Feralis
Biology Notes

Hi! My name is Feralis. I'm a content expert in DAT Biology, and I want to help you ace this section on your DAT.

I took the DAT and scored a 30 in Bio - these are the notes that helped me get there. These notes were designed and written exclusively for students preparing for the DAT.

Students find biology to be one of the most difficult sections to prepare for due to the breadth of information out there. Fear not! I've gone through and picked out exactly what you need to study for the DAT.

If you have any feedback or questions, please email me at feralisbionotes@gmail.com. Your feedback is invaluable to helping me further improve these notes for future generations of pre-dent students.

Feralis Biology Notes

2018 DAT Biology Notes

Table of Contents

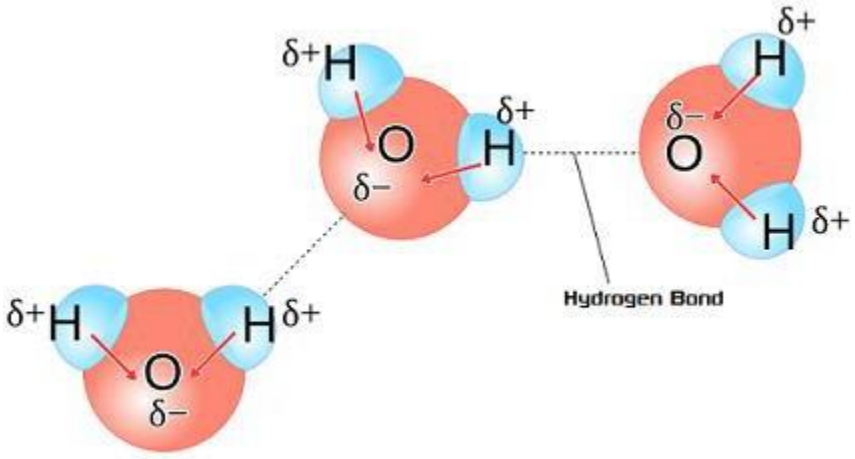
Chapter 1: Molecules and Fundamentals of Biology

Atoms & Bonds

- An **atom** is made up of neutrons, protons, and electrons.
- **Molecules** are groups of 2 or more atoms joined via chemical bonds. Chemical bonds are due to electron interactions.
- **Electronegativity** defines the ability of an atom to attract electrons.
- Ionic and covalent bonds are **intramolecular**, whereas hydrogen bonds are **intermolecular**.

Types of Bonds

Bond Type		Description	Electronegativity of Atoms	Example
IONIC BOND		**Complete transfer** of electrons from one atom to another	Very Different	NaCl
COVALENT BOND Electrons are **shared** between atoms	**Nonpolar Covalent Bond**	**Equal sharing** of electrons between atoms	Equal	Cl_2
	Polar Covalent Bond	**Unequal sharing** of electrons between atoms – forms a **dipole** *(electrons spend more time around one atom, giving that atom slight negative charge and the other a slight positive charge)*	Slightly Different	HCl
HYDROGEN BOND		A weak **intermolecular** bond between molecules that results when a hydrogen attached to a highly electronegative atom is attracted to the negative charge on another molecule (with an F, O, or N atom)	-	Between H_2O molecules



Hydrogen Bonding between water molecules

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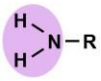
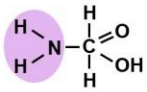
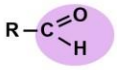
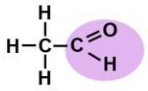
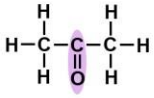
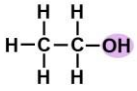
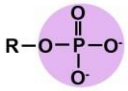
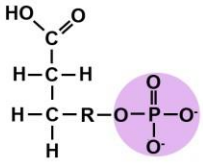
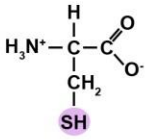
Organic Molecules

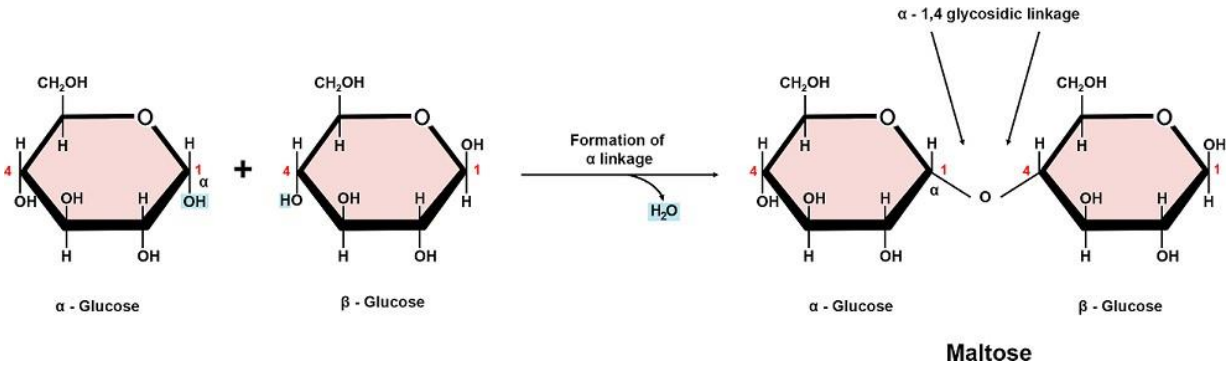
- Organic molecules have carbon atoms. Macromolecules form **monomers** (single unit) which combine to form **polymers** (series of repeating a monomers).
  - 4 of carbon's 6 electrons are available to form bonds with other atoms. These bonds may be single, double, or triple bonds.
- **Functional groups** are a specific cluster of atoms that give molecules unique properties. These are often referred to as **R-groups**

Functional Group	Formula	Family of Molecules	Example
Amino		Amines	 Glycine
Carbonyl		Aldehydes	 Acetaldehyde
		Ketones	 Acetone
Hydroxyl		Alcohols	 Ethanol
Phosphate		Organic phosphates	 3 - Phosphoglyceric acid
Sulfhydryl		Thiols	 Cysteine

Examples of commonly seen functional groups

Carbohydrates

- **Monosaccharides** are single sugar molecules
  - E.g. glucose, fructose, galactose
  - Alpha vs beta carbon is based on the position of H and OH on the 1st (anomeric) carbon (OH ↓ = alpha, OH ↑ = beta).
- **Disaccharides** are two sugar molecules joined together by a **glycosidic linkage** (joined by dehydration)
  - E.g. sucrose (glucose + fructose), lactose (glucose + galactose), maltose (glucose + glucose)
- **Polysaccharides** are a series of connected monosaccharides (an example of a polymer)
  - Bonded together via **dehydration synthesis** and broken down via **hydrolysis**

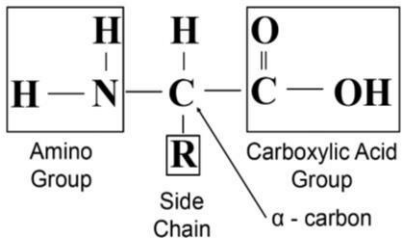


Glycosidic linkage formation via dehydration synthesis

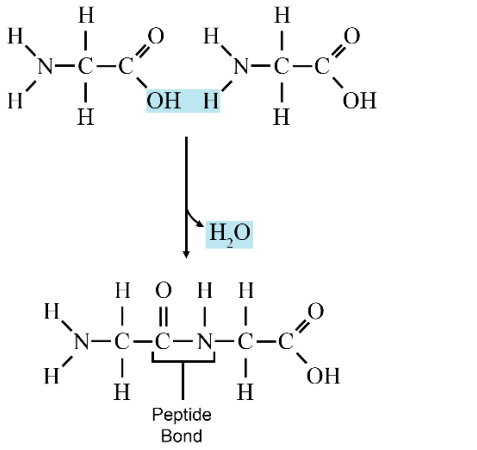
- α-glucose polymer carbohydrates:
  - **Starch**: Functions to store energy in plant cells. Consists primarily of amylose and amylopectin.
  - **Glycogen**: Functions to store energy in animal cells. Differs from starch in its polymer branching.
- β-glucose polymer carbohydrates:
  - **Cellulose**: Functions as a structural molecule for the walls of plant cells and wood
  - **Chitin**: Functions as a structural molecule in fungal cell walls & arthropod exoskeletons. Structurally similar to cellulose but with nitrogen-containing groups attached to each β-glucose ring.

Proteins

- Polymers of amino acids joined by **peptide bonds**
  - Amino acid structure: Central α-carbon bonded to H, NH_2, COOH and a variable R group



Amino acid structure: Central α-carbon bonded to H, NH_2, COOH and a variable R group



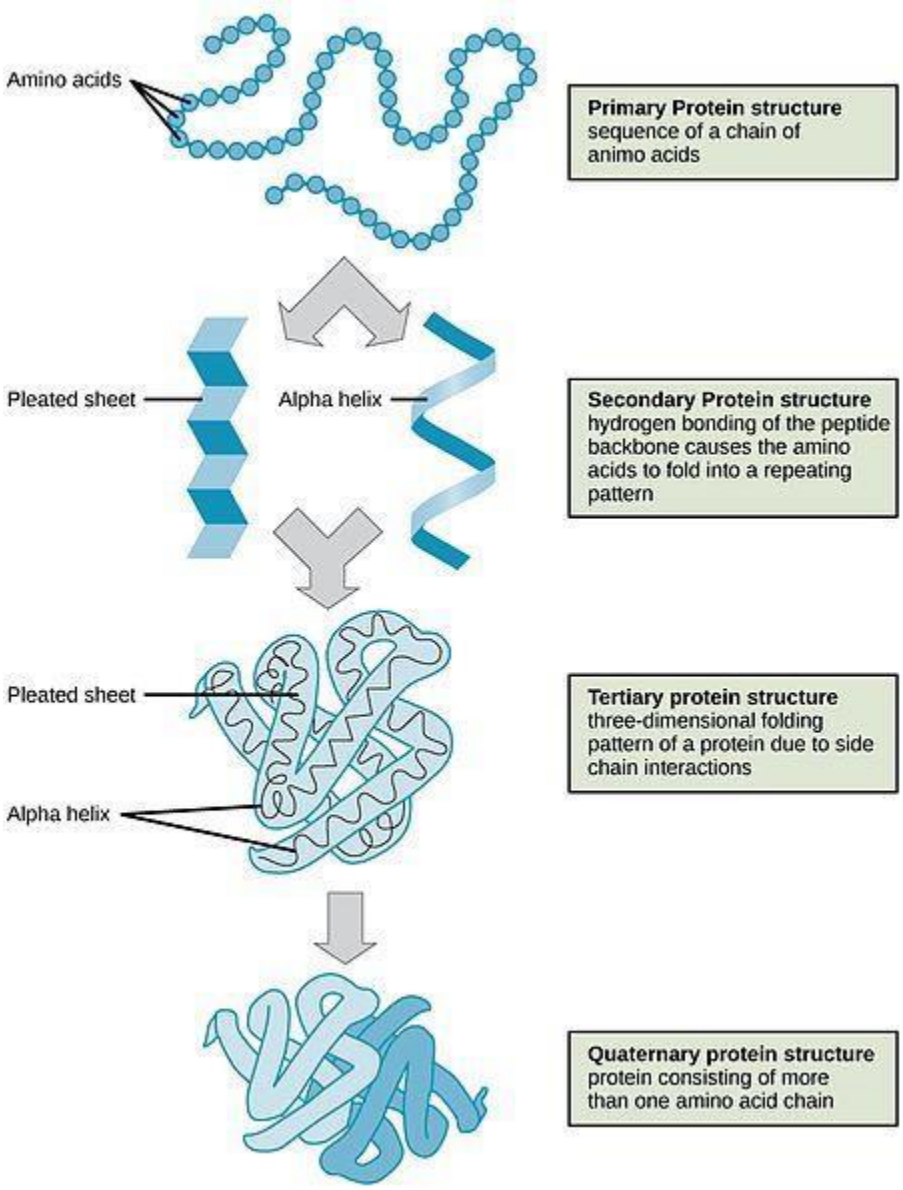
Peptide bond formation via dehydration synthesis

Proteins can be classified based on structure or composition

Classification Type		Description	Example
STRUCTURAL Based entirely on the structure of the protein	**Fibrous**	Insoluble, long polymer fibers/sheets, form structural components of cells	collagen
	Globular	Soluble, folded tightly, perform many functions	albumin
	Intermediate	Soluble, fiber shaped, perform many functions	fibrinogen
COMPOSITION Based entirely on the composition of the protein	**Simple**	Only amino acids	albumin
	Conjugated	Amino acids + non-protein components	glycoprotein (mucin), metalloprotein (hemoglobin), lipoprotein (HDL/LDL)

- The **primary structure** of a protein is its amino acid sequence
- The **secondary structure** of a protein is the 3D shape that results from its hydrogen bonding between amino and carboxyl groups of adjacent amino acids. Secondary structures include the **alpha helix** and **beta sheet**.
- The **tertiary structure** of a protein is the 3D structure due to noncovalent interactions between the R-groups of amino acids. These interactions include hydrogen bonding, ionic bonding, hydrophobic effect (R-groups are pushed away from the water center), disulfide bonds (the covalent exception to tertiary structure), and Van der Waals forces.
- The **quaternary structure** of a protein is the 3D structure from the grouping of two or more separate peptide chains
- All proteins have a primary structure, and most have a secondary structure. Larger proteins can have a tertiary and quaternary structure.

- During **protein denaturation** any secondary, tertiary, and quaternary structure is removed but the amino acid sequence (primary structure) remains intact. Protein denaturation usually occurs from excess temperature, chemical stress, pH variance, heavy metal salts, and radiation. A protein's 3D structure is critical to its function –loss of shape due to denaturation leads to loss of function.



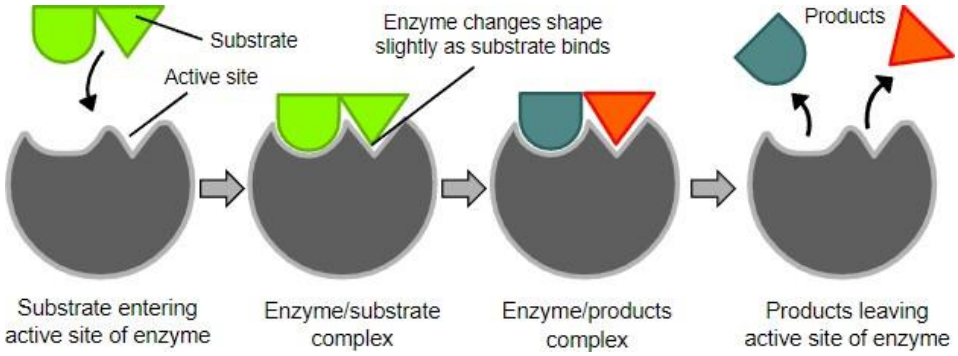
Levels of protein structure

CNX OpenStax (https://commons.wikimedia.org/wiki/File:Figure_03_04_09.jpg), https://creativecommons.org/licenses/by/4.0/legalcode

Proteins can have a wide variety of functions

Protein Category	Function	Example
Storage	biological reserves of amino acids	ovalbumin (egg whites), casein (milk), plant seeds
Transport	movement of substances within and between cells	hemoglobin (transport oxygen), cytochromes (carry electrons)
Hormones	signaling molecules circulated throughout the body to regulate organs	growth hormone, prolactin, glucagon *Covered in more detail in the endocrine section*
Receptors	membrane proteins that bind ions and signaling molecules, causing changes on a cellular level	insulin receptors, ligand-gated ion channels
Motion	movement generated at a cellular or at the level of the entire organism	tubulin (flagella -cell movement), actin and myosin (skeletal muscles – organism movement)
Structure	strengthen and support tissues	collagen (connective tissue), keratin (nails)
Immune Defense	prevent and protect against pathogen attack	antibodies
Enzymes	*See below*	amylase

- **Enzymes** are (usually) globular proteins that act as catalysts, lowering the activation energy and accelerating the rate of reactions.
  - Enzymes can catalyze reactions in both forward and reverse directions based on substrate concentration.
  - Enzymes remain chemically unchanged throughout a reaction (but can undergo conformational changes).
  - Not all enzymes are proteins – some RNA molecules can act as enzymes (**ribozymes**).
  - Enzyme efficiency is determined by temperature and pH
  - Enzymes cannot change the spontaneity of a reaction
  - Enzymes bind at the active site via **induced fit** – enzyme binding is specific to structure.



Active site and induced fit

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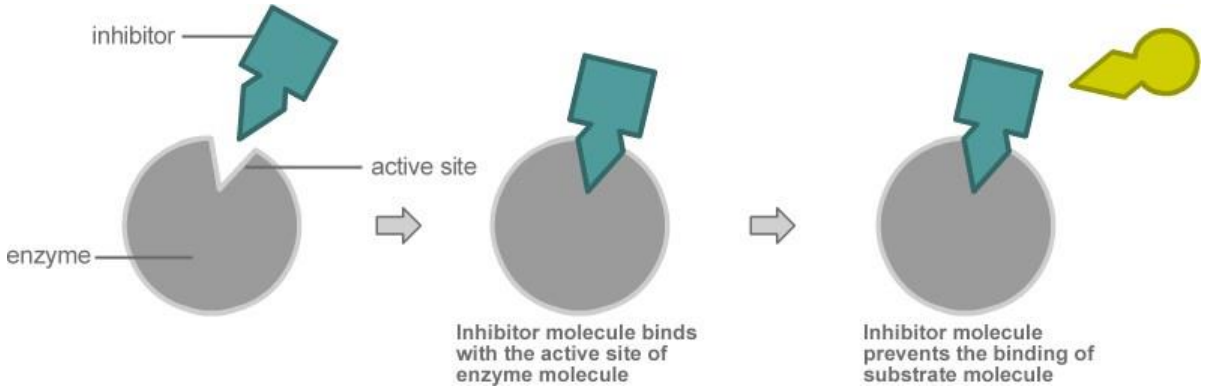
  - **Cofactors** are non-protein molecules that assist enzymes (usually by donating or accepting some component of a reaction like electrons)
    - **Coenzymes** are organic cofactors (e.g. vitamins)

- Inorganic cofactors are usually metal ions (e.g. $Fe^{2+}$and Mg^{2+})
- A cofactor that binds tightly/covalently to an enzyme is referred to as a **prosthetic group**
- **Apoenzyme** = enzyme w/out its cofactor
- **Holoenzyme** = enzyme + cofactor

❖ Enzyme regulation

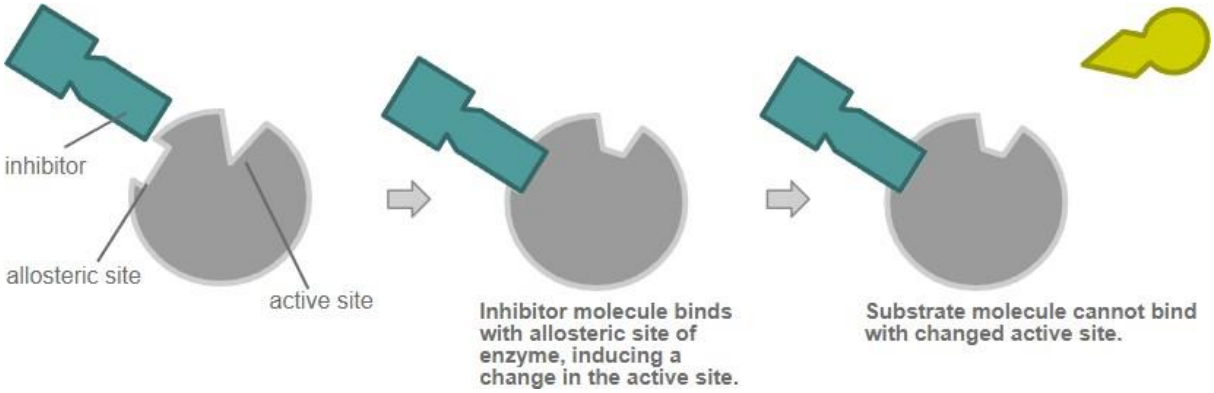
- **Allosteric enzymes** have both an active site (for substrate binding) and an allosteric site (for binding of an allosteric effector – can be an activator or inhibitor)
- **Competitive inhibition** occurs when a substance that mimics the substrate binds at the active site.
  - Competitive inhibition can be overcome by increasing substrate concentration
  - K_m is raised but V_{max} remains the same



Competitive Inhibition

https://commons.wikimedia.org/wiki/File:Comp_inhib.png, Mcy jerry at the English language Wikipedia [GFDL (http://www.gnu.org/copyleft/fdl.html)

- **Noncompetitive inhibition** occurs when a substance inhibits an enzyme by binding at a location other than the active site – the substrate still binds, but the reaction is prevented from completing
  - K_m remains the same but V_{max} is lowered



Noncompetitive Inhibition

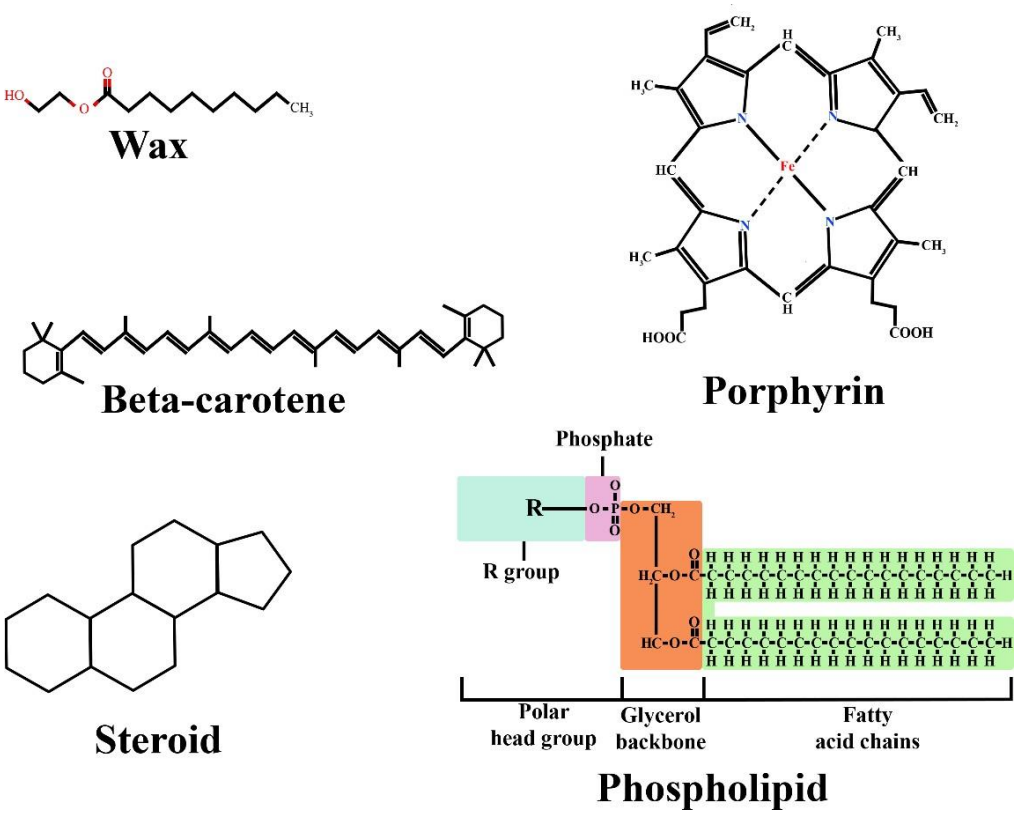
Comp_inhib.svg: PNG version: Jerry Crimson Mann at en.wikipedia (https://commons.wikimedia.org/wiki/File:Allosteric_comp_inhib_2.svg), https://creativecommons.org/licenses/by-sa/3.0/legalcode

- Enzyme **cooperativity** allows for an enzyme to become increasingly receptive to additional substrate molecules after a substrate molecule has attached to an active site
    - Applicable to enzymes w/ multiple subunits that each have an active site
        - E.g. hemoglobin binding additional oxygen (although hemoglobin is not an enzyme!)
- K_m is the Michaelis constant. It represents the substrate concentration at which the rate if reaction is half of V_{max}. It indirectly represents binding affinity.
    - A small K_m indicates the enzyme requires only a small amount of substrate to become saturated (high affinity, maximum velocity is reached at low substrate concentrations). A large K_m indicates the enzyme requires high amounts of substrate to achieve V_{max}.
    - ↑ K_m = worse substrate binding, ↓K_m = better substrate binding
- An enzyme's **specificity constant** measures how efficiently an enzyme converts a substrate into product
    - ↑ specificity constant = ↑ enzyme efficiency and substrate affinity
- ↑ substrate concentration = ↑ rate of reaction (until a point – when all enzyme molecules become fully saturated by substrate)

Lipids

- Lipids are hydrophobic molecules with multiple functions: insulation, energy storage, structure, and endocrine
- **Triglycerides** are three fatty acid chains attached to a glycerol backbone
    - **Saturated**: no double bonds
        - Bad for health: saturated = straight chains = stack densely and form fat plaques
    - **Unsaturated**: double bonds present
        - Better for health: unsaturated = double bonds cause branching = stack less densely
- **Phospholipids** are two fatty acids and a phosphate group (+R) attached to a glycerol backbone
    - These molecules are **amphipathic**: they have both hydrophobic and hydrophilic properties
- **Steroids** are a fused 4 ring structure (three 6-membered rings & one 5-membered ring). They are used as hormones (sex hormones, corticosteroids) and are a structural component of membranes (cholesterol).
- Other Lipid Derivatives:
    - Waxes
        - Structure: esters of fatty acids and monohydroxylic alcohols
        - Function: used as protective coating or exoskeleton (lanolin)
    - Carotenoids
        - Structure: fatty acid carbon chains with conjugated double bounds and 6-membered rings at each end.
        - Function: Pigments which produce colors in plants and animals.
        - Includes the carotenes and xanthophylls (subgroups)

- ❖ Porphyrins (tetrapyrroles)
  - Structure: 4 joined pyrrole rings, often complexed w/ metal ion
  - E.g. Heme (complexes with Fe in hemoglobin), Chlorophyll (complexes w/ Mg)



Lipid structures

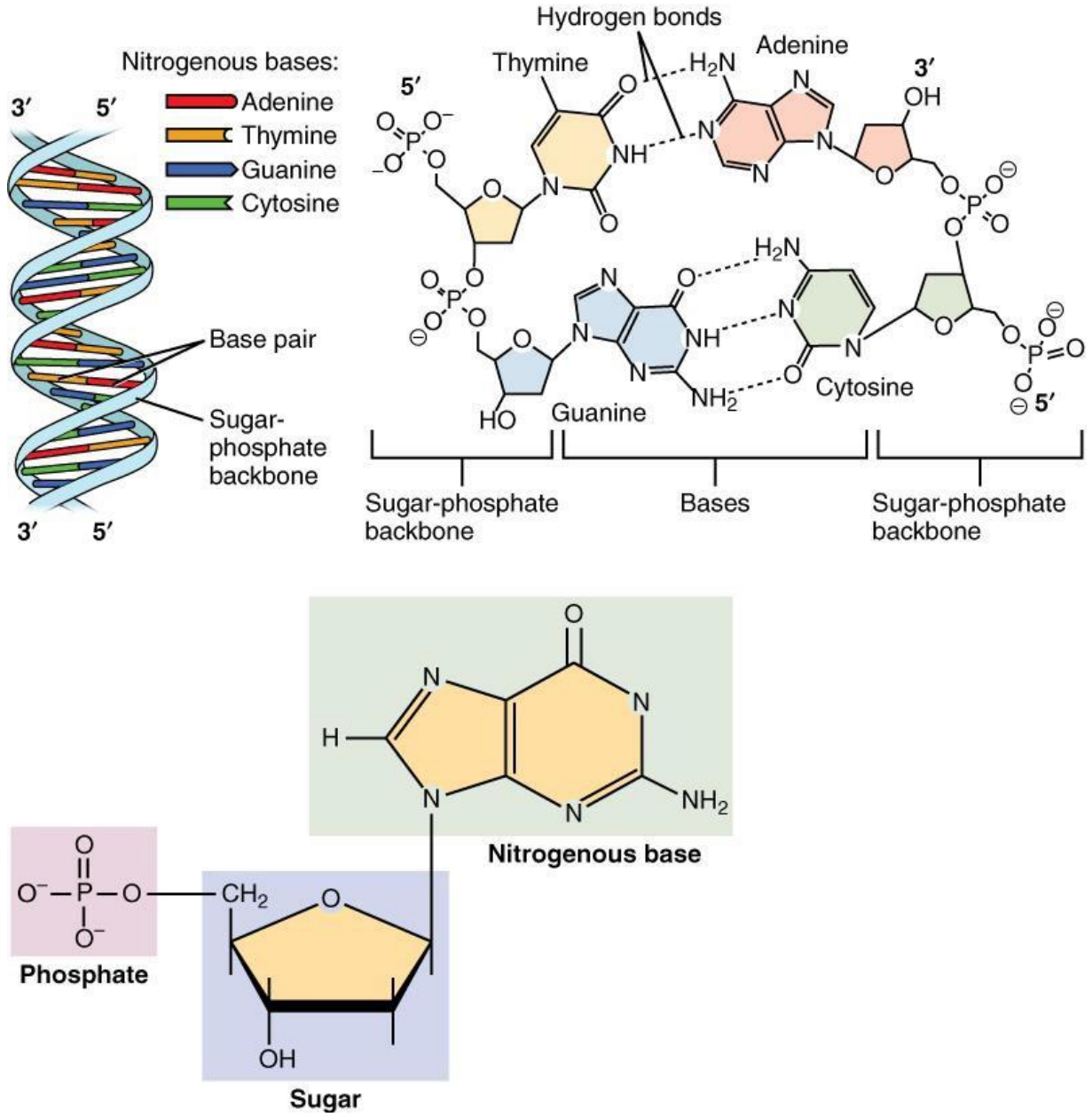
- **Adipocytes** are specialized fat cells
  - White fat cells contain a large lipid droplet composed primarily of triglycerides, with a small layer of cytoplasm around it.
  - Brown fat cells have considerable cytoplasm, with smaller lipid droplets scattered throughout the cell and lots of mitochondria.
- **Glycolipids** are like phospholipids but with a carbohydrate group rather than a phosphate group.
- Lipids are insoluble are must be transported in the blood via **lipoproteins** (a lipid core surrounded by phospholipids and apolipoproteins).
- Lipids play a critical role in **membrane fluidity**. Cells are capable of changing membrane fatty acid composition.
  - ↑ unsaturated fatty acids = ↑ membrane fluidity
    - Unsaturated fatty acid tails have double bonds that introduce 'bends' in the structure that prevent the molecules from packing as closely together
    - In response to cold temperature, a cell would ↑ unsaturated fatty acids in the membrane to maintain fluidity and avoid rigidity
  - ↑ saturated fatty acids = ↓ membrane fluidity
    - Saturated fatty acid tails lack double bonds and are straight chains that pack together closely
    - In response to warm temperature, a cell would ↑ saturated fatty acids in the membrane to increase rigidity and avoid excess fluidity
  - **Cholesterol** in the plasma membrane of animals also influences fluidity
    - In high temperatures, cholesterol molecules prevent phospholipids from excess movement, prevent excess fluidity
    - In low temperatures, cholesterol molecules prevent phospholipids from packing together too closely, preventing excess rigidity
    - **Sterols** provide similar function in plant cells. Prokaryotes use **hopanoids** instead of cholesterol in their plasma membranes.

