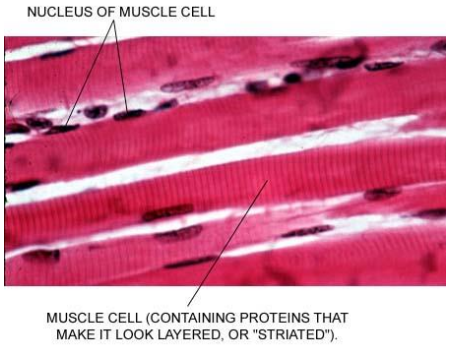
Topic 1 – CELL BIOLOGY

1.1 Introduction to Cells

The Cell Theory:

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

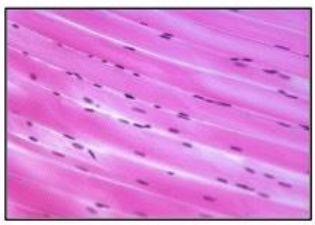
Caveats of the cell theory:



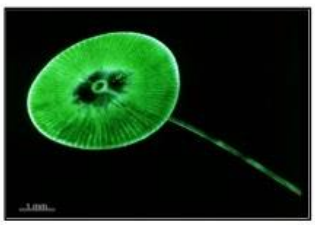
1. Striated muscle cells
   - Muscle cells fuse to form fibres that may be very long -> up to 30 cm in length
   - Consequently they have *multiple nuclei* despite being surrounded by a single, continuous membrane
     - Challenges idea that cells always function as autonomous units

2. Giant algae
   - Certain species of unicellular algae may grow to very large sizes -> up to 10 cm
     - Challenges the idea that larger organisms are always made of many microscopic cells

3. Aseptate fungi
   - Fungi may have filamentous structures called hyphae (separated into cells by internal walls called *septa)*
   - But some fungi are not partitioned by septa -> have a continuous cytoplasm along the length of hyphae
     - Challenges the idea that living structures are composed of discrete cells


Muscle cells form long, multinucleated fibres


Aseptate hyphae have **no** cellular partitions


Giant algae can be very large in size (>70mm)

Non-cellular, uni-cellular, multicellular:

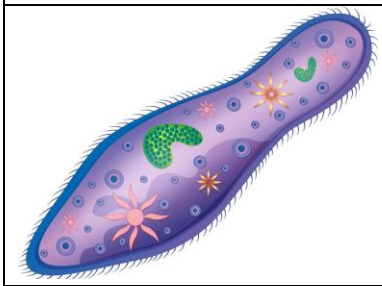
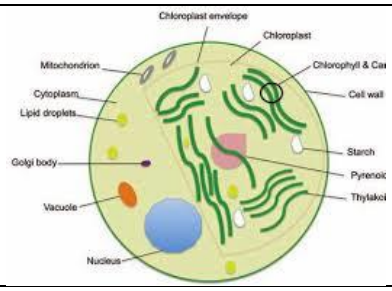
- *Non-cellular:* exists without cell structure, nucleus, cytoplasm or organelles -> e.g. viruses
- *Uni-cellular:* made up of only one cell -> E.coli bacteria, paramecium
- *Multi-cellular:* made of many specialised/ differentiated cells -> each cell carries out a particular function
  - All cells have the same genetic information -> differentiation occurs due to the expression of certain specific genes but not others

7 functions of life

1. **M**etabolism – sum of all chemical reactions within the cell or organism
2. **R**eproduction – production of offspring
3. **S**ensitivity/ Response – sensing and responding to stimulus
4. **H**omeostasis – maintaining the internal environment
5. **E**xcretion – removal of the waste products of metabolism
6. **N**utrition – consuming materials for growth and repair -> nutrients are a source of energy or matter to build the organism
7. **G**rowth – increase in cell number or size

Functions of life in Paramecium and one photosynthetic unicellular organism:

- Paramecium: unicellular member of kingdom Protista -> heterotroph, live in water
- Chlorella: single celled organisms with one very large chloroplast for photosynthesis

Paramecium	**Feature**	**Chlorella**
Metabolic reaction take place in cytoplasm (catalyzed by enzymes	Metabolism	metabolic reactions take place in cytoplasm
Primarily asexual (binary fission)	Reproduction	nuclei can divide by cell division
contractive vacuoles absorb and expel water to maintain balance	Homeostasis	contractive vacuoles absorb and expel water
consumes smaller organisms via food vacuoles -> cytosomes	Nutrition	photosynthesis occurs inside the chloroplasts
waste products diffuse out of the membrane -> solid waste= anal pore, liquid waste= contractile vacuole	Excretion	oxygen diffuses through membrane and cell wall
paramecia are surrounded by small hairs (cilia) which allow movement	Response	light sensitive eye spot sense light -> cell beats cilia to move towards light in response
maximum size is 0.5 mm	Growth	about 0.01 – 0.03 mm in diameter
	Image	

Magnification and Actual Size:

Magnification: $\frac{\text{Image size (with ruler)}}{\text{Actual size (according to scale bar)}}$

OR Magnification: $\frac{\text{scale bar measurement}}{\text{scale bar label}}$

Actual size: $\frac{\text{Image size (with ruler)}}{\text{Magnification}}$

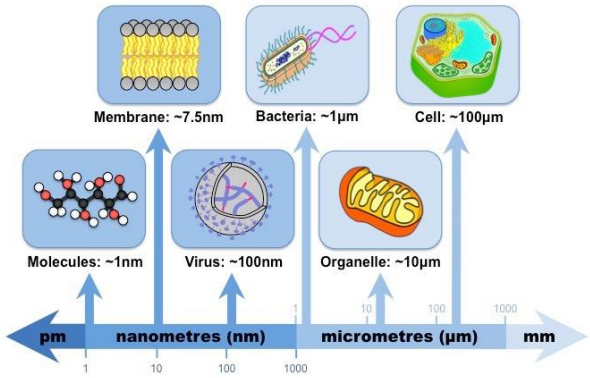
Unit Conversions:

1 cm

10mm

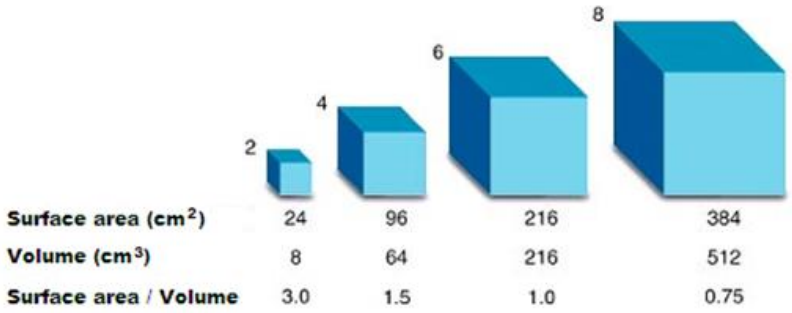
10,000 µm (micrometres)

10,000,000 nm (nanometres)



Surface Area to Volume ratio – limiting cell size

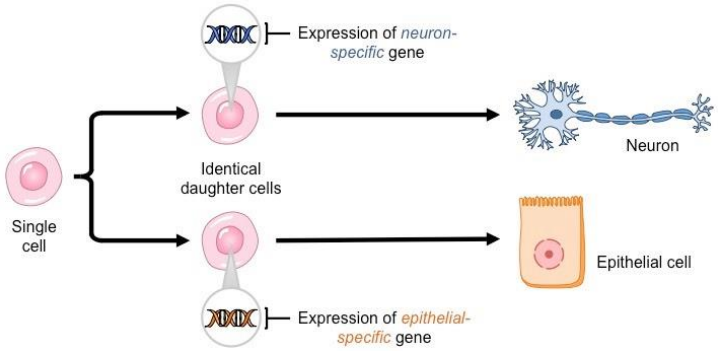
- Cells do not grow to large sizes due to the SA:Vol ratio:
  - The rate at which metabolic reactions occur depends on the volume of the cell -> larger volume = greater metabolic rate
  - However the rate at which raw materials enter the cell and waste materials leave the cell depends on the surface area of the cell
  - As the cell size increases, the volume increases faster than the surface area -> this means as the cell size *increases* the SA:Vo ratio *decreases*
- If metabolic rate exceeds the rate of exchange of vital materials and wastes (low SA:Vol ratio) the cell will eventually die
  - This is why cells tend to divide and remain small in order to maintain a high SA:Vol ratio
- Cells/ tissues that specialize in gas or material exchange will maximise their SA:
  - Intestinal tissue of digestive tract has a ruffled structure (villi), alveoli within lungs have membranous extensions called microvilli



Surface area (cm^2)	24	96	216	384
Volume (cm^3)	8	64	216	512
Surface area / Volume	3.0	1.5	1.0	0.75

Cell reproduction and differentiation:

