V. THE CELL MEMBRANE

V.1. GENERAL CHARACTERISTICS

All human cells are surrounded by a plasma membrane (also called the cell membrane). Even the most complex cell surface irregularities and projections are covered by a plasma membrane. The plasma membrane is a **semi-permeable** boundary between the cell and its external environment that allows certain substances to pass through from the outside while keeping other constituents from escaping from the cell. The semi-permeable plasma membrane has an essential role in controlling the composition of cytoplasm; water, ions, and small molecular weight metabolites as glucose pass through the plasma membrane into the cytoplasm in a controlled fashion.

Cells use another mechanism to internalize extracellular macro-molecules. The cell membrane surrounds and engulfs the macromolecule, and then transports it into the cell.

Under the transmission electron microscope the cell membrane is composed by glycocalyx, plasma membrane, and the membrane cytoskeleton.

V.2. CELL MEMBRANE FUNCTIONS

a) Physical isolation. The cell membrane is a physical barrier that separates the inside of the cell from the surrounding extracellular fluid.
b) Regulation of exchange with the environment. The cell membrane controls the entry of ions and nutrients, the eliminations of wastes and the release of secretory products.
c) Sensitivity. The cell membrane is the first part of the cell affected by changes in the extracellular fluid. It also contains a variety of receptors that allow the cell to recognize and respond to specific molecules in its environment. Any alteration in the cell membrane may affect all celular activities.
d) Structural support. Specialized connections between cell membranes or between membranes and extracellular materials give tissues a stable structure.

V.3. MEMBRANE COMPOSITION

Molecular organisation of cell membrane is represented by inorganic compounds (water-30%, ions) and organic compounds (70%) like proteins, lipids and carbohydrates.
The relations between proteins and lipids are:
a) P/L=1/1 - in surface membranes which perform both a functional and isolation role;
b) P/L=2/1 - in endomembranes with major functional roles (energogenesis, synthesis and secretion);
c) P/L=1/2 - in myelin sheath cell membrane which provides a barrier function.

V.4. GLYCOCALYX

The terms **cell coat** or **glycocalyx** (sweet husk) are often used to describe the carbohydrate-rich peripheral zone at the surface of most eukaryotic cells. This zone can be visualized by a variety of stains, such as ruthenium red-whith photonic microscope. In electronic microscopy, glycocalyx looks like a fibrillar material of 50nm thick, made up of two layers: *an internal layer* which is 20nm thick, less electron-dense; and an *external layer* which is 30nm thick, denser than the first.

V.4.1. MOLECULAR STRUCTURE AND FUNCTION

The carbohydrate consists of the oligosaccharide side chains of plasma membrane -bound glycoproteins and glycolipids, although it often includes, in addition, both glycoproteins and proteoglycans that have been secreted and then adsorbed on the cell surface. Some of these adsorbed macromolecules are components of the extracellular matrix so that it is largely a matter of semantics as to where the plasma membrane ends and the extracellular matrix begins. Although the high concentration of cell-surface carbohydrate must have an important influence on many functions of the plasma membrane, the nature of these influences is not yet known.

Of the more than 100 different monosaccharides found in nature, only 9 occur in membrane glycoproteins and glycolipids. The principal ones are galactose, mannose, fructose, galactosamine, glucosamine, glucose and sialic acid. The sialic acid residues are usually found at the ends of the carbohydrates side chains, and they are mainly responsible for the net **negative surface charge** that characterizes all eukaryotic cells. The oligosaccharide side chains of glycoproteins and glycolipids can be complex. Although they usually contain fewer than 15 sugar residues, they are often branched, with the sugars bonded together by a variety of different linkages. In principle, even 3 sugar residues can be put together to form more than 1000 different trisaccharides. It is still technically very difficult to determine the sequence of complex oligosaccharide side chains on membrane proteins and lipids; thus the details of their structures are in many cases unknown.

