CATALYST ACADEMY OF LIFE SCIENCES [CALS], MUMBAI

Study material for Life sciences - CSIR NET, SET, GATE (biotechnology & Life sciences), DBT-JRF, ICMR, and ARS-NET

Unit: 2 – **CELL BIOLOGY**

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UNIT 2. CELLULAR ORGANIZATION

A) Membrane structure and function (Structure of model membrane, lipid bilayer and membrane protein diffusion, osmosis, ion channels, active transport, membrane pumps, mechanism of sorting and regulation of intracellular transport, electrical properties of membranes).

B) Structural organization and function of intracellular organelles (Cell wall, nucleus, mitochondria, Golgi bodies, lysosomes, endoplasmic reticulum, peroxisomes, plastids, vacuoles, chloroplast, structure & function of cytoskeleton and its role in motility).

C) Organization of genes and chromosomes (Operon, unique and repetitive DNA, interrupted genes, gene families, structure of chromatin and chromosomes, heterochromatin, euchromatin, transposons).

D) Cell division and cell cycle (Mitosis and meiosis, their regulation, steps in cell cycle, regulation and control of cell cycle).

E) Microbial Physiology (Growth yield and characteristics, strategies of cell division, stress response)

2A. INTRODUCTION

TYPES OF CELL:

Two basic classes of cells—prokaryotic and eukaryotic— distinguished by their size and the types of internal structures, or **organelles**, they contain (Figure 2A1). The structurally simpler, **prokaryotic** cells include bacteria, whereas the structurally more complex **eukaryotic** cells include protists, fungi, plants, and animals. We are not sure when prokaryotic cells first appeared on Earth. Evidence of prokaryotic life has been obtained from rocks approximately **2.7 billion years of age**. **Cyanobacteria** almost certainly appeared by 2.4 billion years ago, because that is when the atmosphere becomes infused with molecular oxygen (O_2), which is a by-product of the photosynthetic activity of these prokaryotes.

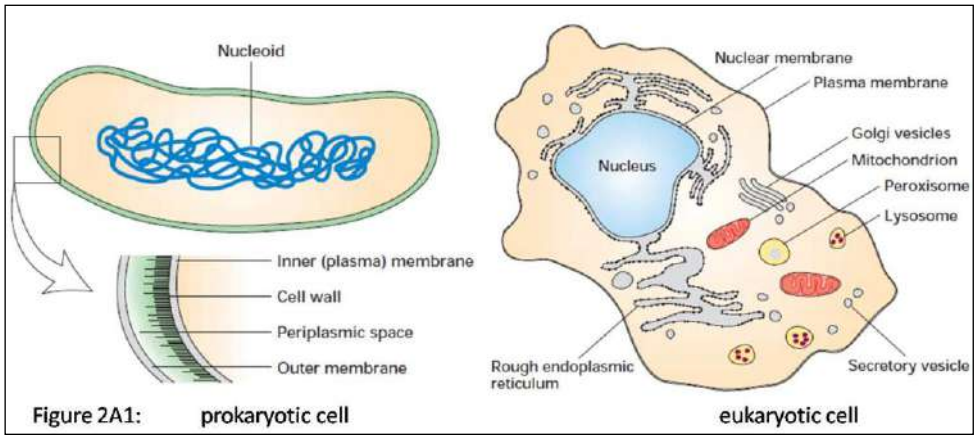


Figure 2A1:

A Comparison of Prokaryotic and Eukaryotic Cells

Features held in common by the two types of cells:

- Plasma membrane of similar construction
- Genetic information encoded in DNA using identical genetic code
- Similar mechanisms for transcription and translation of genetic information, including similar ribosomes
- Shared metabolic pathways (e.g., glycolysis and TCA cycle)
- Similar apparatus for conservation of chemical energy as ATP (located in the plasma membrane of prokaryotes and the mitochondrial membrane of eukaryotes)
- Similar mechanism of photosynthesis (between cyanobacteria and green plants)
- Similar mechanism for synthesizing and inserting membrane proteins
- Proteasomes (protein digesting structures) of similar construction (between archaebacteria and eukaryotes)

Features of eukaryotic cells not found in prokaryotes:

- Division of cells into nucleus and cytoplasm, separated by a nuclear envelope containing complex pore structures
- Complex chromosomes composed of DNA and associated proteins that are capable of compacting into mitotic structures
- Complex membranous cytoplasmic organelles (includes endoplasmic reticulum, Golgi complex, lysosomes, endosomes, peroxisomes, and glyoxisomes)

- Specialized cytoplasmic organelles for aerobic respiration (mitochondria) and photosynthesis (chloroplasts)
- Complex cytoskeletal system (including microfilaments, intermediate filaments, and microtubules) and associated motor proteins
- Complex flagella and cilia
- Ability to ingest fluid and particulate material by enclosure within plasma membrane vesicles (endocytosis and phagocytosis)
- Cellulose-containing cell walls (in plants)
- Cell division using a microtubule-containing mitotic spindle that separates chromosomes
- Presence of two copies of genes per cell (diploidy), one from each parent
- Presence of three different RNA synthesizing enzymes (RNA polymerases)
- Sexual reproduction requiring meiosis and fertilization

The shared properties reflect the fact that eukaryotic cells almost certainly evolved from prokaryotic ancestors. Because of their common ancestry, both types of cells share an identical genetic language, a common set of metabolic pathways, and many common structural features. For example, both types of cells are bounded by plasma membranes of similar construction that serve as a selectively permeable barrier between the living and nonliving worlds. Both types of cells (not animal cells) may be surrounded by a rigid, nonliving *cell wall* that protects the delicate life form within. Although the cell walls of prokaryotes and eukaryotes may have similar functions, their chemical composition is very different.

Internally, eukaryotic cells are much more complex—both structurally and functionally—than prokaryotic cells (Figure 2A1). Both types of cells contain a nuclear region, which houses the cell's genetic material, surrounded by cytoplasm. The genetic material of a prokaryotic cell is present in a **nucleoid**: a poorly demarcated region of the cell that lacks a boundary membrane to separate it from the surrounding cytoplasm. In contrast, eukaryotic cells possess a **nucleus**: a region bounded by a complex membranous structure called the *nuclear envelope*. This difference in nuclear structure is the basis for the terms *prokaryotic* (*pro* = before, *karyon* = nucleus) and *eukaryotic* (*eu* = true, *karyon* = nucleus). Prokaryotic cells contain relatively small amounts of DNA. Most eukaryotic cells contain considerably more genetic information. Both prokaryotic and eukaryotic cells have DNA containing chromosomes. Eukaryotic cells possess a number of separate chromosomes, each containing a single linear molecule of DNA. In contrast, nearly all prokaryotes that have been studied contain a single, circular chromosome. More importantly, the chromosomal DNA of eukaryotes, unlike that of prokaryotes, is tightly associated with proteins to form a complex nucleoprotein material known as **chromatin**.

The cytoplasm of the two types of cells is also very different. The cytoplasm of a eukaryotic cell is filled with a great diversity of structures, as is readily apparent by examining an electron micrograph of nearly any plant or animal cell Even yeast, the simplest eukaryote, is much more complex structurally than an average bacterium, even though these two organisms have a similar number of genes. Eukaryotic cells contain an array of membrane-bound organelles. Eukaryotic organelles include mitochondria, where chemical energy is made available to fuel cellular activities; an endoplasmic reticulum, where many of a cell's proteins and lipids are manufactured; Golgi complexes, where materials are sorted, modified, and transported to specific cellular destinations; and a variety of simple membrane bound vesicles of varying dimension. Plant cells contain additional membranous organelles, including chloroplasts, which are the sites of photosynthesis, and often a single large vacuole that can occupy most of the volume of the cell. Taken as a group, the membranes of the eukaryotic cell serve to divide the cytoplasm into compartments within which specialized activities can take place. In contrast, the cytoplasm of prokaryotic cells is essentially devoid of membranous structures.

The complex photosynthetic membranes of the cyanobacteria are a major exception to this generalization (see Figure 2A2). The cytoplasmic membranes of eukaryotic cells form a system of interconnecting channels and vesicles that function in the transport of substances from one part of a cell to another, as well as between the inside of the cell and its environment. Electron micrograph of a cyanobacterium

showing the cytoplasmic membranes that carry out photosynthesis. These concentric membranes are very similar to the thylakoid membranes present within the chloroplasts of plant cells, a reminder that chloroplasts evolved from symbiotic cyanobacteria.

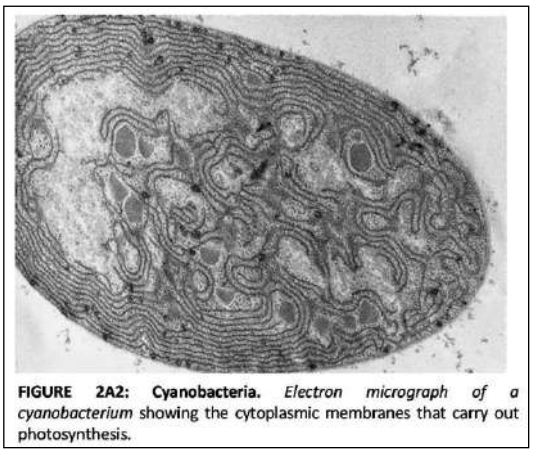


FIGURE 2A2: Cyanobacteria. *Electron micrograph of a cyanobacterium* showing the cytoplasmic membranes that carry out photosynthesis.

Because of their small size, directed intracytoplasmic communication is less important in prokaryotic cells, where the necessary movement of materials can be accomplished by simple diffusion.

Eukaryotic cells also contain numerous structures lacking a surrounding membrane. Included in this group are the elongated tubules and filaments of the cytoskeleton, which participate in cell contractility, movement, and support. It was thought until recently that prokaryotic cells lacked any trace of a cytoskeleton, but **primitive cytoskeletal filaments have been found in bacteria**. It is still fair to say that the prokaryotic cytoskeleton is much simpler, both structurally and functionally, than that of eukaryotes. Both eukaryotic and prokaryotic cells possess ribosomes, which are nonmembranous particles that function as "workbenches" on which the proteins of the cell are manufactured. Even though ribosomes of prokaryotic and eukaryotic cells have considerably different dimensions (those of prokaryotes are smaller and contain fewer components), these structures participate in the assembly of proteins by a similar mechanism in both types of cells.

The cytoplasm of a eukaryotic cell is extremely crowded, leaving very little space for the soluble phase of the cytoplasm, which is called the **cytosol**. Other major differences between eukaryotic and prokaryotic cells can be noted. Eukaryotic cells divide by a complex process of mitosis in which duplicated chromosomes condense into compact structures that are segregated by an elaborate microtubule-containing apparatus. This apparatus, which is called a ***mitotic spindle,*** allows each daughter cell to receive an equivalent array of genetic material. **In prokaryotes, there is no compaction of the chromosome and no mitotic spindle.** The DNA is duplicated, and the two copies are separated accurately by the growth of an intervening cell membrane.

For the most part, prokaryotes are nonsexual organisms. They contain only one copy of their single chromosome and have no processes comparable to meiosis, gamete formation, or true fertilization. Even though true sexual reproduction is lacking among prokaryotes, some are capable of ***conjugation*****, in which a piece of DNA is passed from one cell to another**. However, the recipient almost never receives a whole chromosome from the donor. The cell soon reverts back to possession of a single chromosome. Although prokaryotes may not be as efficient as eukaryotes in exchanging DNA with other members of their own species, they are more adept than eukaryotes at picking up and incorporating foreign DNA from their environment, which has had considerable impact on microbial evolution.

Types of Prokaryotic Cells:

The distinction between prokaryotic and eukaryotic cells is based on structural complexity (as detailed in above) and not on phylogenetic relationship. Prokaryotes are divided into two major taxonomic groups, or domains: the **Archaea** (or archaebacteria) and the **Bacteria** (or eubacteria). **Members of the Archaea are more closely related to eukaryotes than they are to the other group of prokaryotes** (the Bacteria). The domain Archaea includes several groups of organisms whose evolutionary ties to one another are revealed by similarities in the nucleotide sequences of their nucleic acids.

The best known Archaea are species that live in extremely inhospitable environments; they are often referred to as "**extremophiles**." Included among the Archaea are the **methanogens** [prokaryotes capable of converting CO_2 and H_2 gases into methane (CH_4) gas]; the **halophiles** (prokaryotes that live in extremely salty environments, such as the Dead Sea or certain deep sea basins that possess a salinity

equivalent to 5M $MgCl_2$); **acidophiles** (acid-loving prokaryotes that thrive at a pH as low as 0, such as that found in the drainage fluids of abandoned mine shafts); and **thermophiles** (prokaryotes that live at very high temperatures like hydrothermal vents of the ocean floor).