Nucleic Acids

- DNA is a polymer of nucleotides
  - A DNA **nucleotide** contains a nitrogen base, five carbon sugar deoxyribose, and a phosphate group
  - Nucleotides can be further categorized depending on their nitrogen base (purines and pyrimidines)
    - **Purines** include adenine and guanine, and have 2 rings.
    - **Pyrimidines** include thymine and cytosine, and have 1 ring.
      - An easy way to remember the pyrimidines: CUT the PYE (**C**ytosine, **U**racil, **T**hymine).
  - A **nucleoside** contains only a nitrogen base and five carbon sugar
  - DNA forms two antiparallel strands of a double helix. The backbone is held together by **phosphodiester bonds**, while the bases of separate strands are connected via **hydrogen bonds**
    - Adenine and Thymine pair together via 2 hydrogen bonds

- Cytosine and Guanine pair together via 3 hydrogen bonds
- Antiparallel strands refers to the phosphodiester backbone of each strand running in opposite directions, from 5′ to 3′ at either end.

❖ RNA is a polymer of nucleotides that contain ribose sugar, not deoxyribose
  ❖ The nucleotide thymine is not seen in RNA. It is replaced by uracil, which pairs together with adenine via 2 hydrogen bonds.
  ❖ Unlike DNA, RNA is usually single stranded.
  ❖ RNA is less stable than DNA (due to its extra hydroxyl group), making it more likely to participate in chemical reactions



DNA and Nucleotide Structure

OpenStax (https://commons.wikimedia.org/wiki/File:0322_DNA_Nucleotides.jpg), https://creativecommons.org/licenses/by/4.0/legalcode

Water

- Water (H_2O) is a molecule with several important properties:
    1. High Heat Capacity
        - **Heat capacity** is how much a substance changes temperature in response to gain/loss of heat. A high amount of energy must be used to raise the temperature of water.
    2. Cohesion/Surface Tension
        - **Cohesion** is the mutual attraction between like substances. Due to its ability to form many hydrogen bonds, water has strong cohesion which produces a high surface tension.
    3. Adhesion
        - **Adhesion** is the attraction between unlike substances. Water has strong adhesion.
            - E.g. Wetting a finger to flip a page
            - E.g. **capillary action**: the ability of water to flow without external forces, such as against gravity
    4. Unique solid density
        - Water becomes *less* dense as it freezes (the transition from its liquid to solid form), unlike most substances which become more dense as they transition from liquid to solid.
        - Ice is less dense than water because its hydrogen bonds form a crystal lattice structure that keeps the water molecules separated further apart than in their liquid form
    5. Strong Solvent
        - The dipoles of water molecules are excellent for separating charged ionic molecules

Fundamentals of Biology

Cell Theory/Cell Doctrine

- Modern cell theory covers several fundamental principles of biology:
    - All living things are composed of one or more cells
    - Cells are the basic unit of structure, function, and organization in all organisms
    - All cells come from pre-existing, living cells
    - Cells carry hereditary information
    - Energy flow (e.g. metabolism) occurs within cells
    - All cells have the same basic chemical composition
- Note that **viruses** are not considered to be living from a biological perspective

Central dogma of genetics

- The central dogma of genetics states that information flows from DNA → RNA → proteins
- Biological information cannot be transferred backwards from protein to protein or protein to nucleic acid
- An exception to the central dogma of genetics is **prions**, misfolded proteins that cause other proteins to misfold

RNA World Hypothesis

- The RNA world hypothesis suggests that self-replicating RNA molecules were the precursor to current life (which now consists of DNA, RNA, and proteins).
- This hypothesis is supported by two main facts:
  - RNA can store genetic information, like DNA
  - RNA can catalyze chemical reactions, similar to enzymes

Chapter 2: Cells and Organelles

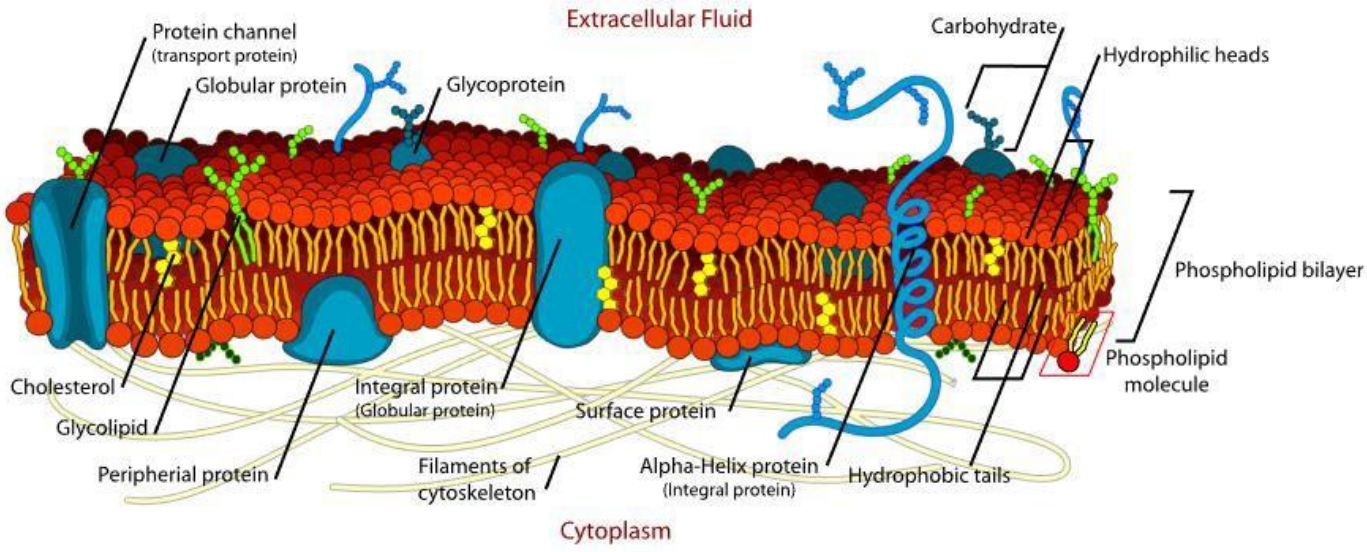
Cell Membranes

Phospholipid membrane permeability

- Small, uncharged, nonpolar molecules (polar molecules can only pass through if small and uncharged) and hydrophobic molecules can freely pass across the membrane.
- All other molecules require a transporter (large, polar, charged molecules).

Classification of Membrane Proteins

- Peripheral: Loosely attached to one surface of the phospholipid bilayer.
- Integral: Embed inside the lipid bilayer.
  - Transmembrane: Spans the entire phospholipid bilayer, going through both sides. This is a subtype of integral membrane protein.



Cell Membrane and associated structures

Source: LadyiofHats (https://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_en.svg), Public Domain

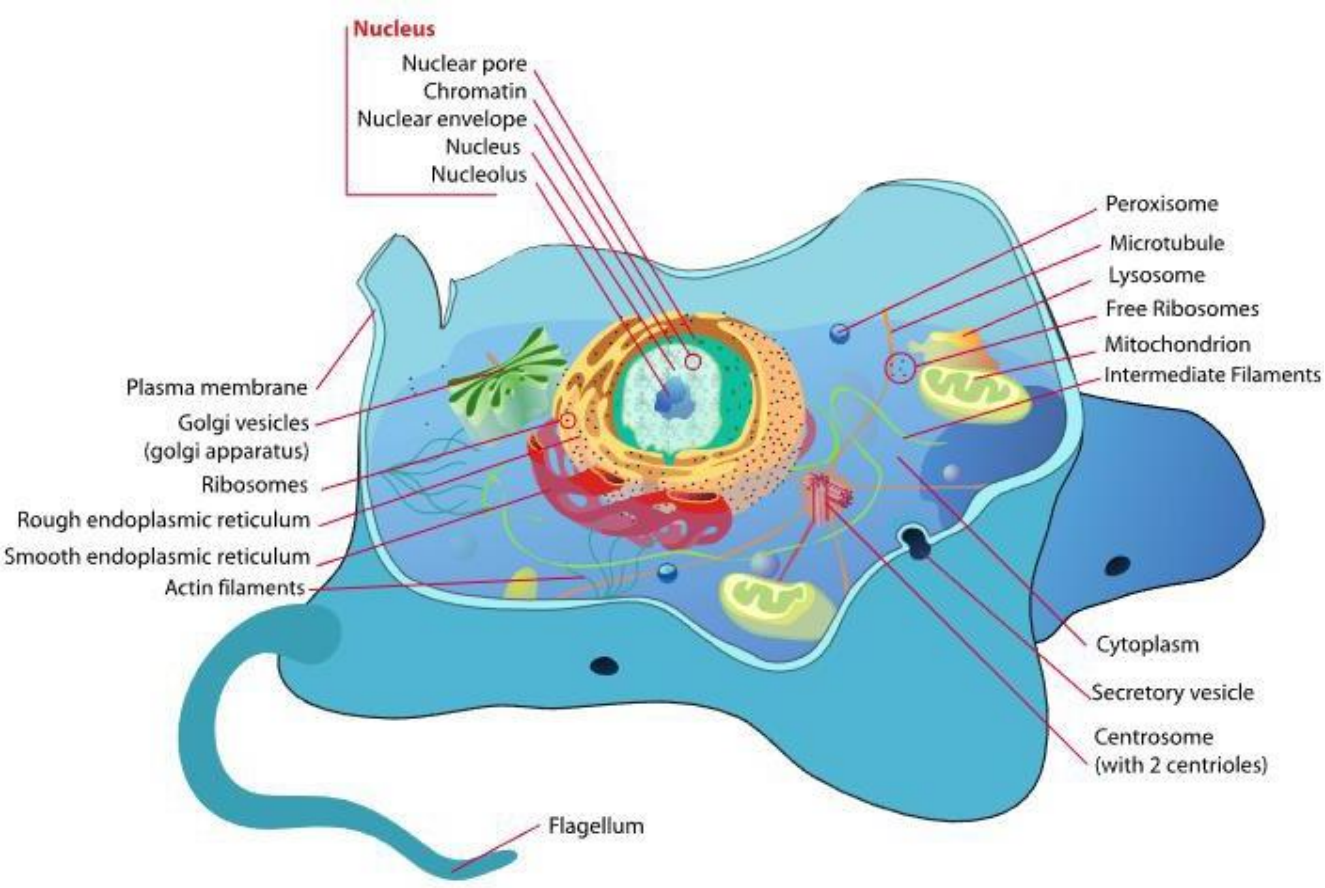
Membrane Proteins

- **Channel proteins**: Provide passageway through membrane for hydrophilic (water-soluble) substances (polar, and charged).
- **Recognition proteins**: These include the major-histocompatibility complex (**MHC**) on macrophages used to distinguish between self and foreign; they are **glycoproteins** due to oligosaccharides attached.
- **Ion channels**: Allow **passage of ions** across membrane. Called gated channels in nerve and muscle cells, respond to stimuli. These can be further classified:
  - Voltage-gated: respond to difference in membrane potential
  - Ligand-gated: chemical binds and opens channel
  - Mechanically-gated: respond to pressure, vibration, temperature, etc.
- **Porins**: Allow passage of certain ions + small polar molecules. These tend not to be specific, they're just large passages. Molecules that fit will diffuse through.
  - **Aquaporins** increase the rate of H_2O passing (kidney and plant root cells).
- **Transport proteins** move substances across a membrane.
  - In **active transport** ATP is used in the movement of substances
    - E.g. Na^+-K^+ pump to maintain gradients.
  - Facilitated diffusion also makes use of transport proteins, but does so via **passive transport** (does not require the direct use of ATP).
  - Transport proteins are a broad category that encompass many of the channels and proteins discussed above
  - **Carrier proteins** are a specific type of transport protein. Unlike channels (which are simultaneously exposed to the extracellular and intracellular environment), carrier proteins are only exposed to one side at a time.
    - Bind to a specific molecule → protein changes shape → molecule (e.g. glucose) passes across into or out of cell.
- **Adhesion proteins** attach cells to neighboring cells and provide **anchors** for internal filaments and tubules (increasing stability).
- **Receptor proteins**: Binding site for hormones and other trigger molecules.

Outer Membrane Components

- **Glycocalyx**: A **carbohydrate** coat that covers outer face of cell wall of some bacteria and outer face of plasma membrane (in some **animal cells**). It consists of glycolipids (attached to plasma membrane) and glycoproteins (such as recognition proteins). It may provide adhesive capabilities, a barrier to infection, or markers for cell-cell recognition.

Organelles



Eukaryotic Cell and Organelles

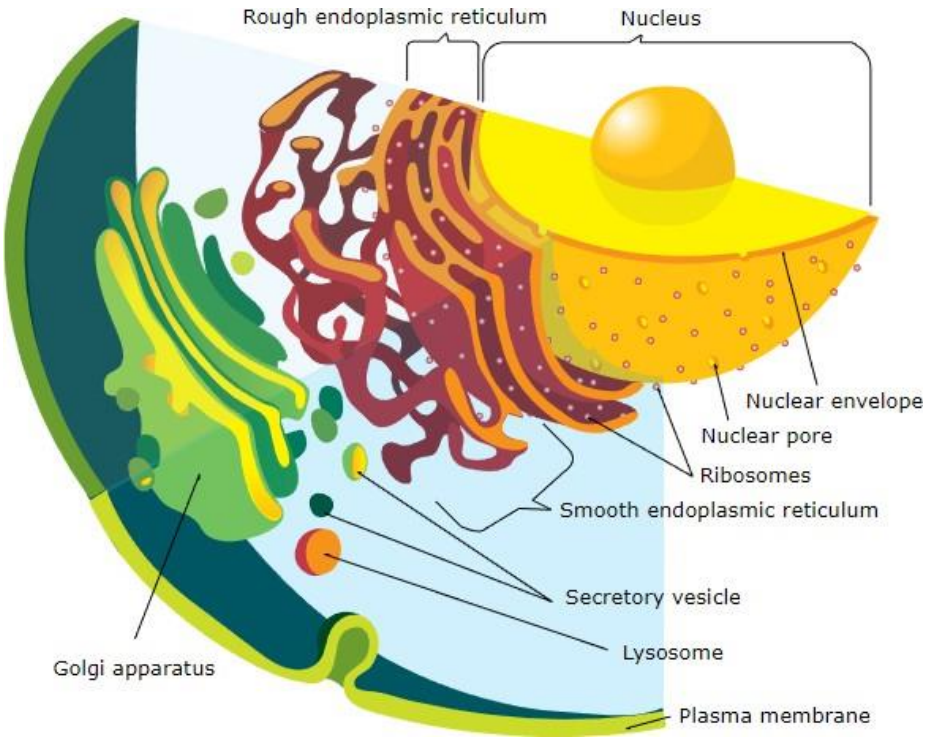
Source: LadyiofHats (https://commons.wikimedia.org/wiki/File:Animal_cell_structure_en.svg), Public Domain

- The **nucleus** is a membrane-enclosed organelle that contains most of its genetic material.
  - **Chromatin** is the general packaging structure of DNA around proteins in eukaryotes. The tightness of the packaging varies depending on cell stage.
    - **Heterochromatin** is tightly packed DNA.
    - **Euchromatin** is loosely packed DNA.
  - **Chromosomes** are tightly condensed chromatin that form when the cell is ready to divide.
  - **Nucleosomes** are basic units of DNA organization composed of 8 histones.
    - **Histones** serve to organize DNA which coil around it into a bundle.
  - **Nucleolus** helps produce ribosomes inside the nucleus.
    - rRNA is synthesized in the nucleolus. Ribosomal proteins are imported from cytoplasm and together with rRNA form ribosomal subunits. These subunits are exported to the cytoplasm for final assembly into complete ribosomes.
  - **Nuclear lamina** is a dense fibrillar network inside the nucleus of eukaryotic cells. It is composed of intermediate filaments and membrane-associated proteins.
    - The nuclear lamina provides mechanical support. It also helps regulate DNA replication, cell division, and chromatin organization.
  - **Nucleoid** is an irregular shaped region within the cell of prokaryotes that contains all/most genetic material (prokaryotes lack a nucleus).

- **Nucleoplasm** is the "cytoplasm" of the nucleus.
- **Nuclear envelope** is a lipid bilayer that surrounds the nucleus. Nuclear pores cross the nuclear envelope for transport in/out (mRNA, ribosome subunits, nucleotides, proteins such as the RNA polymerase and histones, etc.).

- **Cytoplasm**: the site of metabolic activity and transport. It doesn't include nucleus, but does include cytosol, organelles, and everything suspended within the cytosol.
  - The cytoplasm is an area, not a structure!
- **Cytosol**: the **intracellular fluid** inside a cell. It is a *part* of the cytoplasm but does not include the components of the cell suspended within it (such as organelles). If the cytoplasm were a stew, the cytosol would be the liquid.
- **Ribosomes** function in the synthesis of proteins.
  - The **eukaryotic** ribosome is composed of 2 subunits: **60S + 40S = 80s unit**.
    - The two subunits are produced inside the nucleolus, then moved into the cytoplasm where they assembled into a single 80S ribosomes (larger S value indicates heavier molecule).
  - The **prokaryote** ribosome is composed of **50S + 30S = 70S**.
  - Ribosomes are made of rRNA and ribosomal proteins.
  - Free ribosomes (those not attached to the endoplasmic reticulum) tend to make proteins that function within the cytosol of the cell.
- **Endoplasmic reticulum (ER)**:
  - **Rough ER** (with ribosomes) creates glycoproteins by attaching polysaccharides to polypeptides as they are assembled by ribosomes.
    - In eukaryotes, the rough ER is continuous with the outer nuclear membrane.
    - The rough ER tends to make proteins that are part of the membrane, or secreted by the cell.
    - Neurons contain **nissl bodies**, granules of rough ER and free ribosomes that synthesize protein
  - **Smooth ER** (no ribosomes) synthesizes lipids and steroid hormones for export.
    - In liver cells, the smooth ER functions in the breakdown toxins, drugs, and toxic by-products from cellular respiration.
- Muscle cells have smooth ER's called **sarcoplasmic reticulum** that store and release ions, (e.g. Ca^{2+}).

- **Endomembrane system** is the network of organelles and structures, either directly or indirectly connected, that function in **the transport of proteins** and other macromolecules into or out of the cell. Includes plasma membrane, endoplasmic reticulum, Golgi apparatus, nuclear envelope, lysosomes, vacuoles, vesicles, and endosomes but NOT the mitochondria or chloroplasts.



Endomembrane System

Source: LadyiofHats (https://en.wikipedia.org/wiki/File:Endomembrane_system_diagram_en_(edit).svg), Public Domain

- **Lysosomes**: These are **vesicles** produced from Golgi that contain digestive enzymes (low pH for function). They are used to break down nutrients/bacteria/cell debris.
  - Lysosomes function in **apoptosis** when they release their contents into cell and **autophagy** (intracellular breakdown of unneeded/defective cellular components).
- **Golgi apparatus**: Responsible for **transport** of various substances in vesicles (cis face is for incoming vesicles, trans face for secretory vesicles). Has flattened sacs known as **cisternae**. Modifies the products of the ER, e.g. proteins: glycosylation/phosphorylation/sulfation.
- **Peroxisomes** break down various substances ($H_2O_2 + RH_2 \Rightarrow R + 2H_2O$), fatty acids, and amino acids.
  - Common in liver and kidney where they breakdown toxic substances.
  - In plant cell, peroxisomes modify the by-products of photorespiration. In germinating seeds, they are called **glyoxysomes**.
  - Peroxisomes break down stored fatty acids to help generate energy for growth.

- Peroxisomes produce H_2O_2 (**hydrogen peroxide**) which is then used to oxidize substrates. They can also break down H_2O_2 if necessary via **catalase** (H_2O_2 => H_2O + O_2) since H_2O_2 is toxic to cells.

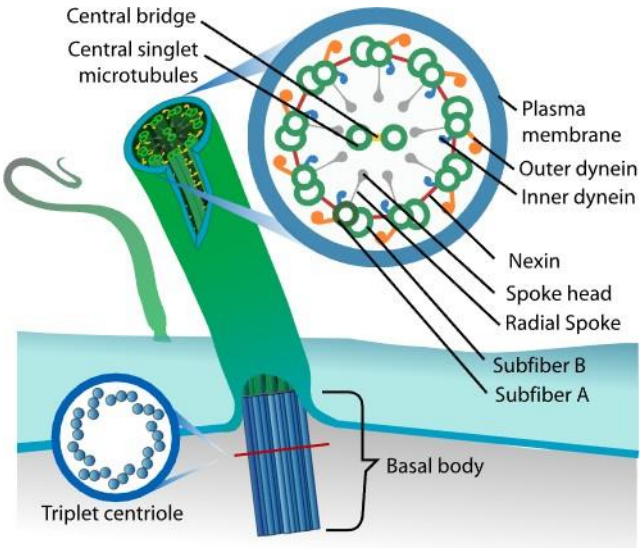
❖ **Vacuoles**
- **Transport vacuoles** move materials between organelles or organelles and the plasma membrane.
- **Food vacuoles** are temporary receptacles of nutrients; merge with lysosomes which break down food.
- **Central vacuoles** are large, and occupy most of plant cell interior. They exert **turgor** when fully filled to maintain rigidity.
  - Central vacuoles also store nutrients and carry out functions performed by lysosomes in animal cells.
  - Have a specialized membrane called the **tonoplast.**
- **Storage vacuoles** in plants store starch, pigments, and toxic substances (e.g. nicotine).
- **Contractile vacuoles** are in single-celled organisms that collect and pump excess water out of the cells (prevent bursting).
  - Utilizes **active transport**.
  - Found in organisms that live in hypotonic environments in which it is necessary to pump out water to prevent lysing.

❖ **Mitochondria** make ATP and participate in fatty acid **catabolism (β-oxidation**) (fatty acids are made in cytosol but broken down in the mitochondria).
- ❖ Have their own **circular DNA** and ribosomes (evidence supporting the **endosymbiotic theory**).
- ❖ Have a **double layered membrane**.
- ❖ Cells with high energy requirements have a lot of mitochondria relative to other cells – e.g. heart and kidney cells.

Cytoskeleton, Extracellular Components and Cell Junctions

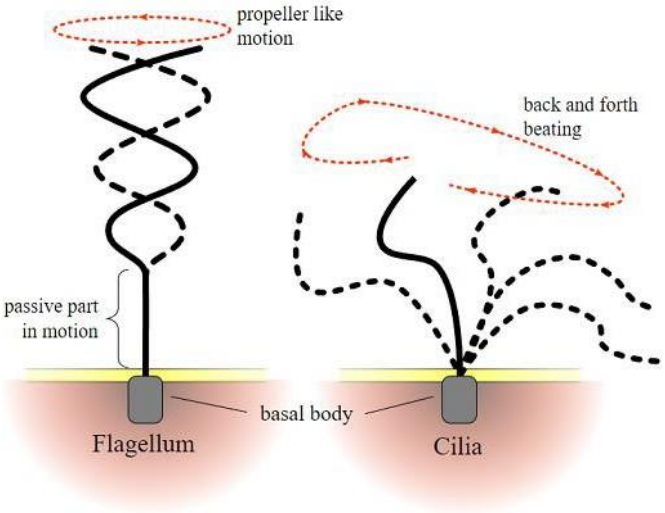
❖ **Cytoskeleton** includes microtubules, microfilaments, and intermediate filaments. In eukaryotic cells, it aids in cell division, cell crawling, and the movement of cytoplasm and organelles. The cytoskeleton is found in both prokaryotes and eukaryotes.
- ❖ **Microtubules** are made up of the protein **tubulin** and provide support and motility for cellular activities
  - E.g. spindle apparatus which guides chromosomes during division;
  - E.g. **flagella** and **cilia** (9+2 array; 9 pairs + 2 singlets in center) in all animal cells and lower plants (mosses, ferns).
  - **Colchicine** is an alkaloid that will inhibit polymerization of microtubules.
- ❖ **Intermediate filaments** provide support for maintaining cell shape. e.g. keratin.
- ❖ **Microfilaments** are made up of **actin** and are involved in cell motility. Structures that are made up of microfilaments include skeletal muscle, amoeba pseudopod and cleavage furrow (a structure associated with cytokinesis).
- ❖ **Microtubule organizing centers (MTOCs)** include **centrioles** and **basal bodies** (found at the base of each flagellum and cilium and organize their development). Organized in a 9x3 array.

- Note: Plant cells lack centrioles and its division is by cell plate instead of cleavage furrow, but plants do have MTOC's.



