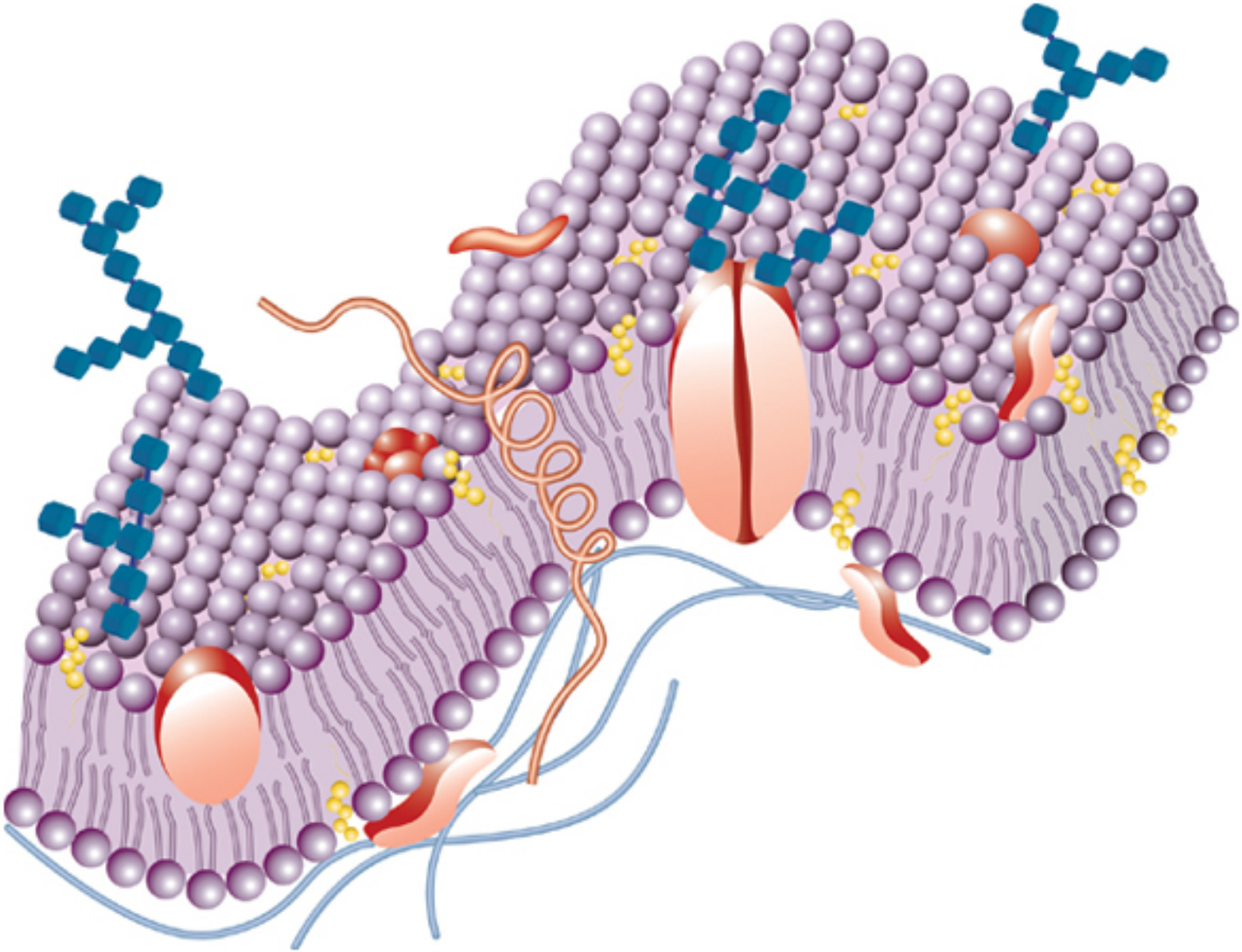

Membrane Biology

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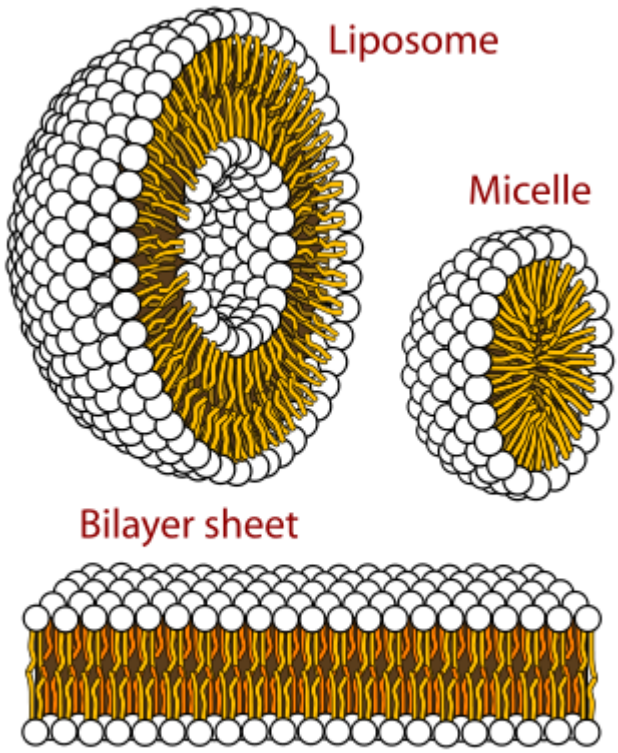
Introduction



Cross section view of the structures that can be formed by phospholipids in aqueous solutions

A **biological membrane** or **biomembrane** is an enclosing or separating membrane that acts as a selective barrier, within or around a cell. It consist of a lipid bilayer with embedded proteins that may constitute close to 50% of membrane content. The cellular membranes should not be confused with isolating tissues formed by layers of cells, such as mucous and basement membranes.

Function

Membranes in cells typically define enclosed spaces or compartments in which cells may maintain a chemical or biochemical environment that differs from the outside. For example, the membrane around peroxisomes shields the rest of the cell from peroxides,

and the cell membrane separates a cell from its surrounding medium. Most organelles are defined by such membranes, and are called "membrane-bound" organelles.

Probably the most important feature of a biomembrane is that it is a selectively permeable structure. This means that the size, charge, and other chemical properties of the atoms and molecules attempting to cross it will determine whether they succeed in doing so. Selective permeability is essential for effective separation of a cell or organelle from its surroundings. Biological membranes also have certain mechanical or elastic properties.

Particles that are required for cellular function but are unable to diffuse freely across a membrane enter through a membrane transport protein or are taken in by means of endocytosis.

Diversity of biological membranes

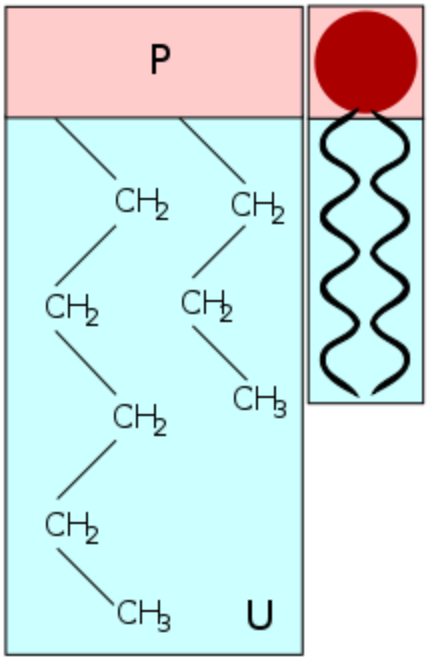
Many types of specialized plasma membranes can separate cell from external environment: apical, basolateral, presynaptic and postsynaptic ones, membranes of flagella, cilia, microvillus, filopodia and lamellipodia, the sarcolemma of muscle cells, as well as specialized myelin and dendritic spine membranes of neurons. Plasma membranes can also form different types of "supramembrane" structures such as caveola, postsynaptic density, podosome, invadopodium, desmosome, hemidesmosome, focal adhesion, and cell junctions. These types of membranes differ in lipid and protein composition.

Distinct types of membranes also create intracellular organelles: endosome; smooth and rough endoplasmic reticulum ; sarcoplasmic reticulum; Golgi apparatus; lysosome; mitochondrion (inner and outer membranes); nucleus (inner and outer membranes); peroxisome; vacuole; cytoplasmic granules; cell vesicles (phagosome, autophagosome, clathrin-coated vesicles, COPI-coated and COPII-coated vesicles) and secretory vesicles (including synaptosome, acrosomes, melanosomes, and chromaffin granules).

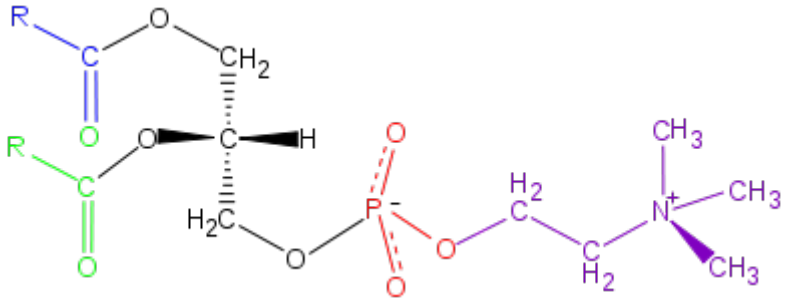
Different types of biological membranes have diverse lipid and protein compositions. The content of membranes defines their physical and biological properties. Some components of membranes play a key role in medicine, such as the efflux pumps that pump drugs out of a cell.

Chapter 1

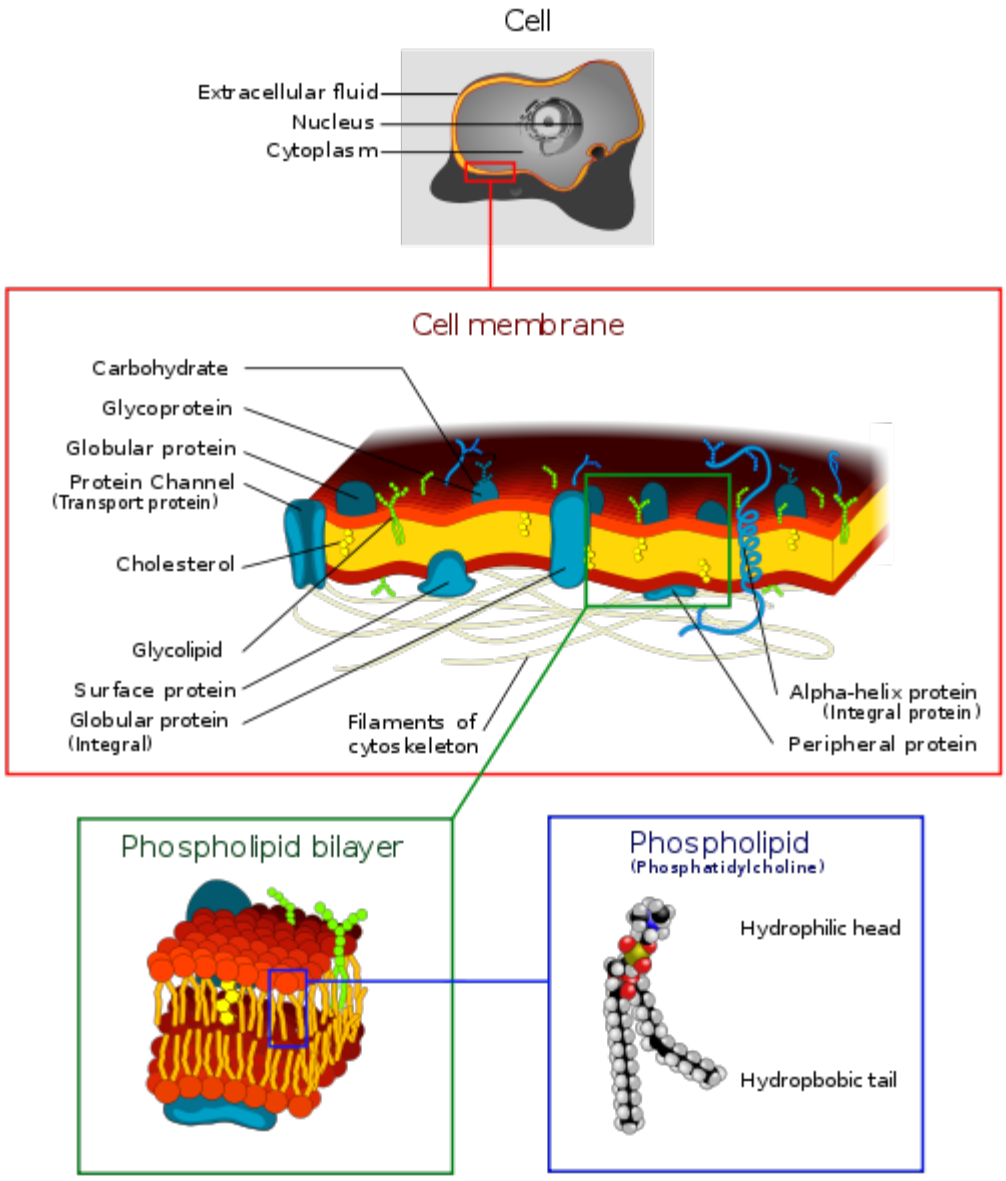
Phospholipid



Polar group of the molecule, highlighted in red.
The **U** indicates the uncharged hydrophobic portion of the molecule, highlighted in blue.



Phosphatidyl choline is the major component of lecithin. It is also a source for choline in the synthesis of acetylcholine in cholinergic neurons.



Cell membranes consist of phospholipid bilayers

Phospholipids are a class of lipids and are a major component of all cell membranes as they can form lipid bilayers. Most phospholipids contain a diglyceride, a phosphate group, and a simple organic molecule such as choline; one exception to this rule is sphingomyelin, which is derived from sphingosine instead of glycerol. The first phospholipid identified as such in biological tissues was lecithin, or phosphatidylcholine, in the egg yolk, by Theodore Nicolas Gobley, a French chemist and pharmacist, in 1847.

Amphipathic character

The 'head' of a phospholipid is hydrophilic (attracted to water), while the hydrophobic 'tails' are repelled by water and are forced to aggregate. The hydrophillic head contains

the negatively charged phosphate group, and may contain other polar groups. The hydrophobic tail usually consists of long fatty acid hydrocarbon chains. When placed in water, phospholipids form a variety of structures depending on the specific properties of the phospholipid. These specific properties allow phospholipids to play an important role in the phospholipid bilayer. In biological systems, the phospholipids often occur with other molecules (e.g., proteins, glycolipids, cholesterol) in a bilayer such as a cell membrane. Lipid bilayers occur when hydrophobic tails line up against one another, forming a membrane with hydrophilic heads on both sides facing the water.

Such movement can be described by the Fluid Mosaic Model, that describes the membrane as a mosaic of lipid molecules that act as a solvent for all the substances and proteins within it, so proteins and lipid molecules are then free to diffuse laterally through the lipid matrix and migrate over the membrane. Cholesterol contributes to membrane fluidity by hindering the packing together of phospholipids. However, this model has now been superseded, as through the study of lipid polymorphism it is now known that the behaviour of lipids under physiological (and other) conditions is not simple.

Types of phospholipid

Diacylglyceride structures

- Phosphatidic acid (phosphatidate) (PA)
- Phosphatidylethanolamine (cephalin) (PE)
- Phosphatidylcholine (lecithin) (PC)
- Phosphatidylserine (PS)
- Phosphoinositides:
    - Phosphatidylinositol (PI)
    - Phosphatidylinositol phosphate (PIP)
    - Phosphatidylinositol bisphosphate (PIP2) and
    - Phosphatidylinositol triphosphate (PIP3).

Phosphosphingolipids

- Ceramide phosphorylcholine (Sphingomyelin) (SPH)
- Ceramide phosphorylethanolamine (Sphingomyelin) (Cer-PE)
- Ceramide phosphorylglycerol

Simulations

Computational simulations of phospholipids are often performed using molecular dynamics with force fields such as GROMOS, CHARMM, or AMBER.

Characterisation

Phospholipids are optically highly birefringent, i.e. their refractive index is different along their axis as opposed to perpendicular to it. Measurement of birefringence can be

achieved using cross polarisers in a microscope to obtain an image of e.g. vesicle walls or using techniques such as dual polarisation interferometry to quantify lipid order or disruption in supported bilayers.

Phospholipid synthesis

Phospholipid synthesis occurs in the cytosol adjacent to ER membrane that is studded with proteins that act in synthesis (GPAT and LPAAT acyl transferases, phosphatase and choline phosphotransferase) and allocation (flippase and floppase). Eventually a vesicle will bud off from the ER containing phospholipids destined for the cytoplasmic cellular membrane on its exterior leaflet and phospholipids destined for the exoplasmic cellular membrane on its inner leaflet.

In signal transduction

Some types of phospholipid can be split to produce products that function as second messengers in signal transduction. Examples include phosphatidylinositol (4,5)-bisphosphate (PIP_2), that can be split by the enzyme Phospholipase C into inositol triphosphate (IP_3) and diacylglycerol (DAG), which both carry out the functions of the G_q type of G protein in response to various stimuli and intervene in various processes from long term depression in neurons to leukocyte signal pathways started by chemokine receptors.

Phospholipids also intervene in prostaglandin signal pathways as the raw material used by lipase enzymes to produce the prostaglandin precursors. In plants they serve as the raw material to produce Jasmonic acid, a plant hormone similar in structure to prostaglandins that mediates defensive responses against pathogens.

Food technology

Phospholipids can also act as an emulsifier, enabling oils to dissolve in water. Phospholipids called lecithin are extracted out of cooking oil and then used as food additives in many things such as bread and can also be purchased separately in a health food store.

Phospholipid derivatives

- Natural phospholipid derivates:

  egg PC, egg PG, soy PC, hydrogenated soy PC, sphingomyelin as natural phospholipids.

- Synthetic phospholipid derivates:
  - Phosphatidic acid (DMPA, DPPA, DSPA)
  - Phosphatidylcholine (DDPC, DLPC, DMPC, DPPC, DSPC, DOPC, POPC, DEPC)

- Phosphatidylglycerol (DMPG, DPPG, DSPG, POPG)
- Phosphatidylethanolamine (DMPE, DPPE, DSPE DOPE)
- Phosphatidylserine (DOPS)
- PEG phospholipid (mPEG-phospholipid, polyglycerin-phospholipid, funcitionalized-phospholipid, terminal activated-phospholipid)

Abbreviations used and chemical information of glycerophospholipids

Abbreviation	CAS	Name	Type
DDPC	3436-44-0	1,2-Didecanoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
DEPA-NA	80724-31-8	1,2-Dierucoyl-*sn*-glycero-3-phosphate (Sodium Salt)	Phosphatidic acid
DEPC	56649-39-9	1,2-Dierucoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
DEPE	988-07-2	1,2-Dierucoyl-*sn*-glycero-3-phosphoethanolamine	Phosphatidylethanolamine
DEPG-NA		1,2-Dierucoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Sodium Salt)	Phosphatidylglycerol
DLOPC	998-06-1	1,2-Dilinoleoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
DLPA-NA		1,2-Dilauroyl-*sn*-glycero-3-phosphate (Sodium Salt)	Phosphatidic acid
DLPC	18194-25-7	1,2-Dilauroyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
DLPE		1,2-Dilauroyl-*sn*-glycero-3-phosphoethanolamine	Phosphatidylethanolamine
DLPG-NA		1,2-Dilauroyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Sodium Salt)	Phosphatidylglycerol
DLPG-NH4		1,2-Dilauroyl-*sn*-glycero-3[Phospho-rac-	Phosphatidylglycerol

		(1-glycerol...) (Ammonium Salt)	
DLPS-NA		1,2-Dilauroyl-*sn*-glycero-3-phosphoserine (Sodium Salt)	Phosphatidylserine
DMPA-NA	80724-3	1,2-Dimyristoyl-*sn*-glycero-3-phosphate (Sodium Salt)	Phosphatidic acid
DMPC	18194-24-6	1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
DMPE	988-07-2	1,2-Dimyristoyl-*sn*-glycero-3-phosphoethanolamine	Phosphatidylethanolamine
DMPG-NA	67232-80-8	1,2-Dimyristoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Sodium Salt)	Phosphatidylglycerol
DMPG-NH4		1,2-Dimyristoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Ammonium Salt)	Phosphatidylglycerol
DMPG-NH4/NA		1,2-Dimyristoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Sodium/Ammonium Salt)	Phosphatidylglycerol
DMPS-NA		1,2-Dimyristoyl-*sn*-glycero-3-phosphoserine (Sodium Salt)	Phosphatidylserine
DOPA-NA		1,2-Dioleoyl-*sn*-glycero-3-phosphate (Sodium Salt)	Phosphatidic acid
DOPC	4235-95-4	1,2-Dioleoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
DOPE	4004-5-1-	1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine	Phosphatidylethanolamine
DOPG-NA	62700-69-0	1,2-Dioleoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Sodium Salt)	Phosphatidylglycerol
DOPS-NA	70614-14-1	1,2-Dioleoyl-*sn*-glycero-3-phosphoserine (Sodium Salt)	Phosphatidylserine

DPPA-NA	71065-87-7	1,2-Dipalmitoyl-*sn*-glycero-3-phosphate (Sodium Salt)	Phosphatidic acid
DPPC	63-89-8	1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
DPPE	923-61-5	1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine	Phosphatidylethanolamine
DPPG-NA	67232-81-9	1,2-Dipalmitoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Sodium Salt)	Phosphatidylglycerol
DPPG-NH4	73548-70-6	1,2-Dipalmitoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Ammonium Salt)	Phosphatidylglycerol
DPPS-NA		1,2-Dipalmitoyl-*sn*-glycero-3-phosphoserine (Sodium Salt)	Phosphatidylserine
DSPA-NA	108321-18-2	1,2-Distearoyl-*sn*-glycero-3-phosphate (Sodium Salt)	Phosphatidic acid
DSPC	816-94-4	1,2-Distearoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
DSPE	1069-79-0	1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine	Phosphatidylethanolamine
DSPG-NA	67232-82-0	1,2-Distearoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Sodium Salt)	Phosphatidylglycerol
DSPG-NH4	108347-80-4	1,2-Distearoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol...) (Ammonium Salt)	Phosphatidylglycerol
DSPS-NA		1,2-Distearoyl-*sn*-glycero-3-phosphoserine (Sodium Salt)	Phosphatidylserine
Egg Sphingomyelin empty Liposome			

EPC		Egg-PC	Phosphatidylcholine
HEPC		Hydrogenated Egg PC	Phosphatidylcholine
HSPC		High purity Hydrogenated Soy PC	Phosphatidylcholine
HSPC		Hydrogenated Soy PC	Phosphatidylcholine
LYSOPC MYRISTIC	18194-24-6	1-Myristoyl-*sn*-glycero-3-phosphocholine	Lysophosphatidylcholine
LYSOPC PALMITIC	17364-16-8	1-Palmitoyl-*sn*-glycero-3-phosphocholine	Lysophosphatidylcholine
LYSOPC STEARIC	19420-57-6	1-Stearoyl-*sn*-glycero-3-phosphocholine	Lysophosphatidylcholine
Milk Sphingomyelin MPPC		1-Myristoyl-2-palmitoyl-*sn*-glycero 3-phosphocholine	Phosphatidylcholine
MSPC		1-Myristoyl-2-stearoyl-*sn*-glycero-3–phosphocholine	Phosphatidylcholine
PMPC		1-Palmitoyl-2-myristoyl-*sn*-glycero-3–phosphocholine	Phosphatidylcholine
POPC	26853-31-6	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
POPE		1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine	Phosphatidylethanolamine
POPG-NA	81490-05-3	1-Palmitoyl-2-oleoyl-*sn*-glycero-3[Phospho-rac-(1-glycerol)...] (Sodium Salt)	Phosphatidylglycerol
PSPC		1-Palmitoyl-2-stearoyl-*sn*-glycero-3–phosphocholine	Phosphatidylcholine
SMPC		1-Stearoyl-2-myristoyl-*sn*-glycero-3–phosphocholine	Phosphatidylcholine
SOPC		1-Stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
SPPC		1-Stearoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine

Chapter 2

Protein Targeting

Protein targeting or **protein sorting** is the mechanism by which a cell transports proteins to the appropriate positions in the cell or outside of it. Sorting targets can be the inner space of an organelle, any of several interior membranes, the cell's outer membrane, or its exterior via secretion. This delivery process is carried out based on information contained in the protein itself. Correct sorting is crucial for the cell; errors can lead to diseases.

Targeting signals

Targeting signals are the pieces of information that enable the cellular transport machinery to correctly position a protein inside or outside the cell. This information is contained in the polypeptide chain or in the folded protein. The continuous stretch of amino acid residues in the chain that enables targeting are called signal peptides or targeting peptides. There are two types of targeting peptides, the presequences and the internal targeting peptides. The presequences of the targeting peptide are often found at the N-terminal extension and is composed of between 6-136 basic and hydrophobic amino acids.In case of peroxisomes the targeting sequence is on the C-terminal extension mostly. Other signals are composed by parts which are separate in the primary sequence. To function, these components have to come together on the protein surface by folding. They are called signal patches. In addition, protein modifications like glycosylations can induce targeting.

Protein translocation

In 1970, Günter Blobel conducted experiments on the translocation of proteins across membranes. He was awarded the 1999 Nobel prize for his findings. He discovered that many proteins have a signal sequence, that is, a short amino acid sequence at one end that functions like a postal code for the target organelle. The translation of mRNA into protein by a ribosome takes place within the cytosol. If the synthesized proteins "belong" in a different organelle, they can be transported there in either of two ways, depending on the protein.

Cotranslational translocation

The N-terminal signal sequence of the protein is recognized by a signal recognition particle (SRP) *while the protein is still being synthesized on the ribosome*. The synthesis pauses while the ribosome-protein complex is transferred to a SRP receptor on the endoplasmic reticulum (ER), a membrane-enclosed organelle. There, the nascent protein is inserted into the Sec61 translocation complex (also known as the translocon) that passes through the ER membrane. The signal sequence is immediately cleaved from the polypeptide once it has been translocated into the ER by signal peptidase in secretory proteins. This signal sequence processing differs for some ER transmembrane proteins. Within the ER, the protein is first covered by a chaperone protein to protect it from the high concentration of other proteins in the ER, giving it time to fold correctly. Once folded, the protein is modified as needed (for example, by glycosylation), then transported to the Golgi apparatus for further processing and goes to its target organelles or is retained in the ER by various ER retention mechanisms.

Posttranslational translocation

Even though most proteins are cotranslationally translocated, some are translated in the cytosol and later transported to their destination. This occurs for proteins that go to a mitochondrion, a chloroplast, or a peroxisome (proteins that go to the latter have their signal sequence at the C terminus). Also, proteins targeted for the nucleus are translocated post-translation. They pass through the nuclear envelope via nuclear pores.

Transmembrane proteins

The amino acid chain of transmembrane proteins, which often are transmembrane receptors, passes through a membrane one or several times. They are inserted into the membrane by translocation, until the process is interrupted by a stop-transfer sequence, also called a membrane anchor sequence. These complex membrane proteins are at the moment mostly understood using the same model of targeting that has been developed for secretory proteins. However, many complex multi-transmembrane proteins contain structural aspects that do not fit the model. Seven transmembrane G-protein coupled receptors (which represent about 5% of the genome of humans) mostly do not have an amino-terminal signal sequence. In contrast to secretory proteins, the first transmembrane domain acts as the first signal sequence, which targets them to the ER membrane. This also results in the translocation of the amino terminus of the protein into the ER membrane lumen. This would seem to break the rule of "co-translational" translocation which has always held for mammalian proteins targeted to the ER. This has been demonstrated with opsin with in vitro experiments. A great deal of the mechanics of transmembrane topology and folding remains to be elucidated.

Sorting of proteins to mitochondria

Most mitochondrial proteins are synthesized as cytosolic precursors containing uptake peptide signals. Cytosolic chaperones deliver preproteins to channel linked receptors in

the mitochondrial membrane. The preprotein with presequence targeted for the mitochondria is bound by receptors and the General Import Pore (GIP) (Receptors and GIP are collectively known as Translocase of Outer Membrane or TOM) at the outer membrane. The preprotein is translocated through TOM as hairpin loops. The preprotein is transported through the intermembrane space by small TIMs (which also acts as molecular chaperones) to the TIM23 or 22 (Translocase of Inner Membrane) at the inner membrane. Within the matrix the targeting sequence is cleaved off by mtHsp70.

Three mitochondrial outer membrane receptors are known: TOM20, TOM22 and TOM70
TOM70: Binds to internal targeting peptides and acts as a docking point for cytosolic chaperones.
TOM20: Binds presequences
TOM22: Binds both presequences and internal targeting peptides
The TOM channel is a cation specific high conductance channel with a molecular weight of 410 kDa and a pore diameter of 21Å.

The presequence translocase23 (TIM23) is localized to the mitochondial inner membrane and acts a pore forming protein which binds precursor proteins with its N-terminal. TIM23 acts a translocator for preproteins for the mitochondrial matrix, the inner mitochondrial membrane as well as for the intermembrane space. TIM50 is bound to TIM23 at the inner mitochondrial side and found to bind presequences. TIM44 is bound on the matrix side and found binding to mtHsp70.
The presequence translocase22 (TIM22) binds preproteins exclusively bound for the inner mitochondrial membrane.

Mitochondrial matrix targeting sequences are rich in positively charged amino acids and hydroxylated ones.

Proteins are targeted to submitochondrial compartments by multiple signals and several pathways.

Targeting to the outer membrane, intermembrane space, and inner membrane often requires another signal sequence in addition to the matrix targeting sequence.

Sorting of proteins to chloroplasts

The preprotein for chloroplasts may contain a stromal import sequence or a stromal and thylakoid targeting sequence. The majority of preproteins are translocated through the Toc and Tic complexes located within the chloroplast envelope. In the stroma the stromal import sequence is cleaved off and folding as well as intra-chloroplast sorting to thylakoids continues. Proteins targeted to the envelope of chloroplasts usually lack cleavable sorting sequence.

Sorting of proteins to both chloroplasts and mitochondria

Many proteins are needed in both mitochondria and chloroplasts. In general the targeting peptide is of intermediate character to the two specific ones. The targeting peptides of these proteins have a high content of basic and hydrophobic amino acids, a low content of negatively charged amino acids. They have a lower content of alanine and a higher content of leucine and phenylalanine. The dual targeted proteins have a more hydrophobic targeting peptide than both mitochondrial and chloroplastic ones.

Sorting of proteins to peroxisomes

All peroxisomal proteins are encoded by nuclear genes.

To date there are two types of known Peroxisome Targeting Signals (PTS):

Peroxisome targeting signal 1 (PTS1): a C-terminal tripeptide with a consensus sequence (S/A/C)-(K/R/H)-(L/A). The most common PTS1 is serine-lysine-leucine (SKL). Most peroxisomal matrix proteins possess a PTS1 type signal.

Peroxisome targeting signal 2 (PTS2): a nonapeptide located near the N-terminus with a consensus sequence (R/K)-(L/V/I)-XXXXX-(H/Q)-(L/A/F) (where X can be any amino acid).

There are also proteins that possess neither of these signals. Their transport may be based on a so-called "piggy-back" mechanism: such proteins associate with PTS1-possessing matrix proteins and are translocated into the peroxisomal matrix together with them.

Diseases

Peroxisomal protein transport is defective in the following genetic diseases:

- Zellweger syndrome.
- Adrenoleukodystrophy (ALD).
- Refsum disease

Receptor-mediated endocytosis

Several molecules that attach to special receptors called clathrin coated pits on the outside of cells cause the cell to perform endocytosis, an invagination of the plasma membrane to incorporate the molecule and associated structures into endosomes. This mechanism is used for three main purposes:

- Uptake of essential metabolites, for example, LDL.
- Uptake of some hormones and growth factors, for example, epidermal growth factor and nerve growth factor.

- Uptake of proteins that are to be destroyed, for example, antigens in phagocytotic cells like macrophages.

Receptor-mediated endocytosis can also be "abused":

- Some viruses, for example, the Semliki forest virus, enter the cell through this mechanism.
- Cholera, diphtheria, anthrax, tetanus, botulinum, and other bacterial toxins enter the cell this way.

Protein destruction

Defective proteins are occasionally produced, or they may be damaged later, for example, by oxidative stress. Damaged proteins can be recycled. Proteins can have very different half lifes, mainly depending on their N-terminal amino acid residue. The recycling mechanism is mediated by ubiquitin.

Protein targeting in bacteria

With some exceptions, Bacteria lack membrane-bound organelles as found in eukaryotes, but they may assemble proteins onto various types of inclusions such as gas vesicles and storage granules. Bacteria may have a single plasma membrane (Gram-positive bacteria), or an inner membrane plus an outer membrane separated by the periplasm (Gram-negative bacteria). Proteins may be incorporated into the plasma membrane, or either trapped in the periplasm or secreted into the environment, according to whether or not there is an outer membrane. The basic mechanism at the plasma membrane is similar to the eukaryotic one. In addition, bacteria may target proteins into or across the outer membrane. Systems for secreting proteins across the bacterial outer membrane may be quite complex and play key roles in pathogenesis. These systems may be described as type I secretion, type II secretion, etc.

In most Gram-positive bacteria, certain proteins are targeted for export across the plasma membrane and subsequent covalent attachment to the bacterial cell wall. A specialized enzyme, sortase, cleaves the target protein at a characteristic recognition site near the protein C-terminus, such as an LPXTG motif (where X can be any amino acid), then transfers the protein onto the cell wall. An system analogous to sortase/LPXTG, termed exosortase/PEP-CTERM, is proposed to exist in a broad range of Gram-negative bacteria.

Secretory pathways

The secretory pathway includes vesicular traffic, secretion, and endocytosis. Secretory proteins follow this pathway.

Early stages

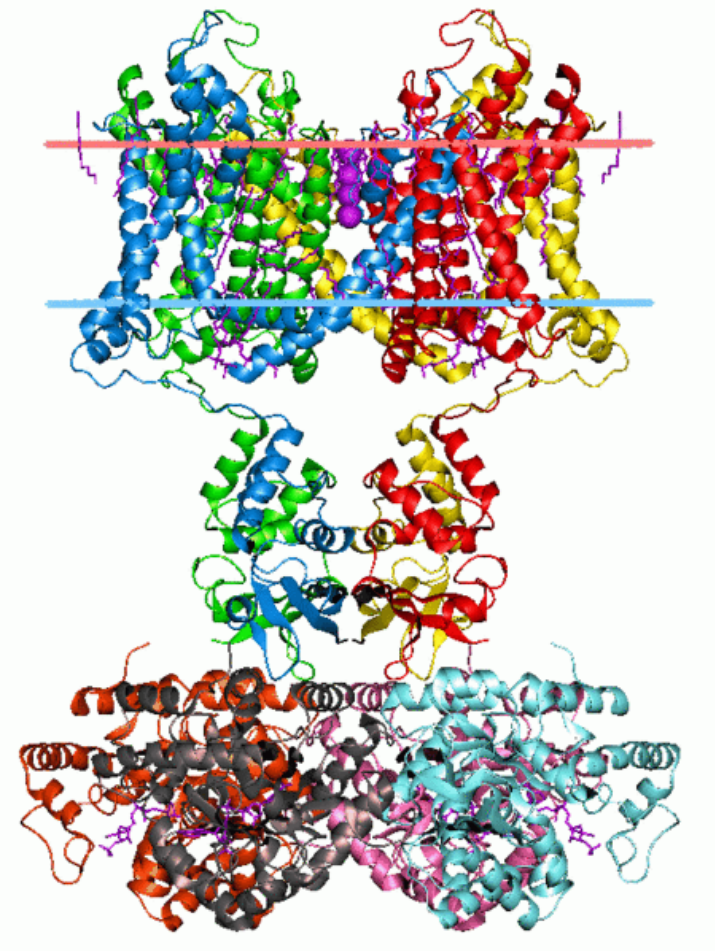
Retrograde transport is common in the early stages. Proteins that have been successfully delivered to the Golgi apparatus advance through cisternal progression.