The function of the oligosaccharide side chains in membrane glycolipids and glycoproteins is unclear. It is possible that those in certain transmembrane glycoproteins **help to anchor and orient the proteins** in the membrane by preventing them from slipping into the cytosol or from tumbling across the bilayer.

The carbohydrate also may play a role in **stabilizing the folded structure of glycoproteins.**

Carbohydrate may play a role in **guiding a membrane glycoprotein** to its appropriate destination in or on the cell. The complexity of some of the oligosaccharides on plasma membrane glycoproteins and glycolipids, taken together with their exposed position on the cell surface, suggests that they may play an important part in sophisticated cell-to-cell recognition processes. Some cells have surface proteins that bind specific oligosaccharides; theoretically such lectins could recognize oligosaccharides on the surface of other cells and thereby play a role in guiding cell-to-cell interactions. While there is evidence for this kind of interaction in plants, it has been difficult to prove that cell-surface carbohydrate functions in the same way in animals, despite increasing evidence that this is the case.

Glycocalyces can be subdivided into two general categories, depending on the degree of connection to the cell surface. An **attached glycocalyx** (or surface coat) is an inherent part of the cell surface that cannot be removed by mechanical means without simultaneously removing a portion of the plasma membrane itself. These coatings often appear in electron micrograph as fuzzy layers of filamentous material covering the cell surface. Since the carbohydrate chains of

plasma membrane glycoproteins and glycolipids are major constituents of this layer, it may be more proper to consider it as part of the plasma membrane.

In contrast, an **unattached glycocalyx** (or extraneous coat) consists of material located external to the plasma membrane that can be readily removed without affecting the viability of the cell or disrupting the plasma membrane. Included in this category are the membranes surrounding most animal eggs, the outer coat of amoebas, and the sarcolemma of muscle fibers. Although the various types of glycocalyx do not provide a rigid enclosure like plant and PK cell walls, they have been implicated in functions such as cell recognition and adhesion, protection of the cell surface, and the creation of permeability barriers.

V.5. PLASMA MEMBRANE

V.5.1. MOLECULAR MODELS OF PLASMA MEMBRANE

In the late nineteenth century C. Nagely, W. Pfeffer, and C.E. Overton studied rates at which various molecules pass into and out the cells. Their discovery that different compounds enter and leave cells at significantly different rates suggested the existence of a surface membrane that regulates the passage of materials into and out of the cell. Overton made the especially important discovery that the rate at which a given substance passes into cells is directly related to its solubility in lipids; the more soluble it is in lipids, the more readily a substance passes into cells. This correlation led Overton to propose that cells are covered by a membrane containing a thin film of lipid.

The next important contribution to our understanding of membrane structure was made by Irving Langmuir, who discovered that phospholipids spread on a water surface spontaneously form a film whose dimensions suggest it to be one molecule thick. Because phospholipids are amphipathic molecules containing both hydrophilic and hydrophobic regions, Langmuir theorized that such monomolecular films involve an organized arrangement in which the hydrophilic or "head "groups of the lipid molecules are aligned next to the water surface while their hydrophobic "tails" extend out toward the air.

Shortly thereafter E. Garter and F. Grendel exploited this phenomenon in their studies on the behaviour of lipids extracted from red blood cells. When these extracted lipids were spread as a monolayer on water, the film was found to cover an area twice that of the calculated surface area of the red cells from which the lipids had been extracted. Gorter and Grendel therefore concluded that the red cell is surrounded by a lipid membrane two molecules thick; such a **lipid bilayer** would be most stable if the hydrophilic head groups were exposed to the aqueous environments at the two membrane surfaces, and the hydrophobic tails were sequestered away from water in the membrane interior.

