Kwak/Sze BSCI330 Spring 2010

1/26/10 (Chapter 1)

- Cell biology is the study of how cells grow, develop, and adapt
- Cell bio is central to understand how cells/organisms go through life cycle
- Three domains of life are based on rRNA analysis
  - Bacteria, Archaea, Eucarya
- What are the relationships of organisms to one another?
  - Primary producers:
    - Algae and plants
- Nitrogen cycle
  - Only plants and microbes are able to obtain Nitrogen from both soil and air
- Prokaryotes
  - Bacterial flagellum, ribosome's, cell wall, plasma membrane, DNA nucleoid, no mitochondria, capsule
  - Some bacteria are aerobic.
  - **Look for differences between eukaryotes and prokaryotes!**
- Yeast
  - A unicellular eukaryote
  - Asexual and sexual reproduction
  - Model organism – very simple genome
- Complex organisms consist of cells types with distinct functions
  - Ex. Humans
- Flowering plants have sexual reproduction
  - Sepal
  - Petal
  - Stamen (male) → Pollen (sperm) →Fuse → zygote
  - Pistil (female) → Ovule (egg)
- Egg cells from different organisms look pretty similar
  - Information in egg cell determines nature of organism
- How did eukaryotic cells form?
  - **Predator symbiosis**
  - Origin of mitochondria
    - Ancestral euk. cell engulfs bacterium(aerobic) to create mitochondria
  - Origin of Chloroplast
    - Photosynthetic bac. engulfed and retains function in cell

1/28/10 (Chapter 1)

- Properties of cells [and organisms]
  - Cells are highly organize
  - Cells possess genetic information for growth and development
  - Cells can produce more cells
  - Cells acquire energy and use energy
  - Cells carry out many chemical reactions
  - Cells are able to sense and respond to stimuli
  - Cells are able to self-regulate
  - Cells evolve
- Prokaryotes vs. Eukaryotes
  - Prokaryotes
    - Genetic material not membrane bound
    - Genome in bound up in cytosolic nucleoid
    - Oxidative phosphorylation and photosynthesis occur in plasma membrane

        - Larger surface area to volume ratio giving them higher metabolism, higher growth rate, and shorter generation time
        - Easily perform horizontal gene transfer through conjugation
    - Eukaryotes
        - Genetic material is in a **nuclear membrane**
        - Genome bound up in nucleic acid/protein complexes called chromatin
        - Oxidative phosphorylation and photosynthesis occur in **mitochondria** and **chloroplasts**
        - Other membrane bound organelles such as **ER, Golgi**, lysosomes, peroxisomes (called endomembrane system)
        - Undergo **mitosis and meiosis**
- Why are Eukaryotic Cells more complex in organization
    - Larger cell than prokaryote surface area/volume ratio decreases. (Much faster in smaller cells)
        - Diffusion of nutrients/molecules takes more time
    - Highly compartmentalized (caused by decreased diffusion rates, much more organized/efficient rxn. Rates)
        - To separate incompatible chem. rxns
        - Keep concentration of molecules high
        - Each organelle has specialized role
        - To keep surface area/cytosol volume large
    - Multicellular organism – is large, but has more cells. This keeps (area/vol ratio)
        - Cells become differentiated to assume its specialized roles
- Origin of Eukaryoic Cell
    - Endosymbiont theory:
        - Predator cell engulfed other cells for food and energy
        - Mitochondria used to be bacteria that had **aerobic respiration**(in their plasma membrane)
            - Still in membrane in mitochondria
        - Chloroplast used to be bacteria that could photosynthesize
        - Host cell developed a symbiont relationship with the bacteria
- Eukaryotic cell evolution
    - Ancestral cell (archaea) which was anaerobic, heterotrophic engulfed aerobic bacteria that evolved in mitochondria
    - Sequence of nuclear envelope formation and bacteria capture is not known
    - The aerobic, heterotrophic prokaryote (w/mitochondria) has invagination of plasma membrane which leads to formation of **nuclear envelope** (formed around genetic material).
    - ER is **connected** to nuclear Envelope, leads to nuclear envelope precursor and proeukaryotic cell
    - Cell then captures photosynthetic bacteria
        - If occurs creates plant cells
        - If not goes to protist, fungal and animal cells
- Know relative size of organelles **(fig. 1.19)**
    - Bacterium size = mitochondria → 1um
    - Eukaryotic cell is 10-20 times bigger
    - Virus < 1/10 size of a bacteria
    - Ribosome (need electron microscope to see – very small)
    - Membrane thickness
        - 5-10 nm
- Cells vs. Viruses
    - Cells are **autonomous** and can generate everything they need from environmental resources, or by eating other organisms
    - Essentially, viruses have to steal this functionality from cells
- Virus is not a cell
    - Not cells. They are particles.
    - Alone they consist of DNA or RNA protected by "protein coat"

