————————————Cell Membrane Overview————————————

CELL MEMBRANE AND PHOSPHOLIPIDS INTRODUCTION

- Cell membranes protect us from the outside world, compartmentalize organelles and reactions, etc.
- They are made largely of phospholipids- which themselves contain a glycerol backbone, phospholipid head, and 2 fatty acid tails
  - Phosphate head is polar, hydrophilic. Fatty acid tails are nonpolar and hydrophobic.
- In aqueous solutions, these phospholipids spontaneously form membrane bilayers with hydrophobic interiors.
  - The formation of a phospholipid bilayer is primarily driven by an increase in entropy of surrounding water molecules.
  - Lipids cause water to arrange in an ordered, unfavorable cage-like structure (called a Clathrate cage). Forcing lipids into a bilayer reduces this effect, thus increasing entropy.
- The membrane is semi-permeable. What can pass?
  - Small nonpolar molecules (mostly gases, such as O2 and CO2) go through fairly quickly. This is called passive diffusion
  - Small polar molecules (such as ethanol, H2O) can also go through, but much more slowly
  - Large, nonpolar molecules (such as benzeze) also go through the membrane, but also slowly
  - Large, polar molecules, such as glucose, cannot pass through the membrane
  - Charged molecules (such as Cl–, Na+, many ions and amino acids) also cannot pass through the membrane
- Phospholipids often bond with another head group via a phosphodiester bond:
  - phosphotidyl serine
  - phosphotidyl choline
  - phosphotidyl ethanolamine
  - phosphotidyl inositol
  - diphosphotidyl glycerol (aka cardiolipin)
- The fatty acid “R” group is made of long fatty acids, which can sometimes have double bonds.
  - If cis double bonds, they’ll have a kink, which affects cell membrane

CELL MEMBRANE PROTEINS AND THE FLUID MOSAIC MODEL

- Recall, the main component of cell membranes is phospholipids, but it can have other molecules in it, as well.
  - cholesterol: maintains fluidity (at low temps, especially) and integrity (at high temps) of membrane
  - proteins: carry out membrane processes. Two main types
    - **integral / transmembrane proteins**: crosses the whole membrane. Strongly bonded (hydrophobic) with the phospholipids and must be removed by a detergent. They also have polar and nonpolar regions (Charged parts are on outside, in aqueous environments.)
    - **peripheral proteins**: noncovalently bonded to outside of membrane. Can come and go as it is needed
    - **lipid bound protein**: found within the interior of the bilayer. These are rare because they don’t really have access to either side of the membrane and thus don’t play a role in membrane performing its duties.
- **Channel Protein: One of the main types of integral proteins that have a** channel / hole that allows things (such as ions) to pass through.
  - can also pump things out

- generally don't require energy, or ATP, and thus go down a concentration gradient
- One type of channel protein is the **aquaporin** protein, which selectively allows water through

- **Carrier Protein**: Will protect the substance so it can carry the molecule in,
  - Can go against the concentration gradient if needed (which uses ATP)
  - Glucose typically enters the cell through facilitated diffusion via a carrier protein
- **Glycoprotein**: membrane proteins that are also also be bound to carbohydrates (glycoproteins); these play a big role in cell-cell communication, and signaling with / recognizing other cells.
  - carbohydrates are always on the outside of the cell membrane for this reason
  - There also can be glycolipids, carbohydrates covalently bonded to a lipid that anchors it
- **fluid mosaic model** (1972) - cell is made of many different components that are relatively fluid, the different "pieces" can undergo rapid lateral diffusion around their layer

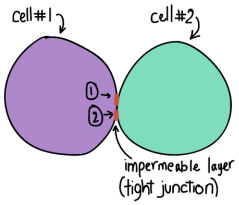
CELL MEMBRANE FLUIDITY

- Temperature:
  - As temperature decreases, fluidity decreases. (The phospholipids will cluster together and not have energy to move around a lot. At very low temps, this is called a crystallized state.)
    - membrane is rigid and may break
  - As temperature increases, fluidity increases. (More space in between phospholipids).
    - membrane won't hold shape
- Cholesterol: sort of a buffer, allows the membrane to maintain a certain level of fluidity
  - At low temps, cholesterol can help maintain fluidity by creating space between phospholipids
  - At high temps, cholesterol can help maintain integrity (b/c phospholipids want to get closer to cholesterol molecules)
- Unsaturated or Saturated fatty acids in the phospholipids:
  - Saturated fatty acid chains pack tighter than unsaturated, and thus will have lower fluidity than the kinked unsaturated fatty acid chains.
  - So at lower temps, longer chains and more saturated fatty acids are desired.

MEMBRANE DYNAMICS: How do phospholipids move in the cell membrane?

- Uncatalyzed movement:
  - *Transbilayer*, or "flip flop" diffusion — when a phospholipid goes from the inner leaflet to outer leaflet, or vice versa. This is very slow and uncommon.
  - *Lateral diffusion* — phospholipids move all around their own leaflet. Very fast and common.
- Catalyzed movement:
  - **Flippase protein** — catalyzes the movement of a phospholipid from *outer* leaflet to the *inner*, using ATP. Fast compared to transbilayer diffusion
  - **Floppase protein** — catalyzes the movement of a phospholipid from *inner* leaflet to *outer* leaflet, using ATP
  - **Scramblase** — catalyzes the simultaneous movement of one phospholipid from inner —> outer leaflet, and a second phospholipid from outer —> inner leaflet. Does not require ATP.

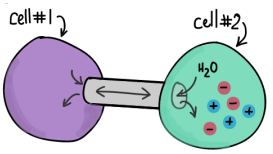
What happens when there is a problem with the cell membrane's ability to uptake/export important molecules or communicate?



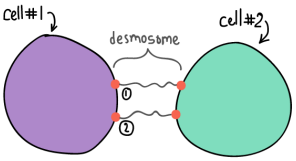
- There are many diseases associated with problems in the ability of the phospholipid b perform these functions. One of these is Alzheimer's disease, characterized by brain shrinkage and memory loss. One idea explaining why Alzheimer's disease occurs is the forming of plaque sticking to the phospholipid bilayer of the brain neurons. These plaques block communication between the brain neurons, eventually leading to neuron death and in turn causing the symptoms of Alzheimer's, such as poor short-term memory.

———————Cell–Cell Interaction———————

CELL JUNCTIONS



- Three main ways for cells to connect to each other: gap junctions, tight junctions, an desmosomes
- **Gap Junctions** are essentially tubes that join two cells together.
  - These tubes create a connection that allows for the transport of water and ions to and from the connecting cells.
  - Gap junctions allow action potentials to spread between cardiac (or neural) cells by permitting the passage of ions between cells, producing depolarization of the heart muscle (or nerve).
- **Tight Junctions** form when cells are squished up against one another.
  - The cell membranes are connected, but the contents of each cell are not connected in any way; there is an impermeable layer in between the cells.
  - Tight junctions are useful in places that need to contain certain fluids, like in the bladder, the intestines or the kidneys.



- **Desmosomes** are thread-like substances that connect cells across the space in be cells.
  - Also called *macula adherens.*
  - Like tight junctions, desmosomes physically hold the cells together, but do not allow fluids or materials to pass from the inside of one cell to the next.
  - These connections are also attached to the cytoskeleton (aka the scaffolding of the cell) to help with structural support.
  - The space in between the cells allows for water and solutes to flow freely between each cell without compromising the connection.
  - This is convenient for areas of our body that experience high stress like in our *skin* or our *intestines* because the space in between the cells offer flexibility that the other junctions can't.
- **What happens when cell junctions don't work properly**?
  - Gap junctions are most commonly found in the skin, so mistakes in their functions can lead to a variety of diseases that make up ectodermal dysplasia, a series of genetic disorders affecting the development or function of the teeth, hair, nails and sweat glands.
  - Additionally, errors in specific gap junction genes called, Cx43 and Cx56.6, can lead to the breakdown of some of our brain tissue called white matter which makes up 60% of our brain.
    - This is involved in diseases such as multiple sclerosis & Huntington's disease
  - Mistakes in our genes that produce desmosomes cause skin blistering.

MEMBRANE RECEPTORS

- Membrane receptors = integral proteins that interact with outside environment

- Signaling molecules (aka ligands) such as neurotransmitters, hormones, etc. bind to the membrane receptor (with specificity) and make a ligand-receptor complex
    - This complex then triggers a response in the cell, explaining how hormones function, how / when cells divide, when they die, etc. Also explains how cells communicate with each other
    - Membrane receptors are a common target for pharmaceutical drugs; this is why some cells can target specific cells (like your liver or heart)
- **Signal transduction** — an extracellular signal molecule (ligand) binds to membrane receptor, which then triggers and intracellular response
    - The binding causes protein to chain, causes conformational chain, causes cascade of signals in the cell, causing it to perform a certain function.
- Each receptor can only bind with specific / certain types of molecules. This is especially important in hormonal signaling.
    - Used to be called lock-and-key, but induced fit is now the model, which means the ligand and receptor can change shape to better fit one another.
- Three main types of membrane receptors:
    - Ligand gated ion channels
    - G-protein coupled receptors
    - Enzyme linked receptors

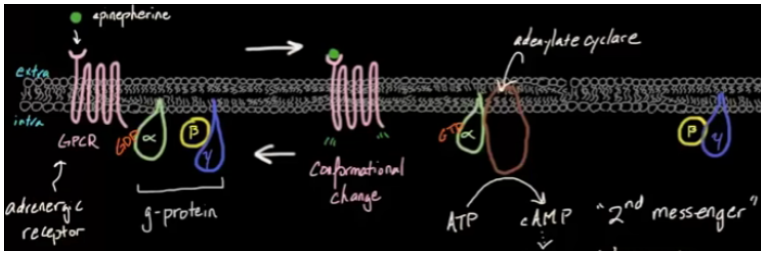
LIGAND GATED ION CHANNELS

- Also called ion channel linked receptors, these are transmembrane ion channels that open or close in response to the binding of a ligand.
- Commonly found in excitable cells like neurons, because these channels react quickly to binding of a ligand, and thus the cells can respond quickly to stimulus.
- Only specific ligands can bind to specific channels (lock and key / induced fit)
- Note, binding site is no where near actual channel.. ligand binds to allosteric site and alters opening / closing by conformational change.
    - The allosteric binding site can be inside the cell, but that's rare.
    - Also possible for there to be multiple binding sites for ligands.
- Once the ions move in or out of the cell, an intracellular electrical signal happens
- Ligand gated ion channels are not the same as **voltage gated ion channels..** those only depend on a different in membrane potential, not the binding of a ligand
- Ligand gated ion channels are also different from **stretch activated ion channels**.. which open / close in response to deformation or stretching of the cell membrane

G-PROTEIN COUPLED RECEPTORS

- Only found in eukaryotes; are the largest class of membrane receptors. Each is specific to a specific function, but are
    - Ligands that bind to these range from light sensitive compounds to pheromones, hormones, neurotransmitters, etc.
    - GCPRs can regulate immune system, growth, sense of smell / taste / behavioral / visual and our moods. Many G-proteins and GCPRs still have unknown functions.
- Most important characteristics:
    - GCPRs have 7 transmembrane alpha helices.
    - They're also linked to G-proteins, which have the ability to bind to GTP / GDP and be activated
        - G-proteins have 3 subunits: G_{α}, $G_{ß}$, and G_{γ}. G_{α} and G_{γ} are attached by lipid anchors.

• (1) When the ligand binds to the GCPR, it undergoes a conformational change.
(2) Because of the conformational change, the G_α exchanges its GDP for a GTP, becoming activated.
(3) The GTP causes the G_α subunit to dissociate and move away from $G_{\beta\gamma}$ dimer.
(4) G_α subunit then goes on to regulate target proteins
(5) Target protein then relays signal via second messenger.
  • So target protein could be an enzyme that produces second messengers, or an ion channel that let ions be second messengers
  • Some G-proteins stimulate activity, others inhibit.
• This chain of events, with a G_α protein subunit dissociating and going on to activate further response in the cell, will happen repeatedly as long as the ligand is bound. So how do we turn it off?
(6) GTP on the G_α is hydrolyzed to GDP
  • This often occurs internally, by GTPase within regulated (accelerated) by an RGS protein
• Ex with epinephrine (aka adrenaline):
  • Epi binds to ß-adrenergic receptor (GCPR), which causes it to undergo conformational change and switch GDP to GTP on the G_α subunit of the G-protein.
  • The G_α subunit dissociates and binds to adenylate cyclase, which then makes the secondary messenger cAMP from AMP.
  • cAMP goes on to increase heart rate, dilate blood vessels, and break down glycogen —> glucose



ENZYME LINKED RECEPTORS

• Are transmembrane proteins that uniquely function as receptors for signaling molecules *and enzymes*
  • also called catalytic receptors
• General structure has extracellular “ligand binding domain” and intracellular “enzymatic domain”
• Most common enzyme linked receptors are **tyrosine kinases (also called RTKs),** which regulate cell growth differentiation and survival; and they can bind and respond to ligands such as growth factors.
  • Unique because Tyrosine is on the enzymatic intracellular receptor. RTKs have ability to transfer phosphate groups to intracellular proteins, which activates them, and they go on to trigger additional change.
• RTKs occur in pairs. When ligands bind, the RTKs come together and act together in a **cross-linked dimer**. This helps activate the Tyr.
• Each Tyrosine in one dimer activates a Tyrosine on the other dimer! This is **cross phosphorylation.**
  • Tyr causes an ATP —> ADP + Pi
  • Other Tyr then pick up the free phosphate group.
  • Once activated, these phosphorylated Tyr allow different proteins to come by and attach themselves to them.
  • The only thing these proteins need to dock is an **SH2 domain**, which allows them to bind.

    - Multiple docking of different proteins allows changes to different intracellular signaling pathways at the same time. It often ends at the nucleus, with the signal from the docking protein affecting transcription.
- RTKs are useful / famous for their role in growth factors, such as in regulating surface proteins called ephrines, which can guide developmental processes in tissue architecture, placement of nerve endings, and blood vessel maturation. Other growth factors (like platelet derived) and hormones (such as insulin) also bind to RTKs.
- When the RTKs fail to regulate properly, they can cause issues in cell growth and differentiation. Many cancers involve mutations of RTKs
    - Many chemotherapies thus target RTKs. For example, the breast cancer drug Herceptin binds to and inhibit an RTK that is overexpressed in many breast cancers.

———————Transport Across a Cell Membrane———————

- Recall, cell membranes are semipermeable, meaning they have control over what molecules can or cannot pass through it.
- The bigger the passenger (molecule) we need to get across the membrane, the bigger car (vesicle / transport mechanism) we'll need.

PASSIVE TRANSPORT

- In passive transport, no energy is required. This includes:
    - diffusion
    - osmosis
    - filtration
    - facilitated diffusion
- **Diffusion** is when molecules move down their gradients (higher concentration —> lower concentration) through the membrane.
    - No matter what the situation, the cell it will try to stabilize / equalize concentrations
- **Simple Diffusion** occurs when small & nonpolar molecules pass through the membrane, down their gradient, without the use of energy or any membrane transporter.
    - This diffusion can be disrupted if the diffusion distance is increased.
    - Ex: if the alveoli in our lungs fill with fluid (pulmonary edema), the distance the gases must travel increases, so their transport decreases. Risk of pulmonary edema can be decreased by decreasing hydrostatic pressure and increasing osmotic pressure
- **Osmosis** is when water undergoes simple diffusion; water can easily pass through cell membranes.
- **Facilitated diffusion** is diffusion with the help of a membrane transport channel.
    - These channels are glycoproteins (proteins w/ carbohydrates attached) that allow molecules to pass through the membrane. They're almost always specific for a certain molecule (or a certain type of molecule, if they're an ion channel), thus are tightly linked to certain physiologic functions.
    - Ex: **K+ leak channel** — potassium has a much higher concentration inside the cell than outside, so K+ ions will flow down their gradient via this K+ leak channel in the membrane.
    - Ex: Chloride transporter that flips its conformation from outside to inside of the cell after binding chloride. Once the chloride is released into the cell, the transporter flips again to outside of cell.
    - Ex: GLUT4 is a glucose transporter found in fat and skeletal muscle.
        - Insulin triggers GLUT4 to insert into membranes of these cells so glucose can be taken in.