Shortly after Gorter and Grendel proposed the lipid bilayer model of the plasma membrane in 1925, its general validity was cast in doubt by surface tension experiments carried out by E. Harvey and J. Danielli. The data obtained by these investigators revealed that the surface tension of biological membranes is considerably higher than that of pure lipid droplets, implying that natural membranes cannot be made solely of lipid. It was subsequently discovered that the addition of protein to pure lipid droplets causes the surface tension to fall to levels comparable to those observed with natural membranes, implicating proteins as a component of membrane structure. Danielli and Davson proposed a detailed molecular model of the plasma membrane in which an inner bilayer of lipid molecules, oriented with their hydrophilic head groups toward the membrane surfaces, is covered on both sides by layers of proteins. The proteins were presumed to be bound to the hydrophilic head groups of the lipid bilayer through

ionic bonds. In order to account for the observed ability of some water-soluble molecules to diffuse through membranes, intermittent protein-lined pores were believed to interrupt the lipid bilayer. **The Danielli-Davson model**, first elaborated in detail in 1934, was to dominate the thinking of cell biologists for many decades to come.

During the early 1950s the application of electron microscopy to cell ultrastructural permitted the first direct visualization of biological membranes. Upon close examination, all membranes were found to measure 7-8 nm in thickness and to exhibit the same staining appearance: two electron-dense lines separated by a more lightly stained central zone. Because of the presence of these three layers, membranes were said to have a "trilaminar" appearance. This trilaminar arrangement, occurs in a broad spectrum of membranes including EK and PK plasma membranes, the endoplasmic reticulum and its derivates; mitochondrial, chloroplast, and nuclear membranes. The widespread occurrence of this morphological appearance led J.D. Robertson to postulate in his **unit membrane hypothesis** that all biological membranes share a common underlying structure.

Of the various models of membrane organization proposed in recent decades, the **fluid mosaic model** of S. J. Singer and G. Nicholson has the greatest impact on our thinking. According to this more recent model, illustrated in figure 33, three principles guide the organization of all biological membranes:

- Membrane lipids are arranged predominantly in the form of a bilayer, but this bilayer may be frequently interrupted by the presence of embedded proteins.
- Membrane proteins exist in two classes, integral proteins embedded in the lipid bilayer and peripheral proteins bound to the bilayer surface.
- The lipid bilayer is fluid, thereby permitting lateral movement of both membrane proteins and lipids.

V.5.2. BIOCHEMICAL COMPOSITIONOF THE PLASMA MEMBRANE

The typical cell membrane is composed of lipids and proteins in approximately equal amounts. Cell membrane structure and chemical composition varies. For example, the red cell membrane contains comparatively few glycolipids and is rich in sphingomyelin, a variety of phospholipid. In contrast, the myelin sheath cell membrane is rich in glycolipids and contains little sphingomyelin.

V.5.2.1. The lipid bilayer

As mentioned earlier, the calculations that originally led Gorter and Grendel to propose the existance of a lipid bilayer were not precise enough to be completely convincing. But as more sophisticated techiniques have gradually been developed, the data have continued to support the theory that membrane lipids are predominantly in the form of a bilayer. The most widespread approach to this problem has been to compare the properties of natural membranes to those of artificially created phospholipid bilayers. Such artificial bilayers can be generated in the laboratory by either placing a drop containing amphipathic lipids in a small hole separating two aqueous compartments or by exposing an amphipathic lipid-water suspension to ultrasonic vibrations. The first procedure generates planar bilayer membranes, while the second produces enclosed bilayer vesicles, or **liposomes**. Among the physical and chemical properties studied in such artificial lipid bilayers are thickness, electrical properties, permeability to water and solutes, temperature-dependent changes in state, X-ray diffraction paterns. In all cases the data obtained

is similar to that derived from studies of natural membranes, suggesting that the majority of lipid in natural membranes is arranged in the form of a bilayer. This does not rule out the possibility, however, that the continuity of the bilayer is occasionally interrupted by the presence of embedded proteins.

The universal presence of lipid bilayers in biological membranes occurs in spite of widespread differences in the particular kinds of lipids involved. The three major types of membrane lipids are phospholipids, cholesterol, and glycolipids.

