Cell Biology and Genetics - Notes on all Lectures

Cell Biology and Genetics (University of Technology Sydney)

Cell Biology and Genetics

Lecture 1 – Introduction to Cells

- Cell Theory
  - Discovered by Robert Hooke ~ coined the term 'cell'.
  - Arises only from pre-existing cells.
  - Cells are an organisms basic unit of structure and function.
  - The cell is the lowest level of organisation that can perform all activities required for life.
    - Single cell (bacteria, protozoa)
    - Multi-cellular (animals, plants)
  - All enclosed by a membrane.
  - DNA – Genetic information.
  - Division of cells ~ basis of reproduction, growth and repair.
  - Cell size is limited ~ as size increases, it takes longer for material to diffuse from the cell membrane to the interior of the cell.
  - SA:V Ratio ~ Cell increases in size, volume increases 10x faster than SA.
- Certain structures in common
  1) Genetic material
     - Information storage ~ DNA
     - Duplicator of information ~ DNA replication enzymes
     - Information translator ~ ribosome
  2) Cytoplasm – Semifluid matrix.
  3) Plasma membrane – phospholipid bilayer
- Grouping Species

Taxonomy – classifies species into groups of increasing breadth.

- Domain Bacteria
- Domain Archaea
- Domain Eukarya

- Types of Cells

PROKARYOTES

EUKARYOTES

- Prokaryotes

These cells thrive almost everywhere, e.g. too acidic, salty, cold or hot.
Mostly microscopic – More in a handful of fertile soil than the number of people who have ever lived.

Structural and functional adaptations contribute to prokaryotic success:

- Unicellular.
- Variety of shapes:
  - Spheres (cocci)
  - Rods (bacilli)

    - Spirals (spirillium)
    - Curved (vibrio)
- Two types of prokaryotes
    1) Bacteria
    2) Archaea

Cell surface structures:

- Cell wall maintains shape, provides physical protection, and prevents the cell from bursting in a hypotonic environment.
- Made of cellulose (chitin).
- Bacterial cell walls contain peptidoglycan (sugar polymers cross-linked by polypeptides).
- Archaea contain polysaccharides and protein but lack peptidoglycan.
- Gram stain – used to classify bacterial species into gram-positive and gram negative groups based on cell wall composition.
    - Gram negative – Less peptidoglycan, outer membrane can be toxic, antibiotic resistant.
    - Gram positive –
- Some prokaryotes have fimbriae – allows them to stick to substrate or other individuals.
- Sex pili are longer than fimbriae and allow DNA exchange.

Reproduction and Adaptation:

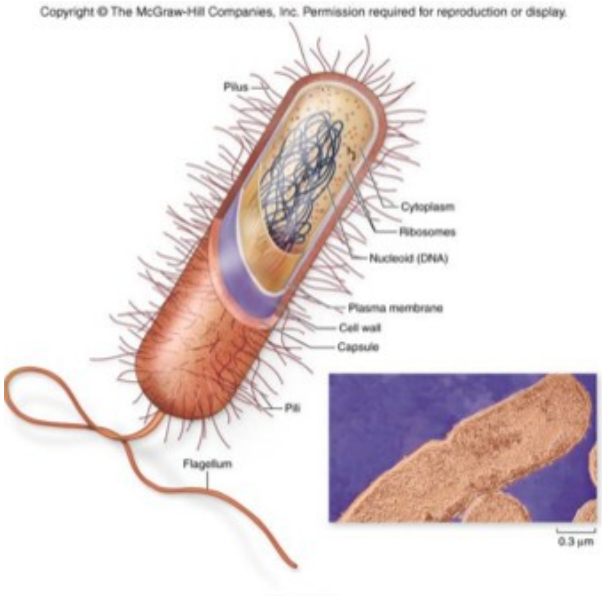
- Reproduce through Binary fission.
- Form endospores, which can remain viable in harsh conditions.
- They do have sex.

Motility (movement):

- Propel themselves by flagella.
- Heterogeneous environment = exhibit taxis, move toward/away from stimuli.

Internal and genomic Organisation:

- Lack complex compartmentalisation.
- Some have specialised membranes that perform metabolic functions.
- Genome has less DNA than eukaryote genome.
- Genome consists mainly of circular chromosome.
- Genome = 1000-4000 genes.

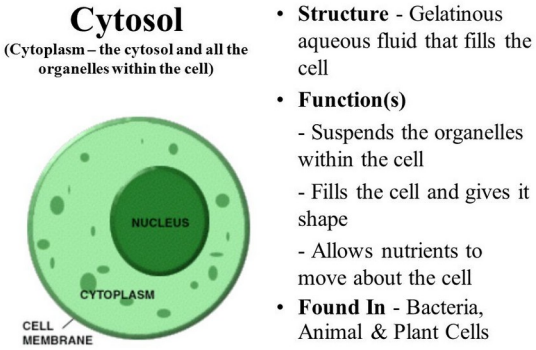


- Eukaryotes

Possess a membrane bound nucleus.
Compartmentalize many cellular functions within organelles and the endomembrane system.
Possess a cytoskeleton for support and to maintain cellular structure.

Cytosol:

- Part of the cytoplasm that is not held by any of the organelles in the cell.
- Functions:
  - ➢ Location of specific chemical reactions.
  - ➢ Storage of fat, carbohydrates as inclusions.
  - ➢ Storage of secretory vesicles.
- Cytoplasm: Cytosol and organelles.

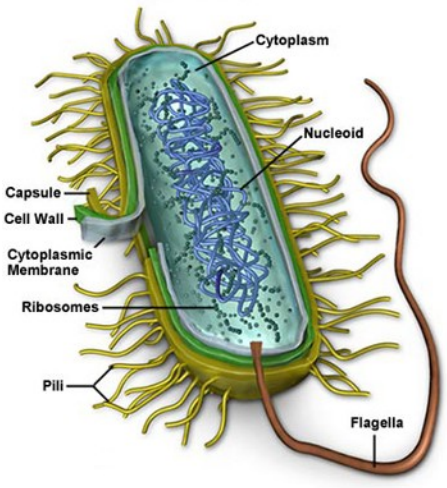


Plasma Membrane:

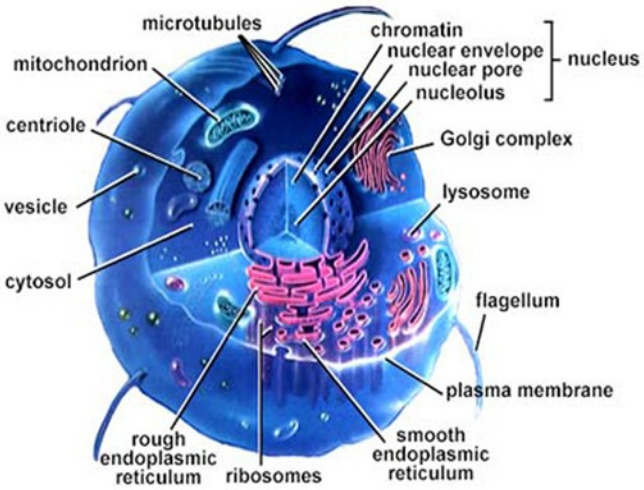
- Surrounds cytoplasm, acts as a physical barrier.
- Site of attachment of cytoskeleton and membrane associated structures.
- Regulates movement of material in/out of cell, thus whole internal environment.
- Such materials include oxygen, waste, nutrients.
- Double layer of phospholipids = structure.

Nucleus of eukaryotes:

- Contains most of the genes.
- Nuclear envelope ~ seperates from cytoplasm.
- Nuclear membrane ~ Double membrane (each consists of lipid bilayer).
- Pores regulate entry and exit of molecules.
- Shape maintained ~ nuclear lamina (composed of protein).



prokaryotic cell
(bacteria)



eukaryotic cell
(protists, fungi, animals, plants)

- DNA

Transmission and expression of genetic information.

- Chromosomes consist of DNA and associated proteins – store genetic code. Chromatin is the non-condensed form.
- DNA transcribed into RNA (mRNA) – necessary to express code.
- Each gene is the DNA code for a particular protein. When cells divide the DNA condenses into chromosomes.

- Ribosomes

Particles made of ribosomal RNA and protein (found in cytosol of cytoplasm).

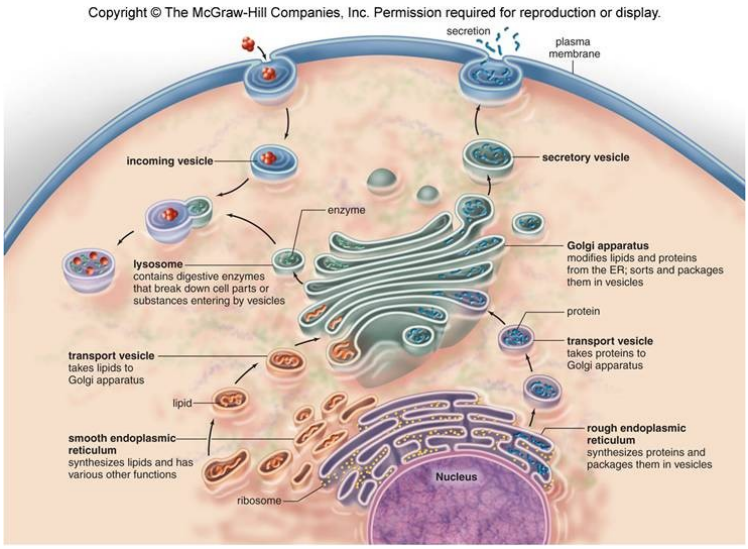
- Carries out protein synthesis in 2 locations:
  1) Cytosol (free ribosomes).
  2) Nuclear envelope or outside of endoplasmic reticulum (bound ribosomes).

- Endomembrane System

Divides cells into compartments where different cellular functions occur.
A series of membranes throughout the cytoplasm.

Components:

- Endoplasmic reticulum
- Golgi Apparatus
- Lysosomes
- Nuclear envelope
- Vacuoles
- Plasma membrane



Endoplasmic Reticulum

- Continuous with the nuclear envelope.
- Two regions:
  1) Smooth ER: Synthesis of membrane lipids, calcium storage, detoxification of foreign substances.
  2) Rough ER: Membranes, ribosomes attached to membrane. Synthesis of proteins (glycoproteins) and distributed in transport vesicles.

Golgi Apparatus

- Flattened stacks of interconnected membranes.
- Synthesis of cell wall components.
- Functions:
  - Modification of ER products.
  - Manufacture of certain macromolecules.
  - Packaging materials into transport vesicles.

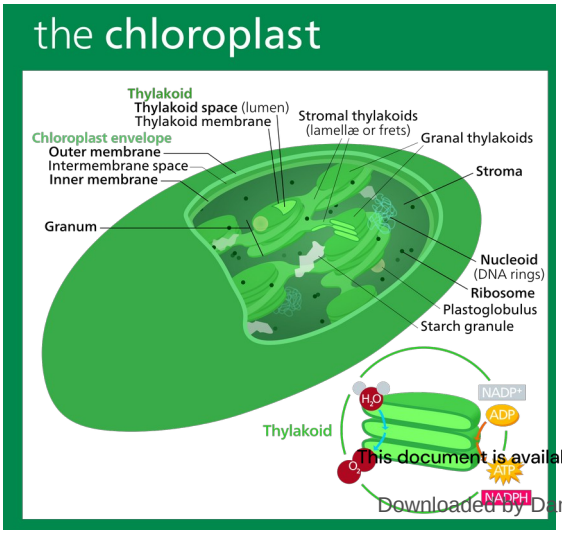
Lysosomes

- Membrane-bound vesicles containing digestive (hydrolytic) enzymes to break down macromolecules (proteins, fats, polysaccharides and nucleic acids).
- Destroy cells or foreign matter that the cell has engulfed by phagocytosis.

Vacuoles

- **Central vacuoles** hold organic compounds and water.
- **Contractile vacuoles** found in many freshwater protists, pump excess water out of cells.
- **Food vacuoles** formed by phagocytosis.

- Microbodies
  - Membrane bound vesicles.
  - Contain enzymes.
  - Not part of the endomembrane system.
  - Glyoxysomes in plants contain enzymes for converting fats to carbs.
  - Peroxisomes contain oxidative enzymes and catalase – produce hydrogen peroxide and convert it to water.
- Mitochondria
  - Contain oxidative metabolism enzymes for transferring the energy within macromolecules to ATP.
  - Surrounded by 2 membranes
    1) Smooth outer
    2) Folded inner with layers of cristae.
  - *Matrix* = within inner membrane.
  - *Intermembrane space* = Between 2 membranes.
  - Contain their own DNA.
- Chloroplasts
  - Work to convert light energy into sugars that can be used by cells. Depends on the green chlorophyll molecules in each chloroplast.
  - Contain chlorophyll for photosynthesis.
  - Surrounded by 2 membranes.
  - *Thylakoids* are membranous sacs within inner membrane of chloroplast.
  - *Grana* are stacks of thylakoids.



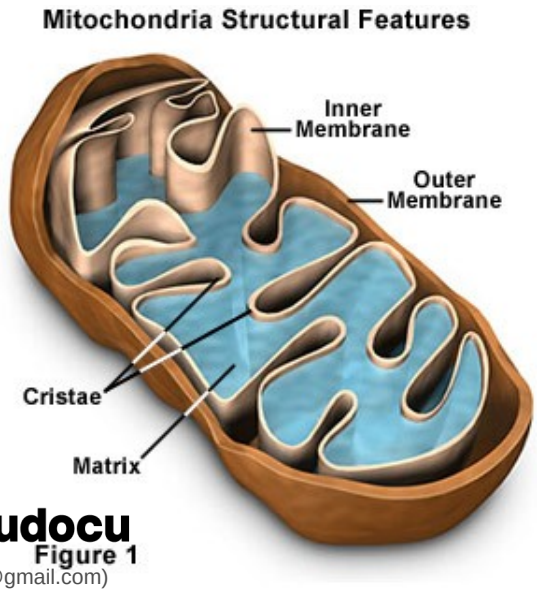


Figure 1

Mitochondria and chloroplasts

- Thought to have evolved through the process of **Endosymbiosis** ~ one cell engulfed a second cell and a symbiotic relationship developed.
- Such evidence:
  - Both have 2 membranes.
  - Possess DNA and ribosomes.
  - Size of a prokaryotic cell.
  - Divide by a process similar to bacteria.

- Cytoskeleton
  - Network of protein fibres found in all eukaryotic cells.
  - Supports shape of cell.
  - Keeps organelles in fixed locations.
  - Helps move materials within the cell.
  - Functions:
    - Mechanical support and structure.
    - Intracellular transport for materials.
    - Suspension of organelles.
    - Contraction.
    - Cell motility. (Cilia and flagella: motile extensions of the plasma membrane with a core of microtubules)

- Filaments of cytoskeleton

*Microfilaments (common type – **Actin**)*

- Muscle contraction
- Amoeboid-like movements
- Separation of cytoplasm during cell division
- Structural support for cell projections

I*ntermediate filaments*

- Vimentin (support cellular membranes and keep some organelles in fixed place within cytoplasm)
- Keratin (found in skin and hair)

Microtubules (composed of tubulin – hollow tubes of spherical protein)

- Strength for cytoskeleton
- Determine overall shape of cell and distribution of cellular organelles
- Mitotic spindle involved in chromosome distribution during cell division

- Extracellular structures

Most cells synthesise and secrete materials that are external to the plasma membrane.

Include:

- Cell walls of plants, fungi, and some protists.
- Extracellular matrix surrounding animal cells.

- Intercellular junctions.
- Bacteria have several extracellular structures.

Cell Walls

- The carbohydrates present in the cell wall vary depending on the cell type.
    - Plant/protist – cellulose
    - Fungi – Chitin
- The cell wall distinguishes plant cells from animal cells.
- Maintains shape, protects and prevents excessive uptake of water.

Extracellular Matrix

- Surrounds animal cells.
- Composition ~ glycoproteins and fibrous proteins such as collagen.
- May be connected to cytoplasm via integrin proteins in plasma membrane.

Intercellular Junctions

- Plasmodesmata are channels that perforate plant cell walls. Water and small solutes pass through this.
- Tight junctions, membranes of neighbouring cells are pressed together preventing leakage of extracellular fluid.
- Desomsomes fasten cells together into strong sheets.
- Gap junctions provide cytoplasmic channels between adjacent cells.

Lecture 2 – Intro to Chemical Compounds

- Chemical Bonds

Ionic Bonds

- Involves the transfer of an electron from one element to another.
- Not common in biology.
- One element becomes positive, one becomes negative.

Covalent Bonds

- Sharing of electrons to make a molecule.
- Common in biology.
- Carbon compounds use covalent bonding.

Polarity

- Unequal sharing of electrons.
- Slightly negative, slightly positive.
- E.g. Water = Oxygen (positive), Hydrogen (negative)
- Polarity of water is essential for life.
- Because oxygen is more electronegative than hydrogen, shared electrons are pulled more toward oxygen.

Polarity and H^+ Bonds

- Hydrogen bonds are weak.

- Slightly negative and positive attracted towards each other.
- They are weak because the molecules need to be close together for an attraction to occur.
- Repulsion – if two slightly negative ends of molecules come close together, they will repel each other.

Interaction with water

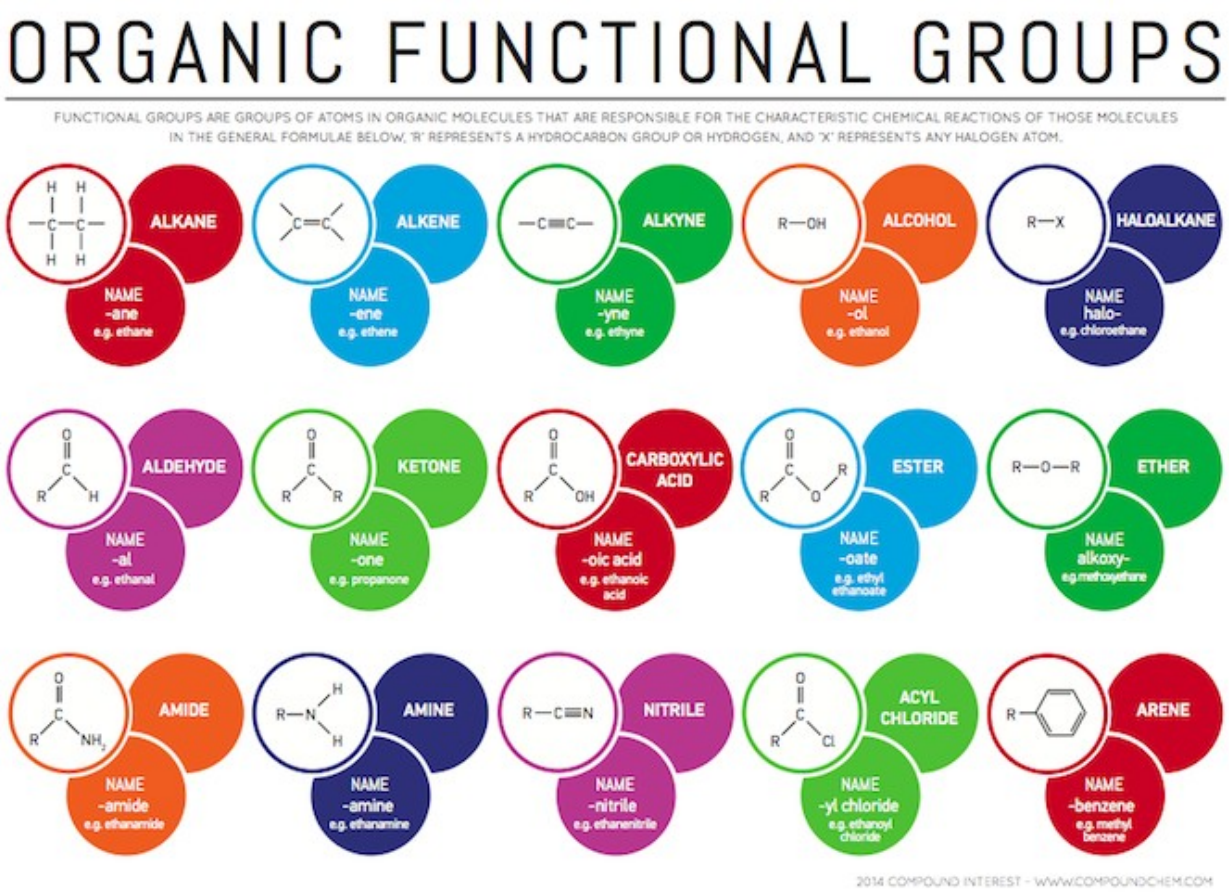
- Loads of hydrogen bonds.
- 70% water = Earth.
- 60% water = Humans.
- Aqueous environment.
- Elements in the human body need to interact with water (ions, compounds, molecules).
- Compounds need to dissolve in water.
- Molecules try to keep their polar regions (charged) on the outside of the molecule – it can interact with water.
- Hydrophobic (non-polar) do not dissolve in water.
- Compounds that are hydrophilic do.

