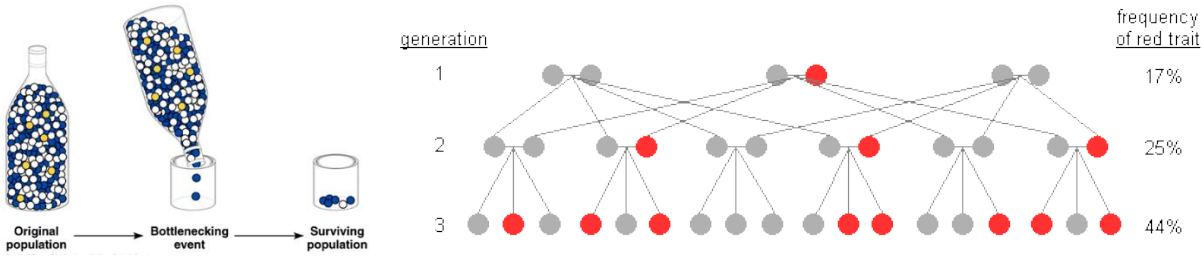
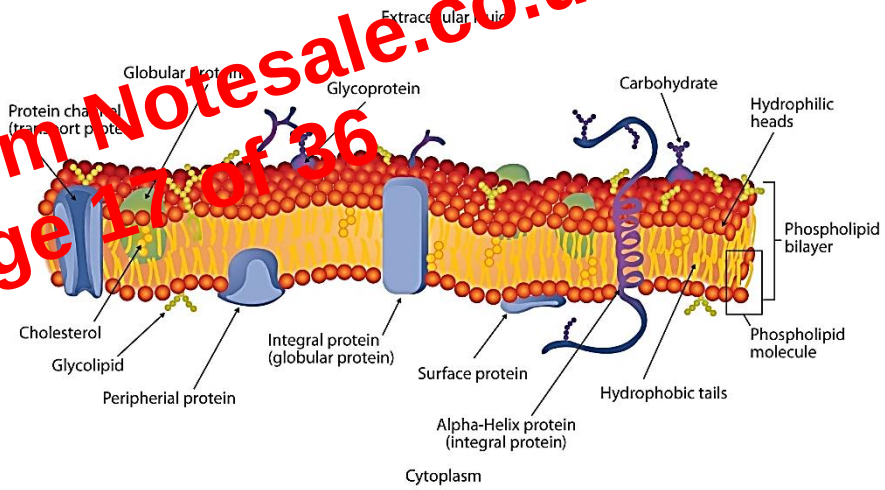


- Oncogenes

These are genes that upon expression induce cancer. Many viruses carry oncogenes however there are also non-virally induced cancers that are the result of oncogenes. Cancer cells have overexpression of oncogenes and so there is large concentrations of the proteins coded for by oncogenes, this can happen in cells without viral action, however as viruses contain genes for DNA for replication and as the viruses replicate, there are multiple copies of the oncogenes being made which increases cell proliferation.

- **<u>Biological membranes</u>**



The membranes of cells are made up of phospholipid bilayers that contain many other molecules such as proteins, lipids and cholesterol molecules. They are all free to move around in the structure and so they are known as having a fluid mosaic model.

More than 50% of the cell membrane is made up of phospholipids. These are amphipathic molecules that have both hydrophobic and hydrophilic properties, they are made of a glycerol group with a phosphate group, which has a choline group attached to it, that bonds to the 3rd carbon of glycerol. There are also 2 fatty acids attached to the 1st and 2nd carbons of the glycerol molecule. Due to the amphipathic nature of the phospholipids the lipophilic tail sits on the inside of the layer and the lipophobic group sits on the outside, thus forming the phospholipid bilayer, or micelles. The arrangement of the phospholipids into these micelles or phospholipid bilayers is due to them being more energetically favourable. The fatty acids may be straight or have a kink in them due to a double bond in the fatty acid.

