Contents

CHAPTER

5

Plasma Membrane and Cell Wall

Diatoms. A glassy outer shell and a selectively permeable plasma membrane help cells maintain relatively constant internal conditions.

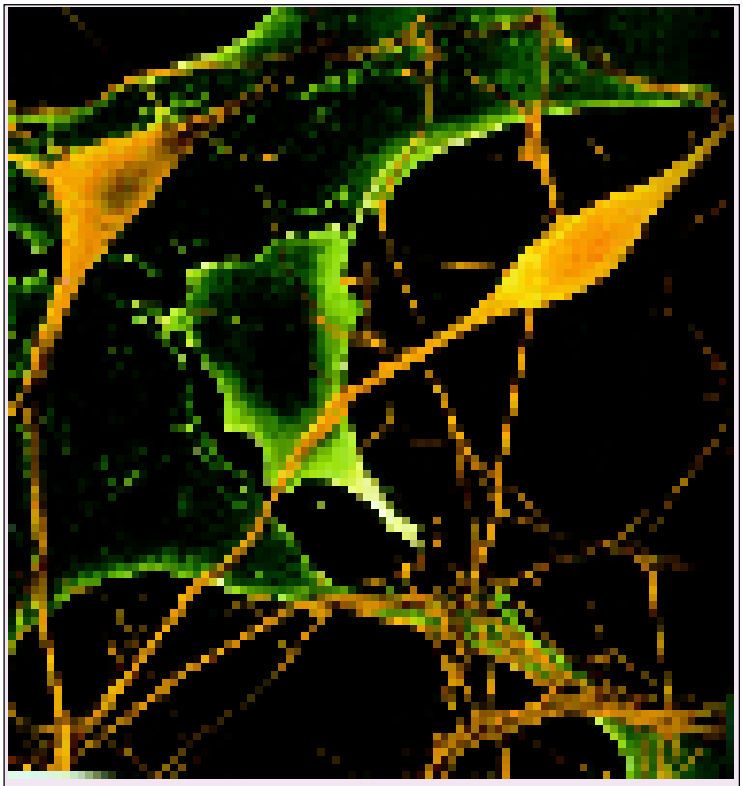
A plasma membrane encloses every type of cell, both prokaryotic and eukaryotic cells. It physically separates the cytoplasm from the surrounding cellular environment. Plasma membrane is a ultrathin, elastic, living, dynamic and selective transport-barrier. It is a fluid-mosaic assembly of molecules of lipids (phospholipids and cholesterol), proteins and carbohydrates. Plasma membrane controls the entry of nutrientes and exit of waste products, and generates differences in ion concentration between the interior and exterior of the cell. It also acts as a sensor of external signals (for example, hormonal, immunological, etc.) and allows the cell to react or change in response to environmental signals. The cells of bacteria and plants have the plasma membrane between the cell wall and the cytoplasm. For cells without cell walls (*e.g.*, mycoplasma and animal cells), plasma membrane forms the cell surface.

All biological membranes including the plasma membrane and internal membranes of eukaryotic cells (*i.e.*, membranes bounding endoplasmic reticulum or ER, nucleus, mitochondria, chloroplast, Golgi apparatus, lysosomes, peroxisomes, etc.) are similar in structure (*i.e.*, fluid-mosaic) and selective permeability but differing in other functions.

The plasma membrane is also called **cytoplasmic membrane**, **cell membrane,** or **plasmalemma**. The term cell membrane was coined by **C. Nageli** and **C. Cramer** in 1855 and the term plasmalemma has been given by **J. Q. Plowe** in 1931.

ISOLATION AND ANALYSIS

The plasma membrane is so thin that it cannot be observed by the light microscope. Structure of the plasma membrane of various cells has been studied by their isolation from the living systems and also by their artificial synthesis by using their constituent molecules (*e.g.*, liposome, see Chapter 4). The pure and isolated membranes are then studied by biochemical and biophysical methods. The purity of isolated membranes is controlled by **electron microscopy**, **enzyme analysis** and the **study of surface antigens**. A variety of cells such as mammalian red blood cell (erythrocytes), medullated nerve fibres, Ehrlich mouse ascites tumor cells, liver cells, striated muscle, *Amoeba proteus*, sea urchin eggs and bacteria, have been used in studying the ultra-structure of the plasma membrane. The mammalian erythrocytes and the myelin sheath of the nerve fibre, however, have provided the bulk of information regarding the structure and properties of the plasma membrane. For such experiments, human red blood cells or erythrocytes have been selected by **E. Gorter** and **F. Grendel** (1925) for following advantages : these cells are easy to obtain and are known to be extremely simple. Since these cells contain no intracellular organelles or membrane, so the only membrane structure to be considered is almost entirely that of the cell surface. Lastly, the plasma membrane of erythrocytes is relatively tough and does not readily fragment (See **Lucy**, 1975)



SEM of neurons. These have been used extensively in studying the ultra-structure of the plasma membrane.

Plasma membranes are more easily isolated from erythrocytes subjected to haemolysis. The cells are treated with hypotonic solutions (to be discussed elsewhere in the chapter) that due to endosmosis produce swelling and then loss of the heamoglobin content (*i.e.*, haemolysis). The resulting membrane is called a **red cell ghost**. If haemolysis is mild, permeability functions of the membrane can be restored by certain treatment, such a ghost is called **resealed ghost**. But if heamolysis is more drastic (*i.e.*, there is complete removal of the haemoglobin) and there is no chance of its resealing, the resulting membrane is called **white ghost**. While the resealed ghosts can be used for the study of physiological as well as biochemical properties, white ghosts can only be used for the study of biochemical properties.

The cell wall of yeast, *Saccharomyces cerevisiae*, can be enzymatically removed by the help of a snail gut enzyme, and the resultant protoplast serves as a source of plasma membrane in a manner similar to that of mammalian erythrocytes.

CHEMICAL COMPOSITION

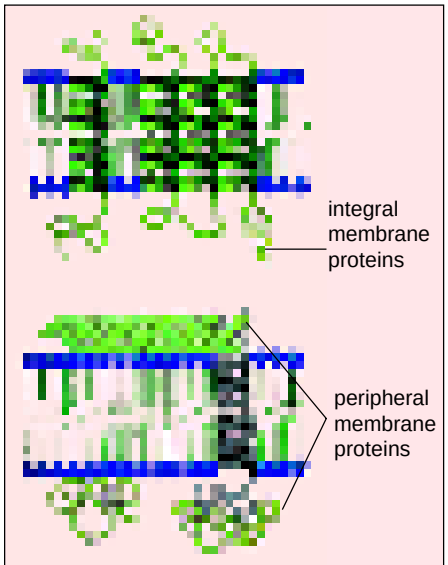
Chemically, plasma membrane and other membranes of different organelles are found to contain proteins, lipids and carbohydrates, but in different ratios (Table 5-1). For example, in the plasma membrane of human red blood cells proteins represent 52 per cent, lipids 40 per cent and carbohydrates 8 per cent.

Table 5-1. **Chemical composition of some purified membranes (in percentages) (Source : Darnell *et al.*, 1986).**

Membrane	Protein	Lipid	Carbohydrate
1. Myelin (Nerve cell)	18	79	3
2. Plasma membrane :			
(i) Mouse liver	44	52	4
(ii) *Amoeba*	54	42	4
(iii) Human erythrocyte	52	40	8
3. Spinach chloroplast lamellae	70	30	0
4. Mitochondrial inner membrane	76	24	0

1. Lipids

Four major classes of lipids are commonly present in the plasma membrane and other membranes : **phospholipids** (most abundant), **sphingolipids**, **glycolipids** and **sterols** (*e.g.*, **cholesterol**) (For more details see Chapter 4). All of them are amphipathic molecules, possessing both hydrophilic and hydrophobic domains. The relative proportions of these lipids vary in different membranes. Phospholipids may be **acidic phospholipids** (20 per cent) such as **sphingomyelin** or **neutral phospholipids** (80 per cent) such as **phosphatidyl choline**, **phosphatidylserine**, etc. Many membranes contain cholesterol. Cholesterol is especially abundant in the plasma membrane of mammalian cells and absent from prokaryotic cells. **Cardiolipin** (diphosphatidyl glycerol) is restricted to the inner mitochondrial membrane (see **Darnell** *et al.*, 1986).

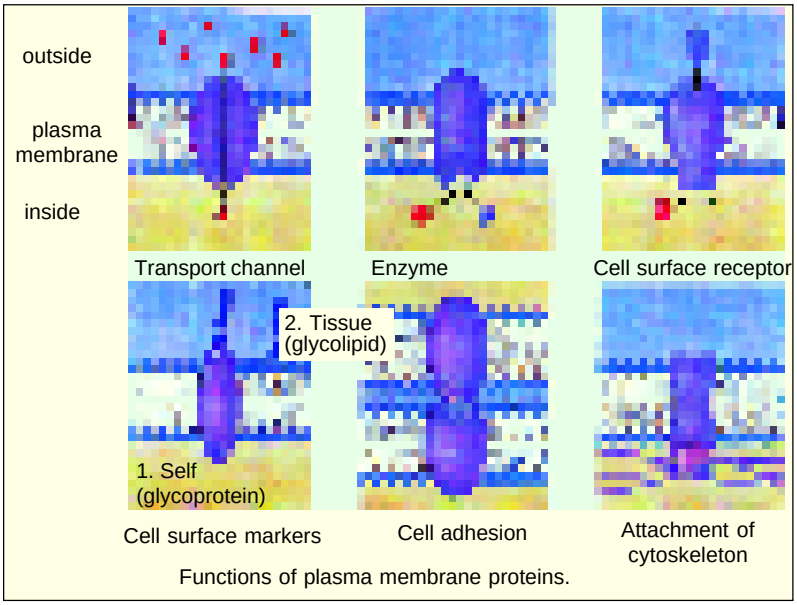


Classes of membrane proteins.

2. Proteins

The amount and types of proteins in the membranes are highly variable : in the myelin membranes which serve mainly to insulate nerve cell axons, less than 25 per cent of the membrane mass is protein, whereas, in the membranes involved in energy transduction (such as internal membranes of mitochondria and chloroplasts), approximately 75 per cent is protein. Plasma membrane contains about 50 per cent protein.

According to their position in the plasma membrane, the proteins fall into two main types : **integral** or **intrinsic proteins** and **peripheral** or **extrinsic proteins**, both of which may be either **ectoproteins**, lying or exposing to external or extracytoplasmic surface of the plasma membrane or **endoproteins**, lying or sticking out at the inner or cytoplasmic surface of the plasma membrane. The intrinsic proteins tend to associate firmly with the membrane, while the extrinsic proteins have a weaker association and are bound to lipids of membrane by electrostatic interaction. On the basis of their functions, proteins of plasma membrane can also be classified into three main types : structural



Functions of plasma membrane proteins.

proteins, enzymes and transport proteins (permeases or carriers). Some of them may act as **antigens**, **receptor molecules** (*e.g.*, insulin-binding sites of liver plasma membrane), **regulatory molecules** and so on. **Structural proteins** are extremely lipophilic and form the main bulk (*i.e.*, backbone) of the plasma membrane. **Enzymes** of plasma membrane are either **ectoenzymes** or **endoenzymes** and are of about 30 types (Table 5-2). **Transport proteins** transport specific substances across the plasma membrane and other cellular membranes.

3. Carbohydrates

Carbohydrates are present only in the plasma mambrane. They are present as short, unbranched or branched chains of sugars (**oligosaccharides**) attached either to exterior ectoproteins (forming **glycoproteins**) or to the polar ends of phospholipids at the external surface of the plasma membrane (forming **glycolipids**). No carbohydrate is located at the cytoplasmic or inner surface of the plasma membrane. All types of oligosaccharides of the plasma membrane are formed by various combinations of six principal sugars (all of which are glucose-derivatives) : **D-galactose**, **D-mannose**, **L-fucose**, **N-acetylneuraminic acid** (also called **sialic acid**), **N-acetyl-D-glucosamine** and **N-acetyl-D-galactosamine**.

Table 5-2. Some important enzymes present in the plasma membrane (Source : Sheeler and Bianchi, 1987).

1. Acetyl phosphatase	11. Cholesterol esterase
2. Acetyl cholinesterase (Ectoenzyme of erythrocyte)	12. Guanylase cyclase
3. Acid phosphatase	13. Monoglyceride lipase
4. Adenosine triphosphatase	14. NAD-ase (Ectoenzyme of erythrocyte)
5. Mg^{2+} ATPase (Endoenzyme of erythrocyte)	15. Protein kinase (Endoenzyme of erythrocyte)
6. Na^{+}-K^{+} ATPase (Ectoenzyme of erythrocyte)	16. Phospholipase A
7. Adenylate cyclase (Endoenzyme of erythrocyte)	17. Lactase
8. RNAase	18. Maltase
9. Alkaline phosphatase	19. Sialidase
10. Aminopeptidase	20. UDP glycosidase

STRUCTURE OF PLASMA MEMBRANE

1. Evolution of Fluid Mosaic Model of Membrane

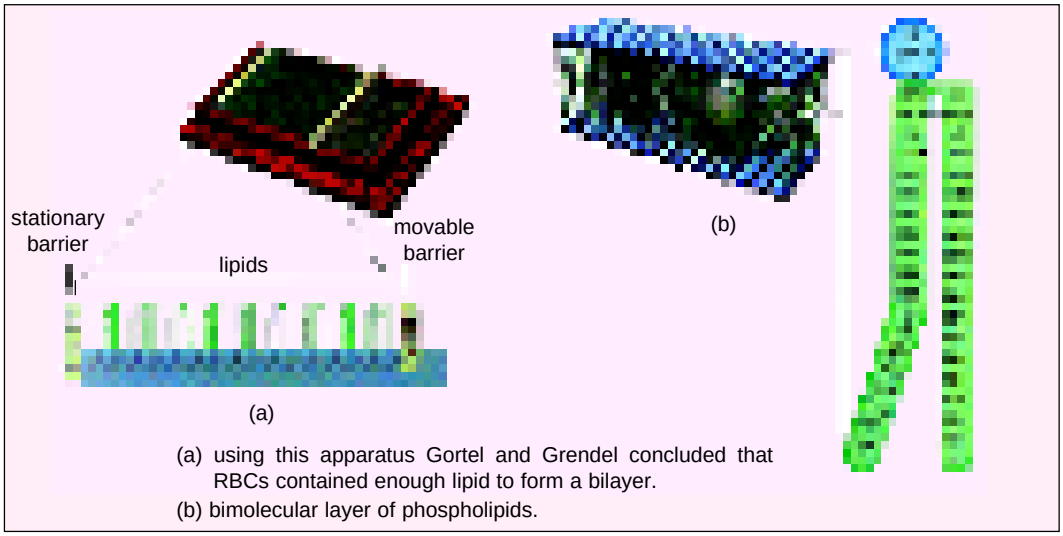
The existence of the plasma membrane of the cell was difficult to prove by direct examination before 1930's (when electron microscopy was invented) because of technological limitations. The membrane is beyond the resolution of the light microscope, rendering a morphological approach of its study quite unfeasible with this instrument. Thus, most of the experimental approaches have been provided by only indirect evidences of the existence of such a membrane around the cells. Let us narrate in brief the saga of evolution of presently well accepted fluid-mosaic model of structure of the plasma membrane :

1. The plasmolysis of plant cells in hypertonic solutions suggests the existence of the plasma membrane in the plants.

2. The very fact that a cell, especially an animal cell which has no cell wall, can exist as a physically defined entity suggests that it must have some sort of boundary around it.

3. The presence of plasma membrane can be inferred because protoplasm leaks out of animal cells when cell surface is punctured.

4. After performing some 10,000 experiments with more than 500 different chemicals, in 1899. **Overton** concluded that the peculiar osmotic properties of living protoplasts are due to a **selective**



(a) using this apparatus Gortel and Grendel concluded that RBCs contained enough lipid to form a bilayer.
(b) bimolecular layer of phospholipids.

solubility mechanism. Hydrophobic compounds entered cells more rapidly than hydrophilic ones. **Overton** believed this was because of an outer lipoid layer in which hydrophobic compounds were more soluble. He correctly speculated that this layer might contain cholesterol, lecithin and fatty oils.

5. **Hober** (1910) and **Fricke** (1925) found that the intact cell had low electrical conductivity, indicating the presence of a lipid layer around it.

6. If a lipid containing **hydrophilic groups** (such as the carboxyl groups of fatty acids or the phosphate groups of phospholipids) is dissolved in a highly volatile solvent (*e.g.*, benzene) and several drops of it are then carefully applied to the surface of the water, the lipid spreads out to form a thin, one-molecule-thick or **monomolecular film**. In this film, it is found that the hydrophilic parts of each molecule project into the water surface and the hydrophobic parts are directed up, away from the water.

7. In 1917, **Langmuir** (Nobel Laureate of 1932 in chemistry) fabricated a **trough** or **film balance** (Fig. 5.1) for measuring the specific minimum surface area occupied by a monomolecular film

of lipid and the force necessary to compress all the lipid molecules into this area. Langmuir trough consists of a shallow trough filled with water on which lipid substance can be spread to make a monomolecular film. A barrier can be pushed across the trough to compress the film.

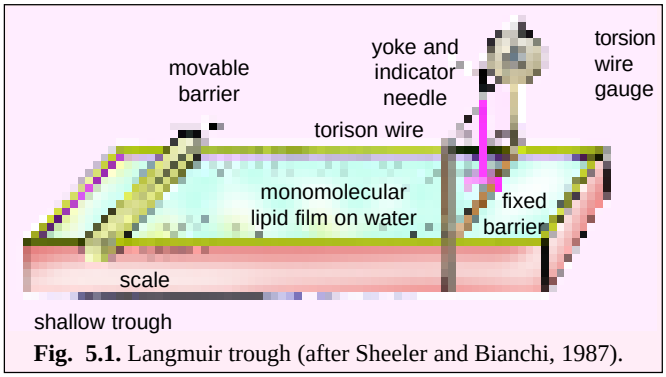


Fig. 5.1. Langmuir trough (after Sheeler and Bianchi, 1987).

8. In 1925, **Gorter** and **Grendel** extracted the lipids from erythrocyte ghosts of a variety of mammals (such as dogs, sheep, rabbits, guinea pigs, goats and humans) and spread them out on monolayers in the Langmuir trough. These investigators discovered that the area covered by the lipid monomolecular layer film was twice than what was needed to cover the surface of the cells from which the lipid was extracted. Consequently, they safely concluded that *erythrocytes were covered by a layer of lipids two molecules thick* (**lipid bilayer** or **bimolecular lipid layer**) oriented with polar groups toward the inside and outside of the cell.

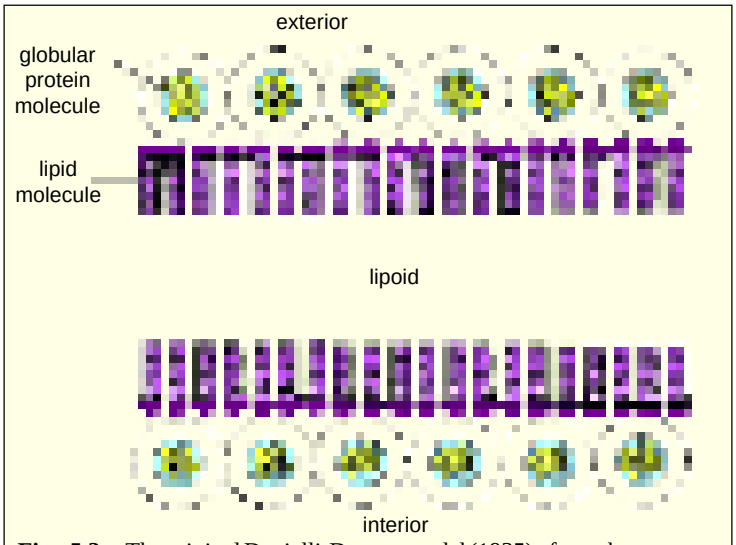


Fig. 5.2. The original Danielli-Davson model (1935) of membrane structure. The bimolecular layer of lipid molecules is of undefined thickness and is covered on each side by a continuous layer of globular proteins (after De Witt, 1977).

9. By studying the surface tension of cells (**Harvey** and **Cole**, 1931, **Danielle** and **Harvey**, 1935) suggested the presence of proteins in the plasma membrane, in addition to the lipids.

10. In 1935, **Danielli** and **Davson**, proposed a model, called **sandwich model**, for membrane structure in which a lipid bilayer was coated on its either side with hydrated proteins (globular proteins). Mutual attraction between the hydrocarbon

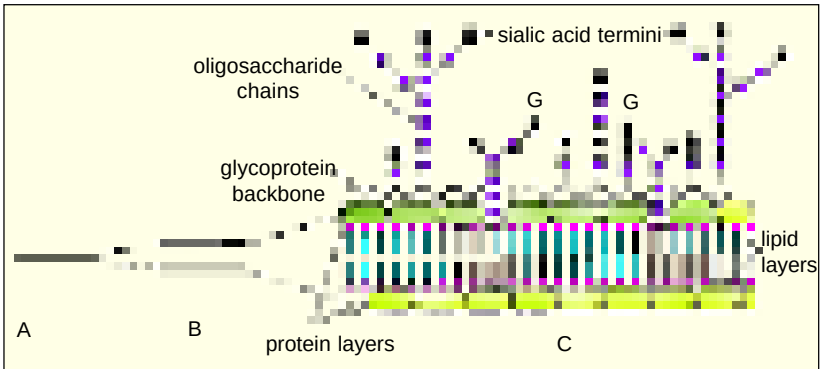


Fig. 5.3. The structure of plasma membrane as observed A—at low magnification of electron microscope; B— at high magnification of electron microscope. C— trilaminar model of plasma membrane showing possible arrangement of the lipid, protein and oligosaccharide molecules in the plasma membrane (after De Robertis *et al.*, 1970).

chains of the lipids and electrostatic forces between the protein and the "head" of the lipid molecules, were thought to maintain the stability of the membrane. From the speed at which various molecules penetrate the membrane, they predicted the lipid bilayer to be about 6.0 nm in thickness, and each of the protein layer of about 1.0 nm thickness, giving a total thickness of about 8.0 nm.

