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# Biology 1140 Lecture Notes

## Introduction to Cell Biology

*What is a Cell?*

- Fundamental unit of life.
- Every organism is a cell or a collection of cells.
- *Key points to describe a cell:* organelles, plants/animals, cytoplasm, network, membrane, walls, compartments, nucleus, power, regulate internal environment, contain genetic information, bounded by a membrane and exert control of what enters and leaves, respond to environment, able to replicate (cells give rise to other cells), evolve, communicate with each other (respond to other cells around them), mutate, carry out metabolic processes by using nutrients, energy transfer, transfer energy with ATP, cytoplasm (semi-liquid consisting of salts and organic matter) is the structural unit.
- **Cell Theory:**
    - All organisms consist of one or more cells
    - The cell is the basic unit of structure for all organisms
        - Schwann (Zoologist) and Schleiden (Botanist)
    - All cells arise only from pre-existing cells (i.e. the cell is the basic unit of reproduction.
        - Rudolf Virchow
- The cell is enormous, and diverse.
- They vary in shape to very complex structures (retinal cells).
- Highly proliferated membrane, better for transportation, can carry out more reactions.
- An ostrich egg is an example of a cell; it's a *coyesent cell* (non-active cell).
- Lots of diversity in cell function: some are unicellular, and some are multicellular (you can have cells that are highly specialized for a single function, such as information transmission, or hormone production).
- They have a common chemistry, made out of same molecules, lipid membranes, same basic types of lipids. Proteins, same collection of 20 or so amino acids. DNA is pretty much the same, in terms of basic chemistry. Metabolism, energy transfer, uses common currency across all cell types in the form of ATP.
- Muscle cells are a good choice to study ATP.
- To work on chloroplasts, use plants.
- Internal Organelles do not affect the surface to volume ratio.

*Size Matters…*

- Most of the things that we will measure are in micron ($1x10^{-6}$m) and a nanometer ($1x10^{-9}$m).
- Refer to slide for more information…
- Nucleus – 0.006mm
- Ribosome – 30nm
- Microfilament – 0.007 micrometer
- Giraffe Axon – $1x10^{6}$ micrometer
- Mitochondrion – $3x10^{-6}$m

*Why are Cells Small?*

- Surface to volume ratio.
- Larger volume, more nutrients, more wastes to get rid of.
- Have to easily transport nutrients and wastes across cell surface.
- Length = L
  - SA:V is 6:1
- Length = 2L
  - SA:V is 3:1
- SA to V provides a limitation to cell size.
- Second limitation comes from the rate of diffusion. They way molecules move around the cell is by diffusion. It is only fast over very short distances, longer distance the longer it takes.
- Concentrations of substrates need to be high for reactions to take place.
- Larger cell needs 8 times as many molecules that the smaller cell to get the same concentration.
- Third limitation is the need to achieve these adequate concentrations. You can overcome this with respect to SA:V Ratio, large organism made up of many small cells. To overcome this problem, contain reaction in a smaller space, such as organelles in mitochondria.

*Eukaryotic vs. Prokaryotic*

- Prokaryotic Cells:
  - Typically small, 1 to 5 microns.
  - Constrained by all the factors we discussed.
  - Small simple organisms
  - Cell membrane, and inside cytoplasm, you'll find ribosomes, nucleoid (DNA) which is free flowing.
  - Because of their simplicity, constrained by factors we discussed, therefore small.
  - *Bacteria* (thousands of species, we only now a few, primarily the ones that cause human disease) and *Archaea* (Extremophiles are included in this group), two groups of organisms that have prokaryotic cells.

- Eukaryotic Cells:
  - A typical eukaryote cell is 10 to 30 microns.
  - A lot of membranes present, a key characteristic.
  - Inside of the cell is divided up into compartments by a series of membranes. One of the way these cells become large, by breaking up interior into compartments.
  - Transport systems, can move solutes around, without relying on diffusion, another reason they can become bigger than prokaryotes.
  - Four groups to look at:
    - The protists, single celled eukaryotes. Such as *amoeba* and *paramecium*. A protist has a nucleus and PROKARYOTES DO NOT.
    - Fungi – *Yeast*, bakers or brewers, commonly used for lab research, model fungus, grows easily in a lab, small, grows in large vats, easy to work on, for bread and beer, and although its simple, shows many characteristics of more complex fungi.
    - Plants – *Arabidopsis*, is a plant that is commonly used in research, small genome, grows easily like growth cabinets in a lab, short generation time, grows to maturity in a few months.
    - Animals:
      – A *fruit fly* is a model organism. Geneticists used them for a long time. A lot of mutants that are easily distinguished.
      – A *mouse* is a popular choice for biomedical research, relatively small, happy living in sawdust, eat rodent chow, reproduce quickly, three months generation time; mammals, basic physiology similar to humans.
      – *Zebra Fish*, external fertilizers, and eggs are clear which is a tremendous advantage, early stages of development take place over 48 hours, only a few centimeters long, reproduce really easily, have some traits that we are interested in figuring out, their heart muscle can heal itself, attractable and easy to keep.

*Eukaryotic Cells*

- *Cytosol* is the aqueous solution, and the *cytoplasm* is the cytosol plus what you find in it, the ribosomes, etc…
- Cytosol the same in prokaryotes and eukaryotes, but the cytoplasm is DIFFERENT, prokaryotes lac the organelles.

*Non-membrane Bound Organelles*

- *Cytoskeleton*
  - Found in eukaryotic cells.
  - Support/shape, internal organization, movement of cell, movement within a cell.
  - Microfilaments, Microtubules, and Intermediate Filaments, make up the cytoskeleton.
  - Why they can become larger because of the role of transport.

- Prokaryotic cells lack the cytoskeleton.
- Prokaryotic cells may have proteins that carry out similar functions, but not the same as the cytoskeleton.

- *Ribosomes*
  - Eukaryotic ribosomes are larger and structurally more complex than those of prokaryotic ones, but serve the same purpose.

*Membrane Bound Organelles*

- *Nucleus*
  - Surrounded by nuclear envelope, double membrane.
  - Within it you find genetic material of the cell.
  - In eukaryotic cell, DNA is structured with protein, number of distinct chromosomes.
  - Number of distinct linear chromosomes.
  - Also the site of ribosome synthesize, synthesized by the nucleolus, migrate out into the cytoplasm, by means of the nuclear pores in the envelope.
  - Between divisions, distinct chromosomes become less obvious.
  - Naked and circular DNA in prokaryotes.
  - Outer membrane of nuclear envelope is continuous with the endoplasmic reticulum.
- *Endoplasmic Reticulum*
  - Series of tubes and sacks made out of membranes.
  - ER is continuous with the outer layer of the nuclear envelope.
  - *Rough*
    - Ribosomes attached to it, site of protein synthesis.
    - Ribosomes assembled here, come out of nucleus as pieces (2).
    - Find a lot in cells that export lots of proteins, like a cell that makes peptide proteins.
  - *Smooth*
    - Lacks ribosomes, serves as a site for lipid and steroid synthesis and detoxification.
    - Large amounts in liver cells, because it is an important site of breaking down toxic compounds, smooth ER plays an important role in breaking those toxins down.
  - *Golgi Complex*
    - Stack of flattened vesicles.
    - Sorting, modification and packaging of proteins.
    - Proteins further modified, i.e. large protein brought down to smaller compounds can be sorted into different types.
    - Proteins can be exported from the cell.
    - Can be inserted into membranes.
    - Golgi complex sets up the proteins to export them to different part of the cell or out of the cell to specified locations.
  - *Vesicles*
    - Transport among organelles and/or to plasma membrane.

- *Lysosomes and Peroxisomes*
  - Contain hydrolases, catalases.
  - Breaks down stuff.
- *Vacuole*
  - Temporary structure.
  - Maintains turgor pressure in plant cells.
    - Water enters the cell and causes the cell to be stiff and makes plant stand up instead of droop down.
  - Turgor pressure
    - When central vacuole fills with water, it pushes contents against cell wall, causes plant to remain upright and strong; and when there is less water, causes plant to droop.
- *Mitochondrion*
  - Around 2 micrometers
  - Double membrane, cristae
  - Oxidative metabolism yielding ATP, cellular respiration
  - Circular m(mitochondrial)DNA, naked chromosomes; have ribosomes, smaller and fewer subunits than standard eukaryotic ribosomes.
  - Reproduce by fission
- *Chloroplast*
  - Around 5 micrometers
  - Double membrane + thylakoids
  - Conversion of light energy into chemical energy (make food, in the form of carbohydrates)
  - Circular cp(chloroplast)DNA, naked chromosomes; have ribosomes, smaller and fewer subunits than standard eukaryotic ribosomes.
  - Reproduce by fission
  - Thylakoids house the chlorophyll
  - Light energy captured by the chlorophyll

    - Mitochondria and Chloroplasts very similar to bacteria.

- *Endosymbiont Theory*
  - Mitochondria from incorporation of aerobic bacteria.
  - Chloroplast from (later) incorporation of cyanobacterium.
  - Life started as a prokaryotic cell. **First event,** at one point it engulfed an aerobic bacterium, and instead of breaking it down, it stayed within cell and functioned in the cell, symbiotic relationship; mutual benefit speculation is that the bacterium found a safe home and the larger home cell benefited from the more beneficial ATP synthesis. Over time, exchange of DNA, bacterium turned into a *mitochondrion*. Still functions in aerobic metabolism.
  - **Second event,** occurred, gave rise to the *chloroplast*. Photosynthetic bacterium gets incorporated.

- Cells cannot synthesize chloroplast and mitochondria.
- Looking at present day examples of endosymbiosis, for evidence to prove endosymbiont theory. An example is the **solar powered sea slug**, it is an animal that carries out photosynthesis, blurs boundary between plant and animal. Animal that carries out photosynthesis. Where do those chloroplasts come from? Baby sea slugs are brown, only become green when feeding on algae. Acquire green color because they steal chloroplasts from algae that they eat; this process is called *kleptoplasty*. In a sea slug, intestines go all through body, where chloroplasts end up. The sea slug eats the algae, breaks it down, chloroplasts are then incorporated into the intestinal cells. Then they start to carry out photosynthesis. Once all chloroplasts are in place, do not have to feed any more. The solar powered sea slug is an example of endosymbiosis, shows evidence of the endosymbiont theory.
    - In the sea slugs, chloroplasts not passed down to generations, but in the chloroplast endosymbiont theory, passed down. Endosymbiotic origins of chloroplasts probably only happened once. In the original case of endosymbiosis, what was ingested was an entire bacterial cell, and the slug ingests only a specific cell.
    - Gives us some confidence that endosymbiosis is possible.

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# Cell Membranes

- *Fluid Mosaic Model*
  - Singer and Nicolson in 1972
  - Fluid lipid molecules in which proteins are embedded and freely floating.
  - Unique complement of proteins, proteins largely responsible for membrane.
  - Proposed in 1972
  - The structure of DNA, eluded in the 1950s.
- *Functions*
  - Define boundaries; selectively permeable barrier (only certain things can pass through)
  - Localization and Organization
    - Scaffold for biochemical activities (enzymes)
    - Example is the mitochondria, within it the enzymes and proteins are all nicely organized for efficient movement for ATP synthesis.
  - Regulation of Solute Transport
    - In and out of cell and/or organelles
    - Uphill and downhill
    - e.g. Na+, K+, -ATPase (sodium potassium pump)
  - Responses to External Signals
    - First point of contact with environment
    - Receptors and signal transduction
    - e.g. B-adrenoreceptor
  - Cell-to-Cell Communication
    - Recognition, adhesion, exchange of materials.
    - Gap junctions (animals), plasmodesmata (plants) that occur at the membrane.

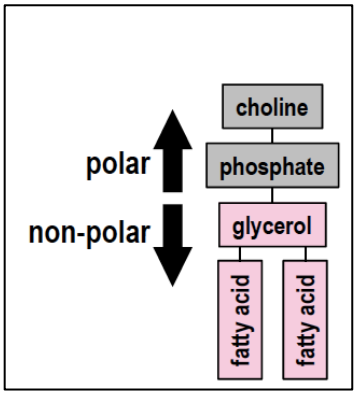
  - There structures, fundamentally similar, all tailor to a specific role.
  - Inner mitochondrial membrane is 76% protein and 24% lipid. And the Schwann cells are 18% protein and 82% lipid.

- *Fluid Mosaic Model*
  - Lipid-protein assembly in which components are held together in a thin sheet by non-covalent bonds.
  - Two fluid lipid layers – structural backbone, permeability barrier.
  - Mosaic of proteins – unique complement, these proteins responsible for specific role of membrane. Not held together by covalent bonds, very fluid
  - Only 10 nanometers thick.
  - One of the early experiments, by Fry and Addinen, they took two cell line (human and mouse cell line), using different fluorescent labels, labeled proteins. They then fused them together. If proteins are not free to move around, it should be half on one flag and half the other, BUT they found that the signal were mixed around the cell, showing how it is a very fluid/dynamic environment.

- Typically now, we use FRAP (*florescence recovery after photobleaching*). Cell surface labeled with fluorescent dye, then laser beam bleaches an area of the cell surface, and the fluorescent-labeled molecules then diffuse back into the bleached area. The data that is generated is plots, which is the intensity of fluorescence over time. It will recover at a rate at how mobile that protein is. If you have a non-mobile protein, it would go down and just stay flat, no recovery. For example, proteins that are anchored to cytoskeleton are often not mobile.

- <u>The Lipid Bilayer</u>
  - Gorter and Grendel, 1925.
  - Key component of permeability barrier.
  - Only had fluid mosaic model since 1972, which was develop by Signer and Nicholson.
  - **Composition:**
    - **Phospholipids**
      - *Phophoglycerides:*
        - Glycerol backbone
        - Hydrophilic head group: phosphate + serine, choline, ethanolamine, inositol. Also, hydrophobic tails.
        - 2 fatty acids; saturated and unsaturated
      - Sphingolipids*:*
        - Sphingosine backbone
        - Hydrophilic head group: phosphate + choline
        - 1 fattty acid
        - Absent from plants, and bacteria

- Kleptoplasty refers to the solar powered sea slug that acquires chloroplasts from the algae it eats. The chloroplasts are then incorporated into the intestinal cells. Important, because it is a present day example of endosymbiosis, which shows how this could have happened in the past.
- Singer and Nicolson developed the fluid mosaic model in 1972.
- Phosphoglyceride:



- Whether it forms a liposome or a micelle depends on the amount of water to lipid ratio.
- **Glycolipids**
  - Similar to phospholipids in structure
  - Can be built on glycerol or sphingosine back bone
  - Difference between phospholipids and glycolips is the removal of the phosphate group and one or more carbohydrates.
  - There are two very abundant glycolipids found in plants based on glycerol.
  - Two of the best-known glycolipids are found in plants.
  - In terms of glycolipids, A B O blood groups, based of glycolipids found in membrane of red blood cells. Important in allowing other cells to recognize each other.
- **Sterols**
  - Particular form of a more general form called steroids
  - Built on a framework of 4 linked carbohydrate rings.
    - Defining characteristic
  - Attached to the 4-ring structure is an OH group attached to one side and on the other side is a hydrocarbon tail.
  - The ring framework and hydrocarbon tail are all hydrophobic, imbeds itself in middle of the membrane with the polar hydroxyl group oriented towards the polar head groups.
  - Sterols differ among different cell types, absent from prokaryotic cells.
  - Within eukaryotic cells, each group has its own characteristic sterol.
    - In *animal* cells, you find cholesterol.
    - *Plants* have phytosterols.
    - *Fungi* have ergosterol.
  - Can distinguish prokaryotic and eukaryotic cell membranes from presence or absence of sterols.
  - What membrane in a eukaryotic cell might be expected to lack sterols? Why?
    - The membrane surrounding either mitochondrion or chloroplasts, because they originated as prokaryotic cells, and prokaryotic cells lack sterols. And the inner membrane would lack the sterols out of the double membrane.

<u>Properties of the Lipid Bilayer</u>

- Lipids are distributed unequally.
- Two leaflets [top half (facing extracellular fluid) and bottom half (facing cytoplasm)] are not the same.
  - Lipids within a leaflet can mover freely but rarely move from one leaflet to the other because the hydrophilic head would have to go through the hydrophobic core.
- Glycolipids are relatively small, and would expect to find them on the outer half of the membrane because they are involved in signaling. There can be some glycolipids on the cytoplasm side, but only small.

- **Fluidity**
  - When cold, cell membranes stiffen up, and receptors that detect sensation for example, go stiff and the sensory receptors stop working, and membrane fluidity decreased.

  - **Factors that affect membrane fluidity:**
    - Temperature
      - At low temperatures, fatty acid chain stick together, less mobile. At higher temperatures, move more freely, and make the membrane more fluid.
    - Composition of the membrane
      - *Length of the fatty acid chains and the degree of saturation.* Longer chains pack together, lower fluidity. Shorter chains (mix of short and long) pack less well and give higher fluidity. Saturated pack together really well, lower fluidity. Unsaturated have double bonds which causes a kink, causes them to not pack together well, increases fluidity. (There is at least one double bond present.) In general, the more fluid the membrane, the more double bonds are present.
    - Head groups that are present
      - Cylindrical in shape, lower fluidity (PhosphotitolCholine Head Group). And cone shape, which spreads out, doesn't pack nicely, raises fluidity (PhosphotitolEthanolanine Head Group).
    - Where there are sterols
      - Have a four ringed framework, much more rigid and stiff than a fatty acid chain. At high temperatures, stiffness is an advantage, decreases fluidity. At low temperatures, unpack, increases fluidity of membrane. Sterols have a buffering effect. *Fluidity increases when temperature low and increases when temperature is high when sterols are present.*

  - **Homeoviscous Adaptation**
    - Organism adjusts composition of membrane to bring fluidity back to desired value, can happen in an individual organism or over evolutionary time. Changes within the organism.
    - Goal is to maintain constant fluidity.
    - Can compare tropical species with Antarctic fish, find more double bonds in the animals that come from colder temperatures.
    - PC and PE are both adjusted at different temperatures, but it is the ratio that matters which affects fluidity.
    - Can add more sterols.

- Diet also affects fluidity
  - Dietary adjustments, tests were carried out in a lab here at uOttawa.
  - Semi palpated sand piper, about 20 grams, hops along shore looking for food. Spends summer in arctic and spends winter in South America. What it does in a series of short hops, then fly's stays at bays of fundy for two weeks. Then solid flight for 3 days. What had been noticed at the two weeks at the Bay of Fundy, they are there to gain fat. Need that fat to power flight, most endurance activities powered by fats. What was curious is they bulk up by eating a mud shrimp, which is 90% of there diet. Why do they focus on mud shrimp? Mud shrimp contain high levels of polyunsaturated fatty acids (PUFAS). Adding unsaturated fatty acids to flight muscle membranes, make it more fluid, which makes it easier to import foods into the wings (Hypothesis). If it was true, there should be selective corporation of those fatty acids into flight muscles, and that is what they found. All because of the mud shrimp diet. But they also noticed that as the birds gained unsaturated fatty acids, showed an increase in enzyme activity, which elevated *citrate synthase* (CS) activity. Important enzyme in ATP synthase. Adding membrane unsaturated fatty acid would prime the muscle. Another master's student (tried to figure out causation) fed the birds unsaturated fatty acids to quails, which are poor fliers. If you can turn this bird into an endurance bird, by just feeding it unsaturated fatty acids, they saw an increase in enzyme activity.
  - Dietary lipids cannot only affect membrane fluidity but signal changes to happen within the cells.

<u>Membrane Proteins</u>

- Thought to be responsible for the unique characteristics of the membrane.
- Looking at integral membrane proteins (embedded), peripheral (linked), and lipid-anchored proteins (anchored but through covalent bonds).
- Functions of proteins:
  - In the membrane are responsible for moving solutes through the membrane, solute transport. i.e. sodium pump
  - Enzymes, such as the ETC, or the proteins of the ETC
  - Serve as receptors, get signals from external environment
  - Forming attachments to extracellular structures or to other cells
- Membrane protein mobility is much more variable than lipids.

