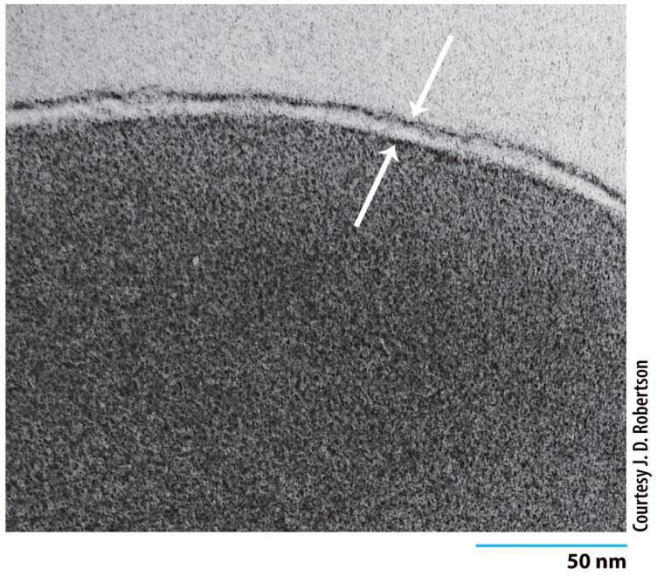
CHAPTER 8

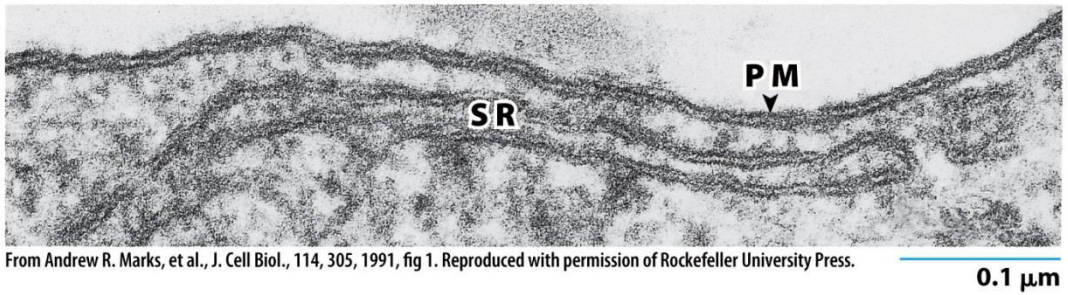
Cellular Membranes

Keys

- Describe the functions of cellular membranes.
- Elucidate the chemical components and properties of cell membranes.
- Describe the development of the models to the Fluid-Mosaic Model.
- Explain the role of carbohydrates in membrane structure.
- Describe the types of proteins found in membranes and their roles.
- Stress the importance and detection of membrane fluidity in living cells.
- Describe biological membrane asymmetry.
- Describe the mechanisms to transport materials across membranes: simple and facilitated diffusion, channel proteins, active transport.
- Explain the process involved in generating an action potential and propagating the signal across the synapse to the postsynaptic cell.

Introduction



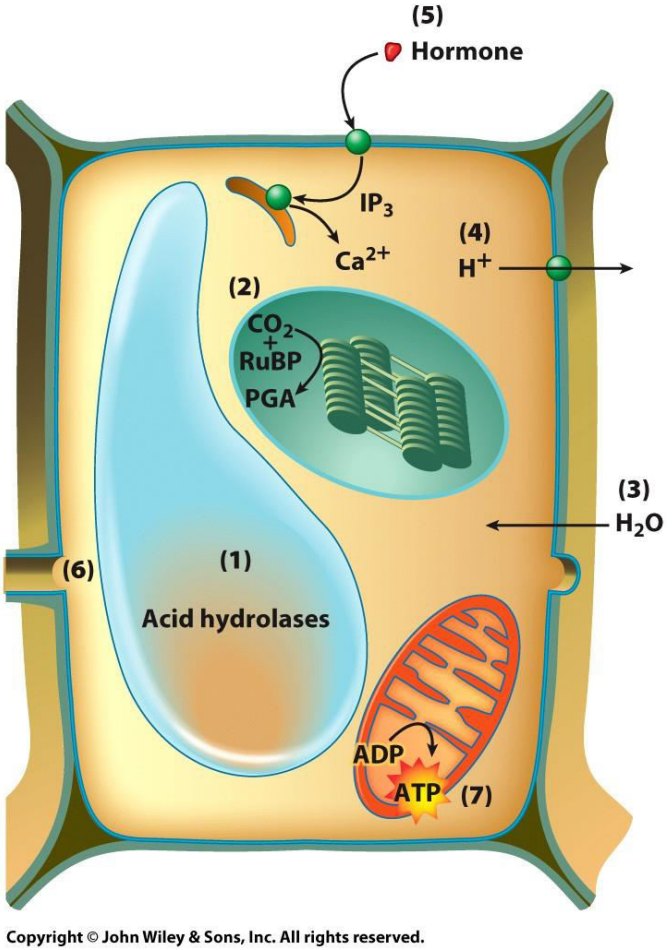


The trilaminar appearance of membranes as revealed by electron micrograph of the plasma membrane and sarcoplasmic reticulum.