The Danielli-Davson model got support from electron microscopy (Fig. 5.3). Electron micrographs of the plasma membrane showed that it consists of two dark layers (electron dense granular protein layers), both separated by a lighter area in between (the central clear area of lipid bilayer). The total thickness of the membranes too turned out to be about 7.5 nm.

11. Using evidence from various electron micrographs, **Robertson** in 1960, proposed the **unit memb-rane hypothesis** (Fig 5.4). This hypothesis states that all cellular membranes have an identical **trilaminar** structure (or dark-light- dark or railway track pattern, see **Thorpe**, 1984). However, thickness of the unit membrane has been found to be greater in plasma membrane (10 nm) than in the intracellular membranes of endoplasmic reticulum or Golgi apparatus (*i.e.*, 5 to 7 nm).

12. **S.J.Singer** and **G.L.Nicolson** (1972) suggested the widely accepted **fluid mosaic model** of biological membranes. According to this model (Fig. 5.5), the plasma membrane contains a bimolecular lipid layer, both surfaces of which are interrupted by protein molecules. Proteins occur in the form of globular molecules and they are dotted about here and there in a mosaic pattern. Some proteins are attached at the polar surface of the lipid (*i.e.*, the extrinsic proteins); while others (*i.e.*, integral proteins) either partially penetrate the bilayer or span the membrane entirely to stick out on both sides (called **transmembrane proteins**). Further, the peripheral proteins and those parts of the integral proteins that stick on the outer surface (*i.e.*, ectoproteins) frequently contain chains of sugar or oligosaccharides (*i.e.*, they are glycoproteins). Likewise, some lipids of outer surface are glycolipids.

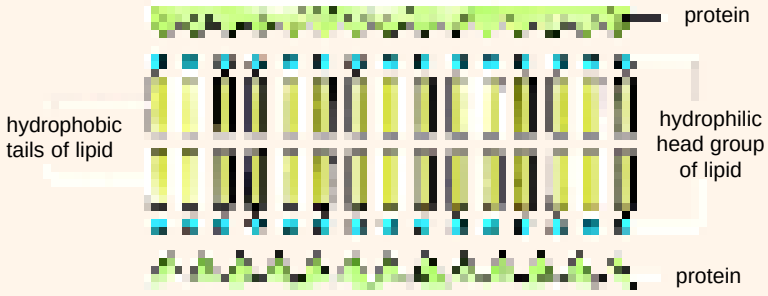
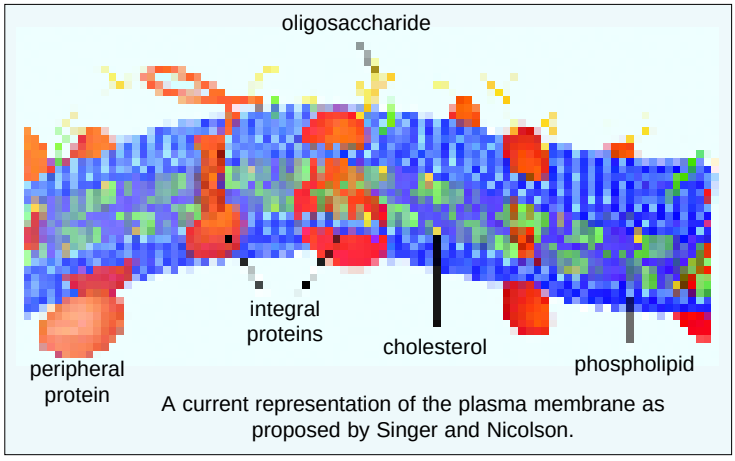


Fig. 5.4. Schematic diagram of the Robertson model of membrane structure. The lipid layer is defined as bimolecular, and the protein is extended but different on the two faces of the membrane (after Thorpe, 1984).

The fluid-mosaic membrane is thought to be a far less rigid than was originally supposed. In fact, experiments on its viscosity suggest that it is of a fluid consistency rather like the oil, and that there is a considerable sideways movement of the lipid and protein molecules within it. On account of its fluidity and the mosaic arrangement of protein molecules, this model of membrane structure is known



A current representation of the plasma membrane as proposed by Singer and Nicolson.

as the "fluid mosaic model" (*i.e.*, it describes both properties and organization of the membrane). The fluid mosaic model is found to be applied to all biological membranes in general, and it is seen as a dynamic, ever-changing structure. The proteins are present not to give it strength, but to serve as enzymes catalysing chemical reactions within the membrane and as pumps moving things across it.

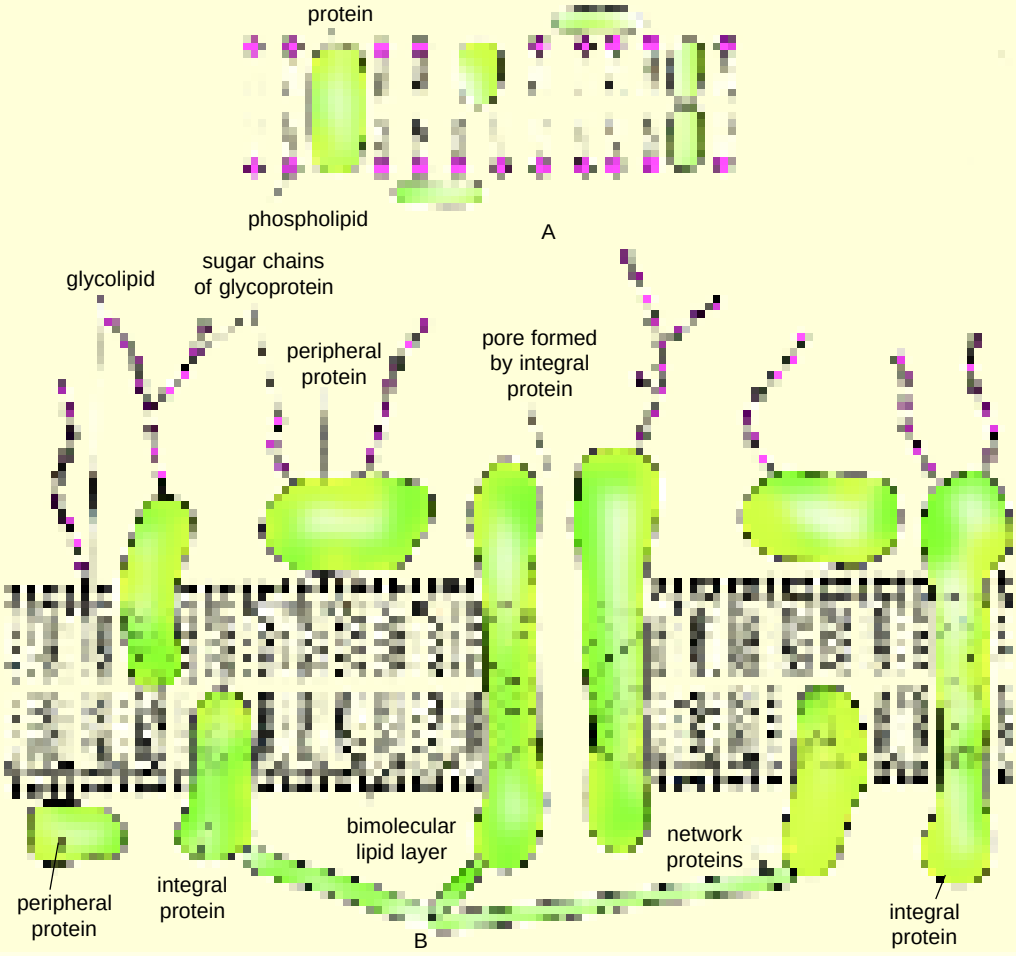


Fig. 5.5. Fluid mosaic model of the plasma membrane. A — Simplistic view; B— Complex view (after Berns, 1983; Sheeler and Bianchi, 1987).

2. Experimental Evidence in Support of Fluid Mosaic Model of Plasma Membrane

There is a good deal of evidence to support the fluid mosaic model of the plasma membrane:

A. Evidence in support of mosaic arrangement of proteins. Freeze-fracture electron microscopy of the plasma membrane by **Branton** (1968) revealed the presence of bumps and depressions (7 to 8 nm in diameter) which are randomly distributed. These were later shown to be transmembrane integral protein particles (Fig. 5.7). (For details of freeze-fracture technique, see Chapter 2).

B. Evidence in support of fluid property of lipid bilayer. Mobility of membrane proteins due to fluid property of lipid bilayer was demonstrated by a classical experiment of **D. Frye** and **M. Edidin** (1970). They fused two different types of cultured cells having different surface antigens (proteins). The **cell fusion** is achieved by the use of some fusogen such as an inactivated parainfluenza

virus, called Sendai virus (named after a city of Japan). A **fusogen** is a membrane fusion promoting factor such as Sendai virus, lysophosphatides, oleic acid and an electric field. Sendai virus facilitates fusion of the plasma membranes and cytoplasms of both cells to produce a **hybrid cell** or **heterokaryon** with two types of nuclei. If the two cells are originally labelled with fluorescent antibodies of different colours, such as **fluorescein** (green) and **rhodamine** (red), it is possible at the onset of fusion to recognise the parts of the plasma membrane corresponding to each cell. However, intermixing occurs as the antigens are dispersed and the two colours become less and less detectable. After 40 minutes (at 37° C) the intermixing of two colours is complete and the two antigens can no longer be distinguished (Fig. 5.6).

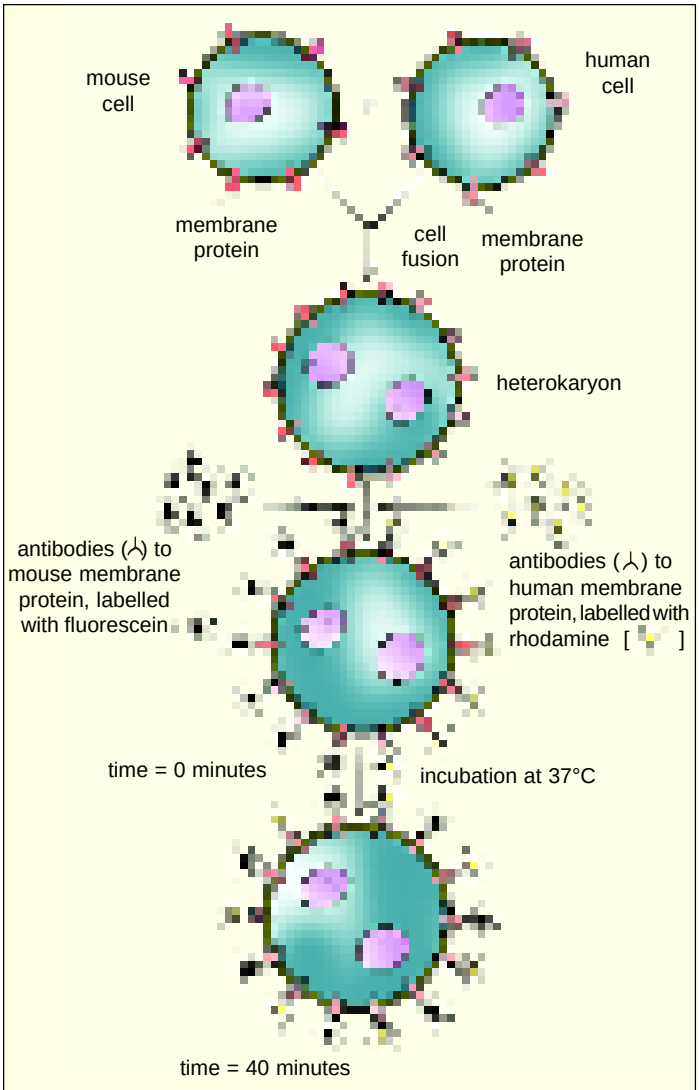


Fig. 5.6. Frye and Edidin's experiment demonstrating the mixing of plasma membrane proteins on mouse-human hybrid cells. In the plasma membrane of newly formed heterokaryon, the mouse and human proteins are intially confined to their own halves, but they intermixed with time (after Alberts *et al.*, 1989).

3. Role of Lipid Molecules in Maintaining Fluid Property of Membrane

(i) Types of movements of lipid molecules. Lipid molecules very rarely migrate from one lipid monolayer to other monolayer of lipid bimolecular layer. Such a type of movement is called **flip-flop** or **transbilayer movement** and occurs once a month for any individual lipid molecule. However, in membranes where lipids are actively synthesized, such as smooth ER, there is a rapid flip-flop of specific lipid molecules across the bilayer and there are present certain membrane-bound enzymes, called **phospholipid translocators** (*e.g.*, flippase) to catalyze this activity (**Bishop** and **Bell**, 1988). On the other hand, lipid molecules readily exchange places with their neighbours within a monolayer (~ 10^7 times a second). This results in their rapid **lateral diffusion**. Individual lipid molecules **rotate** very rapidly about their long axes and their hydrocarbon chains are flexible, the greatest degree of **flexion** occurring near the centre of the bilayer and the smallest adjacent to the polar head groups (Fig. 5.8).

(ii) Role of unsaturated fats in increasing membrane fluidity. A synthetic bilayer made from a single type of phospholipid changes from a liquid state to a rigid crystalline or gel (viscous) state at a characteristic freezing point. This change of state is called a **phase transition** and the temperature at which it occurs becomes lower if the hydrocarbon chains are short or have double bonds. Double bonds in unsaturated hydrocarbon chains tend to increase the fluidity of a phospholipid bilayer by making it more difficult to pack the chains together. Thus, to maintain fluidity of the membrane, cells of organisms living at low temperatures have high proportions of unsaturated fatty acids in their membranes, than do cells at higher temperatures.

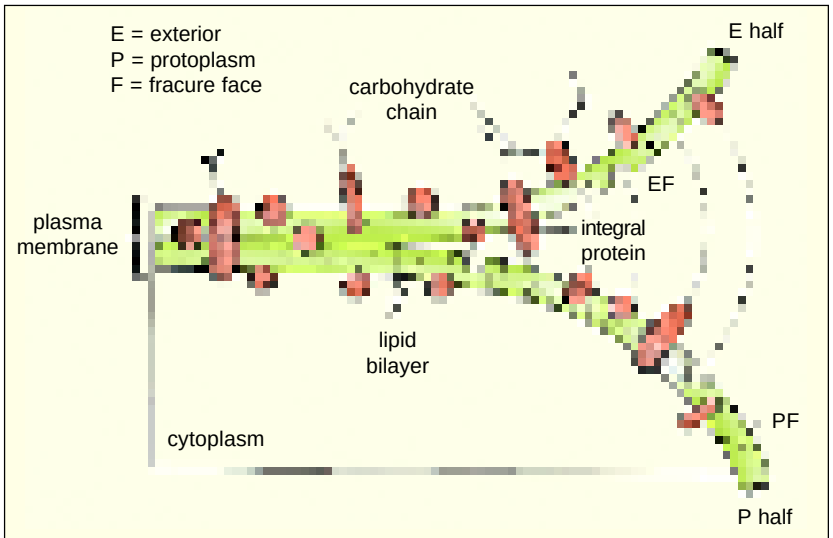
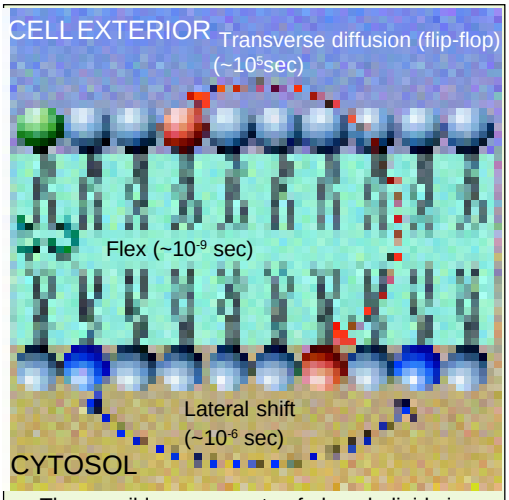


Fig. 5.7. Freeze fracturing of the plasma membrane. The fracture plane occurs at the centre of the lipid bilayer and passes over (or under) the integral membrane proteins. E=exterior, P=protoplasmic (cytosolic) side, F=fracture face (after Sheeler and Bianchi, 1987).



The possible movements of phospholipids in a membrane.

In fact, certain membrane **transport processes** and **enzyme activities** are found to cease when the lipid bilayer's viscosity increases beyond a threshold level (**Kimelberg**, 1977). In contrast, if lipid bilayer's fluidity is increased, the membrane's receptors for the hormone are withdrawn from the cell surface, thereby hampering hormone action (see **Sheeler** and **Bianchi**, 1987).

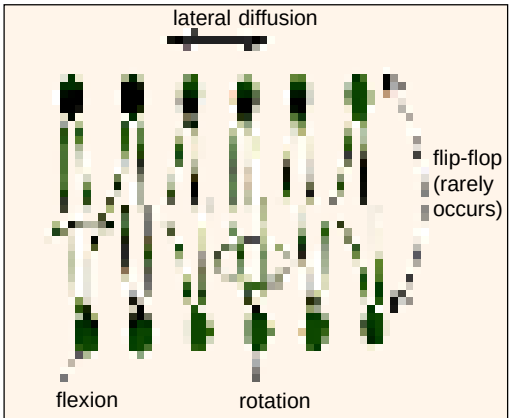
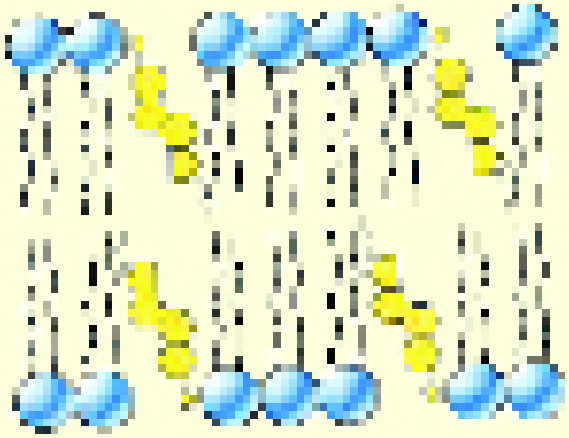


Fig. 5.8. The types of movement possible for phospholipid molecules in a lipid bilayer (after Alberts *et al*, 1989).

(iii) Role of cholesterol in maintaining fluidity of membrane. Eukaryotic plasma membranes are found to contain a large amount of cholesterol; up to one molecule for every phospholipid molecule. Cholesterol molecules orient themselves in the lipid bilayer in such a way that their

hydroxyl groups remain close to polar head groups of the phospholipids, their rigid plate-like steroid rings interact with and partly immobilise those regions of hydrocarbon chains that are closest to the polar head groups, leaving the rest of the chain flexible (Fig. 5.9). Cholesterol inhibits phase transition by preventing hydrocarbon chains from coming together and crystallizing. Cholesterol also tends to decrease the permeability of lipid bilayers to small water-soluble molecules and is thought to enhance both the flexibility and the mechanical stability of the bilayer.



The cholesterol molecules of a membrane are oriented with their small hydrophilic end facing the external surface of the bilayer and the bulk of their structure packed in among the fatty acid tails of the phospholipids.

4. Membrane Asymmetry

Both lipid and protein molecules have irregular distribution in both monolayers of the lipid bilayer, this is called **membrane asymmetry.**

A. Phospholipid asymmetry in plasma membrane. The lipid composition and state of fluidity of two halves of the lipid bilayer are found to be strikingly different. For example, in human erythrocyte's plasma membrane, outer half contains those phospholipids which have more saturated fatty acid chains, and inner half contains those phospholipids which contain terminal amino groups and less saturated fatty acid chains. As a result, inner monolayer is more fluid than the outer lipid monolayer. Such a phospholipid asymmetry is generated in smooth ER. The asymmetry of glycolipids such as galactocerebroside, ganglioside, etc., in myelin sheath of nerves (*i.e.*, they are found only in the outer half of lipid bilayer) is found to be originated in lumen of Golgi apparatus. The specific role of lipid asymmetry of the membrane is still not clear.

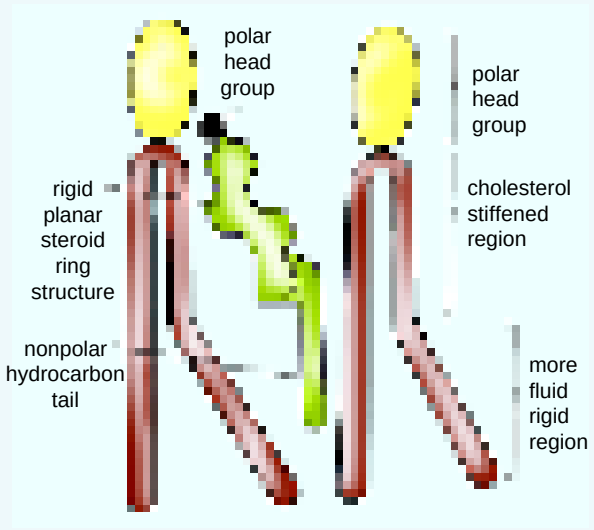
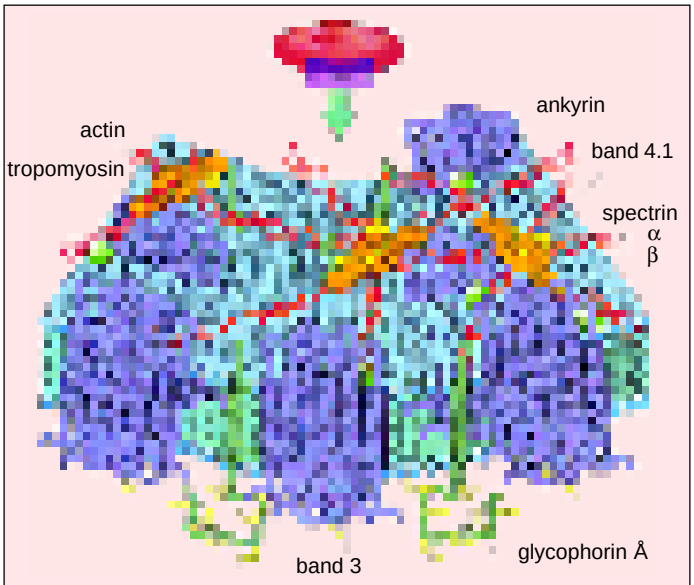


Fig. 5.9. Cholesterol molecule (schematic) interacting with two phospholipid molecules in a monolayer (after Alberts *et al.*, 1989).