General flagella & cilia structure

Source (Public Domain): https://commons.wikimedia.org/wiki/File:Eukaryotic_cilium_diagram_en.svg

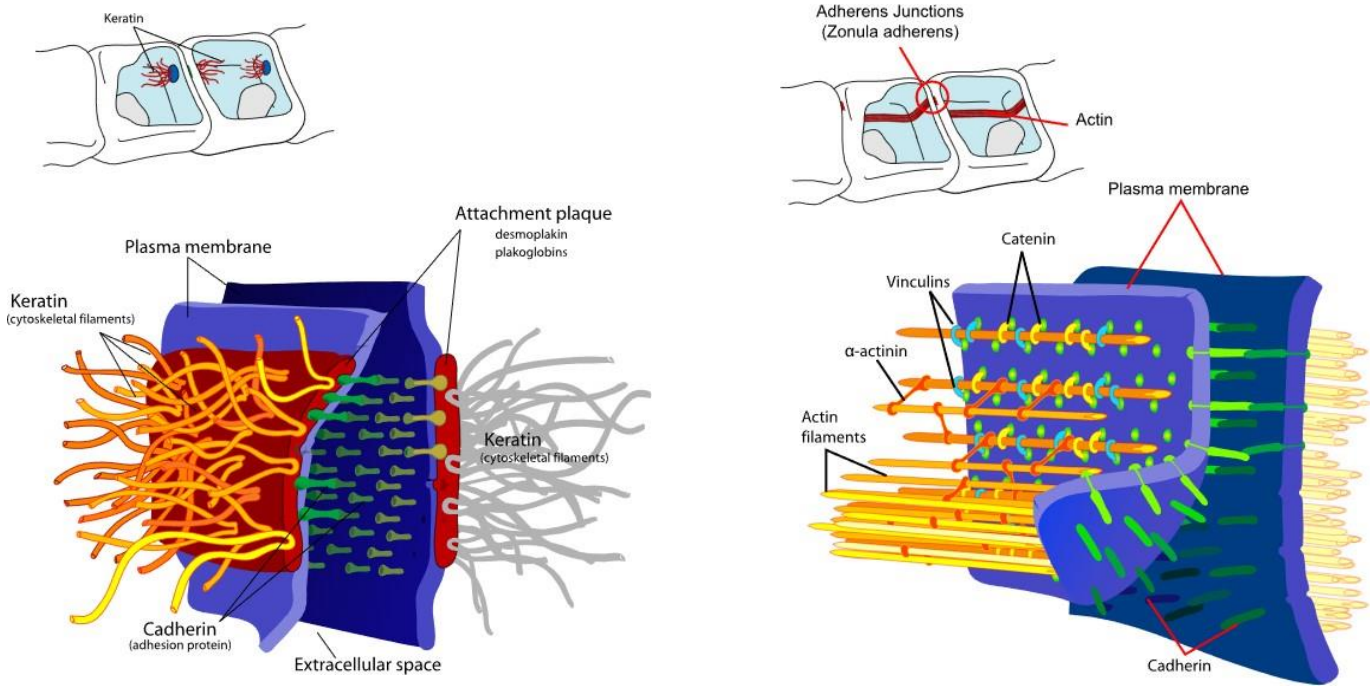


Flagella vs Cilia Motion

Original: Kohidai, L. Vector: Urutseg (https://commons.wikimedia.org/wiki/File:Flagellum-beating.svg), „Flagellum-beating", https://creativecommons.org/licenses/by/3.0/legalcode

- **Cell walls**: These are found in **plants, fungi, protists, and bacteria** (cellulose in plants; chitin in fungi; peptidoglycans in bacteria, polysaccharides in archaea). Provides structural support.
- **Extracellular matrix (ECM)**: provides **mechanical support** and helps bind adjacent cells. Found **in animals**, in areas between adjacent cells (beyond the plasma membrane and glycocalyx). It is often occupied by fibrous structural proteins, adhesion proteins, and polysaccharides secreted by cells. Collagen is *most common* here, we also see **integrin and fibronectin**.
  - **Fibronectin** connect **integrins** to a network of **collagen** and **proteoglycans** in the ECM. This network also functions in transmitting mechanical and chemical signals between outside and inside of cell.
  - **Laminin** can be seen as well acting similarly to fibronectin.
  - **Focal adhesions** are one way cells connect to the ECM. This type of connection uses **actin** filaments in the cell.
  - **Hemidesmosomes** are another way the cell connects to the ECM. This type of connection uses **intermediate filaments** e.g. **keratin**.
  - **Fibroblasts** are the cells which produce collagen and other connective tissue elements.
  - **Integrins** couple the ECM outside of cell to cytoskeleton inside the cell and are involved in cell signaling.
    - Their structure is a heterodimer of α and β subunits.
    - Involved in wound repair

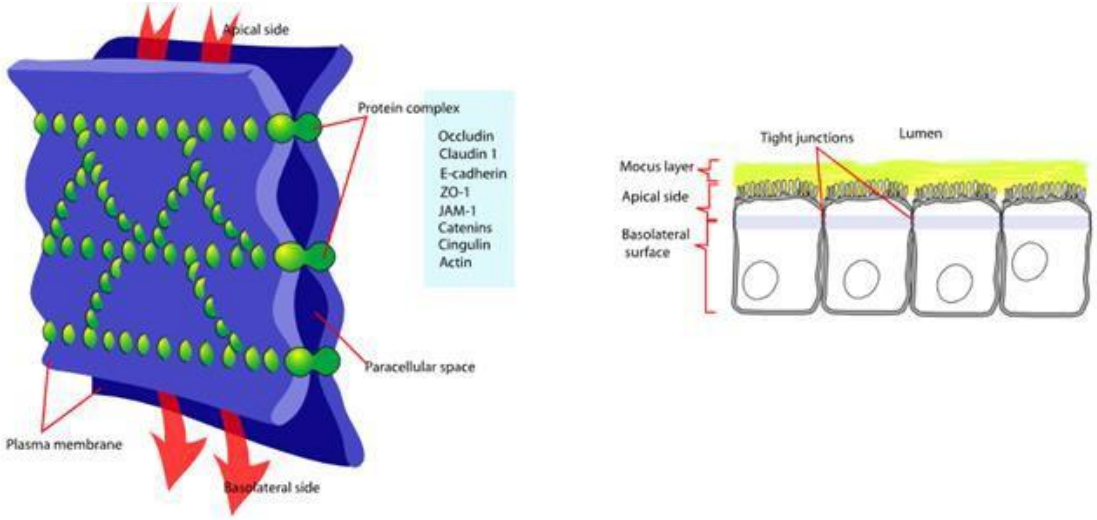
- **Junctions**:
  - **Anchoring junctions** include **desmosomes** (keratin filaments within the cell attached to adhesion plaques which bind adjacent cells together via connecting adhesion proteins), **hemidesmosomes,** and **adherens junctions**.
    - Provide mechanical stability and hold cellular structures together. These are present in animal cells in tissues with mechanical stress such as skin epithelium, cervix and uterus.



***Desmosome** Junction (left)* ***Adherens** Junction (right)*

Source: LadyiofHats (https://commons.wikimedia.org/wiki/File:Desmosome_cell_junction_en.svg and https://commons.wikimedia.org/wiki/File:Adherens_Junctions_structural_proteins.svg), Public Domain

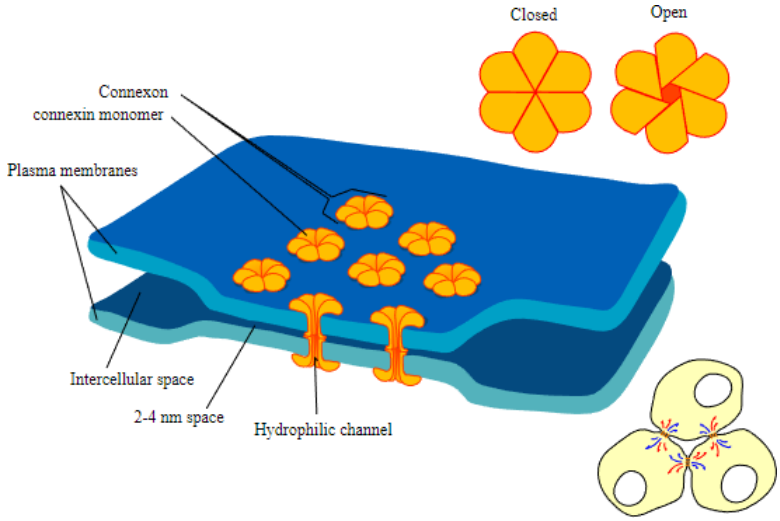
- **Tight junctions** completely encircle each cell, producing a seal that prevents the passage of materials between cells.
  - Prevent the passage of molecules and ions through the space between cells, so materials must actually enter the cells (by diffusion or active transport) in order to pass through the tissue.
  - Present in animal cells**.**
    - Characteristic of cells lining the digestive tract



Tight Junctions

Source: LadyiofHats (https://commons.wikimedia.org/wiki/File:Cellular_tight_junction-en.svg), Public Domain

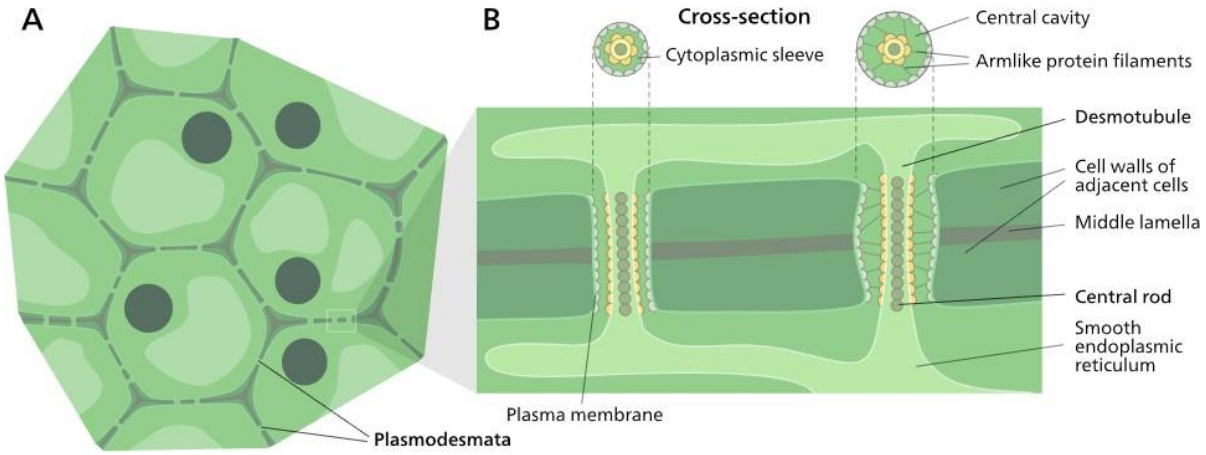
- **Gap junctions** are narrow tunnels between animal cells (formed from **connexin** proteins)
  - Prevent cytoplasms of adjacent cells from mixing, but allow passage of ions and small molecules
  - Essentially channel proteins of two adjacent cells that are closely aligned
  - These are present in **animal cells**.
    - Tissues like the heart have these to pass electrical impulses.



Gap Junctions

Source: LadyiofHats (https://commons.wikimedia.org/wiki/File:Gap_cell_junction-en.svg), Public Domain

- **Plasmodesmata** are narrow tunnels between **plant cells**.
  - **Desmotubules** are narrow tubes of endoplasmic reticulum within plasmodesmata. Plant cells exchange material through cytoplasm surrounding the desmotubule.



Plasmodesmata

Source: Zlir'a (https://commons.wikimedia.org/wiki/File:Plasmodesmata_en.svg), Public Domain

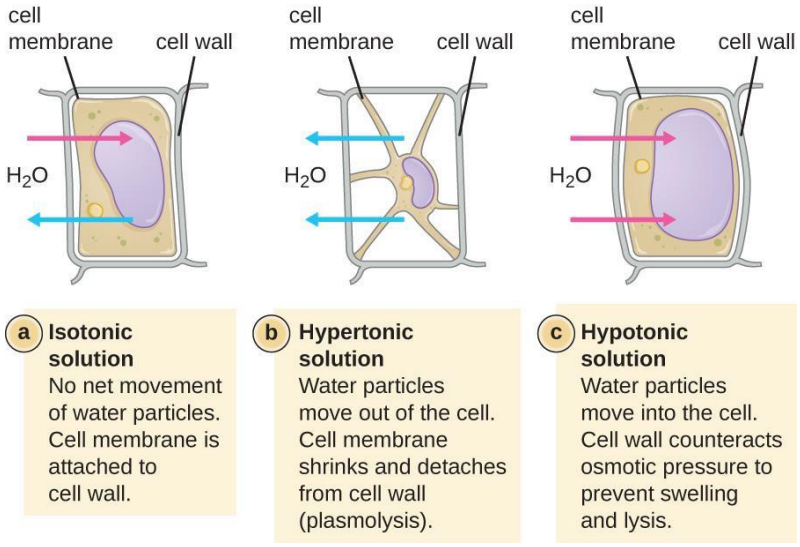
Circulation

	Types of circulation	Description
Intracellular circulation	**Brownian motion**	Random particle movement due to kinetic energy, spreading small suspended particles throughout the cytoplasm.
	Cyclosis/streaming	Circular motion of cytoplasm around cell transport molecules.
	Endoplasmic reticulum	Provides channel through cytoplasm and direct continuous passageway from plasma membrane to nuclear membrane.
Extracellular circulation	**Diffusion**	If cells in close contact with external environment, diffusion can suffice for food and respiration needs. It is also used for transport of materials between cells and interstitial fluid around cells in more complex animals.
	Circulatory system	Complex animals with cells too far from external environment require one. Uses vessels (e.g. the **vascular system**).

Molecular Movement and Transport

- **Tonicity** is the ability of an extracellular solution to cause water to move into or out of a cell

Different states of tonicity	Explanation
Hypertonic	Describes a solution in which there is a higher solute concentration than inside the cell. Cells in this solution undergo **plasmolysis** (shrinkage of cytoplasm from water loss away from the cell wall). Animal cells will shrivel due to water being pulled out of the cell.
Hypotonic	Describes a solution in which there is a lower solute concentration than inside the cell. Plant cells in hypotonic solutions will have their vacuoles swell, resulting in turgid cells - this is their normal state.
Isotonic	Describes a solution in which there is an equal solute concentration with the environment inside the cell. In an isotonic solution, plant cells are flaccid.



Cell Tonicity

CNX OpenStax (https://commons.wikimedia.org/wiki/File:OSC_Microbio_03_03_Plasmolysi.jpg), https://creativecommons.org/licenses/by/4.0/legalcode

Passive and active transport

	Types	Explanation
Passive transport: The movement of molecules down a concentration gradient. This does not require energy input.	**Simple diffusion, osmosis, dialysis**	Diffusion of different solutes across a selectively permeable membrane.
	Plasmolysis	Movement of water out of a cell that results in its collapse.
	Facilitated diffusion	Spontaneous passive transport of molecules across a membrane using transport proteins.
	Countercurrent exchange	Diffusion by bulk flow in opposite directions (e.g. blood and water in fish gills).
Active transport: The movement of molecules against their concentration gradients requiring energy. Usually solutes like small ions, amino acids, monosaccharides are transported.	**Primary active transport**	The process in which energy (ATP) is directly used to move against a concentration gradient.
	Secondary active transport	The process in which energy is indirectly used to move against a concentration gradient (usually by coupling with a 'counter ion' moving down its concentration gradient). Considered secondary active transport because the other substance's gradient was usually established with ATP. Can use **antiporters** (one molecule moves in while another moves out) or **symporters** (both molecules travel in the same direction).
	Group translocation	A process seen in prokaryotes**.** The substances being transported across a membrane is chemically altered in the process (this prevents it from diffusing back out).

Note: Diffusion is a net process overall - some few particles still move against the gradient because molecule movement is random, but net diffusion is generally what we talk about.

Note: Ions have charge and diffuse according to electrochemical gradients determined by both concentration *and* electrical force.

- **Bulk flow** describes a collective movement of substances in the same direction in response to a force or pressure (e.g. blood flow).

Cytosis

- **Endocytosis** transports molecules into the cell (via active transport).
  - **Phagocytosis** is the process in which a cell **engulfs** *undissolved* materials (solid). The plasma membrane wraps outward around the material. Known as "cell eating".
    - E.g. White blood cell engulfing antigens.
  - **Pinocytosis** is the process in which a cell **engulfs** *dissolved* material (**liquid**). Plasma membrane invaginates. Known as "cell drinking".
    - **Receptor-mediated**: A form of pinocytosis triggered when specific molecules (ligands) bind to receptors

- Proteins that transport cholesterol in blood (LDL) and hormones target specific cells via this mechanism

❖ **Exocytosis** transports molecules out of the cell (via active transport).

Eukaryotes vs Prokaryotes

❖ **Eukaryotes** include all organisms except for bacteria, cyanobacteria, and archaebacteria.
❖ **Prokaryotes** have a plasma membrane, DNA molecule, ribosomes, cytoplasm and cell wall (features also present in eukaryotic cells).
❖ Prokaryote vs. Eukaryote characteristics:

	Prokaryotes	**Eukaryotes**
Examples	Bacteria, Cyanobacteria, Archaebacteria	Plants, Animals, Protists, Fungi
Cell Size	Smaller (1-10 um)	Larger (10-100 um)
Cell Number	Usually unicellular (some multicellular Cyanobacteria)	Usually Multicellular
Nucleus	Absent	Present
Genetic Information	Single circular dsDNA Not wrapped around proteins (no chromatin). Present in a region of the cell called the **nucleoid** May also have plasmids	dsDNA wrapped around proteins called **histones** form multiple **chromosomes.** Contained within the membrane-bound nucleus
Membrane Bound Cellular Organelles	Absent	Present (Endoplasmic Reticulum, Golgi Apparatus, Mitochondria, Chloroplasts, etc.)
Cell Wall	Universally Present More complex cell wall structure formed from different molecules (**Peptidoglycan** in Bacteria; Polysaccharides in Archaebacteria). Many have sticky cell capsules surrounding the cell wall.	Present only in Plants (cellulose) and Fungi (chitin)
Ribosomes	Smaller **(70S)** Subunits: 50S + 30S	Larger **(80S)** Subunits: 60S + 40S
Flagella	Smaller Made from one protein filament **Flagellin** Powered by a proton pump in a rotatory movement	Larger Made from **Tubulin** Microtubules arranged as **9 doublets surrounding 2 singlets.** Powered by ATP in a bending movement
Reproduction	Typically via **binary fission**	Typically via **mitosis**

Note: Despite not having mitochondria, prokaryotes still have their own electron transport chain (typically done across their plasma membrane)

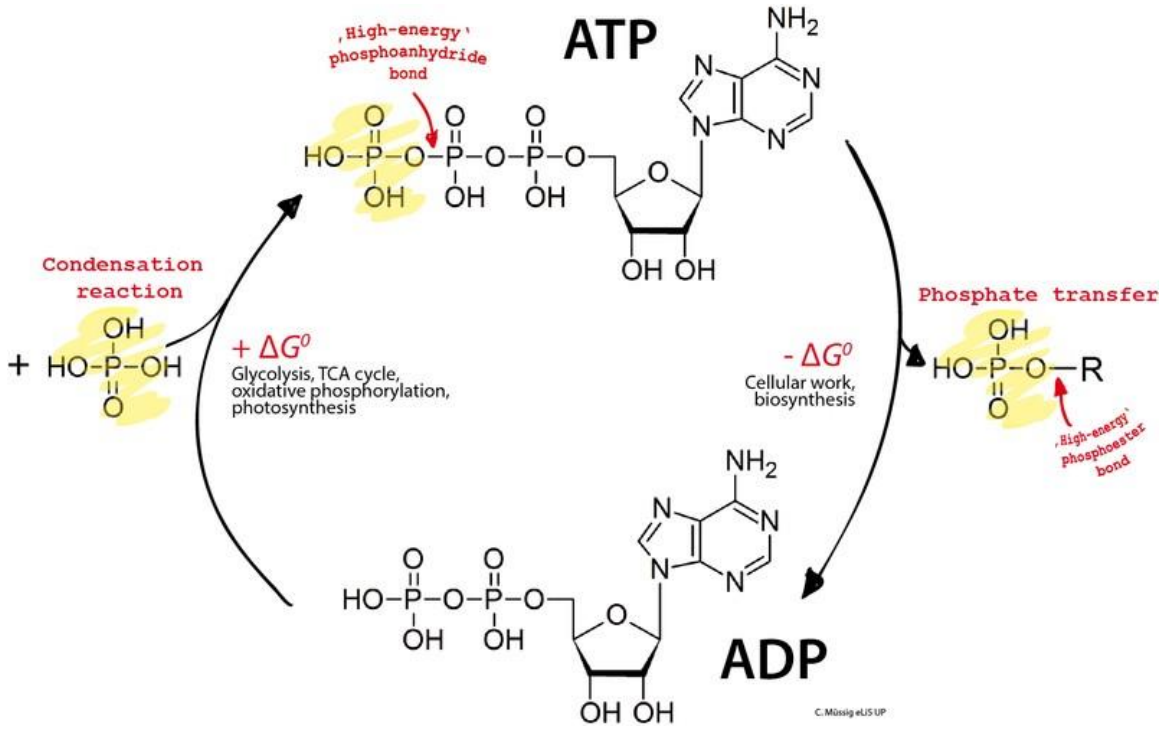
Chapter 3: Cellular Energy

Biothermodynamics

- **Gibbs Free Energy** (G) tells us whether a chemical reaction can occur spontaneously: $\Delta G = \Delta H - T\Delta S$ (H is enthalpy, T is temperature, and S is entropy).
  - If ΔG is negative, the reaction can occur spontaneously.
    - The reaction is **exergonic.** Formation of the products releases energy.
  - If ΔG is positive, the reaction is nonspontaneous.
    - The reaction is **endergonic**. Formation of the products requires energy.
- A system with a high G is considered less stable.
  - Less stable systems will spontaneously change to more stable systems.
  - In a spontaneous change, the ΔG of the system decreases (becomes more negative). This releases free energy that can be used to do work.
- A system with a low G is considered more stable.
  - More stable systems have less work capacity.

Type of System	Less Stable System (high G) ➔ More Stable System (low G)	
Gravitational Motion	Object at high altitude	Object at low altitude
Diffusion	Highly ordered molecules	Randomly dispersed molecules
Chemical Reaction	Molecule of glucose	Breakdown products of glucose

- How does this apply to biology?
  - Chemical reactions can be "coupled" together if they share intermediates.
  - The overall Gibbs Free Energy change is the sum of the ΔG values for each reaction.
    - An unfavorable reaction (positive $\Delta G1$) can be driven by a second, highly favorable reaction (negative $\Delta G2$ where the magnitude of $\Delta G2 > \Delta G$ magnitude of $\Delta G1$).
  - The principle of coupling reactions to alter the change in Gibbs Free Energy is the basic principle behind all enzymatic action in biological organisms, and is how ATP drives chemical work.



ATP in cellular work

Muessig (https://commons.wikimedia.org/wiki/File:ADP_ATP_cycle.png), „ADP ATP cycle", https://creativecommons.org/licenses/by-sa/3.0/legalcode

Relationship of Basal Metabolic Rate (BMR) and Body Size

- As body size of mammals increases, their basal metabolic rate increases.
- Basal metabolic rate *per kilogram of body mass* decreases as body mass increases.
- An increase in body temperature leads to an increase in metabolism (↑ body temp = ↑ metabolism).
- Increasing age leads to a decrease in metabolism (↑ age = ↓ metabolism).

Cellular Respiration

- **Cellular respiration** is overall an oxidative, exergonic process (ΔG = -686 kcal/mole).
  - During respiration, high energy H atoms are removed from organic molecules (dehydrogenation).
  - The chemical formula describing cellular respiration is $C_6H_{12}O_6 + 6O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + energy
- **Aerobic respiration** occurs in the presence of O_2 and is divided into four metabolic processes: glycolysis, pyruvate decarboxylation, Krebs cycle, and the electron transport chain. Water is the final product.

Adenosine Triphosphate (ATP)

- ATP is considered an RNA nucleotide due to its ribose sugar.
- ATP is an unstable molecule because the 3 phosphates in ATP are negatively charged and repel one another.

- When one phosphate group is removed via hydrolysis, a more stable molecule (ADP) results.
- The change from a less stable molecule to a more stable molecule always releases energy.

- ATP provides energy for all cells by transferring phosphate from ATP to another molecule.

Steps of Aerobic Cellular Respiration

STEP 1: Glycolysis

- **Glycolysis** is the decomposition of glucose into pyruvate in the cytosol.
- In these series of reactions **2 ATP** molecules are added to the glucose, **2 NADH** produced, **4 ATP** are produced, and **2 pyruvates** are formed.
  - The NET production is **2 ATP** (made 4 ATP but used 2 ATP), **2 NADH**, **2 pyruvate** (+ 2 H_2O + 2 H^+)
- The ATP produced during glycolysis is via **substrate level phosphorylation**.
  - Substrate level phosphorylation results in the direct enzymatic transfer of a high energy phosphate to ADP and does not require any extraneous carriers.
- The first step of glycolysis involves the addition of a phosphate group to glucose via the enzyme **hexokinase** to produce glucose-6-phosphate. This is important because phosphorylated glucose cannot diffuse outside of the cell and traps the glucose in the cell.
- During the third step of glycolysis, another phosphate is added to an isomer of glucose-6-phosphate to form fructose 1,6-bisphosphate via the enzyme **phosphofructokinase.** This is irreversible and commits the glucose to glycolysis. This is a major regulatory point!

STEP 2: Pyruvate Decarboxylation

- **Pyruvate Decarboxylation** is the conversion of pyruvate to acetyl CoA via the pyruvate dehydrogenase complex (PDC) enzyme.
- This metabolic process occurs in the mitochondrial matrix.
  - In prokaryotes (which lack mitochondria), pyruvate decarboxylation takes place in the cytoplasm
- The product of the reaction is 1 NADH and 1 CO_2.
  - The two pyruvate molecules from glycolysis therefore produce a net of 2 NADH and 2 CO_2.

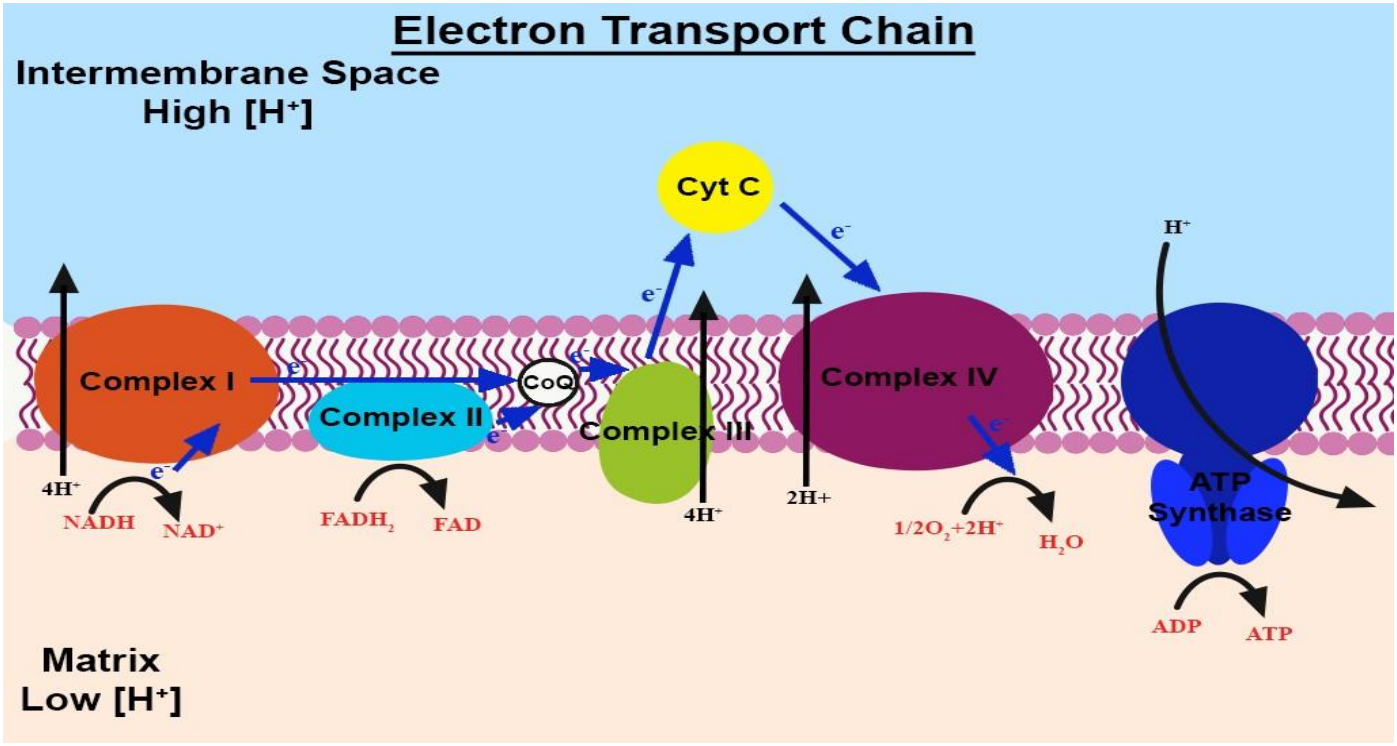
STEP 3: Krebs Cycle (or Citric Acid Cycle or Tricarboxylic Acid Cycle)

- **Krebs Cycle** is the fate of the pyruvate that is produced in glycolysis. It takes place in the mitochondrial matrix.
  - In prokaryotes (which lack mitochondria), the Krebs cycle takes place in the cytoplasm
- The acetyl CoA from pyruvate decarboxylation merges with oxaloacetate to form citrate. The cycle has 7 intermediates.
- Two cycles of the Krebs cycle occur for glucose because 2 pyruvates are made from 1 glucose in glycolysis.

- The final products of the cycle are **3 NADH, 1 $FADH_2$, 1 ATP** (via substrate level phosphorylation), **2 CO_2.**
  - A net production of **6 NADH, 2 $FADH_2$, 2 ATP** (technically GTP), **4 CO_2.**
- The CO_2 produced during the cycle is the CO_2 that the animal exhales when they breathe.

STEP 4: Electron Transport Chain (ETC)

- **Electron Transport Chain** takes place in the inner membrane/crista of the mitochondria. The **cristae** are the folds of the mitochondria and provide greater surface area for ETC to occur.
  - In prokaryotes (which lack mitochondria), the ETC takes place across the cell membrane
  - ETC couples exergonic flow of electrons with endergonic pumping of protons across the cristae membrane of the mitochondria.



Electron transport chain: carrier proteins and organic molecules found in the inner membrane of the mitochondria

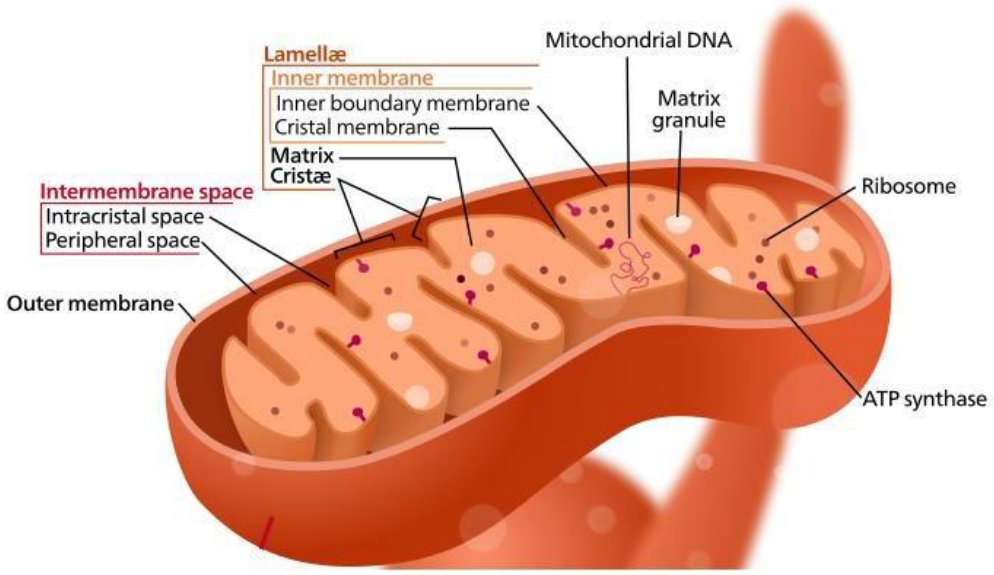
- **Oxidative Phosphorylation** is the process in which ADP forms ATP from NADH and $FADH_2$ via passing of electrons through various carrier proteins.
  - Unlike substrate level phosphorylation, the energy of the phosphate group is not transferred to the ADP. The energy comes from the electron in the ETC establishing an H^+ gradient that supplies energy to ATP synthase.
- Carrier proteins that form the ETC extract energy from NADH and $FADH_2$ while pumping protons (H^+) into the intermembrane space of the mitochondria. ATP synthase uses this gradient (an electrical and pH gradient) to make ATP as H^+ shuttles back into the inner matrix.

