EXAM 1 MATERIAL

Tuesday, September 1, 2020 7:42 AM

There are five universal features of cells on Earth:

- They are the fundamental unit of life
- They store hereditary information in DNA, which may be replicated
- Cells require free energy
- Function as biochemical factories dealing with the same molecular building blocks
- The cell is enclosed in a plasma membrane

Proteins and their roles in the cell

- Proteins act as the "workhorses" of the cell, with numerous different functions, including:
  - Cell signaling (as hormones)
  - Enzymatic reactions
  - Mechanical functions (example - contraction of muscles)
  - Cytoskeleton
  - Membrane transport
  - Cell to cell adhesion
  - Cell cycling
  - Immune responses (antibodies)
- It is energetically favorable for proteins to fold into a conformation requiring the lowest energy.
  - Results in proteins folding into specific shapes, with hydrophilic ends facing toward the outside of the protein whereas hydrophobic amino acids are sequestered away toward the center of the protein.

Cell Biology Methodology

- Microscopy Techniques
  - Light (confocal), FRAT, FRET, EM (Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM))
- Biochemical Techniques
  - Enzyme kinetics
  - In vitro binding
  - Gels and blots
  - Cell fractionation
- Model Organisms
  - We use model organisms due to their cell's observability, their fast generation times, easy ability to maintain and breed in a laboratory, size and annotation of genome, and their conservation of genes.
- Cell culture
  - The in-vitro growth of cells isolated from multicellular organisms.
  - There are two types of growth: primary and immortalized growth
    - Primary growth involves normal cells cultured without any change in their division rate. Due to natural cell death, often times these cell cultures will die off very quickly.
    - Immortalized growth involves the fusing of cancer cells to tissue causing a change to the genome to keep it growing
  - Cells grown in culture have different morphology depending on growth conditions
- Electron Microscopy
  - Scanning Electron Microscopy (SEM)
    - Involves the coating of a surface with some metal, then the shooting of electrons onto the metal. The electrons will bounce off and an image of the object will be shown. The problem is that due to the nature of the SEM, only the outside of an object may be seen through the SEM.
  - Transmission Electron Microscopy (TEM)
    - Involves the splitting of an object in half so that the interior of the object may be seen.
    - TEM has a more powerful resolution than the SEM (1nm vs 10nm, respectively)
  - Difficult sample preparation - samples must be fixed (dead) - invasive procedure
- Light Microscopy
  - Differential Interference Contrast (DIC)
  - Confocal Fluorescence Microscopy
  - Resolution of light microscopy varies greatly and is dependent on microscope setup
  - Relatively non invasive
  - Multi-colored images are possible using light microscopy - many different fluorescent molecules can be images in the same sample.
- Fluorescence Microscopy
  - Fluorescence protein tagging (FP-tagging)
    - Involves the attachment of some fluorophore (usually GFP) to the object being observed.
    - When a light is shined upon the GFP amino acid, the protein will fluoresce.
  - Immunofluorescence
    - May be done directly, with the attachment of fluorophores to the primary antibody with specific epitopes to an antigen on the cell, or indirectly, with the attachment of fluorophores to secondary antibodies which are connected to primary antibodies that attach to antigens on the surface of the cell.
      - The main reason for using indirect immunofluorescence over direct immunofluorescence would be cost reasons - the former is cheaper than the latter.
    - To view the inside of a cell we would need to use paraformaldehyde to permeate the cell membrane, allowing for the antigen to travel into the cell. This requires the cell to be dead.

Antibodies and Cell Biology Research

- A particular antibody has a specific binding site that matches with an epitope. An antigen can be a small portion of a protein
- Monoclonal antibodies
  - mAb are antibodies that are made by identical immune cells which are all clones belonging to a unique parent cell.
- Polyclonal antibodies
  - pAb bind to multiple epitopes and are made by several different plasma cell lineages.

Cell Fractionation

- Used to analyze biochemical role of proteins - cell organelles and internals are extracted, then centrifuged.
- Depending on the amount and strength of centrifugation, different components of the cell will be separated in the supernatant and the pellet. The smaller the component of the cell, the greater strength of centrifugation needed.
- Typically the supernatant is run through a gel for electrophoresis.

