

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 Eukaryotic 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 → 1µm
 - 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 viruses 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
 - Nucleolus
 - rRNA synthesizes 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 lysosomal, 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 degradation 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
 - Alcohol detoxed
- Mitochondria
 - Structure
 - 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 (CO₂ 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
 - Soluble portion of cytoplasm (w/out the membranes and organelles)
 - Contains enzymes, metabolites, 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
 - Integral – 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 contain 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 electronegative atoms
- Fat molecule
 - Fatty acid tail and glycerol backbone
- Triacylglycerols

- 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
 - Amphipathic
 - Sterols (Cholesterol)
 - 50% lipid bilayer in animal membranes
 - Orient small hydrophilic ketone and hydroxyl groups towards outside of membrane
 - Rings are flat/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 liposome (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-Linkages with proteins are via N-containing amino acid residues like asparagines
 - O-Linkages 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 **glycosidic 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 bilayer 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 fact that it 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 vice 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 laser 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
 - Hydrophilic, 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 (allows reaction in chem.. rxns)
 - Form Hydrogen bonds 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 polar molecules diffuse easily
 - High permeability
 - Then H₂O 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
 - V_{max} 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 (V_m is membrane potential)
 - Diffusion of uncharged solute depends on the concentration gradient
 - $\Delta G = RT \ln(C_i/C_o)$
 - Diffusion of charged species depends on electrochemical gradient
 - $\Delta G = RT \ln C_i/C_o + zF\Delta E$
 - At equilibrium $\Delta G = 0$
 - $\Delta E = -RT/zF \ln C_i/C_o \rightarrow$ 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 \rightarrow 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 phosphorylation \rightarrow creates conformation change which lowers affinity for Na⁺ and are released.

- K⁺ then binds, the pump is dephosphorylated 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 as 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
 - $C_6H_{12}O_6(aq) + 6 O_2(g) \rightarrow 6 CO_2(g) + 6 H_2O(l)$
 - The product of this process is energy in the form of ATP (Adenosine Triphosphate), by substrate-level phosphorylation
 - TCA Cycle
 - $Acetyl-CoA + 3 NAD^+ + FAD + GDP + P_i + 2H_2O \rightleftharpoons CoASH + 3 NADH + FADH_2 + GTP + 2CO_2 + 3H^+$
 - 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
 - $NADH + H^+ + \frac{1}{2} O_2 \rightarrow NAD^+ + H_2O$ & 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 helices 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 helices 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 helices and a portion 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 terminal 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 (Acetylcholine) 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, cotransporters, 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

- When water shortage is sensed, Ca²⁺ rushes into the cell, Cl⁻ rushes out
 - K⁺ is no longer influxed, causes reduction in osmotic pressure (turgor) and cells close
- Photosynthesis and the Chloroplast
 - Organisms which depend on an external source of organic compounds are called **heterotrophs**
 - Organisms capable of surviving on CO₂ as principal carbon source are **autotrophs**
 - Chemoautotroph's
 - Use chemical energy stored in inorganic molecules to convert CO₂ to organic compounds
 - Photoautotroph's
 - Use radiant energy of the sun to achieve same result
 - Photosynthesis – process by which sunlight is converted into chem. Energy that is stored in carbs and other organic molecules
 - Photosynthesis Rxn
 - $\text{CO}_2 + \text{H}_2\text{O} \rightarrow (\text{CH}_2\text{O}) + \text{O}_2$
- Chloroplast
 - Cyanobacterium transformed from separate organism living within a host into a cytoplasmic organelle.
 - Polypeptides found within modern chloroplasts are encoded by both the nuclear and chloroplast genomes. (Result of lost of cyano. DNA into host).
- Overview of Photosynthesis
 - Light Absorption
 - Electrons are captured from water
 - NADPH
 - Reducing energy is formed
 - ATP
 - Chemical energy is formed
 - CO₂ → Sugar
 - CO₂ is captured and reduced to sugar
- Chloroplast Structure and Function
 - The light reactions occur on the thylakoid membranes
 - Dark reaction occur in the stroma
 - Outer membrane – selectively permeable
 - Inner membrane – impermeable (movement only through transporters)
 - Thylakoid membrane (energy transducing machinery) – composed of grana stacks
 - Stroma is the cytoplasm (contains small DS circular DNA and ribosomes)
 - Thylakoid membrane (high protein to phospholipid ratio)
- Overview of Photosynthetic Metabolism
 - CO₂ is oxidizing agent (O₂ is derived from H₂O)
 - $6\text{CO}_2 + 12\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} + 6\text{O}_2$
 - Light Reactions
 - Light absorbed and stored as ATP and NADPH
 - Dark Reactions
 - Carbs are synthesized from CO₂ using energy stored from ATP and NADPH.
- Photosynthetic Pigments (Chlorophylls and Carotenoids)
 - Compounds that appear colored because they only absorb light of particular wavelengths within visible spectrum.
 - Pigments contain conjugated double bonds: pi electrons
 - Pi electrons can be excited by light. The energy of excited electron can be transferred to another pigment.
- Chlorophylls
 - Contains a porphyrin ring that functions in light absorption with Mg in center