- NADH pumps more H^+ across the matrix, and produces more energy than $FADH_2$. NADH produces 3 ATP and $FADH_2$ produces 2 ATP.
- The final electron acceptor of the chain is **oxygen**. It combines with native H^+ to form water (H_2O).
- **Coenzyme Q (CoQ)/Ubiquinone** is a soluble carrier that is dissolved in the membrane that can be fully reduced/oxidized. It passes electrons as seen in the diagram above.
    - Note: an oxidizing agent causes *something else* to get oxidized; the oxidizing agent itself is reduced; vice versa for the reducing agent.
- **Cytochrome *c*** is a protein carrier in the ETC, and is common in so many living organisms that is used to test for genetic relations.
    - Cytochromes have non-protein parts such as iron which donate or accept electrons for redox.

Energy Production from Cellular Respiration

- Total Energy from 1 glucose is about 36 ATP, but in prokaryotes it is 38 ATP (not *actual* yield as mitochondrial efficacy varies)
  - A difference exists in the ATP yield between eukaryotes and prokaryotes because prokaryotes have no mitochondria so they do not need to transfer the two NADH molecules into the mitochondrial matrix. In eukaryotes, NADH is transported into the mitochondrial matrix via active transport, costing 1 ATP each. Prokaryotes use the cytoplasm and the cell membrane for cellular respiration.
    - Pyruvate is also actively transported into the mitochondrial matrix (in eukaryotes) but its transport is secondary active transport (symport with protons). It doesn't directly use ATP.

Mitochondria



Mitochondria Structure

Source: Kelvinsong https://commons.wikimedia.org/wiki/User:Kelvin13/Great_board_of_biology#/media/File:Mitocondri_(borderless_version)-ca.svg Public Domain

Mitochondrial Compartments	Definition	Role in Cellular Respiration
Outer Membrane	Phospholipid bilayer that encloses the organelle	-
Intermembrane Space	Space between the outer membrane and the inner membrane	H^+ released during ETC are found here
Inner Membrane	Phospholipid bilayer that is folded into many folds called the cristae	Location of the ETC (oxidative phosphorylation)
Mitochondrial Matrix	Space within the inner membrane	Krebs cycle and Pyruvate Decarboxylation

Chemiosmosis in Mitochondria

- **Chemiosmosis** is the mechanism of ATP generation that occurs when energy is stored in the form of a proton (H^+) concentration gradient across a membrane.
  - The Krebs Cycle produces NADH and $FADH_2$.
  - NADH and $FADH_2$ are oxidized (lose electrons) resulting in the transportation of H^+ from matrix to intermembrane space. This results in the formation of a pH and electric charge gradient (an **electrochemical gradient**).
  - ATP synthase uses the kinetic energy from the flow established by this gradient (proton motive force) to create ATP by letting the protons flow from the intramembrane space back to the matrix.

Anaerobic Respiration

- **Anaerobic Respiration** occurs in the presence of no O_2 in the cytosol. It includes glycolysis and fermentation.
- Aerobic Respiration regenerates NAD^+ via O_2 which is required for the continuation of glycolysis.
  - Without O_2 there would be no replenishing, resulting in the accumulation of NADH. This would result in cell death with no new ATP. Therefore, fermentation occurs.
- **Facultative anaerobes** undergo anaerobic respiration/fermentation when no O_2 is available but they can use O_2 when it is present (more efficient).
- Both fermentation and aerobic cellular respiration use glycolysis and produce a pyruvate.
  - Pyruvate commits to either aerobic cellular respiration or fermentation based on the presence of oxygen.
  - For cells capable of both aerobic cellular respiration or fermentation (facultative anaerobes and muscle cells), the pathway is selected based on the presence of oxygen.
- **Obligate anaerobes** cannot survive in the presence of O_2.
- **Microaerobes** require O_2 but are harmed by increasing amounts of it (e.g. H. pylori).

Alcohol Fermentation

- **Alcohol Fermentation** occurs in plants, fungi (e.g. yeasts), and bacteria (e.g. botulinum)
- The chemical equation can be broken down into two steps.
  1. Pyruvate → Acetaldehyde $+CO_2$
  2. Acetaldehyde → Ethanol (and NADH → NAD^+)
- Acetaldehyde is the final electron acceptor! Acetaldehyde accepts the electrons to form the final product of ethanol. This is similar to O_2 being the final electron acceptor of cellular respiration, thus forming the final product of H_2O.

Lactic Acid Fermentation

- **Lactic Acid Fermentation** occurs in human muscle cells and other microorganisms.
- The chemical equation is Pyruvate → Lactate (and NADH → NAD^+)
- Once an excess amount of ATP is available, lactate is transported back to the liver to be converted back to glucose via the **Cori cycle**.

- Muscles that are actively contracting have higher lactate levels compared to resting muscles.

Alternative Energy Sources

- When glucose supply is low, the body uses other energy source.
  - From highest to lowest priority: carbohydrates > fats > proteins
- These alternative energy sources are first converted to glucose or glucose intermediates, then are degraded in either glycolysis or the Krebs cycle.

Other Carbohydrates

- Disaccharides are hydrolyzed into monosaccharides, most of which can be converted to glucose or glycolytic intermediates.
  - Remember, glucose is not only broken down, but also can be produced via **gluconeogenesis**.
    - Gluconeogenesis occurs in the liver and kidney. The liver is responsible for maintaining the glucose concentration in the blood.
  - Glucose is stored in the body as a polymer called **glycogen** in primarily the liver (2/3) and muscles (1/3).
    - All cells are capable of producing and storing glycogen but only liver cells and muscle cells have large amounts.
  - After large meals, **insulin** stores glucose as glycogen. **Glucagon** has the opposite effect and turns on glycogen degradation.
    - Insulin activates the PFK enzyme, while glucagon inhibits it.
    - Think about it this way: insulin says "hey, we've got a lot of glucose around, so let's chew up," whereas glucagon says "uh oh, not enough glucose around, don't chew it up- we need it for the brain, other tissues can use other energy sources."

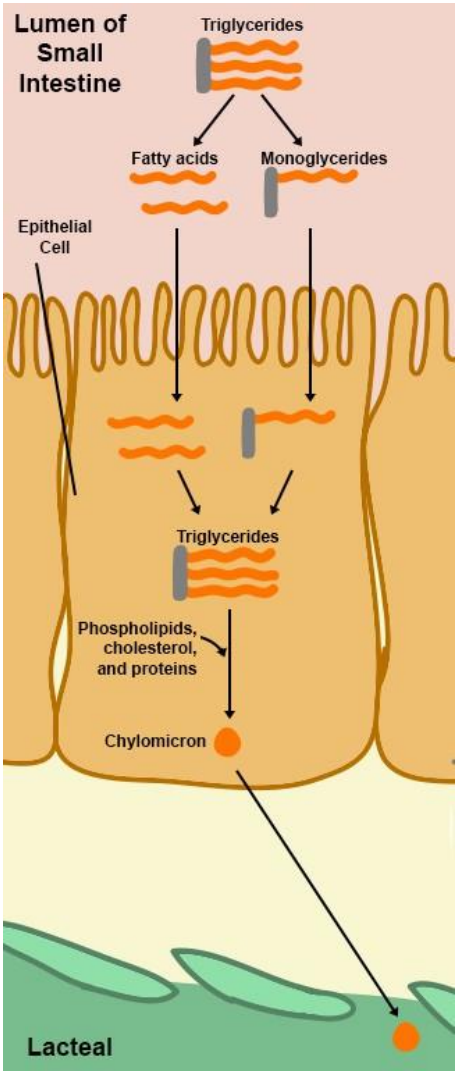
Fats

- Fats store more energy than carbohydrates per carbon as their carbons are in a more reduced state.
  - Hence why fats are 9 cals/g, whereas carbohydrates and protein are 4 cals/g.
- **Lipases** are enzymes that break down fats into fatty acids and glycerol or other alcohols.
  - Lipases in adipose tissue are hormone sensitive (e.g. to glucagon).
  - Fatty acids combine with **albumin** in the blood which carries them.
  - Between meals, most lipids of plasma (mainly fatty acids) are in the form of lipoproteins.
    - Some forms of lipoproteins found are chylomicrons (large microproteins), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).
    - Low density lipoproteins have a low density of proteins and a high fat density. They are considered unhealthy.
    - High density lipoproteins have a high protein density and a low fat density. They are considered healthy.
- Glycerol is converted into PGAL, and then enters glycolysis.
  - PGAL is an alternative name for glyceraldehyde 3-phosphate (G3P).

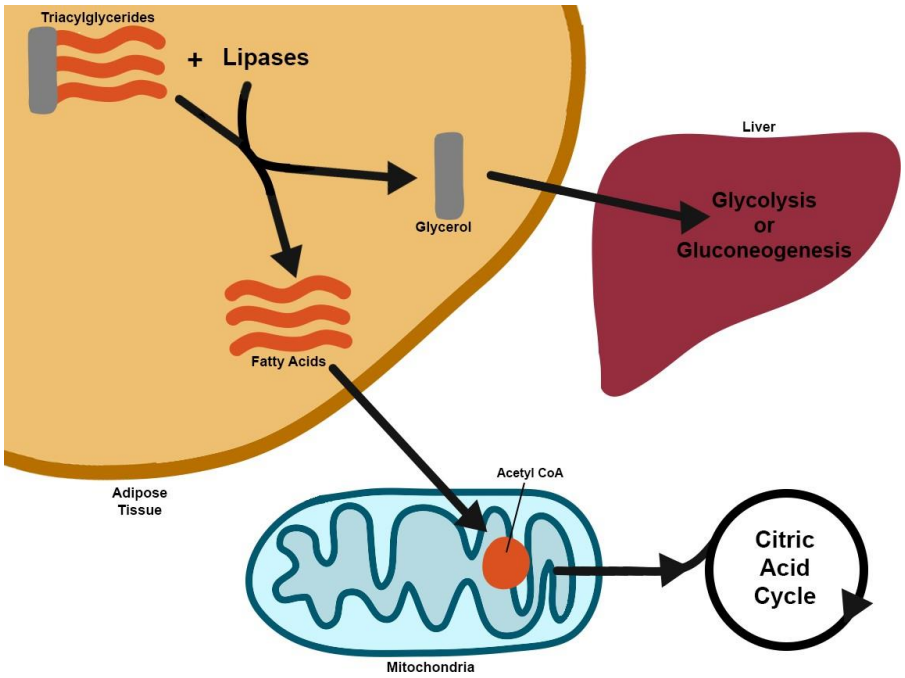
- Fatty Acids are broken down for energy via **beta oxidation**.
  - Beta oxidation takes place in the mitochondrial matrix.
  - Before the fatty acid enters beta-oxidation, it must be activated. Two ATP molecules are spent activating the entire chain.
  - The fatty acid is converted into acetyl CoA, which enters the Krebs cycle.
    - Animals cannot convert fatty acids to glucose. Acetyl CoA, specifically, enters the Krebs cycle.
    - Plants and bacteria use a modified version of the Krebs cycle called **glyoxylate cycle** that produces sugar from acetyl CoA.
  - Every 2 carbons from a fatty acid chain makes an acetyl CoA.
  - Saturated fatty acids produce 1 NADH and 1 $FADH_2$ for every cut into 2 pieces.
    - Note this is NOT the same thing as for every 2 carbons- e.g. 18C chain is 9 2C pieces but cut only 8 times. Each cut is the beta oxidation step.
  - Unsaturated fatty acids produce 1 less $FADH_2$ for each double bond because it can't use the first step of beta oxidation: the double bond forming step.
  - Beta oxidation results in a BIG yield of ATP. It yields more ATP per carbon that carbohydrates. There is more energy in fats than sugars.

Absorption of Fats

- Triglycerides in the lumen of the small intestine (the tube itself) are broken down via lipases into monoacylglycerides and fatty acids.
  - Monoacylglycerides and fatty acids are absorbed into the **enterocytes** (cell lining of the small intestine). There, they are reassembled into triglycerides, and then (along with cholesterol, proteins, phospholipids) packaged into chylomicrons which move on to the lymph capillary for transport to the rest of the body where they are stored as adipose tissue.
- In absorption, nutrients enter the blood steam from the villi of the small intestine and then go to the liver for regulation of blood nutrient content. From the liver, they go to the heart and rest of the body.



Breakdown and absorption of digested fats in the body



Breakdown of stored fat (adipose tissue) in the body

Proteins

- Proteins are the least desirable source of energy. It is used only when carbohydrates and fats are unavailable.
- Most amino acids are deaminated in the liver, and then converted to pyruvate or acetyl CoA or other Krebs cycle intermediates. These metabolic products enter cellular respiration at various points (varies by amino acid).
- **Oxidative deamination** removes ammonia molecules directly from amino acids.
    - Ammonia is toxic to vertebrates.
        - Most aquatic species (and invertebrates) excrete ammonia directly.
        - Insects/birds/reptiles convert ammonia to uric acid and then excrete it.
        - Mammals/sharks/most amphibians convert ammonia to urea for excretion.

Chapter 4: Photosynthesis

Photosynthesis

Description and Overall Reaction

- **Overall reaction**
  - $6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$ (Alternatively: $6CO_2 + \mathbf{12H_2O} \rightarrow C_6H_{12}O_6 + 6O_2 + \mathbf{6H_2O}$)
- **Description**
  - Photosynthesis begins with light-absorbing pigments in plant cells that are able to absorb energy from light: **chlorophyll a, b,** and **carotenoids** (red, orange, yellow). Light is incorporated into electrons and excited electrons are unstable and re-emit absorbed energy. The energy is then reabsorbed by electrons of nearby pigment molecules.
  - The process ends when energy is absorbed by one of two special chlorophyll a molecules (P_{680} & P_{700}). P_{700} forms pigment cluster (PSI) and P_{680} forms pigment cluster (PSII).
- **Pigments**
  - Antenna pigments (chlorophyll b, carotenoids, phycobilins [red algae pigment], and xanthophylls) capture wavelengths that chlorophyll a does not. These pigments pass energy to chlorophyll a where direct light reaction occurs. Chlorophyll a has a porphyrin ring (alternating double and single bonds, double bonds critical for light reactions) complexed with a **magnesium** atom inside.
  - Note: Red and blue light are most effective at promoting photosynthesis, while green light is the least effective.

Noncyclic Photophosphorylation: Light-Dependent Reaction

- Goal of this reaction is to attach a phosphate on ADP to make ATP using light (ADP + P_i + light → ATP)
- Overall reaction of this step
  - H_2O + ADP + P_i + $NADP^+$ + light → ATP + NADPH + O_2 + H^+
- Location: **Thylakoid membranes**, but **photolysis** takes place inside the **thylakoid lumen** (passes electrons to the membrane for noncyclic photophosphorylation).

Steps		Description
1.	**Photosystem II**	Electrons trapped by P_{680} in PSII are energized by light.
2.	**Primary e^- acceptor**	Two excited e^- are passed to primary e^- acceptor; primary because it is the first in chain of acceptor.
3.	**E^- transport chain**	Consists of a plastoquinone complex (PSII) which contains proteins like **cytochrome** and cofactor Fe^{2+}; analogous to oxidative phosphorylation.
4.	**Phosphorylation**	$2e^-$ move down chain → lose energy (energy used to phosphorylate about 1.5ATP).
5.	**Photosystem I**	e^- transport chain terminates with PSI (P_{700}); they are again energized by sunlight and passed on to another primary e^- acceptor. From this point forward it can go to the cyclic or noncyclic path. If noncyclic…
6.	**NADPH**	$2e^-$ then pass down a short electron transport chain (with proteins like **ferredoxin**) to combine $NADP^+ + H^+ + 2e^-$ → NADPH (coenzyme) (this step takes place only in the noncyclic pathway).
7.	**Splitting of water (photolysis)**	The loss of $2e^-$ from PSII (initially) is replaced when H_2O splits into $2e^-$, $2H^+$, and $½O_2$ (The H^+ produced is used for NADPH formation and the ½ O_2 contributes to release as oxygen gas). This occurs at PSII.

Cyclic Photophosphorylation

- **Description:** This replenishes ATP when the Calvin cycle consumes it. When the excited $2e^-$ from PSI join with protein carriers in the first electron transport chain and generate 1 ATP as they pass through, these $2e^-$ are recycled into PSI and can take either cyclic or noncyclic path.
- **Location: Stroma lamellae** (pieces connecting the thylakoids)

Calvin Cycle

- **Description:** Fixes CO_2, repeats 6 times, uses $6CO_2$ to produce $C_6H_{12}O_6$ (glucose).
  - This is the "dark reaction", but it cannot occur without light because it is dependent on the high energy molecules produced from the light reaction (ATP and NADPH).
  - The energy used to drive the light-independent (dark) reactions comes from light (photons). Light energy ultimately drives photosynthesis, and is the original source of energy stored in glucose chemical bonds.
  - Plants do have mitochondria that make ATP. Plant mitochondria that produce ATP are used as energy for general cellular processes, *while the ATP produced from photosynthesis* in the chloroplast is used to *drive photosynthesis* further in the Calvin Cycle. The Calvin cycle then makes glucose for plant cells to break down and use as energy.
- **Location:** Stroma.

Steps	Description
1. **Carboxylation**	$6CO_2$ + 6RuBP → 12PGA. **RuBisCo** (most common protein in the world) catalyzes this reaction. (Thus named because PGA is 3C).
2. **Reduction**	12ATP + 12NADPH converts 12PGA → 12G3P or 12PGAL. Energy is incorporated; by-products ($NADP^+$ and ADP) go into noncyclic photophosphorylation.
3. **Regeneration**	6ATP convert 10G3P → 6RuBP. This allows the cycle to repeat.
4. **Carbohydrate synthesis**	$6\mathbf{CO_2}$ + 18ATP + 12NADPH + H^+ → 18ADP + $18P_i$ + $12NADP^+$ + **1 glucose (2 G3P).** In summary, 2 remaining **G3P** are used to build glucose.

Role of the Chloroplast

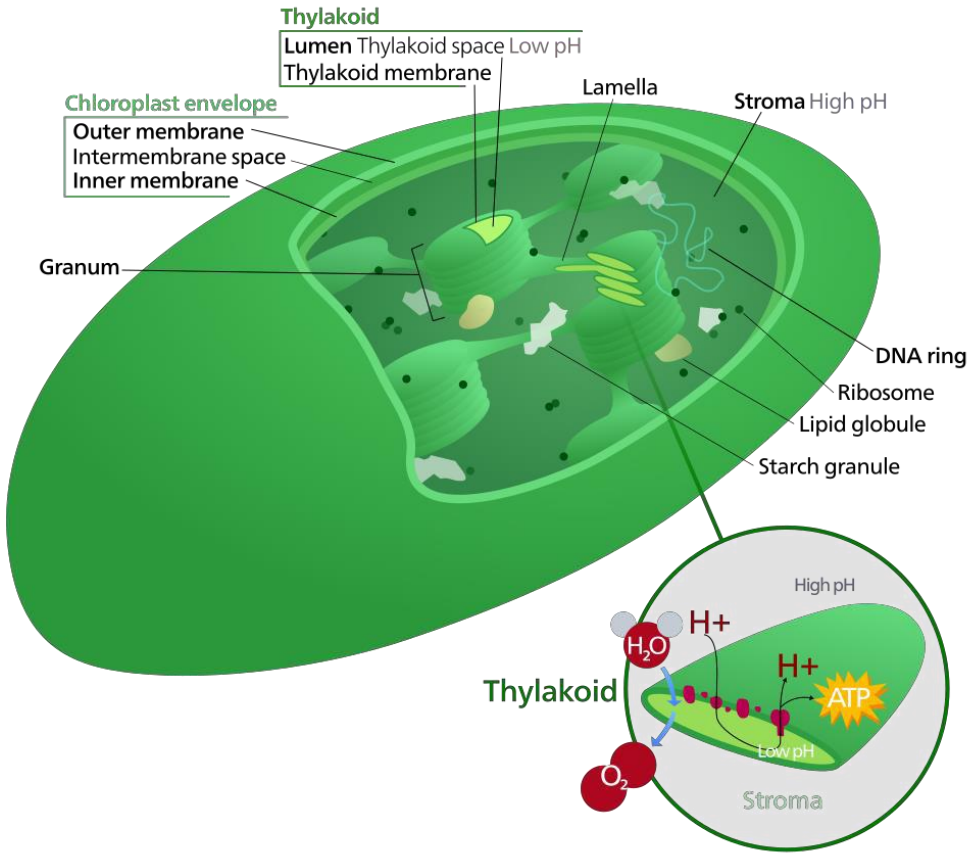
Chloroplast structure

- **Light-dependent** and **light-independent reactions** occur in this organelle. It has a double membrane like the mitochondria and nucleus.

Structure	Description
Outer membrane	The outer plasma membrane composed of a phospholipid bilayer.
Intermembrane space	The space between the outer and inner membranes.
Inner membrane	The inner plasma membrane composed of a phospholipid bilayer.
Stroma	This fluid material fills area inside inner membrane. The **Calvin cycle** occurs here (fixing CO_2 → G3P).
Thylakoids	This phospholipid bilayer structured organelle is suspended within stroma (stacks). The individual membrane layers are thylakoids. An entire stack is called the **granum** membrane of thylakoids. It contains (PSI + PSII), cytochromes, and other e^- carriers.
Thylakoid lumen	This is the interior of the thylakoid. H^+ accumulates here.

Note: Established proton gradient uses ATP synthase to move the accumulated H^+ from inside thylakoid lumen outside to the stroma, generating ATP in the process. In contrast, in oxidative phosphorylation we build up H^+ outside the mitochondria and then shuttle it back in to generate ATP via synthase.

Note: The thylakoid membrane absorbs light. *Not* the outer and inner chloroplast membrane.



Structures of the chloroplast

Source: Kelvinsong (https://commons.wikimedia.org/wiki/File:Chloroplast_II.svg), Chloroplast II, https://creativecommons.org/licenses/by/3.0/legalcode

Chemiosmosis in Chloroplasts

- **Description:** This process uses an H^+ gradient to generate ATP.
- **Location:** Across the **thylakoid membrane**.

Steps	Description and notes
1. **H^+ ions accumulate inside thylakoids**	H^+ are released into lumen when H_2O is split by PSII. H^+ is also carried into lumen from stroma by cytochrome between PSII and PSI.
2. **A pH and electrical gradient is created**	The pH created is about **5**.
3. **ATP synthase generates ATP as H^+ ions move across the thylakoid membrane**	ADP is phosphorylated to create ATP. ADP + P_i → ATP. $3H^+$ ions are required for 1 ATP
4. **Calvin cycle produces 2G3P using NADPH, CO_2, and ATP**	At the end of the e^- transport chain following PSI, $2e^-$ produces NADPH.

Other Photosynthetic Processes

C_2 Photosynthesis (Photorespiration)

- Involves fixation of oxygen by the **rubisco** enzyme but produces no ATP or sugar. Rubisco is not "efficient" or fast because it will fix both CO_2 and oxygen at the same time if both are present.
- The byproducts of photorespiration are metabolized by **peroxisomes**

C_4 Photosynthesis (Hatch-Slack Pathway)

- This process evolved from C_3 photosynthesis. In C_4 photosynthesis, when CO_2 enters a leaf it gets absorbed by mesophyll cells (then moved to bundle sheath cells). Instead of being fixed by rubisco into PGA, CO_2 combines with PEP to form OAA by PEP carboxylase (in mesophyll).
  - OAA has 4C (hence C_4 photosynthesis)
  - OAA → Malate and is then transported through **plasmodesmata** into bundle sheath cells.
  - Malate → pyruvate + CO_2. CO_2 can be used in the Calvin cycle.
  - Pyruvate is moved back to the mesophyll, then pyruvate → PEP (this process requires 1 ATP → AMP).
- Overall, the purpose is to move CO_2 from **mesophyll** to **bundle sheath cell** (this leaf structure = **Kranz** anatomy). Little O_2 presence in bundle sheath cells reduces competition while rubisco is fixing. This minimizes photorespiration and H_2O loss from the **stomata** (leaf pores).
- Found in hot, dry climates (faster fixation speed and more efficient). Requires one additional ATP (which becomes AMP). C_3 typically occurs in mesophyll cells, but in C_4 it occurs in bundle-sheath cells. Examples: corn, sugarcane

CAM Photosynthesis

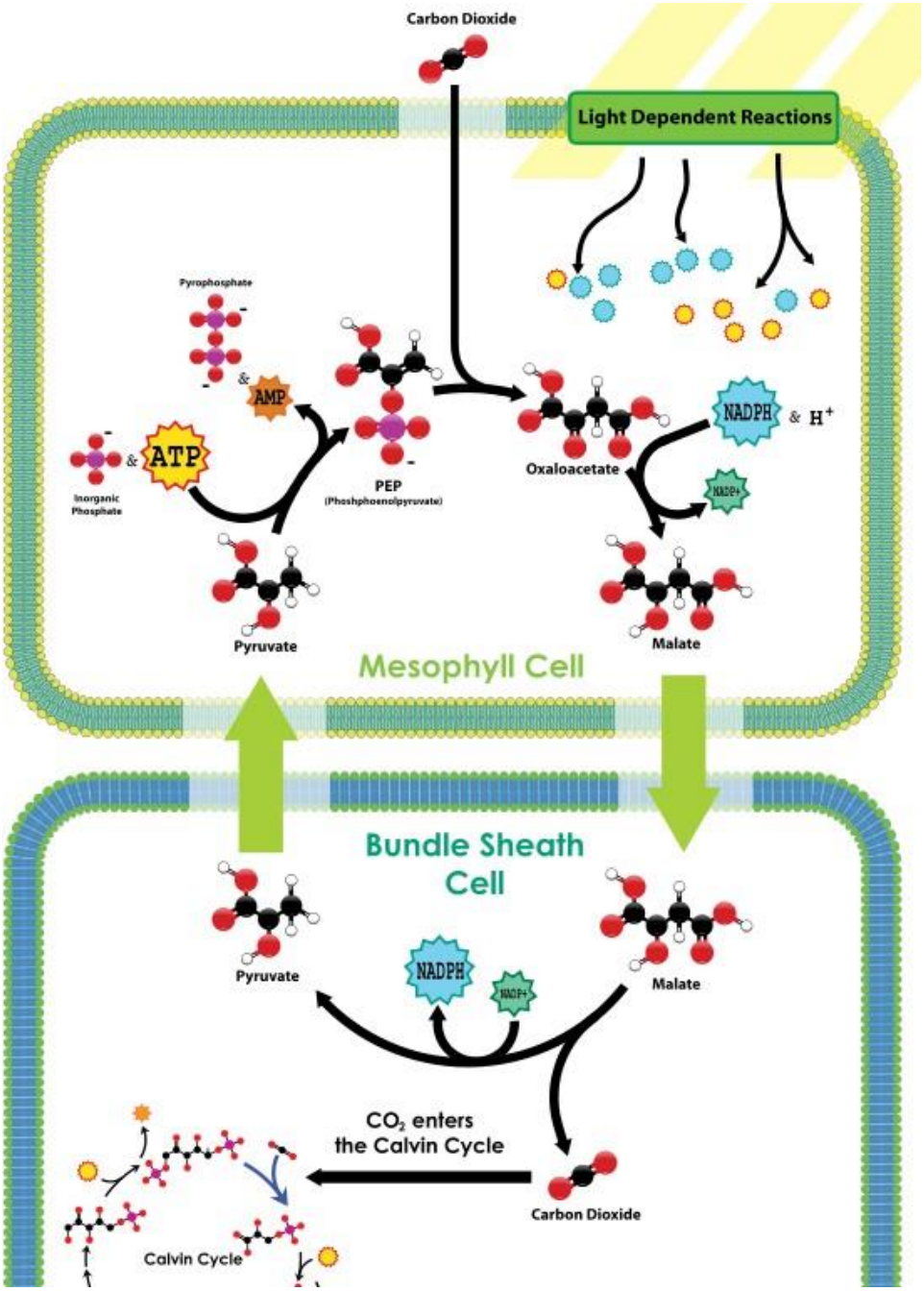
- This is another add-on to C_3, called **crassulacean acid metabolism**; almost identical to C_4.

1. **PEP carboxylase** fixes CO_2 + PEP → OAA; OAA → malic acid.
2. **Malic acid** is shuttled into vacuole of cell.
3. At **night**, stomata are open (opposite of normal), PEP carboxylase is active, malic acid

accumulates in vacuole.