Carbon Compounds

- Carbon has 4 valence electrons – huge capacity to make a variety of compounds.
- Can be single bonds (rotate), double bonds (don't rotate) or triple bonds (for carbon).
- Can combine to itself or to other elements such as oxygen or hydrogen.
- Isomers – A compound that has the same molecular formula but a different arrangement of those elements in 3D space.
    - Structural – form of isomerism in which molecules with the same molecular formula have bonded together in different orders, as opposed to stereoisomerism
    - Geometric – each of two or more compounds which differ from each other in the arrangement of groups with respect to a double bond, ring, or other rigid structure.
    - Enantiomers – are chiral molecules that are mirror images of one another. Furthermore, the molecules are non-superimposable on one another. This means that the molecules cannot be placed on top of one another and give the same molecule.
    - Stereoisomer – Stereoisomers are isomeric molecules that have the same molecular formula and sequence of bonded atoms (constitution), but differ in the three-dimensional orientations of their atoms in space.

Functional Groups

- Also known as R-groups.
- Different groups of elements with different chemical properties.
- Adding the R-groups add the diversity.



- <u>Monosaccharides</u>

Simplest form of carbohydrate, e.g. glucose, fructose, ribose, etc.

$C_nH_{2n}O_n$ e.g. Glucose – $C_6H_{12}O_6$

Physico-chemical Properties

- Colourless crystals – solid.
- Sweet.
- Soluble in water.
- Not soluble in organics.

Chemical Forms

- Known as isomers.
- 2 classes:
  1) Aldohexose (carbonyl group at end of carbon skeleton [chain])
  2) Ketohexose (carbonyl group in the middle of carbon skeleton [chain])

Chain Length

- Most are 3-7 carbons long.
- Most common:
  - 3 Triose – only straight chained (linear)

- 5 Pentose – DNA and RNA
- 6 Hexose – energy sources

Cyclisation

- 5+ carbons can cyclise.

Anomers

- Anomers are stereoisomers that differ at the carbonyl carbon (anomeric carbon)
- The arrangement of the OH group around that anomeric carbon determines whether the anomer is an alpha or beta.
- Stereoisomers have the same chemical formula but a different spatial arrangement of the atoms around a single carbon.

Substitutions

- When we add things to monosaccharides we call them substitutions.
- Involves adding a functional group.

- <u>Complex Carbohydrates</u>

Glycosidic Bond

- Is covalent
- Electrons are shared.
- Forms between the anomeric carbon (C=0) of one monomer and an OH of the other monomer.
- Covalent forms by loss of water – dehydration.

Anomer

- Form of stereoisomer (structural isomer with variation around one single carbon)
- C=0 + OH reacts = molecular movement. Forms with Alpha glucose (opposite side of the C-OC) or beta glucose (same).
- Spontaneous swap in solution = mutarotation.

Alpha Glycosidic Bonds

- h

Beta Glycosidic Bonds

- Beta monomer –OH on the same side as the C-O-C.

Disaccharides

- 2 monomers joined together by a Glycosidic bond.
- E.g. Sucrose, Lactose, maltose.

Polysaccharides

- Polymers, 100-1000's of monomeric units coming together to make a super molecule.
- Glycogen (alpha) = Homopolymer. All glucose monomeric units. Easy access store of glucose. Stored in liver and muscle. All bonds are alpha. 2 types. Alpha 1-4 in the straight chain. Branch is 1-6.

- Starch (Alpha) – Plants use starch as energy store. All monomers are glucose. Straight chain is an Alpha 1-4 linkage. Branch point is alpha 1-6 linkage. 2 types of polymer = Amylose (straight chained) Amylopectin (Branched). They wind up tightly.
- Cellulose (Beta) = structural polysaccharide. Beta linkage. Beta 1-4 linkages. Polar bonds between monomers.
- Structure - Differences between alpha and beta. Spiral alpha helix. Linear beta strands.

- Carbohydrate Conjugates

Chitin
- Linear and unbranched.
- Common in the exoskeleton of insects and spiders.
- Homo-polysaccharide (polymer composed of a single type of sugar polymer).
- N-acetyl glucosamine joined together by beta-linked Glycosidic bond.

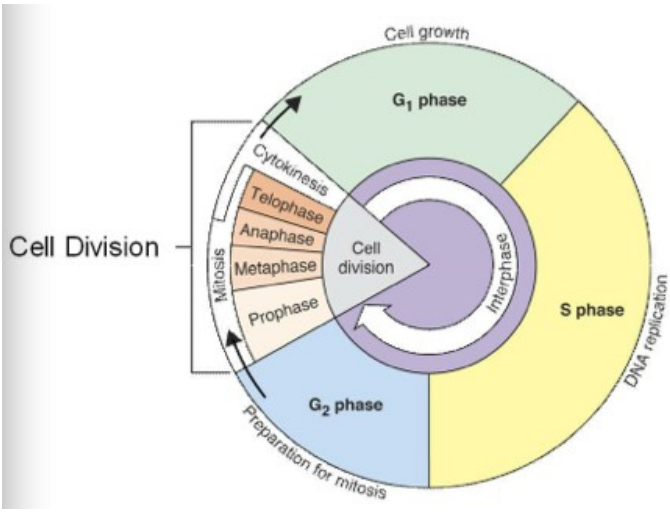
Peptidoglycans
- Components of the bacterial cell wall.
- Polymer of NAM (N-acetyl muramic acid) and NAG (N-acetyl glucosamine) bound together by small peptide bridges.
- Rigid and cross-linked.
- Target for antibiotics.

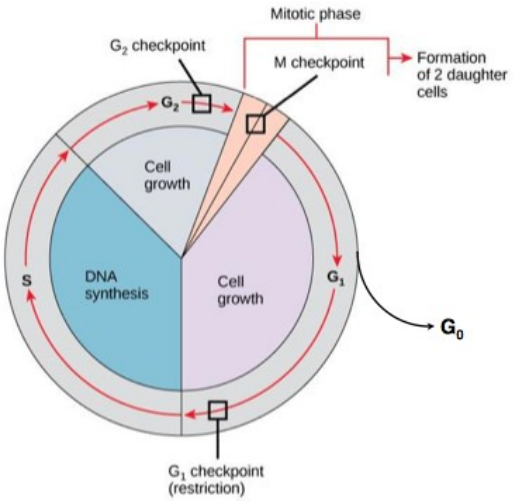
Glycoproteins
- Oligosaccharides (few monomers). Associated with proteins.
- Protein with a short stretch of monosaccharides attached.
- Associated with cell-cell contact. For example, the immune response, viral attack.

Glycosaminoglycans
- Linear hetero polymers. Repeating unit is a disaccharide.
- Hyaluronic acid ~ part of synovial fluid due to its viscosity.
- Chondroitin ~ provides tensile strength to connective tissue.
- Part of the ECM.

Proteoglycans
- Glycosaminoglycan attached to a protein.
- Large, strong and flexible molecules.
- Part of the ECM.
- Core of protein with branches consisting of heteropolysaccharides.

Extracellular Matrix
- Fills space between cells in multicellular organisms.
- Highly complex and diverse.
- Aids in cell-cell communication, an anchor for cell adhesion and is a pathway for cell migration.





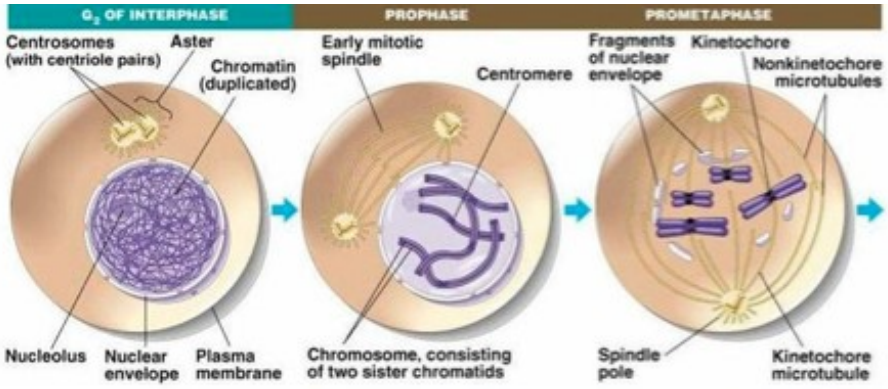
The cell cycle is directed by specific checkpoints:

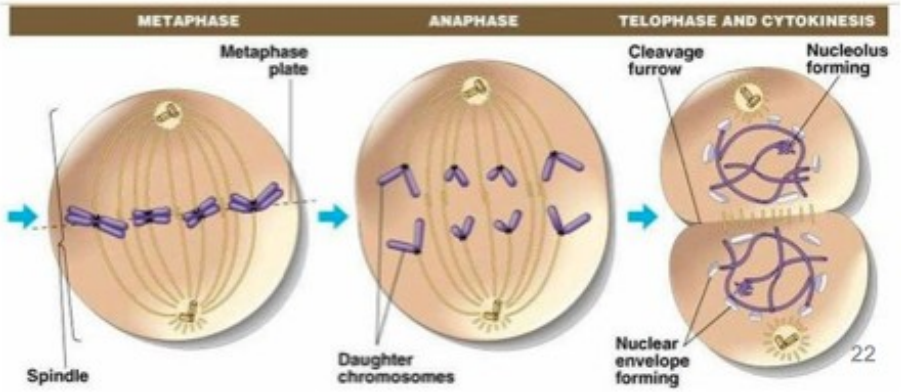
G_1 checkpoint determines whether all conditions are favourable for cell division to proceed, e.g. size, DNA integrity, etc.

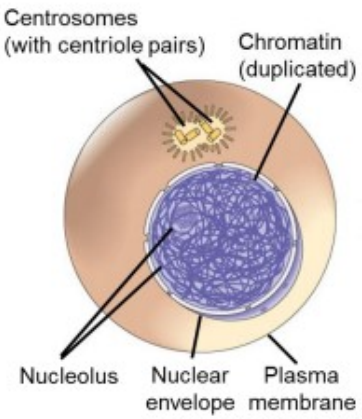
G_2 checkpoint is to ensure that all of the chromosomes have been accurately replicated without mistakes or damage.

M checkpoint, the spindle checkpoint, determines whether all the sister chromatids are correctly attached to the spindle microtubules.

- **Mitosis (somatic cell)**

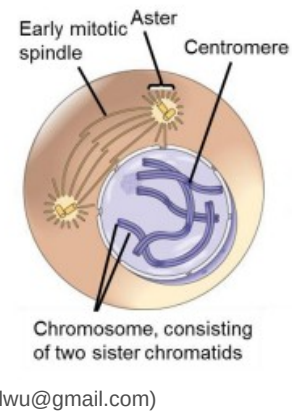






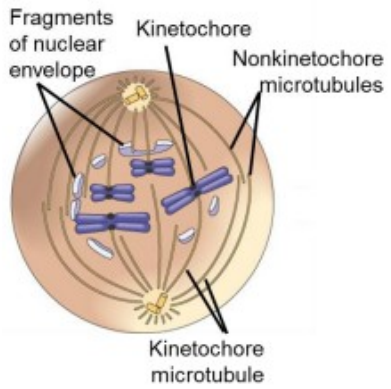
G2 of Interphase

- Chromosomes, in the form of chromatins, duplicated during S phase, cannot be seen individually because they are not yet condensed
- Two centrosomes have formed by replication
- Each centrosome contains two centrioles



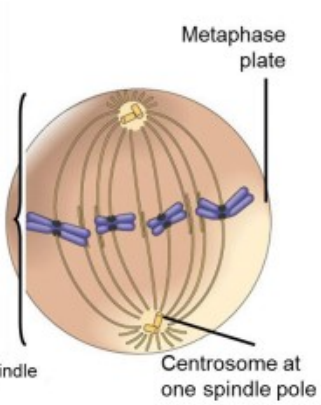
Prophase

- Chromatin fibers become more tightly coiled, condensing into discrete chromosomes of two identical sister chromatids joined together
- Mitotic spindle begins to form. It is composed of the centrosomes and the microtubules that extend from then.
- Centrosomes move away from each other propelled by the lengthening microtubules between them.



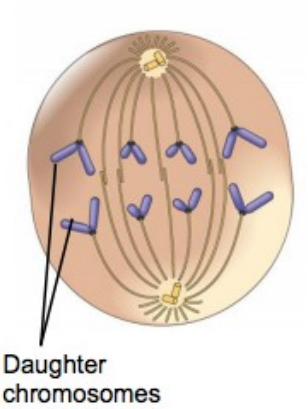
Prometaphase

- The nuclear membrane dissolves.
- The microtubules originating from the centrioles invade the nuclear space
- Proteins get attached to the centromeres forming kinetochores
- Microtubules get attached to the kinetochores



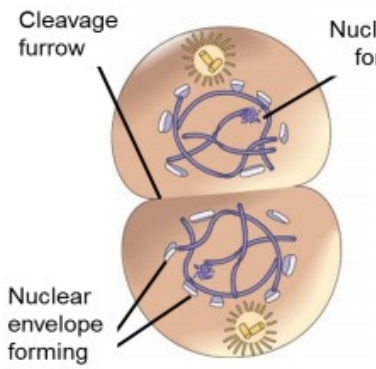
Metaphase

- Centrosomes are now at opposite ends of the cell.
- Chromosomes align along the imaginary equator of metaphase plate between two poles of the spindle.
- For each chromosome, the kinetochores of the sister chromatids are attached to kinetochore microtubules coming from opposite poles.
- The entire apparatus of microtubules is called the spindle.



Anaphase

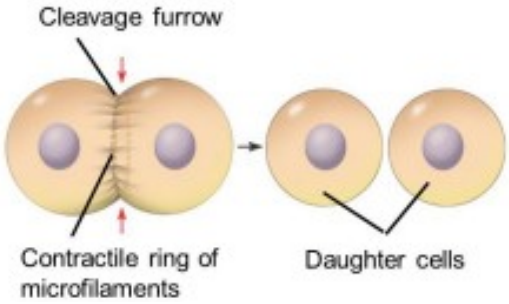
- Anaphase begins when the two sister chromatids suddenly part.
- The two chromatids begin moving towards opposite ends, as their kinetochore microtubules shorten.
- The cell elongates as the non-kinetochore microtubules lengthen.
- By the end of anaphase, the two ends of the cell have equivalent and complete sets of chromosomes.



Telophase

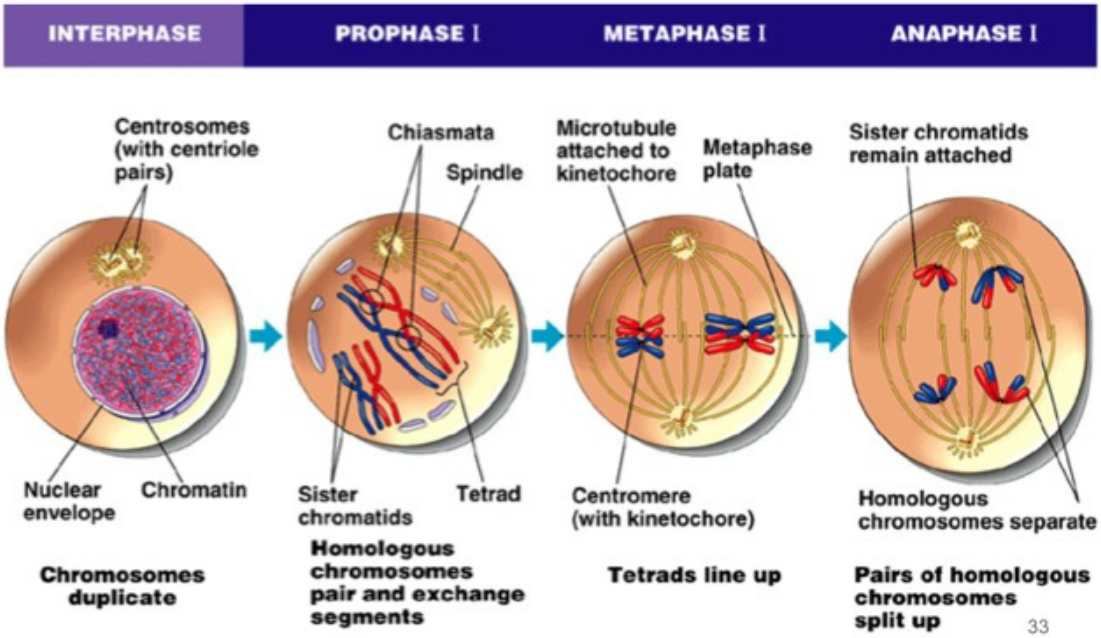
- Two daughter nuclei begin to form, so are nuclear envelopes.
- The chromosomes become less condensed.
- Mitosis, the division of one nucleus into two genetically identical nuclei, is now complete.

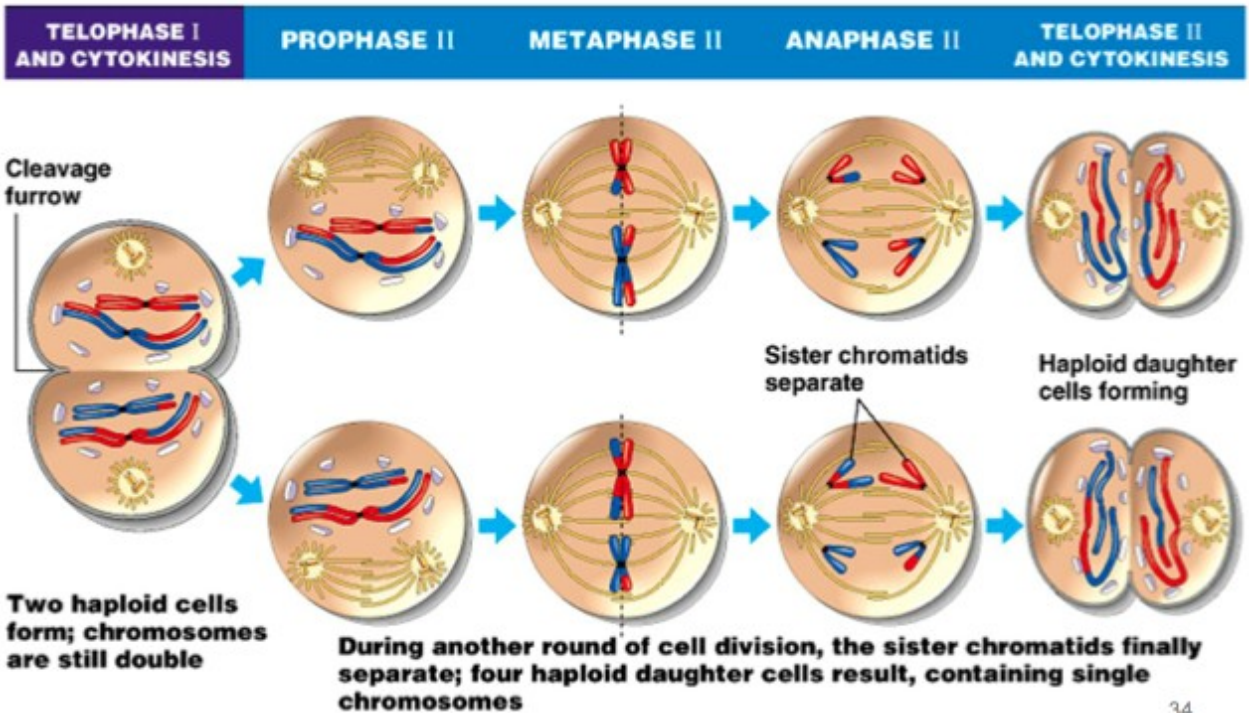
- Formation of the constriction belt or contractile ring from microfilaments
- Cleavage of cell into two identical halves



Meiosis (sex cell)

- Meiosis reduces the chromosome number.
- Diploid cell (2N) gives rise to haploid (N) gametes.
- Genetic Variation – mixture of characteristics from both parents.
- Fertilisation restores the diploid number back to 46 in humans.