- Being a passive mechanism, the amount of sugar entering our cells is proportional to how much sugar we consume, up to saturation point
- In type II diabetes mellitus, cells do not respond as well to the presence of insulin, and so do not insert GLUT4 into their membranes. This can lead to soaring blood glucose levels.

- **Primary Active Transport:** directly uses ATP for energy.
  - Since these are so costly in terms of energy, they're more rare than other kinds of transporters.
  - Ex: Proton-potassium exchanger (H^+/K^+ ATPase) found in the stomach. These proton pumps are responsible for creating the acidic environment of the stomach, and can cause acid reflux. Proton pump inhibitors like omeprazole are used to treat ulcers or acid reflux because they help reduce the acidity of the gut.
  - Ex: sodium-potassium pump (Na^+/K^+ ATPase) helps maintain resting potential in the cell.
- **Sodium-Potassium Pump** (also called Na^+/K^+ ATPase) maintains a voltage gradient across a cell or neuron's membrane.
  - This protein uses the energy released from hydrolysis of ATP to pump three sodium ions out of and two potassium ions into the cell.
  - Three sodiums bond and ATP —> ADP (This is why it's an ATPase). This causes the transport protein to flip and open on other side, where Na^+ ions are released. Once empty (and facing outside), two K^+ ions bind, causing the transport protein to again flip (this time to the inside) and releases K^+ into the cell.
  - **Na^+ concentration is thus higher outside the cell, K^+ concentration is higher inside the cell.**
  - *The outside of the cell is also more positive than inside*, b/c for every exchange, we add a net +1 charge to the outside. This creates an electropotential gradient, which eventually stabilizes because of facilitated diffusion.
- **Secondary active transport** moves multiple molecules across the membrane, powering the uphill movement of one molecule(s) with the downhill movement of the other(s).
  - Uses a gradient (which is set up using energy) instead of ATP for energy
  - Ex: SGLT2 is a glucose symporter transporter that allows glucose into our cells *against* its gradient by bringing in a sodium molecule *down* its gradient at the same time.
    - Na^+ wants to get inside the cell, and the energy released by it traveling *down* its gradient is enough to power glucose into the cell in the same direction (against its gradient).

Transport	Molecules moved	Uses energy?	Example transporter/disease
Simple diffusion	Small, nonpolar	No	Pulmonary edema
Facilitated diffusion	Polar molecules, larger ions	No	GLUT4 / Diabetes Mellitus Type II
Primary active transport	Molecules moving against their gradient coupled to the hydrolysis of ATP	Yes	Sodium-potassium pump, proton pump / atrial fibrillation, acid reflux
Secondary active transport	Molecule going with + molecule going against gradient	Yes	Sodium-calcium exchanger, SGLT2

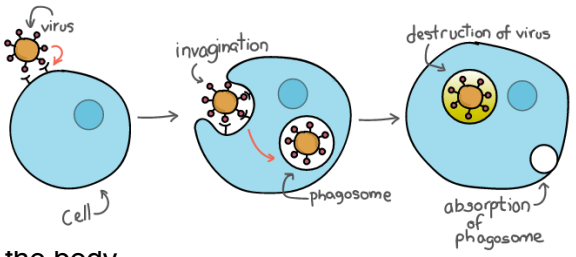
  - Ex: Sodium/calcium exchanger used to restore cardiomyocyte (heart cell) calcium concentrations after an action potential. An influx of calcium causes the heart to contract. The transporter then pushes calcium *out* against its gradient, while bringing *in* a sodium ion to relax the heart.
  - This type of transporter that allow molecules to go in opposite directions is an **antiporter**

- Secondary active transport also occurs in the neurons (see organ systems section for more).

- SSRIs (used to treat depression) block a *specific* sodium-neurotransmitter symporter (why?) in the pre-synaptic neuron to keep the neurotransmitter in the synaptic cleft for a longer period of time.

ENDOCYTOSIS:

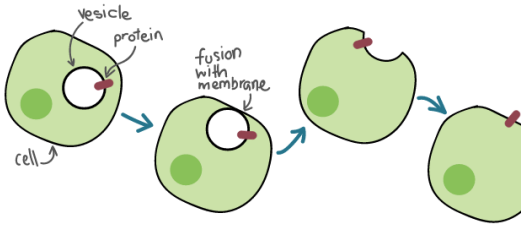
- When an extracellular molecule(s) is too large to pass through the bilayer (but it's still needed in the cell), the membrane folds inward, causing a new vesicle to bud off into the interior of the cell.
- **Phagocytosis** — cell engulfs a molecule in order to move it to the interior of the cell.
  - A molecule binds to specific receptors on the surface of the cell membrane, triggering the cell membrane to reshape and surround the molecule. (This process is specific.)
  - Then, the the two ends of the cell fuse, creating a vesicle that surrounds the molecule. Eventually the membrane around the molecule will be "digested "and its contents will be used.
  - Ex: white blood cells recognize pathogens, such as viruses cell and will use phagocytosis to bring it in to destroy it!



- Phagocytosis has proteins involved that end up getting hijacked virus can then spread its genetic material and infect throughout the body.
  - Ex: our body has helper T-cells (which target and kill bad things in the body) that have a receptor on them call CXCR4. This receptor is targeted by the HIV virus protein GP41, which binds to CXCR4. Some people have a genetic alteration in the CXCR4 that does not allow GP41 to bind; these individuals are immune to HIV!
- **Pinocytosis** — cell engulfs dissolved ions & other solutes in the liquid medium surrounding the cell.
  - This is different than phagocytosis, which brings in full, undissolved or insoluble molecules, but the distortion of the cell membrane is similar.
  - Pinocytosis is *not specific* to what is carried into the cell, just brings in a bunch of ions and solutes. This means that it can accidentally bring in bacteria or pathogens; pinocytosis is how salmonella infects an individual.
- **Receptor-Mediated Endocytosis** — very specific (even more so than phagocytosis) importation of molecules into the cell, lock-and-key
  - Receptors embedded in the cell membrane, when bound by molecules with an exact match in shape, size, or other physical attribute, will allow those molecule to enter into the cell through the same engulfment process as phagocytosis or pinocytosis

EXOCYTOSIS:

- The golgi body (which has its own membrane) releases a waste protein or molecule into a vesicle.
- Once the vesicle has enclosed the waste proteins, it moves towards the cell membrane and merges with it, opening the bubble-like structure and ejecting its conte environment.
- *Exocytosis can also embed proteins in the cell membrane* ————>
  - The new protein is formed inside the cell and becomes a part of a vesicle's membrane
  - The vesicle, now containing the new protein in its phospholipid bilayer, fuses with cell membrane, allowing the proteins to become directly integrated with it.



DETAILS ON PHAGOCYTOSIS:

- Phagocytosis is a when a cell (often an immune system cell) binds to the item it wants to engulf on the cell surface and draws the item inward while engulfing around it.
  - Often happens when the cell is trying to destroy something, like a virus or an infected cell
  - Very specific process that depends on the cell being able to bind by engulfing surface receptors
  - Won't happen unless the cell is in physical contact with the particle it wants to engulf.
- Cell surface receptors used for phagocytosis depends on the type of cell that's phagocytizing.

The most common cell receptors are:

- **Opsonin receptors —** bind bacteria or other particles that have been coated with immunoglobulin G (or "IgG") antibodies by the immune system.
  - Immune system coats potential threats in antibodies so other cells know it should be destroyed.
  - Immune system can use something called the "complement system", a group of proteins used to tag the bacteria… basically another way for the immune system to destroy pathogens and threats.
- **Scavenger receptors —** bind to extracellular matrix produced by bacteria itself.
  - Most bacteria and other cellular species produce a matrix of proteins surrounding themselves… It's a perfect way for immune system to identify foreign species, because human cells do not produce the same protein matrix.
- **Toll-like receptors** — bind to specific molecules produced by bacteria
  - Once they're bound to a bacterial pathogen, the innate immune system recognizes these toll receptors and activates the immune response.
  - Many different types of toll-like receptors are produced by the body, which bind diff. molecules.
- **Antibodies** — Some immune cells make antibodies that can bind to specific antigens.
  - Antigens are specific to their pathogens, they help immune system know what threat it has to fight.
  - This process is similar to how toll-like receptors recognize and identify what type of bacteria is infecting the host.

Details of phagocytosis:

- The virus and the cell need to come into contact with each other.

(1) Sometimes the immune cell accidentally bumps into a virus in the blood stream. Other times, cells move via **chemotaxis**, in response to a chemical stimulus.

- Many immune system cells move in response to **cytokines**, small proteins that signal cells to move to certain area in the body where the particle (e.g. a virus) is found.
- Common in infections that are specific to a certain area (e.g. skin wound infected by bacteria).

(2) The virus binds to the cell surface receptors on the macrophage.

- Recall, different cell types express different receptors. Some are general, meaning they can identify a self-produced molecule versus a potential threat (and that's about it); others are very specific, like toll-like receptors or antibodies.
- The macrophage will not initiate phagocytosis without successful binding of cell surface receptors.
- Viruses can also have surface receptors specific to those on the macrophage.

- Viruses need to access the host cell's cytoplasm or nucleus in order to replicate and cause an infection, so they use their surface receptors to interact with immune system cells and exploit the immune response for entry into the cell.
- Sometimes, when a virus and a host cell interact, the host cell is able to successfully destroy the virus and stop the spread of infection.
- Other times, the host cell engulfs the virus, and the virus tricks the cell, gaining access to what it needs to replicate. Once this happens, the infected cell is identified and destroyed by other cells of the immune system in order to stop viral replication and infection.

(3) The macrophage starts to invaginate around the virus, engulfing it into a pocket.

- Instead of passing it through the membrane, phagocytosis uses invagination (turns it inside out or folds back on itself to form a cavity / pouch) to draw the particle inward while closing in around it.

(4) Invaginated virus becomes enclosed in a **phagasome** (bubble-like structure) within the cytoplasm.

- The lips of the pocket, formed by invagination, extend towards each other to close the gap.
- The plasma membrane then moves around and encases the particle.

(5) The phagosome fuses with a lysosome, becoming a "**phagolysosome**".

- Lysosomes are similar to phagosomes, which process wastes inside the cell.
- "Lysis" means "to break down", making it easy to remember the function of a lysosome.
- Without fusing with a lysosome, the phagosome wouldn't be able to do anything with its contents.

(6) Phagolysosome lowers the pH to break down its contents.

- A lysosome or phagolysosome is able to break down its contents by drastically lowering the pH of its internal environment. The strong acidity is an effective way of killing or neutralizing whatever is inside the phagolysosome so it cannot infect the cell.
- Some viruses actually exploit this lowered pH to escape the phagolysosome and start replicating inside the cell. Ex: influenza uses the drop in pH to activate a conformational change, allowing it to escape into the cytoplasm.

(7) Once the contents have been neutralized, the phagolysosome forms a residual body that contains the waste products from the phagolysosome. The residual body is eventually discharged from the cell.

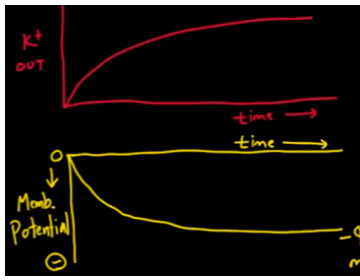
<u>Phagocytosis and the Immune System</u>

- Phagocytosis is a critical part of the immune system, allowing cells to ingest and destroy pathogens (e.g. viruses, bacteria) and infected cells. This limits how quickly the infection can spread and multiply.
- The act of phagocytizing pathogenic or foreign particles also allows immune system cells to know what they are fighting against… then the cells of the immune system can specifically target similar particles circulating in the body.
- Several types of cells of the immune system perform phagocytosis, such as neutrophils, macrophages, dendritic cells, and B lymphocytes.
- Phagolysosomes create an acidic environment to destroy or neutralize its contents, but immune system cells that perform phagocytosis can also use other mechanisms to destroy pathogens inside the phagolysosome, such as:
  - **Oxygen Radicals —** highly reactive molecules that react with proteins, lipids and other biological molecules. During physiological stress, the amount of oxygen radicals in a cell can increase dramatically, causing oxidative stress, which can destroy cell structures.

- **Nitric Oxide —** a reactive substance, similar to oxygen radicals, that reacts with superoxide to create further molecules that damage various biological molecules.
- **Antimicrobial Proteins —** proteins that specifically damage or kill bacteria.
  - ex: antimicrobial proteins such as proteases, which kill various bacteria by destroying essential proteins; and lysozyme, which attacks the cell walls of gram positive bacteria
- **Antimicrobial Peptides —** similar to antimicrobial proteins in that they attack and kill bacteria. Some antimicrobial peptides, like *defensins*, attack bacterial cell membranes.
- **Binding Proteins —** competitively bind to proteins or ions that would have otherwise been beneficial to bacteria or viral replication. They're important players in the immune system
  - ex: *lactoferrin*, a binding protein found in mucosal membranes, binds iron ions, which are necessary for growth of bacteria.

- Some species of amoebas, algae, and other single-celled organisms use endocytosis & phagocytosis to eat. These engulfing mechanisms allow larger species to consume smaller species easily.
  - Many of these single-celled organisms don't express specific binding receptors. Instead, **cilia** (hair-like structures) is used to capture / entangle smaller species and initiate phagocytosis.

MEMBRANE POTENTIALS:



- Recall, cells have a high concentration of K+ (much higher inside then outside).. Let's say it's around 150mMol/L inside, and 5mMol/L outside.
  - This is set up by the Na^+/K^+ ATPase — move 2 K^+ in and 2 Na^+ out.
  - Net charge of a cell is neutral, though, because every cation has an anion.
- The concentration gradient across the membrane makes K^+ move outside the cell via specific ion channels in the membrane.
- When K+ moves out of the cell by these channels, the anions that were bound to it are left, creating a big negative charge within the cell. This membrane potential charge makes K+ want to move back in to neutralize the cell..
- Eventually, an equilibrium is reached such that $K+_{out} = K+_{in}$. This typically happens around –92mV for K+, but is different for every ion (and for different types of cells)
- Note: technically speaking, at some point you will have more K^+ ions outside than inside, but the concentration, which we measure in mols, will stay relatively constant compared to those ion amounts
- **The cell will always revert to its equilibrium potential.**
  - Let's say we pour a bunch of positive charge into the cell that raises the membrane potential to -46mV. The K^+ will continue to leave because of the concentration gradient, but the lesser negative charge means they won't be drawn back in as quick… so the cell returns to -92mV potential.
- As long as you maintain concentration gradient (150mM inside and 5mM outside) and permeability, the cell will stabilize at its equilibrium! Without both a desire to leave and a way to leave, though, the concentration gradient won't happen. (It would be 0mV)
- How do we get calculate V_M (membrane potential)?
  - $V_M = 61.5 \times \log ([K^+_{out}] / [K^+_{in}])$
- Examples of membrane potentials for different ions (for a cell that's was only permeable to one ion):
  - K^+ = -67mV (positive ions moving outside cell) • Na^+ = +67mV (positive ions moving into cell)

- Cl^- = -87mV (negative ions moving into cell)
- Ca^{2+} = 123mV (positive ions moving into cell)
  - note that because Ca^{2+} has a 2+ charge, the 61.5 gets changed to 30.75 in our V_M equation.