- Multicellular organisms show *emergent* properties -> emergent properties can be summarized as *The whole is greater than the sum of the individual parts*
  - Emergent properties arise when the interaction of individual components produce new functions
    - Ex. the heart is an organ who's function it is to pump blood -> however cardiac muscle cells, valves, neurons have to work together -> so the pumping function is an emergent property
- *Differentiation:* differentiation is the process during development whereby newly formed cells become more specialised and distinct as they mature



- All cells of an organism share an identical genome -> every cell in the body has a *full set of genes*
- However the activation or *expression* of certain genes will cause differentiation -> for each type of specialized cell a unique subset of genes will be expressed

Stem cells:

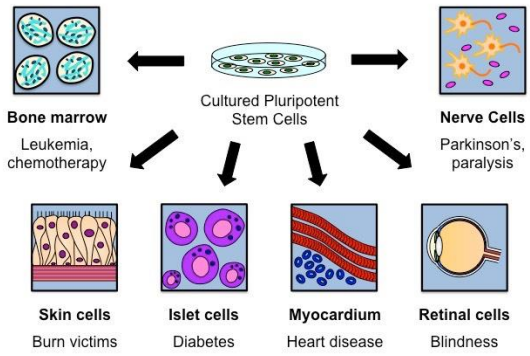
- Stem cells are unspecialised cells that have two distinct qualities:
  - *Self-renewal* – stem cell scan continually divide and replicate so that large numbers of cells (tissues can be made)
  - *Potency* – stem cells undifferentiated (unspecialized) so they have the capacity to differentiate into specialised cell types
- Types of stem cells:
  - Totipotent – can form *any* cell type, as well as embryonic (placental) tissue
  - Pluripotent – can forma any cell type (embryonic stem cells)
  - Multipotent – can differentiate into a number of closely related cell types (haematopoietic adult stem cells)
  - Unipotent – can not differentiate but are capable of self renewal (muscle cells, neurons)
- Importance of stem cells in embryo development:
  - Stem cells are necessary for embryonic development as they are an undifferentiated cell source from which all other cell types may be derived

Therapeutic uses of stem cells:

- Stem cells can be used to replace damaged or diseased cells with healthy, functioning ones -> these are known as *cell based therapies*
  - process includes using biochemical solutions to trigger differentiation of stem cells, surgical implantation into patient's tissue, suppression of patient's immune system and monitoring of cells so they do not become cancerous
- Examples of stem cell therapy:

1. **Stargadt's disease:** is a genetic disease that causes macular degeneration in children and young adults
   - Photoreceptor cells in the retina become damaged and progressively lost -> patients gradually lose their vision
   - Treated by injecting thousands of functioning retina cells that have been developed from embryonic stem cells into patient eyes
2. **Leukemia***:* is a type of cancer that causes a high number of abnormal white blood cells to be produced by bone marrow
   - Fluid is taken from the bone marrow of patient's pelvis -> healthy blood stem cells are identified and frozen (or a donor is used)
   - chemotherapy is used to kill cancerous cells -> patient's bone marrow can no longer produce blood cells
   - The stored/donated blood stem cells are injected into patient and enter the bone marrow -> bone marrow regains its ability to produce healthy blood cells

3. **Other therapeutic examples:**
    - *Parkinson disease:* degenerative disorder of CNS caused by death of dopamine-secreting cells (dopamine is involved in transmission of smooth, purposeful movements) -> dead nerve cells are replaced with living, dopamine-producing ones
    - *Diabetes:* replace non-functioning islet cells with those capable of producing insulin in type I diabetes
    - *Burn victims:* graft new skin cells to replace damaged tissue



Ethical implications:

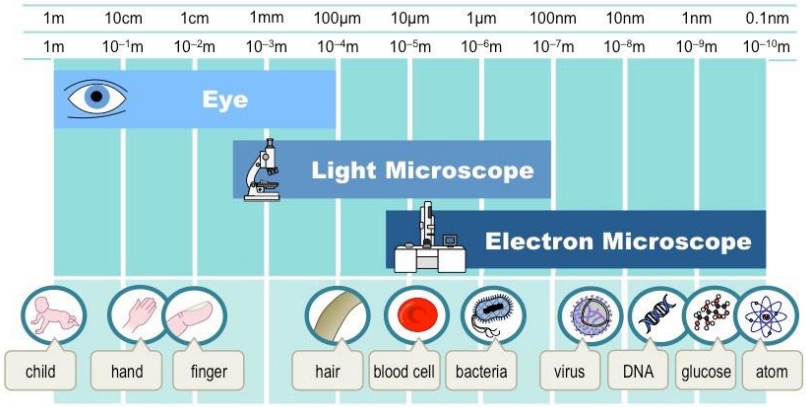
- The main argument for therapeutic use of stem cells that it could result in a significant reduction in human suffering (for otherwise incurable diseases)

Stem cell source	Ethical implication
Adult stem cells	no ethical concerns with consent of donor but limited scope of application
Cord blood stem cells	no ethical concerns, as long as parents give their consent -> however very expensive method
Stem cells from embryos created in vitro (in laboratory)	the greatest yield of pluripotent stem cells comes from embryos, but requires the destruction of *potential* living organism -> some argue killing an embroyo is equivalent to taking a human life, yet other's define life to start at a later stage of embryo development (since embryos lack a nervous system and are little more than balls of cells) -> the creation of embryos just for research is also opposed because human life should never be created/ destroyed

1.2 Ultrastructure of Cells

Electron Microscopy

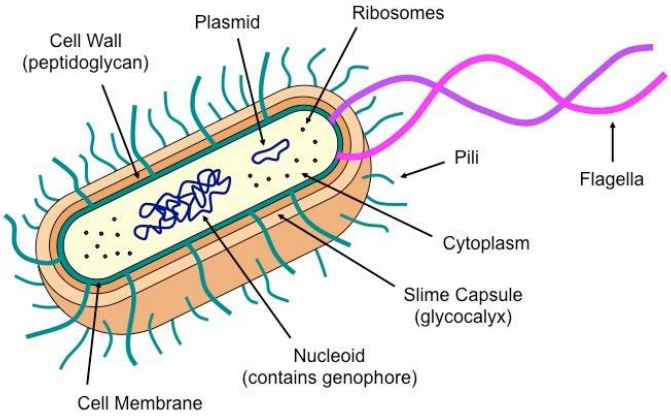
- Electron use electron beams focused by electromagnets to magnify specimens:
    - *Transmission microscopes (TEM)* generate high resolution cross sections of objects
    - *Scanning microscopes (SEM)* map objects in 3D
- Advantages of electron microscopes compared to light microscopes:
    - They have a higher *resolution* since electron beams have a much shorter wavelength -> light= 200 nm, electron = 1 nm (200 times better)
    - They have a higher range of magnification -> light microscopes reveal the structure of cells while electron microscopes reveal the *ultrastructure* (ribosomes, lysosomes, ER)
        - BUT electron microscopes are costly and cannot display living specimens in natural colours

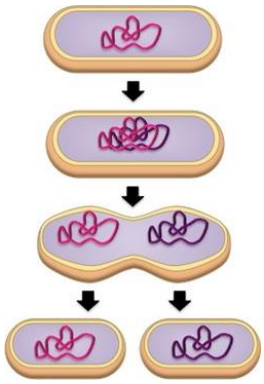


Resolution: the smallest distance apart two objects can be in order for them to appear distinct

Prokaryotes

- Prokaryotes are organisms whose cells *lack a nucleus* (pro= before, karyon= nucleus)
- They are much simpler and smaller than eukaryotic cells -> less than 1 um in diameter
- They can be further classified into two domains:
    - Archaebacteria – found in extreme environments (extremophiles)
    - Eubacteria – traditional bacteria (E.coli, S. Aureus)
- Features of prokaryotic cells:
    - *cytoplasm* – internal fluid component of the cell where all the chemical reactions occur and DNA is located
    - *cell membrane* – semi permeable and selective barrier surrounding cell
    - *cell wall* – rigid outer covering made of peptidoglycan – provides structure, support and protection
    - *flagella* – long, slender projections containing a motor protein that enables movement
    - *pili* – hair like extensions that enable adherence to surfaces (attachment)
    - *ribosomes* – complexes of RNA and protein – responsible for protein synthesis (70S in prokaryotes)
    - *nucleoid* – region of cytoplasm where the (circular) DNA is located
    - *plasmid* –smaller ring of DNA



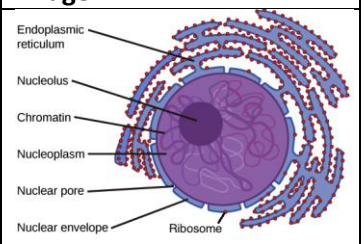
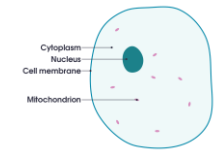
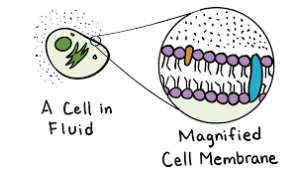
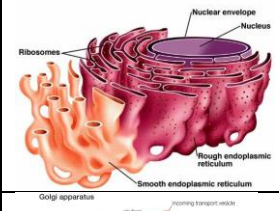
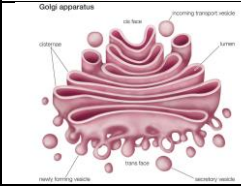