All other prokaryotes are classified in the domain Bacteria. This domain includes **the smallest known cells, the mycoplasma** (0.2 µm diameter), which are **the only known prokaryotes to lack a cell wall and to contain a genome (circular DNA molecule approximately 580,000 base pairs in length,) with as few as 500 genes**. Bacteria are present in every conceivable habitat on Earth, from the permanent ice shelf of the Antarctic to the driest African deserts, to the internal confines of plants and animals. Bacteria have even been found living in rock layers situated several kilometers beneath the Earth's surface. Some of these bacterial communities are thought to have been cut off from life on the surface for more than one hundred million years. The most complex prokaryotes are the cyanobacteria. Cyanobacteria contain elaborate arrays of cytoplasmic membranes, which serve as sites of photosynthesis (Figure 2A2). The membranes of cyanobacteria are very similar to the photosynthetic membranes present within the chloroplasts of plant cells. As in eukaryotic plants, photosynthesis in cyanobacteria is accomplished by splitting water molecules, which releases molecular oxygen.

Many cyanobacteria are capable not only of photosynthesis, but also of nitrogen fixation, the conversion of nitrogen (N_2) gas into reduced forms of nitrogen (such as ammonia, NH_3) that can be used by cells in the synthesis of nitrogencontaining organic compounds, including amino acids and nucleotides. Those species capable of both photosynthesis and nitrogen fixation can survive on the barest of resources—light, N_2, CO_2, and H_2O. It is not surprising, therefore, that cyanobacteria are usually the first organisms to colonize the bare rocks rendered lifeless by a scorching volcanic eruption.

The Sizes of Cells and Their Components:

Two units of linear measure are most commonly used to describe structures within a cell: the **micrometer** (µm) and the **nanometer** (nm). One µm = 10^{-6} meters, and one nm = 10^{-9}meters. The **angstrom** (Å), which is equal to one-tenth of a nm, or one Å = 10^{-10} meters. Å is commonly employed by molecular biologists for atomic dimensions. **One angstrom is roughly equivalent to the diameter of a hydrogen atom**. Large biological molecules (i.e., macromolecules) are described in either angstroms or nanometers.

TABLE 1-2 *Some SI prefixes*

Prefix	Abbreviation	Factor of base unit
giga	G	1,000,000,000 (10^9)
mega	M	1,000,000 (10^6)
kilo	k	1,000 (10^3)
hecto	h	100 (10^2)
deka	da	10 (10^1)
base unit		1
deci	d	0.1 (10^{-1})
centi	c	0.01 (10^{-2})
milli	m	0.001 (10^{-3})
micro	µ	0.000001 (10^{-6})
nano	n	0.000000001 (10^{-9})
pico	p	0.000000000001 (10^{-12})

The eukaryotic cell size ranges from about 10 to 30 µm. There are a number of reasons most cells are so small. (1) Most eukaryotic cells possess a single nucleus that contains only two copies of most genes. Because genes serve as templates for the production of information-carrying messenger RNAs, a cell can only produce a limited number of these messenger RNAs in a given amount of time. The greater a cell's cytoplasmic volume, the longer it will take to synthesize the number of messages required by that cell. (2) As a cell increases in size, the surface area/volume ratio decreases (You can verify this statement by calculating the surface area and volume of a cube whose sides are 1 cm in length versus a cube whose sides are 10 cm in length. The surface area/volume ratio of the smaller cube is considerably greater than that of the larger cube.) The ability of a cell to exchange substances with its environment is proportional to its surface area. If a cell were to grow beyond a certain size, its surface would not be sufficient to take up the substances (e.g., oxygen, nutrients) needed to support its metabolic activities. Cells that are specialized for absorption of solutes, such as those of the intestinal epithelium, typically possess microvilli, which greatly increase the surface area available for exchange. (3) A cell depends to a large degree on the random movement of molecules (*diffusion*). Oxygen, for example, must diffuse from the cell's surface through the cytoplasm to the interior of its mitochondria. The time required for diffusion is proportional to the square of the distance to be traversed. For example, O_2 requires only 100 microseconds to diffuse a distance of 1 µm, but requires 106 times as long to diffuse a distance of 1 mm.

As a cell becomes larger, and the distance from the surface to the interior becomes greater, the time required for diffusion to move substances in and out of a metabolically active cell becomes prohibitively long. Table 1-2 represents prefix the SI units of some common measurements.

VIRUSES:

The first virus to be identified was tobacco mosaic virus (TMV). It is a rod-shaped particle consisting of a single molecule of RNA surrounded by a helical shell composed of protein subunits (figure 2A3).

Viruses are responsible for dozens of human diseases, including AIDS, polio, influenza, cold sores, measles, and a few types of cancer. Generally they are smaller than the bacterium. Viruses occur in a wide variety of very different shapes, sizes, and constructions, but all of them share certain common properties. **All viruses are obligatory intracellular parasites**; that is, they cannot reproduce unless present within a host cell. Depending on the specific virus, the host may be a plant, animal, or bacterial cell. Outside of a living cell, the virus exists as a particle, or **virion**, which is little more than a macromolecular package. The virion contains a small amount of genetic material that, depending on the virus, can be single-stranded or double-stranded,RNA or DNA. Remarkably, some viruses have as few as three or four different genes, but others may have as many as several hundred. The genetic material of the virion is surrounded by a protein capsule, or *capsid*. Virions are macromolecular aggregates, inanimate particles that by themselves are unable to reproduce, metabolize, or carry on any of the other activities associated with life. For this reason, viruses are not considered to be organisms and are not described as being alive.

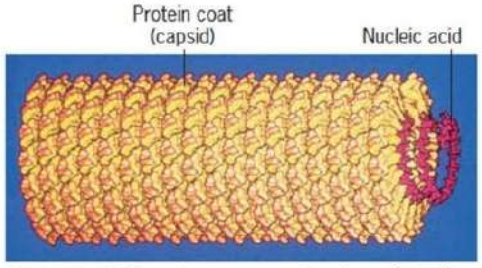


FIGURE 2A3: Tobacco mosaic virus (TMV). *Model of a portion of a* TMV particle. The protein subunits, which are identical along the entire rod-shaped particle, enclose a single helical RNA molecule.

VIROIDS:

It came as a surprise in 1971 to discover that viruses are not the simplest types of infectious agents. An **infectious agent consisting of a small circular RNA molecule that totally lacks a protein coat called a viroid**. The RNAs of viroids range in size from about 240 to 600 nucleotides, one tenth the size of the smaller viruses. No evidence has been found that the naked viroid RNA codes for any proteins. Rather, any biochemical activities in which viroids engage take place using host-cell proteins. For example, duplication of the viroid RNA within an infected cell utilizes the host's RNA polymerase II, an enzyme that normally transcribes the host's DNA into messenger RNAs. Viroids are thought to cause disease by interfering with the cell's normal path of gene expression. The effect on crops can be serious a viroid disease called **cadang-cadang** has devastated the coconut palm groves of the Philippines, and another viroid has wreaked havoc on the chrysanthemum industry in the United States.

PLASMA MEMBRANE STRUCTURE AND FUNCTION

The outer walls of a house or car provide a strong, inflexible barrier that protects its human inhabitants from an unpredictable and harsh external world. You might expect the outer boundary of a living cell to be constructed of an equally tough and impenetrable barrier because it must also protect its delicate internal contents from a nonliving, and often inhospitable, environment. Yet cells are separated from the external world by a thin, fragile structure called the **plasma membrane** that is only 5 to 10 nm wide. It would require about five thousand plasma membranes stacked one on top of the other to equal the thickness of a single page of these notes.

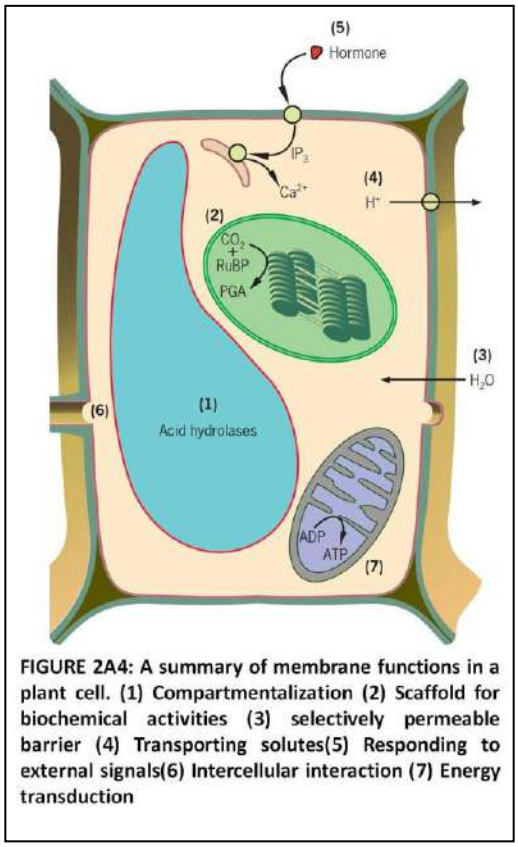


FIGURE 2A4: A summary of membrane functions in a plant cell. (1) Compartmentalization (2) Scaffold for biochemical activities (3) selectively permeable barrier (4) Transporting solutes(5) Responding to external signals(6) Intercellular interaction (7) Energy transduction

AN OVERVIEW OF MEMBRANE FUNCTIONS: (figure 2A4)

1. ***Compartmentalization.*** Membranes are continuous, unbroken sheets and, as such, inevitably enclose compartments. The plasma membrane encloses the contents of the entire cell, whereas the nuclear and cytoplasmic membranes enclose diverse intracellular spaces. The various membrane-bounded compartments of a cell possess markedly different contents. Membrane compartmentalization allows specialized activities to proceed without external interference and enables cellular activities to be regulated independently of one another.

2. ***Scaffold for biochemical activities.*** Membranes not only enclose compartments but are also a distinct compartment themselves. As long as reactants are present in solution, their relative positions cannot be stabilized and their interactions are dependent on random collisions. Because of their construction, membranes provide the cell with an extensive framework or scaffolding within which components can be ordered for effective interaction.

3. ***Providing a selectively permeable barrier.*** Membranes restrict the exchange of molecules from one side to the other. At the same time, membranes provide the means of communication between the compartments they separate. The plasma membrane, which encircles a cell, serve as a general barrier, yet both have gated "bridges" that promote the movement of select elements into and out of the enclosed living space.

4. ***Transporting solutes.*** The plasma membrane contains the machinery for physically transporting substances from one side of the membrane to another, often from a region where the solute is present at low concentration into a region where that solute is present at much higher concentration. The membrane's transport machinery allows a cell to accumulate substances, such as sugars and amino acids, that are necessary to fuel its metabolism and build its macromolecules. The plasma membrane is also able to transport specific ions, thereby establishing ionic gradients across itself. This capability is especially critical for nerve and muscle cells.

5. ***Responding to external signals.*** The plasma membrane plays a critical role in the response of a cell to external stimuli, a process known as **signal transduction**. Membranes possess **receptors** that combine with specific molecules (or **ligands**) having a complementary structure. Different types of cells have membranes with different receptors and are, therefore, capable of recognizing and responding to different ligands in their environment. The interaction of a plasma membrane receptor with an external ligand may cause the membrane to generate a signal that stimulates or inhibits internal activities

6. ***Intercellular interaction.*** Situated at the outer edge of every living cell, the plasma membrane of multicellular organisms mediates the interactions between a cell and its neighbors. The plasma membrane allows cells to recognize and signal one another, to adhere when appropriate, and to exchange materials and information.

7. ***Energy transduction.*** Membranes are intimately involved in the processes by which one type of energy is converted to another type (energy transduction). The most fundamental energy transduction occurs during photosynthesis when energy in sunlight is absorbed by membrane-bound pigments, converted into chemical energy, and stored in carbohydrates. Membranes are also involved in the transfer of chemical energy from carbohydrates and fats to ATP. In eukaryotes, the machinery for these energy conversions is contained within membranes of chloroplasts and mitochondria.