B. Protein asymmetry in plasma membrane. The outer and inner sides of the plasma membrane and other membranes do not contain either the same types or equal amounts of the various peripheral and integral proteins, *e.g.*, erythrocyte's plasma membrane.

Proteins of plasma membrane of erythrocytes. When the extracted proteins of the plasma membrane of human erythrocytes (RBC) are studied by SDS polyacrylamide-gel electrophoresis (SDS = sodium dodecyl sulphate ; a detergent), approximately 15 major protein bands are detected, varying in molecular weight from 15,000 to 25,000. Most of these proteins are found to be peripheral proteins of cytosolic face of the plasma membrane. Important properties of some of these proteins are the following :

(i) Spectrin and other cytoskeleton proteins. Spectrin is the principal component of the protein meshwork (cytoskeleton) that underlies the erythrocyte's plasma membrane (Fig. 5.10). It, thus, maintains the structural integrity and biconcave shape of this membrane (**Branton** *et al.*, 1981). Spectrin is long, thin, flexible rod about 100 nm in length. It constitutes about 25 per cent of the membrane associated protein mass (about 2.5 x 10^5 copies per cell). Spectrin is a **heterodimer** and consists of two non-identical, antiparallel, loosely intertwind, flexible polypeptide chains, *i.e.*, **α–spectrin** (~ 240,000 daltons M.W.) and **β-spectrin** (~ 220,000 daltons M.W.), both being attached non-covalently to each other at multiple points including their ends (*i.e.*, phosphorylated 'head' and 'tail').



The plasma membrane of the human erythrocyte.

The spectrin heterodimers self-associate head-to-head to form 200 nm long **tetramers**. The tail ends of five or six spectrin tetramers are linked together by binding to short **actin filaments** (also called **band 5 proteins**; with 43,000 dalton M.W.) and each with 15 actin monomers and to another protein, called **band 4.1 protein** (82,000 dalton M.W.). These three proteins form the "**junctional complex**" of deformable, net-like meshwork of the cytoskeleton. Further, the binding of spectrin cytoskeleton to the cytosolic face of the erythrocyte's plasma membrane depends on a large intracellular attachment protein, called **ankyrin** (or **band 2.1 protein**; 210,000 dalton M.W.). Ankyrin tends to bind to both β-spectrin and to the cytoplasmic domain of a transmembrane protein, called **band 3 protein** (**Shen** *et al.*, 1986).

(ii) Glycophorin. It is a small transmembrane glycoprotein (single-pass membrane protein) having molecular weight of 55,000 daltons and 131 amino acid residues. This protein bears about 100 sugars on 16 separate oligosaccharide side chains (90 per cent of which is sialic acid). Despite there being more than 6 × 10^5

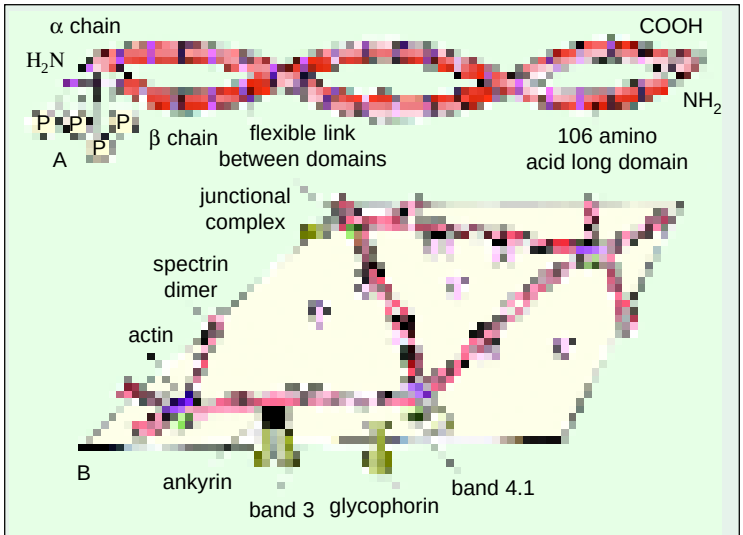
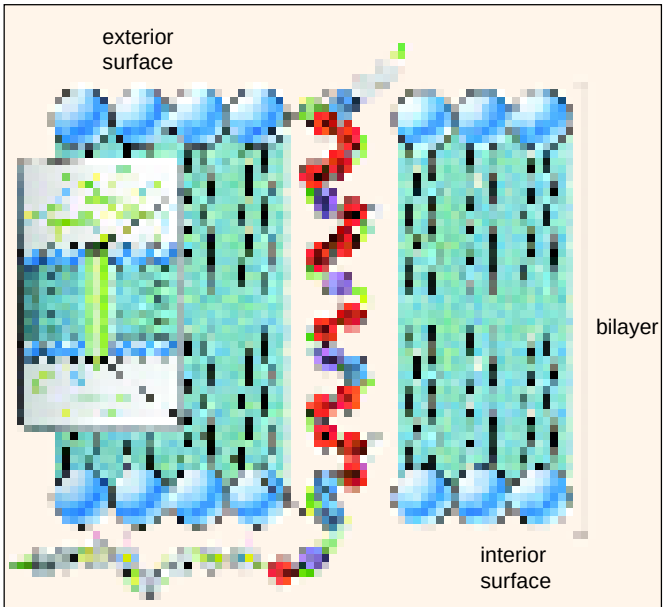


Fig. 5.10. A—One spectrin molecule from human red blood cell; B—Schematic drawing of the spectrin based cytoskeleton on the cytoplasmic side of the human red blood cell membrane.



Glycophorin A, an integral protein with a single transmembrane domain.

glycophorin molecules per cell (*i.e.*, erythrocyte), their exact function is still not known. However, glycophorins are found to contain certain antigenic determinants (carbohydrates) for the **ABO blood groups** and **MN blood groups**. Further, sialic acid confers a high negative charge to the cell surface of erythrocyte. This sugar may be important in the life cycle of the erythrocytes as it has been shown that cells lose sialic acid as they age in the circulatory system. Correlated with this is the observation that *loss of sialic acid is a signal for removal and destruction of an erythrocyte by the spleen and liver*. In this way the life span of red blood cells may be regulated (see **King**, 1986).

(iii) Band 3 protein. Like the glycophorin, **band 3 protein** (93,000 daltons M.W.) is a transmembrane protein, but it is a **multipass membrane protein**, *i.e.*, its highly folded polypeptide chain (about 930 amino acid long) extends across the lipid bilayer at least 10 times. Each human erythrocyte contains about 10^6 and band 3 proteins, each of which forms either a dimer or tetramer in the membrane. Band 3 protein acts as the **anion exchange channels** in the membrane. As the erythrocytes pass through the lungs, they exchange bicarbonate (HCO^-) for chloride (Cl^-) through these hydrophilic channels during the process of CO_2 release (**chloride shift**).

5. Constraints on the Motility of Membrane Molecules

In the fluid mosaic plasma membrane, there is not complete and independent freedom of movement for its different component molecules. The mobility of some part of lipid molecules is constrained since that remains tightly bound to some of the integral membrane proteins. For example, the mobility of lipid molecules surrounding **cytochrome oxidase** (an enzyme involved in the synthesis of ATP) are immobilized by the enzyme and makes boundary lipid layer. The immobilized boundary lipid makes 30 per cent of membrane lipid in the mitochondrial membrane.

In contrast to lipids, the mobility and distribution of protein molecules in the membrane is controlled by various ways : (1) Certain proteins of membrane are constrained by protein-protein interactions to form spe-

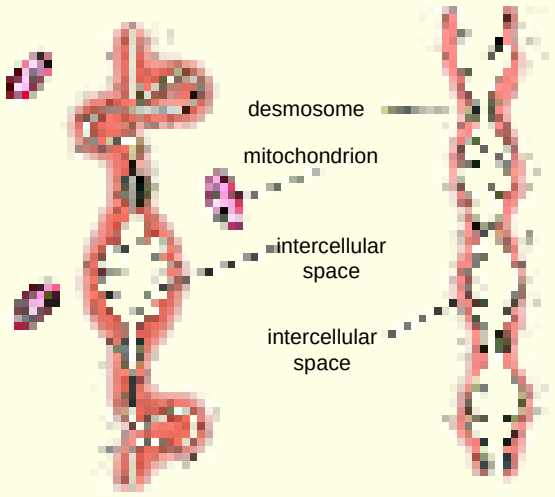


Fig. 5.11. Inter-cellular space.

cialized ordered regions, representing 2 to 20 per cent of the membrane of a system, *e.g.*, gap junctions, synapsis of neurons and plaques of halobacteria. (2) Certain peripheral proteins (endoproteins) may form a bridge-like lattice work between integral proteins and restrict their lateral mobility, *e.g.*, spectrin-ankyrin-actin cytoskeletal meshwork provides a rigidity to the membrane of human erythrocytes and does not permit the clustering or **capping** of integral proteins when the appropriate antibodies or lectins are added. (3) In nucleated eukaryotic cells, the mobility of the peripheral endoproteins and integral proteins is restrained by their attachment to the ectoplasmic cytoskeleton. The cytoskeleton is extensive, including **myosin** filaments, **actin** filaments and **microtubules** (Fig. 5.10). Rearrangement of cytoskeletal components just below the cell surface manifests in the distribution of integral membrane proteins and also in the cellular motions, endocytosis and exocytosis.

The inter-cellular space. In the tissues of multicellular animals, the plasma membranes of two adjacent cells usually remain separated by a space of 10 to 150 A^{o} wide. This inter-cellular space is uniform and contains a material of low electron density which can be considered as a cementing substance. This substance is found to be a mucopolysaccharide (Fig. 5.11).

ORIGIN OF PLASMA MEMBRANE

There is hardly any cell structure more important to the immediate health of the cell than the plasma membrane. If it is weakened or injured, the cell loses its ability to maintain gradients, to carry out the selective transport of nutrients, and to contain the pool of enzymes and organelles essential for the homeostasis. In consequence, new membranes may be added to existing membranes without altering the functions as a barrier and selective transporter. Also for maintaining the characteristic membrane asymmetry, the membrane must be assembled with precisely the correct moleular topography.

Thus, all cellular membranes grow from pre-existing membranes which act as **templates** for the addition of new precursors. All cells divide, daughter cells receive a full complement of membrane systems which undergo growth until the next division, to be passed on to subsequent progeny. Meanwhile the molecules within the membrane undergo continuous replacement.

The protein molecules of the plasma membrane are synthesized on both attached and free ribosomes. Proteins synthesized by free ribosomes may be inserted into the plasma membrane following their completion and release from the ribosomes. Proteins of plasma membrane synthesized on attached ribosomes of rough ER are **inserted** first into the membrane of RER and then **transferred** to the Golgi apparatus, **processed** there (*e.g.*, glycosylation) and ultimately are dispatched to the plasma membrane via the secretory vesicles. Likewise, the synthesis of phospholipid molecules of the plasma membrane takes place by the smooth ER (SER). Like the proteins, newly synthesized lipids are inserted into SER membranes, then they are passed to Golgi apparatus for the processing and ultimately are dispatched to the plasma membrane via small secretory vesicles. The cytosol also contains a number of **phospholipid transport proteins** that function to transfer phospholipid molecules from one cellular membrane to another (*e.g.*, from ER membranes to plasma membranes) (see **Sheeler** and **Bianchi**, 1987).

In fact, the process of glycosylation (or glycosidation, *i.e.*, addition of oligosaccharides containing the sugars such as galactose, fucose and/ or sialic acid, to the molecules of proteins and phospholipids of the plasma membrane) is completed at the level of Golgi apparatus. However, some sugars are added to the proteins in the lumen of RER.

FUNCTIONS OF PLASMA MEMBRANE

The plasma membrane acts as a thin barrier which separates the intra-cellular fluid or the cytoplasm from the extra-cellular fluid in which the cell lives. In case of unicellular organisms (Protophyta and Protozoa) the extra-cellular fluid may be fresh or marine water, while in multicellular organisms the extra-cellular fluid may be blood, lymph or interstitial fluid. Though the plasma

membrane is a limiting barrier around the cell but it performs various important physiological functions which are as follows :

1. Permeability. The plasma membrane is a thin, elastic membrane around the cell which usually allows the movement of small ions and molecules of various substances through it. This nature of plasma membrane is termed as permeability. According to permeability following types of the plasma membranes have been recognised :

(i) Impermeable plasma membranes. The plasma membrane of the unfertilized eggs of certain fishes allows nothing to pass through it except the gases. Such plasma membranes can be termed as impermeable plasma membranes.

(ii) Semi-permeable plasma membranes. The membranes which allow only water but no solute particle to pass through them are known as semi-permeable membranes. Such membranes have not so far been recognised in animal cells.

(iii) Selective permeable plasma membranes. The plasma membrane and other intra-cellular membrane are very selective in nature. Such membranes allow only certain selected ions and small molecules to pass through them.

(iv) Dialysing plasma membranes. The plasma membranes of certain cells have certain extraneous coats around them. The basement membranes of endothelial cells are the best examples of extraneous coats. This type of plasma membrane having extraneous coats around it, acts as a dialyzer. In these membranes the water molecules and crystalloids are forced through them by the hydrostatic pressure forces.

Mode of Transport Across Plasma Membrane

The plasma membrane acts as a semipermeable barrier between the cell and the extracellular environment. This permeability must be highly **selective** if it is to ensure that essential molecules such as glucose, amino acids and lipids can readily enter the cell, that these molecules and metabolic intermediates remain in the cell, and that waste compounds leave the cell. In short, the **selective permeability** of the plasma membrane allows the cell to maintain a constant internal environment (**homeostasis**). In consequence, in all types of cells there exists a difference in ionic concentration with the extracellular medium (Table 5-3). Similarly, the organelles within the cell often have a different internal environment from that of the surrounding cytosol and organelle membranes maintain this difference. For example, in lysosomes the concentration of protons (H^+) is 100 to 1000 times that of

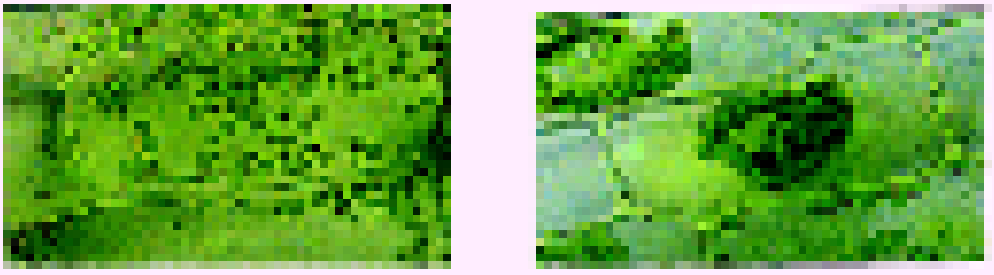
Table 5-3. **Comparison of ion concentration inside and outside of a typical mammalian cell (Source : Maclean and Hall, 1987).**

Component	Intracellular concentration (mM)	Extracellular concentration (mM)
Cations :		
Na^+	5-15	145
K^+	140	5
Mg^{2+}	30	1–2
Ca^{2+}	1-2	2.5–5
H^+	4×10^{-5} (pH 7.4)	4×10^{-5} (pH 7.4)
Anions* :		
Cl^-	4	110

* Since the cell must contain equal positive and negative charge (*i.e.*, be electrically neutral), the large deficit in intracellular anions reflects the fact that most cellular constituents are negatively charged, *e.g.*, HCO_3^-, PO_4^{3-}, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.

the cytosol. This gradient is maintained solely by the lysosomal membrane. Transport across the membrane may be passive or active. It may occur via the phospholipid bilayer or by the help of specific integral membrane proteins, called **permeases** or **transport proteins.**

A. Passive transport. It is a type of **diffusion** in which an ion or molecule crossing a membrane moves down its electrochemical or concentration gradient. *No metabolic energy is consumed in passive transport.* Passive transport is of following three types :

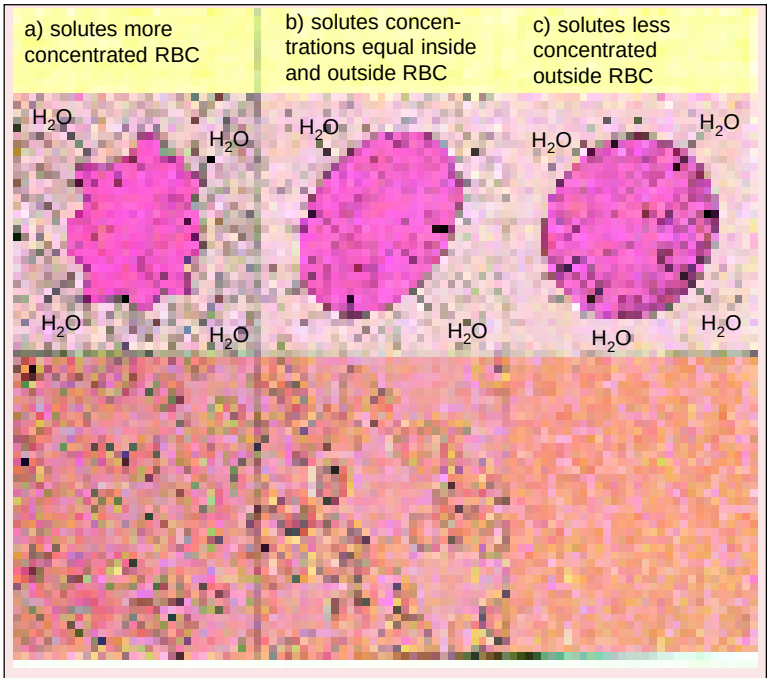


(a) (b)

The effects of osmosis on a plant cell. (a) hypotonic : normal turgor pressure; (b) hypertonic : no turgor pressure.

1. Osmosis. The plasma membrane is permeable to water molecules. The to and fro movement of water molecules through the plasma membrane occurs due to the differences in the concentration of the solute on its either sides. The process by which the water molecules pass through a membrane from a region of higher water concentration to the region of lower water concentration is known as **osmosis** (Gr., *osmos*=pushing). The process in which the water molecules enter into the cell is known as **endosmosis,** while the reverse process which involves the exit of the water molecules from the cell is known as **exosmosis**. In plant cells due to excessive exosmosis the cytoplasm along with the plasma membrane shrinks away from the cell wall. This process is known as **plasmolysis** (Gr., *plasma*=molded, *lysis*=loosing) (Fig. 5.13).

A cell contains variety of solutes in it, for instance, the mammalian erythrocytes contain the ions of potassium (K^+), calcium (Ca^+), phosphate (PO_4^-), dissolved haemoglobin and many other substances. If the erythrocyte is placed in a 0.9% solution of sodium chloride (NaCl), then it neither shrinks nor swells. In such case, because the intra-cellular and extra-celluar fluids contain same concentration and no osmosis takes place. This



Osmosis and the RBC.

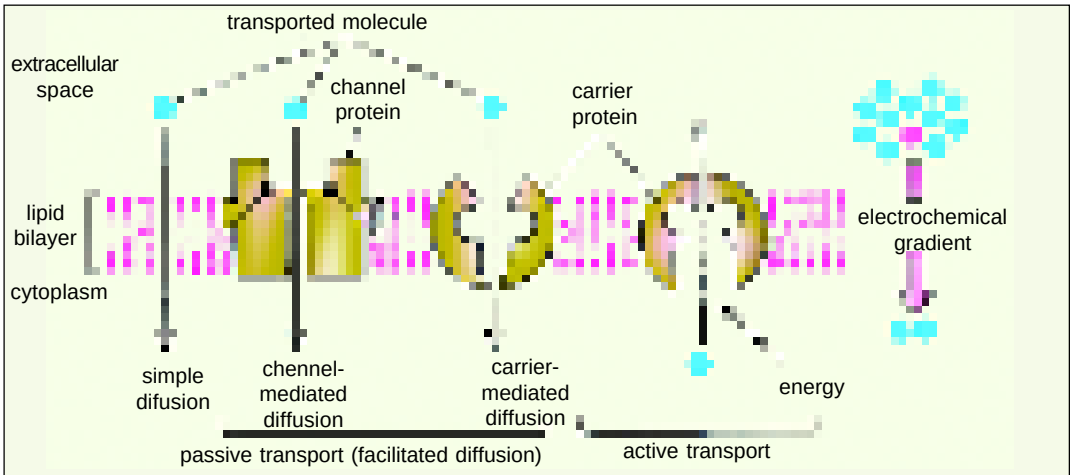


Fig. 5.12. Schematic diagram showing various types of transports across the membrane : simple diffusion, passive transport (down an electrochemical gradient) and active transport (against an electrochemical gradient) (after Alberts, *et al.*, 1989).