Later stages

Coated vesicles mediate several transport steps.

Chapter 3

Membrane Protein



Crystal structure of Potassium channel KvAP. Calculated hydrocarbon boundaries of the lipid bilayer are indicated by red and blue dots.

A **membrane protein** is a protein molecule that is attached to, or associated with the membrane of a cell or an organelle. More than half of all proteins interact with membranes.

Function

Biological membranes consist of a phospholipid bilayer and a variety of proteins that accomplish vital biological functions.

- Structural proteins are attached to microfilaments in the cytoskeleton which ensures stability of the cell.
- Cell adhesion molecules allow cells to identify each other and interact. Such proteins are involved in immune response, for example.
- Membrane enzymes produce a variety of substances essential for cell function.
- Membrane receptor proteins serve as connection between the cell's internal and external environments.
- Transport proteins play an important role in the maintenance of concentrations of ions. These transport proteins come in two forms: carrier proteins and channel proteins.

Main categories

Membrane proteins can be divided into three categories:

- Integral membrane proteins, penetrating the lipid bilayer
- Peripheral membrane proteins, external but bound with noncovalent bonds
- Lipid-anchored protein, external but bound with covalent bonds

An alternative classification is to divide all membrane proteins to integral and *amphitropic*.

- The *amphitropic* are proteins that can exist in two alternative states: a water-soluble and a lipid bilayer-bound. The amphitropic protein category includes water-soluble channel-forming polypeptide toxins, which associate irreversibly with membranes, but excludes peripheral proteins that interact with other membrane proteins rather than with lipid bilayer.
- The *integral* proteins can be found only in the membrane-bound state.

Integral membrane proteins

Integral membrane proteins are permanently attached to the membrane. They can be defined as those proteins which require a detergent (such as SDS or Triton X-100) or some other apolar solvent to be displaced. They can be classified according to their relationship with the bilayer:

- Integral polytopic proteins, also known as "transmembrane proteins," are proteins that are permanently attached to the lipid membrane and span across the membrane (at least once). The transmembrane regions of the proteins are either beta-barrels or alpha-helical. The alpha-helical domains are present in all types of biological membranes including outer membranes. The beta-barrels were found

only in outer membranes of Gram-negative bacteria, lipid-rich cell walls of a few Gram-positive bacteria, and outer membranes of mitochondria and chloroplasts.

- Integral monotopic proteins are proteins that are permanently attached to the lipid membrane from only one side and do not span across the membrane.

Peripheral membrane proteins

Peripheral membrane proteins are temporarily attached either to the lipid bilayer or to integral proteins by a combination of hydrophobic, electrostatic, and other non-covalent interactions. Peripheral proteins dissociate following treatment with a polar reagent, such as a solution with an elevated pH or high salt concentrations.

Integral and peripheral proteins may be post-translationally modified, with added fatty acid or prenyl chains, or GPI (glycosylphosphatidylinositol), which may be anchored in the lipid bilayer.

Polypeptide toxins

Classification of membrane proteins to integral and peripheral does not include some polypeptide toxins, such as colicin A or alpha-hemolysin, and certain proteins involved in apoptosis. These proteins are water-soluble but can aggregate and associate irreversibly with the lipid bilayer and form alpha-helical or beta-barrel transmembrane channels.

Intracellular localization

Proteins are specifically targeted to many different types of biological membranes

Membrane Protein Complexes

Membrane Proteins commonly function as complexes. These complexes are vital to cellular function. Understanding how these complexes are assembled, degraded, and their composition are crucial to understanding their function and regulation. Reoccurring in recent literature are the ideas that: membrane protein complexes assemble in an orderly fashion, chaperones aide assembly by preventing unfavorable interactions, and membrane proteins can be interchanged in existing complexes. Membrane protein complexes assemble through the orderly assembly of intermediates. For example, the simple membrane-embedded four subunit complex, cytochrome bo3 of Escherichia coli, is assembled via two intermediate complexes. This suggests a linearly organized assembly pathway. Although interactions between other subunits could lead to the formation of many intermediates, they do not occur. Ordered assembly could be the cell's protection against harmful intermediates. Chaperones interact with membrane proteins guiding their assembly. They aide in preventing the assembly of dead-end and toxic intermediates, as well as unwanted aggregations. Via chaperones assembly can occur through inactive intermediates potentially preventing damaging interactions they could cause. Membrane protein complexes are not fixed entities. Though a process called dynamic exchange,

membrane proteins are exchanged in and out of exsitisting protein complexes. This has its implications as a repair mechanism and in regulation.

Membrane Protein Structures

The structures of membrane proteins are stabilized by weak interactions and influenced by additional interactions with the solubilizing environment. The influence of the environment on membrane protein structures is especially significant. Despite the significant functional importance of membrane proteins, the structural biology has been particularly challenging as shown by the low number of membrane protein structures determined. Integral membrane proteins are present in a heterogeneous environment that poses major obstacles for existing structural methodologies.

Many of the successful membrane protein structures are characterized by X-ray crystallography and are very large structures in which the interactions with the membrane mimetic environments can be anticipated to be small in comparison to those within the protein structures. The small domains are particularly sensitive to the influence of membrane mimetic environments, potentially leading to non-native structures. Fortunately, there are many sample preparation conditions that can be chosen for crystallization and for solution NMR. All membrane protein structural biology should be subjected to careful scrutiny; through a combination of structural methodologies it should be possible to achieve an understanding of the native functional state for membrane protein structures.

Chapter 4

Cell Membrane

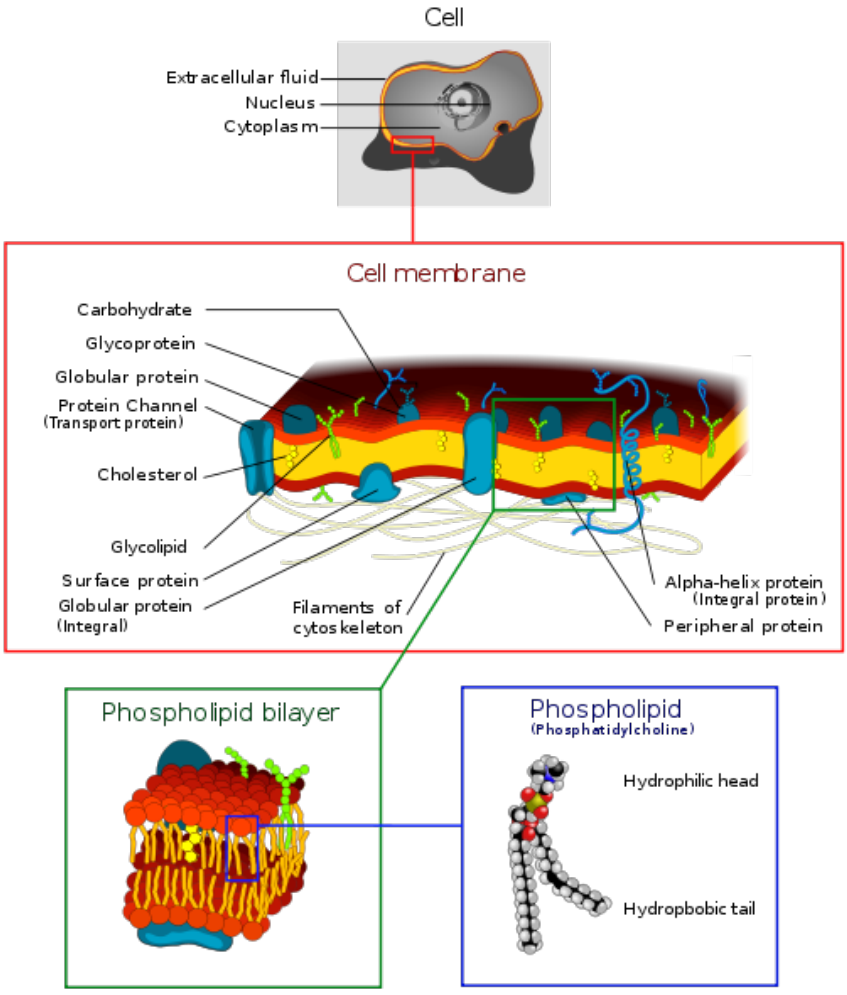


Illustration of a Eukaryotic cell membrane

The **cell membrane** is a biological membrane that separates the interior of all cells from the outside environment. The cell membrane is selectively-permeable to ions and organic molecules and controls the movement of substances in and out of cells. It consists of the

phospholipid bilayer with embedded proteins. Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling and serve as the attachment surface for the extracellular glycocalyx and cell wall and intracellular cytoskeleton.

Function

The cell membrane surrounds the protoplasm of a cell and, in animal cells, physically separates the intracellular components from the extracellular environment. Fungi, bacteria and plants also have the cell wall which provides a mechanical support for the cell and precludes passage of the larger molecules. The cell membrane also plays a role in anchoring the cytoskeleton to provide shape to the cell, and in attaching to the extracellular matrix and other cells to help group cells together to form tissues.

The barrier is differentially permeable and able to regulate what enters and exits the cell, thus facilitating the transport of materials needed for survival. The movement of substances across the membrane can be either *passive*, occurring without the input of cellular energy, or active, requiring the cell to expend energy in moving it. The membrane also maintains the cell potential.

Prokaryotes

Gram-negative bacteria have plasma membrane and outer membrane separated by the periplasmic space. Other prokaryotic species have only plasma membrane. Prokaryotic cells are also surrounded by a cell wall.

Structure

Fluid mosaic model

According to the fluid mosaic model of S. J. Singer and Garth Nicolson 1972, the biological membranes can be considered as a two-dimensional liquid where all lipid and protein molecules diffuse more or less easily. This picture may be valid in the space scale of 10 nm. However, the plasma membranes contain different structures or domains that can be classified as: (a) protein-protein complexes; (b) lipid rafts, and (c) pickets and fences formed by the actin-based cytoskeleton.

Lipid bilayer

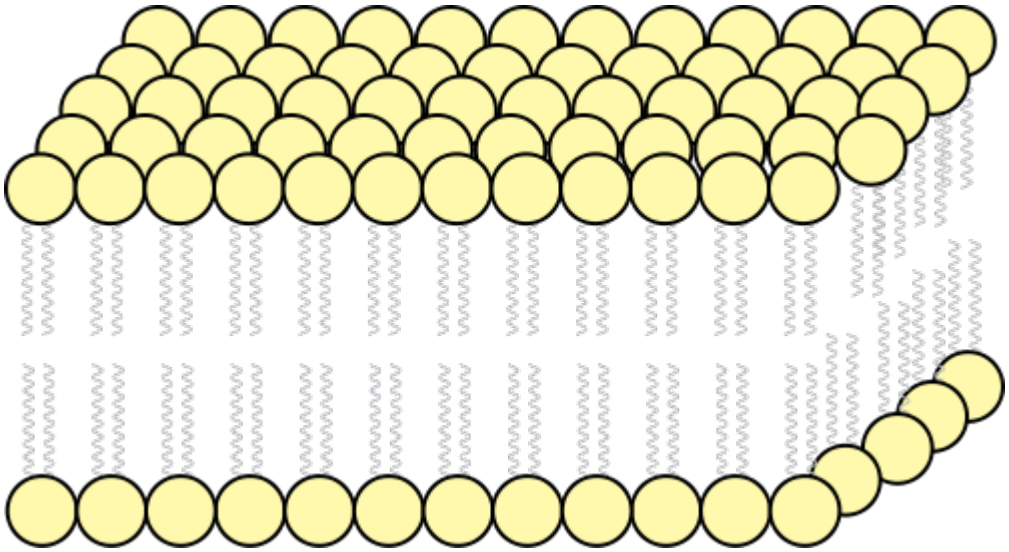


Diagram of the arrangement of amphipathic lipid molecules to form a lipid bilayer. The yellow polar head groups separate the grey hydrophobic tails from the aqueous cytosolic and extracellular environments.

Lipid bilayers go through a self assembly process in the formation of membranes. The cell membrane consists primarily of a thin layer of amphipathic phospholipids which spontaneously arrange so that the hydrophobic "tail" regions are shielded from the surrounding polar fluid, causing the more hydrophilic "head" regions to associate with the cytosolic and extracellular faces of the resulting bilayer. This forms a continuous, spherical lipid bilayer. Forces such as Van der Waal, electrostatic, hyrdogen bonds, and noncovalent interactions, are all forces that contribute to the formation of the lipid bilayer. Overall, hydrophobic interactions are the major driving force in the formation of lipid bilayers.

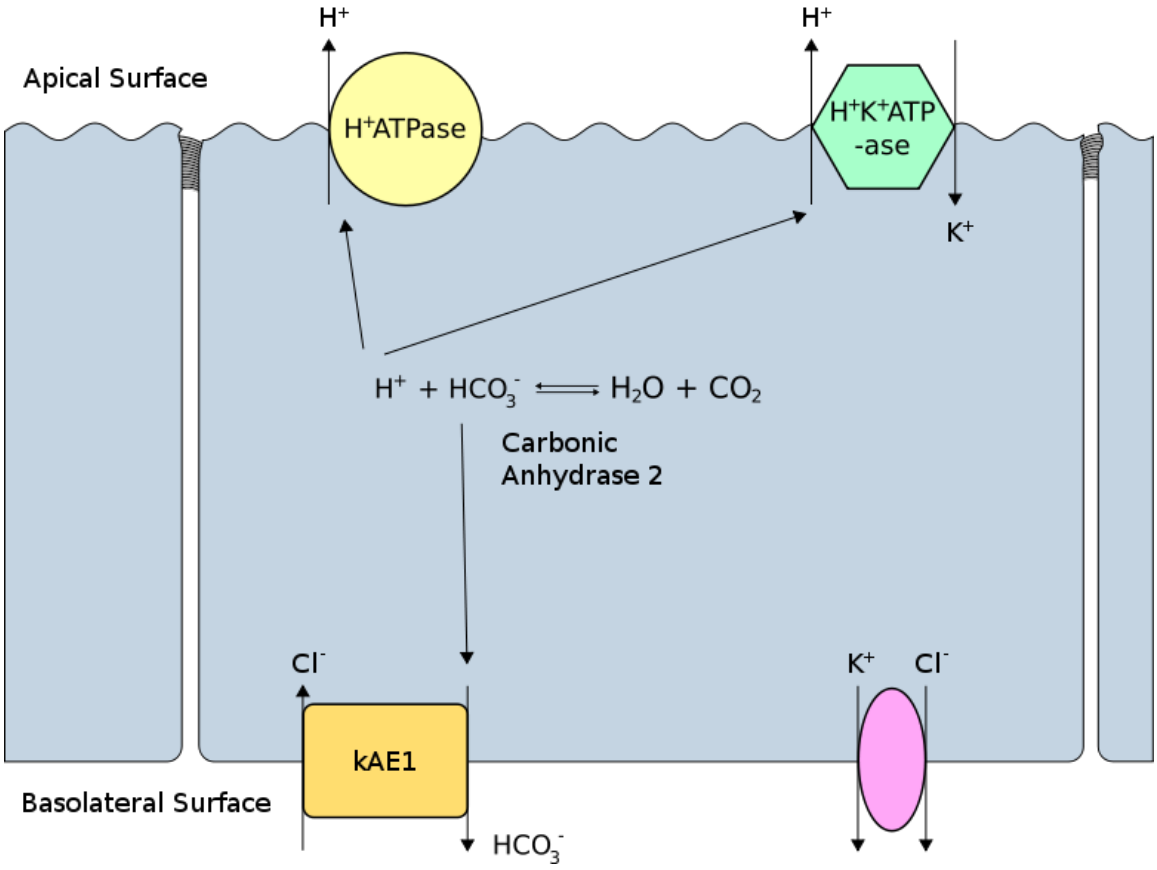
Lipid bilayers have very low permeability for ions and most polar molecules.The arrangement of hydrophilic heads and hydrophobic tails of the lipid bilayer prevent polar solutes (e.g. amino acids, nucleic acids, carbohydrates, proteins, and ions) from diffusing across the membrane, but generally allows for the passive diffusion of hydrophobic molecules. This affords the cell the ability to control the movement of these substances via transmembrane protein complexes such as pores and gates.

Flippases and Scramblases concentrate phosphatidyl serine, which carries a negative charge, on the inner membrane. Along with NANA, this creates an extra barrier to charged moieties moving through the membrane.

Membranes serve diverse functions in eukaryotic and prokaryotic cells. One important role is to regulate the movement of materials into and out of cells. The phospholipid bilayer structure (fluid mosaic model) with specific membrane proteins accounts for the selective permeability of the membrane and passive and active transport mechanisms. In

addition, membranes in prokaryotes and in the mitochondria and chloroplasts of eukaryotes facilitate the synthesis of ATP through chemiosmosis.

Membrane polarity



Alpha intercalated cell

The **apical membrane** of a polarized cell is the surface of the plasma membrane that faces the lumen. This is particularly evident in epithelial and endothelial cells, but also describes other polarized cells, such as neurons.

The **basolateral membrane** of a polarized cell is the surface of the plasma membrane that forms its basal and lateral surfaces. It faces towards the interstitium, and away from the lumen.

"Basolateral membrane" is a compound phrase referring to the terms *basal (base) membrane* and *lateral (side) membrane*, which, especially in epithelial cells, are identical in composition and activity. Proteins (such as ion channels and pumps) are free to move from the basal to the lateral surface of the cell or *vice versa* in accordance with the fluid mosaic model.

Tight junctions that join epithelial cells near their apical surface prevent the migration of proteins from the basolateral membrane to the apical membrane. The basal and lateral

surfaces thus remain roughly equivalent to one another, yet distinct from the apical surface.

Integral membrane proteins

The cell membrane contains many integral membrane proteins, which pepper the entire surface. These structures, which can be visualized by electron microscopy or fluorescence microscopy, can be found on the inside of the membrane, the outside, or membrane spanning. These may include integrins, cadherins, desmosomes, clathrin-coated pits, caveolaes, and different structures involved in cell adhesion. Integral proteins are the most abundant type of protein to span the lipid bilayer. They interact widely with hydrocarbon chains of membrane lipids and can be released by agents that compete for the same nonpolar interactions.

Peripheral membrane proteins

Peripheral proteins are proteins that are bounded to the membrane by electrostatic interactions and hydrogen bonding with the hydrophilic phospholipid heads. Many of these proteins can be found bounded to the surfaces of integral proteins on either the cytoplasimic side of the cell or the extracellular side of the membrane. Some are anchored to the bilayer through covalent bond with a fatty acid.

Membrane skeleton

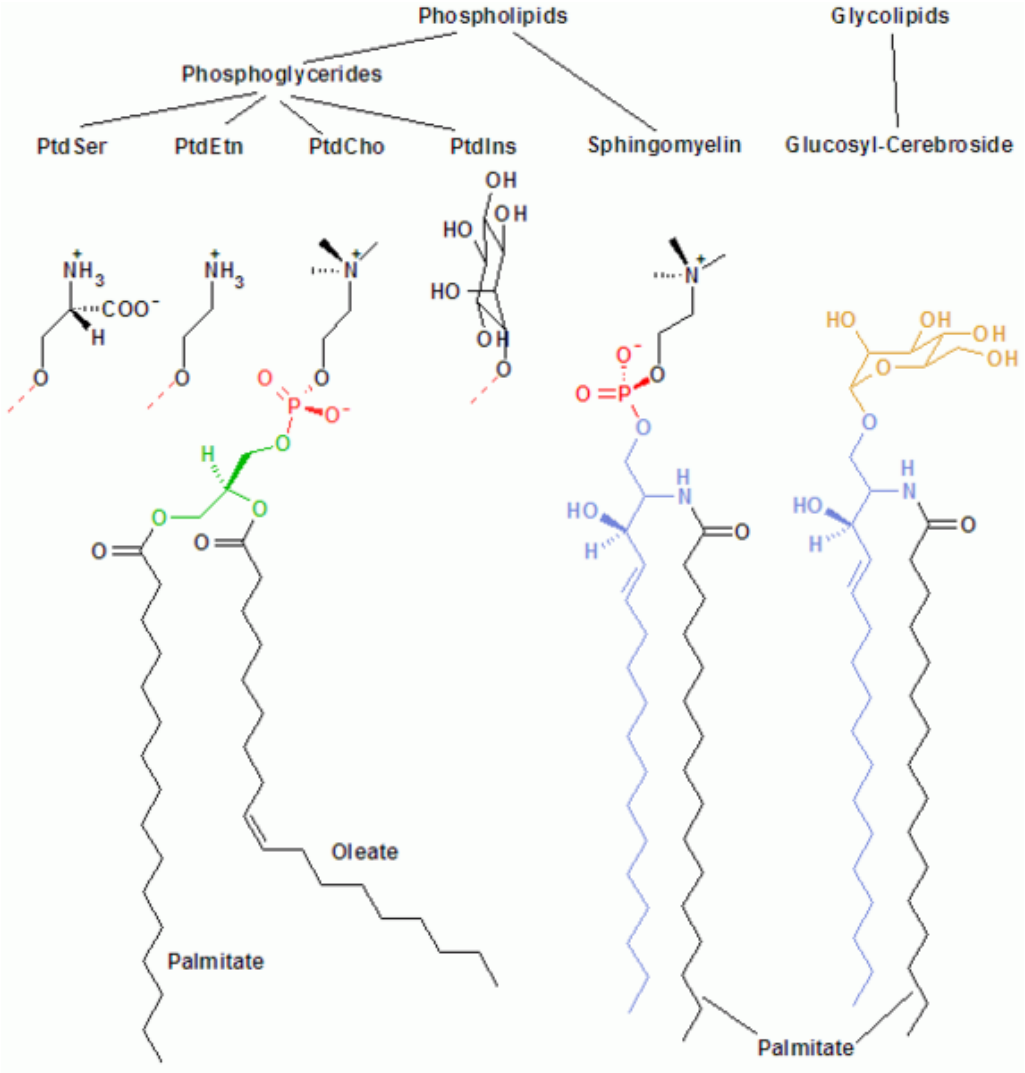
The cytoskeleton is found underlying the cell membrane in the cytoplasm and provides a scaffolding for membrane proteins to anchor to, as well as forming organelles that extend from the cell. Indeed, cytoskeletal elements interact extensively and intimately with the cell membrane. Anchoring proteins restricts them to a particular cell surface — for example, the *apical surface* of epithelial cells that line the vertebrate gut — and limits how far they may diffuse within the bilayer. The cytoskeleton is able to form appendage-like organelles, such as cilia, which are microtubule-based extensions covered by the cell membrane, and filopodia, which are actin-based extensions. These extensions are ensheathed in membrane and project from the surface of the cell in order to sense the external environment and/or make contact with the substrate or other cells. The apical surfaces of epithelial cells are dense with actin-based finger-like projections known as microvilli, which increase cell surface area and thereby increase the absorption rate of nutrients. Localized decoupling of the cytoskeleton and cell membrane results in formation of a bleb.

Composition

Cell membranes contain a variety of biological molecules, notably lipids and proteins. Material is incorporated into the membrane, or deleted from it, by a variety of mechanisms:

- Fusion of intracellular vesicles with the membrane (exocytosis) not only excretes the contents of the vesicle but also incorporates the vesicle membrane's components into the cell membrane. The membrane may form blebs around extracellular material that pinch off to become vesicles (endocytosis).
- If a membrane is continuous with a tubular structure made of membrane material, then material from the tube can be drawn into the membrane continuously.
- Although the concentration of membrane components in the aqueous phase is low (stable membrane components have low solubility in water), there is an exchange of molecules between the lipid and aqueous phases.

Lipids



Examples of the major membrane phospholipids and glycolipids: phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer).

The cell membrane consists of three classes of amphipathic lipids: phospholipids, glycolipids, and cholesterols. The amount of each depends upon the type of cell, but in the majority of cases phospholipids are the most abundant. In RBC studies, 30% of the plasma membrane is lipid.

The fatty chains in phospholipids and glycolipids usually contain an even number of carbon atoms, typically between 16 and 20. The 16- and 18-carbon fatty acids are the most common. Fatty acids may be saturated or unsaturated, with the configuration of the double bonds nearly always *cis*. The length and the degree of unsaturation of fatty acid chains have a profound effect on membrane fluidity as unsaturated lipids create a kink, preventing the fatty acids from packing together as tightly, thus decreasing the melting temperature (increasing the fluidity) of the membrane. The ability of some organisms to regulate the fluidity of their cell membranes by altering lipid composition is called homeoviscous adaptation.

The entire membrane is held together via non-covalent interaction of hydrophobic tails, however the structure is quite fluid and not fixed rigidly in place. Under physiological conditions phospholipid molecules in the cell membrane are in the liquid crystalline state. It means the lipid molecules are free to diffuse and exhibit rapid lateral diffusion along the layer in which they are present. However, the exchange of phospholipid molecules between intracellular and extracellular leaflets of the bilayer is a very slow process. Lipid rafts and caveolae are examples of cholesterol-enriched microdomains in the cell membrane.

In animal cells cholesterol is normally found dispersed in varying degrees throughout cell membranes, in the irregular spaces between the hydrophobic tails of the membrane lipids, where it confers a stiffening and strengthening effect on the membrane.

Phospholipids forming lipid vesicles

Lipid vesicles or liposomes are circular pockets that are enclosed by a lipid bilayer. These structures are used in laboratories to study the effects of chemicals in cells by delivering these chemicals directly to the cell, as well as getting more insight into cell membrane permeability. Lipid vesicles and liposomes are formed by first suspending a lipid in an aqueous solution then agitating the mixture through sonication, resulting in a uniformly circular vesicle. By measuring the rate of efflux from that of the insideof the vesicle to the ambient solution, allows researcher to better understand membrane permeability. Vesicles can be formed with molecules and ions inside the vesicle by forming the vesicle with the desired molecule or ion present in the solution. Proteins can also be embedded into the membrane through solubilizing the desired proteins in the presence of detergents and attaching them to the phospholipids in which the liposome is formed. These provide researchers with a tool to examine various membrane protein functions.

Carbohydrates

Plasma membranes also contain carbohydrates, predominantly glycoproteins, but with some glycolipids (cerebrosides and gangliosides). For the most part, no glycosylation occurs on membranes within the cell; rather generally glycosylation occurs on the extracellular surface of the plasma membrane.

The glycocalyx is an important feature in all cells, especially epithelia with microvilli. Recent data suggest the glycocalyx participates in cell adhesion, lymphocyte homing, and many others.

The penultimate sugar is galactose and the terminal sugar is sialic acid, as the sugar backbone is modified in the golgi apparatus. Sialic acid carries a negative charge, providing an external barrier to charged particles.

Proteins

Proteins within the membrane are key to the functioning of the overall membrane. These proteins mainly transport chemicals and information across the membrane. Every membrane has a varying degree of protein content. Proteins can be in the form of peripheral or integral.

Type	Description	Examples
Integral proteins or *transmembrane proteins*	Span the membrane and have a hydrophilic cytosolic domain, which interacts with internal molecules, a hydrophobic membrane-spanning domain that anchors it within the cell membrane, and a hydrophilic extracellular domain that interacts with external molecules. The hydrophobic domain consists of one, multiple, or a combination of α-helices and β sheet protein motifs.	Ion channels, proton pumps, G protein-coupled receptor
Lipid anchored proteins	Covalently-bound to single or multiple lipid molecules; hydrophobically insert into the cell membrane and anchor the protein. The protein itself is not in contact with the membrane.	G proteins
Peripheral proteins	Attached to integral membrane proteins, or associated with peripheral regions of the lipid bilayer. These proteins tend to have only temporary interactions with biological membranes, and, once reacted the molecule, dissociates to carry on its work in	Some enzymes, some hormones

the cytoplasm.

The cell membrane plays host to a large amount of protein that is responsible for its various activities. The amount of protein differs between species and according to function, however the typical amount in a cell membrane is 50%. These proteins are undoubtedly important to a cell: Approximately a third of the genes in yeast code specifically for them, and this number is even higher in multicellular organisms.

The cell membrane, being exposed to the outside environment, is an important site of cell-cell communication. As such, a large variety of protein receptors and identification proteins, such as antigens, are present on the surface of the membrane. Functions of membrane proteins can also include cell-cell contact, surface recognition, cytoskeleton contact, signaling, enzymatic activity, or transporting substances across the membrane.

Most membrane proteins must be inserted in some way into the membrane. For this to occur, an N-terminus "signal sequence" of amino acids directs proteins to the endoplasmic reticulum, which inserts the proteins into a lipid bilayer. Once inserted, the proteins are then transported to their final destination in vesicles, where the vesicle fuses with the target membrane.

Variation

The cell membrane has different lipid and protein compositions in distinct types of cells and may have therefore specific names for certain cell types:

- Sarcolemma in myocytes
- Oolemma in oocytes
- Historically, the plasma membrane was also referred to as the plasmalemma.

Permeability

The permeability of a membrane is the ease of molecules to pass through it. Permeability depends mainly on the electric charge of the molecule and to a lesser extent the molar mass of the molecule. Electrically neutral and small molecules pass the membrane easier than charged, large ones.

The inability of charged molecules to pass through the cell membrane results in pH parturition of substances throughout the fluid compartments of the body.

Chapter 5

Depolarization and Glycerophospholipid

Depolarization

In biology, **depolarization** is a change in a cell's membrane potential, making it more positive, or less negative. In neurons and some other cells, a large enough depolarization may result in an action potential. Hyperpolarization is the opposite of depolarization, and inhibits the rise of an action potential.

Mechanism

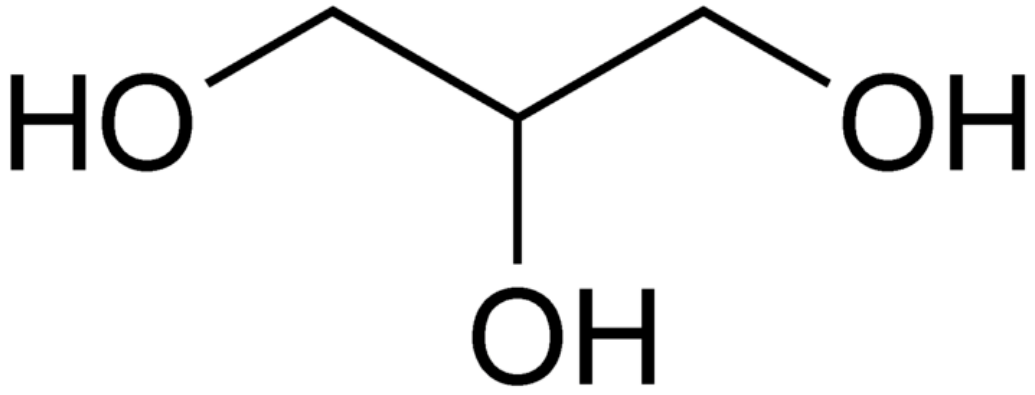
If, for example, a cell has a resting potential of -70mV, once the membrane potential changes to -50mV, then the cell has been depolarized. Depolarization is often caused by influx of cations, e.g. Na^+ through Na^+ channels, or Ca^{2+} through Ca^{2+} channels. On the other hand, efflux of K^+ through K^+ channels inhibits depolarization, as does influx of Cl^- (an anion) through Cl^- channels. If a cell has K^+ or Cl^- currents at rest, then inhibition of those currents will also result in a depolarization.

Because depolarization is a change in membrane voltage, electrophysiologists measure it using current clamp techniques. In voltage clamp, the membrane currents giving rise to depolarization are either an increase in inward current, or a decrease in outward current.

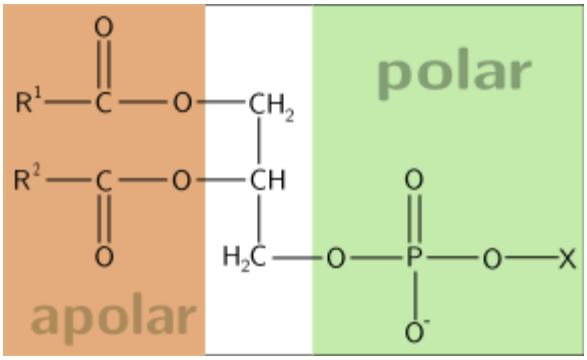
Depolarization blockers

There are drugs, called depolarization blocking agents, that inhibit depolarization, e.g. by blocking the channels responsible for depolarization, or by opening K^+ channels. Examples include the nicotinic agonists suxamethonium and decamethonium.