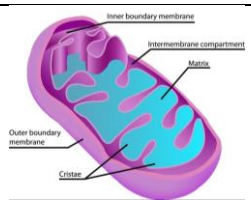
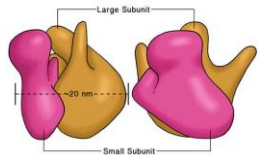
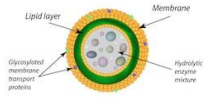
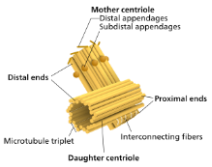
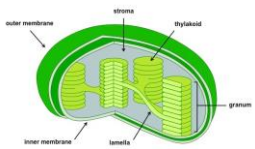
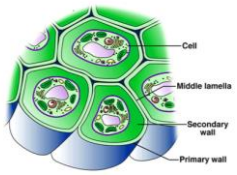
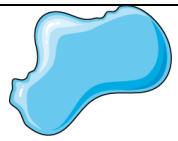
Cell division in prokaryotes:

- prokaryotes divide by binary fission -> asexual reproduction
- in the process of binary fission:
  - the circular DNA is copied in response to a replication signal
  - the two DNA loops attach to the membrane
  - the membrane elongates and pinches off (cytokinesis), forming two genetically identical cells

Eukaryotes:

- Eukaryotes are organisms whose cells *contain a nucleus* (pro= before, karyon= nucleus)
- They have a more complex structure and are believed to have evolved from prokaryotic cells
  - Eukaryotic cells are compartmentalised by membrane bound structures (organelles) that perform specific roles
- Can be divided into four distinct kingdoms: Protista, fungi, plats and animals
- *Organelles:*

Organelle	Structure	Function	Image
Nucleus	double membrane structure with pores, contains an inner region called a nucleolus	stores genetic material (DNA) as chromatin (uncoiled chromosomes) -> nucleus creates	
Nucleolus	Dense solid structure inside thelece nucleus	Involved in ribosome synthesis	
Cytoplasm	Jelly-like substance made out of water and dissolved nutrients	Holds organelles and allows them to move -> helps dissolve waste products	
Plasma membrane	Semi permeable and selective barrier surrounding cell	Protects the cell and holds it in place, controls what enters and exists	
Endoplasmic Reticulum	Membrane network (cisternae) that is either bare (smooth ER) or studded with ribosomes (rough ER)	Transports materials between organelles -> smooth ER: lipids and carbohydrates -> rough ER: proteins	
Golgi Apparatus	An assembly of vesicles and folded membranes (cisternae) located near the cell membrane	Involved in the sorting, storing, modification and export of secretory products	

Mitochondrion	Double membrane structure, inner membrane highly folded into internal cristae – fluid inside called a matrix	Site of aerobic respiration -> ATP production -> powerhouse of the cell	
Ribosomes	Two subunits made of RNA and protein -> larger in eukaryotes (80S) than prokaryotes	Site of polypeptide synthesis (translation)	
Lysosomes (mostly found in animal cells)	Spherical in shape, formed by the Golgi apparatus	Digestive enzymes -> break down harmful chemicals and organisms in cell	
Centrioles	Consist of two groups of nine triple microtubules	Help in the formation of spindle fibres during cell division	
Chloroplast (plant cell)	Double membrane, made up of stack of thylakoids (flattened stacks of membrane)	Produce glucose (and other compounds) via photosynthesis	
Cell wall (plant cell)	External outer covering made of cellulose	Provides support and mechanical strength -> prevents excess water uptake (osmosis)	
Vacuole	Consists of a single membrane with fluid and dissolved nutrients inside	Maintain hydro-elastic pressure -> takes up half of the cell in plant cells, smaller in animal cells	

Compartmentalization in Eukaryotes:

- Eukaryotes are compartmentalized-> they are divided up by partitions (single or double membranes)
- Advantages in being compartmentalized:
  - Enzymes and substrates for a particular process can be concentrated -> this isolation results in increased *efficiency*
  - Toxic/ damaging substances are kept inside the membrane of a organelle -> digestive enzymes of lysosomes
  - Conditions such as pH can be maintained at an ideal level
  - Organelles with their contents can be moved around within the cell

Prokaryotic cells vs. Eukaryotic cells:

Differences between prokaryotic and eukaryotic cells:

	Prokaryotic cells	**Eukaryotic cells**
DNA	DNA in a ring form without protein	DNA with proteins as chromosomes/ chromatin
	DNA free in the cytoplasm (nucleoid region)	DNA enclosed within a nuclear envelope (nucleus)
Organelles	No mitochondria or nucleus	Mitochondria and nucleus present
	70S ribosomes	80S ribosomes
	No internal compartmentalization to form organelles (not membrane bound)	Internal compartmentalization present to form many types of organelles (membrane bound)
Reproduction	Binary fission	Mitosis and Meiosis
	Single chromosome (haploid)	Chromosomes paired (diploid or more)
Size	Size less than 10 micro meters	Size more than 10 micrometres

Similarities:

- Both types of cell have some sort of outside boundary that always involves a plasma membrane
- Both types of cell carry out all the functions of life
- DNA is present in both cell types

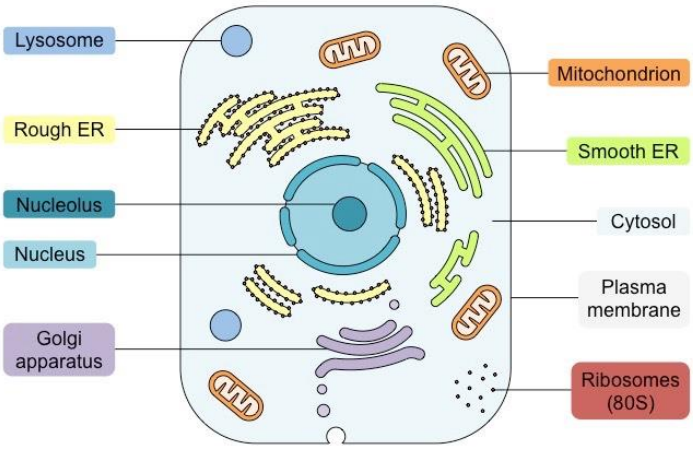
Plant vs. Animals cells:

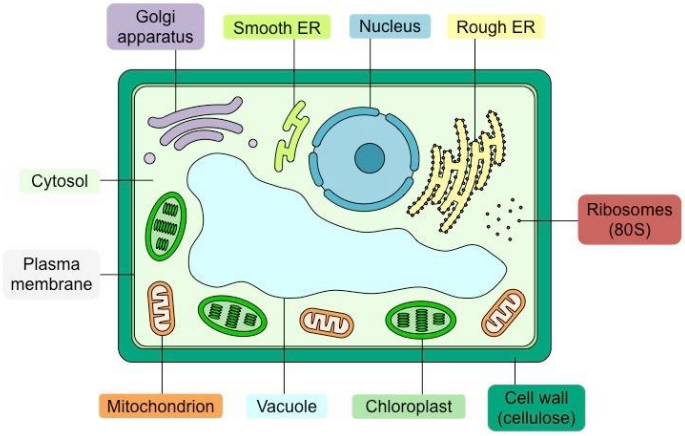
Differences between prokaryotic and eukaryotic cells:

Plant cells	**Animal cells**
the cell exterior includes an outer cell wall with a plasma membrane inside	The exterior of the cell only includes a cell membrane -> no cell wall
Chloroplasts are present in the cytoplasm's	No chloroplasts
Have a large, central vacuole	Have small, temporary vacuoles (if any)
Carbohydrates are stored as starch	Carbohydrates are stored as glycogen
Do not contain centrioles within a centrosome area	Contain centrioles within a centrosome area
Because a rigid cell wall is present, the cells have a fixed, often angular shape	Cells are flexible and more likely to be rounded

Similarities:

- Animal and plant cells are both types of eukaryotic cells, so they share many common features:
    - DNA stores within a nucleus
    - Larger ribosomes
    - Variety of membrane bound organelles


Lysosome
Rough ER
Nucleolus
Nucleus
Golgi apparatus
Mitochondrion
Smooth ER
Cytosol
Plasma membrane
Ribosomes (80S)