4. During the **day**, stomata are closed. Malic acid moves out of vacuole and is converted back to OAA (requires 1 ATP), releasing CO_2 (moves into Calvin cycle with rubisco) and PEP.

- Overall advantage is that CAM photosynthesis can proceed during the day while stomata are closed (reducing H_2O loss). Occurs in cacti, crassulacea, desert plants.



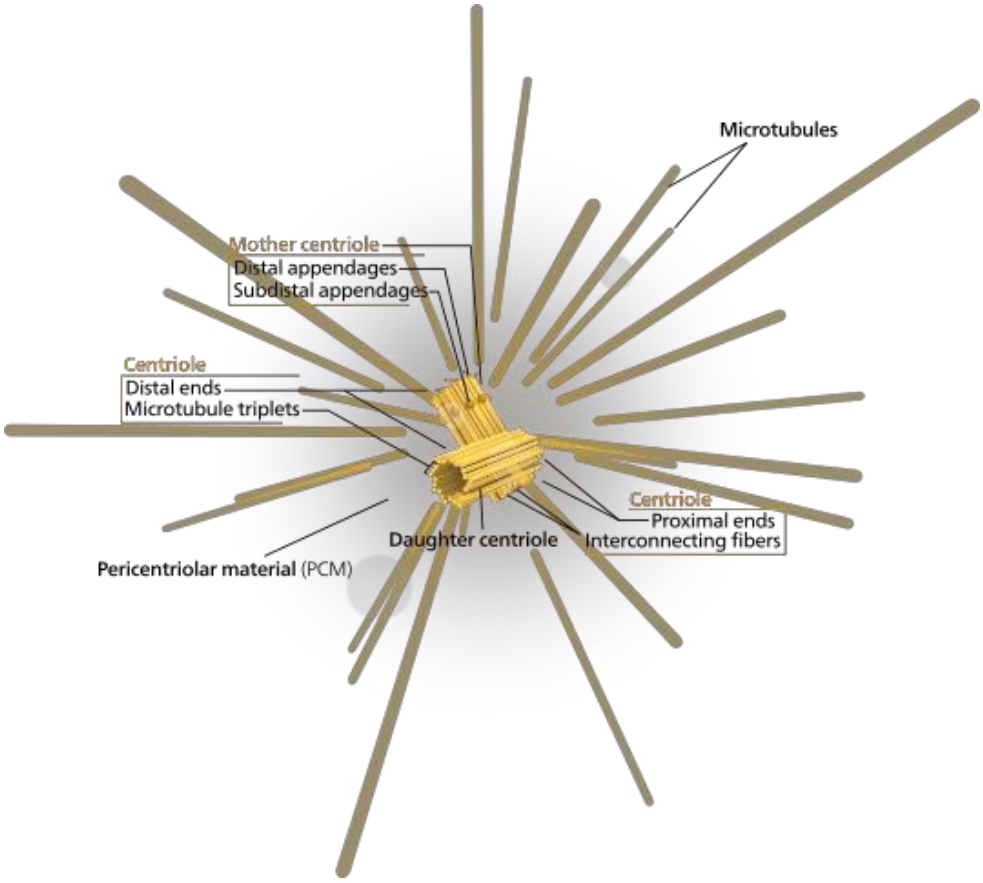
C4 Photosynthesis

Adenosine (https://commons.wikimedia.org/wiki/File:HatchSlackpathway2.svg), "HatchSlackpathway2", https://creativecommons.org/licenses/by-sa/2.5/legalcode

Chapter 5: Cell Division

Cell Division

- **Cell division** is nuclear division (karyokinesis) followed by cytokinesis.
- In diploid cells, there are two copies of every chromosome, forming a pair called **homologous chromosome**.
- Humans have 46 chromosomes, 23 homologous pairs, and a total of 92 chromatids (depending on stage of division).
- **MTOCs** are microtubule organizing centers – in animal cells these are the **centrosomes**. A pair of MTOCs are found outside the nucleus.
    - In animal cells, each MTOC contains a pair of **centrioles**.
    - Plants have MTOCs, but they are not centrosomes.
- Mitosis occurs in somatic cells and meiosis occurs in gametes (egg, sperm, pollen).
    - Fusion of two haploid gametes = fertilization/syngamy = diploid zygote



Structures of the centrosome

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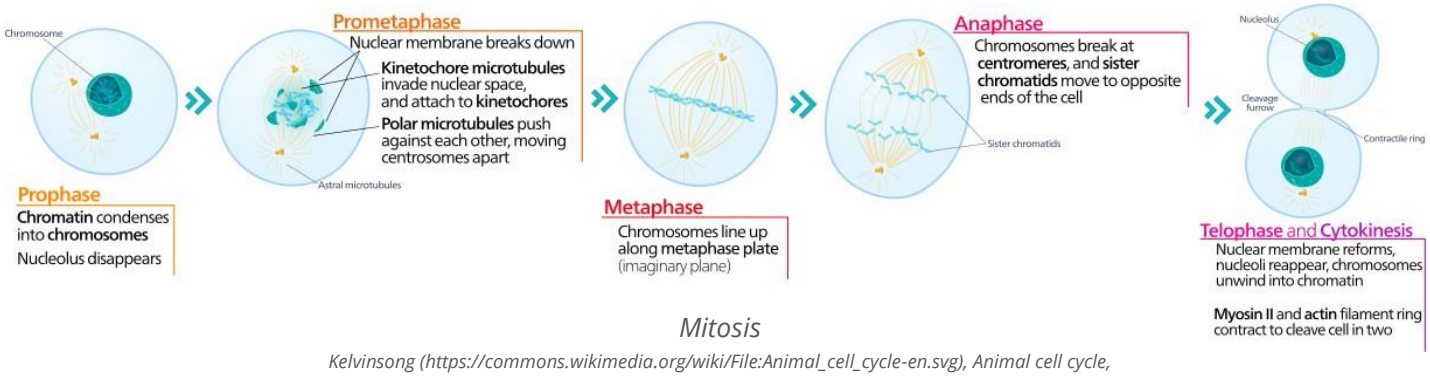
Mitosis



Mitosis

Kelvinsong (https://commons.wikimedia.org/wiki/File:Animal_cell_cycle-en.svg), Animal cell cycle, https://creativecommons.org/licenses/by/3.0/legalcode

Prophase
- Nucleus disassembles, nucleolus disappears
- Chromatin condenses into chromosomes, nuclear envelope breaks down
- Mitotic spindle forms and microtubules (composed of tubulin) begin connecting to kinetichores

Metaphase
- Chromosomes line up in a single file in the center
- Each chromatid is complete with a centromere and attached kinetichore.
- Centrosomes are at opposite ends of the cell
- Karyotyping is performed here

Anaphase
- Microtubules shorten
- Each chromosome is pulled apart into two chromatids (once separated, each chromatid is considered a chromosome)
- Chromosomes pulled to opposite poles (**disjunction**)
- At the end of this phase, each pole has a complete set of chromosomes
- Shortest step of mitosis

Telophase
- Nuclear division
- Nuclear envelope develops
- Chromosomes → chromatin
- Nucleoli reappear

DAT Tips

- Before mitosis, chromatin condenses into chromosomes. Presence of chromosomes means mitosis is occurring.
- Each metaphase chromosome consists of 2 closely attached sister chromatids.
- The end of metaphase is denoted by the presence of centrosomes at opposite ends of the cell.
  - Before moving on to anaphase, the cell checks that each chromosome is attached to microtubules with their kinetochore. This ensures that in anaphase the sister chromatids split evenly.
- In metaphase, to keep track of the total number of chromosomes, count the centromeres!
- In anaphase, the chromosome number doubles.
- At the end of anaphase, each pole has a complete set of chromosomes, same as the original cell before replication.

- There would be a total of 92 chromosomes (92 chromatids) if a cell has 46 chromosomes at the beginning.

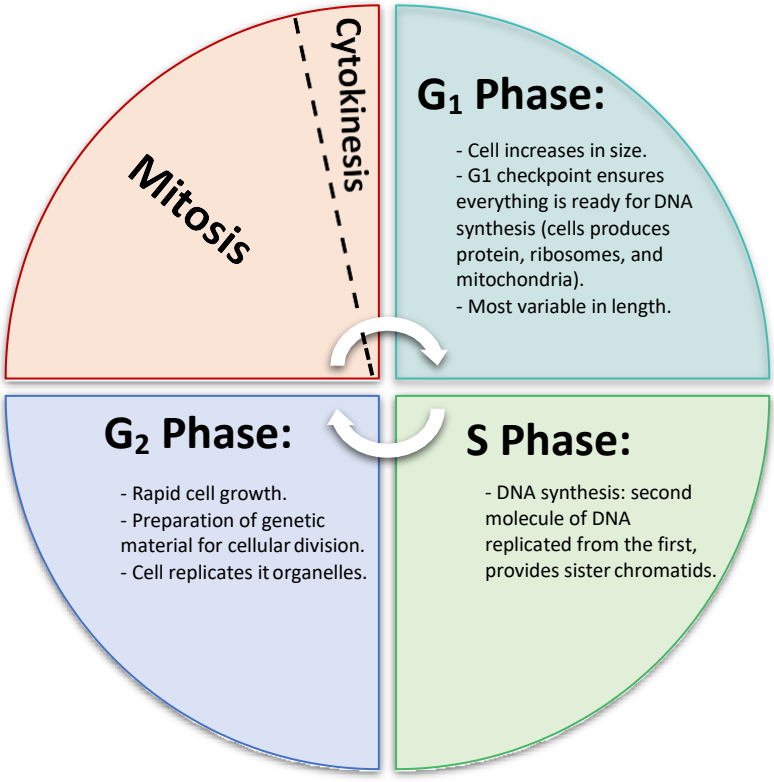
- It is important to note that unlike meiosis, NO genetic variation occurs in mitosis.

Cytokinesis

- **Cytokinesis** begins during the later stages of mitosis (most sources indicate it begins toward the end of anaphase). It is the division of cytoplasm to form 2 cells.
  - Animal cells separate via creation of the **cleavage furrow**.
    - Actin and myosin microfilaments shorten and the plasma membrane is pulled into the center.
  - Plant cells separate via formation of a **cell plate**.
    - Vesicles from Golgi bodies migrate and fuse to form a cell plate, out growth and merge with plasma membrane separating the two new cells.
    - The cells don't actually separate from each other. The middle lamella cements adjacent cells together.

Interphase

- **Interphase** begins after mitosis and cytokinesis are complete. It consists of a G1, S, and G2 phase.
- Interphase is a part of the **cell cycle**.
  - The cell cycle consists of mitotic phases (mitosis, cytokinesis) + interphase (G1, S, G2 phases).
  - 90% of the cell cycle is spent in interphase. Growth occurs in all three interphases, not just G's.
  - Evidence suggests the cell cycle is regulated by molecular signals in the cytoplasm.



Regulation of Cell Cycle

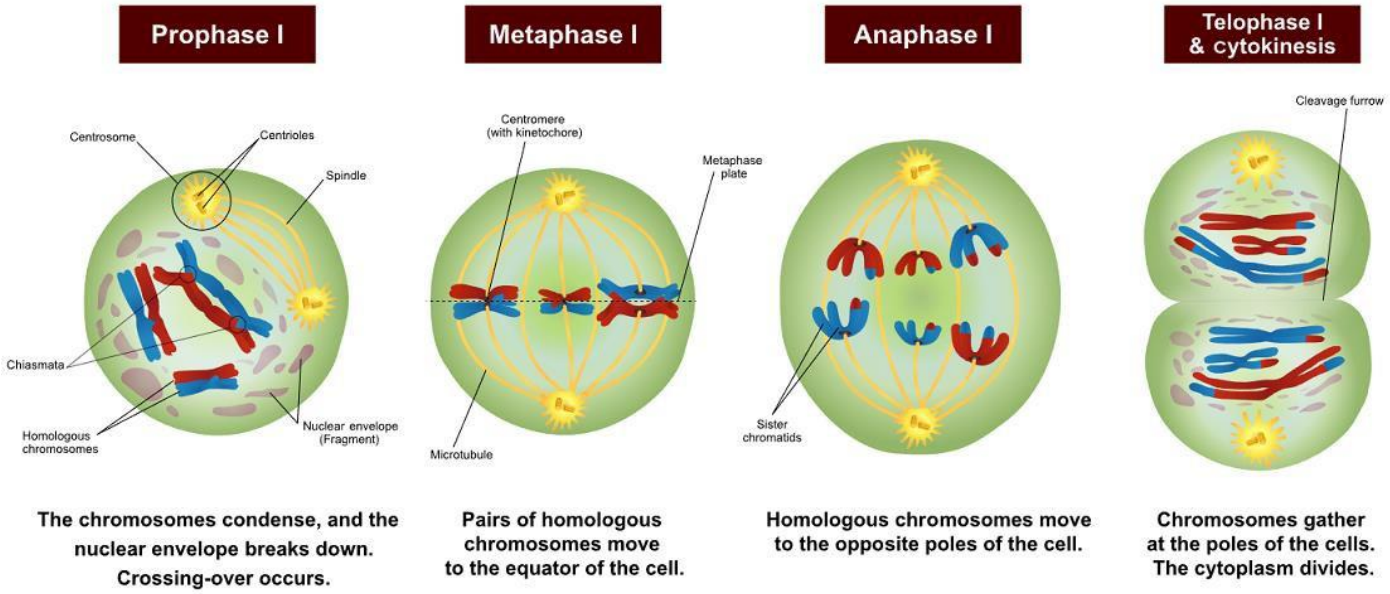
Functional Limitations	
Surface-to-Volume ratio (S/V)	• When a cell grows the volume gets larger more rapidly ($\frac{4}{3}\pi r^2$) vs. surface area ($4\pi r^2$). • When Surface/Volume is large, cellular exchange becomes easier. • When Surface/Volume is small, cellular exchange is hard, and leads to cell death or cell division to increase SA.
Genome-to-Volume ratio (G/V)	• The genome size remains constant throughout life. As the cell grows, only the volume increases. Genome/Volume will be small. • As Genome/Volume decreases, the cell exceeds the ability of its genome to produce sufficient amounts of regulation for cellular activities. Some large cells (paramecium, human skeletal muscle) are multinucleated to deal with this.

Cell Specific Regulations	
Cell Cycle Checkpoints	End of G_1 (Restriction Point) • The most important checkpoint. • Cell growth is assessed and favorable conditions are checked. If checkpoint fails, cell enters G_0. • Some cells (liver, kidney) can be induced out of G_o, some stay permanently (nerve and muscle cells). • Cells can either never proceed or wait until the cell is ready.
	End of G_2 • The cell evaluates the accuracy of DNA replication and signal whether to begin mitosis. • The cell checks for sufficient mitosis promoting factor (MPF) levels to proceed.
	M Checkpoint – during metaphase • Mitosis stops if the chromosomes are not attached to spindle fibers. • If all are attached, cell is allowed to proceed with anaphase.
Cyclin-dependent kinases (Cdk's)	• Cdk enzymes activate proteins that regulate cell cycle by phosphorylation. • Cdk's are activated by the protein cyclin.
Growth Factors	• The plasma membrane has receptors for growth factors that stimulate cells for division (such as damaged cell).
Density-dependent inhibition	• Cells stop dividing when surrounding cell density reaches a maximum.
Anchorage dependence	• Most cells only divide when attached to an external surface such as neighboring cells or a side of culture dish.

- Cancer cells defy the five cell-specific regulations in place. Such cells are called **transformed cells**.
  - Cancerous cells are a manifestation of defective cell differentiation.
  - Cancer drugs that inhibit mitosis do so by disrupting the ability of microtubules to separate chromosomes during anaphase, thus stopping replication.
  - A **myeloma** is a cancerous plasma cell
    - An antibody producing plasma cell can be fused with a myeloma to produce a **hybridoma**
      - The benefit to a hybridoma is the combined longevity of a myeloma and the antibody producing ability of a plasma cell

Meiosis

Meiosis I



Meiosis I

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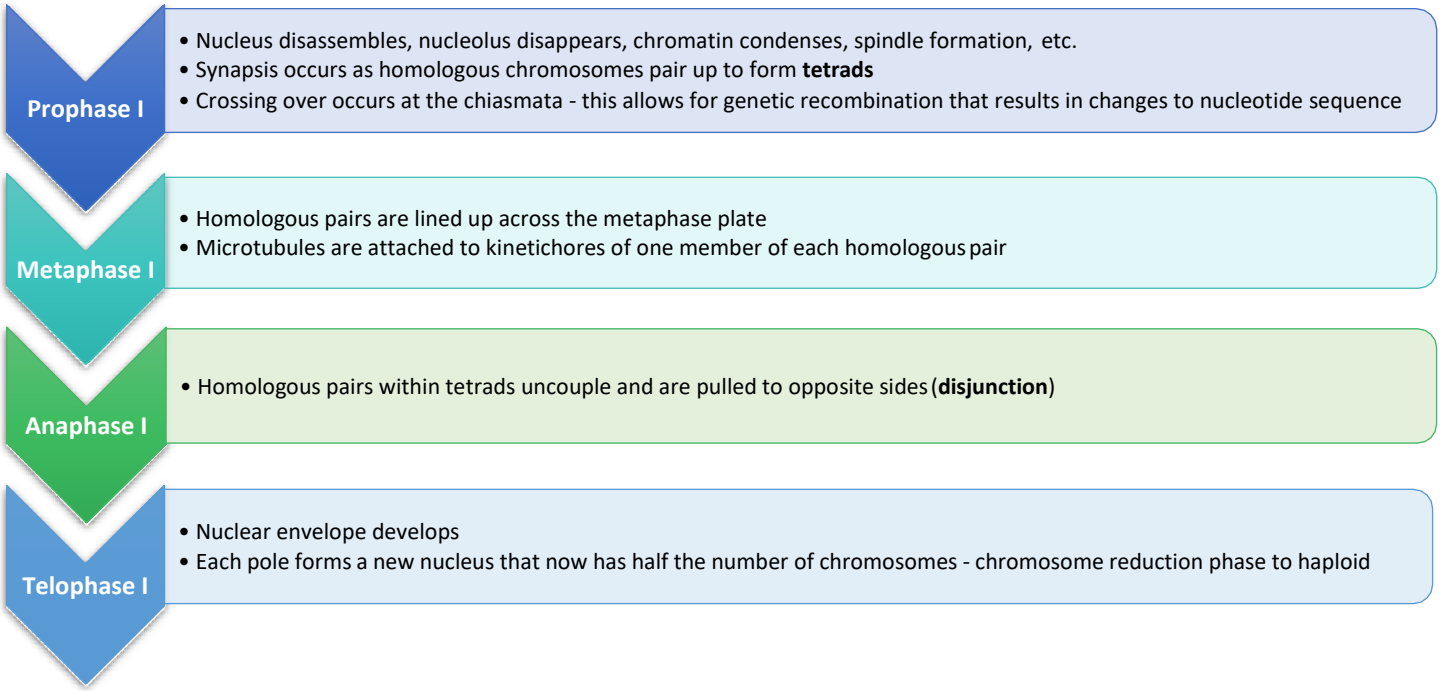
- In **Meiosis I** homologous chromosomes pair at the plate, and migrate to opposite poles. There is no separation of sister chromatids. During Prophase I, the following critical structures and processes are observed:
  - **Synapsis** is when the homologous chromosomes pair up. These pairs are referred to as **tetrads** (groups of 4 chromosomes) or bivalents.
  - The **chiasmata** is the region when crossing over occurs of non-sister chromatids.

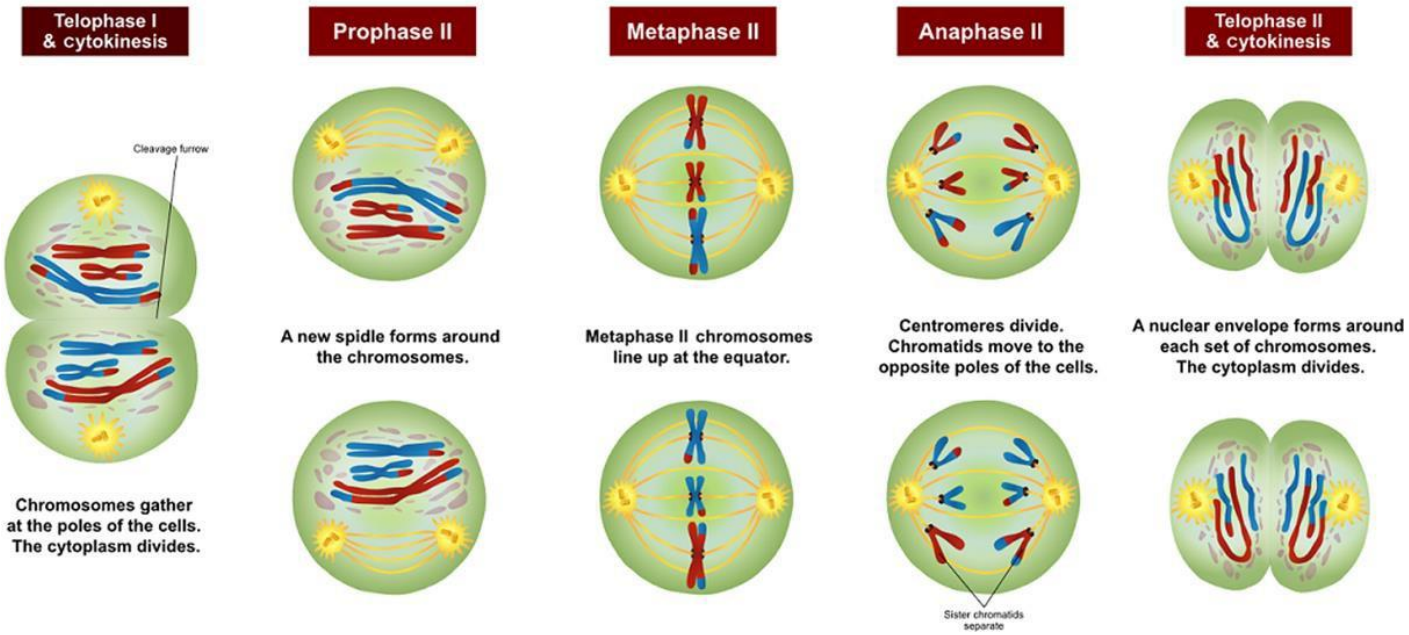
- The **synaptonemal complex** is a protein structure that temporarily forms between homologous chromosomes. This gives rise to the tetrad with chiasmata and crossing over.



DAT Tips

- At the end of anaphase I, there is a total of 46 chromosomes if a cell has 46 chromosomes at the beginning because 23 chromosomes are pulled to each pole by independent assortment and no chromatids are separated at anaphase I.
- Prophase I has 5 sub-steps.
  - **Leptotene** (chromosomes start condensing) → **zygotene** (synapsis begins; synaptonemal complex forming) → **pachytene** (synapsis complete, crossing over) → **diplotene** (synaptonemal complex disappears, chiasma still present) → **diakinesis** (nuclear envelope fragments, chromosomes complete condensing, tetrads ready for metaphase).

Meiosis II



Meiosis II

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Prophase II
- Nuclear envelope disappears, spindle develops, etc.
- No chiasmata
- No crossing over

Metaphase II
- Chromosomes align on metaphase plate like in mitosis, but there are now half the number of chromosomes

Anaphase II
- Each chromosome is pulled into two separate chromatids and migrate to opposite poles of the cell

Telophase II
- Nuclear envelope develops and cytokinesis occurs
- At completion of meiosis II, there are now 4 haploid cells

- In **Meiosis II** chromosomes spread across the metaphase plate and sister chromatids separate and migrate to opposite poles. It is similar to mitosis.
- Interphase may occur in between Meiosis I and Meiosis II. It depends on the species.

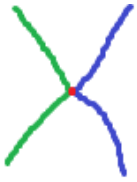
Genetic Variation

- Genetic recombination during meiosis and sexual reproduction originates from three events:
  - Crossing over during prophase I
  - Independent assortment of homologous chromosome during metaphase I
    - The random orientation of homologous chromosomes allows for the production of gametes with many different assortments.
  - Random joining of gametes aka germ cells (which sperm fertilizes which egg)
    - Joining of gametes is random, but some sperm cells have a genetic composition that gives them a competitive advantage – so not all sperm cells are "equally" competitive.

Chromosome and Chromatid Numbers during Mitosis and Meiosis

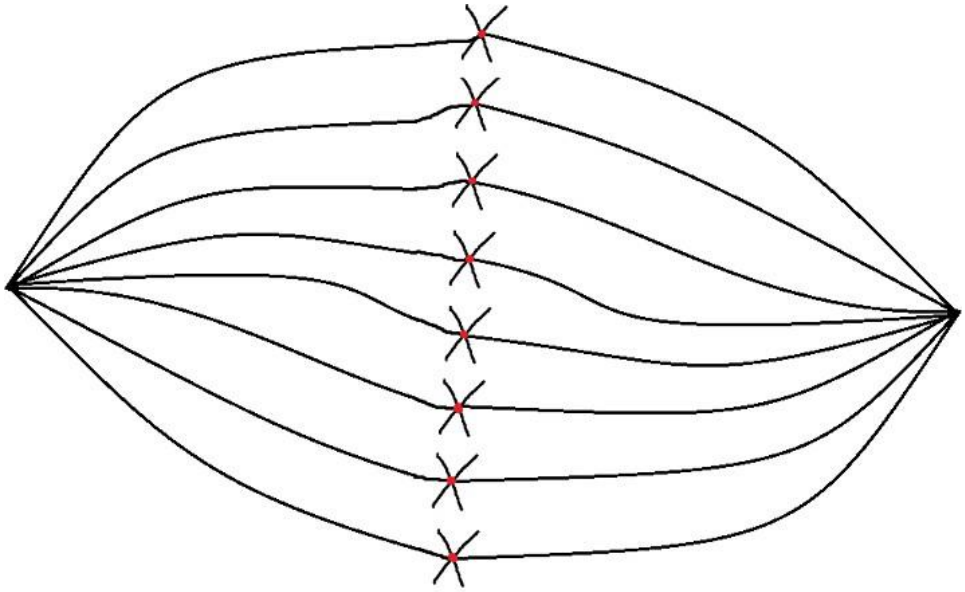
Chromatin is the general packaging of DNA around histone proteins – this arrangement of DNA helps to condense DNA to fit within the nucleus of the cell. Throughout most of the cell cycle, DNA is packaged in the form of chromatin. However, during mitosis and meiosis, chromatin exists in an additional level of organization known as a **chromosome**. Chromosomes are an even denser packaging of chromatin that are visible with a light microscope, particularly during metaphase. Chromosomes can exist in duplicated or unduplicated states. At the beginning of mitosis, for example, a chromosome consists of two sister chromatids – **chromatids** are the term used to describe the chromosome in its duplicated state. Let's try to tie all of this information together and see how it applies to chromosome and chromatid count during the various stages of cell replication.

First, during the S phase of interphase, the genetic material of a cell is duplicated. A human has 46 chromosomes (a set of 23 you inherit from your mother, and a set of 23 from your father). After the genetic material is duplicated and condenses during prophase of mitosis, **there are still only 46 chromosomes** – however, they exist in a structure that looks like an X shape:



A chromosome consisting of two sister chromatids

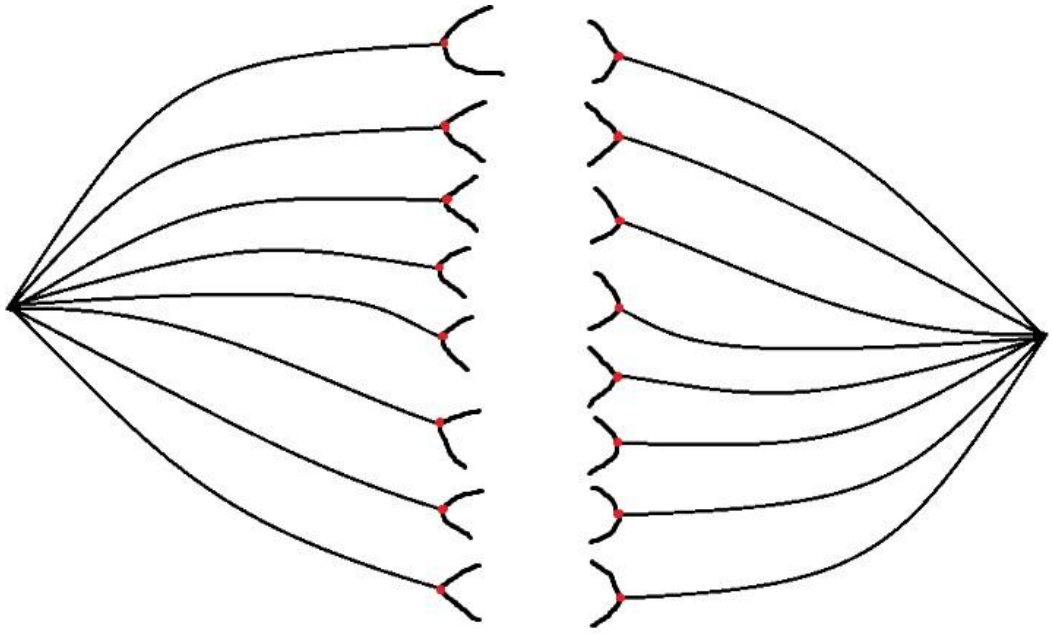
For clarity, one sister chromatid is shown in green, and the other blue. These chromatids are genetically identical. However, they are still attached at the **centromere** and are not yet considered separate chromosomes. Thus, the above picture represents **one chromosome, but two chromatids**. During prophase and metaphase of mitosis, each chromosome exists in the above state. For humans, this means that during prophase and metaphase of mitosis, a human will have 46 chromosomes, but 92 chromatids (again, remember that there are 92 chromatids because the original 46 chromosomes were duplicated during S phase of interphase). It is helpful to see this visualized (for visual simplicity, a 2n=8 arrangement of chromosomes will be demonstrated, rather than the 2n=46 arrangement of chromosomes in humans):



2n = 8 cell during metaphase
Note that there are **8 chromosomes**

As the above image shows, there are 8 chromosomes present, but 16 chromatids. Similarly, in humans (2n=46), there are 46 chromosomes present during metaphase, but 92 chromatids.