    - All viruses are obligate intracellular parasites.
        - They can reproduce if inside the host cell and can take over the host cell machinery to reproduce
    - Most viuses have a **narrow** host range
- **Viroids** even simpler: Only RNA
    - Lack protein coat
    - Naked circular RNA – single stranded
    - RNA doesn't code for protein, but is replicated directly into **new RNA**
- Know all organelles in cells
    - Be able to name from a picture
- Nucleus
    - Purpose
        - Store genome
        - Synthesize DNA and RNA
        - Processes RNA for use elsewhere in cell
    - Nucleus
        - Double membrane, with nuclear pores, DNA in chromosomes
    - Nucleoulus
        - rRNA synthesiszes and ribosome assembly, not delimited by envelope
    - Euchromatin
        - Do not remain condensed (coding regions which expand during interphase)
    - Heterochromatin
        - Remain condensed and inactive during interphase
        - Include telomeres and centromeres
    - Nuclear pores
        - Pores through which transcribed RNA and other proteins enter and exit the nucleus
    - Inner and outer membranes
- ER
    - Continuous with nuclear Membrane
    - Responsible for synthesis (manufacture) of proteins and lipids associated with endomembrane system
        - Carbohydrate synthesis
        - Protein modification
    - Smooth (SER)
        - Synthesis of lipids: phospholipids
        - Detox of liver
        - Store Ca2+ in muscle cells
    - Rough (RER)
        - Synthesis of proteins on membrane bound or free ribosomes
        - Synthesis of secretory lysomal, and plant vacuolar proteins on membrane bound ribosome's
        - Processing of newly synthesized proteins
        - Synthesis of integral membrane bound ribosome's
        - Membrane biosynthesis
- Golgi Complex
    - Stack of membrane sacks (cisternae)
    - Vesicles enter golgi on **cis side** from ER and exit on **trans side**
    - Vesicles move back and forth between different cisternae to modify and transport molecules
    - Functions
        - Sort protein and lipids made in ER and are modified
        - Materials are sorted, modified, and transported to specific cellular destinations
- Lysosomes
    - Found in animals

- Have single membrane
- Part of secretory pathway
- Lysosomes highly acidic because have tubes which pump protons
  - Animals cells digestive organelle
  - Lysosome membrane proteins covered in "sugar" chains which protect proteins from highly acidic proteins inside
  - Important for cell turnover (autophagy) - process involving the degradation of a cell's own components through the lysosomal machinery, maintains balance between degredation and synthesis, and recycling of cellular products. Used by starving cells for reallocation of nutrients to more essential purposes.

- Peroxisomes (single membrane)
  - Multifunctional
    - Make hydrogen peroxide to break down compounds
    - Important in fatty acid oxidation (metabolism)
  - Can be reformed spontaneously from ER (probably has evolutionary origin in ER)
  - Replicate by enlarging and dividing
  - Alchohol detoxed
- Mitochondria
  - Stucture
    - Double membrane
    - Inner membrane folded into structure called cristae
    - Has own DNA inside matrix (evidence it used to be its own bacteria)
    - Can divide and grow
    - Product of endosymbiosis
  - Function
    - Site of TCA cycle (Citric acid cycle) → occurs in mitochondria matrix
    - Site of electron transport and oxidative phosphorylation
      - ATP is product as result
      - O2 consumed in the process
      - Also called aerobic respiration
- Chloroplasts
  - Only in plants
  - Surrounded by double membrane
  - Inside membrane stacks called **grana**.
  - Thylakoid membranes form the stacks (light absorbed her)
    - Stacks within the grana
  - Stroma (cytoplasm) has enzymes
    - Enzymes turn light into chemical energy (CO_2 fixation and glucose formation)
  - Contains DNA
  - Can divide and grow
- Vacuoles
  - Only in plants (many types)
  - Small in dividing cells (large in differentiated plant cells)
  - Origin: Invagination of plasma membrane
    - pH of vacuole is acidic similar to pH outside of cell
    - Vacuole membrane similar proteins to plasma membrane
      - May have pinched off plasma membrane
  - Functions
    - Maintain osmotic balance of cell
    - Most of cells internal pressure, important for cell expansion, is generated here
    - Involved in cell growth and expansion
    - Storage site for most of cells macromolecules and solutes