PERMEABILITY AND MEMBRANE POTENTIALS:

- Permeability is *all* ions crossing back and forth across the membrane. How do we figure out what this membrane potential of the cell is?
- Look at what percentage of the "border crossings" are from each ion?
  - Let's say 95% K^+, 1% Na^+, 2% Cl^-, 2% Ca^{2+}
- Multiply this percentage by the ions ideal membrane potential and add them all together.
  - -87.4mV (K^+) + 0.7mV (Na^+) – 1.7mV (Cl^-)+ 2.5mV (Ca^{2+}) = – 85.9mV cell membrane potential
  - note that equilibrium potential of K^+ most influenced the cell membrane's overall potential, because it has the highest permeability. If Na^+ had the highest permeability by far, the cell's total membrane potential would be positive.

———————————————Cell Theory———————————————

1600s:

- In the 1600s, Anton **Von Leeuwenhoek** ("Father of Biology") was looking at a bunch of stuff under the microscopic, including gunk from his own teeth. He *discovered bacteria* and named them animacules.
- Also in the 1600s, **Robert Hooke** also looked at stuff under a microscopic, including a thin sliver of cork. He noticed the array of their internal structure and *discovered & named cells*.
- Other scientists around this time looked at animal tissues, and also noticed similar cells.
- This all led to 1st tenet of cell theory: **The cell is the basic unit of life**.

Early 1800s:

- Scientists continued to research bacteria and noticed that they all had the same sort of structure.
- In 1830s, a botanist named **Schleiden** also noticed that no matter what kind of plant he was looking at, they all had those cells that Hooke noticed. Him and Schwann discovered sort of the same thing…
- **Schwann** researched animal nervous systems, and noticed that even different species of animals had the same kind of cells. He published a book in 1837 that laid out the 2nd tenet of cell theory:
- **All living organisms are composed of cells.**

Late 1800s:

- It was known that a man and a woman could reproduce, and thus that animals come from animals.
- It was also known that trees produce seeds, which can be planted to make similar trees, and thus plants come from plants.
- But no one really knew where bacteria came from… predominant theory at the time was *abiogenesis*.
- **Abiogenesis** was a theory of *spontaneous generation*… Scientists thought that there was some unknown substance in the air that would combine with non-living material (e.g. a rock) to produce life.

- In late 1800s, scientists studying the cells began to refute this theory. One such scientist was Virchow.
- **Virchow** was a German physician and pathologist who observed that some bacteria, if he was watching them at the right time, divided into 2 bacteria cells that were seemingly identical to the parent. (We know now this is binary fission, and it's how bacteria reproduce.)
  - Virchow published "*ominis cellula e cellula*" — cells produce cells. (Though it's worth noting he didn't coin this phrase and probably wasn't the first / real discoverer of binary fission.
  - Virchow was highly criticized… people thought abiogenesis could still explain what he saw.
- **Louis Pasteur** (1860s) finally really disproved abiogenesis with his *swan-neck bottle experiment*
  - At this time, a well-known experiment that "proved" abiogenesis involved filling a bottle necked flask with broth (which likely contained bacteria). The broth was boiled to kill any bacteria, and then left alone… without anything being added to the flask, scientists noted there was growth/life.
  - Pasteur thought that maybe there was some sort of bacteria particles in the air that were getting into the broth and causing the growth… So he invented his own swan necked bottle flask. This still allowed the broth to be open to the air, but if any microorganisms fell in they would be collected in the curve of the neck instead of going into the broth. Lo and behold, after the boiling / sterilization of broth in this sort of swan-necked bottle, and it was left alone for a while, there was *no growth.*
- This established the 3rd tenet of cell theory: **All cells come from pre-existing cells**.
- *Note: viruses are not considered living organisms because they violate 3rd tenet of cell theory*

——————————————Eukaryotic Cells——————————————

CHARACTERISTICS OF EUKARYOTIC CELLS:

- Eukaryotes are usually much large than prokaryotes, and often found in multicellular organisms.
- Also have compartmentalization, membrane-bound organelles, a nucleus, and divide by mitosis.
  - **Compartmentalization**: different parts of the cell are divided from one another so they have different functions. In prokaryotic cells, everything is just sort of floating around.
  - These compartments are called membrane bound **organelles.**
  - **Nucleus** is very important membrane bound organelle that contains all the genetic material**.**
  - Because prokaryotes are not compartmentalized, they can divide by simply making 2 copies of everything and dividing down the middle through binary fission.
  - Eukaryote division is more complicated, and requires a process called **mitosis.**
- **Nucleus** is the "control" center of the cell. It's where DNA is and where DNA —> mRNA
- **Mitrochondria** is the cells "power plant" it's the site of cell respiration where glucose —> ATP
- **Endoplasmic Reticulum** is a complex pattern of folded membranes that surrounds the nucleus. It is the primary site for protein synthesis, and is thus the "factory" of the cell.
- **Golgi Apparatus** is the "mail room" of the cell. It receives proteins from the ER and then sends them wherever they need to go, to a different organelle or even out of the cell.
- **Lysosomes and Peroxisomes** — cells can have multiple of both of these "recycling centers". The environment inside of these is very different than the cytoplasm. Lysosomes breaks down things. Peroxisomes reduce reactive oxygen species (like peroxides) into non-toxic forms.

THE NUCLEUS

- Most important function of the nucleus is to contain genetic material.
- It is enclosed by two membranes (inner & outer), which allows its nucleoplasm to be separate from the cell's cytoplasm.
- The nuclear membrane has a **nuclear pore** that spans both membranes. This allows complexes in the cytoplasm (such as polymerases) to be transported in, and allows other molecules (like mRNA) to leave. The nuclear pore is very selective, only recognizing/allowing in molecules w/ certain receptors.
    - **Nuclear envelope** = the combination of inner/outer membranes and the nuclear pores.
- Within the nucleus is a very dense region known as the **nucleolus.** This is the site of ribosome assembly, so it's densely packed with regions of DNA that produce ribosomalRNA.
    - Proteins needed for rRNA synthesis can come into the nucleus through the nuclear pore.
    - After synthesis, complete rRNA can exit back through the nuclear pore into the cytoplasm.
- The outer membrane of the nucleus is continuous with the membrane of the endoplasmic reticulum, and thus the intermembrane space of the nucleus is continuous with the lumen of the ER.

MITOCHONDRIA

- Mitochondria are responsible for producing ATP, which provides energy for the whole cell.
- They have a double membrane:
    - Outer membrane is made of a lipid bilayer that is only permeable to small molecules (which pass through via facilitator proteins).
    - Inner membrane is also a lipid bilayer, but it is *not* permeable to small molecules. It also has many folds called **cristae**, which help increase the surface area of this membrane.
        - Increased surface area means more space for membrane proteins involved in ATP production
- In between these two membranes is the intermembrane space
- At the center of the mitochondria is the matrix.
- Let's go through the steps of cellular respiration:
    - (1) Glycolysis splits glucose (6C) into 2 pyruvate (3C each) — in cytoplasm
    - (2) PDH (Pyruvate Dehydrogenase Complex) converts pyruvate to Acetyl-CoA — in mt matrix
    - (3) Krebs Cycle reacts A-CoA in a series of reactions producing NADH and FADH2 — in mt matrix
    - (4) Electron transport chain makes ATP with help of electron carriers — inner mt membrane.
- In the electron transport chain, NADH is oxidized to NAD^+ by complex I, and $FADH_2$ is oxidized to FAD by complex II. In each of these, the resulting electrons from oxidation are delivered to Cyt. Q, which shuffles them through complex III to cyt. c, and the electrons are eventually delivered to complex IV, which uses them to reduce O_2 to H_2O.
- When the electrons move from protein to protein, they're going from a higher energy state to a lower energy state, releasing energy in the process. This energy is used to pump H+ ions out of the matrix into the intermembrane space.
    - The intermembrane thus becomes acidic while the matrix becomes basic, creating a concentration and electropotential gradient. The H^+ ions will want to go back down their

gradient, but remember the inner membrane is impermeable. So the H^+ must go through the **ATP synthase** enzyme.
    - ATP synthase has special channels for H^+ ions. When H^+ goes through them, it causes the axle of ATP synthase to spin, which results in ADP being combined with a phosphate group to make ATP.
    - **chemiosmosis** = H^+ ions passing through channel in ATP synthase to make ATP
- Mitochondria are also unique in the following ways; they…
    - Have their own circular piece of DNA (multiple copies of it), contained in the matrix.
    - Are self-replicating and, because they have their own genome, can make their own rRNA, tRNA, proteins involved in ETC, and some proteins involved in ATP synthesis.
    - Use a different system of transcription and translation
    - Have their own unique genetic code.

ENDOPLASMIC RETICULUM AND GOLGI APPARATUS

- The endoplasmic reticulum is made of the **Rough ER (has ribosomes)** and the **Smooth ER**.
- Rough ER has ribosomes and is thus the site of protein synthesis.
    - Recall: Protein synthesis also happens in the cytoplasm. Proteins made there might end up in the nucleus, mitochondria, peroxisomes, or just stay in the cytoplasm.
    - In contrast, proteins made in the RER will be secreted into the environment, become integral proteins in the cell membrane, or remain in the ER, golgi body, or in lysosomes.
    - The Rough ER is also responsible for post-translational modifications of proteins, such as the formation of disulfide bridges.
- Proteins that are secreted from the cell or that become part of the membrane follow the **secretary pathway** (involves the ER—> golgi —> lysosome or cell membrane). How does it know to follow this?
    - All proteins begin to be translated in the cytoplasm, but those that need to follow the secretory pathway have a **signal sequence** that's detected early on in translation. This sequences causes the protein / polypeptide that's being translated to be pushed into the RER.
- *Smooth ER synthesizes lipids*, including those that end up being part of the cell membrane and those that are eventually secreted from the cell, such as steroid hormones.
    - It also metabolizes carbohydrates and aids in the detoxification of drugs.
    - Smooth ER It is more tubular than rough ER, and may not be continuous with nuclear envelope.
    - Every cell has a smooth ER, but the amount will vary with cell function. (ex: The liver, which is responsible for most of the body's detoxification, has larger amount of smooth ER.
    - Muscle cells contain **sarcoplasmic reticulum**, a modified smooth ER that helps store calcium.
- The **golgi apparatus** is organelle found near the ER; looks like a stack of pancakes. The golgi body modifies proteins made in the RER, sorts and sends proteins to proper destinations, and synthesizes certain molecules for secretion.
- Different molecules have different fates upon entering the Golgi. This determination is done by tagging the proteins with specific sugar molecules that set the protein on one of four paths:
    - *Cytosol*: proteins that enter the Golgi by mistake are sent back into the cytosol
    - *Cell membrane*: proteins destined for the cell membrane are processed continuously. Once the vesicle is made, it moves to the cell membrane and fuses with it. Molecules in this pathway are often protein channels or cell identifiers.

  - *Secretion*: proteins meant to be secreted from the cell must accumulate in number and acquire a special chemical signal before their vesicles can fuse with the cell membrane for release.
  - *Lysosome*: The final destination for proteins coming through the Golgi is the lysosome. Vesicles sent to this acidic organelle contain enzymes that will hydrolyze the lysosome's content.
- Let's look at a protein made in the RER. What will happens to it? For starters, we know it ends up being secreted or going into a lysosome or the cell membrane.
  - First step after synthesis is for the protein to bud off the RER into a vesicle.
  - This vesicle merges with the **cis stack** of the Golgi apparatus (the first part, closest to ER), then goes through the **medial stack**, and eventually ends up in the **trans stack** (furthest from the ER.)
  - From the trans stack, the protein-containing vesicle buds off into the cytoplasm and can take several directions.. It might merge with a lysosome so the protein ends up in it; it might expel its contents via exocytosis, or might merge with the membrane in such a way that the protein is embedded in the cell membrane.

LYSOSOMES AND PEROXIDES

- **Lysosomes** are membrane bound organelles in the cell that digest ("lyse") small molecules and substances via two processes: autophagy and crinophagy.
- **Autophagy = self-eating**. This is when the lysosomes digest parts of the cell itself or other cells.
  - ex: If some organelles in the cell are no longer functional, lysosomes will break them down
  - ex: Macrophages in the immune system break down viruses via lysosome autophagy.
- **Crinophagy** is when lysosomes digest excess secretory product.
  - ex: if cells produce extra of a hormone that needs to be secreted, lysosomes will break down that extra via crinophagy.
- In both processes, after the lysosome breaks down the molecules, it will release the building blocks into the cytoplasm to be reused.
- Enzymes in the lysosomes are known as **acid hydrolases**; they require an acidic environment to work properly. The pH in a lysosome is ~5, which acts as a safety mechanism for the cell.
  - If for some reason a lysosome were to burst, it would release into the cell's cytoplasm all the acid hydrolases that break down organelles and other molecules… but those hydrolases wouldn't be able to work because the pH of cytoplasm is ~7.4!
  - Yes, the lysosome would release some of its internal acid into the cell upon bursting, but not enough to have any significant impact on the pH of the cytoplasm overall.
  - If many lysosomes burst at once, though, that would make the cytoplasm more acidic, and that might make the acid hydrolases start to work, which would not be good for the cell.
- **Peroxisomes** are responsible for variety of metabolic activities… they break down fatty acids, help liver cells detoxify chemical and drugs, and neutralize reactive oxygen species (ROS)
  - ROSs are molecules like oxygen ions or peroxides that are created as a byproduct of normal cellular metabolism, but also by radiation, tobacco, and drugs.
  - They cause **oxidative stress** in the cell by reacting with and damaging DNA and lipid-based molecules like cell membranes.
  - In the peroxisome, an ROS such as H_2O_2 is broken down by catalases into H_2O and O_2.

EPITHELIAL AND CONNECTIVE TISSUE

- Four different types of animal tissues are made of eukaryotic cells:
  - epithelial
  - connective
  - muscle
  - nervous
- Epithelial tissue makes up various linings in the body (both outer and inner) and the glands.
  - Linings: outer layer of skin, outer layer of organs, lumen of organs, inside of an organism's cavities
    - ex: epithelial cells are found in the tissue lining the mouth, esophagus, GI tract, and kidney tubules; as well as in the lining of blood and lymphatic vessels, where it's called **endothelium**
  - Exocrine glands (release hormones / substances directly to target organ)
  - Endocrine glands (release hormones to bloodstream)
- Epithelial tissue can be simple (one layer thick), or stratified (2+ layers):
  - **Simple epithelial tissue** is found where substances needs to diffuse from two different sites
    - ex: Alveoli of lungs are lined with simple epithelium so O_2 and CO_2 can diffuse from alveoli
  - **Stratified epithelial tissue** can be found in places that need to resist chemical/mechanical stress
    - ex: The esophagus intakes food that may be sharp, hot, large, etc.. stratified cells protect it.
- Epithelial cells are attached to a basement membrane that is made of different kinds of fibers (e.g. collagen) rather than cells.
  - The basement membrane is semi-permeable to certain substances, which is important because epithelial cells are avascular; they have no blood vessels.
  - Instead, epithelial cells get nutrients from underlying tissue. Nutrients diffuse from tissue through basement membrane into epithelial cells.