- **Plasma membrane**: The outer boundary of the cell that separates it from the world is a thin, fragile structure about 5 – 10 nm thick.
- Not detectable with light microscope need electron microscope.
- The 2 dark-staining layers in the electron micrographs correspond primarily to the inner & outer polar surfaces of the bilayer
- All membranes examined closely (plasma, nuclear or cytoplasmic) from plants, animals or microorganisms have the same ultrastructure

(8.1) An Overview of Membrane Functions

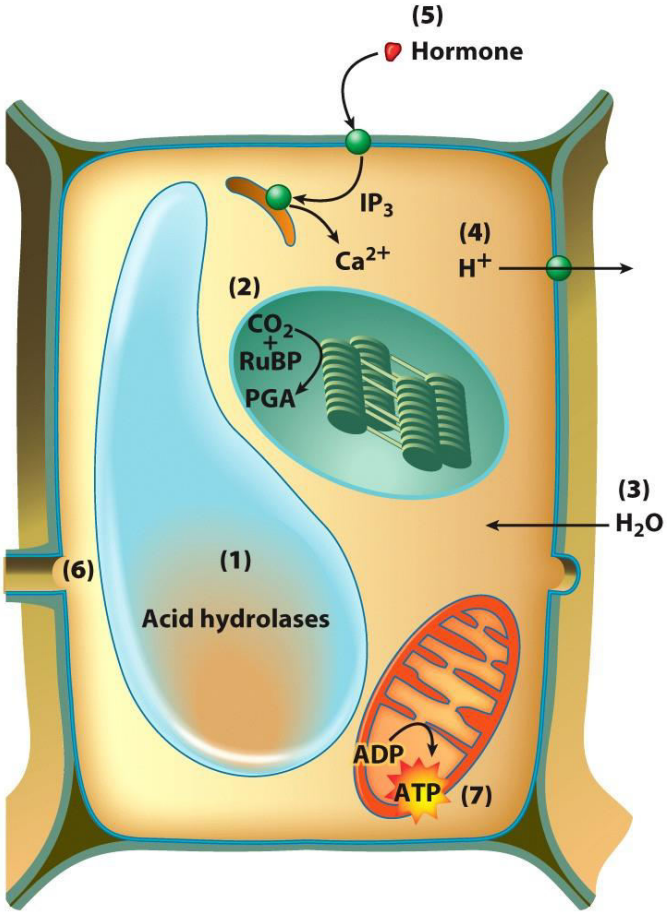
- **Compartmentalization (1)**
  Membranes form continuous sheets that enclose intracellular compartments.
- **Scaffold for biochemical activities (2)**
  Membranes provide a framework that organizes enzymes for effective interaction.
- **Selectively permeable barrier (3)**
  Membranes allow regulated exchange of substances between compartments.



A summary of membrane functions in a plant cell.

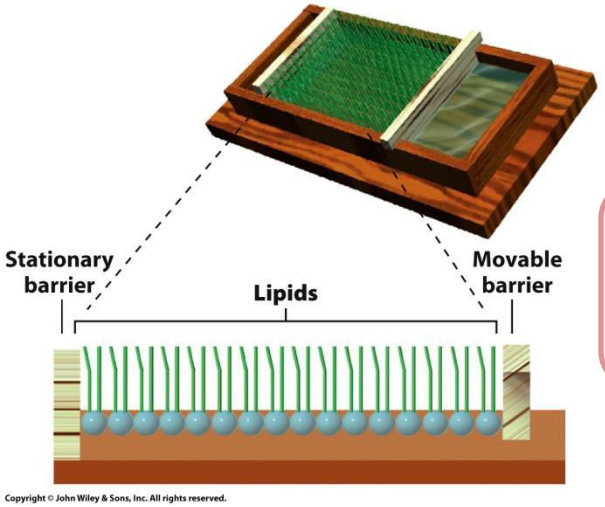
An Overview of Membrane Functions

- **Transporting solutes (4)**
  Membrane proteins facilitate the movement of substances between compartments.
- **Responding to external signals (5)**
  Membrane receptors transduce signals from outside the cell in response to specific ligands.
- **Intracellular interaction (6)**
  Membranes mediate recognition and interaction between adjacent cells.
- **Energy transduction (7)**
  Membranes transduce photosynthetic energy, convert chemical energy to ATP, and store energy.

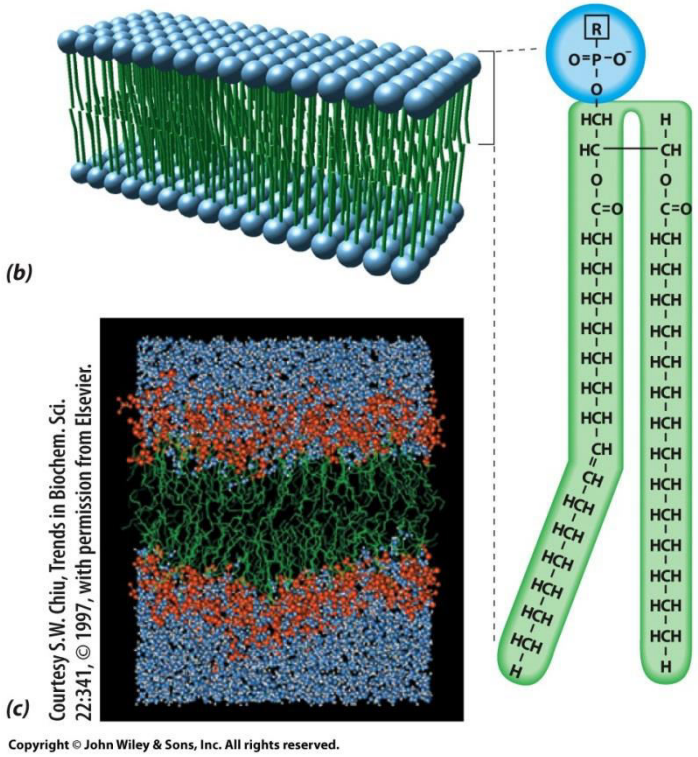


A summary of membrane functions in a plant cell.