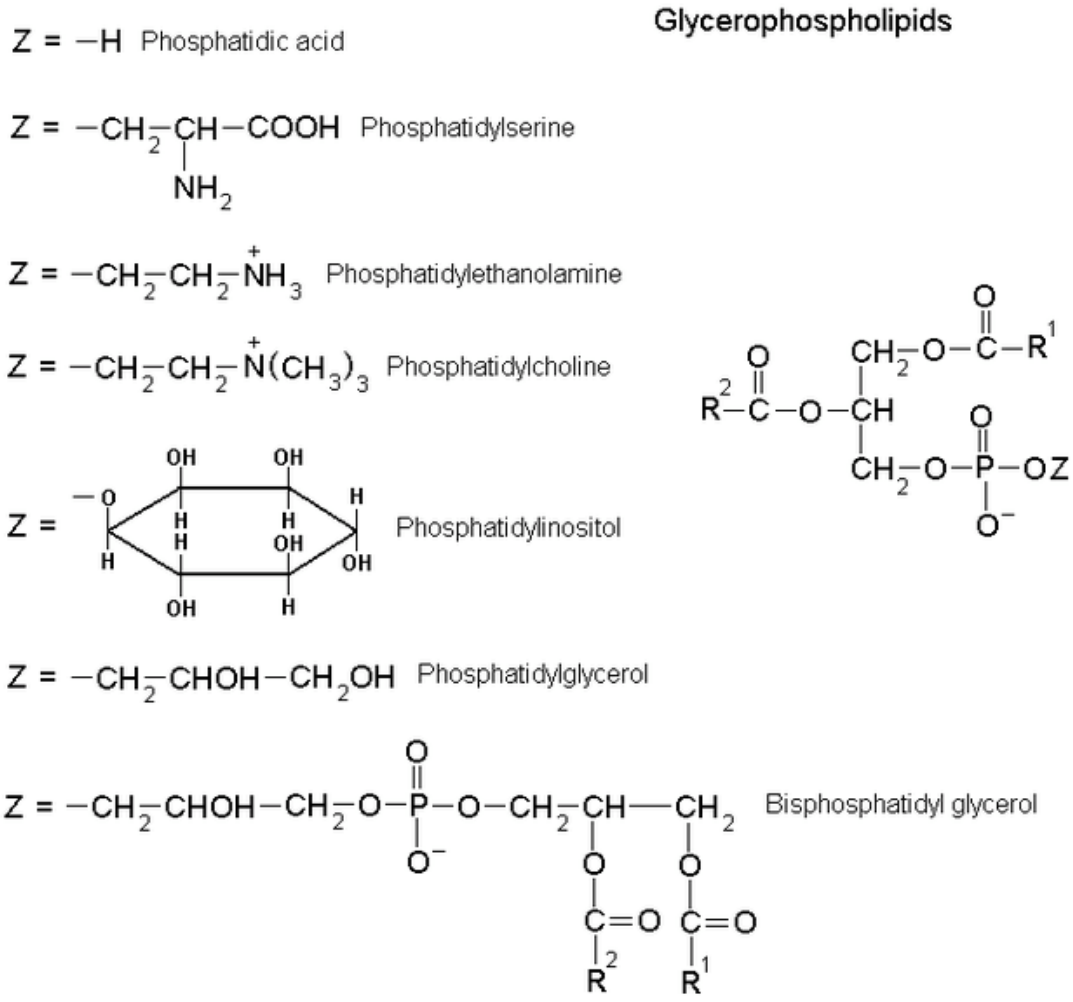
Glycerophospholipid



Glycerol



Phosphoglyceride



Glycerophospholipids or **phosphoglycerides** are glycerol-based phospholipids. They are the main component of biological membranes.

Structures

The term glycerophospholipid signifies any derivative of sn-glycero-3-phosphoric acid that contains at least one O-acyl, or O-alkyl, or O-alk-1'-enyl residue attached to the glycerol moiety and a polar head made of a nitrogenous base, a glycerol or an inositol unit.

It contains a glycerol core with fatty acids. They can be the same or different subunits of fatty acids.

- Carbon 1 (tail, apolar) contains a fatty acid, typically *saturated*
- Carbon 2 (tail, apolar) contains a fatty acid, typically *unsaturated* and in the cis conformation, thus appearing "bent"

- Carbon 3 (head, polar) contains a phosphate group or an alcohol attached to a phosphate group

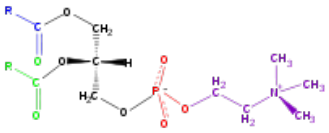
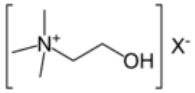
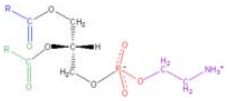
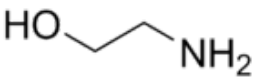
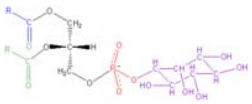
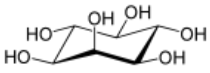
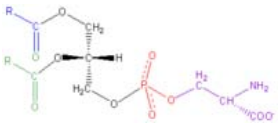
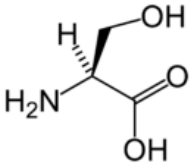
Nomenclature and stereochemistry

In general, glycerophospholipids use a "sn" notation, which stands for stereochemical numbering. When the letters "sn" appear in the nomenclature, by convention the hydroxyl group of the second carbon of glycerol (sn-2) is on the left on a Fischer projection. The numbering follows the one of Fischer's projections, being sn-1 the carbon at the top and sn-3 the one at the bottom.

The advantage of this particular notation is that the spatial conformation (R or L) of the glycero-molecule is determined intuitively by the residues on the positions sn-1 and sn-3.

For example sn-glycero-3-phosphoric acid and sn-glycero-1-phosphoric acid are enantiomers.

Examples of glycerophospholipids

Name	Image	Head	Image	Charge
Phosphatidyl choline (lecithin)		choline		neutral
Phosphatidyl ethanolamine (cephalin)		ethanolamine		neutral
Phosphatidyl inositol		inositol		negative
Phosphatidyl serine		serine		negative
Bisphosphatidyl glycerol (cardiolipin)		-	-	negative

Lecithin and cephalin are more common than the others in most human membranes, but cardiolipin is quite common in the inner membranes of mitochondria.

Uses

Use in membranes

One of a glycerophospholipid's functions is to serve as a structural component of cell membranes. The cell membrane seen under the electron microscope consists of two identifiable layers, or "leaflets", each of which is made up of an ordered row of glycerophospholipid molecules. The composition of each layer can vary widely depending on the type of cell.

- For example, in human erythrocytes the *cytosolic* side (the side facing the cytosol) of the plasma membrane consists mainly of phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol.
- By contrast, the *exoplasmic* side (the side on the exterior of the cell) consists mainly of phosphatidylcholine and sphingomyelin, a type of sphingolipid.

Each glycerophospholipid molecule consists of a small polar head group and two long hydrophobic chains. In the cell membrane, the two layers of phospholipids are arranged as follows:

- the hydrophobic tails point to each other and form a fatty, hydrophobic center
- the ionic head groups are placed at the inner and outer surfaces of the cell membrane

This is a stable structure because the ionic hydrophilic head groups interact with the aqueous media inside and outside the cell, whereas the hydrophobic tails maximize hydrophobic interactions with each other and are kept away from the aqueous environments. The overall result of this structure is to construct a fatty barrier between the cell's interior and its surroundings.

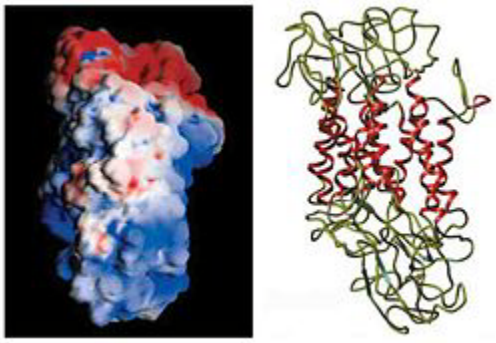
Use in emulsification

Glycerophospholipids can also act as an emulsifying agent to promote dispersal of one substance into another. This is sometimes used in candy making and ice-cream making.

Chapter 6

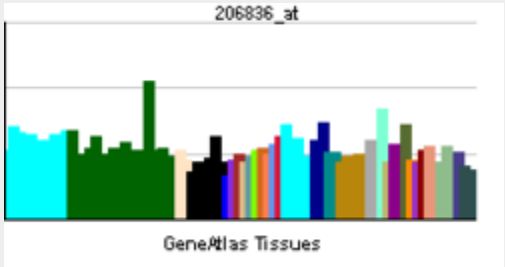
Dopamine Transporter

Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3



Identifiers	
Symbols	SLC6A3; DAT; DAT1
External IDs	OMIM: 126455 MGI: 94862 HomoloGene: 55547 GeneCards: SLC6A3 Gene

RNA expression pattern



Orthologs

Species	Human	Mouse

Entrez	6531	13162
Ensembl	ENSG00000142319	ENSMUSG00000021609
UniProt	Q01959	Q3UVW5
RefSeq (mRNA)	NM_001044	NM_010020
RefSeq (protein)	NP_001035	NP_034150
Location (UCSC)	Chr 5: 1.45 - 1.5 Mb	Chr 13: 74 - 74.04 Mb

The **dopamine transporter** (also dopamine active transporter, DAT, SLC6A3) is a membrane-spanning protein that pumps the neurotransmitter dopamine out of the synapse back into cytosol, from which other transporters sequester DA and NE into vesicles for later storage and release. Dopamine reuptake via DAT provides the primary mechanism through which dopamine is cleared from synapses except in the prefrontal cortex, where dopamine uptake via the norepinephrine transporter plays that role.

DAT is thought to be implicated in a number of dopamine-related disorders, including attention deficit hyperactivity disorder, bipolar disorder, clinical depression, and alcoholism. The gene that encodes the DAT protein is located on human chromosome 5, consists of 15 coding exons, and is roughly 64 kbp long. Evidence for the associations between DAT and dopamine related disorders has come from a genetic polymorphism in the DAT gene, which influences the amount of protein expressed.

Function

DAT is an integral membrane protein that removes dopamine from the synaptic cleft and deposits it into surrounding cells, thus terminating the signal of the neurotransmitter. Dopamine underlies several aspects of cognition, including reward, and DAT facilitates regulation of that signal.

Mechanism

DAT is a symporter that moves dopamine across the cell membrane by coupling the movement to the energetically-favorable movement of sodium ions moving from high to low concentration into the cell. DAT function requires the sequential binding and co-transport of two Na+ ions and one Cl- ion with the dopamine substrate. The driving force for DAT-mediated dopamine reuptake is the ion concentration gradient generated by the plasma membrane Na+/K+ ATPase.

In the most widely-accepted model for monoamine transporter function, sodium ions must bind to the extracellular domain of the transporter before dopamine can bind. Once dopamine binds, the protein undergoes a conformational change, which allows both sodium and dopamine to unbind on the intracellular side of the membrane.

Studies using electrophysiology and radioactive-labeled dopamine have confirmed that the dopamine transporter is similar to other monoamine transporters in that one molecule of neurotransmitter can be transported across the membrane with one or two sodium ions. Chloride ions are also needed to prevent a buildup of positive charge. These studies have also shown that transport rate and direction is totally dependent on the sodium gradient.

Because of the tight coupling of the membrane potential and the sodium gradient, activity-induced changes in membrane polarity can dramatically influence transport rates. In addition, the transporter may contribute to dopamine release when the neuron depolarizes.

Protein Structure

The initial determination of the membrane topology of DAT was based upon hydrophobic sequence analysis and sequence similarities with the GABA transporter. These methods predicted twelve transmembrane domains (TMD) with a large extracellular loop between the third and fourth TMDs. Further characterization of this protein used proteases, which digest proteins into smaller fragments, and glycosylation, which occurs only on extracellular loops, and largely verified the initial predictions of membrane topology.

Location and distribution

Regional distribution of DAT has been found in areas of the brain with established dopaminergic circuitry including: nigrostriatal, mesolimbic, and mesocortical pathways. The nuclei that make up these pathways have distinct patterns of expression.

DAT in the mesocortical pathway, labeled with radioactive antibodies, was found to be enriched in dendrites and cell bodies of neurons in the substantia nigra pars compacta and ventral tegmental area. This pattern makes sense for a protein that regulates dopamine levels in the synapse.

Staining in the striatum and nucleus accumbens of the mesolimbic pathway was dense and heterogeneous. In the striatum, DAT is localized in the plasma membrane of axon terminals. Double immunocytochemistry demonstrated DAT colocalization with two other markers of nigrostriatal terminals, tyrosine hydroxylase and D2 dopamine receptors. The latter was thus demonstrated to be an autoreceptor on cells that release dopamine.

Surprisingly, DAT was not identified within any synaptic active zones. These results suggest that striatal dopamine reuptake may occur outside of synaptic specializations once dopamine diffuses from the synaptic cleft.

In the substantia nigra, DAT appears to be specifically transported into dendrites, where it can be found in smooth endoplasmic reticulum, plasma membrane, and pre- and postsynaptic active zones. These localizations suggest that DAT modulates the intracellular and extracellular dopamine levels of nigral dendrites.

Within the perikarya of pars compacta neurons, DAT was localized primarily to rough and smooth endoplasmic reticulum, Golgi complex, and multivesicular bodies, identifying probable sites of synthesis, modification, transport, and degradation.

Genetics and regulation

The gene for DAT is located on chromosome 5p15. The protein encoding region of the gene is over 64 kb long and comprises 15 coding segments or exons. This gene has a variable number tandem repeat (VNTR) at the 3' end (rs28363170). Differences in the VNTR have been shown to affect the basal level of expression of the transporter; consequentially, researchers have looked for associations with dopamine related disorders.

Nurr1, a nuclear receptor that regulates many dopamine related genes, can bind the promoter region of this gene and induce expression. This promoter may also be the target of the transcription factor Sp-1.

While transcription factors control which cells express DAT, functional regulation of this protein is largely accomplished by kinases. Both MAPK and PKC can modulate the rate at which the transporter moves dopamine or cause the internalization of DAT.

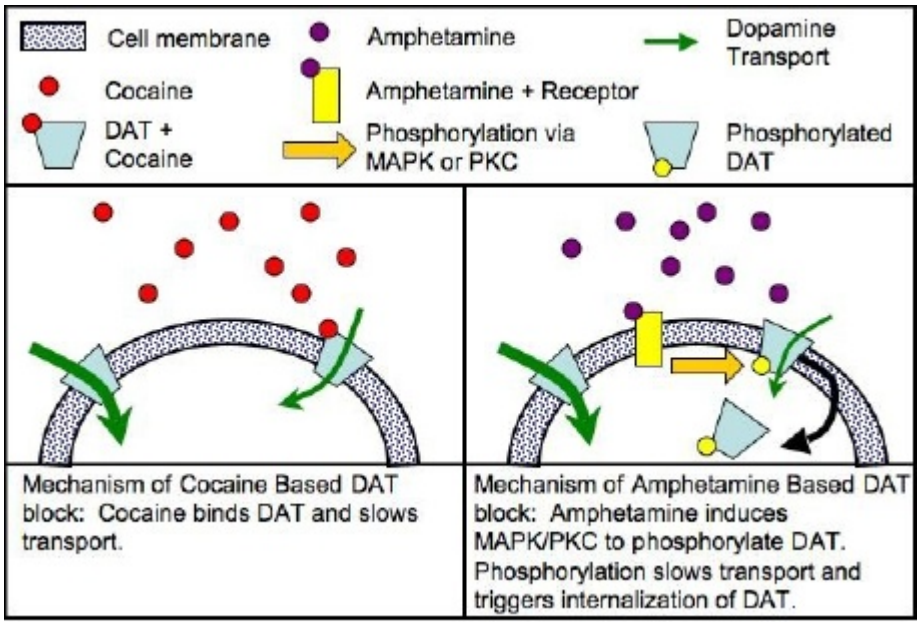
Biological role and disorders

The rate at which DAT removes dopamine from the synapse can have a profound effect on the amount of dopamine in the cell. This is best evidenced by the severe cognitive deficits, motor abnormalities, and hyperactivity of mice with no dopamine transporters. These characteristics have striking similarities to the symptoms of ADHD.

Differences in the functional VNTR have been identified as risk factors for bipolar disorder and ADHD. Data has emerged that suggests there is also an association with stronger withdrawal symptoms from alcoholism, although this is a point of controversy. Interestingly, an allele of the DAT gene with normal protein levels is associated with non-smoking behavior and ease of quitting. Additionally, male adolescents particularly those in high-risk families (ones marked by a disengaged mother and absence of maternal affection) who carry the 10-allele VNTR repeat show a statistically significant affinity for antisocial peers.

Increased activity of DAT is associated with several different disorders, including clinical depression. Decreasing levels of DAT expression are associated with aging, and likely underlie a compensatory mechanism for the decreases in dopamine release as a person ages.

Pharmacology



Mechanisms of Cocaine and Amphetamine DAT blocking

DAT is also the target of several "DAT-blockers" including amphetamines and cocaine. These chemicals inhibit the action of DAT and, to a lesser extent, the other monoamine transporters, but their effects are mediated by separate mechanisms.

Cocaine blocks DAT by binding directly to the transporter and reducing the rate of transport. In contrast, amphetamines trigger a signal cascade thought to involve PKC or MAPK that leads to the internalization of DAT molecules, which are normally expressed on the neuron's surface.

Amphetamine on DAT also has a direct effect in the increased levels of secreted dopamine. Lipophilic AMPH diffuses into the cytoplasm and into the dopamine secretory vesicles disrupting the proton gradient established across the vesicle wall. This induces a leaky channel and DA diffuses out into the cytoplasm. Additionally, AMPH causes a reversal of normal DA flow at the DAT. Instead of DA reuptake, in the presence of AMPH, a reversal in the mechanism of DAT occurs causing an outflow of dopamine released into the cytoplasm into the synaptic space.

Both of these mechanisms result in less removal of dopamine from the synapse and increased signaling, which is thought to underlie the pleasurable feelings elicited by these substances.

Chapter 7

Endomembrane System

The **endomembrane system** is composed of the different membranes that are suspended in the cytoplasm within a eukaryotic cell. These membranes divide the cell into functional and structural compartments, or organelles. In eukaryotes the organelles of the endomembrane system include: the nuclear envelope, the endoplasmic reticulum, the Golgi apparatus, lysosomes, vacuoles, vesicles, and the cell membrane. The system is defined more accurately as the set of membranes that form a single functional and developmental unit, either being connected together directly, or exchanging material through vesicle transport. Importantly, the endomembrane system does not include the membranes of mitochondria, chloroplasts or peroxisomes.

The nuclear envelope is a membrane containing two layers, that encompasses the contents of the nucleus. The endoplasmic reticulum (ER) is a synthesis and transport organelle that branches into the cytoplasm in plant and animal cells. The Golgi apparatus is a series of multiple compartments where molecules are packaged for delivery to other cell components or for secretion from the cell. Vacuoles, which are found in both plant and animal cells (though much bigger in plant cells), are responsible for maintaining the shape and structure of the cell as well as storing waste products. A vesicle is a relatively small, membrane-enclosed sac that stores or transports substances. The plasma membrane, also referred to as the cell membrane, is a protective barrier that regulates what enters and leaves the cell. There is also an organelle known as the spitzenkörper that is only found in fungi, and is connected with hyphal tip growth.

In prokaryotes endomembranes are rare, although in many photosynthetic bacteria the plasma membrane is highly folded and most of the cell cytoplasm is filled with layers of light-gathering membrane. These light-gathering membranes may even form enclosed structures called chlorosomes in green sulfur bacteria.

The organelles of the endomembrane system are related through direct contact or by the transfer of membrane segments as vesicles. Despite these relationships, the various membranes are not identical in structure and function. The thickness, molecular composition, and metabolic behavior of a membrane are not fixed, they may be modified

several times during the membrane's life. One unifying characteristic the membranes share is a lipid bilayer, with proteins attached to either side or traversing them.

History of the concept

Most lipids are synthesized in yeast either in the endoplasmic reticulum, lipid particles, or the mitochondrion, with little or no lipid synthesis occurring in the plasma membrane or nuclear membrane. Sphingolipid biosynthesis begins in the endoplasmic reticulum, but is completed in the Golgi apparatus. The situation is similar on mammals, with the exception of the first few steps in ether lipid biosynthesis, which occur in peroxisomes. The various membranes that enclose the other subcellular organelles must therefore be constructed by transfer of lipids from these sites of synthesis. However, although it is clear that lipid transport is a central process in organelle biogenesis, the mechanisms by which lipids are transported through cells remain poorly understood.

The first proposal that the membranes within cells form a single system that exchanges material between its components was by Morré and Mollenhauer in 1974. This proposal was made as a way of explaining how the various lipid membranes are assembled in the cell, with these membranes being assembled through *lipid flow* from the sites of lipid synthesis. The idea of lipid flow through a continuous system of membranes and vesicles was an alternative to the various membranes being independent entities that are formed from transport of free lipid components, such as fatty acids and sterols, through the cytosol. Importantly, the transport of lipids through the cytosol and lipid flow through a continuous endomembrane system are not mutually exclusive processes and both may occur in cells.

Components of the system

Nuclear envelope

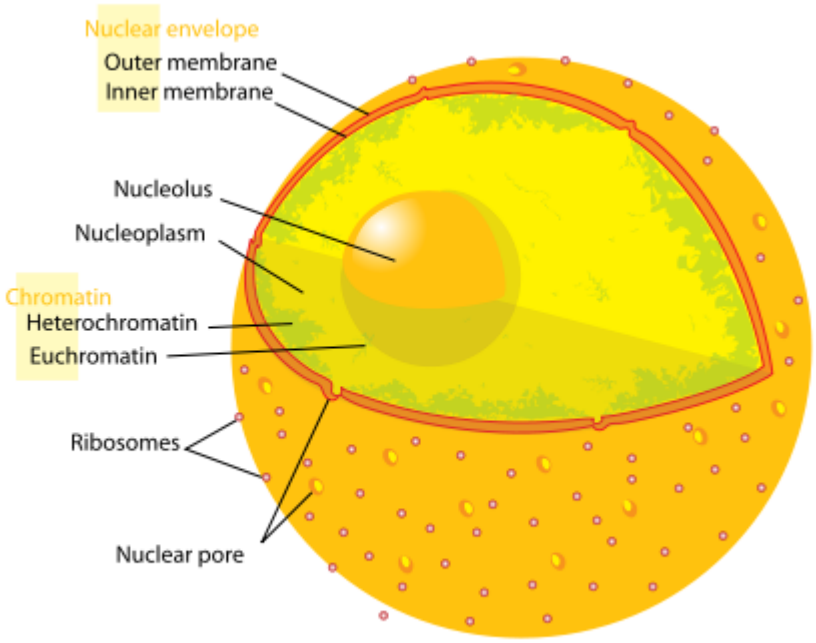


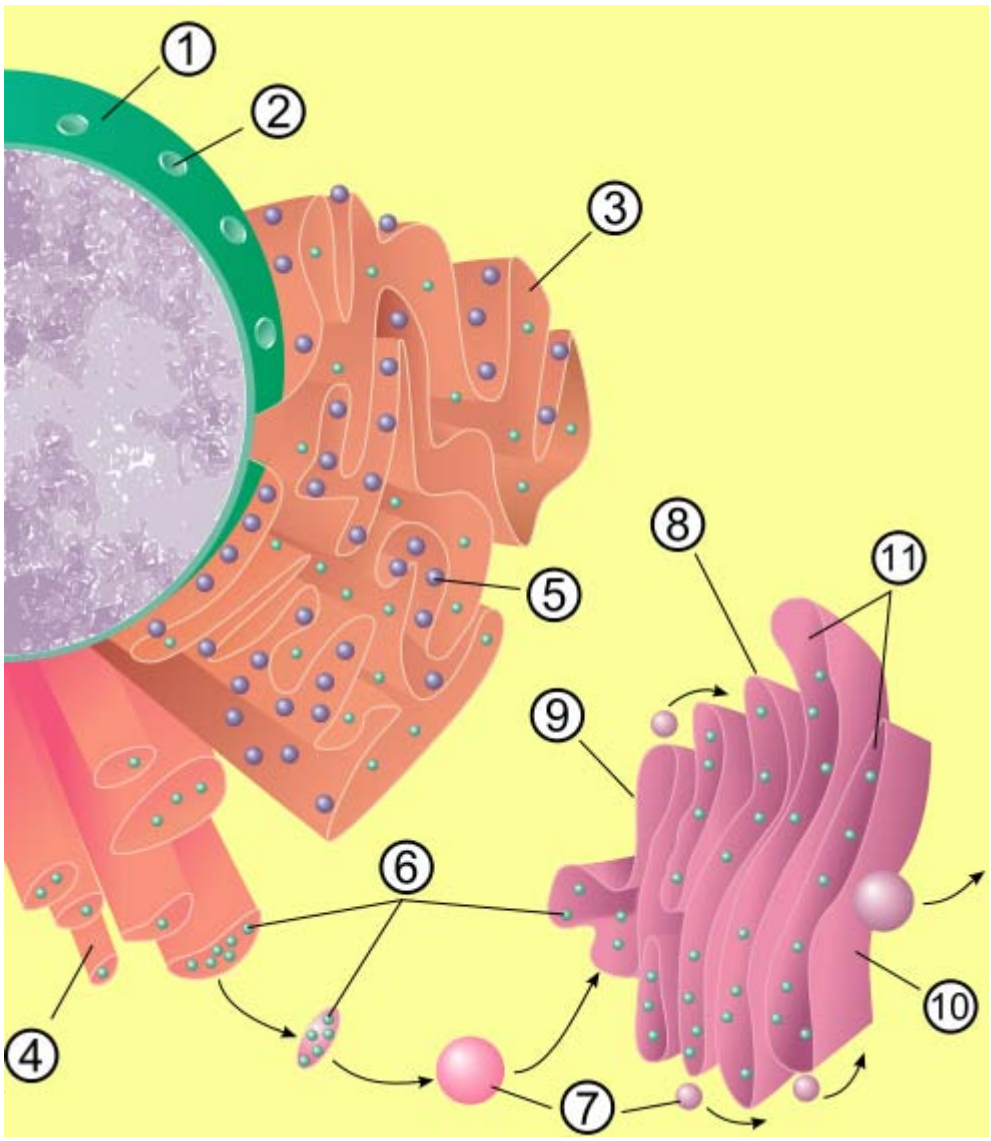
Diagram of the nucleus with the nuclear envelope shown as the orange portion.

The nuclear envelope encloses the nucleus, separating its contents from the cytoplasm. It has two membranes, each a lipid bilayer with associated proteins. The outer nuclear membrane is constant with the rough endoplasmic reticulum membrane, and like that structure, features ribosomes attached to the surface. The outer membrane is also constant with the inner nuclear membrane since the two layers are fused together at numerous tiny holes called nuclear pores that perforate the nuclear envelope. These pores are about 120 nm in diameter and regulate the passage of molecules between the nucleus and cytoplasm, permitting some to pass through the membrane, but not others. Since the nuclear pores are located in an area of high traffic, they play an important role in the physiology of cells. The space between the outer and inner membranes is called the perinuclear space and is joined with the lumen of the rough ER.

The nuclear envelope's structure is determined by a network of intermediate filaments (protein filaments). This network is organized into lining similar to mesh called the nuclear lamina, which binds to chromatin, integral membrane proteins, and other nuclear components along the inner surface of the nucleus. The nuclear lamina is thought to help materials inside the nucleus reach the nuclear pores and in the disintegration of the nuclear envelope during mitosis and its reassembly at the end of the process.

The nuclear pores are highly efficient at selectively allowing the passage of materials to and from the nucleus, because the nuclear envelope has a considerable amount of traffic. RNA and ribosomal subunits must be continually transferred from the nucleus to the cytoplasm. Histones, gene regulatory proteins, DNA and RNA polymerases, and other substances essential for nuclear activities must be imported from the cytoplasm. The nuclear envelope of a typical mammalian cell contains 3000–4000 pore complexes. If the cell is synthesizing DNA each pore complex needs to transport about 100 histone molecules per minute. If the cell is growing rapidly, each complex also needs to transport about 6 newly assembled large and small ribosomal subunits per minute from the nucleus to the cytosol, where they are used to synthesize proteins.

Endoplasmic reticulum



1 Nucleus **2** Nuclear pore **3** Rough endoplasmic reticulum (RER) **4** Smooth endoplasmic reticulum (SER) **5** Ribosome on the rough ER **6** Proteins that are

transported **7** Transport vesicle **8** Golgi apparatus **9** Cis face of the Golgi apparatus **10** Trans face of the Golgi apparatus **11** Cisternae of the Golgi apparatus

The endoplasmic reticulum (ER) is a membranous synthesis and transport organelle that is an extension of the nuclear envelope. More than half the total membrane in eukaryotic cells is accounted for by the ER. The ER is made up of flattened sacs and branching tubules that are thought to interconnect, so that the ER membrane forms a continuous sheet enclosing a single internal space. This highly convoluted space is called the ER lumen and is also referred to as the ER cisternal space. The lumen takes up about ten percent of the entire cell volume. The endoplasmic reticulum membrane allows molecules to be selectively transferred between the lumen and the cytoplasm, and since it is connected to the nuclear envelope, it provides a channel between the nucleus and the cytoplasm.

The ER has a central role in producing, processing, and transporting biochemical compounds for use inside and outside of the cell. Its membrane is the site of production of all the transmembrane proteins and lipids for most of the cell's organelles, including the ER itself, the Golgi apparatus, lysosomes, endosomes, mitochondria, peroxisomes, secretory vesicles, and the plasma membrane. Furthermore, almost all of the proteins that will exit the cell, plus those destined for the lumen of the ER, Golgi apparatus, or lysosomes, are originally delivered to the ER lumen. Consequently, many of the proteins found in the cisternal space of the endoplasmic reticulum lumen are there only temporarily as they pass on their way to other locations. Other proteins, however, constantly remain in the lumen and are known as endoplasmic reticulum resident proteins. These special proteins contain a specialized retention signal made up of a specific sequence of amino acids that enables them to be retained by the organelle. An example of an important endoplasmic reticulum resident protein is the chaperon protein known as BiP which identifies other proteins that have been improperly built or processed and keeps them from being sent to their final destinations.

There are two distinct, though connected, regions of ER that differ in structure and function: smooth ER and rough ER. The rough endoplasmic reticulum is so named because the cytoplasmic surface is covered with ribosomes, giving it a bumpy appearance when viewed through an electron microscope. The smooth ER appears smooth since its cytoplasmic surface lacks ribosomes.

Functions of the smooth ER

In the great majority of cells, smooth ER regions are scarce and are often partly smooth and partly rough. They are sometimes called transitional ER because they contain ER exit sites from which transport vesicles carrying newly synthesized proteins and lipids bud off for transport to the Golgi apparatus. In certain specialized cells, however, the smooth ER is abundant and has additional functions. The smooth ER of theses specialized cells function in diverse metabolic processes, including synthesis of lipids, metabolism of carbohydrates, and detoxification of drugs and poisons.

Enzymes of the smooth ER are vital to the synthesis of lipids, including oils, phospholipids, and steroids. Sex hormones of vertebrates and the steroid hormones secreted by the adrenal glands are among the steroids produced by the smooth ER in animal cells. The cells that synthesis these hormones are rich in smooth ER.

Liver cells are another example of specialized cells that contain an abundance of smooth ER. These cells provide an example of the role of smooth ER in carbohydrate metabolism. Liver cells store carbohydrates in the form of glycogen. The hydrolysis of glycogen leads to the release of glucose from the liver cells, which is important in the regulation of sugar concentration in the blood. However, glycogen hydrolysis requires glucose phosphate, an ionic form of the sugar that can not exit the cell. An enzyme of the liver cell's smooth ER removes the phosphate from the glucose, so that it can then leave the cell.

Enzymes of the smooth ER can also help detoxify drugs and poisons. Detoxification usually involves the addition of a hydroxyl group to a drug, making the drug more soluble and thus easier to purge from the body. One extensively studied detoxification reaction is carried out by the cytochrome P450 family of enzymes, which catalyze water-insoluble drugs or metabolites that would otherwise accumulate to toxic levels in cell membrane.

Muscle cells have another specialized function of smooth ER. The ER membrane pumps calcium ions from the cytosol into the cisternal space. When a muscle cell becomes stimulated by a nerve impulse, calcium goes back across the ER membrane into the cytosol and generates the contraction of the muscle cell.

Functions of the rough ER

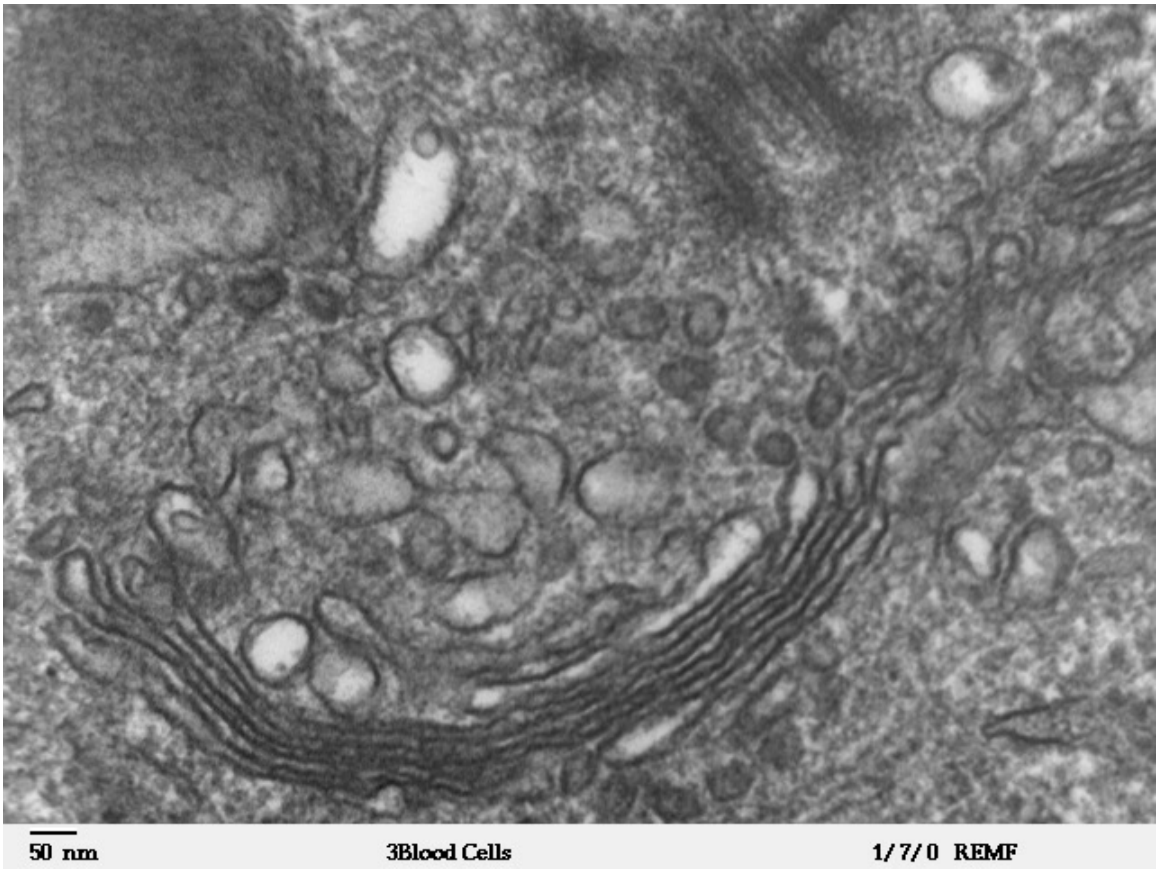
Many types of cells export proteins produced by ribosomes attached to the rough ER. The ribosomes assemble amino acids into protein units, which are carried into the rough ER for further adjustments. These proteins may be either transmembrane proteins, which become embedded in the membrane of the endoplasmic reticulum, or water-soluble proteins, which are able to pass through the membrane into the lumen. Those that reach the inside of the endoplasmic reticulum are folded into the correct three-dimensional conformation. Chemicals, such as carbohydrates or sugars, are added, then the endoplasmic reticulum either transports the completed proteins, called secretory proteins, to areas of the cell where they are needed, or they are sent to the Golgi apparatus for further processing and modification.