- Connective tissue supports tissues, connects tissues, and separates different tissues from each other.
  - ex: bones, cartilage, blood, lymphs, adipose, membranes covering brain and spinal cord
- Connective tissues have 3 key components: cells, ground substance (a viscous type of fluid), & fibers:
  - The ground substance + fiber = matrix. (matrix is usually produced by the connective tissue cells)
- Examples of connective tissue, many of which provide some sort of support for tissues and organs:
- **Areolar tissue** - binds to different types of tissue and provides flexibility and cushioning.
- **Adipose tissue** - basically fat; it provides cushioning from the body and stores energy.
  - Adipose does not have fibers (is an exception to the rule)
- **Fibrous connective tissue** — very strong. provides support and shock absorption for bones and organs.
  - Fibrous connective tissues is found in dermis (middle layer of the skin), tendons, and ligaments
- **Blood** — also doesn't have fibers, and the matrix is the plasma.
- **Osseous-bone** — cells are osteocytes; matrix is bone mineral / *hydroxyapatite* (the latter is basically collagen with different minerals in it such as Mg^{2+}, Cl^-, etc.)
- **Hyaline cartilage** — cells are *chondrocytes*; found in surfaces of joints.

———————————————Cytoskeleton———————————————

INTRODUCTION TO CYTOSKELETON (ANIMAL CELLS):

- Cytoskeleton of the cell provides structural support, helps with cell movement, and helps with transport within the cell.
- Below are three components of the cytoskeleton, which are all made of proteins:
- **Microtubules** have a diameter of ~25nm.
  - Involved in the mitotic spindle of mitosis, they make up cilia (hairlike projections on outside of cell that help sweep things up) and flagella (tail-like projections that help move the cell), and aid in transport across the cell
  - Microtubules are made of two proteins, α-tubulin and ß-tubulin that come together to form a dimer.
  - These dimers then form long chains/ polymers, which then come together to form a sheet, and that sheet is rolled up to form the microtubule.
- **Intermediate filaments** have a diameter of ~10 nm
  - Provide structural support to the cell and help resist mechanical stress. Help cell retain its shape.
  - In contrast to microtubules and microfilaments, these are made of many *different* kind of proteins that are strung together into polymers, which then twist together to make intermediate filaments.
  - Also unlike microtubules and microfilaments, *intermediate filaments are pretty permanent*. Once they're made in the cell they stay put (Microtubules & microfilaments are dynamic)
- **Microfilaments** have a diameter of ~7 nm
  - Involved in the gross movement of the cell. Note that this gross movement comes from within the cell (as opposed to cilia & flagella, which help move the cell from the outside).
  - Microfilaments are found in the cytoplasm.
  - They're composed of a protein called actin. Many molecules of actin join together to form an actin polymer; many actin polymers twist around each other to form actin filaments.

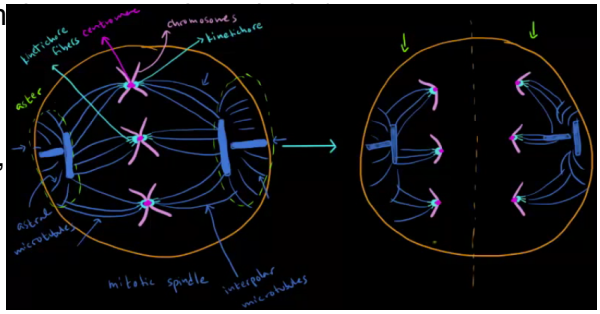
MICROFILAMENTS DETAILS

- Recall, Microfilaments are mainly involved in the *gross* movement of the cell. They can lengthen and shorten very frequently (in that sense they're similar to microtubulues)
  - become longer through **actin polymerization**
  - become shorter through **actin depolymerization**
  - This polymerization / depolymerization process is what moves the cell.
- Actin is both flexible and strong, making it a useful protein in cell movement. In the heart, contraction is mediated through an actin-myosin system.
- ex: Microfilaments help the cell divide; they help make the pinched shape & help actual division occur.
- ex: Microfilament are responsible for the ameboid movement of macrophages (e.g. white blood cell engulfing a pathogen).. An amoeba has projections known as *pseudopods*, which reach out to grab food and ingest it. Pseudopod movement is aided by microfilaments.

MICROTUBULES DETAILS

- These tubules are found in cilia & flagella, and also provide pathways for secretory vesicles to move through the cell. Microtubules are very important in cell division; they help form the **mitotic spindle**.

- One end of the microtubule is anchored to the **microtubule organizing center** (MTOC). At the other end, additional dimers can be added or subtracted to length or shorten the microtubule as needed.
  - Examples of MTOC include **centrosomes and basal bodies**.
- **The centrosome** is an organelle that's found near the nucleus of the cell, it's made of many different proteins, including two very important rods (blue) called **centrioles** (centrosome + proteins).
  - Centrioles are made of triplets of microtubules; 9 triplets m microtubules = 1 centriole
- When a cell is dividing, the centrioles duplicate and one pair ends up on each side of the cell.
  - At the center of the mitotic spindles are the chromosomes, and at the center of those chromosomes is the **centromere**. A protein **kinetichore** structure surrounds the centromere and serves as an anchoring site for the *kinetichore fibers* which come out of the kinetichore.
  - Those kinetichore fibers are what turn into microtubules.



- *Interpolar microtubules* are between different poles of the cell. (anchored by centrosomes)
- *Astral microtubules* are those coming out of the centrioles that bind to the kinetichores — named because the unit of centrioles + fibers coming out of it (/sort of behind it) is called an aster.
- During the next phase of mitosis (anaphase), microtubules become shorter so the chromosomes are pulled apart — one half of each chromosome ends up on either side of the cell and eventually the cell is split down the middle.
- **A Basal body** has pretty much the same structure as a centrioles; they are MTOC in cells that have cilia or flagella anchored to them.
- Cilium and flagellum are made of microtubules in a specific 9 + 2 arrangmenet. Looking at a cross section, we would see 9 pairs around the edge of the tubule, and one pair at the center.
  - Between the pairs of microtubules is a protein called **nexin**, which helps keep them in their place.
  - Coming out of the microtubules is a protein called **dynein**, which breaks down ATP and helps the microtubles move past each other. This is what moves the flagellum or cilium.

Microtubules also play a very important role in neurons.

- Most substances in a nerve cell are made in its soma, and then have to travel through the axon and get out the synaptic terminal… this process also happens with the help of microtubules.
- Microtubules form a network (sort of like a railroad track) from the soma; different substances are moved along this track with the help of two proteins: **kinesin and dynein**.
  - substances that are shuttled down this track include synaptic vesicles (which contain neurotransmitters), proteins, lipids, even organelles.
- Kinesin and Dynein can move substances in either direction: soma <— —> synaptic terminal
  - this is called axonal transport / axoplasmic transport

————————————————Prokaryotes / Bacteria————————————————

OVERVIEW OF ARCHAEA, PROTISTA, AND BACTERIA:

- In the phylogenetic tree of life, one of the main divisions is between prokaryotes and eukaryotes.
    - **Protists** are *eukaryotes* (have nucleus)
    - **Bacteria** and **archaea** are *prokaryotes* ("before nucleus," they don't have one)

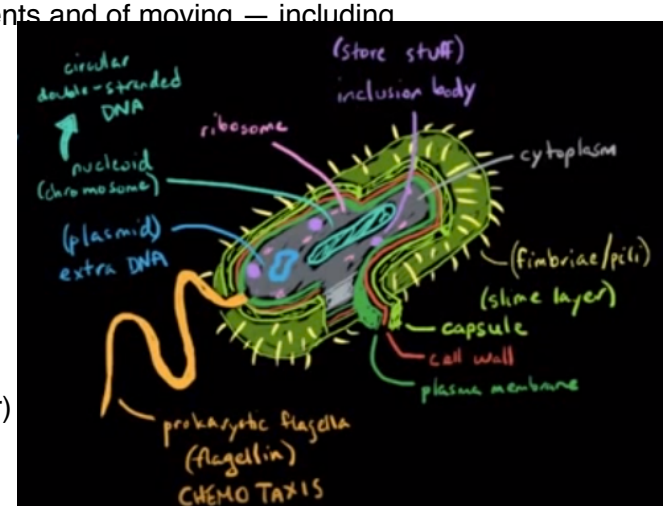
Archaea

- Some of the oldest organisms still in existence, and are thus used to extreme environments
- Three types of archaea: thermophiles (thrive in extreme temperatures), halophiles (thrive in very salty environments), methanogens (thrive in high concentrations of swamp gas)
- In order to live in these types of environments, archaea have very different cell membranes / walls

Protists

- Protists are basically any eukaryote (organism with a nucleus) that is not a plant, fungi, or animal.
- All protists like moist / aquatic environments.
- Characterized as photosynthesizing or non-photosynthesizing
- **Photosynthesizing** protists are closely related to plants, and are called algae.
- Nonphotosynthesizing protists are closely related to fungi (fungi-like) and animals (protozoa)
    - **fungi-like protists** include slime molds
    - **protozoa** include amoebas
- These protists have evolved unique ways of getting nutrients and of moving — including movement by cilia, flagella, and amoeba-like movements

Bacteria



- Can be found in more diverse environments than protists and archaea.. Bacteria can both help and harm us.
- Outside structure of bacteria:
    - Outermost layer is a **capsule or a slime layer**. (Slime layer can be washed off, capsule can't be)
    - Beneath the capsule / slime layer is the **cell wall**. The thickness of this wall is different for gram positive and gram negative bacteria
    - Under the cell wall is **plasma membrane** (lipid bilayer)
    - Attached to the outermost layer of bacteria are structures that aid in movement, such as **prokaryotic flagella** (which is different from eukaryotic flagella in that it's made of the protein **flagellin** rather than microtubules). These help bacteria move toward nutrients via chemotaxis (sensing chemicals and moving towards / away from it)
    - Some bacteria also have hair-like structures on the outside, called **fimbriae / pili**
- Inside the bacteria, we have cytoplasm, ribosomes, etc., and an area *not* bound by the nucleus where the chromosome resides; this is the **nucleoid**
    - Recall: bacterial chromosomes are made of circular double stranded DNA
    - Some bacteria also have plasmids, basically extra pieces of DNA that give bacteria important genetic advantages
- Bacteria also have **inclusion bodies** that store stuff (e.g. nutrients) for the bacteria. This is important in prokaryotes because they have no membrane-bound organelles. Instead of synthesizing energy like the mitochondria make ATP, they must get all their nutrients from the environment and store them in inclusion bodies. (Bacteria use chemotaxis to get to these nutrients or avoid toxins.)

- Aerobes are bacteria that require oxygen in their environment; anaerobes do not. There are several different types of anaerobes:
  - Aerotolerant anaerobes cannot use oxygen, but can tolerate it in the environment.
  - Obligate anaerobes are negatively affected by the presence of oxygen.
  - Facultative anaerobes can make ATP by aerobic respiration if oxygen is available, but are capable of switching to fermentation or anaerobic respiration if oxygen is absent.

BACTERIAL CHARACTERISTICS — GRAM STAINING:

- There are three main shapes of bacteria:
  - Coccus (cocci) bacteria are spherical
  - Bacillus (bacilli) bacteria are rod shaped
  - Spirochete (spirilla) are sort of a squiggle, they actually don't show up in the same way and must be stained differently.
- The gram stain is purple in color. After being exposed to the stain, the bacteria sample is washed.
  - Gram-positive bacteria hold the stain well and thus show up purple under the microscope.
  - Gram-negative bacteria can't old the stain well and thus show up as a pink color.
- The outer layers of bacteria are very different for gram-positive and gram-negative bacteria.
  - Gram-positive have (from inside —> out) a plasma membrane, a very thick cell-wall, & a capsule
    - plasma membrane is lipid bilayer
    - cell wall is thick **peptidoglycan** layer (sugar-chains connected by proteins)
    - capsule / slime layer
  - Gram-negative have *two* plasma membranes + a layer of glycosylated lipids. From inside —> out:
    - inner plasma membrane (lipid bilayer)
    - thin cell wall (peptidoglycan layer)
    - outer plasma membrane
    - lipopolysaccharide, or LPS, layer (lipid area with sugar chains attached)
    - capsule
- Gram-negative bacteria is more likely to cause a systemic effect (such as sepsis) in the body, because its outer membrane may protect if from the effects of antibiotics
- There is some space between the plasma membrane (or inner membrane in gram negative bacteria) and the peptidoglycan layer called the **periplasmic space**.
- Gram stain sticks much better to gram positive bacteria because it can get into the thick cell wall. Gram negative bacteria have such a thin cell wall, it doesn't hold the stain well.

BACTERIAL GROWTH CURVE:

- Bacteria reproduce by an asexual reproduction process called **binary fission**
  - Bacteria do this well because they're small and have a small chromosome
- Because bacterial DNA is circular (and double-stranded) it can have just **one origin of replication.**
  - At this origin, the initiator protein comes in, then 2 DNA polymerases enter to start replication in opposite directions… They eventually just run into each other on the circle.
- After the replication of genetic material, in order to make a copy of themselves, bacteria start growing and making a new cell wall… When that wall is complete, the bacteria pull apart and split.

    - Each resulting bacteria share the ribosomes / cytoplasm of parent, but have their own cell wall.
- How do bacteria grow as a population? Let's look at a bacterial growth chart:
    - Lag phase: Bacteria takes some time to adapt to its environment, so any replication is slow
    - Exponential growth: Once accustomed, the population divides exponentially & growth shoots up
    - Stationary phase: The plateau after grown slows because the bacteria population has grown to capacity and are using all the nutrients available. number of bacteria grown = number that die
    - Death phase: At some point, the population gets so crowded that a toxic environment (with no nutrients) is created and everything dies off.,
- Note: Some gram-positive bacteria can escape the death phase because by forming an **endospore**. Recall, gram-positive bacteria have a super thick cell wall. When they start to sense the toxicity in the environment, some can change their wall chemistry to be super reinforced and seal itself off.. this endospore is then resistant to heat, radiation, toxic chemicals, etc… it can even survive in boiling water for an hour! The endospore "rescues" the bacteria until it gets in an environment that has nutrients again and can be activated

BACTERIAL GENETIC RECOMBINATION:

- How does bacteria get new genetic info? (e.g. pass on antibiotic resistance) — through the **plasmid**
- (1) **Transformation** = the bacteria takes up naked DNA from the environment.
    - This can be forced in the lab by heating up the bacteria or breaking its membrane with chemicals, creating pores that are too small for the big chromosome but ok for the plasmid to get through…
    - Other bacteria can then pick up the plasmid floating around through their pores and then when the heat / chemical is removed, the bacteria repairs its membrane and is perfectly functional.
- (2) **Conjugation** = one bacteria with a plasmid (that is fertility+, it has a fertility factor) and one without plasmid (f-). A bacteria that has a fertility factor in the plasmid and is fertility positive (F+) will create a sex pilus — basically a protein tube — that connects the f+ bacterium to an f- bacterium.
    - Once connected, the plasmid makes a copy of itself, travels across the sex pilus, and is circularized / incorporated again in the next DNA
    - This is good for traits like antibiotic resistance, because it doesn't require that the original plasmid die or be removed like in transformation
- (3) **Transduction** = a bacteriophage (virus that infects bacteria) injects its genetic material into the bacteria and the viral genetic info is taken up by the chromosome or plasmid.
    - The virus eventually will lyse the cell and leave after reproducing. Before doing so, though, it needs to repackage its DNA. In transduction, when the virus repackages its own DNA it will also pick up either the plasmid's or the chromosomal DNA.
    - Then, when the virus goes on to infect the next cell, it will bring either the plasmid's or the chromosome's genetic information into the next cell.