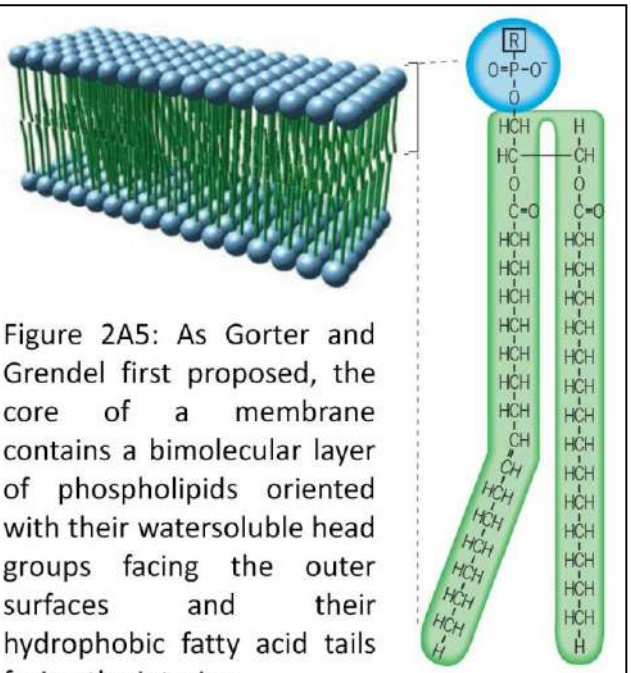


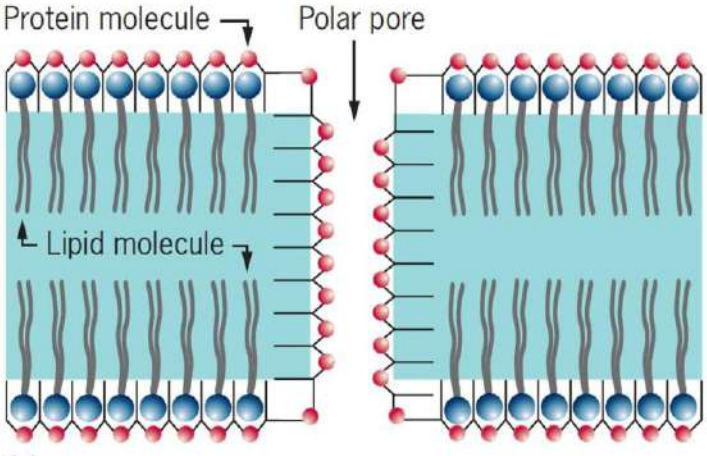
Figure 2A5: As Gorter and Grendel first proposed, the core of a membrane contains a bimolecular layer of phospholipids oriented with their watersoluble head groups facing the outer surfaces and their hydrophobic fatty acid tails facing the interior.

A BRIEF HISTORY OF STUDIES ON PLASMA MEMBRANE STRUCTURE:

To study the plasma membrane, **mature mammalian red blood cells** (RBCs) are used as model system, because it lacks both nuclei and cytoplasmic organelles. Number of scientists contributed to the following conclusions of membrane.

1. First proposal that **cellular membranes might contain a lipid bilayer** was made in 1925 by two Dutch scientists**, E. Gorter and F. Grendel**. These researchers extracted the lipid from human red blood cells and measured the amount of surface area the lipid would cover when spread over the surface of water. The ratio of the surface area of water covered by the extracted lipid to the surface area calculated for the red blood cells from which the lipid is **2:1** this conclude that the **plasma membrane contained a bimolecular layer of lipids**, that is, a **lipid bilayer.** The polar groups of each lipid molecular layer (or *leaflet*) is directed outward toward the aqueous environment, as shown in Figure 2A5. This would be the thermodynamically favored arrangement, because the polar head groups of the lipids could interact with surrounding water molecules.

2. In 1935, **Hugh Davson and James Danielli** proposed that **the plasma membrane was composed of a lipid bilayer that was lined on both its inner and outer surface by a layer of globular proteins**. They revised their model in the 1954 to account for the selective permeability of the membranes they had studied. In the revised version (Figure 2A6), Davson and Danielli suggested that, in addition to the outer and inner protein layers, the lipid bilayer was also duits for polar solutes and ions to enter and exit the cell.



(a)

Figure 2A6: A revised 1954 version of the Davson-Danielli model showing the lipid bilayer, which is lined on both surfaces by a monomolecular layer of proteins that extends through the membrane to form protein-lined pores.

3. Experiments conducted in the late 1960s led to a new concept of membrane structure, as detailed in the **fluid mosaic model proposed in 1972 by S. Jonathan Singer and Garth Nicolson** (Figure 2A7). According to the **fluid-mosaic model**, the bilayer of a fluid-mosaic membrane is present in a fluid state, and individual lipid molecules can move laterally within the plane of the membrane and the proteins are embedded in it. Assume that the fluid state of the lipid bilayer is like ocean and the proteins are embedded in it like icebergs.

THE CHEMICAL COMPOSITION OF MEMBRANES:

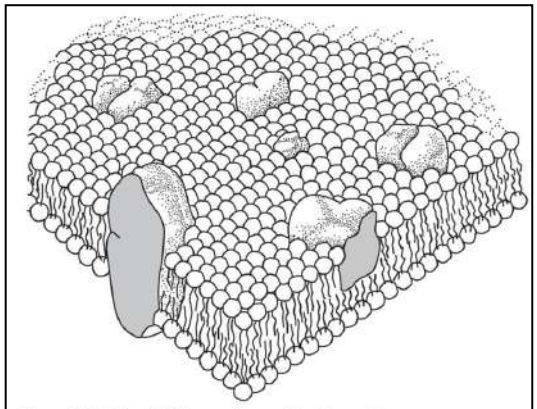


Figure 2A7: The fluid-mosaic model of membrane structure as initially proposed by Singer and Nicolson in 1972. sea of lipid bilayer and embedded proteins it it as icebergs.

Membranes are lipid–protein assemblies in which the components are held together in a thin sheet by **noncovalent bonds**. As noted above, the core of the membrane consists of a sheet of lipids arranged in a bimolecular layer. The lipid bilayer serves primarily as a structural backbone of the membrane and provides the barrier that prevents random movements of water-soluble materials into and out of the cell. The proteins of the membrane, on the other hand, carry out most of the specific functions (Fig 2A4).

Each type of differentiated cell contains a unique complement of membrane proteins, which contributes to the specialized activities of that cell type. The ratio of lipid to protein in a membrane varies, depending on the type of cellular membrane (plasma vs. endoplasmic reticulum vs. Golgi), the type of organism (bacterium vs. plant vs. animal), and the type of cell (cartilage vs. muscle vs. liver).To a large degree, these differences can be correlated with the basic functions of these membranes. Membranes also contain carbohydrates, which are attached to the lipids and proteins as indicated in Figure 2A8.

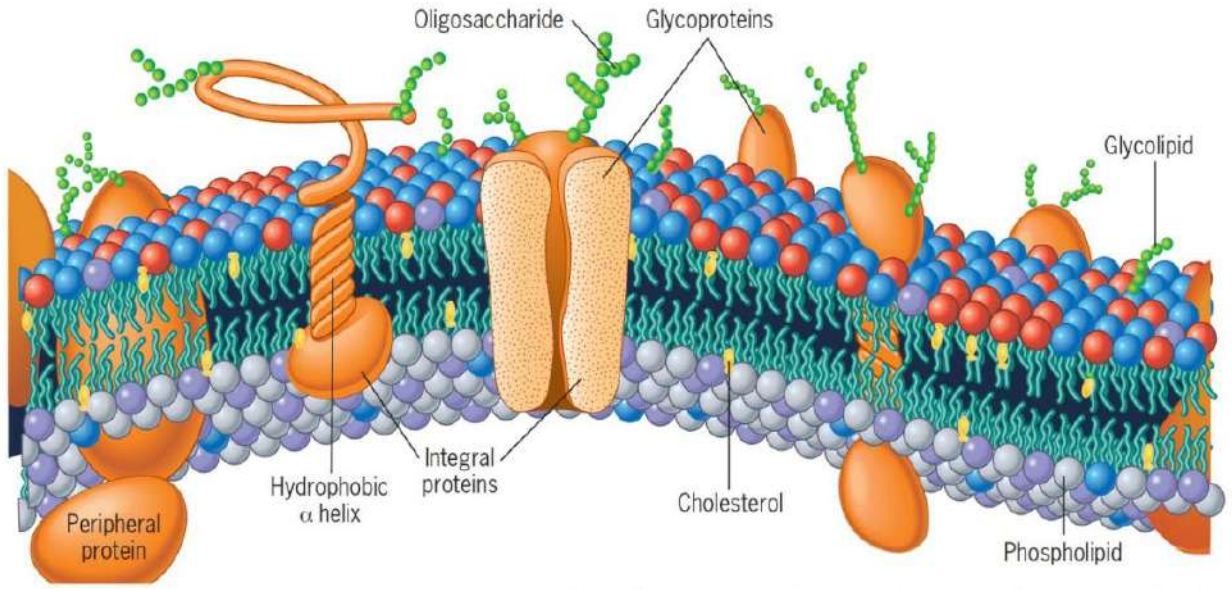


Figure 2A8: A current representation of the plasma membrane showing the same basic organization as that proposed by Singer and Nicolson. The external surface of most membrane proteins, as well as a small percentage of the phospholipids, contain short chains of sugars, making them glycoproteins and glycolipids. Those portions of the polypeptide chains that extend through the lipid bilayer typically occur as helices composed of hydrophobic amino acids. The two leaflets of the bilayer contain different types of lipids as indicated by the differently colored head groups. The outer leaflet may contain microdomains ("rafts") consisting of clusters of specific lipid species.

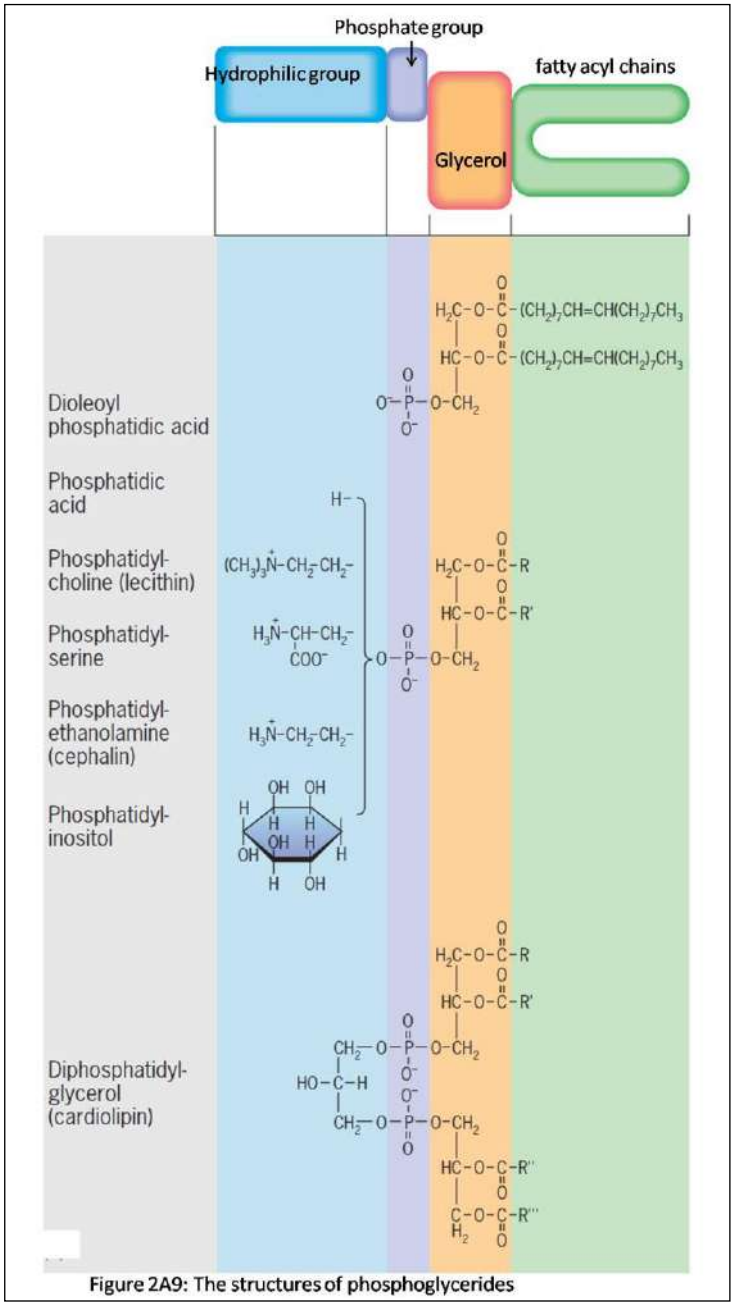


Figure 2A9: The structures of phosphoglycerides

MEMBRANE LIPIDS:

Membranes contain a wide diversity of lipids, all of which are **amphipathic**; that is, they contain both hydrophilic and hydrophobic regions. There are three main types of membrane lipids: **phosphoglycerides, sphingolipids, and cholesterol.**

1. Phosphoglycerides: Most membrane lipids contain a phosphate group, which makes them **phospholipids**. Because most membrane phospholipids are built on a glycerol backbone, they are called **phosphoglycerides** (Figure 2A9).