Separation of proteins using SDS-PAGE

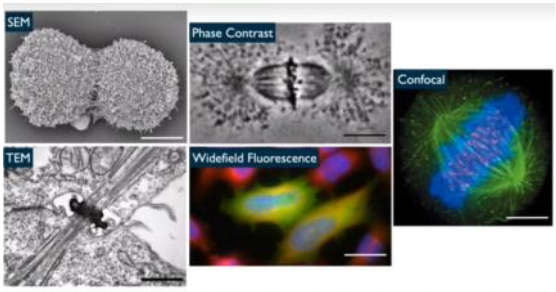
- SDS stands for Sodium Dodecasulfate and is a type of detergent. PAGE - Polyacrylamide Gel Electrophoresis.
- Proteins are separated to components using beta-mercaptoethanol, which breaks down the disulfide bridges present in many protein structures.
- Sodium Dodecasulfate is used to coat the proteins in a negative charge so that they may be run through a gel, as proteins themselves do not inherently have a negative charge.
- Once we separate our proteins on the gel plate, we can use mAb or pAb to identify our protein of interest.
- The gel plate is coated with a dye that identifies the proteins in the gel, the proteins are then transferred onto a nitrocellulose plate, and this plate is then exposed to antibodies. This is known as Western Blotting.

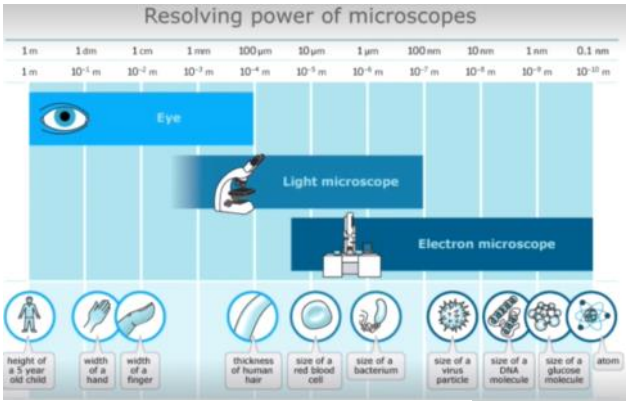
Immunoprecipiation

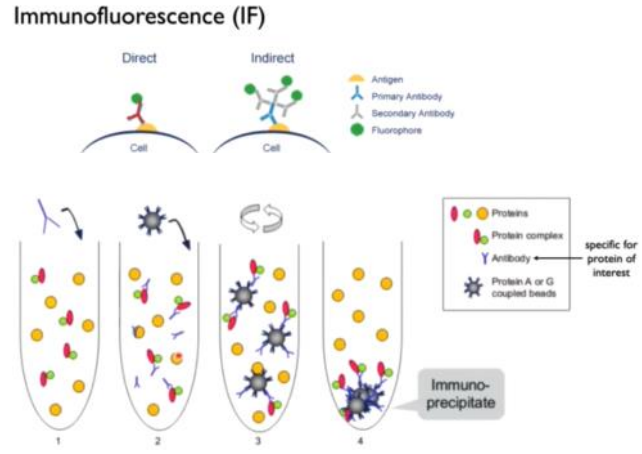
- A method for purifying proteins or identifying members of protein complexes - involves the introduction of antibodies to a solution of homogenized cells, then the addition of a bead that binds to our antibody. We then centrifuge the solution, and the pellet should contain the immunoprecipitate.

The Discovery of the Fluid Mosaic Model of Membrane Proteins

- Originally, two models for protein structure for the membrane existed.
  - The sandwich model proposed that proteins simply sat on the extracellular and intracellular ends of the cell. Observations of proteins that were later falsified from microscopy led researchers to believe this model at first
  - The fluid mosaic model proposed a complex blend of proteins that were both integrated and peripheral in the membrane model.
- Freeze fracture and electron microscopy showed proteins spanning the bilayer.
  - Cells are frozen in liquid nitrogen and a diamond knife is used to cut the membrane from the cytoplasm. The lipid bilayer is then separated in half and the samples are run under an electron microscope