————————————————Viruses————————————————

VIRUSES ARE NOT LIVING (BUT ALSO NOT DEAD)

- Death is what happens when a living organism stops performing biological functions.. this doesn't really apply to viruses, as most biologists would say viruses were never alive.
- Viruses are not made out of cells, they can't keep themselves in a stable state (homeostasis), and they don't grow.
- Viruses also cannot make their own energy or reproduce on their own (they must use a host).
- Even though they have different levels of organization and adapt to their environment, viruses are more like androids than real living organisms.

VIRUS STRUCTURE AND CLASSIFICATION:

- Four things characterize a virus: size, shape, nucleic acids, and type of host
  1) Size.
     - A typical virus is >100x times smaller than a bacterium, and >1000x smaller than a eukaryotic cells. than one
     - Size is also a way to tell viruses apart — some are super tiny, other are just very small
  2) Shape
     - All viruses have a capsid, or protein coat, made of **capsomere** building blocks.
     - Capsomeres are identical for a particular virus, but different types of viruses have different capsomeres, and thus unique capsids. (Although capsids can be categorized by their 3-D shape.)
       - Icosahedral — classic, 6-sided diamond shape
       - Helical shape — looks like a cylinder, but is twisted like a helix
       - Spherical shape — occurs when an envelope covers the capsid and makes it look spherical
     - Some of the capsomeres may help determine the antigenicity of a virus.
  3) Nucleic acids.
     - Viruses can contain one of four types of nucleic acid:
       - dsDNA, dsRNA, ssDNA, ssRNA
     - The different types help identify viruses.
     - This genetic info is stored inside the protein coat. A **nucleocapsid** = protein coat + nucleic acid.
  4) Type of host.
     - A bacteriophage is the name of the viruses that infect bacteria. Viruses that infect eukaryotic cells are all unique and specific and have their own names (e.g. pox virus).
- Viruses are **obligate intracellular parasites**. They're very small (just proteins + nucleic acids), don't have organelles, can't make energy for themselves, and can't replicate. They're not a living thing. Instead they sneak into larger cells and hijack their replication. How do they enter?
  - Bacteriophages have a complex shape in that they're not just icosahedral or helical… They might have nucleocapsid at the top with the head portion shape containing nucleic acid, but viruses also have a sheath that acts like a needle (shoots nucleic acid down it), and a tail that attaches to the bacteria.
  - **Receptor-mediated endocytosis**: If a virus can't attach and inject its nucleic acid like a bacteriophage, it sneaks in by tricking cell receptors.
    - Some receptors can't tell the difference between normal cells and viruses or bacterias. A virus simply enters the cell by binding with (and tricking) receptors and entering via endocytosis.
  - **Direct fusion**: If a virus has that membrane envelope that give the virus a spherical shape, it has an extra option to get in. Enveloped viruses can still enter via receptor-mediated

endocytosis, or they can get in by direct fusion, which is when the envelope around the virus fuses with the membrane and gets virus in that way.

VIRAL REPLICATION: LYTIC VS. LYSOGENIC

- Recall that viruses have to get inside other cells to use their ATP and organelles to copy themselves
- once they get in the cell, viruses either go off to copy themselves or wait awhile.
- **Lytic Pathway** — Impatient viruses go ahead and take over the cells machinery to immediately start making copies of their genetic material and their protein (for the protein coat).
  - These building blocks then self-assemble in the cytoplasm.
  - As the cell continues to make more and more virions, it becomes quite full and eventyally lyses; then the viruses spread to nearby cells to infect them.
  - This cycle is especially good if there are many hosts nearby and viruses need an army fast.
- **Lysogenic Pathway** — Patient viruses wait for a while because maybe their isn't a host nearby, in which case there's no need to destroy their only host by lysing it. Instead, the virus will just combine with the host's genetic info so it can't really tell that it's there.
  - Viral genetic info is combined into the host cell's DNA, but it is repressed, not transcribed; it's **dormant/latent**.. but continues being replicated by the host cell.
  - Every 1/10,000 times that this happens (or if something like exposure to UV light spurs it), the repressor gene malfunctions and the cell will try to respond to this "mistake," or what it thinks is a mutation, by excising or splicing out the "mutated," aka the viral, nucleic acids.
  - When the cell splices these out and sends them into the cytoplasm, the virus is activated; it self-assembles, and again eventually grows in population to lyse the cell.
- All lytic viruses destroy the cell while lysogenic viruses may be replicated along with the host cell. Therefor the host will generate new daughter cells that are infected with the virus, eventually leading to a neoplasm (aka tumor). Thus oncoviruses are lysogenic.

RETROVIRUSES

- Retroviruses don't really fit the box of lytic / lysogenic.
- **Retroviruses are enveloped ssRNA virus**. Three special proteins (reverse transcriptase, integrase, protease) are also carried in the envelope.
- Recall, enveloped viruses can enter in one of two ways, by endocytosis or fusion.
  - HIV, for example, enters via fusion by binding to CD4 receptor cells. This allows then CCR5 receptor to grab hold of the envelope, pull it in, and eventually the virus membrane will fuse with the cell membrane to release its genetic material into the host cell
- After entering the cell, the retroviruses undergoes a step called **uncoating**, meaning the capsid is dissolved. Everything inside this coat is released.
  - **Reverse transcriptase** then binds to the viral RNA and (reading from 3' to 5') makes a complementary DNA strand (cDNA). It then produces a second complementary strand, this time by reading the new strand of cDNA. This creates two DNA strands that can combine to make double-stranded RNA. The viral RNA then gets degraded.
    - Note that reverse transcriptase makes not infrequently makes errors… this mutation of the HIV virus makes it very hard to treat.
  - **Integrase** protein then comes along and clips off each of the 3' ends of the viral DNA, forming sticking ends. It then carries the viral DNA into the nucleus and integrates it into the host RNA, similar to the lysogenic pathway. (This is the *provirus stage*)

- Unlike the regular lysogenic cycle, though, the retroviral cDNA does not have the typical lysogenic repressor gene. It is thus actively transcribed whenever the host DNA is, and turned into mRNA that's exported to the cytosol. Outside the nucleus, the mRNA goes through translation to make campsomere proteins and the other three necessary proteins / building blocks (though they come out as one polypeptide). Viruses are then self-assembled and head to the membrane of the cell with the other three proteins.
- As the immature virus leaves the cell via exocytosis, **protease** cleaves those functional three proteins. The act of leaving via exocytosis means the virion can retain the cell membrane envelope around it.

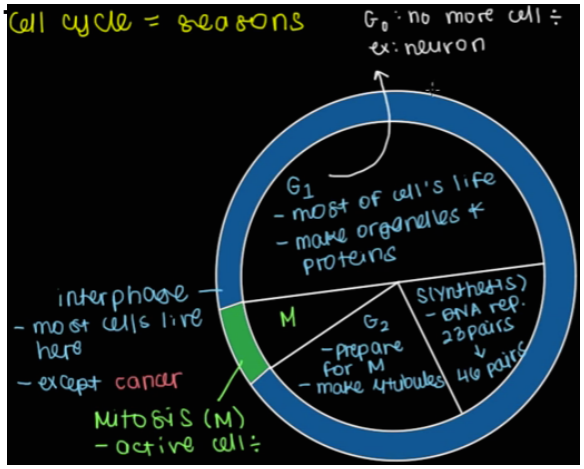
SUBVIRAL PARTICLES: VIROIDS AND PRIONS

- Viruses and subviral particles are categorized as **nonliving infections agents**; they need a host.
- **Viroids** are only made of a single strand of circular RNA
  - Viroids were previously thought to only be in plants, but now they've been found in humans in the case of hepatitis D.
  - Viroids make more of their own genetic material because their **RNA is catalytic**, meaning it can make or break covalent bonds and can thus sel-cleave to make other viroid RNA.
  - Note: don't confuse the *viroid subviral particles* with a *virion*. Virions are what we call whole virus (protein coat + RNA + maybe an envelope)l virions are much smaller and even less complex.
- **Prions** come from the word "proteinacious infections particles"
  - Prions have no genetic material at all! They're only made of proteins.
  - A normal protein is in the shape of an alpha helix. A prion protein (PRP) tends to be in a ß-sheet
  - We don't know much about them but it's thought that because the normal and prion proteins are made of same amino acids, so when the prion ß-sheet comes in contact with alpha helix, it will convert the helix to a ß-sheet.
  - As this happens over and over, and more alpha helices become ß–sheets, protein deposits are created. These are bad in general, but especially bad in something like the brain because the body will want to clean up these protein deposits.. and in "cleaning them up" (via cleavage or other damage repair mechanisms) would leaves holes in your brain and cause disease.

——————————————Cell Division——

CELL CYCLE PHASES

- A cell has a diameter of just 100 μm (1/1000000 of the size of a human)
- The cell cycle can be thought of as seasons in a year.
- Two main "seasons" = interphase and mitosis



- **Interphase** — cell grows but does not divide. Cells spent most of their life in this phase (unless they're cancer cells). There are several sub-seasons of interphase..
  - **G_1** — extra organelles, such as ribosomes and proteins, are produced. This is the longest phase of the cell cycle.
  - After this, if a cell wants to move towards division it enters S phase. If not, it goes into **G_0 phase**, where there is no more division. (This is likely to happen in cells like neurons… Once the brain is formed, there's no need for neurons to divide more; instead they just grow)
  - **S phase** — DNA *synthesis* occurs, and all 23 chromosomes are replicated so we now have 46
  - **G_2 phase** — cell prepares for mitosis through processes like making microtubules (which are used to pull chromatids apart)
- **Mitosis** — time of active cell division
  - Once the cell divides and produces 2 cells, each of those daughter cells enter the daughter G1 phase.

CELL CYCLE CONTROL

- There are two key places or checkpoints of excessive regulation:
  (1) between G1 and S phase, to regulate cell before DNA replication
  (2) between G2 and mitosis, to regulate cell before mitosis / division
- Two main proteins regulate these checkpoints:
  - **Cyclin dependent kinases** (add phosphates to other enzymes to activate / inactivate them)
  - **Cyclins**
- All the different types of CDKs are always present in the cell, but their default function is for them to be inactive. They must be activated by cyclins.
- Specific cyclins are made at specific times. This is important because CDKs are only active when bound to a specific cyclin, so if the cell hasn't reached a certain stage of cyclin production, CDKs are inactive and the cell won't pass the checkpoints.
- In G1, cyclins G (which CDK2 binds to) and D (which CDK4 binds to) are produced. These, specifically CDK4, phosphorylate a protein called Rb.
  - Rb normally inhibits DNA replication, but phosphorylation inactivates it so DNA replication can occur.
- In S phase, Cyclin A is produced, which binds to CDK2. This complex helps activate DNA replication
- In G2 phase, Cyclin B is produced and binds to CDK1. This is able to activate mitosis.
- In order to pass the checkpoints, these cyclin complexes must be present.

LOSS OF CELL CYCLE IN CANCER

- There are higher levels of regulation that occur that occur with a couple of key proteins in addition to Rb. One of these other proteins is **p53**, which is the "guardian of the genome."
  - p53 directly binds to DNA to produce proteins that block production of the cell cycle. This includes proteins like **p21**, which inhibits CDK, and **Rb** which inhibits DNA replication.
  - These proteins are made from **tumor-suppressor genes**.
- If the tumor suppressor genes are defected or have a loss of function, you tend to get cancer.
  - > 50% of tumors have a defect in p53
  - Rb got its name because a defect in this tumor suppressor gene causes a tumor of the eye called retinoblastoma.

- p21 is unusual in that it doesn't actually lead to cancer when it's defective. Instead, mice who lack p21 are able to regenerate limbs!

FERTILIZATION TERMINOLOGY: GAMETES, ZYGOTES, HAPLOID/DIPLOID

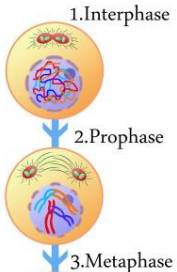
- Fertilization = sperm combines with an ovum. (200-300 million sperm try to get to ovum; only 1 wins)
- Gametes = sex cells (sperm and ovum). Each gamete has just half the number of chromosomes as somatic cells in your body.
  - Sperm cells have a nucleus with 23 chromosomes in it from your father. The last one is sex-determining… if your father contributes an X, you'll be female; if he contributes a Y you'll be male.
  - In the nucleus of the ovum there's also a nucleus with 23 chromosomes; mother contributes an X sex chromosome
- Zygote = fertilized egg, the fused cell of sperm + egg. This has 46 chromosomes (23 from each parent; mom and dad chromosomes pair up). This cell can now keep replicating and differentiating into different cells that make you you.
  - The mom and dad chromosomes pair up as homologous chromosomes. This means they have different variations of codes for the same proteins. 46 chromosomes = 23 homologous pairs.
  - Diploid = having the full number of chromosomes. Zygotes are diploid.
- Haploid refers to when you have *half* the number of chromosomes as a normal cell. For humans, this 23 chromosomes; sperm and ova gametes are haploid.
- When people speak in general about cells, they'll refer to haploid number as n, and diploid as 2n.

ZYGOTE DIFFERENTIATING INTO SOMATIC AND GERM CELLS

- After fertilization, the zygote keeps replicating through mitosis.
- After one round, you have 2 cells that each still have 2n chromosomes. Then those divide over and over… 2 —> 4 —> 8 —> 16 —> 32 —> 64….
  - Eventually you have millions of these cells, all of which have 2n chromosomes!
- These cells can then continue to replicate and differentiate into different parts of a body.. this makes a human fetus!
  - Most of the cells produced via mitosis are body / somatic cells; they differentiate into your heart, lung, brain, etc. Some, though, differentiate into germ cells, into your gonads (ovaries or testes).
  - These differentiated germ cells in the gonads produce, through meosis, sperm or ova.
- If you have a mutation in one of your somatic cells like heart or lung, it might affect you / your ability to survive or reproduce, but it won't effect genetic info in the gametes. You won't pass it on. If a mutation happens in the germ cells, though, that actually has a chance of being pass down to your offspring.

MITOSIS

- Recall, bulk of cell's life cycle is in interphase. Mitosis is the process by which the one nucleus turns into 2 nuclei that each have original genetic info and then splits in 2, so 1 diploid cell—> 2 diploid cells
- Recall, before mitosis is interphase, where the DNA strand of a chromosome is replicated this copied strand is attached to the original strand at the centromere.



    - This new structure is called a *bivalent chromosome*. A bivalent chromosome consists of two sister chromatids. When a chromosome exists as just one chromatid, just one DNA strand (and its associated proteins), it is called a *monovalent chromosome*.
- **Prophase**
    - DNA goes from being in chromatin form, where it's all spread out into their *chromatid* form that is a condensed, distinct shape. (one chromosome = two sister chromatids, connected by centromere.)
    - The nuclear membrane starts to go away, and the centrosomes (organelles that organize microtubules) start to migrate to opposite sides of the cell.
- **Metaphase**
    - Nuclear membrane is completely gone and chromosomes line up at the cell's center.
    - Centrosomes are fully on opposite ends of the cell. (Each centrosome has two centrioles, cylindrical-looking structures that aid in extending microtubules).
    - Microtubules grow out of **centrosomes** and attach at the *centromere*, forming the **mitotic spindle**
- **Anaphase**
    - The microtubules (coming out of the kinetochore) begin pulling on the chromosomes.
    - The sister chromatids separate and are pulled to opposite sides of the cell. (Note: These are still called chromosomes b/c a chromosome unit is defined by centromere)
- **Telophase**
    - The newly separated chromosomes on opposite ends of the cell start to unwind into chromatin
    - Nuclear membrane starts to reform around the chromosomes (centrosome stays in the cytosol)
- When mitosis is complete, the cell has two groups of 46 chromosomes, each enclosed with their own nuclear membrane. It then splits via **cytokinesis**, creating two clones of the original cell.

