CELL BIOLOGY AND MEMBRANE BIOCHEMISTRY

Membrane Biochemistry

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Keywords
Biological membrane, membrane components, lipid vesicles, liposomes, dynamic properties of membranes, lipid rafts, caveolae, fluid–mosaic model; extra cellular membranous structures, Transporters, pumps, channels, Group Translocase, aquaporins or water channels, Constitutive and regulated exocytosis, Receptor mediated endocytosis, Cell Signaling, Receptors, second messengers, Adenergic and Nicotinic Receptors, Receptor Tyrosine Kinase, Receptor Guanylate cyclase, cytokine receptor super family.
Membrane biochemistry deals with the structure, function and physiological significance of biological membranes. Some of these aspects are discussed here.

**Biological Membranes**

Biological membrane is the barriers that separate the cellular content of the cell or that of an organelle from its environment. This barrier is essential for the cell/organelle to sustain life and maintain its identity. Biological membranes are not just inert barriers but are dynamic, semi permeable and have a number of biochemical and physiological functions.

There are two types of biological membranes depending on their location in the cell.

**I. Plasma membranes or cell membranes**

This is present in all cells of the prokaryotes and eukaryotes and encloses the cellular contents and defines the boundary of the cell (Fig. 1).

**II. Intracellular membranes or internal membranous structure**

Eukaryotes contain extensive intracellular membranes that segregate specific regions from the cytoplasm- the sub cellular organelles. These inter cellular membranes in animal cells include those of nucleus, endoplasmic reticulum, golgi apparatus, mitochondria, lysosomes, peroxisomes and vacuoles. Plant cells, in addition to the above, have those of chloroplasts. These intracellular membranes separate the cells into compartments with an identity of their own to carry out specific functions. However, they remain in contact with the cytoplasm of the cell through specific proteins in these membranes. The plasma membrane is ~10% of the total membranes in the eukaryotic cells.

Although prokaryotes lack these intracellular membrane systems, their plasma membrane may be in folded to form structures know as mesosomes. The physiological/biochemical functions associated with the intracellular membranes of eukaryotic organelles are performed by plasma membranes in prokaryotes. However, the photosynthetic bacteria contain internal membrane around the vesicles that contain proteins and photosynthetic assemblies involved in light reactions and initial steps of photosynthesis.

Besides these urological membranes, most cells synthesize and secrete coats of one kind or another that are external to the cell membrane - the extra cellular or external membranous structures/surfaces. These include: cell walls, calyx, fuzzy coat and desmosomes, tight junctions, etc, present in the prokaryotic, plant and some animal cells. These have a specialized structure and a supportive role.

**Important Functions carried out by Plasma and Intracellular Membranes**

Different biological membranes are associated with various functions. To innumerate:

- Permit and in some cases enhance the absorption of essential nutrients into the cell while preventing the diffusion of needed metabolites e.g. Biological Transport, absorption through intestinal epithelial cells and endocytosis.
Fig. 1: Schematic view of (a) typical animal (b) plant
Secrete cellular products eg. excretion through kidney epithelial cells.
Keep out toxic material.
Carry out exocytosis and endocytosis.
Maintain intracellular pH, ionic concentration
Carry out energy transduction processes such as oxidative phosphorylation by mitochondrial inner membrane and photophosphorylation by chloroplast thylakoid membranes.
Control the flow of information between the cells - Signal Transduction - via specific receptors for hormones, neurotransmitters and other cell signals. eg.. Role in nerve impulse transmission, muscle contraction, hormone action etc.
Hold the cells together by cell: cell interactions.
Role in cell recognition and adhesion.
Bind certain cellular constituents particularly enzymes/ proteins in an advantageous location for their specific biochemical functions e.g. Electron transport carriers in mitochondrial and thylakoid membranes, transport proteins in plasma membrane and specific orientation of membranes enzymes.
Compartmentation enables diverse metabolic processes, many of which are incompatible with one another to occur in the cell and thereby prevent futile metabolic cycle e.g. glycolysis (break down of glucose to pyruvate) occurs in cytosol whereas specific gluconeogenic enzymes are located in mitochondria and lysosomes. Thus, Hexokinase, a soluble enzyme, is involved in phosphorylating glucose to glucose-6-phosphate and glucose phosphatase hydrolyzing glucose-6-phosphate to glucose is present in lysosomes.

The supportive, semi permeability, transport (by transport proteins, channels and pumps), compartmentation roles, signal transduction (through receptor proteins), cell: cell recognition and adhesion, exocytosis and endocytosis, essential to the physiological functions of the cell, indicate a complex and a dynamic nature of the biological membranes.

**Common Features of Biological Membranes**

In spite of such diversity in function, the biological membranes i.e. the plasma and intracellular membranes have a number of common characteristic features. To enumerate, they are:
- Few molecules thick (6-10nm),
- Bimolecular sheets with lipids forming a bilayer,
- Closed boundary,
- Dynamic,
- Cooperative,
Lipid-protein assemblies held together by noncovalent interactions.
Self annealing,
Asymmetric,

Each of the lipid bilayer is referred to as a monolayer or leaflet; the exterior is known as the exoplasmic face and the interior as the cytoplasmic face of the membrane.

Membranes, also, contain varying amounts of carbohydrates (0 to 10%) linked covalently to lipids as glycolipids or to proteins as glycoproteins.

Membranes differ in composition and presence of specific proteins which are responsible for their specific functions.

Structure of the Biological Membranes

When purified membrane is treated with organic solvents to extract lipids, the proteins are separated. When the lipids are added back to the proteins under controlled conditions, the membranes can be reconstituted. This simple experiment showed the involvement of noncovalent interactions in stabilizing the membranes. These noncovalent interactions are between lipids: lipids, lipids: proteins and proteins: proteins.

The lipids, proteins as well as the carbohydrates content of most membranes has been studied. The chemical composition of some purified plasma and intracellular membranes are given in Tables 1 and 2. The ratio of protein to the lipid content is characteristic of the membranes. The specific proteins present in the membrane are responsible for its characteristic biochemical function. The higher the biochemical activity of the membrane, the greater is its protein content. Thus depending on their biochemical activity and lipid/protein ratio, there is the simple membrane characterized by the myelin sheath which functions to insulate the nerve axon and is a semi permeability barrier and is relatively biochemically inert. Its lipid content is 80% and the protein 20%. The intermediate one is that of the plasma membrane of eukaryotic cells which in addition to being a semi permeability barrier, has transport functions and receptors for signal transduction etc. where the lipid and the protein content of these membranes is nearly equal. The more complex membranes are those of mitochondrial inner membrane, thylakoid membranes of the chloroplast and bacterial plasma membranes which have 20-30% lipid and 80-70% protein content, consistent with their high biochemical activity.

Components of Biological Membranes

Since the major components of biological membranes are lipids and proteins and to some extent carbohydrates, their structure and properties are discussed below in a bid to understand the complex structure of membranes:

Lipids

Lipids are insoluble in water and soluble in organic solvents. They are amphipathic molecules with a hydrophobic and a hydrophilic region.
Table 1: Chemical composition of some cell membranes
(Source: Zubay, G. Biochemistry, 1984)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Membrane</th>
<th>Protein (%)</th>
<th>Lipid (%)</th>
<th>Carbohydrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Myelin</td>
<td>18</td>
<td>79</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>Human erythrocyte plasma membrane</td>
<td>49</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>3.</td>
<td>Amoeba plasma membrane</td>
<td>54</td>
<td>42</td>
<td>4</td>
</tr>
<tr>
<td>4.</td>
<td><em>Mycoplasma</em> cell membrane</td>
<td>58</td>
<td>37</td>
<td>1.5</td>
</tr>
<tr>
<td>5.</td>
<td><em>Halobacterium</em> purple membrane</td>
<td>75</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Protein and Lipid content of Organellar membranes
(Source: Zubay, G. Biochemistry, 1984)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Membrane</th>
<th>Approximate Protein/Lipid Ratio (wt/wt)</th>
<th>Approximate Cholesterol/Other Lipids (Molar Ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Golgi apparatus</td>
<td>0.7</td>
<td>0.08</td>
</tr>
<tr>
<td>2.</td>
<td>Liver plasma membrane</td>
<td>1.0</td>
<td>0.40</td>
</tr>
<tr>
<td>3.</td>
<td>Endoplasmic reticulum</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>4.</td>
<td>Mitochondrial outer membrane</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>5.</td>
<td>Mitochondrial inner membrane</td>
<td>3.0</td>
<td>0.03</td>
</tr>
<tr>
<td>6.</td>
<td>Nuclear membrane</td>
<td>3.0</td>
<td>0.11</td>
</tr>
<tr>
<td>7.</td>
<td>Lysosomal membrane</td>
<td>3.0</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The major classes of lipids present in biological membrane are:

1. Fatty Acids
2. Glycerophospholipids
3. Sphingolipids
4. Sterols

The other lipids are:
5. Galactolipids and Sulpholipids present specifically in thylakoid membranes in chloroplasts and
6. Glycerol Di Alkyl Tetraether Lipids present only in Archaebacteria.
Fatty Acids

Fatty acids are not present free in biological membranes but as components of phospholipids and galactolipids etc. The fatty acids present in most membrane are

\[ \text{CH}_3-(\text{CH}_2)_n-\text{COOH} \]

Where \( n \) = 12 to 22

The alkyl chains can be saturated or unsaturated. If the fatty acids are unsaturated, the configuration around the double bond is \textbf{cis} in most cases and the number of double bonds in a fatty acid molecule can be 1 to 6. The common fatty acids found in membranes are:

- Myristic acid (C14:0)
- Palmitic acid (C16:0)
- Stearic acid (C18:0)
- Oleic acid (C18:1)
- Linoleic acid (C18:2) and Arachidonic acid (C20:4)

In thylakoid membranes of chloroplasts, the major fatty acid is trans-hexadecanoic acid. In some bacteria, cyclic or branched alkyl chains are present in the fatty acids. Fatty acid is the simplest of the amphipathic lipid molecule where the –COOH group represents the hydrophilic and the alkyl chain, the hydrophobic region. Fatty acids have an important role in the lipid bilayer and are responsible for the barrier properties and the dynamic properties of the membrane.

Glycerophospholipids

Glycerol phospholipids are the major constituents of biological membranes in animal, plant and bacteria. The basic structure of glycerol phospholipids is depicted in Table 3. The two fatty acids are attached in ester linkage to C-1 and C-2 of glycerol-3-phosphate and ‘X’ is an alcohol in ether linkage to phosphate group. The common phospholipids are phosphatidic acid (PA), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS) and phosphatidyl inositol (PI). The two fatty acid chains at C-1 and C-2 of glycerol can be the same or different. One of them, usually at C-1 is saturated while the other at C-2 is unsaturated or cyclic fatty acid (Fig. 2). The chain length and the number of double bonds in the two fatty acyl chains can vary as also the nature of the ‘X’ group. Thus, different combinations of phospholipids molecules can exist in a membrane and their number is very large.

The phospholipids are depicted with a head group comprising of the phosphate along with its attached ‘X’ group and the fatty acyl chains as two tails.

Sphingolipids

Sphingolipids are the second major constituents of the membranes. They are derivatives of sphingosine (Table 4) when the amino group is linked to a fatty acid by an amide linkage, a class of sphingolipids called Ceramides is formed. In sphingomyelin (Sph), a phosphocholine group is attached at C-1 hydroxyl group (Table 4). In some membranes, the lipid, Ceramide, contains saccharides as the ‘head’ group and are known as Glycosphingolipids which constitute the third major class of membrane lipids (Table 4). The glycosphingolipids include Cerebrosides, Gangliosides and are common in brain and nerve cells.
### Table 3: Major Classes of Phospholipids

<table>
<thead>
<tr>
<th>X Substituent</th>
<th>Name of Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Phospatidic acid</td>
</tr>
<tr>
<td>Choline</td>
<td>Phosphatidylcholine (lecithin)</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>Serine</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Phosphatidylglycerol</td>
</tr>
<tr>
<td>Phosphatidyl-glycerol</td>
<td>Diphaspatidylglycerol (cardiolipin)</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>Phosphatidylinositol</td>
</tr>
</tbody>
</table>

(a) Bovine lecithin containing palmitoyl group at C-1 and an oleoyl group at C-2 position of glycerol; (b) A bacterial phospholipid containing a palmitic acid at C-1 position and a cyclopropane fatty acid at the C-2 position
Sterols

Another important constituent of many membranes in animals is Cholesterol (Fig. 3). The fused cyclohexane rings and the hydrocarbon tail attached to one end provide the bulky rigid structure with the hydrophobic while the -OH group at the other end the hydrophilic nature to the molecule. In many membranes, the cholesterol is oriented parallel to the fatty acyl chains of the phospholipids and the OH-group interacts with the hydrophilic groups of the adjacent lipids.

Cholesterol is found in varying degrees in all animal cell membranes but is essentially absent from intracellular membranes. It is also absent in prokaryotes. The plant membranes contain Stigmasterol and the fungi Ergosterol.

Galactolipids and Sulpholipids

They are present predominantly in plant cells in the thylakoid membranes where they constitute the major lipids (70-80%) (Fig. 4).

Table 4: Sphingolipids

<table>
<thead>
<tr>
<th>Name of sphingolipid</th>
<th>Name of X</th>
<th>Formula of X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceramide</td>
<td>—</td>
<td>— H</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>Phosphocholine</td>
<td>P—O—CH$_2$—CH$_2$—N(CH$_3$)$_3$</td>
</tr>
<tr>
<td>Neutral glycolipids</td>
<td>Glucosylceramide</td>
<td>Glucose</td>
</tr>
<tr>
<td>Glucosylceramide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactosylceramide (α-globoside)</td>
<td>Di-, tri-, or tetrasaccharide</td>
<td>Glc—Gal—Neu5Ac—GalNAc</td>
</tr>
<tr>
<td>Ganglioside GM2</td>
<td>Complex oligosaccharide</td>
<td>Glc—Gal—GalNAc</td>
</tr>
</tbody>
</table>
Fig. 3: Cholesterol

Fig. 4: Glycolipids of chloroplast membrane
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)
Glycerol Dialkyl Tetra Ether Lipids

These are characteristically present in Archaebacteria. Two very long branched alkyl chains (32 carbon) are ether linked to glycerol at both ends (Fig. 5). The ether linkage is very stable and enables the bacteria to maintain its structure and survive at high temperatures and high salt concentrations. The glycerol moiety in these lipids is in ‘R’ configuration and not the ‘S’ configuration found in eukaryotes and eubacteria. Archaebacterial lipids differ in the substituents on the glycerols.

![Fig. 5: A typical membrane lipid of Archaebacteria](Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

The composition of individual lipids in almost all membranes has been determined and varies from one membrane to another. The lipid composition of some membranes is shown in Table 5. It is genetically determined and is constant for a membrane type. However, fatty acid component can be influenced by diet in case of animals and by culture conditions in case of prokaryotes.

**Table 5: Lipid Compositions of Membrane Preparations**

*(Source: Zubay, G. Biochemistry, 1984)*

<table>
<thead>
<tr>
<th>Source</th>
<th>Cholesterol</th>
<th>PC</th>
<th>SM</th>
<th>PE</th>
<th>PI</th>
<th>PS</th>
<th>PG</th>
<th>DPG</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic membrane</td>
<td>30.0</td>
<td>18</td>
<td>14.0</td>
<td>11</td>
<td>40</td>
<td>9.0</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Endoplasmic reticulum (rough)</td>
<td>6.0</td>
<td>55</td>
<td>5.0</td>
<td>3.0</td>
<td>16</td>
<td>8.0</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endoplasmic reticulum (smooth)</td>
<td>10.0</td>
<td>55</td>
<td>12.0</td>
<td>21</td>
<td>6.7</td>
<td>-</td>
<td>-</td>
<td>1.9</td>
<td>-</td>
</tr>
<tr>
<td>Mitochondria (inner)</td>
<td>3.0</td>
<td>45</td>
<td>2.5</td>
<td>25</td>
<td>6.0</td>
<td>1.0</td>
<td>2.0</td>
<td>18.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Mitochondria (outer)</td>
<td>5.0</td>
<td>50</td>
<td>3.0</td>
<td>23</td>
<td>13.0</td>
<td>2.0</td>
<td>2.5</td>
<td>3.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Nuclear membrane</td>
<td>10.0</td>
<td>55</td>
<td>3.0</td>
<td>20</td>
<td>7.0</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Golgi</td>
<td>7.5</td>
<td>40</td>
<td>10.0</td>
<td>15</td>
<td>6.0</td>
<td>3.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>14.0</td>
<td>25</td>
<td>24.0</td>
<td>13</td>
<td>7.0</td>
<td>-</td>
<td>-</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>Rat brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myelin</td>
<td>22.0</td>
<td>11</td>
<td>6.0</td>
<td>14</td>
<td>-</td>
<td>7.0</td>
<td>-</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>Synaptosome</td>
<td>20.0</td>
<td>24</td>
<td>3.0</td>
<td>20</td>
<td>8.0</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Rat erythrocyte</td>
<td>24.0</td>
<td>31</td>
<td>8.5</td>
<td>15</td>
<td>2.2</td>
<td>7.0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Rat red cell (outer segment)</td>
<td>3.0</td>
<td>41</td>
<td>-</td>
<td>37</td>
<td>20</td>
<td>13.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. coli cytoplasmic membrane</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>15.0</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em> cytoplasmic membrane</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>69</td>
<td>-</td>
<td>-</td>
<td>30.0</td>
<td>1.0</td>
<td>-</td>
</tr>
</tbody>
</table>

*PC = phosphatidylcholine, SM = sphingomyelin, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PS = phosphatidylserine, PC = phosphatidylethanolamine, DPG = diphasmatidylglycerol (cardiolipin), PA = phosphatidic acid.*
The phospholipids and galactolipids readily form bilayers or bimolecular sheets in aqueous media and are responsible for the barrier function and impermeability to ionic and most polar molecules in the membranes.

**Micelles, Bilayers and Liposomes**

An inherent property of the lipid molecules is to expel water and close upon itself. When salts of fatty acids or soap molecules or phospholipids are added drop wise to aqueous medium (water), they form monolayers initially, as the hydrophilic head interacts with water at the air-water interface and the hydrophobic tail faces outwards to the air. As the concentration of lipid molecules increases, they tend to close and expel water to form MICELLES (Fig. 6) where the hydrophilic heads interact with the aqueous medium and the tails with each other with some aqueous solution trapped inside the micelles which are thermodynamically stable.