Once secretory proteins are formed, the ER membrane separates them from the proteins that will remain in the cytosol. Secretory proteins depart from the ER enfolded in the membranes of vesicles that bud like bubbles from the transitional ER. These vesicles in transit to another part of the cell are called transport vesicles. An alternative mechanism for transport of lipids and proteins out of the ER are through lipid transfer proteins at regions called membrane contact sites where the ER becomes closely and stably

associated with the membranes of other organelles, such as the plasma membrane, Golgi or lysosomes.

In addition to making secretory proteins, the rough ER makes membranes that grows in place from the addition of proteins and phospholipids. As polypeptides intended to be membrane proteins grow from the ribosomes, they are inserted into the ER membrane itself and are kept there by their hydrophobic portions. The rough ER also produces its own membrane phospholipids; enzymes built into the ER membrane assemble phospholipids. The ER membrane expands and can be transferred by transport vesicles to other components of the endomembrane system.

Golgi apparatus



Micrograph of Golgi apparatus, visible as a stack of semicircular black rings near the bottom. Numerous circular vesicles can be seen in proximity to the organelle

The Golgi apparatus (also known as the Golgi body and the Golgi complex) is composed of interconnected sacs called cisternae. Its shape can be related to that of a stack of pancakes. The number of these stacks varies with the specific function of the cell. The Golgi apparatus is used by the cell for further protein modification. The section of the Golgi apparatus that receives the vesicles from the ER is known as the cis face, and is usually near the ER. The opposite end of the Golgi apparatus is called the trans face, this

is where the modified compounds leave. The trans face is usually facing the plasma membrane, which is where most of the substances the Golgi apparatus modifies are sent.

Vesicles sent off by the ER containing proteins are further altered at the golgi apparatus and then prepared for secretion from the cell or transport to other parts of the cell. Various things can happen to the proteins on their journey through the enzyme covered space of the Golgi apparatus. The modification and synthesis of the carbohydrate portions of glycoproteins is common in protein processing. The Golgi apparatus removes and substitutes sugar monomers, producing a large variety of oligosaccharides. In addition to modifying proteins, the golgi also manufactures macromolecules itself. In plant cells, the Golgi produces pectins and other polysaccharides needed by the plant structure.

Once the modification process is completed the golgi apparatus sorts the products of its processing and sends them to various parts of the cell. Molecular identification labels or tags are added by the golgi enzymes to help with this. After everything is organized, the golgi apparatus sends off its products by budding vesicles from its trans face.

Vacuoles

Vacuoles, like vesicles, are membrane-bounded sacs within the cell. They are larger than vesicles and their specific function varies. The operations of vacuoles are different for plant and animal vacuoles.

In plant cells, vacuoles cover anywhere from 30% to 90% of the total cell volume. Most mature plant cells contain one large central vacuole encompassed by a membrane called the tonoplast. Vacuoles of plant cells act as storage compartments for the nutrients and waste of a cell. The solution that these molecules are stored in is called the cell sap. Pigments that color the cell are sometime located in the cell sap. Vacuoles can also increase the size of the cell, which elongates as water is added, and they control the turgor pressure (the osmotic pressure that keeps the cell wall from caving in). Like lysosomes of animal cells, vacuoles have an acidic pH and contain hydrolytic enzymes. The pH of vacuoles enables them to perform homeostatic procedures in the cell. For example, when the pH in the cells environment drops, the H+ surging into the cytosol can be transferred to a vacuole in order to keep the cytosol's pH constant.

In animals, vacuoles serve in exocytosis and endocytosis processes. Endocytosis refers to when particles are taken into the cell. The material to be taken in is surrounded by the plasma membrane, and then transferred to a vacuole. There are two types of endocytosis, phagocytosis (cell eating) and pinocytosis (cell drinking). In phagocytosis, cells engulf large particles such as bacteria. Pinocytosis is the same process, except the substances being ingested are in the fluid form.

Vesicles

Vesicles are small membrane-enclosed transport units that can transfer molecules between different compartments. Most vesicles transfer the membranes assembled in the

endoplasmic reticulum to the Golgi apparatus, and then from the Golgi apparatus to various locations.

There are various types of vesicles each with a different protein configuration. Most are formed from specific regions of membranes. When a vesicle buds off from a membrane it contains specific proteins on its cytosolic surface. Each membrane a vesicle travels to contains a marker on its cytosolic surface. This marker corresponds with the proteins on the vesicle traveling to the membrane. Once the vesicle finds the membrane, they fuse.

There are three well known types of vesicles. They are clathrin-coated, COPI-coated, and COPII-coated vesicles. Each performs different functions in the cell. For example, clathrin-coated vesicles transport substances between the golgi apparatus and the plasma membrane. COPI- and COPII-coated vesicles are frequently used for transportation between the ER and the golgi apparatus.

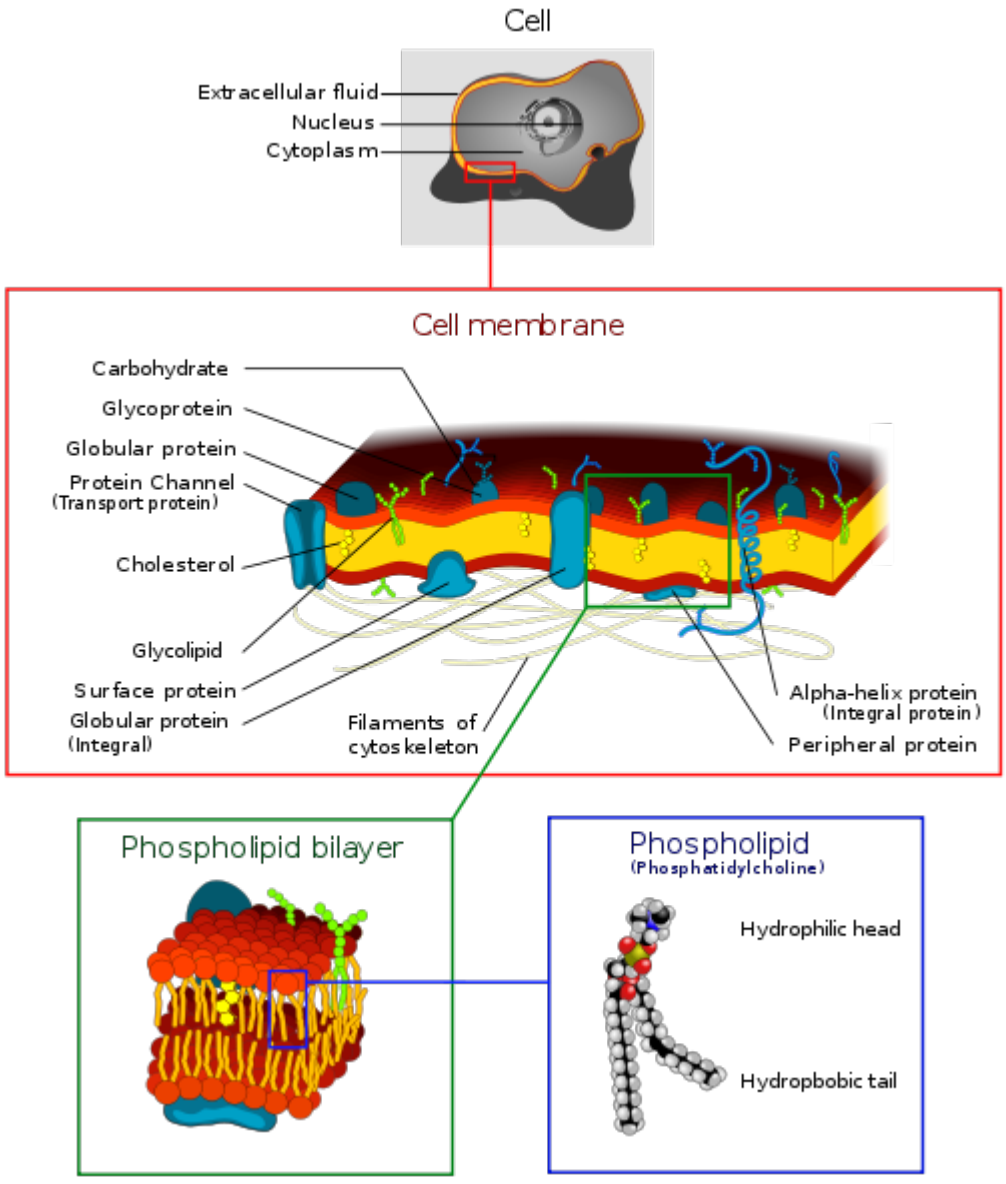
Lysosomes

Lysosomes are organelles that contain hydrolytic enzymes that are used for intracellular digestion. The main functions of a lysosome are to process molecules taken in by the cell and to recycle worn out cell parts. The enzymes inside of lysosomes are acid hydrolases which require an acidic environment for optimal performance. Lysosomes provide such an environment by maintaining a pH of 5.0 inside of the organelle. If a lysosome were to rupture, the enzymes released would not be very active because of the cytosol's neutral pH. However, if numerous lysosomes leaked the cell could be destroyed from autodigestion.

Lysosomes carry out intracellular digestion by fusing with a vacuole and releasing their enzymes into the vacuole. Through this process, sugars, amino acids, and other monomers pass into the cytosol and become nutrients for the cell. Lysosomes also use their hydrolytic enzymes to recycle the cell's obsolete organelles in a process called autophagy. The lysosome engulfs another organelle and uses its enzymes to take apart the ingested material. The resulting organic monomers are then returned to the cytosol for reuse. The last function of a lysosome is to digest the cell itself through autolysis.

Spitzenkörper

The spitzenkörper is a component of the endomembrane system found only in fungi, and is associated with hyphal tip growth. It is a phase-dark body that is composed of an aggregation of membrane-bound vesicles containing cell wall components, serving as a point of assemblage and release of such components intermediate between the Golgi and the cell membrane. The spitzenkörper is motile and generates new hyphal tip growth as it moves forward.



Detailed illustration of the plasma membrane. Including the structure of a phospholipid.

Plasma membrane

The plasma membrane is a phospholipid bilayer membrane that separates the cell from its environment and regulates the transport of molecules and signals into and out of the cell. Embedded in the membrane are proteins that perform the functions of the plasma membrane. The plasma membrane is not a fixed or rigid structure, the molecules that compose the membrane are capable of lateral movement. This movement and the multiple components of the membrane are why it is referred to as a fluid mosaic. Smaller molecules such as carbon dioxide, water, and oxygen can pass through the plasma membrane freely by diffusion or osmosis. Larger molecules needed by the cell are assisted by proteins through active transport.

The plasma membrane of a cell has multiple functions. These include transporting nutrients into the cell, allowing waste to leave, preventing materials from entering the cell, averting needed materials from leaving the cell, maintaining the pH of the cytosol, and preserving the osmotic pressure of the cytosol. Transport proteins which allow some materials to pass through but not others are used for these functions. These proteins use ATP hydrolysis to pump materials against their concentration gradients.

In addition to these universal functions, the plasma membrane has a more specific role in multicellular organisms. Glycoproteins on the membrane assist the cell in recognizing other cells, in order to exchange metabolites and form tissues. Other proteins on the plasma membrane allow attachment to the cytoskeleton and extracellular matrix; a function that maintains cell shape and fixes the location of membrane proteins. Enzymes that catalyze reactions are also found on the plasma membrane. Receptor proteins on the membrane have a shape that matches with a chemical messenger, resulting in various cellular responses.

Evolution

The Golgi, ER, and lysosomes are likely to have evolved as a result of the plasma membrane going through invagination. An increase in the overall volume of a cell would require the plasma membrane to fold in order to maintain a constant surface area to volume ratio. These folds may have led to the specialization of internal membranes to maintain communication with the environment. In the first stages of eukaryotic cell life, the membranes may have been interconnected and attached to the plasma membrane. Later on, as their functions diverged, the membranes may have become separate structures.

Chapter 8

Hydrophobic Mismatch

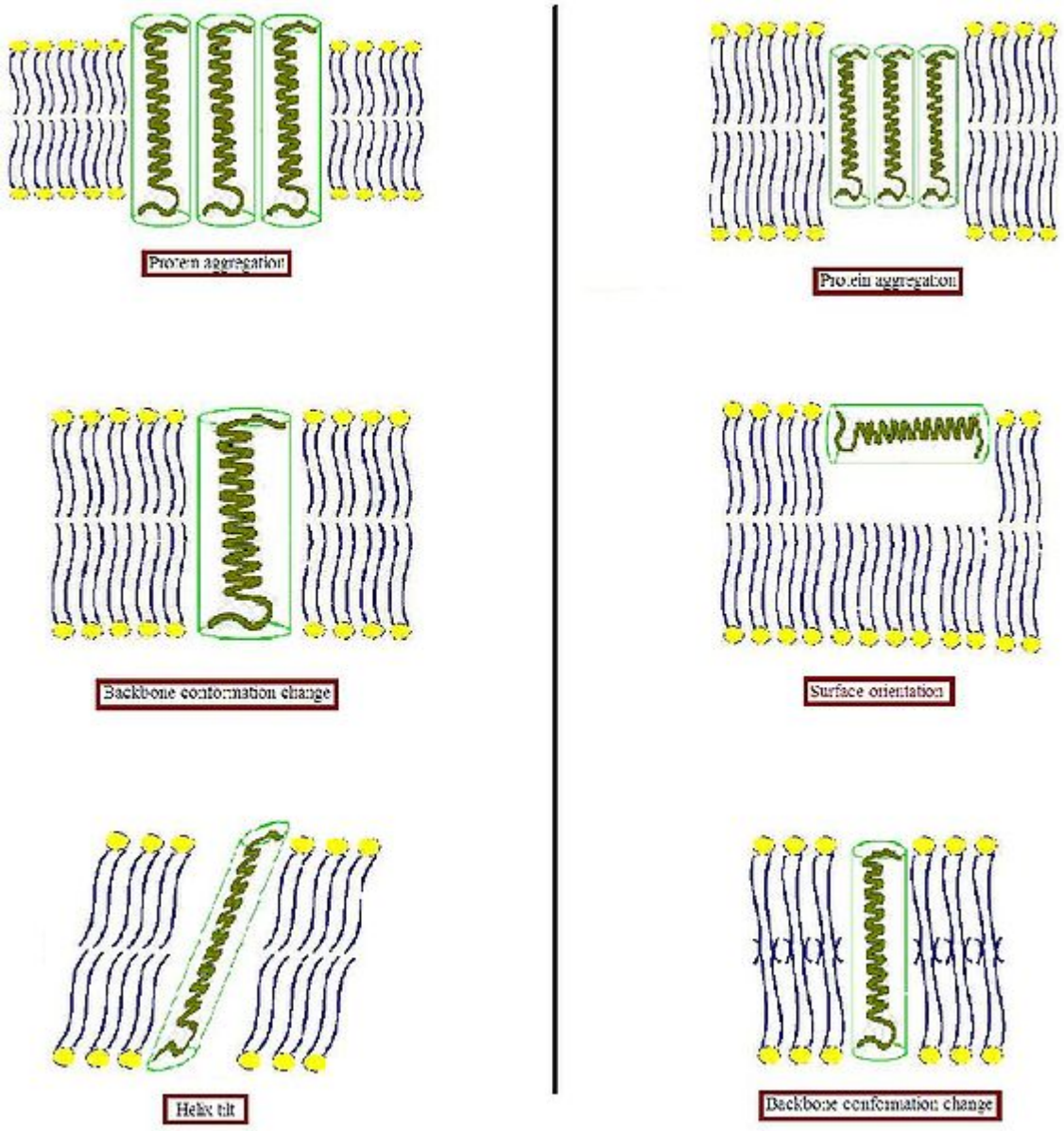
Membrane **hydrophobic mismatch** is the difference between the hydrophobic length of α helices of the integral proteins and the hydrophobic thickness of the membrane they span. Under the consideration of energy requirement, in order to avoid unfavorable exposure of hydrophobic surfaces to a hydrophilic environment, the hydrophobic length of the integral proteins is supposed to be approximately equal to the hydrophobic bilayer thickness.

Structure and organization

A biological membrane is a kind of amphipathic layer that acts as a barrier within or around a cell. In most case, it is a lipid bilayer, composed of a double layer of lipid molecules and proteins that may constitute close to 50% of membrane content. The interaction of integral proteins with the lipids inside membrane bilayer is of great importance for membrane function.

The thickness of biological membrane is approximately same with the hydrophobic core of lipid bilayer. At the same time, the protein embedded inside one membrane could have different hydrophobic length, for example Escherichia Coli, a Gram negative bacterium that is commonly found in the lower intestine of warm-blooded animals, the predicted length of inner membrane enzyme leader peptidase for the second transmembrane helix is only 15 amino acids long. While resides in the same membrane, the helices of lactose permease has the mean length of 24±4 residues long. Vice versa, membrane proteins with the same length can be encountered in bilayers of different thickness. Like in eukaryotic cells, due to their higher content of cholesterol and sphingomyelin, the plasma membrane is much thicker than the membranes of the endoplasmic reticulum. Yet all proteins that are abundant in the plasma membrane are initially integrated in the endoplasmic reticulum upon synthesis on ribosomes.

Possible adaptations to mismatch



Hydrophobic Mismatch.

In order to avoid unfavorable exposure of hydrophobic surfaces to a hydrophilic environment, biological membrane tends to make some adaptations to such mismatch. In various other systems, is that an integral protein tends to surround itself by lipids of matching size and shape. Since proteins are relatively rigid, whereas lipid hydrocarbon chains are flexible, the condition of hydrophobic matching can be fulfilled by stretching, squashing, and/or tilting of the lipid chains

- When the hydrophobic part of a transmembrane protein is too large to match the hydrophobic bilayer thickness (left part of Figure), the protein might aggregate in

the membrane to minimize the exposed hydrophobic area or tilt to reduce their effective hydrophobic length. They could also adopt another conformation by changing the orientation of hydrophobic and hydrophilic side chains near the interface. Lipids in turn could modulate the membrane thickness by stretching their acyl chains or even assemble into another type of aggregate, thereby disrupting the bilayer organization.

- When the hydrophobic part of a transmembrane protein is too small to match the hydrophobic bilayer thickness (right part of Figure), again this might result in protein aggregation, or changes in backbone conformation and/or side chain orientation. Too short peptides in addition might not incorporate and instead adopt a surface localization. Lipids could decrease the effective bilayer thickness by disordering their acyl chains or disrupt the bilayer organization to form an inverted non-lamellar structure. Combinations of these possible modes of adaptation might also occur.

Protein aggregation

Since Mouritsen and Bloom proposed the detailed thermodynamic model, which includes adaptation of the lipids and induction of protein segregation at a more extreme mismatch in their "Mattress Model", more additional insight into mismatch-induced protein aggregation has been obtained. Also some experimental evidence that a hydrophobic mismatch can lead to protein aggregation in fluid bilayer were founded. Electron microscopy studies on bacteriorhodopsin, reconstituted in saturated and unsaturated fluid PC bilayers with varying chain length, showed that protein aggregation occurred only with a rather large mismatch, and that bilayer thicknesses of 4 angstrom thicker and 10 angstrom thinner than the estimated hydrophobic length of the protein are allowed without induction of significant aggregation.

Helix tilt

Tilt is also a possible result if the hydrophobic part of a peptide or protein is too long to span the membrane. A previous study on lactose permease of E. coli showed that upon reconstitution of the protein in PE/PG (3/1) lipid bilayer, an increase in helix tilt occurs at increasing protein content. This tilt was accompanied by a decrease in lipid order, which results in a decrease in bilayer thickness, suggesting that it is a mismatch related response. In large proteins that span the membrane multiple times, changes in helical tilt may occur with little effect on lipid packing. However, for a single transmembrane helix, it is possible that a tilt would cause a strain on the surrounding lipids to accommodate the helix in the bilayer. Thus, a large degree of tilting can be a less favorable option for single transmembrane proteins.

Surface orientation

Relatively small hydrophobic peptides may not be able to integrate into the membrane, and in response adopt an orientation at the membrane surface. The experimental evidence

was shown by a fluorescence study on an artificial peptide with a 19 amino acid long hydrophobic sequence of mainly leucines and flanked on both sides with lysines as anchoring residues. The results indicated that a conversion from a dominant transmembrane to parallel orientation of the peptide could be induced by modulating bilayer thickness via addition of cholesterol or by increasing lipid chain length.

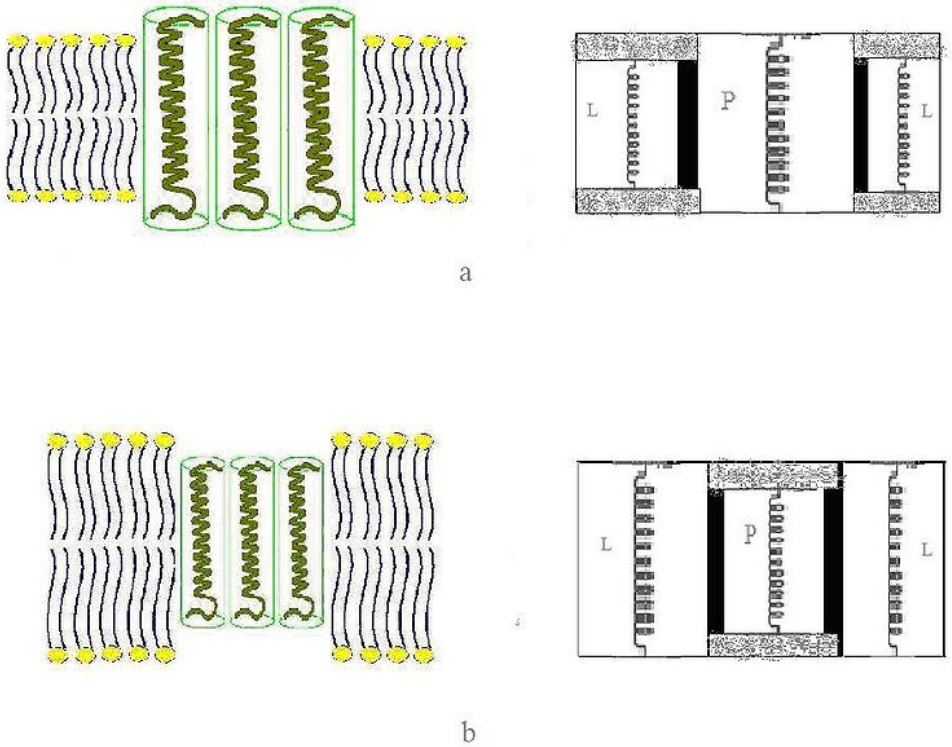
Backbone conformation change

To obtain detailed information on the consequences of mismatch for the conformation of peptides and proteins in lipid bilayer, small membrane-spanning peptides are most suitable. Still need some studies.

Theories for the mismatch effects

Since the diverse possibility of the hydrophobic mismatch effects, there is a need to understand this puzzling behavior and to isolate the various factors which bring about the net result of hydrophobic mismatch. Many theoretical approaches have been applied to the effects of mismatch. Typically two trails have been dominated, phenomenological trail which based on treating the membrane as an elastic sheet is used mostly. together with few previously. but more and more useful microscopic approach. Some important theory approaches used in the history are shown here:

Mattress model



Mattress model

Mattress model was proposed as a phenomenological theory approach in 1984 by Mouritsen and Bloom. It is a two-component real solution theory based on the theory of nonideal solutions and hence allows for phase separation. In their model, they relate the energy stored in the undulations of the membrane surface caused by the mismatch to the elastic properties of the lipids and proteins. They do not include microscopic detail of the lipids, but use as input the known thermodynamic properties of the pure lipid system. They also include indirect lipid-protein interactions induced by the mismatch as well as direct lipid-protein van der Waals-like interactions between the hydrophobic parts of the lipid bilayer and the proteins. The excess "hydrophobic effect" associated with the lipid-protein hydrophobic mismatch, and the elastic deformation free energy of the lipid chains near the protein. The interaction potentials are estimated based on experimental data derived from thermodynamic and mechanical measurements of membrane properties.

Monte Carlo simulation scheme

The mattress model was later replicated in a Monte Carlo simulation scheme by Sperotto and Mouritsen. They allowed for different microstates of the lipids, classified according to Pink's 10-state model. hence enabling a pure lipid bilayer phase transition. This version of the model provides a connection between the microscopic characteristics of the system and its thermodynamic behavior.

Molecular theory

A major theoretical advance was the work of Fattal and Ben-Shaul. who provided a molecular theory for the behavior of the lipid chains of the membrane. The peptides, with their hydrophobic length, were treated as providing a boundary condition on the configuration of the lipid chains. This molecular modeling was combined with phenomenological free energy contributions describing lipid head group repulsion and membrane solvent surface tension. Duque et al. described the effects of an embedded protein in a bilayer via molecular theory, which yielded the free energy of the entire system.

Effect of mismatch

The membrane hydrophobic mismatch has the possible effects in more complex biological membranes and possible significance for biological membrane processes, like in the protein sorting, lipid raft.

Protein sorting

In eukaryotic cells, the level of cholesterol increases through the secretory pathway, from the endoplasmic reticulum to the Golgi to the plasma membrane, suggesting a concomitant increase in membrane thickness. In line with this, the average length of transmembrane domains of plasma membrane proteins typically is five amino acids longer than the average length of proteins from the Golgi. Experimental evidence was obtained that protein sorting in the Golgi may be based on this length difference: for

several proteins that normally reside in the Golgi, it was shown that increasing their hydrophobic length can reroute the proteins to the plasma membrane, or vice versa, that decreasing the hydrophobic length of proteins from the plasma membrane can cause their retention in the Golgi.

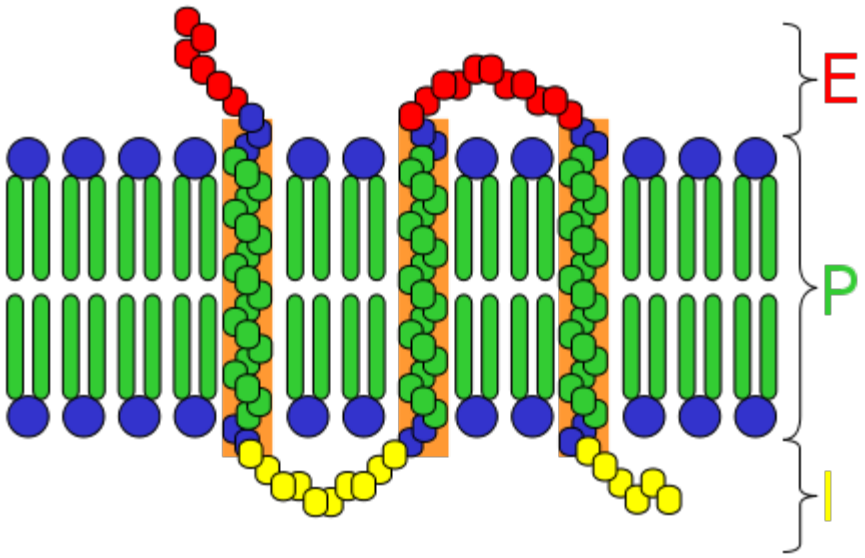
Lipid raft

One type of membrane heterogeneity, termed rafts, is enriched in cholesterol, sphingomyelin (SM), and certain membrane proteins. Rafts have putative roles in many physiological processes, such as signal transduction, endocytosis, apoptosis, protein trafficking, and lipid regulation. Raft lipids typically have saturated hydrocarbon chains. Cholesterol, a molecule that is enriched in lipid rafts, has a shorter hydrophobic length. Hence, because of the differences in lipid thickness between raft and nonraft lipids, and since energetically it can be expected that the lengths of the hydrophobic moieties of neighboring membrane components will be approximately equal to avoid unfavorable exposure of hydrophobic surfaces to a hydrophilic environment, it is reasonable to assume that nonraft lipids should coalesce and predominantly give rise to smaller bilayer thicknesses compared to raft lipids.

Chapter 9

Integral Membrane Protein and Mechanosensitive Channels

Integral membrane protein



E=extracellular space; P=plasma membrane; I=intracellular space

An **integral membrane protein** (**IMP**) is a protein molecule (or assembly of proteins) that is permanently attached to the biological membrane. Proteins that cross the membrane are surrounded by "annular" lipids, which are defined as lipids that are in direct contact with a membrane protein. Such proteins can be separated from the biological membranes only using detergents, nonpolar solvents, or sometimes denaturing agents.

IMPs comprise a very significant fraction of the proteins encoded in an organism's genome.

All transmembrane proteins are IMPs, but not all IMPs are transmembrane proteins.

Structure

Three-dimensional structures of only ~160 different integral membrane proteins are currently determined at atomic resolution by X-ray crystallography or nuclear magnetic resonance spectroscopy due to the difficulties with extraction and crystallization. In addition, structures of many water-soluble domains of IMPs are available in the Protein Data Bank. Their membrane-anchoring α-helices have been removed to facilitate the extraction and crystallization.

IMPs can be divided into two groups:

1. Integral polytopic proteins (Transmembrane proteins)
2. Integral monotopic proteins

Integral Polytopic Protein

The most common type of IMP is the transmembrane protein (TM), which spans the entire biological membrane. **Single-pass** membrane proteins cross the membrane only once, while **multi-pass** membrane proteins weave in and out, crossing several times. Single pass TM proteins can be categorized as Type I, which are positioned such that their amino-terminus is outside of the membrane, or Type II, which have their carboxy-terminus outside of the membrane.

Integral Monotopic Proteins

Integral monotopic proteins, are permanently attached to the membrane from one side. Such domains require detergents for extraction or crystallization, even after removal of their transmembrane helices. Therefore, they are often classified as integral monotopic *proteins*

Determination of Protein Structure

The use of hydropathy plots helps determine integral protein structures based on the hydrophobic and hydrophilic characteristics of alpha helical integral proteins.

Function

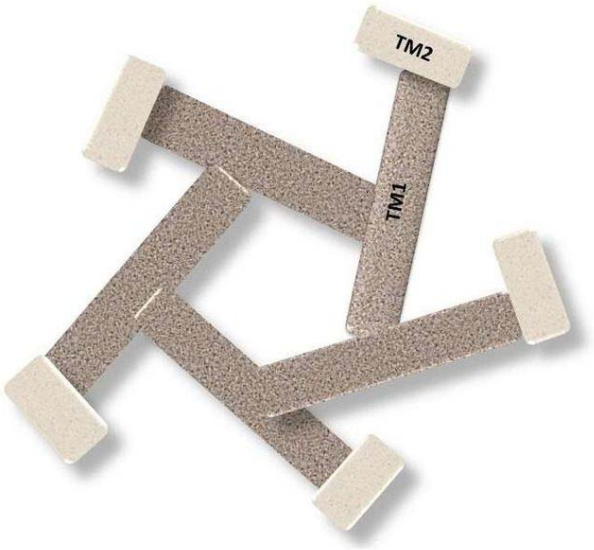
IMPs include transporters, channels, receptors, enzymes, structural membrane-anchoring domains, proteins involved in accumulation and transduction of energy, and proteins responsible for cell adhesion. Classification of transporters can be found in Transporter Classification database.

Examples

Examples of integral membrane proteins:

- Insulin receptor
- Some types of cell adhesion proteins or cell adhesion molecules (CAMs) such as Integrins, Cadherins, NCAMs, or Selectins.
- Some types of receptor proteins
- Glycophorin
- Rhodopsin
- Band 3
- CD36
- GPR30

Mechanosensitive channels



Finite Element Model of MscL transmembrane model. This figure is similar to the Tang et al. .

Mechanosensitive channels or mechanosensitive ion channels are membrane proteins capable of responding over a wide dynamic range to external mechanical stimuli. They are found in prokaryotes and eukaryotes. The channels vary in selectivity for the permeating ions from nonselective between anions and cations, to cation selective allowing passage Ca^{2+}, K^{+} and Na^{+}, a highly selective K^{+} channels.

All organisms and apparently all cell types sense and respond to a mechanical stimulus . MSCs function as mechanotransducers capable of generating either an electrical and ion flux signal as a response to external stimuli. Under extreme turgor in bacteria, non selective MSCs such as MSCL and MSCS serve as safety valves to prevent lysis. In specialized cells of the higher organisms, other types of MSCs create the senses of hearing and touch and sense the stress needed for muscular coordination. MSCs also allow plants to distinguish up from down due to differences in signal rates resulting from the force of gravity. MSCs are not pressure sensitive, but sensitive to local stress, most likely tension in the surrounding lipid bilayer.

History

Mechanosensitive channels were first observed in chick skeletal muscles by Falguni Guharay and Frederick Sachs in 1983 and the results were published in 1984 . Since then MS channels have been found in cells from bacteria to human as well as plants . In the last two decades, the knowledge of the structure and function of MSCs has been increased since its discovery. They are present in different kingdoms such as Archaea, Bacteria, Plants, Fungi and Eukarya.

Classification

MS can be classified based on the type of ion to which they are permeable.

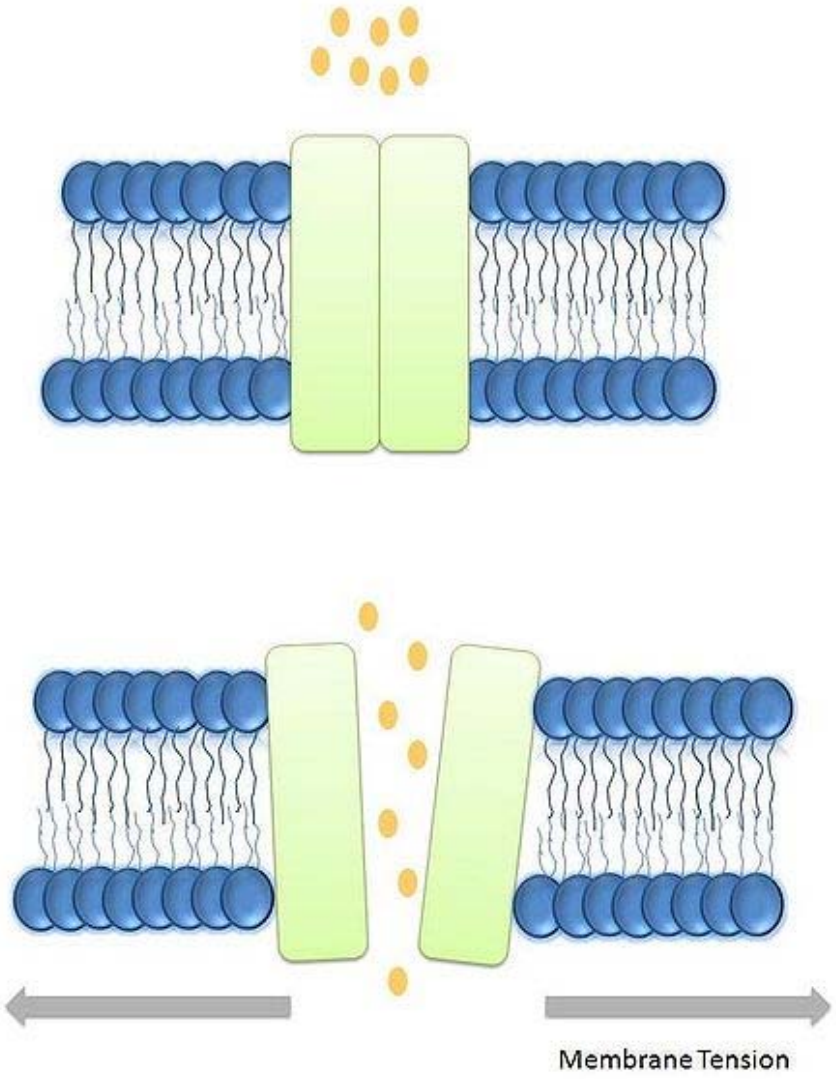
Cation Selective MSCs: As the name suggests, they exhibit a selective permeability for positive ions with the most selective channels being those for K+. The most common eukaryotic MSCs are cation selective passing Na+, K+ and Ca+ but not Mg+. They have a single channel conductance range (25-35 pS) and they are blocked by trivalent ion Gadolinium. The K selective MSCs such as TREK-1 are not blocked by Gd+3..