- **Integral Membrane Proteins**
  - Cross membrane, transmembrane domains are the parts that cross the lipid bilayer. Can be one or multiple (multi-pass transmembrane).
  - Typically they are anthipatic (region of the protein domain are hydrophobic and bits that link them together are hydrophilic).
  - Fairly characteristic structure of transmembrane domain, 20 – 30 amino acids, forms alpha helix.
  - Hydrophobic regions take on form of alpha helix, 20 to 30 amino acid chain (transmembrane domain).
  - Multi-pass proteins has several transmembrane domains.
  - Difficult to get out of membrane.
- **Peripheral Proteins**
  - Found on either surface of the membrane.
  - Held in place by non covalent interactions, van der waals, ionic, hydrophobic interactions, hydrogen bonding, etc… either between protein and lipids or between protein and other proteins.
  - Because they are held in place by weak interactions, dynamic relationship. Can come off easily, and experimentally easy to come off.
  - Examples of these later on when we look at how cytoskeleton is linked to membrane. Hold together proteins of cytoskeleton of the cell to the proteins in the membrane. Connects cytoskeleton with membrane.
  - Not all peripheral membrane proteins react to changes in pH, so change ionic strength, temperature etc…
- **Lipid Anchor Proteins**
  - Found on one side or the other.
  - Held together by covalent linkages.
  - *Fatty acid anchored proteins*, found on cytosolic side. Inserted in membrane. Covalent bond between protein and fatty acid. Example is G proteins.
  - *GPI Anchored Proteins*. Protein anchored to carbohydrate group (Glyco) that is covalently bonded to phosphatidylinositol. Can use *phospholipase C* which cleaves, and removes protein from the membrane. Example is type IV carbonic anhydrase, it's found in the kidney of humans, reabsorption of solutes, in the brain and in the eye.

- **What is membrane asymmetry?**
  - Membrane asymmetry refers to different compositions of the two leaflets of the membrane. Examples, PC is more abundant in the outer membrane than the inner membrane. Glycolipids are also distributed. Sterols tend to be equally distributed, therefore not a good example.
- **Distinguish between Correlation and Causation**
  - Correlation does not equal causation. Correlation, document a trend between two variables. The changes in the variable to be linked. To establish causation, the change in one variable alters the other variable. What is the experiment? Change one variable and see if there is a link, experimentally manipulate it and look for an affect of that manipulation.

- To go from a sequence of amino acids (**Primary Structure**) to a protein in its native conformation, the chain must fold. Can get folding due to hydrogen bonding in the backbone. Alpha helix, **secondary structure**. **Tertiary structure**, referrers to folding of the entire structure, due to primarily interactions of the R groups, can be ionic bonds, hydrophobic interactions, hydrogen bonds, and covalent interactions (two sulfide groups coming together to make a disulfide bridge). Many proteins have more than one polypeptide making it up, the **quaternary structure (**tetramer – 4 subunits and each subunit is a polypeptide chain).

- *How can the different properties of membrane proteins be used to distinguish among integral, peripheral and GPI-linked (lipid-anchored) proteins experimentally?*
    - Add fluorescent marker so that we can find it again. If we want to remove it from the membrane, can use tag to see if still there or not. Alter pH of solution would remove peripheral proteins. If it doesn't come off, then we can add and enzyme, phospholipase C, to see if we can cleave the bond. If you add it and floats of and you solved the problem. If both of these techniques don't come off, then it is an integral protein. To study it further, to get integral protein out of the membrane. Add detergent to the membrane to break up the fats, which will leave us with the protein.
        - Step 1 – play around with pH, ionic strength, temperature.
        - Step 2 – add enzymes.
        - Step 3 – add detergent to break up membrane (don't do first).
            - Step 1 and Step 2 shouldn't matter because they are independent steps, but from a practical point of view start off with easy, cost efficient methods.
- **<u>Movement Across Membranes</u>**
    - Non-polar molecules, especially small ones, cross membrane readily, and small uncharged polar molecules. Polar and quite small, like water, able to get across despite polar nature because of small.
    - Anything larger than 100 Daltons is not able to get across membrane if polar.
    - Anything that is charged, like ions, can't get across membrane. The charge attracts a cloud of water molecules, that molecule, as a whole is too large to cross membrane.
    - When a molecule moves from an area in which it is abundant to less abundant, it is *passive transport*. Downhill movement.
    - If molecule is moving against concentration gradient, uphill, requires energy, it is *active transport.*

    - **<u>Passive Transport</u>**
        - Random Brownian motion of molecules.
        - Movement is driven by gradient, different gradient for different molecules.

- Glucose, the concentration gradient matters, more glucose outside than inside, therefore it can move into the cell passively.
- For gases, for example oxygen, partial pressure gradient, move into cell down partial pressure gradient.
- For ions, chemical gradient, must also consider charge, therefore also electrochemical gradient.
- For water, moves by diffusion through osmosis using osmotic gradient.
- **Hypertonic** solution has more solutes than cell, more concentrated than cell. Leads to shriveled cells.
- **Hypotonic** solution has less solutes than cell, less concentrated than cell. Leads to swollen cells.
- **Membrane Permeability**.
    - **Simple Diffusion** driven by diffusion gradient.
    - If molecule cant move across membrane on its own, relies on carrier proteins or channels and is **Facilitated Diffusion**.
    - Facilitated diffusion, plateaus when all transporters are used, *saturation kinetics*. Follows linear relationship, simple diffusion.
    - *Partition coefficient* describes how well a solute will dissolve.
- **Facilitated Diffusion**
    - *Channels*
        - Integral membrane (transmembrane) proteins
        - Core of protein is a hydrophilic channel, allows solute to move across membrane.
        - These proteins tend to be highly selective, there are also aqua channels which are called *aquaporin*.
            - Aquaporin specific for the movement of water.
        - Outer part is hydrophobic and inner part is hydrophilic.
        - Different types of compounds that can bind, that will inhibit the protein from letting ions, things pass.
        - Some channels are always open, leak channels, such as aquaporin.
        - Some channels open and close, gated channel. Like the potassium channel, opens and closes with changes in membrane potential.
        - Also ligand gated channels, open when something binds to them.

- *Carrier Proteins*
    - Proteins that share many similarities with channels.
    - Integral membrane proteins, can be inhibited, highly selective.
    - Two conformations, and when changing between both they transport a solute.
    - Solute binds, undergoes conformation change, moves solute to cytosol and releases solute, undergoes second conformation change and moves back to original spot.
    - Swinging doors, solutes can go in either direction. Can reverse gradient, and solute reverses direction, using same protein.
    - Direction of movement driven by gradient.
    - Example is a single solute moving across, **uniporter**. Can also get transport proteins that transport two different solutes at same spot, **co-transporter**, one moves glucose and sodium ion at same time. Can also get **exchangers**, two solutes moving in opposite directions; chloride ion moves in one direction bicarbonate ion moves in other direction.

- **Active Transport**
    - Also have carrier proteins, requires energy and refereed to as pumps.
    - Highly selective, integral transmembrane protein.
    - BUT they are unidirectional. Determined by diffusion gradient.
    - Always pumps in same direction. Intrinsically unidirectional.
        - *Primary Active Transport*
            - Sodium-Potassium ATPase, **P-Type pumps**, moves solutes against diffusion gradients, and energy comes from splitting ATP (get inorganic phosphate and that inorganic phosphate attached to pump and comes off later on). Characterized by phosphorylation. Attached for pumping and comes off after. Phosphate group is transiently attached, reversely phosphorylated.
                - P-Type pumps move cations.
            - **V-Type Pumps**, found on vacuoles, move protons (H+). Can also be found on plasma membrane. Not reversely phosphorylated. Key point it is a transport protein itself that is hydrolyzing ATP.
        - *Secondary Active Transport*
            - Downhill transport along gradient established by 1° active transport allows uphill transport of second solute.

- The gradient that is driving movement is the proton gradient, which is stronger than the gradient of the solute. And the proton gradient is driving the transport of the solute, using a co-trasnporter.
- Still requires input of energy, but that is a second step.
- Occurs in both animal and plant cells.
- In animal cells, gradient established by sodium pump.
- In plant cells, gradient established by proton gradient.
- Co-transporter and antiport can be applied to both active and passive transport.

**Pop Quiz**
**Differentiate between two things:**

1- **Peripheral Membrane Protein and Fatty-Acid Anchored Membrane Protein**
Peripheral lay on the outside of the membrane, do not pass through the hydrophobic core. They are also used in signaling and as receptors. The Fatty-Acid anchored membrane protein passes through the whole membrane and the fatty acid and there is a covalent bond between protein and fatty acid. The peripheral membrane protein is non covalent bonds.

2- **Passive Transport and Simple Diffusion**
Passive transport is the broader case, and simple diffusion is a specific case where the molecule is able to pass through the membrane using the diffusion gradient. Passive transport includes simple and facilitated diffusion.

3- **Integral Membrane Protein and Channel Protein**
Channel protein is a specific type of integral membrane protein. Integral membrane proteins are imbedded in the membrane.

4- **Channel and Carrier**
Channel is a protein that simply allows a solute to pass through membrane where as a carrier moves a solute across a membrane because a solute binds to it and the carrier will undergo conformation. Channels can change conformation if gated.

5- **Co-Transporter and Exchanger**
Co-transporter transports two different solutes in the same time in the same direction and an exchanger transports two different solutes in the same time in the opposite direction.

- Pump that hydrolyzes ATP to move protons to outer area to create a gradient, they will fall down the membrane through another channel bringing along another solute against its gradient.

- Energy using step is separated from the transport of the solute of interest.
- Sodium Potassium ATPase gradient in animals, and in plant cells it is a proton pump.
- Can be exchange or co-transport.
- Kidney tubule cell for example. On one side is the filtrate and on the other side is the blood. The role of the cell is to reabsorb solutes. Reabsorbed from filtrate to blood. Because of this, kidney tubule cell are packed full of transporters, good for studying transporters.

| Properties | Simple Diffusion | Facilitated Diffusion | Active Transport |
|---|---|---|---|
| Direction of Movement Relative to Diffusion Gradient | With diffusion gradient. | With diffusion gradient. | Against diffusion gradient. |
| Metabolic Energy Required | No | No | Yes (ATP) |
| Intrinsic Directionality | No (if you reverse gradient, reverse direction). | No (if you reverse gradient, reverse direction). | Yes (pumps can only operate in one direction). |
| Membrane Protein Required | No | Yes (either carrier or channel) | Yes (pump) |
| Saturation Kinetics | Does not show saturation kinetics. Plot is a linear relationship. | Yes, anytime you use a transport protein, you see saturation kinetics. | Yes, uses a membrane transporter. |
| Competitive Inhibition | No | Yes | Yes |
| Solutes Transported | 1) Small non-polar molecules.<br>2) Water.<br>3) Small polar molecules.<br>4) Large non-polar molecules (lipophilic). | 1) Larger polar molecules such as glucose.<br>2) Charged molecules. | Solutes that are getting transported against the concentration gradient. |

- Case Study example using urea transport in Gulf Toadfish
  - The fish excretes urea, unusual. Toadfish excrete urea, in mammals, urea movement requires transporters, is this the case for the fish. First setoff experiments, looked at kinetics, when she did the measurements, shows saturation kinetics. What does that mean? From mammals, we know that urea transporters can be inhibited by phlorotin, and when it was added, rate of urea uptake

dropped. Excrete pulses of urea every few hours, every time it appears, the plasma urea concentrations fall. Under normal conditions, very little in water, gradient to move into water. Can the direction be reversed? Put toadfish in water with urea, then saw the direction of the urea. Every time it excreted urea, plasma urea levels increased. Therefore, the movement can be reversed. Therefore it uses facilitated diffusion. In mammals it is also facilitated diffusion.

- *Can you design an experiment to distinguish between facilitated diffusion and secondary active transport?*
  - Change the gradient, if the direction of movement changes than it is facilitated diffusion, if not than it is active transport.
  - Take away energy source, if solute can still be transported it is facilitated; if it is active it will stop working.

- **<u>Membrane and Cell-to-Cell Signaling</u>**
  - Water-soluble vs. lipid soluble signal molecules.
    - Location of receptor.
    - E.g. stress hormones (adrenaline, cortisol).
    - Hydrophilic signal molecule on outside and hydrophobic signal molecule is on the inside.
      - Cortisol exerts affects on receptors in the cell.
      - Adrenaline is water-soluble and affects receptors on surface of cell.
  - **Steps:**
    - *Reception*
      - Lignand-binding site
      - Only cells with receptor respond to signal
    - *Transduction*
      - Conversion of signal to cellular response
      - Amplification
      - Transduce signaling molecule into response.
    - *Cellular Response*
      - Changes in gene expression
      - Changes in protein activity
      - Responses can be cell type specific
    - *Termination*
      - Have to be able to turn down the activity in a cellular signaling pathway
      - If the mechanism fails, example is cancer, cancer are cells that divide out of control, because signaling pathways have not terminated properly.

- *How can we show experimentally that binding of the signal molecule to a surface receptor is what triggers the cellular response, i.e. that the signal molecule itself does not enter the cell?*
  - If its water soluble, it should react with the cell surface receptor.
  - Add it to the inside of the cell will be without affect.
  - If it's on the outside, the signal needs to be on the outside as well.

- **Membrane receptors are integral membrane proteins**
  - Types that we will be looking at:
    - Ligand-gated channels
    - Enzyme-coupled receptors
    - G protein-coupled receptors
  - Signal binds to receptor, which activates a intracellular domain, which changes in conformation.
- **Receptor Tyrosine Kinases (enzyme-coupled receptors)**
  - Has one transmembrane domain
  - Activation of cytoplasmic protein kinase domain
  - Phosphorylation (tyrosine residues)
  - Has a tyrosine kinase domain
    - Phosphate removed from ATP and added to protein through a covalent bond.
  - A tyrosine kinase phosphorylates tyrosine residues.
  - A receptor tyrosine kinase is an enzyme that phosphorylates tyrosine residues.

**Pop Quiz**

1- *What is "signal transduction" and why is it significant?* Signal binds to cell by means of a receptor, than converted into a cellular response. Conversion of signaling molecule into response. Without it cell cannot respond to signal.
2- *What structural element is absolutely required by a cell for it to respond to a signaling molecule?* A receptor for that signal (a protein sitting in the membrane).
3- *What is a protein kinase?* Transfers a phosphate group from ATP to a protein, its an enzyme that phosphorylates protein. Example is a P Type Pump.

  - Phosphate group can be removed again (dephosphorylation) by phosphatases.
  - When signaling molecule binds to ligand binding molecule, the receptors come together, dimerization, to proteins coming together.
  - The signaling binding to receptor, two coming together, these two coming together, activate protein kinase domain. First thing it does is phosphorylate tyrosine residues within the receptor,

phosphorylates itself. Once that happens, it now can initiate downstream pathways.
    - Most of the pathways initiated are pathways that regulate cell growth and division.
    - Tyrosine kinases important in how cells grow and divide.
    - Nerve growth factor acts through a tyrosine kinase.
- **G Protein Coupled Receptors**
    - Integral membrane proteins.
    - Have 7 transmembrane domains.
    - Binding site inside for a G protein (GTP Binding Protein).
    - When the receptor is activated, it activates G Protein, which activates further steps in cell, usually involving a second messenger (from the cell, it is a chemical intracellularly, serves to turn on inner pathways).
    - G protein leads to production of second messenger, which leads to further action within the cell.
    - **G Proteins**
        - Fatty acid anchored proteins
        - Proteins that are covalently attached to fatty acids, and inserted in cytosolic leaflet side.
        - Have three different sub units (heterotrymaric)
            - Alpha (binds GTP), Beta and Gama form unit.
            - Alpha subunit with GTP attached to it and Beta/Gama complex.
            - Each of these two pieces can go off and imitate things within the cell.
            - Two elements by activating one G Protein.
            - To turn off, GTP must be hydrolyzed to GDP, turns protein back into coyesin from, until it is activated once again.
            - Signal molecule activates receptor, receptor binds G protein, and gets rid of GDP, and allows it to pick up GTP, once it picks up GTP it is activated.
            - Role of receptor is to turn G Protein on, G Proteins acts as an on-off switch.
                - GTP bound = Activated (on)
                - GDP bound = Deactivated (off)
            - Alpha subunit is capable of hydrolyzing GTP, not fast, delay between GDP binding and hydrolyzing occurring, during that delay it is active.
    - *GTP(gamma)S is a non-hydrolysable analogue of GTP. How would use of GTP(gamma)S in a cell affect G protein signaling?*
        - It would never be able to get deactivated, therefore the signal that was received would never be able to get turned off. This could have severe affects, for example it is was a signal for division, it could go out of control, cancer.

- There are a number of G protein, one pathway that is very common is the cyclic AMP pathways.
- **cAMP Pathway**
  - Cyclic AMP acts as a second messenger and is a derivative of ATP.
  - AMP for monophosphate and cyclic because the phosphate is bridged between two molecules.
  - cAMP is the active from the messenger, AMP is not a messenger.
  - Adenyl cyclase turns on pathway by phosphorylating ATP to cAMP then phosphodiesterase hydrolyses cAMP to get AMP.
  - Adenyl cyclase is held in the membrane, G protein binds to it and turns it on and catalyzes the reaction from ATP to cAMP. Then the cAMP activates *protein kinase A* (found in many cells), cAMP binds specifically to *protein kinase A* to activate it and *protein kinase A* does specific things depending on cell your looking at. Then cAMP can be broken down by phosphodiesterase.
- The fight or flight response is initiated by adrenaline acting on its G protein coupled receptors.
  - Example on slide 70 is glucose released into the blood.
  - Activation of Protein Kinase A phosphorylates a kinase, which phosphorylates another kinase (a phosphorylation cascade).
- *What is the advantage of a phosphorylation cascade?*
  - It's easier to turn off; many control points and/or activate it at many places.
  - If one part of the pathway malfunctions, it is easier to fix one small step than one giant step.
  - It can amplify the signal, the possibility to amplify the signal at each subsequent step.
    - Rapid amplification of the signal, important because signaling molecules are low in supply.
- *Caffeine is a phosphodiesterase inhibitor. How might caffeine consumption affect the liver's response to adrenaline?*
  - Phosphodiesterase catalyzes breakdown cAMP to non-active AMP, so you'll end up with cAMP, which will give you a longer and stronger response. And in this case faster heart rate, and an over release of sugar.

| Point of Comparison | Receptor Tyrosine Kinase | G Protein Coupled Receptor |
|---|---|---|
| Receptor Structure | - One Transmembrane Domain<br>- An enzyme (tyrosine kinase) on cytosolic side | - 7 Transmembrane Domains<br>- Binding site for a G protein on cytosolic side |
| Intracellular on-off Switch | Self phosphorylates (to turn on), the kinase domain on the cytosolic side functions as the on-off switch | G Protein |
| Enzyme | Tyrosine Kinase | Adenyl Cyclase (gives second messenger) |
| Second Messenger | Not in the Pathway We Looked At | cAMP |
| Phosphorylation Cascade | Yes | Yes |
| Cellular Responses | Growth and Division | Glucose released from the cell (fight or flight response) |
| Chemical Messengers | Growth factors as the signals | Adrenaline |

- **Lipid Soluble Signals and Intracellular Receptors**
    1- Signals enter cells by diffusion
        a. E.g. steroid hormones
    2- Receptors are ligand-activated transcription factors
        a. Ligand binding domain
        b. DNA-binding domain
    3- Regulate gene expression
        a. Activated receptor binds to a response element.
    4- Cellular response
        a. Reflects genes regulated by the response element.

- Water soluble vs. lipid soluble signals
    - Water soluble
        - Membrane receptor
            - Signal binds on the outside
        - Activation of receptor activates transduction which leads to respons
    - Lipid soluble
        - Membrane receptor in the cell
            - Lipid enters the cell and binds to receptor
            - Changes to gene transcription
            - Response is in the nucleus.