Similar micellar structures are also formed by lysophosphatidic acid or lysophospholipids (phospholipids with one fatty acid attached to C-1of glycerol) i.e. all phospholipids with one tail. The micellar structure is favored when the hydrophilic area of the lipid molecule is greater than its hydrophobic area.

However, with glycerophospholipids and sphingolipids with two fatty acyl chains, the hydrophobic area of the molecules is equal to that of its hydrophilic region, hence; extensive lipid bilayer sheets are formed. As the hydrophobic edges are in contact with water, the bilayer sheet is transiently unstable and it then forms a hollow sphere or a vesicle called a liposome . These are closed lipid bilayer aggregates forming a compartment with an aqueous medium in the centre. The hydrophilic head groups of phospholipids in each of the lipid bilayer or monolayer or leaflet, interact with the aqueous medium on each side, while the hydrophobic tails point towards each other forming a non polar region, not exposed to the aqueous medium (Fig. 6).

**Preparation of Liposomes**

1. When phospholipids (either extracted from biological sources such as lecithin from egg yolks or soya beans and / or synthetic phospholipids) are suspended in water, they form multilamellar vesicles or lipid arrangements very much like those of an onion rings. On sonication of these multilamellar vesicles, liposomes are formed. Under controlled conditions, the diameter as well as the phospholipid composition of the liposomes can be manipulated.
2. Alternately, injecting an ethanolic solution of phospholipids into water, liposomes with a diameter of ~1000 A can be prepared.
3. A solution of phospholipids dissolved in detergent, followed by dialysis also results in the formation of liposome.
4. Liposomes are very stable and can be isolated from the solution from which they are formed by dialysis, gel filtration chromatography, or centrifugation.
5. Planar bilayer sheets are prepared by spreading a bilayer across a small hole in a partition between two compartments (Fig. 7).
Membrane Proteins

The proteins of biological membranes are broadly grouped into two classes on the basis of the ease with which the proteins can be extracted from the membranes.

Peripheral or Extrinsic Protein
Peripheral proteins can be dissociated from isolated membranes by change in pH, ionic concentrations or treatment with EDTA or high salt concentrations under conditions which disrupt ionic or hydrogen bonds.

Integral or Intrinsic Protein
Integral Proteins, on the other hand, require treatment with reagents such as detergents or organic solvents which disrupt hydrophobic interactions.

Isolation and purification of membrane proteins
The peripheral proteins can be easily isolated from the membranes in aqueous solutions and can be purified by standard purification methods used for proteins and enzymes.

The integral membrane proteins are solubilized by using detergents. Detergents are natural or synthetic amphiphatic molecules. Some of the commonly used detergents in purification and stabilizing membrane proteins and enzymes are shown in Fig. 8. Many detergents disrupt membrane structure and form detergent-lipid and detergent-lipid-protein mixed micelles (Fig. 9). As discussed in an earlier section on micelles and bilayers, the geometry of the detergents is such that they form micellar structures and not bilayers. Since ionic detergents can interact with
hydrophilic regions of the protein and affect catalytic or other activity of the protein, usually nonionic detergents are preferred. Detergent solubilized membrane can be electrophoresed on SDS Page Electrophoresis to give a fair idea of the different proteins present in the membrane.

**Fig. 7: Preparations of planar bilayers**  
(Source: Mathews, C.K and Holde K.E. van. Biochemistry, 1996)

The proteins separated from membranes by detergent treatment can be isolated by a variety of separation techniques, if a suitable assay is available for the protein of interest.

**Reconstitution of a Proteoliposome**  
Many membrane proteins, such as enzymes, transport protein and receptors can be incorporated into phospholipid vesicles. It involves sonication of purified membrane protein with phospholipids. Dialysis or dilution or gel filtration of protein detergent complexes in solution
containing phospholipids results in formation of Proteoliposomes (protein phospholipid vesicles) capable of carrying out the functions of the membrane protein (Fig. 10).

Fig. 8: Preparations of erythrocyte ghosts
Fig. 9: Preparations of Proteoliposomes
(Source: Zubay, G. Biochemistry, 1984)
This is a useful technique to study the properties, action and role of membrane proteins in isolation, under varying experimental conditions. Action of transport proteins, F/Fo–ATPase of mitochondria and purified Bacteriorhodopsin was first studied by such reconstitution experiments by E. Racker and his coworkers.

**Preparation of erythrocyte plasma membrane**

Osmotic lysis of erythrocytes placed in distilled water results in union of hemoglobin and other constituents to leave a ghost which can reseal itself to give pure plasma membranes Sealed vesicles with either a right side out or inside out (Fig. 11) can be obtained by manipulating these conditions. These erythrocyte ghosts have been studied extensively.

One important tool useful for separation of membrane proteins is Hydrophobic Interaction Chromatography. In this technique, insoluble support such as agarose or polyacrylamide with covalently linked hydrophobic alkyl or aryl groups are used in a liquid chromatography column. Depending on the relative hydrophobicity, the proteins are eluted from the column by changes in the hydrophobicity, ionic strength etc. of the eluting buffer (Fig. 11).

Another technique the Affinity Chromatography can be used for purifying membrane proteins such as receptors, transport proteins and enzymes which interact with specific metabolite such as agonist, hormone, solute or substrate/inhibitor etc.

However, in case of proteins present in very low concentrations such as some receptors, another useful technique is to synthesize them by DNA recombinant technology, by cloning in a suitable vector.

**Characteristics of Membrane Proteins**

The peripheral proteins are present at the exterior face or the anterior face of the membrane and interact with hydrophilic heads of the lipid bilayer or the hydrophilic residues of the membrane proteins by hydrophilic interactions stabilized by ionic and hydrogen bonds. Ankyrin and Band 4.1 proteins in erythrocyte membrane are peripheral proteins which interact with integral proteins by protein: protein interactions mostly.
The integral proteins are embedded in the lipid bilayer and are stabilized by hydrophobic interactions between the alkyl chains of the phospholipids and the hydrophobic amino acids of the membrane proteins. The integral membrane proteins require a hydrophobic environment for stability and to retain their biologically active structure.

Most of the membrane proteins have been purified and studied. They can be monomers, dimers, trimers, tetramers or multimers, etc. like the soluble proteins. However, these proteins are present in a specific orientation which is maintained throughout. Some membrane proteins are located in the inner face / cytoplasmic face and others in the external / exoplasmic face of the membrane while still others traverse the entire structure of the membrane or have most of the polypeptides embedded in the membrane.

Bacteriorhodopsin of Halobacterium halobium spans the membrane seven times as a α-helix and has a very high content of hydrophobic amino acids (Fig. 12). Band 3 or Anion channel and glycoporphin of erythrocytes plasma membrane are sialoglycoproteins. Band 3 or Anion channel is a transmembrane dimer of identical chains, where each monomer has 12 to 14 trans membrane α-helices. The carbohydrates are attached to residues on the outside and the C-terminal end is embedded in the lipid bilayer while the N-terminal end folds into hydrophilic region protruding into the cytoplasm which is attached to the cytoskeleton proteins. Glycoporphin has a large proportion of its mass protruding into the aqueous phase beyond the hydrophobic interior of the membrane. It is a dimer with each monomer containing a trans membrane α-helix. The two α-
helices form a coiled-coil structure. It has very high content of carbohydrates. However, in Cyclooxygenase, the hydrophobic domain of the α-helix interacts with the acyl chains on one side of the bilayer.

![Fig. 12: Bacteriorhodopsin membrane spanning protein with seven α–helices](Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

The various types of spatial arrangements of integral membrane proteins in a membrane are shown in Fig. 13. Most of the, integral membrane proteins usually have an α-helix conformation in amino acid residues (about 20 to 25 residues) traversing the lipid bilayer thickness (3.0 nm) such as the adrenergic or serpentine receptors, anion channel, bacteriorhodopsin, glucose transporters etc. Some proteins have polypeptide chains with anti parallel β strands arranged in a barrel shape where about 5 to 8 residues are enough to span the membrane e.g. OmpLA porin and maltoporin in E.coli. etc. (Fig. 14). In each case, the hydrophobic residues on the outer surface of the integral membrane proteins interact with alkyl chains of the lipid bilayer.

Thus,  
1. In each membrane, the integral proteins must interact with membrane lipids through hydrophobic interactions,  
2. α-helix is the common intra membrane structure and  
3. In some cases, β-barrel structure motif is also observed in the transmembrane proteins

**Proteins Linked Covalently to Lipids**

The third type of membrane proteins are those which are covalently linked to lipids and are anchored in the lipid bilayer.

These proteins are covalently attached to:  
1. Long chain fatty acids such as palmitic acid and myristic acid.  
2. Isoprenoids such as farnesyl and geranylgeranyl groups and  
3. Glycosylated derivatives of phosphoinositol, GPI.
Fig. 13: Different types of spatial relationships of integral membrane proteins of the plasma membrane. (Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

Fig. 14: β-barrel structural motif in membrane proteins. (a) OmpLA and (b) Maltoporin. (Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

The different types of lipid anchored proteins are shown in Fig. 15.
Fig. 15: Membrane proteins covalently linked to lipid in the membrane bilayer
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

The lipid anchors help to insert the protein into the lipid bilayer such that the protein is free at the membrane surface. Besides, the hydrophobic interactions between the lipid anchor and the various membrane lipids, other interactions between the lipid anchor and between the polar head groups of the lipid bilayer and the hydrophilic groups of protein can also help to stabilize this
protein insertion into the membranes where they have a specific location. Thus, the GPI anchored proteins are always on the exoplasmic surface of the membrane while the palmitoyl-, geranylgeranyl-, farnesy1- proteins are on the inner or cytoplasmic face of the membrane (Fig. 15). The src protein contains a myristoyl group while rhodopsin contains two adjacent palmitoyl groups that serve as membrane anchor.

Proteins participating in signal transduction and protein targeting contain either a farnesyl (C15) or a geranylgeranyl [C20] unit at C-terminal cysteine group, for example, ras protein or p21. The rab family of small GTP binding proteins are geranylgeranylated and are involved in membrane targeting.

The GPI anchor of the protein provides a flexible leash and allows the protein to act on molecules outside the cell, e.g. many cell surface hydrolytic enzymes such as alkaline phosphates and also adhesions involved in cell interaction.

**Carbohydrates**

Membrane carbohydrates are usually branched oligosaccharides (short polysaccharides made up of less than 15 sugars/monosaccharides units). These oligosaccharides can be covalently linked to (1) lipids to form glycolipids or lipo polysaccharides or (2) to proteins to form glycoproteins. Both the glycolipids and glycoproteins are present in plasma membranes of eukaryotic cells. They are however absent from inner membranes of mitochondria, thylakoid membranes of the chloroplasts and other intracellular membranes. However, they are present in endoplasmic reticulum, golgi and nuclear membranes.

The sugar units commonly present in glycoproteins and glycolipids are galactose, mannose, fucose, N–acetyl neuraminic acid (commonly known as sialic acid), N-acetyl glucosamine and N-acetyl galactosamine). Many glycoproteins and glycolipids have a net negative charge due to the presence of sialic acid (Fig. 16) sugar residues. Membrane glycolipids and glycoproteins increase the hydrophilic character of lipids and proteins and help to stabilize the membrane protein conformations. Glycophorin(Fig. 17), a protein of erythrocyte membrane contains as much as 60% carbohydrates (by mass).

![Fig. 16: N-Acetylneuraminic acid a sialic acid (Neu5Ac)](image-url)
Fig. 17: Topography of glycophorin in mammalian erythrocyte membrane. Carbohydrate residues are all on the N-terminal domain on the outside of the cell.
(Source: Zubay, G. Biochemistry, 1984)

Short heterosaccharide oligosaccharides chains synthesized are attached to proteins through two types of linkages i.e. the alkali labile O-linkage and the alkali stable N-linkage. The O-type linkages are between N-acetyl galactosamine of oligosaccharide and the hydroxyl group of serine or threonine of the protein (Fig. 18a). Such oligosaccharide chains are shorter and variables. The N-type linkages are formed between the N-acetyl glucosamine and the amide side chain of asparagine in the protein (Fig.18b). The heterosaccharide chains of N-linked sugars are long, branched and have complex structures. N-glycosylation of proteins occurs in the endoplasmic reticulum while the O-glycosylation in the Golgi. The structures of O-linked oligosaccharide and N-linked oligosaccharids are different and contain different sugars in the glycoproteins. The type and the sequence of carbohydrate units in the oligosaccharides vary from species to species and among individuals of same species and even one cell type to another.

(a) The O-type linkage between N-Acetyl galactosamine of oligosaccharide and the hydroxyl group of serine or Threonine residue in the protein
The N-type linkage between N-Acetylg glucosamine of oligosaccharide and the amide side chain of asparagine residue in the protein.

Fig. 18: Glycoprotein linkage between the oligosaccharide and protein

The oligosaccharide components of the glycoproteins determine their intracellular or extracellular sites i.e. the location of secretory proteins and vesicular transport of membrane proteins.

Carbohydrates are always on the exoplasmic face of the membrane. The oligosaccharide chains are distinguishing markers or tags on the plasma membrane. They distinguish one cell type from another. They are the antigenic determinants on the cell surface. The antigenic determinants, A, B and O, in human blood are due to the differences in the glycoprotein present on red blood cell surface. Some glycoproteins are the transport proteins and membranes receptors e.g. Anion channel of erythrocytes, Na\(^+\)–K\(^-\) ATPase, Insulin receptor, while, some involved in cell-cell recognition and interaction include Integrin, cadherin, selectin, neural cell adhesive molecules (n-CAM) and adhesion proteins etc.

Dynamic Properties of Biomembranes

The biomembranes are dynamic and exhibit a fluid nature which is characterized by the lipid composition. The individual lipids can exhibit lateral and to some extent transverse diffusion while the proteins exhibit lateral diffusion in the plane of the membrane. These properties have been studied by using electron spin resonance (ESR), Fluorescence recovery after photo bleaching (FRAP), Differential scanning calorimetry (DSC) etc.

1. Lipid Bilayer Fluidity

Pure lipid molecules or liposome made of pure phospholipids or that of biological membranes exhibit fluidity. When these lipids are heated over a range of temperatures they show a fluid/liquid phase above their transition temperature and a gel-like/solid phase below the transition temperature. Below the mid point of this transition temperature, Tm, the lipids are in ordered, closely packed structures and above it the molecules are in a fluid, disordered state. This has been termed as order-disordered transition and is depicted in (Fig. 19a). Fluorescent techniques, Electron Spin Resonance (ESR) and Differential scanning calorimetry (DSC) have been used to determine the mid transition temperature, Tm, values of various lipids. The mid transition temperature value of some phospholipids is given in Table 6. Pure lipids exhibit a very sharp narrow range of Tm whereas biological membranes show a broad transition temperature, T_{m} (>= 10 C) as they are made up of a mixture of phospholipids (Fig. 19b).
Fig. 19a: Molecule interpretation of the heat-absorbing reaction during the phase transition (Source: Zubay, G. Biochemistry, 1984)

Fig. 19b: Differential scanning calorimetry of various phospholipids dispersed in water: a, dipalmitoyl phosphatidyl ethanolamine; b, dimyristoyl lecithin; c, dipalmitoyl lecithin; d, egg lecithin (plus ethylene glycol to prevent freezing) (Source: Zubay, G. Biochemistry, 1984)

Table 6: Midtransition Temperatures for Aqueous Suspensions of Phospholipids
(Source: Zubay, G. Biochemistry, 1984)

<table>
<thead>
<tr>
<th>Phospholipid³</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1:14:0 PC</td>
<td>24</td>
</tr>
<tr>
<td>D1:16:0 PC</td>
<td>41</td>
</tr>
<tr>
<td>D1:18:0 PC</td>
<td>38</td>
</tr>
<tr>
<td>D1:22:0 PC</td>
<td>75</td>
</tr>
<tr>
<td>D1:18:1 PC</td>
<td>-22</td>
</tr>
<tr>
<td>1:18:0, 2:18:1 PC</td>
<td>3</td>
</tr>
<tr>
<td>D1:14:0 PE</td>
<td>51</td>
</tr>
<tr>
<td>D1:16:0 PE</td>
<td>63</td>
</tr>
<tr>
<td>D1:14:0 PG</td>
<td>23</td>
</tr>
<tr>
<td>D1:16:0 PG</td>
<td>41</td>
</tr>
<tr>
<td>D1:16:0 PA</td>
<td>67</td>
</tr>
</tbody>
</table>

³ Rose et al.
The $T_m$ value of a lipid depends on the nature of its fatty acid and the phospholipids’ head group. Thus, lipid bi layers consisting of saturated long chains are in a crystalline gel state, closed packed molecules with a high $T_m$. However those with shorter chains and unsaturated fatty acids have a low $T_m$ above which they are in a disordered liquid or fluid state. Short hydrocarbon chains have a smaller area with which to undergo hydrophobic interactions which stabilizes a lipid bilayer. Unsaturation, in the acyl- chain of the fatty acids produces kinks in the molecule (Fig. 20) which prevents close packing and introduces disorder in lipid molecules.

![Fig. 20: Comparison of an saturated fatty acid (left) with an unsaturated fatty acid (right) having a $cis$ double bond which introduces an inflexible kink or bend in the molecule.]

Biological membranes have $T_m$ values in the range of $10^\circ – 40^\circ$ C. They are, thus, in a fluid state at body temperature which allows membrane proteins to interact. Bacteria and poikilothermic (cold blooded) animals modify their fatty acid composition and have a high percentage of unsaturated fatty acids so as to maintain membrane fluidity. Table 7 shows the fatty acid composition of bacteria, E. coli, grown at different temperatures. Membranes with high biochemical activity, such as mitochondrial inner membranes and bacterial plasma membranes, are rich in unsaturated fatty acid and have an high ratio of unsaturated to saturated fatty acids (Table 2).

**Table 7:** Fatty Acid Composition of E. coli Cells Cultured at Different Temperatures  
*(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>10 °C</th>
<th>20 °C</th>
<th>30 °C</th>
<th>40 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid (14:0)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>18</td>
<td>25</td>
<td>29</td>
<td>48</td>
</tr>
<tr>
<td>Palmitoleic acid (16:1)</td>
<td>26</td>
<td>24</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>Oleic acid (18:1)</td>
<td>38</td>
<td>34</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Hydroxymyristic acid</td>
<td>13</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Ratio of unsaturated to saturated$^1$</td>
<td>2.9</td>
<td>2.0</td>
<td>1.6</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Cholesterol in the lipid bilayer helps to control the fluidity of the membranes. Cholesterol intercalates within the lipid bilayer such that it reduces the fluidity by introducing order and close packaging in the membrane with a high fluidity and reducing mobility of the fatty acyl
chains. On the other hand, it increases disorder and leads to mobility in the bi layers with little or less fluidity. Cholesterol is, thus, a regulator of fluidity in the membranes.

