

# MEMBRANES

## Contents:

1. Introduction
2. Membrane Models
3. Membrane Components
4. Biosynthesis and Assembly
5. Membrane Fusion

Membranes are perhaps the most important biological entity, for it is on membranes that most cellular processes occur. These processes include enzyme activities, active transport of substances (ions, electrons, etc.) across and along membranes, reception and recognition of various stimuli, protein synthesis, nucleic acid synthesis, ATP synthesis, and even membrane synthesis. Membranes are composed mainly of lipids and proteins (in approximately equal amounts) with small amounts of asymmetrically distributed carbohydrates and other prosthetic groups. The precise proportions of membrane components and their special arrangement is controlled with extreme precision, dictating the type and specialization of the membrane at the subcellular level. It is this control that determines whether a membrane becomes a nuclear envelope, a mitochondrial cristae, etc.

Even though a single type of membrane can be identified morphologically (such as endoplasmic reticulum), functionally, not all regions are the same. Varied function is often exemplified by differences in membrane thickness. The tonoplast and plasma membrane are usually the thickest, nuclear and rough endoplasmic reticulum are usually the thinnest, and all others are intermediate in thickness. Membranes must be structurally defined in respect to the arrangement of their components in the plane of the membrane having a thickness of 100-125 Å. They must also be defined so as to explain:

- a. passive permeability of substances
- b. passive transport of substances by membrane components with specificity
- c. active transport of substances by membrane components with specificity
- d. the association and disassociation of enzymes including those arranged in a specific order of reaction sequence

- e. the association and disassociation of substances which affect and control the electrical properties of membranes
- f. the alteration of structure or properties as a result of stimuli (e.g. light, temperature, bonding)
- g. the ability of membranes to fuse.

## MEMBRANE ULTRASTRUCTURE AND MEMBRANE MODELS

Around 1950, techniques for viewing the ultrastructure of cells first started to reveal the structure of membranes. At first, membranes were described as something less than 100 Å thick and a bilayer. Early views on membrane structure proposed a bilayer of lipids arranged with hydrophobic tails opposing each other end to end on the inside and the polar (hydrophilic) heads toward the outside. In the early 1930's proteins were reasoned to be associated with the bilayer of lipid.

The formal presentation of this model (first worked on by Danielli and Harvey) was in 1935 and is referred to as the Danielli-Davson model. It proposed the lipid bilayer and a coating of protein on the surface of the lipids. The proteins were proposed to be in an "alpha" configuration.

In 1957 Robertson, on the basis of observations of myelin sheaths of Schwann cells, specifically modified the Danielli-Davson model and called it the "unit membrane" model. He proposed the same lipid bilayer but with proteins in the beta configuration. It was claimed to represent the basic ultrastructure of all biological membranes.

However, membranes vary in composition and function, from species to species, from cell to cell, and from one cell locale to another. They are dynamic, fluid, plastic, elastic structures in a constant state of flux and turnover. It is justifiable to suspect that the known diversity of membrane function and composition may be matched by an equivalent diversity in structure. The unit membrane hypothesis was widely accepted for several years. New information from determining chemical compositions and from characterization of membrane components required a reevaluation of current theories on membrane structure.

The general concepts of the Danielli-Davson (i.e., unit membrane) model are still well founded and well supported. This support can be summarized:

- a. Chemical analysis shows that lipids and proteins are the major components of membranes. Major lipids are phospholipids.

- b. Impedance (resistance) measurements on suspensions of cells and membrane bound organelles are compatible with the prediction that they are bound with a bilayer of lipid.
- c. X-ray diffraction and polarized light data of the myelin sheaths of Schwann cells supports the bilayer model.
- d. Bilayers of isolated membrane lipids and lipoproteins form spontaneously in water and are found to be the most stable form of these compounds under physiological conditions.
- e. When such artificial membranes are prepared for and viewed under the electron microscope, they appear as unit membranes.
- f. When those artificial membrane preparations are finely dispersed in water, they form "vesicular structures of variable size, indistinguishable from natural membrane preparations."
- g. The electrical and permeability properties of model membrane systems are compatible with properties of natural membranes.
- h. Freeze-etching experiments indicate that a preferential cleavage plane parallel to the surface of membranes is located in the center plane. Artificial bilayers of lipids also split (freeze-etch) along this plane.