- *Design an experiment to determine whether activation of the urea excretion mechanism of toadfish relies on a G protein-coupled receptor signal transduction pathway involving cAMP. Briefly describe the experimental approach that would be taken, the rationale for this experimental approach, and the expected results.*
  - Measure urea released by the fish, and manipulate the pathway, and if urea excretion changes than the pathway is involved in the excretion of urea. Then give the fish of coffee and see if urea release increases or if it is unaffected.
  - You can also introduce a non hydrolysable version of GTP, affect the pathway.
  - Inhibit or activate protein kinase A.

**Pop Quiz**
**Distinguish Between**

1- A G protein-coupled receptor and a G protein.
   a. G protein coupled receptor work together. G protein coupled receptor is an integral membrane protein, and has 7 transmembrane domains. And the G protein is a lipid-anchored protein. It is a monomeric receptor vs a trimeric protein (the G protein). G protein coupled receptor has a binding site for the G protein, and when the G protein attaches, it helps to turn on the G protein. The G coupled receptor turned on by a signal. The G protein stays attached to membrane, even when it separates into beta and alpha subunits.

2- A protein phosphatase and phosphodiesterase.
   a. A phosphodiesterase breaks down cyclic AMP to AMP. The proteins phosphatase dephosphorylate proteins, they remove phosphate groups.

3- Protein kinase A and receptor tyrosine kinase.
   a. Receptor tyrosine kinase has 1 transmembrane domain, found in membrane, phosphorylates tyrosine residues. Protein kinase A is found in the cell and is activated by cyclic AMP, part of a signaling cascade.

# Cellular Energetics

- **What energy is?**
  - Energy is defined as the capacity to do work.
  - Most of these types of work involve physical or chemical work.
  - Cells need energy to make these changes.
  - Ex. Whenever cells synthesize macro- or micro molecules requires energy, like the synthesis of glycogen is an energy consuming process.
  - **Mechanical work**, such as movement within the cell, like the cytoskeleton that has motor proteins that move along it requires energy.
  - Cells that move, such as amoeba, require energy because of the physical changes that occur.
  - **Concentration work**, such as moving solute against gradient.
  - **Electrical work**, ions are moved across membrane, and in addition to making concentration gradient, potential/electrochemical gradient is also made. This requires energy. Then that energy can be activated to activate muscle cells or transport nerve impulses.
  - Cells require energy for are specific to types of cells.
    - *Generation of heat*, in mammals and birds.
    - *Generation of bioluminescence,* is found in different types of cells, one of the nicest examples is the firefly, in the firefly there is an enzyme lasiferase, when it uses ATP, light is produced. Some jellyfish and toads are bioluminescent.
    - Only seen in a relatively small amount of cells.
- Most of our energy comes from the sun
  - **Photosynthesis**
    - Transformation of light energy into chemical energy. i.e. plants.
  - **Cellular Respiration**
    - Conversion of chemical energy from carbohydrates/lipids/proteins to ATP (catabolic pathways metabolism). i.e. animals
    - ATP can then be used to power reactions with in the cell.
    - ATP is the energy currency of the cell.
      - Mechanism to move energy around within the cells.
    - Glucose, or carbohydrates in general, fats, lipids or proteins used to generate ATP.
- **ATP Itself**
  - Composed of three separate components.
    - Pentose sugar, such as ribose.
    - Attached to first carbon is a nitrogen carbon, adenine
    - Last carbon, 5, is a string of up to three phosphate groups.
  - Nucleoside, sugar added to base, adenosine when adenine is there.
  - One phosphate group is monophosphate then diphosphate then triphosphate.
  - Nucleotides are also used as other things, not just as energy currency.
    - 5 different bases

        - Involved in cell signaling, GTP, cyclic AMP is a derivative of a nucleotide.
        - Building blocks of nucleic acids.
        - Helping to build the cytoskeletal elements.
    - **Hydrolysis of ATP releases energy**
        - *Charge repulsion*
            - Phosphate group carries negative charge; therefore nucleotides also carry negative charge. To form ATP must bring two negatively charged molecules, in order to form it must put in energy to overcome tendency of both negative molecules to come together. When you hydrolyze it energy released
        - *Increased entropy*
            - More ordered state to a less ordered state, two independent molecules that can move independently, increase in entropy means energy has been released.
        - *Resonance stabilization*
            - Phosphate group on its own is more stable than phosphate groups bound to each other. When phosphate group is on its own, electrons able to distribute over entire molecule, lowest energy state. When its bound to other group, electrons cannot distribute as much, energy increases. Allowing phosphate group to adopt most stable conformation.
    - ATP couples energy-yielding processes to energy-requiring processes.
- **Cellular Respiration**
    - *Glycolysis*
        - ATP production by substrate-level phosphorylation
        - Reduction of NAD+
        - Yields some ATP, produces enough energy to reduce NAD+ to NADH
        - Vitamin B essential to provide NAD.
        - NAD is a coenzyme that is found in the cell, so is FAD (come across later).
        - Glucose split into two three carbon molecules, pyruvate.
        - Occurs in the cytosol.
        - The pyruvate enters mitochondrion and oxidized into ‘acetyl coenzyme A’, which enters the citric acid cycle. Acetyl-coA is an input into the citric acid cycle.
    - *Pyruvate oxidation and the citric acid cycle*
        - ATP production by substrate-level phosphorylation
        - Reduction of NAD+ and FAD
        - Acetyl-coA is an input into the citric acid cycle.
        - At the end of glycolysis and citric acid cycle we have a few ATP.

- *Oxidative phosphorylation*
  - NADH and FADH2 oxidation
  - Electron transport drives ATP production by chemiosmosis.
  - Re-oxidizing them back to oxidized form and using energy to create proton gradient. That gradient is used to drive ATP production the chemiosmotic model. Proton gradient drives ATP synthesis.

- **Glycolysis**
  - Glucose catabolized to pyruvate yielding ATP and NADH.
  - 10 enzyme catalyzed reactions
    - Phosphorylation and cleavage
      - 6 carbon glucose molecule converted to two three molecules (phosphorylated), using two ATP
    - Oxidation yielding ATP and NADH
      - Each of those intermediates phosphorylated a second time, reduction of NAD+ to NADH, production of two molecules of ATP (substrate-level phosphorylation).
    - Pyruvate yielding with ATP generation
      - The phosphorylated molecule looses phosphate group, produces two ATP, and end up with two pyruvate molecules.
  - **Net gain:** of two ATP and two NADH and two H+
  - If oxygen is present, pyruvate enter the mitochondria. If oxygen is not present, one of two fermentation pathways occur:
    - **Lactate fermentation**
      - The pyruvate molecule accepts electrons from NADH and becomes a lactate molecule.
    - **Alcoholic fermentation**
      - The pyruvate molecule is first decarboxylated, looses it as CO2, and then that two carbon molecule gains the electrons from NADH becomes ethanol [important for bread (yeast uses this pathway), bear and wine, and goldfish (goldfish that are deprived of oxygen make use of this pathway, they are capable of turning on alcohol fermentation pathway, and the ethanol diffuses out of the gills].
    - These pathways both regenerate NAD+, that is the purpose.
    - *Fermentation involves the regeneration of NAD+ that was reduced during glycolysis. Why is this regeneration of NAD+ significant?*
      - In the absence of oxygen, the only way to get ATP is glycolysis, therefore the NAD+ is needed to carry out glycolysis to keep that pathway going.
  - In the presence of oxygen, the pyruvate will enter the mitochondria.
  - **Mitochondria**
    - *Outer membrane* contains porins
      - Permeable, more than the inner
      - Contains a type of protein called porins

        - Porins are like channels, they are quite large and non selective. Barrel like structure through the membrane that forms a large nonselective hydrophilic channel.
        - Pyruvate enters through the porins, but to get into matrix, need a pyruvate transporter.
            - Proton pyruvate co-transporter.
        - Porins found in many bacterial membranes, supports theory of bacterial past.
- *Inner membrane*
    - High surface are because of the folding, cristae.
    - 75% protein (pyruvate transporter, electron transport chain, ATP synthase)
- *Matrix* contains enzymes of pyruvate oxidation, citric acid cycle.
    - Find mitochondrial DNA and soluble enzyme and ribosomes.
- *Intermembrane space.*
    - This is the space, particularly in the folds, where you see proton gradient across inner membrane.
- *Discuss structure-function relationships at the mitochondrion.*
    - *Identify structural features.*
    - *Relate these structural features to the function they support.*
        - The cristae (folding of inner membrane), increase surface area of the membrane for more electron transport chains to increase ATP production.
            - More ATP production means more cristae.
        - The inner membrane is more selectively permeable, which allows a proton gradient to be established, to drive ATP production.
        - The porins allow pyruvate to get in and to allow ATP to get out, and a porin is a large nonselective channel.
        - The inner mitochondrial membrane makes it hard for NADH to get out, keeping it closer to the electron transport chain.
        - The matrix contains ribosomes and genetic material, which allows it to synthesize itself and enzymes for the citric acid cycle and pyruvate oxidation.

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**Pop Quiz**

1- How can a cell conserve energy?
   a. Shut down active transport to reduce ATP usage. Move to colder environment, to reduce metabolic rate, only works for organisms whose body temperature affected by environment. Dormant, don't move as much, reduce movement within cell or cell itself. Reduce biosynthesis.
2- What is substrate-level phosphorylation?
   a. Substrate-level phosphorylation is when an inorganic phosphate is transferred from a phosphorylated intermediate to ADP, to generate ATP.
3- What happens in glycolysis?
   a. Glucose molecule is broken down into two three carbon molecules, pyruvate, with a net production of two ATP molecules, and two NADH.
4- When do cells use fermentation? Why?
   a. Fermentation is used under anaerobe conditions to regenerate NAD+.
5- How does pyruvate enter the mitochondrion?
   a. Enters through the porins, which are on the outer membrane, allowing them to get to intermembrane space. Then pyruvate transporters are used to get pyruvate into the matrix, energy used to transfer pyruvate into the matrix.

 ***MIDTERM 1 ENDS HERE*** 

- **Pyruvate Oxidation**
  - Undergoes oxidative decarboxylation
    - Has one carbon taken off as $CO_2$, leaving a two carbon acetyl molecule, that is attached to coA, which gives acetyl coA, and it is an oxidative reaction. Produces an NADH molecule, it yields one molecule of $CO_2$ and NADH. Therefore, for each glucose molecule, two NADH molecules are produced, and two acetyl-coA molecules, which enter the citric acid cycle.
- **Citric Acid Cycle**
  - 8 enzyme catalyzed reactions.
    - Each with its own enzyme, each intermediate binds with acetyl-coA, and initial intermediate is regenerated.
  - First step, acetyl-coA added to first intermediate.
  - Citrate synthase, catalyzes first step, synthesizes citrate.
  - Converts four carbon molecule to oxaloacetate (first intermediate), is what the rest of the citric acid cycle is about.
  - Each turn of the cycle, yields 3 NADH, 1 $FADH_2$, and one molecule of ATP, this circle turns TWICE, for each glucose molecule, therefore double the numbers (6 NADH, 2 $FADH_2$ and 2 ATP for each molecule of glucose).
  - Takes place in matrix (so does pyruvate oxidation), oxidative phosphorylation takes place on the inner mitochondrial membrane.

- **Oxidative Phosphorylation**
  - Two step process, electrons transferred to oxygen, produces energy to pump hydrogen's into intermembrane space, and that gradient is used to drive ATP synthase, to produce ATP.
  - Chemiosmotic model.
  - Each complex, has a higher affinity for electrons, this is used to transfer the electrons down the ETC.
  - Complex II, is a single protein, and is not an integral membrane protein, it is a peripheral protein attached to the mitochondrial matrix side, where FADH2 binds, and releases electrons.
  - NADH electrons are captured by complex I.
  - For both I and II, electrons are transferred to complex III, through the coenzyme UQ (ubiquinone). Hydrophobic, found in interior of membrane.
  - Second electron carrier from complex III to complex IV, and is called cytochrome C, and is a peripheral membrane protein and attached to the membrane on the intermembrane space side.
  - Ultimately going to oxygen.
  - Energy released by these transfers, drive proton pumps in complexes I and IV. Ubiquinone also pumps protons. Releases protons in intermembrane space. Pumping protons from the matrix.
  - Cyanide acts on complex 4, prevents electrons from getting to complex 4, and ATP production is shut down because electrons cant get to electron.
    - Sodium azide also causes this.
  - In final step, proton gradient used to drive ATP synthesis, through complex called **ATP synthase**:
    - One component of protein imbedded in inner-mitochondrial membrane, and one part that extends into matrix.
    - Both parts capable of turning, the *stater stalk* connects both parts.
    - Protons move into part embedded in membrane and causes it to turn, motor/gear, causes bottom portion to turn and results in production of ATP.
      - Mechanism not fully understood.
    - Each *NADH* produces three ATP molecules through ATP synthase.
    - Each *FADH2* produces two ATP molecules through ATP synthase.
    - NADH → 10 protons pumped across membrane → 3 ATP
    - FADH2 → 2 ATP
    - Produce more ATP in prokaryotes (38 ATP) and eukaryotic cells 36 ATP produced, some energy is used to move pyruvate into the matrix of the mitochondrion.
    - 40 – 50% efficient, doesn't sound that high BUT car engines are only 10% efficient.
    - Energy released in all the intermediate steps as heat. This lowers efficiency.
    - Some cells take advantage of this.
      - *Brown adipose tissue*, heat-producing tissue found in newborn mammals and baby mammals.

- In the presence of UCP 1, protons pumped out into mitochondrial space. Protons pumped out and moved back in, completely inefficient, all energy used produced as heat.
- UCP 1 is a transporter, NOT a pump.

- *What experimental evidence could be gathered to support the chemiosmotic model of ATP synthesis?*
  - Break the intermembrane, and if you have protons leaking out, there will be no proton gradient, and no ATP going to ATP synthase, therefore not ATP production.
    - Poke holes in inner mitochondrial membrane, do it chemically, dinitrophenoyl.
  - Could check pH, electron transfer generate electrochemical gradient.
    - Intermembrane space should see fall in pH as protons gets pumped.
  - Later experiments localized membrane proteins.
  - Cyanide would shut down either model.
  - What if you were to drive ATP synthesis, by changing gradient? Lower pH, change gradient, ATP produced using ATP synthase.

- **<u>Regulation of Cellular Respiration</u>**

  - Serves to match ATP supply to ATP demands of the cell
  - Two basic ways in which cellular respiration is controlled.
    - Need ADP to produce of ATP, supply of ADP regulates. If there is lots of ADP around, not much ATP, therefore favour oxidative phosphorylation.
    - Control rate at which NADH and FADH2 are produced, control glycolysis, pyruvate oxidation and citric acid cycle.
      - Regulated by allosteric regulation.
      - Allosteric regulation refers to changes in enzyme activity, regulated by activator molecule that binds to enzyme.
        - Allosteric regulator binds to molecule changes conformation and starts process.
        - Allosteric inhibitors change its shape and reduce activity.
      - Feedback mechanism. Substrates that are used in these processes. If they are readily available ATP is in short supply, they act as allosteric regulators.
      - The products of these reactions can allosterically inhibit these rates. ATP is an allosteric inhibitor. Acetyl-coA can allosterically inhibit glycolysis.
  - **Hibernation and Torpor**
    - When animals encounter low food/temperatures, enter these states.
    - Model that was used is the ground squirrel, good hibernators.

- When they hibernate, lower their metabolic rate to less then 5% of an active molecule and body temperature to around 5°C.
- Metabolic rate falls, so does body temperature.
- They suppress mitochondrial activity.
- They isolated mitochondria from hibernating and active animals and compared their functions.
    - Measured several different things,
        - Substrate oxidation
        - ADP phosphorylation
        - Proton leak
            - If there are any ways for protons to leak across, this causes energy consumption, trying to make membrane less permeable to avoid leaks.
- Values for active animals are significantly higher than torpid animals (top two figures on slide 30).
- Ground squirrels actively shut down cellular respiration as a mean to conserve energy.
- Mitochondrial respiration drops by up to 88%.
- As they are about to enter hibernation, eat PUFA rich food.
    - Why eat those foods?
        - Because it is associated with more fluid membranes. They need this because there body is at 5°C, need fluid membranes.
    - If they have excess PUFA's almost as bad as not having any at all. Excess will cause them to not enter hibernation properly.

- *Suppression of mitochondrial respiration contributes to lowering of metabolic costs during hibernation and torpor. How else could cellular energy costs be reduced?*
    - Cannot be motile, during hibernation animal not moving around. Muscle cells not consuming energy, applies to skeletal muscles and heart, heart rate slows down.
    - Not eating, don't have to digest food; active transport slows down.
    - Less generation of new cells; shut down biosynthesis.
    - Kidney cells, filtering of blood shut down and neural cells, brain activity shut down. Neurons use lots of energy.
    - Lowering heat production, less energy consumed.

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**Pop Quiz**

1- Distinguish between ATPase and ATP synthase.
   a. ATPase removes a phosphate group from an ATP molecule and ATP synthase produces an ATP molecule by combining ADP with an inorganic phosphate.
2- Distinguish between oxidative phosphorylation and substrate-level phosphorylation
   a. Substrate level phosphorylation, phosphate group removed from intermediate to ADP. Oxidative phosphorylation is driven by electron transport and utilizes a proton pump to produce ATP; protons go down gradient through ATP synthase to produce ATP.
3- What is *uncoupling protein 1* and why does it matter?
   a. Allows for return of protons into mitochondrial matrix, and bypasses proton gradient that drives ATP synthesis, futile cycle that drives heat production. It is a transporter located on the inner mitochondrial membrane. Protein that functions as a proton carrier
4- What is allosteric regulation and why does it matter to cellular respiration?
   a. Allosteric regulation is when a compound binds to an enzyme and changes its activity. Can also activate an enzyme allosterically. It matters in cell respiration because many enzymes involved are subject to allosteric regulation by substrates in the pathway, to math ATP production to ATP demand.

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# The Cytoskeleton

- **Functions**
  - The provision of structure and support
    - Supports the cell
  - Intracellular transport
  - The positioning of organelles
  - The generation of force for cell movement
    - Movement of the cell itself, cilia, movement within the cell.
    - Any cuts that you would get would never heal, cytoskeleton involved in healing.
    - Cytoskeleton used for sperm to find egg.
  - Contributing to cell division

## Microtubules

  - Hollow tube, like a straw
  - 25nm found in all eukaryotic cells.
  - Dynamic, growing, shifting and changing shape.
    - Found in the cytoplasm
  - Microtubules that you find in cilia and flagella are called axonemal which are very stable
  - **Structure**
    - Made out of tubulin
    - Alpha and beta tubulin, form a heterodimer
    - Although the two elements of the dimer held together by non-covalent interactions, it is very stable.
    - *Can bind GTP*
      - The GTP that binds to alpha tubulin NEVER hydrolyses
      - The GTP that binds to beta tubulin CAN be hydrolyzed
      - When GTP is bound, dimer can be added to microtubule, when GDP is bound, less likely to be active, dissociates.
      - Dynamic
    - The alpha-beta dimers are simply stacked, and always stacked in same orientation.
      - As a result of that, the two ends are slightly different.
        - Minus end – the alpha tubulin dimer exposed.
        - Plus end – the beta tubulin dimer exposed.
    - To go from protofilament to microtubule, stack 13 protofilaments together, microtubules is 13 protofilaments stacked in a circle.
    - Plus end is called plus because it tends to grow, and vice versa.
  - **Dynamic Character**
    - GTP is bound to beta tubulin, the dimer tends to form microtubule, when it is hydrolyzed, much less likely to be in a microtubule, it falls apart/disassembles
    - Longer microtubule exists, more likely GDP bound

- Can add more rows; as time goes on, the likelihood of GTP hydrolyzing becomes increased, longer the microtubule exists the more likely the GTP will be hydrolyzed at the minus end of the microtubule; as this occurs, more dimers at the minus end have GDP instead of GTP; increases likelihood that they'll fall of at the minus end (shrinks at the minus end).
- Dimers added at the plus end bound GTP; minus end shrinks because it has GDP (increases disassembling). In cilia/flagella, the microtubule is stabilized.
- Rapid addition/loss; microtubules are very dynamic; see rapid addition of tubulin at the plus end but tubulin dimers are los at the minus end, eventually the rates catch up (TREADMILLING)
- In addition to tread milling, microtubules can shrink at the plus end → CATASTROPHE
- If plus end not growing fast enough, GTP at plus end will be hydrolyzed to GDP, nothing to stop plus end from falling a part.
- Plus end protected by addition of GTP dimers
- If tubulin dimers sit there for to long, hydrolyzed, CATASTROPHE occurs.
- Microtubules can grow or shrink at plus end, *the dynamic instability model*.
- Tread milling is growth at plus end and shrinking at minus end.
- **Colchicine** – Binds to tubulin dimers and prevents them from forming microtubules. Microtubules tend to break down
- **Taxol** – U trees, cancer treatment. Binds to microtubules and stabilizes them. The microtubules are no longer dynamic in presence of taxol.