II. Lateral Diffusion

Lateral diffusion of both lipids and proteins has been determined by fluorescence techniques such as Fluorescence recovery after photo bleaching, FRAP, or by ESR techniques. A lipid or a protein molecule is specifically labeled by a fluorescent probe and attached to membrane. An intense laser beam is focused on the membrane to bleach the area. The time taken for the bleached area to recover the fluorescence is monitored by a fluorescence microscope (Fig. 21). Thus, the rates of lateral diffusion of both lipids and proteins are measured.

Fig. 21: Measurement of lateral diffusion rates of lipids by fluorescence recovery after photo bleaching (FRAP)
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)
Since lipids do not have an unpaired electron, probes with an N→O unpaired electron such as Tempo choline (Fig. 22) is commonly used to study the lipid diffusion by ESR. Lipids in bilayers show a fast rate of lateral diffusion (Fig. 23a) and can move over the length of a bacterium, 1µm, in ~ 1s.

Fig. 22: Spin labels containing mitroxide groups (a) Tempo and (b) a spin-label analog of phosphatidylcholine

Fig. 23: Diffusion of phospholipids in a bilayer (Source: Voet, D and Voet J. Biochemistry, 1995)
Proteins can also diffuse in the plane of the lipid bilayer in biomembranes (Fig. 23b). In the classical experiment where a mouse cell labeled with green fluorescent antibody and human cell labeled with red fluorescent antibodies were fused by *sendai* virus. It was observed that after a while the heterokaryon (fused cell) showed a mosaic of intermediate fluorescence indicating lateral diffusion of the integral membrane proteins (Fig. 24).

However, all integral membrane proteins do not show such lateral diffusion. Some of them are immobilized as they may be tightly bound to lipids, cytoskeleton proteins or to other proteins etc.

**Fig. 24: Lateral diffusion of cell surface membrane proteins labeled by differently colored fluorescent dyes** *(Source: Mathews, C.K. and Holde K.E. van. Biochemistry, 1996)*

**III. Transverse Diffusion or Flip-Flop**

Transverse diffusion or flip-flop movement of lipids across the bilayer is very slow (Fig. 23b). It is energetically unfavorable. In biological membranes, enzyme Flippase can carry out this Flip-Flop of membrane lipids at a fast rate. Proteins do not exhibit transverse diffusion in the lipid bilayers at all.

**Asymmetry of Biological Membranes**

The biological membranes are asymmetric and the two mono layers of the membranes exhibit differences with respect to:

1. Specific orientation of proteins
2. Lipid composition and
3. Carbohydrates

1. *Specific orientation of proteins*

Unidirectional and Asymmetric orientation or vectorial arrangement of proteins is largely known for several membrane functions. Thus, the electron transport carrier proteins and F$_{1}$-F$_{0}$ ATPases in the inner membrane of mitochondria and thylakoid membrane in chloroplast, as also, other
membrane proteins have a specific location and orientation in the membranes, determined at the time of their synthesis.

Freeze-Fracture electron microscopy is a valuable tool to observe the proteins within the lipid bilayer. Thus, whole cells or membranes which have been rapidly frozen are sliced with a sharp knife or microtome to fracture the plane of the membrane between the two lipid monolayers (the outer and the inner leaflets) because in this region, the interactions are very weak, thus exposing the inner surfaces of the leaflets. These are then shadowed with heavy metals and viewed under the electron microscope which reveals the location and preferential attachment of proteins to one of the surfaces of the bilayers (Fig. 25).

![Image](image.png)

**Fig. 25: Freeze-Fracture electron microscopy of plasma membrane demonstrating the location of membrane proteins in the membrane lipid bilayer**
(Source: Voet, D and Voet J. Biochemistry, 1995)

2. Lipid composition of the two monolayers

Membranes also show unequal distribution of certain lipids between the inner and the outer leaflet of the bilayers. Each membrane has preferential location of its individual lipids in the two halves of the bilayer (Table 8). The erythrocyte membranes have phosphatidylcholine (PC), sphingomyelin (sph), glycolipids and cholesterol on the outer leaflet and phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS) preferentially on the inner leaflet. Although the lipid composition of each leaflet appears to be different, the total membrane lipid is equally distributed in the outer and the inner mono layers of the erythrocyte membrane. Artificial
membranes made up of pure phosphatidyl choline (PC)/phosphatidyl ethanolamine (PE) show PC on the outside and PE on the inner leaflet.

Table 8: Lipid Asymmetry in Biological Membranes
(Source: Zubay, G. Biochemistry, 1984)

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Preferential Outside</th>
<th>Preferential Inside</th>
<th>Equal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various erythrocytes</td>
<td>PC, Sph, glycolipids, cholesterol</td>
<td>PE, PS</td>
<td>–</td>
</tr>
<tr>
<td>Rabbit sarcoplasmic reticulum</td>
<td>PE</td>
<td>PS</td>
<td>PC, lyso PC</td>
</tr>
<tr>
<td>Mouse LM cell plasma membrane</td>
<td>Sph</td>
<td>PE</td>
<td>PC</td>
</tr>
<tr>
<td>E. coli outer membrane</td>
<td>–</td>
<td>PE</td>
<td>–</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>–</td>
<td>PE</td>
<td>–</td>
</tr>
<tr>
<td>Micrococcus lysodeikticus</td>
<td>PG</td>
<td>PI</td>
<td>DPC</td>
</tr>
<tr>
<td>PC/PE artificial vesicles</td>
<td>PC</td>
<td>PE</td>
<td>–</td>
</tr>
</tbody>
</table>

3. Carbohydrates

Carbohydrates are always present on the exoplasmic surface of plasma membrane and face outside. In the endoplasmic reticulum, also, they are present on the exoplasmic surface and face the lumen of the endoplasmic reticulum as also in the nuclear envelope and the nuclear pores.

Lipid Rafts and Caveolae

Recent studies have shown that some integral membrane proteins are tightly bound to a layer of phospholipids so that the boundary lipids or microdomains are formed. In some plasma membranes, sphingomyelin and cholesterol interact with and bind membrane proteins to form lipid rafts (Fig. 26a). Membranes, when treated with triton-X-100 at low temperatures, are disrupted, leaving behind the cholesterol, sphingolipid and glycolipid region i.e. lipid rafts. Lipid rafts can be disrupted by treatments with cyclodextrin or systems which remove cholesterol. The lipid rafts help to bind proteins in a specific orientation and coordinate and regulate a variety of signaling processes. Certain proteins such as GPI anchored proteins, some of Receptor Tyrosine kinases belonging to src protein family or some proteins after activation such as β-cell receptors and T-cell receptors are associated with lipid rafts. However, Transferrin receptors and a member of ras family are not associated with the lipid rafts. Lipid rafts are implicated in cell signaling, molecular trafficking and in certain diseases such as HIV and malaria.

Caveolae (Latin for little caves) are small (50-100 nm) invaginations of the plasma membrane in many cell types such as endothelial cells and adipocytes. However, neurons may completely lack caveolae. These are flask shaped structures which are rich in proteins where one of the protein is caveolin (hence the name) and contain cholesterol and sphingolipids (Fig. 26b). They are actually a type of lipid rafts containing the protein caveolin along with other proteins. The protein, caveolin, has a cytoplasmic C-terminus and a cytoplasmic N-terminus, linked together
with hydrophobic hairpin that is inserted into the cytoplasmic leaflet of the membrane which results in a change in the morphology of the membrane. They have several functions in signal transduction and play a role in endocytosis and oncogenesis, receptors for insulin and other growth factors, and also, GTP binding proteins and protein kinases may be associated with caveolae.

Fig. 26: (a) Microdomains or rafts and (b) caveola in membranes in the plasma membrane
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)
Evidence that Biological Membranes consist of Lipid Bilayers

1. Phospholipids in aqueous solution form lipid bilayer structure spontaneously i.e. the free energy change for the process is negative. Liposomes are closed, self-sealing, solvent filled vesicles bounded by a single lipid bilayer with a diameter of several hundred Angstrom and resemble biological membranes. However, the living cell is a plasma membrane encapsulated vesicle whose shape is determined by the interaction of proteins with the cytoskeleton or extracellular matrix or with lipids in the membrane. Thus, the cell is not always a sphere like liposomes prepared from phospholipids only.

2. The liposomes prepared from synthetic phospholipids or phospholipids purified from biological membranes as well as purified biological membranes appear as trilamellar structures i.e. two dense regions separated by a less dense region, when viewed under an electron microscope.

3. Liposomes are impermeable to small ionic and polar molecules as are the biological membranes. However, biological membranes are semipermeable due to the presence of specific transport proteins. Proteoliposomes containing transport proteins are also semipermeable depending on the protein incorporated in the vesicle.

4. Both liposomes and biological membranes exhibit similarity in their hydrophobic region, as also, in their dynamic properties such as lateral diffusion and fluidity, asymmetric distribution of lipid molecules and other physical and chemical properties. Liposomes containing membrane protein have been used as models for biological membranes.

Thus, it is concluded that, biological membranes consist of lipid bilayers.

Models for the Structure of Biological Membranes

Early Models

In 1917, Langmuir first made artificial membranes using phospholipids dissolved in benzene. In 1925, E.Gorter and F. Grendal (on the basis of studies on erythrocyte membranes) first said that in membranes phospholipids are arranged in a bilayer which is stable. In a subsequent model of membrane structure, J. Danielli and H. Davson in 1935, proposed a Lipid bilayer separated by “Lipoid” material and globular proteins to be present at the two surfaces of the lipids (Fig. 27a). This was further adapted by J.D. Robertson in 1959, who gave the “Unit membrane” model which showed the lipids arranged as a single bilayer with protein in an extended conformation on the outside at the hydrophilic heads on each side of the bilayer (Fig. 27b). In this model, the integrity of the membrane was ‘lipid derived’. Another model proposed by Benson’ postulated the membrane integrity to be ‘protein derived’ with lipid bilayers interspersed in the proteins.

When electron microscopy was used to study membrane structures, the membranes appeared as two dense regions separated by a less dense region, as a trilamellar structure indicating lipid bilayer in the membrane structure as an integral part. However, these models could not explain all the characteristics and properties of membranes as indicated with more sophisticated techniques e.g. ESR, NMR, X-ray diffraction, high resolution electron microscopy, fluorescent techniques etc.
Fluid-Mosaic Model

In 1972, J.S. Singer and G.I Nicolson gave the Fluid-Mosaic model for the membrane structure. Essentially, it suggests that the phospholipid molecule is the repeating unit in a bilayer
arrangement with a thickness of 5-10 nm. The lipid bilayer has been compared to a bed of sea with the membrane proteins embedded in the lipid bilayer in a random fashion; some proteins are located at one or the other of the two surfaces of the membranes while other proteins have specific transbilayer orientation extending the entire bilayer. Also, the model incorporated the idea that the membrane is a dynamic and not a static structure with lipids and proteins capable of lateral diffusion in the plane of the bilayer unless restricted by specific interactions. Fig. 27c depicts the fluid-mosaic model for membrane structure. The model successfully explained the permeability barrier properties and the lower electron density of the hydrophobic tails than of the hydrophobic heads consistent with the low density x-ray diffraction studies of membranes and also, the other physical and chemical properties observed with other techniques.

**Plasma Membrane Interaction with Cytoskeleton**

Interaction of plasma membrane has been well studied in red blood cells. Mature red blood cells or erythrocytes are independent, flow freely in the blood. They do not contain nucleus, organelles and other cellular constituents and are essentially a bag of hemoglobin enclosed by plasma membrane. They are biconcave in shape, flexible and can pass through narrow capillaries with very small diameters. The shape of the erythrocytes is maintained by several different kinds of proteins that form a meshwork or cytoskeleton which lies below the plasma membrane. When the plasma membranes ghosts of erythrocytes are treated with non-ionic detergents to remove most of the lipids and proteins, the cytoskeleton is left behind as an insoluble fraction containing proteins. Two cytoskeletal proteins, Band 4.1 and ankyrin attach plasma membranes proteins, Band 3 and glycophorin, to the cytoskeleton at certain places. This is responsible for the inability of these plasma membrane proteins to diffuse in the plane of the membrane and also provide flexibility and strength to the erythrocyte cell membrane.

The cytoskeletal proteins include:

*Spectrin*

This forms the major component and makes up to 30% of the proteins associated with plasma membrane. It is a dense scaffolding of proteins. Each spectrin molecule consist of two long polypeptide chains which are loosely wound around each other to form a dimer. Each polypeptide is made up of α- and β- polypeptide chains. The two dimeric subunits link head to head to form tetramers which are 200 nm in length. The ends of the spectrum molecule are bound to other cytoskeletal proteins such as actin filaments, actin and spectrin binding proteins such as tropomyosin and adducin at region known as junctional complex.

*Band 4.1 Protein*

It is part of the junctional complex. It binds to spectrin and also binds to glycophorin, an erythrocyte membrane protein.

*Actin*

This is another important component, a globular protein, from which the actin filaments of cytoskeleton are formed. It also binds to Band 4.1 protein.
**Ankyrin**

It is a peripheral protein which binds to a region of Band 3 protein or Anion channel of erythrocyte membrane and to the β-chains of the spectrin near the center of the tetramer.

A short filament of actin and spectrin binding proteins, adducin and Band 4.1, attach to one end of spectrin tetramer and also to that of the another end at the junctional complex.

The resulting meshwork forms a flexible frame work which is securely anchored to the erythrocyte membrane (Fig. 28).

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**Fig. 28: Organisation of major erythrocyte cytoskeletal proteins and their interactions with Band 3 and glycophorin, the major integral proteins**

(Source: Voet, D and Voet J. Biochemistry, 1995)

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**Plasma Membranes at Intercellular Junctions**

In animals and plants, cells within a tissue or an organ are tightly packed. However adjacent cells often allow direct contacts of various types i.e. they adhere, interact and communicate through contacts between their plasma membranes. Thus, plasmodesmata in plant cell walls and tight junctions, desmosomes and gap junctions in animals are the main types of intercellular junction. Tight junctions and desmosomes are absent in plants.

**Plasmodesmata**

Plasmodesmata (singular, plasmodesma) are channels between the plant cells connecting cytoplasm of adjacent cells (Fig. 29). Their diameter is 30 to 60 nm and appear to be lined by the cell membrane (Fig. 29) i.e. the plasma membranes of adjacent cells are continuous through plasmodesmata in the channel. Water and small solutes can pass freely from cell to cell and probably RNA molecules can also pass by moving along fibers of the cytoskeleton.
Gap Junctions

Adjacent cells have channels which are cytoplasmic connections. These channels are 2nm in diameters. Gap junction is a hexamer and the subunits are arranged in a rosette structure. The two hexamers of adjacent cells juxtaposed to form a channel (Fig. 30a). This permits the passage of ions or small molecules such as sugars, amino acids etc between the cells. The calcium ion concentration in the cells regulate the opening/closing of the gap junctions by tilting of the hexameric subunits (Fig. 30b). Thus, the metabolic activities of the cells in the tissue can be regulated by passage of signaling molecules such as cyclic AMP, Ca++ etc.

Tight Junctions

The bands of plasma membranes proteins of adjacent cells are fused in a tight junction (Fig. 30a) to form a seal around each cell in a layer of tissue and prevent leakage of nutrients between cells and across the cells e.g. the tight junctions around intestinal epithelial cells and those in pancreatic acini prevent leakage of pancreatic proteins and digestive enzymes from central cavities into the blood. Tight junctions occur only in vertebrates while septate junctions perform the same function in invertebrates and are slightly different structures.

Desmosomes

Desmosomes consist of plaques of dense fibrous material between cells. They fasten cells into strong epithelial sheets. Clusters of filaments from the cytoplasm of adjacent cells loop in and out of them. They give tissues mechanical strength and rigidity to the tissue (Fig. 30a).
Plasma Membrane Modification in Specialized Cells

In plants and animals, cells form organized tissues which carry out specific functions. The plasma membranes of specialized cells and tissues exhibit several modifications depending on their specific functions such as those of erythrocytes, epithelial cells of intestines and kidney and acinar cells of pancreas etc.
Intestinal Epithelial Cells

The epithelial cells lining the lumen of the small intestines have two major functions associated with two regions of the plasma membrane:
1. The apical region absorbs the nutrients, that are formed as a result of digestion of food, from the lumen of the intestines and
2. The basal region transports the absorbed nutrients into the blood for delivery to various other organs or tissues

Most of the absorption of nutrients takes place from the small intestines, while only limited absorption occurs from the stomach and the large intestines. The epithelial lining of the small intestines has a very large surface area of about 300m and the large circular folds in the lining have finger like projections called villi (singular villus). The apical region of each of the epithelial cell or villus has many appendages called microvilli which project into the lumen of the intestines (Fig. 30a). This increases the microvillar surface or the absorption area of the epithelial cells, a specialized adaptation for their function. A bundle of actin filaments is present in the center of each microvillus to provide rigidity to the microvillus. These microvilli are surrounded by a loose network forming a fuzzy coat or glycocalyx which contains several digestive enzymes such as lactase, sucrase, maltase and peptidases. These enzymes are bound to the exoplasmic face of the plasma membrane of the microvilli and degrade the nutrients in the intestinal lumen. The resulting carbohydrates and amino acids etc. are absorbed through transport proteins such as Na\(^+\)-glucose- and Na\(^+\)-aminoacids-cotransporters present in the microvillar plasma membrane in the apical region, into the epithelial cell. The sugars and other absorbed molecules are then passed across the basal membrane of the epithelial cell into the blood through a separate set of transport proteins such as glucose transporter. The Na\(^+\)-K\(^+\)-ATPase present in the basal membrane pumps Na\(^+\) to maintain the Na\(^+\) gradient for the Na\(^+\)-cotransporters. The plasma membrane between adjacent cells has specialized regions, the tight junction, gap junction and desmosomes.

Similar microvillar projections are also present in kidney epithelial cells with a similar function to increase the surface area of absorption etc.

Pancreatic Acinar Cells

The plasma membrane of pancreatic acinar cells has two distinct regions - The apical and the basolateral - with specific functions.

The acinar cells of the pancreas synthesize the degradative enzymes such as ribonucleases, proteases and amylases as inactive precursors called zymogens which are stored in vesicles. These vesicles bud off the golgi and migrate to the cell surface where they cluster under the apical or the lumen facing regions of the plasma membrane, which is adjacent to the ductule which is formed when lumen of several acinar cells are connected. The ductule leads eventually into the lumen of the small intestines.

