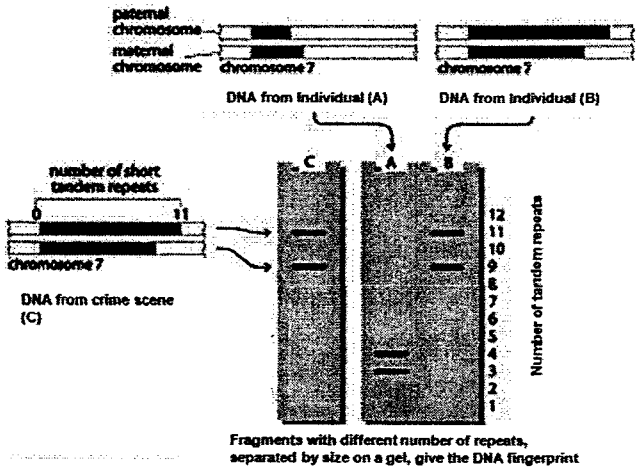


Individual (1): is homozygous (2 copies) for disease causing allele
Individual (2): is homozygous (2 copies) for normal allele
Individual (3): has one normal allele and one disease causing allele

Thus in order to find out if person X has the disease causing allele or not, person X's RFLP analysis needs to be compared with that on the left.

iii) Use of RFLP analysis in DNA fingerprinting

- As no individuals (exception of twins) have the same genome, therefore they will not have the same DNA profile.
- The DNA profile is the restriction banding pattern that identifies individuals.
- How to carry out DNA fingerprinting:
  1. Restriction digestion of genomic DNA followed by gel electrophoresis
  2. Southern blotting (use of nitrocellulose membrane, radioactive *probes and autoradiography). Probes for STRs are usually* used.
- At a particular RFLP locus, different individuals will have different number of STR repeats, hence different length of fragment.
- RFLP alleles of each locus can inherited from parents. Therefore individuals with similar banding patterns should be closely related.



These RFLP alleles are all from the same RFLP locus.
DNA from the crime scene (C) has 2 RFLP alleles, one with 11 repeats and the other with 9 repeats. Individual (A) has two RFLP alleles, one with 3 repeats and the other with 4 repeats. Hence Individual (A)'s DNA fingerprint is different from the DNA at the crime scene.

- The greater the number of RFLP loci used in the DNA fingerprint of an individual, the more unique the fingerprint. In the CODIS (combined DNA index system) used by the FBI, **13 different STR loci** are used to distinguish between different individuals.

iv) Use of RFLP analysis in genomic mapping

- Genomic mapping is: arrangement of genes and genetic markers (are DNA sequences on known locations that can be used to identify individuals or species) relative to each other in genome.
- RFLPs at particular chromosomal locus have alternative forms (i.e. **alleles**) since they differ in nucleotide sequence (due to presence of SNPs or different number of STRs). Therefore RFLPs serve as genetic markers for a particular locus in genome.

How to calculate genetic distance between two loci on the same chromosome:

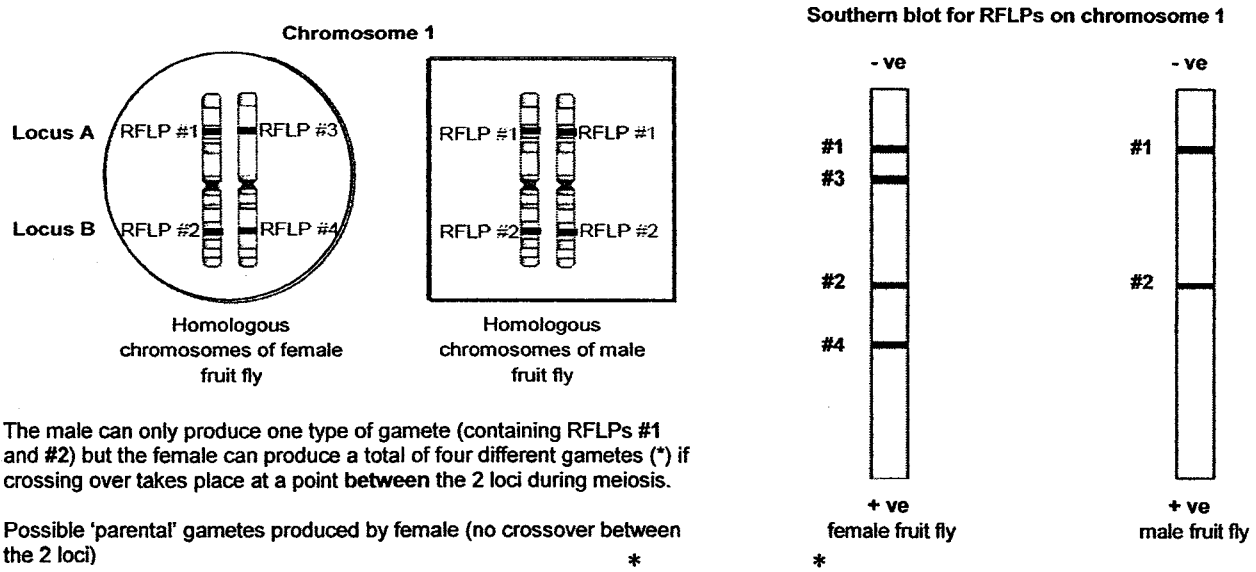
- Calculate the recombination frequency between two RFLP loci on the same chromosome (a.k.a linkage mapping) using:

$$\text{Recombination frequency} = \frac{\text{No. of recombinant progeny}}{\text{Total no. of progeny}} \times 100\%$$

- The **further 2 RFLP loci** are from each other, the **higher the probability that a cross over will occur** between 2 RFLP loci, the **higher the recombination frequency.**