        - Storage site for toxic compounds which are released when cell is under attack
        - **Lytic Vacuoles** – contain enzymes for hydrolysis of macromolecules for recycling
- Cytoplasm also contains (ribosome's and cytoskeleton)
    - Cytosol
        - Souble portion of cytoplasm (w/out the membranes and organelles)
        - Contains enzymes, metabolities, ions
    - Structures w/out membrane
        - Ribosome: makes proteins from amino acids
            - Made from complexes of RNA and proteins
    - Cytoskeleton (made of actin and tubulin filaments)
        - Fibers that make up the cell's skeleton
        - Interconnected network of filamentous proteins
        - Determines cell shape
        - Organizes cytoplasm
        - Aids in transport of vesicles and chromosomes
            - Brings about motility
        - All actin and tubulin filaments can be assembled and disassembled
        - Actin used for resisting tension and maintaining cellular shape
        - Tubulin plays role in intracellular transport and mitotic spindle
- Extracellular Matrix (ECM) in animal cells
    - Organized network of materials outside the plasma membrane
    - Proteins and polysaccharides
    - Holds cells together
    - Determines shape and activities of the cell
    - Barrier
- Connections between animal cells
    - Tight junctions
        - PM proteins that cement epithelial cells together so water or molecules in gut cannot leak between cells
            - Causes materials to actually enter the cell in order to pass through tissues. Very high control of which substances get through
    - Desmosomes
        - Holds cells together to strengthen tissues. Connect the cytoskeletons of cells
        - Cell to cell adhesion, help resist shearing forces
    - Gap Junctions
        - PM proteins that form channels so ions and molecules can flow between two cells. Lines of communication between cells.
        - Molecules/ions can pass freely between two cells (cell cytoplasm's are attached)
- Cell walls in plant cells and connections between cells
    - Wall outside the PM of all cells, made of cellulose
    - Provides support to cell and whole plant (external skeleton)
    - Glues cell together, gives cell shape, protects against pathogens, conducts water and ions
- Connections between plant cells
    - Plasmodesmata
        - Narrow path formed when dividing cells did not separate completely.
        - Thus cytoplasm of two cells is interconnected.
        - Allows macromolecules to move freely between cells, strong communication between cells.

Lecture 3 (2/2/10)

- Membranes
    - Compartmentalizes the cell
    - Keep organelles separate from cytosol

    - Provides selectively permeable barrier
        - Not everything crosses membrane
        - Components of membrane determines what crosses
    - Transports solutes
    - Responds to external signals
    - Facilitates intracellular interactions
        - Gap junctions
        - Plasmodesmata
    - Transduces (converts)energy
        - Mitochondria, chloroplasts
    - Provides scaffold for biochemical activities
        - Membrane itself is a compartment
- Membrane compartmentalize cells and organelles
    - Segregate reactions
    - Permit multiple functions to occur simultaneously
- History: Experiment-based models of the membrane
    - Davson and Danielli's 1954 model
        - Polar heads of phospholipids are all coated with proteins
        - Protein-lined pores permit flow of materials across the membrane
    - Singer and Nicholson's model (1972)
        - Fluid mosaic model - hydrophobic integral components (lipids and proteins) move laterally while it is mosaic due to the fact that it is made up of many different parts
        - Lipid bilayer is more of model (50% mass)
            - 50% lipids
            - 50% proteins
            - Little to no carbohydrates
        - Proteins make rest of membrane
            - Intergral – span the bilayer
            - Peripheral – associated with the outside of the bilayer
            - Proteins can move freely(diffuse) in the bilayer
- Current model
    - Lipids and proteins
    - Two layers of bilayer conatin different types of phospholipids
    - Integral and peripheral proteins (noncovalent)
    - Portions of integral proteins that span the membrane are typically alpha helices
    - External surface of membrane is decorated by glycosylated proteins and phospholipids
        - Glycoproteins: Glycoproteins are often important integral membrane proteins, where they play a role in cell-cell interactions
        - Glycolipids: are carbohydrate-attached lipids. Their role is to provide energy and also serve as markers for cellular recognition.
    - The outer layer may contain "lipid rafts", which are microdomains defined by lipid composition and which float through the membrane independently
    - Lipid raft: contain twice as much cholesterol as bilayer
- Membrane under electron microscopy
    - Membranes have three layers (trilaminar) under EM
- Review of lipids
    - Diverse nonpolar compounds
    - Mostly composed of C and H – hydrophobic
    - Contain few elctronnegative atoms
- Fat molecule
    - Fatty acid tail and glycerol backbone
- Triacylycerols

- No double bonds = high melting point
- Double bonds = lower melting point (liquid at room temp)