- Contains hydrophobic phytol chain that keeps chlorophyll embedded in photosynthetic membrane
 - Stabilized by protein complexes in lipid bilayer of thylakoid membrane
- Carotenoids
 - Orange but are able to absorb green light
- Important photosynthetic pigments
 - Chlorophylls
 - *a* is found in all oxygenic organisms
 - *b* and *c* are found in some
 - Carotenoids
 - Beta-carotene, lutein, Fucoxanthin
 - Photocobilins (found in red algae and cyanobacteria)
 - Phycoerythrin
 - Phycocyanin
 - Pigments bind to protein to form a Light Harvesting Complex
 - Embedded in the thylakoid membrane (can consist of up to 250-300 Chl)
 - Chlorophyll absorbs in blue and red (B-Carotene absorbs in the blue)
- Basic Concepts about light
 - Amount of energy is dependent on wavelength of light. Light has properties of a wave and a particle (photon)
 - Quantum Energy = $h\nu = hc/\lambda$
 - When pigments absorb light, they change their electron state. Plants contain pigments that absorb the energy of photons
 - Principle of Gotthaus-Draper: Only light absorbed can be active in photochem. Reactions
 - Einstein-Stark Law: A single photon can excite only one electron
- Many pigments are packed to form a LHC
 - Antenna Pigments – consist of Chlorophylls and carotenes (most pigments in complex are antennas)
 - Absorb light energy and pass excited energy from one pigment to another if very close
 - Purpose of antenna pigments is to ensure light energy reaches rxn. center pigment
 - Chlorophyll can absorb photon → get excited → give up high energy electron (oxidized)
 - What is light used for
 - Excitation energy transfer
 - Photochemistry or photo-oxidation
 - 1st electron acceptor is Pheophytin
- Light Reactions
 - Light absorption and water spilling
 - $2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$
 - Transfer of electrons from H₂O to NADP⁺ forming NADPH + H⁺
 - $\text{NADP}^+ + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{NADPH} + \text{H}^+$ (net reaction of excitation and transfer of electron through both photosystems is reduction of NADPH)
 - Electron transport and water splitting generate a H⁺ electrochemical gradient that is used to make ATP.
 - $\text{ADP} + \text{P}_i \rightarrow \text{ATP}$
- Photosynthesis is a light-driven redox process
 - Entire process in several steps, insufficient energy to boost e⁻ from H₂O directly into NADP⁺
 - O₂ evolving organisms have 2 photosystems
 - PSII : pull electrons from H₂O
 - PSI : e⁻ reduce NADP⁺ to form reducing power
 - Both reactions centers are connected by an electron transport chain
 - Electron transport and water splitting generates a proton gradient that is used to make ATP
 - A single photon leads to the excitation of an electron in the reaction center chl to a higher energy state
 - In PSII : P680 → P680* (primary electron acceptor)

- In PSI : P700 → P700* (primary electron acceptor)
 - P680 is strong reducing agent, donates electron downstream to pheophytin
 - After losing electron (referred to as P680+ and P700+ in oxidized states or oxidizing agents), act as attractants for electrons. Separation of charges sets stage for flow of electrons along chain of carriers
 - P680+ is able to pull electrons from H₂O forming O₂ (in atmosphere)
 - P700+ donates electron to NADPH and accepts electron from chain
- Overall....
 - Light is absorbed by the antenna pigments of **photosystems II and I**.
 - The absorbed energy is transferred to the reaction center chlorophylls, **P₆₈₀** in photosystem II, **P₇₀₀** in photosystem I.
 - Absorption of 1 photon of light by Photosystem II removes 1 electron from **P₆₈₀**.
 - With its resulting positive charge, P₆₈₀ is sufficiently electronegative that it can remove 1 electron from a molecule of water.
 - When these steps have occurred 4 times, requiring 2 molecules of water, 1 molecule of oxygen and 4 protons (H⁺) are released
 - The electrons are transferred (by way of **plastoquinone — PQ** in the figure) to the **cytochrome b₆/f** complex where they provide the energy for **chemiosmosis**.
 - Activation of **P₇₀₀** in photosystem I enables it to pick up 4 electrons from the cytochrome b₆/f complex (by way of **plastocyanin — PC** in the figure) and raise them to a sufficiently high redox potential that, after passing through **ferredoxin (Fd** in the figure), they can reduce NADP⁺ to **NADPH**.
- Photosystem II and LHCII complex
 - Light excited pigments pass energy to reaction center pigment (P680 → P680*)
 - Excited P680* gives e⁻ to pheophytin (P680* → P680+)
 - Pheophytin is PSII's primary electron acceptor (Pheo + e⁻ → Pheo⁻)
 - Electron is quickly passed to plastoquinone PQ_A and then to PQ_B⁻.
 - Second photon sent down path forming PQ_B²⁻ which combines with two protons to form plastoquinol (PQH₂).
 - Protons utilized in the formation of PQH₂ are derived from the stroma causing formation of proton gradient. The reduced PQH₂ molecules and diffuses into the lipid bilayer.
 - The displaced PQH₂ is replaced by a fully oxidized PQ molecule derived from a small "pool" of plastoquinone in the bilayer.
 - The splitting of H₂O during photosynthesis is called photolysis.
 - Formation of 1 O₂ molecule is thought to require loss of 4 electrons from two molecules of water
 - $2\text{H}_2\text{O} \rightarrow 4\text{H}^+_{\text{lumen}} + \text{O}_2 + 4\text{e}^-$ (overall PSII reaction)
 - P680+ is very good at stripping electrons from water
 - However the oxygen contained in water have to be held in place until they are able to form O₂
 - At water oxidation site, there is a reaction center with 4 Mn and 1 Ca that holds the H₂O molecules in place
- Electron transfer between PSII and PSI
 - 4 every 4 electrons through PSII, 4 H⁺ are transferred via the plastoquinone cycle to thylakoid membrane
 - Cytochrome b₆f complex re-oxidizes plastoquinol
 - The resulting plastoquinone returns to PSII
- Photosystem I and LHC I absorb light energy to pass electrons to NADP⁺
 - Light absorbed by antenna pigment and transfer energy to P700 chlorophyll (PSI reaction center)
 - P700* (excited) passes electron to A₀ (primary electron acceptor)
 - Goes through A₁, F_{x,a,b} (iron sulfur center).
 - Electron is then passed to ferredoxin (small iron-sulfur protein) that is external (stroma) to PSI complex