It is only when sister chromatids separate – a step signaling that **anaphase** has begun – that each chromatid is considered a separate, individual chromosome. Pictured below, we see how the 2n=8 cell from above has progressed from having 8 chromosomes to 16 chromosomes:



2n = 8 cell during anaphase
Note that there are now **16 chromosomes**

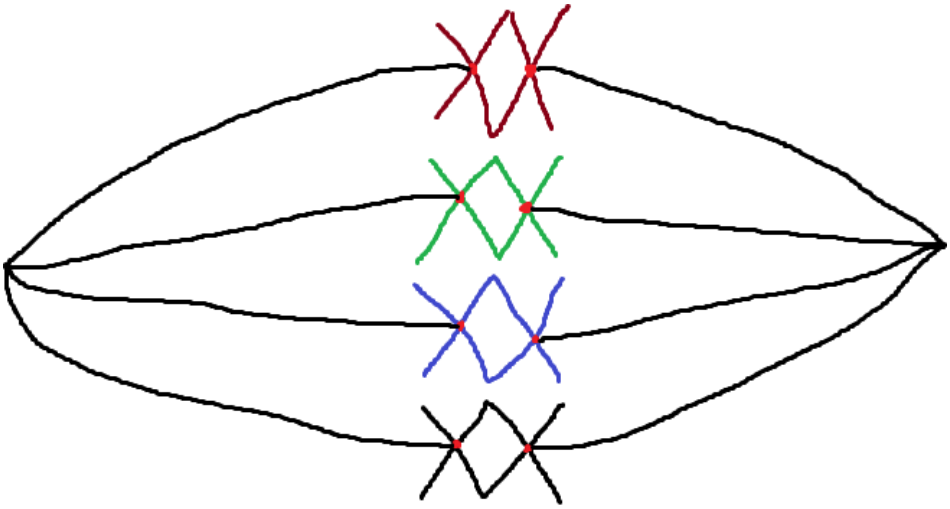
Now that the sister chromatids have separated, each chromatid is also considered a chromosome. During anaphase, we now have a total of 16 chromosomes and 16 chromatids – in short, each chromatid is now a chromosome. Similarly, in humans, there are 92 chromosomes present and 92 chromatids during anaphase. These numbers remain the same during telophase. It is only after the end of mitosis – when the dividing cells have fully separated and the membranes have reformed – that the normal chromosome number is restored to the cell. Below is a table summarizing the chromosome and chromatid number during mitosis in humans:

Phase (Mitosis)	# Chromosomes	# Chromatids
Prophase	46	92
Metaphase	46	92
Anaphase	92	92
Telophase	92	92
End of Mitosis (separated cells)	46	46

The chromosome and chromatid count during meiosis works a bit differently. Recall that there are two divisions during meiosis: meiosis I and meiosis II. The genetic material of the cell is duplicated during S phase of interphase just as it was with mitosis resulting in 46 chromosomes and 92 chromatids during Prophase I and Metaphase I. However, these chromosomes are not arranged in the same way as they were during mitosis. Rather than each chromosome lining up individually across the center of the cell, homologous pairs of chromosomes line up together (forming **tetrads**, also known as **bivalents**):

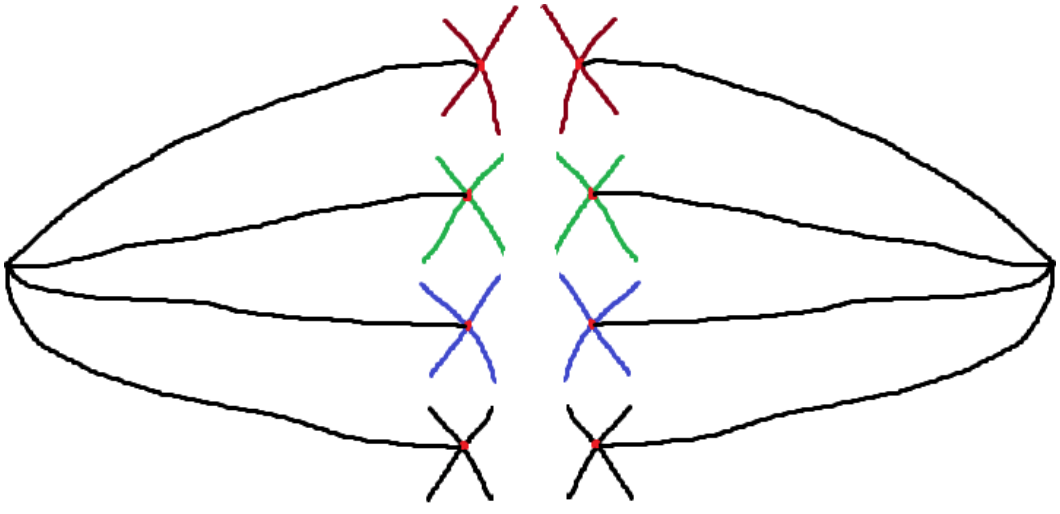
A **tetrad** consisting of 2 attached homologous chromosomes (a total of 4 chromatids)

For visual consistency, let us look at the hypothetical 2n=8 cell from earlier during metaphase I. Here, the homologous chromosome pairs have been color coded:



2n = 8 cell during Metaphase I
There are **8 chromosomes** and **16 chromatids**

When anaphase I begins, you may expect the chromosome number to change, but it does not. Remember – it is only after the **sister chromatids** separate that the chromosome number changes. Since anaphase I only separates the homologous chromosomes, neither the chromosome number nor the chromatid number changes during anaphase. Visualized below:

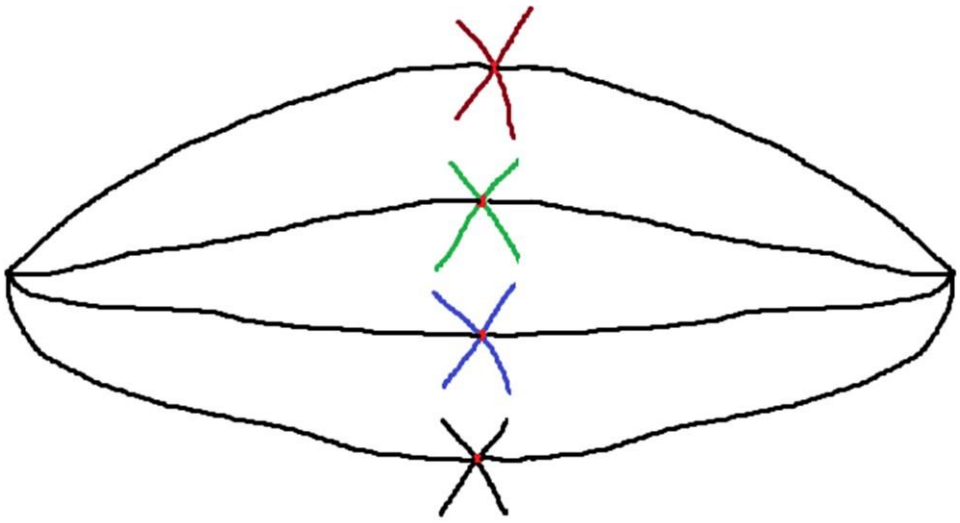


2n = 8 cell during Anaphase I
There are **8 chromosomes** and **16 chromatids**

As you can see, the separation of homologous chromosomes does not change the chromosome number or the chromatid number. There are still 8 chromosomes and 16 chromatids. In fact, until the completion of meiosis I, the chromosome and chromatid numbers remain the same through all stages. Similarly in a human, we do not see a change in chromosome or chromatid number until the end of meiosis I (when division of the cell in two results in half the chromosome and chromatid count). Below is a table summarizing the chromosome and chromatid number during meiosis I in humans:

Phase (Meiosis I)	# Chromosomes	# Chromatids
Prophase I	46	92
Metaphase I	46	92
Anaphase I	46	92
Telophase I	46	92
End of Meiosis I (separated cells)	23	46

The second division of meiosis (meiosis II) appears similar to mitosis, with the only difference being that there are now half as many chromosomes as before. Continuing with the 2n=8 cell example from above, we will observe a cell during metaphase II:



2n = 8 cell during Metaphase II
There are **4 chromosomes** and **8 chromatids**

During metaphase II, the chromosomes are lined up individually across the center of the cell. Due to the reduction division of meiosis I, there are now half as many chromosomes (and chromatids) as there were before. When anaphase II begins, however, the sister chromatids split apart, which once again doubles the chromosome number:

Below is a table summarizing the chromosome and chromatid number during meiosis II in humans:

Phase (Meiosis II)	# Chromosomes	# Chromatids
Prophase II	23	46
Metaphase II	23	46
Anaphase II	46	46
Telophase II	46	46
End of Meiosis II (separated cells)	23	23

A quick tip: notice that during the stages of meiosis and mitosis, the chromatid count never changes. Only the number of chromosomes changes (by doubling) during anaphase when sister chromatids are separated. During meiosis I, neither the chromosome number nor the chromatid number change until after telophase I is complete.

Full Mitosis and Meiosis Summary Chart

Phase (Mitosis)	# Chromosomes	# Chromatids
Prophase	46	92
Metaphase	46	92
Anaphase	92	92
Telophase	92	92
End of Mitosis (separated cells)	46	46
Phase (Meiosis I)	# Chromosomes	# Chromatids
Prophase I	46	92
Metaphase I	46	92
Anaphase I	46	92
Telophase I	46	92
End of Meiosis I (separated cells)	23	46
Phase (Meiosis II)	# Chromosomes	# Chromatids
Prophase II	23	46
Metaphase II	23	46
Anaphase II	46	46
Telophase II	46	46
End of Meiosis II (separated cells)	23	23

Chapter 6: Heredity

Key Terminology

- A **gene** is a distinct unit, or sequence, of genetic material that codes for a trait.
- The term **locus** refers to where the gene is located within the genome.
  - For humans, this would indicate on which of the 23 chromosomes a gene is located, and its physical location on that gene.
- An **allele** is an alternative form of a gene.
  - An example of an allele is eye color: blue, green, and brown eyes are different alleles of the eye color gene.
  - A **wild type** allele is the 'normal' copy of an allele
  - A **mutant** allele has an altered DNA sequence that can affect a gene's phenotype
- **Genotype** refers to the actual DNA sequence of a gene, while **phenotype** refers the observable characteristics of that gene's expression.
  - E.g. the genotype for blue eyes could be ACGGT, while the phenotype would be the blue color
  - For the purpose of Punnett Squares, genotypes are often simplified to dominant and recessive genotype forms as single letters rather than the entire nucleotide sequence
    - An example genotype for eye color (Bb): B represents the dominant allele for brown eyes, and b represents the recessive allele for blue eyes.
- **Homologous chromosomes** are a pair of chromosomes (one maternal and one paternal) that contain all the same genes in the same location (but not necessarily the same alleles, as each parent may contribute different alleles for a given gene).
  - Humans have 22 pairs of autosomal chromosomes and a pair of sex chromosomes. In females (XX), the sex chromosomes are homologous but in males (XY) they are not.
- An organism is considered to be **homozygous** for a given gene if an identical allele is present on each homologous chromosome. **Homozygous-dominant** individuals carry two copies of the dominant allele (e.g. BB), while **homozygous-recessive** individuals carry two copies of the recessive allele (bb).
- An organism that is **heterozygous** for a given gene carries a copy of the recessive allele on one of their homologous chromosomes and a copy of the dominant allele on their other homologous chromosome (e.g. Bb).
- **Hemizygous** refers to the condition of having a single copy of a gene instead of two.
  - E.g. men have two different sex chromosomes (XY), and are therefore hemizygous for the genes present on each chromosome.

Term	Definition	Examples
Gene	Genetic material on a chromosome for a trait	Eye color, blood type
Allele	Variance of genes	Blue eyes, brown eyes, Type A blood, Type B blood
Locus	Location on chromosomes where gene is located	2
Homozygous	Two copies of the same allele	AA or aa
Heterozygous	Different alleles of the same gene	Aa
Hemizygous	One single copy of a gene instead of two	Male sex chromosome (XY)
Genotype	Set of genes that are responsible for the trait	BB, Bb, bb
Phenotype	Physical appearance of a trait	BB and Bb → brown eyes bb → produces blue eyes

Mendel's Three Laws of Inheritance

The Law of Segregation

- During meiosis (anaphase 1), homologous chromosomes separate from one another. This results in haploid gametes that contain only one allele per gene.
  - Thus, an offspring inherits only one of each type of allele per parent.
  - What does this law really mean, in practical terms? The law of segregation basically says you'd only pass one set of your alleles down to your kid (you produce haploid gametes), not both. Using the gene for eye color as an example, say you specifically carry two different alleles: one for blue eyes and one for brown eyes. When you make your gametes, you can't pass both the blue allele and the brown allele down to your kid - only one or the other. Why? Because at Anaphase I, the homologous chromosomes pairs split up. One of those homologues in the pair has your blue allele on it and the other has the brown allele on it. Since they separate at Anaphase I, any gametes produced thereafter are guaranteed to only have one or the other.

The Law of Independent Assortment

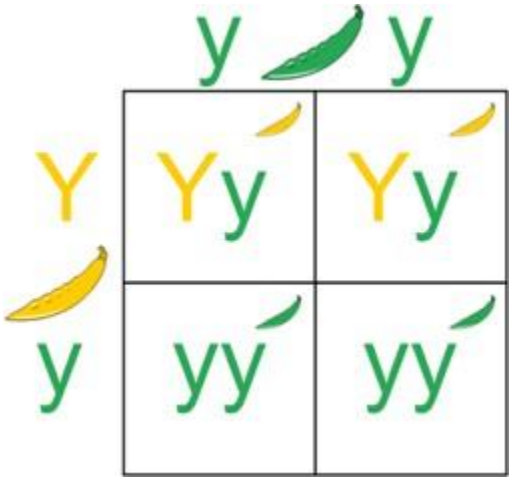
- During meiosis (prophase 1), the lining up and subsequent separation of one pair of homologous chromosomes does not influence that of a different pair of homologous chromosomes.
  - Thus, the separation of one pair of chromosomes is completely independent from the separation of another; therefore, alleles for different traits are passed on independently of one another.
  - What does this law really mean, in practical terms? The law of independent assortment basically says your different chromosome pairs separate completely independently of one another. Let's go back to the above example: say your different eye color genes were on the first chromosome pair, and your hair color genes (blonde and brown) were on a completely different chromosome pair. Independent assortment says that during Anaphase I, the way that your first chromosome pair separates (e.g. whether your blue eye color separated to the left or to the right), has

no impact whatsoever on where that completely different chromosome pair with hair color will separate (e.g. whether blonde hair will go to the left or to the right).

The Law of Dominance

- A dominant alleles masks the effect of a recessive allele.
- For example, an organism with a dominant eye color allele (brown) on one chromosome and a recessive eye color allele (blue) on the other chromosome will simply display the phenotype of the dominant allele (brown eyes).

The Testcross



Test Cross – in this example above, Y (yellow) is dominant to y (green)

KatieAnn127 (https://commons.wikimedia.org/wiki/File:Punnett_Square_Test_Cross.PNG), Cropped to one test cross, https://creativecommons.org/licenses/by-sa/4.0/legalcode

- The objective of a testcross is to determine an organism's genotype for a given trait.
  - A **monohybrid** cross is used to test a single gene.
  - A **dihybrid** cross tests two different genes simultaneously.
- Testcross generations are labeled as follows
  - **P**: parental
  - **F1**: Filial 1 hybrid
    - First generation of offspring
    - The result of breeding the parental organisms
  - **F2**: Filial 2 hybrid
    - Second generation of offspring
    - The result of breeding the F1 organisms
- During a **monohybrid cross**, the phenotypically-dominant organism in question is bred, or "crossed," with another organism that is homozygous-recessive for the given trait.
  - If the offspring display a dominant phenotype, then the parent organism is homozygous-dominant.
  - If the offspring display a combination of dominant and recessive phenotypes, then the parent organism is heterozygous-dominant.
- A **dihybrid cross** is identical to a monohybrid cross, except two different genes are studied concurrently.
  - Gene loci must be on separate chromosomes for this experiment to be successful.

- To determine probabilities in a dihybrid cross, it is often easier to calculate the probability of each gene separately, then multiply the results together.

Patterns of Inheritance

- Not all genes are inherited and expressed in terms of simple dominance or recessiveness - there are other forms of gene expression that are more complex (known as non-Mendelian inheritance). The critical patterns you should know for the DAT have been collected with examples in the table below.

Term	Definition	Example
Incomplete Dominance	The expression of alleles is blended, producing a unique heterozygous phenotype	Flower petals: R red x R' white = RR' comes out pink
Codominance	Both alleles are completely expressed	- Blood Type: AB blood type expresses both A and B antigens on RBC surface - Flower petals: R red x W white = RW alternating patches of red and white
Multiple Alleles	Multiple alleles (more than two possibilities) exist for a given gene.	- ABO blood type: A, B, O allele possibilities - Rabbit coat color has four different allele possibilities
Epistasis	One gene affects the phenotypic expression of a second, separate gene	Fur pigmentation: 1st gene controls (turns on/off) the production of pigment and the 2nd gene controls pigment color. If 1st gene codes for no pigment → 2nd gene has no effect, regardless of pigment color gene. CCBb → black fur ccBb → no fur pigment
Pleiotropy	Single gene has more than one phenotypic expression	- Gene in pea plants that expresses seed texture also influences phenotype of starch metabolism and water uptake. - Gene causing sickle cell anemia leads to multiple health conditions.
Polygenic inheritance	The interaction of many genes to shape a single phenotype with continuous variation	Height, skin color

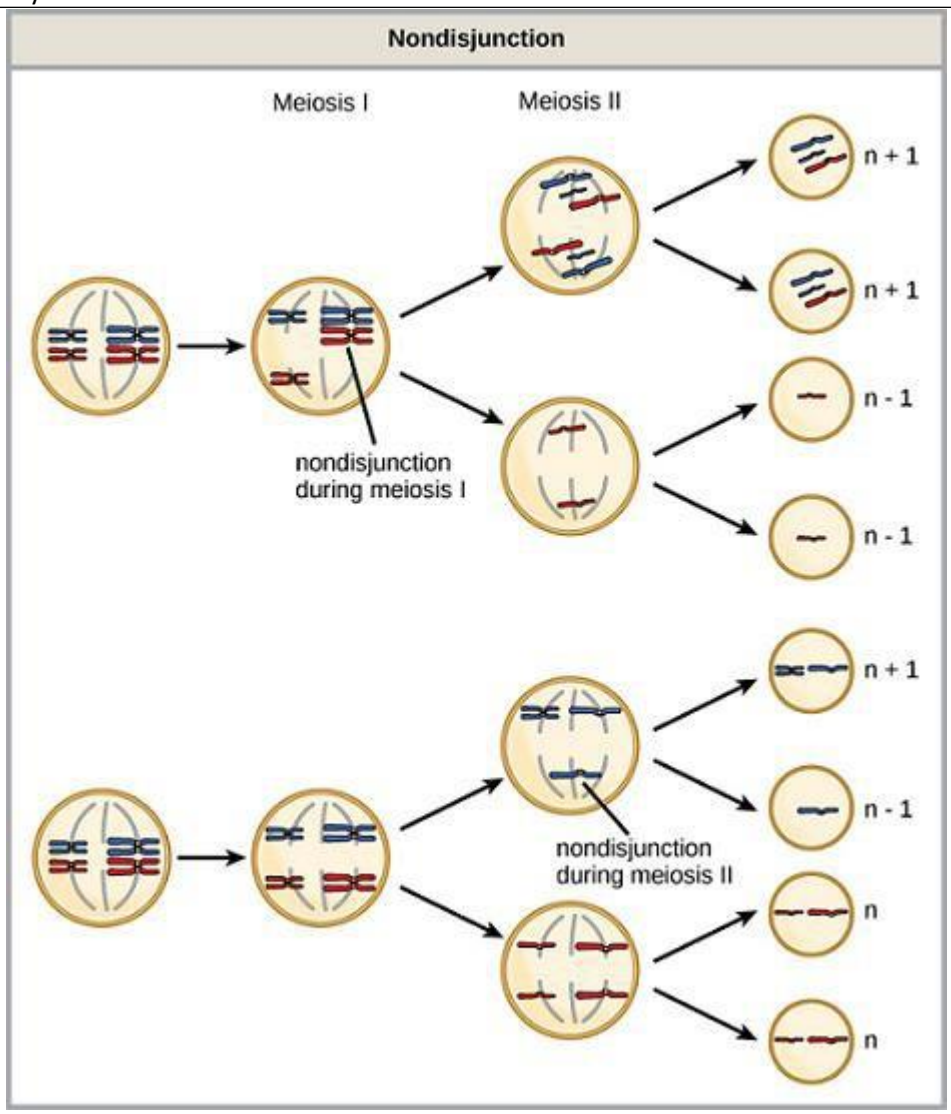
- **Sex-linked genes** are genes that reside on a sex chromosome.
  - Example: the gene that causes color blindness is located on the X-chromosome, so it is said to be sex-linked.
  - A consequence of women having two X chromosomes (XX) and men having just one (XY) is that men are more likely to have X-linked diseases; when a male inherits an affected X allele from his mother the affected gene will be expressed regardless of whether is dominant or recessive because there is no second copy of the gene on the Y chromosome to potentially mask its effect.
- **Sex-influenced genes** can be influenced by sex of individual carrying trait (e.g. a Bb female not bald, Bb male is).

- **Genomic imprinting** is a similar phenomenon in which a specific allele is expressed (or not) depending on whether or not it is maternal or paternal. This is distinct from sex-influenced genes because in genomic imprinting the effect also occurs on autosomal chromosomes.

- **Penetrance** is the probability that an organism with a specific genotype will express the corresponding phenotype.
  - E.g. In a fully penetrant gene, 100% of individuals that carry the allele for blindness would be blind. In a gene that is not fully penetrant, not all individuals that carry the allele for blindness will be blind.
- **Expressivity** is the term that describes the variation of a phenotype for a specific genotype.
  - E.g. Assume Bb is the genotype for body hair, and two individuals are both Bb but differ in their expressivity. In the individual with high expressivity, there would be a significant amount of body hair while the individual with low expressivity would have less body hair.
- **X-inactivation**: During embryonic development in female mammals, one of two X chromosomes does not uncoil. Instead, it forms a dark and coiled compact body chromosome (**Barr body**) that is not expressed. The genes on the other X chromosome will be expressed.
  - The X chromosome that is inactivated can differ from cell to cell – as a result, not all cells in a female mammal are necessarily functionally identical.
    - This phenomenon is why calico cats have multiple patches of different fur color, as their fur pigment is X-linked and subject to X-inactivation.
    - Hemophilia is an X-linked recessive disease. A woman that is heterozygous for hemophilia (X^HX^h) is normally just a carrier for the disease, but if X^H is inactivated, X^h can be expressed.
- **Linked genes** are two or more genes that reside close together on a chromosome, and are therefore less likely to be separated by recombination during meiosis, and are more likely to be inherited together.
  - A **linkage map** uses recombination frequency to show the positions of genes rather than physical distance units.
    - The closer together the genes are, the less likely separation by recombination is.

Human Genetic Defects

- **Nondisjunction** is the failure of chromosome pairs or chromatids to separate during mitosis (failure of two chromatids of a single chromosome during anaphase) or meiosis (homologous chromosomes can fail to separate during meiosis I, or sister chromatids can fail to separate during meiosis II).
  - Meiotic nondisjunction can result in gametes with missing or extra chromosomes, creating conditions such as **trisomy** (three copies of a chromosome) or **monosomy** (only one copy of a chromosome).
  - **Mosaicism** occurs in cells that undergo mitotic nondisjunction during embryonic development; as a result a fraction of body cells have extra or missing chromosomes
  - **DAT Tip:** Know the resulting gametes of nondisjunction during meiosis I vs meiosis II
    - Meiosis I non-disjunction: n+1, n+1, n-1, n-1
    - Meiosis II non-disjunction: n, n, n+1, n-1

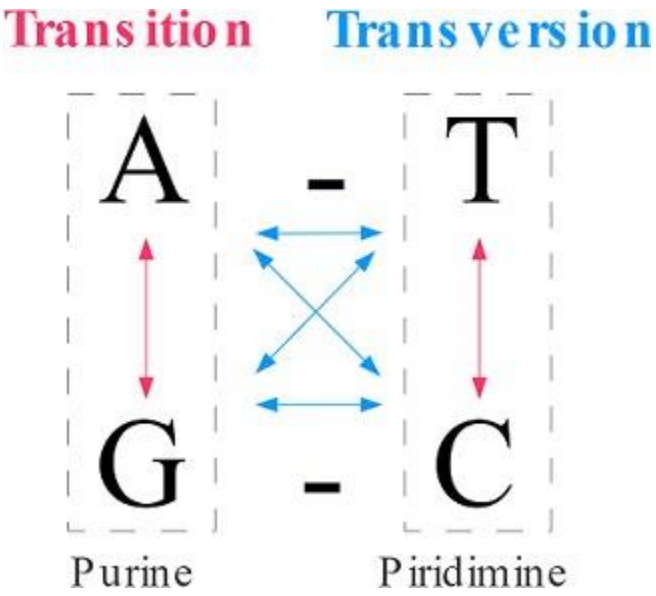


Meiotic Nondisjunction Gametes

CNX OpenStax (https://cnx.org/resources/39c69b00e262390556bdec7fafc748c7f0ec611c/Figure_13_03_02.png)
https://creativecommons.org/licenses/by/4.0/legalcode

- A **point mutation** is a single nucleotide change causing substitution (the change of one nucleotide to a different nucleotide), insertion (the addition of a nucleotide), or deletion (the removal of a nucleotide)
  - Both insertion and deletion of nucleotides can cause **frameshift mutation.** A frameshift mutation results in the 'reading frame' of an RNA transcript being shifted, causing different amino acids to be translated and resulting in impaired protein structure.
    - Why does a frameshift mutation occur? Recall that **codons** are nucleotide triplets that specify an amino acid during translation (every three nucleotides in sequence corresponds to an amino acid in the protein). If nucleotides are added or removed, the nucleotide sequence will be offset, resulting in different triplets than the original sequence.
    - If the number of nucleotides added or removed is a multiple of 3, then a frameshift is avoided (because the codons are still read correctly but an entire amino acid will have been added or removed).

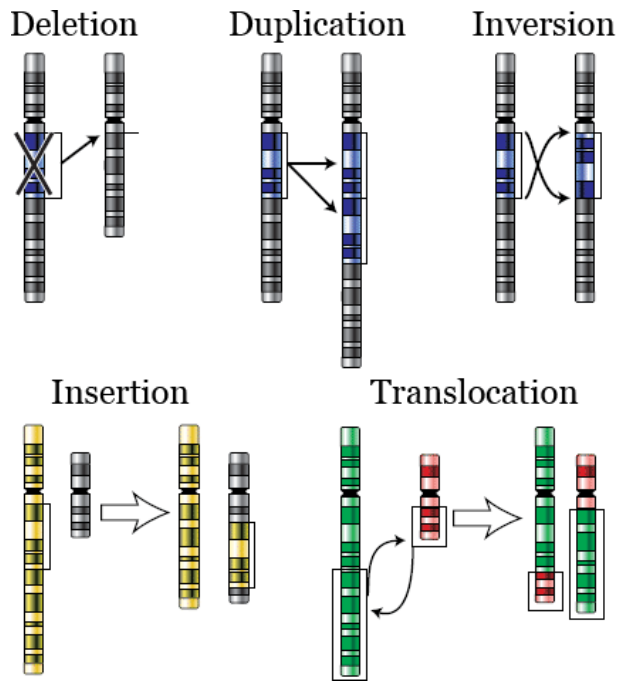
- A **transition mutation** is when a purine nucleotide is converted to another purine, or a pyrimidine is converted to another pyrimidine.
- A **transversion mutation** is when a purine nucleotide is converted to a pyrimidine nucleotide or vice versa.
- A **forward mutation** changes a wild type allele to a mutant allele
  - A **backwards mutation** reverts a mutant allele to a wild type allele



Meiotic Nondisjunction Gametes

Sara (https://commons.wikimedia.org/wiki/File:Point_mutations.svg), "Point mutations", https://creativecommons.org/licenses/by-sa/3.0/legalcode

- **Aneuploidy** is the condition of a genome having extra or missing chromosomes, often caused by nondisjunction.
  - E.g. Down Syndrome (Trisomy 21) – characterized by intellectual disability
  - E.g. Turner Syndrome (XO, missing sex chromosome) - results in sterility and physical abnormalities. This is a monosomy.
  - E.g. Klinefelter Syndrome (XXY) – results in sterility
  - E.g. Edwards Syndrome (Trisomy 18) – characterized by heart defects
- **Chromosomal aberrations** involve changes to segments of DNA
  - **Duplications** occur when a chromosome segment is repeated on the same chromosome, and can occur from unequal crossing over
  - **Inversions** occur when a chromosome segment is rearranged in the reverse of its original orientation
  - **Translocations** occur when a chromosome segment is moved to another chromosome. This can be reciprocal (meaning two non-homologous chromosomes swap segments) or nonreciprocal (one chromosome segment is transferred to a different chromosome – this can also be referred to as an **insertion**).
    - A Robertsonian translocation involves two different chromosomes breaking and rejoining near their centromeres.