The basolateral membrane covers the sides and the base of an acinar cell (Fig. 31). It contains receptors for various peptide hormones which are released into the blood when food is present. When these harmones bind to their receptors in the pancreass, the secretory vesicles carrying the
various zymogens fuse only with the apical region of plasma membrane, adjacent to the ductules, so that the enzymes are released directly into the intestines by the process known as exocytosis.


Tight junctions separate and seal off the apical and the basolateral regions of plasma membranes of intestinal epithelium and pancreatic acinus. They, thus, prevent lateral diffusion of proteins and lipids between the apical and the basolateral regions of the exoplasmic leaflet and enable the two regions to retain their specific proteins to carry out their functions. The lipid components can, however, diffuse in the cytoplasmic leaflet.

**Extra Cellular Membranous Structures**

As mentioned earlier, the membranous structures, external to the plasma membrane of the cell include cell walls, extra cellular matrix, calyx or fuzzy coal, tight junctions and desmosomes etc.

**Plant Cell Walls**

The distinguishing feature between a plant and an animal cell is the presence of cell walls around the plant cells. The cell walls protect the cell, maintain its shape, provide rigidity and prevents excessive uptake of water. These strong cell walls enable the plant to stand upright. These cell walls are much thicker than the plasma membrane and the thickness ranges from 0.1 um to several micrometers.

The exact chemical composition of the wall varies from species to species and from one cell type to another in the same plant. However, the basic design of the cell wall is consistent. The basic
architecture involves ground substance or matrix consisting of polysaccharides and proteins in which the microfibrils made of polysaccharide cellulose are embedded. Hemi cellulose, a highly branched polysaccharide made up of $50\beta (1 \rightarrow 4)$ linked sugars of a single type are hydrogen bonded to n cellulose microfibrils and helps to bind microfibrils to each other and to other components of the matrix. One of these is pectin which is crosslinked to hemicellulose to form a complex network present in the principal cell wall components and binds adjacent cells together. Major protein, extensin, is a glycoprotein rich in serine–hydroxy proline–hydroxy proline–hydroxyproline sequences which are glycosylated.

Another component is lignin complex, an insoluble polymer of phenolic residues. This is present in all cell walls and is the strengthening material. The cell walls are highly impermeable to diffusion of particles with diameter greater than $\sim 4$ nm but water and ions can diffuse freely in cell walls.

A young plant cell first secretes a thin and flexible wall - the primary cell wall (Fig. 29). Between primary walls of adjacent cells is the middle lamella, a thin layer rich in sticky polysaccharides called pectin. When the cell mature and stops growing, it strengthens its wall by secreting hardening substances into the primary wall of some plant cell, while others add a secondary cell wall between the plasma membrane and the primary wall which makes the cell strong and protects it. Wood consists mainly of secondary walls.

**Bacterial Cell Walls**

All prokaryotes contain a cell wall which is external to the plasma membrane or cell membrane. The cell wall maintains the shape of the cell protects the animal and prevents lysis in hypotonic environment. However they can plasmolyze and die in hypertonic environment. This is the basis of preservation of meat and pickles where high salt concentrating protects from bacterial infections.

These cell walls differ in molecular composition and construction from those of plants and fungi. The classification of bacteria is on the basis of their cell walls taking up the gram stain which is a dye-iodine complex. Thus, the gram positive bacteria retain the stain while the gram negative does not. The cell walls of the gram positive bacteria are simple and contain large amount of peptidoglycan while those of gram negative have a more complex structure, less peptidoglycan and an outer lipid bilayer membrane and also contain lipoproteins and lipopolysaccharides (Fig. 32a and b) which are more toxic, protect the bacteria from the defenses of the host and render them more resistant to antibiotics than the gram positive bacteria.

**Gram-positive Bacteria**

Their cell wall consists of a thick peptidoglycan layer made up of alternating N-acetyl glucosamine and N-acetyl muramic acid polysaccharide chains and short peptides linked by glycine pentapeptides. The short peptides (tetra peptides) have unusual structure with the sequence $L$-ala-$D$-glu-$L$-lys-$D$-ala and having some $D$–amino acids and the linkage of glutamic acid in the chain is via its $\gamma$–carboxyl group. The $\epsilon$–NH$_2$ group of each lysine (marked as a * in Fig. 32c) is linked to a glycine pentapeptide which is bonded at the other end to the terminal $D$-Ala residue (marked as a ** in Fig. 30c) of an adjacent chain (Fig. 32c).
This results in a covalent cross linked structure covering the bacterial cell (Fig. 32a). In addition, elongated lipid-oligosaccharide complexes called lipoteichoic acids protrude from the membrane into the peptidoglycan wall. Plant cell, while others add a secondary cell wall between the plasma membrane and the primary wall which makes the cell strong and protects it. Wood consists mainly of secondary walls.

**Fig. 32: Bacterial cell walls.** These schematic diagrams show (a) The cell wall of a representative a. gram-positive bacterium Staphylococcus aureus; (b) Gram-negative bacterium, Escherichia coli
Gram Negative Bacteria

Their cell wall has thin peptidoglycan layer and an outer membrane as already mentioned. The basic polysaccharide structure is the same but the peptide chains and their linkages are different (Fig. 32b). The structure and diversity of these macromolecules among different bacterial species is very large. The outer membrane is permeable because of its channel forming proteins – porins.

Fig. 32c: Bacterial cell walls. These schematic diagrams show the peptidoglycan layer of gram positive bacteria.

The antibiotic such as penicillin and others interfere in the formation of the peptidoglycan linkage or the cell wall synthesis. However, lysozyme which is a naturally occurring antibiotic
present in bacteriophages, egg white and human tears etc acts by catalyzing the hydrolysis of glycosidic linkage between N-acetyl glucosamine (glc N Ac) and N-Acetyl muramic acid (Mur N Ac) in the polysaccharide and thus, dissolve the cell wall and lead to bacterial death.

**Extracellular Matrix (ECM) of Animal Cells**

Animal cell do not have cell walls like plant cells. They have an elaborate extracellular matrix (Fig. 33). This functions in support, adhesion, movement and regulation. Its molecular composition and structure varies from one cell type to another. The extracellular matrix contains largely glycoproteins which are secreted by the cells. Collagen is the most abundant glycoprotein of extracellular matrix of most animal cells and forms strong fibers outside the cells. It constitutes about half of the total protein in the human body. The proteoglycans, which are rich in carbohydrates (containing as much as 95%) form large complexes. The proteoglycan network contains the attached collagen fibers and other kinds of glycoproteins specially fibronectin. The plasma membrane contains integrins which are transbilayer receptors and able to bind fibronectin on the outer surface of the cell. Integrin binds on the cytoplasmic side of the membrane to the microfilaments of the cytoskeleton. Thus, changes in the extracellular matrix are transmitted to the cytoskeleton via integrin and vice-versa. The extracellular matrix plays a vital role in a cell’s behaviour and can influence the activity of the gene by various mechanical and signaling pathways. The signaling pathways involve fibronectin, integrin and the cytoskeleton which in turn triggers chemical signaling pathways inside the cell.

![Extracellular Matrix](image)

**Fig. 33: Extra cellular matrixes (ECM) of an animal cell**

Biological Transport

The lipid bilayer of the membranes is permeable to only hydrophobic molecules and small uncharged molecules such as CO₂, N₂, O₂, NO, Urea, ethanol, anaesthetics and to some extent water by simple diffusion. However, the lipid bilayer is impermeable to most ions, polar molecules such as Na⁺, K⁺, Ca²⁺, Cl⁻, HPO₄²⁻, HCO₃⁻, Glucose, other sugars, amino acids, ADP₃⁻, ATP₄⁻ and other organic molecules of biological importance. These ions and hydrophilic organic molecules are required by all living cells to carry out cellular activities and meet their metabolic requirements.

Living cells have, thus, evolved systems which enable them to transport ions and molecules of interest into and out of the cell and intracellular organelles. The biological transport is mediated by transmembrane integral proteins which are of three types (Fig. 34):

(I) Passive Transporters, which carries out facilitated or passive biological transport.
(II) Active transporters or pumps, which carry out active transport.
(III) Channels.

Fig. 34: Schematic diagram illustrating the action and types of membrane transport proteins (Source: Lodish, H., Baltimore, et al. Molecular Cell Biology, 1995)

Passive and Active transporters are also termed as mediators or Facilitators. They have specific binding sites for the molecules or the solute to be transported and are saturable and show other characteristics discussed below.

Channels are usually less specific or non specific and do not have a specific binding site for the solute / molecule which flows through the channel passively i.e. down the concentration gradient.

On the other hand, ionophores (ion bearing) are antibiotics, natural or synthetic, low molecular weight compounds that mediate ion transport down an electrochemical gradient. They are either mobile carriers or form channels. They have been widely used as models systems to study membrane functions.

Simple Passive Diffusion or Transport

Transport of molecules across planar lipid bilayer sheets or membrane separating two compartments, A1 and A2 is studied by adding radioactively labeled compounds in one
compartment, say A1, and determining the radioactivity that appears in the second compartment as a function of time.

If the concentration of the solute / compound in compartment A₁ is C₁ and its concentration in compartment A₂ is C₂, then according to Fick’s law, the movement of solute C across a barrier is dependent upon the concentration gradient of the solute in the two compartments. If [C₂] > [C₁] the movement is from A₂ to A₁ till the gradient is abolished and an equilibrium is reached.

The net rate of transport, J (in moles per square centimeter per second, moles/cm²/s) is given by:

\[ J = \frac{KD_1}{l} x \{(C_2) - (C_1)\} \]  

(i)

where, \( l \) is the thickness of the membranes,  
\( D_1 \) is the diffusion coefficient of the diffusing molecule, C.  
\( K \) is the partition coefficient for the diffusing molecule between lipid and water (the ratio of solubilities of the molecule in lipid and water).

For ions and other hydrophilic molecules, \( K \) is a very small number, so that these molecules diffuse across the membrane at an extremely slow rate or not at all.

If \( C_1 \) and \( C_2 \) are expressed in mole/cm³ and \( l \) in cm, then \( D_1 \) has the unit cm²/s (\( D_1 \) is not the same as \( D \) (Diffusion coefficient) of the molecule in an aqueous solution). \( D_1 \) depends on the shape and size of the molecule as well as the viscosity of the membrane lipid.

Since \( K \), \( D_1 \) and the exact thickness of the membrane are not known; the rate of passive diffusion is given by Permeability coefficient, \( P \). This can be measured experimentally:

\[ J = P \{(C_2) - (C_1)\} \]  

(ii),

where, \( P = \frac{KD_1}{l} \) with units of cm²/s.

The permeability coefficients for few molecules through membranes are given in Table 9.

**Table 9: Permeability coefficients from some ions and molecules through membrane**  
*(Source: Mathews, C.K. and Holde K.E. van. Biochemistry, 1996)*

<table>
<thead>
<tr>
<th>Permeability Coefficient (cm/s) for</th>
<th>Membrane</th>
<th>Phosphatidylserine</th>
<th>Human Erythrocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>&lt;9 × 10⁻¹³</td>
<td>2.4 × 10⁻¹⁰</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>&lt;1.6 × 10⁻¹³</td>
<td>10⁻¹⁰</td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>1.5 × 10⁻¹¹</td>
<td>1.4 × 10⁻⁴⁺</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>4 × 10⁻¹⁰</td>
<td>2 × 10⁻³⁺</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>5 × 10⁻⁴</td>
<td>5 × 10⁻⁷</td>
<td></td>
</tr>
</tbody>
</table>

*Facilitated transport. Note that whenever facilitated transport is encountered, the permeability coefficient rises dramatically.*
**Thermodynamics of Transport**

According to the thermodynamic principles, the transfer of a 1 mole of a substance from compartment one to the other separated by a membrane or a barrier is given by

\[
\Delta G = RT \ln \left( \frac{C_2}{C_1} \right) \quad \text{.....(iii)}
\]

Where \( C_1 \) and \( C_2 \) are the concentrations of the free substance in Compartment 1 and Compartment 2 respectively and the substance is transferred from Compartment 1 to 2.

If \( C_2 \) is less than \( C_1 \), \( \Delta G \) is negative and the process is thermodynamically favourable. The substance can be transferred till the concentration in the two compartments is the same, i.e. if \( C_2 = C_1 \), \( \Delta G \) equals to zero and the system is in equilibrium, the rate of transport in the two directions is the same and no net transport occurs. Thus, movement of a solute can occur spontaneously down a concentration gradient.

For a charged molecule with a charge \( Z \), the free energy of transport across a membrane involves besides the concentration term given in (iii), an additional contribution due to the diffusion of the ion across a potential difference:

\[
\Delta G = RT \ln \left( \frac{C_2}{C_1} \right) + ZF \Delta \Psi \quad \text{....(iv)}
\]

Where \( F \) is Faraday and is equal to \( 96.5 \text{ kJ mol}^{-1} \text{Vol}^{-1} \) and \( \Delta \Psi \) is difference in membrane potential in volts in the two compartments, \( Z \) is the charge on the molecule. However, if \( C_2 > C_1 \), then an energy input is required to transport the molecules uphill or against the concentration gradient, as this process will not occur spontaneously. This is then referred to as Active Transport and the general equation now becomes

\[
\Delta G = \Delta G^0 + RT \ln \left( \frac{[C_2]}{[C_1]} \right)
\]

If there is ten fold difference in concentration between two compartments, the cost of moving 1 mol of an uncharged solute at 25ºC across a membrane separating the two compartments is therefore :

\[
\Delta G = (8.315 \text{ J/mol}) (298)(\ln 10)/1
\]

\[
= 5.7 \text{ kJ/mol}
\]

If the molecule is charged, then the cost of moving it will be

\[
\Delta G = RT \ln \left( \frac{[C_2]}{[C_1]} \right) + ZF \Delta \Psi
\]

an additional contribution due to the moving of the ions across a potential difference.

**Facilitated Passive Transport**

When transport of biological membranes is studied using purified membranes protein, certain common features are observed.

1. The transport of the solute / molecule can be in either direction depending on the concentration gradient of the solute, from a higher to a lower concentration, i.e. there is a down hill movement.
2. The transport is highly specific. The specificity for a particular transport system is akin to that of enzyme for its substrate. Thus, the transport system for D–glucose will not transport any other sugar or at a very low rate.

3. The transport is not only specific for a particular molecule, it also shows stereospecificity i.e. can differentiate between D– and L– Sugars or between L– and D– aminoacids etc.

4. The transport shows saturation kinetics i.e. the rate of transport reaches a maximum as the concentration of the solute is increased till a maximum transport rate is reached. This distinguishes biological transport from simple diffusion or unfacilitated transport where the rate is directly proportional to concentration gradient (Fig. 35).

5. The transport is inhibited by known protein reagents that react with specific groups of proteins as in the case of enzymes e.g. p–chloromericuribenzoate or other mercurials that react with –SH groups (cysteine residue); fluorodi nitrobenzene, FDNB, which reacts with –NH₂ group etc.

![Fig. 35: Kinetics of (a) Passive Transport and (b) Facilitated Transport](Source: Zubay, G. Biochemistry, 1984)

The kinetics of facilitated passive transport are similar to that of enzyme kinetics exhibiting maximum transport rate viz. Vmax for enzymes and a high affinity binding site for the solute transported across biological membranes.

These characteristics showed the involvement of proteins with specific binding sites and that the transport is not by simple passive diffusion but is facilitated by proteins.

These proteins have been called as mediators, carriers, porters, transporters by different workers as they were studied and the transport system as Facilitated Passive Transport. However, presently it is referred to as Passive transport or Facilitated transport carried out by transport proteins or transporters. In some cases the original names have been retained such as Anion channel or Cl⁻/HCO exchanger, glucose carriers etc.
**Active Transport**

This type of transport is also carried out by transporters and is referred to as PUMPS. Active transport shows similarity to Passive Transport in

1. Saturation kinetics.
2. Specificity
3. Stereospecificity
4. Inhibition by specific protein reagents.

However, they differ

1. in the movement of the solute across the biological membranes which is
   (a) unidirectional and in a specific direction and
   (b) against the concentration gradient or uphill.
2. and require an energy input i.e. are accompanied by hydrolysis of ATP or any other source of energy for the uphill movement of the solute and hence named as Pumps.
3. are inhibited by cyanide and such reagents that inhibit energy production.

**Primary Active Transport**

The type of active transport where ATP is the source of energy is known as Primary Active Transport (Fig. 36a). Examples of active transport are Na⁺/K⁺ ATPase or calcium pumps.

**Secondary Active Transport**

In this case, the solute is transported uphill coupled to the downhill transport of another different solute which has been originally pumped uphill by the Primary Active Transport (Fig. 36b). Thus, a concentration gradient of Na⁺ maintained by Na⁺/K⁺ ATPase or a proton gradient is used to transport a second molecule. Examples are Na⁺–glucose transport and H⁺ Lactose transport. Some cells may utilize 30% to 50% of ATP on active transport.

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![Fig. 36: Two types of Active transport (a) Primary Active Transport, (b) Secondary Active Transport](Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)
Group Translocase

Another type of Active Transport for sugar is known as Group Translocase which is present in unaerobic bacteria such as E.coli where the sugar is released as sugar phosphate in the cell, utilising phosphoenol pyruate (PEP) as the energy source, such as PEP; glycose phospho-transferase system (PTS) for glucose, fructose, mannose etc. This is a means to conserve energy as the same high energy compound (PEP) is used to actively transport the sugar molecule and to prime it to a form (Sugar Phosphate) which can directly enter the metabolic pathways (bypassing the hexokinase step which involves phosphorylation of sugar by ATP).

There are three general types of Transport (Fig. 37):

1. A Uniport is the transport of a single molecule at a time. The transport by glucose carrier of erythrocyte is an uniport.
   Co-transport is the transport of two different types of molecules at the same time.
2. When two different molecules are transported in the same direction, the transport is symport e.g. Na+ glucose transporter of intestinal epithelial cells.
3. When the two different molecules are transported in opposite directions, the transport is antiport e.g. Na+/K+ ATPase and ADP3-/ATP4- transporter.

The transport can be:
1. Electroneutral if there is a charge neutralization by symport of oppositely charged ions or antiport of similarly charged ions. e.g. (H+ – K+) – ATPase of gastric mucosal parietal cells.
2. Electrogenic if a charge separation results during transport. Thus, ADP3-/ATP4- Transporter is an electrogenic antiporter.