- Phospholipids: Basic structure (Amphipathic)
  - Fatty acid tail (chain – non polar), glycerol backbone, phosphate and choline (polar head)
- Lipid Content of Membranes
  - Phospholipids
    - Glycerol backbone
    - Two fatty acid chains
    - Hydrophilic phosphate group
    - Additional head group usually attached to phosphate group **(KNOW STRUCTURES)**
      - Phosphatidic acid (H-)
      - Phosphatidylcholine **(PC)** – at pH 7 = neutral
      - Phosphatidylserine **(PS)** – at pH 7 = negatively charged
      - Phosphatidylethanolamine **(PE)** – at pH 7 = neutral
      - Phosphatidylinositol **(PI)** – at pH 7 = negatively charged
        - Each group is small and hydrophilic
  - Sphingolipids
    - Much less abundant
    - Amphiphatic
  - Sterols (Cholesterol)
    - 50% lipid bilayer in animal membranes
    - Orient small hydrophilic ketone and hydroxyl groups towards outside of membrane
    - Rings are plat/rigid
    - Interfere with free movement (fluidity) of fatty acid tails of other lipids (rigidity)
- Nature of bilayer
  - Not symmetric bilayer
    - One side has more lipids than the other
  - Variability in composition results in different properties of membranes
    - Types of lipids
    - Head groups
    - Fatty acid chains
  - Nature
    - 60 ampheres thick
    - Continuous sheets throughout cell with no exposed edges (hydrophobicity)
    - Flexible, can change shape
- Self assembly into lipsome (occurs when membrane is disrupted)
  - Phosphatidylcholine in aqueous solution forms lipid bilayer filled with water (liposome)
  - Liposomes are used to deliver certain vaccines, enzymes, or drugs (e.g., insulin and some cancer drugs) to the body
- Membrane Carbohydrates
  - Glycoproteins – proteins with attached carbohydrates
  - Glycolipids – lipids with covalently attached carbohydrates
  - Glycosylations are always on extracellular side (process of attaching sugars)
  - Only a few sugars attached
    - Oligosaccharides (3 to 10)
    - They determine blood types as antigens (when attached to lipids)
  - Types of linkages to proteins
    - N-Likages with proteins are via N-containing amino acid residues like asparagines
    - O-IInkages with proteins are via O-containing residues lie serine or threonine
  - Simple sugars (monosaccharides)
    - Glucose, fructose
    - Glyceraldehyde is a triose (3 carbon atoms)

        - Disaccharides
            - Sucrose, lactose
                - These sugars are attached by **glycosdic bonds**
        - Polysaccharides
            - Glycogen
            - Starch
            - Cellulose
- Membrane Proteins
    - Integral, peripheral, lipid-anchored
    - Proteins do all the work and give membrane its identity
    - Properties
        - Each protein has a specific orientation with one side always facing the cytoplasm (sidedness)
- Peripheral
    - Can be solubilized by high-salt solution (polar reagent)
    - Weak electrostatic bonds
    - Structural function
        - E.g. Inner membrane skeleton at cytoplasmic face where they form a fibrillar network
    - Signaling role
        - Protein can attach or dissociate from the membrane in response to signals
- Lipid-anchored proteins
    - GPI – linked
        - It is composed of a phosphatidylinositol group linked through a carbohydrate (oligosaccharides)
        - Found when membrane proteins were released by an enzyme that cleaves inositols
        - Include receptors, enzymes, cell-adhesion proteins
- Integral membrane proteins
    - Penetrate lipid bilayer
        - Some have single spanning domain
        - Some are multi-domain (transporters)
        - Transmembrane domains are usually alpha-helices
        - 20-30% of all encoded proteins
        - Residues on transmembrane domains are hydrophobic
            - Form van der waals interactions with fatty acid of bilayer
            - Form tight seal between protein and lipids
    - Functions
        - Transporters
        - Receptors of external signals
- Challenges presented by integral membrane proteins
    - They are imbedded in lipid bilayer so portion of protein is hidden
    - Needs to be solubilized from membrane with **detergent (SDS)**
    - Detergents are amphiphatic molecules
- Freeze fracture analysis to view Integral membrane proteins
    - Split lipid bilayer
    - Block of frozen tissue is split by a knife
    - Fracture follows middle of bilyer but does not split protein
    - **Proteins remain intact**
        - The protoplasmic face (cytoplasmic side)
        - Ectoplasmic face (opposite side)
    - Proteins can be visualized using a metallic replica of the surface
- Identifying Transmembrane domains
    - Amino acid sequence shows regions of hydrophobic residues
    - Transmembrane region has 20 **nonpolar** amino acids

- Hydrophobicity
  - The free energy change associated with taking those residues from inside the bilayer to its hydrophilic exterior is positive, thus it requires energy
  - Values of hydrophobicity higher than a certain value indicate good candidate regions from transmembrane domains
- Hydrophilic residues
  - Other regions contain positively charged residues (2 lysines and 2 arginines). These may interact with hydrophilic heads of the phospholipids.
  - Glycophorin has a single transmembrane alpha-helix (ppt pic)