Unlike triglycerides, which have three fatty acids and are not amphipathic, **membrane glycerides are *diglycerides*—** only two of the hydroxyl groups of the glycerol are esterified to fatty acids; the third is esterified to a hydrophilic phosphate group. Without any additional substitutions beyond the phosphate and the two fatty acyl chains, the molecule is called ***phosphatidic acid***, which is virtually absent in most membranes. Instead, membrane phosphoglycerides have an additional group linked to the phosphate, most commonly either **choline (forming *phosphatidylcholine*, PC), ethanolamine (forming *phosphatidylethanolamine*, PE), serine (forming *phosphatidylserine*, PS), or inositol (forming *phosphatidylinositol*, PI)**. Each of these groups is small and hydrophilic and, together with the negatively charged phosphate to which it is attached, forms a highly water-soluble domain at one end of the molecule, called the **head group**. At physiologic pH, the head groups of PS and PI have an overall negative charge, whereas those of PC and PE are neutral. In contrast, the fatty acyl chains are hydrophobic, unbranched hydrocarbons approximately 16 to 22 carbons in length (Figure 2A9). A membrane fatty acid may be fully **saturated (i.e., lack double bonds), monounsaturated (i.e., possess one double bond), or polyunsaturated (i.e., possess more than one double bond)**. Phosphoglycerides often contain one unsaturated and one saturated fatty acyl chain. With fatty acid chains at one end of the molecule and a polar head group at the other end, all of the phosphoglycerides exhibit a distinct amphipathic character.

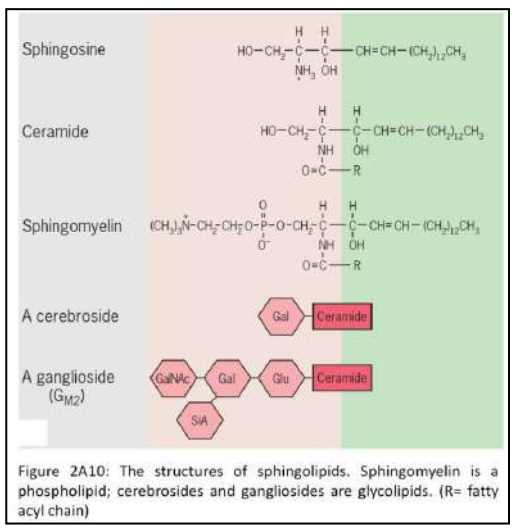


Figure 2A10: The structures of sphingolipids. Sphingomyelin is a phospholipid; cerebrosides and gangliosides are glycolipids. (R= fatty acyl chain)

2. Sphingolipids: A less abundant class of membrane lipids, called **sphingolipids**, are derivatives of sphingosine, an amino alcohol that contains a long hydrocarbon chain (Figure 2A10). Sphingolipids consist of sphingosine linked to a fatty acid (R of Figure

2A10) by its amino group. This molecule is a ***ceramide***. The various sphingosine-based lipids have additional groups esterified to the terminal alcohol of the sphingosine moiety. If the substitution is phosphorylcholine, the molecule is ***sphingomyelin*, which is the only phospholipid of the membrane that is not built with a glycerol backbone**. If the substitution is a carbohydrate, the molecule is a **glycolipid**. If the carbohydrate is a simple sugar, the glycolipid is called a ***cerebroside***; if it is a small cluster of sugars, the glycolipid is called a ***ganglioside***. Since all sphingolipids have two long, hydrophobic hydrocarbon chains at one end and a hydrophilic region at the other, they are also amphipathic and basically similar in overall structure to the phosphoglycerides. **Glycolipids** are interesting membrane components. Relatively little is known about them. The nervous system is particularly rich in glycolipids. The myelin sheath contains a high content of a particular glycolipid, called **galactocerebroside** (shown in Figure 2A10), which is formed when a galactose is added to ceramide. Humans who are unable to synthesize a particular ganglioside (GM3) suffer from a serious neurological disease characterized by severe seizures and blindness. Glycolipids also play a role in certain infectious diseases; the toxins that cause cholera and botulism both enter their target cell by first binding to cell-surface gangliosides, as does the influenza virus.

TABLE 4.1 Lipid Compositions of Some Biological Membranes*

Lipid	Human erythrocyte	Human myelin	Beef heart mitochondria	E. coli
Phosphatidic acid	1.5	0.5	0	0
Phosphatidylcholine	19	10	39	0
Phosphatidyl-ethanolamine	18	20	27	65
Phosphatidylgycerol	0	0	0	18
Phosphatidylserine	8.5	8.5	0.5	0
Cardiolipin	0	0	22.5	12
Sphingomyelin	17.5	8.5	0	0
Glycolipids	10	26	0	0
Cholesterol	25	26	3	0

*The values given are weight percent of total lipid.

3. **Cholesterol:** Another lipid component of certain membranes is the sterol **cholesterol**, which in certain animal cells may constitute up to 50 percent of the lipid molecules in the plasma membrane. **Cholesterol is absent from the plasma membranes of most plant and all bacterial cells**. Cholesterol molecules are oriented with their small hydrophilic hydroxyl group toward the membrane surface and the remainder of the molecule embedded in the lipid bilayer (Figure 4.7). The hydrophobic rings of a cholesterol molecule are flat and rigid, and they interfere with the movements of the fatty acid tails of the phospholipids.

THE NATURE AND IMPORTANCE OF THE LIPID BILAYER:

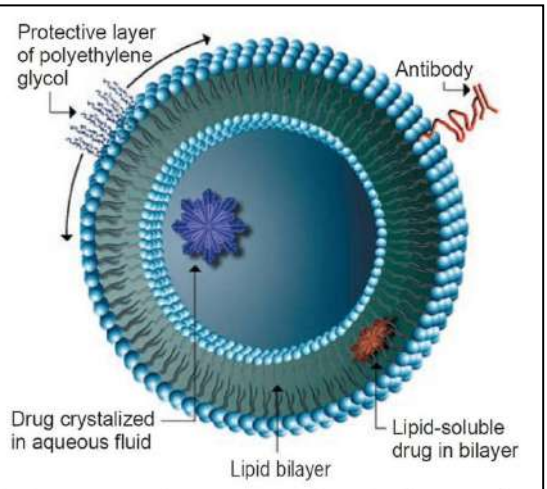


FIGURE 2A11: Liposomes. A schematic diagram of a stealth liposome containing a hydrophilic polymer (such as polyethylene glycol) to protect it from destruction by immune cells, antibody molecules that target it to specific body tissues, a water-soluble drug enclosed in the fluid-filled interior chamber, and a lipid-soluble drug in the bilayer.

Each type of cellular membrane has its own characteristic lipid composition, differing from one another in the types of lipids, the nature of the head groups, and the particular species of fatty acyl chain(s). Lipid bilayer has ability to self-assemble, which can be demonstrated more easily within a test tube than a living cell. If, for example, a small amount of phosphatidylcholine is dispersed in an aqueous solution, the phospholipid molecules assemble spontaneously to form the walls of **fluid-filled spherical vesicles**, called **liposomes** (figure 2A11). The walls of these liposomes consist of a continuous lipid bilayer that is organized in the same manner as that of the lipid bilayer of a natural membrane. Liposomes have proven invaluable in membrane research. Liposomes have also been developed as vehicles to deliver drugs or DNA molecules within the body. The drugs or DNA can be linked to the wall of the liposome or contained at high concentration within its lumen (Figure 2A11). In these studies, the walls of the liposomes are constructed to contain specific

proteins (such as antibodies or hormones) that allow the liposomes to bind selectively to the surfaces of particular target cells where the drug or DNA is intended to go. Most of the early clinical studies with liposomes met with failure because the injected vesicles were rapidly removed by phagocytic cells of the immune system. This obstacle has been overcome with the development of so-called stealth liposomes (e.g., Caelyx) that contain an outer coating of a synthetic polymer that protects the liposomes from immune destruction (Figure 2A11).

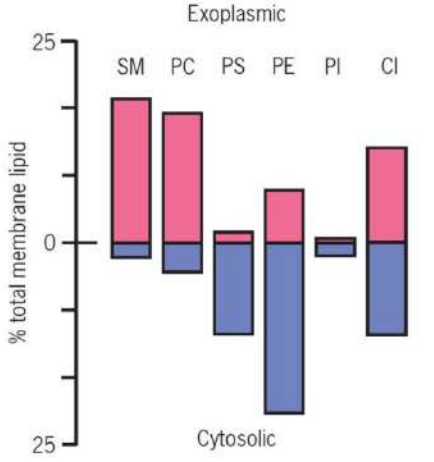


Fig. 2A12: **The asymmetric distribution of phospholipids (and cholesterol) in the plasma membrane of human erythrocytes. (SM,** sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; Cl, cholesterol.)

THE ASYMMETRY OF MEMBRANE LIPIDS:

The lipid bilayer consists of two distinct leaflets that have a distinctly different lipid composition. One line of experiments that has led to this conclusion that **outer leaflet have (sphingomyelin) SM and (phosphatidylcholine) PC; whereas inner leaflet has (phosphatidylethanolamine) PE, (phosphatidylserine) PS, and (Phosphatidylethanolamine) PI** (Figure 2A12). The cholesterol molecules are present in both the leaflets. All the glycolipids of the plasma membrane are in the outer leaflet where they often serve as receptors for extracellular ligands. **Phosphatidylethanolamine**, which is concentrated in the inner leaflet, tends to promote the curvature of the membrane, which is important in membrane budding and fusion. **Phosphatidylserine**, which is concentrated in the inner leaflet, has a net negative charge at physiologic pH, which makes it a candidate for binding positively charged lysine and arginine residues, such as those adjacent to the membrane-spanning α helix of glycophorin A (protein).

MEMBRANE CARBOHYDRATES:

The plasma membranes of eukaryotic cells also contain carbohydrate. Depending on the species and cell type, the carbohydrate content of the plasma membrane ranges between 2 and 10 percent by weight. More than 90 percent of the membrane's carbohydrate is covalently linked to proteins to form **glycoproteins**; the remaining carbohydrate is covalently linked to lipids to form **glycolipids**. As indicated in Figure 2A8, all of the carbohydrate of the plasma membrane faces outward into the extracellular space (It can be noted that even though phosphatidylinositol contains a sugar group, it is not considered to be part of the carbohydrate portion of the membrane in this discussion). The carbohydrate of internal cellular membranes also faces away from the cytosol. The addition of carbohydrate, or **glycosylation**, is the most complex of these modifications. The carbohydrate of glycoproteins is present as short, branched hydrophilic **oligosaccharides**, typically having fewer than about 15 sugars per chain. In contrast to most high-molecular-weight carbohydrates (such as glycogen, starch, or cellulose), which are polymers of a single sugar, the oligosaccharides attached to membrane proteins and lipids can display considerable variability in composition and structure.

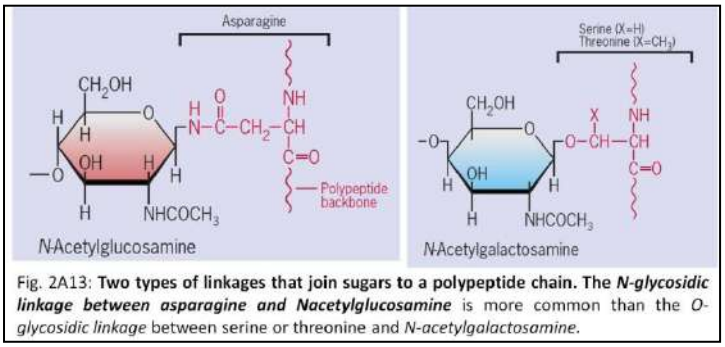


Fig. 2A13: **Two types of linkages that join sugars to a polypeptide chain. The *N-glycosidic linkage between asparagine and Nacetylglucosamine*** is more common than the *O-glycosidic linkage* between serine or threonine and *N-acetylgalactosamine.*

Oligosaccharides may be attached to several different amino acids by two major types of linkages (Figure 2A13).These carbohydrate projections play an important role in mediating the interactions of a cell with its environment and sorting of membrane proteins to different cellular compartments.