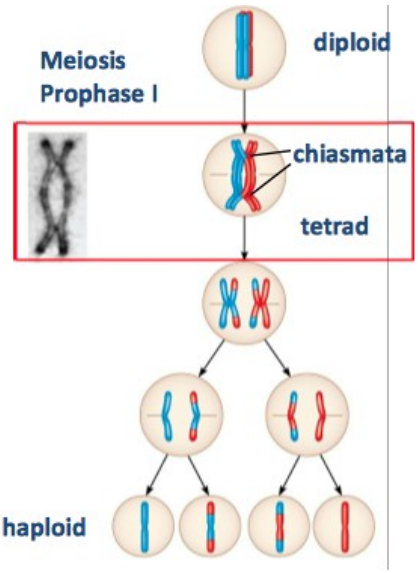




- Creating Variation (Meiosis)

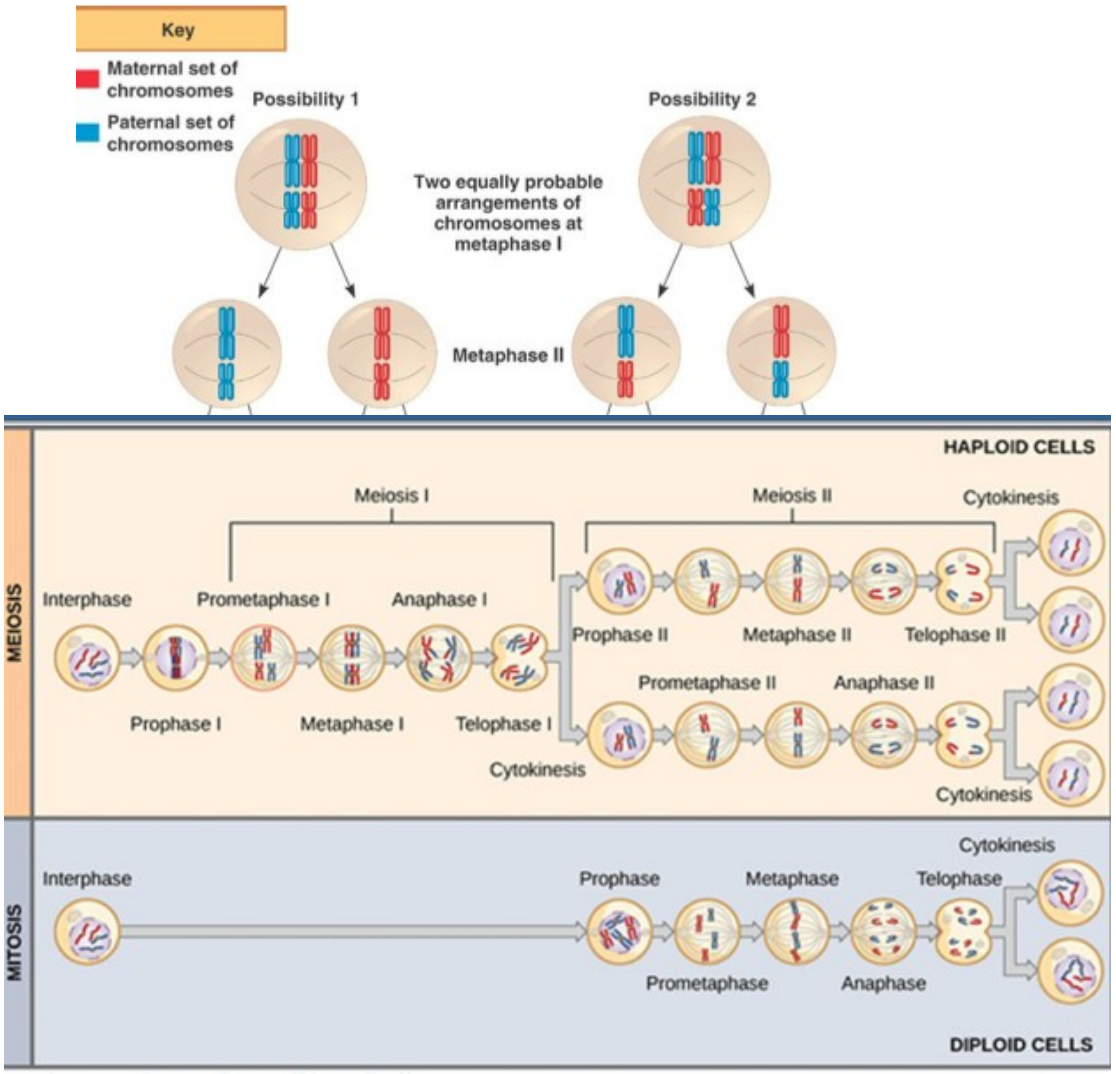
Crossing Over (recombination)

- Homologous chromosome forms a tetrad in prophase 1.
- **Synapsis** occurs – Alleles swap at chiasma between non-sister chromatids.



Independent Assortment

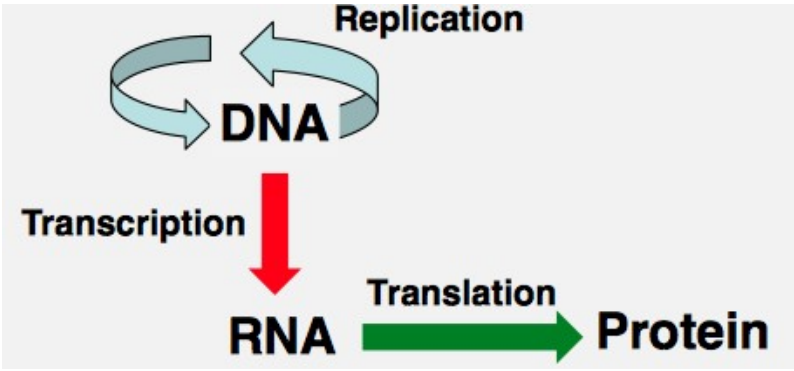
- The orientation of the homologous chromosome pairs during metaphase I and II is random which leads to a 50% chance that a specific resultant haploid cell will receive the maternal copy of a chromosome, likewise for the paternal copy.



Lecture 4 – Nucleic Acids, DNA Structure and DNA replication

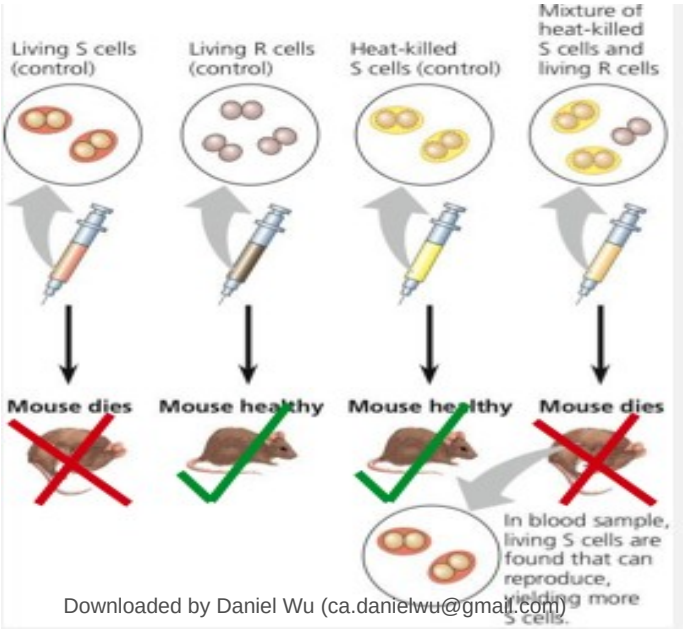
- <u>Nucleic Acids</u>

- Macromolecules composed of chains of nucleotides.
- Found in all cells.
- Most well known are:
  - DNA (deoxyribonucleic acid) – Contains genetic information of the cell.
  - RNA (Ribonucleic Acid) – Plays roles in translating the genetic information in the DNA into proteins.



- <u>Frederick Griffith</u>

- Studied Streptococcus pneumonia, a pathogenic bacterium causing pneumonia.
- 2 strains of streptococcus:
  - S strain is *virulent*
  - R strain is *novirulent*
- Infected mice with each of the 2 strains to understand the difference between the strands.
- Conclusions:
  - Information specifying virulence was passed from the dead S strain cells into the live R strain cells.
  - Griffith called the transfer of this information transformation (it transformed cells from being non-virulent to being virulent)



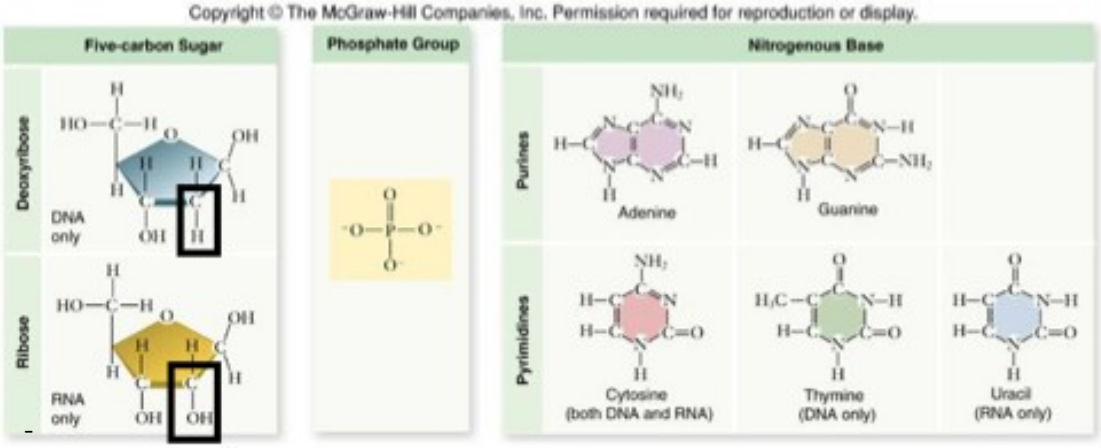
- Avery, Macleod and McCarty

- Repeated Griffith's experiment but used purified cell extracts (DNA and protein)
- They discovered:
  - Removal of all protein from the transforming material did not destroy its ability to transform R strain cells.
  - DNA-digesting enzymes destroyed all transforming ability.
  - **Transforming material is DNA**.

- Structure of DNA

Building blocks of DNA (and RNA) are nucleotides, each composed of:

- A 5-carbon sugar
- A phosphate group (PO^{-}_{4})
- A nitrogen-containing base that may be a purine (Adenine or guanine) or a pyrimidine (thymine or cytosine)

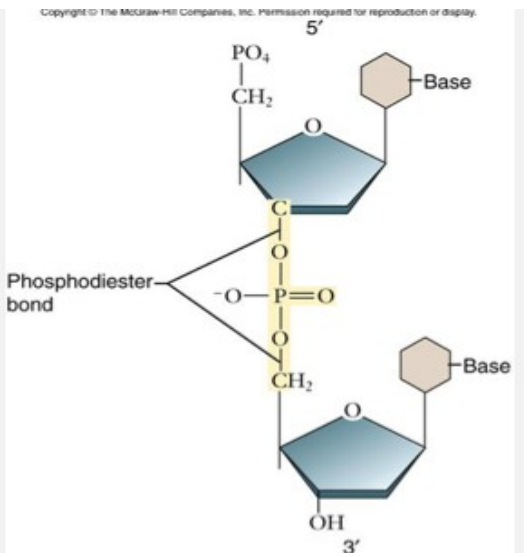


Structure of Nucleotides:

- Nitrogenous base attached to the 1' carbon deoxyribose.
- Phosphate group attached to the 5' carbon of deoxyribose.
- Free hydroxyl group (-OH) at 3' carbon of deoxyribose.

Structure of DNA:

- Nucleotides are connected to each other to form a long chain (polymer)
- Phosphodiester bond. Formed between the 5'phosphate group and the 3'-OH of the next in a 5' to 3' orientation.



Determining the structure of DNA

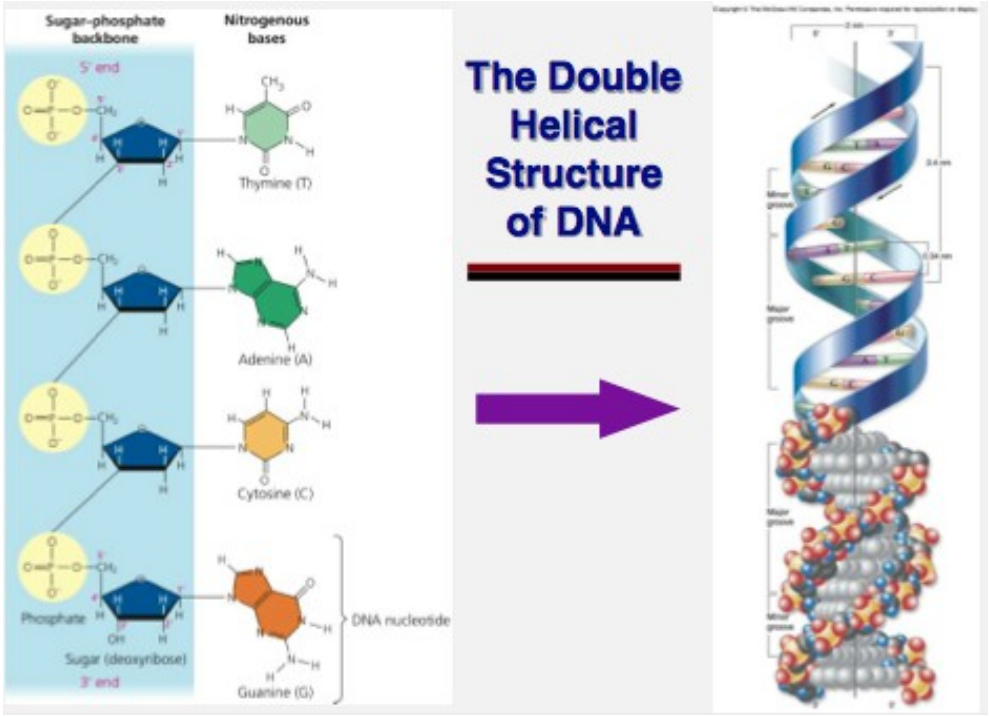
- Chargaff's Rules: Amount of adenine = amount of thymine. Amount of cytosine = amount of guanine.
- Franklin and Wilkins: Franklin performed X-ray diffraction to identify the 3-D

structure. Discovered DNA is helical. Diameter of 2nm, complete turn of helix every 3.4nm.

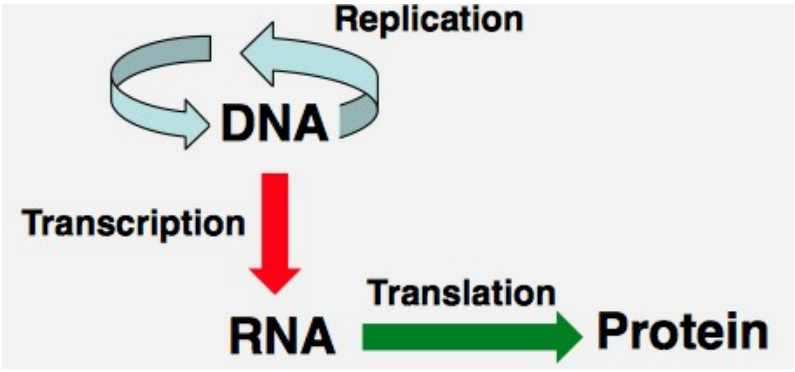
- Watson and Crick: Deduced the structure of DNA using others evidence. Proposed a double helical structure.

Structure

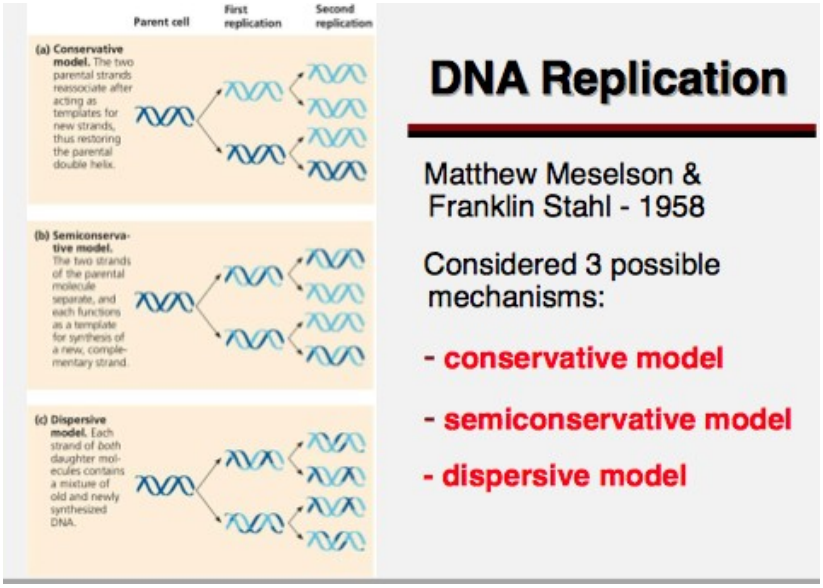
- 2 sugar phosphate backbones.
- Nitrogenous base face toward the interior of the molecule.
- Bases form hydrogen bonds with complementary bases on the opposite sugar-phosphate backbone.
- 2 DNA strands held together by hydrogen bonds.
- A-T and G-C.



- <u>DNA Replication</u>



- Required prior to cell division.
- One parental helix with 2 DNA strands yields 2 daughter helices and 4 strands.



- Meselson and Stahl considered 3 possible mechanisms.

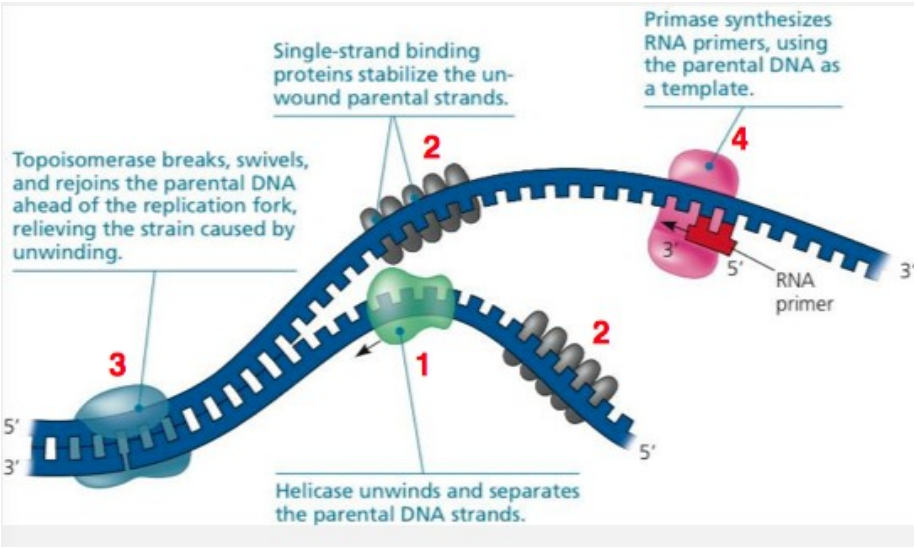
- DNA replication is **SEMICONSERVATIVE**!! Each strand of DNA acts as a template for the synthesis of a new strand.

Basic Steps of DNA Replication:

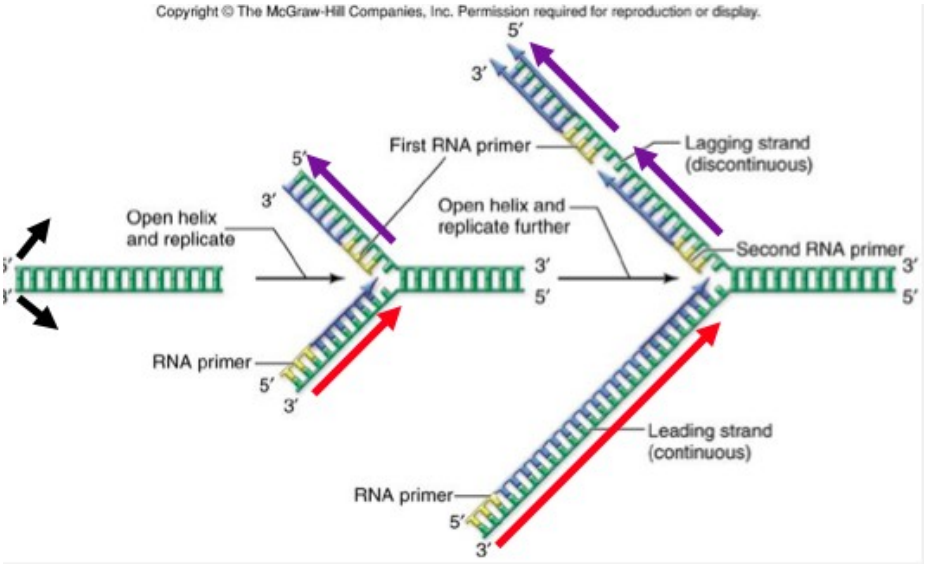
1) **Initiation –** Begins at an origin of replication (oriC).
2) **Elongation** – new strands of DNA are synthesised by DNA polymerase.
3) **Termination** – different in eukaryotes and prokaryotes.

DNA Replication in **Prokaryotes**

- Double helix is unwound by the enzyme – helicase.
- DNA polymerase III adds nucleotides to the 3' end of the daughter strand of DNA.
- The torsional strain of unwinding is relieved by the enzyme DNA gyrase (Topoisomerase enzyme).



- The single strand of DNA produced by helicase are stabilised by the binding of single-strand binding protein. (SSB)
- DNA polymerase requires a primer *(strand of short nucleic acid sequences that serves as a starting point for DNA synthesis)* and can only synthesise DNA in one direction (5' to 3')
- DNA strands are *antiparallel* to each other and run in opposite directions. Thus DNA polymerases on the 2 strands polymerise DNA in opposite directions.
- Leading strand = Continuous.
- Lagging strand = Discontinuous (creates okazaki fragments)



- Lagging strand: DNA polymerase 1 removes primer (RNA) segments and replaces them with DNA. DNA ligase then seals the gaps between okazaki fragments with phosphodiester bonds.
- Enzymes needed for replication are stored in replisome.