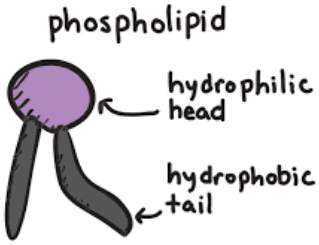

Golgi apparatus
Smooth ER
Nucleus
Rough ER
Cytosol
Plasma membrane
Ribosomes (80S)
Mitochondrion
Vacuole
Chloroplast
Cell wall (cellulose)

1.3 Membrane Structure

Cell membranes:

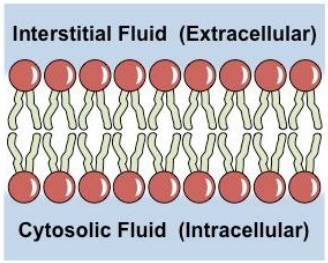
- *Function:* to keep the cell contents separate from the outside, control movement of substances in and out of the cell
- *Key structures:*
  - Phospholipids
  - Proteins
  - Glycoproteins
  - Cholesterol (animal cells only)

1. Phospholipids – structure:



- Consist of polar head -> composed of a glycerol and a phosphate molecule and is *hydrophilic* (attracted to water)
- Consist of two non-polar tails -> consist of fatty acid (hydrocarbon) chains -> *hydrophobic* (repelled by water)
  - Because phospholipids contain both hydrophilic and lipophilic (fat-loving) regions, they are classed as *amphipathic*

Arrangement in membranes:



- When phospholipids are mixed with water the phosphate heads are attracted to water but the hydrocarbon tails are attracted to each other -> are arranged in a *double layer*
  - Hydrophobic tails face inwards (shielded from fluids) while the two hydrophilic heads face the water on either side
  - These double layers are called *phospholipid bilayers* and form the bases of all cell membranes

Properties of phospholipid bilayer:

- Bilayer is held together by weak hydrophobic interactions between the tails
- Hydrophilic/hydrophobic layers restrict the passage of many substances -> selectively permeable
- Individual phospholipids can move within the bilayer, allowing for membrane fluidity and flexibility -> allows for spontaneous breaking/reforming of membranes

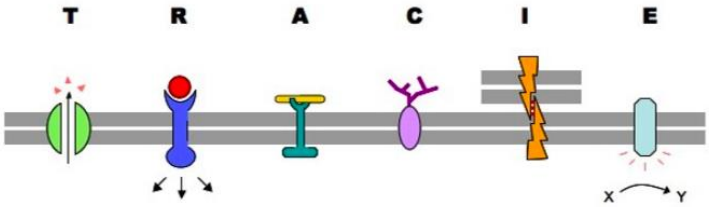
2. Membrane proteins:

- However, the plasma membrane not only forms a barrier for the cell but also carries out a whole range of other functions through the proteins in its membrane:
  - *Integral proteins* are permanently attached to the membrane (typically transmembrane – extend across membrane) -> mainly involved in transport of molecules
  - *Peripheral proteins* are temporarily attached by non-covalent interactions -> associate with one surface of the membrane - primary function is cell-to-cell communication

Functions of proteins:

- *Hormone binding sites (or hormone receptors):* hormones transported by the blood will only act on cells that have the appropriate protein receptor on the outside of cells (recognition)

- *Immobilised enzymes:* these enzymes are bound to the membrane in order to make it easier for sequences of reactions to occur -> ex. epithelial cells in the small intestine
- *Cell adhesion:* integral proteins can stick out and bind to specific protein molecules in adjacent cells (cytoskeleton) and extracellular matrix (form an anchorage)
    - Provide permanent or temporary connection -> include gap junctions and gap junctions
- *Cell-to-cell communication:* either via direct contact between proteins of adjacent cells or via signals such as neurotransmitters or hormones
- *Cell-to-cell recognition:* antigens, for example ABO blood group
- *Transport proteins:*
    - Some proteins contain channels to allow substances to passively move through the membrane
    - Or pumps for active transport of substances across the membrane by changing shape



Transport: Protein channels (facilitated) and protein pumps (active)

Receptors: Peptide-based hormones (insulin, glucagon, etc.)

Anchorage: Cytoskeleton attachments and extracellular matrix

Cell recognition: MHC proteins and antigens

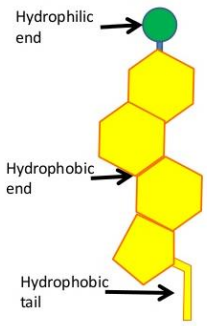
Intercellular joinings: Tight junctions and plasmodesmata

Enzymatic activity: Metabolic pathways (e.g. electron transport chain)

3. Glycoproteins:

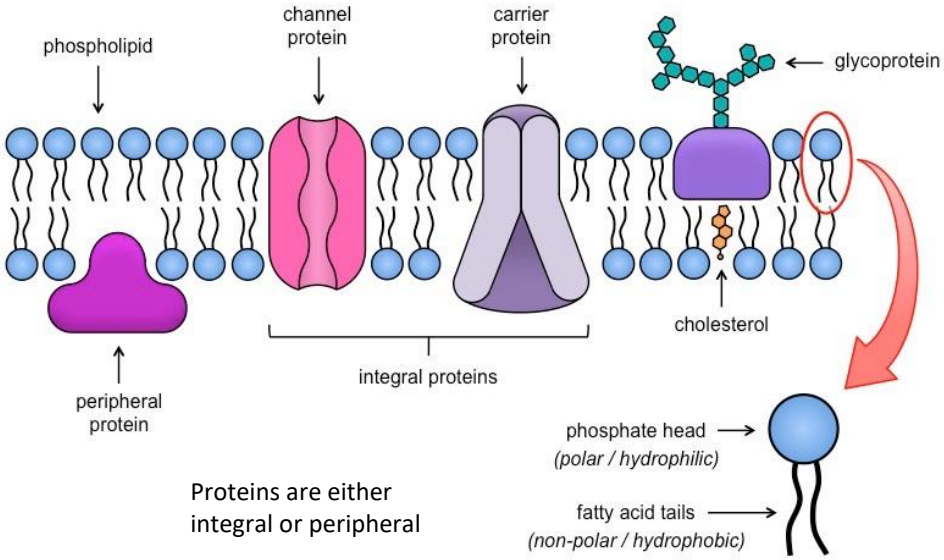
- Are proteins with an oligosaccharide (sugar) chain attached -> are important for cell recognition by immune systems and as hormone receptors

4. Cholesterol:



- Cholesterol is a component of animal cell membranes -> functions to regulate the fluidity and flexibility of the membrane, maintain mechanical stability
    - Absent in plant cells as plasma membranes are supported by cell wall
- Cholesterol is an *amphipathic* molecule (both hydrophilic and hydrophobic regions)
    - Cholesterol's hydroxyl group is hydrophilic - aligns with phosphate head of phospholipids
    - Remainder (steroid ring and tail) is hydrophobic, associates with phospholipid tails
- Cholesterol moderates the properties of the membrane:
    - It regulates membrane fluidity, allowing membranes to function at a wider range of temperatures:
        - Cholesterol's presence restricts movement of phospholipid tails, reducing flexibility (and increasing stability)
        - also disrupts regular packing of hydrocarbon tails -> increases flexibility by preventing crystallization/solification
    - It reduces permeability to some water-soluble molecules (sodium, hydrogen)

Fluid mosaic model:



Cholesterol improves stability and reduces fluidity

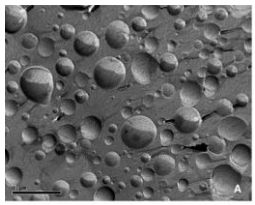
Proteins are either integral or peripheral

Form a bilayer (phosphate heads face outwards, fatty acid tails inwards)

Previous membrane models:

Davson- Danielli Model or sandwich model (1935)

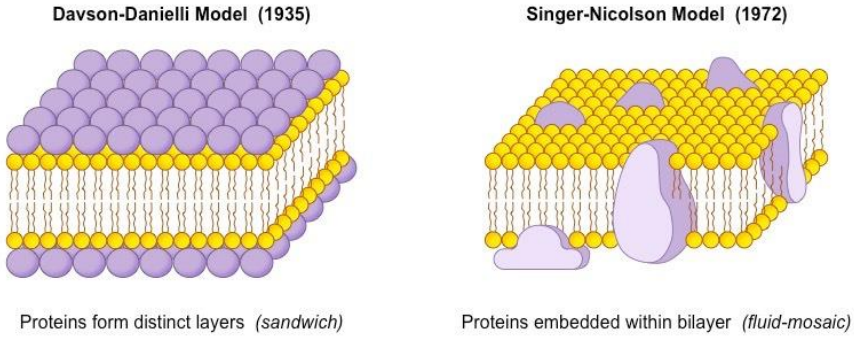
- Proposed a model whereby a central phospholipid bilayer was covered on both sides b a thin layer of globular protein -> called the lipo-protein sandwich
    - Dark segments seen under electron microscope were identified (wrongly) as two protein layers
- *Problems with the Sandwich model:*
    - Not all membranes are identical (uniform thickness, constant lipid-protein ratio etc.) or have symmetrical inner and outer surfaces (are bifacial)
    - Membranes with different functions also have a different composition and different structure -> not possible if all had the same structure
    - A protein layer is not likely because it is largely non-polar and would not interface with water
- *Falsification evidence*
    - *Structure of membrane proteins:* improvements in biochemical techniques showed that proteins were varied in size and insoluble in water
        - Such proteins would not be able to form a uniform and continuous layer around surface of a membrane
        - Proteins had hydrophobic regions would therefore embed in the membrane not the outside