The carbohydrates of the glycolipids of the red blood cell plasma membrane determine whether a person's blood type is A, B, AB, or O (Figure 2A14). A person having blood type A has an enzyme that adds an ***N*-acetylgalactosamine** to the end of the chain, whereas a person with type B blood has an enzyme that adds **galactose** to the chain terminus. These two enzymes are encoded by alternate versions

of the same gene, yet they recognize different substrates. People with AB blood type possess both enzymes, whereas people with O blood type lack enzymes capable of attaching either terminal sugar.

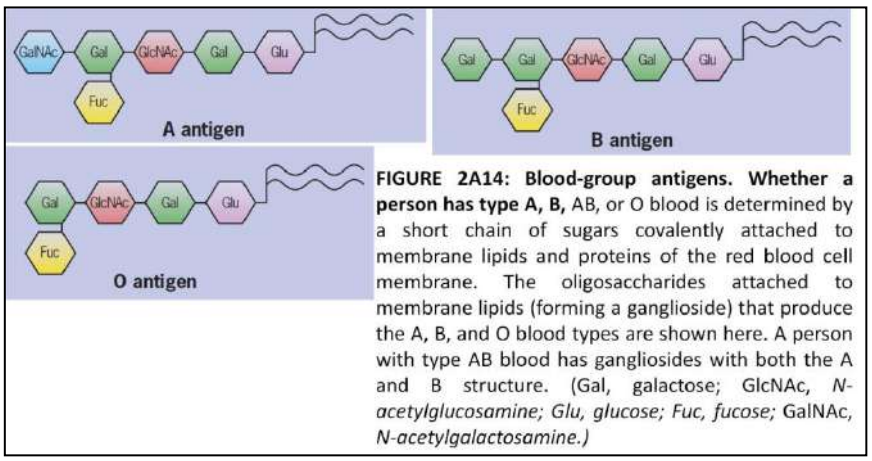


FIGURE 2A14: Blood-group antigens. Whether a person has type A, B, AB, or O blood is determined by a short chain of sugars covalently attached to membrane lipids and proteins of the red blood cell membrane. The oligosaccharides attached to membrane lipids (forming a ganglioside) that produce the A, B, and O blood types are shown here. A person with type AB blood has gangliosides with both the A and B structure. (Gal, galactose; GlcNAc, *N-acetylglucosamine; Glu, glucose; Fuc, fucose;* GalNAc, *N-acetylgalactosamine.)*

THE STRUCTURE AND FUNCTIONS OF MEMBRANE PROTEINS:

Depending on the cell type and the particular organelle within that cell, a membrane may contain hundreds of different proteins. Each membrane protein has a defined orientation relative to the cytoplasm, so that the properties of one surface of a membrane are very different from those of the other surface. This asymmetry is referred to as membrane "**sidedness**." In the plasma membrane, for example, those parts of membrane proteins that interact with other cells or with extracellular substances project outward into the extracellular space, whereas those parts of membrane proteins that interact with cytoplasmic molecules project into the cytosol. Membrane proteins can be grouped into three distinct classes distinguished by the intimacy of their relationship to the lipid bilayer (Figure 2A15).

1. **Integral membrane proteins:** That penetrates the lipid bilayer. Integral proteins are **transmembrane proteins**; that is, they pass entirely through the lipid bilayer and thus have domains that protrude from both the extracellular and cytoplasmic sides of the membrane. Some integral proteins have only one membrane-spanning segment, whereas others are multispanning. Genome-sequencing studies suggest that integral proteins constitute 20–30 percent of all encoded proteins.

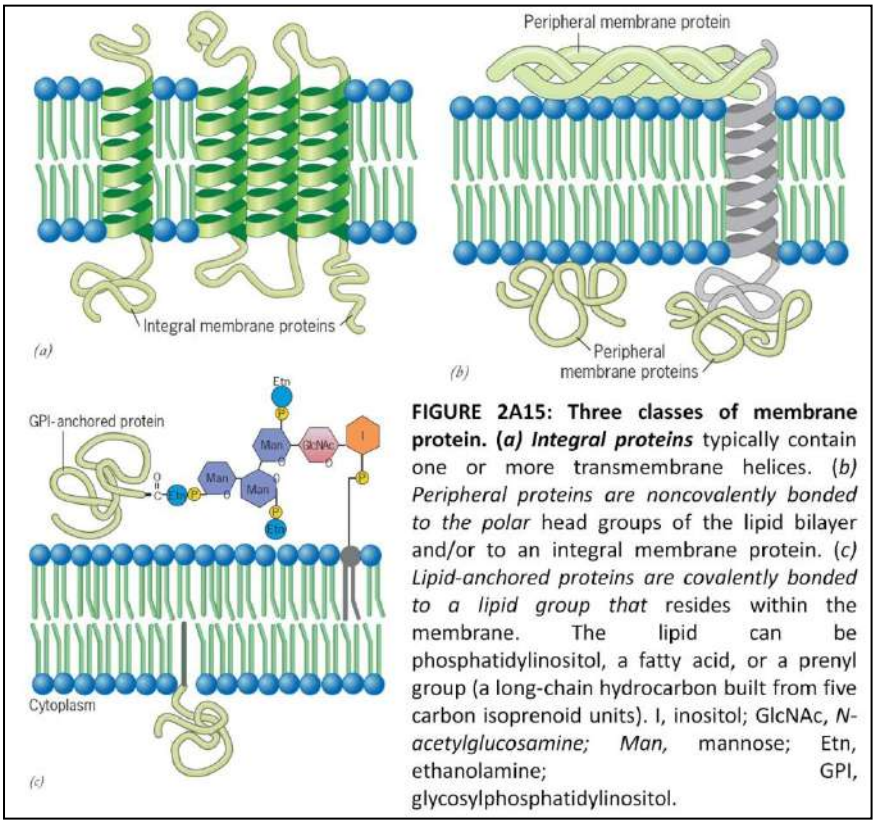


FIGURE 2A15: Three classes of membrane protein. (*a*) *Integral proteins* typically contain one or more transmembrane helices. (*b*) *Peripheral proteins are noncovalently bonded to the polar* head groups of the lipid bilayer and/or to an integral membrane protein. (*c*) *Lipid-anchored proteins are covalently bonded to a lipid group that* resides within the membrane. The lipid can be phosphatidylinositol, a fatty acid, or a prenyl group (a long-chain hydrocarbon built from five carbon isoprenoid units). I, inositol; GlcNAc, *N-acetylglucosamine; Man,* mannose; Etn, ethanolamine; GPI, glycosylphosphatidylinositol.

Most integral membrane proteins function in the following capacities: as receptors that bind specific substances at the membrane surface, as channels or transporters involved in the movement of ions and solutes across the membrane, or as agents that transfer electrons during the processes of photosynthesis and respiration. Like the phospholipids of the bilayer, integral membrane proteins are also amphipathic, having both hydrophilic and hydrophobic portions. As discussed below, those portions of an integral membrane protein that reside within the lipid bilayer tend to have a **hydrophobic character**. Amino acid residues in these transmembrane domains form **van der Waals interactions** with the fatty acyl chains of the bilayer, which seals the protein into the lipid "wall" of the membrane. As a result, the permeability barrier of the membrane is preserved and the protein is brought into direct contact with surrounding lipid molecules. Lipid molecules that are closely associated with a

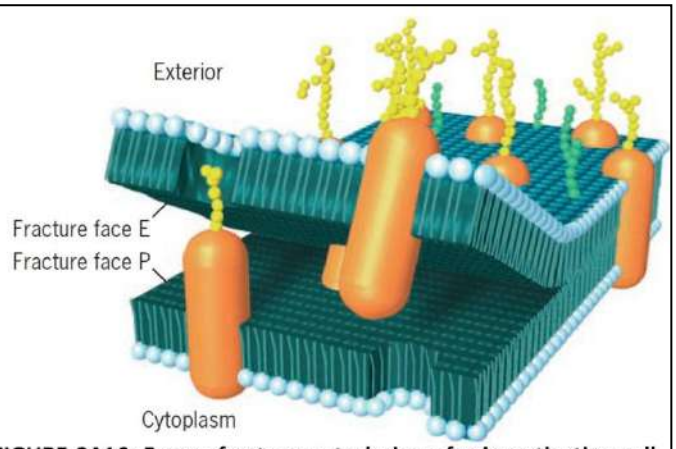


FIGURE 2A16: Freeze fracture: a technique for investigating cell membrane structure. (*a) When a block of frozen tissue is struck by a knife* blade, a fracture plane runs through the tissue, often following a path that leads it through the middle of the lipid bilayer. The fracture plane goes around the proteins rather than cracking them in half, and they segregate with one of the two halves of the bilayer. The exposed faces within the center of the bilayer can then be covered with a metal deposit to form a metallic replica. These exposed faces are referred to as the E, or ectoplasmic face, and the P, or protoplasmic face.

membrane protein can play an important role in the activity of the protein, although the degree to which a particular protein requires specific interactions with particular lipid molecules remains unclear. Those portions of an integral membrane protein that project into either the cytoplasm or extracellular space tend to be more like the globular proteins. These nonembedded domains tend to have hydrophilic surfaces that interact with water-soluble substances (low molecular-weight substrates, hormones, and other proteins) at the edge of the membrane. Several large families of membrane proteins contain an interior channel that provides an aqueous passageway through the lipid bilayer. The linings of these channels typically contain key hydrophilic residues at strategic locations. Integral proteins need not be fixed structures but may be able to move laterally within the membrane.

Distribution of Integral Proteins: Freeze-Fracture Analysis

The concept that proteins penetrate through membranes, rather than simply remaining external to the bilayer, was derived primarily from the results of a technique called **freezefracture replication**. In this procedure, tissue is frozen solid and then struck with a knife blade, which fractures the block into two pieces. As this occurs, the fracture plane often takes a path between the two leaflets of the lipid bilayer (Figure 2A16). Once the membranes are split in this manner, metals are deposited on their exposed surfaces to form a shadowed *replica*, which is viewed in the electron microscope. The replica resembles a road strewn with pebbles, which are called *membrane-associated particles*. Since the fracture plane passes through the center of the bilayer, most of these particles correspond to integral membrane proteins that extend at least halfway through the lipid core. When the fracture plane reaches a given particle, it goes around it rather than cracking it in half. Consequently, each protein (particle) separates with one half of the plasma membrane, leaving a corresponding pit in the other half. One of the great values of the freeze-fracturing technique is that it allows an investigation of the microheterogeneity of the membrane. Localized differences in parts of the membrane stand out in these replicas and can be identified. Biochemical analyses, in contrast, average out such differences.

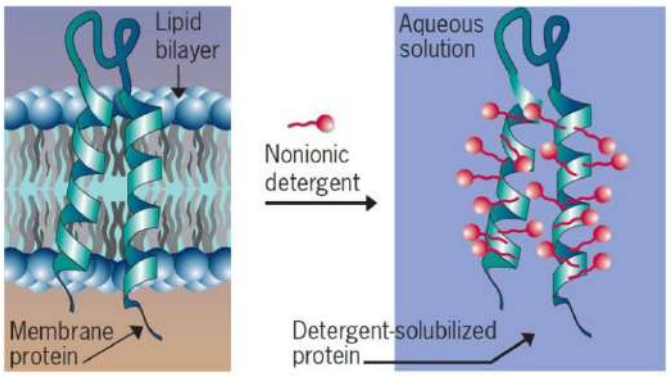


FIGURE 2A17: Solubilization of membrane proteins with detergents. The nonpolar ends of the detergent molecules associate with the nonpolar residues of the protein that had previously been in contact with the fatty acyl chains of the lipid bilayer. In contrast, the polar ends of the detergent molecules interact with the surrounding water molecules, keeping the protein in solution.Nonionic detergents, as shown here, solubilize membrane proteins without disrupting their structure.

Studying the Structure and Properties of Integral Membrane Proteins: (Fig 2A17)

Because of their hydrophobic transmembrane domains, integral membrane proteins are difficult to isolate in a soluble form. Removal of these proteins from the membrane normally requires the use of a detergent,

such as the ionic (charged) detergent SDS (which denatures proteins) or the nonionic (uncharged) detergent Triton X-100 (which generally does not alter a protein's tertiary structure).