Chromosomal Aberrations

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- A **chromosomal breakage** can occur spontaneously or be induced as a result of mutagenic agents. The fragments of a chromosomal breakage can rejoin or may remain unrepaired.
  - **Mutagenic agents** include cosmic rays, X-rays, UV rays, radioactivity, and chemical compounds
  - E.g. **colchicine** which inhibits spindle formation and can induce polyploidy
  - E.g. mustard gas
- **Proto-oncogenes** (e.g. ras gene) stimulate normal growth, but if mutated they can become **oncogenes** (genes that can cause cancer).
- Similarly, **tumor suppressor genes** (e.g. p53) help prevent growth, and if mutated in a way that decreases their activity, can contribute to the onset of cancer.
- Genetic disorders can follow a number of inheritance patterns:
  - **Autosomal dominant**: a dominant allele on an autosome. If the allele is inherited from either parent, the offspring will be affected.
  - **Autosomal recessive**: a recessive allele on an autosome. The offspring will only be affected if the recessive allele is inherited from both parents.
  - **Sex-linked** genetic disorders are carried on either the X or Y chromosome:
    - **X-linked dominant**: a dominant allele on the X chromosome. If the allele is inherited from either parent, the offspring will be affected. Cannot be passed from father to son.
    - **X-linked recessive**: a recessive allele on the X chromosome. If the allele is inherited from both parents, daughters would be affected. Inheriting a single copy of the allele will result in sons being affected (as men only have one copy of the X chromosome). Cannot be passed from father to son.
    - **Y-linked**: an affected allele on the Y chromosome. Men only carry one copy of the Y chromosome, so if the allele is inherited from the father, any sons will be affected. Can only be passed from father to son.

- Other genetic disorders can be due to:
  - Chromosomal: the chromosome is damaged, duplicated, or missing in some way leading to disorders)
  - **Extranuclear inheritance**: In eukaryotes, genes also exist outside the nucleus, and can be found in mitochondria and chloroplasts. Defects in mitochondrial DNA can reduce cell's ATP production. Mitochondria passed to the zygote all come from the mother, so all mitochondrial related diseases are maternally inherited.
    - **DAT Tip**: Mitochondria have their own 70S ribosomes that make mitochondrial proteins within the mitochondrial matrix!
  - A **lethal gene** prevents survival of an organism.
    - Lethal alleles can be dominant, recessive, or conditional
    - In a standard Aa x Aa cross, we would expect to see a 1:2:1 genotype ratio of AA:Aa:aa. If a recessive allele were lethal, we would see a characteristic 1:2 ratio of AA:Aa (since the aa condition would not survive).
  - A **maternal effect gene** is a gene that when mutated in the mother results in a mutant phenotype *in the offspring* regardless of the offspring's own genotype.
    - How does this happen? The mRNA or protein products of a maternal effect gene are placed in the egg while in the mother's ovary. If the mother has a mutated maternal effect gene → defective gene product → eggs end up defective.
      - E.g. Egg-polarity genes (control the orientation of the egg) in flies.
    - Maternal effect mutations are usually embryonic lethal.

Genetic Disorders

- The table below summarizes several important genetic disorders and their inheritance patterns.

Inheritance Pattern	Disorder	Description
Autosomal Recessive	PKU	Inability to produce proper enzymes for phenylalanine breakdown → degradation product phenylpyruvic acid accumulates → mental retardation
	Cystic fibrosis	Thick mucus buildup in respiratory tracts
	Tay-Sachs	Lysosome defect: cannot breakdown lipids for normal brain function (fatal within the first 5 years of life)
	Sickle-Cell Anemia	Defective hemoglobin due to substitution
	Galactosemia	Cannot breakdown galactose properly
Autosomal Dominant	Huntington's Disease	Degenerate nervous system disease
	Achondroplasia	Dwarfism
	Hypercholesterolemia	Excess cholesterol in blood → Heart disease
X-Linked Recessive	Hemophilia	Abnormal blood clotting
	Color blindness	Inability to distinguish between different colors
	Duchenne	Muscular dystrophy
X-linked Dominant	Fragile X Syndrome	Intellectual disability
Chromosomal	Downs Syndrome	Trisomy 21; causes mental retardation
	Turner Syndrome	XO; sterility; typically doesn't cause mental retardation
	Klinefelter Syndrome	XXY; sterility; causes mental retardation
	Cri du Chat	Deletion on chromosome 5; causes mental retardation

*A fetus can be tested for genetic disorders via **amniocentesis** or **chorionic villus sampling** (CVS).

DAT Tips

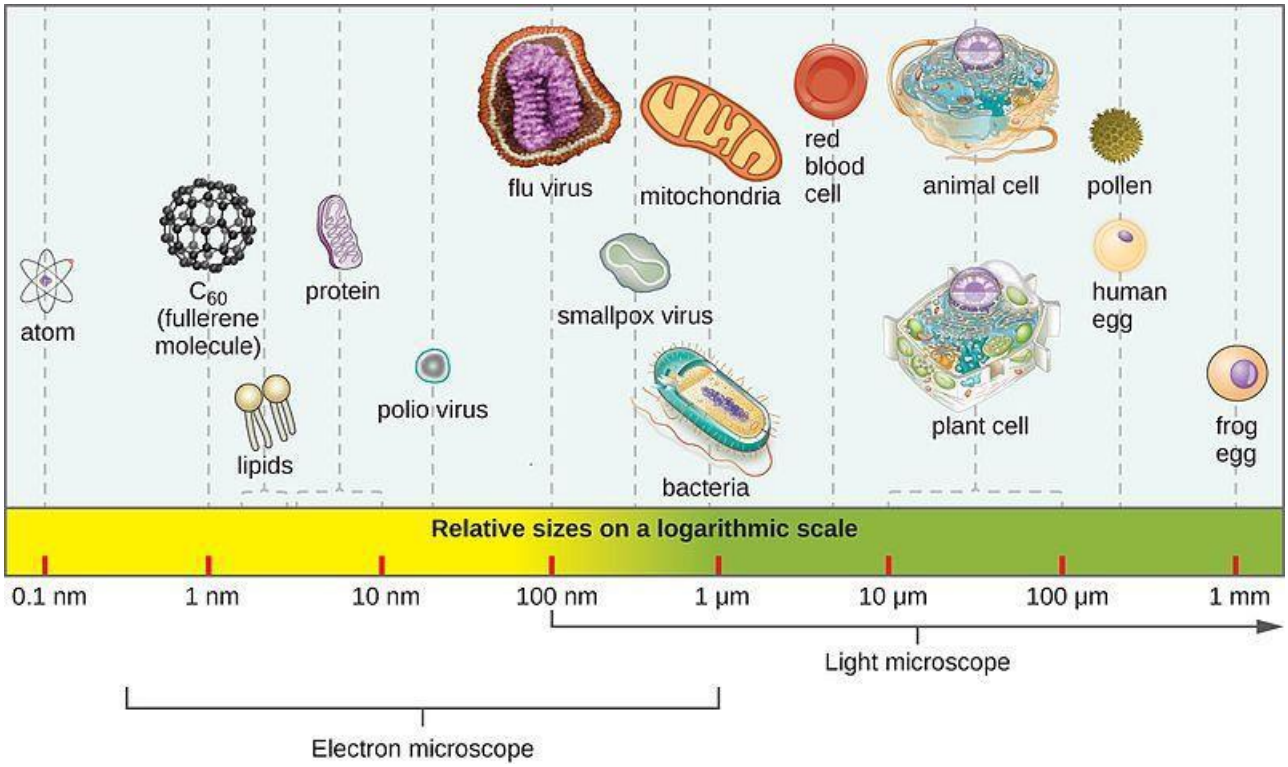
- Codominance is typically represented with two capital letters, e.g. AB blood type
- Incomplete dominance is typically represented as capital letters, with one letter having an additional apostrophe, e.g. AA' for alleles A and A'
- Phenotype ratios and genotype ratios are not necessarily the same.
  - Take the cross of Aa x Aa for example. The offspring *genotype* ratio would be 1 AA: 2 Aa: 1 aa, but the *phenotype* ratio would be 3:1 (AA and Aa produce the same dominant phenotype, while aa represents the recessive phenotype).
- A heterozygote cross resulting in a 1:2:1 phenotype ratio of offspring is characteristic of **incomplete dominance**
  - E.g. using the earlier in complete dominance flower color example, RR' x RR' → 1 RR (red) : 2 RR' (pink) : 1 R'R' (white) phenotype ratio
- To determine the probability of two or more independent events occurring together multiply the probabilities of each separate event.
  - Say you were asked to calculate the probability of a specific outcome (aaBb) in the cross of AaBB x aabb. Instead of doing a complex probability calculation, it is easier

to do two separate crosses of Aa x aa and BB x Bb, then multiply the probability outcomes of each separate cross together to find the solution.

- The probability of aaBb is (probability of aa) * (probability of Bb)
  - (½) * (1) = ½

❖ In a pedigree analysis, if a phenotype "skips" generations, be suspicious of an autosomal recessive disorder. If the phenotype does not skip a generation, it is more likely to be an autosomal dominant disorder. Be suspicious for X-linked recessive disorders as well – if a father doesn't have the phenotype, neither will any of his daughters.

Chapter 7: Microscopy & Lab Techniques



Biological Size and Scale

CNX OpenStax (https://commons.wikimedia.org/wiki/File:OSC_Microbio_01_03_sizes.jpg), https://creativecommons.org/licenses/by/4.0/legalcode

Microscopy

- Microscopy is an important tool in biology that allows us to view objects that could not be seen with the naked eye. In optical microscopy, visible light is focused on an object and then reflected back through lens to magnify the view of a sample. Electron microscopy is similar, but a focused beam of electrons is used rather than visible light.

***Critical Review*:**

- In general, electron microscopy allows for significantly higher magnification than light microscopy, but it cannot be used to view living specimens due to the necessary preparation steps
- Note that in the image above, cellular organelles (such as the mitochondria) are approximately the same size as bacteria.
- Most viruses are so small that they must be viewed using electron microscopy.

	Microscopy	Description	Advantages	Disadvantages
Optical Microscopy	**Stereomicroscope (Light)**	Visible light is focused to produce a 2D image of surface of sample	Living samples	Low resolution
	Compound Microscope (Light)	Visible light is focused to produce a 2D image of a thin slice of sample	Some living samples (single cell layer)	May require staining (kills samples)
	Phase-Contrast	Uses light phase changes and contrast to produce a 2D image of thin samples	Detailed observation of living organisms (including internal structures) Good resolution and contrast	Ineffective on thick samples "Halo Effect" around sample edges
	Confocal Laser Scanning and Fluorescence	Tag certain structures with fluorescent marker, then use laser light to scan specimen. 2D image is displayed digitally.	Living samples - can look at thin slices (keeping sample intact) Can look at specific parts of cell (e.g. view chromosomes during mitosis)	Fluorescence can cause artifacts
	Dark field microscopy	Excludes light that is not reflected from the sample	Excellent contrast on living samples	Low light intensity
Electron Microscopy	**Scanning Electron Microscopy (SEM)**	Sample must first be dehydrated. Scans sample with a beam of electrons → electrons interact with surface atoms → produces a 3D image of a sample's surface	High resolution	Costly Extensive sample preparation (kills sample)
	CryoSEM	Like SEM, but sample is frozen rather than dehydrated to produce a 3D image of sample's surface.	High resolution Sample presented in a more natural form	Extensive sample preparation (kills sample) Freezing can cause artifacts
	Transmission Electron Microscopy (TEM)	Beam of electrons passed through a very thin section of a sample to product a 2D image of the thin slice	Can view internal structures High resolution	Costly Extensive sample preparation (kills sample)
	Electron Tomography	Not a form of microscopy, but the 3D model build up using TEM data (multiple slices are integrated into a 3D model).	Can look at objects and their relative positions in 3D	Costly Requires extensive sample preparation (kills sample)

Note: to help distinguish between **S**EM and TEM, think **S** for **S**urface.

Cell Fractionation

- In **cell fractionation**, the process of differential **centrifugation** (spinning at high speeds) is used in order to separate the contents of cells based on density, size, and shape.
  - First, cells must be broken apart to create cellular homogenate, which can be thought of as the contents of a cell without being contained in its membrane.
  - The cell homogenate is passed through a filter, and then spun at a relatively lower speed, creating a dense pellet layer of nuclei. This layer appears the most quickly because nuclei are the most dense cell content.
  - The remaining homogenate is then poured out and spun again at a higher speed. This creates the second most dense layer, containing mitochondria, chloroplast, lysosomes, and peroxisomes.
  - The remaining homogenate is once again poured out and then spun at an even higher speed, creating the next most dense layer containing plasma membrane and endoplasmic reticulum fragments.
  - The process repeats, creating a layer of ribosomes. The remaining homogenate contains only the cytosol (the aqueous portion of the cytoplasm).
- In contrast to differential centrifugation, **density centrifugation** only separates cell contents on the basis of density. Unlike the multiple spin steps of differential centrifugation, density centrifugation involves one spin step that creates multiple layers separated by density.
- Centrifugation can also be used to separate proteins based on solubility (insoluble proteins pellet out, soluble proteins remain in the supernatant).

***Critical Review*:**

- Cell fractionation separates cell components via centrifugation based on density, size and shape. From most dense to least dense: nuclei > mitochondria/chloroplast > ER fragments > ribosomes.

Biology Lab Techniques

The DAT can test numerous topics related to biological techniques, equipment, and historically significant experiments. I have collected the most high yield topics here for review.

DNA Technology

- **Recombinant DNA** is DNA containing different segments from multiple sources. These DNA segments can be transferred from viral transduction, bacterial conjugation, transposons, or through artificial recombinant DNA technology.
- Recombinant DNA technology uses **restriction endonucleases** (aka restriction enzymes) to cut up specific segments of DNA. The cut ends of DNA are called **sticky ends**, and are left unpaired. These sticky ends allow for new DNA pieces (cut at the same points with the same restriction endonuclease) to bind, creating a DNA molecule from multiple sources.
  - Restriction enzymes (e.g. EcoRI and BamHI) are normally used by bacteria to protect against viral DNA. Their own DNA is protected via DNA methylation.
  - When restriction enzymes are applied to the DNA of individuals, fragments of different sizes are created due to individual differences in DNA. These fragments are called **restriction fragment length polymorphisms (RFLPs).** RFLPs are inherited in

a Mendelian fashion, and can be used in paternity suits or in crime scenes to match DNA to suspects (sometimes referred to as **DNA fingerprinting**).
    - Recall that a **polymorphism** is when two or more different phenotypes exist for a trait – for example, there are multiple human blood types, and each is considered a different polymorphism). In RFLPs, the polymorphisms are the fragments of different length.
    - Another aspect of DNA used to identify individuals in crime or paternity are **short tandem repeats (STRs)**. These are repeats of 2-5 nucleotides that are different between all individuals except identical twins. This differs from RFLP analysis in that restriction enzymes aren't used. Instead, probes and PCR amplification are used to discover STR lengths

- A **vector** functions to transfer foreign DNA into another cell.
    - Examples of vectors include plasmids and bacteriophages
- To introduce foreign DNA into a bacterium, we must first get the foreign DNA into a plasmid. The plasmid is treated with the same restriction enzymes as the foreign DNA so the same sticky ends to bind. DNA ligase stabilizes the attachments; then the plasmid is introduced into bacterium by transformation.
    - To ensure efficient production of a gene that has been added to a plasmid, **expression vectors** are often used – these vectors contain a highly active promoter upstream of the restriction site where new genes are added for maximum output.
- Next, bacteria must then be "made competent" to take up the plasmid, accomplished via **electroporation** (a brief electrical pulse applied to a solution with cells, creating temporary holes in plasma membrane through which DNA can enter) or a combination of heat shock + $CaCl_2$.
- Once the plasmid has entered the bacteria, the bacteria can now can be grown to produce a product, form a clone library, etc.
    - An antibiotic resistance screen is used to filter out bacteria that did not successfully uptake the plasmid. A gene for antibiotic resistance is included in the plasmid; bacteria that have taken up the plasmid (and by extension have the antibiotic resistance gene) will successfully survive antibiotic treatment, while those without the plasmid will perish.

- **Gel electrophoresis** can be used on DNA molecules to separate them on the basis of charge and size. In gel electrophoresis, different DNA molecules are added to an agarose gel that is under an electric field. The negatively charged DNA moves away from the negative end towards the positive end. Shorter DNA molecules moves further than larger DNA.
    - After electrophoresis, DNA can be sequenced or probed to identify the location of a specific DNA sequence.
        - A **DNA probe** is a radioactively labeled single strand of nucleic acid used to tag a specific DNA sequence.
    - Note that gel electrophoresis can also be applied to proteins. In the case of proteins, we must first add SDS (a compound that denatures, linearizes, and adds a negative charge to protein).

- **Nucleic acid hybridization** is when the nucleic acids of one strand of DNA (or RNA) form base pairs with the complementary nucleic acids of a different strand. This is used in many

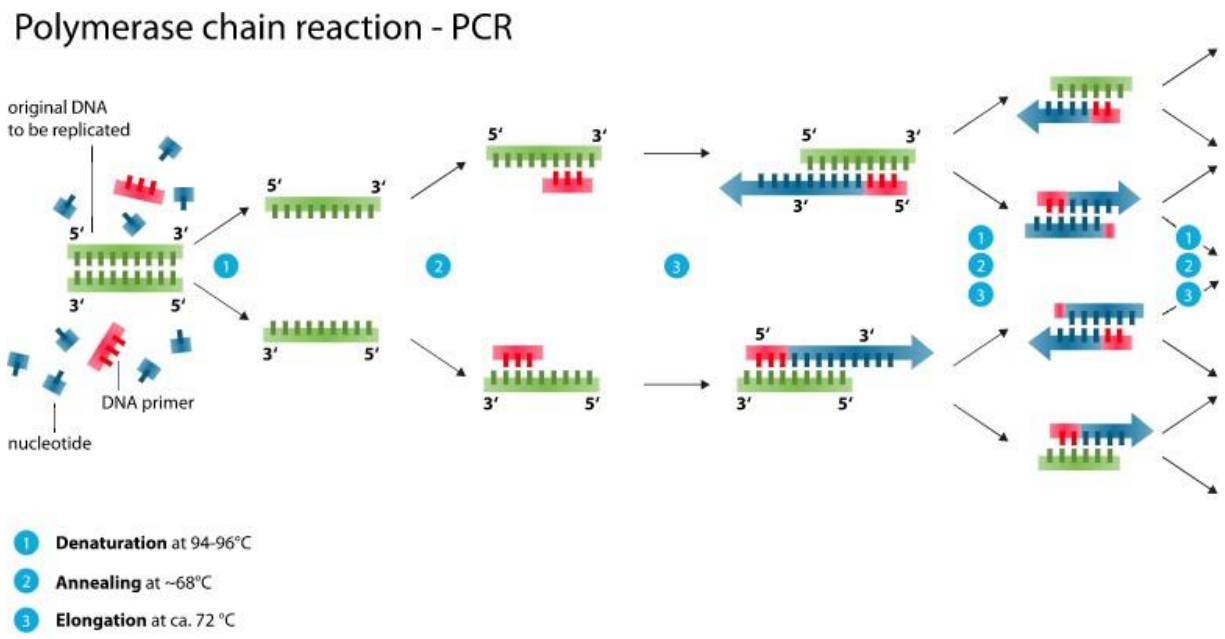
of the techniques discussed in this section (for example, DNA probes mentioned above hybridize with their specific DNA sequence target).

- The expression of a single gene can be tested using a nucleic acid probe via a fluorescently labeled single stranded complementary nucleic acid for an mRNA of interest. This can be used 'in place' on an intact organism, a technique known as **in situ hybridization** that lets us view gene expression in tissues and small embryos.

- **DNA sequencing**, as the name implies, is used to determine the sequence of base pairs in a DNA or RNA molecule.
  - Dideoxy chain termination was the early method of sequencing DNA – its specifics are unimportant for the DAT.
  - Next generation sequencing is currently used, and records which nucleotide is added to a growing strand during its synthesis.
- Amino acid sequencing can be performed via **Edman degradation** (its specifics are also unimportant for the DAT).

- **Reverse transcriptase** is used to synthesize DNA molecules off an RNA molecule template.
  - A reverse transcriptase is naturally used by some viruses, such as HIV.
  - Reverse transcriptase is used in lab procedures to create **complementary DNA** (**cDNA**) off of mRNA templates.
    - Why make cDNA? Recall that eukaryotic RNA contains **introns** that are removed in RNA processing before the final mRNA molecule is translated to protein. Prokaryotic RNA does not contain introns, so they cannot process them out. If you wanted to create recombinant DNA in a bacteria produce a desired gene product, the foreign DNA introduced to the bacteria cannot contain introns. Therefore, cDNA is created off the final mRNA template of the desired gene so that it can be efficiently transcribed and translated after insertion.
  - ***Critical Review*:** Reverse transcriptase is used to make cDNA which lacks the introns that suppress transcription in prokaryotes.

- **P**olymerase **C**hain **R**eaction (**PCR**), created by Kary Mullis, is an important technique for the amplification of DNA. The necessary ingredients of PCR are a heat resistant polyermase (e.g. Taq polymerase), nucleotides, primers, and buffer salts. The basic steps of a PCR are:
  1. Denaturation: the double-stranded DNA molecule is heated to a high temperature (>90° C) to separate it into separate strands.
  2. Annealing: As the temperature is cooled down (~55-65° C), the primers are able to attach to the separate strands. This will facilitate the next step…
  3. Elongation: The temperature is raised (~70° C) and the heat resistant polymerase synthesizes complementary strands.
     - The cycle then repeats to exponentially increase the number of DNA molecules.
     - Reverse transcriptase can be combined with PCR to create large amounts of cDNA for various genes.



Polymerase Chain Reaction (PCR)

Enzoklop (https://commons.wikimedia.org/wiki/File:Polymerase_chain_reaction.svg), Polymerase chain reaction https://creativecommons.org/licenses/by-sa/3.0/legalcode

- The genome of humans differs roughly one every 1000 nucleotides. These differences are called **single nucleotide polymorphisms** (**SNPs**). SNP's can be used as genetic markers for disease-causing alleles (the SNP's don't cause disease, but are physically close enough be linked to their presence).

- A **DNA microarray assay** can be used to monitor the expression of large groups of genes across the entire genome – this is useful for seeing which genes are transcribed in different tissues or at different stages of development. In a DNA microarray assay, tiny amounts of a large number of single-stranded DNA fragments representing different genes are fixed to a glass slide in array on a grid in wells. Then, mRNAs are isolated from a cell and reverse transcriptase is used to make cDNA. In the microarray assay, the cDNAs are labeled fluorescently and then allowed to hybridize to a DNA microarray. So the wells that light up tell you which gene is expressed and color tells you where it is expressed (we usually differentiate samples/tissues with different color labels). Using this, expression of genes across the entire genome can be analyzed simultaneously.

Blotting Techniques

- Blotting techniques allow for the identification of target fragments of DNA, RNA, or protein.
- **Southern Blotting** initially begins with the process of **gel electrophoresis** (see above). After electrophoresis is performed, the DNA fragments are separated into single strands and then transferred to a nitrocellulose membrane. A probe is then added that will hybridize and mark the target DNA fragment.
- **Northern Blotting** is just like Southern Blotting, but for RNA molecules.
- **Western Blotting** is a similar method but for proteins. Rather than a probe attaching via nucleic acid hybridization, a primary antibody specific to a protein is added. Then, a

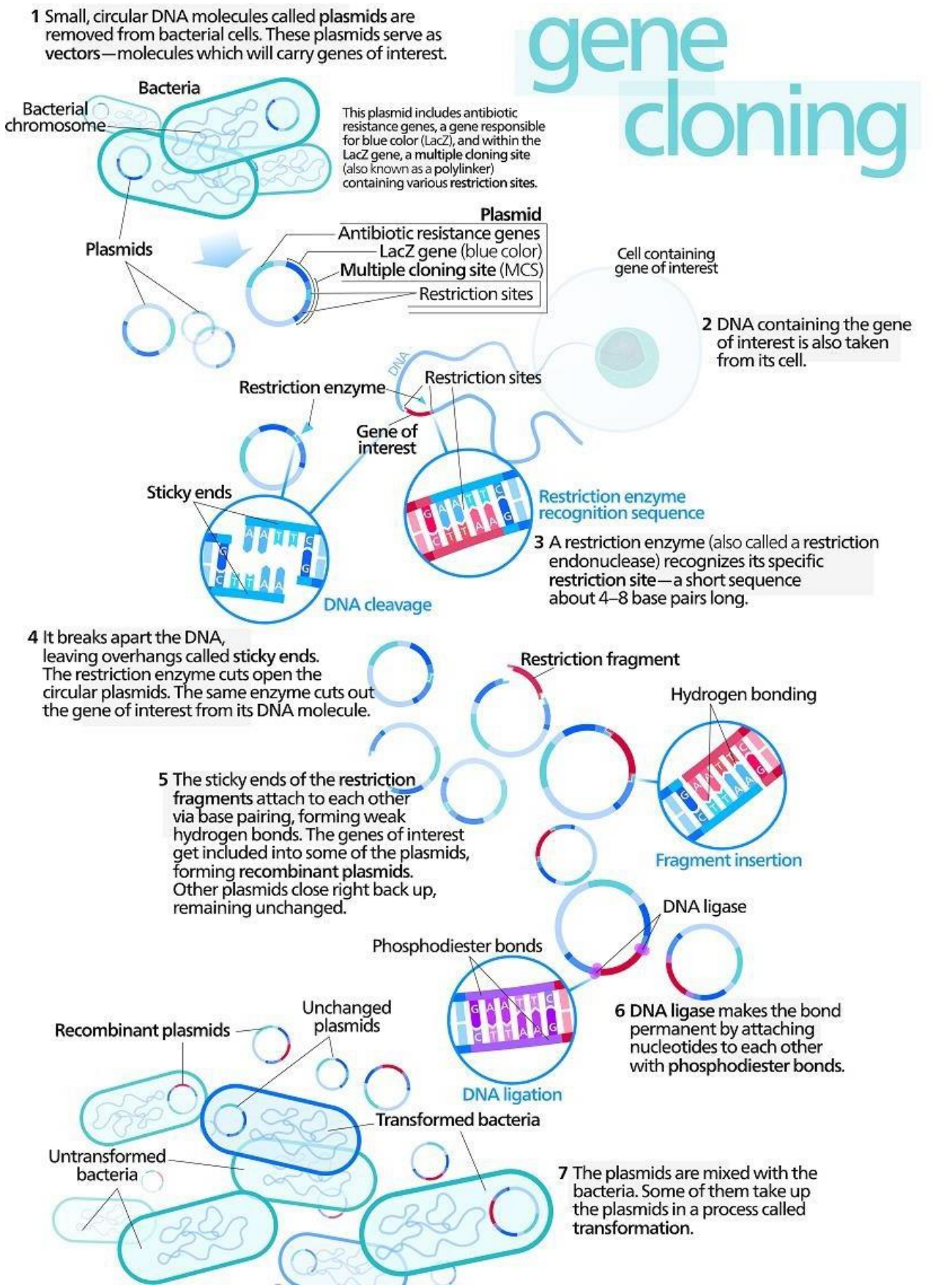
secondary antibody-enzyme conjugate will bind to the primary antibody to mark it for visualization.

