CH1131 Biomolecular Engineering

Syllabus

Plasma Membrane

2017

THE PLASMA MEMBRANE

LECTURE OUTLINE

Summary of Membrane Functions

I. Compartmentalization - membranes enclose entire cell or diverse intracellular spaces in which occur specialized activities that proceed with little outside interference & are regulated independently
   A. They are continuous, unbroken sheets
   B. A cell's various membrane-bound compartments have markedly different contents

II. Scaffold for biochemical activities – membranes are also distinct compartments themselves
   A. They provide cell with extensive framework (scaffolding) within which components can be ordered for effective interaction

III. Provide selectively permeable barrier – membrane can be compared to moat around a castle; a general barrier that has gated "bridges" that allow desirable things to enter & leave space they surround
   A. They prevent the unrestricted exchange of molecules from one side to the other - control what gets into & out of cell; H_2O moves easily
   B. They also provide the means of communication between the compartments they separate

IV. Transporting solutes – they have transport machinery to move substances from one side to the other
   A. Can transport substances (ions, sugars, amino acids, etc.) up or down concentration gradient; sugars & amino acids taken up since they are needed to fuel metabolism & build macromolecules
   B. Can establish ionic gradients across itself (critical for nerves, muscles, maybe helps all cells respond to their environment) by transporting specific ions

V. Response to external signals (**signal transduction**) – plays critical role in response to external stimuli (hormones, growth factors, neurotransmitters)
   A. Receptors in membrane combine with specific molecules (**ligands**) having complementary structure & then initiate response
      1. Different cells have different receptors; can therefore recognize & respond to different ligands in environment
   B. Interaction of receptor with external ligand causes generation of new signal (**second messenger**); stimulates or inhibits internal cell activities like:
      1. Making more glycogen, preparing for cell division, concentrating particular compounds, releasing calcium from internal stores, even committing suicide

VI. Intercellular interactions - allows cells to recognize & signal one another, adhere when appropriate & exchange materials & information; mediates interactions between cells of multicellular organisms

VII. Energy transduction – intimately involved in processes by which one type of energy is converted to another type (**energy transduction**); done by membranes of chloroplasts & mitochondria

A. Electron transport site in mitochondria

1. Mitochondrial membranes transfer chemical energy from carbohydrates & fats to ATP

B. Allows storage of energy in gradients

The Chemical Composition of Membranes

I. Membranes - lipid-protein assemblies held together in thin sheet by noncovalent bonds

A. Lipid bilayer is structural backbone of membrane & barrier preventing random movements of water-soluble materials into & out of cell

B. Proteins of membrane carry out most of its specific functions

C. Often include carbohydrates attached to membrane lipids & proteins

II. Lipid:protein ratio varies greatly depending on membrane type (cell membrane vs. ER vs. Golgi), organism (prokaryote vs. plant vs. animal) & cell (cartilage vs. muscle vs. liver)

A. These differences largely relate to the particular functions of the membranes, e. g. inner mitochondrial vs. myelin sheath

B. Example: inner mitochondrial membrane – very high protein:lipid ratio relative to RBCs that are high relative to myelin sheath (multilayered wrapping around nerve cell axon)

III. Membrane lipids – wide diversity of amphipathic lipids with both hydrophobic & hydrophilic portions

A. Most have phosphate groups & are phospholipids (except cholesterol, glycolipids)

IV. Cholesterol – a sterol that can be up to 50% of animal membrane lipids; it is smaller & less amphipathic than other membrane lipids; it is missing from most plant & all bacteria cell membranes

The Nature and Importance of the Lipid Bilayer

I. Each type of cell membrane has its own characteristic lipid composition

A. Differ from each other in types of lipids, nature of head groups & particular species of fatty acyl chain(s)

II. Lipid composition can influence biological properties of membrane; not just structural elements
A. Can influence activity of particular membrane proteins
B. Can determine physical state of membrane

III. Membranes never have free end due to cohesion & spontaneous formation (closed bimolecular sheets); always continuous, unbroken structures; form extensive interconnected networks within cell
A. Bilayer is flexible, deformable, can change shape (as in locomotion & cell division)
B. Facilitates regulated fusion or budding of membranes – events of secretion (cytoplasmic vesicles fuse to plasma membrane; exocytosis), endocytosis or fertilization (2 cells fuse to form single cell)