- **Microtubules Organizing Centers (MTOC)**
  - The structure of a microtubule very complex, likelihood of forming spontaneously is rare.
  - MTOC increase this likelihood.
  - MTOC include centrosomes.
  - MTOCs include another form of tubulin, gamma tubulin that forms templates on which microtubules can grow. The minus end is ALWAYS anchored to the MTOC. This is how cell controls orientation.
  - You end up with minus end at MTOC and plus end extending out from the MTOC.
  - Centrosome is typically found in periods of cell division near nucleus, and serves as a MTOC.
  - Neurons, centrosomes near cell nucleus and plus ends extend down axons.
  - Cilia, plus ends extend down to the ends of the cilia.
  - Centrosome is made up of two centrioles at right angles to each other.

- Pericentriolar material in the center of the centrosome attracts gamma tubulin to bind.

- **Functions**
  - Movement of cell and within the cell, functions as highways within the cells.
  - Things that move along microtubules or microfilaments are motor proteins or "mechanoenzymes".
  - There is a motor domain, hydrolyses ATP, and a tail, which is a place to attach things to the motor protein.
  - Motor protein hydrolyzes ATP, and drags whatever is attached to it.
  - Although they are small, more efficient than a car, and the rate of movement is faster of a car.
  - Motor proteins found in pairs, so that they can walk instead of hop, because if they hop they could never reattach, fly fly away.
    - Each step involves hydrolysis of ATP
      - Provides energy for motor protein to move
  - **Motor Proteins**
    - *Kinesins*
      - Found in pairs, globular motor domain, and they have some kind of cargo attachment point.
      - They are plus end directed proteins, walk to end of microtubules.
      - Carry out bound cargo.
    - *Cytoplasmic Dynein*
      - Found in pairs, globular motor domain, and they have some kind of cargo attachment point.
      - They are minus end directed proteins.
      - Carry cargo back towards nucleus of the cell, inbound cargo.
    - Can be turned off, signal transduction pathways that turn them on and off.
- **Transport**
  - *Axonal Transport*
    - Tends to happen in cell body, transports down length of axon.
    - Involves motor proteins walking along microtubules.
  - *Chomatophores (in fish, amphibians, and reptiles)*
    - Animals have control of where pigment vesicle is located.
    - Pinpoints of pigment surrounded by light cell gives a light coloured animal.
    - Can be dispersed, dark coloured cell and therefore dark animal.
    - Paler colour in stressed animal, mechanism for animal to control coloration.

- Urea Excrettion in Toadfish
  - How does the signal transduction pathway activate the facicilated diffusion mechanism?
    - When it excretes urea, facilitated diffusion activated.
    - When the toadfish goes to get rid of urea, vesicles move to cell membrane and fuse with it, inserts transporters into plasma membrane and fuse, putting facilitated diffusion transporters where they need to be.
      - If correct, movement relies on microtubules.
        - Use colchicine, microtubules will break a part, and urea excretion would be reduced, and that's what happened. Plasma urea levels in fish were increased.
        - This suggests microtubules involved in excretion.

- **Cell Division and Microtubules**
  - Microtubules that extend across entire length of cell = polar microtubules.
  - Microtubules will attach to duplicated chromosomes at the kinetochore, there are kinetochore microtubules that attach to the kinetochores of the chromosomal pairs.
    - If not they are polar microtubule cells.
  - Chromosomes must get to two ends of the cell, driven by motor proteins associated with kinetochore. Not walking along microtubules, chewing up microtubule as they go, the microtubule disssintegrates.
  - Kinesin is involved, but it is bipolar Kinesin, two kinesin molecules that are bound together at the center. Kinesin will attach to two microtubules, one from each end of the cell, and walk in a plus end directed fashion, and when they walk tends to push it apart.
  - Net affect in plant cells is much the same.

- *Taxol is used in cancer chemotherapy. Why?*
  - Used as anti-cancer drug
  - Stabilized by taxol; whatever forms the microtubules, they stay in that form; in mitotic spindle they are no longer dynamic; effects at the cells in the body.

- **Cellular Motility**
  - Cilia and flagella arrangement the same inside.
  - Distinguish between them on external characteristics
  - Cilia = shorter than flagella at a length of about 10 microns
  - Flagella = longer than cilia at a length of about 200 microns
  - Typically only one to two flagella.
  - There will be many cilia.
  - Flagella always move the cell around
  - Cilia may move the cell, but they may also move fluid over cell, the cilia beat back and forth to move fluid over cell.

- Flagella, long undulation that goes along the length.

**Pop Quiz**
**True or False**

1- The building block of MTs is a tubulin heterodimer with two molecules of ATP.
   a. False, it is GTP not ATP
2- According to the dynamic instability model, MTs shrink at the plus end when the rate of addition of tubulin dimers is too slow.
   a. True
3- The centromere is a MT organizing center.
   a. False, it is a centrosome.
4- Kinesin transports cargo to the periphery of the cell owing to the typical orientation of MTs and kinesin's tendency to move towards the plus end.
   a. True
5- Cilia are shorter and more numerous than flagella, and use a short, stiff power stroke to move the cell or move fluid over the cell.
   a. True

- **Structure of The Cilia/Flagella**
  - What connects the doublets together is *nexin*.
  - The *radial spokes* between doublets and center of the axoneme.
  - Element that is the most important is the motor protein
    - *Axonemal Dynein* is responsible for this:
      - Dynein is attached to A Tubule in one doublet and walks along the adjacent doublet (the B Tubule) and it moves in the minus direction.
  - Protein connections between doublets.
    - Connections hold them together, motor protein tries to move proteins against the other, it causes bending.
      - The whole structure bends.
      - You see bending in one direction, then the other, then the other, giving rise to waving motions going back and forth.
    - Coordination causes movement.

## Microfilaments

- Smallest, only a diameter of 7nm
- Not a hollow tube like MT, string/thread form
- Made up out of different protein
  - Made out of actin, and it is a monomeric protein

- G-Actin (Globular Actin)
  - Central cavity binds ATP
  - Stack together get a filament or F-Actin
- F-Actin (Filamentous Actin)
  - Wind together make a microfilament
  - It is a polymer of G-Actin

- **Dynamic Character**
  - Actin with ATP and Actin with ADP have different binding affinities
    - ATP present actin added to filament
    - ADP present it tends to disassemble
  - ATP actin added to plus end, tends to grow
  - ATP tends to hydrolyze at the minus end, break down/dissociate
  - Growth at plus end, and tendency at minus end, TREADMILLING
  - Microfilaments a marginally more stable than microtubules
  - The cell wants to have control over where they form, there stability, how they grow, and that is controlled by Actin Binding Proteins, that regulate all aspects of microfilament formation.
    - Determine how quickly they grow, how stable they are (capping proteins), determine if they are in networks or bundles.
  - **Useful Drugs**
    - *Cytochalasin*
      - Prevents actin from binding to microfilaments.
      - Since they cant grow, tend to break down.
      - Derived from fungi.
    - *Phalloidin*
      - Stabilizes microfilaments, whatever arrangement they had, they'll keep.
      - Derived from fungi as well.

- **Functions**
  - Structural Support
    - Cell cortex, network of meshwork of microfilaments, beneath cell membrane, supporting cell membrane.
  - Allows cell to take on different shapes
    - Microvilli (microvillus), they are small finger like projections off cell membrane, used to increase surface area.
    - Cells that line intestine, used to increase absorption.
    - Inside a microvillus, bundle of microfilaments, parallel bundle, minus ends are at the base of microvillus, and the plus ends are up at the tip. Held by bundling proteins, and linked to plasma membrane.
    - A protrusion of cell membrane to increase membrane surface area.
  - At junctions between cells
    - Adherens junctions
    - Focal adhesions

- *Contrast and compare microvilli and cilia?*
  - Cilia are composed of microtubules and are mobile, where as microfilaments are stable and just used for structural support and to increase surface area.
  - Microvilli are composed of actin and cilia are composed of tubulin.
  - They both include a plus and a minus end, BUT the minus end of the microfilament hydrolyzes ATP and the minus end of the cilia hydrolyzes GTP.
  - Cilia are used to move the cell around or to move fluid over the cell, and microfilaments increase surface area, for example microvilli are used to increase absorption surface area for intestinal cells. The cilia are motile because they have motor proteins.
  - Cilia have a 9+2 arrangement and microvilli are just bundled.
  - They are both finger like projections of the cell membrane.

- **Microfilaments and Movement**
  - One way involves motor protein and one involves polymerization
  - **Motor Protein way of Movement**
    - Myosin's, long tail, and like kinesin, plus end directed motor.
    - Muscle contractions.
    - A single sarcomere, extends from Z line to adjacent Z line
      - Z line includes protein called cap Z, stabilizes microfilaments.
    - Myosin is organized into thick filaments, located in center of sarcomere.
    - Tails are anchoring myosin molecules together, and globular heads sticking out towards microfilaments. Walk along microfilaments.
    - The myosin heads called cross bridges, walk along thin filaments.
    - They do so by typical motor protein fashion.
      - Hydrolyzing ATP, providing energy for movement, binding to microfilament.
      - Called cross bridge cycle, myosin motor domain called the cross bridge.
      - Happen repeatedly as long as ATP is present.
      - There is a control element built into this, calcium ions.
      - Calcium is present myosin can bind to microfilament, when it is not present it cannot bind.
    - Within the sarcomere, myosin heads walking along thin filaments, they pull ends of sarcomeres together, two Z lines brought closer together. Another example of sliding filaments.
    - Very dynamic process.
    - Similar and smaller arrays in other cell types.
      - An example is in cytokinesis, in animal cells, cell splits into two daughter cells.

- **Cell Crawling.**
  - Cell moving on its own, amoeboid movement is an example and another example is phagocytosis.
  - Lamella podium, large plate-like projection.
  - Filapoida, small plate-like projection.
  - Basic process relies on actin polymerization.
  - Initial projection is achieved by actin polymerization.
  - Actin filaments grow, give protrusion, anchor and cell body gets pulled in utilizing motor proteins. Initial part relies on polymerization.

- *Design an experiment to determine whether vesicle movement in a cell relies on myosin or kinesin motor proteins. Briefly describe the experimental approach that would be taken, the rationale for this experimental approach, and the expected results.*
  - Myosin only walks on microfilaments, get rid of them, and movement stops, it had to be on microfilaments, but if movement continues, then it must by kinesin. To confirm this, break down microtubules, and if it stop then you know for sure it was kinesin.

## Intermediate Filaments

- Intermediate in diameter.
- Structurally, rather different, made up of proteins that are fibrous, long string like proteins, instead of globe like proteins.
- To build in take to fibrous proteins, wind them together, gives us a dimer, put two dimers side by side, a protofilament. Staggered arrangement is an intermediate filament.
- Whole range of proteins that can be used to make them. Made up of 1 to 60 proteins, one of the most common is keratin.
- Strong rope like cytoskeletal elements, not as dynamic, more stable.
- Functions reflect stability, based on structural support.
- Only found in cytoplasm of animal cells, and found in nuclear membrane of all eukaryotes.
- Role in cytoplasm is structural.

- *Example of where they are found:*
  - Play a structural role outside the cell, like the slime in hagfish.
  - Lacks jaws.
  - Hagfish, when disturbed, produce large quantities of slime. Can drown themselves in there own slime. Purpose of the slime, its though to be anti-predator mechanism.
  - What's interesting about slime, it is a mix of proteins. Two different components, glycoproteins and intermediate filaments.
  - Slime glands all down length of fish, one cell produces glycoproteins, and the other type of cell releases a wound up intermediate filament. When the hagfish releases slime, intermediate bundle released with glycoproteins.

Mix with seawater and smashing of fish, and intermediate filaments mix with glycoproteins and produce slime.

**Pop Quiz**

1- Distinguish among actin, tubulin, and keratin.
   a. Keratin found in intermediate filaments, and actin is found in microfilaments and tubulin is found in microtubules. Globular actin molecules; two tubulin building blocks form microtubules and is globular; and keratin is a fibrous protein.
2- What is the crossbridge cycle and why does it matter?
   a. The cycle refers to myosin motor protein in muscle. Refers to the motor protein cycle of myosin. Refers to myosin in muscle reaching out from thick filament to walk along thing filament. It matters because without it muscles do not contract.
3- What is the sliding filament model and why does it matter?
   a. Bigger scale, looking at level of sarcomere, instead of individual myosin molecule. Refers to actin microfilaments sliding over myosin thick filaments. Because of sliding filaments, sarcomere shrinks. Z lines pulled in towards into the middle.
4- What structural features of MFs and MTs contribute to their dynamic nature? Why is this dynamic nature important to their function?
   a. Made up of small units, and held together by non-covalent interactions. Proteins are held together by non-covalent bonds, allowing them to be dynamic. What causes them to break down, the hydrolysis of the nucleotide that is bounded to them. When it is hydrolyzed, changes shape of building block so it can no longer stay attached. Formation of mitotic spindle, must be built up and removed again, and also the actin and myosin contraction that pinches off the two cells. Need to put highways where you want things to go. Cell gliding/crawling.

*AP.PNP is a non-hydrolysable analogue of ATP (modification of the phosphate groups prevents ATP from being hydrolyzed to ADP). How would use of AP.PNO in a cell affect…*

*a) Microfilaments?*
   *a.* Actin binds ATP, when ATP bound, polymerize to form microfilaments, cannot hydrolyze, microfilaments will not break down. Effect is to stabilize microfilaments in the cell.

*b) Microtubules?*
   *a.* No effect, nucleotide is GTP.

*c) Intermediate Filaments?*
   *a.* No effect, don't bind any nucleotide.

*d) Motor Proteins?*
   *a.* Motor proteins hydrolyze ATP for energy to move, everything would grind to a halt.

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## Extracellular Interactions

- **Plant Cell Walls and Plasmodesmata**
  - Rigidity and protection, and regulates permeability.
  - Structure involves 3 classes of molecules
    - *Structural fibers*
      - Provide mechanical strength, such as cellulose microfibrils.
      - Cellulose long chain of glucose monomers, covalently bonded together.
      - Cellulose is an un-branched chain, gives a linear molecule.
      - Can stack a number of cellulose together = cellulose microfibril → gives structural support to cell wall.
      - Unlike most components of extracellular structures, cellulose is synthesized on outside of cell.
        - Integral membrane proteins in plasma membrane, go to external, and synthesize molecules.
    - *Matrix*
      - Polysaccharides (e.g. hemicellulose, pectins)
    - *Adhesive Molecules*
      - Pectins
        - Carbohydrate, lots of negative charges, attracts water, jelly like mass.
        - Gives plant cell wall matrix.
        - Cell walls of adjacent cells held together by pectins.
    - *Which of the following treatments would affect the pattern of cellulose fibrils in the cell wall?*
      - Treatment of the cell with colchicine.
        - Need microtubules to guide the cellulose to give strength to where it is needed.
        - Microtubules help orient the enzymes.
  - **Plasmodesmata**
    - Lined with plasma membrane.
    - Route for ions, small molecules (e.g. nucleotides, small RNA molecules, etc…)
    - Allows two cells to be in chemical communication with each other.
    - Whole through cell wall, lined with plasma membrane.
    - To cells cytoplasm's in contact with each other.

- **Animal Cells: Cell to Cell Connections**
  - Variety of types of cell to cell connections:
    - ***Adhesive junctions***
      - Anchoring junctions
      - Hold cells together, in an animal cells, many cells held together, main junction types are adhesive/anchoring junctions.

- *Cadherins*
  - Integral membrane proteins, large extracellular structure, holds two cells together.
  - Transmembrane proteins.
  - Inside of the cell, cytoskeleton helps to support
- *Desmosomes* (like buttons)
  - Strong points, strong structure
  - Connect to IFs
  - Protein plaque for strong adhesion
- *Adherens* (like velcro)
  - Weaker than desmosomes
  - Connect to MFs
  - Small areas or continuous zones of attachment in epithelial.
  - Fewer linking proteins, still strong but no dense structure of peripheral proteins.

- ***Tight junctions***
  - Seal spaces between cells
  - Sealing junctions, seal spaces (paracellular space) between cells).
  - Don't want things to get through that space, like intestine, stomach, blood vessels, different fluids on either side, don't want them to mix.
  - Always between cells.
- ***Gap junctions***
  - Communicating junctions which are like Plasmodesmata.
  - Have a rather different structure, made of protein complexes, integral membrane proteins, connexin's.
  - Membranes come close together, connexin's form non-covalent bond, creating cytoplasmic channel form one cell to the other.
  - Gap junctions in animal cells, smaller than Plasmodesmata.
    - Nucleotides can go through, but RNA cannot.
  - Electrical and chemical communication.
  - Heart wouldn't beet without them; allow electrical communication from one cell to the next.
    - Neurons and muscle cells.
      - Best example is the heart.

- **The Extracellular Matrix of Animal Cells**
  - Bone, cartilage, connective tissue, basement membrane, cornea.
  - Secreted by cells it surrounds and organized locally.
  - Plant cell wall and animal extracellular matrix, lots of similarities of how they are put together.

- Structure involves 3 classes of molecules:
  - *Structural Proteins*
    - *Collagens*
      - Provides strength
      - Like cellulose, it is a linear molecule, get fibrils.
      - Made up of three collagen molecules; a trimer. Then they are stacked together to give collagen fibrils.
    - *Elastin*
      - Need flexibility, like in skin; blood vessels that expand and shrink; lungs, that inflate.
      - Protein that gives elasticity.
      - Somewhat unusual, individual molecules held together by covalent bonds, pull on it doesn't break apart, but release pull snaps back to original shape.
  - *Matrix*
    - Proteoglycans (protein-polysaccharide complexes)
      - Carry strong negative charge (like pectin in cell wall), attract water molecules, gives us hydrated matrix.
  - *Adhesive Glycoproteins*
    - Fibronectin, for example.
      - Glycoprotein, capable of binding both to proteins of extracellular matrix and membrane proteins, called integrin's.
      - Don't hold cells together!
      - It is a type of adhesive protein.
- *Integrins*
  - Integrate ECM with cytoskeleton
    - Extracellular binding site for adhesive glycoproteins.
    - Intracellular binding sites for linking proteins.
  - Focal adhesions and hemidesmosomes.
    - Connections between cell and ECM
    - Membrane protein is integrin (not cadherin)

**Summary**

| Organism | Extracellular Structure | Structural Fiber | Matrix | Adhesive Molecules |
|---|---|---|---|---|
| Animal | ECM | Collagen | Proteoglycans | Fibronectin |
| Plant | Cell Wall | Cellulose | Pectins | Pectin |

*Cell Wall Composition:* Animal Cells → Proteins | Plant Cells → Carbohydrates

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# DNA & RNA Structure

**General Overview**

- Most basic structure is the DNA structure, double stranded in cells most of the time. Not double stranded during replication.
- Adenine, Guanine, Thymine, and Cytosine are the four common bases.
    - During replication, only taking about these four bases, only found in natural DNA.
    - Right after DNA synthesis, these can be chemically modified.
        - Same thing happens in transcription, when taking about RNA.
- Uracil replaces Thymine in transcription.
- Guanine and Adenine are PURINES.
- Thymine and Cytosine are PYRIMIDINES.
- All of these are found in primary DNA and primary RNA (the bases).
- Bases are heterocyclic, aromatic rings.