    - *Freeze fracturing* was used to split open the membrane and remove the outer phospholipid layer
        - Micrographs revealed rough areas within the membrane -> these were interpreted as being *transmembrane* proteins
    - *Fluorescent antibody tagging:* Membrane proteins from two different cells were tagged with red and green fluorescent markers -> when the two cells fused the markers became mixed through the membrane of the fused cell
        - This demonstrated that proteins were *mobile* and not fixed in a peripheral, static layer (as per Davson-Danielli)

Singer Nicholson model or Fluid mosaic model (1972)

- In light of these limitations a new model was proposed in 1972
- According to this model, proteins are embedded within the lipid bilayer rather than existing as separate layers
    - Proteins form a mosaic in a fluid layer of phospholipids so they are also free to move and cross membranes from one side the other



Fluid – the phospholipids and proteins can move so the membrane is fluid and flexible

Mosaic – globular proteins are arranged randomly in the phospholipid bilayer -like a mosaic

1.4 Membrane Transport

Types of transport:

- Cellular membranes are *semi-permeable* (only certain materials may freely cross) and *selective* (membrane proteins regulate passage of materials)
    - Phospholipid bilayer is permeable to: water, CO_2, Oxygen, Fatty acids, urea
    - Phospholipid bilayer is *not* permeable to: Amino acids and glucose (too large) hydrogen ions, chloride ions (as they are charged)

Cellular transport occurs through:

- *Passive transport:* involves the movement of material along a concentration gradient (high -> low concentration) and does not require expenditure of energy
    - There are three main types:
        - *Simple diffusion* – movement of small/liphilic molecules (O_2, CO_2)
        - *Osmosis* – movement of water molecules
        - *Facilitated diffusion* – movement of large or charged molecules via membrane proteins (ions, sucrose)

- *Active transport:* involves the movement of materials *against* a concentration gradient (requires energy) and includes:
    - *Primary (direct) active transport* – involves direct use of metabolic energy
    - *Secondary (indirect) active transport* – involves coupling the molecule with another moving along an electrochemical gradient

1. Simple diffusion

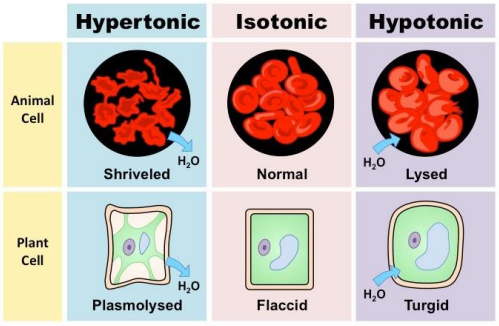
- *Diffusion:* the passive movement of particles from a region of higher concentration to a region of lower concentration as a result of the random motion of particles
- The rate of diffusion is influenced by: temperature (affects kinetic energy), molecular size (larger size = more resistance) and steepness of gradient

2. Osmosis:

- *Osmosis* is the passive movement of water molecules across a semi permeable membrane from a region of lower solute concentration to a region of higher solute concentration (until equilibrium is reached)
    - solutes cannot cross a cell membrane unaided, so water will move to equalize the two solutions -> at higher solute concentrations there are less free water molecules -> so osmosis is the diffusion of *free water molecules*
- *Osmolarity:* measure of solute concentration in a solution -> number of osmoles of a solute per litlre (pure water = osmolarity of zero)
    - Solutions with a higher osmolarity are *hypertonic* (high solute concentration)
    - Solutions with a lower osmolartiy are *hypotonic* (low solute concentration)
    - Solutions that have the same osmolarity are *isotonic*

Avoiding osmosis in donor organs:

- In human tissues or organs osmosis can cause cells to swell up and burst (lysis) or shrink/shrivel (crenation)
- Therefore tissues or organs must be kept in *isotonic* solutions to prevent osmosis from occurring
- In plant cells the effects of uncontrolled osmosis are reduced by the cell wall



Estimating osmolarity – LAB

-> osmolarity of a tissue is found by bathing the sample in solutions with known osmolarities

Method:

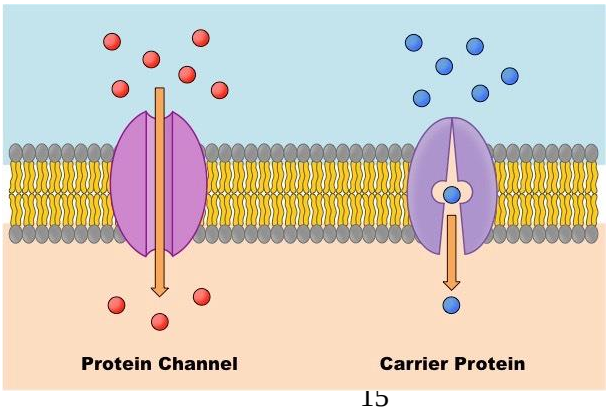
1. prepare a series of solutions with a suitable range of solute concentrations -> 0.0, 0.1, 0.3, 0.5 moles/litre
2. cut the tissue into samples equal size and shape (ex. potato cores)
3. find the mass of each sample, then bathe tissue samples in range of solutions (10 - 60 minutes)
4. remove tissue samples and dry them, then find their mass again
5. % change= (final mass – initial mass)/initial mass x 100
6. to find tissue osmolarity identify concentration of solution at which there is no weight change (isotonic)

3. Facilitated diffusion

- Some substances are unable to freely cross the phospholipid bilayer (large, polar molecules and ions) using simple diffusion
    - Therefore membrane proteins are needed to recognize a particular molecule and help it across the membrane -> this is called *facilitated diffusion*
    - This process is mediated by channel and carrier proteins

Channel proteins:

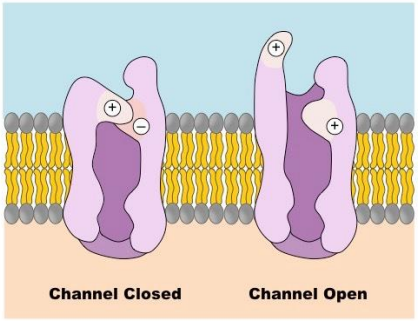
- Are lipoproteins which contain a passage for ions to cross
- Channel proteins are *specific* -> only allow one type of substance to pass through -> chloride channels only allow Cl ions to pass through
    - Different cell types can very the number and types of channel proteins depending on their function



- Channel proteins can only move molecules along the concentration gradient (not used in active transport) but have a much *faster rate of transport* than carrier proteins

Potassium Channels – voltage gated channels:

- Voltage gated channels control the flow of ions in neurons
- *Stage 1:* the channel is closed
    - The paddle region of the channel proteins is positively charged and is attracted to the negative interior of the axon -> closing the channel
- *Stage 2:* the channel is open
    - There is a change in the voltage across the membrane -> becomes depolarized
    - Paddle region of channel proteins is now repelled by positive charges inside axon -> attracted to negative charge outside the axon -> channel opens, allowing K^+ ions to diffuse out

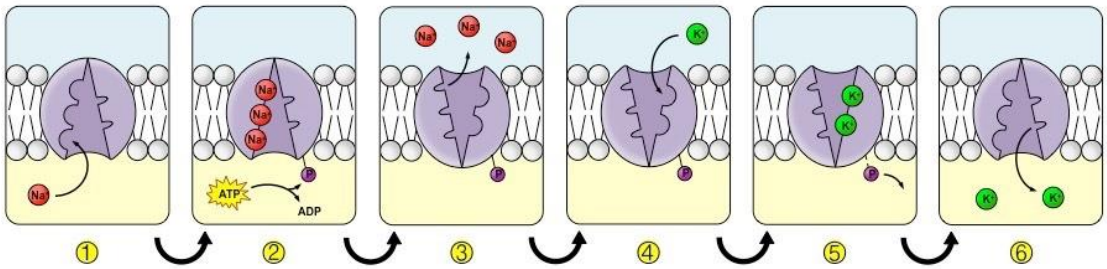


Active transport:

- Active transport uses *energy* to move molecules against a concentration gradient and involves the use of *carrier proteins or protein pumps*
- *Carrier proteins:* are integral glycoproteins which bind to a solute and undergo a conformational change to translocate the solute across the membrane
    - Will only bind to a specific molecule -> like enzyme substrate interaction
    - Proteins pumps only transport substances in one direction (either in or out of the cell)