Anion Channels: they exhibit a significant permeability for negative ions, and are not predominant as cation MS. They have a large conductance range (> 300pS).

Non Selective ion channels: As the name indicates, they do not differentiate between positive and negative channels those are more common to Archaea and Bacteria, but rarely found in Eukarya.

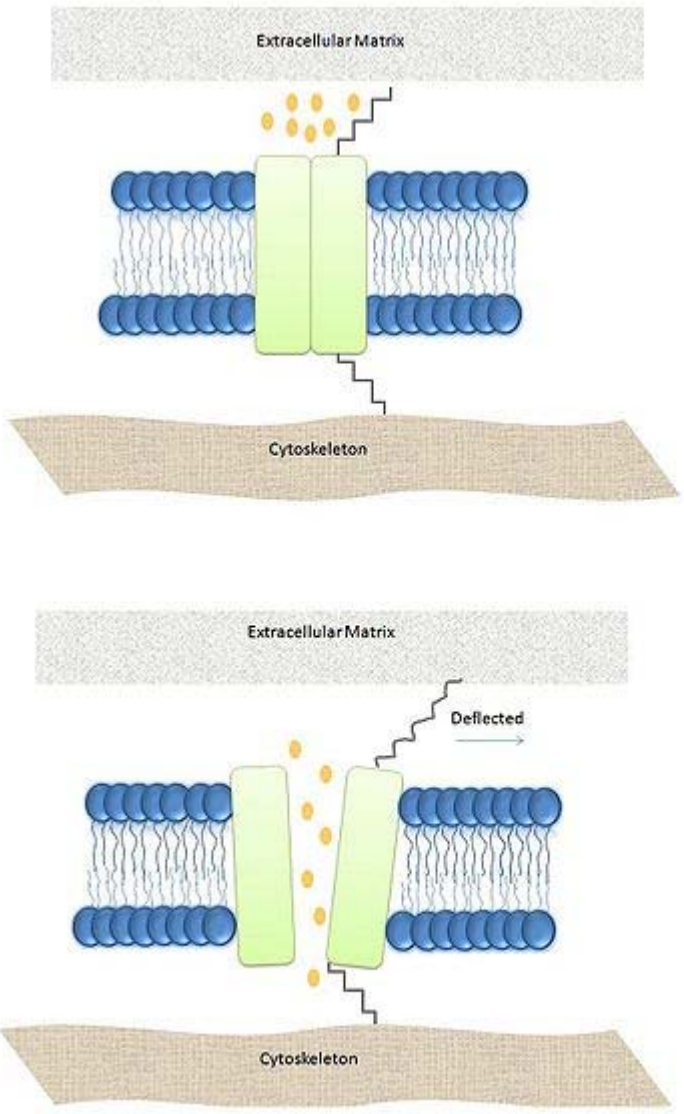
Gating Mechanism

Although MS are different in many aspects, structures and function between them, all the MS studies up to date share the important feature gating meaning they all open as a pore-like when protein-channel is activated by a mechanical stimuli. To understand how membrane-activated ion channels open currently there are three models which explained the gating process.



Gating Mechanism of MS.Stretch activated model, tension in the lipid bilayer triggers conformational changes which open the channel. Figure adapted from lumpkin et al., .

Lipid bilayer Tension or stretch model: In this model tension in the lipid bilayer triggers conformational changes, thus leading to the opening of the channels. The tension perceived by the protein comes from the lipids. It has been demonstrated that the tension/stretch profile in the lipid bilayer is originated by membrane curvature and bilayer-protein hydrophobic mismatch.



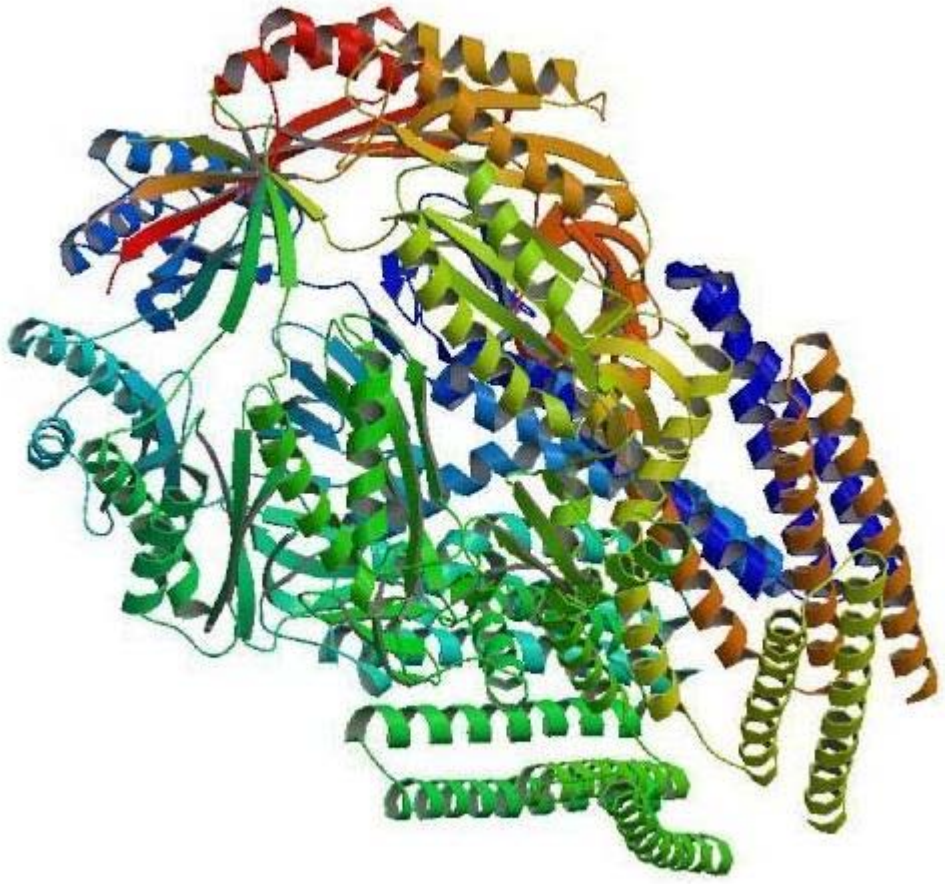
Gating Mechanism of MSC:Spring-like tether model - The tethers are attached to the channel proteins and are connected to the cytoskeleton. The tethers act like spring mechanisms of a shutter. Figure adapted from lumpkin et al., .

Spring-like Tether model: In this model a spring-like tether is attached directly to the MS channel and can be present in either the cytoskeleton or the extracellular matrix linking these elements together. When external stimuli deflect the tether the displacement opens the channel. This particular mechanism has been demonstrated to be the responsible for gating hair cells which are responsible for hearing in vertebrates.

Bacteria MS

MS channels were first discovered by patch-clamp experiments in E. coli. They have been classified based on their conductance as mini (MscM), small (MscS) and large (MscL). These channels function in tandem-mode and are responsible of turgor regulation in bacteria; when activated by changes in the osmotic pressure. MscM is activated first at really low pressures followed by MscS, and finally MsCL being the last chance of survival during osmotic shock. Their task was demonstrated when bacteria missing both MscS and MscL was lysed after exposure to osmotic downshocks.

MscS: Small conductance mechanosensitive channel.



The close structure of MscS

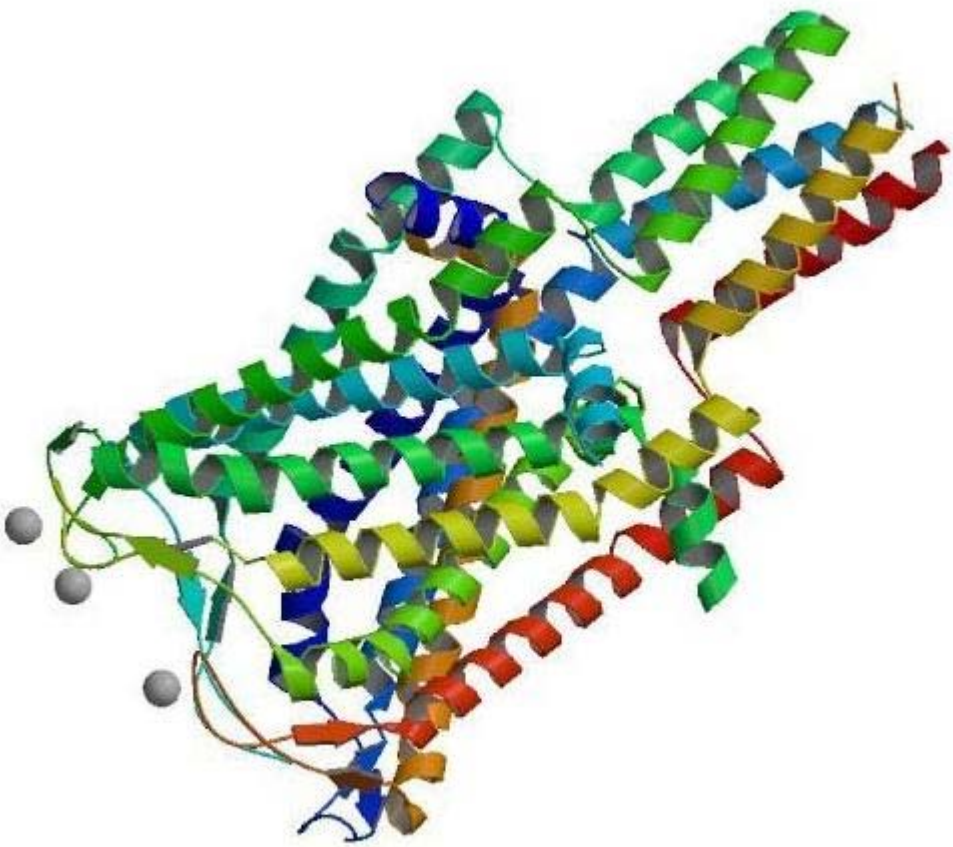
The main conductance is 1nS in buffer solution. Channel-proteins have been found in gram positive and gram negative bacteria, archaea and plants. MscS channel was found after studies in E. coli spheroplasts[10]. The identification of the gene family necessitated for MS of small conductance was as two different channels. YggB encoding MscS and

KefA encoding MscK in E. coli further confirm its role osmotic regulation. Mutagenesis studies showed that when both genes YggB and KefA were deleted MscS lost its function, but maintain MscL and MscM, but mutants deficient of YggB and MscL showed that the function of those channel is to open in respond to pressure range right before cell rupture.

The 3D structure of this channel at closed state was elucidated after the crystallography study by Bass et al. which showed that at resolution of 3.9 Å this 31kDa protein is an homoheptamer forming a channel with 80 Å of diameter and 120 Å in length, each subunit contains three transmembrane domains (TM1, TM2, and TM3) with the N-terminal facing the periplasm and the C-terminal embedded in the cytoplasm. The TM3 is highly conserved in MscS family and it is thought to play an important role in MS prokaryotic gating. MscS is an small protein composed of 286 amino acid residues activated by both tension in the lipid bilayer and voltage; in 2002 Vasquez et al. detailed this process and showed that during the change from close state to open state the TM1 tilt and rotate making TM2 being exposed to the membrane and the TM3 helices expand, tilt, and rotate. During the rearrangement the confined part of the pore was measured as 11 Å, and water molecules were more accessible to the TM3. The two transmembrane domains are in continuous contact with the lipid bilayer and are thought to be the sensor for the tension in the lipid bilayer as well as sensor for voltage because of the three arginine residues present in those domains.

Although MscS is activated by voltage it has been demonstrated that, voltage itself is insufficient to open the channel, thus functioning in a cooperative manner with the channel. The more positive voltage, the higher the probabilities of opening the channel as long as pressure over the threshold is still applied in the system; the performance of this channel at higher voltage has not been completely understood. MscS has a small affinity for negative ions including Cl-, and glutamate.

MscL: Large conductance mechanosensitive channel.



The close structure of MscL

In bacteria MscL was the first MS channels cloned and sequenced, and is by far one of the most studied channels. The gene encoding MscL protein is trkA and it is located in the inner membrane of the E. coli. The protein is 17 KDa, and consists of 136 amino acids; mostly hydrophobic residues resulting in two hydrophobic segments, however molecular weight of the functional channel is presumed to be 60-70 KDa from gel filtration experiments, suggesting oligomerization. As a common feature no cysteines residues are present in this channel.

In 1998 the homolog MscL from mycobacterium tuberculosis Tb-MscL was elucidated at closed state by X ray crystallography at 3.5 Å resolution. The protein is a homopentamer composed mostly of helical regions trans orientation of the helices with respect to the bilayer, with two domains: the cytoplasmic and the transmembrane. The channel is 85 Å in length, 35 Å and 50 Å for the cytoplasmic transmembrane domain respectively and 50 Å in diameter. The helices cross the membrane twice with both the C-terminal and the N-terminal, thus having two transmembrane domains TM1 and TM2 being TM1 the most conserved region among MscL proteins especially at the N-terminal region. It is located

in the cytoplasm and forms a α-hydrophobic helix called S1; the region between the transmembrane domains form a loop that is divided into two regions: S2 a glycine-proline rich region and S3 a short helical section. Also interestingly the secondary structure of the protein is resistant to thermal denaturation still in the presence of SDS.

During the activation of the prokaryotic MscL by tension in the lipid bilayer an intermediate state was determined. The S1 segments form a bundle when the structure is in the closed state, and the crosslinking of S1 segments prevents the opening of the channel. When tension is applied to the membrane the transmembrane barrel-like structure expand and stretch apart the region S1-TM1 allowing the channel to open. The size of the pore at open state is approximately 25Å. The transition from close to intermediate state is accompanied by small movements of the TM1; further transitions to the open stated are characterized by big rearrangements in both the TM1 and TM2.

Role of lipid bilayer in MS

The lipid bilayer is an important structure in all living cells; it has many functions such as separation of compartments, and signaling among others. In the case of the prokaryotic protein channels MscS and MscL both are gated by tension in the lipid bilayer, thus suggesting an important role in such a complex structures.

The tension in the membrane bilayer has been extensively studied, simple intrinsic properties of the lipids can account for the contributions in the free energy of the open, intermediate, and close state of the MS channels. The bilayer possess different features that allows it to transducer tension and to prevent exhaustive deformations, the first one is "in plane fluidity of the lipid bilayer" meaning that any in plane tension in the lipid bilayer is felt homogenously in the absence of cytoskeleton interactions. The lipid molecules have a specific space in between them, preventing the bilayer from any changes.

The contribution of membrane deformation in the gating of MS channels can be divided in two types: the deformation of the plane of the bilayer, and the deformation of the thickness of the bilayer. Also during any process involving changes in the structure, the free energy of the process itself is also an important factor. During gating the major processes that account for this event are: hydrophobic mismatch, and membrane curvature. It has been calculated that the free energy of the tension in the lipid bilayer is similar to the energy needed for gating the channels.

A different study showed that the length of the hydrophobic tail affects its functioning as well as supporting the different states, Phosphatidylcholine (PC) 18 stabilizes better the open state of the MscL channel, PC 14 stabilizes the intermediate state, and a mixture of PC 18 and lysophosphatidylcholine (LPC) stabilizes the closed state[23], suggesting that the bilayer thickness (for carbon tail lengths of 16, 18 and 20) affects channel function. In conclusion the energy from the environment of the membrane plays an important role in the total energy of channel gating.

Physiological role of MS

Table 1. List of some MS channels that have been cloned and characterized.

CHANNEL	SOURCE	GATING MECHANISM	PHYSIOLOGICAL ROLE	REFERENCE
MscL	Bacteria	Lipid Bilayer	Turgor regulation and cell growth	Sukharev et al., 1999; Levina et al., 1999
MscS	Bacteria	Lipid Bilayer	Turgor regulation and cell growth	Martinac et al., 1990; Sukharev et al.,1993
MscMJ	Archaea	Lipid Bilayer	Turgor regulation	Kloda and Martinac, 2001a
MEC4	C. elegans	Tether	Touch	Tavernarakis et al 1997; Hamill et al, 2001.
TRPY	Fungi	Bilayer	Turgor regulation	Zhou et al., 2003, 2005
TRECK-1	Mammalian	Bilayer	Resting membrane potential	Patel et al., 1998, 2001
TRP-1	Hair cell	Tether	Hearing	Corey et al., 2004; Lin and Corey, 2005

Table 1. List of some MS channel that have been cloned and characterized. Data adapted from Martinac, 2001.

MS channels are ubiquitously expressed in the membrane of prokaryotes suggesting their significance. In Bacteria and Archaea the function of these channels is conserved and it has been demonstrated that they play a role in turgor regulation. In Eukarya MS channels are involved in all five senses. The main family is TRP, and one good example is hair cells involved in the hearing process. When a wave of sound deflects the stereocilia, the channel opens. This is an instance of the Spring-like Tether gating mechanism. Recent studies have revealed a new role of mechanosensitive pathways in which naive mesenchymal stem cells are committed to a particular lineage based on the elasticity of its surrounding matrix.

MS have also been suggested as a potential target for antibiotics, the reasoning behind this idea is that both McsS and MscL are highly conserved among prokaryotes, but their homologs have not been found in animals making them an exceptional potential for further studies.

Techniques used to study MS

This is a short list of the most frequently techniques used to study the properties, function, mechanism and other features of these channels.

• Patch-clamp: Single cell recording.

• EPR

• Molecular dynamics simulation: determination of the atomic fluctuation of the system.

• Atomic force Microscopy: mechanical forces of the membrane.

• Micropipette Aspiration: Pressure to cells.

• 3D simulations

• Mutagenesis

MS Diseases

• Polycystic kidney disease.

• Atrial fibrillation

Abnormalities in the function of MS channels can cause[5]:

I. Neuronal disease

II. Muscular degeneration.

III. cardiac arrhythmias

IV. Hypertension.

Chapter 10

Membrane Curvature

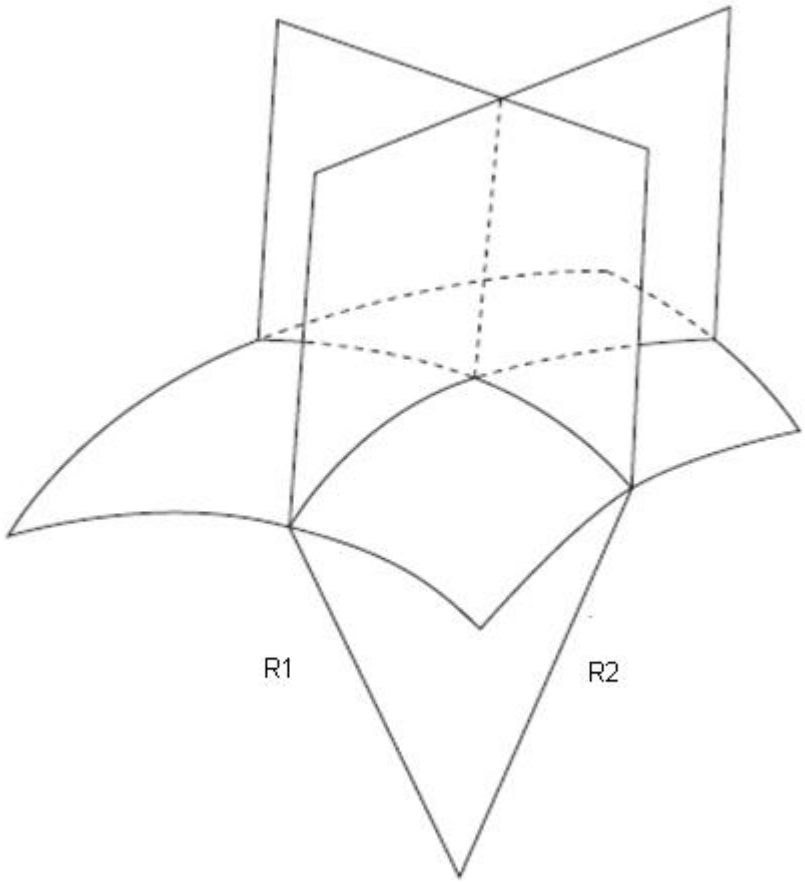
Membrane curvature is the geometrical measure or characterization of the curvature of membranes. The membranes can be naturally occurring or man-made (synthetic). An example of naturally occurring membrane is the lipid bilayer of cells, also known as cellular membranes. Synthetic membranes can be obtained by preparing aqueous solutions of certain lipids. The lipids will then "aggregate" and form various phases and structures. According to the conditions (concentration, temperature, ionic strength of solution, etc.) and the chemical structures of the lipid, different phases will be observed. For instance, the lipid POPC (palmitoyl oleyl phosphatidyl choline) tends to form lamellar vesicles in solution, whereas smaller lipids (lipids with shorter acyl chains, up to 8 carbons in length), such as detergents, will form micelles if the CMC (critical micelle concentration) is reached.

Basic Geometry of Curvature

A biological membrane is commonly described as a two-dimensional surface, which spans a three-dimensional space. So, to describe membrane shape, it is not sufficient to determine the membrane curling that is seen in a single cross-section of the object, because in general there are two curvatures that characterize the shape each point in space. Mathematically, these two curvatures are called the principal curvatures, c1 and c2, and their meaning can be understood by the following thought experiment. If you cross-section the membrane surface at a point under consideration using two planes that are perpendicular to the surface and oriented in two special directions called the principal directions, the principal curvatures are the curvatures of the two lines of intercepts between the planes and the surface which have almost circular shapes in close proximity to the point under consideration. The radii of these two circular fragments, R1 and R2, are called the principal radii of curvature, and their inverse values are referred to as the two principal curvatures.

$c1 = 1 / R1$

$c2 = 1 / R2$



The principal curvatures C1 and C2 can vary arbitrarily and thereby give origin to different geometrical shapes, such as cylinder, plane, sphere and saddle. Analysis of the principal curvature is important, since a number of biological membranes possess shapes that are analogous to these common geometry staples. For instance, prokaryotic cells such as cocci, rods, and spirochette display the shape of a sphere, and the latter two the shape of a cylinder. Erythrocytes, commonly referred to as red blood cells, have the shape of a saddle, although these cells are capable of some shape deformation. The table below lists common geometric shapes and a qualitative analysis of their two principal curvatures.

Shape	C1	C2
Plane	0	0
Cylinder	+	0
Sphere	+	+
Saddle	+	-

Even though often membrane curvature is thought to be a completely spontaneous process, thermodynamically speaking there must be factors actuating as the driving force for curvature to exist. Currently, there are some postulated mechanisms for accepted

theories on curvature; nonetheless, undoubtedly two of the major driving forces are lipid composition and proteins embedded and/or bound to membranes.

Driving forces for membrane Curvature

Lipid Spontaneous Curvature

Perhaps the most simple and intuitive driving force in membrane curvature is the natural spontaneous curvature exhibited by some lipids. This is because, depending on their chemical structures, lipids tend to curve with a slight spontaneously negative or positive curvature. Lipids such as DOPC (dioleyl phosphatidyl choline), diacyl glycerol, dioleyl phosphatidylethanolamine (DOPE) and cholesterol exhibit a negative spontaneous curvature. On the other hand, lipids with smaller acyl chain area to polar head group area ratio tend to curve positively, in other words they exhibit positive spontaneous curvature. The table below lists experimentally determined spontaneous curvatures for different lipids in DOPE (dioleyl phosphatidyl ethanolamine).

Lipid	J_s (nm^{-1})
Lysophospholipids	
L-lyso PC	1/5.8
O-lyso PC	1/3.8
P-lyso PC	1/6.8
L-lyso PE	<1/40
O-lyso PE	<1/40
S-lyso PE	<1/40
Other Lipids	
DOPS	1/14.4
DOPC	-1/20
PA	-1/4.6
DOPE	-1/3
Cholesterol	-1/2.9
DCG	-1/1.3

The energy requirements to generate a cylinder shaped cell from an originally flat membrane can be expressed as

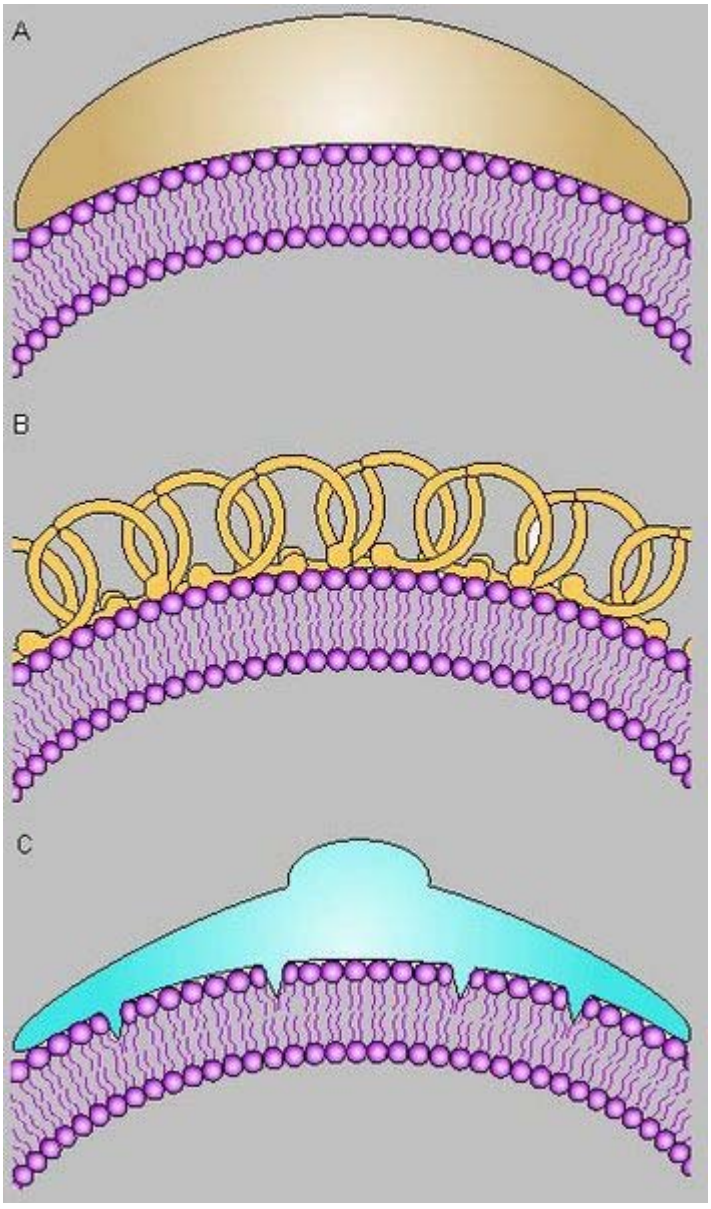
$FCyl = \pi x L x K_b(1/R - 2J_B)$ where L is the length of the cylinder, J_B is the difference between the spontaneous curvature, J_s, for the lipids in the inner and outer leaflet divided by two, and K_b is the bending modulus of the bilayer.

The radii of membrane cylinders that form in intracellular membrane-transport pathways are typically ~25–30 nm. So, the spontaneous curvature necessary to generate such cylinders equals ~(1/50) nm–1. As J_B results from a difference in the spontaneous curvatures of the monolayers, an unusual membrane lipid composition would be required to produce such curvature. The lipids cholesterol, dioleoylphosphatidylethanolamine(DOPE) and diacylglycerol are characterized by strongly negative spontaneous curvatures (figure 1) and therefore have the potential to generate a large membrane curvature. However, even for these lipids, the required J_B can be reached only if they are extensively concentrated in the internal monolayer.

Proteins can Induce Curvature

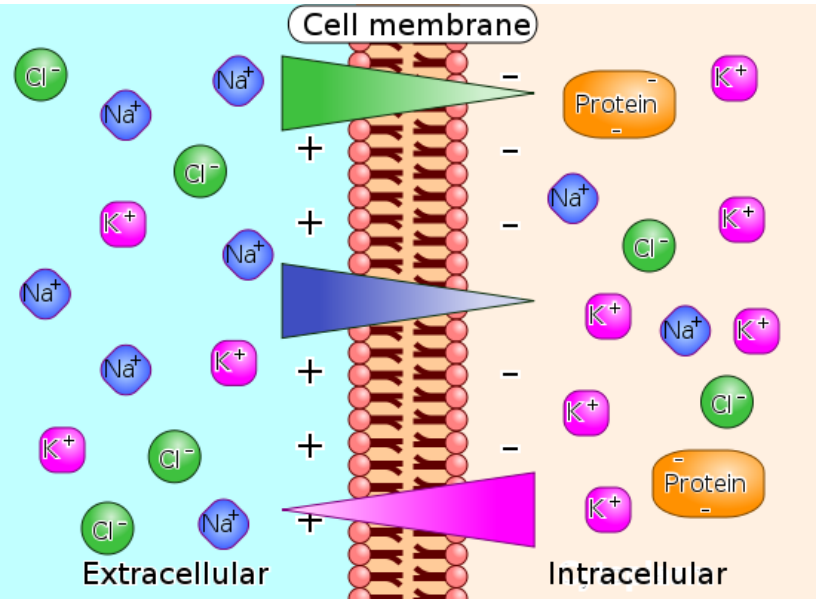
As mentioned previously, some biologically occurring lipids do exhibit spontaneous curvature which could explain the shapes of biological membranes. Nevertheless, calculations show that spontaneous lipid curvature alone is either insufficient or would require conditions that are unrealistic to drive the degree of curvature observed in most cell. From calculations similar to the one showed above, it was evident that lipid curvature alone would not be sufficient and further research was necessary to shed more light on the phenomenon. It is now known that lipid curvature is "aided" by protein structures in order to generate complete cellular curvature. A classical example of such interactions is the activity of the protein clathrin. Clathrin is involved in cellular exocytosis and is sequestrated by specific signaling molecules. Clathrin can attach to clathrin receptors on the cellular membrane and it polymerizes to drive greater curvature resulting in exocytosis of a vesicular unit. Another example of protein interactions that directly affect membrane curvature is that of the BAR (Bin, amphiphysin, Rvs') domain. The BAR domain is present in a large family of proteins. This domain is rigid, relative to the cellular lipid bilayer and it exhibits a "banana" shape. Upon binding, the membrane's curvature is increased by the rigid domain.. One more case of protein interaction that induces and/or aids curvature is the class of proteins such as epsin. Epsin has several alpha helices that possess amphipathic properties, which allows it to partition between the hydrophobic core of the membrane and the hydrophilic aqueous environment. Another interesting characteristic of epsin and other proteins that bind to membranes is the fact that it shows high binding affinity for a fairly common membrane lipid, phosphatidylinositol-4,5-bisphosphate (PI-4,5-PPi). Unlike other proteins that simply bend the membrane through sheer rigidity, epsin is a globular soluble protein and thus not rigid. The insertion of its helices into the membrane force the neighboring lipids of the leaflet that has been bound to expand laterally. This displacement of lipids on only one of the leaflets increases the bilayer's curvature. The figure below illustrates the different mechanisms through which proteins can aid and/or induce membrane curvature. In **A**, an illustration of a BAR domain present in a number of proteins. The curvature is induced by the very shape of this proteic region. This domain attaches to the lipid bilayer through strong coulombic interactions. This idea is supported by the existence of positively charged amino acid residues in the concave region of the BAR domain. These amino acids would come into contact with the negatively charged polar head groups of lipids in the bilayer. This form phenomenon is also referred to as the "scaffold mechanism" **B** shows a protein coating that induces curvature. As mentioned above proteins such as

clathrin are recruited to the membrane through signaling molecules and assemble into larger polymeric structures that form a rigid structure which serves as a frame for the membrane. Clathrin binds to its receptors that are present in the membrane. **C** illustrates a slightly different mechanism, in this case the membrane-bending protein does not exhibit intrinsic rigidity, instead they are often globular and soluble. The protein epsin is an example. Epsin has an ENTH (epsin N-terminal homology) domain which inserts its amphipathic alpha helix into the membrane. Epsin has high binding affinity for the mebrane if PI-4,5-PPi is present.



Chapter 11

Membrane Potential



Differences in concentration of ions on opposite sides of a cellular membrane produce a voltage difference called the membrane potential. The largest contributions usually come from sodium (Na^+) and chloride (Cl^-) ions which have high concentrations in the extracellular region, and potassium (K^+) ions, which along with large protein anions have high concentrations in the intracellular region. Calcium ions, which sometimes play an important role, are not shown.

Membrane potential (or **transmembrane potential**) is the difference in voltage (or electrical potential difference) between the interior and exterior of a cell ($V_{interior} - V_{exterior}$). All animal cells are surrounded by a plasma membrane composed of a lipid bilayer with a variety of molecular structures embedded in it. The membrane potential arises from the interaction of ion channels and ion pumps embedded in the membrane,

which maintain different ion concentrations on the intracellular and extracellular sides of the membrane.

The membrane potential has two basic functions. First, it allows a cell to function as a battery, providing power to operate a variety of "molecular devices" embedded in the membrane. Second, in electrically excitable cells such as neurons, it is used for transmitting signals between different parts of a cell. Opening or closing of ion channels at one point in the membrane produces a local change in the membrane potential, which causes electric current to flow rapidly to other points in the membrane.