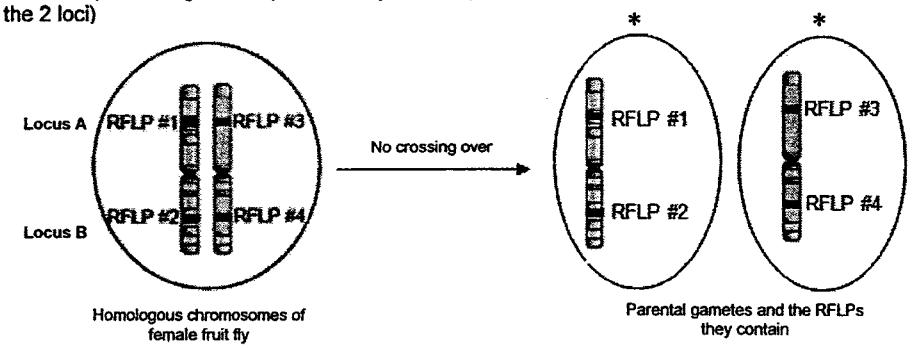
iv) Use of RFLP analysis in genomic mapping (cont'd)

- It is possible to measure the genetic distance between two loci by studying their RFLP patterns
- Example:
  - 2 RFLP loci, **A** and **B**, on the chromosome **1**
    RFLP locus **A** has 2 RFLP alleles **#1** and **#3**
    RFLP locus **B** has 2 RFLP alleles **#2** and **#4**
  - In the southern blot pattern, the male is homozygous for band **#1** at Locus **A** and homozygous for band **#2** at the Locus **B**. The female is heterozygous at both loci.

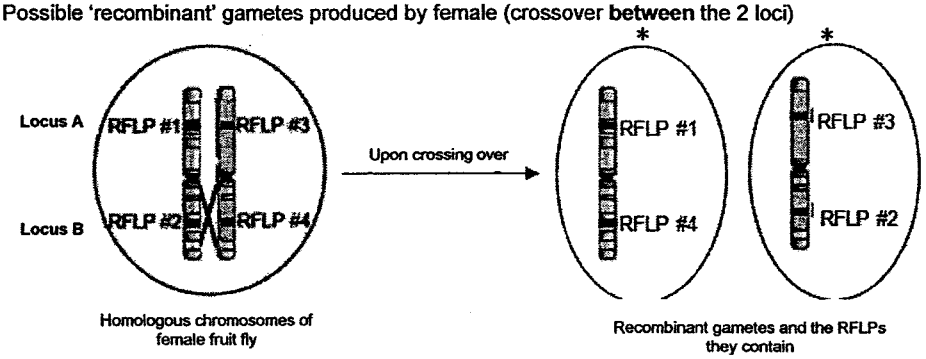


- The male can only produce one type of gamete (containing RFLPs **#1** and **#2**) but the female can produce a total of four different gametes (*) if crossing over takes place at a point **between** the 2 loci during meiosis.

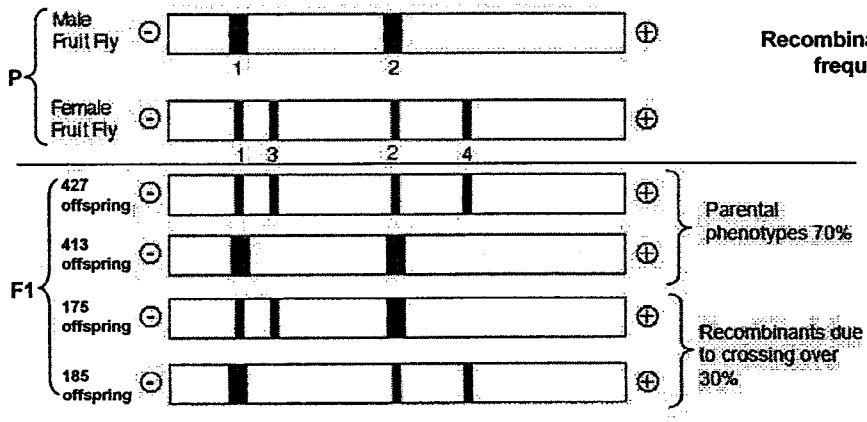
- Possible 'parental' gametes produced by female (no crossover between the 2 loci)



- Possible 'recombinant' gametes produced by female (crossover **between** the 2 loci)



- When the male and female flies mate, the frequency of the four possible progenies can be measured. (Refer to southern blotting pattern below.)



Recombination frequency = $\frac{\text{No. of recombinant progeny}}{\text{Total no. of progeny}}$ X 100%

= $\frac{175 + 185}{1200}$ X 100%

= $\frac{175 + 185}{1200}$ X 100%

= 30%

This translates to the two RFLP loci being **30 centiMorgans (cM)** apart from each other.

Stems Cells

a) are **unspecialized/undifferentiated** i.e. they do not have any tissue-specific structure for it to perform a particular function
b) are **able to differentiate** to produce specialised cells upon receiving appropriate **molecular signals** (e.g. hormones, growth factors)
c) undergo **extensive proliferation** and **self-renewal** i.e. they can divide many times by mitosis, with the daughter cells possessing the same developmental and replicative potential as the parent cell

d) can undergo
1) symmetrical division → produces 2 identical daughter stem cells →to **ensure a constant pool of stem cells**
2) asymmetrical division→produces a) 1 daughter stem cell → **to ensure a constant pool of stem cells**
& b) 1 **progenitor** cell →**to renew/replace a population of specialised cells** in a specific tissue
→occurs in the presence of appropriate **molecular signals**

e) can be
1) **totipotent** → can **differentiate into all of the cell types** that make up an **entire** organism **including** the extraembryonic tissue e.g. the **placenta**
→ e.g. **fertilised egg to 8 cell stage** (These cells are **zygotic** stem cells.)
2) **pluripotent** → can **differentiate into all of the cell types** that make up an organism **except** the extraembryonic tissue such as the **placenta**
→ e.g. **inner cell mass of blastocyst** (The cells in the inner cell mass are **embryonic** stem cells.) (Draw & label a blastocyst here please.)
3) ***multipotent*** → *can develop into only a limited and related range of cell types and tissues in an organism*
→ e.g. **haematopoietic stem cells**, which are found in the bone marrow & give rise to all of the cells found in the blood, including red blood cells, white blood cells, and platelets. Haematopoietic stem cells are **adult** stem cells. They are also found in babies.)

Note: 1) Stem cells can differentiate into different cell types due to the presence of molecular signals that cause **differential switching on of genes**.
2) A progenitor cell is an early descendent of a stem cell that can only differentiate. It cannot renew itself.