MEIOSIS

- Germ cells (cells in the gonads) can either undergo mitosis to produce other germ cells, or they can undergo meiosis to produce gametes. (This is not part of the cell cycle.)
- The purpose of meiosis is to make haploid gametes
- Like mitosis, meiosis starts with a cell that has a diploid number of chromosomes (because during interphase the DNA replicates).
    - Meiosis I yields 2 germ cells that each have a haploid number of chromosomes.
    - Meiosis II yields 2 haploid cells from each of the original daughter cells. Now we have 4 haploid cells that may not have the same genetic info (because of crossover).
        - If these are egg cells, only 1 of those 4 will become an ova, the other will become polar cells.
- **Oogenesis** & **spermatogenesis** describe the process of meiosis in females and males, respectively.

Meiosis I:

- Let's start with a germ cell that has 2 chromosomes from the father and two from the mother. (2n = 4)
- With interphase, the DNA and centrosomes are replicated.
- **Prophase I**:

    - Chromosomes condense into distinct homologous pairs of sister chromatids attached by a centromere. Note: In each pair, you have *four* chromatids (and two centromeres), so a homologous pair is sometimes called a tetrad.
    - These chromosomes are homologous in that they might contain different sequences, but they code for the same genes / characteristics (hair color, eye color, height, etc.)
    - Nuclear envelope begins to dissolve, and centrosomes start to migrate to the ends of the cell.
    - Also in prophase, **recombination** occurs! Homologous chromosomes switch gene sequences. This is a way to get more variation into the offspring population. (See Biomolecules document for details on the different types of recombination).
- **Metaphase I**:
    - Nuclear membrane is gone and the centrosomes are on opposite sides of the cell
    - Homologous pairs of chromosomes line up along the center axis of the cell, aka bivalent chromosomes lined up two-by-two. Different from mitosis metaphase which has them single file.
    - Microtubules extend from kinetichore fibers and attach to the chromosome centromeres.
- **Anaphase I:**
    - The two homologous pairs (aka the tetrad) get pulled apart. This is unlike the anaphase in mitosis, which splits into sister chromatids rather than full chromosomes!
    - How they split is random, which also adds to genetic variation. The gametes produced will all have different gene sequences.
- **Telophase I:**
    - Homologous pairs are fully separated into opposite ends of the cell and begin to unravel into their chromatin state.
    - Nuclear membranes reform and microtubules dissolve.
    - Cytokinesis occurs, creating two haploid germ cells from the one diploid germ cell. The centrosomes replicate themselves again.

<u>Meiosis II (very similar to what happens in mitosis b/c it preserves # of chromosomes)</u>:

- Sometimes, after meiosis I, we might have an interphase II that's basically a rest period between the two phases of meiosis. Otherwise the two daughter cells go right into meiosis II.
- **Prophase II**:
    - Nuclear envelope dissolves again and the chromosomes again condense into sister chromatids.
    - Centrosomes start to migrate to opposite ends of the cell.
- **Metaphase II**
    - Our centrosomes are on opposite ends of the cell, our nuclear membrane is gone, and the chromosomes line up at the cell's equator.
    - Microtubules grow out of the kinetochores and attach to the centromeres.
- **Anaphase II**
    - The sister chromatids get pulled apart by the microtubules to opposite ends of the cell (different from anaphase I!).
- **Telophase II**
    - Daughter chromosomes begin to unravel into chromatin form, and nuclear envelope re-forms.
    - Microtubules dissolve and cytokinesis begins, yielding a total of 4 haploid gametes.
    - Note that the resulting daughter chromosomes are not homologous; they code for different genes. If they merge with a sperm (or egg) to form a zygote, only then will they have 23

homologous pairs, aka the diploid 46 chromosomes. In addition, because of recombination all the gametes will have different genomes!

- If the chromosomes aren't split correctly during anaphase II, you get **aneuploidy** — a term that refers to mistakes in the number of chromosomes in an organism. For example, if one gamete has an extra copy of chromosomes 21 (as a result of the bivalent chromosome not being separated into sister chromatids in anaphase II), the resulting offspring will have Down syndrome.
- Cells can be **karotyped** during metaphase to check for certain chromosomal mutations.

EMBRYONIC STEM CELLS

- How does the fertilized egg develop after it becomes a diploid zygote?
- Immediately after fertilization, the zygote undergoes several rounds of mitosis to become a mass of cell (all with the same genetic info) called a **morula.**
- Once the morula gets to 16 cells or so (takes 4-5 days), it starts differentiating into a **blastomere**.
  - **trophoblasts** = layer of outer cells
  - **embryoblasts** = inner cells
- The cells keep dividing as fluid then comes in between the trophoblasts and embryoblasts
  - Trophoblasts form an outer circle of cells called a **trophosphere**, while embryoblasts clump together inside it, forming an **inner cell mass** against one side of the trophoblast sphere**.**
  - The rest of the space is filled with fluid (called blastocoel). This whole structure is called the **blastocyst** in humans (or blastula, blastosphere in other animal… those don't necessarily have the inner cell mass there.)
  - *This inner cell mass is what turns into the organism*! The outer cells, troposphere, is what turns into the placenta.
- Cells (blastospheres) of the inner call mass, which have not yet differentiated, are called **embryonic stem cells**. These have *plasticity,* which means they could potentially turn into *any* type of cell!
  - The theory is that if you have some damage in your body, stem cells could go that site and grow/differentiate to replace this damage. Very exciting and promising research for all sorts of diseases!
  - We also have somatic stem cells throughout out body (e.g. in the bone marrow to make red blood cells), but those are not as plastic.. they can't make *any* cell in the human body. Somatic stem cells won't turn into human beings if implanted, embryonic cells can.
- Stem cells are controversial because to harvest them (for research, etc), you must kill the developing embryo. Some think this is bad because that embryo had the potential to turn into a human.
  - If you get just one stem cell, you can put it in a petri dish and grow it into an embryonic stem cell line. This does destroy the embryo, but so do other processes such as in vitro fertilization.
- In vitro fertilization is when some eggs are taken out of the mother, fertilized with semen so they all become zygotes, and then allow them to develop to the blastocyst stage (such that they have embryo and blastocoel space). Then a few of the healthiest looking blastocysts are implanted into the mother.
  - The embryos they don't implant also have the potential to create human beings, but most places just destroy those embryos!

CANCER

- In regular circumstances: cells undergo mitosis and grow until they get a little crowded.. then they recognized that they're crowded and stop dividing. (This is called **contact inhibition**.)
  - If something were to mutate in one of those cells, it recognizes this error and destroys itself via **apoptosis.**
  - There are ~100 billion new cells in the cell every day! and ~100 trillion total cells in a human body.
- If you have a mutation in a cell and it doesn't destroy itself, though, this causes problems. Let's say a the cell has *two* mutations, one that prevents apoptosis and one that makes the cell defective in another way.
  - This cell will start dividing more and more and not realize contact inhibition that normally stops / slows cell growth… instead you'll have an ever-growing cluster of these mutated cells called a **neoplasm**. If this group of cells form a lump of abnormal cells, it's called a **tumor.**
- A **benign tumor** will continue to replicate itself, but not really affect anything around it.
- **Cancer** occurs when the mutation causes the neoplast of cells to become invasive and start infiltrating everything, including other tissues, organs. This is invasive super-growth; cancer cells crowd out other cells and use up nutrients and continue replicating at high speeds. Note that as the cell continues replicating, it will not only pass on its own mutation, but probably also develop other mutations (especially if one of the first mutations affects transcription).
  - One mutation might eventually cause those cells to break off and travel to infect other parts of the body. This is **metastasis**.
- Cancer is thus especially hard to cure because it's not like a virus or bacterial infection where we can target a specific protein or pathogen, it's a whole system of mutations… Even if you target one specific type of the cells, they can mutate and then that medicine won't be effective for those cells.
  - Common theme behind chemotherapy is to attack things that are quickly growing.

———————————Cellular Development———————————

STEM CELLS

- The zygote starts to divide until it reaches the blastocyst stage. Recall, this stage involves an inner grouping of cells called the inner cell mass, which go on to become the embryo.
  - This inner grouping is made of embryonic stem cells, which are **pluripotent**, meaning they can go on to differentiate into any different cell type (if/when given proper stimulation)
- We also have *somatic stem cells,* which are used more as a repair system for the body. They aren't quite pluripotent and thus can't repair everything, but can help some things recover / replenish.
  - ex: epidermal stem cells regenerate our skin as we slough of the outer layer of epidermis.
- Mature cells are not the same as stem cells; they are already specialized but stem cells are not.
- In order to be considered a stem cell (of any kind), you must possess two main qualities:
  (1) Continuous production of stem cells — As the cell divides over and over, at least one of the
  offspring must still be a stem cell
  (2) Differentiation into specialized cells when the time comes
- There are several different types of stem cells, and some are more potent than others in terms of what they can specialize into
  - ex: Epidermal cells are **unipotent**; they can only differentiate into more epidermal cells

    - ex: Our red blood cells have a lifespan of about 4 months, and thus need to be replenished. Hematopoietic stem cells in bone marrow are **multipotent**, meaning they can differentiate into many different types of cells, but only ones within a specific family. (in this case, blood cells)
    - In blood diseases like leukemia, certain blood cells grow uncontrollably within a patient's bone marrow and crowd out the stem cells. To treat this, the blood cells are removed from marrow by chemo or radiation, and then doctors can put more hematopoietic stem cells in the bone marrow to replenish the good blood cells.
        - We also have multipotent neural stem cells (give rise to neural types of cells) and multipotent **mesenchymal** cells (which give rise to bone, cartilage, adipose)
- Why aren't these stem cells being used up as they divide? Two mechanisms:
    - **Obligate asymmetric replication**: when stem cells divide, they divide into one cell that's identical to the mother cell, and one daughter cell that is differentiated. The mother is still a plastic stem cell, while the daughter can go on to divide to make more specialized cells.
    - **Stochastic differentiation**: If a stem cell messes up and divides into two daughter cells, another stem cell will recognize that difference and make up for it by undergoing mitosis to make two mother stem cell copies.
- **Induced pluripotent stem cells (iPS)** are a third kind of stem cells… they arise when specific genes are introduced into the genes of already specialized somatic cells genes. This causes the cells to sort of forget what kind they are, and revert back into a pluripotent stem cell like an embryonic stem cell.
    - This is hugely important / exciting in medicine! iPS cells are the core of regenerative medicine (a sort of new field where the goal is to repair damage in tissues of a person by giving them their own line of pluripotent stem cells). Not only would a patient be able to get a new organ/tissue, but there'd be no immune system rejection because the organ is made of their own cells!
- **Cord blood** is another source of stem cells; it comes from blood taken from the umbilical cord and placenta after a baby is born.

What causes stem cells to differentiate?

- Every cell in our body has originated from a common group of stem cells during development. They have the same DNA, but a neural cell looks very different from a muscle cell, for example, because it is **expressing different genes**.
- In order to differentiate, stem cells must **turn on** one set of "muscle / neural / etc." genes, and turn off other genes. Once the cell specializes into a certain type, it can't go back and re-specialize (at least not on its own).
- *How does a cell turn off and on genes and know what to become?* Cues from the environment:
- **Asymmetrical Segregation of Cellular Determinants** (Internal environmental cue):
    - Zygotes, have **transcription factors** (and mRNA precursors) floating around in the cytoplasm.
    - These TFs, which activate certain genes and turn them on, are clustered in one region of the cell.
    - This means that when the zygote divides, some of the resulting daughter cells will have those TFs to turn on certain genes so they'll specialize into a certain type of cell; other cells won't have those TFs and thus won't specialize to that certain cell.
- **Inductive Signaling**: peer pressure… one group of cells can influence another group to differentiate

    - The influencing group can send signals to other cells via **diffusion** (paracrine signaling) where they bind receptors on the other group and cause differentiation
    - The group of cells can also influence by **direct contact**, or by passing chemical signals via **gap junctions** (collectively connexon proteins) to induce differentiation.
- The goal of the transcription factors and of the signals of induction is to get cells to change their gene expression, which is ultimately what causes them to differentiate.

CELLULAR COMMUNICATION

- Evolutionarily, cells being able to communicate is a major reason we're so complex.
- Cells communicate directly with one another via **direct cell-cell communication**, aka **direct binding**:
    - Ex: When macrophages see a pathogen, then can ingest it and break it down. They then show off a little piece of the pathogen, called an antigen, on their surface. This antigen is basically a a note to pass to friends; it sends a message that this type of pathogen is around.
    - Another white blood cell such as a helper T-cell may come along, then, and grab that antigen with one of its own membrane receptor proteins. The T-cell thus receives the message and, depending on what antigen it is, can decide whether or not to start a full-blown immune response.
- Cells communicate over short distances by neural communication or paracrine signaling:
    - **Neural Communication**: Neurons need to communicate with other neurons close by, but the end of one doesn't quite touch the head of the next neuron; it leaves a gap called the **synaptic cleft**. So neurons can **release neurotransmitters** that travel from the axon of the first neuron, through the synaptic cleft, and are then absorbed into the dendrite of the next neuron.
    - **Paracrine Signaling: T**his is how one cell can also talk to a small group of cells locally (huddle). Ex: Just under our skin in, e.g. in our nose, we have skin cells called **mast cells** that are very important in mediating allergic reactions. When you breathe in something you're allergic to, like pollen, that allergen will bind to receptors in our mast cells. Mast cells then release **histamines** that travel to other cells in the area and lets them know to start preparing for an allergic reaction.
- Cells communicate over long distances via **endocrine signaling** with hormones.
    - Ex: Hormones are created by cell bodies and released into the blood stream. They can then travel through the body and get to any place needed! Not every organ is responsive to them, of course, because not all have the appropriate receptors, but this allows cells to communicate long-distance

MITOCHONDRIA, APOPTOSIS, AND OXIDATIVE STRESS

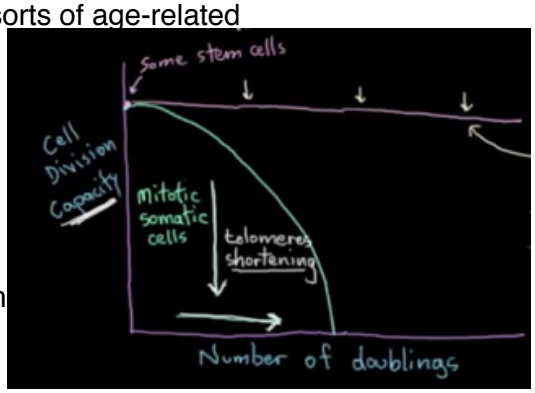
- The mitochondria is most well known for producing ATP, but it also plays a big role in **apoptosis** — programmed cell death.
    - There's another type of cell death called **necrosis**, which is more of an "uncontrolled" cell death that usually occurs in response to extreme stress like an infection or extreme trauma.
    - Apoptosis, in contrast, is considered to be more controlled. Even though the cell is dying it will likely benefit the body / system in some way.