**Basic Experiments that DNA is the Genetic Information within Cells**

- *Transformation experiments*
    - Griffith's Experiment
        - Two strains, S and R; refers to how they looked under microscope.
        - S cells are virulent/lethal, and R cells are nonvirulent/nonlethal.
        - Heat and activated the lethal S cells, release the contents, it was no longer lethal.
        - Transforming Principle, it is now called transformation.
        - Refer to Slide 6
    - Experiment of Avery-MacLeod-McCarty
        - Did a series of treatments with the transforming principle.
        - Took replicates of lysase and treated with extracts, treat with protease, degrade protein, and if protein was transforming material, loose transformation, but it wasn't the case.
            - Conclusion was that the transforming principle was not protein, and if it worked properly then it would degrade and you would not get transformation.
        - Next using RNAse, which degrades RNA, and still had transformation.
        - Next using DNAse, which degrades DNA and lost transformation.
        - Therefore, DNA is transforming principle.
        - Today transformation is taking up DNA; DNA gets incorporated, and is stable. When cell reproduces, it reproduces with the new DNA as well.
- *Chargaff's rules*
    - A with T and G with C.
    - Experimentalist, interested in DNA and components of the bases.
    - Developed methods to degrade DNA to A, C, T, G and look at there properties.
    - Got ratios of how much there are.

    - Found out A and T percentages are the same and C and G are the same percentages.
    - Then he looked at different organisms, even percentages were different, A and T matched up as well as C and G.
    - Hence, base pairing.
    - However, occasionally, Chargaff would make some DNA, or some bring DNA to him, he would find exceptions, and Watson and Crick would have to ignore them so that their model would still be correct.
- *Phage infection experiment*
    - Bacteriophages affect bacteria.
        - Chosen virus with this shape.
        - Works by binding to host, it injects its DNA, and protein part stays outside. DNA alone codes for all the genes so that virus gets made.
            - DNA leads to production of more viruses, by getting replicated and transcribed.
    - Grew a culture, labeled S-35 (found in protein not in DNA), grew another culture P-32 (found on the DNA, DNA labeled with this), can monitor the DNA and protein differently, by labeling them differently.
    - Only find the radioactive P-32, showing that the DNA got replicated and not the protein, if protein would have been, we would have seen S-35. This suggests that the protein does not get inside.
    - Supports the transformation experiment.
    - Here you have viable phage, and the DNA they inject is the only thing needed to replicate more viral phage.
    - Main caveat, if they have chosen virus with different properties this may not have worked.
    - Transformation
        - Separate DNA from protein, DNA can be transformed, get same result for virus.
- *X-ray analysis*
    - One of the most complicated to explain.
    - Taken a picture of DNA, thought it had to do with X-Rays.
    - Maurice Wilkins, had a collaborator working in lab, and she took DNA samples and blasted them with X-Rays, and noticed a pattern.
        - Somewhere diffracted, noticed diffraction patterns.
    - Watson and Crick (recognized fiber diffraction picture was a helix) looked at this and new immediately what it was.
    - Fiber diffraction, DNA being stretched out.
    - Measure distance from center, works out to be a base-to-base difference of 3.4 Angstroms.
        - Pattern does not fall on x- and y-axis, fall on about axis of about 45°.
    - Looked like a helix.
    - Conclusion, had a helix, bases pairs separated by small distance, and had 10 of them before it repeated again.
        - Could not tell any detail using this picture.

- *Standard model called "B-DNA" and other DNA forms*
  - Why do we need a standard model?
    - When we say there is a standard human genome, there is something we can refer to.
  - Natural DNA forms a right-hand helix
  - The two strands are "anti-parallel" (read 5' to 3' but in opposite directions).
  - Not a perfect spiral, identify regions that are further apart (Major Groove) and regions that are closer together (Minor Groove).
  - Anti-Parallel plays a key role in storing information and replication.
  - DNA structure is NOT always the same.
    - Make different forms in the test tube, but is it inside the cell.
  - **A DNA** is a bit thicker and grooves are a bit different, still right handed, angles different.
  - **Z DNA**, zig-zag and not smooth, and it is a left-handed helix. Still have major and minor groove, but angles different from standard model.
  - Are the different forms inside the cell, and is it inside the cell at different times of the cell cycle?
  - Some viruses have double stranded RNA.
    - When DNA being transcribed into RNA, part will have DNA and RNA, RNA-DNA hybrid at some parts.
  - A forms do occur inside the cell.
  - Z DNA associated with G, C rich DNA. People made antibodies that react with Z DNA and they found it in cells. It could exist inside the cell.
  - Microheterogeneity → Different forms of DNA within cells.
- *Model Building*
  - Variety of strange structures but do they exist in nature.
  - Can cause DNA to transform into loop structure.
  - G Tetraplex is hydrogen bonded.
    - All made up of G bases
  - DNA Aptamer can recognize specific parts of proteins. Functions like protein.
  - Structures are not fixed, change on conditions.
    - Going to change during replication and transcriptions
    - Ends are going to be different.
- *The modern "synthesis"*

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**Questions from Class**

1- Using table on slide 14 can your create a hypothesis about
   a. The experiment of Avery-MacLeod-McCarthy, the 'transforming principle was DNAse-sensitive'. What would conclude if in a similar experiment done today the; ""transforming was principle RNAse-sensitive"?; was "protease-sensitive""?
      i. If RNA sensitive, it degrades RNA, and if you do not get transformation, the transforming principle must be RNA. Therefore, it must be a virus or viroid.
      ii. Protein must be the transforming principle. Therefore, it would be a protein; it is a prion.
   b. The Experiments of Chargaff, A was not the same as T; the amount of G was not the same as C. There are multiple possible answers.
      i. Single stranded → DNA virus → if they were looking at ATUG then it would be an RNA virus.
      ii. If he did experiment with RNA, even if double stranded, the U would not appear as T, not quantified in the same way.
      iii. If you have GATC, and bases are modified, different chemically, hard to quantify. DNA in bacteria is modified, and in everything is modified. Some viruses have modified DNA. Modifications control something. If Chargaff took modified DNA, would not match up.
   c. The experiments of Hershey-Chase, both P-32 and S-35 were found in progeny?
      i. Both parts have gone in, and both parts could be reassembled and be reused.
      ii. Protein gets phosphorylated, then it was
2- What DNA helix is this? L or R?
   a. Left

- **The Basic Structural Forms of DNA**
  - More DNA loops than virus, more DNA in bacteria than virus.
  - Non-histoned protein, makes up structure which DNA can fold on → scaffold or matrix. DNA attached to it by proteins. Structure is bound to nuclear matrix under nuclear membrane.
- RNA structures are highly organized
  - Primary (5' GGGCGUG…etc)
    - Order of the bases on the DNA, molecular biologists have a short hand notation; G=G-ribose monophosphate, run of G → don't know if its RNA or DNA; U here indicates that it is RNA.
  - Secondary
    - Has regions that are double and single stranded.
    - Has stems that are hydrogen bonded.
    - Red dots indicated modified bases.

        - First RNA example has a lot of different bases, for example inocine (resembles A or G, is a purine).
        - Emphasizes hydrogen bonding and has stems and loops
    - Tertiary
        - 3D Structure
        - In tertiary structure, folds up and forms hydrogen bonds not seen in secondary structure.
        - Amazed because some interactions between sugar and the base.
            - Ribose sugar can form hydrogen bond.
        - Is important for correct binding of proteins.
        - Cannot be predicted
    - Slide 19 is a secondary structure.
    - Difficult to predict secondary and tertiary.
    - Secondary emphasizes a lot of bases that have been modified
        - Hundreds of them in RNA.
- **The Basic Structural Forms for DNA and RNA**
    - **Post Replication**
        - 5 Methyl C is important in regulation of eukaryote genes.
        - Control of genes from generation to generation → epigenetics
        - Importance in final structure of DNA/protein complex
    - **Post Transcription**
        - Proteins are made they are modified, by addition of something else.

- Many structures for DNA, DNA Protein, RNA and RNA Protein in the cell.
- Structure are dynamic, changing based on what's happening in the nucleus. Ex) condense to fit in the nucleus → de-condense to get to the genes and transcribe.
- Modification to bases
    - One modification Cytosine at 5 position can put methyl there, change regulation, genetics.
    - Chemical modifications
- **TRANSFORMATION DEFINITION**

In molecular biology transformation is genetic alteration of a cell resulting from the direct uptake, incorporation and expression of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membrane(s).

**Questions**

DNA and RNA Structures:

1. What is the possible role of transformation *in vivo*?
    a. Assuming transforming principle is DNA, what is the role in life (in vivo). Its role of transformation in vivo is genetic diversity, the DNA contains genes, taken up by conjugation (between bacteria), bacteria to plants, eat plants, possible. All of these are biological molecules (contains nitrogen, carbon), plants need these to grow, DNA massive source for things to grown on.

2. Is DNA always the same structure?
    a. No, because there can be A Form and Z Form.
3. What is microheterogeneity?
    a.
4. What are the different possible RNA structures?
    a. Three RNA sturctures: primary(Linear), secondary (Hyrdorgen bonds, stems and loops) and tertiary (Three dimensional).

# Chromatin a Dynamic Structure

- DNA is organized by a repeating structure.
- **The Basic Principles**
  - DNA Packaging involves specific proteins.
    - Very few of them, because folding and packaging used by repeating subunits.
    - Using principle of repeating units, reduce the amount of proteins; use same mechanism regardless of where you are.
    - Need a lot of proteins not a lot of genes.
  - Repeating Subunits
    - Don't need specificity for DNA sequence.
  - Complex structures based on simpler structures
    - Fold up simpler structure
      - Hierarchical organization
    - In Eukaryote hierarchy of structure
      - Simple → Nucleosome
      - Second Level → Solonoid
      - Twist solenoid get to another level
      - So on and so one until you get to histones and nonhistones.
  - Subunit assembly must be dynamic to allow for metabolic functions to occur, has to be dynamic
    - Also going to imply that it is controlled someway, only going to happen when you want it to happen.
  - Protein modification can affect chromosome structure.
    - Proteins that form basic structure, when modified, or others that lead to modifications of these, cause changes in structure.
    - Can now predict some of these changes, the histone code.
  - In eukaryotes this is called the "histone code"
  - *Viruses*
    - Use basic proteins that may be a part of the virus particle (capsid).
    - Most common organism on the planet is a virus, affects the most common living thing, the bacteria.
  - *Bacteria*
    - Use basic proteins to form a series of loops called nucleoid.
    - Relatively simple structure, amount of folding is relatively small, don't have to make up as compact.
    - Use basic proteins such as "HU", "IHF", "H-NS".
  - *Eukaryotes*
    - Use basic proteins called histones AND a hierarchical organization.
  - Why are all these proteins basic?
    - Have positive change at neutral pH and DNA is a polyanion.
    - Basic because in both meaning of the word, basic because they are fundamental to the organization; basic because DNA is negatively charge, going to have positive charges, amino side chains.

        - Just key bases get modified.
    - DNA is a polyanion, only way to compact is neutralize the charge and salt.
- **DNA Organization in Eukaryotes and Prokaryotes**
    - In prokaryotes, organization simple, less DNA, non-defined structure, follow same basic principles.
    - *Histones* pack eukaryotic DNA at successive levels of organization.
    - Many *nonhistone* proteins have key roles in the regulation of gene expression.
        - Nonhistone structural proteins, can still bind to DNA, involved in matrix.
        - Some are a part of chromatin and non-structural.
- **The Bacterial Chromosome**
    - Sizes range from 500 to 10,000 kilobase pairs (kb)
    - Called a nucleoid, to differentiate from chromosome
        - One bid circle, all twisted up.
    - Replication or transcription, structure has to come a part and then refold. Begins from single spot at proceeds in both directions.
    - Plasmids (in many bacteria) replicate independently of the host chromosome.
        - They are not part of the structure, only the chromosome.
- **The Bacterial Nucleoid**
    - Each loop is a double stranded DNA.
    - Naming of proteins not as logical as histones.
    - More DNA more loops, less DNA less loops.
        - Loops secured by matrices (protein-protein and protein-DNA interactions).
    - Basic proteins, are binding to DNA, double stranded DNA is covered by proteins (supercoiled)
    - Secured at base by basic proteins.
    - Basic in terms of charge, loops are secured by matrix type at the bottom.
    - Consequences of simple model
        - To replicate, you must remove matrix, proteins on DNA, to go further remove the next matrix and so on, structure has to come apart and remove proteins, and then reform the structure.
        - Doubling amount of DNA and doubling amount of protein to form same structure in daughter.
    - In test tube you can isolate complexes, or break the loop with restriction enzymes. *Topologically independent*, one loop is independent of the other because proteins cause them to not interact with each other.
- **Eukaryotic Chromosome**
    - Histone and nonhistone proteins.
    - First Level
        - The parts of the histone that acts with the DNA are on the outside of the protein.
        - The amino acid residues that are basic are on the outside.
    - The Next Level is the Solenoid

- Nonhistone proteins help control the expression of individual genes.

- **Nucleosomes and Chromatin Fiber**
  - Nucleosomes form a left-handed helix
  - Do not have histone H1
  - This nucleosome consists of 8 proteins, octomer. Two copies of each of these (H2A, H2B, H3, H4).
  - All nucleosomes are octomers.
  - Core nucleosome is the octomer plus the DNA wrapped around it (approximately 140 base pairs).
  - Nucleosome would be from one position in the repeat to the next position (approximately 200 base pairs).
  - A 10 nm fiber is when you remove H1, and see beads on a string.
  - H1 is involved in the formation of another structure.
- **The Nucleosome (lowest level) is Conserved**
  - *Nucleosome Parameters*
    - Adding histone H1 brings nucleosomes together to form the 10nm fiber.
    - 2 each of 4 histones named H2A, H2B, H3 and H4 (the "octomer")
    - Need H1 to make it look like a continuous fiber, remove H1, beads on a string.
    - DNA (~146 base pairs) is wrapped around the outside.
    - Sequences that are distant can now be closer together!
      - Reality of what occurs in side the cell.
      - Distant apart depends on structure.
- **The Solenoid or 30nm Fiber (the next level)**
  - 6 to 8 nucleosomes per turn.
  - This structure is not as precise as a nucleosome; it is made up of nucleosomes.
  - Not a well-defined structure.
  - In electron micrograph, see a fiber, nucleosomes close together, around a 30nm fiber.
  - Sequences that are distant can now be close together.
- **Higher Order Structures: the Looped Structure and the Chromosome**
  - Solenoids used to build this level.
  - Have looped structures, central bit and loops come out; loops are the solenoid.
  - Because situation is dynamic, don't expect it to maintain position during cell cycle.
- **General Properties of Chromatin**
  - In the interphase cell, chromatin can be experimentally divided into heterochromatin and euchromatin.
  - *Heterochromatin*
    - Dark structures on slide 14 are heterochromatin
    - More highly condensed
    - Genes are generally inactive
      - Constitutive

        - Facultative, might be heterochromatin but in a different cell type it is less condensed.
    - DNA is more highly methylated
    - 15% or higher of genome
    - Centromeric and telomeric regions
        - Usually highly condensed.
    - Highly enriched in DNA repeats.
        - Highly condensed, plants have more of them
  - *Euchromatin*
    - Less dark structures on slide 14 are euchromatin.
    - Less condensed, more dispersed.
    - Genes are generally active.
        - Regions in which genes are active.
        - Active either means it is being transcribed, or in a structure that can potentially be transcribed.
    - DNA is less highly methylated.
        - Transcribed genes are less methylated.
- **Chromatin Remodeling**
  - Eukaryotic DNA wraps around histones, to form nucleosomes, etc.
  - *Promoters* are less accessible.
    - A promoter is a region of a DNA molecule that forms the site at which transcription of a gene starts.
  - *Chromatin Remodeling* makes gene promoters more accessible.
    - Activators recruit remodeling complexes that displace nucleosomes.
        - A remodeling complex is a collection of proteins and enzymes that leads to modification of histones, and the structure where histones are bound to is removed.
        - Need a remodeling complex to build it up again.
    - Activators recruit enzymes modify histones and loosed their association with DNA. The best-studied system is *actelyation of Lysine.*
    - Other modifications can include methylation of Lys, methylation of ARG and His, phosphorylation of Ser and His.
  - Methylase to go one-way demethylase to go the other way.
- **The Histone Code and Consequences**
  - *Euchromatin*
    - Higher histone acetylation
    - Lower histone methylation
    - Lower DNA methylation
    - Active genes
  - *Heterochromatin*
    - Lower histone acetylation
    - Higher histone methylation
    - Higher DNA methylation
    - Inactive genes.

    - Acetylation describes a reaction that introduces an acetyl functional group into a chemical compound.
- **Chromatin Structure: Summary**
    - Structure of chromatin influences transcription, replication, repair and recombination.
    - Chromatin remodeling can convert accessible to inaccessible.
    - Remodeling is influenced by alteration of the cell physiology
    - Remodeling influences the expression of many genes, thus the "transcriptome".

# Replication

- **Central Dogma**
  - DNA (Replication) → RNA (Transcription) → Protein (Translation)

- Copying not making a copy, replication is semi conservative, and synthesis follows base pairing rules.
- One strand is made continuously and one strand is made discontinuously.
- Synthesis is always 5' to 3'
- DNA polymerases are the primary enzymes of DNA replication.
  - It is a multi-protein complex, only interested in part that does synthesis.
- DNA replication begins at replication origin [*ori, ars (in eukaryotes – autonomously replicating sequence)]*.
- Cannot replicate ends of a chromosome, it is a problem.
  - Use a special system called a telomerase to solve a specialized replication problem at the ends of linear DNA.
  - Some organisms don't linear DNA, which is a problem; the organisms have circular DNA.
- All DNA synthesis, starts with an RNA primer, give it a piece of DNA, can start making RNA.

- **DNA Polymerase cannot start synthesis *de novo (starting new)***
  - DNA polymerases require a primer to initiate synthesis.
  - RNA polymerases can synthesize a primer (RNA) *de novo*.
    - RNA polymerases are used to initiate replication.
  - This leads to other problems that relate how the cell deals with these short RNAs.
  - DNA polymerase cannot synthesize circular single stranded DNA, because there is no primer. The primer can be a suitable DNA molecule, one that has a 3' OH, which can be extended.
  - GAP REPAIR – Filling in a little spot
    - DNA Repair, first thing enzymes do is create a gap (remove problem) then fill it in. Enzymes create gap, then gap repair.

- *Why do you need 3 types of DNA synthesis for a chromosome? (and multiple DNA polymerases in the cell?)*
  - Need different polymerases, to do different types of polymerizing with different substrates.
  - Location and specialization
- *What happens when you need more or faster DNA synthesis?*
  - Can only go a certain speed, cannot go faster that optimal rate.
  - Start from multiple origin points.
- *Do all DNAs have ends? What happens to them?*
  - Answered later on…
- *When DNA replicates what happens to the chromosome?*
  - Answered later on…

- *What signals the cell to replicate?*
  - Answered later on…

- **Enzymes of DNA Replication**
  - Helicase – Unwinds DNA
  - Primase – Synthesis RNA primer (starting point for nucleotide assembly by DNA polymerases.
  - DNA polymerases – Assemble nucleotides into a chain remove primers
  - RNA primers
  - Telomerase

- **Assembling Antiparallel Strands**
  - Leading strand is continuous, as long as the helix keeps opening up, can go on. Can continue right till end of molecule.
  - Bottom strand is discontinuous, open up a bit more, prime some more, then go from there. Discontinuous, open up prime, synthesis, then prime and so on…
  - Leading strand starts from 3' end and runs 5' to 3'
  - Lagging strand starts from 5' end and runs 3' to 5'
  - Synthesis has to be from 5' to 3' whether it is RNA or DNA.