Gene Therapy

a) involves the introduction of a **normal, functional allele** into **cells** with defective mutant alleles so that the cells can produce a **functional protein**
b) treats **single gene recessive**[1]**disorders** (e.g.cystic fibrosis,SCID) where affected individuals have two copies of the recessive allele in their genome
c) can target 1) **somatic** (non-gametic) cells → thus treatment is effective for patient but effect is not heritable
2) **germline** cells→ gamete or fertilized egg is treated → allele present in all cells from modified embryo
→ treatment is only effective for individual that arose from modified embryo and all subsequent generations
d) treatment can be 1) ***ex vivo*** : cells are removed from the body, modified outside the body and then transplanted back in again } depending on
OR 2) ***in vivo*** : cells are modified while still in the body } accessibility of cells
e) involves a few steps: (1) **Normal, functional allele** isolated & linked to appropriate promoter (to ensure expression in host cell)
(2) inserted into vector (e.g. virus[2]/liposome) (NB: Direct gene transfer into cell by microinjection, electroporation also possible)
(3) which is then introduced into target[3] cells that need correcting.
(4) The introduced allele expresses the protein that is lacking.

[1] When treating dominant disorders, dominant, disease -causing allele/s must be inactivated before introducing normal, functional allele. This is because the dominant allele/s will mask the effect of the functional recessive allele.
[2] When using viral vectors, disease causing genes (i.e. genes that code for components of the virus that are needed for the assembly of the virus/ genes that allow the virus to replicate) of viral genome must be removed so that virus does not cause disease in host. This will also allow for larger DNA fragments to be inserted into the genome.)
[3] When stem cells are used for gene therapy, treatment is long lasting/ not transient.

Severe Combined Immunodeficiency Syndrome (SCID)

* An **inherited disorder** where affected individuals **T- and B- lymphocytes** are **reduced in numbers or malfunctioning.**
* Two types of SCID

~~name of disorder~~	**X-linked SCID**	**Adenosine deaminase (ADA) deficient SCID**
type of disorder	**X-linked recessive** disorder	**autosomal recessive** disorder
location of mutation	due to **mutations** in gene coding for **common gamma chain** (a cell receptor for interleukins) in **X chromosome**	due to **mutations** in the gene responsible for the enzyme **adenosine deaminase on chromosome 20**
effect	Without functional receptors for interleukins (proteins that stimulate our T & B lymphocytes) on the T & B lymphocytes **→T & B lymphocytes fail to proliferate & differentiate.**	ADA, in normal individuals breaks down adenosine. Without ADA, deoxyadenosine which is **toxic** to cells will accumulate. **→T & B lymphocytes die**

Cystic Fibrosis (CF)

* inherited **autosomal recessive disorder**
* due to a **deletion mutation** (3 nucleotides on **chromosome 7**), resulting in the **loss of phenylalanine** in the protein
* **cystic fibrosis transmembrane conductance regulator (CFTR)**, an **ATP- gated channel protein**, is either **not produced** or **defective**
(NB: ATP binding to CFTR causes a conformations change that allows the channel to open. Cl^- move across by **facilitated diffusion** not active transport.)
* Effects in various parts of body
a) With **functional CFTR** in the **lungs**: Cl^- **transported out** of lung epithelial cells → Na^+ transported out subsequently to maintain electrical neutrality→thus Ψ_w in the lumen of the alveoli more negative → water transported out of epithelial cells →watery mucus (which can be coughed out)
When **CFTR missing/defective** in lung epithelial cells → Cl^- not transported out of epithelial cells → Na^+ retained too
→ Ψ_w more –ve in cell → water retained in cell → mucus lining in lumen undiluted → thick & cannot flow → congestion
→ reduced gaseous exchange→ mucus remains too long in respiratory tract making it conducive for bacteria growth → lung infection
→ **severe breathing difficulty**

b) When **CFTR missing/defective** in the **pancreas**: **pancreatic duct is choked by thick mucus** preventing release of enzymes → **indigestion** (can be treated with pancreatic extract); also, thick mucus layer in intestines reduces absorption of digested food

c) With functional CFTR In **sweat glands** → as the salty sweat rises up the duct towards the pore, upper duct reabsorbs Cl^- while Na^+ **enters** the duct cells via Na^+ channels. →excessive water loss is minimised.
With defective CFTR, no reabsorption of NaCl takes place → **copious salty sweat** is exuded from the sweat glands
→basis of diagnosis measure $[Cl^-]$ in sweat
(NB: The **orientation of CFTR is reversed in the sweat duct** as compared to the lung epithelium & pancreas.)

name of disorder	SCID	CF
treatments not involving gene therapy	1) **stem cell transplant** from normal individual to affected individual → new cells can rebuild immune system BUT patient immune system may reject introduced cells 2) **weekly ADA replacement therapy** to hinder dATP build up so that B & T lymphocytes can mature BUT constant treatment necessary & efficacy of treatment varies in different patients	1) **daily physiotherapy** to remove mucus 2) **daily medication** including antibiotics, mucolytics, pancreatic enzymes etc. → above not a cure but improves the quality of life of patient
type of gene therapy	*ex vivo* approach	*in vivo* approach
vector	virus: retrovirus	liposome: spherical str. enclosed by a phospholipid bilayer
steps in approach	1)remove T cells from child with SCID 2)insert normal, functional gene into T lymphocytes using retroviral vector 3)transplant cells back into patient 4)recombinant T lymphocytes will produce ADA (NB: In the case of X-linked SCID, the functional interleukin receptor will be produced)	1)insert normal, functional CFTR allele into **plasmid vector** 2)package vector into liposome and deliver liposomes into lungs using aerosol spray (lipofection) 3)liposome fuses with cell membrane and releases DNA into cell. Plasmid with functional CFTR allele may enter nucleus. 4)normal CFTR protein inserts into plasma membrane
disadvantages	1) production of ADA (or in the case of X-linked SCID, production of functional receptor for interleukins) is **transient despite intergration** of allele into the genome due to **short life span of** T **cells**. →**regular infusion** of recombinant T lymphocytes necessary	1)treatment is **transient** as lung epithelial cells are **constantly shed** →**regular treatment** with aerosol spray necessary 2)liposomes have **low transfection efficiency** 3)liposomes have **low rate of stable integration** 4)liposomes have **low gene expression**
advantages	1) virus can **target specific cells** (T lymphocytes) 2) target cells are **easily removed** 3) **high transduction/transfection efficiency** 4) virus uses **integrase** to integrate the normal functional allele into host cell genome, providing stable gene expression 5) can **monitor expression of normal functional allele before transfer** 6) **no introduction of retrovirus into patient** (as the treatment in *ex vivo*) which can randomly insert normal functional allele into host cell genomes & cause insertional inactivation of tumour suppressor genes or activation of proto-oncogenes	**1)non-pathogenic** **2)no immunity problems,** **3)no limit to size of allele to be inserted**
alternative methods	insert the normal, functional allele into **haematopoietic stem** cells for long lasting results. This is because stem cells can **proliferate** and **self-renew**, ensuring a constant supply of new replacement cells with the ability to produce ADA.	use a **modified adenovirus** as vector * it will not cause cancer as it can **allow the expression** of the normal functional allele in the cell even though it is **not integrated into the genome** * but **remove disease causing genes** (i.e. genes that code for components of the virus that are needed for assembly of the virus [e.g. viral capsid genes]/genes that allow the virus to replicate [e.g. viral polymerases])