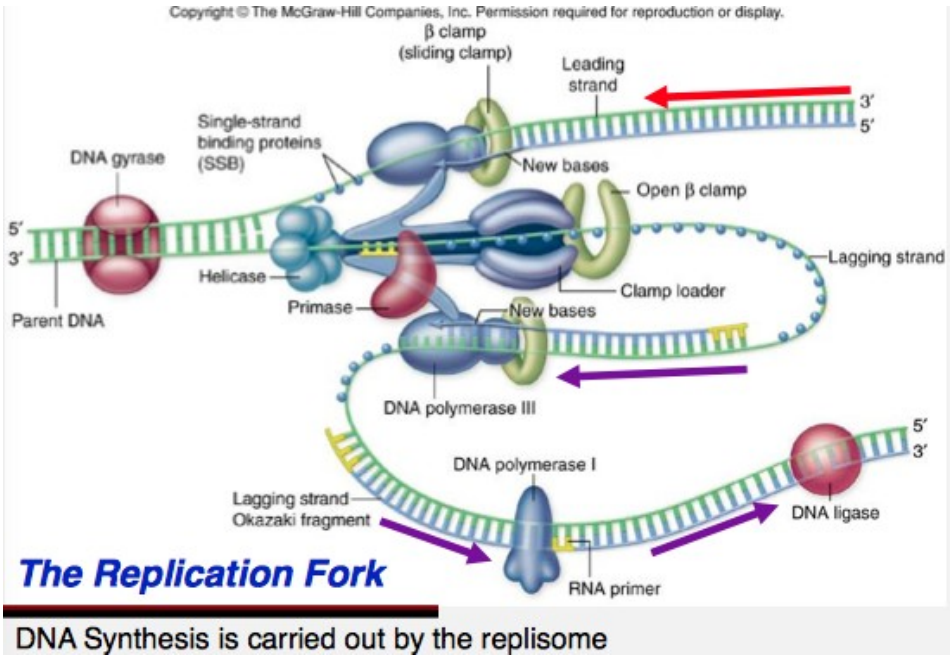


Table 16.1 Bacterial DNA Replication Proteins and Their Functions

Protein	Function
Helicase	Unwinds parental double helix at replication forks
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template
Topoisomerase	Relieves "overwinding" strain ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase	Synthesizes an RNA primer at 5′ end of leading strand and of each Okazaki fragment ofl agging strand
DNA pol III	Using parental DNA as a template, synthesizes new DNA strand by covalently adding nucleotides to the 3′ end of a pre-existing DNA strand or RNA primer
DNA pol I	Removes RNA nucleotides of primer from 5′ end and replaces them with DNA nucleotides
DNA ligase	Joins 3′ end of DNA that replaces primer to rest of leading strand and joins Okazaki fragments of lagging strand

DNA replication in **Eukaryotes**

- Replicated from multiple origins of replication due to complexity.
- Synthesising the ends of the chromosomes is difficult because of the lack of a primer.
- Thus with each round of DNA replication, the linear eukaryotic chromosome becomes shorter.
- This is prevented through the action of an additional enzyme – telomerase.

Lecture 5 – Lipids and membranes

Lipids

- Fats, acids.
- Properties given by their chemical structure ~ chemistry.
- Hydrophobic (non-polar).
- Fat molecule (triacylglycerol)
- Different lengths of hydrocarbon chains.
- 14-24 carbons long.

Uses:

- Energy storage.
- Protection.
- Insulation.
- Lipid membrane.

Types:

- Non-polar e.g. Triacylglycerol.
- Polar e.g. Phospholipid.

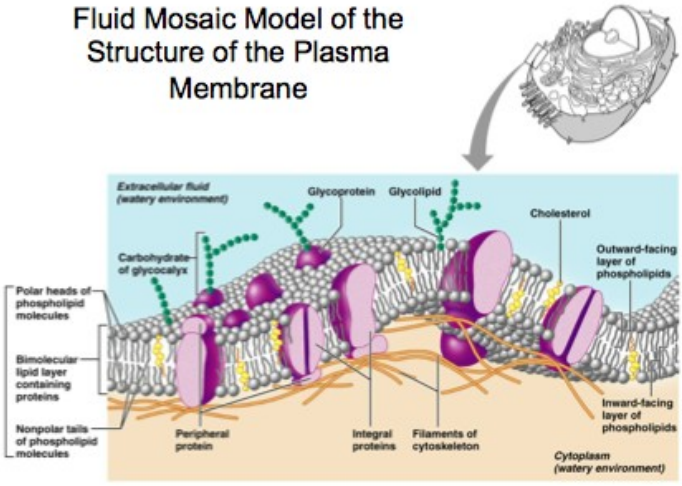
Groups:

- **Fatty Acids**
  - Energy source.
  - Saturated = All single C-C bonds
  - Unsaturated = 1+ double C bond.
  - Poorly soluble.
  - Free fatty acid – carboxyl acid head group (COOH).
- **Triacylglycerol's**
  - 3 fatty acids bound to a glycerol. Highly reduced, soluble in organic solvents.
  - Non-polar.
  - Saturated = C-C
  - Unsaturated = at least 1 double c bond.
- **Phospholipids**
  - Amphipathic.
  - Bridge between non-polar and polar solvents.
- **Sterols**
  - Steroid nucleus.
  - Fatty acyl chain with small polar head.

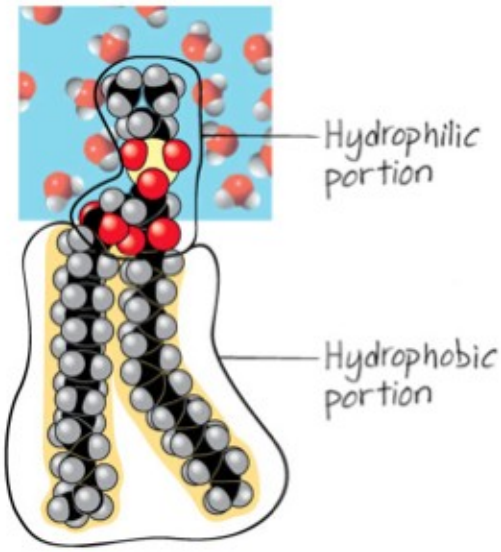
- <u>Cell Membrane Transport</u>

The plasma membrane:

- Selective barrier that allows sufficient passage of oxygen, nutrients, and waste to service the volume of every cell.
- Double layer of phospholipids.



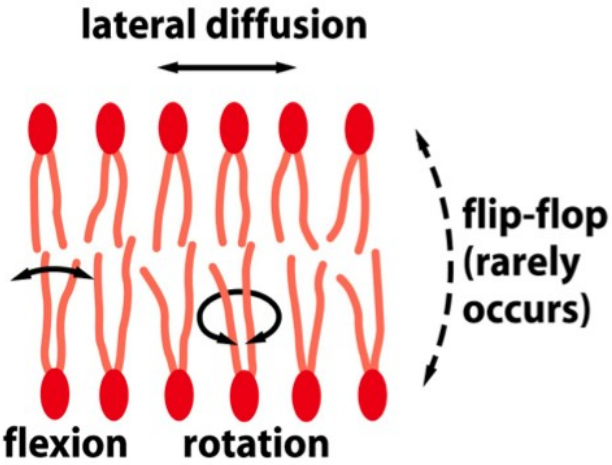
Cellular membranes are fluid mosaics of lipids and proteins:



- **Phospholipids** = most abundant in the plasma membrane.
- **Phospholipids** = amphipathic molecules (containing hydrophobic and hydrophilic regions)
- Fluid mosaic model states: *Membrane = fluid structure with a mosaic of proteins embedded in it.*

Fluidity of Membranes:

- Phospholipids in the plasma membrane can move within the bilayer.
- Greater proportion of unsaturated phospholipids tend to increase membrane fluidity.
- Drift laterally, rarely flip over.
- Membranes must be a fluid to work properly.
- Cooling; Fluid to a solid state. The temperature at which a membrane solidifies depends on the type of lipid.

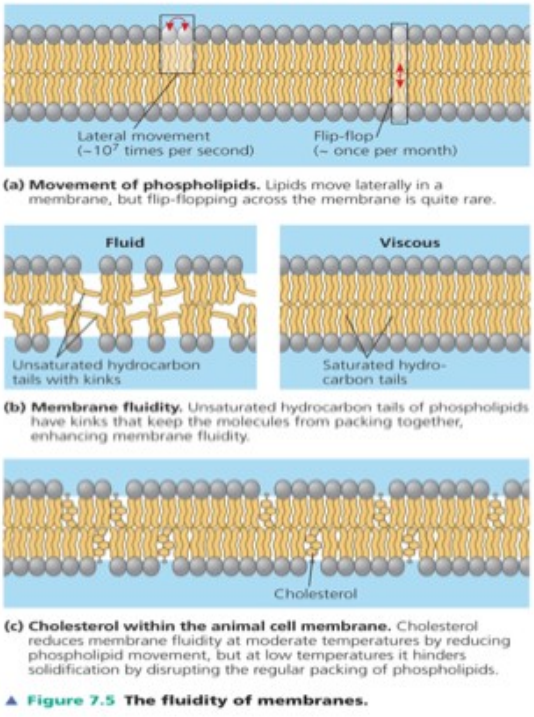


- Cholesterol has different effects on the membrane fluidity at different temperatures ~ *Warm*=Restrains movement of phospholipids. *Cool*=Maintains fluidity by preventing tight packing.

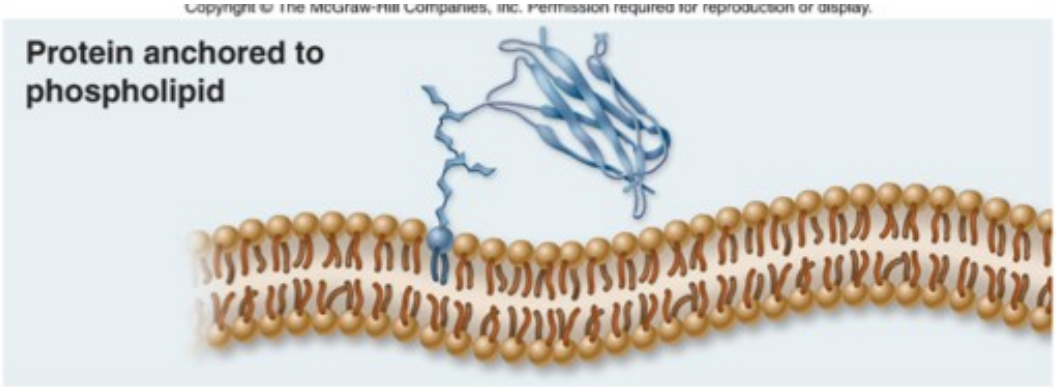
Membrane Proteins and their Functions:

- Proteins determine most of the membrane's specific functions.
- **Peripheral proteins**
  - Anchored to a phospholipid in one layer of the membrane.
  - Possess non-polar regions that are inserted in the lipid bilayer.
  - Free to move throughout one layer of the bilayer.

- **Integral proteins** penetrate the hydrophobic core.
  - Integral proteins that span the membrane are called **transmembrane proteins.**
  - Non-polar regions – Embedded in the interior of bilayer.



▲ Figure 7.5 **The fluidity of membranes.**



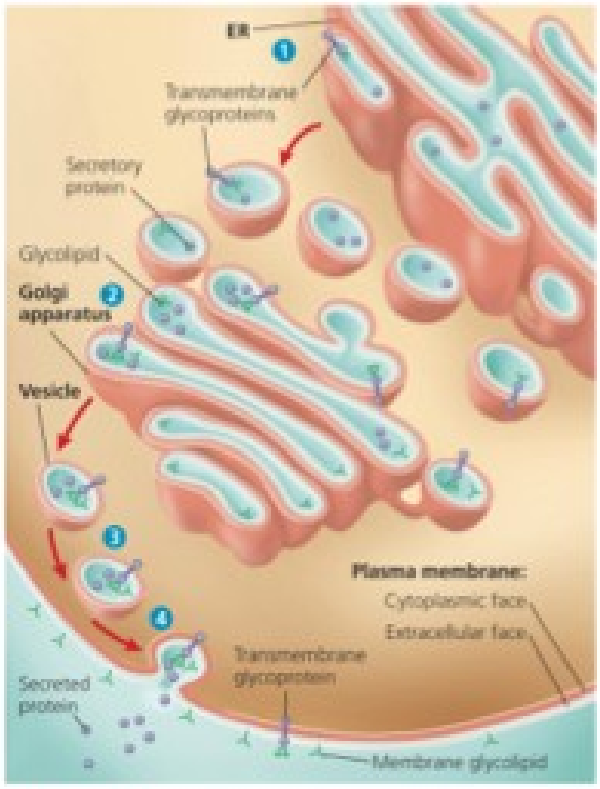
- Polar regions – Protude from both sides of the bilayer.
- The hydrophobic (non-polar) regions of an integral protein consist of one or more stretches of non-polar amino acids, often coiled into alpha helices.

Functions of membrane proteins:

- **Transport**
  -
- **Enzymatic activity**
  - A protein built into the membrane may be an enxzyme with its active site exposed to substances in the adjacent solution, In some cases, several enzymes in a membrane are organized as a team that carries out sequential steps of a metabolic pathway.
- **Signal transduction**
  -
- **Cell-Cell recognition**
  -
- **Intercellular joining**
  -
- **Attachment to the cytoskeleton and extracellular matrix (ECM).**
  -

Synthesis and sidedness of membranes:

- Distinct inside and outside faces.
- Asymmetrical distribution of proteins, lipids, and associated carbohydrates in the plasma membranes is determined when the membrane is built (by the ER and Golgi apparatus).

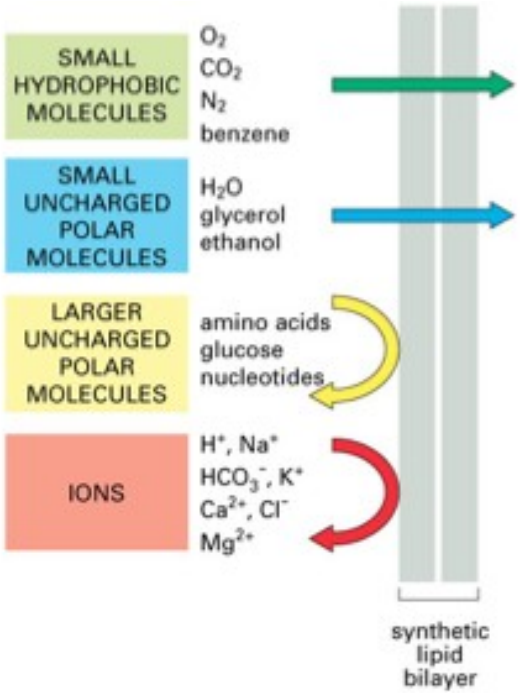


▲ **Figure 7.10 Synthesis of membrane components and their orientation on the resulting membrane.** The plasma membrane has distinct cytoplasmic (orange) and extracellular (aqua) faces, with the extracellular face arising from the inside face of ER, Golgi, and vesicle membranes.

- <u>Movement of materials across cell membranes</u>

Plasma membranes are selectively permeable.
Permeability of the lipid bilayer:

- Hydrophobic (non-polar) molecules, such as hydrocarbons, can dissolve in the lipid bilayer and pass through the membrane rapidly.
- Polar molecules do not cross the membrane easily.



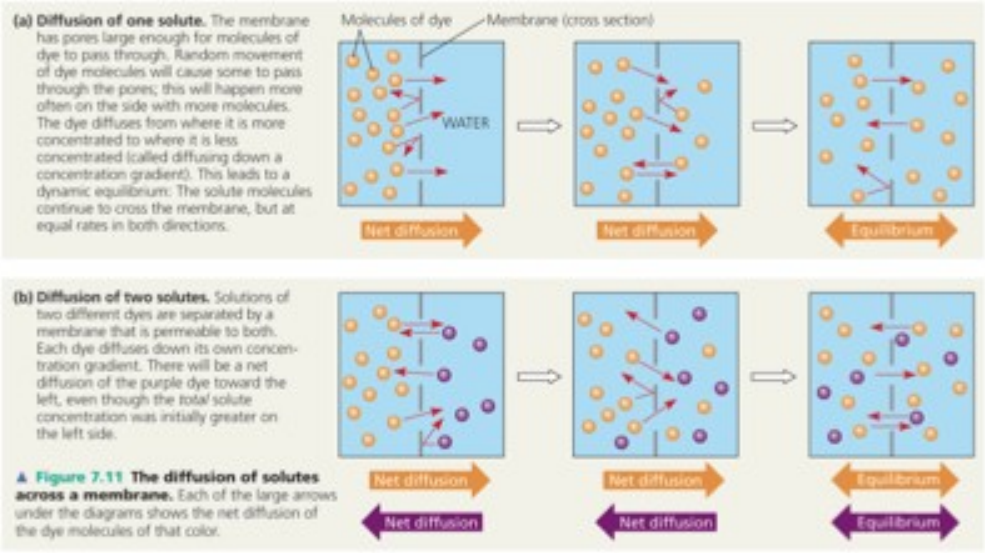
From a thermodynamic point of view there are only **3 types of transport processes:**

1) **Passive Transport**:

- Passive transport is movement of molecules through the membrane in which;
  - ➢ No energy is required.
  - ➢ Molecules move in response to a concentration gradient.
- Passive transport is diffusion of a substance across a membrane with no energy investment.

Diffusion is movement of molecules from high to low concentration. It's the tendency for molecules to spread out evenly into the available space.

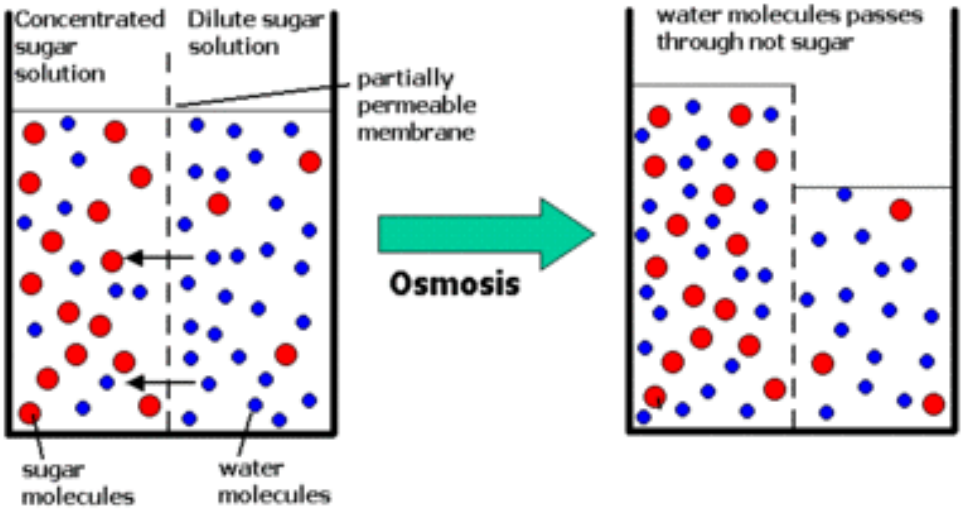
- Substances diffuse down their concentration gradient, which requires no work.
- Diffusion across a biological membrane is passive transport.



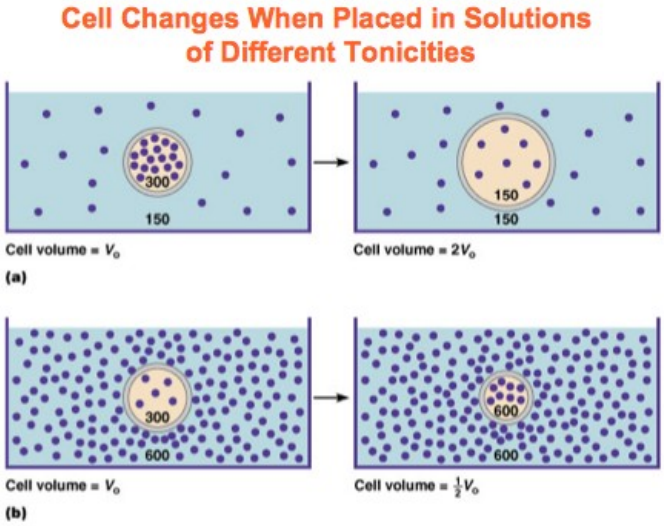
▲ Figure 7.11 **The diffusion of solutes across a membrane.** Each of the large arrows under the diagrams shows the net diffusion of the dye molecules of that color.

Osmosis is the diffusion of water across a selectively permeable membrane.

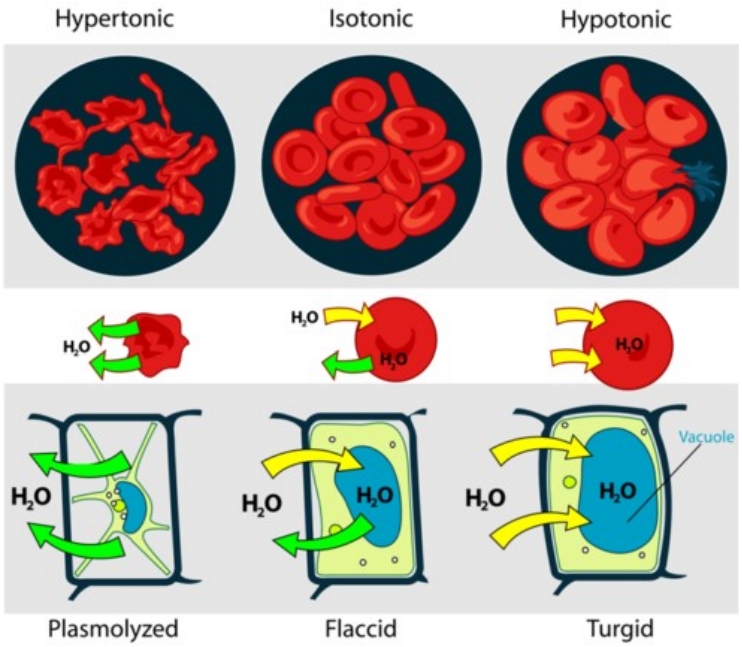
- Water diffuses across a membrane from the region of lower solute concentration to the region of higher solute concentration until the solute concentration is equal on both sides.
- Water diffuses from high to low water concentration.
- Water diffuses from low to high solute concentration.