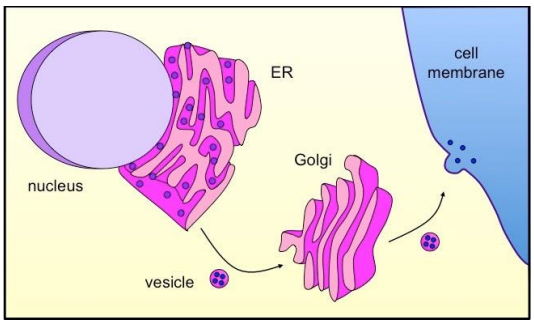
Sodium – Potassium pumps:

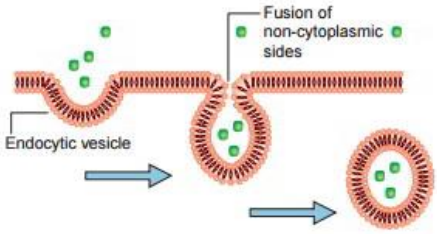
- Integral proteins exchange 3 sodium ions (move out of cell) with two potassium ions (move into cell)
- *Stage 1:* the pump is open towards the inside of the axon -> three sodium ions bind at specific binding sites
- *Stage 2:* ATP is hydrolysed to ADP and inorganic phosphate -> the energy released causes the protein to change shape
    - This means the pumps is now open towards the outside of the axon
- *Stage 3:* the three sodium ions are released
    - Two potassium ions bind at specific binding sites
- *Stage 4:* phosphate group is released which causes the pump to return to its original shape
    - This translocates the potassium ions across the membrane, completing the ion exchange



Vesicular transport:

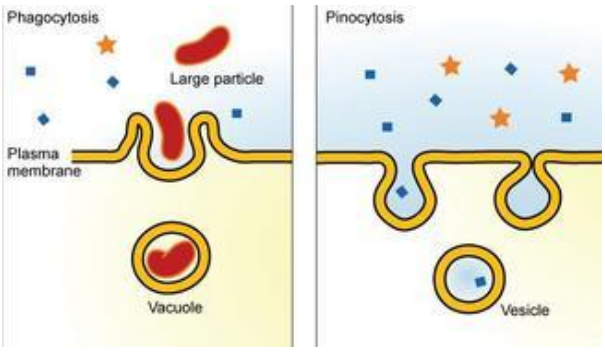
- Materials destined for secretion are transport around the cell in membranous containers called vesicles
- Proteins are secreted by the following steps:
    1. Proteins are synthesized by ribosomes on the rough endoplasmic reticulum
    2. Vesicles containing newly synthesized proteins bud off the RER and carry the proteins to the Golgi Apparatus
    3. The vesicles fuse to the internal (cis) face of the complex -> materials move via vesicles from cis face to externally oriented trans face
    4. At the Golgi Apparatus proteins are structurally modified (ex. carbohydrates are added)
    5. Vesicles bud off Golgi Apparatus and carry modified proteins to the plasma membrane, where they fuse with the membrane -> this results in the secretion of the contents of the vesicle into the extracellular fluid (exocytosis)
    6. Vesicles form at the plasma membrane, bring in material from outside the cell (endocytosis)

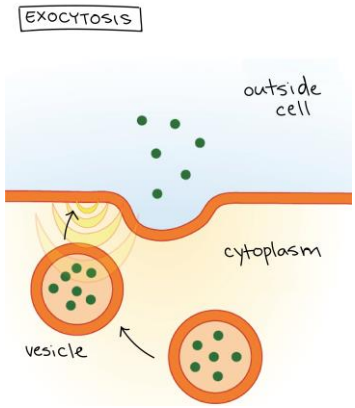




Endocytosis and Exocytosis

- *Endocytosis:* the process by which large substances (or bulk amounts of smaller substances) enter the cell without crossing the membrane
    - This occurs through an *invagination* (bulging in) of the plasma membrane -> a vesicle forms inside the cell which contains extracellular material
    - The small piece of membrane then pinches off and the two ends reattach (due to hydrophobic and hydrophilic properties of phospholipids)
    - There are two types of endocytosis:
        - Phagocytosis – process by which solid substances are ingested
        - Pinocytosis – process by which liquids/dissolved substances are ingested
- *Exocytosis:* process by which large substances exit the cell without crossing the membrane
    - A vesicle inside the cell fuses with the plasma membrane , expelling its contents into the extracellular environment
    - The membrane of the vesicle (along with proteins embedded in it) is now part of the plasma membrane (replacing phospholipids lost via endocytosis)
- The fluidity of the membrane is essential to allow fusion and subsequent secretion of the vesicle contents





1.5 The origin of cells

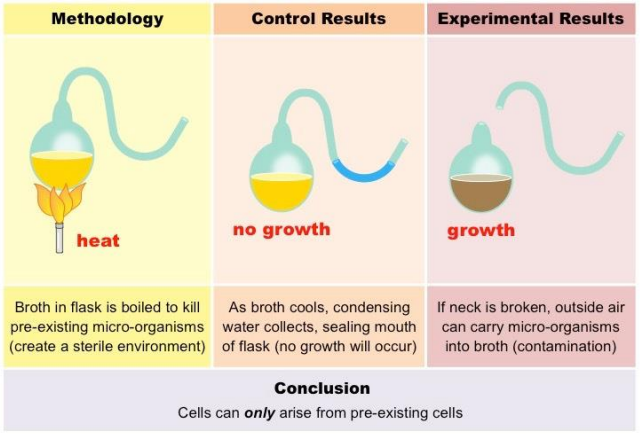
Origins of first cells:

- The cell theory states that all cells come from pre-existing cells (*biogenesis)*
  - however since before the first cells no life existed on Earth, the first living cells must have evolved from non-living matter
  - the formation of the first cells is therefore still a unanswered question today
- Until the 19th century some biologists believed in *spontaneous generation:* the formation of living organisms from non living matterä
  - However, Louis Pasteur's swan necked flask experiments demonstrated that spontaneous generation does not now occur on earth

Pasteur's Experiments:

1. Pasteur boiled a nutrient broth to kill pre-exiting micro-organisms (sterile environment).
2. The sterile nutrient broth was then placed in two swan necked flasks (did not allow dust to enter).
3. Then, Pasteur broke off the swan neck in experiment 1, exposing the contents to contaminants from the outside.

Result: the broth in experiment 1 turned cloudy, while the broth in experiment 2 remained clear -> microbe growth only occurred in experiment 1.
Conclusion: emergent bacterial growth comes from external



LUCA – last universal common ancestor

- All life on earth can be traced back through multiple generations to the first single-celled organisms
  - This is because all organisms currently living on Earth share the same genetic code -> universal
  - The genetic code has 64 different codons and their meaning (how they code for amino acids) is the same
    - The universality of the genetic code suggests strongly that all life evolved form the same original cells

The Endosymbiotic theory:

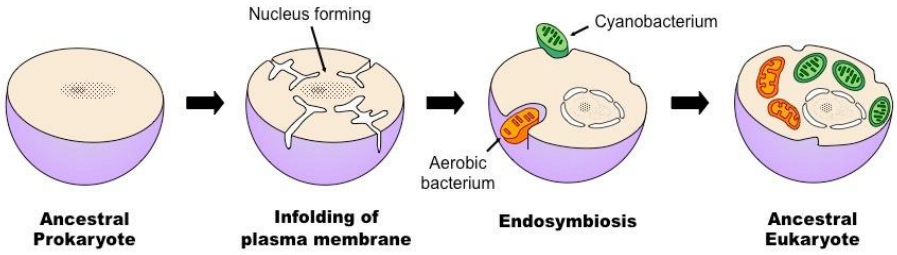
- The endosymbiotic theory explains the origin of eukaryotic cells
- An endosymbiont is a cell which lives inside another cell with mutual benefit -> eukaryotic cells are believed to have evolved from early prokaryotes (mitochondria, chloroplasts) that were engulfed by phagocytosis
  - the engulfed prokaryotic cell remained undigested as it contributed new functionality to the engulfing cell (photosynthesis, energy production)
  - over generations the engulfed cell lost some of its independent utility and became a supplemental organelle

Symbiosis is when two organisms mutually help each other to survive
Endosymbiosis is when one smaller organisms lives inside one larger organisms

Evidence for the Endosymbiotic theory:

- Mitochondria and chloroplasts are both organelles suggested to have arisen via endosymbiosis -> remember MAD DR

Component	Evidence
Membranes	some organelles have double membranes -> this is a result of being ingested by endocytosis
Antibiotics	susceptible to antibiotics -> indicates organelles may have bacterial origins
Division	reproduction occurs via binary fission like process
DNA	have their own DNA which is naked and circular (like prokaryotic DNA structure)
Ribosomes	Have ribosomes that are more similar to 70S than 80S ribosomes (like prokaryotes)