- When 2 ferredoxin molecules have accepted an electron, act together to reduce a molecule of NADP⁺ to NADPH.
- Electron deficient P700⁺ is reduced (accepts electron) from plastocyanin.
- A proton gradient is formed in electron transport. ATP is made through **chemiosmosis**
 - The energy released as electrons pass down the gradient between photosystem II and photosystem I is harnessed by the cytochrome b₆/f complex to pump **protons (H⁺) against** their concentration gradient from the stroma of the chloroplast into the interior of the thylakoid (an example of active transport). (Increase pH of thylakoid)
 - As their concentration increases inside (which is the same as saying that the pH of the interior decreases), a strong diffusion gradient is set up.
 - The only exit for these protons is through the ATP synthase complex. As in mitochondria, the energy released as these protons flow down their gradient is harnessed to the synthesis of **ATP**. The process is called **chemiosmosis** and is an example of facilitated diffusion.
- Photosynthesis 2: CO₂ fixation
 - All plants use C₃ pathway to fix CO₂ to make sugars (carbs)
 - C₃ reduction or Calvin cycle
 - 3 CO₂ → C₃ sugar (2x) → C₆ carbon sugar (glucose)
 - C₄ plants maximize CO₂ fixation using C₄ pathway that increases [CO₂]
 - CAM plants minimize water loss by fixing CO₂ at night
- The Calvin Benson Cycle (stroma)
 - Discovery dependent on use of ¹⁴C-radioactive isotopes
- 3 stages of the C₃ reduction pathway (Calvin Cycle)
 - Carboxylation (3RuBP + 3 CO₂ → 6 PGA)
 - CO₂ combines with the phosphorylated 5-carbon sugar **ribulose bisphosphate**.
 - Reduction of P-glycerate (6 PGA → 6 GAP → 5 GAP + 1 GAP)
 - This reaction is catalyzed by the enzyme **ribulose bisphosphate carboxylase oxygenase (RUBISCO)** (most abundant protein on earth).
 - The resulting 6-carbon compound breaks down into two molecules of **3-phosphoglyceric acid (PGA)**. (NADPH and ATP are used)
 - The **PGA** molecules are further phosphorylated (by ATP) and are reduced (by NADPH) to form **GAP** (Glyceraldehyde-3-P)
 - **GAP** serves as the starting material for the synthesis of **glucose and fructose**.
 - Glucose and fructose make the disaccharide **sucrose**, which travels in solution to other parts of the plant (e.g., fruit, roots).
 - Glucose is also the monomer used in the synthesis of the **polysaccharides starch and cellulose**.
 - Regeneration of CO₂ Acceptor (5 GAP + 3 ATP → 3 RuBP)
 - GAP and hydrolysis of ATP are used to regenerate RuBP. (1 ATP = 1 RuBP)
- Experimental evidence for C₃ Cycle
 - Unicellular green alga (Chlorella) was given light
 - Fed with ¹⁴CO₂ for a few seconds
 - Killed quickly with hot ethanol
 - Identified first spots labeled with ¹⁴C using 2-D chromatography
- Calvin cycle stoichiometry
 - Each CO₂ (C₁) is bound to RuBP (C₅) to make two PGA's (2x C₃)
 - Each PGA (C₃) is activated by ATP and reduced by NADPH to make 1 GAP (C₃)
 - 1 out of every 6 GAP come out of the cycle to make sucrose
 - The remaining 5 GAP are reactivated by ATP to make 3 RuBP and fix more CO₂.
- Quick Notes
 - 3 ATP consumed for every 2NADPH
 - The stoichiometry is balanced for the entire cycle with high numbers
 - All reactions are occurring at the same time and all the time