There are 4 major phospholipids in mammalian plasma membranes:

- Phosphatidylethanolamine
- Phosphatidylserine
- Phosphatidylcholine

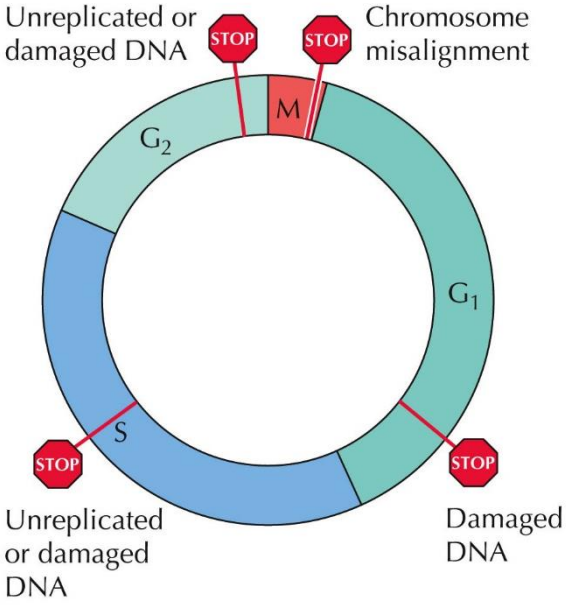
} Phosphoglycerides which are derived from glycerol, only vary in the group attached to the phosphate

- Sphingomyelin ---------- Sphingolipid, derived from sphingosine

- TGF-β receptors
- Integrins
- Monitors of cell metabolism

In order to intervene in cancer there are several antibodies that intervene in the tyrosine kinase receptor action, such as kinase inhibitors and antibodies that can attack the receptors and ligands.

There are also several checkpoints within the cell cycle:

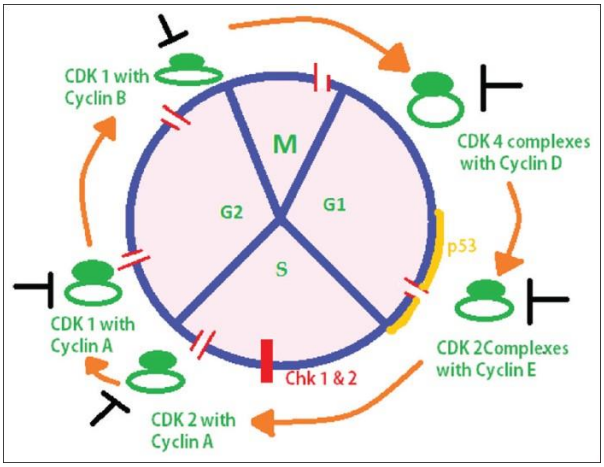


The first check is at the end of the first growth phase, where DNA is checked for any damage before it is replicated and if there is any fault it will not enter S phase. There is a similar check part way through S phase and all replication will cease if a mutation is found. If some of the DNA has not been completed and not been picked up in S phase then the check in G2 checks for unfinished replicated DNA and stops mitosis. The final check point is before anaphase and if the chromatids are not properly arranged on the spindle fibres.

A loss of these check points is very detrimental to the cell. If G_1 is lost then inappropriate proliferation occurs and the others result in a loss of the integrity of the genome and an increased rate of mutation. A loss of the S check point means that the cell has the wrong amount of DNA.

Mutations occur in different places along the DNA strand and thus have different severities on the genome. Those on the introns have no effect on the phenotype but increases polymorphic diversity. Mutations within the coding region or exon are more easily detectible by the cell and are lethal mutations that can kill the individual if expressed.

In order to control the cell cycle, cyclin dependent kinases and cyclins work together. The cyclins control cyclin dependent kinases to regulate their action as CDK's are at a constant level within the cell so it is the level of cyclin within the cell that controls the rate of action.