(8.2) A Brief History of Studies on Plasma Membrane Structure



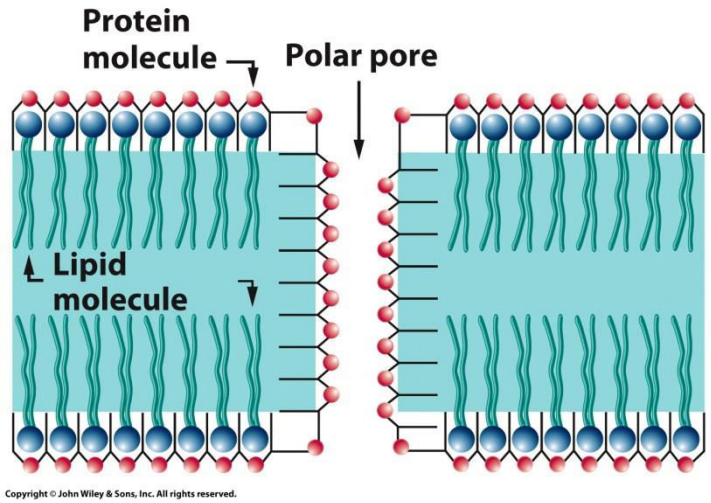
Calculating the surface area of a lipid preparation



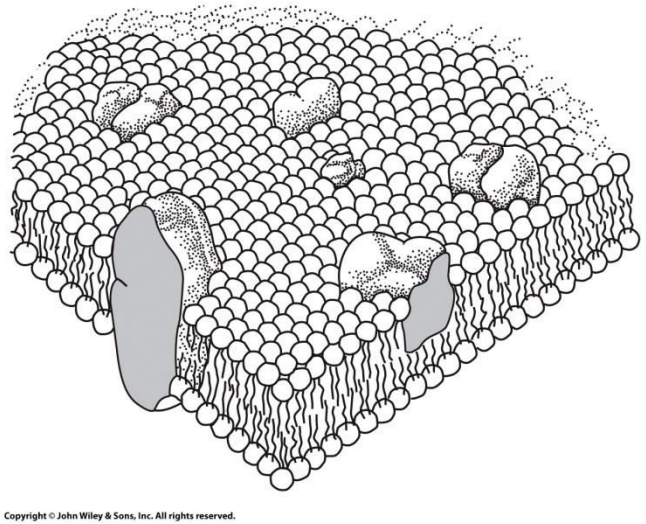
- Membranes were found to be mostly composed of lipids because their dissolving power matched that of oil.
- The **lipid bilayer** accounted for the 2:1 ratio of lipid to cell surface area
- The most energetically favored orientation for polar head groups is facing the aqueous compartments outside of the bilayer