- **Enzyme Activities 1**
  - Once DNA is unwound, single stranded structure (can be stem-loop), so we need proteins (SSBs) to stop DNA from winding back up again.
    - In reality there are a lot of SSBs.
  - We can synthesize from 5' to 3', open → prime → synthesize → open → prime → synthesize → and so on…
  - Okazaki fragment, each one is primed by little RNA.
  - Need a lot of nucleosomes, if DNA is doubled (double the amount of nucleosomes).
  - As helix is opened, push forward more and more until it becomes to impossible to open, need enzymes to open further.
    - Topoisomerases solves this problem
      - Enzyme that regulate the over winding or under winding of DNA.

- **Enzyme Activities 2**
  - Blue ring is a clamp; collection of protein to make sure DNA stays together.
  - A whole complex opens, prime again and come back.
  - Length of Okazaki, 500 to 1000 bases.
  - Activity that removes RNA primer is an RNAse, that RNAse has to recognize particular substrate, one strand RNA and one Strand DNA.
  - One polymerase starts DNA synthesis, and one fills in the gap, specialization.

- **Midterm Type Questions**
  - *Why are there multiple copies of histone encoding genes?*
    - A vast number of histone copies is needed during early development, requiring several hundred genes.
      - General rule need more of something have more genes, gene dosage effect.
  - *How does the association of DNA with histones change during replication and transcription?*
  - *What are the basic principals for the structure of the bacterial and eukaryotic chromosomes?*

- **Telomeres**
  - Not added once, could be added a thousand times.
  - Short sequences repeated hundreds to thousands of times (TTAAGGG)
  - Repeats protect against chromosome shortening during replication.
    - Purpose is to protect against chromosome shortening.
  - Chromosome shortening is prevented in some cell types which have a telomerase enzyme (adds telomere repeats to chromosome)
  - Dynamic, lot of functions associated with DNA, such as replication, transcription and translation.
  - Telomeres, cannot by the model we discussed, replicate to the end, can on one strand but not on the opposite. That synthesis has to be RNA primed, and remove RNA primer, cant fill it in, fix by filling in repeats (TTAGGG…)
  - Happens at both end to protect at chromosome shortening; add on DNA that can get removed, so that actual chromosome unaffected.

- **DNA Ends the Problem**
  - Origins in middle of molecule, generally.
  - Remove primer cannot fill it in, still will have RNA, remove it still have a gap. Cannot fix problem unless primer comes from end of molecule.
  - Telomerase extends, subsequently loose DNA, just loose junk that DNA put there.
  - Telomerase is going to make DNA using an RNA template.
  - Several in our cells, this evolved as the function to fix the end of chromosomes.
  - *Adding Telomere Repeats*
    - Strategy is to take a small sequence, and make copies of that and attach them to the end of the top strand.
      - It does it by using a ratchet mechanism.
    - Every time it shifts, the gap repair adds the right number of nucleotides.
    - Add and add until it decides to stop.
    - Added an extension to the end of the chromosome, extension of the simple repeat of junk (doesn't code for anything).

        - Still get a gap at the end, but don't care anymore, because the chromosomes have been protected by extending the 'junk'
        - Always 3' strand that is the primer.
    - Put primer, extend DNA, remove primer
    - Eventually the gap degrades and is stopped by something, than begins to remove sequences further on (genes further on) than genes are getting deleted.
        - Simultaneously at both ends.
        - Repeat this mechanism.
        - Some cells are turned off.
    - Solve problem by using telomerase
        - It carries its own template!
        - We don't have to worry about making a primer, its already there.
        - That template is RNA, second unique property, RNA dependent polymerase.
        - Things that you find in retroviruses.
        - Automatically complementary, sequence on template generated by same mechanism from previous generation.
        - Then perform gap repair, making a small sequence (in our case its 6 nucleotides.
        - Sequence is also a repeat. It can shift one unit to the right to form another structure.
        - Shift one to the right, gap, fill it in, shift, fill it in… ratchet mechanism.
        - Does this for some length of time, don't know what controls it.
            - Might do it 100 times
            - When it does it 100 times in our system, adds 600 base pairs, extend it by 600 base hairs, roughly the same size as an Okazaki fragment.
            - Created a single strand of DNA that goes out. Now we can prime by primase and do synthesis, like what we would do in discontinuous synthesis, work towards the original DNA, and then join together with ligase.
        - Creating small repeats over and over to protect ends of chromosome, as long as you have telomerase can do this at the end of S Phase.
- **DNA Synthesis**
    - IF cell cycle is going quickly, have to replicate DNA more quickly, and vice versa, happens in S Phase.
    - In our cells, DNA synthesis has structure like Slide 16.
    - Those start at sequence at an origin, and goes bi-directionally.
    - Origins (*ori, ars*)
    - If you want to go faster, have to origins, cut down time by half approximately.
    - Lots of these origins.
    - Once they come together, fuse together and join.

- When piece of DNA being synthesized that meets up with another piece of DNA that has been synthesized, joins together using DNA Ligase.
- The faster you go in cell cycle (S Phase becomes smaller amount of time) use more and more origins.
- The faster you want to go, start at more origin, distance between origins is smaller and smaller.
- Synthesis is exactly what we saw in the model.
- How do eukaryotes solve the problem>
  - More origins.
- How do prokaryotes solve the problem (look at what happens in E. Coli).

- **DNA Replication in Bacteria**
  - Replication starts at origin (ori), and goes bi-directionally and finishes at a termination site (ter).
  - In us, finishes at an origin or at an end.
  - Piece that gets replicated gets bigger and bigger and comes apart and goes into daughter cells.
  - All proteins in nucleoid have to come off, need a lot more proteins.
  - Theta structures.
  - Can divide really, really quickly.
  - Bacteria growth is assessed by the doubling time.
  - Can grow at rates ranging from 18 to 180 minutes, depending on conditions.
  - Start at the origin, replicate and before termination, initiate relocation again from the origin.
    - Continues to go to the end, but initiates again.
    - More initiations → faster
    - Consequences → when you finally get to termination, what its going to inherit, not a molecule that has been replicated, and further behind half replicated, and further behind half replicated. Therefore, in the next generation, only have to finish off what you started in the last generation. Therefore, not finishing than dividing, keeps initiation before first round is finished.
    - What conditions are optimal? How we grow them in the land. Nutrient limited conditions (not optimal).
- **DNA Repair Mechanisms**
  - Absolutely necessary, DNA being damaged all the time.
  - Lots of agents that can damage DNA, oxygen free radicals (we create during oxidative phosphorylation), X-Rays.
  - When you eat spices, eating alkylating agents.
  - You also create things inside cells, during normal metabolic processes.
  - *Proofreading*
    - Recognizing a problem → recognize errors in the structure of DNA → Remove problem → then fill it in
    - Some structures can't be removed by these mechanisms, it'll just continue if it doesn't hurt gene.

        - Some that are so serious, you cannot repair them, just repair them anyway, even is you make a mistake.
- **Proofreading by DNA Polymerase**
    - Starts to copy, and instead of copying A – T, makes a mistake and puts in C. Can occur 1 in ever million.
    - Recognizes mismatched region, and reverses itself and fizes it
    - Polymerizes in one direction, depolymerizes in the reverse direction.
    - Puts the correct one back and then continues on.
    - Adherent rate of making a mistake, due to DNA polymerase.
    - How does it recognize
        - Structural change
        - Recognizes mismatches that don't form perfect helix
- **Mismatch Repair: General Model for Post-Replication Damage**
    - Somehow recognize structural integrity is not right, and remove that region, remove nucleosomes and everything else. Uses nuclease to take it out.
    - Then, region here is a gap, use gap repair to fill it in, then ligate it.
    - These systems work very efficiently, but not completely and function differently in people.
    - Repair for us is efficient.
- **Summary**
    - Leading and lagging strand, continuous and discontinuous, have different polymerases.
    - More DNA synthesis, recruit more origins.
    - Telomerase explains what happens to the ends.
    - In S Phase, what are the signals that tell it to start (looking at that later on).

# Transcription

- When you transcribe, it's only a small region.
- 5' to 3'; only for a short region, and does not require a primer.
    - Using RNA polymerase directly.
- **Transcription in Eukaryotes**
    - What is a gene?
        - DNA is organized into "functional" DNA and "non-functional" DNA (genic and non-genic).
        - A gene codes for a protein.
            - Does it?
            - Most of genome is made up of genes, which codes for genes and some regions that don't code for anything.
            - Some genes just code for RNA, for many genes no translation (ribosomal).
        - We need to have these definitions, without them have problems talking about what transcription is recognizing.
- **Modern view of a "gene"**
    - Part of the n code project.
    - Boxes represent subunits of polymerases.
    - Recognizing some sequence and making a copy, its not making an exact copy, making the compliment of the sequence (from 5' to 3')
    - The sense sequence, want to make a copy of that in RNA and then other strand is the anti-sense.
    - Using anti-sense strand to make sense strand.
    - Region that gets transcribed (5' end is upstream and 3' end is downstream)
    - Regulatory sequence that is not very far away by invoking structure of chromosome.
    - Transcribed region (message will have 5' upstream and a 3' downstream).
    - Sequences that bind proteins needed for transcriptional control and RNA synthesis are "cis-acting". The protein themselves are "trans-acting".
    - Controls are cis-acting.
    - Some RNAs are trans-active (know of some).
- **At the level of the genome, is the genome what we think it is?**
    - Most of the genome in "higher eukaryotes" consists of repeated sequences and mobile elements called transposons or retrotransposons (retroposons) and small RNAs and tandem repeats, etc…
    - Proteome → the entire complement of proteins that is or can be expressed by a cell, tissue, or organism.
    - The protein coding regions are only ~2% of the total.
        - This recognizes sequences that will be transcribed and translated.
        - Two parts → Part that doesn't code for proteins and the part that doesn't code for proteins (introns).
    - Genes weren't what we thought they were.
    - LINEs and SINEs
        - 20% is LINEs and 13% are SINEs

        - Sequence throughout gene.
        - SINEs are small and LINEs are long
            - Short Interspersed DNA Element (SINE)
            - Long Interspersed DNA Element (LINE)
        - Some are mobile, can cause mutation by moving.
        - They require same enzyme activity as telomerase, RNA depended DNA polymerase.
        - Move via RNA, this group has a structure that is related with many variants.
    - LTR Retrotransposons
        - Similar structure to retroviruses
        - Can move
        - Impossible to get rid of.
        - Sperm production requires genes that resemble these.
        - Nonfunctional at a high rate.
        - Treat them as sequences that you don't want to be expressed.
            - Heterochromatin → Highly condensed, non-active.
    - DNA Transposons
        - Move via DNA
    - A lot of the rest are duplications. Others are simple sequence repeats (dimers or trimers) these are CSI or RCMP use to tag you. In some lineages have more than others.
    - Misc. don't make up any category, don't really know what they do, make up 20%.
- Look at proteome; get around 10 proteins on average.
- Some regions have a lot of genes, some do not, not equally spread out. Gene Islands → Where there are many genes.
- LINES are elements that move, if they are capable, via a DNA intermediate to move via by transposition, it can also be an RNA intermediate. They can move anywhere and can accumulate as a mutation. If they integrate into functional genes too much, that linage of the gene will be lost.
- Most have accumulated mutations, cant move, heterochromatinized
    - LINES, SINES, LTR Transposons and DNA Transposons.
    - Mostly non functional, some are, cause mutations.
- Repeats (form of repeats, much bigger)
    - Simple Sequence, dimer or trimer and repeated several times.
    - Inherited
    - Another name is Variable Number of Tandem Repeats (VNTR)
    - Two repeats or hundred repeats, still next to each other, still tandem repeats.
- Segmental Duplications
    - Have part of a chromosome duplicated and inserted somewhere.
- Heterochromatin and Miscellaneous Sequences
    - Probably genes that weren't identified in Human Genome Project
        - Human Genome Project very narrow view of what a gene is.
- Different components tell us that we must change our view on what a gene is.

- **Transcription**
  - Copy DNA into RNA (single stranded)
  - Making 5' to 3' polymer
  - Like continuous DNA replication, but making an RNA strand
  - In a typical prokaryote cell, transcription and translation occur in only one compartment.
  - In eukaryotic, functions sequestered in cytoplasm and nucleus. Translation in cytoplasm and transcription in nucleus.
  - Not all messages are processed in the same way, only talking about common processing events.
  - As your transcribing RNA, can start translation on that RNA before you finish transcription. Co-Translation Transcription.
  - *RNA: General Characteristics*
    - Usually single0stranded (one polynucleotide) but double stranded regions
    - Complimentary to part of a DNA polynucleotide
    - Has ribose instead of deoxyribose
    - Has uracil instead of thymine
    - May contain modified bases
    - Made by RNA polymerases (multiple subunit enzymes) instead of DNA polymerase
    - 3 Types of RNA Polymerase in nucleus eukaryotes (actually more if you consider organelles)
      - Localization and specialization of function is why there is more than one
    - In bacteria, have the same RNA polymerase, buy change one of the subunits for different recognition (the signal factors)
- **Why are there multiple RNA Polymerases?**
  - Recognize, and transcribe different RNA
  - Recognizes particular regulatory sequences, and not others "so its very specific".
  - Can distinguish between them because different sequence in the promoter region that are recognize by the different RNA polymerase → ex. mRNA. snRNA = particular sequences
  - Think gene, RNA polymerase, RNA requires sequence = one group.
  - RNA Polymerase I
    - Transcribes rRNA in the nucleolus (part of the nucleus)
  - RNA Polymerase II
    - Transcribes mRNA and most snRNAs
    - Transcibes almost all the messages, protein coding
  - RNA Polymerase III
    - Transcribes tRNA, 5sRNA, some snRNAs and scRNAs
      - s = small and n = nuclear from snRNA
      - 5sRNA involved in ribsosome
      - s = small and c = cytoplasmic from scRNA

- **Overview of Transcription**
  - RNA polymerase, unlike DNA polymerase, doesn't need a primer to start. Regulatory sequences are upstream, 5'.
  - Promoter → Control sequence initiates transcription
  - Transcription Unit → Portion of gene that is copied into RNA
  - Terminator → Signals the end of transcription of a gene
  - Polymerizing 5' to 3' (Being read from 3' to 5')
    - Sense sequence in the RNA (strand getting transcribed)
    - Anti-Sense Strand is the strand getting read
  - Synthesis is always 5' to 3' therefore template is 3' to 5'
  - *Initiation*
    - Functional gene that has a transcriptional unit, and from point of transcription, indicates first nucleotide.
    - First nucleotide upstream from +1 to -1
      - First RNA base is +1
    - TATA box is where RNA polymerase binds.
    - The protein that binds here is trans-acting, and the site is called cis-acting
    - TATA Box → TATAAAA
      - Shows variability in population
    - In one sequence, that T could be an A, but its still called a TATA box.
    - Typically, you have variability, limit or else gene wont work.
    - Consensus sequence → Understood by looking at row going to back of room, each individual represents a base, first position, always a T, then next is an A.
      - *Definition:* Is the calculated order of most frequent residues, either nucleotide or amino acid, found at each position in a sequence alignment.
    - Like continuous DNA synthesis, start without primer, start keep going, gets bigger and bigger (elongation)
  - *Elongation*
    - RNA polymerase II moves along the DNA, unwinding it and adding new RNA nucleotides to the transcript in the 5' to 3' direction. Behind the enzyme, he DNA strands reform into a double helix.
  - *Termination*
    - The complete RNA molecule is released from the template DNA< RNA polymerase II leave the DNA, and the DNA double helix reforms in eukaryotes, the transcript of a protein-coding gene is a pre-mRNA molecule; it is processed to produce the translatable mRNA.
  - When RNA gets transcribed, it gets bigger. Must start from smaller and get bigger, you know that the promoter is where the RNA is smaller.

- **What about Prokaryotes?**
  - There is usually single RNA polymerase consisting of multiple subunits. Different promoters may be recognized by using different subunits especially "sigma".
    - Sigma factor, as part of RNA polymerase recognizes the promoter.
  - In prokaryotes, only one RNA polymerase, cant recognize promoters, but you can tell them, just not by looking at RNA polymerases.
    - Sigma factor → specificity
      - Some RNA have different sigma factors when they want to express different genes.
  - There are two termination of transcription systems.
    - RHO Dependent → Particular sequence, cis-acting sequences, consensous sequence (defined sequence with some variability)
    - Intrinsic or Rho Independent → Recognizes signal that doesn't have particular sequence BUT has particular structure, and that structure is GC rich.
  - **Production and Processing of mRNAs in Eukaryotes**
    - Processing is the process by which the primary transcript in the nucleus is transcribed.
    - These modification can include RNA cleavage, RNA splicing (remove introns and join exons), RNA addition (some examples, after primary transcript, add RNA to it, and RNA is not codded by gene), nucleotide modification, RNA capping, polyadenylation, and RNA editing (gene transcript is made, at particular sites that bases are changed).
- **Questions from Class**
  - During replication in a eukaryote, DNA synthesis from one ARS meets DNA synthesis from a neighboring ARS.
    - *True, because an ARS is an origin and you need origins to increase the speed of replication during the cell cycle.*
  - During replication histone octomers dissociate from the DNA. Following replication these octomers associate only with newly replicated DNA.
    - *False, all nucleosomes have to come off of the DNA during replication. Associates with new and old, part of the same strand, semi-conservative.*
  - Bacteria have multiple types of DNA polymerases so that if one is lost due to mutation, another can substitute and the cell can survive.
    - *False.*
- **Pre-mRNA Processing**
  - *RNA Cleavage*
    - rRNA is a large piece, S is denoted as the speed
    - Some tRNAs, the actual transcript is bigger than the mature RNA.
    - RNAse P Cleaves off the little bit, not all, not common.
    - In many species rRNA genes are transcribed as one long precursor containing several genes and intergenic regions. This precursor is

cleaved into the mature rRNAs while the intergenic regions are discarded.
    - Pieces are thrown away (recycled) if not used.
- *RNA Addition*
    - After synthesis, the non-templated addition of extra ribonuleotides.

- *RNA Splicing*
    - Specific sites to cut, unlike cleavage, things are joined together, such as the exons, and introns are discarded.
    - Remove introns and use exons.
    - Through away introns and recycle.
    - Introns are the non-protein coding sequences in the pre-mRNA.
        - Different classes, what we will find, in some tissues, the same message gets slightly processed than other messages, depends on composition of spliceosomes.
    - Spliceosomes must recognize sequences close to the boundaries.
    - After message is processed, translation is going to initiate CLOSE to the end, not at the end. Regions that don't code for the protein are called untranslated region (UTR), at both ends. There will be a 5' and 3' UTR (or upstream and downstream, respectively).
    - Since there are multiple introns, how are they removed, removal starts somewhere depending on structure, it is experimentally determined. There are cases where you do NOT remove ALL introns, alternative splicing.
    - Exons are the amino acid coding sequences in pre-mRNA
    - A complex of proteins and snRNAs caries out the splicing reactions.
        - This is the "Spliceosome"
    - In a non-intron world (bacteria), in our genes, same after splicing.
    - Depends on secondary and not primary structure to determine what to take out first.
    - If splicing did not remove all the introns, what's produced is not functional.
        - *Alternative splicing*, splice one way get a gene product, and splice another way get another gene product.
            - Related but not the same.
            - Alternative splicing of the alpha-tropomyosin (gene that consists of 12 exons and 11 introns) pre-mRNA to distinct mRNA forms found in smooth muscle and skeletal muscle.
            - In skeletal muscle, are missing exons 2 and 12, i.e. Spliceosome in skeletal muscle processes it differently.
            - In smooth muscle, exons 3, 10 and 11 are not present.