IV. Membrane can self-assemble in aqueous solutions
A. If a small amount of phosphatidylcholine is dispersed in aqueous solution, the phospholipid molecules assemble spontaneously to form the walls of liposomes (fluid-filled spherical vesicles)
1. Their walls made of single continuous lipid bilayer organized in same way as natural membrane
2. Valuable in membrane studies - insert membrane proteins, study their function in simpler environment than that of a natural membrane
B. Liposomes containing DNA/drugs are potential delivery system to specific target cells in body; can be linked to liposome walls or placed at high concentrations in central cavity (lumen) of liposome
1. Build liposome walls to contain specific proteins (antibodies, hormones)
2. The proteins allow liposomes to bind selectively to surfaces of particular target cells where drug or DNA is supposed to go
3. When first tried, immune system phagocytes removed them - now stealth liposomes are given synthetic polymer coating that protects them from immune destruction
4. Now being used against some cancers – stealth liposomes containing doxorubicin are approved for Kaposi's sarcoma treatment

Membrane Carbohydrates

I. Eukaryotic plasma membranes have carbohydrate content covalently linked to both lipid & protein membrane components
A. Depending on species & cell type, carbohydrate content of plasma membrane ranges between 2 - 10% by weight, e. g., RBC membrane - ~52% protein, 40% lipid, 8% carbohydrate
B. <10% of membrane carbohydrate is covalently linked to lipids to form glycolipids & >90% of membrane carbohydrates are covalently linked to protein to form glycoprotein)

II. All membrane carbohydrates face toward outside of cells into extracellular space or toward organelle interior (carbohydrates of internal cellular membranes); in both cases, they face away from cytosol

III. Glycoproteins - carbohydrates are short, branched oligosaccharides with < ~15 sugars per chain
- A. Oligosaccharides vary in composition & structure; sialic acid usually on end giving negative charge
- B. Attach to several different amino acids by two major types of linkages
- C. May play role in mediating interactions of cell with other cells & nonliving environment & sorting of membrane proteins to different cell compartments

Structure and Function of Membrane Proteins: Overview

I. Membranes may contain hundreds of different proteins depending on cell type or particular organelle
- A. Parts of proteins interacting with hormones, growth factors, other cells, extracellular matrix elements face out; those interacting with cytoplasmic molecules face inward (G proteins, protein kinases)

II. Three classes of membrane proteins distinguished by intimacy of their relationship to lipid bilayer
- A. Integral proteins - penetrate into lipid bilayer; they pass entirely through bilayer (transmembrane)
  1. Have domains that protrude from both sides of membrane (extracellular & cytoplasmic)
  2. Some have only one membrane-spanning segment; others are multispanning
  3. Genome-sequencing studies suggest that integral membrane proteins constitute ~30% of all encoded proteins
- B. Peripheral proteins – located entirely outside of bilayer on extracellular or cytoplasmic surface; associated with membrane surface by noncovalent bonds
- C. Lipid-anchored proteins – located outside bilayer on either extracellular or cytoplasmic side, but they are covalently linked to membrane lipid situated within bilayer

Structure and Function of Membrane Proteins: Integral Membrane Proteins

I. Integral membrane proteins - amphipathic; hydrophobic parts contact fatty acids in bilayer & seal proteins into membrane "wall"; hydrophilic portions on outside or coating aqueous channel through it

A. Intimate contact of membrane & integral proteins preserves permeability barrier & protein is brought into direct contact with surrounding lipid molecules
1. Lipid molecules that are closely associated with a membrane protein may play an important role in the protein's activity
2. Protein portions that protrude into cytoplasm or extracellular space are more like globular proteins
3. These nonembedded domains tend to have hydrophilic surfaces that interact with water-soluble substances (low MW substrates, hormones, other proteins) at the edge of the membrane

B. Several large families of membrane proteins have an interior channel that provides an aqueous passageway through the lipid bilayer

C. Integral proteins need not be fixed structures but may move laterally within membrane

II. Studying structure & properties of integral membrane proteins – difficult to isolate in soluble form due to their hydrophobic transmembrane domains

A. Extraction from membrane normally requires the use of detergents
1. Ionic (charged) detergents like SDS, which denatures proteins
2. Nonionic (uncharged) like Triton X-100, which usually does not alter protein tertiary structure