Missing: Miller-Urey experiment, LUCA, origin of first cells (spontaneous generation – 4 point summary), origins of life (volcanoes, vents etc.),

1.6 Cell division

The role of mitosis:

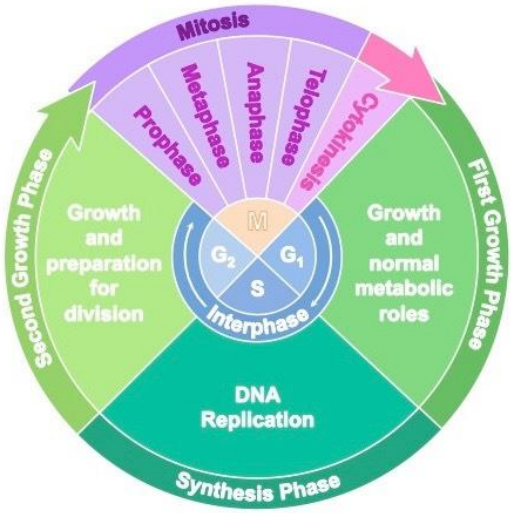
- Mitosis is the division of the nucleus into two genetically identical daughter nuclei
- Mitosis is involved whenever cells with genetically identical nuclei are required in eukaryotes:
    - during tissue repair/ replacement, organismal growth, asexual reproduction and embryonic development
- Mitosis is preceded by interphase in which the DNA is replicated and is divided into four distinct stages: prophase, metaphase, anaphase, telophase

The cell cycle:

- the cell is an ordered set of events which culminates in the division of a cell into two genetically identical daughter cells
- it can be divided into two main stages:

1. Interphase – very active phase of the cell cycle with many processes occurring in the nucleus and cytoplasm, stage in the development of a cell between two successive divisions
    - G1 – first intermediate gap stage in which the cell grows and prepares for DNA replication
    - S – synthesis stage in which the DNA is replicated
    - G2 – second intermediate gap stage in which the cell finishes growing and prepares for cell division
        - Organelles may increase in number, DNA begins to condense from chromatin to chromosomes, microtubules begin to form

2. M phase – period of the cell cycle in which the cell and contents divide to create two genetically identical cells
    - Mitosis – *Nuclear division*, whereby DNA (as condensed chromosomes) is separated into two identical nuclei
        - Occurs in four stages: prophase, metaphase, anaphase and telophase
    - Cytokinesis – *Cytoplasmic division*, whereby cellular contents are segregated and the cell splits into two



Remember that in interphase the following key processes occur:

DNA replication – DNA is copied during the S phase of interphase
Organelle duplication – Organelles must be duplicated for twin daughter cells
Cell growth – Cytoplasmic volume must increase prior to division
Transcription / translation – Key proteins and enzymes must be synthesised
Obtain nutrients – Vital cellular materials must be present before division
Respiration (cellular) – ATP production is needed to drive the division process

Mnemonic: DOCTOR

Supercoiling of chromosomes:

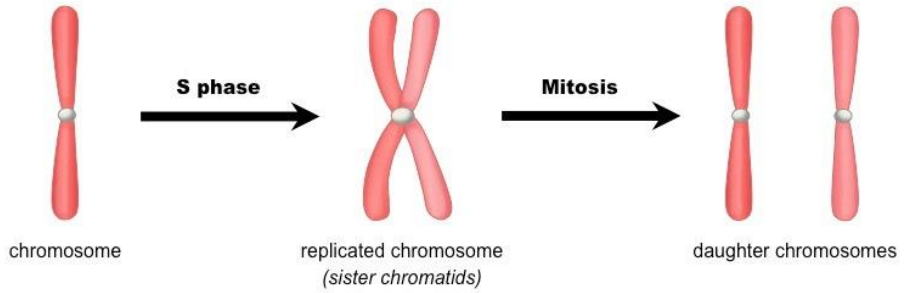
Chromosomes condense by supercoiling during mitosis

- During the second growth phase, G2, the chromatin (elongated DNA and histones) begins to condense – this condensation is accomplished via *supercoiling*
    - First DNA wraps around histones to produce nucleosomes
    - The nucleosomes are then further wrapped into a solenoid, which then group together until a final coiling occurs to produce the chromosome

Chromatin:	Chromosome:
- DNA is loosely packed within the nucleus as unravelled chromatin - in this form, the DNA is accessible to transcriptional machinery - genetic information can be translated - DNA is organised as chromatin throughout interphase in all non-dividing cells	- DNA is temporarily packaged into a tightly wound and condensed chromosome prior to division - DNA is able to be easily segregated but inaccessible to transcriptional machinery - DNA is organised as chromosomes during mitosis

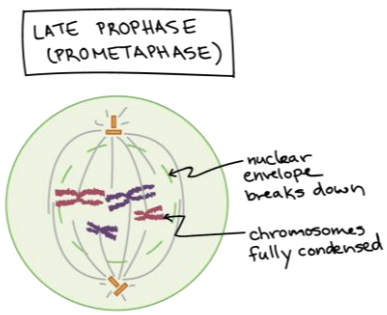
Chromosome vs chromatid

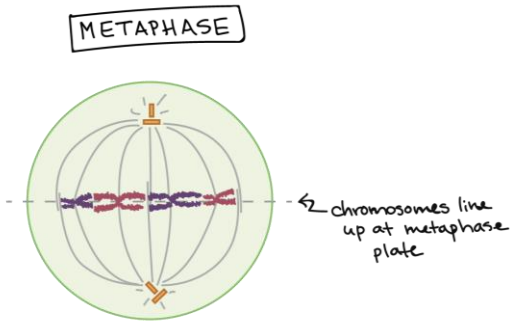
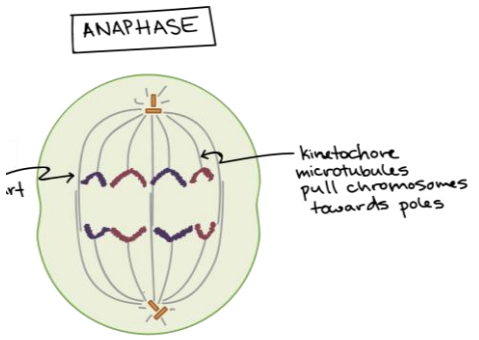
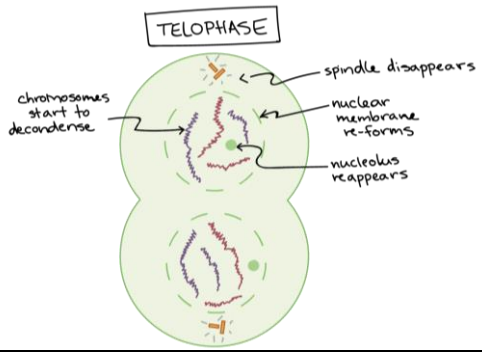
- As the DNA is replicated during the S stage of interphase, the chromosome initially contains two identical DNA strands
    - These genetically identical strands are called *sister chromatids* and are held together by a central region called the centromere
    - When they separate during mitosis, they form independent chromosomes

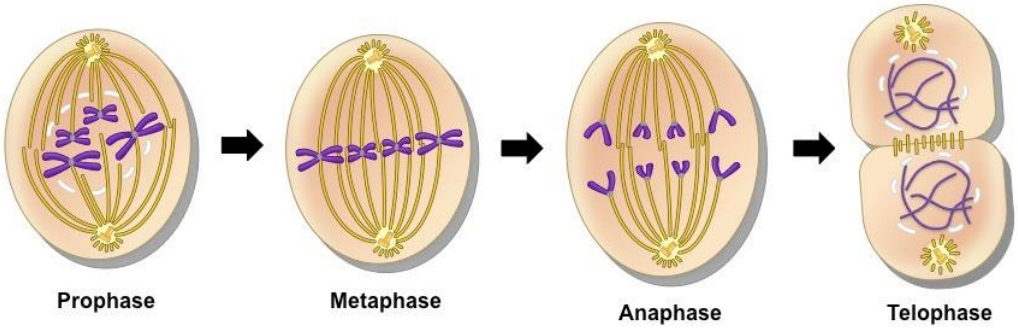


Stages of mitosis in detail

Identification of phases of mitosis viewed with a microscope or in a micrograph.