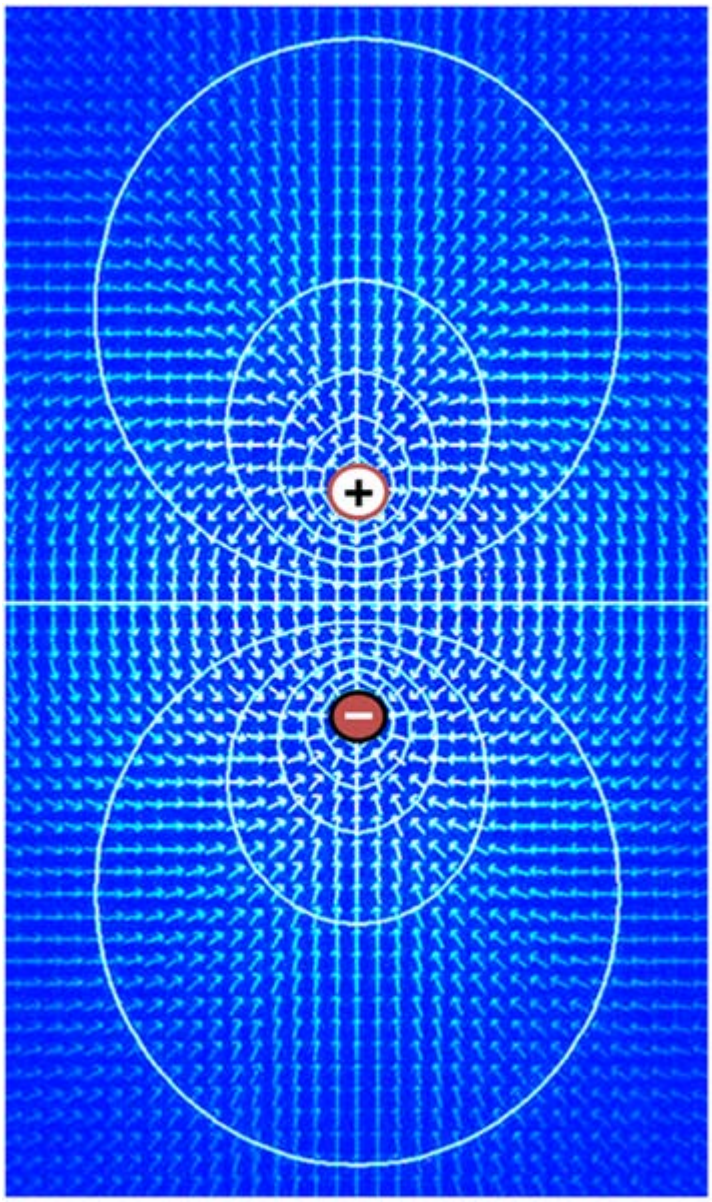
In non-excitable cells, and in excitable cells in their baseline states, the membrane potential is held at a relatively stable value, called the resting potential. For neurons, typical values of the resting potential range from –70 to –80 millivolts; that is, the interior of a cell has a negative baseline voltage of a bit less than one tenth of a volt. Opening and closing of ion channels can induce a departure from the resting potential, called a depolarization if the interior voltage rises (say from –70 mV to –65 mV), or a hyperpolarization if the interior voltage becomes more negative (changing from –70 mV to –80 mV, for example). In excitable cells, a sufficiently large depolarization can evoke a short-lasting all-or-nothing event called an action potential, in which the membrane potential very rapidly undergoes a large change, often briefly reversing its sign. Action potentials are generated by special types of voltage-dependent ion channels.

In neurons, the factors that influence the membrane potential are diverse. They include numerous types of ion channels, some that are chemically gated and some that are voltage-gated. Because voltage-dependent ion channels are controlled by the membrane potential, while the membrane potential itself is partly controlled by these same ion channels, feedback loops arise which allow for complex temporal dynamics, including oscillations and regenerative events such as action potentials.

Physical basis

The membrane potential in a cell derives ultimately from two factors: electrical force and diffusion. Electrical force arises from the mutual attraction between particles with opposite electrical charges (positive and negative) and the mutual repulsion between particles with the same type of charge (both positive or both negative). Diffusion arises from the statistical tendency of particles to redistribute from regions where they are highly concentrated to regions where the concentration is low.

Voltage



Electric field (arrows) and contours of constant voltage created by a pair of oppositely-charged objects. The electric field is at right angles to the voltage contours, and the field is strongest where the spacing between contours is the smallest.

Voltage, which is synonymous with *electrical potential*, is the ability to drive an electric current. If a voltage source such as a battery is placed in an electrical circuit, the higher the voltage of the source, the greater the amount of current that it will drive. In a functioning circuit, each point can be assigned a voltage level—the voltage difference between any two points determines the amount of current that would flow through a wire hooked directly from one point to the other. In practical electronics, the voltage difference between two points can be measured by connecting them to the two leads of a volt meter (voltmeter).

The functional significance of voltage lies only in voltage *differences*—the absolute value of voltage has no significance. A volt meter can measure the voltage difference between two locations in a circuit, but there is no instrument that can measure the voltage at a single point: the concept has no meaning. It is conventional in electronics to assign a voltage of zero to some arbitrarily chosen element of the circuit, and then assign voltages for other elements on the basis of the measured or calculated voltage differences, but there is no significance in which element is chosen as the zero point—the function of a circuit depends only on the differences, not on voltages *per se*.

The same principle applies to voltage in cell biology. In electrically active tissue, the voltage difference between any two points can be measured by inserting an electrode at each point and connecting both electrodes to the leads of a volt meter. There is no way, however, to measure the voltage of a single point. Thus, a statement that the voltage difference across the membrane of a cell is 60 millivolts can be verified by placing electrodes inside and outside the cell—but whether the exterior is assigned a voltage of 60 mV and the interior 0 mV, or the exterior is assigned a voltage of 0 mV and the interior –60 mV, has no significance; only the difference between the two matters, not the absolute number assigned to either.

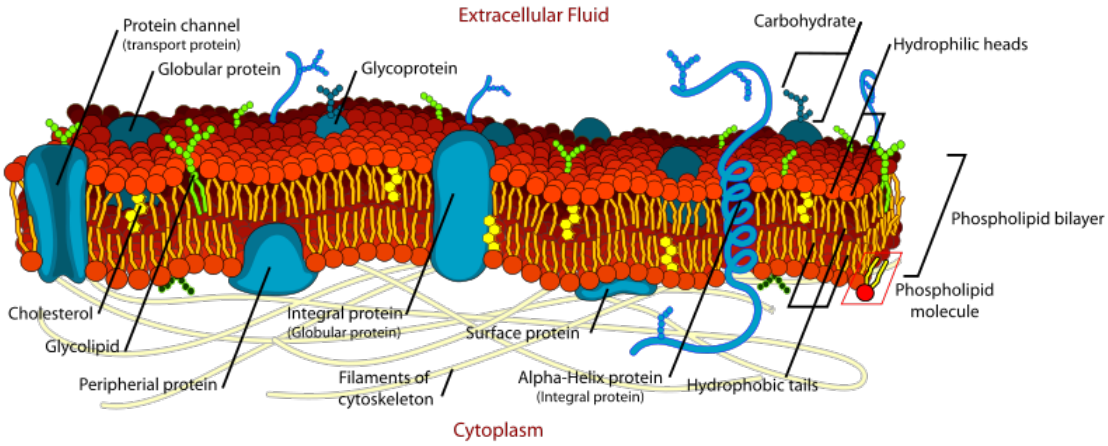
In mathematical terms, the definition of voltage begins with the concept of an electric field **E**, a vector field assigning a magnitude and direction to each point in space. In many situations, the electric field is a conservative field, which means that it can be expressed as the gradient of a scalar function V, that is, $\mathbf{E} = -\nabla V$. This scalar field V is referred to as the voltage distribution. Note that the definition allows for an arbitrary constant of integration—this is why absolute values of voltage are not meaningful. In general electric fields can only be treated as conservative if magnetic fields do not significantly influence them, but this condition usually applies well to biological tissue.

Because the electric field is the gradient of the voltage distribution, rapid changes in voltage within a small region imply a strong electric field; conversely, if the voltage remains approximately the same over a large region, the electric fields in that region must be weak. A strong electric field, equivalent to a strong voltage gradient, implies that a strong force is exerted on any charged particles that lie within the region.

Salts and ions in an aqueous medium

The fluid both inside and outside of animal cells (intracellular and extracellular) contains a high concentration of dissolved salts. When salts dissolve in water, they break apart into ions—for example sodium chloride (NaCl) breaks up almost entirely into positively charged sodium ions (Na^+) and negatively charged chloride (Cl^-) ions. Small ions such as sodium (Na^+), potassium (K^+), calcium (Ca^{++}), and chloride (Cl^-) are present in high concentrations, and are capable of diffusing freely from place to place, unless some type of barrier impedes them.

Plasma membrane



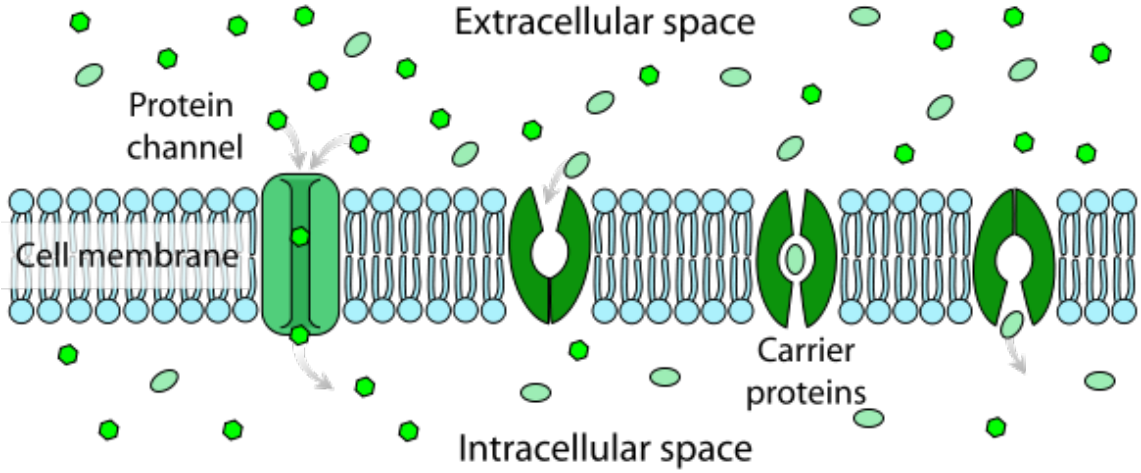
The cell membrane, also called the plasma membrane or plasmalemma, is a semipermeable lipid bilayer common to all living cells. It contains a variety of biological molecules, primarily proteins and lipids, which are involved in a vast array of cellular processes.

Every animal cell is enclosed in a plasma membrane, which has the structure of a lipid bilayer with many types of large molecules embedded in it. Because it is made of lipid molecules, the plasma membrane intrinsically has a high electrical resistivity, in other words a low intrinsic permeability to ions. However, some of the molecules embedded in the membrane are capable either of actively transporting ions from one side of the membrane to the other, or of providing channels through which they can move.

In electrical terminology, the plasma membrane functions as a combined resistor and capacitor. Resistance arises from the fact that the membrane impedes the movement of charges across it. Capacitance arises from the fact that the lipid bilayer is so thin that an accumulation of charged particles on one side gives rise to an electrical force that pulls oppositely-charged particles toward the other side. The capacitance of the membrane is relatively unaffected by the molecules that are embedded in it, so it has a more or less invariant value estimated at about 2 $\mu F/cm^2$ (the total capacitance of a patch of membrane is proportional to its area). The conductance of a pure lipid bilayer is so low, on the other hand, that in biological situations it is always dominated by the conductance of alternative pathways provided by embedded molecules. Thus the capacitance of the membrane is more or less fixed, but the resistance is highly variable.

The thickness of a plasma membrane is estimated to be about 7-8 nanometers. Because the membrane is so thin, it does not take a very large transmembrane voltage to create a strong electric field within it. Typical membrane potentials in animal cells are on the order of 100 millivolts (that is, one tenth of a volt), but calculations show that this generates an electric field close to the maximum that the membrane can sustain—it has been calculated that a voltage difference much larger than 200 millivolts could cause dielectric breakdown, that is, arcing across the membrane.

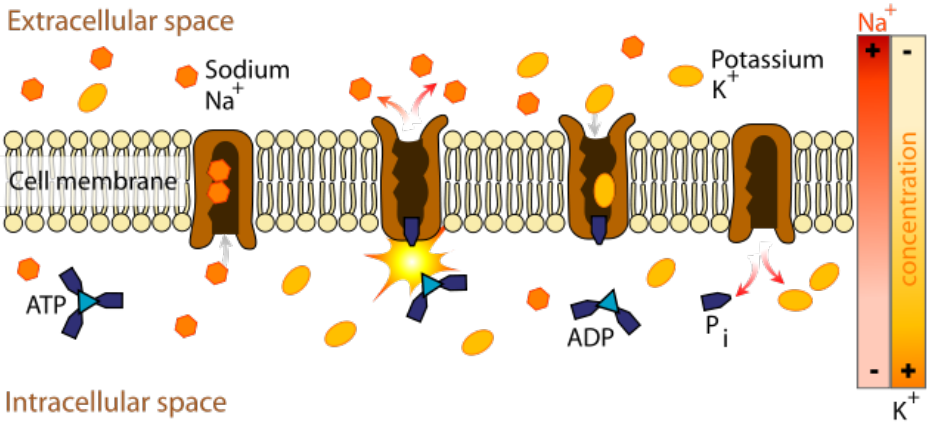
Facilitated diffusion and transport



Facilitated diffusion in cell membranes, showing ion channels and carrier proteins

The resistance of a pure lipid bilayer to the passage of ions across it is very high, but structures embedded in the membrane can greatly enhance ion movement, either actively or passively, via mechanisms called facilitated transport and facilitated diffusion. The two types of structure that play the largest roles are ion channels and ion pumps, both usually formed from assemblages of protein molecules. Ion channels provide passageways through which ions can move. In most cases an ion channel is only permeable to specific types of ions (for example sodium and potassium but not chloride or calcium), and sometimes the permeability varies depending on the direction of ion movement. Ion pumps, also known as ion transporters or carrier proteins, actively transport specific types of ions from one side of the membrane to the other, sometimes using energy derived from metabolic processes to do so.

Ion pumps



The sodium-potassium pump uses energy derived from ATP to exchange sodium for potassium ions across the membrane.

A major contribution to establishing the membrane potential is made by the sodium-potassium exchange pump. This is a complex of proteins embedded in the membrane that derives energy from ATP in order to transport sodium and potassium ions across the membrane. On each cycle, the pump exchanges three Na^+ ions from the intracellular space for two K^+ ions from the extracellular space. If the numbers of each type of ion were equal, the pump would be electrically neutral, but because of the three-for-two exchange, it gives a net movement of one positive charge from intracellular to extracellular for each cycle, thereby contributing to a positive voltage difference. The pump has three effects: (1) it makes the sodium concentration high in the extracellular space and low in the intracellular space; (2) it makes the potassium concentration high in the intracellular space and low in the extracellular space; (3) it gives the extracellular space a positive voltage with respect to the intracellular space.

The sodium-potassium exchange pump is relatively slow in operation. If a cell were initialized with equal concentrations of sodium and potassium everywhere, it would take hours for the pump to establish equilibrium. The pump operates constantly, but becomes progressively less efficient as the concentrations of sodium and potassium available for pumping are reduced.

Another functionally important ion pump is the sodium-calcium exchanger. This pump operates in a conceptually similar way to the sodium-potassium pump, except that in each cycle it exchanges three Na^+ from the extracellular space for one Ca^{++} from the intracellular space. Because the net flow of charge is inward, this pump runs "downhill", effectively, and therefore does not require any energy source except the membrane voltage. Its most important effect is to pump calcium outward—it also allows an inward flow of sodium, thereby counteracting the sodium-potassium pump, but because overall sodium and potassium concentrations are much higher than calcium concentrations, this effect is relatively unimportant. The net result of the sodium-calcium exchanger is that in the resting state, intracellular calcium concentrations become very low.

Ion channels

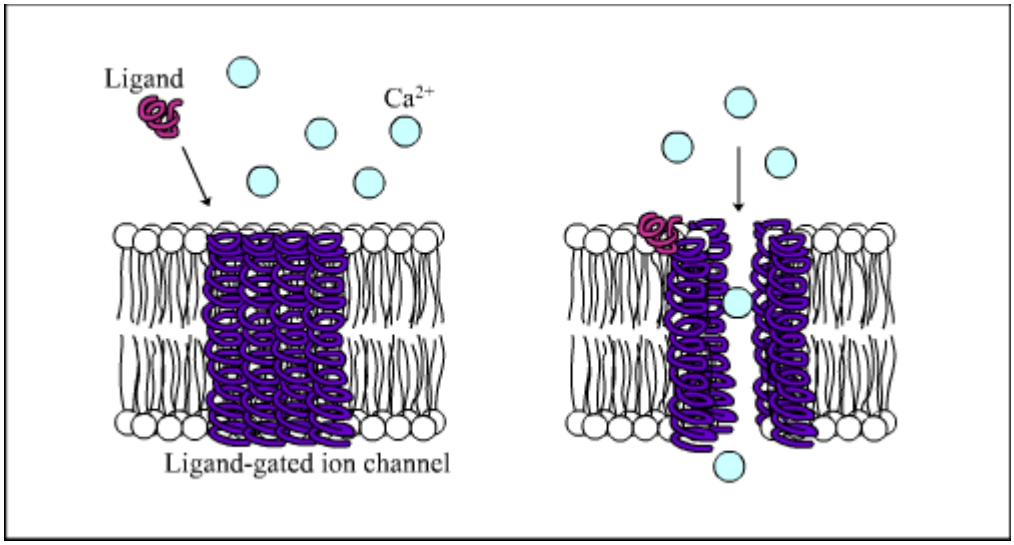
As explained above, a pure lipid bilayer has a very low permeability to ions of any type. However, animal cell membranes contain a very diverse set of ion channels, which are protein structures embedded in the membrane that allow passage of specific types of ions under specific conditions. These can be divided into three types: leakage channels, ligand-gated channels, and voltage-dependent channels. This categorization is not exhaustive—it leaves out sensory receptors, many of which depend on ion channels that are activated by physical stimuli such as light, temperature, or stretching.

Leakage channels

Leakage channels are the simplest type, in that their permeability is more or less constant. The types of leakage channels that have the greatest significance in neurons are potassium and chloride channels. It should be noted that even these are not perfectly constant in their properties: first, most of them are voltage-dependent in the sense that

they conduct better in one direction than the other (in other words, they are rectifiers); second, some of them are capable of being shut off by chemical ligands even though they do not require ligands in order to operate.

Ligand-gated channels



Ligand-gated calcium channel in closed and open states

Ligand-gated ion channels are channels whose permeability is greatly increased when some type of chemical ligand binds to the protein structure. Animal cells contain hundreds, if not thousands, of types of these. A large subset function as neurotransmitter receptors—they occur at postsynaptic sites, and the chemical ligand that gates them is released by the presynaptic axon terminal. One example of this type is the AMPA receptor, a receptor for the neurotransmitter glutamate that when activated allows passage of sodium and potassium ions. Another example is the $GABA_A$ receptor, a receptor for the neurotransmitter GABA that when activated allows passage of chloride ions.

Neurotransmitter receptors are activated by ligands that appear in the extracellular area, but there are other types of ligand-gated channels that are controlled by interactions on the intracellular side.

Voltage-dependent channels

Voltage-gated ion channels, also known as *voltage dependent*, are channels whose permeability is influenced by the membrane potential. They form another very large group, with each member having a particular ion selectivity and a particular voltage dependence. Many are also time-dependent—in other words, they do not respond immediately to a voltage change, but only after a delay.

One of the most important members of this group is a type of voltage-gated sodium channel that underlies action potentials—these are sometimes called *Hodgkin-Huxley*

sodium channels because they were initially characterized by Alan Lloyd Hodgkin and Andrew Huxley in their Nobel Prize-winning studies of the physiology of the action potential. The channel is closed at the resting voltage level, but opens abruptly when the voltage exceeds a certain threshold, allowing a large influx of sodium ions that produces a very rapid change in the membrane potential. Recovery from an action potential is partly dependent on a type of voltage-gated potassium channel which is closed at the resting voltage level but opens as a consequence of the large voltage change produced during the action potential.

Some voltage-dependent ion channels are also at the same time ligand-gated. One of the best known of these is the NMDA receptor, a type of calcium channel that is gated by the neurotransmitter glutamate but also requires the membrane potential to be elevated substantially above baseline in order to open.

Reversal potential

The reversal potential (or *equilibrium potential*) of an ion is the value of transmembrane voltage at which diffusive and electrical forces counterbalance, so that there is no net ion flow across the membrane. This means that the transmembrane voltage exactly opposes the force of diffusion of the ion, such that the net current of the ion across the membrane is zero and unchanging. The reversal potential is important because it gives the voltage that acts on channels permeable to that ion—in other words, it gives the voltage that the ion concentration gradient generates when it acts as a battery.

The equilibrium potential of a particular ion is usually designated by the notation E_{ion}.The equilibrium potential for any ion can be calculated using the Nernst equation. For example, reversal potential for potassium ions will be as follows:

$$E_{eq,K^+} = \frac{RT}{zF} \ln \frac{[K^+]_o}{[K^+]_i},$$

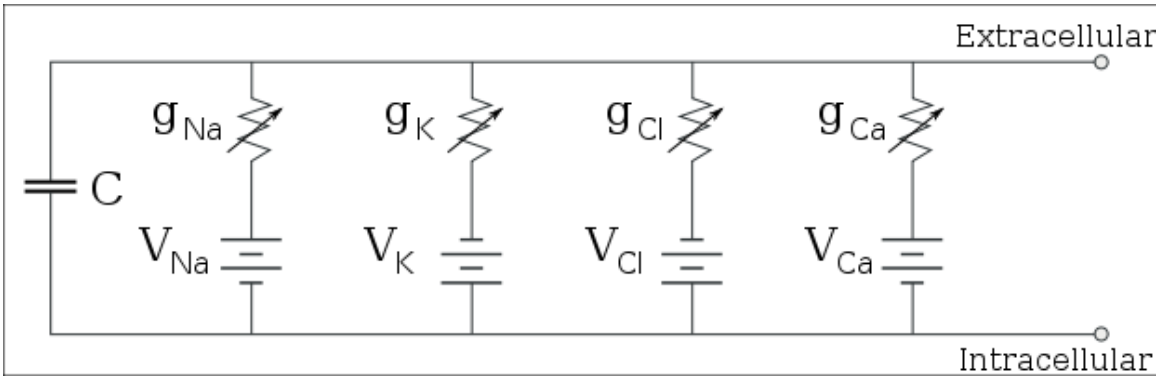
where

- E_{eq,K^+} is the equilibrium potential for potassium, measured in volts
- R is the universal gas constant, equal to 8.314 joules·K^{-1}·mol^{-1}
- T is the absolute temperature, measured in kelvins (= K = degrees Celsius + 273.15)
- z is the number of elementary charges of the ion in question involved in the reaction
- F is the Faraday constant, equal to 96,485 coulombs·mol^{-1} or J·V^{-1}·mol^{-1}
- $[K^+]_o$ is the extracellular concentration of potassium, measured in mol·m^{-3} or mmol·l^{-1}
- $[K^+]_i$ is the intracellular concentration of potassium

Even if two different ions have the same charge (i.e. K^+ and Na^+), they can still have very different equilibrium potentials, provided their outside and/or inside concentrations

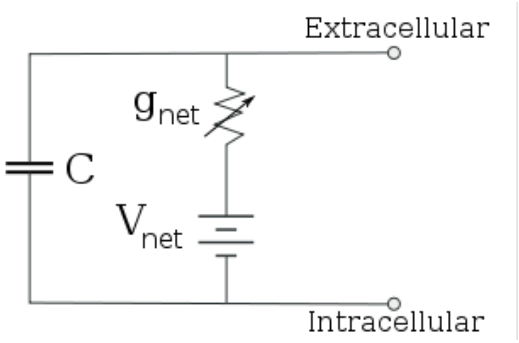
differ. Take, for example, the equilibrium potentials of potassium and sodium in neurons. The potassium equilibrium potential E_K is -84 mV with 5 mM potassium outside and 140 mM inside. The sodium equilibrium potential, on the other hand, E_{Na} is approximately +40 mV with approximately 12 mM sodium inside and 140 mM outside.

Equivalent circuit



Equivalent circuit for a patch of membrane, consisting of a fixed capacitance in parallel with four pathways each containing a battery in series with a variable conductance

Electrophysiologists model the effects of ionic concentration differences, ion channels, and membrane capacitance in terms of an equivalent circuit, which is intended to represent the electrical properties of a small patch of membrane. The equivalent circuit consists of a capacitor in parallel with four pathways each consisting of a battery in series with a variable conductance. The capacitance is determined by the properties of the lipid bilayer, and is taken to be fixed. Each of the four parallel pathways comes from one of the principal ions, sodium, potassium, chloride, and calcium. The voltage of each ionic pathway is determined by the concentrations of the ion on each side of the membrane. The conductance of each ionic pathway at any point in time is determined by the states of all the ion channels that are potentially permeable to that ion, including leakage channels, ligand-gated channels, and voltage-dependent channels.



Reduced circuit obtained by combining the ion-specific pathways using the Goldman equation

For fixed ion concentrations and fixed values of ion channel conductance, the equivalent circuit can be further reduced, using the Goldman equation as described below, to a circuit containing a capacitance in parallel with a battery and conductance. Electrically this is a type of RC circuit (resistance-capacitance circuit), and its electrical properties are very simple. Starting from any initial state, the current flowing across either the conductance or capacitance decays with an exponential time course, with a time constant of $\tau = RC$, where C is the capacitance of the membrane patch, and $R = 1/g_{net}$ is the net resistance. For realistic situations the time constant usually lies in the 1—100 millisecond range. In most cases changes in the conductance of ion channels occur on a faster time scale, so an RC circuit is not a good approximation; however the differential equation used to model a membrane patch is commonly a modified version of the RC circuit equation.

Resting potential

When the membrane potential of a cell can go for a long period of time without changing significantly, it is referred to as a resting potential or resting voltage. This term is used for the membrane potential of non-excitable cells, but also for the membrane potential of excitable cells in the absence of excitation. In excitable cells, the other possible states are graded membrane potentials (of variable amplitude), and action potentials, which are large, all-or-nothing rises in membrane potential that usually follow a fixed time course. Excitable cells include neurons, muscle cells, and some secretory cells in glands. Even in other types of cells, though, the membrane voltage can undergo changes in response to environmental or intracellular stimuli. For example, depolarization of the plasma membrane appears to be an important step in programmed cell death.

The interactions that generate the resting potential are modeled by the Goldman equation. This is similar in form to the Nernst equation shown above, in that it is based on the charges of the ions in question, as well as the difference between their inside and outside concentrations. However, it also takes into consideration the relative permeability of the plasma membrane to each ion in question.

$$E_m = \frac{RT}{F} \ln \left(\frac{P_{\mathrm{K}}[\mathrm{K}^+]_{\mathrm{out}} + P_{\mathrm{Na}}[\mathrm{Na}^+]_{\mathrm{out}} + P_{\mathrm{Cl}}[\mathrm{Cl}^-]_{\mathrm{in}}}{P_{\mathrm{K}}[\mathrm{K}^+]_{\mathrm{in}} + P_{\mathrm{Na}}[\mathrm{Na}^+]_{\mathrm{in}} + P_{\mathrm{Cl}}[\mathrm{Cl}^-]_{\mathrm{out}}} \right)$$

The three ions that appear in this equation are potassium (K^+), sodium (Na^+), and chloride (Cl^-). Calcium is omitted, but can be added to deal with situations in which it plays a significant role. Being an anion, the chloride terms are treated differently than the cation terms; the intracellular concentration is in the numerator, and the extracellular concentration in the denominator, which is reversed from the cation terms. P_i stands for the relative permeability of the ion type i.

The Goldman formula essentially expresses the membrane potential as a weighted average of the reversal potentials for the individual ion types, weighted by permeability. In most animal cells, the permeability to potassium is much higher in the resting state

than the permeability to sodium. Consequently, the resting potential is usually close to the potassium reversal potential. The permeability to chloride can be high enough to be significant, but unlike the other ions, chloride is not actively pumped, and therefore equilibrates at a reversal potential very close to the resting potential determined by the other ions.

Values of resting membrane potential in the most animal cells usually vary between the potassium reversal potential (usually around -80 mV) and around -40 mV. The resting potential in excitable cells (capable of producing action potentials) is usually near -60 mV—more depolarized voltages would lead to spontaneous generation of action potentials. Immature or undifferentiated cells show highly variable values of resting voltage, usually significantly more positive than in differentiated cells. In such cells, the resting potential value correlates with the degree of differentiation: undifferentiated cells in some cases may not show any transmembrane voltage difference at all.

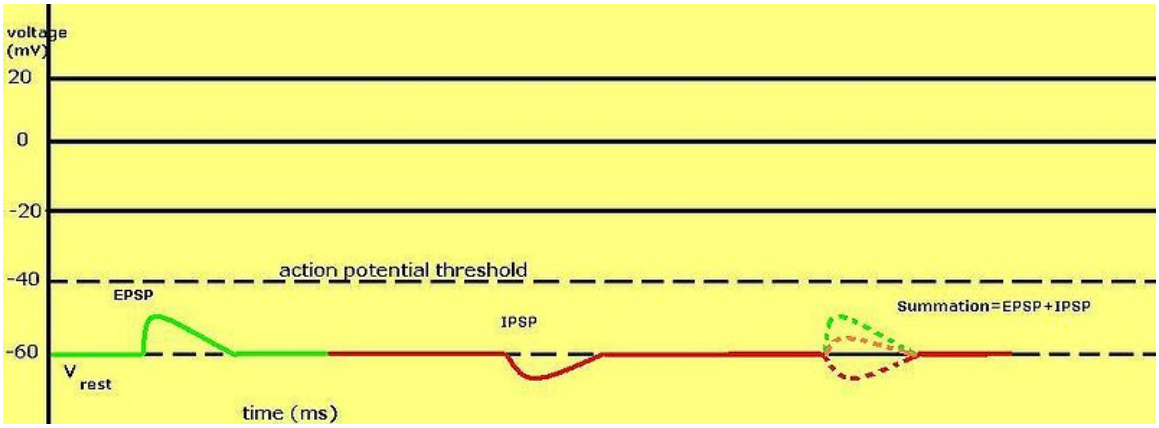
Maintenance of the resting potential can be metabolically costly for a cell because of its requirement for active pumping of ions to counteract losses due to leakage channels. The cost is highest when the cell function requires an especially depolarized value of membrane voltage. For example, the resting potential in daylight-adapted blowfly (*Calliphora vicina*) photoreceptors can be as high as -30 mV. This elevated membrane potential allows the cells to respond very rapidly to visual inputs; the cost is that maintenance of the resting potential may consume more than 20% of overall cellular ATP.

On the other hand, the high resting potential in undifferentiated cells can be a metabolic advantage. This apparent paradox is resolved by examination of the origin of that resting potential. Little-differentiated cells are characterized by extremely high input resistance which implies that few leakage channels are present at this stage of cell life. As an apparent result, potassium permeability becomes similar to that for sodium ions, which places resting potential in-between the reversal potentials for sodium and potassium as discussed above. The reduced leakage currents also mean there is little need for active pumping in order to compensate, therefore low metabolic cost.

Graded potentials

As explained above, the potential at any point in a cell's membrane is determined by the ion concentration differences between the intracellular and extracellular areas, and by the permeability of the membrane to each type of ion. The ion concentrations do not normally change very quickly (with the exception of calcium, where the baseline intracellular concentration is so low that even a small inflow may increase it by orders of magnitude), but the permeabilities can change in a fraction of a millisecond, as a result of activation of ligand-gated or voltage-gated ion channels. The change in membrane potential can be large or small, depending on how many ion channels are activated and what type they are. Changes of this type are referred to as graded potentials, in contrast to action potentials, which have a fixed amplitude and time course.

As can be derived from the Goldman equation shown above, the effect of increasing the permeability for a particular type of ion is to shift the membrane potential toward the reversal potential for that ion. Thus, opening sodium channels pulls the membrane potential toward the sodium reversal potential, usually around +100 mV. Opening potassium channels pulls the membrane potential toward about -90 mV; opening chloride channels pulls it toward about -70 mV. Because -90 to +100 mV is the full operating range of membrane potential, the effect is that sodium channels always pull the membrane potential up, potassium channels pull it down, and chloride channels pull it toward the resting potential.



Graph displaying an EPSP, an IPSP, and the summation of an EPSP and an IPSP.

Graded membrane potentials are particularly important in neurons, where they are produced by synapses—a temporary rise or fall in membrane potential produced by activation of a synapse is called a postsynaptic potential. Neurotransmitters that act to open sodium channels cause the membrane potential to rise, while neurotransmitters that act on potassium channels cause it to fall. Because the membrane potential in a neuron must rise past the threshold value to produce an action potential, a rise in membrane potential is excitatory, while a fall is inhibitory. Thus neurotransmitters that act to open sodium channels produce a so-called excitatory postsynaptic potential, or EPSP, whereas neurotransmitters that act to open potassium channels produce an inhibitory postsynaptic potential, or IPSP. When multiple types of channels are open within the same time period, their postsynaptic potentials summate.

All other values of membrane potential

From the viewpoint of biophysics, the *resting* membrane potential is merely the membrane potential that results from the membrane permeabilities that predominate when the cell is resting. The above equation of weighted averages always applies, but the following approach may be more easily visualized. At any given moment, there are two factors for an ion that determine how much influence that ion will have over the membrane potential of a cell.

1. That ion's driving force and,

2. That ion's permeability

Intuitively, this is easy to understand. If the driving force is high, then the ion is being "pushed" across the membrane hard (more correctly stated: it is diffusing in one direction faster than the other). If the permeability is high, it will be easier for the ion to diffuse across the membrane. But what are 'driving force' and 'permeability'?

- Driving force: the driving force is the net electrical force available to move that ion across the membrane. It is calculated as the difference between the voltage that the ion "wants" to be at (its equilibrium potential) and the actual membrane potential (E_m). So formally, the driving force for an ion = E_m - E_{ion}

- For example, at our earlier calculated resting potential of −73 mV, the driving force on potassium is 7 mV : (−73 mV) − (−80 mV) = 7 mV. The driving force on sodium would be (−73 mV) − (60 mV) = −133 mV.

- Permeability: is simply a measure of how easily an ion can cross the membrane. It is normally measured as the (electrical) conductance and the unit, siemens, corresponds to 1 $C \cdot s^{-1} \cdot V^{-1}$, that is one charge per second per volt of potential.

So in a resting membrane, while the driving force for potassium is low, its permeability is very high. Sodium has a huge driving force, but almost no resting permeability. In this case, potassium carries about 20 times more current than sodium, and thus has 20 times more influence over E_m than does sodium.

However, consider another case—the peak of the action potential. Here permeability to Na is high and K permeability is relatively low. Thus the membrane moves to near E_{Na} and far from E_K.

The more ions are permeant, the more complicated it becomes to predict the membrane potential. However, this can be done using the Goldman-Hodgkin-Katz equation or the weighted means equation. By simply plugging in the concentration gradients and the permeabilities of the ions at any instant in time, one can determine the membrane potential at that moment. What the GHK equations says, basically, is that at any time, the value of the membrane potential will be a weighted average of the equilibrium potentials of all permeant ions. The "weighting" is the ions relative permeability across the membrane.