Tonicity: The ability of a surrounding solution to cause a cell to gain or lose water.

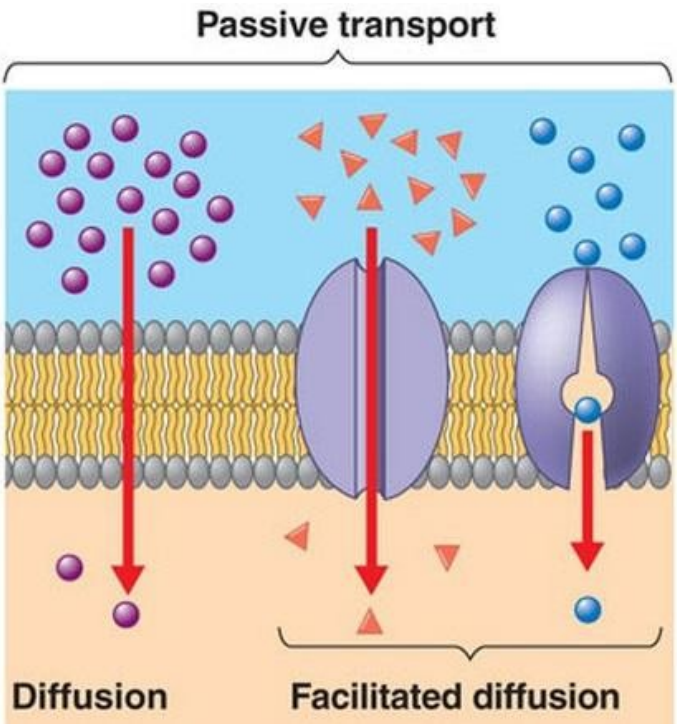


Isotonic solution: Solute concentration is the same as that inside the cell; no net water movement across the plasma membrane.
Hypertonic solution: Solute concentration is greater than that inside the cell; cell loses water.
Hypotonic solution: Solute concentration is less than that inside the cell; cell gains water.
Osmoregulation: The control of solute concentrations and water balance, is a necessary adaptation for life in such environments.



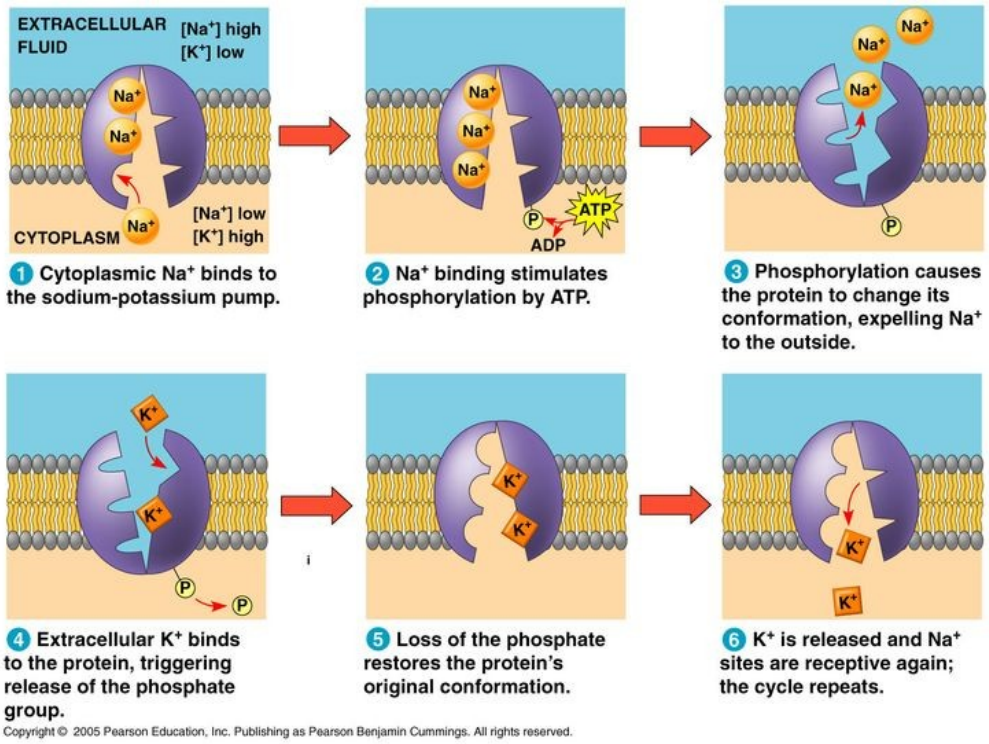
2) **Facilitated Diffusion: passive transport aided by transport proteins**

- Proteins speed up the passive movement of molecules by allowing the passage of hydrophilic substances across the membrane.
- Channel proteins – provide corridors that allow a specific molecule or ions to cross the membrane via their hydrophilic channel.
    - E.g. Aquaporins for facilitated diffusion of water; ion channels that open or close in response to a stimulus and allow ions to cross the membrane.
- Carrier proteins - bind to molecules and change shape to shuttle them across the membrane. Specific for the substance it moves.



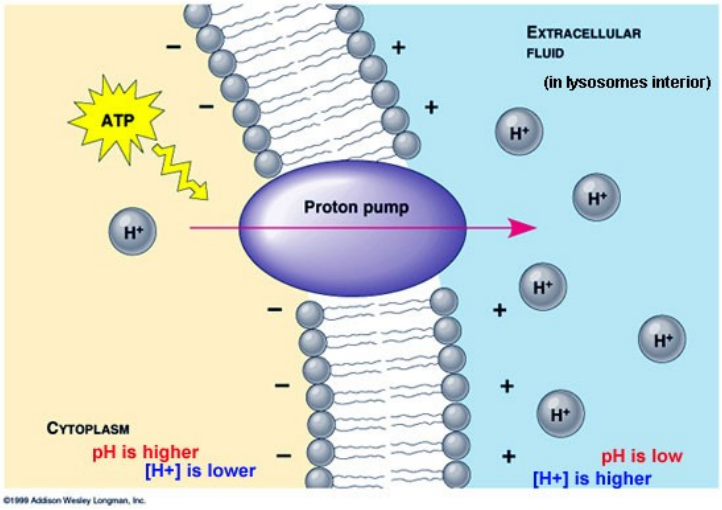
3) **Active Transport**

- Uses energy to move solutes against their gradients.
- Energy in the form of ATP (Adenosine Triphosphate).
- Performed by specific proteins embedded in the membranes.
- Active transport allows cells to maintain concentration gradients that differ from their surroundings.
- E.g. Sodium-Potassium Pump.
    - Uses an antiporter to move 3 Na+ out of the cell and 2 K+ into the cell.
    - ATP energy is used to change the conformation of the carrier protein.
    - The affinity of the carrier protein for either Na+ or K+ changes so the ions can be carried across the membrane.



How ion pumps maintain membrane potential

- Membrane potential is the voltage difference across a membrane.
- Voltage is created by differences in the distribution of positive and negative ions.
- The electrochemical gradient drives the diffusion of ions across a membrane:
  - Chemical force (the ionic concentration gradient)
  - Electrical force (the effect of the membrane potential on ionic movement)



- An **electrogenic pump** is a transport protein that generates voltage across a membrane
- The sodium-potassium pump is the major electrogenic pump of animal cells
- The main electrogenic pump of plants, fungi, and bacteria is a **proton pump**
- Electrogenic pumps help store energy that can be used for cellular work

Bulk transport across the plasma membrane occurs by exocytosis and endocytosis

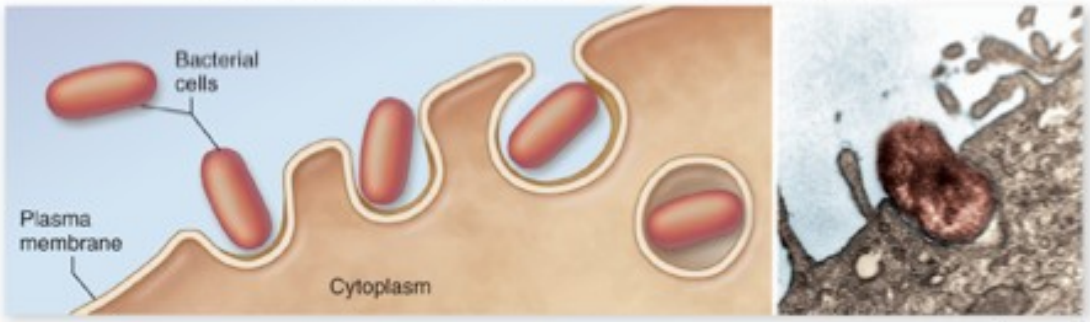
- Large molcules, such as polysaccharides and proteins, cross the membrane in bulk via vesicles.
- Bulk transport requires energy.

Exocytosis

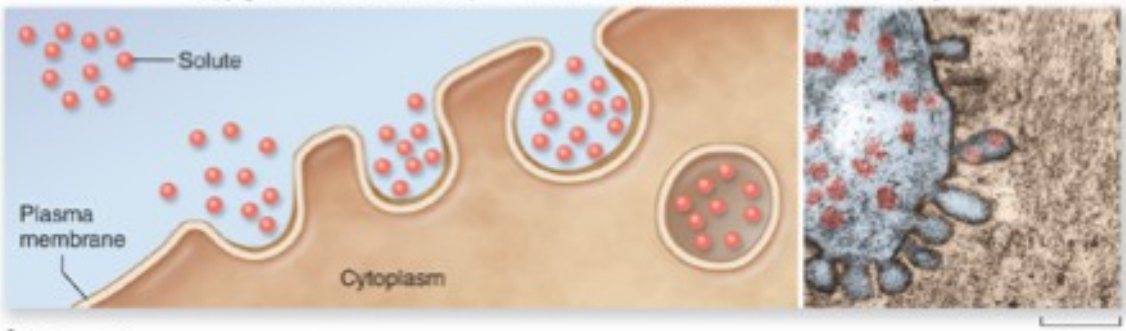
- Transport vesicles migrate to the membrane, fuse with it, and release their contents.
- Many secretory cells use exocytosis to export their products.

Endocytosis

- The cell takes in macromolecules by forming vesicles from the plasma membrane.
- ***Reverse of exocytosis.***
- 3 types:
  - Phagocytosis (cellular eating)
  - Pinocytosis (cellular drinking)
  - Receptor-mediated endocytosis.



a. Phagocytosis © Micrograph Courtesy of the CDC/Dr. Edwin P. Ewing, Jr



b. Pinocytosis © 17b:BCC Microimaging, Inc., Reproduced with permission

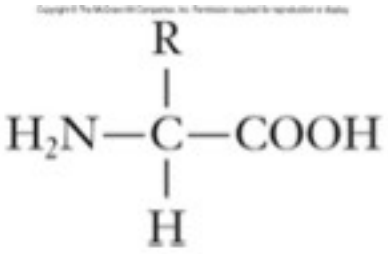
Lecture 6 – Proteins

Function:

- Some proteins are enzymes, antibodies (defense against foreign invaders such as bacteria or viruses), transporters.
- Proteins have plenty of functions. Strong role in homeostasis.

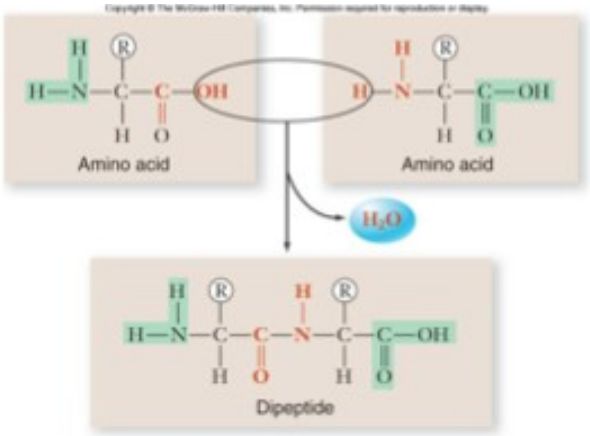
Structure:

- Polymers (1 protein is many monomers [amino acid] joined together to make a macromolecule)
- Amino acid



Peptide bonds:

- Bonds in proteins, monomers joined together.
- A peptide bond is a chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule, releasing a molecule of water (H2O).
- C-N peptide bond – cannot rotate giving proteins specific structures.

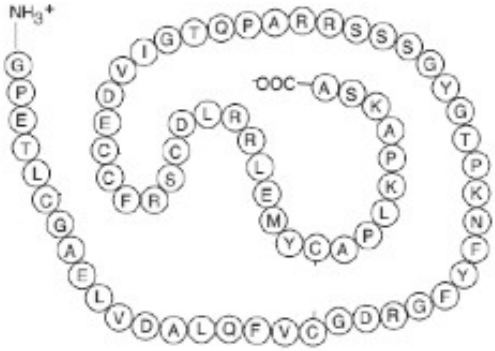


Polypeptide relationship:

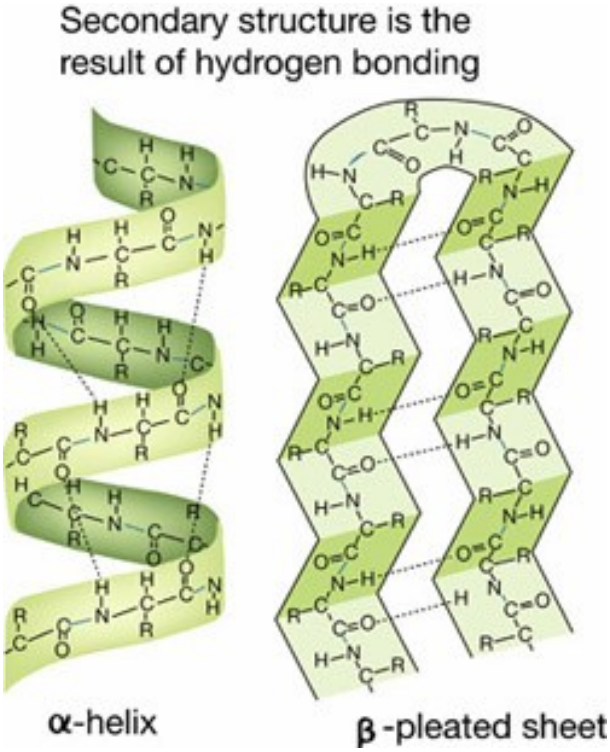
- Proteins are a polypeptide and a polypeptide is lots of amino acids joined together with lots of peptide bonds.
- Polypeptide is an unbranched sequence of monomers.
- Proteins have specific sequences, which gives specifc structure and function.

Structure:

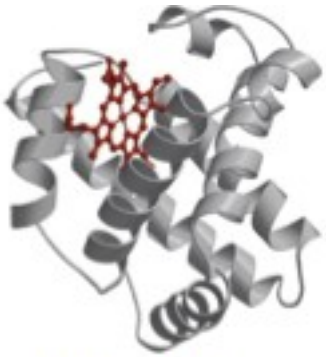
- 3D shape (Fold)
- Sequence gives structure gives function.
- Must be properly folded to have function
- 4 levels of structure:
  1) **Primary** – linear sequence of amino acids.



  2) **Secondary** – Is stabilised by hydrogen bonds between peptide bonds. Localised. Two forms – alpha helix or beta sheets (parallel [same] or anti-parallel [opposite]).

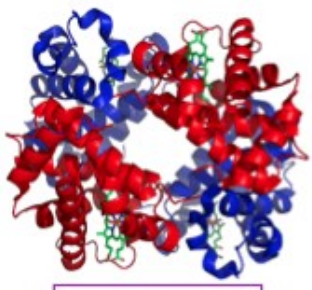


3) **Tertiary** – Overall final fold of a protein. It's a 3D shape and is also known as a globular fold. Stabilised mostly via hydrophobic exclusion (hydrophobic = inside and hydrophilic = outside). Interaction between functional groups, which are the side chains on amino acids.



Single chain
Whale myoblobin

4) **Quaternary** – Two or more polypeptides come together to make a super macromolecule. Required to be functional. Haemoglobin = tetramer (4mer). Homopolymers, different polypeptides are the same. Heteropolymers, where the polypeptide that come together to make the functional unit are different.

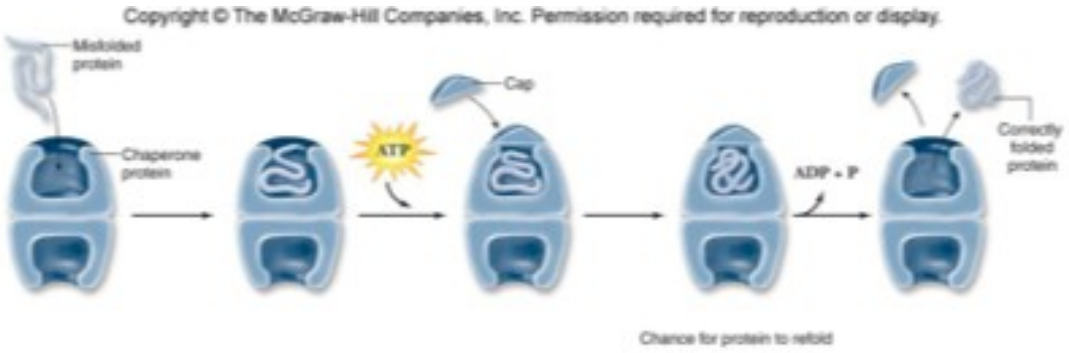


Haemoglobin:
2α globin chains
2β globin chains

- Proteins needs shape to be functional
- Synthesised off a ribosome, reading off the mRNA, which has come of DNA (genes).
- As the mRNA is read on ribosome, individual amino acids join to by peptide bonds 1 by 1. Protein folds as its synthesised. In some cases, mis-folds off the ribosome. Mis-folded doesn't have correct shape = not

functional. Chaperones help other mis-folded proteins to get to correct shape – require energy (ATP).

- Protein can denature. Lost its structure = inactive.
- Denatured – loses shape = loses function.
- Can happen in a number of ways – changing pH, raising temperature, addition of reducing agents, ionic strength of solutions, changes in polarity of solution.



Sickle Cell Anaemia

- Error in RBC
- Error in shape, they become elongated and flat. Decreased oxygen transport, can clot in blood vessels.
- Comes about due to an error in haemoglobin – tetramer, heteropolymer (two alpha and beta chains).
- Comes about due to a single amino acid change.
- Valine – Hydrophobic amino acid. Gets situated outside of protein making it reacting to water but it doesn't want to. Therefore, these valine residues interact with each other causing the molecules of haemoglobin to come together to form strands. These sit in the cytoplasm and push out the sides of the cell, which gives the "sickle" shape.

Cystic Fibrosis

- Error in ion transport
- Symptom = increase of mucous in lungs.
- Single amino acid change in the CTFR protein. This amino acid change is phenylalanine. Due to a single mutation in a codon. A codon is 3 nucleotides. This phenylalanine mutation alters the sequence, which alters the function. Function = moves chloride ions inside to outside of cell. When protein is mutated, we lose this movement. Decrease in movement of water leads to increased thickness of mucous leading to an increased risk of bacterial infections.

Enzymes:

- All enzymes are proteins.
- Not all proteins are enzymes.
- Biological catalyst – They increase reaction rates.
- Binds to and transforms specific molecules. These molecules are known as substrates. Substrates bind at the active site.
- Enzymes are specific. 1 enzyme, 1 substrate, 1 reaction.
- Named based on functions.
- "ase" means enzyme.
- Enzymes are needed for bodily processes to help maintain homeostasis.
- E.g. Amylase helps digest starch (complex carbohydrate). Used as an energy store in plants. Cellulase breaks down the cellulose cell wall of plants. Made by bacteria in the gut of cows and sheep. Lactase helps digest milk.

Enzyme Structure:

- 3D shape or a fold.
- Need shape to be functional. Hydrophobic exclusion – balance between hydrophobic and hydrophilic amino acids and interaction with water.
- Enzymes are selective. Only bind to 1 substrate.
- Enzymes are specific- catalyse 1 reaction.
- Occurs at active site. Match the shape of the substrate, size and hydrophobicity and hydrophilicity state of substrate.