Bimolecular layer of phospholipids with water soluble head groups facing outward

A Brief History of Studies on Plasma Membrane Structure

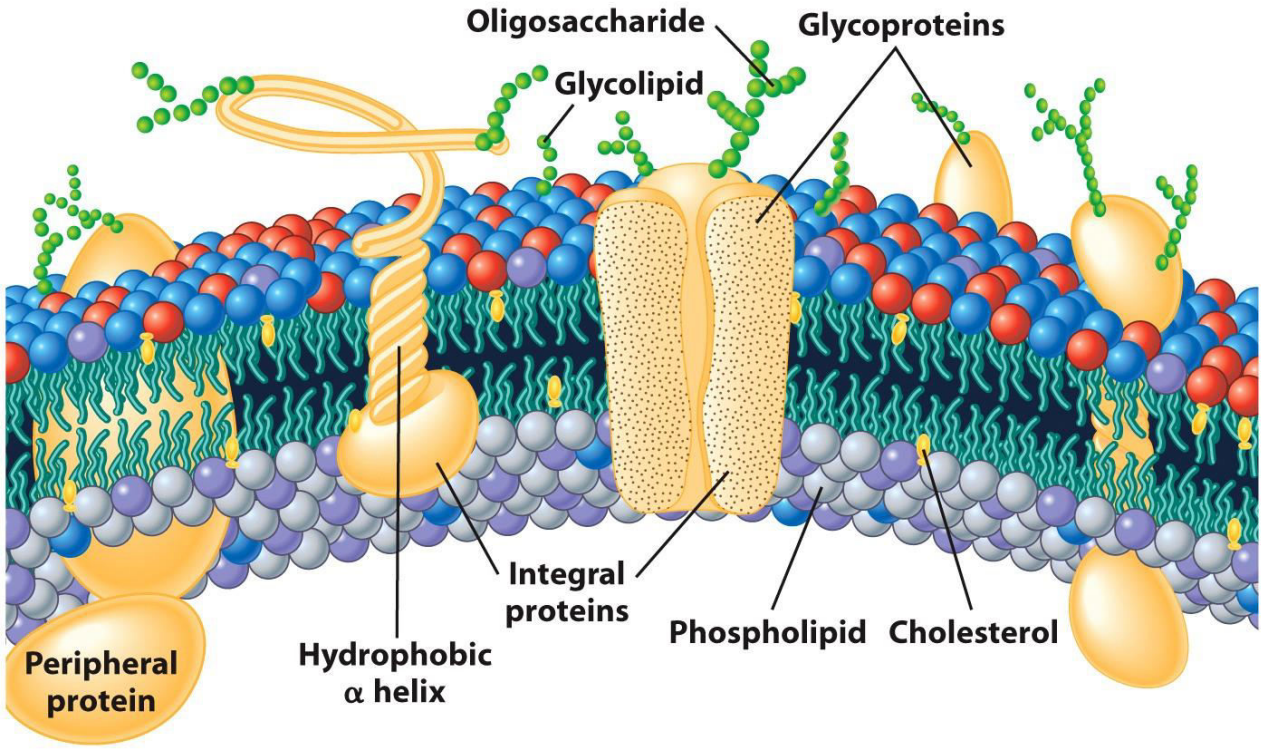


Early models representing the lipid bilayer



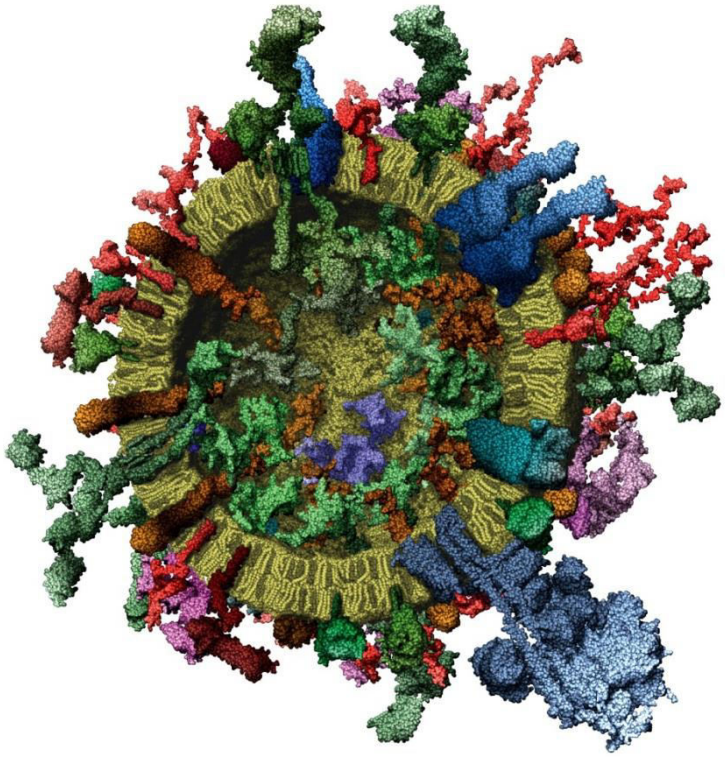
- The nature and importance of the lipid bilayer:
  - Lipid composition can influence the activity of membrane proteins and determine the physical state of the membrane.
  - The cohesion of bilayers to form a continuous sheet makes cells deformable and facilitates splitting and fusion of membranes.
- Protein-lined pores in the membrane account for the movement of polar solutes and ions across cell boundaries.

A Brief History of Studies on Plasma Membrane Structure



- The **fluid-mosaic model**
  - Core lipid bilayer exists in a fluid state, capable of movement.
  - Membrane proteins form a mosaic of particles penetrating the lipids.

A Brief History of Studies on Plasma Membrane Structure



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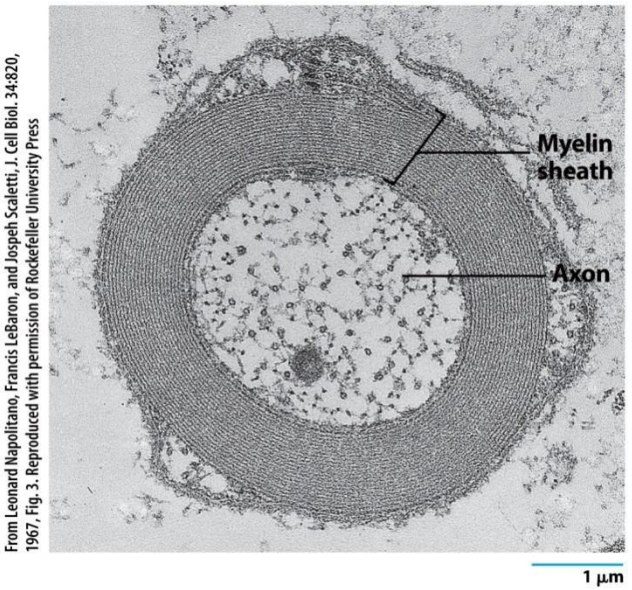
Molecular model of the membrane of a synaptic vesicle constructed with various proteins embedded into the lipid bilayer

- The **fluid-mosaic model**
  - Core lipid bilayer exists in a fluid state, capable of movement.
  - Membrane proteins form a mosaic of particles penetrating the lipids.

(8.3) The Chemical Composition of Membranes

- Membrane composition
  - The lipid and protein components are bound together by non-covalent bonds.
  - Membranes also contain carbohydrates.
  - Protein/lipid ratios vary among membrane types.



From Leonard Napolitano, Francis LeBaron, and Jospeh Scaletti, J. Cell Biol. 34:820, 1967, Fig. 3. Reproduced with permission of Rockefeller University Press

- Membrane lipids are **amphipathic** with three main types:
  - **Phosphoglycerides** are diacylglycerides with small functional head groups linked to the glycerol backbone by phosphate ester bonds.
  - **Sphingolipids** are ceramides formed by the attachment of sphingosine to fatty acids.
  - **Cholesterol** is a smaller and less amphipathic lipid that is only found in animals.