- Ways to measure spatial relationships
  - Site-directed mutagenesis
    - A research technique to modify a gene in a predetermined way so as to produce a protein with a specifically altered amino acid sequence
    - Change specific alpha helix residues to cysteines → 2 cysteine residues are able to form a disulfide bridge with one another, then these helices must reside in close proximity to one another.
  - Electron Paramagnetic Resonance (EPR)
    - Detects **open vs. closed channels**
    - Glycine residue is replaced by cysteine → Cysteine is labeled with nitroxide and are detected due to having unpaired electron
    - Shapes of spectra depend on distances between unpaired electrons in the nitroxides on different subunits
    - Channel is open at 6.5 pH, closed at 3.5 pH
    - This indicates that the nitroxide charges are separated at pH 3.5
- Test membrane sidedness
  - Parts of integral membrane protein protrude outside the membrane, and therefore are susceptible to protease digestion
  - Partially digested protein will have lower molar mass
  - Proteins can be separated by size and charge using electrophoresis
    - Method
      - Shave off proteins from external membrane using Trypsin using an intact cell and permeabilized cell
      - Mixtures of proteins separated using SDS gel
        - Proteins based on sizes will be separated
        - Smaller digestion will move further
        - Proteins on external side will have change in molar mass due to digestion
- Separating different organelles and membranes
  - Different organelles have different sizes and densities
  - Can separate by centrifugation
    - Break cells → filter and save filtrate
    - Centrifuge filtrate at 20 k xg. Pellet
    - Separate by centrifugation with a density gradient (different organelles have different densities)
  - Cell fractionation can be used to study activity of organelles inside the cell
- Membrane fluidity
  - Phospholipids
    - Saturated fatty acids packed more closely together
    - The close packed (the more saturated) the higher the transition temp.
    - The shorter the fatty acid chains, the lower the transition temp
      - 1 double bond will decrease melting point by 50 degrees Celsius
    - Membranes with mixtures of phospholipids and fatty acids chains have indistinct transition temp as individual lipids undergo their transition one by one

        - Transition temp = lipid liquid crystalline converted to frozen crystalline gel which restricts movement of the phospholipid fatty acid chains
      - Cholesterol interferes with fatty acid packing, making transition temps indistinct
        - It reduces membrane fluidity
        - Decreases membrane fluidity
        - Increases membrane durability
    - Fluidity important due to the fcat that is provides perfect compromise between rigid ordered with no mobility and completely fluid membrane with no structural organization.
    - Permits intra-membrane interactions
      - Complexes of proteins can come together and assemble at particular sites to form intracellular junctions.
      - Membrane molecules can come together, carry out reactions and then move apart
    - Important for membrane assembly
      - Important in cell division, growth, secretion and endocytosis
      - Gives flexible and dynamic membrane as cells respond to cues
- Phospholipid Asymmetry:
    - Inner and outer leaflets are not the same
      - Outer leaflet (red blood cell)
        - High in PC
        - Low PS and PE
      - Inner leaflet
        - High in PS and PE
- Flip-flop is restricted (movement from inner to outer leaflet or vise versa)
    - Lateral diffusion of phospholipids and sphingolipids is fast
    - However, flip-flop is very slow
      - Flip-flop to outer side is most restricted
      - Hydrophilic head group of the lipid must pass through the internal hydrophobic sheet of the membrane (unfavorable)
      - Cholesterol lacks polar head so can more easily flip-flop
- Lipid rafts (quick signaling)
    - Microdomains with different lipid compositions
    - Seen primarily in artificial membranes
    - Certain types of lipids and proteins tend to group together (transiently)
    - Postulated to serve as floating rafts that collect certain proteins (strongly favored by GPI-anchored proteins)
    - Could provide local environment for cell-surface receptors to interact with membrane proteins in other cells
- Evidence protein moves in the membrane
    - Red fluorescent on human proteins were segregated from mouse proteins with green fluorescent
    - After 40 min....fluorescence's are fully mixed, reveal protein movement
- Protein diffusion rates vary (FRAP)
    - Membrane proteins labeled with dye
    - A small region of the cell surface is exposed to a lser beam. Laser bleaches the dye in that spot.
      - Membrane still intact
      - Proteins still there, dye is just gone from bleached region
    - If fluorescence label reappears in bleached region, then proteins in that area are mobile
    - The rate of fluorescence recovery provides a direct measure of rate of diffusion.
- Types of motility
- Epithelial cell polarity (distinct functions of the PM)
    - Apical PM
      - Regulation of nutrient and water intake
      - Regulated secretion