Enzyme Requirements:

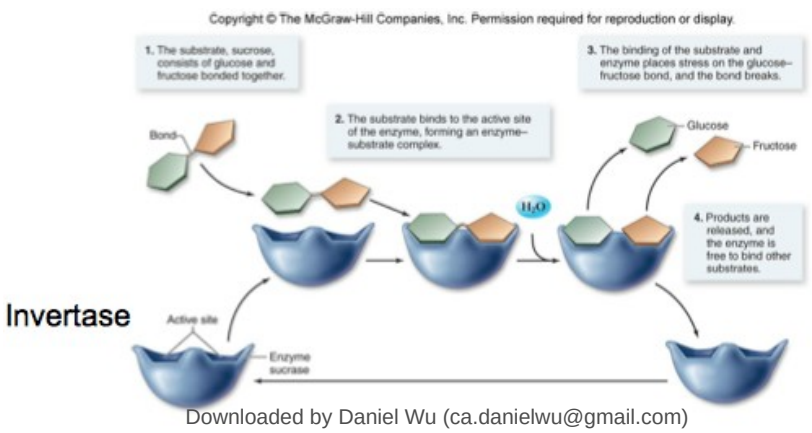
- Specific to their environment.
- E.g. Ionic strength (salt dissolved in solution), organic or aqueous solution, pH, temperature.

Induced Fit – Not exact fit. Meaning there is molecular movement or a conformational change, which is a change in 3D shape. Non-substrates cannot induce this fit.

Reaction rate increases and activation energy decreases. Therefore providing another path for the reaction to go through by stabilising the high-energy state intermediate (transition state). By doing this, we decrease activation energy, and an increase in reaction rate.

Catalysis.

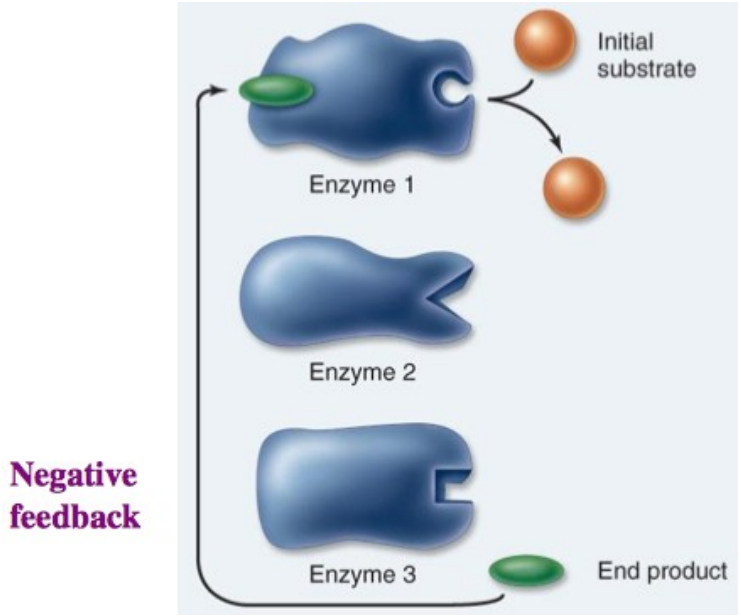
Enzymes don't partake in reaction, always regenerated.



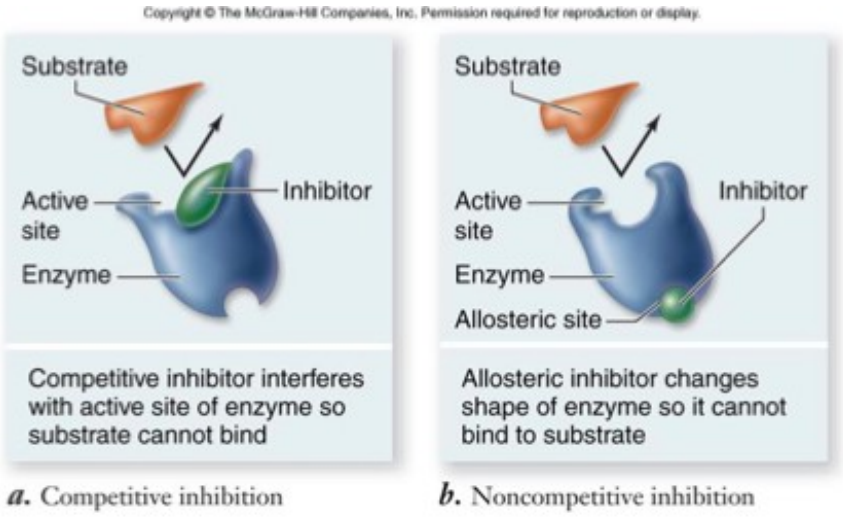
Enzymes are molecular machines. Within cell, many reactions occur at the same time.

Enzyme Regulation:

- Negative feedback control – turns off a process.
- End product acts on enzyme 1 to turn it off. Turns it off by a conformational change in the catalytic site.
- Allosteric site meaning “other”. End products acts like this. Allosteric modulation means the end product binds on a different enzyme at a different site, alters the catalytic site, now the initial substrate cannot bind.



Enzyme Inhibition:



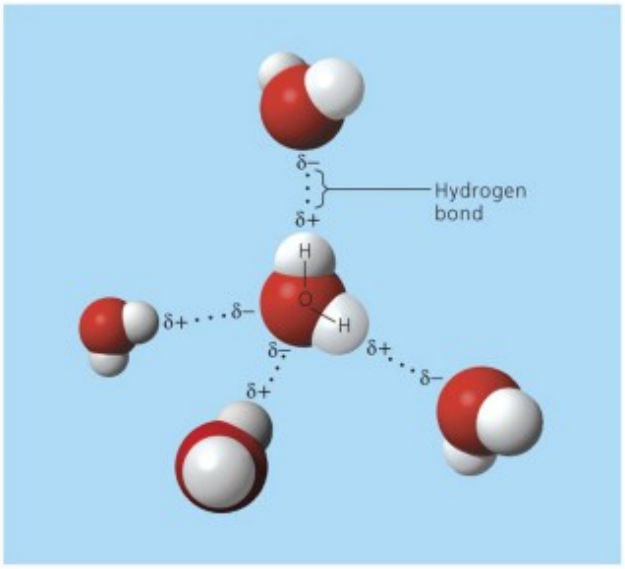
a. Competitive inhibition *b.* Noncompetitive inhibition

Cytoskeleton:

Lecture 7 – Water and Buffers in Cells

- <u>Water</u>

- Polar molecule ~ opposite ends have opposite charges.
- Polarity allows water molecules to form hydrogen bonds with each other.



▲ **Figure 3.2 Hydrogen bonds between water molecules.** The charged regions of a polar water molecule are attracted to oppositely charged parts of neighboring molecules. Each molecule can hydrogen-bond to multiple partners, and these associations are constantly changing.

- In biomolecules, H-bonds form within the same molecule, or between molecules to maintain stability, structure and/or activity.
- <u>Four properties</u>:
  1) *Cohesion behaviour*
  2) *Ability to moderate temp.*
  3) *Expansion upon freezing.*
  4) *Versatility as a solvent.*

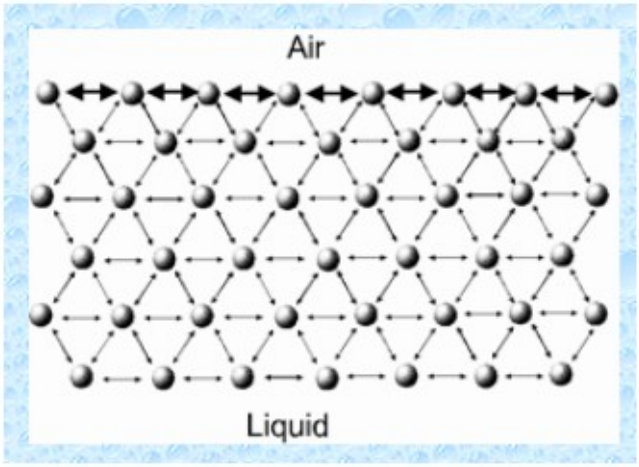
Cohesion

- H-bonds hold water molecules together ~ cohesion.
- Helps transport of water against gravity in plants.
- **Adhesion** is an attraction between different substances (water and plant cell walls).

Surface Tension

- Measure of how hard it is to break the surface of a liquid.
- Related to cohesion.
- Property of the surface of a liquid.
- Water have strong intermolecular interactions = high surface tensions.

- Imbalance of forces at the surface so there will be a net attractive force towards the bulk. The air/water interface will spontaneously minimise its area and contract.
- Common units for surface tension = mN/m
- Any factor, which decreases the strength of this interaction, will lower surface tension. E.g. raising temperature.



Moderation of Temperature

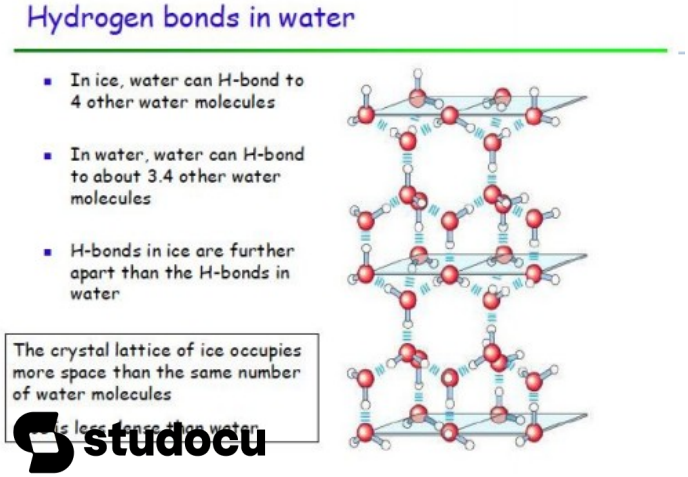
- Water absorbs heat from warmer air and releases it to cooler air.
- Can absorb large amounts with only a slight change to its own temperature.
- The specific heat of a substance is the mount of heat that must be absorbed or lost for 1g of that substance to change its temperature by 1 degree Celsius. Water = 1cal/g/degree Celsius.
- Water has high specific heat.
- Heat is absorbed when hydrogen bonds break.
- Heat is absorbed when hydrogen bonds form.

Evaporative Cooling

- Liquid to gas.
- Heat of vaporisation is the heat a liquid must absorb for 1g to be converted into gas.
- As a liquid evaporates, its remaining surface cools = helps stabilise temperature in organisms.

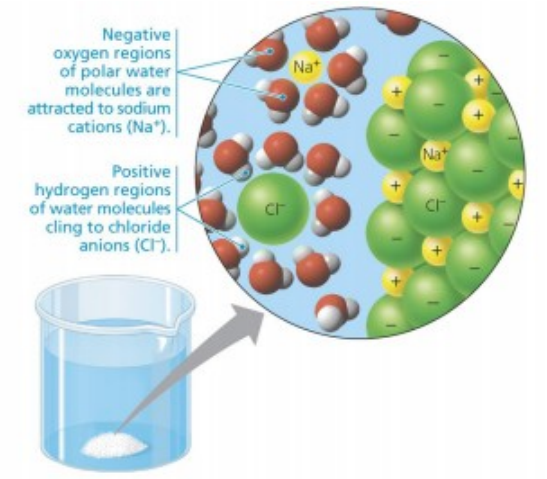
Insulation of bodies of water by floating ice

- Ice floats in liquid because hydrogen bonds in ice are more ordered, making ice less dense.



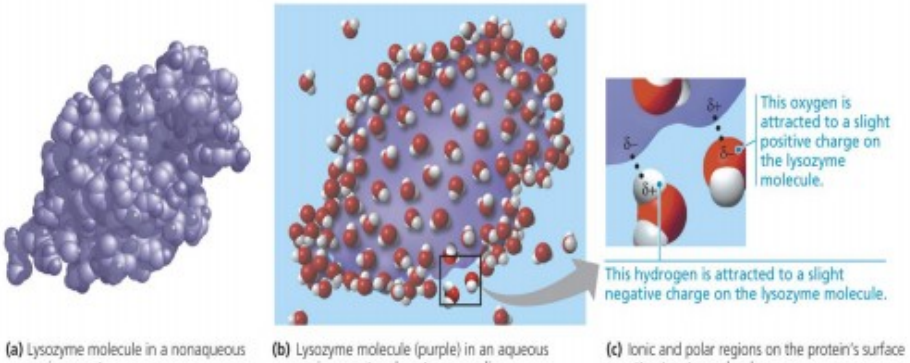
The Solvent of Life

- A solution is a liquid that is a homogenous mixture of substances.
- A solvent is the dissolving agent of a solution.
- The solute is the substance that is dissolved.
- An aqueous solution is one in which water is the solvent.
- Water is a versatile solvent is due to its polarity, which allows it to form hydrogen bonds easily.
- When an ionic compound is dissolved in water, each ion is surround by a sphere of water molecules called a hydration shell (hydrating the ions).



▲ **Figure 3.7 Table salt dissolving in water.** A sphere of water molecules, called a hydration shell, surrounds each solute ion.
? *What would happen if you heated this solution for a long time?*

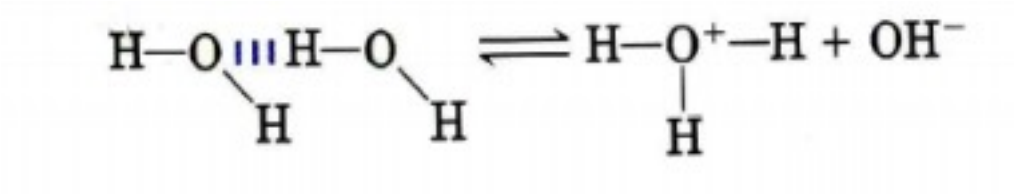
- Water can dissolve compounds of non-ionic polar molecules.
- Large polar molecules such as proteins can dissolve in water, if they have ionic and polar regions.
- Colloid = a stable suspension of fine particles in a liquid.
- Water is partially ionised ~ property of importance to biomolecules.
- Water is a metabolite ~ degradation of glucose in metabolism and energy production by photosynthesis.



▲ **Figure 3.8 A water-soluble protein.** This figure shows human lysozyme, a protein found in tears and saliva that has antibacterial action.

Acidic and basic conditions affect living organisms

- A hydrogen atom in a hydrogen bond between two molecules, can shift from one to another:
  ~ The hydrogen atom leave its electron behind and is transferred as a proton, or hydrogen ion (H+)
  ~ The molecule with the extra proton is now a hydronium ion (H3O+), although it is often represented as H+.
  ~ The molecule that lost the proton is now a hydroxide ion (OH-)



- Water = in a state of dynamic equilibrium. Molecule dissociate at the same rate they are reformed.
- Changes in H+ and OH- can drastically affect the chemistry of a cell ~ changes the pH.
- pH scale.

Biological Systems

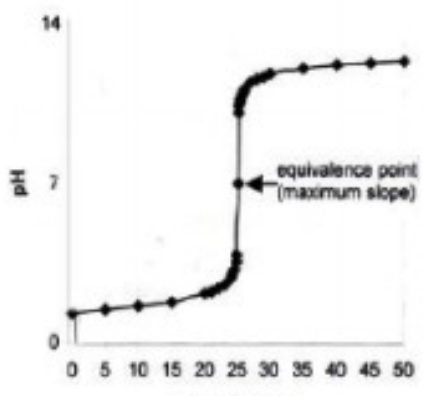
- pH dependant.
- Acid = increase H+
- Bases = Decrease H+
- Contain mainly weak acids and bases.
- Ionic state is dependant on surrounding pH.

Dissociation of compounds

- Strong acids completely dissociate in water.
  - HCl
  - H2SO4
- Strong bases completely dissociate in water.
  - NaOH
  - KOH

Titration Curves

- Measures amount of acid in a solution.
- Uses a known concentration of base to find out the concentration of the acid in the original solution.



Buffers

- Substances that minimise changes in concentration of H+ and OH- in a solution.
- Consist of an acid-base pair that reversibly combines with H+.
- Buffers resist changes in pH.
- Buffers are mixtures of weak acids and their conjugate (matches) bases.
- Aqueous solutions.
- Effective between 10% and 90% neutralisation of the proton donor species.
- E.g. **Buffer in blood**
  ~ carbonic acid (acid) and bicarbonate (base).
  ~ Increased H+ (lactic acid) = increase H_2CO_3.
  ~ Leads to increased CO_2 in the blood plasma.
  ~ Leads to increased CO_2 in lungs.
  ~ CO_2 is exhaled.
  ~ the rate of inhaling or exhaling CO_2 adjusts quickly and keeps blood pH constant.

Lecture 8 – Cellular Processes, Respiration, Photosynthesis

- Energy Flow

- Autotroph (plant)
  Photosynthesis and **Respiration**.
- Heterotroph (animal/fungus)
  Respiration (not breathing) = oxidation of organic compounds to release energy (ATP).
- We use energy for active processes. Expend energy in the form of atp in order to get something done. E.g. making complex molecules from monomers.
- Energy for movement. E.g. segregation of chromosomes, muscle contraction.
- Active transport uses energy. Moving compounds or ions against their concentration gradient.
- Energy for production of heat = Body uses the law of thermodynamics.

- Thermodynamics

1st LAW = Energy can neither be created nor destroyed but it can be transferred or transformed.
2nd LAW = The universe tends to disorder (entropy).

- Gibbs Free Energy

"The energy available to do work."
Measured as g. Change in free energy = change in heat – temperature in Kelvin x change in entropy.

$$\Delta G = \Delta H - T\Delta S$$

Favourable reaction	Unfavourable reaction
$\Delta H < 0$	$\Delta H > 0$
$\Delta S > 0$	$\Delta S < 0$
$\Delta G < 0$	$\Delta G > 0$

Favourable = Spontaneously (not instantaneous). Will go forward without energy input.
Unfavourable = Not spontaneous. Need energy to go in in order for the reaction to go forward.

Endergonic Reaction

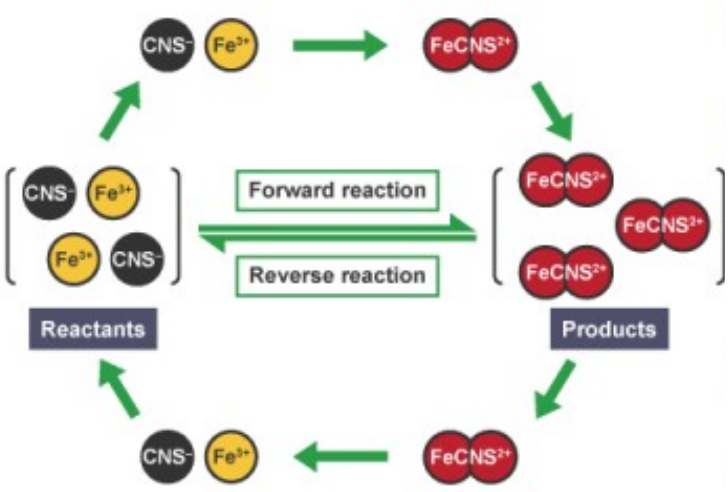
- Unfavourable – not spontaneous.
- Reaction favours reactants.
- +ve Delta G
- Delta G > 0

Exergonic Reaction

- Energy is released
- Favourable – Spontaneous (no energy is needed for the reaction to go forward).
- -ve delta G
- Delta G < 0

- Equilibrium

- Reversible reaction.
- Does not go to completion.



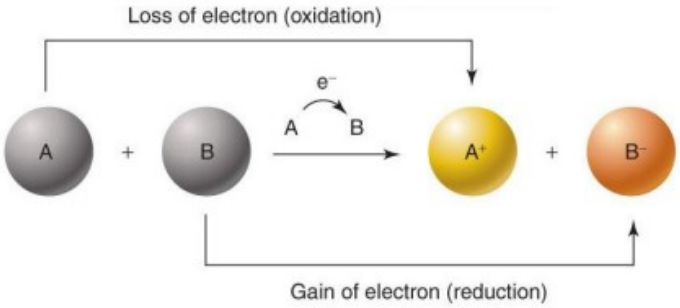
- No net change. No change in amount of products and reactants. Occurring at the same rate ~ Dynamic equilibrium (forward and backward reaction at same rate).

- Le Chatelier's Principle

" When a system at equilibrium is changed, the system adjusts to absorb that change."

- Oxidation and Reduction

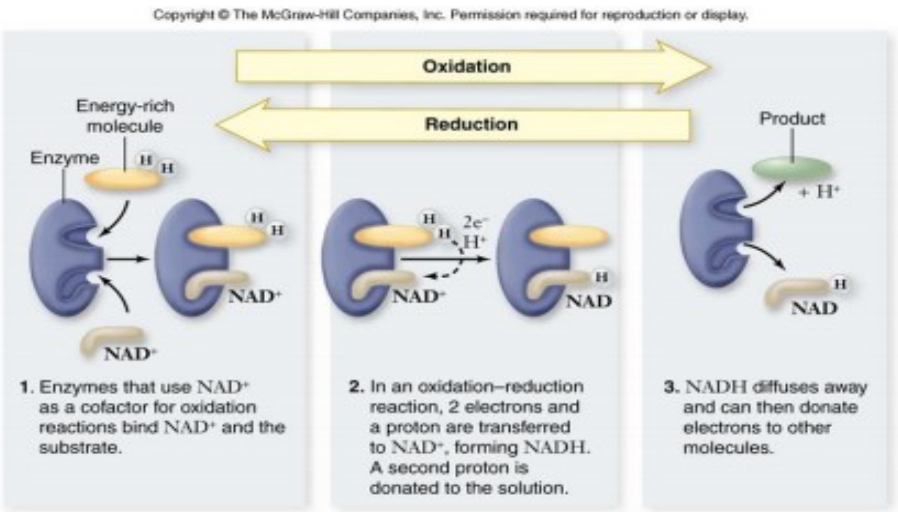
Oxidation = Loss.
Reduction = gain.