The Chemical Composition of Membranes

Chemical structure of membrane lipids

- **Phosphoglycerides** are diacylglycerides with small functional head groups linked to the glycerol backbone by phosphate ester bonds.
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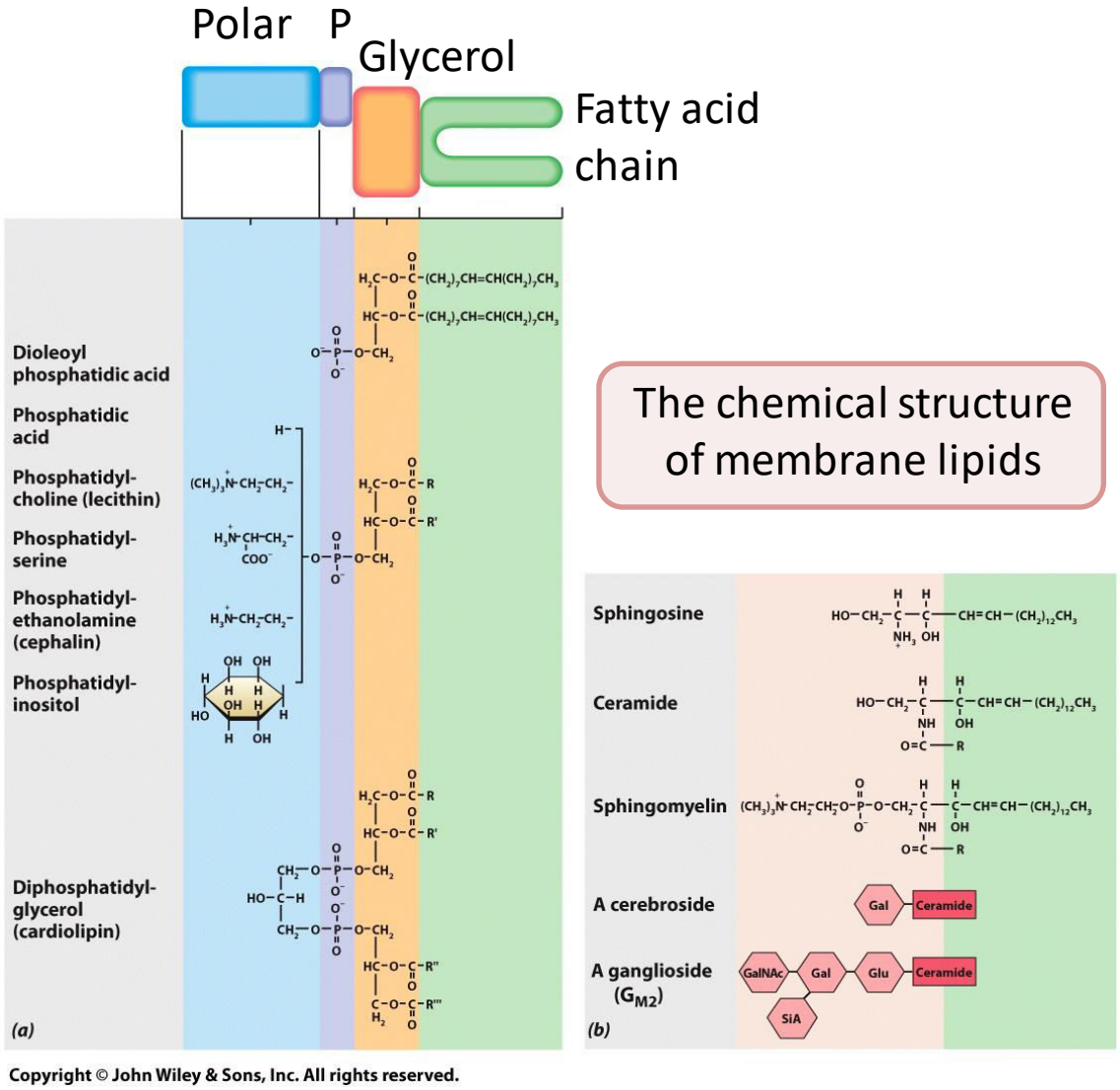
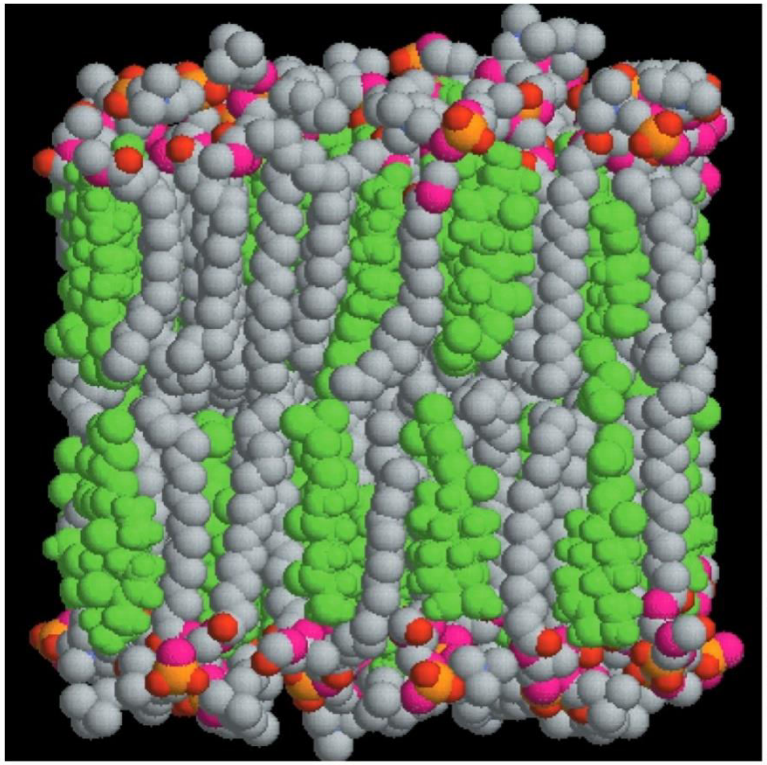


The chemical structure of membrane lipids

The Chemical Composition of Membranes

Chemical structure of membrane lipids

- **Cholesterol** is a smaller and less amphipathic lipid that is only found in animals.
- A sterol that makes up to 50% of animal membrane lipids.
- The -OH group is oriented toward membrane surface
- Carbon rings are flat and rigid; interfere with movement of phospholipid fatty acid tails



From H. L. Scott, Curr. Opin. Struct. Biol 12: 499, 2002, Figure 3. © 2002, with permission from Elsevier.