        - Protection
    - Lateral PM
        - Cell contact and adhesion
        - Cell communication
    - Basal PM
        - Cell-substratum contact
        - Generation of ion gradients
- The red blood cell
    - Best studied plasma membrane
        - Cell readily available
        - Lack internal membranes
        - Isolate pure membranes easily
    - Extensive membrane protein contacts
        - 2 most abundant proteins called band 3 and glycophorin A (integral proteins)
            - Provide charges so red blood cells repel each other (prevents clumps)
        - Band 3 heavily glycosylated – prevents cell-cell interaction
        - Peripheral proteins on internal surface – play major role in determining biconcave shape of cell
- Review of lipids
    - Diverse group of non-polar compounds
    - Mostly composed of C-H – generally hydrophobic
- Sterols
    - Have basic 4-ring structure
    - Cholesterol important in membrane structure
        - Membranes high in sterols tend to be thinner and **less flexible**
- Starch has 1-4 alpha linkages (carbohydrate)
- Cellulose has 1-4 beta linkages (carbohydrate)
- Protein
    - 20 amino acids
        - Polymer is made up of AA units joined by a peptide bond
- 4 groups of AA
    - Polar AA (side chain is negative or positive)
        - Depends on pH
        - Asp, Glu, Lys, Arg, His
        - Hydrophillic, act as acids or bases which are fully charged
        - Form ionic bonds, often involved in chemical reactions
    - Polar Uncharged
        - Ser, Thr, Gln, Asn, Tyr
        - Side chains have partial positive and negative charge (aloows reaction in chem.. rxns)
        - Form Hydrogen dons and associate with water
        - Side chains tend to have balanced charges but contain more than C and H
        - Often quite reactive
    - Nonpolar
        - Hydrophobic
        - Ala, Val, Leu, Ile, Met, Phe, Trp
        - Side chains have almost entirely C and H
        - Inner core of soluble proteins, buried away from aqueous medium
        - Associate with lipid bilayer
    - Unique side chains
        - Glycine
            - Side chains consists only of one hydrogen atom
        - Cysteine

        - Polar, uncharged, bonds with another cysteine to form a disulfide link
    - Proline
        - Hydrophobic side chain, creates kinks in polypeptide chains and disrupting ordered secondary structures
- Amino Acid Structure
    - Amino group, Carboxyl group and a side chain
- Transport
    - Phospholipid bilayer is a remarkable barrier to ions and metabolites
    - CO2, O2, N2, and small uncharged poplar molecules diffuse easily
        - High permeability
    - Then H20 is partially permeable (also uses aquaporins)
    - Then is large uncharged polar molecules such as glucose, tryptophan
    - Next are ions such as K+, Cl-, HCO3-
    - Last are charged polar molecules
        - Amino Acids, ATP, Glucose 6-phosphate
        - Low permeability
- Terms and Definitions
    - Simple Diffusion (passive)
        - Diffusion is movement down a gradient. It is passive movement across a lipid bilayer.
        - No proteins involved
    - Facilitated Diffusion (passive)
        - This diffusion is mediated by a protein:
            - Channel mediated (faster)
            - Passive transporter or carrier mediated (slower)
            - Still high to low movement
    - Transport via a transporter can be active or passive
    - Active Transport (active)
        - **Movement against gradient**
        - **Requires energy** (ATP or ion gradient)
        - Mediated by a protein transporter or pump
- Two classes of Transport Proteins
    - Transporter
    - Transport proteins catalyze transport similar to enzymes in chemical reactions
        - Alternates between two conformations
        - Solute binding site is accessible to one side and then the other
    - Channel
        - Forms continuous pore across the bilayer through which a solute can diffuse
        - Always downhill
    - Vmax is limited by the number of channels = limited transport
    - Substrate affinity is extremely specific
- Transport
    - Substrate doesn't change during transport
        - It binds, gets transported, and is then released
- Types of active transport
    - Primary
        - Ion pump is directly coupled to an energy-yielding reaction
        - ATP hydrolysis
    - Secondary
        - Ion pumped is driven by downhill movement of another ion
        - Na+ or H+
        - No direct coupling to ATP
        - Releases energy, this energy is used to move another molecule against gradient

- Types of transporter-mediated transport
  - Uniport (facilitated)
    - One solute at a time
  - Symport (coupled transport)
    - Secondary active transport
    - Two molecules across membrane in same direction
    - Co-transporters
  - Antiport (coupled transport)
    - Secondary active transport
    - Two molecules in separate directions
- Energetics of solute transport (Vm is membrane potential)
  - Diffusion of uncharged solute depends on the concentration gradient
    - $\Delta G = RT\ln(Ci)/(Co)$
  - Diffusion of charged species depends on electrochemical gradient
    - $\Delta G = RT\ln Ci/Co + zF\Delta E$
  - At equilibrium $\Delta G = 0$
    - $\Delta E = -RT/zF \ln Ci/Co \rightarrow$ Nernst Equation
    - Reversal potential
- How is membrane potential formed
  - Diffusion of ions
  - Ion pump
  - Movement of ions generates an electrical gradient
- How do mammalian cells maintain low Na inside cell
  - Na will always enter will down concentration gradient
  - It is then pumped out by the Na/K-ATPase (sodium potassium pump)
  - Na and K gradients are maintained by Na_{out}/K_{in} pump
- Plants Fungi
  - Electric potential is maintained by a H+ extrusion pump
  - K+ maintained → K+ comes into cell passively via K+ channel
- Bacteria
  - Electron potential generated by H+ extrusion pump
- Roles of primary H+ or Na+ pumps
  - Central theme of bioenergetics is ion coupling
  - Generate and maintain electric and chemical gradient
  - Provide driving force for transport of various ions and metabolites
  - Generate electric and ion changes that serve as stimuli
- Transport of glucose across epithelial cells
  - Na/Glucose transporter brings in 2Na+/Glucose (Na/Glucose symport protein)
    - Secondary active transport
  - Glucose moves to blood through GLUT2 (Facilitate)
  - Na exits cell through Na+/K+ ATPase, brings K+ into cell
- Primary Active Transport Pump
  - H+ pump
  - Na+/K+ pump
  - Vacuolar H+-ATPase acidify endomembrane compartments
- Na/K-ATPase pumps $3Na_{out}/2K_{in}$
  - Plasma membrane of animal cell
  - Na extrusion pump
  - **Electrogenic pump**: pump that generates an electrical difference or membrane potential
  - 3Na+ picked up by pump,ATP which is attached which is then hydrolyzed, this leads to phosporylation→creates conformation change which lowers affinity for Na+ and are released.