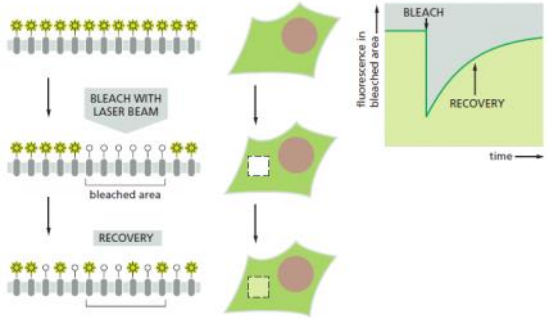


FRAP

- Stands for fluorescent recovery after photobleaching.
- Used to determine how much a particular protein moves in a membrane, or the diffusion of molecules in a protein

How it works

- When a fluorophore protein is struck by a strong laser beam, the proteins are bleached, meaning that they will be unable to emit light.
  - If the proteins in question do not move, then the area which is bleached will remain in the same place.
  - On the other hand, if the proteins do move, then the proteins will be displaced. However, since there are so many more fluorophore proteins than bleached proteins in a cell membrane, the displacement of the proteins will not be obvious under the microscope - the cell will undergo "recovery" of the bleached area:
  - If the protein is static, then we would not see an increase in the fluorescence in bleached area over time.
  - The mobile fraction, or the difference from absolute minimum resultant from bleaching to the asymptote of recovery, is a relative scale of how mobile a protein is.

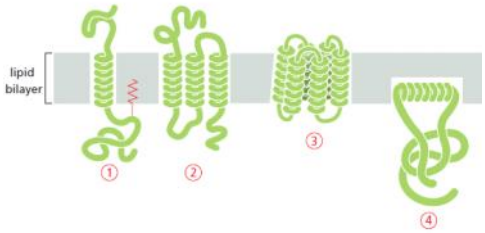


model at first
    - The fluid mosaic model proposed a complex blend of proteins that were both integrated and peripheral in the membrane model.
- Freeze fracture and electron microscopy showed proteins spanning the bilayer.
    - Cells are frozen in liquid nitrogen and a diamond knife is used to cut the membrane from the cytoplasm. The lipid bilayer is then separated in half and the samples are run under an electron microscope.
    - Researchers discovered from the uneven relief map generated by electron microscopy that the lipid bilayer was not smooth and had many areas of protrusion only explicable by proteins which were integrated into the membrane of the cell, disproving the sandwich model.

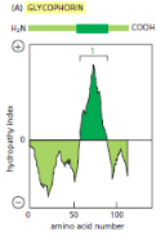
Integral Proteins

- Integral proteins are defined as proteins which "lock themselves into" a membrane. We will focus primarily on 4 types of integral proteins:

1. Single-pass transmembrane protein
2. Multi-pass transmembrane protein
3. Beta barrel protein
4. Alpha helix anchored



- Typical integral membrane proteins consist of a - helix regions comprised of various hydrophobic amino acids to span the length of the membrane.
    - Hydropathy is commonly used to determine potential transmembrane regions of a protein, where the higher the hydropathy index, the more hydrophobic the amino acids are.
    - A 20 amino acid long transmembrane region is typical of an integral protein.

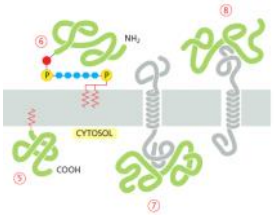


Peripheral Proteins

- Many peripheral proteins serve the function of regulation.
- As opposed to integral proteins, peripheral proteins interact on the surface of the membrane
- Note that despite the protein of interest being on the outside of the membrane, the protein still relies on an integral protein to be anchored to the membrane.
- Peripheral proteins can be easily identified as they separate easily from the integral protein which anchors them.
    - If the cell membrane was washed with a mild detergent, the peripheral proteins will detach from the membrane. On the other hand, the integral proteins on the inside need a stronger detergent in order to be detached from the membrane.

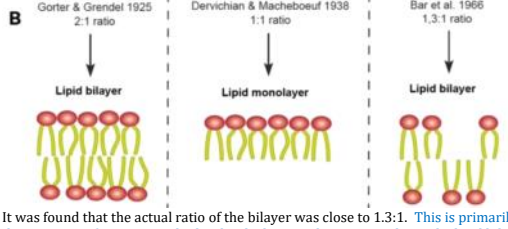
GPI anchored and Fatty Acid Linked Proteins

- Neither peripheral nor integral, these proteins rely on fatty acid interactions to anchor themselves to the membrane. (5) is an example of a fatty acid linked protein, whereas (6) is an example of a GPI anchored protein.



