BCH 4053 Summer 2001 Chapter 9 Lecture Notes

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Chapter 9

Membranes and Cell Surfaces

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Membranes

- Plasma membrane separates "inside" from "outside" of cell
- Organelles in eukaryotic cells surrounded by specific membranes
- Membranes serve as barriers to contain most substances on one side or the other
- Only small, lipid soluble, molecules are permeable to membranes

Membranes of organelles are different in composition from that of the plasma membrane. Mitochondria have two membranes, an outer membrane which has some pores that allow passage of medium sized molecules, and an inner membrane which serves as the permeability barrier as well as the energy transducing structure in oxidative phosphorylation.

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Membranes, con't.

- Membranes have many functions
  - Allow specific movement of substances into or out of cell, and into or out of organelles
  - Provide mechanism of energy storage and coupling for both transport and metabolic processes
  - Involved in cell-cell recognition and interactions
  - Signal transduction from external stimuli
  - Locomotion, reproduction

Chloroplasts have a membrane separating the oranelle from the rest of the cell, but also contains a set of layered membranes called thylakoid membranes within the chloroplast structure.

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Membrane Composition

- Membranes are composed of lipids and proteins
- Lipids provide the organizational backbone
- Proteins provide most of the specific functional features of membranes

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Lipid Aggregate Structures

- The hydrophobic effect is the main factor causing lipids to aggregate
- Aggregation can take several forms
  - Monolayers
  - Micelles
  - "Reverse" micelles
  - Bilayers
    - (See Figure 9.2)

As lipid molecules aggregate, there is a repulsion between neighboring polar head groups, particularly those with a net charge. In fatty acid salts, this repulsion causes the head groups to occupy the maximum area possible, which is a sphere, so the micelles of fatty acid salts are spherical. For phosphoglycerolipids with two hydrocarbon chains, the cross-sectional area of the two chains is large enough that the head group repulsion is minimized, and the aggregates form sheet-like structure instead.

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Lipid Aggregate Structures, con't.

- Micelles are characterized by a "critical micelle concentration" (CMC)
  - This is the concentration of monomer in equilibrium with the micelle
- The CMC decreases as the hydrophobic part of the molecule gets larger (i.e. as MW increases)
- Detergents that form such micelles are used to disrupt membrane and protein structures
  - See Figure 9.3

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Lipid Aggregate Structures, con't.

- Bilayer structures wrap to form "vesicles", which can be unilamellar or multilamellar.
- Unilamellar structures are called **liposomes**.
  - See Figure 9.4
- Liposomes are stable and can be "purified", creating structures with different contents inside and outside
- Liposomes serve as good models for membranes
- Liposomes have been used for drug delivery to specific locations

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Early Membrane Models

- Lipid bilayer structure was postulated early
  - Quantity of lipid in red cell membrane would form a monolayer about twice the area of the cell surface
  - Electrical and permeability properties of membrane were similar to those of artificial lipid bilayers
  - Electron micrographs showed a sandwich-like structure with low electron density in the middle, high on the edges, about the width of two lipid molecules
- Early models had proteins associating with polar surface groups of the bilayer

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The Fluid Mosaic Model

S. J. Singer and G. L. Nicolson

- The phospholipid bilayer is the organizational feature
- Proteins are imbedded in the bilayer like **mosaics** in a tile
- The hydrocarbon region of the bilayer is in a **fluid** or **liquid crystalline** state
- Proteins and lipids are free to rotate and move laterally
  - See Figure 9.6

Both monolayers and liposome bilayers have been used as models to study permeability of various substances across the bilayer and other properties related to natural membranes.

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Evidence for Membrane Fluidity

- Frye and Ediden experiment
  - See Figure 9.7
- Lipid lateral diffusion demonstrated by NMR and EPR measurements.
- Membrane "phase transition" similar to that in pure phospholipid bilayers, but broader.
  - See Figures 9.12 and 9.13
- Presence of unsaturated fatty acids in natural membranes lowers the transition temperature.
  - Low temperatures stimulate an increase in fatty acid content of some organisms.

Frye and Edidin used fluorescent labeled antibodies to bind specifically to membrane proteins. Antibodies for human cell antigens had a red fluorescent tag. Antibodies for mouse cell antigens had a green fluorescent tag. When human and mouse cell hybrids were produced, one could visualize under the microscope the half of the membrane coming from each. After a short time, though, the fluorescent labels mixed. However, if the cells were cooled to low temperature, the lateral diffusion was greatly slowed.

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Membrane Asymmetry

- Lateral asymmetry
  - Lipids can sometimes aggregate to form "phase separations" (See Figure 9.8)
  - Some proteins might cluster because of self-association (as for **bacteriorhodopsin** in *Halobacterium halobium*—Fig. 9.9) Some may aggregate through interaction with cytoplasmic proteins.

In intact erythrocytes, trypsin will only digest the amino terminal section of glycophorin, and protein reagents will only react with the amino terminal section. In erythrocyte membrane fragments, both the amino terminal section and the carboxyl terminal section can react with the reagents. Most membrane glycoproteins are found in the plasma membrane of cells and have the carbohydrate residues on the external face.

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Membrane Asymmetry, con't.

- Transverse asymmetry
  - Protein asymmetry first demonstrated for **glycophorin**, a major red cell glycoprotein (See Figure 9.14)
  - Lipid asymmetry is also demonstrated for most membranes (See Figure 9.10)
- Rate of lipid "flipping" is slow, but does occur. "Flippases" can accelerate the flipping rate. (See Figure 9.11)

While flipping of proteins is unlikely to occur, the flipping of lipids does occur slowly, perhaps on the order of days. Some still incompletely understood process must account for the continued asymmetry of lipids—otherwise they would equilibrate with equal concentrations on both surfaces. Differential rates of synthesis on the two surfaces, and differential binding of lipids to asymmetrically aligned proteins might partly account for the difference.