III. Identifying transmembrane domains – which segments are embedded in membrane?

A. Transmembrane domains can be predicted from amino acid sequence, which is deduced from the
1. Segments thought to span membrane usually consist of string of 20–30 predominantly nonpolar amino acids that adopt an α-helical secondary structure
2. Example: glycophorin A, the major erythrocyte cell membrane integral protein – of 20 amino acids of its lone α-helix, all but 3 have hydrophobic R groups; some (glycine residues) have H atom
3. The maximum number of H bonds between neighboring amino acids allowed by α-helix creates a highly stable (low-energy) configuration
4. This is important for a membrane-spanning polypeptide that is surrounded by fatty acyl chains and is thus unable to form H bonds with an aqueous solvent anyhow
5. Since each amino acid occupies 1.5 Å of polypeptide length & the hydrophobic core of bilayer is 30 Å wide, it takes at least 20 amino acids to span the hydrophobic part of membrane

B. Transmembrane segments usually identified using hydropathy plot; each site along polypeptide is assigned value giving a measure of the hydrophobicity of amino acid at that site & its neighbors
1. Gives a running average of hydrophobicity of short sections of polypeptide & guarantees that one or a few polar amino acids in a sequence do not alter the profile of the whole stretch
2. Hydrophobicity is determined by various criteria: lipid solubility or energy required to transfer them from an aqueous into a lipid medium

3. Transmembrane segments usually identified as a jagged peak extending well into hydrophobic side of spectrum
4. Flanking amino acid residues give clues about transmembrane segment orientation in bilayer; transmembrane segment cytoplasmic flank usually more "+" than extracellular flank amino acids

Structure and Function of Membrane Proteins: Peripheral Membrane Proteins

I. Peripheral membrane proteins - attach by noncovalent (weak electrostatic) bonds to hydrophilic head groups of lipids or to hydrophilic portions of integral proteins protruding from bilayer

A. Can usually be solubilized by extraction with aqueous salt solutions

B. In multisubunit proteins, some subunits may be peripheral & others integral (blurs distinction between integral & peripheral proteins)

II. Best-studied peripheral proteins are located on cytosolic membrane surface where they form fibrillar network that acts as membrane skeleton

A. These proteins give mechanical support to membrane & function as an anchor for integral proteins

B. Other peripheral proteins on internal membrane surface function as enzymes, specialized coats or factors that transmit transmembrane signals

C. Peripheral proteins on external surface of membrane are typically part of extracellular matrix

III. Typically have dynamic relationship with membrane, being recruited to or released from membrane depending on prevailing conditions

Structure and Function of Membrane Proteins: Lipid-Anchored Membrane Proteins

I. Lipid-anchored proteins - marked by lipid anchor types & surface on which they are exposed

A. GPI-anchored proteins - on external face of plasma membrane; bound to membrane by short oligosaccharide linked to molecule of glycophosphatidylinositol (GPI) in membrane outer leaflet;

B. Another group on cytoplasmic side of membrane is anchored to membrane by long hydrocarbon chains embedded in bilayer inner leaflet

1. At least two, Src & Ras, are implicated in transformation of a normal cell to a malignant state

The Movement of Substances Across Cell Membranes: Diffusion and Osmosis

I. Membrane has dual function – it retains dissolved materials of cell so they do not leak out into the environment & it must allow the necessary exchange of materials into & out of the cell

A. Lipid bilayer is ideally suited to prevent loss of charged & polar solutes (ions, sugars, amino acids)

1. Must make special provisions for movement of nutrients, respiratory gases, hormones, wastes, etc.

B. Membranes are selectively permeable barrier - how movement controlled? - two means for movement both of which lead to net flux of ions/compounds (influx - into cell; efflux - out of cell)

1. Passively by diffusion
2. Actively by an energy-coupled transport process

C. Several different processes by which substances move across membranes

1. Simple diffusion through lipid bilayer
2. Simple diffusion through an aqueous, protein-lined channel
3. Facilitated diffusion via a protein transporter
4. Active transport via an energy-driven protein pump capable of moving substances against a concentration gradient

II. Energetics of solute movement - depends on magnitude of concentration gradient

A. Diffusion is a spontaneous process in which substance moves from region of high concentration to region of low concentration, eventually eliminating concentration difference between the 2 regions