Phospholipids

- Phospholipids are a biological macromolecule consisting of a hydrophilic phosporus head and a hydrophobic lipid tail.
- Phospholipids spontaneously form bilayers due to energetic favorability- if there is a single tail, a micelle will be formed. If there are two tails, a lipid bilayer will be formed.
    - Phospholipids will spontaneously form into a sealed compartment, from a flat lipid bilayer to a spherical compartment, enclosing whatever is inside the bilayer.
- Experiments determining the composition of the bilayer:
  1.) Isolated erythrocytes (RBC), which don't have nuclei and don't have internal membrane compartments
  2.) Estimated the total surface area of the red blood cell using microscopy
  3.) Extracted the lipids from the cell then measured the length of the cell lipid surface
  4.) Compared the ratio of the surface area to the length of the cell lipid surface.
    - Several different hypotheses were given as to the composition of the membrane, including a lipid bilayer, a lipid monolayer, and a fragmented lipid bilayer, as shown below:



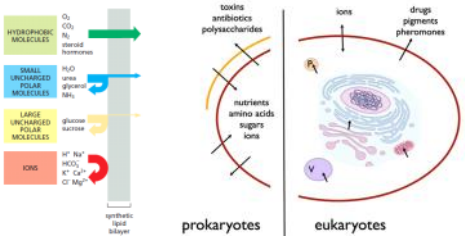
It was found that the actual ratio of the bilayer was close to 1.3:1. This is primarily because of

Types of Transport

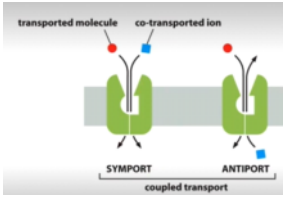
- Membrane transport and membrane trafficking are two distinct ideas. Membrane trafficking occurs when vesicles bud off of a membrane source and are moved (or trafficked) to another part of the cell.
- ER translocation involves moving protein in the endoplasmic reticulum. This process involves specific sets of proteins and a specified biological pathway.
- Mitochondrial transport
- Nuclear transport
- Transport across the plasma membrane - this will be the focus for this week.

Passive and Active Transport

- Membrane transport comes in two major types: passive and active transport.
- Passive transport - solutes move down concentration gradient, no additional energy input required.
    - Channel proteins - simple channels that allow for certain solutes to move down their concentration gradient with no input of energy.
        - In neurons, there is a greater amount of potassium channels than sodium channels.
    - Carrier proteins - requires a confirmational change which allows for solutes to transport down their concentration gradient.
- Active transport
    - Primary active transport - ATP driven pumps
        - Three classes of ATP-driven pumps:
            - P - type pump - directly gets phosphorylated to pump ions through
            - ABC transporter - (ATP Binding Casette) - many species of MDR bacteria use ABC transporters (transports small molecules)
            - V-type proton pump, F- type proton pump - found in bacteria
        - ABC transporters - responsible for transporting a wide variety of molecules across different membranes. In prokaryotes, widely found in clinical settings in multidrug resistant bacteria (MDR bacteria) to remove toxins, antibiotics, and polysaccharides from the cell.



    - Secondary active transport - also known as coupled transporters, use a concentration gradient created by a primary active transport source to move another solute against its concentration gradient.
        - Symporter - moves both solutes in the same directions
            - Example: cotransport of glucose and Na+ across the gut epithelium - in an environment where sodium concentrations are high on one side and glucose concentrations are high on the other side, primary active transport (Na+ gradient) is established by Na+ / K+ ATPase (Pump), then secondary active transport is utilized in ion driven carriers which co-transport Na+ and glucose down the concentration gradient of Na+, but up the concentration gradient of glucose.
        - Antiporters - moves solutes in opposite directions (usually transports charged molecules)