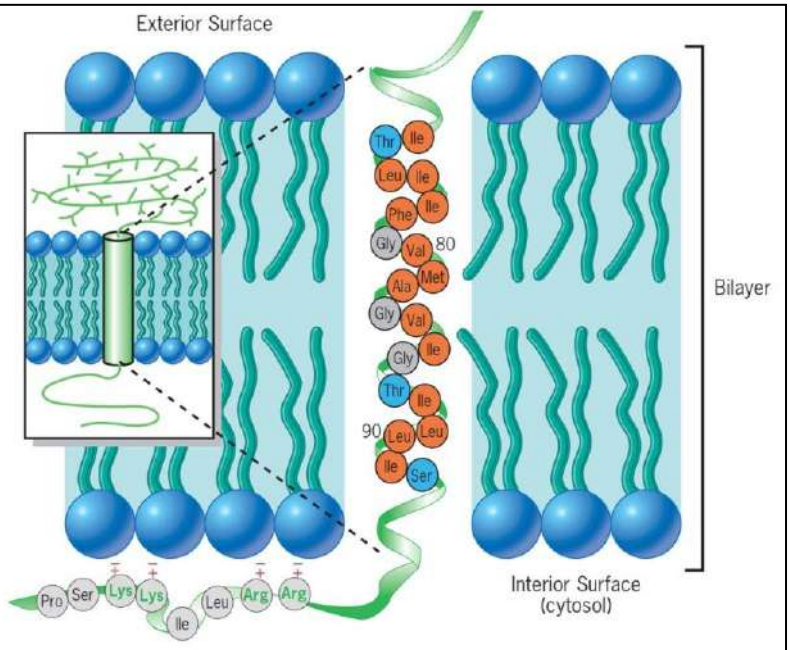


FIGURE 2A18: Glycophorin A, an integral protein with a single transmembrane domain. The single helix that passes through the membrane consists predominantly of hydrophobic residues (orange-colored circles). The four positively charged amino acid residues of the cytoplasmic domain of the membrane form ionic bonds with negatively charged lipid head groups. Carbohydrates are attached to a number of amino acid residues on the outer surface of the protein (shown in the inset). All but one of the 16 oligosaccharides are small *O-linked* chains (the exception is a larger oligosaccharide linked to the asparagine residue at position 26). Glycophorin molecules are present as homodimers within the erythrocyte membrane (Figure 4.32*d*). *The two helices cross over one another in the* region between residues 79 and 83. This Gly-X-X-X-Gly sequence is commonly found where transmembrane helices come into close proximity.

Like membrane lipids, detergents are amphipathic, being composed of a polar end and a nonpolar hydrocarbon chain. As a consequence of their structure, detergents can substitute for phospholipids in stabilizing integral proteins while rendering them soluble in aqueous solution (Figure 2A17). Once the proteins have been solubilized by the detergent, various analyses can be carried out to determine the protein's amino acid composition, molecular mass, amino acid sequence, and so forth.

Identifying Transmembrane Domains:

Which segments of the polypeptide chain are actually embedded in the lipid bilayer? Those segments of a protein embedded within the membrane, which are described as the **transmembrane domains**, have a simple structure; they consist of a string of about 20 predominantly nonpolar amino acids that span the core of the lipid bilayer as an α helix. (*the α helix is a favored conformation because it allows for a maximum number of hydrogen bonds to be formed between neighboring amino acid residues, thereby creating a highly stable (low-energy) configuration. This is particularly important for a membrane-spanning polypeptide that is surrounded by fatty acyl chains and, thus, cannot form hydrogen bonds with an aqueous solvent. Transmembrane helices are at least 20 amino acids in length, because this is the minimum stretch of polypeptide capable of spanning the hydrocarbon core of a lipid bilayer of 30 Å width. A few integral membrane proteins have been found to contain loops or helices that penetrate but do not span the bilayer. An example is the P helix*). The chemical structure of a single transmembrane helix is shown in Figure 2A18, which depicts the two-dimensional structure of glycophorin A, the major integral protein of the erythrocyte plasma membrane. Of the 20 amino acids that make up the lone α helix of a glycophorin monomer (amino acids 73 to 92 of Figure 2A18), all but three have hydrophobic side chains (or an H atom in the case of the glycine residues). The exceptions are serine and threonine, which are noncharged, polar residues. A portion of a transmembrane helix with a threonine residue, not unlike those of glycophorin A. The hydroxyl group of the residue's side chain can form a hydrogen bond with one of the oxygen atoms of

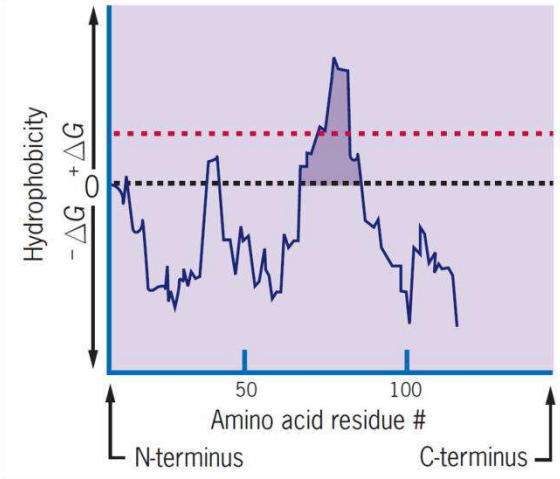


FIGURE 2A19: Hydropathy plot for glycophorin A, a single membranespanning protein. Hydrophobicity is measured by the free energy required to transfer each segment of the polypeptide from a nonpolar solvent to an aqueous medium. Values above the 0 line are energyrequiring (+Δ*Gs), indicating they consist of stretches of amino acids* that have predominantly nonpolar side chains. Peaks that project above the red-colored line are interpreted as a transmembrane domain.

the peptide backbone. Fully charged residues may also appear in transmembrane helices, but they tend to be accommodated in ways that allow them to fit into their hydrophobic environment.

HYDROPATHY PLOT:

Knowing the amino acid sequence of an integral membrane protein, we can usually identify the transmembrane segments using a ***hydropathy plot***, in which each site along a polypeptide is assigned a value that provides a measure of the *hydrophobicity* of the amino acid at that site as well as that of its neighbors. This approach provides a "running average" of the hydrophobicity of short sections of the polypeptide, and guarantees that one or a few polar amino acids in a sequence do not alter the profile of the entire stretch. Hydrophobicity of amino acids can be determined using various criteria, such as their lipid solubility or the energy that would be required to transfer them from an aqueous into a lipid medium. A hydropathy plot for glycophorin A is shown in Figure 2A19.

Transmembrane segments are usually identified as a jagged peak that extends well into the hydrophobic side of the spectrum. A reliable prediction concerning the orientation of the transmembrane segment within the bilayer can usually be made by examining the flanking amino acid residues. In most cases, as illustrated by glycophorin in Figure 2A18, those parts of the polypeptide at the cytoplasmic flank of a transmembrane segment tend to be more positively charged than those at the extracellular flank. **Not all integral membrane proteins contain transmembrane α helices**. A number of membrane proteins contain a relatively large channel positioned within a circle of membrane-spanning β strands organized into a barrel. To date, aqueous channels constructed of β barrels have only been found in the outer membranes of bacteria, mitochondria, and chloroplasts.

SITE DIRECTED CROSS LINKING:

Determining Spatial Relationships within an Integral Membrane Protein Suppose you have isolated a gene for an integral membrane protein and, based on its nucleotide sequence, determined that it contains four apparent membrane-spanning α helices. You might want to know how these helices are oriented relative to one another and which amino acid side chains of each helix face outward toward the lipid environment. Although these determinations are difficult to make without detailed structural models, considerable insight can be gained by site-directed mutagenesis, that is, by introducing specific changes into the gene that codes for the protein. For example, site-directed mutagenesis can be employed to replace amino acid residues in neighboring helices with cysteine residues.

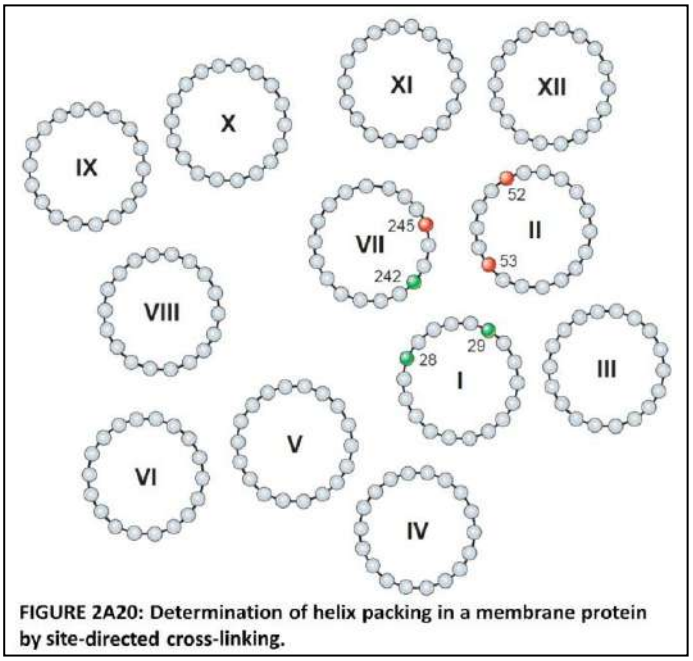


FIGURE 2A20: Determination of helix packing in a membrane protein by site-directed cross-linking.

As we know two cysteine residues can form a covalent disulfide bridge. If two transmembrane helices of a polypeptide each contain a cysteine residue, and the two cysteine residues are able to form a disulfide bridge with one another, then these helices must reside in very close proximity. The results of one site-directed cross-linking study on lactose permease, a sugar-transporting protein in bacterial cell membranes, are shown in Figure 2A20. It was found in this case that helix VII lies in close proximity to both helices I and II.

In figure 2A20, (the experiments), pairs of cysteine residues are introduced into the protein by site-directed mutagenesis, and the ability of the cysteines to form disulfide bridges is determined. Hydropathy plots and other data had indicated that lactose permease has 12 transmembrane helices. It was found that a cysteine introduced at position 242 of helix VII can cross-link to a cysteine introduced at either position 28 or 29 of helix I. Similarly, a cysteine at position 245 of helix VII can cross-link to cysteines at either 52 or 53 of helix II. The proximity of these three helices is thus established.

2. **Peripheral proteins:** Those are located entirely outside of side, yet are associated with the surface of the membrane by **noncovalent** bonds. Peripheral proteins are associated with the membrane by weak electrostatic bonds (refer to Figure 2A15*b*). Peripheral proteins can usually be solubilized by extraction with **high-concentration salt solutions** that weaken the electrostatic bonds holding peripheral proteins to a membrane. The best studied peripheral proteins are located on the internal (cytosolic) surface of the plasma membrane, where they form a fibrillar network that acts as a membrane "skeleton". These proteins provide mechanical support for the membrane and function as an anchor for integral membrane proteins. Other peripheral proteins on the internal plasma membrane surface function as enzymes, specialized coats, or factors that transmit transmembrane signals. Peripheral proteins typically have a dynamic relationship with the membrane, being recruited to the membrane or released from the membrane depending on prevailing conditions.

3. **Lipid-anchored proteins:** They are located outside the lipid bilayer, on either the extracellular or cytoplasmic surface, but are **covalently** linked to a lipid molecule that is situated within the bilayer. Several types of lipid-anchored membrane proteins can be distinguished. Numerous proteins present on the external face of the plasma membrane are bound to the membrane by a small, complex oligosaccharide linked to a molecule of phosphatidylinositol (PI) that is embedded in the outer leaflet of the lipid bilayer (refer to Figure 2A15*c*). Peripheral membrane proteins containing this type of **glycosyl-phosphatidylinositol** linkage are called **GPI-anchored proteins**. They were discovered when it was shown that certain membrane proteins could be released by a phospholipase that specifically recognized and cleaved inositol-containing phospholipids. A rare type of anemia, **paroxysmal nocturnal hemoglobinuria**, results from a deficiency in GPI synthesis that makes red blood cells susceptible to lysis.

Another group of proteins present on the *cytoplasmic* side of the plasma membrane is anchored to the membrane by one or more long hydrocarbon chains embedded in the inner leaflet of the lipid bilayer (refer to Figure 2A15*c* and accompanying legend). At least two proteins associated with the plasma membrane in this way (Src and Ras) have been implicated in the transformation of a normal cell to a malignant state.