    - ex: early on in our development, our hand just sort of looks like a blob of knuckles.. through apoptosis, the cells in between those 'knuckles' die and we get 5 distinct fingers.
- **Reasons / situations in which apoptosis is triggered:**
    - *Development* (like example above)
    - *DNA damage* — the cell has mechanisms in place to repair damage, but the DNA damage may be too extensive or our repair mechanisms may not be working, in which case our last resort is apoptosis. Of course, apoptosis is also good here because we wouldn't want a damaged cell passing on damaged DNA to offspring cells.
    - *Infection* — especially by viruses. Often times, our immune cells can recognize specific proteins on cells surface that indicate is has been infected with a virus(es). Immune cells then send a signal to the infected cell to undergo apoptosis and prevent the virus from spreading.
    - *Stress* — due to deprivation of oxygen, nutrients, or even disruption of cell-to-cell connections
        - In order to continue surviving, cells need to receive signals that it is attached or in close proximity to other cells around it. If those are taken away, it might undergo cell death.
        - Many cells are also often receiving signals from growth factors. If these are removed somehow, the cell might see it as a sign to go into programmed cell death.
        - Ultimately, cells can undergo cell death if their environment is unfavorable.
    - **Reactive oxygen species (ROS)** — These are oxygen species that have acquired extra electrons are are unstable.
- Oxygen is important in the cell because its the final electron acceptor in oxidative phosphorylation. However, about 1/4 are improperly (partially) reduced in this process.
    - This leads to the production of ROS, such as superoxides like O_2^-, OH (no negative charge = only 1 lone electron), and H_2O_2
- How do cells prevent interaction between ROS and important stuff? Cells have enzymes and antioxidants try to turn ROS into less reactive species or trap it and neutralize / destroy it.
- All ROS will have an effect on mitochondria, though, which play a role in initiating apoptosis. ROS make the mitochondria membrane *more permeable* than before. The proteins that regulate the permeability of this outer mitochondrial membrane are known as the **BCL-2 family of protein**.
    - BCL-2 proteins can be pro-apoptotic or anti-apoptotic; these activate and inhibit the process, respectively. When things are going well in the cell and its not receiving apoptotic signal, the balance of these proteins is in favor of anti-apoptic ones; they prevent mitochondria from initiating apoptosis. When the mitochondria receive signals downstream from apoptotic signals, the balance shifts in favor of pro-apoptotic proteins, which facilitate increased permeability of outer membrane.
- **Why is the permeability of the mt membrane increased to initiate apoptosis**? Doing so allows cytochrome c to exit the intermembrane space and enter the cytoplasm. Recall, **cytochrome C** is part of oxidative phosphorylation; it helps shuttle electrons between complex III and IV.
    - Cyt C in the cytoplasm also activates a family of enzymes called **caspases** (c-asp-ase = break down cytoplasmic proteins by attacking their aspartate residues with a caspase cysteine residue).
    - **Apoptosis utilizes caspase enzymes,** but necrosis doesn't. These caspase enzymes set off a controlled cascade of action; their activation of one protein will cause that protein to go on and activate/degrade another, and on and on. Caspases will activate other types of enzymes like nucleases (break down DNA).

- These degraded polymers inside the cell can then be recycled to neighboring cells, which will use phagocytosis to eat up the "building blocks" and use them in their own cell.

TELOMERES AND CELL SENESCENCE

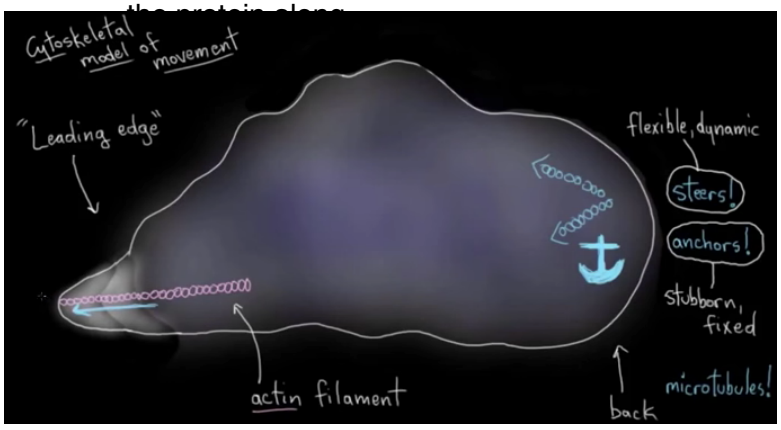
- In your body, you have a couple overarching types of cells called mitotic and post-mitotic.
  - **Mitotic cells**: actively able to divide by mitosis to replenish / regenerate tissues they're a part of
    - Ex: epithelial cells (skin), fibroblast cells (make up scaffolding of organs), endothelial cells (line blood vessels), stem cells
  - **Post-mitotic cells**: don't undergo mitosis and are thus incapable of proliferation. They have a limited ability to repair / regenerate the systems they're a part of. They can do it with the help of stem cells, but it happens very slowly.
    - Ex: neurons, heart muscle cells
- Recall that in mitotic division, DNA must be replicate. And every time DNA is replicated, their telomeres get slightly shorter.
  - This happens because of the way DNA must be synthesized in the 5' —> 3' direction. The lagging strand is made of Okazaki fragments separated by RNA primers, which are later turned replaced with actual DNA by DNA polymerase. The final RNA primer, however, cannot be converted to DNA because there's no where for the polymerase to bind. Thus that last primer is just degraded and the copy of DNA is slightly shorter than its template.
- Telomeres on the end of DNA strands prevent actual coding genes from being shortened / mutated, but a cell can only divide 60-70 times before its telomeres are too short and important coding DNA is at risk. Once this happens, cell initiates a DNA damage response and sort of gives up its ability to divide; it becomes a **senescent cell**.
- Senescence is a change from a happy, active, often dividing cell state — to a state of non-division that may or may not be happy. The senescent cell starts expressing genes it wasn't expressing before and starts to look different / respond differently to cells around it.
  - If a cell reaches senescence because its telomere is too short (as described above), it is called **replicative senescence**.
  - A cell can also go into senescence if their telomeres malfunction in some way, or if DNA mutates (even in a post-mitotic cell), etc
  - The number of times a cell can divide before it reaches senescence is called **Hayflick limit** (~60)
- **Cells transform to a senescent state to stop division & prevent impending DNA damage**
  - On one hand, this prevents tumors and cancer from happening by preventing division of damaged cells. On the other hand, as we age we get more senescent cells that can't divide and our tissue can't repair itself as well. This leads to all sorts of age-related diseases, like cataracts.
- On a graph of cell division capacity vs. number of doublings:
  - Somatic/mitotic cells start out with a high cell division capacity and move down.. As they continue to divide, their capacity to divide lessens because telomeres are getting shorter.
  - Stem cells maintain a high capacity to divide because they express the **telomerase** enzyme!
- Every time a stem cell undergoes replication / division and the telom telomerase will replenish it.

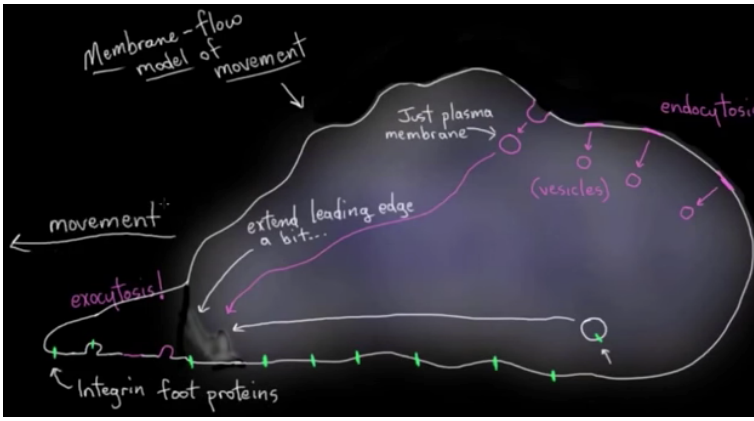


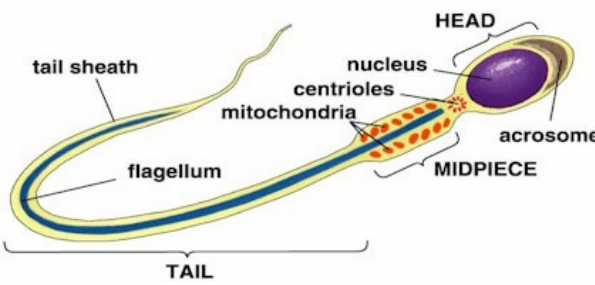
- Sometimes somatic cells can have a mutation that allows them to express telomerase, and then their cell division capacity gets increases and they escape senescence. This can cause cancer.

CELLULAR MOVEMENT

- Some cells have to move around the body (not blood cells, they're just pumped along) on their own.
- Cell migration / movement is super important
  - In development, cells move around the embryo to form distinct organs and limbs.
  - As an adult, cell movement is critical for the immune system
- Recall, a sperm's life is pretty much all about movement, which they do through a **flagellum**.
  - The flagellum is made of microtubules connected by **dynein proteins**; that all work together to create a whipping movement of the flagellum
  - There are prokaryotic flagellae in bacteria and archaea too, but they're made slightly differently, from a protein called flagellin.
- Movement of other cells, such as the **neutrophil** (a white blood cell, major part of our immune system) is dependent on the cytoskeleton.
  - Neutrophils respond to signals from tissues "in danger" by sticking to the endothelial cells lining the blood vessel and rolling along for a bit until, at some point, they duck between two endothelial cells and go into the tissue where they're needed. There are two major theories / mechanisms for how these immune system cells roll along the endothelium:
- **Cytoskeletal model of movement**: The cell quickly polymerizes / puts together actin filaments (made of actin protein) on the leading edge of the cell. This is how the front of the cell is advanced. In the back of the cell, we have microtubules that sort of act as a rudder to steer the cell where it needs to go, and can act as an anchor
  - If microtubules are in their flexible / dynamic state, they steer the cell.
  - If microtubules are in their stubborn / fixed state, they anchor the cell and it won't move even if there's actin filaments pushing the leading edge forward.
- **Membrane-flow model**: Bits of the plasma membrane enter the cell in the back via endocytosis, and they move to the front of the cell and recombine via exocytosis. These vesicles can just be plasma membrane, or they can contain integrin proteins that act as feet and sort of anchor the cell where they are; the combination of these types of vesicles move the protein along



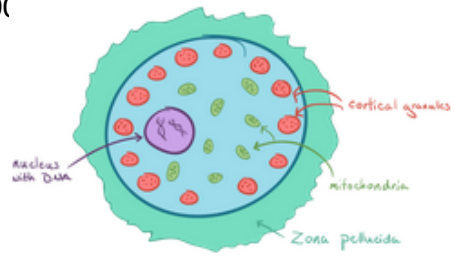




————————————————Embryology————————————————

——Egg and Sperm Structure:

- The entire purpose of the male's gamete, the sperm, is to transfer the male's genetic material into the woman's gamete, the ovum. The structure is like a torpedo, conducive to this aim:
  - Pointed head allows it to travel in the forward direction
  - Flagellum tail propels the sperm. It requires a lot of energy and thus remains dormant until sperm enters the vagina.
  - At the base of the head, wrapped around the flagellum, is a middle section full of mitochondria that provide energy to propel the sperm towards the egg.
  - Inside the head of the sperm is the genetic material, within a nuclear envelope
  - The head also has a "warhead" at its front called an **acrosome**, important for fertilization
- Recall, the sperm is haploid; it contains one set of 23 chromosomes. Meiosis creates 4 sperm from a single germ cell. Sperm are ejaculated in semen, a basic fluid with a pH of about 7.4.
- The ovum is round, not designed for active mobility. It's also about 10,000 [illegible] sperm, so big that it's often visible to the human eye!
  - Like sperm, the egg cell has its genetic material within a nuclear envelope.
  - It also has a thick, protective layer of glycoproteins (peptides with chains of sugars coming off of them) called the **zona pellucida.**
  - Beneath the zona pellucida is the plasma membrane. Once the sperm penetrates that, fertilization has occurred.
  - The egg is the source of cytosol and organelles, particularly mitochondria (of which is has 100,000 - 200,000 present), for the future zygote.
  - Unlike sperm, *the egg has not completed meiosis* - it's stuck in the Metaphase II stage of division. This means that the egg is haploid but with sister chromatids still attached to each other.
  - Also unlike sperm, the meiotic division to create eggs, oogenesis, only makes one viable egg.



FERTILIZATION

- Egg and sperm travel in opposite directions to ultimately meet in (most often) the fallopian tubes.
- During ovulation, ovaries release an egg into one of the fallopian tubes, and the egg proceeds down the tube toward the uterus, which is being prepared for possible implantation.
  - Part of this preparation involves elevated levels of **estrogen** and **luteinizing hormone** (LH).
  - LH triggers the ovaries to release the egg, while higher blood estrogen levels stimulate the vaginal membrane to secrete glycogen, which is then metabolized to lactate.

- Cervical mucus may prevent sperm from passing into the uterus, but during ovulation when estrogen is released and the egg is released from the ovaries, the mucus gets thinner and lower in pH.
- This lower vaginal pH (to as low as 3.8), creates an acidic environment hostile to pathogens (like the ones that cause sexually transmitted infections).
  - However, this environment can also be toxic to sperm, though the semen (a basic fluid) can buffer the vaginal acidity to preserve sperm cells.
  - As the semen mixes with vaginal secretions, the pH settles at a point that is not harmful for sperm, and this new environment is the trigger to activate sperm flagella and increase sperms' motility.
- Only about 1 in a million sperm that are ejaculated into the vagina will reach the site of fertilization.
- Increased estrogen causes cervical mucus to become watery and more alkaline, as well as relaxes the cervix, and stimulates uterine contractions – all of which help sperm reach the egg.

Steps of fertilization:

1. **Sperm binding**: A sperm comes in contact with the zona pellucida and becomes bound to it
2. **Acrosome Reaction**: The binding of the sperm to the zona pellucida sets off release of acrosomal enzymes (from the head of the sperm) into the zona pellucida, where they start to digest it.
   - This allows the sperm head to dive in deeper towards the plasma membrane
   - Without the acrosomal reaction allowing the sperm to penetrate the egg, sterility is expected.
3. **Cortical Reaction (blocks polyspermy):** Eventually the sperm head makes it to the plasma membrane, and when the two make contact, it it triggers a release of calcium ions, which then cause **cortical granules** (right under the plasma membrane) to fuse with the egg's membrane.
   - These granules then release their enzymes into the zona pellucida, and digest its glycoproteins, specifically the ones that allow other sperm to bind.
   - This makes the zona pellucida unable to bind more sperm, while other molecules found in the granules create a new protective layer around the fertilized egg.
   - The cortical reaction thus prevents polyspermy, or the fertilization of a single egg by multiple sperm. Any sperm that torpedo into the egg after the first one binds will just bounce off now.
   - If cortical reaction does not occur, more than one sperm may enter, increasing the risk of trisomy.
4. **Genetic transfer**: The plasma membranes of the sperm and egg cell become fully used and all the genetic material within the nucleus of the sperm is released into the egg. This is fertilization.

- A note on **Copper IUDs**: IUDs in general trigger a mild inflammatory reaction that brings in immune cells that make it even harder for the sperm to complete their journey.
  - The copper released by copper IUDs, specifically, is a natural spermicide (and ovicide, though it more strongly affects sperm). The copper ions reduce sperm's motility, ability to trigger the acrosomal reaction, and general viability.
  - Though the devices release less copper than what could be found in our diets, the copper build-up in the mucous lining of the cervix and uterine is enough to halt the movement of sperm.

EARLY EMBRYOGENESIS: CLEAVAGE, BLASUTLATION, GASTRULATION, AND NEURULATION:

- Let's start with a **zygote**, an egg that's just been fused with a sperm and received its genetic material.