Different cyclin proteins and CDK's are needed at each stage for the next stage in mitosis to occur.

Cyclin D's are needed to start the cell cycle and their concentration within the cell is directly altered by growth factors which are constantly informing the cell of its environment, the type of growth factor is dependent on the location of the cell: smooth muscle cells and fibroblasts have receptors from platelet derived growth factor whilst epithelial cells only have receptors for epidermal growth factor. This cyclin is formed in the cytoplasm so must migrate to the nucleus to have its effect. Following cyclin D activating the cell cycle the cycle is autonomous and no longer relies on external factors, instead they detect internal stimuli such as mutations of the DNA.

Cyclin levels are also regulated by CDK inhibitors. **$p27^{kip1}$** is produced in the resting phase of the cell cycle and inhibits CDK1 and CDK2 by binding to the cyclins, however when growth factors reach the cell they stimulate **P13K** which is a transporter which moves $p27^{kip1}$ out of the nucleus and into the cytoplasm which prevents it from inhibiting the action of CDK1 and CDK2.

INK4 proteins inhibit CDK4 action by binding to cyclin D preventing it forming a complex with CDK4. **TGF-β** is capable of increasing the level of INK4 and thus further inhibiting the action of cyclin D and CDK4, thus preventing G1 to occur and keeping the cells in G_0.

$p21^{cip1}$ also inhibits CDK1 and CDK2 action, it plays a key role in detecting DNA damage and will not let the cell cycle progress if it detects damage.

Rb is a tumour suppressor gene that becomes more and more phosphorylated as the cell cycle continues, with it being unphosphorylated in G0, weakly phosphorylated in G1 and Hyperphosphorylated after passing the R point then dephosphorylated at the end of mitosis. As Rb becomes phosphorylated it changes conformation and releases E2F transcription factors from its structure allowing transcription to occur. A lack of this gene results in retinoblastoma. Many cancers also cause the hyperphosphorylation of the Rb protein, releasing the growth factors and leading to tumour development.

P53 monitors the integrity of the genome within the cell and controls apoptosis of the faulty cells. In addition to information from the genome P53 also gets information about any metabolic disorders within the cell. If any abnormalities are detected then it increases $p21^{cip1}$ action, stopping CDK1 and CDK2 action, thus preventing the cell cycle from occurring, allowing repair to occur to the genome. If the damage is beyond repair then P53 activates apoptosis. If there is a bi mutation in the P53 gene then it no longer works and allows cells to divide with a mutation in them, this makes mutations in the P53 gene common in many cancers. The main triggers for an increase in P53 levels are breakages in double stranded DNA (often caused by γ-rays), stalled replication forks as a result of UV light, hypoxia and dysregulated growth factor signalling. Also if the cell loses adhesion to the extracellular matrix then it also goes through apoptosis.

The level of each cyclin increases up until the point where it is used in the cell cycle and then it drops to ensure that mitosis only occurs once, and when required.

- Apoptosis the controlled death of a cell in contrast to necrosis which is uncontrolled bursting which damages surrounding cells by releasing lysosomal hydrolases.

Caspases are cysteine aspartate specific proteases and cleave all proteins within the cell at the cysteine aspartate peptide bond. No one cleavage has been proven to be more important than another but a typical one is the cleavage of iCAD (a Dnase enzyme) which activates it and cleaves DNA at exposed nucleosomes which results in the DNA ladder effect in apoptotic cells.

As well as the P53 protein signalling $p21^{cip1}$ to increase action and stop the cell cycle there are also extracellular signals that can stimulate apoptosis. Tumour necrosis factors bind to trimer receptors on the surface of the cell, such receptors are TNF1, Fas and TRAIL. When the ligand binds to the trimer receptors, many of the complexes cluster together, FADD and other adaptor proteins bind their death domain to the death domain of the receptors on the cell surface, the adaptor proteins are then able to bind to the Caspases and activate them.