MEMBRANE LIPIDS AND MEMBRANE FLUIDITY:

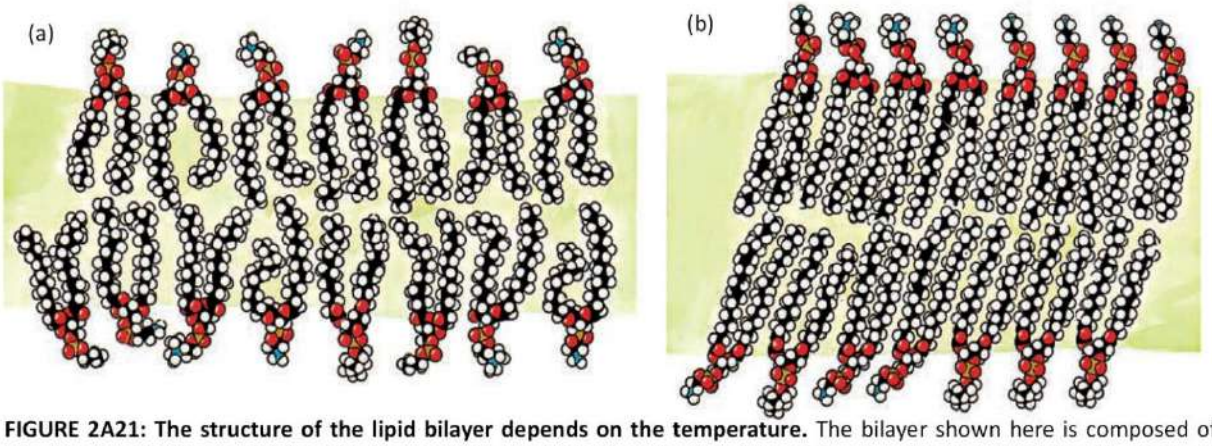


FIGURE 2A21: The structure of the lipid bilayer depends on the temperature. The bilayer shown here is composed of two phospholipids: phosphatidylcholine and phosphatidylethanolamine. (*a) Above the transition* temperature, the lipid molecules and their hydrophobic tails are free to move in certain directions, even though they retain a considerable degree of order. (*b) Below the transition temperature, the movement of the* molecules is greatly restricted, and the entire bilayer can be described as a crystalline gel.

The physical state of the lipid of a membrane is described by its fluidity or viscosity (*Fluidity and viscosity are inversely related; fluidity is a measure of the ease of flow, and viscosity is a measure of the resistance to flow*). Consider a simple artificial bilayer composed of phosphatidylcholine and phosphatidylethanolamine, whose fatty acids are largely unsaturated (having double bond). If the temperature of the bilayer is kept relatively warm (e.g., 37^{o}C), the lipid exists in a relatively fluid state (Figure 2A21a). At this temperature, the lipid bilayer is best described as a two-dimensional liquid crystal. As in a crystal, the molecules still retain a specified orientation; in this case, the long axes of the molecules tend toward a parallel arrangement, yet individual phospholipids can rotate around their axis or move laterally within the plane of the bilayer. If the temperature is slowly lowered, a point is reached where the bilayer distinctly changes (Figure 2A21*b*). The lipid is converted from a liquid crystalline phase to a frozen crystalline gel in which the movement of the phospholipid fatty acid chains is greatly restricted. The temperature at which this change occurs is called the **transition temperature**.

The transition temperature of a particular bilayer depends on the ability of the lipid molecules to be packed together, which depends in turn on the particular lipids of which it is constructed. Saturated fatty acids have the shape of a straight, flexible rod. ***Cis*-unsaturated fatty acids**, on the other hand, have crooks (kink/bend) in the chain at the sites of a double bond (Figure 2A21a). Consequently, **phospholipids with saturated chains pack together more tightly than those containing unsaturated chains**.

TABLE 4.2 Melting Points of the Common 18-Carbon Fatty Acids

Fatty acid	*cis* Double bonds	M.p.(°C)
Stearic acid	0	70
Oleic acid	1	13
Linoleic acid	2	−9
Linolenic acid	3	−17
Eicosapentanoic acid (EPA)*	5	−54

*EPA has 20 carbons.

Factors that influences bilayer fluidity is fatty acid chain length and degree of saturation. The organism living in **high temperature have long length saturated fatty acyl chain to maintain the membrane rigidity; whereas those organisms living in low temperature must have unsaturated short length fatty acyl chains to maintain membrane fluidity.** The organisms living at moderate temperature have almost equal quantity of saturated and unsaturated fatty acyl chains with moderate length. The shorter the fatty acyl chains of a phospholipid, the lower its melting temperature. The physical state of the membrane is also affected by cholesterol. Because of their orientation within the bilayer, cholesterol molecules disrupt the close packing of fatty acyl chains and interfere with their mobility. **The presence of cholesterol tends to abolish sharp transition temperatures and creates a condition of intermediate fluidity**. In physiologic terms, cholesterol tends to increase the durability while decreasing the permeability of a membrane.

The effect of fatty acid saturation on melting temperature is illustrated by familiar food products. Vegetable oils remain a liquid in the refrigerator, whereas margarine (butter) is a solid. Vegetable oils contain polyunsaturated fatty acids, whereas the fatty acids of margarine have been saturated by a chemical process that hydrogenates the double bonds of the vegetable oils used as the starting material.

The Importance of Membrane Fluidity:

What effect does the physical state of the lipid bilayer have on the biological properties of the membrane? Membrane fluidity provides a perfect compromise between a rigid, ordered structure in which mobility would be absent and a completely fluid, nonviscous liquid in which the components of the membrane could not be oriented and structural organization and mechanical support would be lacking. In addition, fluidity allows for interactions to take place within the membrane. For example, membrane fluidity makes it possible for clusters of membrane proteins to assemble at particular sites within the membrane and form specialized structures, such as intercellular junctions, light-capturing photosynthetic complexes, and synapses. Because of membrane fluidity, molecules that interact can come together, carry out the necessary reaction, and move apart.

Fluidity also plays a key role in membrane assembly. Membranes arise only from preexisting membranes, and their growth is accomplished by the insertion of lipids and proteins into the fluid matrix of the membranous sheet. Many of the most basic cellular processes, including cell movement, cell growth, cell division, formation of intercellular junctions, secretion, and endocytosis, depend on the movement of membrane components and would probably not be possible if membranes were rigid, nonfluid structures.

Maintaining Membrane Fluidity:

The internal temperature of most organisms (other than birds and mammals) fluctuates with the temperature of the external environment. Since it is essential for many activities that the membranes of a cell remain in a fluid state, cells respond to changing conditions by altering the types of phospholipids of which they are made. Maintenance of membrane fluidity is an example of homeostasis at the cellular level and can be demonstrated in various ways. For example, if the temperature of a culture of cells is lowered, the cells respond metabolically.

The initial "emergency" response is mediated by enzymes that remodel membranes, making the cell more cold resistant. Remodeling is accomplished by (1) desaturating single bonds in fatty acyl chains to form double bonds, and (2) reshuffling the chains between different phospholipid molecules to produce ones that contain two unsaturated fatty acids, which greatly lowers the melting temperature of the bilayer. Desaturation of single bonds to form double bonds is catalyzed by enzymes called ***desaturases***. Reshuffling is accomplished by ***phospholipases***, which split the fatty acid from the glycerol backbone, and ***acyltransferases***, which transfer fatty acids between phospholipids. In addition, the cell changes the types of phospholipids being synthesized in favor of ones containing more unsaturated fatty acids. As a result of the activities of these various enzymes, the physical properties of a cell's membranes are matched to the prevailing environmental conditions. Maintenance of fluid membranes by adjustments in fatty acyl composition has been demonstrated in a variety of organisms, including hibernating mammals, pond-dwelling fish whose body temperature changes markedly from day to night, cold-resistant plants, and bacteria living in hot springs.

Lipid Rafts:

Every so often an issue emerges that splits the community of cell biologists into believers and nonbelievers. The issue of lipid rafts falls into this category. When membrane lipids are extracted from cells and used to prepare *artificial* lipid bilayers, cholesterol and sphingolipids tend to self-assemble into microdomains that are more gelated and highly ordered than surrounding regions consisting primarily of phosphoglycerides. Because of their distinctive physical properties, such microdomains tend to float within the more fluid and disordered environment of the artificial bilayer (Figure 2A22*a*).

As a result, these patches of cholesterol and sphingolipid are referred to as **lipid rafts**. When added to these artificial bilayers, certain proteins tend to become concentrated in the lipid rafts, whereas others tend to remain outside their boundaries. GPI-anchored proteins show a particular fondness for the ordered regions of the bilayer (Figure 2A22*a*).

The controversy arises over whether similar types of cholesterol-rich lipid rafts, such as that illustrated in Figure 2A22*b*, exist within living cells. Most of the evidence in favor of lipid rafts is derived from studies that employ unnatural treatments, such as detergent extraction or cholesterol depletion, which makes the results difficult to interpret. Attempts to demonstrate the presence of lipid rafts in living cells have

generally been unsuccessful, which can either mean that such rafts do not exist or they are so small (5 to 25 nm diameter) and short-lived as to be difficult to detect with current techniques.

The concept of lipid rafts is very appealing because it provides a means to introduce order into a seemingly random sea of lipid molecules. Lipid rafts are postulated to serve as floating platforms that concentrate particular proteins, thereby organizing the membrane into functional compartments (Figure 2A22*b*). For example, lipid rafts are thought to provide a favorable local environment for cell-surface receptors to interact with other membrane proteins that transmit signals from the extracellular space to the cell interior.

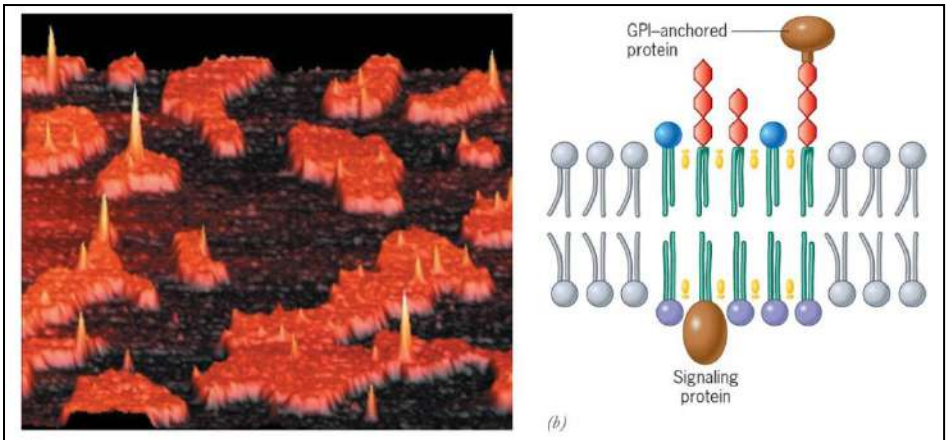


FIGURE 2A22: Lipid rafts.

[FIGURE 2A22 description: (*a*) Image of the upper surface of an artificial lipid bilayer containing phosphatidylcholine, which appears as the black background, and sphingomyelin molecules, which organize themselves spontaneously into the orange-colored rafts. The yellow peaks show the positions of a GPI-anchored protein, which is almost exclusively raft associated. This image is provided by an atomic force microscope, which measures the height of various parts of the specimen at the molecular level. (*b*) Schematic model of a lipid raft within a cell. The outer leaflet of the raft consists primarily of cholesterol (yellow) and sphingolipids (red head groups). Phosphatidylcholine molecules (blue head groups) with long saturated fatty acids also tend to concentrate in this region. GPI-anchored proteins are thought to become concentrated in lipid rafts.] The lipids in the outer leaflet of the raft have an organizing effect on the lipids of the inner leaflet. As a result, the inner-leaflet raft lipids consist primarily of cholesterol and glycerophospholipids with long saturated fatty acyl tails. The inner leaflet tends to concentrate lipid anchored proteins, such as Src kinase, that are involved in cell signaling.

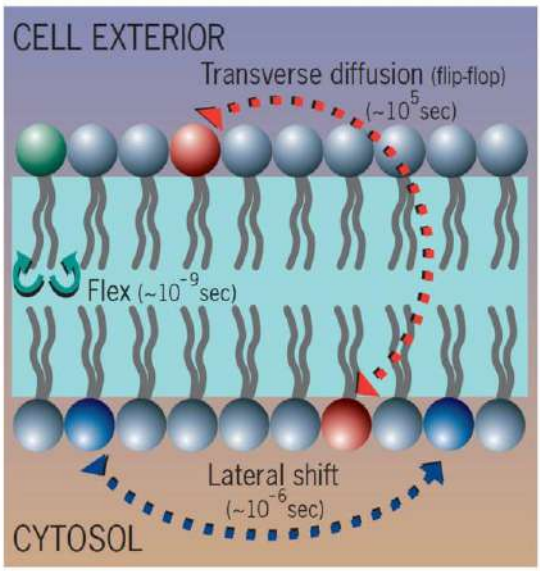


FIGURE 2A23: The possible movements of phospholipids in a membrane.