Cleavage:

- You want to become an embryo and need to divide really fast… so fast you don't have time to grow.
- Cleavage occurs, where the zygote splits into two cells, over and over (still within zone of pellucida).
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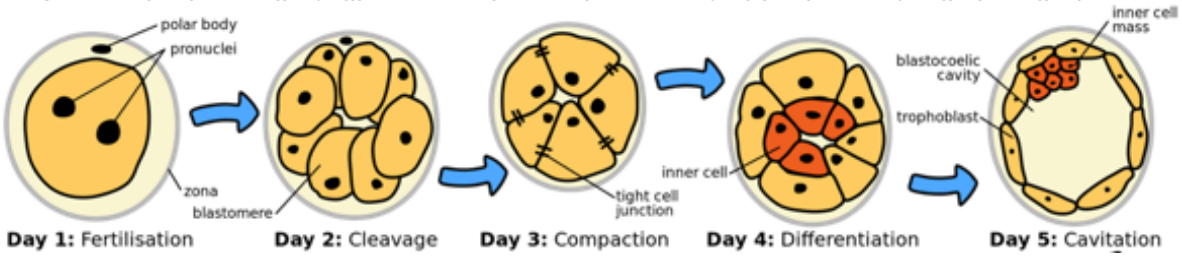
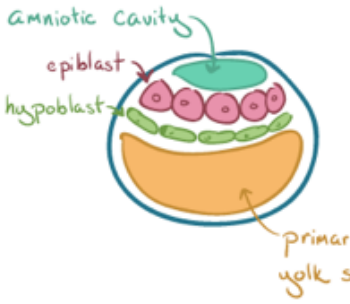


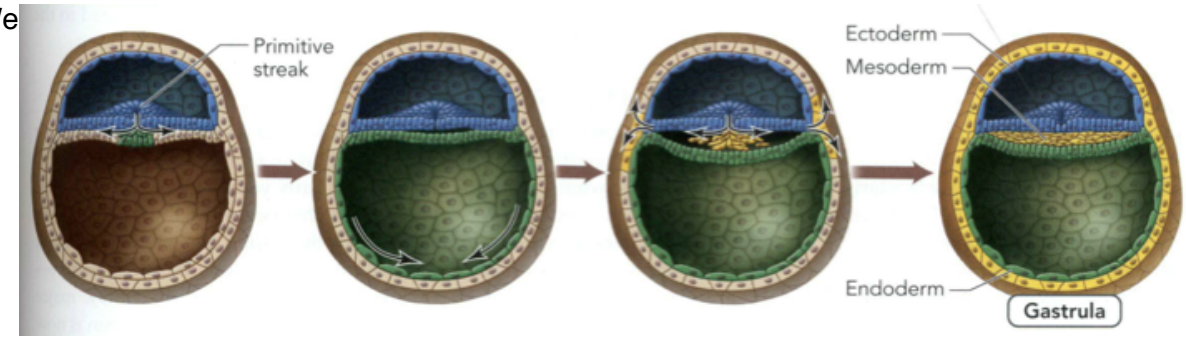
Blastulation: mass of cells forms a hollow ball; cells begin to differentiate and form cavities.

- The cells of the morula, still stuck within the zone of pellucida, start to get closer to each other, compact, and the cells start to differentiate.
- Two layers develop: an outer shell layer known as the **trophoblast**, and an inner collection of cells called **embryoblasts**, which make up a clump called the **inner cell mass**.
- Rather than being arranged in a solid sphere of cells, the inner cell mass is pushed off to one side of the sphere formed by the trophoblast. The rest of the fluid-filled cavity is called the **blastocoel.** The whole setup now resembles a snow globe, and is collectively called the **blastocyst**.
  - Outer trophoblast will develop into structures that help the growing embryo implant in the uterus.
  - The inner cell mass will continue to differentiate and parts of it will eventually b
    embryo.
  - At this point, cells in the inner cell mass are pluripotent, meaning they can eventually turn into the cells of any body tissue (muscle, brain, bone, etc).
- This is also the time when the zona pellucida begins to disappear, allowing the blastocyst to grow and change shape.
- During the 2nd week, these cells differentiate further into the **epiblast** and the **hypoblast**, which make up the two layers of the **bilaminar disc**. This disc is a flat slice across the developing sphere; it splits the environment into two cavities.
  - The hypoblast is the layer facing the blastocoel; epiblast is on the other side.
- Cavities then expand within the hypoblast and epiblast cells to fill the space.
- The **primary yolk sac** develops where the blastocoel used to be, on the side of the hypoblast**.**
- The **amniotic cavity** develops on the side of epiblast, and is what will eventually surround the fetus.



Gastrulation: Three germ layers form; the primitive streak forms

- A **primitive streak** of epiblasts then forms (~ day 16) on the bilaminar disc. This streak is actually the movement of epiblast cells towards the hypoblast layer. The cells (and thus the streak) start from the caudal (anus) end and migrate toward the end that will eventually become the head.
  - This streak determines the midline of the body, and separates the left and right sides.
  - This streak will end up as a "waterfall" of cells in between the epiblast layer and the hypoblast.
- If we imagine this motion of the primitive streak formation as a waterfall, the first layer to invaginate dives the deepest and ends up closest to the hypoblast – this is the **endoderm**.
- The next layers will become the **mesoderm**, and the cells of the epiblast that continue to border the amniotic cavity are the **ectoderm**.
- We



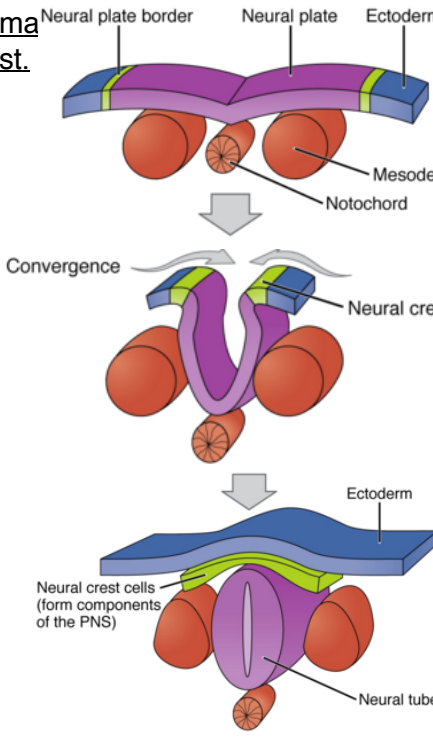
- Directly beneath the primitive streak, the mesoderm (middle germ layer) then forms a thin rod of cells known as the **notochord**.
  - The notochord helps define the major axis of our bodies, and its main purpose is in inducing the next step of embryogenesis, when we finally start to make our tubes!
  - The notochord is also what eventually becomes our intervertebral discs in adults

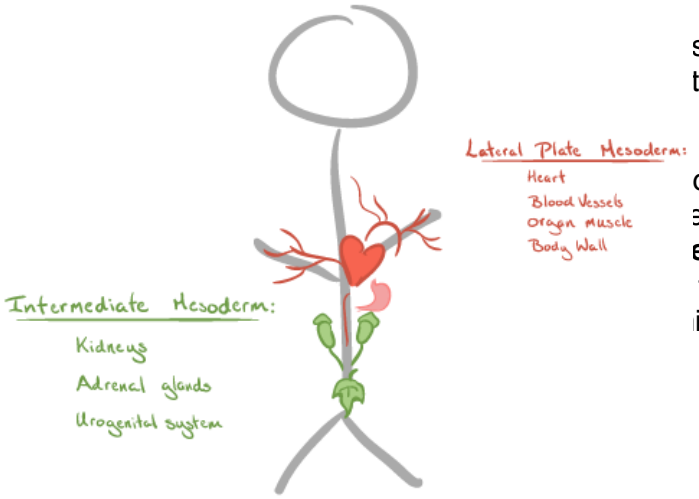
<u>Neurulation: Notocord forms; tubes form into a neurula; notochord induces forma neural plate, which folds back on itself to make the neural tube and neural crest.</u>

1. The formation of the notochord causes a sort of thickening within the ectoderm, forming a thick flat plate of cells called the **neural plate**. The neural plate extends the length of the rostral-caudal axis.
   - The neural plate border separates the ectoderm from the neural plate.

2./3. The neural plate then bends back on itself and seals itself into a tube known as the **neural tube,** that fits underneath the ectoderm. The neural tube will become the brain and spinal cord.

- In the process, the borders of where the neural plate get pulled under with the ectoderm and become the **neural crest**.
- The closure of the neural tube disconnects the neural crest from the epidermis. Neural crest cells differentiate to form most of the peripheral nervous system — the sympathetic and parasympathetic nervous systems, melanocytes, Schwann cells, even some of the bones and connective tissue of the face.





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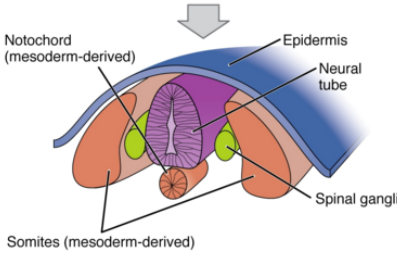
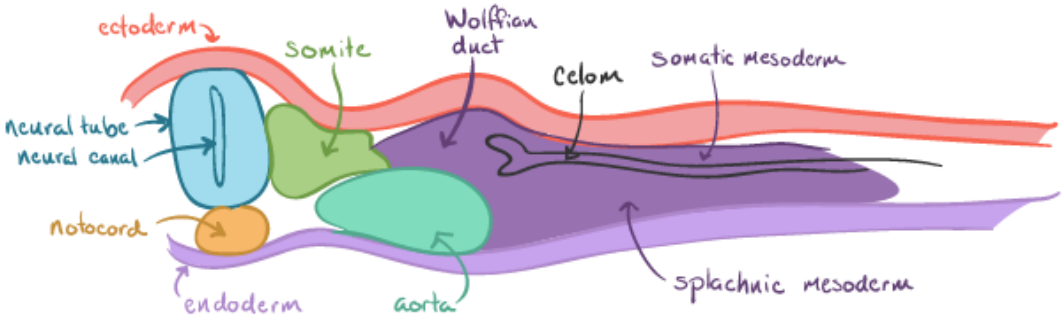
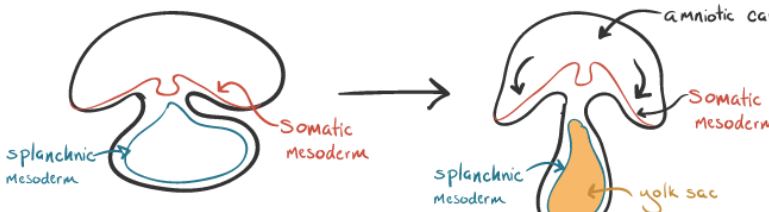


- The **intermediate mesoderm** is the origin of our urogenital system – our kidneys, gonads, adrenal glands, and the ducts that connect them.
- The **lateral plate mesoderm** will give rise to the heart (the first organ to develop!), blood vessels, the body wall, and the muscle in our organs.

- Also at the same time, the endoderm is rolling into a tube as well – the digestive tract. The digestive tract is subdivided into the foregut, midgut, and hindgut. Each subdivision has its own nerve and blood supply. Organs related to the GI tract actually start off as outpouchings of this tube.
  - The **foregut** gives rise to the esophagus, stomach, part of the duodenum, and the respiratory bud, which will eventually develop into the lungs.
  - The second half of the duodenum through to the transverse colon arise from the **midgut**.
  - The remainder of the GI tract, including the rest of the transverse colon, the descending colo





- Consider: The gut tube is the only developmental tube that is supposed to remain an open cylinder. If the neural tube does not close, it creates a life-threatening condition known as **spina bifida**.
  - Spina bifida can occur due to genetic factors, but may also be caused by a lack of folic acid during pregnancy or if the mother has uncontrolled diabetes.
  - Spina bifida can lead to weakness and paralysis of the legs, bladder and bowel control issues, and other physical problems. Children with spina bifida often struggle academically, potentially due to problems in development of the central nervous system.
  - While there is no known cure for spina bifida, the introduction of folic acid into everyday foods like cereal and bread has drastically reduced the incidence of neural tube defects in newborns.

IMPLANTATION:

- As the zona pellucida around a blastocyst begins to disintegrate (blastulation), the endometrial lining of the uterus anticipates implantation and begins to grow. It develops valleys called **crypts**, in which the blasocyst eventually rests. **Apposition** = process of a blastocyst coming into contact with a crypt.
- The blastocyst is not firmly embedded at this point, though, and must become really bound before it can start nutrient transfer. So trophoblasts on the outside of the blastocyst (now that zona pellucida is dissolved) start to multiply and invade surrounding endometrium in a process called **adhesion**.
- The endometrium cells also continue to divide so pretty soon the blastocyst is entirely surrounded by endometrial tissues.
- In the endometrium are **blood vessels** (fed by vessels from uterine arteries) that start to grow and coalesce and form large pools of blood.
- At the same time, the trophoblasts continue dividing and getting bigger and start to fuse to form multinuclear cells that extend out *into the endometrium* — called **syncitiotrophoblasts**. The remaining trophoblasts are then called **cytotrophoblasts** (they've maintating their unicellularity)
- Syncitiotrophoblasts continue to grow and form finger-like projections called **villi** within the endometrium. The blood vessels also continue to grow together.
- Within the villi, tiny **fetal blood vessels** start to grow as well. These are in really close contact to the uterine blood vessels.
  - The vessels aren't actually mixing together (because there's a membrane of trophoblasts between them), but they're close enough that nutrients from uterine blood vessels can be transferred to fetal blood vessels, and waste from fetal blood vessels can be transferred to uterine vessels.

- Eventually this structure of villi and coalesced uterine blood vessels near fetal blood vessels forms around almost the whole uterus. This is called the **placenta**.

GESTATION:

- Gestation is often used synonymously with pregnancy; it lasts about 9 months and ends at birth.
- Gestation can be divided into three trimesters, or into weeks.
  - Week 0 = Last menstrual period (LMP)
  - Week 2 = Fertilization.. sperm meets egg and genetic material is combined
  - Weeks 2-10 = Embryogenesis. (+ **organogenesis**, because in this stage all organs are formed.
  - After week 10, the embryo is considered a fetus, and it undergoes **fetal development** for the remainder of gestation.
- At ~24 weeks, we hit a milestone of 50% survival outside the womb. If you're born at 24 weeks, you have 50% chance of survival. After this, the rate of complications significantly decreases
- **Full term** is considered to be at 40 weeks (with ~2-3 weeks on either side). If you're born before this, it's considered *pre-term*. If you're born after this period, it's considered *post-term*.
  - There are complications to being born both pre and post term.

GERM LAYER DERIVATIVES (RECAP):

- After gastrulation, you've formed your three major germ layers: endoderm, mesoderm, and ectoderm.
- Endoderm turns into the gastrointestinal and pulmonary systems.
  - At the top of the gastrointestinal track/tube are holes from which the lungs actually form, as well as the liver, and the pancreas.
  - And of course that tube goes on to form the stomach, esophagus, large intestines and small intestines.
- The mesoderm forms the muscle, skeletal, and genitourinary systems.
  - Some of the inner layers of skin, as well as muscles and bones, including cardiac muscle, the kidneys, bladder, and ovaries/testes.
- The ectoderm forms the outer layer of skin, and some skin related items like sweat glands and hair. It also forms our nervous system.
- The order of formation of germ layers is first ectoderm, then endoderm, then mesoderm. Thus the order of embryological cells —> tissues is spine formation first, followed by the lungs, then muscle.