Factors that keep gene therapy from becoming an effective treatment for genetic diseases

[NB: Gene therapy is successful when the normal, functional allele is stably integrated into to nucleus and is expressed to give a functional protein and it does not have any ill effects on the patient.]

1. **Short-lived nature of gene therapy** (and hence the need for multiple rounds of gene therapy to have long term benefits.)
   →due to **death of cells** containing normal functional allele.(This can be overcome by transforming stem cells. However, this is not always possible.)
   →due to **inability to stably integrate** normal, functional allele into genome

2. **Multigene disorders** (e.g. diabetes, heart disease)
   →are difficult to treat as they are caused by the **combined effects of several genes**
   →disorders that arise from mutations in a **single gene** are the best candidates for gene therapy

3. **Precise level of gene regulation required for some diseases** (e.g. thalassemia)
   →overexpression of a transferred gene can be problematic while low levels of expression will be ineffective

4. **Viral vectors**
   a) **can trigger an immune response**
   → use of viral vectors can elicit an immune response which can destroy **the vector before it can deliver the normal functional allele to target cells. Subsequent treatment** with the same virus will elicit a **faster & stronger immune response** making repeated treatment difficult.
   → immune responses can also include **allergic, inflammatory** or **toxicity** responses to the viral vectors resulting in tissue damage & discomfort.
   → can be minimized with use of non-viral gene delivery systems.

   b) **can target only specific cells**
   → thus **different delivery systems** need to be **developed for targeting different cells.**

   c) **can possibly recover its ability** to cause disease once in the host.

   d) **can induce tumour formation by insertional mutagenesis.**
   → when **retroviruses** are used for delivery, **random integration** of the viral genome into the target cell genome **(i.e insertional mutagenesis)** may
   1) inactivate tumour suppressor genes → increased chance of getting cancer (by disruption of tumour suppressor genes)
   2) activate proto-oncogenes → increased chance of getting cancer (as strong regulatory sequence in the vector can upregulate proto-oncogenes)

Social and ethical considerations for the use of gene therapy

For	Against
• **Somatic gene therapy** corrects or alleviates the genetic defect present in the individual alone, without impacting the genetic information transmitted to the next generation.	• **Germ line gene therapy** involves alteration to the genes of germ-line cells affect offspring and successive generations.
• Arguments specifically <u>for</u> the development of **germline gene therapy** include: 1. It offers a **true cure**, and not simply palliative or symptomatic treatment. 2. It may be the **only effective way** of addressing some genetic diseases. e.g. for diseases that affect an **extensive or *inaccessible area*** *of the body like the nervous system such as* neurodegenerative diseases. At least the next generation need not suffer the same fate as the parent. 3. By preventing the transmission of disease genes, the **expense and risk** of somatic-cell therapy for **multiple generations** are avoided. 4. Prospective **parents** at risk for transmitting serious genetic diseases have an option of having normal children where previously they would simply refrain from having children for fear of passing on the disease.	• Arguments specifically <u>against</u> the development of **germline gene therapy** include: 1. It is difficult to follow patients in long-term clinical research. Gene therapy patients would need to be under **surveillance for decades** to monitor the **long-term effects** of the therapy on future generations. 2. *It would open the door to attempts at* ***altering human traits not associated with disease***, which could exacerbate problems of social discrimination. There are many ethical dangers in pursuing **genetic enhancement**, including increased social inequality and a lowered tolerance for human diversity. 3. Because germ-line gene therapy involves research on early embryos and affects the offspring, it essentially creates **generations of unconsenting research subjects**.
• The scientific community has a **right to free inquiry**, within the bounds of acceptable human research. Techniques in gene therapy help us to **study gene function**.	• **Technique is new and unreliable** and there needs to be a good enough justification for its use when there are other tried and tested modes of treatment. e.g. Enzyme replacement therapy in ADA deficiency.
• Potential for **treating desperately ill patients** or preventing the onset of terrible illnesses. Where **conventional treatment has failed**, gene therapy may offer the only effective way to treat such patients. Compared to the hardship and death faced by these patients, the risks and uncertainties of gene therapy are acceptable. **Civic, religious, scientific, and medical groups have all agreed in principle that somatic-cell gene therapy is appropriate for humans.**	• There is concern that many gene therapy candidates are **children** who are too young to understand the ramifications of gene therapy treatment and can become **unwitting participants.** • There are issues of **justice and resource allocation.** Gene therapy is very expensive. There are concerns that this treatment is being made available only to those who can afford it. Also, there are those who argue that research funding could be better spent on improving existing proven therapies than on such a high risk, costly 'experiment'. • Such experiments would involve too much scientific uncertainty and clinical risk, and the **long term effects** of such therapy are **unknown**.