V.5.2.1.1. Phospholipids are represented by: ***a) phosphoglycerides*** Examples of phosphoglycerides: phosphatidylcholine (PC) or lecithin, phosphatidyle-thalonamina (PE) or cephalin, phosphatidylserine (PS), phosphatidylthreonine, phosphatidylglycerol (PG), phosphatidylinositol (PI), cardiolipin and ***b) sphingophospholipids,*** ex: sphingomyelin.

In most membranes the majority of the lipid is phospholipid, although glycolipids predominate in myelin and chloroplast membranes. Even among membranes containing mostly phospholipids, the kinds of phospholipids vary significantly. Phosphoglycerides are generally the most abundant, but the various types of phosphoglycerides (phosphatidylcholine, phosphatidyle-thanolamine) occur in different proportions. Cardiolipin, which contains two phosphoglyceride molecules linked together by a glycerol bridge, occurs in mitochondrial inner membranes, lysosomal membranes, and bacterial plasma membranes. Sphingomyelin is an important phospholipid of animal plasma membranes, but is scarce or absent in mitochondrial, chloroplast, and bacterial plasma membranes.

Phospholipids are amphipatic molecules and therefore spontaneously form planar bilayered sheets or spherical **micelles** in an aqueous environment because these configurations represent minimum free energy conditions. The hydrophilic portions interact strongly with water and the hydrophobic portions interact strongly with one another.

V.5.2.1.2. Cholesterol is amphipathic and becomes intercalated between phospholipids in membranes. It increases the stability of the bilayers and prevents the loss of membrane liquidity at low temperature. The concentration of phospholipids and cholesterol varies in the membranes of organisms that live at temperature extremes. Presumably, this variation maintains membrane fluidity above crucial threshold levels. If membrane fluidity falls bellow these hypothetical thresholds, vital functions such as selective membrane transport may cease and the cell will die.

V.5.2.1.3. Glycolipids are represented by cerebrosides, sulfatides, and gangliosides. They are lipids that are covalently bonded to complex side chains containing various combinations of sugar residues. Glycolipids are amphipathic constituents of membranes, which constitute about 5% of all membranes lipids.

Their chemical composition varies considerably among species and among tissues within a species. For example, antigenic differences between human blood group substances are partially due to differences in glycolipid composition. Complex glycolipids mediate cell-to-cell and cell-to environment interaction.

In view of the above differences in lipid composition, it may seem surprising that all membranes exhibit a similar bilayer organisation. This common bilayer structure is possible, even in the face of such diversity among the lipids are amphipathic molecules exhibiting a hydrophilic “head” and a hydrophobic “tail”. Hence in spite of their chemical differences, membrane lipids can all be arranged in the form of a bilayer. This does not mean that the differences are of no significance. Many of the physical and biological properties of membranes, such as permeability, fluidity, and enzymatic activities, may be significantly influenced by the nature of the lipids present.

V.5.2.2. Arrangement of membrane proteins

The fluid mosaic model divides membrane proteins into two general categories, integral and peripheral. The principal classes of membrane proteins are illustrated in Table XI.

Integral proteins are embedded in the lipid bilayer and can only be removed by relatively harsh treatments, such as exposure to detergents or organic solvents. Integral proteins are bound to the membrane primarily by hydrophobic interactions with the tails of the lipid bilayer, and may either span the bilayer completely (transmembrane proteins) or be embedded in one side of the bilayer or the other. In order for such an arrangement to be thermodynamically stable, integral proteins must be amphipathic molecules whose hydrophobic regions are buried in the membrane interior and hydrophilic regions are exposed at the membrane surfaces. Such molecules account for the bulk of the membrane proteins, including most membrane-associated enzymes, receptors, and antigens.