Arguments against the unit membrane hypothesis:

- a. Natural membranes vary in biological activity, in the nature and amount of phospholipids and proteins.
- b. Removal of 95% of the lipid from mitochondrial inner membrane doesn't destroy the triple layered appearance seen with the EM.
- c. The x-ray diffraction studies agree with the unit membrane structure but give no information on the orientation of the phospholipid or the nature of the lipid-protein bonds.
- d. The exact structure of the chemical reactions of osmium and  $\text{KMnO}_4$  fixatives is unknown, thus little can be said to indicate the orientation of lipids in membranes.
- e. Since osmium reacts chemically with unsaturated fatty acids (or other double bonds) it could well alter the configuration of the lipid and perhaps the entire membrane.
- f. Myelin is so functionally different from plasma (and other) membranes that ultrastructural information cannot be extrapolated to other membranes.
- g. Studies with lipids show many less stable forms other than lamella.
- h. Our current understanding of genetic control and molecular biology proposes that proteins determine specificity and order. Thus it would seem improbable that a bilayer of lipids would

impart specificity and order to the attachment of proteins to its surface.

- i. Protein configuration is predominantly  $\alpha$  - helix or random coil instead of the  $\beta$  - structure proposed by Robertson's unit membrane model.
- j. Various studies support various other models, especially the subunit and mosaic theories.

Other models and hypothesis

### SUBUNIT MODEL

The subunit model is based upon the initial work of Blasie, et al. (JMB 14: 143-152, 1965). Using low angle X-ray diffraction and EM of frog retina, the work demonstrated subunits making up the plane of the membrane of rod cell outer segments. The subunits were globular and 40-50 A in diameter.

Other investigations have shown, however, that the subunits were actually rhodopsin and were imbedded to one half their diameter in the bilayer of lipid when light-bleached, and appeared to stand out even further upon dark adaptation.

Other proponents of a subunit model were Green and Perdue. They studied mitochondria in relation to their function and proposed several statements supporting a subunit membrane structure. Most were derived from their work on the dissociation and reassociation of functional subunits (i.e., those with enzyme or energy transfer activity) on the inner mitochondrial membrane.

They stated that:

1. There is evidence for predominance of hydrophobic interaction between lipids and proteins of membranes.
2. Electron transfer complexes (or subunits) lose their activity upon removal of lipids of the membrane and regain the activity upon reintroduction of the lipid.
3. Almost all (about 95%) of the lipid can be removed without changing the trilayered appearance under the EM.
4. Subunits of some sort are seen in several membranes under the EM after positive or negative staining.

5. There is very little beta configuration in membrane proteins.

However, evidence for extensive hydrophobic interactions does not rule out a lamellar structure. The fact that lipids are required for electron transfer is not incompatible with the unit membrane model. Lipids may well help to stabilize proteins in an active configuration as well as visa-versa. Removing (i.e., unbonding) lipids from proteins with which they associate could change the configuration and energetics of the proteins. Helical proteins are still compatible with the unit membrane hypothesis. The various staining procedures and their interpretations can be misleading. There is no reason to doubt that proteins on the surface or embedded in a lipid bilayer could give the appearance of subunits under the electron microscope.

#### MICELLAR MODEL

Supported by the work of F.S.Sjostrand and Lucy, among others.

Sjostrand used KMnO<sub>4</sub> fixed sections of mitochondria and smooth ER. Electron micrographs showed globular components separated by septa about 10 A thick. Freeze etched preparations showed the same.

Sjostrand states that "the membranes would consist of the lipids in the form of small micells. The polar ends of the lipid micells are located at the surface of the micell. The stained material we propose to consist of protein, which, by covering the surface of the lipid particles, would prevent a fusion of the lipid particles to form either a bimolecular leaflet or larger lipid droplets."

Lucy's proposal was based on his work with an artificial system consisting of various mixtures of lecithin, cholesterol, and saponin. When examined under the EM, they revealed micells 35-40 A in diameter which aggregated to form a lamellar array of hexagonally packed units.