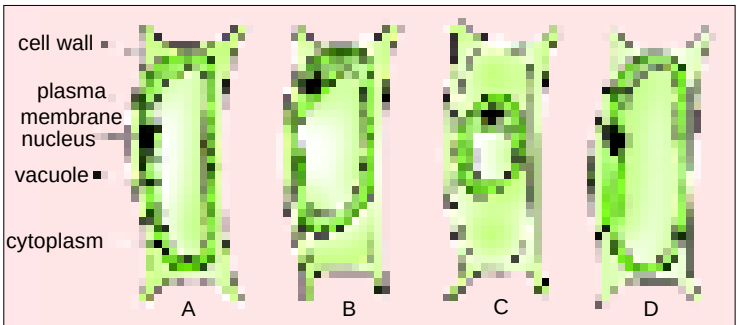


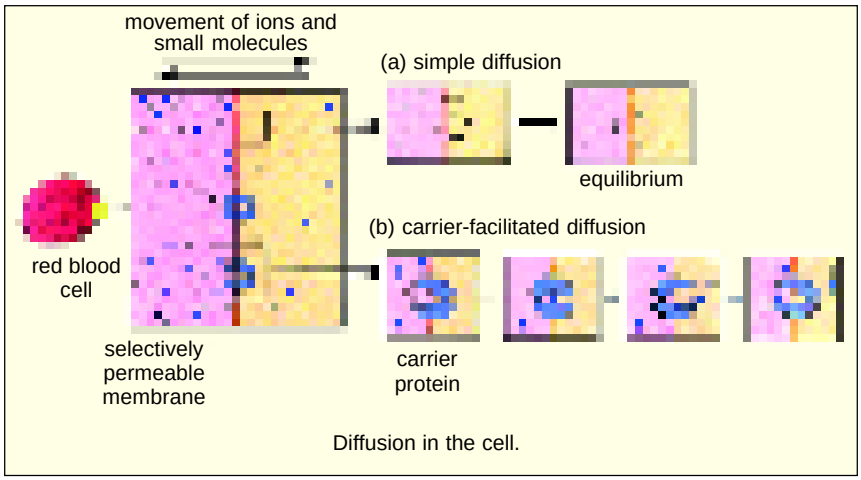
Fig. 5.13. Plasmolysis and deplasmolysis in plant cells. Plasmolysis occurs when a normal plant cell (A) is placed in a hypertonic solution. Water leaves the cell and the plasma membrane shrinks away from the cell wall (B,C). If solutes can penetrate the plasma membrane, the cell will eventually regain water—a process termed **deplasmolysis** (D) (after De Witt, 1977).

type of extra-cellular solution or fluid is known as **isotonic solution** or **fluid.** If the concentration of NaCl solution is increased above 0.9% then the erythrocytes are shrinked due to excessive exosmosis. The solutions which have higher concentrations of solutes than the intracellular fluids are known as **hypertonic solutions.** Further, if the concentration of NaCl solution decreases below 0.9% the erythrocytes will swell up due to endosmosis. The extra-cellular solutions having less concentration of the solutes than the cytoplasm are known as **hypotonic solutions.**

Due to endosmosis or exosmosis the water molecules come in or go out of the cell. The amount of the water inside the cell causes a pressure known as **hydrostatic pressure.** The hydrostatic pressure which is caused by the osmosis is known as **osmotic pressure.** The plasma membrane maintains a balance between the osmotic pressure of the intra-cellular and inter-cellular fluids.

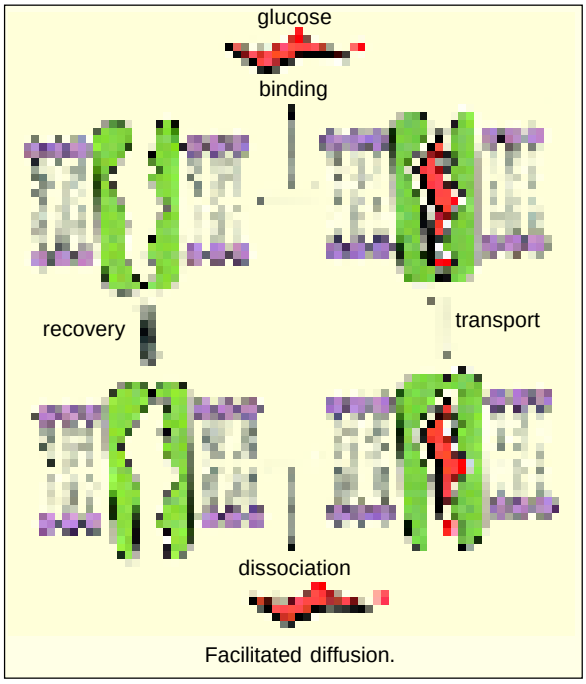
2. Simple diffusion. In simple diffusion, transport across the membrane takes place unaided, *i.e.*, molecules of gases such as oxygen and carbon dioxide and small molecules (*e.g.*, ethanol) enter the cell by crossing the plasma membrane without the help of any permease. During simple diffusion, a small molecule in aqueous solution dissolves into the phospholipid bilayer, crosses it and then dissolves into the aqueous solution on the opposite side. There is little specificity to the process. The relative rate of diffusion of the molecule across the phospholipid bilayer will be proportional to the concentration gradient across the membrane.

3. Facilitated diffusion. This is a special type of passive transport, in which ions or molecules cross the membrane rapidly because specific permeases in the membrane facilitate their crossing. Like



Diffusion in the cell.

the simple diffusion, facilitated diffusion does not require the metabolic energy and it occurs only in the direction of a concentration gradient. Facilitated diffusion is characterized by the following special features: (1) the rate of transport of the molecule across the membrane is far greater than would be expected from a simple diffusion. (2) This process is specific; each facilitated diffusion protein (called **protein channel**) transports only a single species of ion or molecule. (3) There is a maximum rate of transport, *i.e.*, when the concentration gradient of molecules across the membrane is low, an increase in concentration gradient results in a corresponding increase in the rate of transport. Currently, it is believed that transport proteins form the **channels** through the membrane that permit certain ions or molecules to pass across the latter (see **Darnell** *et al.*, 1986).

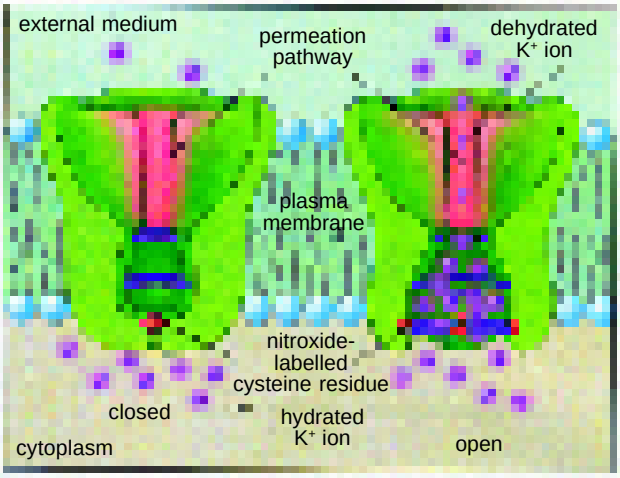


Facilitated diffusion.

Examples of Facilitated Diffusion

(i) Ionic transport through charged pores. Nerve conduction is propagated along the axonal membrane by **action potential** which regulates opening and closing of two main types of **ion channels** (*i.e.*, channel proteins with water filled pores) : **Na^+ channels** (or **voltage-gated Na^+ channels**) and **K^+ channels** (or **k^+ leak channels**). At the point of stimulation there is a sudden and several hundred fold increase in permeability to Na+, which reaches its peak in 0.1 millisecond (*i.e.*, the membrane potential may depolarise from –90 mV and overshoot to + 50 mV). At the end of the period, the membrane again becomes essentially impermeable to Na^+, but the K^+ permeability increases and this ion leaks out of the cell, repolarising the nerve fibre. In other words, during the rising phase of the spike, Na^+ enter through the Na^+ channels, and in descending phase K^+ is extruded through the K^+ channels.

Such ion channels also occur in other types of cells such as muscle, sperm and unfertilized ovum. They are not coupled to an energy source (ATP), so the transport they mediate is always passive ("down hill"), allowing specific ions mainly Na^+, K^+, Ca^{2+} and Cl^- to diffuse down their electrochemical gradient across the lipid bilayer (**Hille**, 1984). Further, an ion channel is made of integral proteins of neural membrane. This protein has two functional elements : (1) a **selective filter** which determines



Use of EPR spectroscopy to monitor changes in conformation of a bacterial K^+ ion channel as it opens and closes.

the kind of ion that will be transported ; (2) a **gate** which by opening and closing the channel, regulates the ion flow. In both Na^+- and K^+- channels, the gating mechanism is electrically driven and is controlled by the membrane potential, without the need of other energy source. In the resting condition (steady state) both Na^+ and K^+ channels are closed. With depolarisation, the Na^+ channel is opened and during repolarisation, it closes again and K^+ channel opens.

Calcium ion channels (**Ca^{2+}-channels**) occur in axonal membranes and other membranes for the entrance of Ca^{2+} ions in the cell. Ca^{2+} ions have a fundamental role in many cellular activities such as exocytosis, endocytosis, secretion, cell motility, cell growth, fertilization and cell division. In the neuronal membrane, there are a number of Ca^{2+} channels that are driven by the membrane potential and are essential in the release of neurotransmitters (acetylcholine).

2. D-hexose permease of erythrocyte. The plasma membrane of mammalian erythrocytes and other body cells, contain specific channel proteins for the facilitated diffusion of glucose into the cells. They are called **glucose transporter**, **glucose permease** or **D-hexose permease**. After the glucose is transported into the erythrocyte, it is rapidly phosphorylated (by **hexokinase** enzyme and ATP) to form **glucose-6-phosphate**. Once phosphorylated, the glucose no longer leaves the cell; moreover, the concentration of the simple glucose in the cell is lowered. As a result, the concentration gradient of glucose across the membrane is increased, allowing the facilitated diffusion to continue to import glucose. Since no cellular membrane (except the mitochondrial membranes) contains any permease for facilitated diffusion of phosphorylated compounds, so a cell can retain any type of molecule by **phosphorylating** them, *e.g.*, ATP and phosphorylated nucleosides are never released from the cells containing a normal intact plasma membrane. However, permeases for ATP and ADP do exist in a mitochondrial membrane to allow these molecules to move across it.

The D-hexose permease of the erythrocyte is an integral and transmembrane protein of 45,000 daltons M.W. It accounts for 2 per cent of erythrocyte membrane protein. D-hexose permease, most probably operates in the following way : the binding of glucose to a site on the exterior surface of the permease triggers a conformational change in the polypeptide. This change somehow generates a pore in the protein that allows the bound glucose to pass through the membrane.

3. Anion exchange permease of erythrocyte. Band 3 polypeptide of plasma membrane of the erythrocytes and other cells is an **ion exchange permease protein** which catalyzes an one-for-one exchange of anions such as chloride (Cl^-) and bicarbonate (HCO_3^-) across the membrane (called **chloride shift**; erythrocyte has 100,000 times more permeability of Cl^- than other cells). The rapid flux of anions in the erythrocyte facilitates the transport in the blood of CO_2 from the tissues to the lungs. Waste CO_2 that is released from cell into the capillary blood, diffuses across the membrane of erythrocyte. In its gaseous from, CO_2 dissolves poorly in aqueous solutions such as blood plasma, but inside the erythrocyte the potent enzyme **carbonic anhydrase** converts it into a bicarbonate anion :

$$CO_2 + H_2O \underset{\text{anhydrase}}{\overset{\text{carbonic}}{\rightleftharpoons}} \underset{\text{proton}}{H^+} + \underset{\text{bicarbonate}}{HCO_3^-}$$

This process occurs while the haemoglobin in the erythrocyte is releasing its oxygen into the blood plasma. The removal of oxygen from haemoglobin induces a change in its conformation that enables a globin histidine (amino acid) side chain to bind to the proton produced by carbonic anhydrase enzyme. The bicarbonate anion formed by carbonic anhydrase is transported out of the erythrocyte in exchange for a chloride (Cl^-) anions:

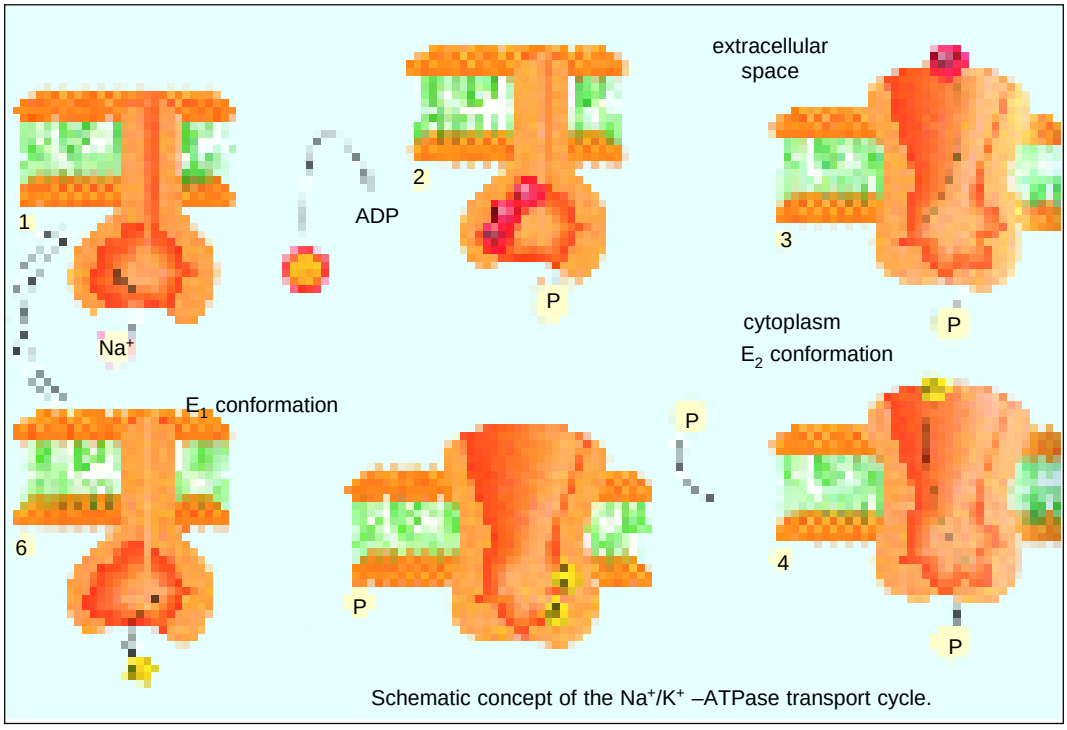
$$\underset{\text{(in)}}{HCO_3^-} + \underset{\text{(out)}}{Cl^-} \rightleftharpoons \underset{\text{(out)}}{HCO_3^-} + \underset{\text{(in)}}{Cl^-}$$

As the total volume of the blood plasma is about twice that of the total erythrocyte cytoplasm, this exchange triples the amount of bicarbonate that can be carried by blood as a whole. Without the presence of an anion exchange protein (*i.e.*, band 3 protein), bicarbonate anions generated by carbonic anhydrase would remain within the erythrocyte and blood would be unable to transport all of the CO_2 produced by tissue. The entire exchange process is completed within 50 millisecond (ms) during which time 5×10^9 HCO_3^- ions are exported from the cell. The process is reversed in the lungs : HCO_3^- diffuses into the erythrocyte in exchange for a Cl^-. Oxygen binding to haemoglobin causes release of the proton from haemoglobin. The CO_2 diffuses out of the erythrocyte and is eventually expelled in breathing. The exact mechanism of anion transport by the Band 3 protein is still unknown.

B. Active transport. Active transport uses specific transport proteins, called **pumps**, which use metabolic energy (ATP) to move ions or molecules against their concentration gradient. For example, in both vertebrates and invertebrates, the concentration of sodium ion is about 10 to 20 times higher in the blood than within the cell. The concentration of the potassium ion is the reverse, generally 20 to 40 times higher inside the cell. Such a low sodium concentration inside the cell is maintained by the sodium-potassium pump. There are different types of pumps for the different types of ions or molecules such as calcium pump, proton pump, etc.

Examples of Active Transport

1. Na^+- K^+- ATPase. It is an **ion pump** or **cation exchange pump** which is driven by energy



Schematic concept of the Na^+/K^+ –ATPase transport cycle.

of one ATP molecule to export three Na^+ ions outside the cell in exchange of the import of two K^+ ions inside the cell. Electrical organs of eels are found to be very rich in this enzyme or pump. N^+- K^+- ATPase is a transmembrane protein which is a dimer having two subunits : one smaller unit which is a glycoprotein of 50,000 daltons M.W., having a unknown function ; and another larger unit having 1,20,000 daltons M. W. The larger subunit of Na^+- K^+- ATPase performs the actual function of cation transport. It has three sites on its extracytoplasmic surface : two sites for K^+ ions and one site for the inhibitor **ouabain.** On its cytosolic side, the larger subunit contains three sites for three Na^+ ions and also has one catalytic site for a ATP molecule. It is believed that the hydrolysis of one ATP molecule somehow drives conformational changes in the Na^+- K^+- ATPase that allows the pump to transport three Na^+ ions out and two K^+ ions inside the cell (Fig. 5.15).

2. Calcium ATPase. Calcium pump or Ca^{2+}-ATPase pumps Ca^{2+}-ions out of the cytosol, maintaining a low concentration of it inside the cytosol. In some types of cells such as erythrocytes, the calcium pumps are located in the plasma membrane and function to transport Ca^{2+} ions out of the cell. In contrast, in muscle cells, Ca^{2+}-ion pumps are located in the membrane of ER or **sarcoplasmic reticulum**. The Ca^{2+}-ATPase transports Ca^{2+} from the cytosol to the interior of the sarcoplasmic reticulum for causing the **relaxation** of the muscle cells. Release of Ca^{2+} ions from the sarcoplasmic reticulum into the cytosol of muscle cells causes **contraction** of the muscle cells. Sarcoplasmic reticulum tends to concentrate and store Ca^{2+} ions by the help of following two types of reservoir proteins : (1) **Calsequestrin** (44,000 daltons M.W. ; highly acidic protein) which tends to bind up to 43 Ca^{2+} ions with it. (2) **High affinity Ca^{2+}- binding protein** which binds Ca^{2+} ions and also reduces the concentration of free

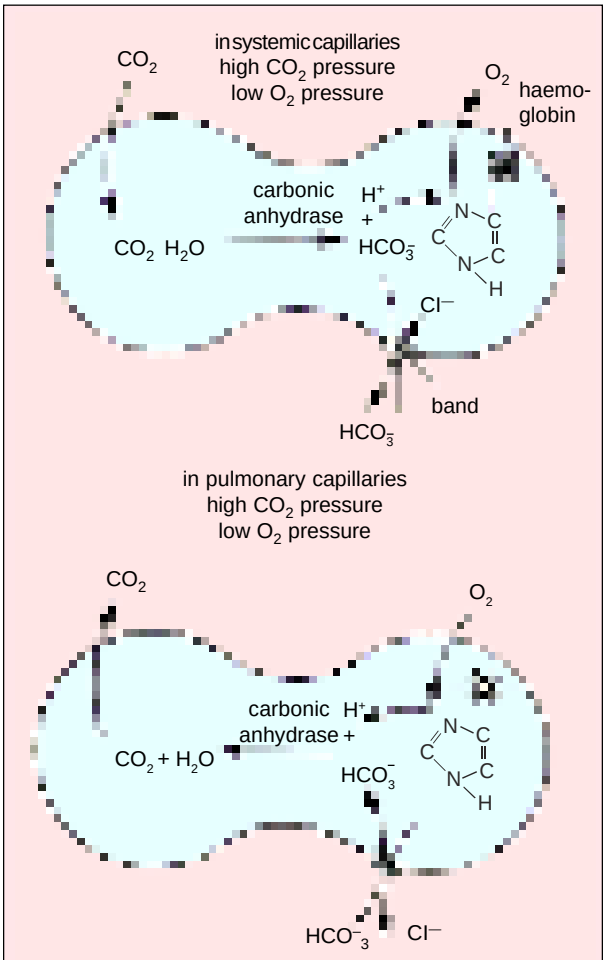
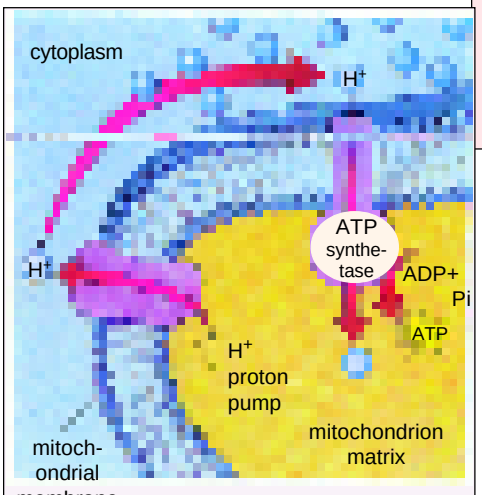


Fig. 5.14. Schematic drawing showing anion transport through the erythrocyte membrane in the capillaries and in the lungs. Band 3 protein (= anion exchange permease) catalyzes the exchange of the anions : Cl^- and HCO_3^- across the erythrocyte membrane (after Darnell *et al*., 1986).



The proton pump.

Ca^{2+} ions inside the sarcoplasmic reticulum vesicles and decreases the amount of energy needed to pump Ca^{2+} ions into it from the cytosol.

A calcium pump is a 100,000 M.W., polypeptide, forming 80 per cent of integral membrane protein of sarcoplasmic reticulum. In it, hydrolysis of one ATP molecule transports two Ca^{2+} ions in the counter-transport of one Mg^{2+} ion.

3. Proton pump or H^+- pump. The lysosomal membrane contains the ATP-dependent proton pump that transports protons from the cytosol into the lumen of the organelle, keeping the interior of lysosomes very acidic (pH 4.5 to 5.0). The pH of the cytosol is about 7.0 (Fig. 5.16).