- Biological Oxidation and Reduction

Hydride Ions H+.

Oxido-reductase = enzyme that carries out reduction and oxidation. E^- transfer.

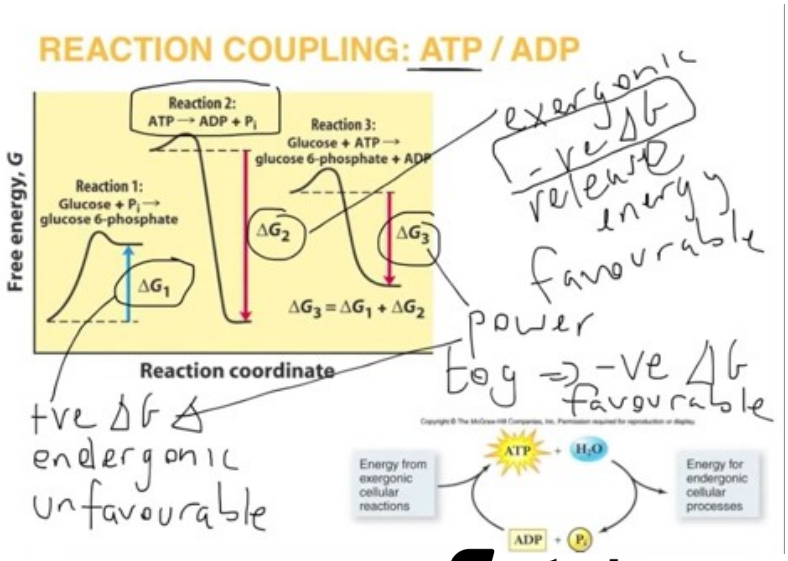


NAD (oxidised) = Nicotinamide Adenine Dinucleotide. Used in catabolic processes (breaking down).
NADH (reduced) = Picked up electrons in form of hydride ion.

- Reaction Coupling

ATP = Adenosine Triphosphate – 3 phosphates
ADP = Adenosine Diphosphate – 2 phosphates
AMP = Adenosine Monophosphate – 1 phopshate
All negatively charged.

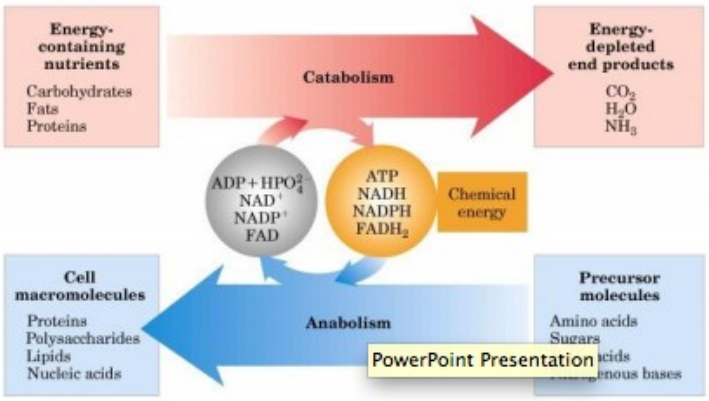
Hydrolysis of ATP to make ADP has –ve Delta G
Hydrolysis of ATP can be used to power other reactions.



- Metabolism

Sum of all reaction occurring in your body at any 1 time.

- Catabolism (Complex – simple) Release energy
- Anabolism (simple – complex)



- Respiration

Harvest energy from chemical compounds
Complete oxidation of glucose.
Organic glucose gets oxidised to power ATP synthesis.

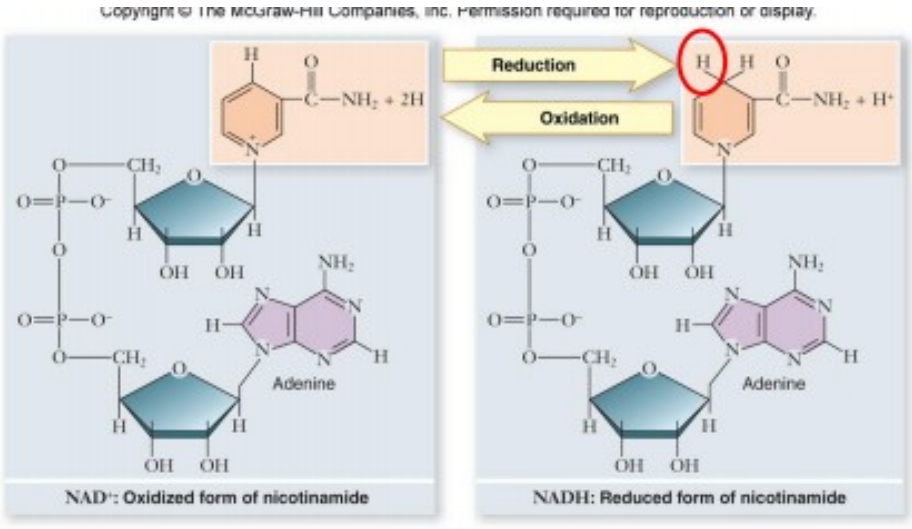
- Catabolism of Glucose

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

- Making ATP

2 ways:

1) Substrate level phosphorylation
2) Oxidative phosphorylation (uses electrons) NADH –- NAD (loses electron)



- Fates of Glucose

Glucose has multiple uses.

- Energy store e.g. glycogen is animals and starch in plants.
- Oxidation via glycolysis
- Oxidation via pentose phosphate pathway.

- Cellular location (eukaryotes)

Mitochondrion – citric acid cycle, electron transport chain, oxidative phosphorylation.
Cytoplasm – Glycolysis.

- Glycolysis

Anaerobic (without oxygen).
Occurs in cytoplasm.
6 carbon glucose convert to 2 x 3 carbon pyruvate.
4 ATP get made. 2 are needed to go in. 2 ATP net gain.
Make reduced electron carriers.

- Pyruvate Dehydrogenase Complex

Within matrix, inside mitochondria.
Quaternary structure.
3 different reactions, using 3 enzymes.

- E1 (pyruvate dehydrogenase)
- E2 (dihydrolipoyl transacetylase)
- E3 (dihydrolipoyl dehydrogenase)

Respiration Pt 2 and 3.. to be summarised...

- Photosynthesis

Occurs in the chloroplast. Has a double membrane. Inside membrane is a highly folded is the thylakoid membrane. Thylakoid is folded into grana. Inside grana is the matrix or stroma.
Occurs in 3 stages:

1) Capturing light energy.
2) Converted into chemical energy.
3) Chemical energy used to make organic carbon compounds.

$$6CO_2 + 6H_2O \xrightarrow{\text{Light}} C_6H_{12}O_6 + 6O_2$$

$$\Delta G^{o} = 2840 \text{ kJ Mol}^{-1}$$

Inorganic carbon (oxidised) to organic carbon (reduced).

- Chlorophyll

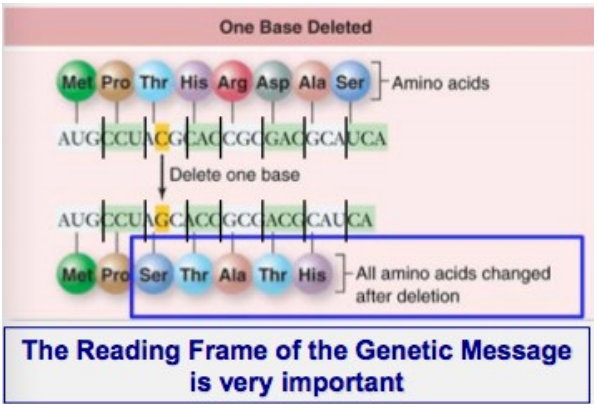
Chlorophyll absorbs light energy.
400-500nm range.
600-700nm range.
Reflect light 500-600nm range which is why we see plants as green.
Pigment apart of the thylakoid membrane.
Consists of a porphyrin ring, which captures light energy and uses it to transfer to electrons raising the energy of the electron.

Lecture 9 – Transcription and Translation

Messenger RNA (mRNA) – is transcribed from a segment of DNA (a gene) – carries the message of that gene to the protein-making machinery of the cell.
Ribosomal RNA (rRNA) – are part of the ribosome, the "machinery" that translates the mRNA into a protein sequence.
Transfer RNA (tRNA) – carries individual amino acids into the ribosome to be assembled into a protein.
DNA is TRANSCRIBED to make mRNA, which is TRANSLATED to make a protein.
Transcription – the flow of information from DNA (the gene) to RNA.
Translation – the flow of information from RNA to protein.

Codons (sets of 3 nucleotides) specify the different amino acids.
64 codons in total.
3 codons are stop codons.
AUG is the start codon.
Some amino acids are encoded by more than one codon.

The genetic code is read in groups of 3.

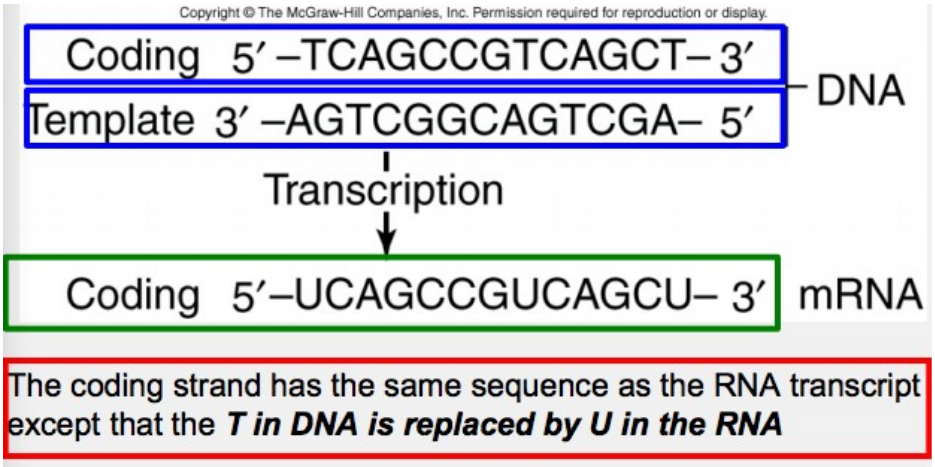


- Transcription and the production of RNA

3 steps:

1) Initiation – RNA polymerase identifies where to begin transcription of the DNA template.
2) Elongation – RNA nucleotides are added to the 3' end of the new RNA.
3) Termination – RNA polymerase stops transcription when it encounters terminators in the DNA sequence.

Transcription is the DNA-directed synthesis of RNA.
Only 1 of the 2 strands of DNA is copied (template strand).
The nucleotide sequence of the RNA copy is complementary to the template strand sequence.
The RNA transcript used to direct synthesis of proteins is messenger RNA (mRNA).



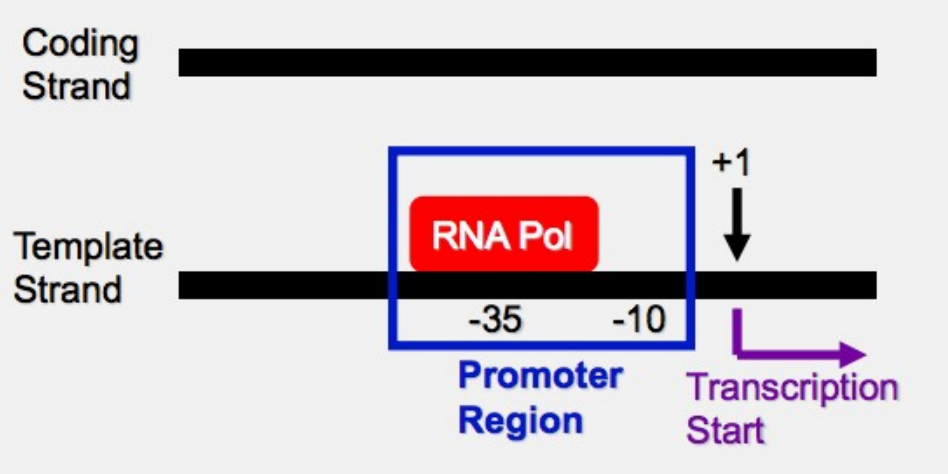
- Transcription in Prokaryotes

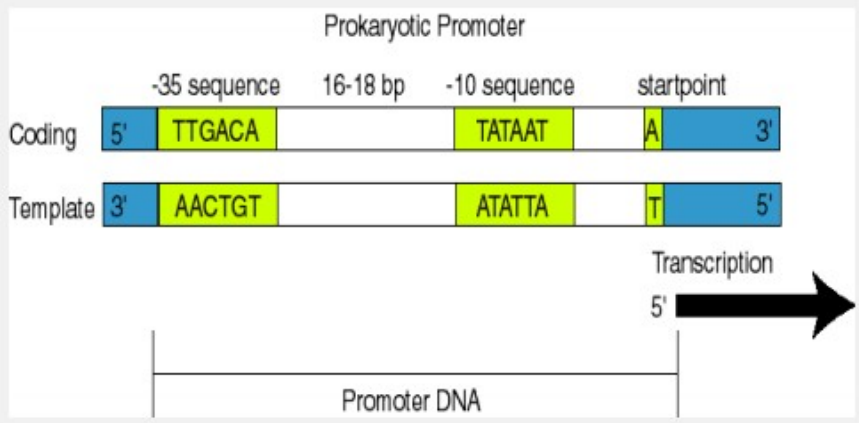
RNA polymerase found in 2 forms.

- Holoenzyme – Composed of the core enzyme and the sigma factor, which is required for transcription initiation (5 subunits).
- Core polymerase – Capable of RNA elongation but not initiation (4 subunits).

Step 1) – Initiation
Requires 2 sites:
1 – The promoter – A DNA sequence for binding of RNA polymerase.
2 – The start site – first base to be transcribed.

A transcriptional unit extends from the promoter to the terminator (signal to end transcription).
The promoter is a short sequence occurring before the start site.
Two 6-base sequences common to prokaryotic promoters.
-35
-10
Once bound to the promoter, RNA polymerase begins to unwind the DNA helix at the -10 site.





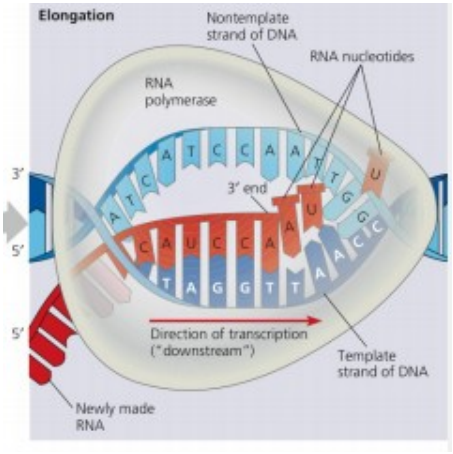
Step 2) – Elongation
RNA strand grows in the 5' to 3' direction as ribonucleotides are added to the growing RNA chain.
During transcription, DNA is unwound by RNA polymerase and then rewound behind the enzyme.
The unwound region called the transcription bubble.
Transcription bubble consists of:

1) DNA template.
2) RNA polymerase.
3) Growing RNA transcript.

Transcription bubble moves down the DNA template at a rate of 50 nucleotides/sec.

Growing RNA strand protrudes from the bubble.

The transcribed DNA is rewound after the bubble passes.



Step 3) – Termination

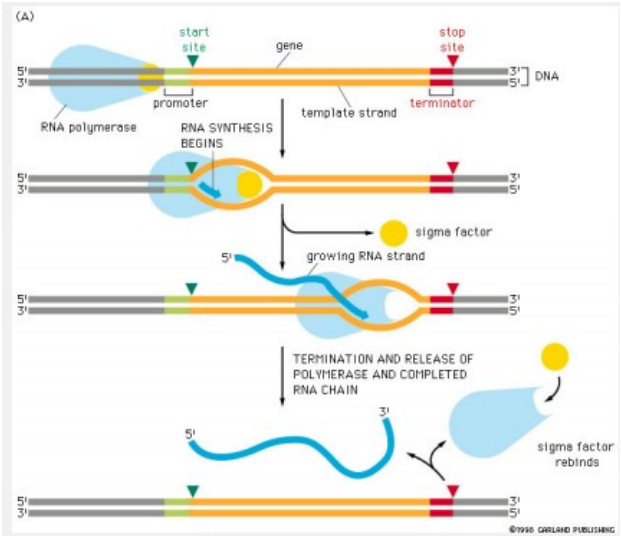
Transcription stops when the transcription bubble encounters terminator sequences.

This includes a series of G-C base pairs followed by a series of A-T base pairs.

RNA transcript can form a double stranded structure called a hairpin in the GC region.

Formation of the hairpin causes RNA polymerase to pause placing it directly over the run of 4 uracils.

Pairing of U with A is weak and RNA strand dissociates from DNA.



- Transcription in Eukaryotes

3 RNA polymerases

- I – Transcribes rRNA
- II – Transcribes mRNA and snRNA.
- III – Transcribes tRNA and some other small RNA's.

Initiation requires as series of transcription factors (proteins that act to bind RNA polymerase to the promoter and initiate transcription).
Termination differs from that in prokaryotes.
After transcription in the nucleus, the primary transcript (pre-mRNA) produced is modified by:

1) Addition of a 5' cap (7-methyl-guanosine).
2) Addition of a 3' poly-A tail.
3) Removal of non-coding sequences (introns).

- Translation and Protein synthesis

Translation also proceeds in 3 stages:

1) Initiation – mRNA, tRNA, and the ribosomes come together.
2) Elongation – tRNA's bring amino acids to the ribosome for incorporation into the new polypeptide.
3) Termination – Ribosome encounters a stop codon and releases the polypeptide.

More complex than transcription.
Uses tRNA as an adaptor molecule interacting with both RNA and amino acids.
Translation takes place on the ribosome, the cellular protein synthetic machinery.

Structure and role of tRNA

- tRNA molecules carry amino acids to the ribosome for incorporation into a polypeptide.
- Aminoacyl-tRNA synthetases (there are 20 different ones, specific) are enzymes that add amino acids to the acceptor arm of tRNA.
- The anticodon loop contains 3 nucleotides complementary to mRNA codons.

Ribosome

- Multiple binding sites
  1. Aminoacyl site – binds the tRNA carrying the amino acid to be added to the growing peptide.
  2. Peptidyl site – binds the tRNA to which is attached the growing peptide chain.
  3. Exit site – binds he tRNA that carried the last amino acid to the growing peptide chain.
- Structure
  - 2 subunits and 3 tRNA binding sites.
  - Subunits are complexes of ribosomal RNA molecules and proteins.
- Role

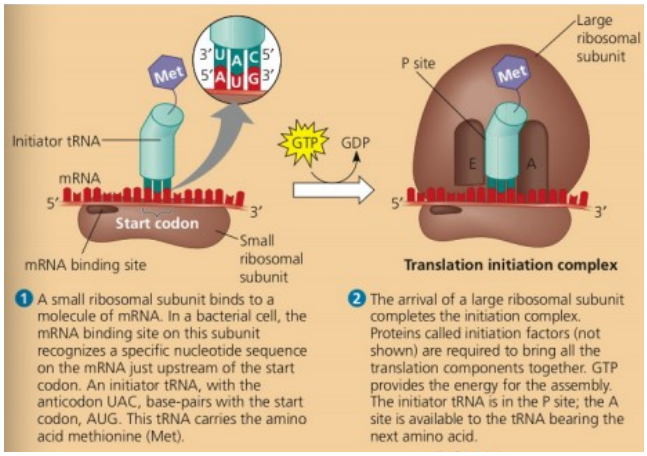
- To decode the mRNA.
- Form peptide bonds between amino acids.

Step 1 – Initiation

In prokaryotes, initiation of translation requires the formation of the initiation complex including:

- An initiator tRNA charged with N-formylmethionine
- The small ribosomal subunit
- mRNA strand (start codon)

The ribosomal binding sequence of mRNA is complementary to part of the rRNA sequence.

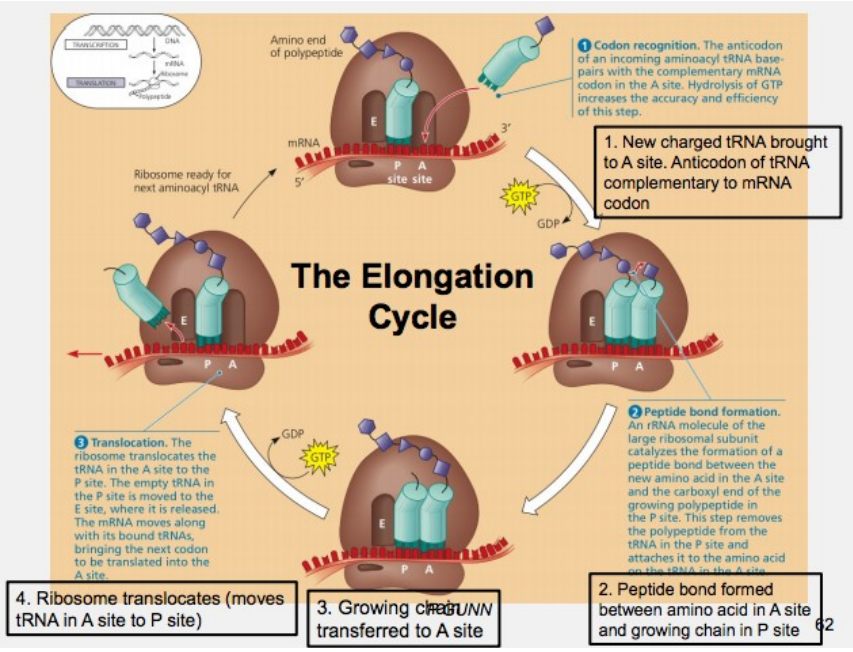


Step 2 – Initiation

Involves the addition of amino acids.