However, the evidence comes from EM alone and attempts to bolster this model with X-ray diffraction and infrared dichroism have failed. Freeze etched views of the middle of these membranes would be difficult to extrapolate to total membrane structure.

#### FLUID MOSAIC MODEL

This model, most forcefully proposed by S.J. Singer (A.R. Biochem. 1974, 43: 805-826), is the currently most favored model. Some consider it the

most current modification of the original Danielli-Davson model. It proposes:

1. A phospholipid bilayer with the hydrophobic (nonpolar) fatty acid chains directed inwards and the polar hydrophilic heads lining the surfaces.
2. Proteins, glycoproteins, and lipoproteins, associate with the inner and outer surfaces of the membranes. The polar portions of the proteins associate with the polar surface and may protrude from it while portions of the proteins may be embedded in the bilayer of lipid. It is proposed that the nonpolar portions of these proteins associate with the nonpolar fatty acid chains. Proteins having both polar and nonpolar areas (residues) are termed amphipathic.
3. Under physiological conditions the membrane components are not rigidly fixed, but exist in a fluid and dynamic state. Lipids of the membrane are fluid at physiological temperatures, thus there may be fluid movement of the lipid molecules and lateral movement (diffusion) of the proteins that are embedded in the membrane. "Protein icebergs in a sea of lipid." (Singer) This forms a heterogeneous mosaic of proteins which may be continuously rearranged (but often with specificity.)
4. Not only is there lateral mobility of lipids but there is also exchange from one monolayer of the bi-leaflet to the other monolayer. This exchange (flip-flop) is much slower than the lateral exchange, detected by nuclear resonance studies. This process is facilitated by specific proteins (enzymes) called flippases.
5. Proteins may be integral or peripheral, probably most being integral and displaying various different secondary and tertiary forms. The proteins, being capable of secondary, tertiary, and quaternary structure changes in response to stimuli, may reorient in relation to the phospholipid bilayer.

The protein reorientation may be demonstrated by the following method:

Rhodopsin studies - photoinduced infrared dichroism changes of frog rod cell outer segments of retina.

Rhodopsin is a simple protein molecule which is an amphipathic integral protein of the outer rod segment membranes. As such, it is embedded in the lipid bilayer surface. The technique of IR dichroism detects the

uniform orientation changes of molecules. It was found that the rhodopsin molecules of frog retina rod cells adapted to darkness would change their orientation within the membrane to a position parallel to the plane of polarized monochromatic light.

The lipid bilayer of the membrane must be fluid in order for the reorientation of rhodopsin to occur. The response was blocked by lowering the temperature below the phase transition temperature such that the lipid bilayer assumed a crystalline (frozen) state. Fixing the rhodopsin with a bifunctional fixative (gluteraldehyde) also inhibited the change in dichroism. However, monofunctional fixatives had little effect on the response

The lateral fluidity can be demonstrated using an elegant immunochemical experiment:

Fluorescent labeling of antibodies to two different cells.

Frye and Edidin (1970) - mouse and human cells were used as antigens in rabbits to produce antibodies which would bind to each cell selectively. The antibodies were then labeled, one with fluorescein (which fluoresces green under the UV microscope), and the other with rhodamine (which fluoresces red under the UV microscope). The two cell types were then fused in vitro (using Sendai virus). When the fused cells were exposed to the antibody mixture, one cell appeared red and the other green. Mixing of the antigen sites (proteins) embedded in the membrane was observed at 37 C after 40 minutes and the hybrid cells showed a green and red speckled mosaic. This mixing occurred independent of protein synthesis but did not occur at low temperatures. (Phase transition later). This fluid movement was also prevented by fixation of the cells with glutaraldehyde.

#### LIPIDS OF MEMBRANES:

There are a variety of different phospholipids associated with the structure of membranes. Associated with each class of phospholipids is a distribution of fatty acids of varying chain length and unsaturation. Other components include protein, water, metal ions, and sometimes cholesterol. Mitochondrial lipids have been studied greatly. Protein:lipid ratios vary.