- *mRNA Capping*
  - A cap attached to the 5' end of the mRNA results in more efficient translation
  - Cap consist of a guanine, guanine is methylated
    - 7-methylguanosine (7MeG Cap)
  - If you cap your messages, it will be more stable, protects 5' end from being degraded.
  - Sometimes, ribosome recognizes end of message and that's what it recognizes.
  - It occurs because of two reasons (1) it helps to protect end from digestion and (2) it results in a more efficient translation.
  - Scanning Model, start at the end and 'scan' down along the bases.
  - AUG is starting point for translation.
- *Polyadenylation*
  - At end of message, run of A's that are not codded → poly-A tail
  - Adds 5- to 250 adenine nucleotides to form the tail.
  - More poly-A, more stable, protects from digestion.
  - More stable, can translate more and more; more copies of protein.
  - Go past end of gene, have polyadenylation, during it you have cleavage, which tells it to stop than keep going.
  - Transcription gone past that, once you have cleavage it stops it.
- *RNA Editing*
  - Processing by changing a base.
  - Uncommon in our genes, common in plants.
  - Translation stops there (where one codon was changed to a stop codon by changing a base) produce a truncated protein.
  - After RNA has been made, and some time during processing (editing and splicing sometimes linked), have RNAs made, and certain bases are changed.
  - Change code, and you can change to a termination codon, sometimes it changes to another codon that affects structure/function.
  - Inosine has property to form hydrogen bonds with several other bases.
  - Some forms of editin involved deamination
    - C becomes a U (cytidine deaminase)
    - A becomes Inosine (adenosine deaminase)

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# Translation

- Message been processed, now in cytoplasm.
- Functions of transcription and translation (in eukaryotes) are segregated, whereas in prokaryotes/organelle, no segregation, can have transcription and translation occurring at the same time.

- **Overview**
  - tRNAs are small RNAs of a highly distinctive structure that bring amino acids to the ribosome "adaptive hypothesis".
  - All assembly is done on rRNA-protein complexes
    - Has two subunits.
    - A simple reaction that occurs over and over.
  - Assembled by bringing together the ribosomal subunits, an mRNA and the first aminoacyl-tRNA together.
  - Start with an AUG, and read three at a time, and finish at a terminator, Open Reading Frame.
  - 88% of proteins are modified.
- **Translation is mRNA-Directed Polypeptide Synthesis**
  - Polysome, one message is being translated simultaneously by many ribosomes all at once.
    - Happens when you want a lot of protein.
- **Translation Overview**
  - Assembly of amino acids into polypeptides occurs on ribosomes.
  - P, A, and E sites on ribosome used for stepwise addition of amino acids to polypeptide as directed by mRNA.
  - mRNA always read three at a time.
  - E site where tRNA has no amino acid and leaves
  - A site is where a new tRNA comes in with an amino acid
  - P site is where the previous one in the A site exists. On the P site, that is where protein chain is growing. Amino and carboxyl terminal end. Amino terminal end attacks carboxyl terminal end.
  - All steps require protein factors:
    - Initiation factors [IF (for prokaryotes) or eIF (eukaryotic initiation factor)]
      - To get it going.
    - Elongation factors [EF (for prokaryotes) or eEF (eukaryotic elongation factor)]
      - To keep protein growin.
    - All energy is supplied by GTP hydrolysis, NOT ATP hydrolysis.

- **Genetic Code**
  - DNA, three-letter code: Triplet
  - RNA, three-letter code: Codon
  - A three letter code with 4 bases allows 64 combinations, more than enough for 20 amino acids, start codon and stop (codon). 61 that can code for amino acids. Redundancy in code, different codons, can give rise to insertion of SAME amino acid. The genomic code is degenerate, different codons can give rise to insertion of the same amino acid. Relates to tRNAs, there is NOT 61 tRNAs.
    - Wobble hypothesis used to explain this.
- **Genetic Code: Conventions**
  - Convention, sequences are always written 5' to 3'
  - The codon in DNA is written the same as the codon in RNA, except that:
    - T becomes U
  - If the codon is 5'GAC3', then the anticodon is 5'GUC3' to maintain the H-bonding rules.
  - By convention 5'GAC3' is simple GAC.
- **Feautres of Genetic Code**
  - Start or intiator codon
    - First amino acid recognized during translation
    - Specifiies Amino Acid (methionine)
  - Sense Codons
    - 61 codons specify amino acids
  - Stop codons (or nonsense codons)
    - End of polypeptide-encoding mRNA sequence
- **Codon Table**
  - Left hand side is first base, top is second base and right hand side is third base.
  - Only one codon for methionine
  - Three stop codons: UAA, UAG, and UGA.
  - No correspondence between tRNA molecules and number of amino acids
  - The code is redundant.
  - Redundancy can mitigate mutation (at third position – less effect)
  - Some codons are used for different purposes
  - Conservative mutations relate to grouping by amino acid function.
    - Resulting product has same properties, basic to basic or acidic to acidic, or neutral to neutral.
    - Ex) Asp. To Glu. → acidic to acidic.
  - Different organisms show codon bias.
    - Refers to in different organisms, different codons are preferred.
  - If a base is changed, can get a different amino acid, could be bad. Or if a base is changed, it could be the same amino acid, this is due to the redundancy (many codons can code for an amino acid).

- **Is the genetic code Universal?**
  - The same codons specify the same amino acids in all living organisms and viruses; however, there are exceptions. For example:
    - Slight differences in mitochondrial and chloroplast mRNA which have their own transcription and protein synthesis system.
  - If you have an exception, it cannot be universal.
  - In Eubacteria AUG encoded for fMET-tRNA (has a formal group); in Archaea and eukaryotes AUD encodes for MET-tRNA (no formal group).
- **tRNAs**
  - Contains *anticodon* sequence that pairs with codon in mRNA.
  - Has stem-loop structure, clover leaf.
  - Anti codon at bottom.
  - Contains modified bases, without them would not fold up into its structure.
    - One to look at is at the bottom, anticodon.
  - The third position of codon is most variable, and that binds to first position of tRNA and it is inocine (as an example).
  - 61 different sense codons do *not* require 61 different tRNAs.
  - First two nucleotides of anticodon and codon must match exactly. The third nucleotide has more flexibility. This discovers lead to the "Wobble Hypothesis".
    - Interactions non-standard hydrogen bonds. Proteins stabilize these interactions.
    - A G can form a hydrogen bond with a U, (Wobble Pairing or Non-Standard) can be seen in crystal structures.
  - Inocine has capable of forming three hydrogen bonds with three different bases (refer to isoleucine; first two are standard then inocine can bind to U, C or A, and still get same amino acid, using same tRNA molecule).
- **Aminoacylation**
  - Aminoacyl-tRNA synthetase charges the tRNA with correct amino acid.
- **Ribosomes**
  - Made of ribosomal RNA (rRNA) and proteins
    - Two subunits: Large and Small
- **Reading Frame**
  - Reading three, three, three, …
  - Determine correct reading frame, start with base 123, the next codon is 456, the next is 789, …
  - Must have correct reading frame to make correct protein.
- **Stages in Translation**
  - *Initiation*
    - Initiator tRNA (Met-tRNA) binds to small subunit.
    - Complex binds to 5' cap of mRNA, scans along mRNA to find AUG start codon (scanning or cozax model).
      - Another way is to enter directly, what bacteria and some eukaryotic gene use.

            - This establishes the correct reading frame (when it finds AUG).
        - Large ribosomal subunit binds to complete initiation.
        - 5' end and 3' end are fairly close.
        - Carboxy end forms a acyl bond with the tRNA and the amino end sticks out of the amino acid.
        - E site is where all tRNAs leave, no amino acid attached.
        - A site is where new tRNA comes in.
        - Energy is GTP hydrolysis; requires GTP.
        - After complex form, can start translation, it has now been initiated.
        - Aminoacyl-tRNA enters the aminoacyl site (or the charged tRNA.
        - Initiator codon goes directly into P site, to leave A site free for next tRNA.
    - *Elongation*
        - Aminoacyl-tRNA matching the next codon enters A site.
        - Pepidyl transferase catalyzes reaction in the A site to join the amino acids together.
            - Peptide bond between two amino acids.
            - Amino acids distinguished by side chains.
            - Amino terminal acts as a nucleophile.
        - Next step is to recreate free position in A site; therefore whatever was in A site moves to P site and whatever was in P site moves to E site. Once in E site, it exits.
    - *Termination*
        - Begins when A site reaches stop codon.
        - Release Factor (RF) or termination factor binds to A site.
        - Polypeptide chain released from P site.
        - Remaining parts of complex separate.
- **Polysomes**
    - Wanted to make a lot of protein, how would you make more of that protein?
        - More transcription, more genes (gene dosage), more translation…
    - Can see ribosomes all along length of mRNA; follow one after another.
    - Can initiate many or a few times.
    - Making same protein multiple times.
- **Simultaneous Transcription and Translation**
    - Can occur where no nuclear envelope is present; prokaryotes, organelles.
    - Can detect protein being made on the Polysome.
    - Soon after transcription, ribosome can come and start translation.
    - Each ribosome making a protein.
- **Polypeptide Processing**
    - Processing reaction convert polypeptides into finished form.
        - Removal of one or more amino acids from the protein chains.
            - Cleave off an amino acid.

        - Addition of organic or inorganic groups.
            - Some can be amino acids, inorganic groups, the most important one is phosphorylation.
        - Folding guided by chaperons.
            - Accelerated by chaperons.
            - Proteins when made have to fold up properly, may fold up improperly. Folding before peptide released. Chaperon keeps it in proper way.
        - Alternative pathways to different mature polypeptides.
            - Basically, as with modifications of RNA, no one pathway.
- **Summary**
    - RNA polymerase II is responsible for making mRNA and some snRNAs.
    - Many cases, no corresponding tRNAs for each codon, invoke wobble hypothesis. Protein factors stabilize.
    - Standard genetic code.
    - Translation is a complex process; the ribosome is the scaffold.
    - Translation can occur in multiple locations.
    - Following translation, you must have processing.
- **Practice Questions**
    - *Transcription*
        - *What does the term "gene island" mean?*
            - Where many genes are found in a DNA strand.
            - Cluster of genes.
            - Don't find in bacteria.
        - *The amount of DNA in a plant genome containing transposable elements can increase with time. Yes or no?*
            - Yes, because multiple transposable elements, they move via RNA to DNA intermediate, increase amount with time.
    - *Translation*
        - *Codons can be synonymous. Explain.*
            - Since the genetic code is redundant, many codons can code for the same amino acid. One codon, one amino acid, multiple codons, one amino acid; they are synonymous. The Wobble Hypothesis, helps us to understand why they are synonymous, such as inosine that can form three bonds.
        - *Why is the variability of the third nucleotide in a codon less significant than variability in the 1$^{st}$ or second codons?*
            - The first and second must be precise, but in a tRNA molecule the third base can change to another base, such as inocine, which can form three different bonds between A, U and C, which for non-standard hydrogen bonds.
            - First two dictate what amino acid is, and some variability in three.

**END OF MIDTERM**

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# Protein Targeting

- **Life Cycle of a Protein**
  - Protein synthesis occurs on ribosomes. When proteins released from the ribosome (or even before), their fate is still uncertain.
- **Protein Targeting: Examples and Mechanism**
  - There are LOTS of locations inside an animal cell that require the protein to cross a membrane.
  - Every time might be a combination of signal and receptor, complicated when you have to cross more and more membranes.
- **Protein Targeting: Examples**
  - If you want to target ER, protein has to get into ER. Protein synthesis occurring on membrane of ER, see ribosomes speckled on ER, protein not going back into cytoplasm, going into ER; it is a vesicle. No dots, just smooth ER.
  - Experimentally, proteins going into ER, which require signal and receptor. Signal within first amino acids, amino terminal end. WE can take gene for protein and clone it and put it in a different gene, gets fluorescent green colour. Green colour tells us where original protein went. Can now see fluorescent green colour inside ER.
- **Targeting to the Exterior of the Cell**
  - Plant apoplast.
- **Targeting to Organelles**
  - Has to get inside, and cross two membranes or three.
  - Take red for chloroplast.
  - Take green for mitochondria.
  - Tells us that a typical plant cell, lot of DNA and mitochondria.
  - GFP is a good marker because it will not kill the cell.
- **Targeting the Nucleus**
  - Not specific but can be done.
- **Principal for Targeting!**
  - Translation
  - Interaction with receptor and unfolding. There are signals on the protein ( a “signal peptide”) that interacts with the receptor. Proteins aid unfolding (chaperons).
  - Translocation across membrane.
  - Refolding/processing inside the organelle. Proteases may remove signal and chaperons aid refolding.
    - Signal peptide cleaved to remove it → signal peptidases. Remove signal it cant go back (double or triple membrane, remove first signal, can bring about next signal).

- **General Principles for Targeting**
  - Protein has a signal, a signal peptide, cross one membrane has on signal peptide, cross more than one, can have more than one signal peptide.

- **Protein Targeting, Cartoon Version**
  - Folding and unfolding, mediated by chaperons.
  - Binding to receptor, amino acids help.
  - Once it translocate's, folds up, and signal is removed.
- **Targeting: ER**
  - Has to get into lumen of the ER.
  - Going to the lumen, only first step of going to many other locations.
    - All of these (golgi and so on), require going to the lumen first.
  - Default pathway, goes into ER, and all other locations are possible without any signal.
  - Default pathway takes you outside unless you have information to go somewhere else. By default you go out.
  - *How it works?*
    - First drag ribosome to ER, occurs in two to three steps. Series of ribosomes, Polysome, different stages of translation.
    - Translation starts, then all the other amino acids starting at amino terminal end, amino terminal end made first. If this ribosome translates in cytoplasm; but here starts at beginning, start at amino terminal end, and once its been pushed out far enough (above ribosome), it can interact with the signal recognition particle (consist of a protein and on scRNA). If that recognizes the signal, binds to it and causes translation to pause. Complex now can interact with receptor in membrane. Then binds to receptor. Signal recognition particle binds to receptor, SRP Receptor. Now the possibility to resume translation once bound to translation. Two things happen: 1) product made is pushed into the lumen of the ER and 2) the signal peptide is cleaved by a signal peptidase.
    - Then continuing to make protein until termination codon appears.
    - *Signal* is a collection of amino acids, which are specific to each receptor.
  - The signal peptides vary in length, depending on what system and receptor you are using. Different signal → different amino acids.
    - The signal peptide in this system is about 20 to 50 amino acids.
  - Signal cleaved off after, then reused.
  - When translation resumes the signal peptide is cleaved by the signal peptidase. We refer to the original protein as the preprotein. The preprotein becomes the protein.
    - Ex) Pre-lysozyme becomes lysozyme when the signal peptide is removed.
  - We would expect the signal peptide to be a consensus sequence, but this one is NOT, this one rich in hydrophobic amino acids. No basic or acidic amino acids.
  - *What else happens the lumen of the ER?*
    - Going to fold with aid of chaperons, when you fold or unfold, you need chaperons.

        - Might want to stay in golgi, default pathway is to keep going, therefore need additional information to retain it.
            - Two ways that you retain things
                - Have a signal for retention (H/KDEL at the carboxy end)
                - No retention signal, bind something strong there to retain you from going out.
        - Glycosylation, adding sugars. Pre-formed collection that are added. Sugars that are modified are added there. Can be anywhere within the protein.
    - How does it get from ER to golgi? → Use of vesicles.
        - Vesicles bud off of ER, just see at the end, an these carry protein to the next target, the golgi, which has a series of stacks. Each time it has to bud off from one and fuse to the next one.
        - Flow = going forward
        - Retrograde = going backwards
    - Rough ER, lots of ribosome, means lots of exports of protein.
    - Then you produce secretory vesicles, which fuse with membrane and release.
        - This is the flow/default pathway.
    - When it buds off of ER, its going to fuse with the part of golgi close to ER, and the ER is contiguous with the nucleus. The part that is close to ER is called the *cis-face* and the part that is far away is called the *trans-face*.
    - Outside could mean sticking in membrane. How does that occur and how do you target to a lysosome?
    - *How do you get into membrane?*
        - Use anchor sequences. If you have an anchor sequence near amino terminal, short amino terminal and long carboxy terminal, or vice versa. Multiple anchor sequence, going in and outside, cross membrane.
        - Can fuse in membrane at original vesicle.
    - *Targeting to the Lysosome or Vacuole?*
        - Pathway diverted due to additional information.
        - Example to use is going to lysosome:
            - If no signal, would go out through default pathway.
            - In cis-face there will be a modification. It is a modification to the sugar NOT the protein. One of the sugars, called manose (glycosylation), and you add phosphate to it, and that is the signal. Signal is sugar plus phosphate.
            - When it buds off, reacts with receptor of trans-face receptor. When it buds off, it goes to the golgi.
            - The vesicle goes to lysosome, releases contents, and then the vesicle and receptor goes back to golgi through retrograde movement.

- *Targeting to the Mitochondria*
  - Receptor is “Tom” and “Tim” 1
  - In this case, going to talk about to proteins.
  - DHFR, protein necessary to make nucleic acids, favorite target for cancer research.
  - Two receptor, one in outer and inner membrane.
  - DHFR ends up in matrix.
  - Comes in, recognized by receptor, the signal peptide, once cleaved, sometimes more cleavage, called spacer.
  - The top (involved in recognizing signal peptide) is called TOM and the one below is called TIM
    - TOM = translocase outer membrane mitochondria
    - TIM = translocase inner membrane mitochondria
  - Cytochrome C can go back into intermembrane space.
- *Targeting to the Chloroplast*
  - Goes through two receptors. First signal cleaved, revelas second signal.
  - TOC = translocan outer membrane chloroplast
  - TIC = translocan inner membrane chloroplast
- *Targeting to the Nucleus*
  - In the nucleus, protein made in cytoplasm has to get inside, use of nuclear pores.
  - No hole, filled with protein, protein act to provide specificity.
  - To get in you must have a signal or there is another way.
  - Receptor is inside pore, signal (nuclear localization signal = signal peptide). React with it and go inside.
  - Signals, tend to not be at amino or carboxy terminal end, they are in the middle. If there is a protein with a signal in the middle, not going to have a peptidase, because if it is in the middle, do not want to cut the protein up.
  - If you don’t have a signal, grab on to protein that has a signal to get in.
  - Sometimes, the signal, all those signals are a patch/run of amino acids, nuclear localization signals can be a patch, must be available to receptor. Sometimes you find it is two regions, a few here and a few there (bipartite), how could that be? Folding.

- **Protein Targeting: Targeted Proteins with GFP**
  - GFP gene gives rise to GFP protein.
  - No signal, it will end up in the cytoplasm, no signals for anything.
  - Has a ER signal, but to ER retention signal, it will end up outside the cell.
  - Has a ER signal and a ER retention signal, it will stay in the ER.

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# Gene Regulation

- Refers to the regulation of activity and may occur at any level.
- Important, because organism needs t conserve energy.
    - Do so by regulating expression of genes.
    - Gene is not going to be on or off, going to be in levels in between.
    - Lac Operon is a good system to understand a simple case, only two regulatory circuits.
    - Transcriptional level, easier to study.
    - Post Transcriptional levels, like RNA processing, those events can be regulated.
        - Alpha-Tropomyosin, spliced differently at different levels.
    - Translational.
    - Post-Translational, targeting.
- *Reasons for Gene Regulation:*
    - Changes in environment or changes in nutrients.
    - Changes in make up of cell is response to signals.
    - In a multicellular organism, cellular differentiation.
        - Bacteria can differentiate, usually terminal. We can, produce many different cell types, form different organs.
    - Conserve energy.