- Some Calvin Cycle/Glycolysis intermediates are the same
 - Glycolysis is run in reverse as gluconeogenesis
 - Dark reactions generate GAP and PGA
 - PGA and GAP are gluconeogenesis and glycolysis intermediates
 - 2 GAP molecules are needed per glucose molecule
- Calvin Cycle is tightly regulated
 - CO₂ fixation continues as long as ATP and NADPH are available
 - In the absence of light, the Calvin Cycle must be shut down or all ATP in the cell would be hydrolyzed and all NADPH would be oxidized
 - How do chloroplasts sense and tell enzymes if energy is adequate?
 - Enzymes are sensitive to and regulated by:
 - pH
 - oxidation/reduction status
- Light via ferredoxin to thioredoxin – reduces disulfide bond
 - In the light, ferredoxin is reduced and a fraction of these electrons are transferred to the small protein thioredoxin.
 - Reduces disulfide groups of certain Calvin cycle enzymes, maintaining them in an active state.
 - **In the dark**, electron flow to thioredoxin ceases, the sulfhydryl groups of the regulated enzymes become oxidized to the disulfide state and the enzymes are inactivated (sulfide bridge reforms).
- Fixation of CO₂ by Rubisco is not efficient because O₂ competes
 - Rubisco catalyzes the addition of CO₂ to RuBP
 - However, also has oxygenase activity which will bind O₂ to RuBP instead of CO₂
 - Binding of O₂ → Glycolate, it is transferred to peroxisome and release of CO₂
 - It is an extremely inefficient enzyme (3CO₂/sec) but makes up for it due to large quantities
 - Most abundant protein on earth
 - Evolved when [O₂] was very low
- Rubisco is a carboxylase and an oxygenase
 - Rubisco binds RuBP and exposes it to “attack” by CO₂ but can also be attacked by O₂ (oxygenase activity and photorespiration)
 - 2 carbons lost due to oxygenase activity
 - Has oxygenase activity due to evolving in low O₂ atmosphere
- Photorespiration
 - Process where O₂ is consumed and CO₂ is released off in light by photosynthetic tissues
 - O₂ is attached to rubisco instead of CO₂, consuming part of RuBP in a useless reaction
 - O₂ and CO₂ compete for Rubisco’s active site
 - The relative concentrations of the two determine which reaction (carboxylation or oxygenation) prevails
 - The result of oxygenation is the production of:
 - 2-phosphoglycolate (at the expense of PGA (C₃))
 - Can account for 50% of loss of fixed CO₂ by plants growing in high light intensity
 - Cells waste energy to do one of two things:
 - Return 2-phosphoglycerate to the Calvin cycle (using energy)
 - Limit the oxygenase activity of Rubisco
 - All plants attempt to limit O₂ concentration near rubisco using anatomical and biochemical modifications.
 - In high CO₂ concentrations, plants are capable of much more rapid growth by virtue of elevated CO₂ fixation
- Two ways to increase Rubisco efficiency
 - C₄ metabolism: A mechanism to concentrate CO₂ in the chloroplasts to reduce RuBP oxygenase activity.

- C4 plants: Corn, sugar cane, weeds
 - CAM
 - Take up CO₂ at night. [CO₂] is high during the day
 - Reduce water loss by separating reactions in time
 - CAM plants are suited to dry habitats
- C4 Pathway (Occurs in outer mesophyll cells)
 - Linkage of CO₂ to PEP
 - PEP carboxylase continues to operate at much lower levels than does Rubisco and is not inhibited by O₂.
 - C4 plants are adapted to hot climates, can close stomata to prevent water loss and can simultaneously still have CO₂ uptake to fuel photosynthetic activity at a maximal rate.
- After entering through stomata, CO₂ diffuses into a **mesophyll cell**.
 - Being close to the leaf surface, these cells are exposed to high levels of O₂, but have no RUBISCO so cannot start photorespiration (nor the dark reactions of the Calvin cycle).
 - Instead the CO₂ is inserted into a **3-carbon** compound (C₃) called **phosphoenolpyruvic acid (PEP)** forming the **4-carbon** compound **oxaloacetic acid (C₄)**.
 - Oxaloacetic acid is converted into malic acid or aspartic acid (both have 4 carbons), which is transported (by plasmodesmata) into a **bundle sheath cell**. Bundle sheath cells are deep in the leaf so atmospheric oxygen cannot diffuse easily to them; often have thylakoids with reduced photosystem II complexes (the one that produces O₂).
 - Both of these features keep oxygen levels low.
 - Here the 4-carbon compound is broken down into:
 - **Carbon dioxide**, which enters the Calvin cycle to form sugars and starch.
 - **Pyruvic acid (C₃)**, which is transported back to a mesophyll cell where it is converted back into PEP.
- CAM Plants
 - These are also C₄ plants but instead of segregating the C₄ and C₃ pathways in different parts of the leaf cells, they separate CO₂ fixation and light-dependent reactions in **time** instead.
 - Keep stomata closed during the day, open at night when rate of water loss is reduced.
 - At night open stomata and fix CO₂ by means of PEP carboxylase.
 - The CO₂ joins with PEP to form the 4-carbon oxaloacetic acid.
 - This is converted to 4-carbon **malic acid** that accumulates during the night in the central vacuole of the cells.
 - In the morning, the stomata close (thus conserving moisture as well as reducing the inward diffusion of oxygen).
 - The accumulated malic acid leaves the vacuole (goes to chloroplast) and is broken down to release CO₂.
 - The CO₂ is taken up into the Calvin (C₃) cycle (converted to carbohydrate using energy from ATP and NADPH generated by light-dependent reactions).
- Intracellular Compartments and Protein Sorting
 - Intracellular compartments of Eukaryotic cells
 - Divided into functionally distinct compartments
 - Ex. Root cap cell is specialized to secrete (has an active endomembrane system)
 - Endomembrane system (Golgi, endosomes, lysosomes, ER, and vacuoles)
- Compartment Characteristics and specific proteins
 - Each compartment contains its characteristic set of enzyme and molecules
 - Proteins give each compartment its special structure and function
 - Enzymes, transporters, surface markers
 - Thus the synthesis of proteins and its delivery to specific locations is critical to growth, differentiation, and functions of the cell.
 - Cell organization and function depend on sorting proteins to right destinations
- Origin of intracellular compartments