	Density	Protein:Lipid
Mitochondrial inner membrane	1.21	1.0:0.275
Mitochondrial outer membrane	1.13	1.0:0.829
Smooth ER	1.13	1.0:0.385

All mitochondria seem to have a similar total lipid content--about 27% by weight of which 90% is phospholipid. Other lipids include cholesterol, coenzyme Q, tocopherol, and carotenoids. Mitochondrial phospholipids appear to be of 3 major classes. In beef heart mitochondria they are listed with % total phospholipids:

1. Lecithin (phosphatidyl choline) - PC
2. Phosphatidyl ethanolamine [Note PE + PC = 76-78%]
3. Cardiolipin (diphosphatidyl glycerol) - CL 20%
4. Phosphatidyl inositol - minor component - PI 3-5%

The phospholipids are synthesized by the microsomal fraction and by mitochondria (recall that fatty acids are synthesized in the cytosol). The same phospholipid may be synthesized at either or both sites. In most plants and animal cells, mitochondria are also able to synthesize the fatty acids, phosphatidic acid, and cardiolipin while microsomes (endoplasmic reticulum) appear to contain the enzymes for the *de novo* synthesis of PC, PS, and PI. However, this is not conclusive and there may be several exceptions.

Mitochondrial fatty acids seem to have a very high degree of unsaturation.

Much greater variation is found in the fatty acid chain length and unsaturation (see table), showing considerable species and organ specificity. These characteristics seem to change in response to dietary and environmental factors.

In rat liver and bovine heart cell mitochondria, cardiolipin had the highest percentage of unsaturated acyl chains on its fatty acids (about 84% linoleic acid- 2 double bonds= 18:2). PC and PE fractions had variable amounts of saturation, unsaturation, and polyunsaturation of the acyl chains.

Rat liver mitochondrial membranes had varying amounts of cholesterol:

### Molar ratio of cholesterol to protein

inner membrane..... 1:53 low cholesterol  
 outer membrane..... 1:9 high cholesterol

### ACYL CHAIN DISTRIBUTION OF RAT LIVER AND COW HEART MITOCHONDRIA

Fatty acid	Form	Cardiolipin		PE fraction		PC fraction	
		RAT	COW	RAT	COW	RAT	COW
Palmitic	16:0	4		18		16	24
Stearic	18:0			25	38	20	5
Oleic	18:1	11.5	5	9	4	13	19
Linoleic	18:2	79	84	8	15	20	37
Linolenic	18:3		6				4
Aracadonic	20:4			21	33	16	4
Docosahexaenoic	22:6			12.5		5	

values are % of total fatty acid.