Prophase	
■ DNA supercoils and chromosomes become shorter and fatter (visible under the microscope) ■ Chromosomes are comprised of genetically identical sister chromatids ■ Paired centrosomes move to the opposite poles of the cell as a result of lengthening microtubules ■ The nuclear membrane breaks down and the nucleus dissolves	

Metaphase ■ Microtubules continue to grow and attach to the centromeres on each chromosome ■ Microtubule depolymerisation causes spindle fibres to shorten in length and contract ■ This causes chromosomes to align along the centre of the cell – equatorial plane or metaphase plate	
Anaphase ■ At the start of anaphase, each centromere divides, allowing the pairs of sister chromatids to separate ■ The spindle microtubules pull them rapidly towards the poles of the cell ■ Once the chromatids separate they are considered an individual chromosome	
Telophase ■ Once the two chromosome sets arrive at the poles, spindle fibres dissolve ■ Chromosomes decondense or uncoil ■ Nuclear membranes reform around each chromosome set ■ Cytokinesis occurs concurrently, splitting the cell into two	



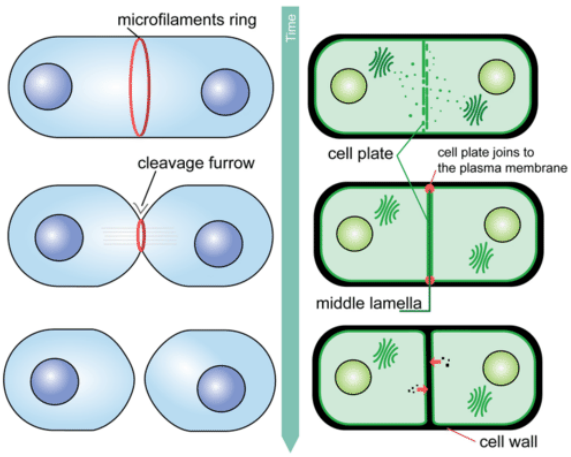
Cytokinesis

Cytokinesis occurs after mitosis and is different in plant and animals cells.

- Cytokinesis is the process of cytoplasmic division, whereby the cell splits into two identical daughter cells
- Cytokinesis occurs during telophase and is different in plant and animal cells
- *Cleavage of an animal cell:*
    - In animal cells the plasma membrane is pulled inwards around the equator of the cell to form a cleavage furrow

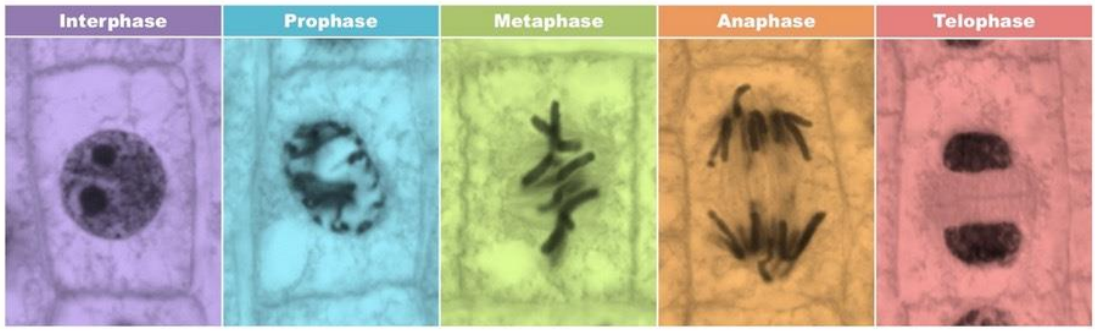
- This is accomplished using a ring of contractile protein that forms a concentric ring around the centre of the cell
- When the cleavage furrow reaches the centre, the cell is pinched apart into two daughter cells

- *Cell plate formation in a plant cell:*
  - In plant cells vesicles are moved to the equator where they fuse to form tubular structures across the equator
  - As more vesicles fuse together a cell plate begins to form, which extends outwards and fuses with the cell wall, dividing the cell into two distinct cells



The mitotic index

- The mitotic index is a measure of the proliferation status of a cell population
  - proportion of dividing cells or ratio between number of cells in mitosis and total number of cells
  - It can be used to identify abnormal activity in a tissue – high mitotic index in a human adult cell could indicate a tumour
- Mitotic index= $\frac{\text{number of cells in mitosis}}{\text{total number of cells}}$



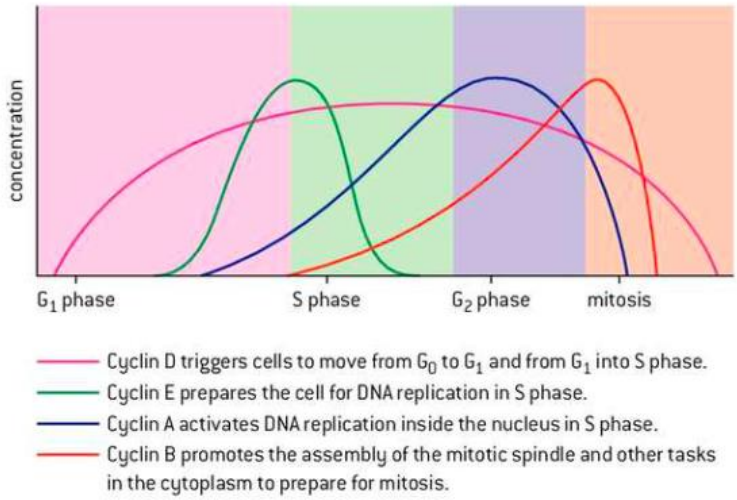
Identifying mitotic cells:

Prophase – chromosomes condensed but still confined to a nuclear region
Metaphase – chromosomes aligned along the equator of the cell
Anaphase – two distinct clusters of chromosomes apparent at poles of the cell
Telophase – two nuclear regions present within a single cell (difficult to see as cytokinesis occurs concurrently)

Cyclins and the control of the cell cycle

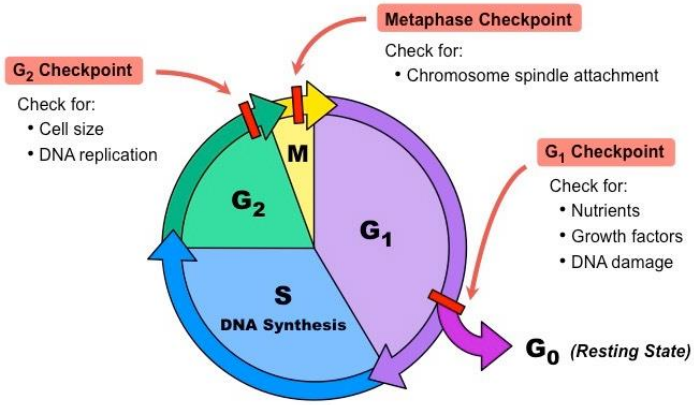
Cyclins are involved in the control of the cell cycle

- Cyclins are a family of regulatory proteins that control the progression of the cell cycle – ensure that tasks are performed at the correct time
- Cyclins bind to enzymes called cyclin-dependent kinases (CDKs) – these kinases become active and attach phosphate groups to other proteins in the cell
    - The attachment of protein triggers the other proteins to become active and carry out tasks specific to one of the phases in the cycle



Cell checkpoints

- *G1 checkpoint* - determines appropriate growth conditions (cell size, nutrients), asses level of DNA damage (from radiation of UV)
- *G2 checkpoint* - determines state of pre-mitotic cell, identifies any replication faults
- *Metaphase checkpoint* - ensures proper spindly assembly and correct attachment to centromeres



Tumour formation and cancer

Mutagens, oncogenes and metastasis are involved in the development of primary and secondary tumours.

Tumours: tumours are abnormal cell growths resulting from *uncontrolled cell division* and can occur in any tissue or organ

- *Benign tumours* remain in their original location and do not invade nearby tissues
- *Malignant tumours* spread and invade neighbouring tissues, developing into a secondary tumour
    - Diseases due to the growth of malignant tumours are known as *cancers* and have diverse causes
    - Metastasis is the movement of cells from a primary tumours to set up secondary tumours in other parts of the body

Mutagens:

⇒ a mutagen is an agent that changes the genetic material of an organism and may be physical, chemical or biological in origin:
- *Physical* – sources of radiation including X-rays, UV light and radioactive decay
- *Chemical* – DNA interacting substances including reactive oxygen species (ROS) and metals (eg. arsenic)
- *Biological* – viruses, certain bacteria and mobile genetic elements

⇒ Mutagens that lead to the formation of cancer are further classified as *carcinogens*

Oncogenes: an oncogene is a gene that has the potential to cause cancer

- *Proto-oncogenes* code for proteins that stimulate the cell cycle and promote cell growth and proliferation (dividing) – once they mutate/ increase in expression they become cancer causing oncogenes
- *Tumour suppressor genes* code for proteins that repress cell cycle progression and promote apoptosis – their normal function prevents cancer

Application: **Smoking and cancer**

- A significant body of scientific data exists which provides strong link between smoking and the incidence of cancers
  - Cigarette smoke contains over 4000 chemical compounds, 60 of which are known to be carcinogenic
- The risk of lung cancer is strongly correlated with smoking, with 90% of lung caners attributable to tobacco use
  - Smoking also increases the risk of other cancers including mouth, throat, stomach, liver, pancreas, kidneys or bladder