B. Depends on random thermal motion of solutes; an exergonic process driven by entropy increase

III. Diffusion of substances through membrane

A. 2 qualifications must be met before substances can diffuse passively across a membrane

1. Substances must go down gradient (must be present at higher concentration on one side of membrane than the other
2. Membrane must be permeable to the substance

B. Membranes are permeable to a given solute in two ways

1. Solute can pass directly through bilayer **or**
2. Solute can traverse an aqueous channel (pore) that spans the membrane & prevents the solute from coming into contact with lipids of bliayer

IV. Factors that determine the ability of molecules to pass directly through membrane

A. Polarity of a solute – a measure of polarity or nonpolarity is its partition coefficient

1. A solute's partition coefficient is the ratio of its solubility in a nonpolar solvent (octanol, vegetable oil) to that in H2O under conditions where the nonpolar solvent & H2O are mixed together
2. Higher nonpolar solvent (e. g., oil) : water solubility ratio —> solute more able to pass bilayer (first clue that membrane has lipid layer)
3. In other words, greater lipid solubility leads to faster penetration of the membrane

B. Size - smaller molecules pass through membrane faster - small inorganic substances penetrate rapidly; bigger polar molecules do not pass easily or at all
1. If two molecules have approximately equivalent partition coefficients, the smaller one tends to penetrate a lipid bilayer more rapidly than the larger one
2. Very small, uncharged (inorganic) molecules (O2, CO2 NO, H2O) penetrate very rapidly through membranes; these smaller molecules may slip between adjacent phospholipids
3. Larger polar molecules (sugars, amino acids, phosphorylated intermediates, etc.) can't penetrate membrane
4. Thus, the lipid bilayer of cell membrane is an effective barrier that keeps these larger essential metabolites from diffusing out of the cell
5. Some of these molecules (sugars, amino acids) must enter cells from bloodstream, but cannot do so by simple diffusion; special mechanisms must be available to allow their penetration
6. The use of such mechanisms allows a cell to regulate the movement of substances across its surface barrier

V. Diffusion of H2O through membranes – since H2O moves faster through membranes than solutes (dissolved ions, small polar organic solutes; essentially nonpenetrating), membranes called semipermeable

A. Osmosis – ready movement of water through a semipermeable membrane from a region of lower solute (high water) concentration to a region of higher solute (low water) concentration
1. Water moves toward hypertonic (higher solute concentration, hyperosmotic) environments & away from hypotonic (lower solute concentration, hypoosmotic) environments
2. 2 solutions with equal solute concentrations - isotonic (no net water movement; isoosmotic)

B. Response of cells to nonisotonic environments
1. Animal cells in hypotonic environments take on water (swell) & eventually lyse (RBCs hemolyze)
2. Plant cells in hypotonic environment take in H2O; no lysis due to cell wall - internal pressure (turgor) builds up; important for support for nonwoody plants & nonwoody plant parts
3. Plant cells in hypertonic environment lose water (volume shrinks) - membrane pulls away from cell wall (plasmolysis); without water, plants wilt

C. The above observations show that a cell's volume is controlled by the difference between the solute concentration inside the cell & that in the extracellular medium

D. Swelling or shrinking of cells in slightly hypotonic or hypertonic media are usually temporary events; within a few minutes, cells recover & return to original volume
1. In hypotonic medium, cells recover as they rid themselves of ions (primarily K+ & Cl-)
2. In hypertonic medium, cells recover as they gain ions (mostly Na+ & Cl-) from medium
3. Once [internal solute] (including a high concentration of dissolved proteins) equals [external solute], external & internal fluids are isotonic (no net movement of H2O into or out of cells)

The Movement of Substances Across Cell Membranes: Diffusion of Ions

I. Ions go through ion channels (integral proteins surrounding aqueous pores) since core of lipid bilayer is highly impermeable to charged substances like Na+, K+, Ca2+, & Cl-

A. Most channels are very selective allowing only one ion through in a downhill direction

B. Ion conductance (rapid movement across membranes) is critical in many cell activities like:

II. Wide variety of ion channels identified, each with integral protein(s) surrounding aqueous pore; most are highly selective in allowing one particular type of ion to pass through pore