- Clue from plastid development
 - Development of proplastid to differentiated plastid (chloroplast) involves membrane invagination
 - Proplastids are precursor organelles (chloroplasts) present in immature plant cells
 - Inner membrane invaginated and then pinched off from inner membrane leading to thylakoid compartments.
- Origin of organelles
 - Nucleus: PM with DNA invagination
 - ER: PM invaginated just not around DNA but connected with nucleus
 - Mitochondria: Plastid engulfed by PM of primitive cell
 - Inner membrane (PM of bacteria)
 - Outer membrane (PM of host)
- Topological relationships of compartments in endomembrane system
 - Only 2 sides to each membrane
 - Cytosol side
 - Lumen side (exterior of cell)
 - Communication via transport vesicles
- Road map of protein traffic
 - All proteins made in the cytosol
 - Fates depend on sorting signals
 - 3 types of protein transport
 - Gated: Nuclear pore
 - Transmembrane: crosses a membrane (ER, mitochondria)
 - Vesicular: Vesicles bud off, move, then fuse
- What determines the destination?
 - Sorting signals built into the protein
 - Encoded in the AA sequence (N-terminus) of the protein or the attached oligosaccharides
 - Complementary sorting receptors recognize these signals
- Sorting signals sequences
 - Mitochondria
 - Mitochondrial matrix targeting sequences are rich in **positively charged amino acids and hydroxylated ones.**
 - Nucleus
 - Import positively charged AA, export hydrophobic AA
 - Plastid
 - Import hydroxylated AA
- Destination through ER ribosomes
 - Signal sequence is recognized by and is bound by a **signal recognition particle (SRP)** defines ER membrane
 - The complex of ribosome with its nascent polypeptide and the SRP binds to a receptor and translocon (protein lined channel) on the surface (facing the cytosol) of the ER.
 - The growing polypeptide chain is extruded through translocon in the ER membrane and into the **lumen** of the ER. The signal sequence is usually clipped off the polypeptide unless the polypeptide is to be retained as an integral membrane protein. SRP leaves.
 - Other proteins, called **molecular chaperones**, present in the lumen of the ER, bind the growing polypeptide chain and assist it to fold into its correct tertiary structure.
 - Includes secreted proteins to outside of cell, soluble proteins in lumen of ER, Golgi, lysosome, endosomes, vesicles, and plant vacuoles, integral membrane proteins of endomem. system
- Destination of Proteins Synthesized by Free Ribosomes (occurs cotranslationally)

- Includes proteins destined for inner surface of PM, transported to nucleus
- Mitochondria
 - Although the mitochondrion has its own genome and protein synthesizing machinery, most of the proteins used by mitochondria are encoded by genes in the nucleus of the cell, synthesized in the cytosol, and must be imported into the mitochondrion.
 - Proteins destined for mitochondrion contain a characteristic signal sequence (N-terminus).
 - Signal peptide is amphiphatic alpha helix with no homology to other mito signals
 - This is recognized and bound by a **chaperone** called **mitochondrial stimulation factor (MSF)**.
 - MSF targets the protein to a receptor embedded in the outer membrane of the mitochondrion. Other factors and receptors shepherd proteins through the intermembrane space to the inner mitochondrial membrane and the matrix.
- Chloroplasts
 - Chloroplasts, like mitochondria, have their own genome and their own protein-synthesizing machinery.
 - Like mitochondria, most of the proteins used in chloroplasts are encoded by genes in the nucleus of the cell, are synthesized by ribosomes in the cytosol, and must then be imported into the chloroplast.
 - Proteins destined for chloroplasts are recognized by their characteristic **transit sequence**. Chaperones are also needed to get them to their final destination: stroma, thylakoid membrane, etc.
- Peroxisomes
 - Proteins destined for peroxisomes are synthesized with a peroxisomal targeting signal (PTS) that binds to a receptor molecule that takes the protein into the peroxisome and then returns for another load.
- Structures of Mitochondria and Chloroplast
 - Both organelles contain DNA, ribosomes, and protein synthesis machinery
 - Many genes have been transferred to the nuclear genome
 - Proteins encoded in nucleus, synthesized in cytosol, and imported
- Uptake of Proteins into mitochondria
 - Posttranslationally
 - Protein must be presented in unfolded state.
 - Signal sequence located at N-terminus for matrix protein and internally for inner membrane protein
 - Cytosolic Hsp70 molecules unfold polypeptide prior to entry
 - Proteins recognized by membrane receptors and translocated through OMM by pores in TOM complex on OMM.
 - Complex includes receptor which bind mitochondrial protein
 - Protein-lined channel which unfolded polypeptides can translocate through OMM
 - Proteins destined for IMM or matrix must pass through intermembrane space and engage IMM TIM complex.
 - TIM22: Binds integral membrane proteins of IMM and inserts them into lipid bilayer of mitochondria IMM.
 - TIM23: binds matrix proteins and translocates them completely through the IMM into the matrix.
 - Once in matrix, bound to mitochondrial chaperone which pulls protein into matrix
 - Once in matrix, protein becomes folded with help of Hsp60 chaperones
 - Movement into the matrix is powered by electrical potential across the IMM
 - Chaperones can act as force-generating motors that use energy derived from ATP hydrolysis to pull polypeptide through
 - Chaperones can aid in biased diffusion by acting as a “Brownian ratchet”
 - Brownian = random diffusion
 - Ratchet = allows movement in only one direction