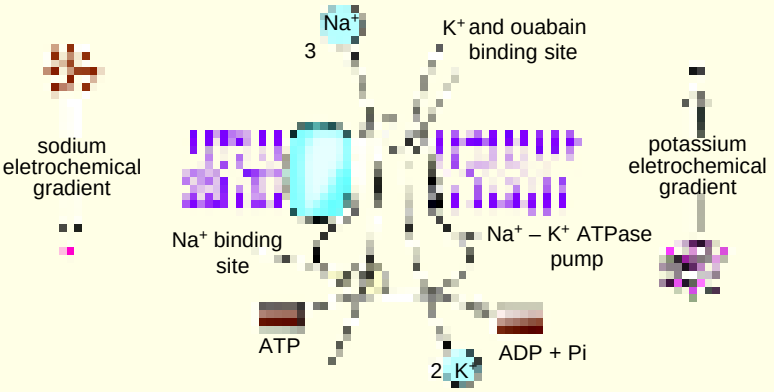


Fig. 5.15. The Na^+- K^+ ATPase in the plasma membrane actively pumps Na^+ out and K^+ into a cell against their electrochemical gradients. For every molecule of ATP hydrolyzed inside the cell, 3 Na^+ ions are pumped out and $2K^+$ ions are pumped in (after Alberts *et al.*, 1989).

Proton pumps also occur in mitochondria and chloroplasts where they participate in the generation of ATP from ADP. They also cause acidification of the mammalian stomach. In the apical membrane of a **parietal cell** or **oxyntic cell** (which sercete HCl or H^+ Cl^-) are located ATP-dependent proton pumps. Hydrolysis of ATP is coupled to the transport of H^+ ions out of the cell (into stomach lumen). HCl production, thus, involves three types of transport proteins : 1. anion-exchange protein; 2. chloride (Cl^-) permeases; and 3. ATP- dependant proton (H^+) pump.

Uniport, symport and antiport. Those carrier proteins which simply transport a single solute from one side of the membrane to the other ; are called **uniports.** Others function as **coupled transporters**, in which the transfer of one solute depends on the simultaneous transfer of a second solute, either in the same direction (**symport**) or in the opposite direction (**antiport**). Both symport and antiport collectively form the **cotransport**. Most animal cells, for example, must take up glucose from the extracellular fluid, where the concentration of the sugar is relatively high, by passive transport through the glucose carriers (such as **D-hexose permease**) that operate as the uniports. By contrast, intestinal and kidney cells must take up glucose from the lumen of the intestine and kidney tubules, respectively, where the concentration of the sugar is low. These cells actively transport glucose by symport with Na^+ ions whose extracellular concentration is very high. The anion exchange permease of human erythrocytes operates as an antiport to the exchange of Cl^- for HCO_3^- (**Alberts** *et al.*, 1989).

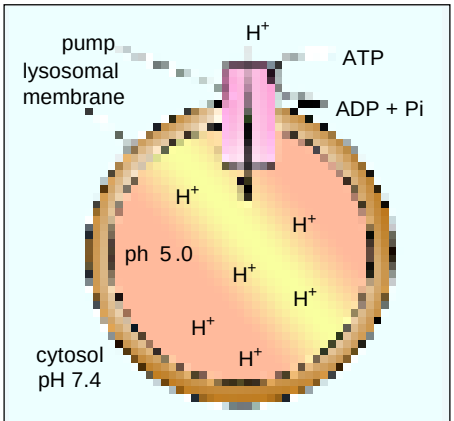


Fig. 5.16. The proton pump of the membrane of the lysosome (after Darnell *et al.*, 1986).

C. Bulk transport by the plasma membrane. Cells routinely import and export large molecules across the plasma membrane. Macromolecules are secreted out from the cell by **exocytosis** and are ingested into the cell from outside through **phagocytosis** and **endocytosis.**

1. Exocytosis. It is also called **emeiocytosis** and **cell vomiting**. In all eukaryotic cells, **secretory vesicles** are continually carrying new plasma membrane and cellular secretions such as proteins, lipids and carbohydrates (*e.g.*, cellulose) from the Golgi apparatus to the plasma membrane or to cell exterior by the process of exocytosis. The proteins to be secreted are synthesized on the rough endoplasmic reticulum (RER). They pass into the lumen of the ER, glycosidated and are transported to the Golgi apparatus by ER-derived **transport vesicles**. In the Golgi apparatus the proteins are modified, concentrated, further glycosidated, sorted and finally packaged into vesicles that pinch off from trans Golgi tubules and migrate to plasma membrane to fuse with it and release the secretion to cell's exterior. In contrast, small molecules to be secreted (*e.g.*, histamine by the mast cells) are actively transported from the cytosol (where they are synthesized on the free ribosomes) into preformed vesicles, where they are complexed to specific macromolecules (*e.g.*, a network of proteoglycans, in case of histamine; **Lawson** *et al.*, 1975), so that, they can be stored at high concentration without generating an excessive osmotic gradient.

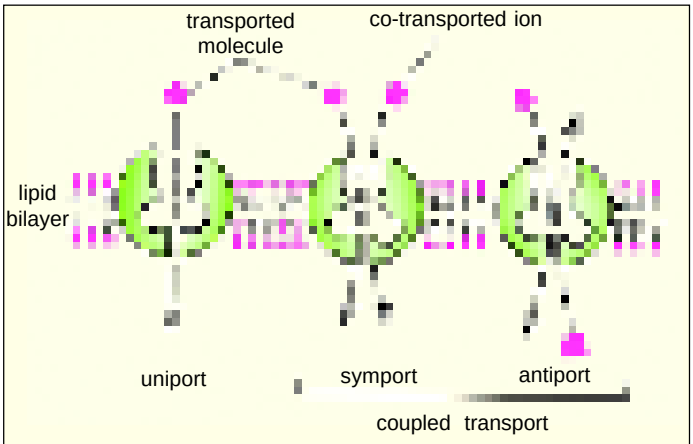
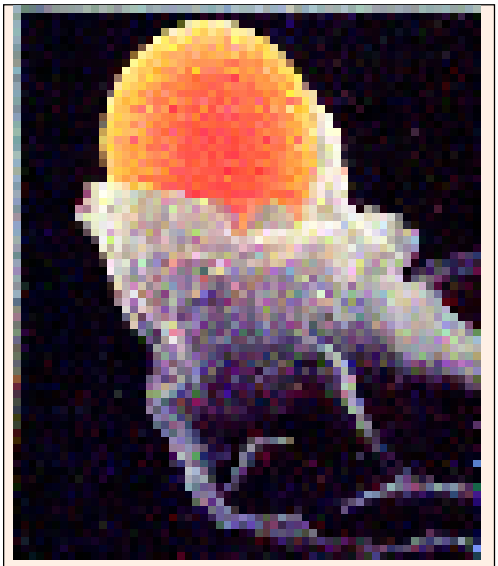


Fig. 5.17. Carrier proteins of membrane functioning as uniports, symports and antiports (After Alberts *et al.*, 1989).

During exocytosis the vesicle membrane is incorporated into the plasma membrane. The amount of secretory vesicle membrane that is temporarily added to the plasma membrane can be enormous : in a pancreatic acinar cell discharging digestive enzymes, about 900 μm^2 of vesicle membrane is inserted into the apical plasma membrane (whose area is only 30 μm^3) when the cell is stimulated to secrete.

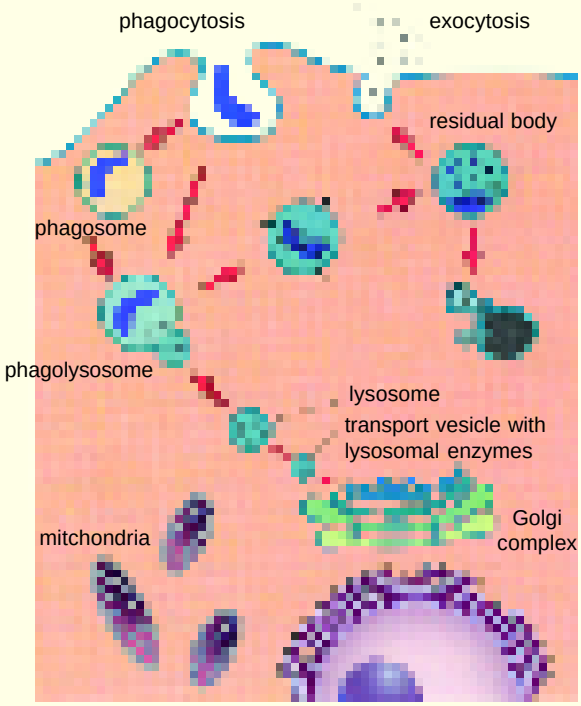


The process of engulfment by a leucocyte ingesting a yeast particle.

2. Phagocytosis. Sometimes the large-sized solid food or foreign particles are taken in by the cell through the plasma membrane. The process of ingestion of large-sized solid substances (*e.g.*, bacteria and parts of broken cells) by the cell is known as **phagocytosis** (Gr., *phagein*=to eat, *kytos*=cell or hollow vessel).

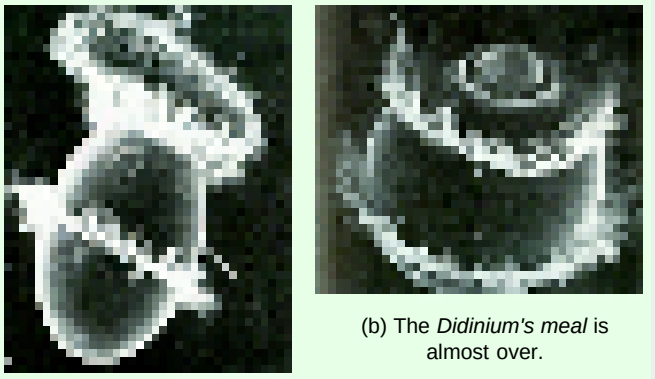
Occurrence of phagocytosis. The process of phagocytosis occurs in most protozoans and certain cells of multicellular organisms. In multicellular organisms such as mammals, the phagocytosis occurs very actively in granular leucocytes and in the cells of mesoblastic origin. The cells of the mesoblastic origin are collectively known as the cells of **macrophagic** or **reticuloendothelial system**. The cells of macrophagic system are histiocytes of the connective tissue,

the reticular cells of the hemopoietic organs (bone marrow, lymph nodes and spleen) and the endothelial cells which form the lining of capillary sinusoid of the liver, adrenal gland and hypophysis. The cells of macrophagic system can ingest bacteria, Protozoa, cell debris or even colloidal particles by the process of phagocytosis.



A summary of the phagocytic pathway.

Process of phagocytosis. In phagocytosis, first the target particle is bound, to the specific receptors on the cell's surface (process is called **adsorption**),then the plasma membrane expands along the surface of the particle and eventually engulfs it. Vesicle formed by phagocytosis is called **phagosome** and it is typically 1 to 2 µm or larger in diameter, much larger than those formed during pinocytosis and receptor-mediated endocytosis. The phagosomes migrate to the interior of the cell and fuse with the pre-existing lysosomes (to form phagolysosome). The food is digested by the hydrolytic enzymes (acid hydrolase) of the lysosomes and the digested food is ultimately diffused to the surrounding cytoplasm. In addition to the normal set of lysosomal hydrolases, macrophage's lysosomes contain enzymes that generate hydrogen peroxide (H_2O_2) and other toxic chemicals that aid in the killing of the bacteria. The undigested food is expelled from the plasma membrane by the process of **ephagy** or **egestion**. In macrophages, the undigested parts of ingested material such as the cell walls of micro-organisms, accumulate within lysosomes as residual bodies. Accumulation of residual bodies may be one reason why macrophages have a very short life time (*i.e.*, less than a few days).



(b) The *Didinium's meal* is almost over.

(a) Here an egg-shaped protist *Didinum* illustrates phagocytosis by ingesting the smaller *paramecium*.

Kinds of phagocytosis. According to the physical and chemical nature of foreign substance following types of phagocytosis have been recognised :

(i) Ultraphagocytosis or colloidopexy. The process in which plasma membrane ingests smaller colloidal particles is known as **colloidopexy** or **ultraphagocytosis**, *e.g.*, leucocytes and the macrophagic cells of mammals.

(ii) Chromopexy. When the cell ingests colloidal chromogen par-

ticles phagocytotically the process is known as **chromopexy**, *e.g.*, some mesoblasitc cells.

3. Endocytosis. In endocytosis, small regions of the plasma membrane fold inwards or **invaginate**, until it has formed new intracellular membrane limited vesicles. In eukaryotes, the following two types of endocytosis can occur : pinocytosis and receptor-mediated endocytosis.

(i) Pinocytosis. Pinocytosis (Gr., *pinein* = to drink; 'cell drinkng') is the non-specific uptake of small droplets of extracellular fluid by **endocytic vesicles** or **pinosomes**, having diameter of about 0.1 µm to 0.2 µm. Any material dissolved in the extracellular fluid is internalized in proportion to its concentration in the fluid. The process of pinocytosis was first of all observed by **Edward** in *Amoeba* and by **Lewis** (1931) in the cultured cells.

The light microscopy has shown that in *Amoeba* tiny **pinocytic channels** are continually being formed at the cell surface by invagination of the plasma membrane. From the inner end of each channel small vacuoles or pinosomes are pinched off, and these move towards the centre of the cell, where they fuse with primary lysosomes, to form **food vacuoles**. Ultimately, ingested contents are digested, small breakdown products such as sugars and amino acids diffuse to cytosol.

Micropinocytosis. Electron microscopic observations have been made on the pinocytotic process at sub-cellular or sub-microscopic level in the cells. The pinocytosis which occurs at sub-microscopic level is known as **micropinocytosis**. In the process of micropinocytosis, the plasma membrane invaginates to from small vesicles of 650 A° diameter. These closed vesicles are not coated by clathrin protein and they move across the cytoplasm of endothelial cells (which line the blood capillaries) to fuse with opposite plasma membrane discharging their contents. This is called **transcytosis** (**Simionescue**, 1980). Such transcellular passage of fluids is also found to occur in other types of cells such as Schawn and satellite cells of nerve ganglion, macrophages, muscle cells and reticular cells, etc., but in them vesicles are coated by clathrin (see **Alberts** *et al.*, 1989).

(ii) Receptor-mediated endocytosis. In this type of endocytosis, a specific receptor on the surface of the plasma membrane "recognizes" an extracellular macromolecule and binds with it. The substance bound with the receptor is called the **ligand**. Examples of ligands may include viruses,

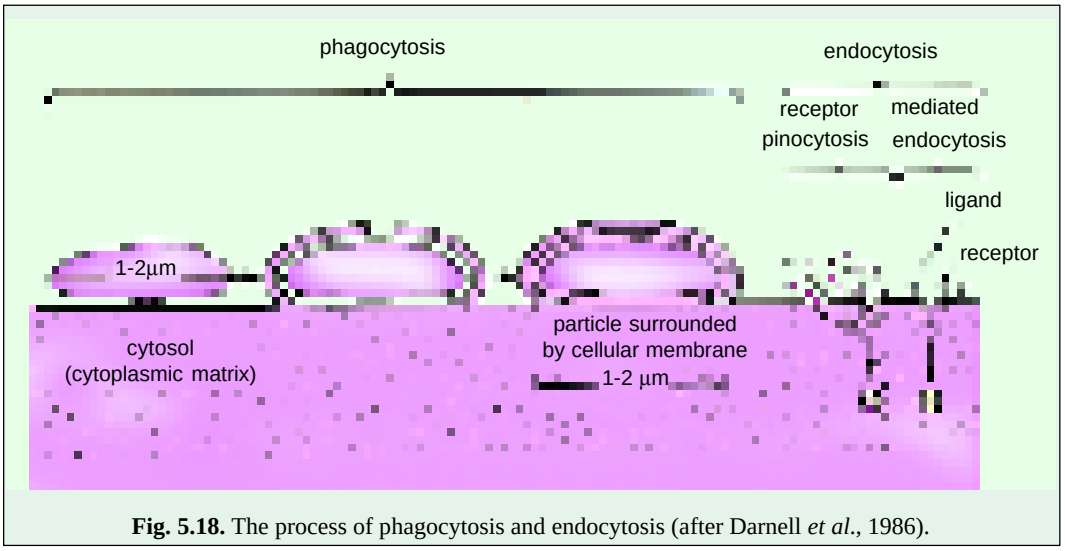


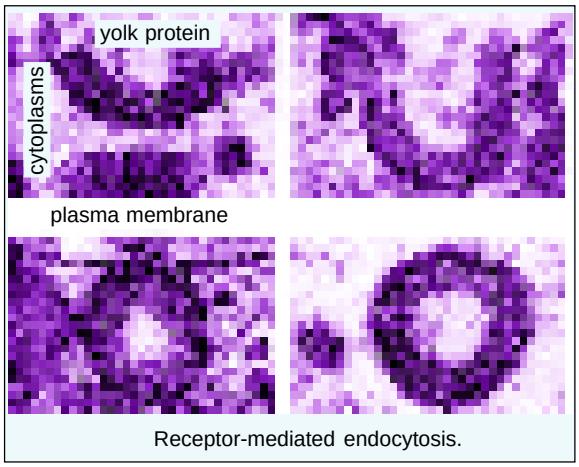
Fig. 5.18. The process of phagocytosis and endocytosis (after Darnell *et al.*, 1986).

small proteins (*e.g.*, insulin, vitellogenin, immunoglobin, transferrin, etc.), vitamin B_{12}, cholesterol containing LDL or low density lipoprotein, oligosaccharide, etc. The region of plasma membrane containing the receptor-ligand complex undergoes endocytosis. The whole process of receptor-mediated endocytosis, includes the following events :

1. Interaction of ligands and cell surface receptors. The macromolecules (ligands) bind to complementary cell-surface receptors. There are more than 25 different types of receptors which are involved in receptor-mediated endocytosis of different types of molecules. Such a receptor is a trans-membrane protein which contains two specific binding sites : (1) **ligand-binding site** at the external surface of plasma membrane ; and (2) **coated-pit binding site** at the inner or cytosolic face of the plasma membrane.

2. Formation of coated-pits and coated-vesicles. The endocytic cycle begins at specialized regions of the plasma membrane, called **coated-pits**. Coated-pits are depressions of plasma membrane having a coat of bristle-like structure towards their cytosolic side. The ligand-loaded receptors diffuse into these coated-pits. A coated-pit may accommodate about 1000 receptors of assorted variety. In fact, coated-pits serve as molecular filters and selective concentrating devices, since, they tend to collect certain receptors and leave others. They increase the efficiency of internalization of a particular ligand more than 1000-fold and also carry minor components of extracellular fluid. The life-time of each coated-pit is quite short—within a minute or so of being formed, it invaginates into the cell and pinches off to form the **coated-vesicles**. The coat of coated pits and coated vesicles is made up of protein, called **clathrin** and certain other proteins. A molecule of clathrin is composed of three large polypeptide chains and three smaller polypeptide chains, all of which together form a three-legged structure, called **triskelion**. A number of triskelions assemble into a basket-like network of hexagons and pentagons on the cytoplasmic surface of the membranes (**Pearse** and coworkers, 1981, 1987).

3. Fusion of endocytic vesicle and endosome. Once a coated vesicle is formed, the clathrin and associated proteins dissociate from the vesicle membrane and return to the plasma membrane to form a new coated-pit (**Schmid** and **Rothman**, 1985). The resultant **endocytic vesicle** gets fused with pre-existing endosomes and ultimately its contents are utilized by the cell.



Receptor-mediated endocytosis.

Endosome or receptosome. Recently it has been found that in the cells exists a complex set of heterogeneous membrane-bound tubes and vesicles, called **endosome**, which extends from the periphery of the cell to the perinuclear region, where it lies quite close to Golgi apparatus. Thus, endosomes may be of two types : (i) **peripheral endosomes** just beneath the plasma membrane and (ii) **perinuclear** or **internal endosomes**. The interior of the endosome is acidic (pH 5-6) due to the presence of ATP-driven **proton (H^+) pumps** in its membrane that pumps H^+ ions into the lumen from the cytosol (**Sly** and **Doisy**, 1984). Endosomes lack in degradative enzymes.

Thus, via receptor-mediated coated-vesicles, the ligands are delivered to the peripheral endosomes which slowly move inward to become perinuclear endosomes. These perinuclear endosomes are converted into **endolysosomes** and then into **lysosomes** due to following three activities : 1. the fusion of transport vesicles from the Golgi apparatus, (**Note. Transport vesicles** capture a cargo of molecules, *e.g.*, proteins, from the lumen of one compartment as they pinch off from its membrane and then discharge that cargo into another compartment as they fuse with it. Thus, in such vesicular transport, the transported proteins do not cross any membrane and they are transferred from lumen to lumen). 2. continuous membrane retrieval, and 3. increased acidification (**Helenius** and coworkers, 1983, 1987). The endosomal compartment also acts as the main **sorting station** in the endocytic

pathway. The acidic environment of the endosome causes dissociation of ligands from their receptors. Such ligands are destined for destruction in the lysosomes along with the other non-membrane-bound contents of the endosome. The receptor-proteins are either returned to the same plasma membrane domain from which they come or they go to lysosomes and are degraded.