![Fig. 37: Schematic diagram illustrating three groups of biological transport systems](Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

Reconstitution of Transport Proteins

Purified transport proteins can be reconstituted in vesicles as discussed for integral membrane proteins. Their characteristics can be determined and studied by using analogs of solute to be transported as also the various inhibitors. Genetic engineering, site directed mutagenesis has also
been used to study the amino acid residues of transport proteins and channels involved in the transport.

**Ionophores**

Ionophores are compounds which transport ions across cell membranes. They may be synthetic or natural compounds. The natural ionophores are non-ribosomally synthesized by some bacteria. The ionophores have the ability to bind and transport ions across the plasma membrane and disrupt ionic concentration gradients and thus destroy bacteria, e.g. Gramicidin, Valinomycin and Monensin etc. Ionophores are valuable tools in studying membrane transport and influence of ions on membrane functions such as energy transduction etc.

These ionophores are either:
1. Channel formers as in the case of Gramicidin, or
2. Mobile carrier molecules as in the case of Valinomycin.

**Gramicidin**

Gramicidin A is synthesized by *Bacillus brevis*. It acts as a cation ionophore. It is a 15 residue linear polypeptide containing both L– and D– amino acids (Fig. 38) and exists in open helical conformation. Two molecules of gramicidin linked in a head to head dimer form a transmembrane channel through which Na+ and K+ can diffuse (Fig. 39).

![Fig. 38: Gramicidin](Source: Voet, D and Voet J. Biochemistry, 1995)

![Fig. 39: Schematic diagram of transmembrane helix channel formed by two gramicidin molecules in a head to head dimer](Source: Voet, D and Voet J. Biochemistry, 1995)
Valinomycin
Valinomycin is produced by *Streptomyces*. It is a cyclic polypeptide like molecule and has 3 repeats of the sequence – [D-Val – L-Lactate – L-Val – D-hydroxy isovalerate] (Fig. 40a). Its folded conformation is like a ‘donut’ shaped molecule with a hydrophobic exterior and 6 to 8 oxygen and several nitrogen atoms protruding into the central cavity (Fig. 40b). These oxygen atoms can chelate ions. Valinomycin is highly specific for K⁺ which can fit in its central cavity. The larger hydration shells around other cations exclude them from the central cavity of Valinomycin.

The hydrophobic exterior makes the ionophore soluble in the membrane so that it can diffuse within the membrane. Thus, valinomycin binds K⁺ on one side of the membrane, chelate it inside its central cavity diffuses in the membrane and then releases it to the other side. Valinomycin has 20,000 fold preference for K⁺ over Na⁺ and Monensin prefers Na⁺ by 10 fold.

These two modes of transport of ions by these ionophores can be distinguished by measuring the rate of transport of K⁺ as a function of temperature in Phospholipid vesicle containing valinomycin in one case and gramicidin in another. The transport by gramicidin is independent of temperature whereas that by valinomycin is almost negligible below the mid-transition temperature (Tm) because, for a molecule to diffuse in the vesicle, the lipids should be in a fluid state.

**Mechanism of Biological Transport**
Membrane Transport in most cases, is by pore or channel formation and not by mobile carrier mechanism. The only known case for mobile carrier mediated mechanism beside ionophores is the transport of protons by nonprotein lipid soluble carriers, ubiquinone and plastoquinone, in the mitochondrial inner membrane and chloroplast thylakoid membrane respectively.
Model for the Mechanism of Biological Transport

The exact mechanism for biological transport is not yet elucidated. Since the integral membrane transport or facilitator proteins can neither rotate, diffuse or flip-flop within the lipid bilayer of the membrane. It is postulated that these proteins undergo conformational changes during transport, where the solute to be transported binds to the transporter specifically on one side of the membrane and is released on the other side. Thus,

1. The protein must at least exist in 2 states A and B.
2. In state A, it has a high affinity binding site for the specific solute molecule facing one side of the membrane.
3. Binding of the solute molecule to the protein in state A changes it to the conformational state B.
4. In state B, the binding site faces the other side of the membrane and has little or no affinity for the solute, with the result that the solute is released to the other side.
5. On dissociation of the solute, the transporter switches back to the original conformation State A.

This alternate access and release mechanism has been postulated for major facilitator transport proteins where solute acquisition or release provides the requisite energy and controls the conversion of one conformational state to another. This postulated mechanism is illustrated in Fig. 41.

![Fig. 41: Generalized molecular model for transport conformational rearrangement of subunits of transport proteins](Source: Zubay, G. Biochemistry, 1984)

In case of Active Transport or pumps, the solute binding in conformational state A leads to the phosphorylation of a specific residue on the protein by ATP which leads to a change in conformation to State B when the solute is released to the other side. Dephosphorylation reverts the conformation to State A; Such a model has been postulated for Na⁺ / K⁺ ATPase an antiport where Na⁺ is pumped out of and K⁺ is pumped into the cell.

Passive Transport

Anion Channel or Exchanger or Cl⁻/HCO₃⁻ Exchange Protein

This is a 89–KD dimeric protein which traverses the membrane 12 times. It is an Cl⁻ /HCO₃⁻ electro neutral antiport). Its N-terminal domain extends towards the cytosol and is associated with the peripheral protein, ankyrin which binds it to the cytoskeleton.
There is a one to one exchange of $\text{HCO}_3^-$ for $\text{Cl}^-$. Transport of $\text{HCO}_3^-$ out of the cell is coupled to inward transport of $\text{Cl}^-$. Carbon dioxide accumulated in the erythrocytes from respiring tissues is rapidly diffused out of the red blood cells in the lung capillaries (Fig. 42). Its role is to increase the $\text{CO}_2$ carrying capacity of the blood.

**Fig. 42: Chloride–bicarbonate antiporter of the erythrocyte membrane**  
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

**Glucose Transporter (GLUT–1)**

Glucose is a universal energy source and is taken up by a variety of transport proteins by different types of cells.

Glucose Transporter (GLUT–1) of erythrocyte is a 55–KD glycoprotein having:

1. A bundle of 12 membrane spanning $\alpha$–helices arranged such that they form a cylinder with a central channel lined by hydrophilic residues and the hydrophobic residues arranged on the exterior or outside the cylinder.
2. A highly charged cytoplasmic domain between helices 6 or 7.
3. The carbohydrate domain on the outside between helices 1 and 2, while the C–terminal domain is on the cytoplasmic side.

A proposed model for glucose transporter is shown in Fig. 43.

Glucose transporter is a uniport, transporting glucose in either direction down the concentration gradient. It thus helps to maintain glucose concentration (levels) in the blood.
Various glucose transporters, depending on their locations in tissues, are known in humans. Thus,

- GLUT–1 is present in most tissues
- GLUT–2 mostly in pancreatic B cells while
- GLUT–4 in muscle and fat cells which are responsive to insulin.

The number of GLUT–4 increases in the plasma membrane, when insulin binds to its receptor by exocytosis or movement of vesicles containing GLUT–4 from the cytoplasm to the plasma membrane on receiving the appropriate signals conveyed by insulin binding to insulin receptor. Glucose transport of GLUT–1 like all other transport proteins, as discussed in the earlier section, is by alternating conformational model or “rocking banana model” (Fig. 44).

**ADP–ATP Exchanger / Transporter of Mitochondria**

In eukaryotes, ATP is synthesized in the mitochondria while it is utilized mostly by processes occurring in the cytosol. Since the inner mitochondrial membrane is impermeable, ATP is transported out of mitochondria by the ADP–ATP exchanger which obligatory transports ADP into the mitochondria. This is an antiport, there is one to one exchange of ATP\(^+\) for ADP\(^-\) and is electrogenic thus, the membrane potential difference or proton gradient of actively respiring

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**Fig. 43: Predicted model for human erythrocyte membrane glucose transporter**
(Source: Voet, D and Voet J. Biochemistry, 1995)
mitochondria exports ATP out and imports ADP into the mitochondria and is, thus, an active transport.

**Fig. 44: Molecular model for ATP/ADP exchanger** *(Source: Zubay, G. Biochemistry, 1984)*

ADP–ATP exchanger is a dimer of identical 30 KD polypeptides. The monomers cannot transport ADP or ATP. The dimeric protein has a single nucleotide binding site which has a high affinity for ATP on the matrix or inside of the mitochondria in one conformation. This site is specifically inhibited by bongkrekic acid (Fig. 45a). The binding site on the outside has high affinity for ADP and is inhibited by the respiratory poison, atractyloside (Fig. 45b). The binding site is never on both sides of the membranes simultaneously.

**Fig. 45: (a) Atractyloside; (b) Bongkrekic acid**
It must bind ATP or ADP to change from one conformation to other (Fig. 44).

**Active Transport**

The major types of active transport are:

**I. Primary Active Transport**

(i) *ATP as energy source*

(a) P–type ATPases
e.g. Na\(^+\) – K\(^+\) ATPase, Ca\(^{2+}\) – ATPase, H\(^+\) – ATPase

(b) F–type ATPases
eg. F\(_1\)–F\(_0\) ATPase of mitochondrial inner membrane.
F\(_1\)–F\(_0\) ATPase of chloroplast thylakoid membrane.

(c) V–type ATPase in plant vacoules.

(d) A–ATPase in bacteria

(ii) *Respiratory electron transport chains which pump H\(^+\) out of mitochondrial matrix.*

(iii) *Light driven H\(^+\) pumps of Halobacterium, bacteriorhodopsin and in photosynthesis.*

**II. Secondary Active Transport**

(a) H\(^+\) – PO\(_4\)\(^{2–}\)  antiport in mitochondria

(b) H\(^+\) – K\(^+\)  antiport in mitochondria

(c) H\(^+\) – lactose  symport in bacteria

(d) Na\(^+\) – glucose  symport in intestinal and kidney epithelial cells

(e) Na\(^+\) – aminoacid  symport in intestinal and kidney epithelial cells

(f) Na\(^+\) – Ca\(^{2+}\)  Antiport in heart muscle

**III. Group Translocase**

Phosphoenol pyruvate:glycose phospho-transferase system (PTS) of bacteria where sugar is transported and released inside the cell as sugar phosphate.

Some of the examples of active transport are discussed below:

*Na\(^+\) – K\(^+\) ATPase or Sodium Pump of Plasma Membranes*

The classical example of Primary Active Transport is Na\(^+\) –K\(^+\) ATPase, also, known as sodium pump and is the most thoroughly studied active transport system.
It is ($\alpha\beta$) dimer, each consisting of $\alpha$ and $\beta$ subunits. The 110 KD $\alpha$–submit contains the binding sites for the ions and the site phosphorylated by ATP. The 55 KD $\beta$–subunit is glycoprotein whose function is not known (Fig. 46).

The $\alpha$–subunit has around 8 $\alpha$-helices forming the transmembrane domain and two large cytoplasmic domains. An aspartyl group in $\alpha$-subunit is phosphorylated by ATP to form a reactive aspartyl phosphate intermediate which has been isolated by reduction with borohydride to homoserine.

The $\beta$-subunit has a large extra cellular domain with carbohydrate moieties on the exterior side and a single Transmembrane helix.

Its known inhibitors cardiotonic steroids and ouabain (wah bane, an arrow poison used in East Africa) binds on the external side, so also the K+, while the Na+ and ATP binding sits are on the cytoplasmic side (Fig. 46).

![Fig. 46: The putative dimeric structure of Na+–K+ ATPase](Source: Voet, D and Voet J. Biochemistry, 1995)

It is called sodium pump as it pumps Na+ out of and K+ into the cell against a concentration gradient.

It is, thus, an electrogenic antiport as 3Na+ are pumped out and 2K+ pumped in. The molecular model given for this pump involves two conformational states $E_1$ and $E_2$ where

1. $E_1$ faces cytoplasmic side and has a high affinity Na+ binding site.
2. Na+ binding to $E_1$ – [E1 3 Na+] promotes phosphorylation of aspartyl residue by ATP [E1 – P . 3Na+].
3. Phosphorphylation of $E_1$ results in a change of conformation to [E2 – P . 3Na+].
4. $E_2$ site faces outside and has a low affinity for Na+ which is released to form [E2–P] where the site has a high affinity for K+.
5. Binding of K+ forms [E2 – P . 2K+] which results in dephosphorylation of and release of Pi into the cytoplasm and reversal of the conformation [E1 – 2K+].
6. $E_1$ having a low affinity for K+, releases K+ in the cytoplasm.
This model is depicted in Fig. 47.


**Inhibition by Cardiac Glycosides**

The cardiotonic steroids and ouabain (Fig. 48) bind specifically to the sodium pump on the outside in the [E₁~P] state. This results in an increase in the intracellular concentration of Na⁺ which stimulates Na⁺–Ca²⁺ antiport of cardiac cells to pump in Ca²⁺. The intracellular increase in Ca²⁺ concentration leads to muscle contraction of heart. These cardiac glycosides induced, increase in the Ca²⁺ concentration leads to muscle contraction of heart and thus are effective in treating congestive heart failure. Initially it was believed that they were present in fox plant and East African Ouabio tree. Cardiac glycosides have been recently discovered to be produced by adrenal cortex, as an animal hormone that regulates Na⁺ concentration of the cell and overall body salt and water balance.

![Ouabain](source: Voet, D and Voet J. Biochemistry, 1995)
Significance of Na⁺ – K⁺ ATPase

It is responsible for maintaining the intracellular concentrations of Na⁺ and K⁺ (Fig. 49) and generally transmembrane electrical potential which is essential for electrical excitability of nerve cells. The sodium gradient is also used to drive the cotransport of several solutes in many cell types e.g. Na⁺–glucose and Na⁺–amino acids symports in intestinal and kidney epithelial cells, Na⁺–Ca²⁺ antiport in muscle cells etc. All cells utilize a large fraction of the produced ATP (70% in nerve cells) to maintain the required Na⁺ and K⁺ concentration.

![Na⁺–K⁺ ATPase](image)

Fig. 49: Na⁺–K⁺ ATPase of animal cell primarily responsible for setting and maintaining of intracellular concentration of Na⁺ and K⁺ and for generating transmembrane electric potential (Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

Ca²⁺ – ATPase or Calcium Pumps

These are present in the plasma membrane and Endoplasmic reticulum and sacroplasmic reticulum of the muscle cells. It regulates the Ca²⁺ concentration in the cells which is crucial for a number of cellular responses and activity e.g. muscle contraction, release of neurotransmitters, and as a second messenger.

Plasma membrane Ca²⁺ – ATPase actively pumps Ca²⁺ out of the cytosol. Their mechanism is very similar to Na⁺ – K⁺ ATPases and belongs to the P–type ATPases. The sarcoplasmic and endoplasmic reticulum calcium (SERCA) pumps are closely related and are inhibited by tumor promoting agent thapsigargin which does not inhibit the plasma membrane Ca²⁺–pump.

Ca²⁺ Pump of sacroplasmic reticulum is a 100 KD single polypeptide having 10 -helices, traversing the membrane. It has 3 cytoplasmic domains connected to transmembrane helices (Fig. 50). The Ca²⁺ pump has 2 Ca²⁺ binding sites near the middle of the membrane layer, away
from the aspartyl residue which is phosphorylated by ATP and the pump undergoes conformational changes from $E_1$ to $E_2$ as in the case of sodium pump.

![Diagram of Ca²⁺ pump of sarcoplasmic reticulum](image)

**Fig. 50: Structure of Ca²⁺ pump of sarcoplasmic reticulum**  
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

**ATP–Binding Cassette (ABC) Transporters or Multidrug ABC Transproter**

These are integral membrane proteins that actively transport chemically diverse substrates across the lipid bilayer of cellular membrane in *E. coli*, human, plants and simple animals etc. The substrates transported include lipids aminoacids, peptides, proteins, metal ions and drugs against a concentration gradient. The multidrug transporter (MDR–I) in human exports cytotoxic drugs across cell membranes and cause tumor cells resistance to drugs used in chemotherapy such as adriamycin, doxorubicin and vinblastine. Their presence confers resistance in pathogenic microbes.

These homodimeric ABC Transporters in *E. coli* consists of 12 transmembrane helices in an arrangement similar to multidrug resistant protein MDRI and have two transmembrane domains and exist in 2 conformation. In the outward facing conformations, ATP binding occurs with the two nucleotide binding domains (NBD) (that hydrolyse ATP) in close contact towards the cytoplasm and the two transmembrane domain from a central cavity through which the drug translocation occurs and is closed from the inner leaflet of the lipid bilayer and the cytoplasm but is open to the outer leaflet and extracellular space (Fig. 51). The tight interaction of NBDs in
ATP binding state is coupled to outward facing conformation of transmembrane domain when the bound substrate escapes to the outside into the aqueous medium surrounding the cell. ATP hydrolysis returns it to its inward facing conformation.

Fig. 51: Structure of two ABC Transporters of E.Coli. (a) The lipid A flippase, MsbA, (b) vitamin B12 importer Bt4 CD
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

Thus, the ABC transporters use the same mechanism as all major facilitator transport proteins but herein ATP binding and hydrolysis and not substrate binding controls the conversion of one state to another.

Most ABC transporters bind and hydrolyze two ATP molecules in each reaction cycle. The number of bound substrates can vary and depends upon the size of the molecular mass. In human MDR1 and MDR2 up to two substrates have been found to enter the binding pockets. Thus, for small substrates, the stoichiometry of ATP hydrolysis is one whereas for a single large molecule, it will be two.

Most ABC transporters are in the plasma membrane while some types are also found in endoplasmic reticulum, in mitochondrial membrane and lysosomes. Most ABC transporters act as pumps but some members act as ion channels that open and close by ATP hydrolysis e.g. CFTR / cystic Fibrosis Transmembrane Conductor Regulator) is a Cl⁻ channel. Flippases which move membrane lipids from one leaflet to another leaflet of the bilayer are ABC Transporters.

**Secondary Active Transport**

The ion gradients established by primary transport of Na⁺ or protons can provide energy for cotransport of other solutes such as another ion, sugar, amino acids or dicarboxylic acids.

**Na⁺–Glucose Symport**

Na⁺ glucose and Na⁺ amino acids cotransport system are present in the specialized plasma membranes of intestinal and kidney epithelial cells. The extracellular high concentration of Na⁺ drives the symport of glucose and amino acids by specific symporters in the apical region of the
cells. The Na\(^+\) concentration is maintained by the pumping of Na\(^+\) into blood by Na\(^+\)–K\(^+\) ATPase present in the basal region of these cells (Fig. 52).