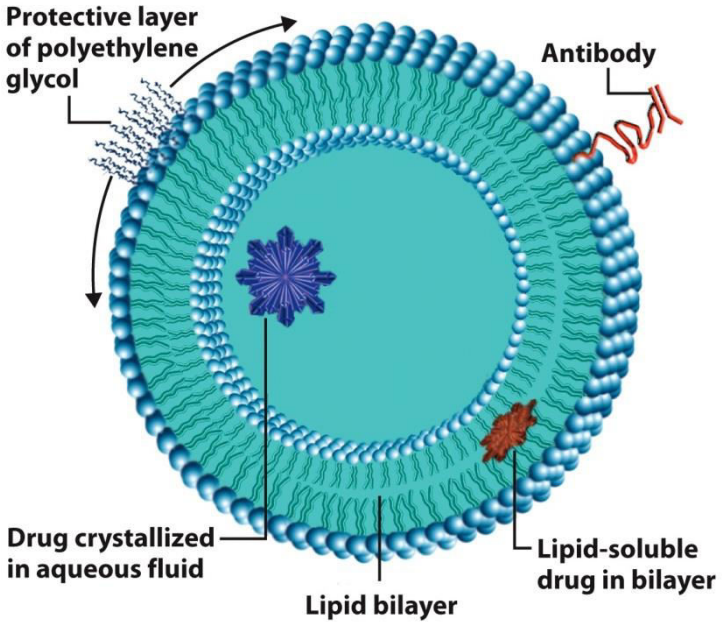
Cholesterol molecules (green) oriented with their small hydrophilic end facing the external surface of the bilayer

The Chemical Composition of Membranes

***Table 8.1** Lipid Compositions of Some Biological Membranes**

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

*The values given are weight percent of total lipid.
Source: C. Tanford, *The Hydrophobic Effect*, p. 109, copyright 1980, John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.

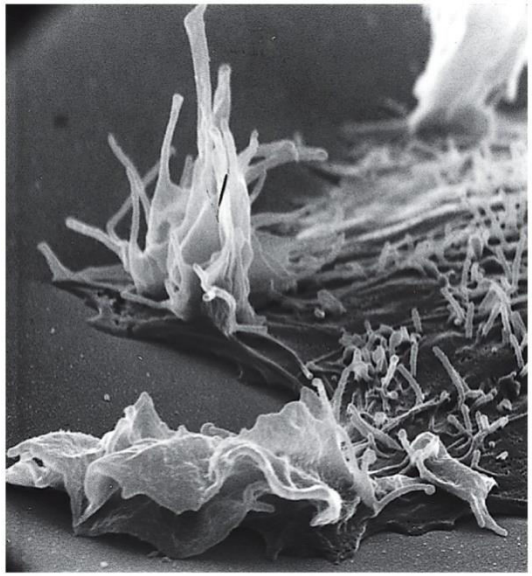


Liposomes: synthetic vesicles

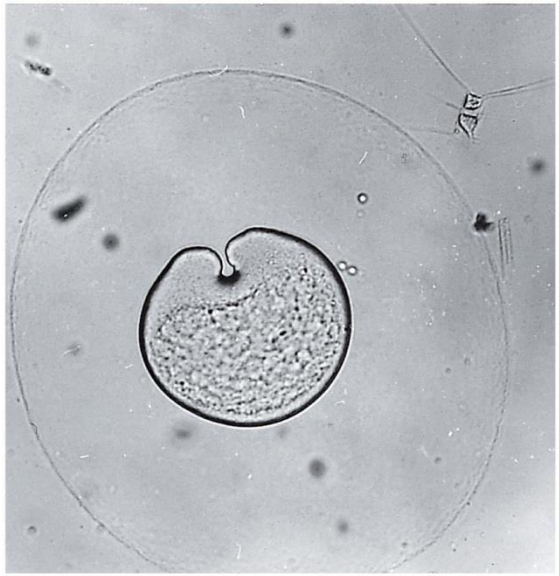
- The Nature and Importance of the Lipid Bilayer
  - Membrane lipid composition is characteristic of specific membranes.
  - Lipids give membranes the ability to fuse, form networks, and separate charge.
  - Lipid bilayers assemble spontaneously in aqueous solutions as in **liposomes**.

The Chemical Composition of Membranes

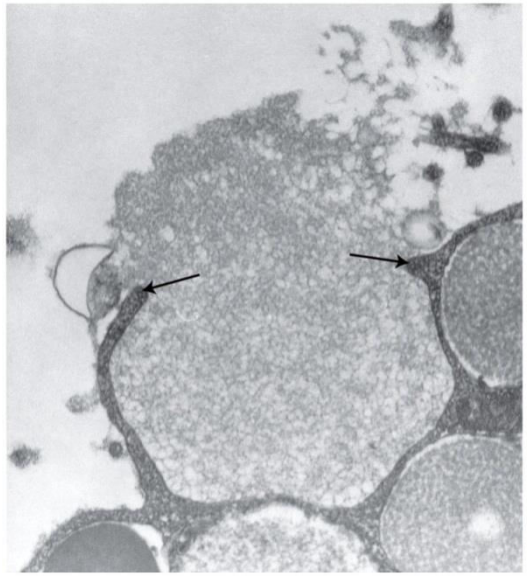
The dynamic properties of plasma membranes