Effects and implications

While cells expend energy to transport ions and establish a transmembrane potential, they use this potential in turn to transport other ions and metabolites such as sugar. The transmembrane potential of the mitochondria drives the production of ATP, which is the common currency of biological energy.

Cells may draw on the energy they store in the resting potential to drive action potentials or other forms of excitation. These changes in the membrane potential enable communication with other cells (as with action potentials) or initiate changes inside the cell, which happens in an egg when it is fertilized by a sperm.

In neuronal cells, an action potential begins with a rush of sodium ions into the cell through sodium channels, resulting in depolarization, while recovery involves an outward rush of potassium through potassium channels. Both these fluxes occur by passive diffusion.

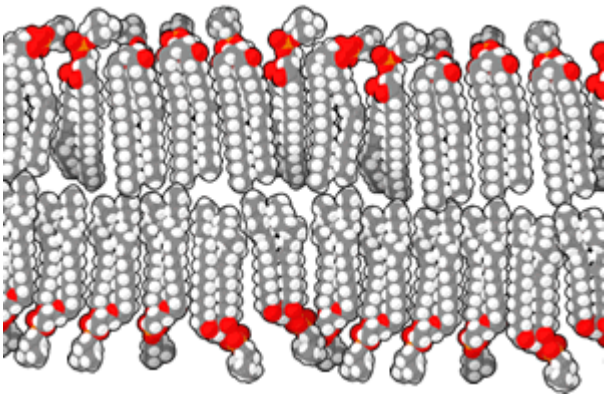
Chapter 12

Lipid Bilayer

The **lipid bilayer** is a thin membrane made of two layers of lipid molecules. These membranes are flat sheets that form a continuous barrier around cells. The cell membrane of almost all living organisms and many viruses are made of a lipid bilayer, as are the membranes surrounding the cell nucleus and other sub-cellular structures.

The lipid bilayer is the barrier that keeps ions, proteins and other molecules where they are needed and prevents them from diffusing into areas where they should not be. Lipid bilayers are ideally suited to this role because, even though they are only a few nanometers thick, they are impermeable to most water-soluble (hydrophilic) molecules.

Bilayers are particularly impermeable to ions, which allows cells to regulate salt concentrations and pH by pumping ions across their membranes using proteins called ion pumps.



This fluid lipid bilayer cross section is made up entirely of phosphatidylcholine.

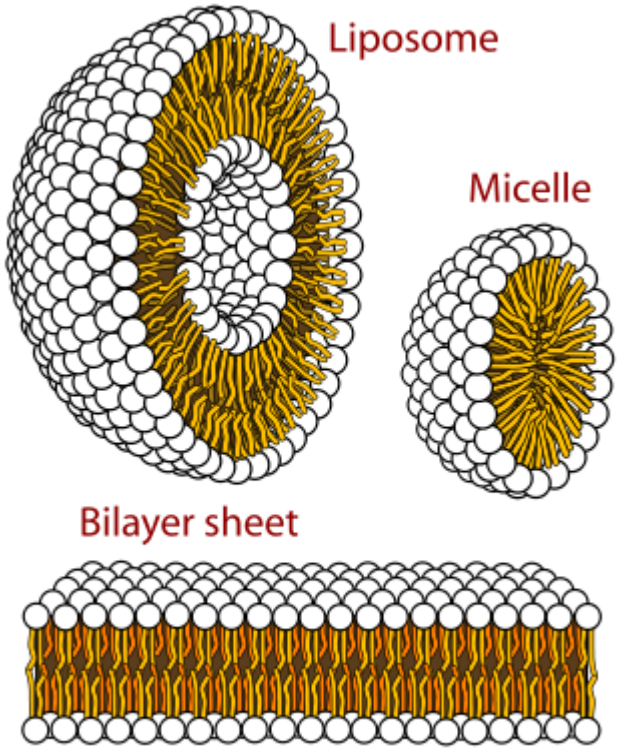
Properties

Natural bilayers are usually made mostly of phospholipids, which have a hydrophilic head and two hydrophobic tails. When phospholipids are exposed to water, they arrange themselves into a two-layered sheet (a bilayer) with all of their tails pointing toward the

center of the sheet. The center of this bilayer contains almost no water and also excludes molecules like sugars or salts that dissolve in water but not in oil. This assembly process is similar to the coalescing of oil droplets in water and is driven by the same force, called the hydrophobic effect. Because lipid bilayers are quite fragile and are so thin that they are invisible in a traditional microscope, bilayers are very challenging to study. Experiments on bilayers often require advanced techniques like electron microscopy and atomic force microscopy.

Phospholipids with certain head groups can alter the surface chemistry of a bilayer and can, for example, mark a cell for destruction by the immune system. Lipid tails can also affect membrane properties, for instance by determining the phase of the bilayer. The bilayer can adopt a solid gel phase state at lower temperatures but undergo phase transition to a fluid state at higher temperatures. The packing of lipids within the bilayer also affects its mechanical properties, including its resistance to stretching and bending. Many of these properties have been studied with the use of artificial "model" bilayers produced in a lab. Vesicles made by model bilayers have also been used clinically to deliver drugs.

Biological membranes typically include several types of lipids other than phospholipids. A particularly important example in animal cells is cholesterol, which helps strengthen the bilayer and decrease its permeability. Cholesterol also helps regulate the activity of certain integral membrane proteins. Integral membrane proteins function when incorporated into a lipid bilayer. Because bilayers define the boundaries of the cell and its compartments, these membrane proteins are involved in many intra- and inter-cellular signaling processes. Certain kinds of membrane proteins are involved in the process of fusing two bilayers together. This fusion allows the joining of two distinct structures as in the fertilization of an egg by sperm or the entry of a virus into a cell.



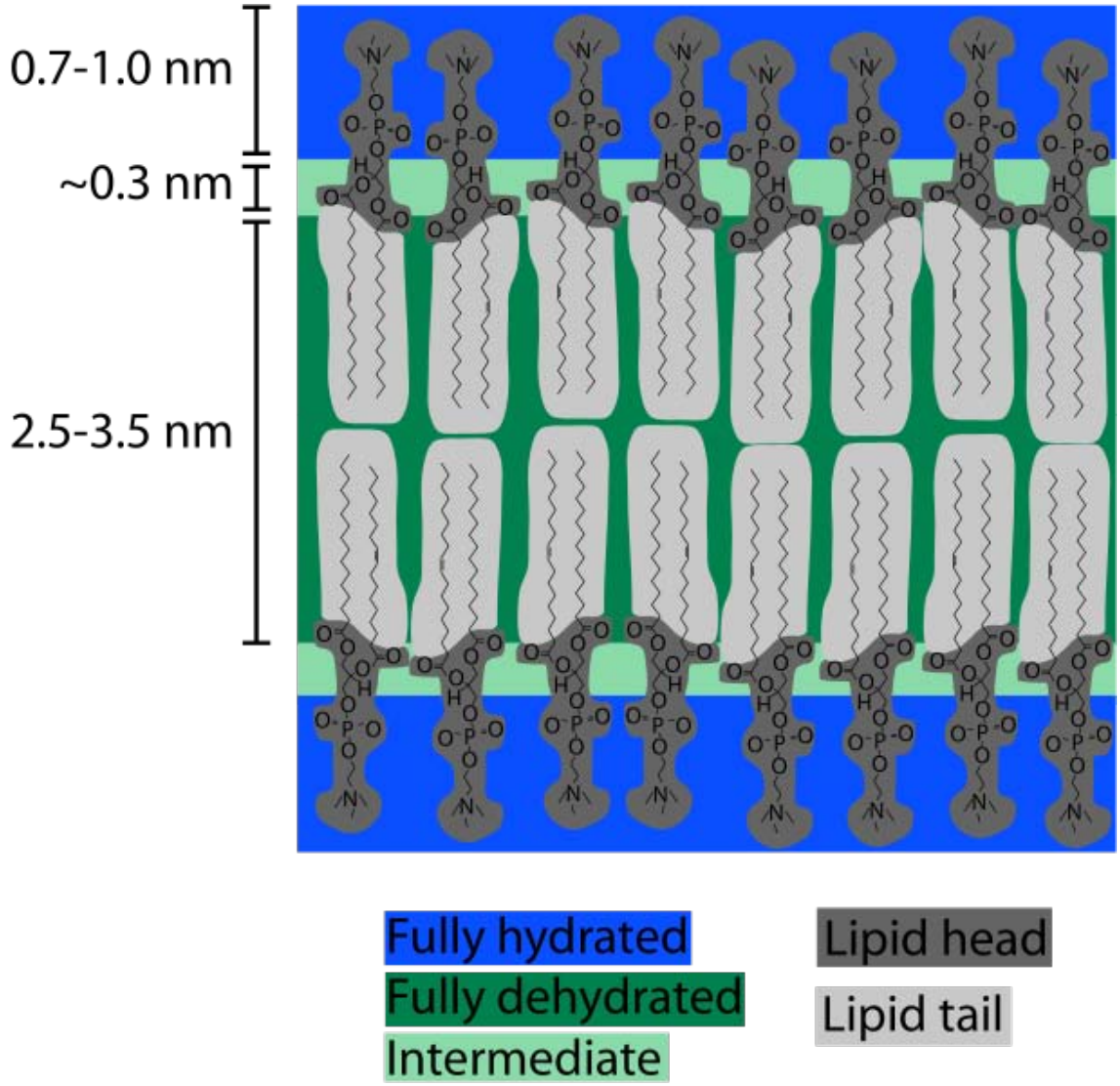
The three main structures phospholipids form in solution; the liposome (a closed bilayer), the micelle and the bilayer.

Structure and organization

A lipid bilayer is a sheet of lipids two molecules thick, arranged so that the hydrophilic phosphate heads point “out” to the water on either side of the bilayer and the hydrophobic tails point “in” to the core of the bilayer. This arrangement results in two “leaflets” which are each a single molecular layer. Lipids self-assemble into this structure because of the hydrophobic effect, which creates an energetically unfavorable interaction between the hydrophobic lipid tails and the surrounding water. Thus, a lipid bilayer is typically held together by entirely non-covalent forces that do not involve formation of chemical bonds between individual molecules.

There are some similarities between this structure and a common soap bubble, although there are also important differences. As illustrated, both structures involve two single-molecule layers of an amphiphilic substance. In the case of a soap bubble, the two soap monolayers coat an intervening water layer. The hydrophilic heads are oriented “in” toward this water core, while the hydrophobic tails point “out” to the air. In the case of a lipid bilayer, this structure is reversed with heads out and tails in. Another important difference between lipid bilayers and soap bubbles is their relative size. Soap bubbles are typically hundreds of nanometers thick, on the same order as the wavelength of light, which is why interference effects cause rainbow colors on a bubble surface. A single lipid

bilayer, on the other hand, is around five nanometers thick, much smaller than the wavelength of light and is therefore invisible to the eye, even with a standard light microscope.



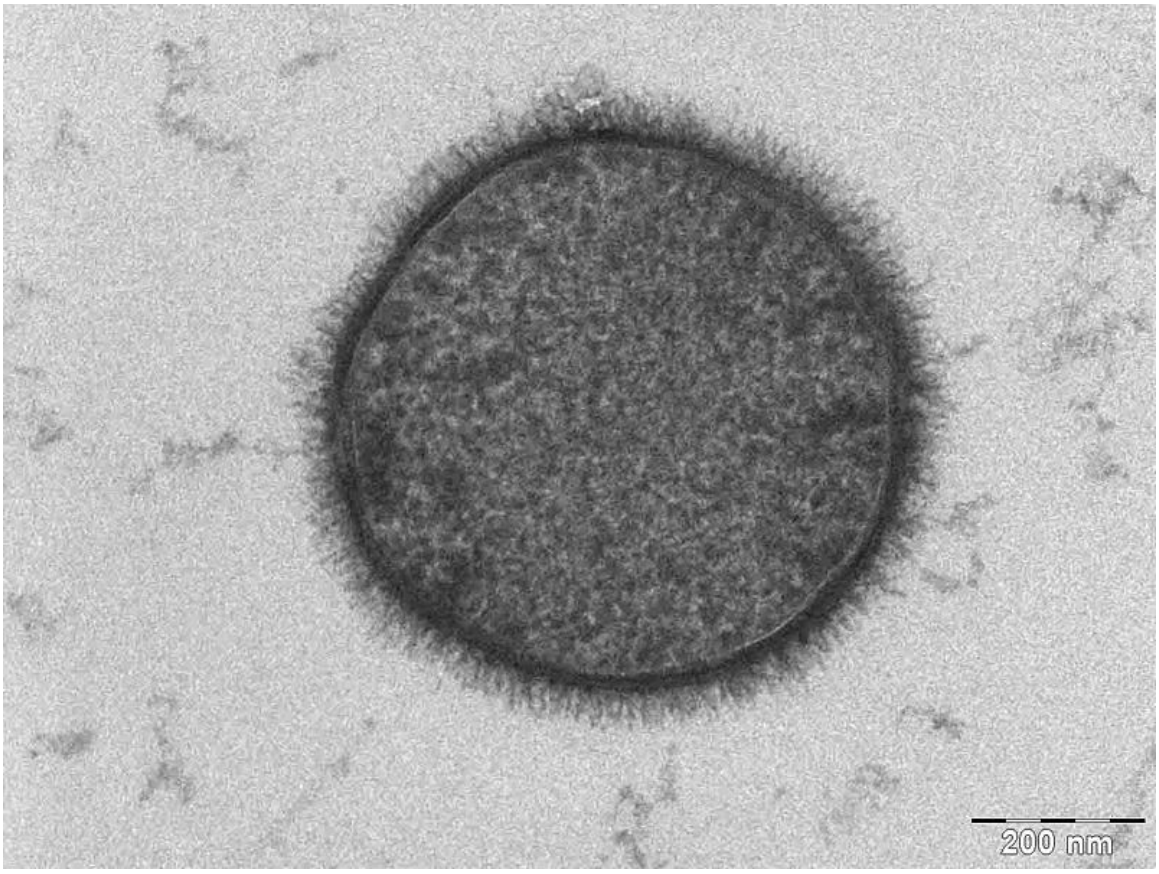
Schematic cross sectional profile of a typical lipid bilayer. There are three distinct regions: the fully hydrated headgroups, the fully dehydrated alkane core and a short intermediate region with partial hydration

Cross section analysis

The lipid bilayer is very thin compared to its lateral dimensions. If a typical mammalian cell (diameter ~10 micrometre) were magnified to the size of a watermelon (~1 ft/30 cm), the lipid bilayer making up the plasma membrane would be about as thick as a piece of office paper. Despite being only a few nanometers thick, the bilayer consists of several distinct chemical regions across its cross-section. These regions and their interactions

with the surrounding water have been characterized over the past several decades with x-ray reflectometry, neutron scattering and nuclear magnetic resonance techniques.

The first region on either side of the bilayer is the hydrophilic headgroup. This portion of the membrane is completely hydrated and is typically around 0.8-0.9 nm thick. In phospholipid bilayers the phosphate group is located within this hydrated region, approximately 0.5 nm outside the hydrophobic core. In some cases, the hydrated region can extend much further, for instance in lipids with a large protein or long sugar chain grafted to the head. One common example of such a modification in nature is the lipopolysaccharide coat on a bacterial outer membrane, which helps retain a water layer around the bacterium to prevent dehydration.



TEM image of a bacterium. The furry appearance on the outside is due to a coat of long chain sugars attached to the cell membrane. This coating helps trap water to prevent the bacterium from becoming dehydrated.

Next to the hydrated region is an intermediate region which is only partially hydrated. This boundary layer is approximately 0.3 nm thick. Within this short distance, the water concentration drops from 2M on the headgroup side to nearly zero on the tail (core) side. The hydrophobic core of the bilayer is typically 3-4 nm thick, but this value varies with chain length and chemistry. Core thickness also varies significantly with temperature, particularly near a phase transition.

Asymmetry

In many naturally occurring bilayers, the compositions of the inner and outer membrane leaflets are different. In human red blood cells, the inner (cytoplasmic) leaflet is largely composed of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol and its phosphorylated derivatives. By contrast, the outer (extracellular) leaflet is based on phosphatidylcholine, sphingomyelin and a variety of glycolipids, In some cases, this asymmetry is based on where the lipids are made in the cell and reflects their initial orientation. The biological functions of lipid asymmetry are imperfectly understood, although it is clear that it is used in several different situations. For example, when a cell undergoes apoptosis, the phosphatidylserine — normally localised to the cytoplasmic leaflet — is transferred to the outer surface: there it is recognised by a macrophage which then actively scavenges the dying cell.

Lipid asymmetry arises, at least in part, from the fact that most phospholipids are synthesised and initially inserted into the inner monolayer: those that constitute the outer monolayer are then transported to the inner monolayer by a class of enzymes called flippases,. Other lipids, such as sphingomyelin, appear to be synthesised at the external leaflet. Flippases are members of a larger family of lipid transport molecules which also includes flopases, which transfer lipids in the opposite direction, and scramblases, which randomize lipid distribution across lipid bilayers (as in apoptotic cells). In any case, once lipid asymmetry is established it does not normally dissipate quickly because spontaneous flip-flop of lipids between leaflets is extremely slow.

It is possible to mimic this asymmetry in the laboratory in model bilayer systems. Certain types of very small artificial vesicle will automatically make themselves slightly asymmetric, although the mechanism by which this asymmetry is generated is very different from that in cells. By utilizing two different monolayers in Langmuir-Blodgett deposition or a combination of Langmuir-Blodgett and vesicle rupture deposition it is also possible to synthesize an asymmetric planar bilayer. This asymmetry may be lost over time as lipids in supported bilayers can be prone to flip-flop.

Phases and phase transitions

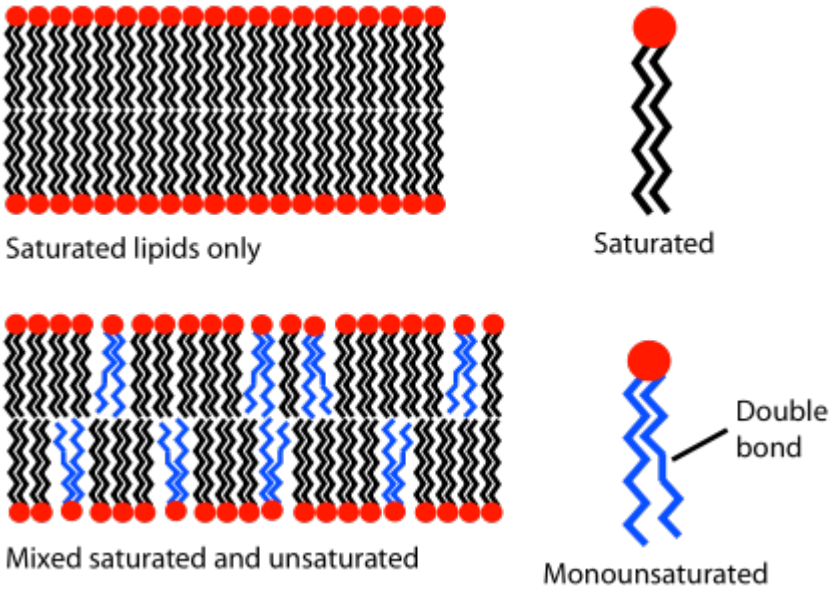


Diagram showing the effect of unsaturated lipids on a bilayer. The lipids with an unsaturated tail (blue) disrupt the packing of those with only saturated tails (black). The resulting bilayer has more free space and is consequently more permeable to water and other small molecules.

At a given temperature a lipid bilayer can exist in either a liquid or a gel (solid) phase. All lipids have a characteristic temperature at which they transition (melt) from the gel to liquid phase. In both phases the lipid molecules are prevented from flip-flopping across the bilayer, but in liquid phase bilayers a given lipid will exchange locations with its neighbor millions of times a second. This random walk exchange allows lipid to diffuse and thus wander across the surface of the membrane. Unlike liquid phase bilayers, the lipids in a gel phase bilayer are locked in place.

The phase behavior of lipid bilayers is largely determined by the strength of the attractive Van der Waals interactions between adjacent lipid molecules. Longer tailed lipids have more area over which to interact, increasing the strength of this interaction and consequently decreasing the lipid mobility. Thus, at a given temperature, a short-tailed lipid will be more fluid than an otherwise identical long-tailed lipid. Transition temperature can also be affected by the degree of unsaturation of the lipid tails. An unsaturated double bond can produce a kink in the alkane chain, disrupting the lipid packing. This disruption creates extra free space within the bilayer which allows additional flexibility in the adjacent chains. An example of this effect can be noted in everyday life as butter, which has a large percentage saturated fats, is solid at room temperature while vegetable oil, which is mostly unsaturated, is liquid.

Most natural membranes are a complex mixture of different lipid molecules. If some of the components are liquid at a given temperature while others are in the gel phase, the two phases can coexist in spatially separated regions, rather like an iceberg floating in the

ocean. This phase separation plays a critical role in biochemical phenomena because membrane components such as proteins can partition into one or the other phase and thus be locally concentrated or activated. One particularly important component of many mixed phase systems is cholesterol, which modulates bilayer permeability, mechanical strength and biochemical interactions.

Surface chemistry

While lipid tails primarily modulate bilayer phase behavior, it is the headgroup that determines the bilayer surface chemistry. Most natural bilayers are composed primarily of phospholipids, although sphingolipids such as sphingomyelin and sterols such as cholesterol are also important components. Of the phospholipids, the most common headgroup is phosphatidylcholine (PC), accounting for about half the phospholipids in most mammalian cells. PC is a zwitterionic headgroup, as it has a negative charge on the phosphate group and a positive charge on the amine but, because these local charges balance, no net charge.

Other headgroups are also present to varying degrees and can include phosphatidylserine (PS) phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). These alternate headgroups often confer specific biological functionality that is highly context-dependent. For instance, PS presence on the extracellular membrane face of erythrocytes is a marker of cell apoptosis, whereas PS in growth plate vesicles is necessary for the nucleation of hydroxyapatite crystals and subsequent bone mineralization. Unlike PC, some of the other headgroups carry a net charge, which can alter the electrostatic interactions of small molecules with the bilayer.

Biological roles

Containment and separation

The primary role of the lipid bilayer in biology is to separate aqueous compartments from their surroundings. Without some form of barrier delineating “self” from “non-self” it is difficult to even define the concept of an organism or of life. This barrier takes the form of a lipid bilayer in all known life forms except for a few species of archaea which utilize a specially adapted lipid monolayer. It has even been proposed that the very first form of life may have been a simple lipid vesicle with virtually its sole biosynthetic capability being the production of more phospholipids. The partitioning ability of the lipid bilayer is based on the fact that hydrophilic molecules cannot easily cross the hydrophobic bilayer core, as discussed in Transport across the bilayer below.

Prokaryotes have only one lipid bilayer- the cell membrane (also known as the plasma membrane). Many prokaryotes also have a cell wall, but the cell wall is composed of proteins or long chain carbohydrates, not lipids. In contrast, eukaryotes have a range of organelles including the nucleus, mitochondria, lysosomes and endoplasmic reticulum. All of these sub-cellular compartments are surrounded by one or more lipid bilayers and, together, typically comprise the majority of the bilayer area present in the cell. In liver

hepatocytes for example, the plasma membrane accounts for only two percent of the total bilayer area of the cell, whereas the endoplasmic reticulum contains more than fifty percent and the mitochondria a further thirty percent.

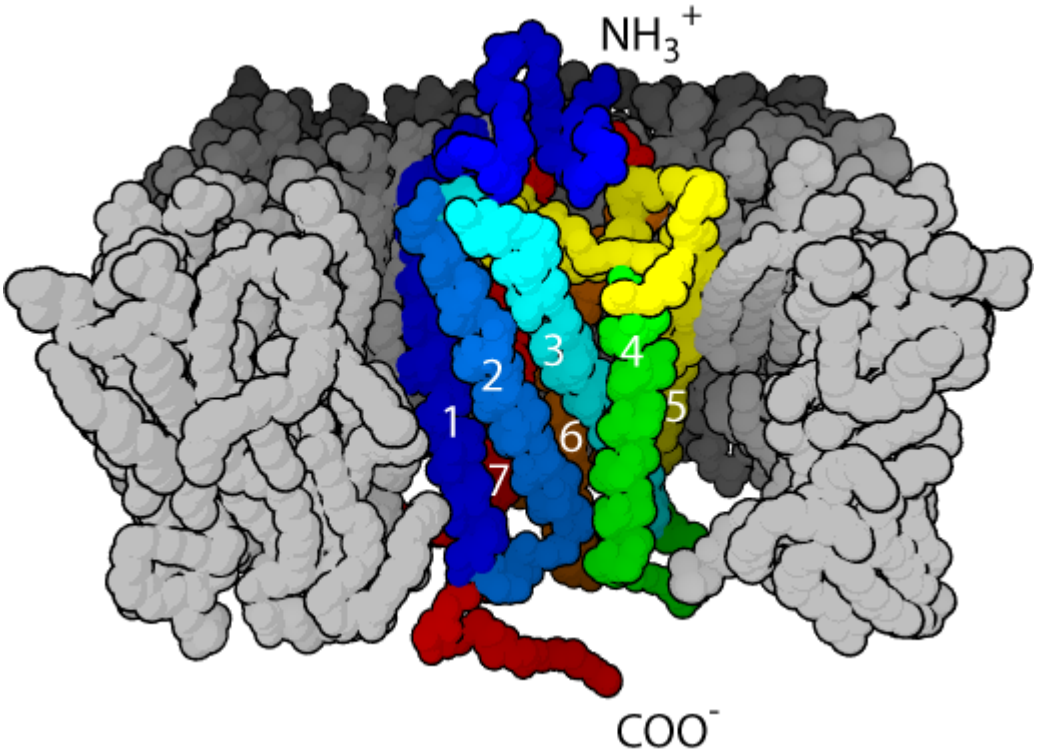


Illustration of a GPCR signaling protein. In response to a molecule such as a hormone binding to the exterior domain (blue) the GPCR changes shape and catalyzes a chemical reaction on the interior domain (red). The gray feature is the surrounding bilayer.

Signaling

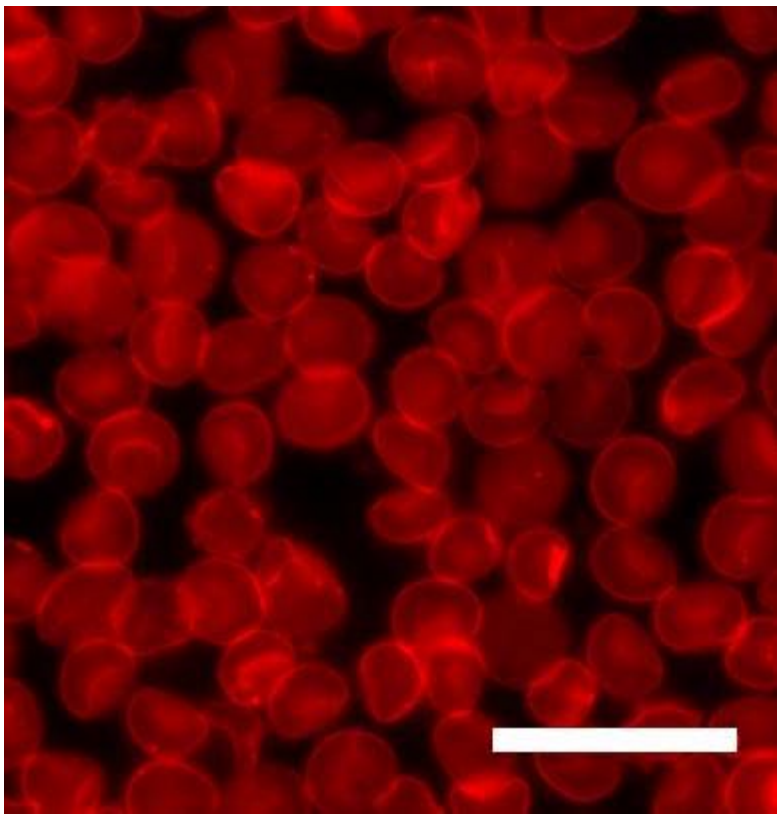
Probably the most familiar form of cellular signaling is synaptic transmission, whereby a nerve impulse that has reached the end of one neuron is conveyed to an adjacent neuron via the release of neurotransmitters. This transmission is made possible by the action of synaptic vesicles loaded with the neurotransmitters to be released. These vesicles fuse with the cell membrane at the pre-synaptic terminal and release its contents to the exterior of the cell. The contents then diffuse across the synapse to the post-synaptic terminal.

Lipid bilayers are also involved in signal transduction through their role as the home of integral membrane proteins. This is an extremely broad and important class of biomolecule. It is estimated that up to a third of the human proteome may be membrane proteins. Some of these proteins are linked to the exterior of the cell membrane. An example of this is the CD59 protein, which identifies cells as “self” and thus inhibits their destruction by the immune system. The HIV virus evades the immune system in part by grafting these proteins from the host membrane onto its own surface. Alternatively, some

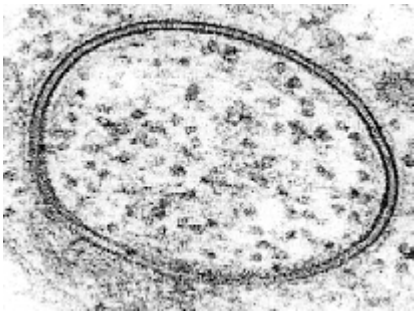
membrane proteins penetrate all the way through the bilayer and serve to relay individual signal events from the outside to the inside of the cell. The most common class of this type of protein is the G protein-coupled receptor (GPCR). GPCRs are responsible for much of the cell's ability to sense its surroundings and, because of this important role, approximately 40% of all modern drugs are targeted at GPCRs.

In addition to protein- and solution-mediated processes, it is also possible for lipid bilayers to participate directly in signaling. A classic example of this is phosphatidylserine-triggered phagocytosis. Normally, phosphatidylserine is asymmetrically distributed in the cell membrane and is present only on the interior side. During programmed cell death a protein called a scramblase equilibrates this distribution, displaying phosphatidylserine on the extracellular bilayer face. The presence of phosphatidylserine then triggers phagocytosis to remove the dead or dying cell.

Characterization methods



Human red blood cells viewed through a fluorescence microscope. The cell membrane has been stained with a fluorescent dye. Scale bar is 20μm.

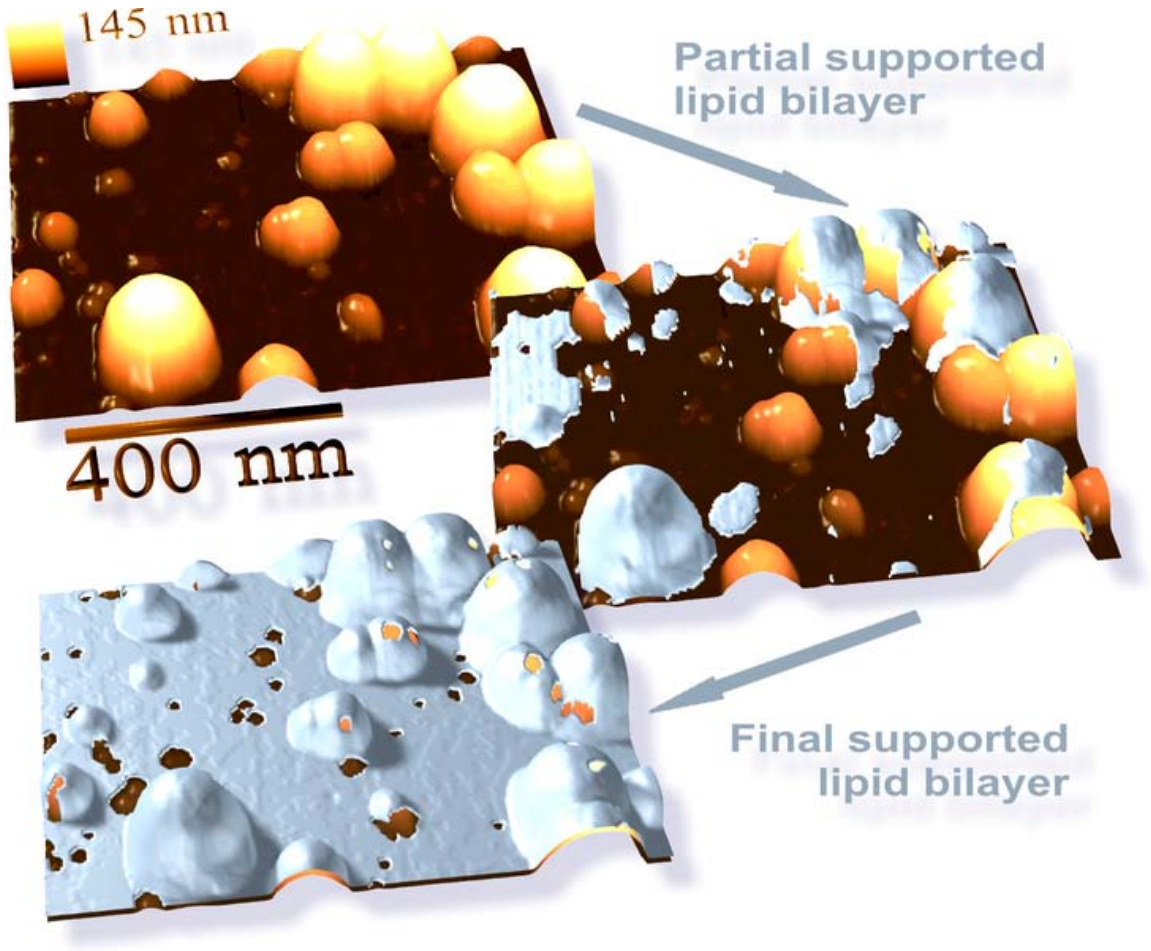


Transmission Electron Microscope (TEM) image of a lipid vesicle. The two dark bands around the edge are the two leaflets of the bilayer. Historically, similar images confirmed that the cell membrane is a bilayer

The lipid bilayer is a very difficult structure to study because it is so thin and fragile. In spite of these limitations dozens of techniques have been developed over the last seventy years to allow investigations of its structure and function.

Electrical measurements are a straightforward way to characterize an important function of a bilayer: its ability to segregate and prevent the flow of ions in solution. By applying a voltage across the bilayer and measuring the resulting current, the resistance of the bilayer is determined. This resistance is typically quite high since the hydrophobic core is impermeable to charged species. The presence of even a few nanometer-scale holes results in a dramatic increase in current. The sensitivity of this system is such that even the activity of single ion channels can be resolved.

Electrical measurements do not provide an actual picture like imaging with a microscope can. Lipid bilayers cannot be seen in a traditional microscope because they are too thin. In order to see bilayers, researchers often use fluorescence microscopy. A sample is excited with one wavelength of light and observed in a different wavelength, so that only fluorescent molecules with a matching excitation and emission profile will be seen. Natural lipid bilayers are not fluorescent, so a dye is used that attaches to the desired molecules in the bilayer. Resolution is usually limited to a few hundred nanometers, much smaller than a typical cell but much larger than the thickness of a lipid bilayer.