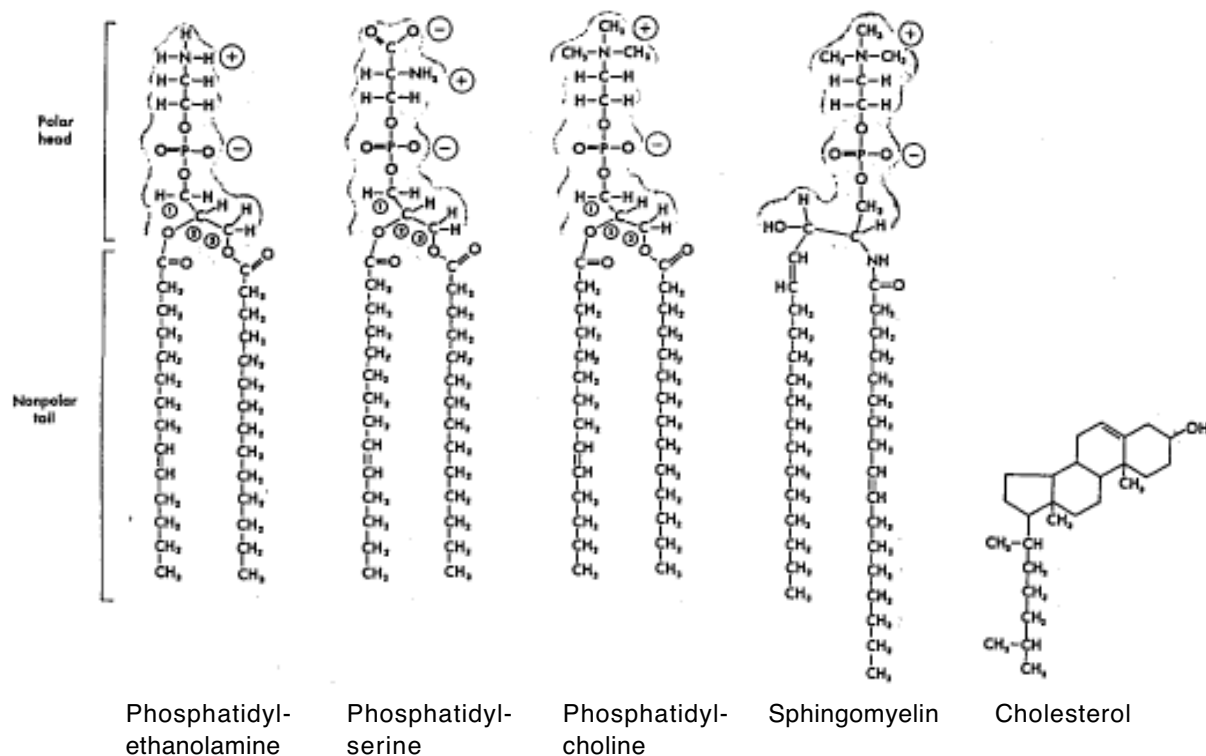
### PHOSPHOLIPID DISTRIBUTION AMONG CELLULAR MEMBRANES

#### CELL MEMBRANE

PHOSPHOLIPID	Plasma	Nuclear	RER	Golgi	Mito In	Mito Out	Lyso
Phosphatidylcholine	34.9	61.4	60.9	45.3	45.4	49.7	33.5
Phosphatidylethanolamin	18.5	22.7	18.6	17	25.3	23.2	17.9
Phosphatidylinositol	7.3	8.6	8.9	8.7	5.9	12.6	8.9
Phosphatidylserine	9	3.6	3.3	4.2	0.9	2.2	8.9
Phosphatidylglycerol	4.8				2.1	2.5	
Phosphatidic acid	4.4	1	1		0.7	1.3	6.8
Cardiolipin	trace	0			17.4	3.4	6.8
lyso Phosphatidylcholine	3.3	1.5	4.7	5.9			0
Sphingomyelin	17.7	3.2	3.7	12.3	2.5	5	32.9

values are %

## COMMON PHOSPHOLIPID STRUCTURES:



The multi step (25) enzyme system responsible for the conversion of squalene to cholesterol (in animal and plant cells) is a component of microsomal membranes. The biosynthetic enzymes up to spualine are probably soluble. Plant membranes may contain some cholesterol and various other phytosterols. Chloroplast membranes have modified lipids such as chlorophylls, carotenoids, xanthrophylls and quinones, which are non-saponifiable lipids.

Phospholipids can exist in several forms or phases with varying phase transition temperatures from lipid to lipid. They can exist in a solid-state (crystalline) form where they may be polymorphic (different shaped crystals). The crystals are commonly in a lamellar or bilayer form but assume other shapes as well (in vitro).

Phospholipids (anhydrous or with water) can exhibit the property of **thermotropic mesomorphism**, i.e., the property of forming a liquid-crystalline phase from a crystalline phase (by increasing temperature). In

the liquid-crystalline phase the hydrocarbon chains appear to be mobile (fluid) and more closely packed. Polar heads are still crystalline (ice). The transition temperature from crystal (or gel) to the liquid-crystalline phase depends upon:

1. length of hydrocarbon chain of lipid
2. degree of unsaturation
3. class of phospholipid
4. presence of cations associated with the phospholipid
5. presence or absence of HOH
6. cholesterol concentration

Cholesterol interacts mostly with the hydrocarbon chains of the phospholipids and thus change the phase transition temperature.

It can be noted that with membranes with little or no cholesterol, the phase transition temperature from crystal to liquid-crystalline form (or to liquid form) is low (<37 C) due to unsaturation of the FA chain (or one of the other factors). This is apparently the case in mitochondria.

The binding of Ca<sup>++</sup> and other ions is related to the net excess negative charge of the phospholipid. No bonding at pH=5.5.

It is probable that the fatty acid distribution serves to match membrane fluidity, environmental temperature, and membrane mediated processes (and rates thereof).