Courtesy Jean Paul Revel



Courtesy Gary Freeman



Courtesy Susan Jo Burwen

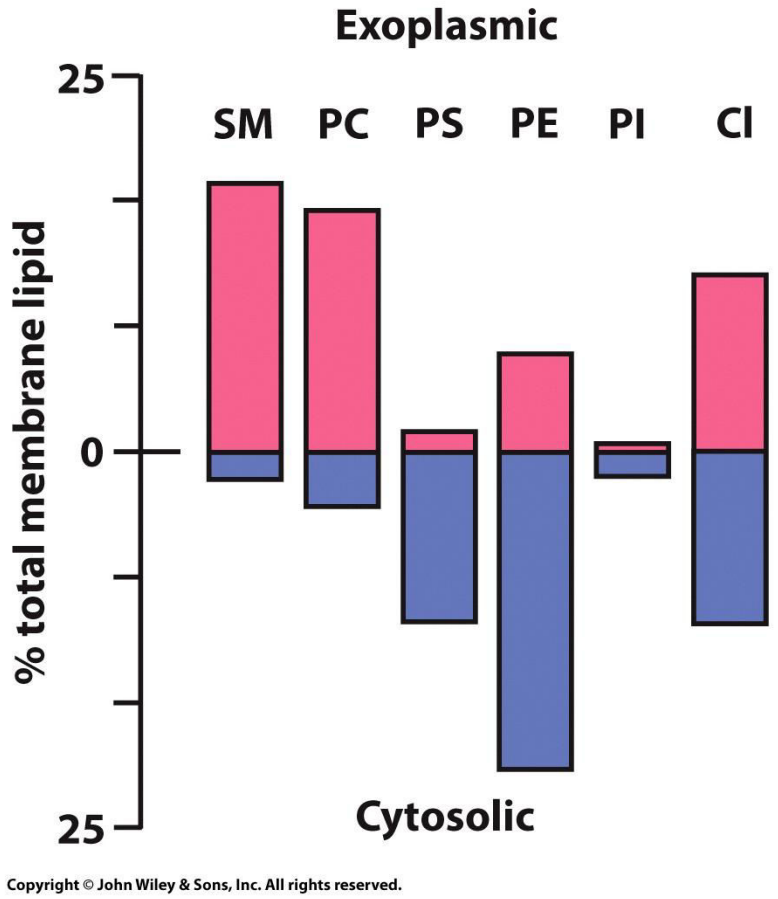
Movement: ruffling of the plasma membrane of a migrating cell

Division: invagination of the plasma membrane towards the cell center during cell division

Fusion: plasma membranes of sperm and egg unite

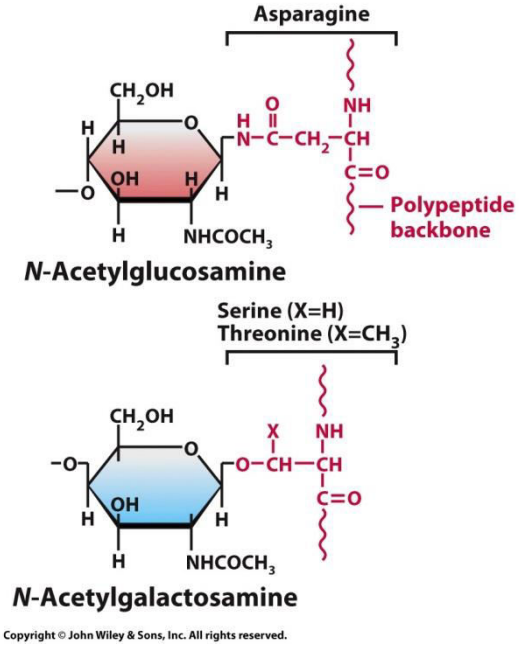
The Chemical Composition of Membranes

- The Asymmetry of Membrane Lipids
  - Inner and outer membrane leaflets have different lipid compositions.
  - Provides different physico-chemical properties appropriate for different interactions
  - Membrane lipids move easily within a leaflet but only rarely “flip-flop”

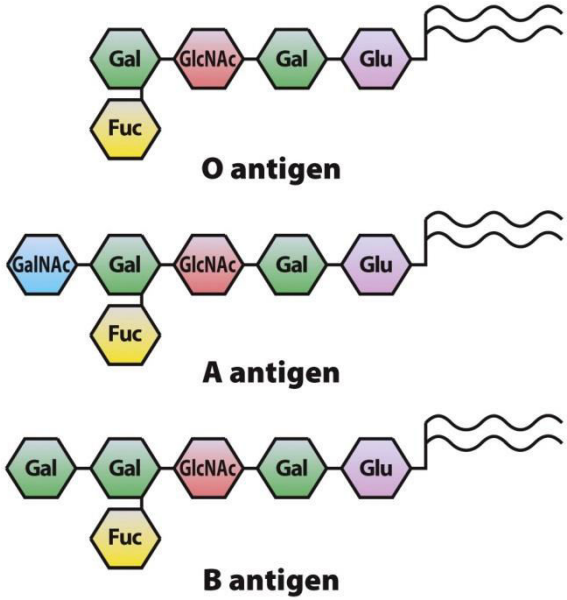


SM: sphingomyelin
PC: phosphatidylcholine
PS: phosphatidylserine
PE: phosphatidylethanolamine
PI: phosphatidylinositol
Cl: cholesterol

The Chemical Composition of Membranes



Two types of linkages that join sugars to a polypeptide chain

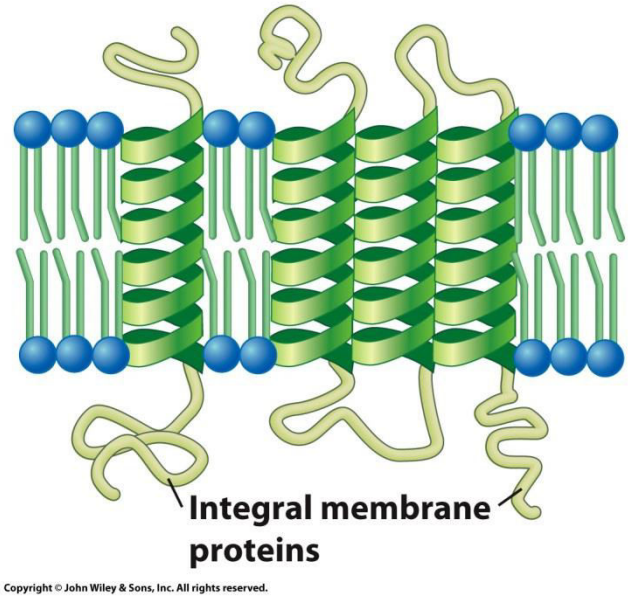


Blood-group antigens

- Membrane Carbohydrates
  - Membranes contain carbohydrates covalently linked to lipids and proteins on the extracellular surface of the bilayer.
  - Glycoproteins have short, branched carbohydrates for interactions with other cells and structures outside the cell.
  - Glycolipids have larger carbohydrate chains that may be cell-to-cell recognition sites.