Summary of different gene delivery systems

Gene delivery system	Advantages	Disadvantages
Retrovirus	• high transfection efficiency • integrates (as it contains intergrase) into host cell genome providing stable gene expression	• random integration may cause insertional mutagenesis • can trigger an immune response • limit to size of insert • safety concerns as virus is dangerous • infects dividing cells only (see ^^^ below Lentivirus)
~~Adenovirus~~	• ~~high transfection efficiency~~ • can inject DNA through the nuclear pore and deliver into nucleus • no integration into the genome→ no insertional mutagenesis • infects dividing and non-dividing cells	• ~~no integration into the genome~~→ transient gene expression → DNA not passed on to daughter cells • can trigger an immune response • limit to size of insert
Adeno-associated virus	• high transfection efficiency • non-pathogenic • does not trigger immune response • insert into specific site in chromosome 19 → no random integration into genome → no insertional mutagenesis • infects dividing and non-dividing cells	• limit to size of insert (smaller than other viruses)
Liposomes	• non-pathogenic • does not trigger an immune response • no limit to size of insert • can be targeted for specific cells or tissues by modification of liposome membrane by the addition of specific glycoproteins	• low transfection efficiency • low rate of stable integration • low levels of expression of normal functional allele

^^^ Lentivirus is a retrovirus which can infect both dividing and differentiated non-dividing cells, shows long term stable expression of the intergrated allele and causes only low immune response

* Transduction: introduction of foreign DNA by a viral vector
Transfection: introduction of foreign DNA into eukaryotic cells using non-viral methods e.g. liposomes
Transformation: direct uptake of foreign DNA from the surrounding

Plant tissue culture→ used to clonally produce thousands of plantlets, i.e. used for **micropropagation**.
(Micropropagation can be combined with genetic engineering to give rise to new types of plants)

Callus Culture (e.g. carrot, tobacco)

STAGE 1 – Choose the explant

* Explant
→ portion of plant tissue excised from donor plant used to initiate a culture
→ preferably taken from ***apical meristems*** (e.g. root or shoot tip) which are1) actively dividing, 2) disease free 3) responsive to callus formation
* Donor plant must 1) have desirable characteristics (e.g.high yielding)
2) disease free

STAGE 2 – Establish aseptic callus cultures

Surface sterilize explant using sodium hypochlorite to kill surface pathogens (bacteria, fungi)
→ Bacterial/fungal pathogens can outgrow the plant cells & use up nutrients in the growth medium.
* Explant cut into smaller pieces
Aseptic transfer *of explant to* ***sterile nutrient agar***
→ Nutrient egs.: Sucrose/glucose as carbon source for energy production via respiration as photosynthesis cannot occur, Mg for chlorophyll synthesis, nitrates and sulphur for protein synthesis, nitrates, sulphur and phosphorus for nucleic acid synthesis, Ca for cell wall synthesis *(must remember egs. + purpose)*
→**intermediate *auxin*: *cytokinin* ratio stimulates *callus* formation** (auxins & cytokinins are plant growth regulators)

Callus
→ amorphous mass of ***undifferentiated*** tissues
→ ***totipotent*** cells which can be induced to develop roots and shoots / embryoids to form plants.
Uses of calli:
→ cells can be easily **subcultured** for **mass propagation** of a plant ie. ***micropropagation***
Effective for plants that (1) have been genetically modified, (2) have desirable characteristics, (3) do not produce seeds or do not respond to other vegetative propagation methods.
→ thinner cell wall can be digested to provide a **source of *protoplasts*** *(link to protoplast culture)*
→can be subjected to **genetic modification** and **screening** for successfully transformed cells

STAGE 3 – Plantlet establishment (i.e. organogenesis)

* Callus can be induced to form plantlets by using different concentration ratios of plant growth regulators
1) high auxin:cytokinin ratio → stimulates root development
2) high cytokinin:auxin ratio → stimulates shoot development

STAGE 4 – Acclimatisation

* Healthy plantlet is removed from culture vessel, soaked in fungicide & first grown in sterile soil
* Initial conditions (in the greenhouse are similar to the culture vessel)
→ low-light, high temperatures, high humidity conditions
* Humidity is gradually decreased and after about 4-8 weeks, the plants can be grown in the field.

Meristem Culture (e.g. soyabean, potato)

Method:
* **Apical meristems** are used as explants. All other steps are similar to callus culture.
Purpose:
* Meristem culture allows the propagation of **disease-free plants** as apical meristems
1) produce **inhibitors** which prevent **viral infection**
2) are **fast growing** & hence **do not have established vascular bundles** through which viruses/bacteria can spread .

Embryo Culture (e.g. peach, grapes)

Method:
* A **whole immature embryo** is excised from a seed and used as the explant.
* It is induced to develop shoots & roots by growing it appropriate media..
Purpose:
1) allows the propagation of **hybrids where embryos fail to develop**
2) **rescues embryos from dormancy** as the endosperm may produce compounds that encourage dormancy
[NB: Embryo culture can also be used to rescue diseased plants.]

Protoplast Culture (e.g. strawberries, potato)

* Protoplasts → plant cells with **cell walls removed**
Method:
1) Obtain ***callus*** (similar to stages1 & 2 of callus culture)
2) ***Plasmolyse*** cells in sucrose solution (which will cause the cell membrane to pull away from the cell wall)
3) **Remove cell wall** by mechanical disruption and enzymatic treatment with **pectinases, hemicellulases & cellulases**
4) Protoplasts cultured in isotonic medium (same Ψw as cell sap)
5) **Polyethylene glycol (PEG)** is added to makes protoplasts 'sticky' & induces them to fuse
6) **Fused protoplasts** (now called somatic hybrids) are cultured in medium where they **resynthesise cell walls** & start to divide
7) Cells induced with plant growth regulators form callus which is then used to generate new plants (similar to stages 3-4 of callus culture)
Purpose:
* Removal of cell wall:
1) **enables easier introduction of genetic material into cells**
→ This can give rise to transformed protoplasts & hence transformed plants
2) **enables the fusion of 2 or more protoplasts resulting in somatic hybrids**
→ This enables breeders to:
a) **overcome reproductive barriers** (e.g. habitat/ temporal/ mechanical) & **combine good traits of two plant species**
e.g. *Solanum brevidens* (wild potato variety) has been used to transfer genes that confer resistance to potato leaf curl virus to *Solanum tuberosum* (commercial potato variety)
b) **generate *polyploids* with increased vigour** (i.e. grow faster & more resistant to disease)

Anther Culture (e.g. tobacco, rice)

Method:
* **Sterilised *anthers*** are used as explants
* **Pollen grains** (haploid cells) develop into ***haploid*** **callus**
→ callus cells are **not genetically identical** as pollen grains are a product of ***meiosis***.
* Mutations can be induced by exposure to UV rays
→ this allow for generation of new alleles
* Cells can be made diploid by addition of ***colchicine*** which **disrupts spindle formation & separation of chromatids**
Purpose:
* Callus cells are ***haploid***
→ phenotypic effect of all genes can be seen (no masking of phenotype since every cell only has 1 copy of each gene)
This allows lethal alleles to be eliminated & plants with desired characteristics can be selected for.
* Diploid callus cells that are generated are homozygous at all loci
→diploid plants homozygous for desired allele.