Table XI – The membrane proteins

Class	***Function***	***Example***
Receptor proteins	Sensitive to specific extracellular materials that bind to them and trigger a change in cell′s activity.	Binding of the hormone insulin to membrane receptors increases the rate of glucose absorption by the cell.
Channel proteins	Central pore, or channel, permits water and solutes to bypass lipid portion of cell membrane.	Calcium ion movement through channels is involved in muscle contraction and conduction of nerve impulses
Carrier proteins	Bind and transports solutes across the cell membrane. This process may or may not require energy.	Carrier proteins bring glucose into the cytoplasm and also transport sodium, potassium, and calcium ions.
Enzymes	Catalyze reactions in the extracelllar fluid or within the cell.	Dipeptides are broken down into amino acids by enzymes on the membranes of cell lining the intestinal tract.
Anchor proteins	Attach the cell membrane to other structures and stabilize its position.	Inside the cell, bound to the network of supporting filaments (the cytoskeleton); outside, attach the cell to extra cellular protein fibers or to another cell.
Identifier proteins	Identify a cell as self or nonself, normal or abnormal, to the immune system	One group of such recognition proteins is the major histocompatibility complex (MHC).

Peripheral proteins - In contrast to integral proteins, peripheral proteins are bound to membranes by relatively weak ionic interactions with the hydrophilic head groups of the lipid bilayer. Such proteins are therefore easily removed from membrane surfaces by raising the ionic

strength. Peripheral proteins do not cover the entire surface of the bilayer, leaving many vacant areas where the lipid head groups are exposed at the membrane surface.

The arrangement of integral and peripheral proteins in the fluid mosaic model overcomes many of the criticisms of the Danielli-Davson model mentioned earlier. For example, the presence of integral proteins embedded in the lipid bilayer is consistent with the protein particles observed within the membrane interior in freeze-fracture micrographs; it is also compatible with the observation that most membrane proteins are not readly extracted by increasing the ionic strength. The presence of proteins embedded in the bilayer is also consistent with the known existence of globular membrane proteins containing alpha-helical structure, while the exposure of lipid head groups at the membrane surface is compatible with their observed susceptibility to phospholipase digestion. Finally, the fluid mosaic model is thermodynamically reasonable because the hydrophilic regions of the membrane lipids and proteins are exposed to the aqueous environment, while their hydrophobic regions are buried in the membrane interior away from contact with water.

V.6. THE MEMBRANE CYTOSKELETON

The membrane cytoskeleton consists only of proteins, and has 5-9 nm thickness. It forms a dense fibrillar shell that underlies the entire plasma membrane to the erythrocyte cytoskeleton. This cytoskeletal network is attached to integral membrane proteins at many points, giving the erythrocyte plasma membrane its great strength and flexibility. This structure differs from that found in most other mammalian cells whose cytoskeleton typically courses throughout the cytoplasm and is anchored to the plasma membrane at relatively few points.

By one-dimensional SDS polyacrylamide-gel electrophoresis, approximately 15 major proteins bands are detected, varying in molecular weight from 15000 to 250000. Three of these proteins - spectrin, glycophorin, and band III - account for more than 60% (by weight) of the total membrane protein.

Spectrin is a pair of 240 kD and 220 kD proteins loosely bound to the inner leaflet of the plasma membrane in a complex near the contractile protein **actin**. Although spectrum is present in highly purified membrane preparations, it is involved in the maintenance of the biconcave-disk shape of the erythrocyte. Spectrin also interacts indirectly, via a protein called ***ankyrin,*** with the cytoplasmic end of band III molecules.

Glycophorin is a transmembrane protein with a molecular weight of 30 kD. A complex group of sugar molecules are attached to its N-terminus, which projects into the pericellular domain. It also has a short segment composed of hydrophobic amino acids and a second hydrophilic region on the C-terminus facing the cytoplasmic watery domain.

Band III protein is a 100 kD dimmer. It is a globular protein embedded in the hydrophobic domain. Because of the peculiarities of the folding of the globular portion of band III protein, a hydrophilic pore exists inside the molecule, providing an aqueous ionic channel.

Other proteins find in the membrane cytoskeleton are actin, band 4.1 protein, calmoduline, gelsolina, filamina, fimbrina.

The membrane cytoskeleton performs the following roles: supporter of membrane surface gives membrane tensile and resistance, role in cell adhesion, movement of the membrane surface, role in reception and transition of the messengers.