3d-Adapted AFM images showing formation of transmembrane pores (holes) in supported lipid bilayer

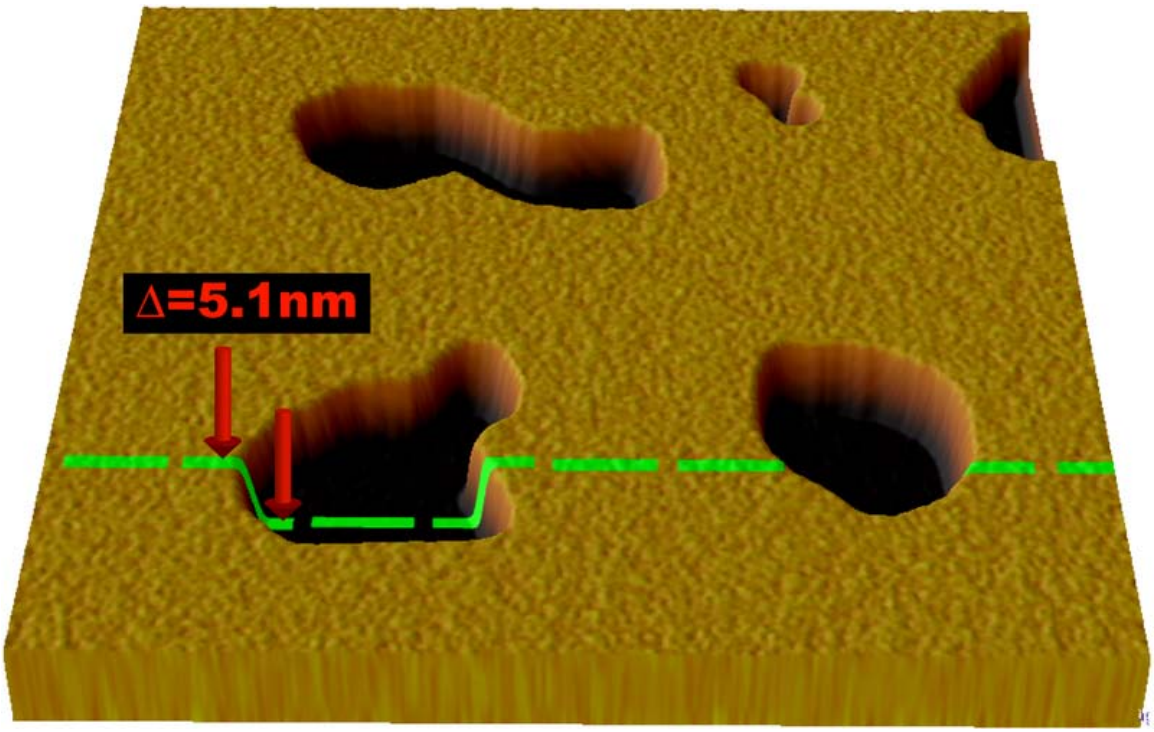


Illustration of a typical AFM scan of a supported lipid bilayer. The pits are defects in the bilayer, exposing the smooth surface of the substrate underneath.

Electron microscopy offers a higher resolution image. In an electron microscope, a beam of focused electrons interacts with the sample rather than a beam of light as in traditional microscopy. In conjunction with rapid freezing techniques, electron microscopy has also been used to study the mechanisms of inter- and intracellular transport, for instance in demonstrating that exocytotic vesicles are the means of chemical release at synapses.

^{31}P-NMR(nuclear magnetic resonance) spectroscopy is widely used for studies of phospholipid bilayers and biological membranes in native conditions. The analysis of ^{31}P-NMR spectra of lipids could provide a wide range of information about lipid bilayer packing, phase transitions (gel phase, physiological liquid crystal phase, ripple phases, non bilayer phases), lipid head group orientation/dynamics, and elastic properties of pure lipid bilayer and as a result of binding of proteins and other biomolecules.

In addition, a specific H-N...(O)-P experiment (transition by scalar coupling 3JH-P~5Hz) could provide a direct information about formation of hydrogen bonds between amid protons of protein to phosphate of lipid headgroups, which is useful in studies of protein/membrane interactions.

A new method to study lipid bilayers is Atomic force microscopy (AFM). Rather than using a beam of light or particles, a very small sharpened tip scans the surface by making physical contact with the bilayer and moving across it, like a record player needle. AFM is a promising technique because it has the potential to image with nanometer resolution at room temperature and even under water or physiological buffer, conditions necessary

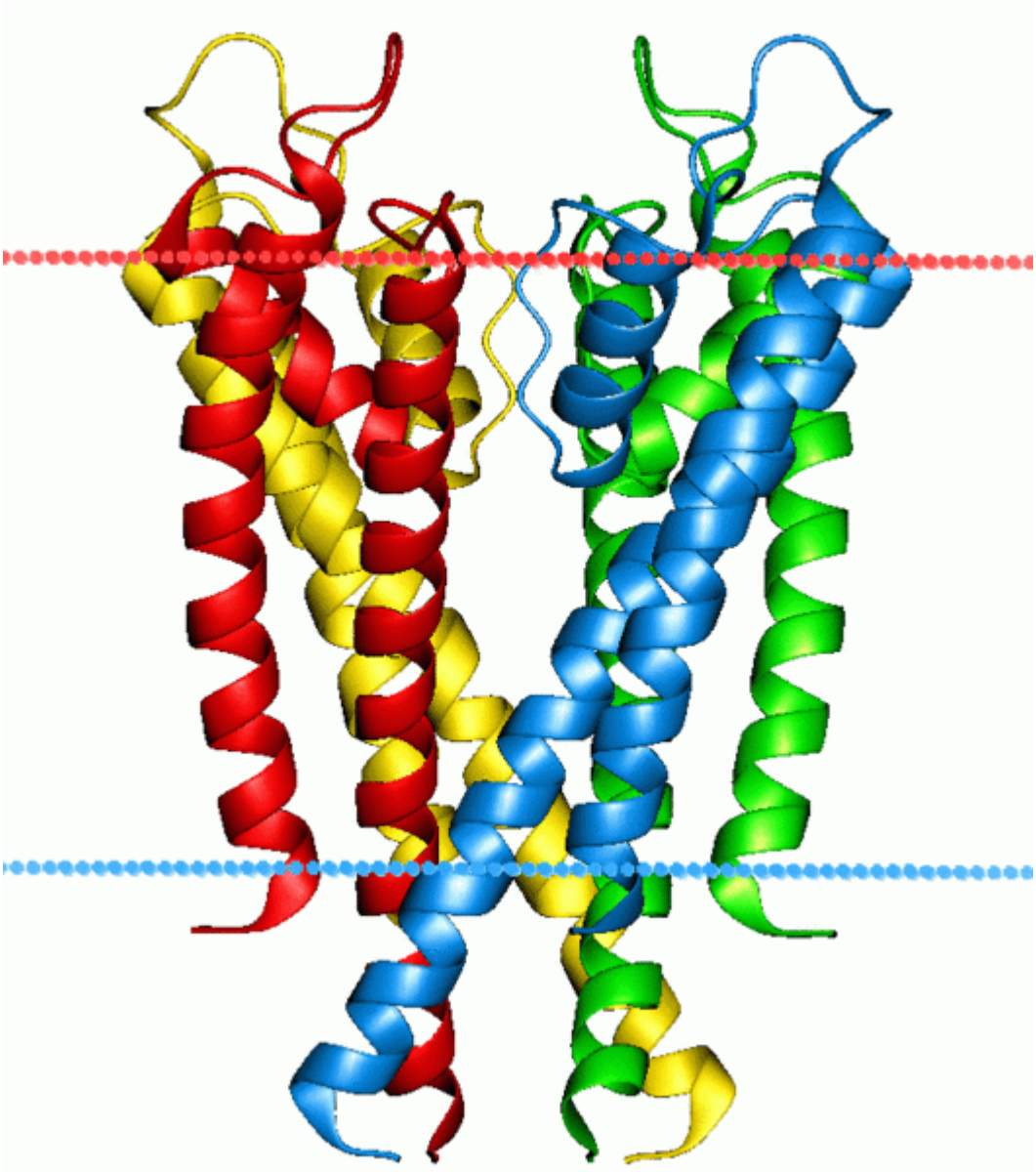
for natural bilayer behavior. Utilizing this capability, AFM has been used to examine dynamic bilayer behavior including the formation of transmembrane pores (holes) and phase transitions in supported bilayers. Another advantage is that AFM does not require fluorescent or isotopic labeling of the lipids, since the probe tip interacts mechanically with the bilayer surface. Because of this, the same scan can image both lipids and associated proteins, sometimes even with single-molecule resolution. AFM can also probe the mechanical nature of lipid bilayers.

Lipid bilayers exhibit high levels of birefringence where the refractive index in the plane of the bilayer differs from that perpendicular by as much as 0.1 refractive index units. This has been used to characterise the degree of order and disruption in bilayers using dual polarisation interferometry to understand mechanisms of protein interaction.

Transport across the bilayer

Passive diffusion

Most polar molecules have low solubility in the hydrocarbon core of a lipid bilayer and consequently have low permeability coefficients across the bilayer. This effect is particularly pronounced for charged species, which have even lower permeability coefficients than neutral polar molecules. Anions typically have a higher rate of diffusion through bilayers than cations. Compared to ions, water molecules actually have a relatively large permeability through the bilayer, as evidenced by osmotic swelling. When a cell or vesicle with a high interior salt concentration is placed in a solution with a low salt concentration it will swell and eventually burst. Such a result would not be observed unless water was able to pass through the bilayer with relative ease. The anomalously large permeability of water through bilayers is still not completely understood and continues to be the subject of active debate. Uncharged apolar molecules diffuse through lipid bilayers many orders of magnitude faster than ions or water. This applies both to fats and organic solvents like chloroform and ether. Regardless of their polar character larger molecules diffuse more slowly across lipid bilayers than small molecules.



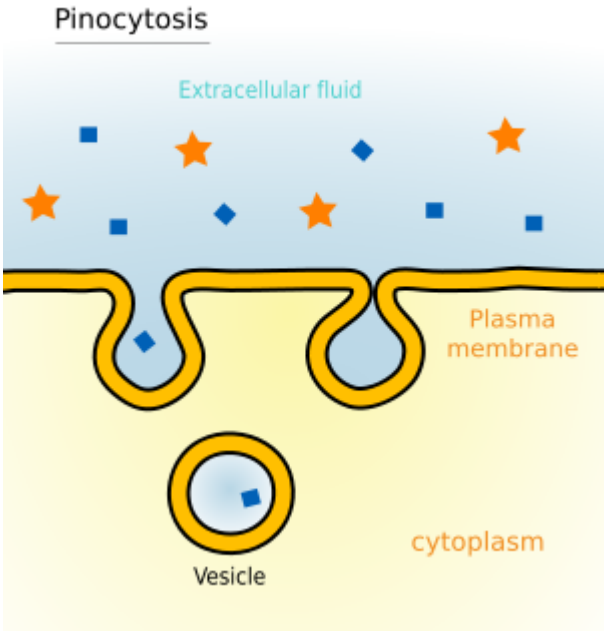
Structure of a potassium ion channel. The alpha helices penetrate the bilayer (boundaries indicated by red and blue lines), opening a hole through which potassium ions can flow

Ion pumps and channels

Two special classes of protein deal with the ionic gradients found across cellular and sub-cellular membranes in nature- ion channels and ion pumps. Both pumps and channels are integral membrane proteins that pass through the bilayer, but their roles are quite different. Ion pumps are the proteins that build and maintain the chemical gradients by utilizing an external energy source to move ions against the concentration gradient to an area of higher chemical potential. The energy source can be ATP, as is the case for the Na^+-K^+ ATPase. Alternatively, the energy source can be another chemical gradient

already in place, as in the Ca^{2+}/Na^{+} antiporter. It is through the action of ion pumps that cells are able to regulate pH via the pumping of protons.

In contrast to ion pumps, ion channels do not build chemical gradients but rather dissipate them in order to perform work or send a signal. Probably the most familiar and best studied example is the voltage-gated Na^{+} channel, which allows conduction of an action potential along neurons. All ion pumps have some sort of trigger or "gating" mechanism. In the previous example it was electrical bias, but other channels can be activated by binding a molecular agonist or through a conformational change in another nearby protein.



Schematic illustration of pinocytosis, a type of endocytosis

Endocytosis and exocytosis

Some molecules or particles are too large or too hydrophilic to effectively pass through a lipid bilayer. Other molecules could pass through the bilayer but must be transported rapidly in such large numbers that channel-type transport is impractical. In both cases these types of cargo can be moved across the cell membrane through fusion or budding of vesicles. When a vesicle is produced inside the cell and fuses with the plasma membrane to release its contents into the extracellular space this process is known as exocytosis. In the reverse process a region of the cell membrane will dimple inwards and eventually pinch off, enclosing a portion of the extracellular fluid to transport it into the cell. Endocytosis and exocytosis rely on very different molecular machinery to function, but the two processes are intimately linked and could not work without each other. The primary mechanism this interdependence is the sheer volume of lipid material involved. In a typical cell, an area of bilayer equivalent to the entire plasma membrane will travel through the endocytosis/exocytosis cycle in about half an hour. If these two processes

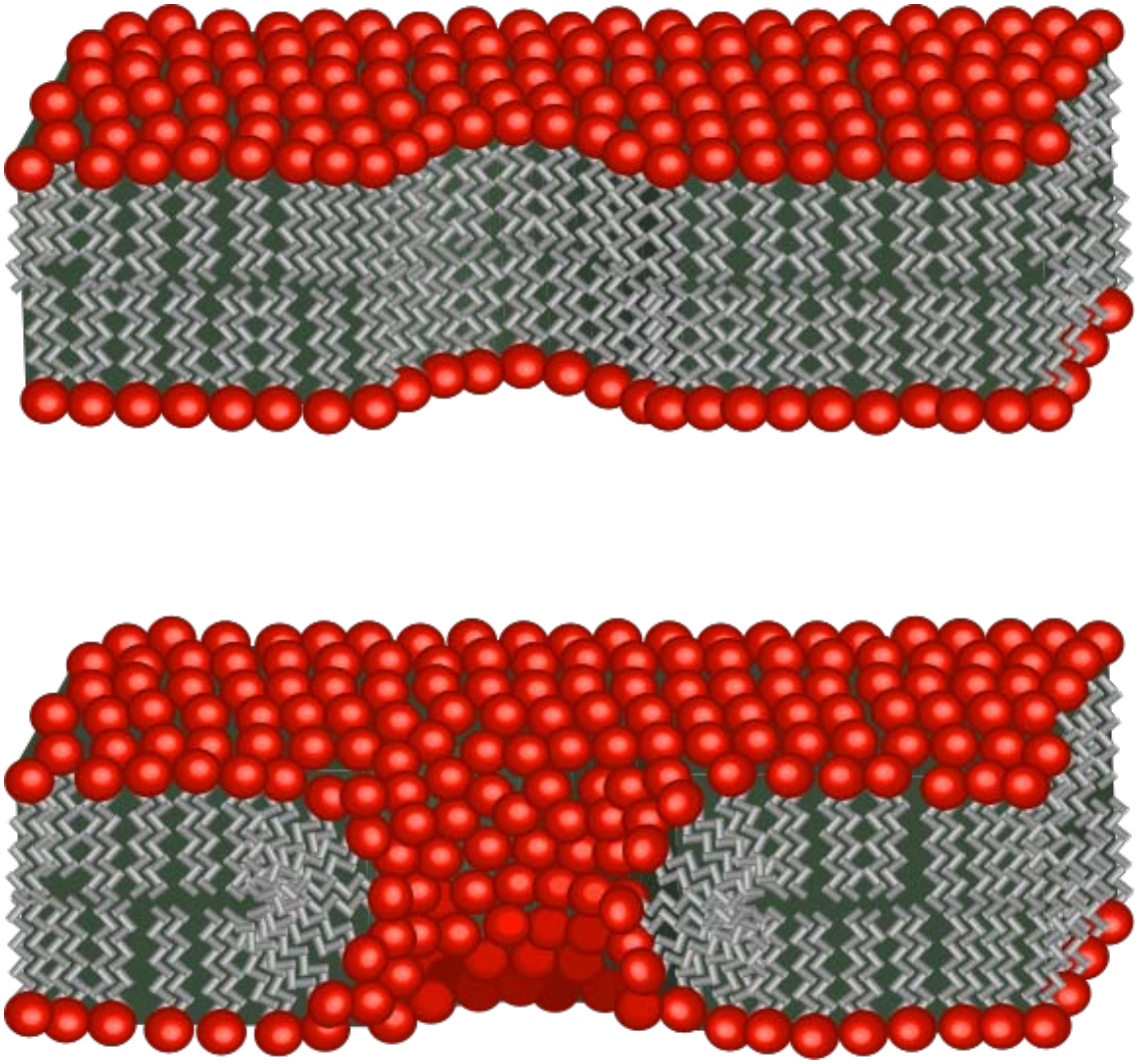
were not balancing each other the cell would either balloon outward to an unmanageable size or completely deplete its plasma membrane within a matter of minutes.

Electroporation

Electroporation is the rapid increase in bilayer permeability induced by the application of a large artificial electric field across the membrane. Experimentally, electroporation is used to introduce hydrophilic molecules into cells. It is a particularly useful technique for large highly charged molecules such as DNA which would never passively diffuse across the hydrophobic bilayer core. Because of this, electroporation is one of the key methods of transfection as well as bacterial transformation. It has even been proposed that electroporation resulting from lightning strikes could be a mechanism of natural horizontal gene transfer.

This increase in permeability primarily affects transport of ions and other hydrated species, indicating that the mechanism is the creation of nm-scale water-filled holes in the membrane. Although electroporation and dielectric breakdown both result from application of an electric field, the mechanisms involved are fundamentally different. In dielectric breakdown the barrier material is ionized, creating a conductive pathway. The material alteration is thus chemical in nature. In contrast, during electroporation the lipid molecules are not chemically altered but simply shift position, opening up a pore which acts as the conductive pathway through the bilayer as it is filled with water.

Mechanics



Schematic showing two possible conformations of the lipids at the edge of a pore. In the top image the lipids have not rearranged, so the pore wall is hydrophobic. In the bottom image some of the lipid heads have bent over, so the pore wall is hydrophilic.

Lipid bilayers are large enough structures to have some of the mechanical properties of liquids or solids. The area compression modulus K_a, bending modulus K_b, and edge energy Λ, can be used to describe them. Solid lipid bilayers also have a shear modulus, but like any liquid, the shear modulus is zero for fluid bilayers. These mechanical properties affect how the membrane functions. K_a and K_b affect the ability of proteins and small molecules to insert into the bilayer, and bilayer mechanical properties have been shown to alter the function of mechanically activated ion channels. Bilayer mechanical properties also govern what types of stress a cell can withstand without tearing. Although lipid bilayers can easily bend, most cannot stretch more than a few percent before rupturing.

As discussed in the Structure and organization section, the hydrophobic repulsion between lipid tails and water is the primary force holding lipid bilayers together. Thus, the elastic modulus of the bilayer is primarily determined by how much extra area is exposed to water when the lipid molecules are stretched apart. It is not surprising given this understanding of the forces involved that studies have shown that K_a varies strongly with solution conditions but only weakly with tail length and unsaturation. Because the forces involved are so small, it is difficult to experimentally determine K_a. Most techniques require sophisticated microscopy and very sensitive measurement equipment.

In contrast to K_a, which is a measure of how much energy is needed to stretch the bilayer, K_b is a measure of how much energy is needed to bend or flex the bilayer. Formally, bending modulus is defined as the energy required to deform a membrane from its intrinsic curvature to some other curvature. Intrinsic curvature is defined by the ratio of the diameter of the head group to that of the tail group. For two-tailed PC lipids, this ratio is nearly one so the intrinsic curvature is nearly zero. If a particular lipid has too large a deviation from zero intrinsic curvature it will not form a bilayer and will instead form other phases such as micelles or inverted micelles. Typically, K_b is not measured experimentally but rather is calculated from measurements of K_a and bilayer thickness, since the three parameters are related.

Λ is a measure of how much energy it takes to expose a bilayer edge to water by tearing the bilayer or creating a hole in it. The origin of this energy is the fact that creating such an interface exposes some of the lipid tails to water, but the exact orientation of these border lipids is unknown. There is some evidence that both hydrophobic (tails straight) and hydrophilic (heads curved around) pores can coexist.

Fusion

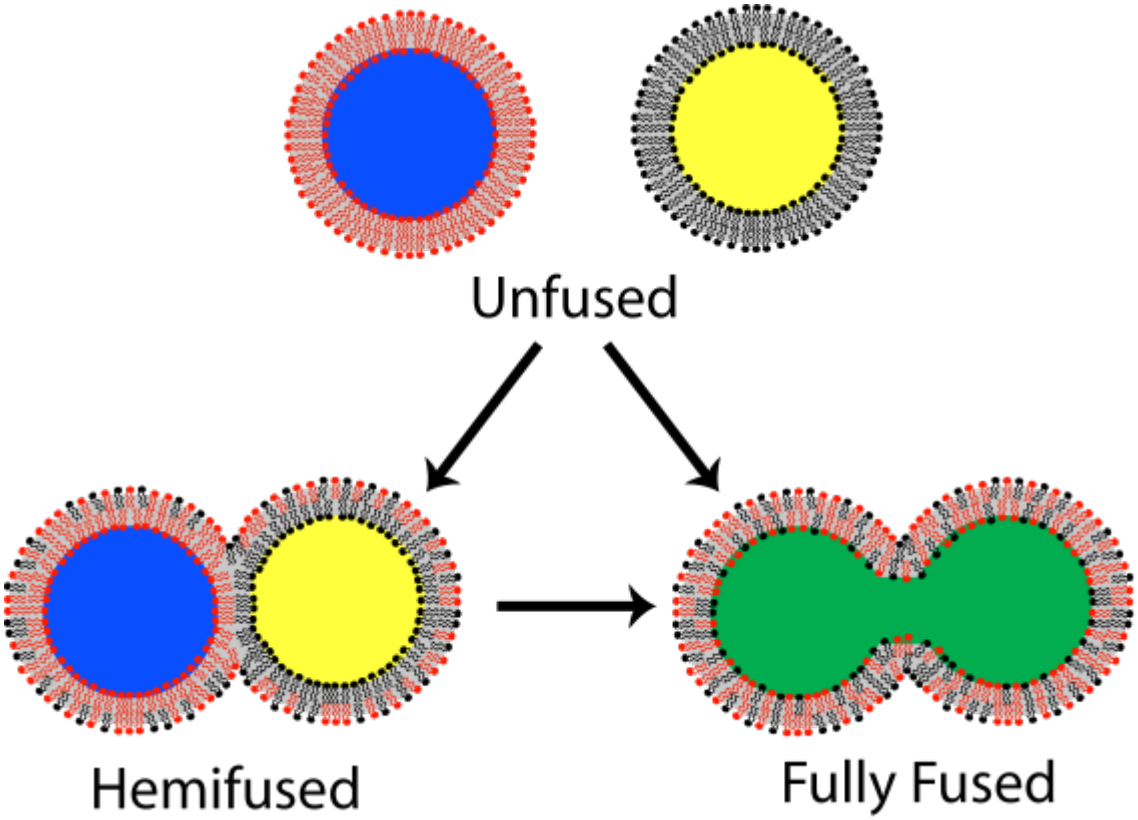
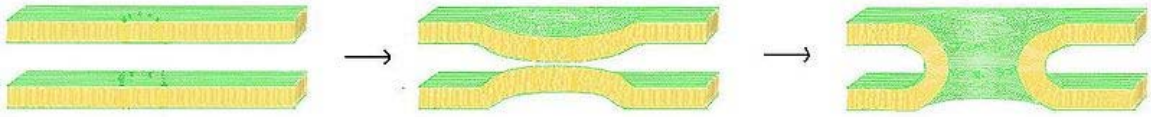


Illustration of lipid vesicles fusing showing two possible outcomes: hemifusion and full fusion. In hemifusion only the outer bilayer leaflets mix. In full fusion both leaflets as well as the internal contents mix.

Fusion is the process by which two lipid bilayers merge, resulting in one connected structure. If this fusion proceeds completely through both leaflets of both bilayers, a water-filled bridge is formed and the solutions contained by the bilayers can mix. Alternatively, if only one leaflet from each bilayer is involved in the fusion process, the bilayers are said to be hemifused. Fusion is involved in many cellular processes, particularly in eukaryotes since the eukaryotic cell is extensively sub-divided by lipid bilayer membranes. Exocytosis, fertilization of an egg by sperm and transport of waste products to the lysozome are a few of the many eukaryotic processes that rely on some form of fusion. Even the entry of pathogens can be governed by fusion, as many bilayer-coated viruses have dedicated fusion proteins to gain entry into the host cell.

There are four fundamental steps in the fusion process. First, the involved membranes must aggregate, approaching each other to within several nanometers. Second, the two bilayers must come into very close contact (within a few angstroms). To achieve this close contact, the two surfaces must become at least partially dehydrated, as the bound surface water normally present causes bilayers to strongly repel. The presence of ions, particularly divalent cations like magnesium and calcium, strongly affects this step. One of the critical roles of calcium in the body is regulating membrane fusion. Third, a

destabilization must form at one point between the two bilayers, locally distorting their structures. The exact nature of this distortion is not known. One theory is that a highly curved "stalk" must form between the two bilayers. Proponents of this theory believe that it explains why phosphatidylethanolamine, a highly curved lipid, promotes fusion. Finally, in the last step of fusion, this point defect grows and the components of the two bilayers mix and diffuse away from the site of contact.



Schematic illustration of the process of fusion through stalk formation.

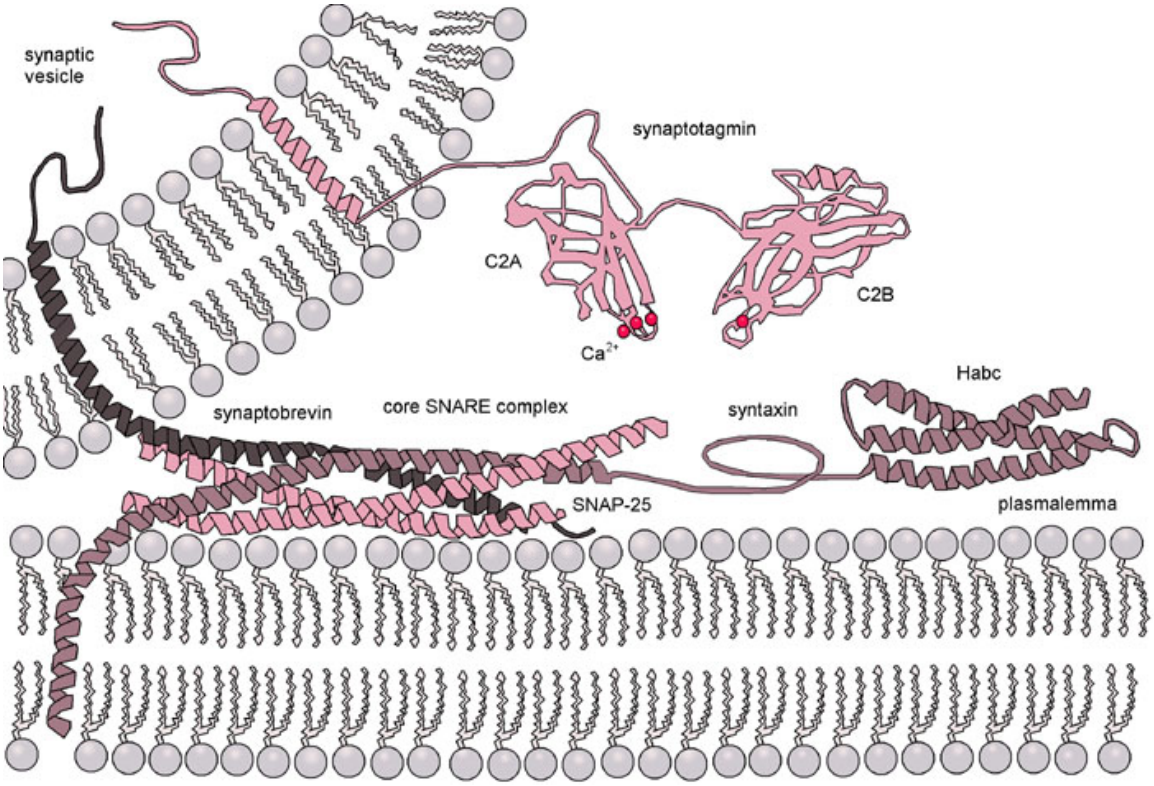


Diagram of the action of SNARE proteins docking a vesicle for exocytosis. Complementary versions of the protein on the vesicle and the target membrane bind and wrap around each other, drawing the two bilayers close together in the process.

The situation is further complicated when considering fusion *in vivo* since biological fusion is almost always regulated by the action of membrane-associated proteins. The first of these proteins to be studied were the viral fusion proteins, which allow an enveloped virus to insert its genetic material into the host cell (enveloped viruses are

those surrounded by a lipid bilayer; some others have only a protein coat).Eukaryotic cells also use fusion proteins, the best studied of which are the SNAREs. SNARE proteins are used to direct all vesicular intracellular trafficking. Despite years of study, much is still unknown about the function of this protein class. In fact, there is still an active debate regarding whether SNAREs are linked to early docking or participate later in the fusion process by facilitating hemifusion.

In studies of molecular and cellular biology it is often desirable to artificially induce fusion. The addition of polyethylene glycol (PEG) causes fusion without significant aggregation or biochemical disruption. This procedure is now used extensively, for example by fusing B-cells with melanoma cells. The resulting "hybridoma" from this combination expresses a desired antibody as determined by the B-cell involved, but is immortalized due to the melanoma component. Fusion can also be artificially induced through electroporation in a process known as electrofusion. It is believed that this phenomenon results from the energetically active edges formed during electroporation, which can act as the local defect point to nucleate stalk growth between two bilayers.

Model systems

Lipid bilayers can be created artificially in the lab to allow researchers to perform experiments that cannot be done with natural bilayers. These synthetic systems are called model lipid bilayers. There are many different types of model bilayers, each having experimental advantages and disadvantages. They can be made with either synthetic or natural lipids. Among the most common model systems are:

- Black lipid membranes (BLM)
- Supported lipid bilayers (SLB)
- Tethered Bilayer Lipid Membranes (t-BLM)
- Vesicles

Commercial applications

To date, the most successful commercial application of lipid bilayers has been the use of liposomes for drug delivery, especially for cancer treatment. (Note- the term "liposome" is essentially synonymous with "vesicle" except that vesicle is a general term for the structure whereas liposome only refers to artificial, not natural vesicles) The basic idea of liposomal drug delivery is that the drug is encapsulated in solution inside the liposome then injected into the patient. These drug-loaded liposomes travel through the system until they bind at the target site and rupture, releasing the drug. In theory, liposomes should make an ideal drug delivery system since they can isolate nearly any hydrophilic drug, can be grafted with molecules to target specific tissues and can be relatively non-toxic since the body possesses biochemical pathways for degrading lipids.

The first generation of drug delivery liposomes had a simple lipid composition and suffered from several limitations. Circulation in the bloodstream was extremely limited due to both renal clearing and phagocytosis. Refinement of the lipid composition to tune

fluidity, surface charge density and surface hydration resulted in vesicles that adsorb fewer proteins from serum and thus are less readily recognized by the immune system. The most significant advance in this area was the grafting of polyethylene glycol (PEG) onto the liposome surface to produce "stealth" vesicles which circulate over long times without immune or renal clearing.

The first stealth liposomes were passively targeted at tumor tissues. Because tumors induce rapid and uncontrolled angiogenesis they are especially "leaky" and allow liposomes to exit the bloodstream at a much higher rate than normal tissue would. More recently work has been undertaken to graft antibodies or other molecular markers onto the liposome surface in the hope of actively binding them to a specific cell or tissue type. Some examples of this approach are already in clinical trials.

Another potential application of lipid bilayers is the field of biosensors. Since the lipid bilayer is the barrier between the interior and exterior of the cell it is also the site of extensive signal transduction. Researchers over the years have tried to harness this potential to develop a bilayer-based device for clinical diagnosis or bioterrorism detection. Progress has been slow in this area and, although a few companies have developed automated lipid-based detection systems, they are still targeted at the research community. These include Biacore Life Sciences, which offers a disposable chip for utilizing lipid bilayers in studies of binding kinetics and Nanion Inc which has developed an automated patch clamping system. Other, more exotic applications are also being pursued such as the use of lipid bilayer membrane pores for DNA sequencing by Oxford Nanolabs. To date, this technology has not proven commercially viable.

A supported lipid bilayer (SLB) as described above has achieved commercial success as a screening technique to measure the permeability of drugs. This **p**arallel **a**rtificial **m**embrane **p**ermeability **a**ssay PAMPA technique measures the permeability across specifically formulated lipid cocktail(s) found to be highly correlated with Caco-2 cultures, the gastrointestinal tract, blood-brain barrier and skin.

History

By the early twentieth century scientists had come to believe that cells are surrounded by a thin oil-like barrier, but the structural nature of this membrane was not known. Two experiments in 1925 laid the groundwork to fill in this gap. By measuring the capacitance of erythrocyte solutions, Hugo Fricke determined that the cell membrane was 3.3 nm thick.

Although the results of this experiment were accurate, Fricke misinterpreted the data to mean that the cell membrane is a single molecular layer. Prof. Dr. Evert Gorter (1881–1954) and F. Grendel of Leiden University approached the problem from a different perspective, spreading the erythrocyte lipids as a monolayer on a Langmuir-Blodgett trough. When they compared the area of the monolayer to the surface area of the cells, they found a ratio of two to one. Later analyses showed several errors and incorrect assumptions with this experiment but, serendipitously, these errors canceled out and from

this flawed data Gorter and Grendel drew the correct conclusion- that the cell membrane is a lipid bilayer.

This theory was confirmed through the use of electron microscopy in the late 1950s. Although he did not publish the first electron microscopy study of lipid bilayers J. David Robertson was the first to assert that the two dark electron-dense bands were the headgroups and associated proteins of two apposed lipid monolayers. In this body of work, Robertson put forward the concept of the “unit membrane.” This was the first time the bilayer structure had been universally assigned to all cell membranes as well as organelle membranes.

Around the same time the development of model membranes confirmed that the lipid bilayer is a stable structure that can exist independently of proteins. By “painting” a solution of lipid in organic solvent across an aperture, Mueller and Rudin were able to create an artificial bilayer and determine that this exhibited lateral fluidity, high electrical resistance and self-healing in response to puncture, all of which are properties of a natural cell membrane. A few years later, Alec Bangham showed that bilayers, in the form of lipid vesicles, could also be formed simply by exposing a dried lipid sample to water. This was an important advance since it demonstrated that lipid bilayers form spontaneously via self assembly and do not require a patterned support structure.