- Uptake of Proteins into Chloroplast (posttranslationally)
 - Many chloroplast proteins are nuclear encoded
 - Proteins destined for the stroma contain a stroma-targeting domain at N-terminus
 - Proteins destined for thylakoid contain both stroma-targeting domain and a thylakoid transfer domain at N-terminus.
 - Stromal proteins (Rubisco) remain in stroma and signal targeting sequence is removed.
 - Thylakoid transfer proteins can be translocated either into or completely through the thylakoid membrane (into the lumen).
 - Chloroplast-encoded genes are encoded by chloroplast genes and synthesized by chloroplast ribosomes bound to surface of thylakoid membrane.
- Approaches to study mechanism of translocation
 - In vitro assay: To determine transport mechanism using in vitro synthesis and import assay
 - Transfection approach—define signal sequence
 - Find putative sorting signal for organelle
 - Fuse targeting signal with reporter protein and transfect a cell
 - Genetic approach to identify essential players
 - Ex. Yeast mutants defective in one protein of the recognition, binding or uptake machinery cannot take up mitochondria-destined proteins.
 - Identify gene product and its function
- Endomembrane system is dynamic and protein sorting
 - Endomembrane system includes
 - ER, Golgi, lysosome (vacuole), secretory vesicles, endosomes, and PM
 - All proteins of endomembrane system are synthesized in the ER
 - Membranes of endomembrane system arose from the ER
 - **Biosynthetic pathway:** new proteins synthesized at the ER is modified and sorted to their right destination
 - **Secretory pathway:** proteins destined for secretion move to outside of the cell (**Exocytosis**)
 - Constitutive: synthesized in ER, sorted in golgi, and secreted to extracellular space in continuous manner
 - Regulated: materials synthesized in ER, appropriate stimulus induces a response for secretory vesicle discharge
 - Sec. vesicles become stored in densely packed **secretory granules**
 - **Endocytic pathway:** materials move out to in
- Protein trafficking
 - Proteins must be directed to right destinations
 - Sorting signals encoded in proteins are recognized by receptors in budding vesicles
 - Vesicles are recognized by markers in destination membrane
- Proteins are sorted and moved by vesicular transport
 - **Budding:** how vesicles are formed
 - **Vesicular transport:** moves proteins
 - **Fusion with correct target membrane:** proteins in vesicle membrane incorporated into membrane of target.
- Definitions
 - **Exocytosis:** expulsion of materials out of cell
 - **Endocytosis:** receptor mediated uptake(nutrients) from outside via vesicles (usually clathrin coated)
 - **Phagocytosis:** uptake of large particles (bacteria or cells) non-receptor mediated
 - **Autophagy:** breakdown of cellular compartments and reutilization within the cell
- Autoradiography
 - a **pulse-chase analysis** is a method for examining a cellular process occurring over time by successively exposing the cells to a labeled compound (pulse) and then to the same compound in an unlabeled form (chase). Follow labeled protein through biochemical pathways.
- Polarized structure of secretory cell