![Intestinal lumen](image)

**Fig. 52: Transport of glucose in intestinal epithelial cells.** The brush border cells concentrate glucose from intestinal humen by Na\(^+\)–glucose symport which is driven by Na\(^+\)–K\(^+\)–ATPase located in the basal membrane. Glucose is exported to blood stream by facilitated uniport (Source: Voet, D and Voet J. Biochemistry, 1995)

Phlorizin inhibits Na\(^+\)–glucose symporters whereas cytochalasin B inhibits glucose carriers or transporters and thus the two types of glucose transports can be differentiated.

The Na\(^+\)–glucose symporter in the epithelial cells of the intestine is a two Na\(^+\) - one glucose symporter. Its exact mechanism is not elucidated as such but conformational changes in the symporter are important and a model analogous to glucose transporter has been proposed.

**Lactose – H\(^+\) Symporter**

Lactose–H\(^+\) symporter in *E. coli* has been studied in considerable detail. Proton gradient established by proton pump drives the uphill movement of Lactose (Fig. 53). When the energy yielding oxidation reductions are inhibited by cyanide (CN\(^-\)), Lactose permease transport lactose passively down a concentration gradient till equilibrium is attained.

![Lactose transporter](image)

**Fig. 53: Lactose–H\(^+\) Symporter in E.coli**
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)
The lactose transporter belongs to Major Facilitator Super family (MFS) which has 28 members. The *E.coli* lactose transporter has 12 membrane spanning $\alpha$-helices (in some cases 14), the cytoplasmic domains and the extraperiplasmic domains exhibit two fold symmetry. The MFS proteins have similar secondary structure and topology even though there is no sequence homology. The transport of lactose is proposed to be through rocking banana model between the two halves of the transporter which undergo conformational change during one cycle of transport. In one state the lactose binding site is exposed to the periplasmic. Space (Fig. 54b) where lactose binds. In other state (Fig. 54a) lactose is released. The interconversion between the two states is due to protonation in the pairing of charged side chain residues such as Glu 323 and Arg 302 occurring by transmembrane proton gradient.

![Fig. 54: Structure of Lactose transporter of *E.coli* showing the rocking of the two halves of the transporter undergoing conformational changes which occur during one transport cycle from the form shown in (a) to that in (b)](Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

Genetic studies involving mutation show that six residues are essential for lactose–H$^+$ co transport, Glu 325 and Arg 302 are necessary for proton symport as mutation in these residues converts this transporter to a facilitated transporter of lactose and it cannot couple proton gradient to transport lactose against a concentration gradient. The conformational change in the states of Lactose symporter is due to charge pairing between Glu 325 and Arg 302

**Group Translocase**

This is a type of Active Transport where phosphoenol pyruvates, PEP, is used by many bacteria to transport sugars into the cell and also to modify the sugars chemically to sugar-phosphates. The transport most studied is the phosphoenol pyruvate–glucose phosphotransferase system, (PEP – PTS) of *E. coli*, first shown by Roseman in 1964. The high energy compound, Phosphoenol pyruvate (PEP) is the energy source and the sugar is released as sugar-phosphate inside the cell. The sugar phosphate is retained since the cell membrane is impermeable to them. There are no transporters for sugar phosphates in the membranes. Some of the PTS–transported sugars are glucose, mannose, mannitol etc. The PTS system of *E.coli* and *Salmonella typhimurium* are well studied.

This transport involves several soluble and membrane bound proteins catalyzing the reactions as depicted in Fig. 55.
Enzyme I and HPr, (Histidine containing Phosphocarrier protein) are soluble proteins required for all sugars transported. Enzyme II is an integral membrane protein specific for type of sugar to be transported and requires diacyl glycerol as a cofactor. In *E.coli*, there are seven different proteins of Enzyme II, each specific for a sugar molecule. In some cases, a sugar specific Enzyme III protein is attached loosely to enzyme II – on the cytoplasmic side of the membrane for some sugars and cytoplasmic for others, is involved in phosphorylation of sugar as it is transported (Fig. 55).

**Fig. 55: Transport of glucose by the PEP–glycose phosphotransferase system (PTS)**
(Source: Voet, D and Voet J. *Biochemistry*, 1995)

1. Enzyme I is a dimer of 70 KD subunits.
2. HPr is a monomer of 9.5 KD. A specific Histidine residue in Enzyme I, HPr and Enzyme III is phosphorylated by PEP during the transport reaction. Enzyme II, or mannitol permease, is a monomer of 68 KD, while its subunit structure is not known for other sugars.
3. A cysteine residue of EII$_{glu}$ is phosphorylated.

Four phosphorylated intermediates have been identified (Fig. 55)

$$\text{PEP} \rightarrow \text{EI} \rightarrow \text{HPr} \rightarrow \text{EIII}^{\text{glu}} \rightarrow \text{EII}^{\text{glu}} \rightarrow \text{glucose}$$

This is an energy efficient system as only one ATP equivalent transports as well as phosphorylates the sugar.

The PTS is a complex system and is genetically regulated by *catabolite* repression mediated by c–AMP concentrations.
Channels

These are transmembrane proteins which form channels or pores through which ions and molecules can flow down their concentration gradient passively depending upon the pore size and amino acid residues lining the channel. Here, the movement of ions or water is very fast, $10^7$ to $10^9$ s$^{-1}$. The channels do not exhibit the characteristics of biological transporters i.e. specific binding and saturation kinetics.

1. Aquaporins or Water Channels

Aquaporins are a family of integral membrane proteins which act as specific water channels. They are involved in rapid movement of water molecules across all plasma membrane in a number of animals and plant tissues and in bacteria and were discovered by Peter Agre. Ten Aquaporins are known in human such as in erythrocytes, renal medulla, nephrons etc. Aquaporin I (API) plays a central role in water reabsorption of kidney. Its structure is shown in Fig. 56.

![Fig. 56: Structure of an aquaporin, AQP–1 tetramer viewed in the plane of the membrane](Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

Human Aquaporin exists as a homotetramer homologous protein in the plasmamebrane. It has a unique fold referred to as “hour glass” or “dumb bell”. The protein contains four pores, one per subunit. Each 28 KD monomer contains four transmembrane $\alpha$-helices. Each pore is formed from two half hemipores. Each of the two halves has three bilayer spanning domains where the cytoplasmic chamber (loop $\beta$) connects within the membrane to an extra cellular loop to form a single narrow aqueous pathway. The motif ‘asn – pro – ala (NPA) at residue 76 to 78 in cytoplasmic loop $\beta$ and 192 to 194 in extracellular loop are oriented at 180º to each other. The second NPA motif is near the Cys 189 which reacts with mercurials inhibiting water permeability. Arg 195 and His180 in the channel give a positive charge to the channel while Phe 56 and Glu 142 are important residues in the pore region. These highly conserved amino acid residues stabilize the novel fold of Aquaporins. The subunit oligomerisation may provide vertical symmetry necessary for residence within the lipid bilayer.

The conserved hydrophobic residues lining the channel allow a rapid, passive water transport selectively due to pore diameter of 0.2 to .3 nm over a span of one residue. This permits passage of water molecules in a single file at a rate of about $10^9$ s$^{-1}$ in a continuous stream in the direction of osmotic gradient. Protons are unable to move down the pore because of their hydration to
H$_3$O$^+$ which is excluded by the narrow pore size. Thus, water selectivity is due to the pore size. These channels increase the permeability to water by as much as ten fold. The structure of API explains why membranes are permeable to water and impermeable to protons.

In some plants, aquaporins may close by the blocking of the pores by the cytoplasmic loops in response to signals such as draught stress or triggered by cellular signals such as pH change or dephosphorylation.

2. **Bacterial Porins**

Porins are present in outer membranes of bacteria. Also, mitochondria and chloroplast outer membrane contains pores which are nonspecific and allow movement of certain small molecules to pass through.

Bacterial porins have been studied in details. They are channel forming proteins whose X–ray structure is known. They are trimeric transmembrane proteins. Each identical subunit consists of 16–stranded anti–parallel β–barrel which forms a channel along its barrel axis through which solutes can pass. (Fig. 57a).

In *E. coli* Omp F porin (Fig. 57b), the channel is 5 nm long and is constricted near its center to form a pore with a minimum cross-section of 0.7 x 1.1 nm which allows only molecules of upto 600D to pass through. This omp F porin is weakly cation selective whereas *E. coli* Pho E porin is weakly anion selective. The difference in ion selectivity is due to the presence of specific Lys 131 in PhOE and Gly 131 in Omp F which protrude in the channel. If lys 131 is altered by mutation to Glu 131, the anion selectivity of PhOE is abolished. The lysine residues present at the mouth of the Channel in PhOE have no role in ion selectivity as their mutation does not alter the anion selectivity. Thus, probably the lysine residues at the mouth function to attract anions and Lys 131 facilitates the passage of anion through the channel.

![X-ray crystal structure of E.coli OmpF porin](image1)

![Longitudinal section through the pore of OmpF porin subunit](image2)

*Fig. 57: (a) X–ray crystal structure of E.coli OmpF porin. The Ribbon diagram of the monomer; (b) Longitudinal section through the pore of OmpF porin subunit as seen in neurons (Source: Voet, D and Voet J. Biochemistry, 1995)*
3. Gap Junction

As mentioned earlier, Gap junctions are present in the plasma membrane connecting the adjacent cells in several tissues. These are hexameric protein channels which appear as ‘Rosettes’. Molecules upto 600D such as glucose, ATP, ions and other small molecules can diffuse through these channels to regulate metabolic activities between the cells. The opening and closing of these gap junctions is regulated by Ca\(^{2+}\) concentration which induces a conformational change by tilling of the hexameric units (Fig. 31b).

4. Ion–Channels

Gated ion-channels are multimeric proteins which open in response to a specific signal such as (i) voltage or membrane potential changes and (ii) ligands such as neurotransmitters and c–AMP and c–GMP (Fig. 58).

![Fig. 58: Types of ion channels in neuron plasmamembrane](Source: Lodish, H., Baltimore, et al. Molecular Cell Biology, 1995)

Voltage gated, Na\(^{+}\)–Channels and K\(^{+}\) Channels are present in brain and nervous tissues. The voltage gated Ca\(^{2+}\) channels are present in muscle, heart and neuromuscular junction where they have a role in muscle contraction and release of acetylcholine from vesicles.

Ligand gated channels are the neurotransmitter receptors which open on binding of a specific ligand such as Acetylcholine, Gama-amino butyric acid, GABA. They are present in brain and nerve tissues. They have an important role in conduction of nerve impulse and maintaining ion gradient in the nervous tissue; acetylcholine receptor is a cation channel whereas GABA receptor is an anion channel.

The c–GMP and c–AMP gated ion channels are abundant in the sensing cells and have a role in visual and olfactory systems.

Another class of Ca\(^{2+}\) channels are the IP\(_3\) induced, present in sarcoplasmic reticulum and also the calcium release activated calcium (CRAC) channels present in Drosophila and mammals. Single ion channels can be studied by Patch–Clamp technique.
**Exocytosis and Endocytosis**

Cells release or take up large molecules or material in a controlled mechanism by forming membrane enclosed vacoules or vesicles around them in a process known as Exocytosis or Endocytosis respectively. (Fig. 59a, b). The flexibility and self sealing properties of biological membranes as also the fusion of membranes mediated by specific proteins play an important role in exocytosis and endocytosis and viral invasion. Formation of vesicles and their transport within the cell plays on important role in these processes. These vesicles are targeted to different destinations where active guided transport of vesicles may be involved. Microtubules and associated ATP driven motors appear to have a role in the intracellular transport of vesicles. Molecular motor, kinesin and dynein, transport vesicles along microtubules in the axon of a nerve cell.

![Diagram of (a) exocytosis and (b) endocytosis](source: Elliot, Witt; Elliot, D.C. Biochemistry and Molecular Biology, 1997)

**Exocytosis**

This is the process where the cells releases large molecules such as secretory proteins and waste products contained in a membrane vesicle or vacoules to the outside of the cells or to the plasma membrane. The latter provides a mechanism to form and remodel plasma membrane i.e. deliver membrane proteins (receptors, enzymes etc.) and lipids to the plasma membrane. Exocytosis is a common secretory mechanism in eukaryotes.

Exocytosis is of two types:
I. Constitutive Exocytosis  
II. Regulated Exocytosis
Constitutive Exocytosis

This type is independent of $\text{Ca}^{2+}$ and other signals and is performed by all cells. It serves to release:

(i) Components to the extracellular matrix such as carbohydrate chains and proteins from golgi vesicles to the outside of plant cells during cell wall synthesis.
(ii) Serum proteins from liver.
(iii) Waste products from the cell by protozoa.
(iv) And to delivers newly synthesized membrane proteins and lipids to be incorporated in the plasma membrane.

Regulated Exocytosis

Here, the vesicle containing secretory proteins releases its contents or membrane receptors etc. to the outside of the cell or to the plasma membrane on receiving a signal such as $\text{Ca}^{2+}$ increase or hormone etc.

The regulated exocytosis includes:

(i) The transport of Glut–4 (Glucose Transporter–4) containing vesicles to plasma membrane of adipose and muscle cells when insulin binds to its receptor in these cells (Fig. 60).

![Fig. 60: Insulin stimulation of GLUT–4 transporters of plasma membrane of myocytes by exocytosis of GLUT–4 membranous vesicles. When insulin is withdrawn, the process is reversed by endocytosis.](Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)
(ii) Secretion of digestive enzymes enclosed in vesicles by acinar cells of pancreas directly into the lumen of the intestines on receiving hormonal signal.

(iii) Release of acetylcholine from vesicles containing acetylcholine which fuse with cell membrane at synaptic when \( \text{Ca}^{2+} \) level rises as a result of the opening of voltage gated \( \text{Ca}^{2+} \) channels in neurons.

(iv) In the \( \text{Ca}^{2+} \) induced formation of a barrier to further sperm penetration after fertilization of an ovum.

Exocytosis is thus a general term used to denote vesicle transport and fusion at plasmamembrane and the release of its contents. It is the final step in the secretory pathway that begins at endoplasmic reticulum, passes through the Golgi apparatus and ends outside the cell. Sorting of contents in the vesicles occurs in the endoplasmic reticulum, golgi or post golgi compartments and are modified enzymically into their mature form ready for delivery (Fig. 61).

Vesicle formation for transport between golgi involves a mechanism in which GTP–protein complexes bind to the vesicle membrane which results in binding of COP (for coat protein) molecules and four other proteins to form a complex called Coatamer around the vesicle. Budding of the vesicle at the binding site leads to the formation of a coated (non is clathrin coated) vesicle which play a role in intra golgi transport (Fig. 62 a, b, c).

These coated vesicles uncoat at the target membrane as a result of GTP hydrolysis to GDP by Rab proteins in mammals, which are GTPases. The Rab proteins play a crucial role where GTP hydrolysis is the signal for uncoating, docking and fusion of vesicles to its target membrane. Recognition of the vesicle for the target membrane sites is via special recognition proteins such as:

v–SNARE (where v is for vesicle and SNARE, soluble NSF attachment protein receptor, located in the external surface of the vesicle). t–SNARE (t is for target vesicle) is the partner protein located on the cytosolic face of the target membrane which is plasma membrane in case of exocytosis. Two other proteins, NSF, N–ethyl maleimide sensitive factor) and SNAP (a soluble NSF attachment Protein) besides other proteins are important in docking and fusion of vesicle in animals and yeast cells. NSF may act to prime SNAREs before docking and to recycle SNAREs after fusion. Specific v– and t–SNARE are associated with exocytosis. Different combinations of SNAREs and other proteins are characteristic of other docking events (Fig. 62c).

Docking is the process by which the vesicle is fixed beneath the target membrane or plasma membrane on the cytosolic side before fusion requiring molecular recognition between vesicle and the target membrane (Fig. 62a). The basic model predicts that while docking a dimer or more likely multimeric complexes are formed with one or more SNAREs from each of the two fusing membrane.
Fig. 61: Diagram showing vesicle transport from endoplasmic reticulum to golgi. The vesicles bud from rough endoplasmic reticulum to the cis face of golgi. Primary lysosomal vesicles bud from transgolgi
(a) Steps in the transport between cis and medial golgi vesicles

(b) Formation of non–clathrin–coated vesicle from the cis golgi
The fusion of the vesicles at the plasma membrane leads to the formation of a fusion pore which is a channel that passes through the vesicle and the plasma membrane and allows the content of the vesicle to move out to the extracellular milieu or compartment. After the transient fusion pore opening, as the contents of the vesicle are released, there is a rapid closure of the pore. Alternatively, the pore formation is followed by full pore opening with incorporation of vesicle membrane into the plasma membrane. Both transient and permanent fusion can be found in the same animal (Fig. 63).

In presynaptic nerve terminal when there is an increase in $\text{Ca}^{2+}$, signals the release of acetylcholine, the core complex which is trimeric, comprising of two t–SNAREs Syntaxin and SNAP–25, (synaptosome associated protein of 25 KD) and synaptobrevim, a v–SNARE is formed which is a bundle of four. This pulls the two membranes together and the bilayer is disrupted at that point which leads to membrane fusion, fusion pore formation, and the release of the neurotransmitter at a synapse (Fig. 64). The complex of SNAREs and SNAPs is digested by the Clostridium botulinum toxin which is a protease and thus blocks neurotransmission causing death of the organism.

**Endocytosis**

Endocytosis is the internalization of material from the extracellular milieu. It is the process by which a portion of the cell membrane forms a pit or invagination around large molecules (food particle, protein, virus, bacteria etc.), engulfs or encloses it by forming a new intracellular membrane enclosed vesicles which is released inside the cell (Fig. 59b). Also, membrane proteins, receptors and transporters are endocytosed e.g. GLUT–4, a glucose transporter in muscle and adipose cells is endocytosed when glucose concentration in blood falls and insulin dissociates from its receptor (Fig. 60).
Fig. 63: Diagram showing exocytosis by fusion of vesicles with the plasmamembrane
Fig. 64: Fusion during neurotransmitter, acetyl choline, release at a synapse
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)
Endocytosis is of 3 types:

(i) Phagocytosis (Cell Eating) when the substance ingested is a solid such as food in case of protozoa or bacterial cells in case of white blood cells.

(ii) Pinocytosis (cell drinking) when liquid is taken in e.g. human egg cell takes nutrients from the “nurse cells” by pinocytosis.

(iii) Receptor Mediated endocytosis: In this type material to be taken up, first binds to a specific protein receptor in the plasma membrane and then the receptor-material (ligand) complex is engulfed as such in a vesicle — the clathrin coated vesicle which is endocytosed.

Most cells in the body take up external particles or molecules by receptor mediated endocytosis and is a widely used pathway for cholesterol, ferritin, cell growth factors, some viruses etc.