Pollen Culture (e.g. tobacco, rice)

* An **isolated pollen grain** is used as the **explant**
(all other steps are similar to anther culture)

Somatic Embryogenesis (e.g. carrot, asparagus)

Method:
* **Somatic cells** used as the explants
* Callus induced to form **genetically identical embryoids** which can be **encapsulated in a protective, hydrated gel** containing nutrients, growth regulators, fungicides, etc to form **artificial seeds**.
(Hence the process **does not involved fusion of gametes**
Purpose:
*Artificial seeds produced can be **transported easily & sown in soil directly**.

Somatic Embryogenesis e.g. carrot, asparagus	Meristem Culture e.g. soyabean, potato	Embryo Culture e.g. peach, grape	Callus Culture e.g. carrot, tobacco	Protoplast Culture e.g. strawberries, potato	Pollen Culture e.g. tobacco, rice	Anther culture e.g. tobacco, rice
Explant: **somatic cell** e.g. meristem ↓ PGR Callus ↓ PGR to stimulate embryo-genesis **Somatic embryos (embryoids)** ↓ Encapsulate somatic embryos in hydrated gel e.g. alginate ↓ Plantlets ↓ *A Plants	Explant: **apical meristem** e.g. shoot tip ↓ PGR Callus → PGR Plantlets ↓ Acclimatisation(*A) ↓ Plants	Explant: **embryo** taken from a seed ↓ PGR Callus ↓ 1 plantlet ↓ *A 1 plant	Explant: **meristem** e.g. shoot tip ↓ PGR Callus ↓ PGR Plantlets ↓ Acclimatisation(*A) ↓ Plants	Explant: **somatic cell or meristem** e.g. shoot tip ↓ PGR Callus ↓ enzymes Remove cell wall ↓ Protoplasts ↓ Genetic manipulation ↓ PGR to stimulate callus formation	Explant: **an isolated pollen grain** ↓ PGR 1 Haploid plantlet ↓ *A 1 haploid plant ↓ PGR Haploid callus ↓ Many genetically identical haploid plantlets ↓ *A Many **genetically identical** plants produced	Explant: **anther** (many pollen grains) ↓ PGR Haploid callus no uv | uv to create mutants ↓ PGR ↓ PGR Many genetically different haploid plantlets ↓ Select for desired phenotype ↓ Colchicine ↓ Homozygous diploid plantlets ↓ *A Homozygous diploid plants
Genetically identical plants produced	**Genetically identical** plants produced	**1 plant** produced (However, if callus formation is induced from one embryo, many **genetically identical** plants can be produced.See 2nd pathway))	**Genetically identical** plants produced	**Genetically identical** plants produced	**1 plant** produced (However, if callus formation is induced from one pollen grain, many **genetically identical** plants can be produced. See 2nd pathway)	**Genetically different** plants produced
Advantages						
Mass clonal production of **artificial seeds** which are easily	Production of **disease free plants** from diseased plants as the apical meristems are normally disease free as 1) they produce **inhibitors** which **prevent viral infections** 2) their **vascular bundles** via which viruses spread are **not established**	1) **Embryo rescue** of hybrids where immature embryo fails to develop 2) **Overcoming seed dormancy** due to compounds produced by endosperm	1) **Mass clonal production of plants** 2) **Genetic manipulation – e.g. inserting of desirable genes using gene gun**	1) **Genetic manipulation** 2) Overcome reproductive barriers betw. different species 3) Formation of **polyploids** with increased vigour	**Select for desirable characteristics** in plants **without** having to **crossbreed** two different plants that are **true breeding** i.e. homozygous at all loci	

NB: 1) Genetic manipulation can be carried out at callus stage. Callus can be repeatedly subcultured to produce more callus.
2) Sometimes in **embryo culture, callus formation is induced**. This can then be used to produce many **genetically identical plants**.
3) Sometimes, **many different pollen grains are used in pollen culture**. In that case, many **genetically different plants** will result.
4) Embryos develop from the fusion of male and female gametes. The term embryoids or somatic embryos are used to describe "embryos" that are derived from somatic cells. The term embryoid can also be used to describe "embryos" that develop from haploid pollen.
5) Tissue culture media contain nutrients for protein synthesis (N & S), nucleic acid synthesis (N, P & S), cell wall synthesis (Ca), chlorophyll synthesis (Mg) and to activate various enzymes (K).
6) * A represents the acclimatisation step, PGR represents plant growth regulators.e.g. auxins and cytokinins

Advantages of Micropropagation	Disadvantages of Micropropagation
1) High multiplication rate results in high reproductive rate (fercundity) 2) Produces genetically uniform plants (unlike conventional breeding) 3) Produces disease-free plants 4) Produces rooted plantlets ready for growth (& not seeds or cuttings) 5) Good way of multiplying plants that are sterile,with seeds that are difficult to germinate, etc. 6) Independent of climatic changes (i.e. not affected by seasonal changes) 7) Takes up less place as they are grown close together in small sterile containers on racks 8) Can be send via air-freight quickly as they are in sterile boxes & can pass quarantine rules 9) Useful tool used in genetic engineering.as it can be used to multiply transgenic plants 10) Secondary metabolites can be extracted from cell cultures or plants	1) Expensive due to skilled labour cost & expensive technology & facilities 2) An infected plant sample will produce infected progeny 3) Losses occur during the acclimatization stage 4) Somaclonal variation is possible

Significance of genetic engineering in improving quality and yield of ANIMALS in solving the demand for food in the world