- Mucus secreting cell from rat
 - Basal end contains nucleus and RER, protein move to close golgi then to membrane bound carriers.
 - Apical end filled with cell filled with secretory granules with proteins ready for release
- Smooth versus Rough ER
 - RER
 - Protein synthesis
 - SER
 - Lipid synthesis, detox. of liver enzymes, site of Ca²⁺ accumulation in muscle cells. Release from SER triggers contraction
- Synthesis of integral protein at the ER membrane (RER)
 - Occurs cotranslationally
 - Contain one or more hydrophobic transmembrane segments and are placed directly into lipid bilayer through lateral gate. More positive end faces cytosol.
 - N-terminal signal peptide binds to SRP which is bound by SRP receptor. Protein inserts into the translocon and protein synthesis continues until becomes alpha helix.
 - If helix charges match, helix is released laterally through translocon into hydrophobic membrane
 - If charges don't match it is filled around
 - Protein then dissolved in lumen
 - If C-terminus is in lumen, charges reversed to transmembrane segment flipped 180° before it can exit translocon.
- Processing of newly synthesized proteins in ER lumen
 - Several proteins are found near translocon that immediately process newly translated polypeptides
 - Signal peptidase cleaves signal sequence (proteolytic cleavages)
 - Oligosaccharyltransferase adds carbohydrates
 - Chaperones permit proper folding
 - PDI catalyzes formation of disulfide bridges
 -
- Glycosylation in the ER (No N-glycosylation causes death of embryos prior to implantation).
 - Carbohydrates important in function of glycoproteins because act as binding sites in interactions with other macromolecules
 - Also aid in proper folding of protein
 - 1st 7 sugars are transferred one at a time to dolichol phosphate (embedded in ER membrane) on cytosolic side of ER.
 - With attached oligosaccharides flips across membrane so carb. is in lumen.
 - More sugars are added to cytosolic side and flips again bring more sugars to oligosaccharide chain.
 - Once completely assembled, transferred to asparagine residue on new polypeptide, d-phosphate flips again to accept more sugars.
 - Quality control
 - Ensures misfolded glycoproteins do not proceed forward to next compartment of biosyn. pathway
 - Recognized by GT which adds a glucose to end of oligosaccharide chains.
 - Given opportunity to refold and if not achieved, protein is exported and degraded.
- Golgi Complex and Functions
 - Structure: Cis, medial, and trans golgi
 - Cis contains CGN which acts as a sorting station that distinguishes between proteins to be shipped back to ER and those which proceed to next golgi network
 - Trans consists of TGN which is also a sorting station. Proteins segregated into different vesicles heading to PM or intracellular destinations.
 - Bulk of Golgi consists of series of large, flattened cisternae.
- Glycosylation in the Golgi
 - Newly synthesized proteins leave ER and enter golgi at cis face and depart at trans face.

- Golgi is a processing plant
- Glycosylation builds on core oligosaccharides added in ER, but differs from process in ER because end products (carbohydrate domains) vary tremendously.
- Site of synthesis of most of cells complex polysaccharides
- Steps
 - Phosphorylation of carbohydrate on protein, then addition of new carbohydrates
- Movement of proteins through golgi
 - Vesicular Transport Model
 - Each compartment has its own enzymes
 - Cargo is shuttled through golgi stack from CGN to the TGN, in vesicles that bud from one membrane compartment and fuse with neighboring compartment farther along the stack
 - Cargo is carried in an anterograde direction while cisternae remain stable elements
 - Cisternal maturation model (retrograde)
 - Golgi is constantly renewed by transport carriers from ER and ERGIC
 - Some materials found in Golgi are never found in golgi vesicles
 - Packages of processing enzymes and newly made proteins that originate in the ER fuse together to form the Golgi. As the proteins are processed and mature, they create the next Golgi
 - This model proposes that the cisternae progress through the Golgi, gradually moving through the stack as new layers form at the cis face and old layers disperse from the trans face, and that they carry the secretory proteins with them.
 - Suggests composition of an individual Golgi cistern can change over time.
 - From one that contains early (cis) golgi proteins to one that contains late (trans) golgi proteins.
- Experimental approaches to study synthesis and insertion
 - Using cells:
 - In vivo: Label protein in cells with isotope and follow fate with microscopy –EM over time, follow protein by cell fractionation, Immuno-ppte
 - Transfection: Follow GFP-protein dynamics in living cells
 - Cell free:
 - In vitro expts: synthesize and label protein using isolated ER membranes and test its fate. Identify players and understand their roles
 - Genetic approach:
 - Identify players and their roles in living cells using yeast mutants defective in sorting and secretion
 - Combination of 1-3
- Pancreatic cell synthesizes and secretes digestive enzymes (filled with RER)
 - Pancreatic acinar cells belong to the exocrine pancreas and secrete digestive enzymes into the gut via a system of ducts.
- Experimental evidence for protein secretion pathway (pulse-chase)
 - Cells exposed to radioactive AA for 3 min
 - These labeled AA are incorporated into proteins and the radioactivity can be tracked through the cells.
 - After 3 min, isotope in ER
 - 17 min chase, isotope in Golgi
 - 120 min chase, isotope is in secretory vesicles and outside cell
 - Release to cells exterior is called exocytosis
- Evidence for protein-GRP in the ER and its movement to the golgi in a living cell
 - GFP fused to end of protein to be studied, has no effect on protein function