Example of receptor-mediated endocytosis. Most animal cells are found to have a regulatory pathway for the uptake of cholesterol. Most cholesterol is transported in the blood in the form of particles of **low-density proteins** or **LDL**. Each of these large spherical particles (22 nm in diameter) contains a **core** of about 1500 cholesteryl ester molecules surrounded by a **lipid monolayer** and also contains a single large protein molecule (apoprotein). When the cell needs cholesterol for membrane synthesis, it synthesizes receptor proteins for LDL particles and inserts them into its plasma membrane. The human LDL receptor is a single-pass transmembrane glycoprotein which is composed of about 840 amino acid residues, only 50 of which stick out from cytoplasmic side of plasma membrane to form the **coated-protein-binding site**. The **LDL - binding site** of the receptor is exposed to cell surface. The LDL receptors move laterally within lipid bilayer, until they become associated to the newly formed coated-pits. Since coated-pits constantly pinch off to form coated-vesicles, the LDL particles are bound to receptors in the coated-pits and are rapidly internalized. After shedding their clathrin-coats the endocytic vesicles deliver their contents to endosomes. In endosomes, the LDL particles and their receptors are separated from each other ; the receptors are returned to the plasma membrane, while LDL ends up in the lysosomes. In the lysosomes, the cholesteryl esters in the LDL

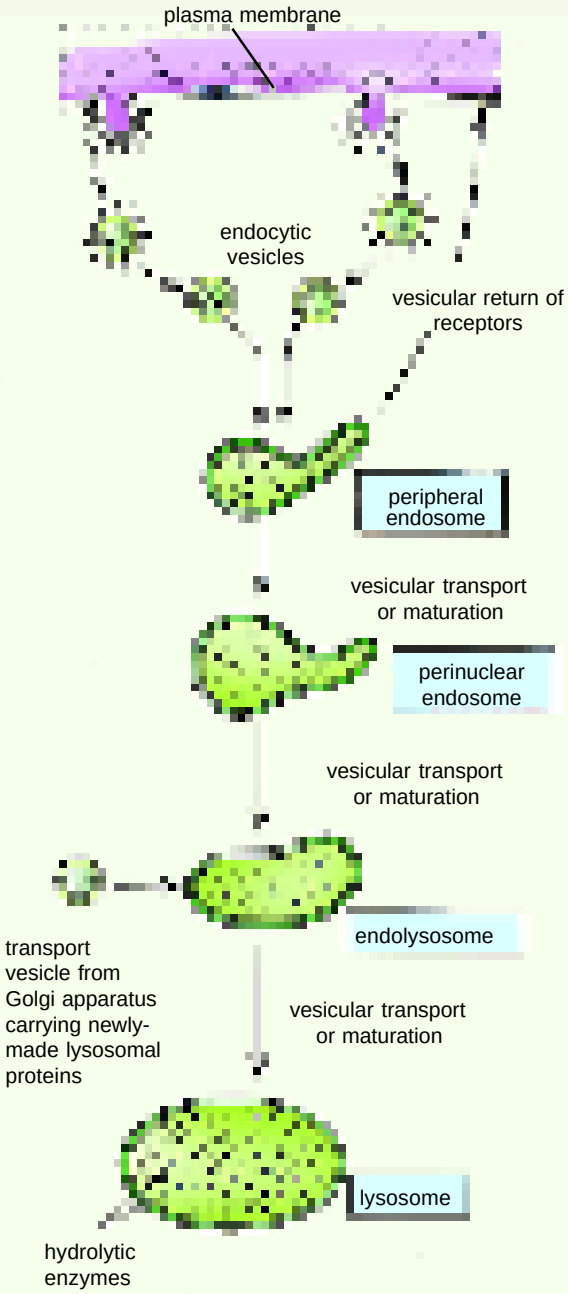


Fig. 5.19. An endocytic pathway : ligands which are endocytosed via coated pits, are delivered to the peripheral endosomes and then move sequentially to perinuclear endosomes, endolysosomes and lysosomes (Alberts *et al.*, 1989).

particles are hydrolyzed to free cholesterol molecules, which thereby become available to the cell for new membrane synthesis. If too much free cholesterol accumulates in a cell, this stops cell's own cholesterol synthesis and the synthesis of LDL-receptor proteins, so that less amount of cholesterol is made and less amount of it is taken up by the cell (**Brown** and **Goldstein**, 1984, 1986).

Energy utilisation by phagocytosis and endocytosis. Unlike pinocytosis, which is a constitutive process that occurs continuously, the phagocytosis is a triggered process in which activated receptors transmit signals to the cell interior to initiate the response (**Wright** and **Silverstein**, 1983). Both phagocytosis and pinocytosis are active mechanisms in the sense that the cell requires energy for

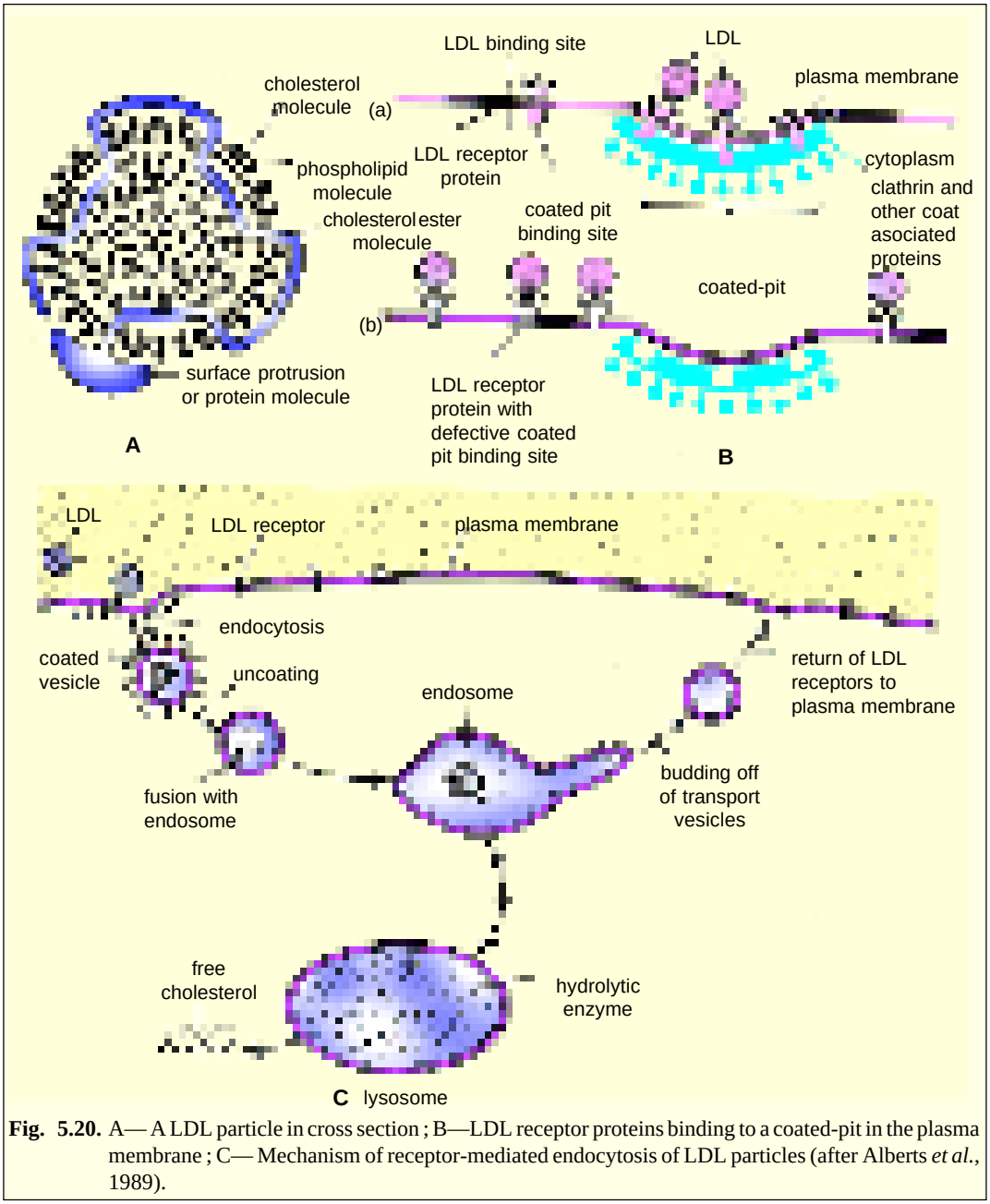


Fig. 5.20. A— A LDL particle in cross section ; B—LDL receptor proteins binding to a coated-pit in the plasma membrane ; C— Mechanism of receptor-mediated endocytosis of LDL particles (after Alberts *et al.*, 1989).

their operation. During phagocytosis by leukocytes oxygen consumption, glucose uptake and glycogen breakdown all increase significantly. The mechanism of endocytosis is found to involve the contraction of **microfilaments** of actin and myosin present in the peripheral cytoplasm (ectoplasm) which causes the plasma membrane to invaginate and to form the endocytic vacuole (pinosome/phagosome). Involvement of actin microfilaments is demonstrated by the action of the drug **cytochalasin B** which inhibits endocytosis and disorganizes actin microfilaments.

Membrane fusion during exocytosis and endocytosis. Both exocytosis and endocytosis involve the fusion of initially separate regions of lipid bilayer and occur in at least two steps : first the two bilayers come into close apposition, it is called **bilayer adherence**, and then they fuse together (This is called **bilayer joining**). Both of these steps are believed to be mediated by some types of specific proteins, called **fusogenic proteins** (**Blumenthal**, 1987).

DIFFERENTIATIONS OF CELL SURFACE

The cell surface of certain cells performs various physiological activities such as absorption, secretion, transportation, etc. To perform such specialized functions certain modifications are inevitable in the plasma membrane of such cells. Such cell surface differentiations may include microvilli, invagination, basement membrane and many types of cell-to-cell interconnections or junctions (Table 5-4).

Table 5-4. **Types of junctions between animal cells (Source : Maclean and Hall, 1987).**

1. Impermeable junctions :	(i) Tight junctions (ii) Septate junctions
2. Adhering junctions :	(iii) Belt desmosomes (iv) Spot desmosomes (v) Hemidesmosoms
3. Communicating junctions :	(vi) Gap junctions (vii) Chemical synapsis

1. Invaginations

The bases (inner ends) of certain cells, such as the cells of the kidney, perform active transportation and contain many **invaginations** or **infoldings** of the plasma membrane. At the base of these folds, there develops a septa and, thus, narrow compartments of basal cytoplasm are formed. These infoldings contain many mitochondria. These mitochondria along with the enzymes of plasma membrane possibly provide energy rich compound, *viz.*, ATP to the plasma membrane for the active transportation of solutes.

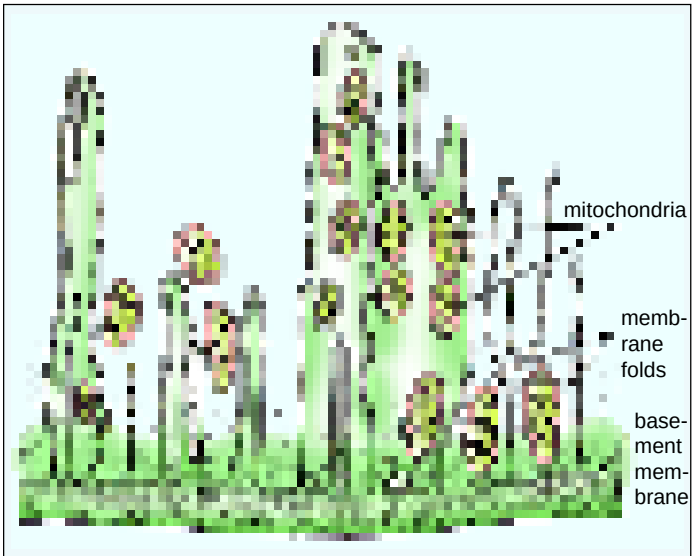


Fig. 5.21. Infoldings of plasma membrane.

2. Microvilli

Microvilli are finger-like,

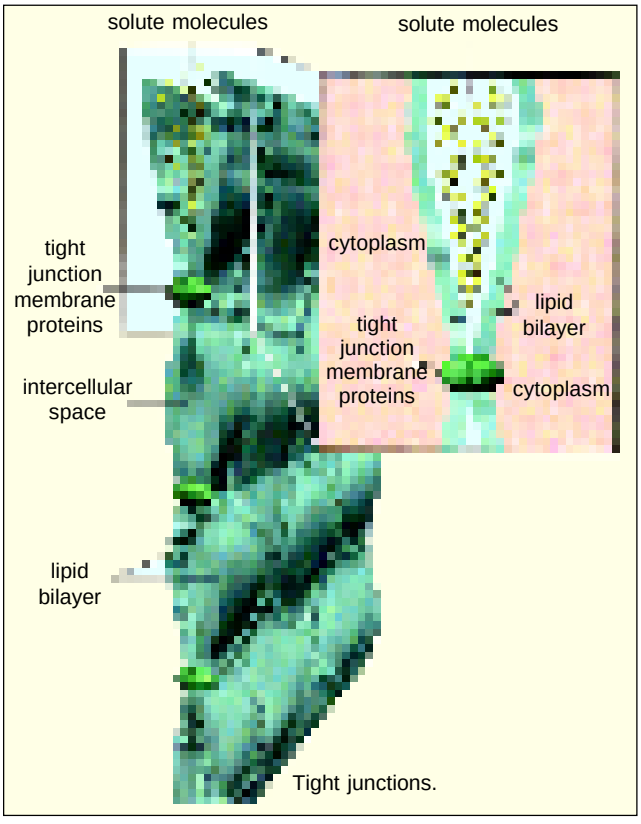
slender projections of plasma membrane which are found in mesothelial cells, hepatic cells, epithelial cells of intestine (**striated border**), uriniferous tubules (**brush border**), gall bladder, uterus, growing oocyte and yolk sac. Microvilli increase the effective surface of absorption. For example, a single epithelial cell of intestine may have as many as 3000 microvilli and in a square millimetre of intestine there may be 200,000,000. These microvilli are 0.6 to 0.8 µm long and 0.1 µm in diameter. The narrow spaces between the microvilli form a kind of sieve through which substances may pass during the process of absorption. Within the cytoplasmic core of a microvillus fine microfilaments are observed which in the underlying cytoplasm form a **terminal web**. The microfilaments contain actin and are attached to the tips of the microvilli by α-actinin ; their function is to produce contraction of microvilli.

3. Basement Membrane

The interface between all epithelia and underlying connective tissue is marked by a non-cellular structure called **basement membrane**. This membrane comprises two basic layers : 1. **Basal lamia** which is in contact with the epithelial basal plasma membrane and is composed of fine feltwork of fibrils of collagen of Type IV that are embedded in an amorphous matrix. It is secreted by the epithelial cells. 2. **Reticular layer** exists just beneath the basal lamina and is composed of fine reticular fibres of reticulin protein. The reticular fibres are embedded in a ground substance. The reticular layer is synthesized by underlying connective tissue into which it is merged (**Wheater** *et al*., 1979). The basement membrane provides structural support for epithelia and may constitute an important barrier to the passage of materials between the epithelial and connective tissue compartments.

4. Tight Junctions (Zonula Occludens)

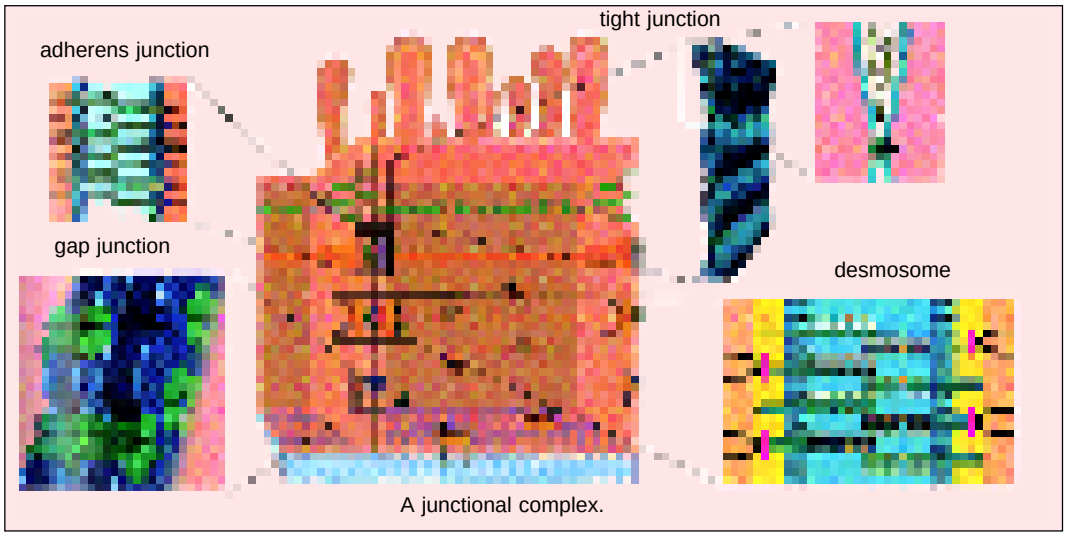
The cells of both vertebrate and invertebrate animals display junctions that are designed to prevent or reduce the flow of even small molecules between the lateral surfaces of adjacent cells. Such junctions are particularly characteristics of epithelial tissues. In higher animals these are termed **tight junctions** and in invertebrates these are called **septate junctions** (see **Maclean** and **Hall**, 1987).



Tight junctions.

Tight junctions are situated below the apical border (often below the microvillar surface) of the epithelial cells and act as permeability barriers. Thus, all nutrients are absorbed from the intestine into one side of the epithelial cell and then released from the other side into the blood because tight intercellular junctions do not allow small molecules to diffuse directly from the intestine lumen into the blood. Also in pancreatic acinar tissue, they prevent the leakage of pancreatic secretory proteins, including digestve enzymes, into the blood.

Tight junctions are composed of thin bands that completely encircle a cell and are in contact with thin bands of



A junctional complex.

adjacent cells. In a thin section, in the tight junction two adjacent plasma membranes appear to be fused at a series of points. However, in three-dimensional structure, revealed by freeze-fracture technique (**Pinto da Silva** and **Kachar**, 1982), the tight junctions appear as a network of ridges on the cytoplasmic half of the membrane, with complementary grooves in the outer half. The ridges appear to be composed of two rows of protein particles, as in zipper, each one belonging to the adjacent cells. The lines of these particles produce the sealing and for this reason have been named **sealing strands**. Often, sealing strands form a series of interconnected and anastamosing lines, like a row of stitches in a quilted surface.

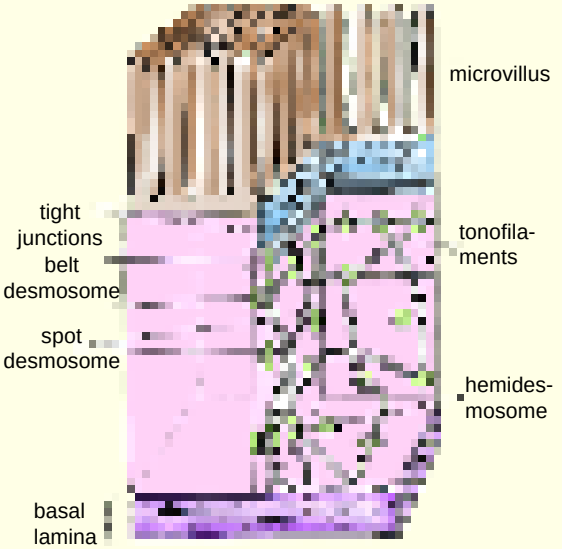


Fig. 5.22. Schematic diagram of the principal types of cell junctions, as found in the intestinal epithelial cell (after Darnell, *et al.*, 1986).

In invertebrates, **septate junctions** perform the functions similar to tight junctions. They differ from the tight junctions in that the proteins that straddle the gaps, occur in parallel rows or **septae**. Also in them adjacent plasma membrane surfaces are not in direct contact, so that the junctional proteins themselves form the seal.

5. Desmosomes

Desmosomes are abundantly found in tissues that have to withstand severe mechanical stress, such as skin epithelia, bladder, cardiac muscle, the neck of uterus and vagina. Their presence in such tissues allows the tissues to function as elastic sheets without the individual cells being torn one from another. Desmosomes are of following three types :

(i) Belt desmosomes (Zonula adherens). They are generally found at the interface between columnar cells, just below the region of tight junctions. They form a band that form a girdle around the inner surface of the plasma membrane. This band contains a web of 6 to 7 nm actin microfilaments and another group of interwoven intermediate filaments of 10 nm. Actin microfilaments are contractile and

intermediate filaments play a structural role. At belt desmosome, the plasma membranes of adjacent cells are parallel, thicker than usual and 15 to 20 nm apart. The intercellular space between them is filled with an amorphous material.

(ii) Spot desmosomes (Macula adherens). The spot desmosomes act like rivets or "spot welds" to hold epithelial cells together at points of contact. They represent localized circular areas of contact about 0.5 µm in diameter, in which the plasma membranes of two adjacent cells are separated by a distance of 30 to 50 nm. The intercellular core or **central stratum** between the two membranes consists of specific desmosomal material rich in proteins and mucopolysaccharides. Under each facing plasma membrane of the spot desmosome, there is a discoidal intracellular **plaque**, 15 to 20 nm thick, having non-glycosylated proteins such as **desmoplakins I**, **II**, and **III** (**Muellar** and **Franke**, 1983). Numerous 10-nm thick intermediate filaments of keratin protein, called **tonofilaments**, converge towards the plaque. These filaments form a loop in a wide arc and course back into the cytoplasm. In addition, there are thinner filaments that arise from each dense plaque and traverse the plasma membrane to form "**trans-membrane linkers**" in the intercellular space. These linkers provide mechanical coupling and chemically are made of glycosylated proteins, called **desmogleins I** and **II**, with the carbohydrate moiety exposed toward the intercellular space (**Steinberg**, 1984). While the tonofil-aments provide the intracellular mechanical support, the cellular adhesion at the desmosome depends mainly on the extracellular coating material.