#### LIPID PROTEIN INTERACTIONS:

The following are candidates for explaining the bonding forces between protein and lipids:

1. covalent bonds
2. electrostatic bonding
3. polarization interaction
4. dispersion interaction
5. hydrophobic bonding
6. other-miscellaneous

Covalent - Little evidence exists for this except for the occurrence of some lipo-amino acids. However, the carboxylic and phosphoric acid groups of

phospholipids are ionized and available for bonding (perhaps through anion bridge) at physiological pH.

Electrostatic bonding - Certain complexes are probably of this type- eg. phosphatidyl serine and basic proteins. There is sufficient evidence accumulating to suggest that there is probably a significant amount of electrostatic interaction.

Hydrophobic interactions - Hydrophobic interactions are indeed possible (on the basis of entropy changes) with the right orientation of phospholipids in relation to the polar surroundings. If we at present base our membrane model on the bilayer of lipid as the inner component of membranes, hydrophobic bonding on a large scale seems reasonable. There is the possibility of hydrophobic bonding with proteins which are completely or partially embedded in the bilayer of lipid. Many proteins are inactive till they bind (a cofactor) to a membrane. This binding could involve a shift of a hydrophobic region of a protein to the surface of the protein molecule in order to associate with a non-polar hydrocarbon chain of a fatty-acid. This reorientation of the tertiary configuration of the protein could serve to activate it for its proposed function.

Hydrogen bonding - It is difficult to express the importance of hydrogen in protein-lipid interactions. There is undoubtedly a significant amount of hydrogen bonding (as well as Van der Waals forces).

#### PROTEINS OF MEMBRANES:

The diversity of membrane associated proteins is immense. Some of the proteins are active in a large variety of processes and their activities are easily measured while other proteins may be extremely difficult to isolate (bound tightly and require harsh treatment for removal) and have no identifiable activity. There have been several attempts to classify membrane proteins. An early attempt was made to class them as structural and enzyme (functional) proteins. Structural proteins were generally defined as the residue of insoluble protein remaining after the readily soluble (dissociable) proteins had been removed by washing and solubilizing with various agents.

Although this fraction was distinctly different from membrane enzyme protein, it doesn't mean that these proteins were inactive. They could function by:

1. defining the binding sites that may determine the spatial relationships of enzymes participating in reaction sequences.
2. undergoing conformational changes (energetic states) in order to control and participate in active transport.
3. determining the architecture of particular areas of the membrane (or of different membranes) by contributing specific sites for binding (i.e. adsorption) of lipids, CHO's, ions, and RNA to the protein matrix.

Usually enzymes were stripped off (but perhaps not all) and lipids were extracted with solvents. This step probably exposed the hydrophobic portion of the proteins perhaps causing a conformational change or making the protein insoluble in HOH.

The purity of the structural protein isolation was based on enzyme activity (i.e., the lack of it). This check for purity was questionable under insoluble conditions. Some feel that this fraction represented denatured enzymes left over from solubilization attempts. There is a need for completely new techniques designed specifically to study these proteins. Due to the inability to resolve these proteins, there is no or little data on the chemical and physical nature of the beast (i.e., AA sequence or content, etc.). An insoluble fraction does exist in all membranes studied so far with many general characteristics in common. Progress is needed.

Membrane functional proteins were thought to be enzymes. This thinking was logical since enzyme reactions occur at the surface of membranes.

Instead of classifying proteins as structural and functional a large number of researchers now refer to two broad categories termed **peripheral** and **integral**. (Many researchers refer to intrinsic and extrinsic proteins, however, the distinction between these terms and peripheral-integral are very narrow and most scholars use the terms as analogies.) The criteria for distinguishing between the two types of proteins are based on association and dissociation of the proteins with membranes, and on solubility after dissociation. Generally, those proteins which are only weakly bound and do not appear to interact directly with membrane lipids are termed peripheral. They associate probably by protein to protein interactions. Those proteins which are strongly bound to the membrane and exhibit functional interactions with the lipid bilayer are termed integral. (see table)

Criteria for distinguishing peripheral and integral membrane proteins

PROPERTY	PERIPHERAL PROTEIN	INTEGRAL PROTEIN
Requirements for dissociation from membrane	Mild treatments sufficient: high ionic strength, metal ion chelating agents	Hydrophobic bond-breaking agents required: detergents, organic solvents, chaotropic agents
Association with lipids when solubilized	Usually soluble free of lipids	Usually associated with lipids when solubilized
Solubility after dissociation from membrane	Soluble and molecularly dispersed in neutral aqueous buffers	Usually insoluble or aggregated in neutral aqueous buffers

Thus the integral and peripheral proteins are attached in distinctively different ways to the membrane.