- Virus (VSVG) fused to GFP. (Viruses turn infected cells into factories for viral proteins.
- Massive amounts are produced in ER and travel to golgi then exported to PM
- Use of virus allows investigators to follow a relatively synchronous wave of protein movement.
- Genetic Approach: Studying mutants
 - Yeast mutants that are defective in secretion at non-permissive temps (does not support replication of mutant gene)
 - Revealed proteins from many species are interchangeable
 - Find mutants defective in one step of secretion
 - Identify the mutated gene to find what protein is critical for each step of the secretory pathway.
- Protein Coats
 - Cause membranes to curve and form a budding vesicle
 - Provide a mechanism for selecting the components to be carried by the vesicle
- Vesicle Transport: Types of Protein coats
 - COPII-coated vesicles
 - Move materials from the ER lumen “forward” to the ERGIC and golgi complex
 - Sar1-GDP molecules recruited to ER membrane by GEF protein
 - Sar1-GDP goes to Sar1-GTP
 - Conformational change - has finger like alpha helix (N-terminal) that extends into membrane and induces curvature
 - Sar1-GTP recruits two polypeptide bind to Sar1-GTP and form dimer.
 - Dimer provides pressure for bending
 - Remaining subunits bind to form coat and vesicle buds of ER membrane
 - COPII coat disassembled and released into cytosol before vesicle can bind to golgi.
 - GTP → GDP
 - COPI-coated vesicles
 - Move materials in retrograde direction from ERGIC and golgi stack “backwards” toward the ER and from trans golgi to cis golgi
 - ARFI, GTP binding protein, used to trigger coat assembly and disassembly.
 - How does each compartment retain its identity?
 - Retention of resident molecules by exclusion from transport vesicles
 - Retrieval of “escaped” molecules back to the ER
 - Proteins both in lumen and membrane of ER have retrieval signal at c-terminus of AA sequence. (Eg. Chaperones and protein disulfide isomerase “lys-asp-glu-leu”, also known as KDEL signal).
 - Retrieval done by specific receptors (KDEL integral membrane protein) that capture molecules and return them to ER in COPI coated vesicles
 - SRP receptor also has retrieval signal at C-terminus (KXXX) K=Lys, X=any AA
 - Clathrin-coated vesicles
 - Move materials from TGN to endosomes, lysosomes, and plant vac.
 - Also move materials from PM to sites within endomembrane system (endocytic pathway)
 - In CGN
 - Lysosomal enzymes tagged with phosphorylated GlcNAc residues which are cleaved leaving phosphorylated mannose which act as recognition signals
 - Export from TGN to Lysosome
 - Mannose Phosphorylation in CGN
 - Selectively transported to clathrin coated vesicle in TGN
 - Outer lattice composed of clathrin protein and inner shell composed of protein adaptors GGA (vesicle membrane)

- Lysosomal enzymes bind to MPR and ARF1 used to create budding action to form vesicle.
 - Clathrin coat is then lost, MPR release and return to TGN, and vesicle goes to early or late endosomes (lysosome) or plant vacuole.
- Moving and binding of vesicles to target membrane
 - How does a transport vesicle recognize the right target membrane?
 - **SNARE**
 - Provide specificity and catalyze fusion
 - **Rabs = Targeting GTPases**
 - Control specificity of docking and tethering, movement and fusion
 - Selective fusion ensures directed flow
 - Transport of vesicles in cytoplasm mediated by microtubules which travel on predetermined route
 - Tethering vesicles to target membrane
 - Early stage in fusion process, much of specificity lies with GTPase called **Rabs** (60 in humans)
 - Associate with membrane by lipid anchor
 - Largely associated with **recruiting tethering proteins**
 - Docking vesicles to target compartment
 - Membrane of vesicle and target come very close, key protein which engage in interactions are called **SNARES** (all contain SNARE motif which allows complex to form with other SNARES).
 - SNARES can be divided into 2 categories
 - V-SNARE
 - Incorporated into membrane of transport vesicle
 - T-SNARE
 - Located in membrane of target compartment
 - Snares form bundle to bring two membranes close and fusion proceeds
- Receptor-mediated endocytosis (selective and efficient uptake)
 - Uptake of specific extracellular molecules (ligands) following binding to receptors on external surface of PM
 - Two kinds of receptors
 - Housekeeping receptors
 - Responsible for uptake of materials (LDL)
 - Signaling receptors
 - Bind extracellular ligands for signaling in cells (hormones)
 - Vesicle bound materials are delivered to endosomes
 - Early endosomes recycle "housekeeping" receptors back to PM
 - They also deliver ligands and "signaling" receptors to late endosomes
 - Late endosomes sorts ligands and Lysosomal enzymes to lysosomes
 - Late endosomes also handles traffic within TGN
- Phagocytosis
 - Foreign cell enclosed in phagosome
 - Phagosome and lysosome fuse and enclosed material is digested
 - Materials either saved by cell or released outside of cell if toxic
- Autophagy
 - Cell turnover – regulated destruction of cells own organelles and their replacement
 - Envelops the materials to be degraded into a vesicle called an **autophagosome**.
 - The autophagosome then fuses with a lysosome forming an **autolysosome** whose hydrolytic enzymes degrade the materials.

Kwak Lectures

Chapter 18: Techniques in Cell and Molecular Biology

- Light Microscope
 - Condenser Lens
 - A condenser is a lens that serves to **concentrate light** from the **illumination** source that is in turn focused through the object
 - Objective Lens
 - Collects light rays focused on the specimen
 - 2 sets of rays: 1 forming background light (not altered by specimen), 1 altered by specimen, These rays are brought into focus by the Ocular lens.
 - Ocular Lens
 - Forms an enlarged and virtual image.
 - When focusing knob turned, relative distance between specimen and obj. lens changes, allowing final image to become focused on the plane of the retina.
 - Total Magnification: product of the magnifications produced by objective and ocular lens.
- Resolution
 - Changing oculars (5x to 10X) increases magnification and resolution
 - Switching to even more increase (20X) does not provide any additional detail
 - Known as **Empty Magnification**
 - **Resolving power**: ability to distinguish two neighboring points as two distinct entities.
 - Limited by diffraction, light emanating through a point in specimen never seen as a point but only as a small disc. If discs overlap distance between cannot be determined.
 - Limited by aberrations, others lenses in objective piece compensate for errors in first lens.
 - Ultimately limited by the **wavelength of light**