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Classes of Membrane Proteins

- Singer and Nicolson model postulated two classes of membrane proteins:
  - Integral (intrinsic) proteins
  - Peripheral (extrinsic) proteins
- A newer class called "lipid-anchored proteins.

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Peripheral Proteins

- Not strongly bound to the membrane
- Can be dissociated with salt or chelating agents, perhaps mild detergent.
- Association is through polar interactions with polar head groups of lipids and external portions of integral proteins.

Determination of the structure of integral membrane proteins has been more difficult than for globular proteins, but methods of obtaining crystals of membrane complexes has begun to yield x-ray structures.

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Integral Membrane Proteins

- Imbedded in the lipid bilayer, with hydrophobic association with the lipid hydrocarbon chains
- Can only be removed from the membrane with organic solvents or detergents
- Pure protein in absence of detergent is insoluble
- Can be transmembrane, or can face only one side of membrane
- Glycophorin (Fig. 9.14), bacteriorhodopsin (Fig. 9.15), maltoporin (Fig. 9.16) are examples

Some proteins span the membrane with only a single helix (glycophorin), some have several helices that traverse the membrane several times (bacteriorhodopsin), some form beta-barrel like structures (maltoporin).
For many membrane proteins the sequence but not the structure is known, but efforts have been made to predict which parts of the protein might be imbedded in the membrane by looking for stretches of hydrophobic amino acids that could form an alpha helix with an apolar surface.

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Lipid-Anchored Proteins

- Four types have been found:
  - Amide-linked myristoyl anchors
  - Thioester-linked fatty acyl anchors
  - Thioether-linked prenyl anchors
  - Glycosyl phosphatidylinositol anchors

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Amide-Linked Myristoyl Anchors

- Always myristic acid
- Always N-terminal Gly residue
  - (See Fig. 9.18)
- Examples: cAMP-dependent protein kinase, $pp60^{src}$ tyrosine kinase, calcineurin B, alpha subunits of G proteins, gag protein of HIV-1

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Thioester-linked Acyl Anchors

- Broader specificity for lipids - myristate, palmitate, stearate, oleate all found
  - (See Fig. 9.18)
- Examples: G-protein-coupled receptors, surface glycoproteins of some viruses, transferrin receptor triggers and signals

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Thioether-linked Prenyl Anchors

- Prenylation refers to linking of "isoprene"-based groups
- Always Cys of CAAX (C=Cys, A=Aliphatic, X=any residue)
- Isoprene groups include farnesyl (15-carbon, three double bond) and geranylgeranyl (20-carbon, four double bond) groups
  - See Fig. 9.19
- Examples: yeast mating factors, $p21^{ras}$ and nuclear lamin

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Glycosyl Phosphatidylinositol Anchors

- GPI anchors are more elaborate than others
- Always attached to a C-terminal residue
- Ethanolamine link to an oligosaccharide linked in turn to inositol of PI
  - See Figure 9.20
- Examples: surface antigens, adhesion molecules, cell surface hydrolase

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Lipid Anchors are Signaling Devices

- Recent evidence indicates that lipid anchors are quite transient in nature
- Reversible anchoring and de-anchoring can control (modulate) signalling pathways
- An example is Ras, a GTP binding protein involved in growth regulation, and in which mutations are responsible for some cancers.
  - See box Page 278

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Bacterial Cell Walls

- Peptidoglycan, cross linked polymer of $(GluNAcMurNac)_n$ (See Fig. 9.21)
  - Gram-positive: One bilayer and thick peptidoglycan outer shell
  - Gram-negative: Two bilayers with thin peptidoglycan shell in between
    - See Figure 9.23
  - Gram-positive: pentaglycine bridge connects tetrapeptides
  - Gram-negative: direct amide bond between tetrapeptides
- Gram positive bacteria also contain Teichoic Acid, a polymer of glycerol phosphate or ribitol phosphate
  - See Figure 9.25

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Animal Cell Surface Polysaccharides

- Many types of cell-cell interactions are mediated through oligo- and polysaccharides
  - Heart cells beating in synchrony
  - Contact inhibition causing cells in culture to stop growing
  - Leukocytes "rolling" on endothelial cells of vascular walls
  - Association with extracellular matrix of a tissue

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Glycoproteins

Many structures and functions!

- May be O-linked or N-linked
- O-linked saccharides are attached to hydroxyl groups of serine, threonine or hydroxylysine
- N-linked saccharides are attached via the amide nitrogens of asparagine residues
  - See structures in Figure 9.26 and 9.29

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O-linked Saccharides of Glycoproteins

- Function in many cases is to adopt an extended conformation
- These extended conformations resemble "bristle brushes"
- Bristle brush structure extends functional domains well above the membrane surface
  - See Figure 9.27

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N-linked Oligosaccharides

Many functions known or suspected

- Oligosaccharides can alter the chemical and physical properties of proteins
- Oligosaccharides can stabilize protein conformations and/or protect against proteolysis
- Cleavage of monosaccharide units from N-linked glycoproteins in blood targets them for degradation in the liver - see Figure 9.30

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Proteoglycans

- Glycoproteins whose carbohydrates are mostly glycosaminoglycans
- Found in extracellular matrix
- Variety of functions in binding cells together in tissues, communicating between cells, cushioning in joints, etc.
- Don’t worry about details of structure, but recognize names as belonging to this class