Integral Proteins: 70-80% of membranes are integral. They include:

1. most membrane associated enzymes
2. antigenic proteins
3. transport proteins
4. drug and hormone receptors
5. receptors for lectins (later)
6. receptors for antibodies

Enzymes that are integral proteins usually require lipids (often specific) for activity. Rh antigen proteins of the human erythrocyte are an example.

Care must be used in classification--many integral proteins may be mistaken as peripheral since they are naturally released from membranes in the normal regeneration processes of the cell, etc. See Singer for examples - p. 807 Ann. Rev. Biochem. 43: 1974.

Glycoproteins are common in animal membranes--in human erythrocyte plasma membranes, the major glycoprotein consists of 60% CHOs, 40% peptide - MW = 55,000. The peptide is a single peptide chain. CHOs are in the form of multiple short oligosaccharides which are confined to the NH terminus half of the peptide chain. This is the portion of the protein which is exposed to the surface of the membrane while the half of the peptide void (mostly) of the CHOs is embedded in the membrane.

**PERIPHERAL PROTEINS:**

Peripheral proteins, those attached to integral proteins or other proteins, are quite numerous. For many, the binding to specific sites on the membrane serves to "activate" the protein. It is believed to hold for many enzymes and with certain components of the serum complement system in mammals. Some peripheral proteins and complexes include:

PROTEIN	LOCATION
Cytochrome C	outer surface of inner mito memb.
Spectrin	plasma membrane of RBC
alpha-lactalbumin	Mammary gland
Aldolase	plasma membrane of RBC
Ribosomes	rough ER, nuclear memb., etc.

**MEMBRANE BIOSYNTHESIS - Site of Cellular Membrane Synthesis**

Most of the "site of synthesis" information has been derived from isotope incorporation time studies.

**Membrane Protein Synthesis**

In vivo experiments have shown that radioactively labeled amino acids (e.g., <sup>14</sup>C-leucine) and labeled proteins are rapidly incorporated into membranes.

In eukaryotic cells, the label first appears in the rough microsomal fraction and in the outer (ribosome binding) nuclear membrane. It is not surprising that the two membranes are a functional and structural continuum. It appears that membrane proteins are mostly made on ribosomes attached to ER but some may be made on polysomes free in the

cytoplasm. After labeling of rough ER and the nuclear envelope, radioactive proteins appear in smooth ER, Golgi membranes, then in plasma membranes and tonoplast. The time-table is variable between cell types. However, the lag period for insertion of labeled proteins into the rough ER and nuclear envelope is about 1 min. (or less) where as the appearance of label in the plasma membrane may require anywhere from a few minutes to more than 20 minutes.

The time course of labeling of mitochondrial and chloroplast membrane proteins also shows an interesting pattern. For mitochondria, the insoluble proteins of the membranes label first. However, after a few hours, the soluble proteins of the matrix are more intensely labeled. This is probably due to the extraorganellar synthesis of many soluble proteins. In vivo, the outer mitochondrial membrane does not label at all and the inner membrane is labeled rapidly. Similar results are obtained with chloroplasts. Most work has been done in vitro using isolated chloroplasts. In these experiments the matrix and outer membrane do not label heavily.

#### MEMBRANE LIPID SYNTHESIS:

Several labeled lipid precursors are readily incorporated into all classes of membrane phospholipids. The time pattern for membrane lipid labeling is ER (rough) > dictyosomes > mitochondria (and their proplastids) > smooth membranes (i.e. plasma membranes, tonoplast). Note, however, that the labeling pattern of phosphatidic acid is just the reverse of the above pattern. The outer membranes of mitochondria (and probably chloroplasts) label before the inner membranes.