A. Diffusion is always downhill (from higher to lower energy state; higher to lower concentration)

B. Comparisons of amino acid sequences of different types of ion channels in diverse organisms (bacteria, plants, animals) show that they are all members of a small number of giant superfamilies

C. Most ion channels can exist in either an open or a closed conformation; said to be **gated**
1. Opening & closing of gates is subject to complex physiological regulation
2. Can be induced by a variety of factors depending on the particular channel

III. Two major categories of gated channels

A. Ligand-gated channels – conformational state depends on binding of specific molecule (the ligand), which is usually not the solute that passes through the channel

B. Voltage-gated channels – conformational state depends on difference in ionic charge on the 2 sides of membrane

IV. Some ligand-gated channels are opened (or closed) after the binding of a molecule to outer surface of the channel; others are opened (or closed) after binding of ligand to inner surface of channel

V. Structure & function of K+ ion channels –first atomic-resolution image of an ion channel protein, a bacterial K+ ion channel called KcsA

A. This feat preceded investigations that revealed mechanism by which these molecules overwhelmingly select K+ ions over Na+ ions while allowing incredibly rapid K+ ion conductance through membrane

1. The mechanisms of ion selectivity & conductance in this channel are thought to be virtually identical to those operating in the much larger mammalian channels
2. Evidently, the basic challenges in operating an ion channel were solved relatively early in evolution, although many refinements appeared over the next billion or 2 years

B. KcsA channel consists of 4 subunits; each subunit contains 2 membrane-spanning helices (M1 & M2) & a pore region (P) at the extracellular end of the channel

C. The KcsA channel has a gate, like eukaryotic channels, but its opening & closing is regulated by the pH of the medium, rather than by the voltage across the membrane or the binding of a ligand

D. Comparison of MthK open structure & the homologous protein KcsA closed structure strongly suggests that their gating is accomplished by shape changes of inner (M2) helices cytoplasmic ends

1. In closed conformation, the M2 helices are straight & cross over one another to form a "helix bundle" that seals the cytoplasmic face of the pore
2. The channel opens when the M2 helices bend by ~30° at a specific hinge point

VI. Example of eukaryotic voltage-gated channels: K+ ion channels; genes encoding a variety of distinct voltage-gated K+ (or Kv) channels have been isolated & their molecular anatomy scrutinized

A. The more complex eukaryotic versions are thought to perform in a manner similar to the prokaryotic channels

B. Members of this protein family have their N- & C-terminal domains situated on the cytoplasmic side of membrane; the middle portion of polypeptide contains 6 membrane-spanning segments (S1–S6)

1. Helices S5 & S6 of the eukaryotic channel subunit correspond to helices M1 & M2 of the bacterial KcsA channel
2. Like the M2 helices of KscA, the S6 helices line much of the pore

C. Single Kv channel consists of 4 homologous polypeptides (subunits) arranged symmetrically around the central ion-conducting pore

1. Walls of the pore at its narrowest portion are lined by a segment of the polypeptide called P that connects the S5 & S6 transmembrane helices & is homologous to the P region of KscA channel
2. The P segments from the 4 subunits dip in toward center of protein forming the selectivity filter of the eukaryotic channel; all known K+ channels have a similar pore structure

VII. Voltage-gated K+ channels exist in at least 3 distinct conformations: closed, open, & inactivated; mechanism of operation

A. Kv channels are opened by a change in voltage; the gate that opens & closes Kv channel is thought to be formed by the inner ends of the S6 helices

1. The opening of the Kv channel gate is regulated by S4 transmembrane helix, which contains several positively charged amino acid residues spaced along the polypeptide chain
2. This part of the protein is thought to act as a voltage sensor

B. Under resting conditions, the negative potential across the membrane keeps the gate closed; S4 helix is in conformation in which pore is closed

C. A change in the potential to a more positive value (depolarization) exerts an electric force on S4 helix
1. The force is thought to cause S4 helix rotate so its "+"-charged residues move from a position where they are exposed to cytoplasm to a new position where they are exposed to cell exterior
2. Movement of S4 helix in response to depolarization initiates a conformational change within the protein that leads to the opening of the S6 gate

D. Once pore opened, ~100 K+ pass through channel/msec (nearly rate that would occur by free diffusion)
1. Due to large ion flux, opening of a relatively small number of K+ channels has significant impact on the membrane electrical properties