THE DYNAMIC NATURE OF THE PLASMA MEMBRANE:

It is apparent from the previous discussion that the lipid bilayer can exist in a relatively fluid state. As a result, a phospholipid can move laterally within the same leaflet with considerable ease. The mobility of individual lipid molecules within the bilayer of the plasma membrane can be directly observed under the microscope by linking the polar heads of the lipids to gold particles or fluorescent compounds. It is estimated that a phospholipid can diffuse from one end of a bacterium to the other end in a second or two. In contrast, **it takes a phospholipid molecule a matter of hours to days to move across to the other leaflet**. Thus, of all the possible motions that a phospholipid can make, its flip-flop to the other side of the membrane is the most restricted (Figure 2A23). This finding is not surprising. For flip-flop to occur, the hydrophilic head group of the lipid must pass through the internal hydrophobic sheet of the membrane, which is thermodynamically unfavorable. However, cells contain enzymes that actively move

certain phospholipids from one leaflet to the other. These enzymes play a role in establishing lipid asymmetry and may also reverse the slow rate of passive transmembrane movement.

[**Description of Fig 2A23**, The types of movements in which membrane phospholipids can engage and the approximate time scales over which they occur. Whereas phospholipids move from one leaflet to another at a very slow rate, they diffuse laterally within a leaflet rapidly. Lipids lacking polar groups, such as cholesterol, can move across the bilayer quite rapidly]

Enzyme flippases (P type ATPase) causes lipid movements from outer leaflet to inner leaflet; whereas floppase (ABC transporters) enzyme flip lipids from inner leaflet to the outer leaflet. Both these enzymes use ATP. Another enzyme called Scramblase, which move lipids in either way towards equilibrium.

THE DIFFUSION OF MEMBRANE PROTEINS AFTER CELL FUSION:

The first experiments to demonstrate that membrane proteins could move within the plane of the membrane utilized cell fusion, and they were reported in **1970 by Larry Frye and Michael Edidin** of Johns Hopkins University. In their experiments, mouse and human cells were fused, and the locations of specific proteins of the plasma membrane were followed once the two membranes had become continuous. **Cell fusion** is a technique whereby two different types of cells, or cells from two different species, can be fused to produce one cell with a common cytoplasm and a single, continuous plasma membrane. Cells are induced to fuse with one another by making the outer surface of the cells "sticky" so that their plasma membranes adhere to one another. Cells can be induced to fuse by addition of certain **inactivated viruses** that attach to the surface membrane, by adding the compound **polyethylene glycol**, or by a **mild electric shock**.

[**Description of Figure 2A24:** Outline of the experiment in which human and mouse cells were fused (steps 1–2) and the distribution of the proteins on the surface of each cell were followed in the hybrids over time (steps 3–4). Mouse membrane proteins are indicated by solid circles, human membrane proteins by open circles. Locations of human and mouse proteins in the plasma membranes of the hybrid cells were monitored by interaction with fluorescent red and fluorescent green antibodies, respectively].

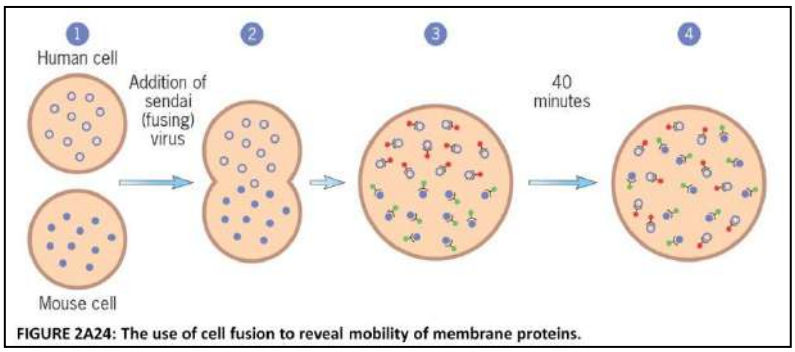


FIGURE 2A24: The use of cell fusion to reveal mobility of membrane proteins.

To follow the distribution of either the mouse membrane proteins or the human membrane proteins at various times after fusion, antibodies against one or the other type of protein were prepared and covalently linked to fluorescent dyes. The antibodies against the **mouse proteins were complexed with a dye that fluoresces green** and the **antibodies against human proteins with one that fluoresces red**. When the antibodies were added to fused cells, they bound to the human or mouse proteins and could be located under a fluorescence light microscope (Figure 2A24). At the time of fusion, the plasma membrane appeared half human and half mouse; that is, the two protein types remained segregated in their own hemisphere (step 3, Figure 2A24). As the time after fusion increased, the membrane proteins were seen to move laterally within the membrane into the opposite hemisphere. By about 40 minutes, each species' proteins were uniformly distributed around the entire hybrid cell membrane (step 4, Figure 2A24). If the same experiment was performed at lower temperature, the viscosity of the lipid bilayer increased, and the mobility of the membrane proteins decreased. These early cell fusion experiments gave the impression that **integral membrane proteins were capable of virtually unrestricted movements**.

RESTRICTIONS ON PROTEIN AND LIPID MOBILITY OR FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING (FRAP)

Several techniques allow researchers to follow the movements of molecules in the membranes of living cells using the light microscope. In a technique called **fluorescence recovery after photobleaching**

(FRAP), which is illustrated in Figure 2A25*a*, integral membrane components in cultured cells are first labeled by linkage to a fluorescent dye. A particular membrane protein can be labeled using a specific probe, such as a fluorescent antibody.

Once labeled, cells are placed under the microscope and irradiated by a sharply focused laser beam that bleaches the fluorescent molecules in its path, leaving a circular spot (typically about 1 µm diameter) on the surface of the cell that is largely devoid of fluorescence. If the labeled proteins in the membrane are mobile, then the random movements of these molecules should produce a gradual reappearance of fluorescence in the irradiated circle. The rate of fluorescence recovery (Figure 2A25*b*) provides a direct measure of the rate of diffusion (expressed as a diffusion coefficient, *D*) of the mobile molecules. The extent of fluorescence recovery (expressed as a percentage of the original intensity) provides a measure of the percentage of the labeled molecules that are free to diffuse. Early studies utilizing FRAP suggested that

(1) **Membrane proteins moved much more slowly in a plasma membrane than they would in a pure lipid bilayer** and (2) a significant fraction of membrane proteins (30 to 70 percent) were not free to diffuse back into the irradiated circle. But the FRAP technique has its drawbacks. FRAP can only follow the average movement of a relatively large number of labeled molecules (hundreds to thousands) as they diffuse over a relatively large distance (e.g., 1 µm). As a result, researchers using FRAP cannot distinguish between proteins that are truly immobile and ones that can only diffuse over a limited distance in the time allowed. To get around these limitations, alternate techniques have been developed that allow researchers to observe the movements of individual protein molecules over very short distances and to determine how they might be restrained.

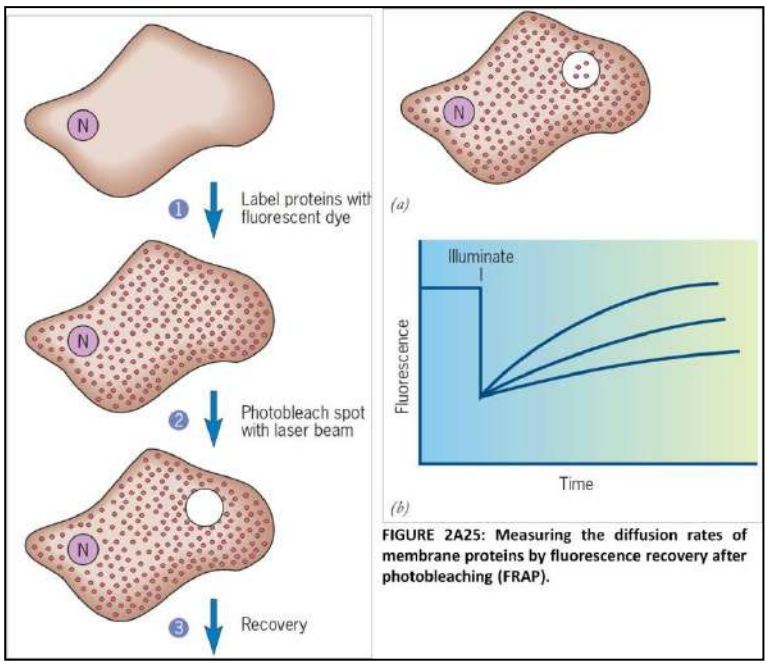


FIGURE 2A25: Measuring the diffusion rates of membrane proteins by fluorescence recovery after photobleaching (FRAP).

[**Description of Fig 2A25:** (*a*) In this technique, a particular component of the membrane is first labeled with a fluorescent dye (step 1). A small region of the surface is then irradiated to bleach the dye molecules (step 2), and the recovery of fluorescence in the bleached region is followed over time (step 3).

(N represents the cell nucleus.) (*b*) The rate of fluorescence recovery within the illuminated spot can vary depending on the protein(s) being followed. The rate of recovery is related to the diffusion coefficient of the fluorescently labeled protein.]

In **single-particle tracking (SPT)**, individual membrane protein molecules are labeled, usually with antibody-coated gold particles (approximately 40 nm in diameter), and the movements of the labeled molecules are followed by computer-enhanced video microscopy. The results of these studies often depend on the particular protein being investigated. So it is depend on the type of protein being labeled; most of the labeled proteins show random movements.

Membrane Domains and Cell Polarity:

For the most part, studies of membrane dynamics, such as those discussed above, are carried out on the relatively homogeneous plasma membrane situated at the upper or lower surface of a cell residing on a culture dish. Most membranes, however, exhibit distinct variations in protein composition and mobility, especially in cells whose various surfaces display distinct functions. For example, the epithelial cells that line the intestinal wall or make up the microscopic tubules of the kidney are highly polarized cells whose different surfaces carry out different functions (Figure 2A26). Studies indicate that the apical plasma membrane, which selectively absorbs substances from the lumen, possesses different enzymes than the

lateral plasma membrane, which interacts with neighboring epithelial cells, or the basal membrane, which adheres to an underlying extracellular substrate (a *basement membrane*). In other examples, the receptors for neurotransmitter substances are concentrated into regions of the plasma membrane located within synapses (see Figure 2A52), and receptors for low-density lipoproteins are concentrated into patches of the plasma membrane specialized to facilitate their internalization. Of all the various types of mammalian cells, sperm may have the most highly differentiated structure. A mature sperm can be divided into head, midpiece, and tail, each having its own specialized functions. Although divided into a number of distinct parts, a sperm is covered by a *continuous* plasma membrane which, as revealed by numerous techniques, consists of a mosaic of different types of localized domains.

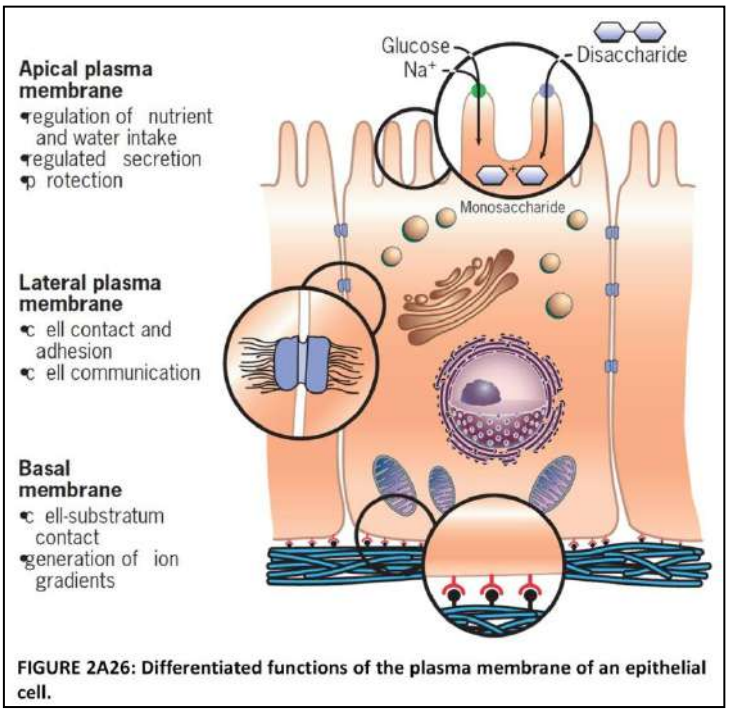


FIGURE 2A26: Differentiated functions of the plasma membrane of an epithelial cell.

[**Description of fig. 2A26:** The apical surface of this intestinal epithelial cell contains integral proteins that function in ion transport and hydrolysis of disaccharides, such as sucrose and lactose; the lateral surface contains integral proteins that function in intercellular interaction; and the basal surface contains integral proteins that function in the association of the cell with the underlying basement membrane].