Receptor mediated endocytosis of LDL

LDL—receptor is a glycoprotein. It specifically binds apolipoprotein B–100 and apoE. The receptor synthesized in the endoplasmic reticulum, matured in the golgi complex, migrates to plasma membrane by exocytosis. It is located in an invagination pit or coated pit where the most abundant protein is clathrin. Clathrin is made up of triskelion units which are self interacting and form a cage like structure (Fig. 65).

![Fig. 65: Three dimensional map, generated from electron micrographs, of a clathrin coated vesicle](Source: Voet, D and Voet J. Biochemistry, 1995)

LDL particle made up of cholesterol esters and apoproteins, binds to the LDL—receptor and the entire LDL—LDL receptor complex is engulfed and taken up by fusion of the plasma membrane
as an endocytotic vesicle. Several such vesicles form an endosome (uncoated vesicle) which fuses with a lysosome or sorting vesicle to form a compartment where the receptor dissociates from LDL. The receptor is recycled and returns to the plasma membrane by exocytosis. The LDL–apoprotein is hydrolyzed to amino acids and the cholesterylesters are hydrolyzed to cholesterol and taken up by the cell (Fig. 66).

Cell Signaling and Membrane Receptors

Cell Signaling

Cell signaling is the process by which a signal on the surface of the cell is relayed across its membrane to a specific cellular response inside the cell. This has been conserved during evolution and developed in prokaryotes and adopted by multicellular organisms or animals.

The activities of different cells within tissues or organs need to be regulated or coordinated in a controlled pathway by specific communications. This type of control is dependent on release of signal molecules from one cell and its recognition and the delivery of the signals to the target cells. This is possible when the target cell has the necessary receptor molecules to receive the signals. Such receptor molecules are allosteric proteins and the signal is referred to as a ligand, whose binding to the receptor protein is highly specific and induces a conformational change (Fig. 67a) in the receptor proteins or causes dimerization of the receptor by bringing the

Fig. 66: Diagram showing the sequence of events in the receptor mediated endocytosis of LDL particle (Source: Voet, D and Voet J. Biochemistry, 1995)
cytoplasmic domains together (Fig. 67b) which triggers the relay of message to affect a change in cellular or biochemical function or gene expression.

Thus (i) only the cell types which contain the specific receptors for the ligand (signaling molecules) can respond and cause changes in the cellular function or activities. (ii) The changes in the metabolic or cellular activity depend on the type of intracellular response initiated by binding of the ligand to its receptor.

Fig. 67: Diagram of a signal transduction showing the binding of a signal (ligand) to a receptor
(a) leading to the postulated change in conformation of the receptor protein molecule
(b) causing the dimerization of the membrane receptors bringing the two cytoplasmic domains together which leads to biochemical response
(Source: Elliot, Witt; Elliot, D.C. Biochemistry and Molecular Biology, 1997)

In some case, binding of different signals or hormones to their specific receptors may lead to the same response e.g. epinephrine and glucagon binding to their respective receptors, cause an increase in blood sugar. On the other hand, the same ligand, e.g. acetyl choline causes different responses by binding to different receptors. It binds to
1. nicotinic receptor which is an ion channel involved in nerve conduction and
2. muscarinic receptor which acts through Gq protein and Phospho lipase C and inositol triphosphate, IP$_3$.

The process by which the message from the ligand or signal molecule is transmitted to the target cell to modify its activity or gene expression is called signal transduction.
Signal transduction is modular and consists of several components such as:

1. **Signaling Ligand** (the first messenger).
2. Receptor which binds the signal (ligand) specifically to form receptor – ligand complex and conveys the message to induce metabolic response.
   In some cases, an intermediate component such as G proteins (GTP binding proteins) relays the message from Receptor-ligand complex to an effector enzyme.
3. In some cases, a second messenger or an activated protein or enzyme carries out the desired effect on the enzyme activities of a metabolic pathway or may affect gene expression.

The modular nature of signal transduction allows an amplification of the message at each successive step and diversity of metabolic responses on the same and different operating systems.

In each case
(i) the receptor protein is characterized by a binding specificity for a particular ligand (similar to that of an enzyme for its substrate, but here, no enzyme reaction occurs and neither the ligand nor the receptor undergo any chemical change) and
(ii) the resulting receptor-ligand complex exhibits effector specificity i.e. mediates a specific cellular response. The cooperative or allosteric changes in receptor on binding its specific ligand results in large changes in receptor activation,
(iii) Amplification is by an enzyme cascade which results when an enzyme associated with the receptor is activated which in turn activates second enzyme and so on.

In most receptor–ligand systems, the only function of the ligand is to bind to the receptor and change its properties and provide information for the presence of the specific signal in the environment. The ligand is neither metabolized to any useful products nor has any enzymatic activity.

The general principal in cell signaling, therefore, involves ultimately a series of phosphorylation and dephosphorylation steps of either (i) enzymes to convert them from an inactive to an active state on a cascade pathway or (ii) nonenzymic protein to eventually lead to the desired effect (Fig. 68). The importance of phosphorylation and dephosphorylation by kinases / phosphatase in the control of metabolic pathways or cellular response to hormones was elucidated by Sutherland for the effect of epinephrine on glycogenolysis (Fig. 69) at molecular level.

The cellular response to a signal can be terminated when the ligand is removed from its receptor or the ligand binding receptor is inactivated or by degradation of the second messenger or the ligand.

**Signal Molecules**

Some of the signaling molecules are:
(i) Hormones  (ii) Neurotransmitters  (iii) Pheromones
(iv) Nutrients  (v) Developmental Signals  (vi) Interleukin
(vii) Light  (viii) Antigens  (ix) Cytokines
(x) Nitric Oxide
The signaling molecules can thus include proteins, large and small peptides, catecholamines, thyroxine, steroids, eicosanoid, vitamin D₃, prostaglandin, nitric oxide etc.

**Fig. 68:** Simplified diagram of main events in membrane receptor mediated signaling. An arrow may represent several steps in the process
(Source: Elliot, Witt; Elliot, D.C. Biochemistry and Molecular Biology, 1997)

**Fig. 69:** Effect of epinephrine on glycogenolysis illustrating the cascade pathway
In animals, the extra cellular, secretory signaling molecules can be classified based on the distance over which the signal acts in the following types illustrated in Fig. 70.

(i) Endocrine Signaling: Where the ligand released by the cell affects target cells distant from their site of synthesis in ductless glands and released directly in the blood and carried by blood to the target cells e.g. insulin hormone, epinephrine, glucagon etc. (Fig. 70a).

(ii) Paracrine Signaling, when the signaling molecule released by the cell only affects target cells which are nearby e.g. neurotransmitters and neurohormones in nerve conduction and prostaglandin, polypeptide growth hormones (Fig. 70b).

(iii) Autocrine Signaling, where the cell respond to the signaling molecules they themselves release e.g. growth factors, T–cell proliferation (Fig. 70c).

(iv) In addition, some membrane, bound proteins on one cell can directly signal on adjacent cell by interacting with receptors on adjacent cells (Fig. 70d).

Beside these intracellular or intercellular signaling, there is another type of signaling, that by Pheromones. Pheromones are chemicals released by many organisms and are sexual attractants. They alter the behaviour and gene expression of other organisms of the same species. Some have other functions in species such as ants that have complex social interactions. Yeast mating type factors, the small polypeptides, or pheromones, are well understood pheromone mediated cell-cell signaling. Some algae and animals also release pheromones into air or water to attract members of the other sex.

**Receptors**

Receptors for cell signaling are broadly classified as:
(i) Intracellular receptors
(ii) Membrane or Cell Surface Receptors.

**Intracellular Receptors**

Signaling molecules which are lipophilic such as steroid hormones and the thyroxine, and nitric oxide (NO) are permeable and can enter the cell and bind to intracellular receptors. The steroids bind to cytosolic or nuclear protein receptors and modulate specific gene transcription (usually at the initiation of transcription) (Fig. 71). Nitric Oxide (NO) binds to a cytosolic receptor which is a guanylate cyclase and produces cGMP.

**Cell Surface Receptors**

Signaling molecules or ligands that are hydrophilic such as proteins, amino and derivatives etc. are impermeable to cell membrane and thus transmit their messages by binding to cell surface receptors e.g. epinephrine, glucagon, insulin, epidermal growth factor, neuro transmitters. Prostaglandin and NO also bind to cell surface receptors which are broadly grouped as:

1. G–Protein Coupled Receptors
2. Ion Channel Receptors
3. Receptors with intrinsic enzyme activity:
Receptor guanylate cyclase; Receptor tyrosine kinases (RTKs); Receptor tyrosine phosphatase and Receptor serine–threonine kinases

4. Cytokine Receptor Super Family
5. Adhesion protein receptors in the plasma membrane of the cell

Fig. 70: General scheme of intercellular signaling in animals; (a) Endocrine signaling, (b) Paracrine signaling, (c) Autocrine signaling, (d) Proteins attached to plasmamembrane (Adhesion proteins) can directly interact with receptors in an adjacent cell

These cell adhesion protein receptors are involved in cell–cell recognition and also carry information between extracellular matrix and cytoskeleton.

No receptors for a plant signaling molecule has been identified so far and the plant signaling is not so well understood as that for animals and microorganisms.

**Intracellular Second Messengers**

Binding of the ligand to some of the cell surface membrane receptors induces formation of the second messenger whereas ligand binding to others does not.

The intracellular second messengers are soluble, low molecular weight, non protein, diffusible molecules. Among the important second messengers are:

(i) 3’,5’–Cyclic AMP (cAMP)  
(ii) 3’,5’–Cyclic GMP (cGMP)  
(iii) Inositol–1, 4, 5–triphosphate (IP$_3$)  
(iv) 1,2–diacylglycerol (DAG)  
(v) Ca$^{2+}$.

The elevated concentration of one or more of each of the second messenger triggers a rapid change in the activity of one or more enzyme(s) or non–enzymic protein(s) which affect
metabolic pathways such as uptake and utilization of glucose by insulin or release of glucose by epinephrine.

The second messenger can also control proliferation and differentiation of cells, regulating specific gene transcription.

Detection, Identification and Purification of Receptors

Receptor are proteins with a specific binding sites for their ligands and are inhibited by compounds competitively and non competitively. Some of the inhibitors are deadly poison. The substrate analogs which mimic the effects of hormones / ligands are called agonists and those which decrease or block the effects of hormones / ligands are termed antagonists (or antihormones). In some cases the binding affinities of these agonists can even be more than the ligand itself. The agonist and antagonist have been used to study the receptor characteristics; as ligand in affinity chromatography and also as drugs

Detection of Receptors

Binding Assay

Since hormone and other ligand receptors are present in minute amounts, it is difficult to identify and purify them. The surface of a typical cell contains 10,000 to 20,000 receptors for a particular hormone which is only \( \sim 10^{-6} \) of the total protein in the cell or \( \sim 10^{-4} \) of the plasma membrane proteins.

The high specificity and the very high affinity with which the receptors bind, as also, their ability to distinguish between closely related substrate analogs, is used to detect them. This receptor–ligand binding is similar to that of enzyme to its substrate and is characterized by Kd which is a measure of its affinity for the ligand.

Hormone binding is represented by the simple reversible equation

\[
R + H \rightleftharpoons RH
\]

And is described by the following equation:

\[
Kd = \frac{[R][H]}{[RH]} \tag{1}
\]

Where [R] and [H] are concentrations of free receptor and hormone (ligand), respectively, and [RH] is the concentration of Receptor–Ligand Complex, Kd, the dissociation constant of the receptor–ligand complex and is a measure of the affinity of the receptor for its ligand. The lower the Kd value, the higher the affinity. The binding equation can be rewritten in a form similar to Michaelis–Meuten equation used in analysis of enzyme reactions.

\[
\frac{[RH]}{RT} = \frac{1}{1 + Kd / [H]} \tag{2}
\]

Where RT is the sum of free and bound receptors, [R] + [RH] or total binding sites in the preparation, Kd value is equivalent to the ligand concentration at which half of the total receptors are bound or the receptors are 50% saturated.
Thus, when \([\text{RH}] = 0.5 \, \text{RT}\), \(K_d = \text{[H]}\)

The \(K_d\) values of hormones range from \(10^{-7}\) to \(10^{-12}\) M and thus \([\text{RH}]\) can be measured directly by using radioactive hormones binding to receptor and rapidly separating unbound hormone. Scatchard analysis of receptor–hormone binding is used for determination of \(K_d\) and number of receptor binding sites in a given preparation. Equation (i) can be rewritten as

\[
\frac{1}{K_d} = \frac{[\text{RH}]}{[\text{H}][B_{\text{max}} - \text{RH}]} \tag{1}
\]

\(\text{RT}\), the total number of binding site on hormone, when occupied to saturation gives \(B_{\text{max}} = [\text{R} + \text{RH}]\).

Rearranging, we get

\[
\frac{[\text{RH}]}{[\text{H}]} = \frac{1}{K_d} \left( B_{\text{max}} - [\text{RH}] \right) = \frac{\text{Bound Ligand}}{\text{Free Ligand}}
\]

Thus a plot of \(\frac{[\text{RH}]}{[\text{H}]}\) against \([\text{RH}]\) gives a straight line with a slope of \(-1/K_d\) and an intercept on the abscissa of \(B_{\text{max}}\), the total number of binding sites.

Typically, increasing concentrations of radiolabelled hormone (e.g. \(^{125}\text{I}{-}\text{insulin}\)) are added to cell suspension or membrane preparation and incubated for 1 hr at 4°C (low temperature prevents endocytosis of the receptor). The amount of hormone bound to receptor is determined by separating the receptor–hormone complex (RH) from free hormone (by filtration through nitrocellulose filter and washing it thoroughly to remove adhering free hormone) and determining the radioactivity of the RH. A plot of \(^{125}\text{I}\) insulin (nM) against \(^{125}\text{I}\) insulin bound (in molecules per cell) gives the binding curve shown in Fig. 72a which represents insulin bound specifically to its receptor and also the insulin bound nonspecifically to other molecules on cell surface. The nonsaturable, nonspecific insulin binding is determined in a separate series of binding experiment in presence of a large (100 fold) excess of unlabelled insulin, in addition to the dilute solution of labelled hormone. The unlabelled molecule competes with the specific binding sites, saturating them, but not for the non–specific binding sites. The true value for the specific binding is obtained by subtracting non specific binding from total binding (Fig. 72a).

On plotting the ratio of bound hormone to free hormone \([\text{RH}]/[\text{H}]\) against bound hormone \([\text{RL}]\) in a Scatchard Plot, the linear plot gives \(K_d\) and \(B_{\text{max}}\) or RT. (Fig. 72b).

For hepatoma cell insulin receptor, \(K_d = 20\) nM (2 x \(10^{-8}\) M) and the number of receptor molecules per single hepatoma cell, \(RT = \sim 30,000\). The binding affinity of the erythropoietin receptor on erythrocyte precursor cells (\(K_d = \sim 1 \times 10^{-10}\) M) is even greater than that of insulin receptor.

**Identification of Receptors**

Hormone receptors can be identified by affinity labeling. A radioactively labelled hormone is covalently cross-linked with its receptor by using a bifunctional reagent that reacts with both the hormone and the receptors. Alternately, the hormone is modified so that it is chemically reactive and forms a covalent bond when it binds in the active site of the receptor. Thus, a latent chemical group is introduced into the hormone that can be activated by light only after it has bound to its
receptor; photo activation reduces the chemical activity with other protein. Such methods have been utilized to identify receptors for insulin, luteinizing hormone, thyroid hormone and β-adrenergic hormones.

![Fig. 72: (a) Identification of a specific insuline receptor on the cell surface by their binding of 125I labelled insulin (Source: Lodish, H., Baltimore, et al. Molecular Cell Biology, 1995) (b) A linear plot of \([RL]/[L]\) versus \([RL]\) gives \(K_d\) and \(B_{\text{max}}\) for the receptor-hormone complex](image)

**Purification of Receptor Proteins**

Cell surface receptors can be purified by affinity chromatography as discussed for integral membrane proteins. Thus, a hormone or its analog such as agonist or antagonist is chemically linked to polystyrene beads. Non-ionic detergent solubilized preparation of membranes is passed through a column containing these beads, when, only the receptors for the ligand bind to these beads and the other proteins are washed down the column by excess fluid. The receptor is eluted from the column by passing an excess of a agonist of higher affinity. In some cases, the receptor can be purified as much as 100,000 fold in a single affinity chromatography step. Receptors for estrogens, progestin, glucocorticoids have been purified by this technique. Likewise, Acetyl choline receptors have been purified by using snake venom toxin linked to solid support.

Like other integral membrane proteins, receptors can be purified by cloning techniques.

**Reconstitution of Receptors**

Purified receptors have been incorporated in vesicles to get reconstituted receptor–vesicles and their characteristics studied by using, ligands, agonists, antagonists and other inhibitor etc.

The activity of single ion channels can be studied using Patch–Clamp technique developed by Neher and Sackman.

Some of the cell surface receptors are discussed below:
**β-Adrenergic Receptors (G protein coupled receptors)**

β–adrenergic receptors bind epinephrine (adrenalin is another name for epinephrine, hence the name adrenergic receptors) and are one of the best studied. They act via G–proteins (GTP, binding proteins).

There are four types of adrenergic receptors, a₁, a₂, b₁ and b₂ which have been identified on the bases of its binding of agonists and antagonists of epinephrine. They have similar structure and are present in different target cells to mediate different responses to epinephrine.

The β-adrenergic receptor is an integral membrane protein with seven transmembrane α-helices linked with E1–E4 extra cellular loops and intracellular loops (C1–C4) which appear like a snake and hence are also known as serpentine receptors. Other names for these receptors are G–protein coupled receptors (GPCR) or 7 transmembrane segment (7 tm) receptors. The segment E1 is the N–terminal end while the segment C4 is the C–terminal end of the receptor. The loop C3 in the receptor between helices 5 and 6 and the C4 facing the cytosol are important in G–protein interaction (Fig. 73). Epinephrine binds to a cleft formed by the transmembrane helices on the extracellular surface.

![Schematic diagram of the general structure of β-adrenergic receptor or G-protein linked receptors](Source: Lodish, H., Baltimore, et al. Molecular Cell Biology, 1995)

Associated with the cytosolic face of the surface is the trimeric G–protein with α-, β- and γ-subunits. The subunit can bind either GTP, when it is in an active form, or GDP when it is in an inactive form. In the absence of a hormone, GDP is bound to the Gα- subunit (Fig. 74).