- K+ then binds, the pump is dephosphorlated and conformation change takes place which releases K+ into the cell

- H/K-ATPases in stomach epithelial cells control acid secretion
  - Primary active transport
- V-ATPase pump
  - Vacuolar H+-pumping ATPase acidifies the vacuolar lumen

- Secondary Active Transport
  - Animals (Na+-coupled)
    - Glucose(uphill)/Na+(downhill) → symport
    - Na/H antiport
    - Uptake of neurotransmitters into synaptic vesicles
  - Plants (H+-coupled)
    - Uptake of lactose, sugars, AA, anions
- Model of 2Na/1 Glucose co-transporter (secondary active transport)
  - Cooperative binding
    - Na binding induces binding of glucose.
    - Binding of both Na/Glucose to sites causes conformational change
    - Na diffuse down concentration gradient causing conformational change
    - Affinity for glucose decreases an Glucose is released into the cytosol
    - Most secondary active transporters have 12 transporter proteins
- Passive Transport
  - Aquaporins (water channels)
    - Evidence for protein channels
  - Diffusion of Ions
    - Voltage gated
    - Ligand gated
    - Mechano-sensitive
- How do cells maintain osmotic concentration as cells increase in volume?
  - Take up more ions/solutes during growth – using pumps, co-transporters and channels
  - Take up water, some cells will lyse in dilute solution
  - Plant, yeast, bacteria have rigid walls, so they do not burst in hypotonic solutions
  - Water moves from region of high water potential to low water potential (passively)

- Water moves passively
  - Hypotonic Solution
    - When a cell is placed in a hypotonic solution, the water diffuses into the cell, causing the cell to swell and possibly explode.
  - Hypertonic Solution
    - When a cell is placed in a hypertonic solution, the water diffuses out of the cell, causing the cell to shrivel.
  - Isotonic Solution
    - When a cell is placed in an isotonic solution, the water diffuses into and out of the cell at the same rate. The fluid that surrounds the body cells is isotonic.
- Effects of osmosis on a plant cell
  - Fresh water plants are surrounded by a hypotonic environment
  - Water therefore tends to flow into the cells creating turgor pressure
  - If the plant is placed in hypertonic solution(seawater), the cell loses water, and the plasma membrane pulls away from the cell wall (No turgor pressure)
- Water moved faster than scientists thought, could only be explained through there being another type of protein channel (aquaporin).

- Ion Channels
  - Channel forms a continuous pore across the bilayer so ions can diffuse through it
  - Channels are regulated and can be gated by:
    - Voltage or ligand
    - Rate of channel is very rapid
      - 1-10 million ions/sec per channel
  - Ion carries a charge, so its movement can be measured by conductance
- Glycolysis (anaerobic respiration)
  - 1 Glucose (C6) --> 2 pyruvate (C3) + 2 ATP + 2NADH
  - Occurs in the cytoplasm
  - Takes place in cytoplasm of cells
  - 2 net ATP produced
- Aerobic Respiration
  - $C_6H_{12}O_6$ (aq) + 6 O_2 (g) → 6 CO_2 (g) + 6 H_2O (l)
  - The product of this process is energy in the form of ATP (Adenosine Triphosphate), by substrate-level phosphorylation
  - TCA Cycle
    - Acetyl-CoA + 3 NAD^+ + FAD + GDP + P_i + 2$H_2$0 <==> CoASH + 3 NADH + $FADH_2$ + GTP + 2CO_2 + 3H^+
    - Oxidizes 1 pyruvate, so it takes 2 turns to completely oxidize 1 glucose. Two turns produce 8 NADH, 2 FADH2, and 2 ATP. NADH and FADH2 are then oxidatively phosphorylated, **resulting in 28 more ATP**
    - Pyruvate converted in Acetyl-CoA
    - Occurs in inner membrane of mitochondria
  - ETC
    - An electron transport chain (ETC) couples a chemical reaction between an electron donor (such as NADH) and an electron acceptor (such as O2) to the transfer of H+ ions across a membrane.
    - Occurs in mitochondria in cells
    - NADH is oxidized via the electron transport chain. Energy released pumps H+ out
    - NADH + H+ + ½ O2 ---> NAD+ + H2O & H+ gradient
    - **38 ATP in prokaryotic cells**
    - **36 ATP in eukaryotic cells**
  - Proton Gradient is used to form ATP
    - Mitochondria (inner membrane)
      - H+ move down proton gradient into matrix creating energy (3 H+), which causes ADP and a phosphate to bind generating ATP molecule
      - pH gradient develops