Electrochemical Gradients and Membrane Potential

- Electrochemical gradient has an electrical and a chemical gradient component.
    - Electrical component - almost all plasma membranes have a membrane potential, a difference in the charge inside of a membrane vs. the outside of a membrane. If the intracellular side of a membrane is opposite of the charge of the ions on the extracellular side of the membrane, the ions will be more inclined to be transported into the membrane.
- The voltage difference across the membrane is established by the sodium-potassium pump in animal cells. It is an ATP driven pump that drives sodium and potassium against their electrochemical gradients.
    - Normally there is a high concentration of sodium on the extracellular side whereas there is a high concentration of potassium on the intracellular side of the membrane. The process is as follows:
        i. 3 sodium ions bind to the intracellular side of the transport protein
        ii. ATP is cleaved, forming a high energy phosphate ion bond with the protein and ADP
        iii. Conformational change caused in the protein
        iv. Sodium ions are released to the extracellular side of the membrane
        v. 2 potassium ions bind to transport protein
        vi. Phosphate group is released
        vii. Conformational change caused in the protein
        viii. Potassium ions move into intracellular side.

Specificity of Ion Channels

- Ion channels recognize specific molecules (such as Na+ or K+) through a vestibule structure and a selectivity filter, in which the ions in the selectivity filter interact with oxygen ended side chains based on the size of the ions.
- Ion channels may also be selective based on specific gating mechanisms:
    - Voltage gated ion channel
    - Ligand gated ion channel

It was found that the actual ratio of the bilayer was close to 1.3:1. This is primarily because of the presence of proteins and other lipids that may be integrated into the lipid bilayer which decreases the ratio of cell lipid surface to total cell surface area.

- There are 4 different types of phospholipids which vary in concentration from one organism to another:
  - phosphatidylcholine (found only on the extracelluar side of the lipid bilayer)
  - sphingomyelin (found only on the extracelluar side of the lipid bilayer)
  - phosphatidylserine (negatively charged, signal for apoptosis, found on the intracellular side of bilayer)
  - phosphatidylinositol (found on the intracellular side of bilayer)

Fluid Mosaic Model of Membranes

- Types of lipids in the lipid bilayer include the phospholipids (phosphoglycerides and sphingolipids), glycolipids, and cholesterol.
  - Glycolipids are found on the surface of all eukaryotic plasma membranes, and consist of a carbohydrate chain attached to the lipid. On the other hand, a glycoprotein is a carbohydrate chain attached to a membrane protein.
- "Fluid Mosaic" model of membranes proposed in 1972 by Singer and Nicholsons.
- The fluidity of membranes is affected by:
  - Temperature
  - Phospholipid composition
  - Concentration of cholesterol
- Phospholipids move in the membrane through lateral diffusion (side to side movement), rotation of individual phospholipids, and very rarely, a flip / flop of the phospholipid from one side of the bilayer to the other side of the bilayer.
- Generally, more unsaturated hydrocarbon chains with cis double bonds in the phospholipid bilayer result in greater cell membrane fluidity due to the kinks in the tail leaving more gaps between the phospholipids, whereas saturated chains result in decreased fluidity due to a straighter tail shape allowing for more compact structure in the membrane.
  - If the lipid tails are shorter, the membrane will also be more fluid.
- Lipid asymmetry is functionally important within a membrane, with different types of lipid composition forming the membrane.
  - Scramblases and flipases use energy to flip the lipids in the membrane, allowing for more mobility.
- Despite the fluidity of the membrane, lipid bilayers may form domains of different compositions - this meaning that a group of membrane proteins with interdependent functionality may stick together as one unit in what is known as a "raft domain" (shown to the right)

Cholesterol

- Important constituent of eukaryotic membranes - moderates fluidity of the membrane.
  - Acts as a temperature activated buffer - if the temperature is low, causing the fluidity of the membrane to decrease due to lower average kinetic energy, the cholesterol disrupts the close packing of acyl chains, increasing fluidity of the membrane. On the other hand, if the temperature is high, and the membrane becomes too fluid, so much so that it is almost falling apart, the cholesterol decreases the fluidity of the membrane by constraining the motion of acyl chains.
  - The result of this buffer increases the temperature range at which an organism can live.
- Consists of a polar head group attached to steroid ring structure attached to hydrophobic tail.