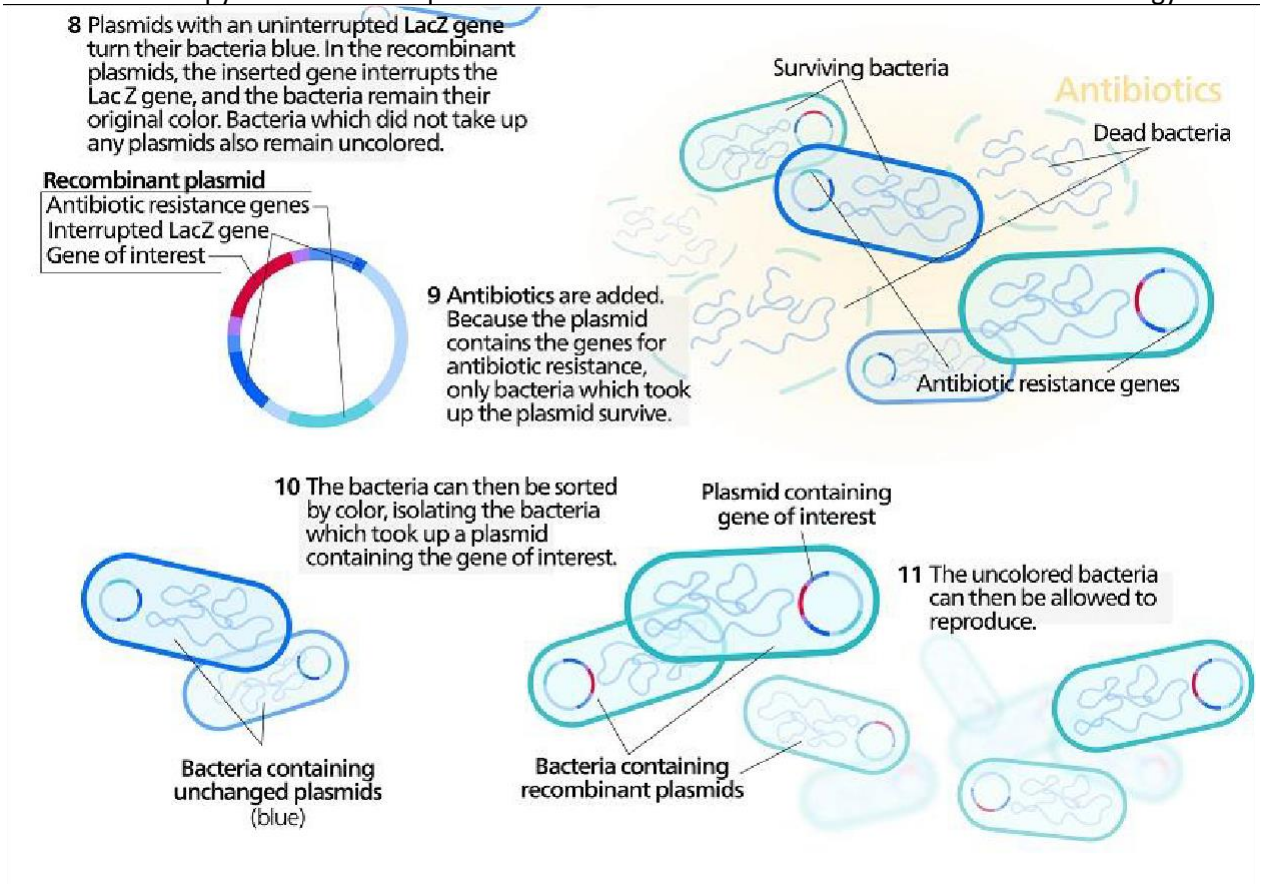
- An easy way to remember the blotting techniques is SNOW DROP:

SNOW	DROP
Southern	**D**NA
Northern	**R**NA
O	**O**
Western	**P**roteins

Gene Technology

- A **genomic library** is a collection of cloned DNA pieces from a genome. The library can then be screened to locate a gene of interest. The formation of a genomic library is a good opportunity to recap many of the techniques discussed above into a practical example:
  - To create a genomic library, we first create multiple copies of the genes we are interested in cloning via PCR. Next, we must insert the gene(s) of interest into a plasmid: the plasmids (and the DNA copies containing the genes of interest) are cut with the same restriction enzymes. The sticky ends of the new DNA will then bind with the matching sticky ends of the plasmid, and the plasmid will be closed using DNA ligase – at this point the resulting plasmid is a recombinant DNA molecule. Note that the plasmid will also contain a gene for antibiotic resistance for screening purposes. Now that the plasmid is complete, we must add it to a bacteria to replicate it – in order for the bacteria to take up the plasmid, the bacteria must first be made competent via electroporation. Not all bacteria will take up the plasmids, so we use the plasmid's antibiotic resistance gene to determine which bacteria were "transformed" into recombinant bacteria that will survive treatment with the antibiotic.


gene cloning
1 Small, circular DNA molecules called plasmids are removed from bacterial cells. These plasmids serve as vectors—molecules which will carry genes of interest.
Bacteria
Bacterial chromosome
Plasmids
This plasmid includes antibiotic resistance genes, a gene responsible for blue color (LacZ), and within the LacZ gene, a multiple cloning site (also known as a polylinker) containing various restriction sites.
Plasmid
Antibiotic resistance genes
LacZ gene (blue color)
Multiple cloning site (MCS)
Restriction sites
Cell containing gene of interest
2 DNA containing the gene of interest is also taken from its cell.
DNA
Restriction enzyme
Restriction sites
Gene of interest
Sticky ends
Restriction enzyme recognition sequence
3 A restriction enzyme (also called a restriction endonuclease) recognizes its specific restriction site—a short sequence about 4–8 base pairs long.
DNA cleavage
4 It breaks apart the DNA, leaving overhangs called sticky ends. The restriction enzyme cuts open the circular plasmids. The same enzyme cuts out the gene of interest from its DNA molecule.
Restriction fragment
Hydrogen bonding
5 The sticky ends of the restriction fragments attach to each other via base pairing, forming weak hydrogen bonds. The genes of interest get included into some of the plasmids, forming recombinant plasmids. Other plasmids close right back up, remaining unchanged.
Fragment insertion
DNA ligase
Phosphodiester bonds
6 DNA ligase makes the bond permanent by attaching nucleotides to each other with phosphodiester bonds.
DNA ligation
Recombinant plasmids
Unchanged plasmids
Transformed bacteria
Untransformed bacteria
7 The plasmids are mixed with the bacteria. Some of them take up the plasmids in a process called transformation.



Gene Cloning: Visual Summary

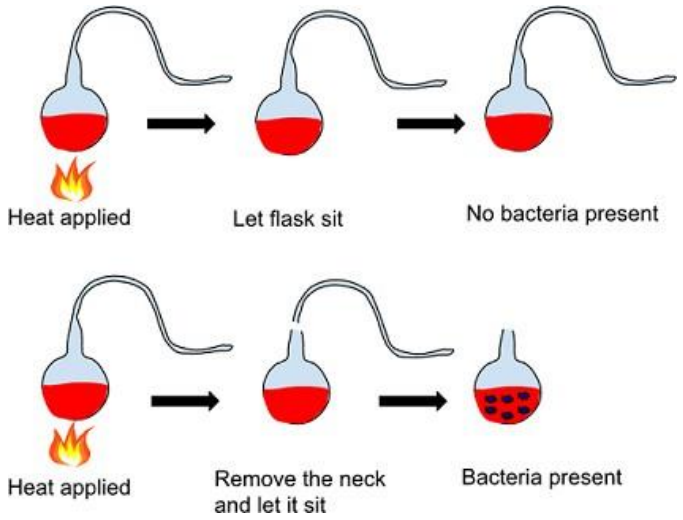
Kelvinsong (https://commons.wikimedia.org/wiki/File:Gene_cloning.svg), "Gene cloning", https://creativecommons.org/licenses/by-sa/3.0/legalcode

- To determine the function of a gene, **in vitro mutagenesis** introduces specific mutations into a cloned gene, then that gene is returned to a cell and the mutant cell is observed for phenotype that may indicate the function of the missing normal protein (often an embryonic stem cell, so that it will develop into adult tissues with the disruption observed). A frequently used example of this technology in research are "knockout mice".
  - In some cases RNAi can be used to achieve the same goal.
- To test for the presence of a specific gene sequence in someone's DNA:
  - Method 1 – Take a drop of their blood, cut up the DNA into smaller pieces, and use PCR with a primer specific for the region that gene sequence appears. If that gene is present, lots of copies will be made via PCR. If the gene is not present, no copies will be made.
  - Method 2 – Amplify the cDNA of the gene sequence, then use a DNA microarray assay to detect the presence of fluorescence, indicating whether or not the gene is there.
- **Gene therapy** is the introduction of genes into an afflicted individual for therapy (e.g. via a retroviral vector to insert genome material into chromosomal DNA).
- **Transgenic** animals have a gene introduced from the genome of another individual (often a different species).
- **Genomics** is the study of whole sets of genes and their interactions. Bioinformatics is the application of computational methods to the storage and analysis of biological data.

- The whole-genome shotgun approach is a sequencing strategy for the entire genome. In this approach, DNA from a chromosome is cut up into many pieces, which are then cloned and sequenced. A computer than analyzes the sequences and places them into an order.

Important Experiments/Discoveries

- **Pasteur's swan neck flask experiment** proved that spontaneous generation was invalid – life cannot be created from non-life.
  - Pasteur's experiment: a broth was kept in a flask with a curved neck (to prevent microorganisms in the air from entering the solution). The solution was then boiled to destroy all microorganisms. When the curved neck remained on, the broth remained free of microorganisms. If the curved neck was removed, microorganisms began to grow in the broth. This established that microorganisms did not arise spontaneously, but came from existing microorganisms (contamination in the air).

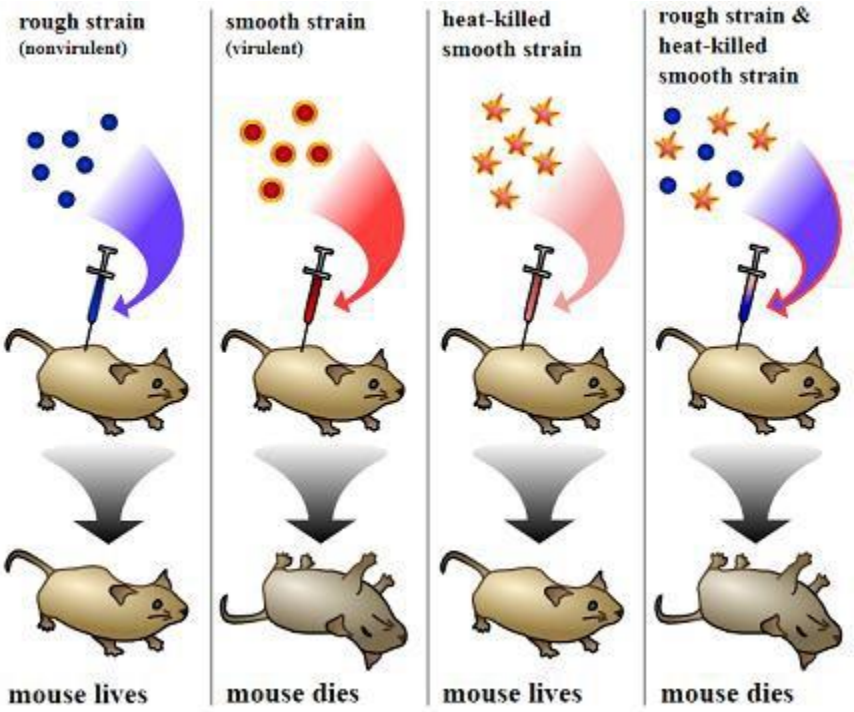


Pasteur's Swan Neck Flask Experiment

Kgerow16 (https://commons.wikimedia.org/wiki/File:Louis_Pasteur_Experiment.svg), https://creativecommons.org/licenses/by-sa/4.0/legalcode

- Griffith showed that genetic traits could be transferred between different bacterial strains (**bacterial transformation**) via an 'unknown heritable substance'.
  - **Griffith's experiment** used two strains of pneumonia: a smooth (S) strain (which had a protective capsule shielding it from the immune system, allowing it to be virulent) and a rough (R) strain (which lacked the protective capsule, and was therefore nonvirulent). Smooth strain injection would kill a mouse, while a rough strain injection would not. If a smooth strain was heat-killed and injected, it would also not kill the bacteria. But when the smooth strain was heat-killed and added to a solution with living rough strain bacteria, and the solution was then injected into a mouse, the mouse would be killed.
    - What killed the mouse? Recall that **bacterial transformation** allows bacteria to absorb DNA from its surrounding and incorporate it into its genome. When the smooth strain was heat-killed and its remains were added to the rough strain bacteria, the rough strain bacteria were transformed when they absorbed the smooth strain's DNA. This new genetic information allowed the rough strain to produce the protective capsule that

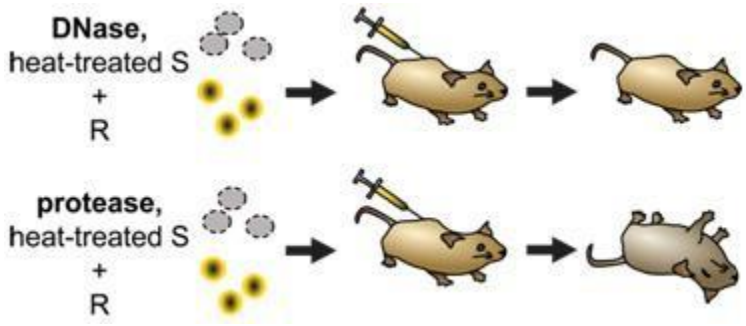
shielded it from the immune system and allowed it to be virulent, resulting in the death of the mouse.



Griffith's Experiment

Madeline Price Ball (https://commons.wikimedia.org/wiki/File:Griffith_experiment.svg), "Griffith experiment", https://creativecommons.org/licenses/by-sa/3.0/legalcode

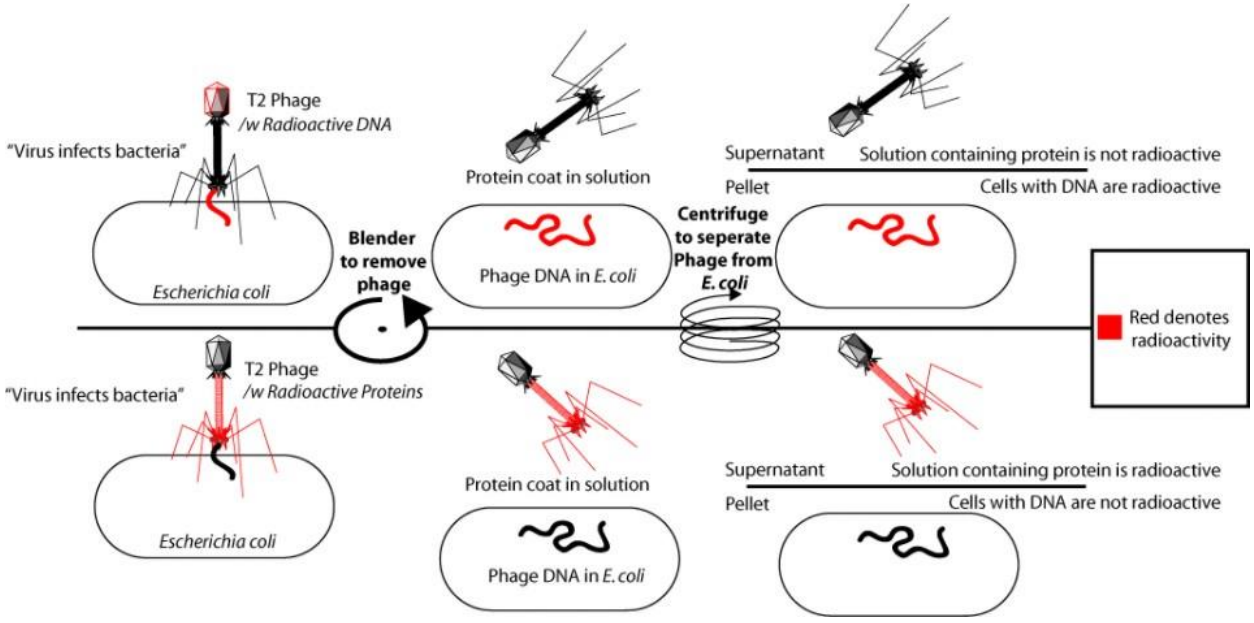
- The **Avery-MacLeod-McCarty experiment** demonstrated that DNA was the 'heritable substance' causing bacterial transformation.
  - At the time of Griffith's experiment, it was not known what component of the killed bacteria allowed for bacterial transformation. The Avery-MacLeod-McCarty experiment was similar to Griffith's experiment, but various digestive enzymes were separately added to the remnants of the heat-killed smooth bacteria: DNAse, proteinase, lipase, etc. Most of these mixtures had no effect, but when DNAse was added to the remnants of the heat-killed smooth bacteria, the mouse survived.
    - Why did adding DNAse prevent the mouse from dying, but not the other enzymes? Remember that DNA is the genetic material containing the information for producing the protective capsule that the rough bacteria absorbed to become virulent. When an enzyme such as proteinase is added to the heat-killed smooth bacteria, proteins would be broken down, but the DNA would be unaffected, allowing the bacteria to still be transformed. DNAse digested the DNA remaining from the heat killed smooth bacteria, preventing bacterial transformation from taking place – as a result the rough bacteria never gained the ability to produce the protective capsule, and remained nonvirulent.



Avery-MacLeod-McCarty Experiment

Deyholos (http://www.cubocube.com/files/images/opengenetics/chapter1/image6-7.png), "Figure 1.4" http://creativecommons.org/licenses/by-nc-sa/2.5/ca/

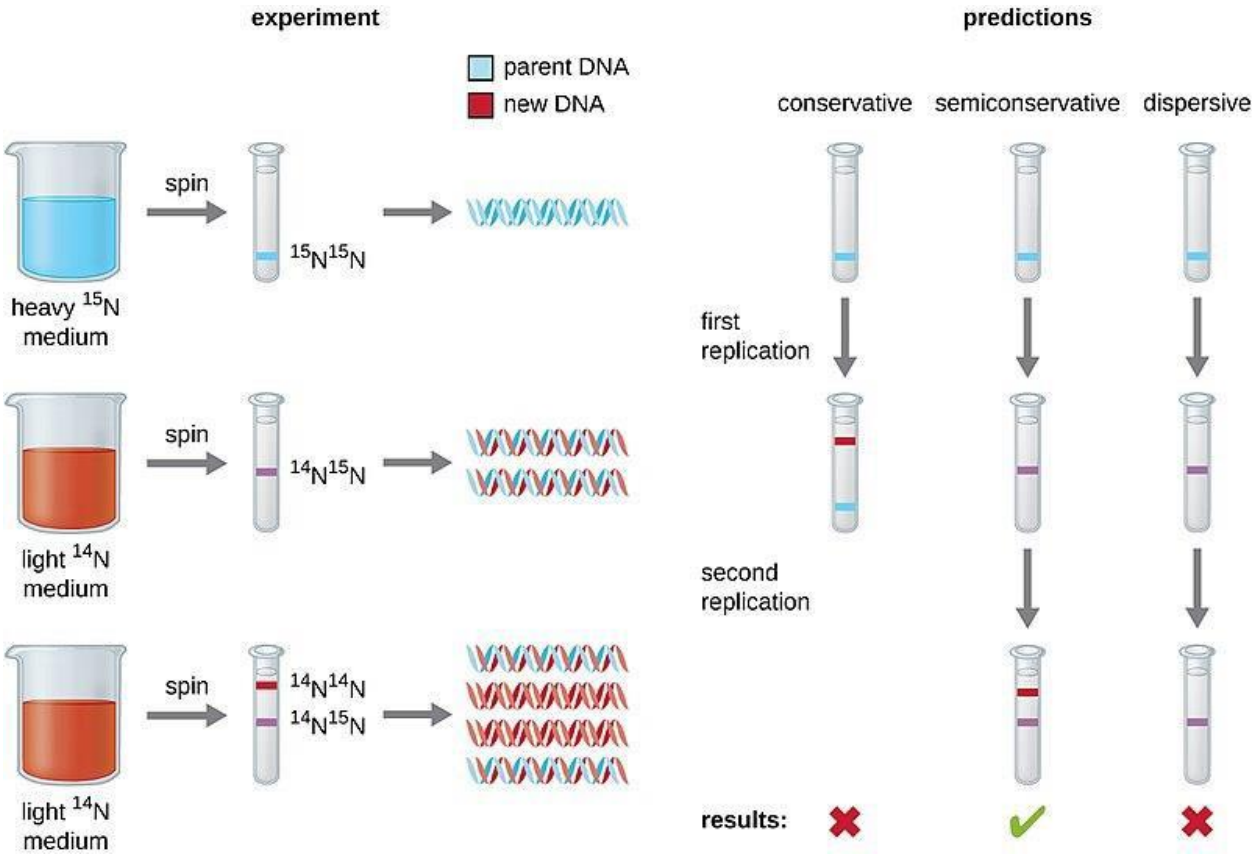
- **Hershey & Chase** showed that DNA, not proteins, was the genetic material of Phage T2 (a virus).
  - Phage T2 is a bacteriophage (a virus that infects bacteria). In order to determine what the genetic material of a virus was, Hershey and Chase ran two separate trials: one in which they placed a radioactive label on phosphorus in DNA of the virus, and one in which they placed a radioactive label on sulfur in protein of the virus. They then observed which radioactively labeled substance appeared within the bacteria. Result: only the radioactively labeled DNA appeared inside the bacteria, confirming DNA was the genetic material of the virus.
    - Why did this confirm DNA was the genetic material? Recall that bacteriophages infect bacteria by attaching and injecting their genetic material into the bacteria, where it is then replicated and assembled into new viruses. When only the radioactively labeled DNA (and not protein) appeared within the cell, DNA was confirmed as the genetic material.



Hershey-Chase Experiment

Adenosine (https://commons.wikimedia.org/wiki/File:HersheyChaseEx.png), "HersheyChaseEx", https://creativecommons.org/licenses/by-sa/2.5/legalcode

- Rosalind Franklin used X-ray diffraction to create the photo that allowed Watson & Crick to deduce DNA was a double helix structure.
- Watson & Crick believed that **semiconservative replication** was the valid DNA model. The **Meselson & Stahl's experiment** proved this was true.
  - Meselson and Stahl grew *E. coli* in medium containing nucleotides with ^{15}N (an isotope of nitrogen). These bacteria were then transferred to medium with ^{14}N nucleotides. In both cases, bacteria will incorporate the nucleotides of the medium they are in when replication. When the ^{15}N bacterial DNA was replicated in the new medium, it was observed that the resulting DNA was not as dense as the original ^{15}N DNA, but not as light as ^{14}N DNA – instead it was a density between the two (implying that semiconservative replication took place: one strand of DNA contained the original ^{15}N nucleotides, while the newly synthesized strand contained the new ^{14}N nucleotides). When replication continued for another round in the new media, the results were further consistent with semiconservative replication.

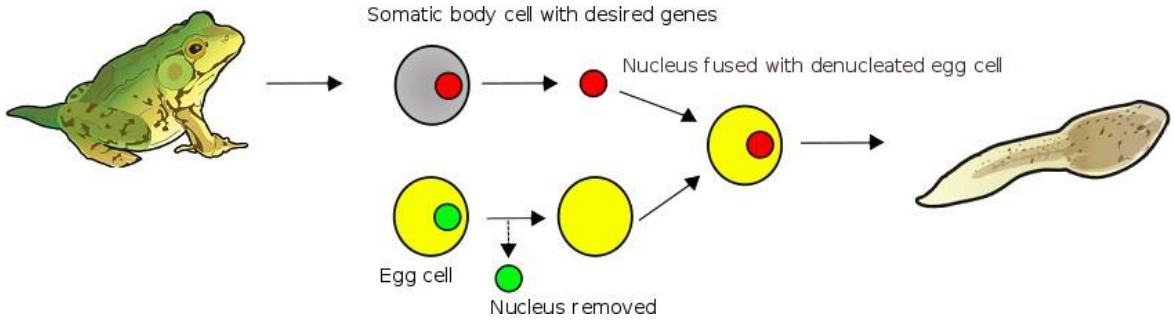


Meselson-Stahl Experiment

CNX OpenStax (https://commons.wikimedia.org/wiki/File:OSC_Microbio_11_02_MesStahl.jpg), https://creativecommons.org/licenses/by/4.0/legalcode

- A common misconception is that Watson & Crick discovered DNA. While they are responsible for deducing its structure, t was Johann Friedrich Miescher who first isolated what he called 'nuclein' (what we now know as nucleic acids) from cell nuclei in 1869.
- **Gurdon's nuclear transfer experiment** showed that when the nucleus from a differentiated frog cell was placed into an enucleated egg cell, it gave rise to a new frog (although this ability decreased as the donor cell became more differentiated).

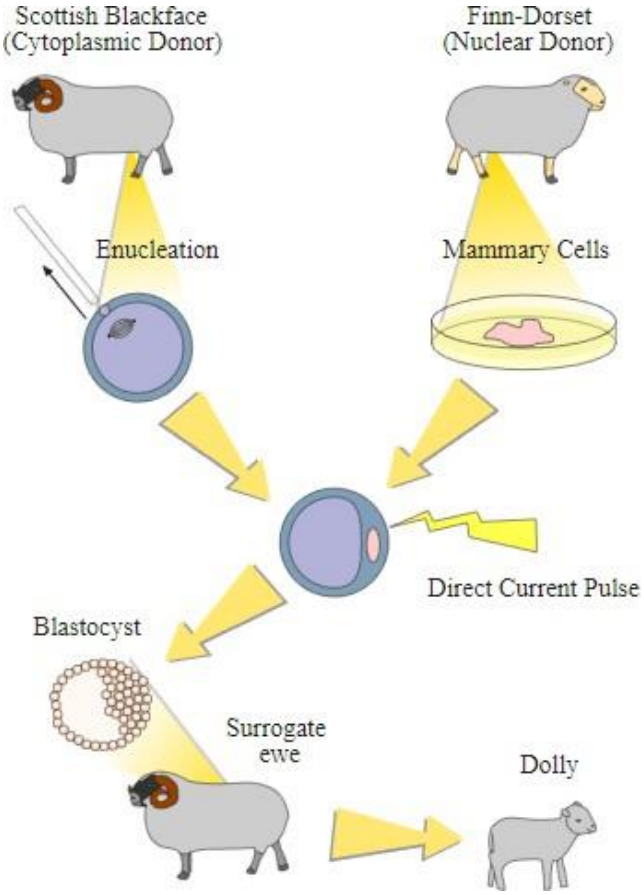
- What is this significance of Gurdon's experiment? It showed that fully differentiated cells *don't* lose their genetic information – they retain the full genome. No DNA is lost when a cell becomes differentiated – only its expression is altered.



Gurdon's Nuclear Transfer Experiment

Derived from an image drawn by Dr. Jürgen Groth, converted to SVG by Belkorin, modified and translated by Wikibob (https://commons.wikimedia.org/wiki/File:Cloning_diagram_english.svg), "Cloning diagram english", Modified to emphasize John Gurdon's experiment using frog diagrams from LadyOfHats, https://creativecommons.org/licenses/by-sa/3.0/legalcode

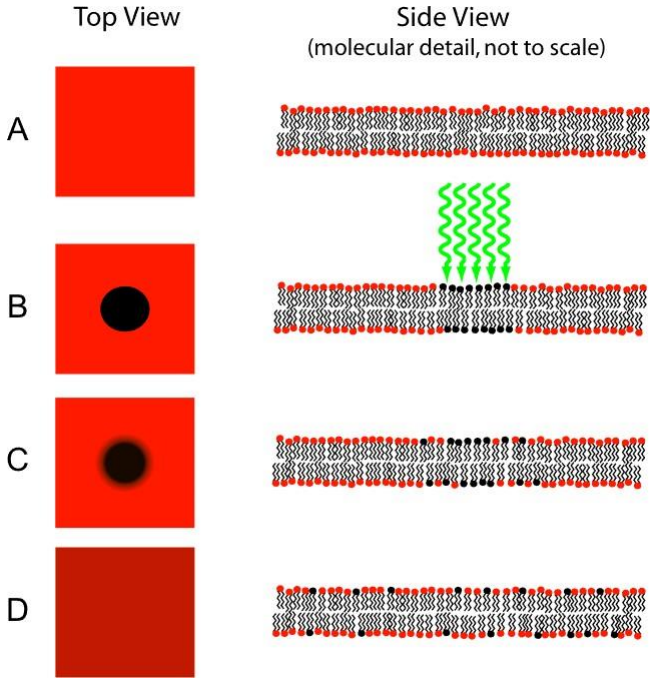
- Reproductive cloning of a mammal by nuclear transplantation (somatic cell nuclear transfer) was first accomplished with Dolly the sheep using a mammary cell nucleus. Note that the embryo is identical to the animal that supplies the nucleus – not the (enucleated) egg donor. Cloning from nuclear transplantation can result in defects – potentially due to epigenetic changes in the chromatin that have occurred in the donor nucleus over its life.



Reproductive Cloning: Dolly the Sheep

Squidonius (https://commons.wikimedia.org/wiki/File:Dolly_clone.svg), Public Domain

- The **pulse chase experiment** can be used to track the movement of proteins in a cell.
  - In this experiment, radioactively labeled amino acids are added to cells which are then incorporated into proteins during synthesis - this is the "pulse". Next, non-radioactively labeled amino acids are added to the cells – the "chase". The purpose of the pulse is to allow for tracking of the radioactive proteins as they move through the cell, while the purpose of the chase is to prevent all subsequent proteins from being radioactive as well (which would make tracking impossible).
- Size-exclusion chromatography is a method for protein separation on the basis of size. Larger proteins elute more quickly than smaller proteins.
- FRAP (**F**luorescence **R**ecovery **A**fter **P**hotobleaching) is used in the observation of cell membrane diffusion. In FRAP, the whole cell is fluorescently tagged, then a special light is shone on a specific spot to remove fluorescence (photobleaching), resulting in a dark spot. We can then determine (based on how long that black spot remains and how much of the brightness recovers) the mobility and the motion of molecules over time in the cell membrane. Two important pieces of information are acquired as the fluorescence returns to this photobleached area:
  1. How much light returns relative to the amount of light that was there before the photobleaching? This is the 'percent recovery'.
  2. How fast did the fluorescent molecules migrate back into the photobleached area? This is a measurement of the 'diffusional mobility'.
  - Application: FRAP can determine if a protein is able to move within a membrane (high percent recovery with fast mobility), or whether it is tethered other structural components of the cell (low percent recovery with slow mobility).



Fluorescence Recovery After Photobleaching
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