Intrinsic apoptosis is regulated within the cell and is activated when the cell identifies a fault, upon identifying the fault the cell increases the membranes permeability through involved mitochondrial outer membrane permeabilization (MOMP). With the increase in permeability cytochrome C is able

Pemphigus this is an acquired auto immune disease that results in a blistering of the skin thanks to a defect in cell-cell adhesion, there are 2 types:

- Pemphigus foliaceus is a fault in the interaction between DSG1 proteins causing problems in the adhesion of the epidermal cells in the upper layers. This means that the blistering is contained to the upper epidermis and so there is only minor damage to the epidermis as the mucous membrane is unaffected.
- Pemphigus vulgaris is a fault in the interaction between DSG3 proteins which results in blistering in the lower levels where there is a high concentration of the DSG3 proteins, leading to a severely compromised epidermis which can affect the mucous membranes and almost always leads to death by infection or water loss if left untreated.

Epidermolysis bullosa is an inherited blistering disorder caused by defective adhesion of the cells to the extracellular matrix, it is split into 3 categories:

- Epidermolysis bullosa simplex: fairly mild, blistering caused by abrasion on hands and feet
- Junctional epidermolysis bullosa: severe blistering causing children to be born with large areas of their body completely devoid of skin, very poor prognosis and mortality rate is 87% in the first year, those who survive suffer from enamel hypoplasia
- Dystrophic epidermolysis bullosa: repeated blistering and scarring of the body causing potential fusion of the digits as well as microstomia which limits oral access and in conjunction with sufferers being unable to brush due to the blistering within the mouth, rampant dental caries ensues. Those that survive childhood are at an increased risk of squamous cell carcinoma.

- Fluid compartments

The body is roughly 42L of water depending on body fat, weight gender and age, with it being split between intracellular fluid (67%), interstitial fluid (25%) and plasma (8%), with water moving freely between all of these compartments. Water is important as it is the solvent for all solutes of the body but we must regulate the total body water in order to prevent damage to our cells and to maintain osmotic and chemical gradients for physiological function and the transport of nutrients and ions for cell communication. Without the gradient substances cannot be transported between compartments by diffusion.

Water is able to move into and out of cells through Aquaporins*:* these are channels within the membrane of cells which allow the passive transport of water without allowing ions to pass through, even hydrated ones as they are too large. Each aqua porin is made up of 4 monomers which are all individual channels and are all 6 pass membrane spanning proteins.

The endothelial cells of the blood vessels allow the flow of water and ions but not proteins to leave and enter the plasma.

All the compartments have different compositions with the intracellular fluid having high protein and potassium content in contrast to the interstitial fluid which only has a high sodium and low K and no protein and the plasma is different still with high sodium and protein but low K. despite this they all have the same osmolarity so are osmotically stable.

Osmosis – the diffusion of water molecules from an area of high water concentration to a low concentration across a semipermeable gradient.
Osmotic pressure – the applied pressure that prevents the net flow of water, the higher difference in the solute concentration, the higher the osmotic pressure

- Hypokalaemia: low concentration of potassium extracellularly and so there is a larger efflux of potassium ions than normal, creating a more negative membrane potential which means that the cells are less excitable
- Hyperkalaemia: high extracellular concentration of potassium so K^+ ions diffuse into the cell more readily and results in a less negative membrane potential, increasing the cells excitability.