When epinephrine binds to the β-adrenergic receptor, it undergoes conformational change in the cytoplasmic domain which in turn affects the G–protein by inducing a conformational change; thereby the Gsα protein (The G–protein being stimulatory in this case, is known as Gs) exchanges a GTP for GDP. This exchange can occur only when the hormone binds to the receptor.
Fig. 74: Activation of adenylate cyclase following the binding of a hormone (e.g. epinephrine, glucagon) to its receptor
As a result, the Gsa subunit dissociates from Gs–protein and migrates to and activates an adenylate cyclase to produce 3’, 5’–cAMP from ATP (Fig. 75a).

G–protein is the intermediate in Epinephrine induced formation of c–AMP, the 2nd messenger. Thus, G–protein is a transducer in this signaling system.

The G-proteins are timed proteins or a timer devise. The Gα has an intrinsic GTPase activity and thus after activating the adenylate cyclase, it hydrolyzes GTP to GDP and Gsa-GDP, formed then dissociates from adenylate cyclase and returns to join subunits to form Gs–GDP.

This G–protein cycle can continue till the receptor has bound hormone, that is, goes on producing several Gα - GTP molecule and the cycle goes on.

One molecule of hormone bound to a receptor can induce activation of one molecule of G–proteins after another and Gsa-GTP can in turn produce large number of c–AMP and thus results in amplification of the signal.

Fig. 75: (a) Synthesis of cAMP from ATP catalyzed by adenylate (adenylyl) cyclase; (b) Hydrolysis of 3’, 5’–cAMP by cAMP phosphodiesterase

Cholera toxin causes inactivation of Gsa in the intestinal mucosal cells which secrete Na⁺ into intestinal lumen. This inactivation results in continuous production of c–AMP which induces loss of Na⁺ and water and thereby causing diarrhea. Cholera toxin prevents the conversion of
Gsα-GTP to Gsα-GDP and the switching off of the effect of Gsα stimulation of adenylate cyclase.

Another type of receptor for epinephrine is known as α1 receptor which activates an inhibitory Gi protein released on binding of epinephrine to α receptor, inhibits adenylate cyclase and lowers cAMP levels and thus negates the effect of α-adrenergic receptor (Fig. 76) Other Hormones such as somatostatin, adenosine and prostaglatndin E also activates Gi protein and acts by lowering cAMP.

The Gβγ subunits in both stimulatory and inhibitory G-proteins are identical while the Ga and the receptors differ.

![Diagram of Hormone Induced Activation of Adenylate Cyclase](image)

**Fig. 76: Hormone induced activation of adenylate cyclase is mediated by and inhibition by .**
**Binding of . GTP to adenylate cyclase activates the enzyme (see Fig. 74) whereas binding of inhibits adenylate cyclase. The subunit is identical in both cases**


**Cyclic AMP Activation Pathway**

The cAMP activates an inactive form of tetrameric (R2C2) protein Kinase A (PKA), which consists of two regulatory (R) and two catalytic units (C), by binding to its regulatory subunits to dissociate the catalytic subunit, the active form of protein kinase A (Fig. 77). The active protein Kinase A (PKA) is a ser-thr kinase and mediates the phosphorylation of inactive kinase to form an active kinase in a cascade leading to the metabolic response such as glycogenolysis (Fig. 69) or lipolysis.

The active PKA can move into the nucleus and also phosphorylate CREB protein to form an active transcription factor for specific genes (Fig. 78).

The cAMP is hydrolyzed by phosphodiesterase enzyme to form AMP and thus the signal response is terminated (Figure 75b).
Fig. 77: Activation of cAMP dependent protein kinase (PKA) by cAMP. The PKA contains two regulatory (R) subunits which have binding sites for cAMP and two catalytic (C) subunits. The tetrameric (Source: Lodish, H., Baltimore, et al. Molecular Cell Biology, 1995)

The caffeine and theophyllin content of tea and coffee inhibits the phosphodiesterase. Thus, there is a continuous increased level of cAMP.

Besides epinephrine, other hormones that use cAMP as second messenger are glucagon, corticotropin (ACTH), Histamine, Serotonin, Tastants (Bitter and sweet), thyroid stimulating factor (TSH), odorants (many) to name a few.

The $\alpha_2$ and $\beta_2$ adrenergic receptor mediates different response to epinephrine and norepinephrine (catechol amines), the former inhibiting and the latter activating adenylate cyclase.

Fig. 78: Diagram illustrating the $\beta$-adrenergic receptor mediated stimulation of adenylate cyclase via G-protein. The c–AMP activates protein kinase A (PKA) to modulate phosphorylation of cAMP–responsive element binding protein (cREB) which produces an active transcriptional factor for specific genes.
Epinephrine bound to $\beta_2$-adrenergic receptors of:

(i) Muscle, liver and adipose tissue mediate increased breakdown of glycogen (glycogenolysis) and fat (lipolysis) resulting in an increase in blood sugar in times of stress.

(ii) Heart muscle increases the contraction rate which increases the blood supply to tissues.

(iii) Smooth muscle cells of intestines causing them to relax.

Epinephrine bound to $\alpha_1$ receptors in smooth muscle cells of the lining of the blood vessels in intestinal tract, skin and kidney causes arteries to constrict, thereby cutting the blood supply to the peripheral organs. This is the reason, a person appears pale during flight or stress.

The $\alpha_1$ adrenergic receptor does not use cAMP as the second messenger but rather causes an increase in $\text{Ca}^{2+}$ by another mechanism known as phosphoinositide pathway. Thus, the hormone can exert different physiologic responses depending on the type of receptors it binds.

**The phosphoinositide signaling pathway**

Some hormone signals act by activating membrane enzyme, phospholipase C (PLC) which hydrolyze phosphatidylinositol to inositol 1,4,5, tri phosphate, 1P$_3$, and diacyl glycerol, DAG (Figure ). The IP$_3$ signaling pathway can be coupled to seven spanning G-linked receptors and receptor tyrosine kinases.

Epinephrine binding to $\alpha_1$-adrenergic receptors activate a Go or Gq protein to release Go or Gq–GTP, or signal binding to Receptor Tyrosine Kinase, activates phospholipase C which produces the second messengers, IP$_3$ and DAG–which are released in the cytosol.

The inositol triphosphate, IP$_3$, binds to $\text{Ca}^{2+}$ channels in the sarcoplasmic reticulum to increase the $\text{Ca}^{2+}$ concentration which mediates the cellular response (Fig. 79) on binding to Calmodulin to a form $\text{Ca}^{2+}$–calmodulin which activates protein kinases. DAG and $\text{Ca}^{2+}$ activate protein kinase ‘C’, PKC, which phosphorylate cellular enzymes and proteins to mediate cellular response. PKC also plays a key role in many aspects of cellular metabolism and growth. PKC also phosphorylates various transcription factors and may induce or suppress synthesis of certain mRNAs depending on the cell types. DAG also mediates inhibition of glycogen synthesis by activating PKC.

Phorbol esters (Fig. 80), analogs of DAG act as tumor promoters since they continuously activate PKC, being long lived, whereas DAG is metabolized to terminate the response.

Hydrolysis of IP$_3$ (Inositol 1,4,5, triphosphate to Inositol 1,4 bis phosphate results in an in active form. IP$_3$ can also be phosphorylated to Inositol tetraphosphate which is hydrolysed to form Inositol 1,3,4, triphosphate inactive in signal transduction. These products can be recycled to produce phosphatidyl inositol 4,5, bis phosphate P$_1$P$_2$ (Fig. 81).
Fig. 79: The phosphoinositide signaling pathway. Binding of a signal to a G-linked receptor or a tyrosine kinase receptor activates a phospholipase C (PLC) which cleaves PIP$_2$ to form inositol 1,4,5 triphosphate (IP$_3$) and 1,2, diacyl glycerol (DAG), the second messengers


Fig. 80: DAG and its analog phorbol esters
Some signals that act through phospholipase C and IP₃, DAG and Ca²⁺ pathway include Acetylcholine (muscarinic M₁) α₁- adrenergic agonists, Angiotensin II, gastrin-releasing peptide, Glutamate, oxytocin, serotonin etc.

**Ion Channel Receptors**

Neurotransmitter receptors, which bind the neurotransmitters, are important in conduction of nerve impulse. The synapse, where acetyl choline is the neurotransmitter, is called cholinergic synapse.

Acetyl choline can bind to different receptors (i) Nicotinic receptors are ligand gated channels for Na⁺ and K⁺. (ii) Muscarinic receptors because muscarine, a mushroom alkaloid causes the same response as does acetyl choline. There are several subtypes of muscarinic receptors M₁, M₂, M₃, M₄ and M₅ all of which act through G–proteins but cause different effects.

Thus, M₂ receptor present in heart muscle activates Gi protein causing opening of K⁺ channel. M₁, M₂, and M₃ found in other cells are coupled to G₀ or Gₗ proteins and activate phospholipase C while M₄ subtypes Gᵢ and inhibits adenylate (adenylyl) cyclase.

**Acetylcholine Receptor or Nicotinic Receptors**

Neurotransmitter receptors are ion channels. One of the best neurotransmitter receptor studied is that of Acetylcholine, also known as Nicotinic receptor as it binds to Nicotine, a plant alkaloid and causes the same excitatory response lasting only ms i.e. rapid depolarisation. Electric eel and electric ray (tarpedo) organs are rich in this receptor and are used for their purification.
The nicotinic receptor is a 250 Kd transmembrane, glycoprotein containing four subunits with a stoichiometry of $\alpha_2$, $\beta$, $\gamma$, $\delta$ and is a cation channel.

Electron crystallographic studies by Nigel Unwin showed that in its closed form and it has an 9 nm diameter and is a 12.5 nm long cylinder which protrude 6 nm into the synaptic space at 2 nm in the cytoplasmic space. Its five subunits $\alpha_2$, $\beta$, $\gamma$, $\delta$ are rod like and arranged in a quasi five fold symmetry over a considerable region of their length. The central water filled channel or pore is tapered and has a diameter of 2 nm (Fig. 82a). The channel is constricted and gated near the middle of the bilayer, to a narrow region of 0.65 to 0.80 nm. This is sufficient to allow passage of both $\text{Na}^+$ and $\text{K}^+$ with their hydration shells.

Each of the subunit contains four -helices, M1, M2, M3 and M4. The actual cation channel is formed by the M2 helix from each of the five subunits (Fig. 82b). Each of the two $\alpha$-subunits binds one molecule of acetylcholine cooperatively which induces the opening of the channel (Fig. 82c). The snake venom toxin, bungarotoxin and cobra-toxin, bind specifically and irreversibly to the acetylcholine receptors and are potent inhibitors and lethal poison.

Positively charged molecule such as chlorpromazine inhibits the receptor function by “plugging’’ the ion channel. Chlorpromazine can be covalently cross-linked to Ser$^{254}$ in $\beta$ subunit and Ser$^{262}$ in $\delta$ subunit in the middle of the M2 helices.

(a) The arrangements of the five subunits
(b) four helices, M1, M2, M3 and M4 in the five subunits where the M2 helices from each of the subunits line the channel
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

(c) Binding of 2 acetyl choline molecules results in a change in conformation of the receptor and opening of the channel
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

Fig. 82: Schematic model of the nicotinic acetylcholine receptor

Enzymic hydrolysis of acetyl choline is used to terminate the signaling. In case of most other neurotransmitters, the termination of the signal is by uptake of the transmitter by presynaptic neuron.
Acetylcholine is hydrolysed to acetate and choline by acetyl cholinesterase localized in the synaptic cleft between the neuron and muscle cell membrane to terminate the depolarization signal (Fig. 83).

\[
\text{CH}_3 - \text{C} - \text{O} - \text{CH}_2 - \text{CH}_2 - \text{N} -(\text{CH}_3)_3 + \underset{\text{Acetylcholinesterase}}{\text{O}} \rightarrow \text{CH}_3 - \text{C} - \text{O}^- + \text{HO} - \text{CH}_2 - \text{CH}_2 - \text{N} -(\text{CH}_3)_3
\]

**Fig. 83: Hydrolysis of acetylcholine by acetylcholinesterase**

A large number of nerve gases and other neurotoxins inhibit acetylcholine esterase by reacting with its active site Serine. These toxins thus prolong the action of acetylcholine i.e. the period of membrane depolarization. Such inhibitors are lethal when they prevent relaxation of muscle necessary for breathing.

GABA and Glycine Receptors are Cl\textsuperscript{−} channels (anionic) and induce a slow, inhibitory postsynaptic response. These receptors have been purified, cloned and sequenced. Their overall structure resembles that of Acetylcholine receptor. All are pentamers but GABA and glycine receptors are made up of only one or two different type of subunits.

**Receptors with Guanylate (Guanylyl) Cyclase Activity**

There are two types of guanylate cyclase receptors involved in signal transduction via c–GMP as second messengers. Atrial natriuretic factor (ANF) and intestinal peptide guanylin stimulate receptor guanylate cyclase (Fig. 84). Cyclic GMP carries different messages in different tissues.

ANF is released in the atrium of the heart when the heart is stretched by increased blood volume. The blood carries ANF to kidney where it activates guanylate cyclase in the cells of the collecting ducts. The rise in cGMP triggers increased secretion of Na\textsuperscript{+} and water by the kidney cells. Water loss reduces blood volume.

The ANF receptor of vascular smooth muscles also triggers release of cGMP which causes relaxation (vasodilation) of the blood vessel and thus, increases blood flow and decreases blood pressure.

A similar guanylate cyclase receptor in the intestinal epithelial cells is stimulated by intestinal peptide, guanylin, which regulates Cl\textsuperscript{−} secretion in the intestine. The endotoxin produced by E.coli and ether gram negative bacteria act at this receptor to increase cGMP levels which increase Cl\textsuperscript{−} secretion and produce diarrhea ultimately.

Cyclic GMP in heart signals relaxation of smooth vessels and in brain, it may be involved both in development and adult brain function.
The raised GMP level mediates cell response by activating specific protein kinases known as cGMP dependent protein kinase, also called protein kinase G or PKG.

Fig. 84: cGMP linked Receptors activating guanylate cyclase in (a) the plasma membrane, (b) the soluble guanylate cyclase activated by NO
(Source: Nelson, D.L and Cox, M. Lehninger Principles of Biochemistry, 2005)

Another signal nitric oxide, NO, is formed from Arginine by the action of NO synthase. NO can diffuse through membranes and enter adjacent cells and it binds to another type of cytosolic heme associated guanylate cyclase to produce cGMP (Fig. 84b).

In the heart, cGMP reduces forcefulness of contractions i.e. induces relaxation of cardiac muscle. This is the basis for giving nitroglycerine tablets and other vasodilators to relieve angina a condition caused by blocked arteries, leading to contraction of heart which is deprived of oxygen.

Nitric oxide is short lived and unstable and within seconds of its formation is oxidized to nitrite or nirate.

Cyclic GMP synthesis decreases after the stimulation ceases, as cGMP is converted to 5’-GMP by a specific phosphodiesterase–(cGMP PDE) (Fig. 85).

The isoform of cGMP PDE in the blood vessels of penis is inhibited by the drug sildenafil (Viagra) causing cGMP levels to remain elevated, once the cGMP concentration is increased by an appropriate stimulus. This is the basis of the use of this drug in the treatment of erectile dysfunction.
Receptor Tyrosine Kinase

These receptors have intrinsic tyrosine kinase activity and most growth factors such as epidermal growth factor and insulin bind to such receptors.

Binding of Epidermal Growth Factor (EGF) to the receptor causes the receptors to dimerise and the tyrosine kinase activity of the cytosolic domain of the receptor is activated and causes autophosphorylation of the tyrosine residues on the cytoplasmic domain of the dimeric receptors (mutually phosphorylate each other on dimerisation). These phosphorylated tyrosine residues on the receptor can bind proteins with SH2 domains (SH2 derived from the fact that these proteins have a domain homologous with region 2 of the Src protein coded for by the oncogene of the Rous sarcoma virus, hence src homology region 2) and results in the activation of a signaling pathway from membrane receptors to nucleus resulting in activation of transcription factors.

The Ras Pathway

One of the widespread signaling pathway from membrane to gene is the Ras Pathway via a protein Ras present in all eukaryotes. Ras is a small monomeric GTP binding protein with GTPase activity. There are no small molecular weight second messengers in this pathway. All the components involved in this pathway are proteins.

In the cytoplasm, the growth factor receptor binding protein (GRB) is associated with a protein SOS (son of sevenless, where sevenless is the receptor mutant in fruitflies and the protein required for the receptor function was called son of sevenless), found to be present in all eukaryotic cells. The SH2 domain of GRB binds to the phosphorylated tyrosine residues of the activated (dimerized) receptor. Thus GRB–SOS binding to the activated receptor causes Ras protein to change to its active form by exchanging its GDP by GTP (Fig. 86) Ras has a low GTPase activity and is also a timer devise. Thus, Ras GTP then activates a ser-thr type kinase, the Raf protein (possibly the activation of Raf kinase is not direct) which initiates a cascade of phosphorylation by ser-thr kinases to phosphorylate transcription factor proteins and their activation to promote binding to their responsive elements and specific gene transcription and cell proliferation.

It is possible that the activated tyrosine kinase type of receptor may bind SH2-proteins of other signalling pathways so that one receptor might activate several pathways.
Fig. 86: Simplified diagram of the activation of the Ras pathway by EGF. (a) Inactive without EGF (b) Activated by EGF, GRB, growth factor receptor binding proteins, SOS, son of seven less, a protein involved in the pathway named after a sevenless mutation in *Drosophila* (*Raf, Ras, Fun and FOS are named from their oncogenes*)
(Source: Elliot, Witt; Elliot, D.C. Biochemistry and Molecular Biology, 1997)

Tyrosine kinase linked Receptors or Cytokine Receptor Superfamily
These Tyrosine kinase linked receptors are activated by Interferon, erythropoietin, Human growth factor etc. Two (inactive) cytoplasmic tyrosine kinases (JAK kinases) are associated with these receptors. Ligand binding causes dimerisation and activation of the JAK Kinases which activate STAT protein by phosphorylating its tyrosine residues. The STAT protein then forms an active transcription factor (Fig. 87).
Fig. 87: Diagram of Tyrosine kinase linked receptors activated by interferon which activates specific gene transcription.

STAT, signal transducer and activator of transcription; GAS, –interferon-activated sequence element. The cytoplasmic tyrosine kinases activated are known as Janus kinases (JAK kinases) which have two kinase sites
(Source: Elliot, Witt; Elliot, D.C. Biochemistry and Molecular Biology, 1997)
Suggested Reading

I. Biological Membrane


II. Biological Transport


III. Exocytosis and Endocytosis

IV. Cell Signaling and Receptors