The Movement of Substances Across Cell Membranes: Facilitated Diffusion

I. Facilitated diffusion - diffusion during which substance binds selectively to a membrane-spanning protein (facilitative transporter), which facilitates diffusion process

A. The term facilitative transporter distinguishes these proteins from active transporters whose activity is coupled to a process that releases energy
1. Technically, transporter applies to membrane protein that can only bind a solute from one side of membrane at a time & in which shape change is mechanism for solute movement across membrane
2. Definition distinguishes transporters from channels, which, if open, can bind solutes from either side of membrane at same time; distinction between them is becoming blurred as more learned

B. Solute binding on one side of membrane changes protein shape, exposing solute to other surface from where it can diffuse down its concentration gradient
1. Since they are passive (not coupled to energy release), they mediate solute movement equally well in both directions; net flux direction depends solely on gradient direction (down gradient)
2. Facilitated diffusion is very important in mediating the entry & exit of polar solutes (amino acids, sugars) that cannot penetrate the lipid bilayer

II. Example: glucose transporter

A. Glucose is body's primary source of direct energy; most mammalian cells have a membrane protein that facilitates the diffusion of glucose from the bloodstream into the cell

1. Gradient favoring glucose diffusion into cell from bloodstream is maintained by phosphorylating glucose after it enters cytoplasm, thus lowering intracellular glucose concentration

B. Increase in blood glucose levels triggers insulin secretion by pancreas, which stimulates glucose uptake into various target cells (most notably skeletal muscle, adipocytes or fat cells)

The Movement of Substances Across Cell Membranes: Active Transport

I. Many ion gradients are very strong & are generated by the expenditure of energy (active transport)

A. [K+] inside a mammalian cell - ~100 mM; outside - ~5 mM —> K+ ions "want" to leave cell

B. [Na+] - ~150 mM outside cell; inside cell - ~10 - 20 mM —> Na+ ions "want" to enter cell

C. [Ca2+] - cytosol concentration 10-7 M; outside cell - 10,000 times higher than that inside cell; this is an even greater difference than with Na+ or K+

II. Establishment of such steep gradients depends on integral proteins that selectively bind solute & move it against a gradient (endergonic process); proteins that carry out active transport are often called pumps

A. Such steep gradients cannot occur by either simple or facilitated diffusion

B. Driven by conformational shift along with energy expenditure; coupled to exergonic reaction like ATP hydrolysis, light absorption, electron transport, flow of other substances down their gradients

III. Coupling active transport to ATP hydrolysis: the Na+-K+ pump; only in animals; primary means to maintain cell volume & establish the steep gradients needed for nerve-muscle impulses

A. Pump is unidirectional & pumps 3 Na+ ions out of cell for every 2 K+ ions pumped in & is electrogenic, contributing directly to the separation of charge across the membrane

1. It is a P-type ion pump that requires phosphorylation of pump during cycle
2. Phosphate is transferred from ATP after its hydrolysis to an aspartic acid residue of pump, causing an essential conformational change within the protein
3. Changes alter pump's affinity for the 2 cations so it can pick up Na+ & release K+ & vice versa
4. To pick either ion up from a low concentration region, protein must bind them with relatively high affinity; to release them into much higher concentration region, affinity for ion must drop
5. Thus, the affinity for each ion on the two sides of the membrane must be different

6. This is achieved by phosphorylation, which changes the protein's shape & also serves to expose the ion binding sites to the different sides of the membrane

C. The Na+-K+ pump cycle repeats - uses ~33% of most animal cells' energy (~67% in nerve cells)

1. Pump binds 3 Na+ ions on inside of cell
2. A bound ATP molecule is hydrolyzed & phosphate transferred to an aspartate residue of pump
3. Pump's shape shifts from the E1 to the E2 conformation; it opens to outside, exposing Na+-binding sites there; since E2 conformation has reduced affinity for Na+ ions, they are released outside cell
4. Once the 3 Na+ ions are released, the pump now picks up 2 K+ ions
5. Pump is then dephosphorylated
6. A molecule of ATP then binds to the protein, causing it to shift back to original E1 conformation; pump is now open to inside of cell, its affinity for the 2 K+ ions is reduced & they are released
7. The cycle is then repeated