- Drugs

The active compounds of drugs are isolated to:

- Avoid harmful effects of the original substance
- It also helps to standardise dosing as the amount of active ingredient changes in unisolated compounds
- The medicines produced are also better
- It also allows industrial scale synthesis

Drugs have 3 names:

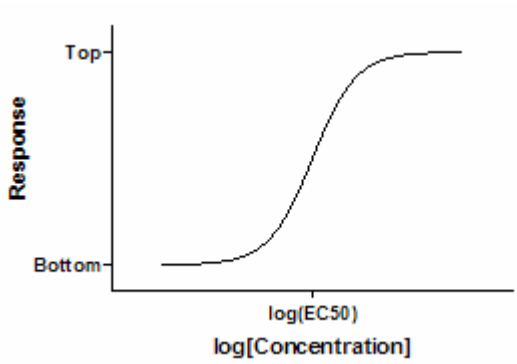
1. Chemical name (IUPAC)
2. Generic name that is internationally agreed
3. Brand name, specific to a company's method of processing or the composition

The rate at which drugs bind to receptors is proportional to the concentration of the drug. However the rate at which the drug detaches is unique to the chemical.

Percentage of receptors occupied by a drug = $\frac{\text{Concentration of the Drug}}{\text{Conc. of Drug + ratio of K-/K+}}$ K=rate constant

By knowing what percentage of receptors drugs are bound to at what concentration helps us know the correct concentration to administer the drug at. EC_{50} is the molar concentration of agonist that causes 50% of the maximum response.

p EC_{50} = $-\log_{10}(EC_{50})$ drugs with larger p EC_{50} are more potent.



If an antagonist is added to the mixture then the curve would shift to the right as more receptors are being inhibited so more agonist is needed for the same response. However if the antagonist is irreversible then the presence of the antagonist decreases the maximal response.

Partial agonists bind to the receptor and activate them but only have partial efficacy meaning that they can act as a competitive antagonist.

Efficacy: the degree to which an agonist produces a response when binding to a given proportion of receptors. A full agonist has an efficacy of 1.
Affinity: the probability of the drug occupying a receptor at a given time
Selectivity: degree to which a drug acts on a given site relative to other sites
Potency: the measure of drug activity expressed in terms of the amount required to produce an effect of given intensity, so they create a greater response at lower concentrations.

Tolerance: if a patient is treated repeatedly with the same drug they become tolerant to it and require a greater dosage to have the same effect, due to decreased number of receptors, decreased binding affinity or a modulation of the downstream response to the initial signal.

There are a variety of different receptors that drugs can bind to:

- Receptors within the cytosol that move into the nucleus, such as those that growth factors bind to.
- Receptors on channels which when bound the permeability changes and substances are allowed into the cell
- G-protein coupled receptors that upon binding activate or inhibit a second messenger system, such as with the adaptor proteins that transfer the signal from tumour necrosis factor to caspases
- Enzyme linked receptors, agonist binds to enzyme which stimulates an action, usually phosphorylation.

When prescribing drugs, we need to achieve maximum effectiveness at minimum risk to the patient whilst ensuring no wastage of resources and ensuring patient's wishes are respected at all times.

Drugs have both specific and non-specific actions:

- Non-specific action
    - Effects on the body are a result of the drugs physicochemical properties and their effects do not require receptor binding e.g. antacids, bulk laxatives, osmotic laxatives, GA – tend to need high concentrations
- Specific actions
    - Chemically sensitive proteins react to the drug
    - The drugs aim to restore normal function in diseased cells
    - Receptors that already exist on the cell react to the drug binding,
    - Can be used in lower concentrations
    - Chemical composition is important in ensuring that chemical bonds can form between the drug and receptor and to be sure there is no electrochemical repulsion

Routes of administration of drugs:

- Topical – administered directly to where it is needed, local effect and slow method
- Enteral – desired effect is systemic as the drug is administered by the digestive tract and carried to the site, slow method of drug administration
- Parenteral – systemic effect as drug is administered not to the direct site and not through the digestive tract, this is a fast method and IV IM AC and IP all deliver the drug fast
- Percutaneous – 'by way of the skin' such as inhalation, sublingual

Despite the different routes of administration all drugs have to cross a membrane to reach their point of action. This can be by passive diffusion, carrier mediated transport or vesicular transport. The level at which a drug is ionised at a certain pH determines the solubility of the drug to pass through the membrane.