Category	YIELD	YIELD & QUALITY	YIELD	QUALITY
Example	1. Atlantic Salmon	2. Porcine Somatotropin (PST)	3. Bovine Somatotropin (BST)	4. Heart-healthy meat
Type of Genetic Engineering	* **Promoter from ocean pout anti-freeze gene** spliced with **Chinook salmon growth hormone cDNA** * This construct was **microinjected** into fertilised eggs of Atlantic salmon * The transgene gets incorporated into the genome and is expressed only in 2-3% of the resulting fry *N.B: The resulting **salmon** are **transgenic*** **1. Growth rate :** → GH causes transgenic Atlantic salmon to grow 3-6x faster than their wild counterparts and hence reach maximum size faster. (NB: They do not grow bigger than non-transgenic salmon.) The higher growth rate is due to: **2. Feed efficiency:** → higher feed conversion efficiency **3. Environmental Tolerance** → GH expressed continuously throughout the year (not just during warmer months as in normal Atlantic salmon)	* PST gene is ligated to a vector and this recombinant vector is used to transform *E. coli* * Successfully transformed *E. coli* which express the PST gene are used to produce large commercial quantities of PST * PST is extracted, purified and then injected into pigs *N.B: The resulting **pig is not transgenic**; however, the transformed E.coli are referred to as recombinant bacteria* **1. Feed efficiency:** → PST increases feed efficiency by 15-20% **2. Fat content** → PST reduces fat deposition in pigs, producing leaner meat	* BST gene is ligated to a vector and this recombinant vector is used to transform *E. coli* * Successfully transformed *E. coli* which express the BST gene are used to produce large commercial quantities of BST * BST is extracted, purified and then injected into lactating cows *N.B: The resulting **cow is not transgenic**; however, the transformed E.coli are referred to as recombinant bacteria* **1. Milk-to-feed ratio:** → BST increases milk to feed ratio by 5-15% **2. Milk yield** → BST increase milk yield up to 25% **3. Weight of cattle** → BST increase cattle weight by 10-15%	* fat-1 gene from roundworm, *Caenorhabditis elegans* transferred into pigs *N.B: The resulting pigs are **transgenic.*** Transgenic pork contains **8% omega-3 fatty acids** compared to typical pork that contains only **1%**
Positive Significance	1. Salmon meat is made **more readily available** and **cheaper** to consumer 2. Increased **profits** for salmon breeders	1. Increases **growth rate** 2. Improves **nutritional qualities** of pork – less fat, more lean meat 3. Increase **profits** for farmers over same period of time	1. Increase **milk yield** in cows 2. Increase **profits** for farmers over same period of time	**1. Higher level of omega-3 fatty acids** protect against heart disease
Negative Significance (food safety, environmental protection, animal welfare)	1. **Escape** of transgenic salmon **into the wild** may **outcompete the wild fish** and affect ecological balance 2. Making only **sterile, triploid female fish** could solve the above problem; however, screening procedure to identify failed cases must be stringent	1. Pigs have increased **joint and skeletal problems** 2. PST may incur **more costs** due to **constant injections** 3. Critics say we show no respect for animals	1. Increased incidence of **mastitis** (infection of the udder) 2. BST may incur **more costs** due to **constant injections** 3. Critics say we show no respect for animals	

Significance of genetic engineering in improving quality and yield of PLANTS in solving the demand for food in the world

Category	INSECT-RESISTANCE	IMPROVE NUTRITIONAL QUALITY	HERBICIDE-RESISTANCE	IMPROVED SHELF-LIFE
Example	1. Bt-Corn/Tomato/ Cotton	2. Golden Rice	3. Glyphosate-resistant Soybean (Roundup Ready™)	4. Flavr Savr™ Tomatoes
Type of Genetic Engineering	* Gene coding for **Bt-toxin** from *Bacillus thuringiensis* is transferred into corn/cotton/tomato plants by a Ti plasmid by 1. ***Agrobacterium*-mediated gene transfer into protoplasts** 2. **bombardment of callus using a gene gun** 3. **electroporation of protoplasts** *The Bt gene has been expressed in all /some parts of the plant depending on the **promoter** it is coupled to.	* Genes coding for enzymes that convert a **natural compound in rice to beta-carotene** transferred into rice embryos in tissue culture *via* ***Agrobacterium*-mediated gene transfer** * This plant is crossed with local rice varieties *Construct details: **Endosperm specific promoter +phytoene synthase gene from daffodil or maize +carotene desaturase gene from soil dwelling bacterium, *Erwinia uredovora***	* **EPSP gene** coding for **resistance to herbicide** Roundup™ (a glyphosate-based herbicide), from *Agrobacterium* strain CP4, was introduced into soybeans.	* **Polygalacturonase (PG)** is an enzyme responsible for **ripening**; it hydrolyses pectins in plant cell walls → flaccid cells → softer fruits * **Antisense gene coding for PG** is transferred into tomato plants via *Agrobacterium*-mediated gene transfer
Effect	1. Transgenic plant produces the **Bt-toxin** due to presence of recombinant plasmid 2. Affects only **specific pests** e.g. **caterpillars** die when they eat the plant as they have specific enzymes in their gut that that activate the Bt-toxin causing the **gut wall to breakdown**	1. Transgenic rice produces **beta-carotene which is a precursor of Vitamin A** in endosperm.	1. Transgenic crop plants express enzymes that **degrade glyphosate** 2. **Weeds** (which do not have the gene for the glyphosate-degrading enzyme) **are affected** by the herbicide	1. Transgenic plant produces **antisense PG mRNA** which binds to the mRNA transcript for PG, hence (a) ribosome cannot gain access to mRNA, (b) duplex RNA quickly degraded by ribonucleases in the cell **2. Prevents translation of PG mRNA into PG protein**
Positive Significance	**1. Higher yield & quality** of crop plants (less leaf damage) 2. Negative effects of **pesticides** are **avoided** e.g. high cost, laborious application, indiscriminate killing (including beneficial organisms) 3. Does **not harm humans** (who do not have the enzyme)	1. The poor in developing countries who can't afford supplements in form of pills can obtain Vitamin A, which **prevents night blindness**, from Golden Rice **2. Rice well-adapted to local conditions** (as they were **crossed with local varieties**)	**1. Higher yield and quality** of crop plants **2. Herbicides** can be freely **applied to kill weeds** without danger of harming crop plants	**1. Delays ripening** so that they can ripen on the vine for a longer time → fuller flavour 2. Fruits still quite **firm after transportation** → reduce loss and increase profits of farmer **3. Increases shelf-life** → increase profits of retailer