- **Regulation of Gene Expression in Prokaryotes**
    - Look at expression, has to respond to environment.
    - *Basics of Gene Expression*
        - Typically RNA polymerase binds to a DNA sequence 5' to the gene called the promoter. Within the promoter may be the consensus sequence 5'-TATAAT-3' called a TATA Box.
        - Repressor proteins binding to other regulatory DNA sequences may prevent the gene from being expressed.
            - System that is repressed/off.
            - Trans-Acting Protein is called a repressor, binds to a regulatory sequence (which is cis-acting), and prevents the gene from being expressed.
            - In order to get expressed must be a signal, induction/de-repression. Inducer is no longer present, then you re-establish repression.
        - Activator proteins binding to other regulatory DNA sequences may turn on expression of the gene.
            - Trans-acting protein, the sequence it binds to is cis-acting.
            - Gene is poorly transcribed, and then when activator protein, and responds to some signal, gets a boost in transcription, remove signal, no longer activated.
            - Goes back to basal level, where it was before, the base level.

Two Basic Circuits

- *Operons*
  - At a smaller scale, many genes are organized into operons (one or more Operons may be found within a transcription unit).
  - Can be considered as a unit of transcription with several genes controlled by a single promoter.
  - In effect an operon is a cluster of genes and DNA sequences involved in their regulation.
  - RNA polymerase binds at the promoter and transcribes all the genes in the operon into one mRNA (called polycistronic because it contain several cistrons).
    - Polycistronic message, means several genes, more than two.
  - In e-coli, large number of operons. At least 30% of the genes are organized into operons. Organized in this way because of regulation, regulation at one promoter can effect all the rest.
- *Lac Operon*
  - Three genes 5'-lacZ lacY lacA -3'
    - Three genes that are on polycistronic message.
    - Can be translated.
      - lacZ → Beta-Galactosidase
      - lacY → Permease
      - lacA → Transacetylase
      - All independently translated.
  - LacZ most important.
    - It is an enzyme.
    - Can easily measure the enzyme. Amount of enzyme (Beta-Galactosidase) is proportional to polycistronic message.
  - Lac operon operator sequence is between promoter and lacZ.
  - Sequence (cis-acting) where repressor protein binds is the operator.
  - Promoter → Operator → Genes
  - Distil, far away, which is the regulator gene → lacI
    - Going to transcribe the repressor.
  - The operator is between the promoter, where RNA polymerase binds, and the start point of transcription.
    - When repressor binds, and then RNA Polymerase binds, RNA Polymerase cannot move past the repressor, and therefore cannot transcribe the genes.
  - Galactose can be converted to glucose. Then two molecules of glucose for one molecule of galactose.
    - Currency of the cell is glucose.
  - When lactose enters the cell, going to cause de-repression, because it binds to repressor, and repressor will no longer bind to the operator.
    - *Inducer* of lac operon by binding to lac repressor → lactose.
    - *Inducible operon* because inducer increases expression.

- Can use something as a substitute instead of lactose, to make it easier to measure.
    - IPTG and X-gal, is what we made to help us understand the system.
        - *X-gal*, X where there used to be a glucose molecule, and when X is produced, makes things blue.
        - *IPTG*, important because if your investigating system, going to have induction with lactose, inducer that you put there is being metabolized, if your metabolizing lactose, level goes to and ability to induce goes down. Hard to investigate when things go up and down. Therefore, IPTG, analog of lactose, doesn't get metabolized.
- *What happens if you mutate lacI gene?*
    - No repressor, cannot block it, lac operon will be on all the time.
- *What if you mutate lacI and one that's not on a plasmid?*
    - Off, because functional copy works, functional copy provides repressor, therefore, lacI is dominant.
- *What happens if you mutate the operator sequence?*
    - Will be on, repressor cant bind to sequence.
- *What happens if mutate operator, and have another sequence somewhere else?*
    - Still on, therefore operator mutant is dominant.
- More lactose, induce more and more, until you get maximum synthesis.
    - Add lactose, gives de-repression.
- Glucose and lactose together, cell prefers to use glucose, cell metabolizes glucose, and when that's used up, will turn to lactose.
    - Glucose is the currency of the cell.

- Another system that is a positive regulatory system.
    - Involves cAMP, and involves the protein called CAP, which binds cAMP.
    - That's going to give us positive regulation.
    - cAMP is inversely correlated wit glucose. Low glucose → relatively high cAMP, and that can bind to CAP and act as an activator.
    - How does this work with the lac operon?
- *Positive Regulation of lac Operon*
    - Second step, whether this system works or not, response to glucose levels; general sensing mechanism for glucose in the cell.
    - Inducer binds to repressor, repressor going to be inactive, and its no longer bound to the operator.
        - Can now have synthesis.
    - Activation kicks in, high cAMP, no glucose. Going to produce even more lacZ.

        - Then beta-galactosidase metabolizes lactose.
    - When cAMP is high, can boost transcription even more.
    - As you metabolize lactose: 1) start producing glucose, then cAMP will go down and 2) eventually restore repressor.
    - No glucose boosts process even more.
    - Glucose is high, will not have CAP site.
    - Produce glucose, activation diminishes, goes down and vice versa.
- *Regulation of the lac Operon and other operons in E. coli: thought experiment.*
    - E. coli uses a general sensing system to positively regulate many possible genes or operons.
    - De-repressed or induced when lactose is present.
- *Regulation of Transcription in Eukaryotes*
    - Occurs at several levels: transcriptional, posttranscriptional, translational, and posttranslational.
    - Using RNA to regulate expression of RNA.
    - Chromatin structure, global phenomenon, plays an important role in whiter a gene is active or inactive.
    - Can regulate transcription, E. coli.
    - Methylation of DNA, consequence of heterochromatin, heterochromatin; chromatin is highly methylated.
- *Organization of Eukaryotic Gene*
    - Eukaryotic gene organization allows regulation
        - Promoter includes TATA box that binds transcription factors
        - Promoters proximal region upstream of promoter increases transcription
        - Enhancer further determine max transcription rate, are positive.
        - Now have exons and introns, promoter region (helps bind RNA polymerase to start transcription – transcribe introns, exons, 5’ UTR and 3’ UTR), regulatory sequence, 5’ and promoter proximal. 5’ point of first nucleotide is +1, to the left is upstream and to the right is downstream. Sometimes TATA box is -10, 10 nucleotides to the left.
        - Silencers negative.
        - Can have enhancers past transcripted message and will still effect.
- *Transcription Complex form on Promoters*
    - Regulatory proteins influence it.
        - Different about eukaryotes and prokaryotes, eukaryotes, regulatory proteins bind to DNA, and in prokaryotes, regulatory proteins also bind to DNA bu also many proteins that influence and don’t bind to DNA, instead they bind to proteins that are bound to the DNA.

        - Activators bind to promoter proximal elements and increase transcription rate.
    - Proteins that bind to them, however, still have problem, have silencers upstream and gene downstream, how can it influence it? Sequences in genome are not arranged in a linear array, folds up, and things that are far a part are now closer together.
    - Co-activators, form bridges between them, now close together.
  - *Regulation of Transcription Initiation*
    - Co-activators bride enhancer and promoter
      - Interactions between co-activator, proteins at promoter and RNA polymerase increase transcription.
      - Repressors oppose effect of activators.
  - *Combinatorial Gene Regulation*
    - Work like a complex of proteins that have a specific DNA binding site for the sequence.
    - Each complex works as an activator, because it is responding to a particular signal, two types of proteins that bind to them, each responding to a different signal cAMP and lactose.
    - Key point, for any situation, not all of them are used at the same time or at the same level.
    - First way, contrast one gene with another gene.
      - One controlled with four systems, but are different from the other four systems. Some are the same, but some are different. For example activators 5 and 8 are shared, but there are also two unique activators for each one.
      - Not every gene has its own unique circuit, some share circuits, but are affected at different levels, unique.
    - Can also express in another way, can just look at Gene A under two different conditions, like under a developmental pathway. So all of these sequences would be the same, but signal would be different.
- **Methylation of DNA: An Epigenetic Mark**
  - DNA Methylation adds –CH3 to cytosine
    - Gene silencing occurs when DNA methylation is locate in promoters.
    - Example) Barr Bodies, which is when you have an entire X chromosome that is completely inactivated by heterochromatization.
  - Genomic Imprinting
    - Methylation is NOT is response to forming heterochromatin, but signal at the DNA level for the expression of a gene.
    - Permanent silencing of a maternal or paternal allele
    - Inherited methylated allele is silenced
    - Methylation maintained as DNA is replicated.
    - Methylation regulating one gene for example.
    - Not global methylation.

        - Inheriting methylation state is what we call imprinting.
        - What is controversial is what may cause the changes.
- **Steroid Hormone Regulation**
    - Regulation in a system that is a hormone.
    - Cell responds to the steroid hormone (testosterone, estrogen…)
    - Circulates in the blood, delivers hormone to cell, and cell is hormone responsive.
    - *How does it regulate?*
        - Steric hormone enters cell, has chemistry/structure that allows it to partition the cell.
        - Binds to receptor inside, receptor inside.
        - When it binds to receptor, receptor is now active, it can now migrate to nucleus, bound to the hormone receptor, and second, can bind to sequence in DNA and activate a whole series of genes.
        - The sequence it binds to in DNA is called hormone responsive element (HRE).
        - Going to have transcription, when hormone level goes down, reverses itself.
        - Depends on chemistry of hormone, if it cant enter cell, mechanism is preposterous.
        - *Why cant it get in the nucleus?* Not activated by hormone.
        - *What is the mechanism it enters the nucleus?* Needs NLS (Nuclear localization signal).
        - Chemical Q cant get in, because it cant get in, but to manifest its signal into the cell it uses a secondary messenger which is a membrane protein. Signal is bound on the outside and gets transported via the receptor or can change the receptor on the inside of conformation.
- **Posttranscriptional, Translational, and Posttranslational Regulation**
    - *Posttranscriptional*
        - Splicing, 5’ Cap, Poly A Tail.
        - Control initial assembly of ribosomes.
    - *Posttranscriptional Regulation*
        - Regulation depending on RNA.
        - Mashing proteins bind to mRNA to prevent translation.
        - Micro-RNA (miRNA) regulates gene expression through RNA interference (RNAi)
            - mRNA binds to any complementary mRNA sequence and silences it.
        - Small interfering RNA (siRNA) is from RNA encoded outside the cell’s genome.
            - Often used by viruses during infection.
            - Viruses can sometimes use it.
        - RNA Interference Mechanism
            - mRNA, cap on 5’ end and Poly A tail on 3’ end, message capable of being translated, but want to prevent translation,

do so by using miRNA. If message codes for viral proteins, targeting it, to diminish infection or transposition.
- Top part is producing siRNA, and when it is produced from normal transcript, processed and binds to protein complex (that presents miRNA to complex) RISK complex.
- Have a sense strand RNA, and have something complementary to it, form small region, double stranded RNA, and dsRNA has two consequences: 1) is you cant translate through double stranded region, stops expression of that gene at posttranscriptional level and 2) is when it is blocked, can now be degraded, message target for degradation, when it degrades, can no longer be used as a template.

- *Translational and Posttranslational Regulation*
  - Translational regulation controls rate at which mRNAs are used in protein synthesis.
  - Posttranslational regulation controls functional proteins.

- **Practice Questions from Past Exams**
  - Protein Targeting
    - *Why is protein targeting so important?*
      - To determine where proteins will go in the cell. Without this, there would be no direct place for proteins to go, therefore the message would not be relayed to the important area.
      - Protein must go into chloroplast, if it does not get there, will not function in the chloroplast.
      - Have to get into right organelle, by using targeting signals on the protein, and they are removed sometimes. If removed, will change sequence all the time. Sometimes, processing events, changes signal, to change location.
      - Go to ER, protein folds up, and they are modified, for example, the addition of sugar. This is necessary for targeting.
    - *What is the role of chaperones in protein translocation?*
      - Aids in the unfolding and refolding of the proteins.
      - As it goes through the ER, co-translational transport, then folds up in the ER with the aid of ER.
      - Also occurs in other locations as well.
      - Unfolding is important in the mitochondria, or the chloroplast. This occurs because protein made in cytoplasm, has to cross membrane, and uses a translocase to cross membrane, when it goes across, gets unfolded, needs a chaperon to do so.

- Regulation
  - *Regulation of the lac operon is positive and negative. Explain?*
    - Lac operon, negative regulation is repressor and positive is activation, and the protein is CAP and the signal is cAMP and what regulates that is glucose.
    - The interaction, repression or deprepression with glucose, when it is deprepressed, CAP can bind and boost transcription.
  - *What is combinatorial regulation?*
    - Series of combination of regulatory circuits could be a combination of silencers and enhancers. Sum of the interactions between them that give rise to the proper expression.
    - DNA binding proteins that can bind other proteins to interact.
    - Responding to cell signal.

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# The Cell Cycle

- In interphase, divide into G1, S and G2.
  - S is DNA synthesis
  - Some cells in G1 that stop and go into hybridization = G0.
  - G1 is growth period.
  - G2 is period after DNA synthesis and cell prepares for mitosis.
  - Has to be a control from G1 to S, something at the boundary that controls whether you can go or not. Also, from going from S to G2 and from G2 to M.
- **Cell Cycle**
  - Goes through a period of phases.
  - Interested in what regulates transition from one phase to another.
    - Two proteins involved, variants of them; can use them to regulate.
    - Gene duplication and divergence.
  - Will be using both antibodies and GFP to tag and look at stages.
  - Going from interphase to mitosis, huge change, easily studied.
    - But going from G1 to S, and mitosis to G1, and G2 to S, not as big of a change.
  - After main picture was described, realized that some cells can fall out of G1 and are coyesent for a period of time, G0.
  - *Interphase Stages*
    - G1 the cell is growing, not yet replicating DNA.
    - S is the replication of DNA.
    - G2, getting ready for mitosis.
  - If looking at this when not knowing anything, wed propose that regulation would happen at each point where it changes phases.
    - Might also postulate, not enough to get to next stage, must sense all processes in previous stage has been complete, *checkpoint*.
  - Hartwell, Hunt and Nurse worked in regulation.
  - Cyclin, protein part of cell cycle.
    - Not one regulation for cell cycle, different points of regulation.
  - Regulation that we will be discussing is evolutionary conserved. Can grow yeast with mutations in these processes, and clone in our gene and will complement mutation and complete cell cycle.
  - Also looking at cyclin dependent kinase, phosphorylates ATP, only does so when it forms a complex with cyclin.
- **Cell Cycle: Regulation**
  - *Cyclin* and *cyclin dependent kinases* (CDK) are the internal controls that directly regulate cell division.
  - *Maturation Promoting Factor (MPF)* is a heterodimeric protein composed of cyclin B or cyclin A AND cyclin dependent kinase that stimulates the mitotic and meiotic life cycles.
    - Promotes going from G2 to M; the one that was first discovered.
  - Internal checkpoints stop cycle if stages are incomplete. i.e. if DNA synthesis not completed CANNOT go from S to G2.

- Kinase will add a phosphate group, does so to target proteins.
- After these two things have come together (cyclin and CDK), can phosphorylate target proteins.
- *CDK's*
  - Add phosphate groups to target proteins
  - Activated when combined with cyclin.
- To remove signal, reverse back to basal setting.
- *External Controls*
  - So many of them, process has to incorporate signals from many places.
- *Cyclin Activity*
  - Increasing during interphase.
- MPF (made out of CDK and cyclin) peaks during mitosis, MPF now can phosphorylate, acts as a kinase, phosphorylates many targets.
- Cyclin increases during the cell cycle.
  - At beginning, start accumulating protein, until you reach checkpoint; accumulation of protein is not enough, other levels.
  - Sub-cellular location is important, has to be modified and in right location.
  - Goes down, regulated mechanism destruction of protein, actively being targeted for destruction (was not predicted for a long time).

- **Cell Cycle Control: Cyclin/CDK Control**
  - Specific cyclin's for certain phases.
  - Form active complex with appropriate CDK, when you get into right phase, cyclin going to be degraded.
    - Each phase has specific cyclin, when you go into the next phase, you will need the specific cyclin for that phase; so old cyclin (from previous stage) will degrade to allow for specific cyclin to bind to CDK complex.
  - Cyclin accumulates before you have activity.
  - What regulates the regulators?
    - We know that they have to integrate signals from within the cell, then out of the cell, and so on…
  - Factors that control cyclin concentration
    - Transcription of genes, thus mRNA and translation.
  - Another form of regulation you see in yeast is SIC1, it binds to CDK and regulates it; it is an inhibitor.
  - *Two We Will Look At*
    - <u>Subcellular localization</u>
      - Cyclin B1 → localization in cell is correlated with function.
    - <u>Controlled Proteolysis</u>
      - Many proteins are turned over in this way.
  - **First Set of Experiments**
    - Cyclin B1 in animal's shuttles between nucleus and cytoplasm.
    - Don't fuse proteins together, join DNA sequences together, and then transform cells. Where does it go as you follow cell division?

        - In picture shown on slide 13, translocation of cyclin into nucleus.
        - Can do experiment with an antibody instead of using GFP.
            - Antibody has a chromofore attached to it that is red.
        - By both techqniques, we can see transition of cytoplasmic to nuclear, associated with going from G2 to M, maturation factor.
        - This implies that it can go into nucleus, it actually shuttles back an forth, it only stays in nucleus once it has been phosphorylated (phosphorylated in nucleus).
        - Why are these cells not looking all the same? All the cells are not synchronous. Synchronous means when you have a cell culture, all cells are in the same state when it goes through the cycle). Hard to make a synchronous culture.
    - **Second Set of Experiments (Degradation of p27)**
        - P27, focus of a lot of regulation (p=protein - 27=27kilodaltons)
        - It is regulated at several levels.
        - This complex control serves to integrate many proliferative and anti-proliferative signals.
        - Regulated in many ways because it has to control so many signals.
        - Looking at G1 to S, thing that is being changed. We would expect prior to that is to have low activity.
            - CDK goes from low level to high level.
            - P27 goes from high level to low level.
            - What controls level of p27?
                - First look at transcription, but it doesn't change.
                    - In this particular experiment, not important.
                - Then look at translation, but it also doesn't change.
                    - Measured level of translation of protein, and we found that there was no change; therefore something else must be controlling level of p27.
                - Specific system that specially degrades proteins.
        - *System that specially degrades proteins.*
            - System that targets proteins for destruction.
            - Two components:
                - Proteasome, a big collection of proteins that degrade targeted proteins, and recycle amino acids.
                - How doe you know which one to target?
                    - Proteins with ubiquitin.
                        - Enzymes add ubiquitin.
                    - Ubiquitin is not targeted, recycled.

- **Cell Cycle Control: Checkpoints**
    - No checkpoints, just keep going, cancerous state.
    - In yeast, checkpoint is called a start, before end of G1 and in us it is called a restriction point.

- **Cell Division in Prokaryotes**
  - Replication occupies most of the cell cycle in rapidly dividing prokaryotic cells.
    - No S or G phases.
  - Chromosomes divided into daughter cells.
  - Can reinitiate many times before separation.
  - Start to synthesize at origin, get theta structure, eventually replicate whole thing, one goes to one daughter and one goes to the other daughter.
  - Pull them apart, can separate them by membrane in between, septum, then you get two daughter cells.
  - Process is binary fission.
- **Mitosis and Binary Fission**
  - Might be something to help pull them a part.
  - In cytokinesis, have filaments to pull them a part. Anything similar to this?
    - We can isolate mutations in the process.
    - FtsZ resembles fibers throughout the cell, no nucleus, nucleoid.
      - FtsZ = filament temperature sensitive
    - Z ring gets smaller and smaller, and then separate, in prokaryotes.
    - Reason we know this is because we can make a GFP fusion. In time the ring gets smaller and smaller.
    - FtsZ is distributed along outer surface of ring and overlap, correlation of an event that looks like cytokinesis and filaments that cause it.
    - If we look at FtsZ looks like tubulin.
    - MreB and FtsA are similar to actin.
- **Summary**
  - Seen how cell cycle was controlled.
  - Found two types of regulation: localization and selective degradation by ubiquitin proteasome system.