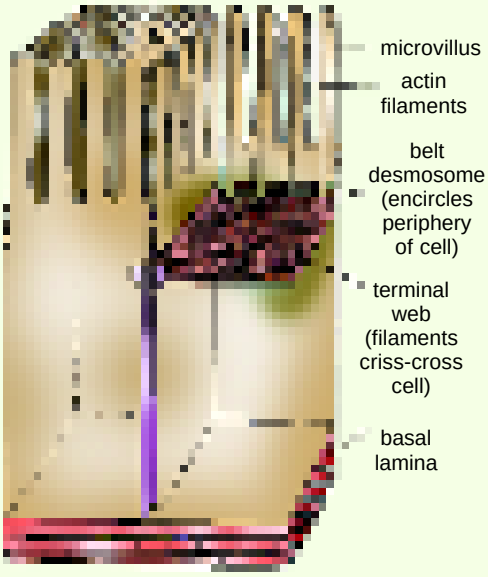
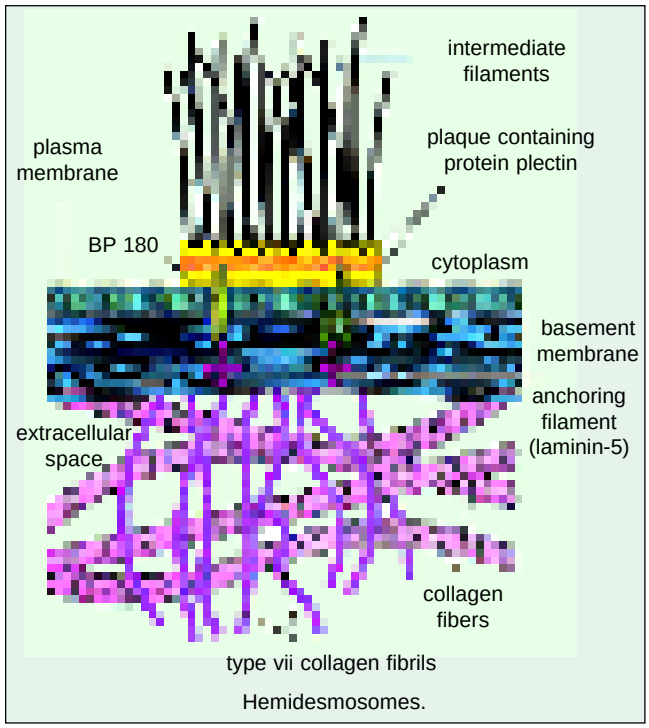


Fig. 5.23. Microvilli and terminal web (after Darnell, *et.al*.,1986).



Hemidesmosomes.

(iii) Hemidesmosomes. They are half desmosomes which resemble spot desmosomes but join the basal surface of an epithelial cell to a basal lamina. They anchor extracellular proteins such as collagen and other proteins to the cell.

6. Gap Junctions (Nexus)

Many cells of the tissues of higher animals are coupled together by interconnecting **gap junctions**, **nexus** or **communicating junctions**. The presence of gap junctions explains the ionic or

electronic connections between adjacent cells, *i.e.*, there are some cells which are **electrically coupled** and have regions of low resistance in the membrane through which there is a rather free flow of electrical current carried by ions. Such electrical coupling is found extensively in embryonic cells. In adult tissues it is usually found in epithelia, cardiac cells and liver cells. Skeletal muscles and most neurons do not show electrical coupling.

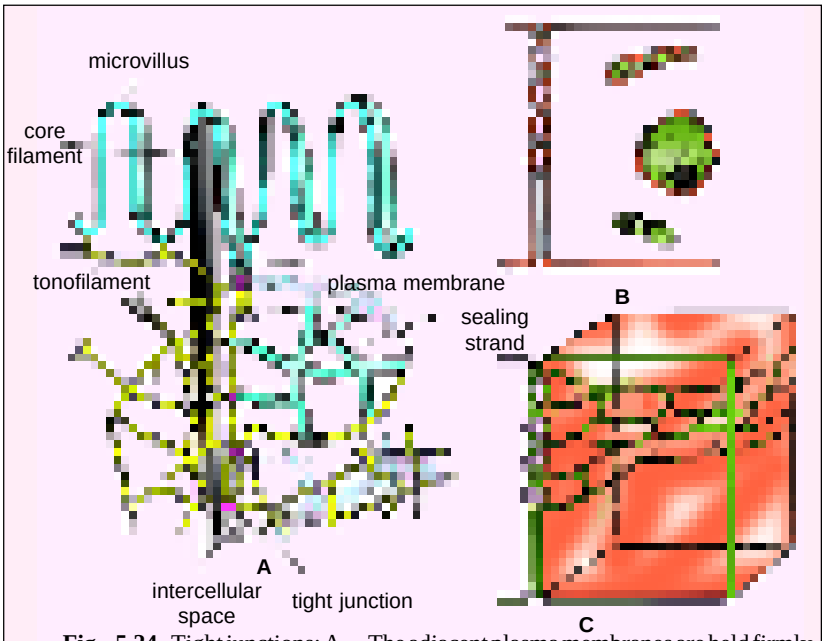
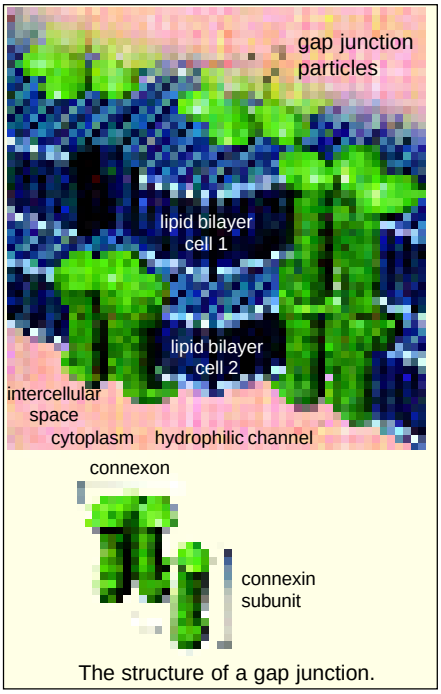


Fig. 5.24. Tight junctions: A— The adjacent plasma membranes are held firmly at the sealing strand which is composed of two rows of particles, as in zipper ; B— Three-dimensional representations (after De Robertis and De Robertis Jr., 1987; Maclean and Hall, 1987).

In fact, gap junctions are found to permit molecules such as inorganic ions, sugars, amino acids, nucleotides and vitamins to pass with comparative freedom between one cell and another within a tissue, but they prevent larger molecules, such as proteins, nucleic acids and polysaccharides from being transferred. This observation also explains the phenomenon of **metabolic cooperation** or **metabolic coupling** between cells, *i.e.*, cells can transfer to neighbouring cells, the molecules which cannot be synthesized by the recipient cells. For example, in the tissue-culture experiments, the mutant cells which are deficient in the enzyme **thymidine kinase** can be shown by autoradiography to be capable of DNA synthesis only when grown in a culture vessel together with the wild type cells (**Hooper** and **Subak-Sharpe**, 1981). This observation shows that required thymidine has been passed from a wild-type cell to a mutant cell, presumably via gap junctions. There are certain other molecules such as AMP, ADP, ATP and cAMP that can pass through gap junctions.



The structure of a gap junction.

A gap junction appears as a plaque-like contact in which the plasma membranes of adjacent cells are in close apposition, separated by a space of only 2 to 4 nm. Structurally, gap junctions consist of hollow channels round which a series of six protein subunits are located ; a channel has a diameter of about 1.5 to 2 nm. A single major protein (a macromolecular unit, called **connexon**) of 27000 daltons has been isolated from rat liver preparations consisting of almost

pure gap junction material (**Hertzberg** *et al.*, 1981), A connexon appears as an annulus of six subunits surrounding the channel. It is believed that the sliding of the subunits caused the channel to open and close. The permeability of channel of gap junction is regulated by Ca^{2+} ions; if the intercellular Ca^{2+} ion level increases, the permeability is reduced or abolished. The gap junctions or connexons of adjacent cells are believed to line up to provide a continuous channel, made up of two connexons opposed end to end (Fig. 5.26).

CELL COAT

The plasma membrane is surrounded and protected by the **cell coat**. Sometimes, the cell coat is also called **glycocalix**, because it contains sugar units in glycoproteins and polysaccharides. The cell coat is found to be equivalent to the oligosaccharide side chains of glycolipids and glycoproteins that stick out from the cell surface and are covalently attached to protein moieties. However, in many type of cells, there is a separate "fuzzy layer", beyond the cell coat, which is composed mainly of carbohydrates and is secreted by the cell.

The cell coat can be stained with PAS (see Chapter 2) or Alcian blue for the light microscopy and with **lanthanum** or **ruthenium red** for the electron microscopy. By the use of **lectins**, the carbohydrates can be specifically observed. Lectins are proteins which are normally derived from the plants and they tend to bind to the cell surface and cause agglutination. They are used as recognition molecules for the sugar components of glycoproteins. **Concanavalin A** is a lectin which has been isolated from jack beans and is specific for the glucose and mannose residues. Another lectin, called **germ agglutinin**, is specific for N-acetylglucosamine. These lectins may be labelled with fluorescent dyes or with electron-dense materials for electron microscopic observation.

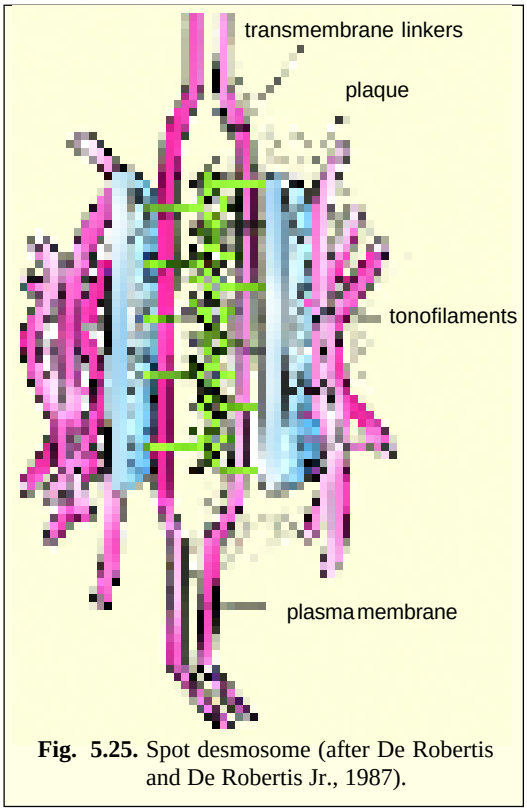


Fig. 5.25. Spot desmosome (after De Robertis and De Robertis Jr., 1987).

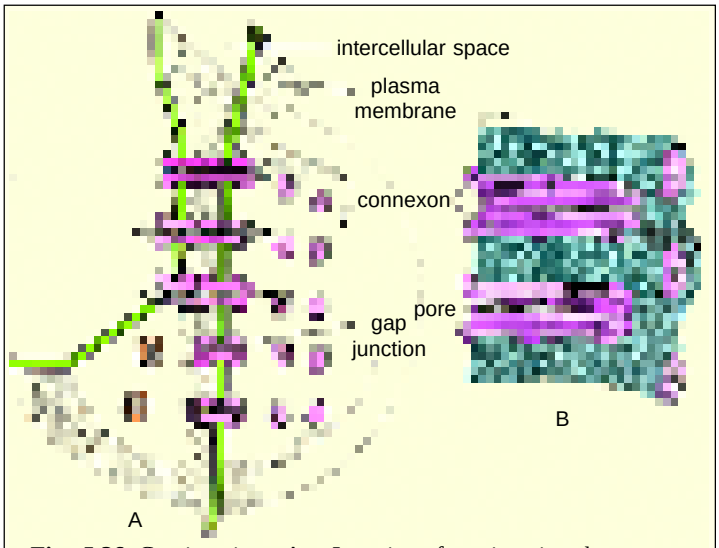


Fig. 5.26. Gap junctions. A— Location of gap junctions between two cells. The channels are made by particles in each membrane that traverse the intercellular space. The flow of fluid between the cells is indicated by arrows. B—Finer structure of unit structure or connexion of gap junction. The channel has a pore about 2 nm and is formed by two hexamers (six subunits) traversing the lipid bilayers of two plasma membranes (after De Robertis and De Robertis Jr., 1987).

The cell coat is a 10 to 20 nm thick layer and is in direct contact with the outer leaflet of the plasma membrane. In *Amoeba*, the cell coat is formed by fine filaments — 5 to 8 nm thick and 100 to 200 nm long. Chemically, the cell coat has negatively charged sialic acid termini, on both glycoproteins and gangliosides. This acid tends to bind the ions of Ca^{2+} and Na^{+}. The strength of the cell coat varies from cell to cell. For example, the cell coat of the intestinal epithelium is quite strong—it resists vigorous mechanical and chemical attacks; in other cells the coat is labile and may be depleted by washing or enzyme exposure (**Luft**, 1976).

The cell coat is the secretion product of the cell that is incorporated into the cell surface and undergoes continuous renewal. As already discussed, the glycoproteins of glycocalyx are synthesized on the ribosomes of RER and their final assembly with oligosaccharide moiety is attained in the Golgi apparatus.

Extracellula Materials

In certain cells, outside the cell coat proper and the fuzzy layer exist the **extracellular materials**, *e.g.*, jelly coat of eggs of fishes and amphibians, the basal laminae of epithelia, the matrix material in which cartilage and bone cells are embedded and the cell wall of plant cells. In these extracellular materials the most conspicuous components are **collagens** and **mucopolysaccharides** (glycosaminoglycans).

Functions of Cell Coat

In addition to the protection of the plasma membrane, the cell coat performs the following important functions :

(i) Filtration. The extraneous coats sometimes act as filters. For instance, the extraneous coats surrounding the blood capillaries of most vertebrates, especially the kidney glomerulus act as filter and regulate the passage of molecules through it. The extracellular coats of connective tissues contain the chemical compound **hyaluronate** which controls the diffusion.

(ii) Maintenance of the micro-environment of the cell. The extraneous coats of animal cells can affect the concentrations of different substances at the surface of the cell. For example, a muscle cell with its excitable plasma membrane which is surrounded by a glycocalyx is found to maintain the micro-environment of muscle cell by trapping the sodium ions.

(iii) Enzymes. The cell coat of intestinal microvilli are found to contain a variety of enzymes which are involved in the terminal digestion of carbohydrates and proteins. For example it contains the enzyme alkaline phosphatase.

(iv) Immunological properties of the extraneous coats. Some substances of extraneous coats provide immunological properties to the cell. As for instance the plasma membrane of mammalian erythrocytes is found to contain some specific, genetically determined substances (carbohydrates and proteins) corresponding to the A, B and O blood groups. The major sialoglycoproteins of the red blood cell membrane carry the M and N antigens that appear infrequently in man. The cell coat also contains the receptor sites for the influenza virus and for various lectins.

(v) Histocompatibility. The cell coats of some cells contain some antigens which provide **histocompatibility**, *i.e.*, they permit the recognition of the cells of one organism and rejection of other cells that are foreign to it (*e.g.*, the rejection of grafts from another organism).

CELL WALL

The plant cell is always surrounded by a **cell wall** and this feature distinguishes them from animal cells. The cell wall is a non-living structure which is formed by the living protoplast (A plant cell without its cell wall is called a **protoplast** ; **Alberts** *et al*.,1989). In most of the plant cells, the cell wall is made up of cellulose, hemicellulose, pectin and protein. In many fungi, the cell wall is formed of chitin and in bacteria, the cell wall contains protein-lipid-polysaccharide complexes. Thus, the cell wall is a rigid and protective layer around the plasma membrane which provides the mechanical support to

the cell. The cell wall also determines the shape of plant cells. Due to the shape of cell walls many types of plant cell as the parenchymatous, collenchymatous, etc., have been recognised.

Chemical Composition

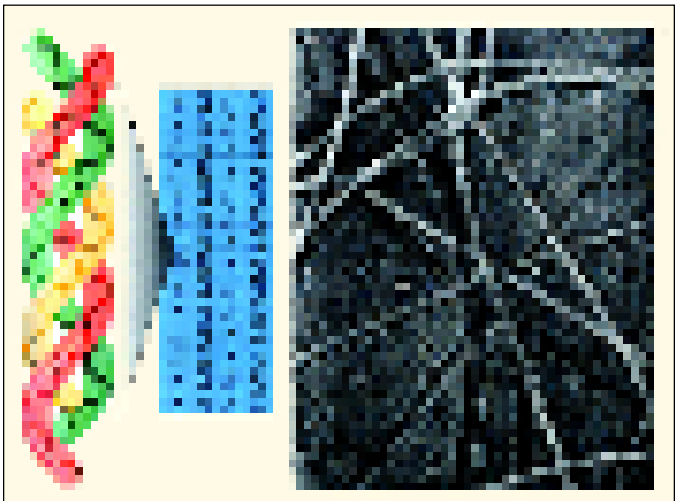
Chemically speaking, the plant cell wall is composed of a variety of polysaccharides (carbohydrates), lipids, proteins and mineral deposits, all exhibiting distinct staining reactions (Table 5-5).

Table 5-5. **Chemical nature and staining reaction of various components of plant cell wall.**

Substance	Chemical unit	Staining reaction
1. Cellulose	Glucose	Chlorzinc iodide (stains violet)
2. Hemicellulose	Arabinose, xylose, mannose, glucose and galactose	No specific stain
3. Pectin	Glucuronic and galacturonic acid	Ruthenium red
4. Lignin	Coniferyl alcohol (*e.g.*, hydroxyphenyl propane)	Phloroglucinol hydrochloride (stains rose); chlorzinc iodide (stains yellow)
5. Cuticular substances	Fatty acids	Sudan III (stains orange)
6. Mineral deposits	Calcium and magnesium as carbonates and silicates	–

The polysaccharides of cell wall include cellulose, hemicelluloses, pectin compounds and lignins.

(1) **Cellulose** is a linear, unbranched polymer, consisting of straight polysaccharide chains made of glucose units linked by 1-4 β- bonds (called **glycosidic bonds** ; see Chapter 4) (**Note :** Complete hydrolysis of cellulose yields D-glucose and its partial hydrolysis yields disaccharide units, **cellobiose**.) These are the glucan chains which by intra-and intermolecular hydrogen bonding produce the structural units known as **microfibrils**, observable under electron microscopy and having toughness like the rubber. Each microfibril is ribbon-like flat fibre being 10 nm wide and 3 nm thick (or 25 to 30 nm in diameter) and is composed of about 2000 glucan chains in it. According to a classical estimate, each cellulose microfibril comprises three **micelles** or **elementary fibrils** : each elementary fibril contains about 100 cellulose molecules and each cellulose molecule is made up of 40 to 70 glucan chains (see **Thorpe**, 1984) (*i.e.*, One microfibril = $3 \times 100 \times 70 = 21000$ glucan chains). Often numerous microfibrils get associated to form the **macrofibrils** having up to 0.5 µm diameter and observable under the light microscopy. Cellulose is synthesized by a wide variety of cells that include bacteria (*e.g.*, acetobacter,agrobacter and rhizobium), algae, fungi, cryptogams and seed plants. (2) **Hemicelluloses** are short but branched heteropolymers of various kinds of monosaccharides such as arabinose, xylose, mannose, galactose, glucose and uronic acid. Some of the common hemicelluloses go under the names xylans, arabinoxylans, glucomannans, galactomannans and xyloglucans.



The structure of collagen.

(3) **Pectins** are water soluble, heterogeneous branched polysaccharides that contain many negatively charged D-galacturonic acid residues along with D-glucuronic acid residues. Because of their negative charge, pectins are highly hydrated and intensely bind cations. When Ca^{2+} is added to a solution of pectin molecules, it cross-links them to produce a semirigid gel. Such Ca^{2+} cross-links are thought to help hold cell-wall component together. (4) **Mannan** is a homopolysaccharide of mannose and is found in the cell wall of yeast, fungi and bacteria. (5) **Agar** is a polysaccharide, found in the cell wall of sea weeds and containing D-and L-galactose residues. (6) **Lignin** is a biological plastic and non-fibrous material. It occurs only in mature cell walls and is made of an insoluble hydrophobic aromatic polymer of phenolic alcohols (*e.g.*, hydroxyphenyl propane). (7) The **chitin** is a polymer of glucosamine.

Glycoproteins (present up to 10 per cent in primary cell wall) are hydroxyproline- rich proteins (like the collagen). In them, many short oligosaccharide side chains are attached to hydroxyproline and serine side chains. Thus, more than half the weight of glycoprotein is carbohydrate. These glycoproteins are known to act like the glue to increase the strength of the wall.

Cutin is also a biological plastic and is made of fatty acids (waxes). **Suberin** is a water-resistant substance, comprisig of fatty acids and found in the cork and cell wall of many plants. **Sporopollenin** is a lipoidal polymer forming tough wall (with species-specific patterns) of pollen grains.

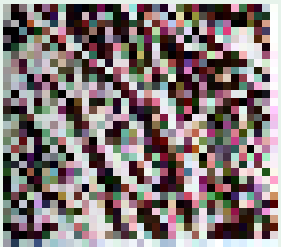
Mineral deposits occur in cuticle in the form of calcium and magnesium carbonates and silicates. Deposits of calcium compounds are found in the cell wall of cruciferous and cucurbitaceous plants. Silicate deposits are common in the cell wall of Graminae family.

Structure

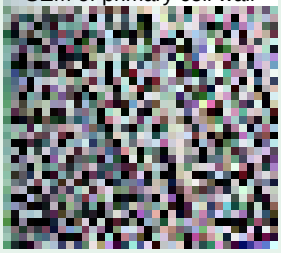
The cell wall is complex in nature and is differentiated in the following layers :

(i) Primary cell wall; (ii) Secondary cell wall; (iii) Tertiary cell wall.

(i) Primary cell wall. The first formed cell wall is known as primary cell wall. It is the outermost layer of the cell and in the immature meristematic and parenchymatous cells it forms the only cell wall. The primary cell is comparatively thin and permeable. Certain epidermal cells of the leaf and the stem also possess the cutin and cutin waxes which make the primary cell wall impermeable. The primary cell wall of the yeast and the fungi is composed of the chitin.



SEM of primary cell wall



SEM of a secondary cell wall

(ii) Secondary cell wall. The primary cell wall is followed by secondary cell wall. The secondary cell wall is thick, permeable and lies near the plasma membrane of the tertiary cell wall, if the latter occurs. It is composed of three concentric layers (S_1, S_2 and S_3) which occur one after another. Chemically the secondary cell wall is composed of compactly arranged macrofibrils of the cellulose, in between which sometimes occurs lignin as a interfibrillar material.