More details on:

GENERAL TECHNIQUE FOR GENETICALLY ENGINEERING PLANTS:

* *Genetic engineering releases constraints associated with interspecific barriers (i.e. traits from plants of different species cannot be transferred through normal means) and improves efficiency of expression of the desired trait*

* Common vector: *Agrobacterium tumefaciens*

1) **Soil-dwelling** bacterium which contains Ti (tumour-inducing) plasmid that has a special region known as **T-DNA which it can insert into the genome** of an infected plant cell; this causes the plant to
   a) secrete special amino acids (opines) which *A. tumefaciens* feeds on
   b) synthesis of plant hormone which disrupts the hormone balance in the cell → causes the formation of crown gall (tumours)
2) Thus suitable for transferring foreign genes (after removing tumour-inducing properties) into the genome of plant cells

* Common technique used in Genetic Engineering

1) **Ti plasmid** isolated from *A. tumefaciens*
2) Tumour-inducing genes are removed from the Ti plasmid
3) Gene of interest and Ti plasmid cut with the **same** restriction enzyme → **complementary** sticky ends → gene **anneals** with Ti plasmid via H-bonds
4) **DNA ligase** → form **phosphodiester bonds** between gene and plasmid
5) The gene of interest is linked to a plant promoter so that the gene can be expressed in the plant cell.
6) Recombinant plasmids introduced into plant genome directly via **protoplast culture**
7) Ti plasmid **integrates T-DNA region** (containing gene of interest) into plant **chromosomal DNA**
8) Only some cells are transformed, hence **selection** is needed.
9) Selectable marker genes used are usually **herbicide- or antibiotic-resistance genes**
   e.g. The kanamycin resistance gene is usually used as a selectable marker.
   It is usually coupled to the gene of interest in the Ti plasmid.
   Most antibiotics only kill bacteria. However, kanamycin can also kill plant cells. Hence plant cells that are transformed will grow in selection medium containing kanamycin while cells that are not transformed will die in kanamycin containing media.
10) **Transformed cells** can then be induced to form **calli** which are used to **generate whole plants**.

TECHNIQUE FOR ANTISENSE TECHNOLOGY [using example of Flavr Savr: inhibition of production of the enzyme polygalacturonase (PG)]:

More details on:

Gene 1
5' XYZ ATTACG 3'
3' Prom TAATGC 5' → template strand of PG gene

5' AUUACG 3' → PG mRNA

3' UAAUGC 5' → antisense PG mRNA

PG mRNA and antisense PG mRNA can anneal, preventing the translation of the PG mRNA, thus delaying ripening.

Translation is prevented
1. as duplex is degraded by ribonucleases
2. as ribosomes cannot gain access to nucleotides in mRNA

Gene 2 (has the same sequence as gene 1 but the promoter is on the end)
5' ATTACG Prom 3' → template strand of PG gene
3' TAATGC ZYX 5'

Ethical and social implications of consuming transgenic food products

	Concerns	Implications	Examples	Possible Solutions/Measures
ETHICAL CONCERNS	Exploitation of animals for genetic engineering	- Animals may not be biologically capable of withstanding addition stress in↑ growth rate & yield - Medical experiments may/will cause suffering in animals	1. Bovine Somatotrophin (BST): ↑ risk of mastitis (disease of udder) 2. Oncomouse" (transgenic mouse with oncogene introduced) develops tumours more frequently	
	Cloning	- Cloning techniques used in Dolly the sheep can be applied to humans	No evidence to support the claims that humans have been cloned.	
	Religious Implications in food choices	- Some religious & ethnic groups avoid eating certain food → GM food may have genes from the prohibited type of food	1. Vegetarians: GM vegetables with animal gene 2. Muslims: Genes from pig in GM foodstuff	Label GM food
ENVIRONMENTAL CONCERNS	**Concerns**	**Implications**	**Examples**	**Possible Solutions/Measures**
	Pest Resistance	- ↑ Exposure to BT plants has conferred resistance in target pests (Process will take a very long time though)	Bt-resistant pink bollworms, major cotton pests	Changed a small part of the toxin → resulting in toxins that the pests are now susceptible to
	Effects on Non Target Organisms	- Accumulation of toxins in predators of BT plant's pests - Loss of food source of predators due to death of pests (prey) from BT toxin - BT Pollen might be transferred to other plants, killing insects that feed on the plants	Death of monarch butterflies after feeding on milkweed leaves with transgenic maize pollen (however, there is no **conclusive evidence**)	
	Gene Flow and Superweeds	- New genes may be passed from transgenic plants to related weeds via pollen transfer → superweeds		1. Having greater isolation dist (planting of unrelated plants) 2. Buffer zones around GM crops to reduce gene flow
	Biodiversity	- GM crops may have selective advantage → outgrow native species → disrupt natural ecosystem		
MEDICAL CONCERNS	**Concerns**	**Implications**	**Potential Examples**	**Possible Solutions/Measures**
	Toxicity of food stuff	- GM plants may produce high quantities of 2° metabolites that may be toxic to humans/livestock - Toxins not normally harmful to human might turn harmful after reacting with body chemicals		Safety assessments and regulations
	Allergies	- Allergenic properties of food proteins from a source with known allergic effect might be conferred on the host plant	Nuts e.g. peanuts etc → reactions: mild (rash) to severe (anaphylactic shock)	
	Nutritional qualities	- Nutritional qualities of GM food could change into form that cannot be metabolised/ absorbed → disruption in balance of nutrients		Set standard for food safety → equivalent to conventional food in nutrition, composition
	Antibiotic Resistance Markers	- Antibiotic resistant genes used as markers in GM food might be passed to bacteria	From GM plant to *E.coli* found in the gut	1. Removal of marker genes before commercialisation 2. Use other markers e.g. X-gal, fluorescent tags

Overall Measures: 1. Educate consumers; 2. Develop international and national regulatory frameworks → Responsibility of international bodies e.g. *Food and Agricultural Organisation (FAO), World Health Organisation (FAO)*