1. Charged tRNA bind to the A site if its anticodon is complementary to the mRNA codon at the A site.
2. Peptidyl transferase forms a peptide bond between the amino acids on adjacent ribosome binding sites.
3. Ribosome moves down the mRNA in a 5’ to 3’ direction 3 nucleotides at a time.

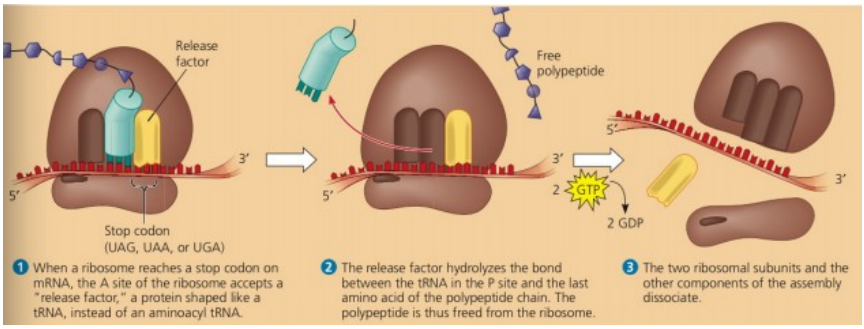
Step 3 –



Termination

Elongation continues until the ribosome encounters a stop codon.

Stop codons are recognised by release factors, which release the polypeptide from the ribosome.



Lecture 10 – Genetics

- Traits

Traits = Inherited characteristic of an individual.

Mendel used the scientific approach to identify two laws of inheritance

1) Principle of Segregation
2) Principle of Independent Assortment

Mendel produced true-breeding pea strains for 7 different traits

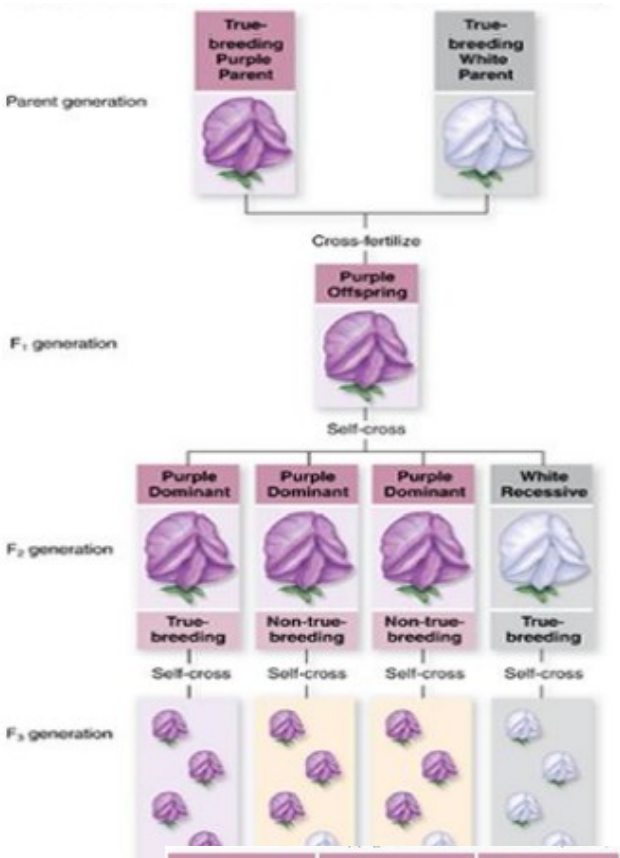
- Each trait had alternate forms (e.g. purple and white flower colour)
- Mendel cross-fertilized the 2 true-breeding strains for each trait.

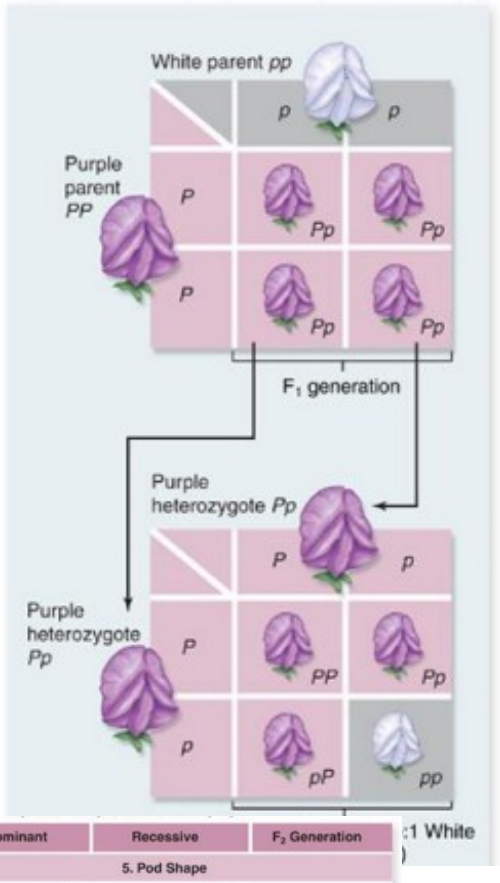
Monohybrid crosses

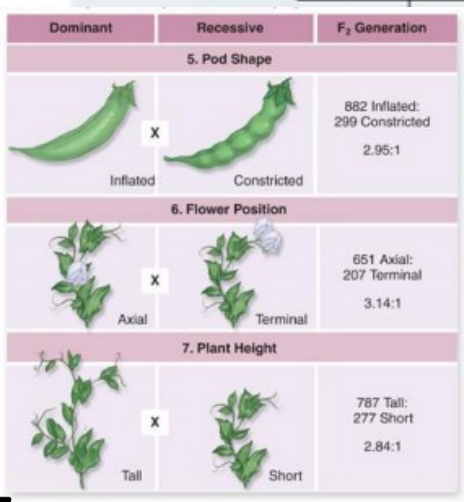
- F1 generation: offspring produced by crossing 2 true breeding strains.
- F2 generation: offspring resulting from the self-fertilization of F1 plants.

Terminology

- Gene: information for a trait passed from parent to offspring.
- Alleles: alternate forms of a gene.
- Genotype: total set of alleles of an individual.
- Phenotype: outward appearance of an individual.







Mendel's first Law of Inheritance:
Principle of Segregation

- Two alleles for a gene segregate during gamete formation and are re-joined at random, one from each parent, during fertilization.

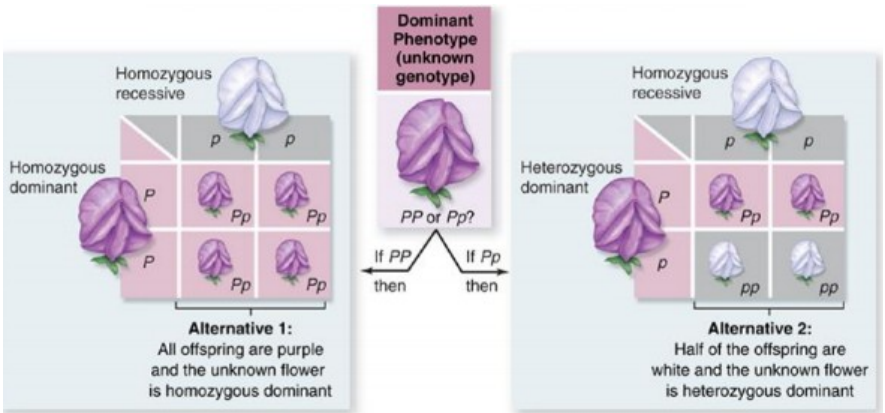
Dihybrid crosses: Examination of 2 separate traits in a single cross. E.g. RR YY x rr yy.
The F1 generation of a Dihybrid cross (RrYy) shows only the dominant phenotypes of each trait.
The F2 generation is produced by crossing members of the F1 generation with each other or allowing self fertilization of the F1/ E.g. RrYy x RrYy
Ratio – 9:3:3:1

Mendel's 2nd Law of Inheritance:
Principle of Independent Assortment

- In a Dihybrid cross, the segregation of allele pairs for different genes is independent.

Testcross: a cross used to determine the genotype of an individual with dominant phenotype.

- Crossed the individual with unknown genotype (e.g. P_) with a homozygous recessive (pp).
- The phenotypic ratios among offspring are different, depending on the genotype of the unknown parent.



Polygenic Inheritance occurs when multiple genes are involved in controlling the phenotype of a trait.
Pleiotropy refers to an allele, which has more than one effect on the phenotype.

- Chromosomes, Mapping, and the Meiosis-Inheritance Connection.

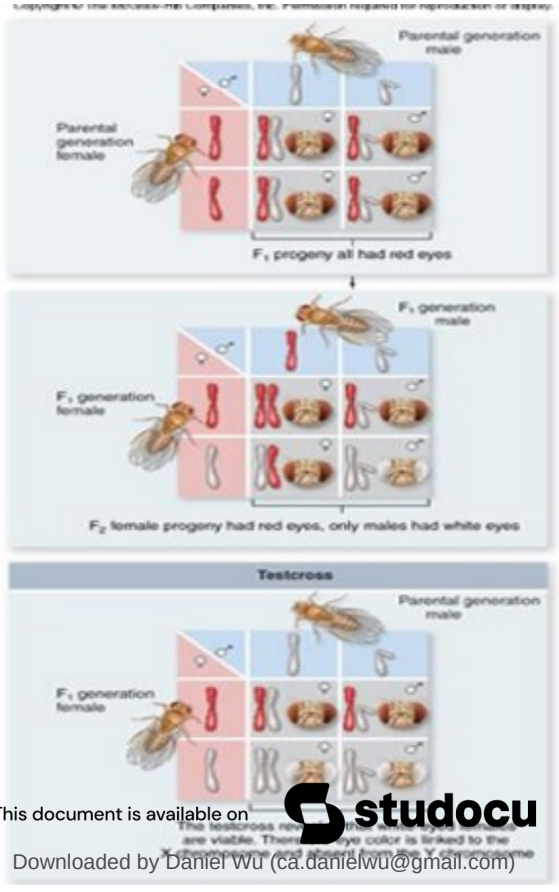
Genes are located on chromosomes.
The location of a particular gene can be seen by tagging isolated chromosomes with a fluorescent dye that highlights the gene.

Chromosome Theory

- Chromosomal Theory of Inheritance.
- Sutton and Boveri both independently proposed that genes are present on chromosmes.

Morgan and Fruit flies

- Isolated a mutant white-eyed drosophilia.
- Red-eyed female X white-eyed male gave a F1 generation of all red eyes.
- Concluded red eyes are dominant.
- Morgan crossed F1 females X F1 males.
- F2 generation contained red and white-eyed flies but all white-eyed flies were made.
- Testcross of a F1 female with a white-eyed male showed the viability of white-eyed females.
- Morgan concluded that the eye colour gene is linked to the X chromosome.



Sex Chromosomes

- Sex determination in humans is based on the presence of a Y chromosome.
- XX chromosomes = female
- XY chromosomes = male
- Sex linked traits: controlled by genes present on the X chromosome.
- Sex-linked traits show inheritance patterns different than those of genes on autosomes.

- Evolution

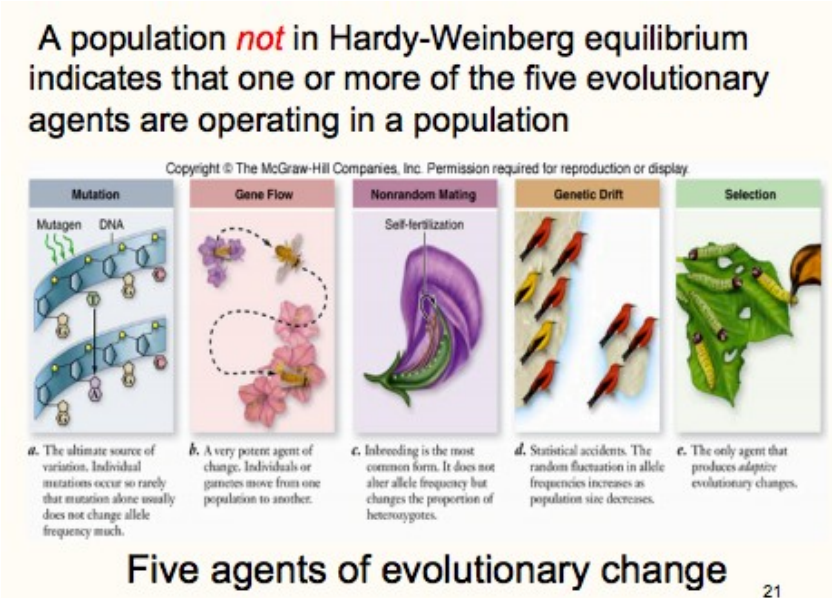
Natural Selection – If some individuals in a population, which possess certain inherited characteristics, produce some surviving offspring than ones lacking these, the population slowly changes to includes more individuals with these specific advantageous characteristics.
3 conditions for natural selection to occur:

1) Variation must exist among individuals in a population.
2) Variation among individuals must result in differences in the number of offspring surviving.
3) Variation must be genetically inherited.

Polymorphisms – Many allelic forms.

Hardy-Weinberg Principle (equilibrium)
Concluded that the original proportions of alleles in a population would remain constant from generation to generation provided that:

- No mutation takes pace
- No genes are transferred to or from other sources
- Random mating is occurring
- The population size is very large
- No selection occurs.



Lecture 11 – Cell Communication

4 Basic mechanisms for cell communication:

1) Direct contact – molecules on the surface of one cell are recognised by receptors on the adjacent cell.
2) Paracrine signalling – signal released from a cell has an effect on neighbouring cells.
3) Endocrine signalling – hormones released from a cell affect other cells throughout the body.
4) Synaptic signalling – nerve cells release the signal (neurotransmitter) which binds to receptors on nearby cells.

Communication between cells requires:

- Ligand - signalling molecule.
- Receptor protein – the molecule to which the receptor binds.

Receptor Types:

- Intracellular Receptor – located within cell.
- Cell surface or Membrane Receptor – locate on the plasma membrane to bind a ligand outside the cell.
    - Ion Channel Linked Receptors – opens in response to a ligand/when the receptor changes shape. When a signal molecule

binds as a ligand to the receptor, the gate allows specific ions through a channel in the receptor.

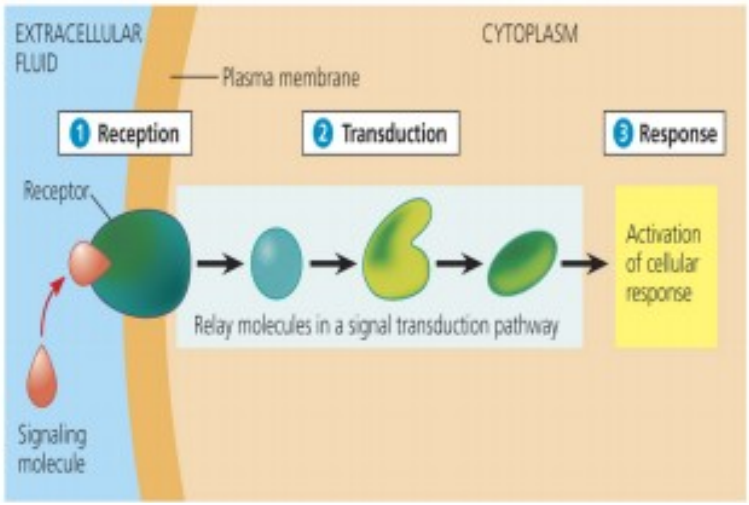
- Enzyme Linked Receptors/Receptor tyrosine kinases - Receptor is an enzyme that is activated by the ligand. Membrane receptor. When bound by a ligand, the receptor is activated by dimerization and autophosphorylation. Activated receptor adds a phosphate to tyrosine on a response protein.
- G protein-coupled Receptors – Assists in transmitting the signal. Plasma membrane receptor protein that works with the help of a G protein.

Reception: a signal molecule binds to a receptor protein, causing it to change shape.

- Binding of ligand and receptor is highly specific.
- A change in shape of the receptor is often the initial transduction of the signal. Signal transduction = the events within the cell that occur in response to a signal. A signal transduction pathway is a series of steps by which a signal on the cell surface is converted into a specific cellular response.
- Most signal receptors are plasma membrane proteins.
- Different cell types can respond differently to the same signal.

- Cell Signalling

1) **Reception**
2) **Transduction**
3) **Response**



Effects of Insulin Binding:

- Insulin promotes glucose transport from the bloodstream across the cell membrane to the cytoplasm of the cell in most cells except brain cells.
- The insulin receptor is a tyrosine kinase enzyme.
- After glucose enters a cell, insulin binding to its receptor triggers enzymatic activity that:
  - Catalyzes the oxidation of glucose for ATP production.

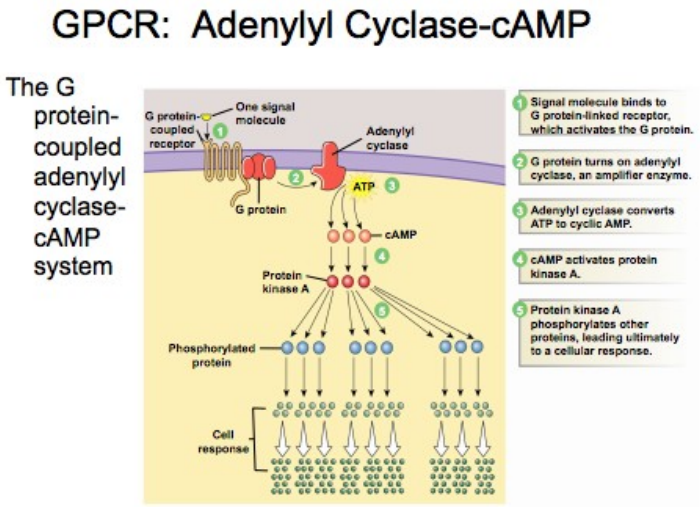
- Polymerizes glucose to form glycogen.
- Converts glucose to fat.

Receptor Kinases

Kinase cascade – a series of protein kinases that phosphorylate each other in succession. Amplifies the signal because a few signal molecules can elicit a large cell response.

G-Protein Coupled Receptors

Effector protein produces a second messenger. The second messenger generates the cellular response to the original signal. One common effector protein is **adenylyl cyclase,** which produces **cAMP** as a second messenger from ATP by dropping off two phosphates. Other second messengers; inositol phosphates, calcium ions.



Cell-Cell Interactions

Cells within tissue are connected to each other by cell junctions.

- Tight junctions: create sheets of cells.
- Anchoring Junctions: Connect the cytoskeletons of adjacent cells.
- Communicating junctions: Permit small molecules to pass between cells.
  - Gap junctions – In animal cells.
  - Plasmodesmata – In plant cells.

Signal Transduction Pathways

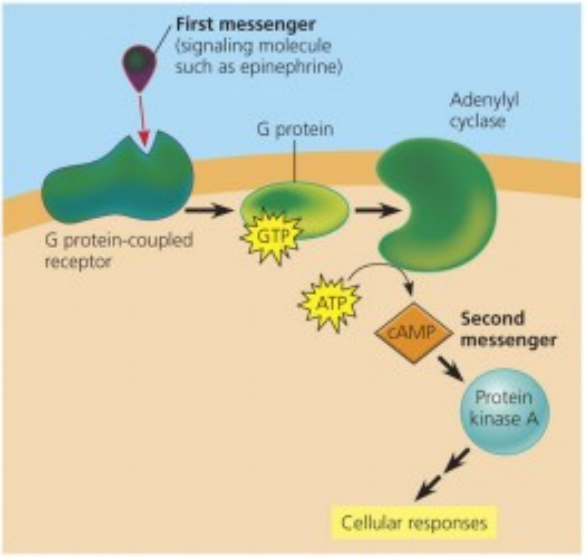
At each step the signal is transduced into a different form, usually a shape change in a protein. In many pathways, the signal is transmitted by a cascade of protein phosphorylations.

- Protein kinases transfer phosphates from ATP to protein (phosphorylation).

- Protein phosphatases remove the phosphates from proteins, a process called dephosphorylation.

These two processes act as molecular switches, turning activities on and off.

cAMP – usually activates protein kinase A, which phosphorylates various other proteins.



▲ **Figure 11.11 cAMP as a second messenger in a G-protein-signaling pathway.** The first messenger activates a G protein-coupled receptor, which activates a specific G protein. In turn, the G protein activates adenylyl cyclase, which catalyzes the conversion of ATP to cAMP. The cAMP then acts as a second messenger and activates another protein, usually protein kinase A, leading to cellular responses.