(iii) Tertiary cell wall. In certain plant cells, there occurs another cell wall beneath the secondary cell wall which is known as tertiary cell wall. The tertiary cell wall differs from the primary and secondary cell wall in its morphology, chemistry and staining properties. Besides the cellulose, the tertiary cell wall consists of another chemical substance known as the xylan.

Middle lamella. The cells of plant tissues generally remain cemented together by an intercellular matrix known as the **middle lamella**. The middle lamella is mainly composed of the pectin, lignin and some proteins.

Ultrastructure

Electron microscopy has shown that the cell wall is constructed on the same architectural principle which applied well in the construction of animal bones and such common building materials as fibre glass (plastic + glass fibres) or reinforced concrete (concrete + metal framework), *i.e.*, strong fibres (*e.g.*, cellulose microfibrils) resistant to tension embedded in an amorphous matrix (comprising hemicellulose, pectin and proteins) resistant to compression. In the primary cell wall, the fibres and matrix molecules are cross-linked by a combination of covalent bonds and non-covalent bonds to form a highly complex structure whose composition is generally cell-specific (Fig. 5.28). In fact, hemicellulose molecules (*e.g.*, **xyloglucans**) are linked by hydrogen bonds to the surface of the cellulose microfibrils. Some of these hemicellulose molecules are cross-linked in turn to acidic pectin molecules (*e.g.*, rhamnogalacturonans) through short neutral pectin molecules (*e.g.*, arabinogalactans). Cell wall glycoproteins are tightly woven into the texture of the wall to complete the structure of matrix.

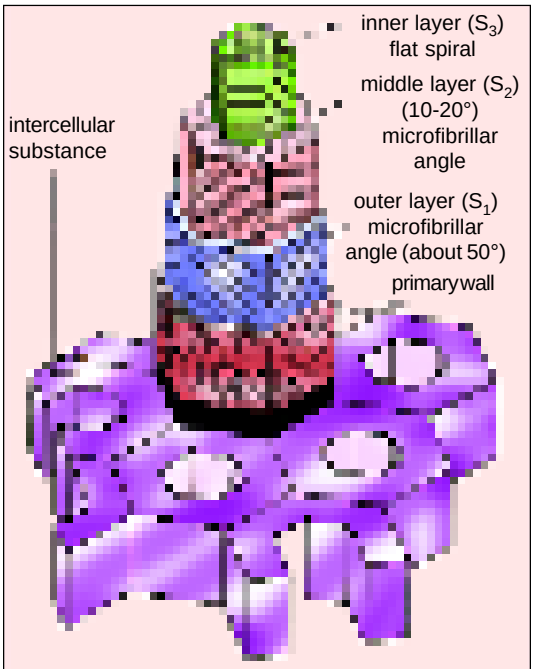


Fig. 5.27. Structure of a cell wall showing middle lamella, primary cell wall and three regions (S_1, S_2 and S_3) of the secondary cell wall (after Thorpe, 1984).

In the multilamellar secondary cell wall, cellulose microfibrils are laid down in layers, the microfibrils of each layer running roughly parallel with each other but at an angle to those in other layers (Fig. 5.27).

Plasmodesmata. Every living cell in a higher plant is connected to its living neighbours by fine cytoplasmic channels, each of which is called a **plasmodesma** (Gr., *desmos* = ribbon, ligament; plural, **plasmodesmata**) which pass through the intervening cell walls. The plasma membrane of one cell is continuous with that of its neighbour at each plasmodesma. A plasmodesma is a roughly cylindrical, membrane-lined channel with a diameter of 20 to 40 nm. Running from cell to cell through the centre of most plasmodesmata is a narrower cylindrical structure, the **desmotubule**, which remains, continuous with elements of the SER membranes of each of the connected cells. Between the outside of the desmotubule and the inner face of the cylindrical plasma membrane is an **annulus of cytosol**, which often appears to be constricted at each end of the plasmodemata. These constrictions may regulate the flux of molecules through the annulus that joins the two cytosols (**Gunning**, 1976, 1983).

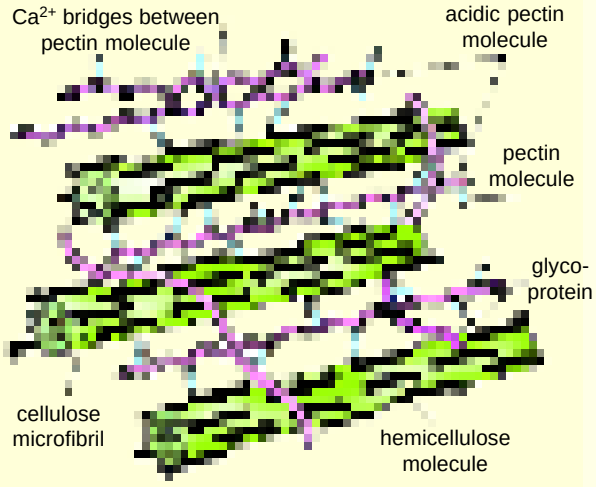


Fig. 5.28. Ultrastructure of primary cell wall showing interconnections between the two major components of the primary cell wall : the cellulose microfibrils and the matrix (after Alberts *et al.*, 1989).

Plasmodesmata are formed around the elements of smooth endoplasmic reticulum that become trapped during cytokinesis (of mitotic cell division) within the new cell wall that will bisect the parental cell. Plasmodesmata function in intercellular communication, *i.e.*, they allow molecules to pass directly from cell to cell. For example, plasmodesmata are especially common and abundant in the walls of columns of cells that lead toward sites of intense secretion, such as in nectar-secreting glands (trichomes of *Abutilon* nectaries). In such cells there may be 15 or more plasmodesmata per square micrometer of wall surface, whereas there is often less than 1 per square micrometer in other cell wall (**Gunning** and **Hughes**, 1976).

In fact, experimental evidence has suggested that the plasmodesmata mediate transport between adjacent plant cells, much as gap junctions of animal cells. They allow the passage of molecules with molecular weights of less then 800 daltons. Transport through the plasmodesmata is also found under complex regulations which may involve Ca^{2+} and protein phosphorylation. Thus, no dye movement is observed between the cells of the rooot cap and the root apex or between epidermal and cortical cells in either roots and shoots. However, certain plant viruses such as TMV can enlarge plasmodesmata in order to use this route to pass from cell to cell. Tobacco mosaic virus is known to synthesize a protein, called **P30** (30,000 dalton M.W.) that nullifies the normal regulatory mechanisms of plasmodesmata (**Zaitlin** and **Hull**, 1987).

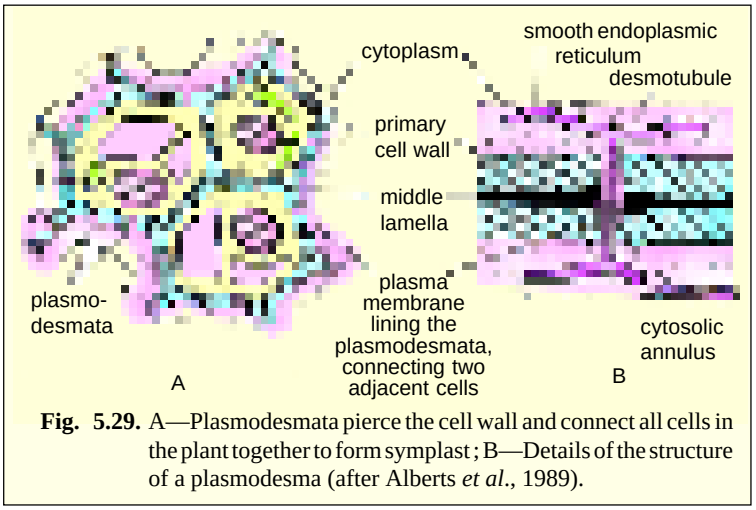
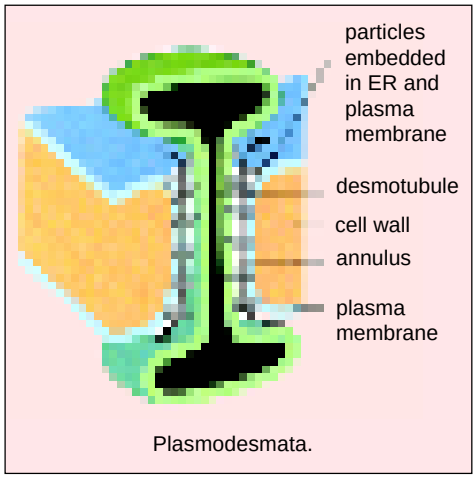


Fig. 5.29. A—Plasmodesmata pierce the cell wall and connect all cells in the plant together to form symplast ; B—Details of the structure of a plasmodesma (after Alberts *et al*., 1989).



Plasmodesmata.

Lignification. The structure of cell wall is stabilized by the deposition of lignin in the cell wall matrix. Such a process of lignification was required in connection with the transition from aquatic to the terrestrial plant life during organic evolution of plants. A lignified cell wall is composed of microfibrils of cellulose embedded in the matrix containing large amount of lignin. Usually the primary cell wall becomes more lignified than secondary cell wall.

Functions of Cell Wall

The chief function of cell wall in plant cells is that it provides mechanical strength to the latter. Like the exoskeleton or endoskeleton of animals, cell wall acts like a skeletal framework of plants. Particularly in vascular plants, the cell walls provide the main supporting framework.

Despite its strength, the plant cell wall is fully **permeable** to water and solutes. This is because the matrix is riddled with minute water-filled channels through which free diffusion of water and water soluble substances such as gases, salts, sugars, hormones and like can take place. Moreover, the molecules of the matrix are strongly hydrophilic ("water-loving") with the result that in normal

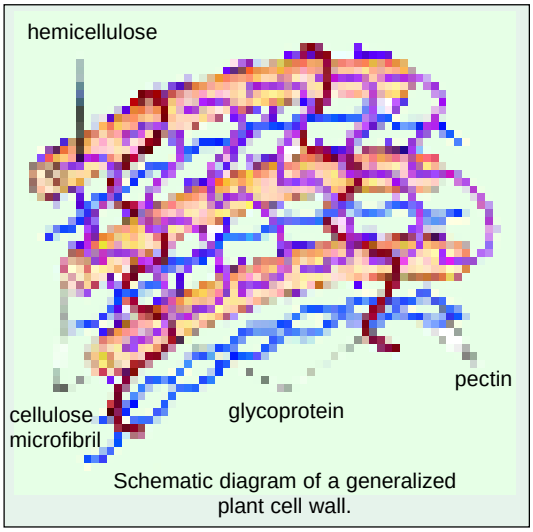
circumstances the cell wall is saturated with water like a sponge (*e.g.*, primary cell wall is 60 per cent water by weight). The cross-linked structure of the cell wall is, however, found to slightly impede the diffusion of small molecules such as water, sucrose and K^+. The average diameter of the spaces between the cross-linked macromolecules in most cell wall is about 5 nm, this is small enough to make the movement of any globular macromolecules with a M.W. much above 20,000 daltons extremely slow. Therefore, plants must subsist on molecules of low molecular weight, and any intercellular signaling molecules that have to pass through the cell wall must also be small and water soluble. In fact, most of the known plant signaling molecules, such as growth regulating substances—auxins, cytokinins and gibberellins—have molecular weights of less than 500 daltons.

During lignification, lignin is deposited in spaces between the cellulose molecules, making the cell wall much more rigid, and rendering it **impermeable**. Once lignification is complete the protoplasm can no longer absorb materials from outside the cell, which, therefore, dies. Hence, *lignified tissue is always dead*. Thus, a lignified tissue becomes well adapted for two types of functions: (1) It provides the mechanical strength due to its ligno-cellulose composition. (2) It transports water and salts, since, lignification involves loss of the protoplasm resulting in the formation of a hollow waterproof tube.

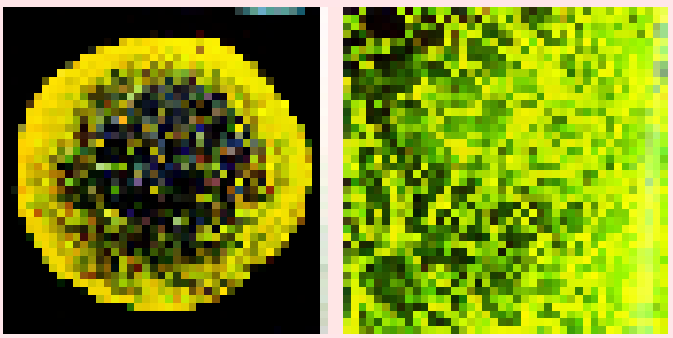
Origin and Growth of Cell Wall

The mechanism of cell wall formation includes the following steps :

1. Formation of matrix. Most cell wall matrix components are transported via vesicles derived from the Golgi apparatus and secreted by exocytosis at the plasma membrane. Golgi apparatus of the plant cells is involved mainly in producing and secreting a very wide range of extracellular polysaccharides, rather than the glycoproteins typical of animal cells. This is perhaps due to following facts : (1) Each of the polysaccharides of cell wall matrix is made of two or more sugars ; (2) At least 12 types of monosaccharides are used in their polymerization ; (3) Most of these polysaccharides are branched ; and (4) Many covalent modifications are introduced in the polysaccharides after they are synthesized. It is estimated that several hundred different enzymes are engaged in the assembly of the polysaccharide components needed to form a typical primary cell wall. Most of these enzymes are found in the endoplasmic reticulum and Golgi apparatus. Some enzymes, which are concerned with later covalent modifications of the polysaccharides, are found in the cell wall itself.



Schematic diagram of a generalized plant cell wall.


Cell wall growth studied in a moss leaf cell; progressive growth of cell wall microfibrils after 4 hours (left) and after 10 hours (right).

Since, the cell wall varies in composition and morphology at different locations around the cell, the Golgi-derived vesicles are di-

rected at specific regions of the plasma membrane by the help of cytoskeleton (*i.e.*, microtubules and microfilaments). For example, we can consider the case of formation of new primary cell wall that separates two daughter cells after the karyokinesis of mitosis. At the end of telophase, a barrel-shaped or disc-like region, called **phragmoplast** (Gr., *phragma* = hedge, enclosure; *plasso* = to form) forms in the plane of former spindle equator. The phragmoplast comprises a double-ring of short microtubules on either side of, and terminating at, the division plane, and a set of microfilaments are coaligned with the microtubules. Golgi derived vesicles containing cell wall precursors, especially **pectin**, are guided inward along these oriented microtubules until they reach the phragmoplast, where they fuse with one another to form the **cell plate** (Fig. 5.30). Cell plate, at this stage, comprises following structures : (1) central middle lamella; (2) primary cell walls on both sides of middle lamella ; and (3) plasma mem-

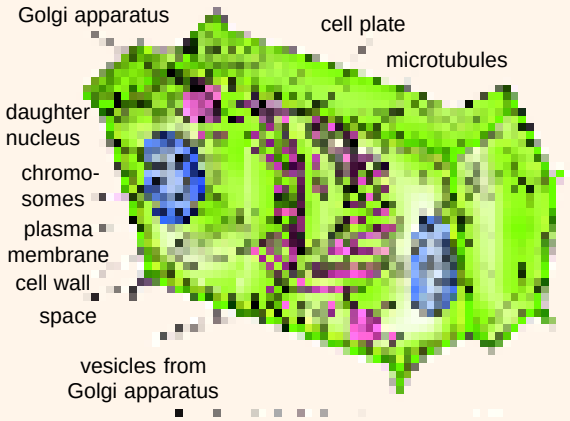


Fig. 5.30. Schematic representation of a cell of a higher plant as seen at telophase in mitosis. In the phragmoplast region, a region of membranes and microtubules, a cell plate forms and grows until it separates the cytoplasm into two daughter cells. The cell plate develops as a membrane-delimited structure enclosing a space in which new cell-wall will form. The Golgi apparatus contributes many vesicles to the phragmoplast membrane; the vesicle membranes apparently are incorporated into the membrane of the cell plate and the vesicle contents enter the forming cell wall (after Novikoff and Holtzman, 1970).

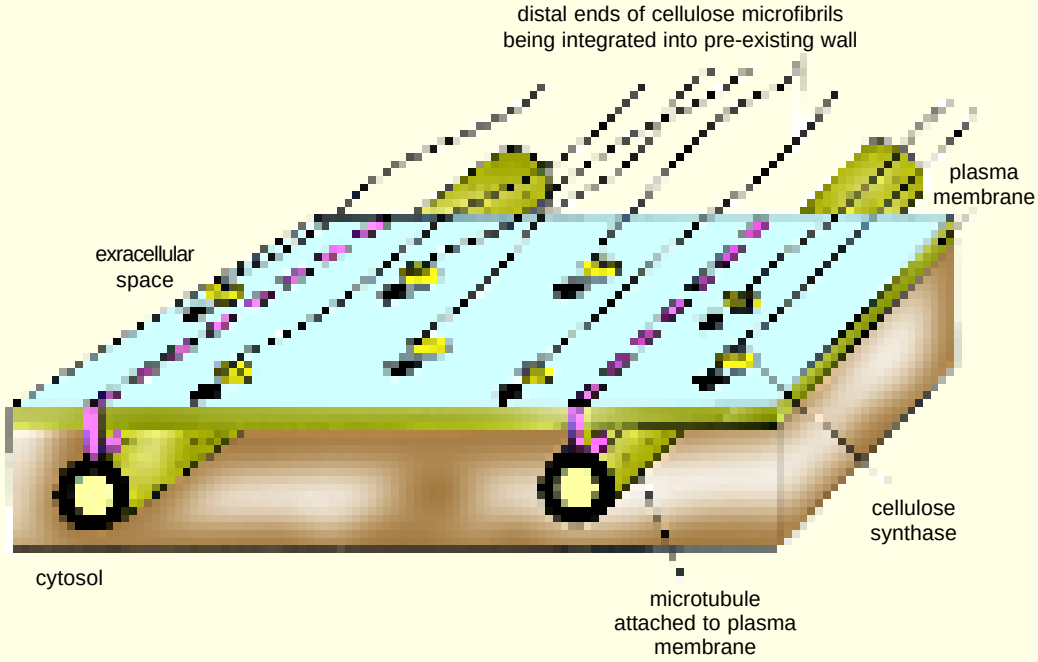
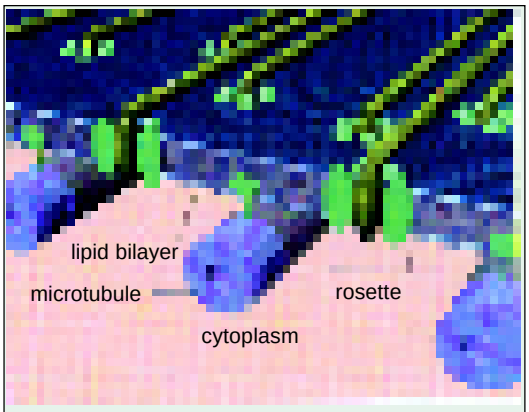


Fig. 5.31. A model explaining the mode of orientation of newly deposited cellulose microfibrils according to orientation of cortical microtubules (after Alberts *et al*, 1989).

brane lining cytoplasm of each daughter cell. Ultimately, the microtubular ring moves centrifugally outward as Golgi vesicles continue to add precursors to the growing cell plate. The cell plate fuses with the mother cell wall to create two separate daughter cells. It is not clear which component of the phragmoplast—the microtubules or the actin filaments (or both)—are responsible for the movement and guidance of the Golgi derived vesicles (see **Alberts** *et al*, 1989).

2. Synthesis and orientation of cellulose microfibrils. In most plants, cellulose is synthesized on the external surface of the cell by a plasma membrane bound enzyme complex, called **cellulose synthetase** which uses a sugar nucleotide precursor supplied from the cytosol, probably UDP- glucose (**Delmer**, 1987). As they are being synthesized, the nascent cellulose chains spontaneously assemble into **microfibrils** that form a layer on the surface of the plasma membrane (a lamella) in which all the microfibrils have more or less the same alignment (**Note.** Each cellulose molecule has a polarity, having a 1' and a 4' end). Cellulose synthetase complexes are thought to be associated with the ends of growing microfibrils and the sugars present in the extracellular matrix are polymerized into cellulose at these "terminal complexes". Extension of a cellulose microfibril is presumably achieved by lateral movement of the enzyme complex in the fluid phase of plasma membrane, with the microfibril "spun out" on the outer surface of the membrane behind the moving enzyme complex (Fig. 5.31). The direction in which the complex moves and the orientation of the microfibril depend on some interactions between the membrane complex and the underlying cytoplasmic microtubules (*i.e.*, microtubules of cell, cortex, **Herth**, 1985). Because the cellulose is synthesized at the plasma membrane, each new wall lamella forms internally to the last formed lamella. The cell wall, therefore, consists of concentrically arranged lamellae, with the oldest on the outside (**Brown**, 1985).



A model of cellulose fibril deposition

REVISION QUESTIONS

1. Describe various models of plasma membrane and explain which of these models is dynamic and why ?
2. Describe the 'Fluid mosaic model' of the plasma membrane. On the basis of this model explain different functions of the plasma membrane.
3. Describe the chemical composition of plasma membrane.
4. Discuss in detail various functions of the plasma membrane.
5. What is cell wall ? Describe the chemical composition, structure, origin and function of the plant cell wall.
6. Write short notes on the following : 1. Cell wall : its structure and function ; 2. Desmosome ; 3. Active transport ; 4. Endocytosis ; 5. Ion pumps ; 6. Nexus ; 7. Receptor-mediated endocytosis ; 8. Plasmodesmata.
7. Differentiate between the following :
   (i) Phagocytosis from pinocytosis ;
   (ii) Passive transport from active transport ;
   (iii) Macula adherens from macula occludens ;
   (iv) Primary cell wall from secondary cell wall.
8. Draw a well labelled diagram of "Fluid mosaic model" of the plasma membrane.
9. Describe the mode of origin and growth of cell wall.