- Membrane Potentials and Nerve Impulses
  - Selectivity of the bacterial KcsA K+ channel protein
    - K+ in solution is surrounded by water molecules
    - At selectivity filter, **only K+ ion** (with no water shell) can fit into the filter
    - Most widely distributed type of ion channel and are found in virtually all living organisms
    - Vast majority of ion channels that are open in a resting nerve cell are selective for K+
      - ➢ These are referred to as K+ leak channels
  - KcsA K+ channel protein
    - Consists of 4 sub units, 2 K+ move across at a time
    - M2 helicies from each subunit bend outward at a specific glycine residue, which pens the intracellular end of the channel to K+ ions.
    - Conformational changes of cytoplasmic ends of inner (M) membrane
      - ➢ When open the M2 helicies bend at hinge point

- Eukaryotic voltage-gated K+ (Kv) channels
  - In plants play role in water balance
  - Important for muscle and nerve function in animals
  - Contains 6 transmembrane helicies and a potion of the pore helix(P) that dips into the protein to form part of the channel wall
  - S5,S6 and P: Channel pore domain(Homologous to M1/M2 and P segment of KcsA)
    - Contains selectivity filter that permits selective passage of K+ ions. (Conformational changes)
  - S1-S4 (Voltage-sensing domain)
    - Senses voltage across plasma membrane
    - Under resting conditions, negative potential keeps gates closed, change in potential to positive (depolarization) exerts force on S4.
    - Once open significant amount of K+ come into cell. Stopped after few milliseconds due to extreme rush. (inactivation)
    - Inactivation caused by peptide which dangles from cytoplasmic portion of complex
      - Moves up into mouth of pore and ion passage is blocked
  - Voltage gated K channel has 3 states
    - Open
    - Closed (rest)– release of peptide from mouth
    - Inactivated – peptide inserts into mouth of pore
- Membrane Potentials and Nerve Impulses
  - All organisms respond to stimuli, nerve cells collect, conduct, and transmit information
  - Dendrites receive incoming info
  - Axon conducts outgoing impulse away from cell body towards target cells (split at ends to form terimal knobs)
  - Terminal knob is site where impulses are transmitted to target cell (neuron to target)
- Measuring Membrane potential with electrodes
- Action potential
  - An action potential occurs when a stimulus is sensed
  - Stimulus causes depolarization (influx Na+) -70mV→-50mV
    - Decrease in polarity between both sides
  - Na channel opens then closes, threshold is reached and K+ open (down gradient)
  - Voltage activated K+ channels open to return membrane to resting potential (hyperpolarization)
    - K+ rush out of cell
  - Whenever action potential occurs muscle fiber will undergo maximum level of contraction **(all or none law)** and below certain stimulus level no action potential is produced
  - Action Potential is propagated (transmitted) down length of cell to terminals
    - The impulse reaches the Terminal knob which releases NT (Acetylcholine) to target cell
- Muscle cell contraction
  - Nerve impulse reaches terminal knob of axon and Ca2+ open VG channels (influx of Ca2+), NT (Acetlcholine) is released from synaptic vesicles and binds to receptors on postsynaptic membrane
    - Bound NT can either
      - Cause depolarization an a nerve impulse will be generated (action potential)
      - Or cause hyperpolarization (influx Cl-), no action potential reached
- Guard Cells
  - Stomata are pores on leaf surface (under), either in open or closed state
  - Pores on leaf surface can open and close to regulate CO2 uptake for photosynthesis
  - Opening and closing of pores (stomates) is controlled by turgor pressure of two guard cells
    - Regulated by pumps, cotrabsporters, channels
  - When opening, K+ (lots)and Cl-(little) rush into cell due to H+ pump which drives protons out of cell (negative potential) and water rushes in through osmosis (increase in turgor pressure)
  - Due to limit of swelling (cellulose), cells bow allowing gas exchange