#### Turnover of Membrane Components

In general, different proteins of a membrane may degrade at different rates. The same is true for lipids. More so, the entire phospholipid molecule may be replaced, or only part of it (i.e., fatty acid moiety) may be regenerated. The glycerol portion alone is not exchanged, however.

Half lives for most membrane proteins of animal cells are about 2-4 days (note, however, that some may have half lives of only a few minutes or of several days).

Membrane lipid degradation is probably due to phospholipases located in microsomes, mitochondria, lysosomes, plasma membranes, or free in the cytoplasm. Individual classes have shown different rates and routes of degradation and turnover.

Phospholipids usually turnover much more rapidly than proteins. The half-life of phospholipids is on the order of two to several hours. However, this is complicated by the fact that the entire phospholipid molecule may not be exchanged.

Labeling studies using labeled glycerol are more accurate in determining total phospholipid turnover. This does not yield any meaningful information concerning cholesterol turnover.

The occurrence of membrane exchange between different organelles also complicates the interpretation of data.

#### MEMBRANE ASSEMBLY:

The mechanism of membrane assembly is virtually unknown. There are two main mechanisms proposed:

1. a "single step" mechanism proposed that all the components; protein, lipid, and carbohydrate, are assembled simultaneously.
2. a "multistep" mechanism proposes the assembly of a basic membrane of phospholipid and integral (intrinsic) proteins occurs first with the sequential addition (assembly line) of peripheral (extrinsic) proteins and sugar moieties.

Most evidence supports the multistep mechanism, especially in the formation of glycoproteins and glycolipids. We are quite sure that at least some components of the inner membranes of mitochondria and chloroplast are derived from within those organelles (especially proteins). Some evidence that for plasma membranes, a pool of precursor proteins exists for incorporation into membranes. With ER, the membranes appear to be a heterogeneous mosaic of units, all of which need not be present at one time; or, there is a common or fundamental (i.e., homogeneous) membrane structure to which additional specific functional units (i.e. proteins) can be selectively added. Rough and smooth ER seem to have many common proteins (without ribosomes) but lipid fractions tend to vary in rates of turnover (radioactive half-life). For information on the origin and synthesis of specific membranes, see: Morre, D.J. (1975) Membrane biogenesis, *Ann. Rev. of Plant Physiol.* 26: 441-81. (Excellent reference)

## MEMBRANE FUSION:

Fusion events include:

- intracellular (organelle to organelle – trafficking)
- intercellular, endo/exo cytotosis, virus-cell fusion

Although artificial lipid membrane can be induced to fuse, in vivo, most if not all fusion is facilitated and ordered via proteins. There is a large variety of fusion proteins reported in the literature which is not surprising given the immense variety of different fusion events and the stimuli that induce or regulate those events. Many of the proteins involved in fusion have some similar properties.

They:

- a. have some transmembrane (hydrophobic) domains
- b. are constitutive and activated/inactivated by binding ions.
- c. peptide sequence homologies i.e., w/in families.

These fusion proteins are distinct from membrane junctions such as gap junctions, tight junctions, desmosomes, plasmadesmata, etc.

Some examples:

Annexins are a class or group (family) of proteins involved in membrane contact and fusion. Synexin (now called annexin VII) was the protein of this family to be isolated in the late 1970's, several others since have been added. They bind to membranes when complexed in  $Ca^{++}$ . We know that  $Ca$  gradients and concentration are highly regulated in most cells. Thus it is not surprising that it also helps regulate membrane events such as fusion. This accounts for much of the intracellular trafficking. The annexins appear to promote fusion by linking membranes in contact with one another in the presence of other "cofactors" such as free fatty acids and other membrane proteins.

Annexins include:

Chromabindins, lipocortins, calpactins, coelectrins, endonexins, (annexin IV), calcimedins, some anticoagulants (for blood), and some collagen binding proteins.

These all have some sequence homology, especially in the core domains where there is a conserved 17 consensus sequence repeated 4 to 8 times (called the "endonexin fold" – 1<sup>st</sup> found in endonexin). Sequences near the amino termini are variable and are involved in regulation and include sites

for phosphorylation by protein kinases such as protein kinase C or tyrosine specific kinases.

These seem to be involved in trafficking vesicles for exocytosis.