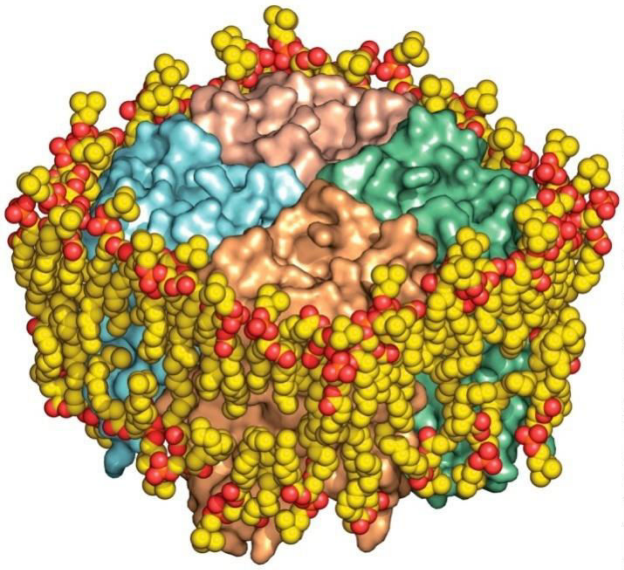
(8.4) The Structure and Functions of Membrane Proteins

- Membrane proteins attach to the bilayer asymmetrically, giving the membrane a distinct "sidedness"
- Membrane proteins can be grouped into three distinct classes:
  1. **Integral proteins** - penetrate and pass through lipid bilayer; make up 20 -30% of all encoded proteins
     - Are amphipathic, with hydrophilic domains anchoring them in the bilayer and hydrophilic regions forming functional domains outside of the bilayer.
     - Channel proteins have hydrophilic cores that form aqueous channels in the membrane-spanning region.



Integral proteins

The Structure and Functions of Membrane Proteins



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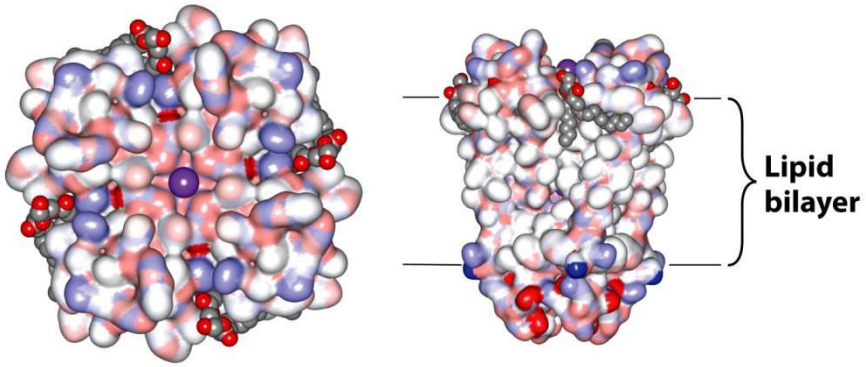


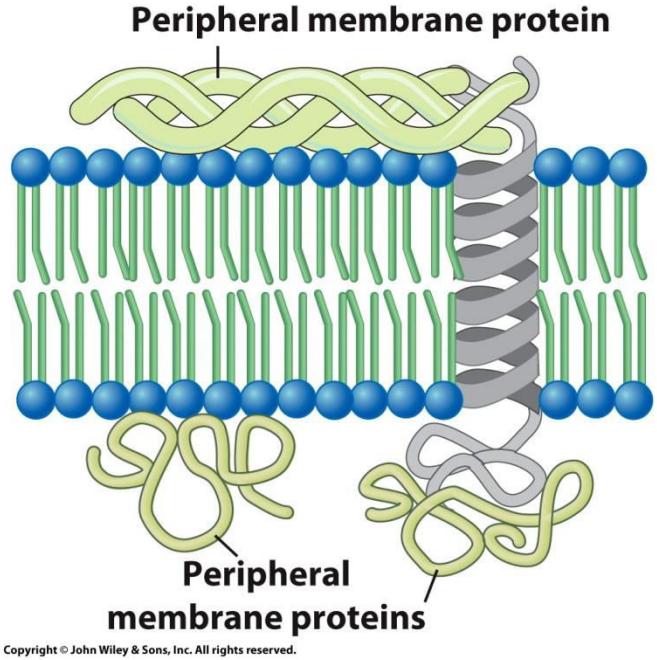
From A.G. Lee, Trends Biochem. Sci. 36:497, 2011, © 2011; with permission from Elsevier.

Driven by van der Waals forces between amino acids and lipids, proteins can be surrounded by a closely applied shell of lipid molecules.

1. **Integral proteins** - penetrate and pass through lipid bilayer; make up 20 -30% of all encoded proteins
   - Are amphipathic, with hydrophilic domains anchoring them in the bilayer and hydrophilic regions forming functional domains outside of the bilayer.
   - Channel proteins have hydrophilic cores that form aqueous channels in the membrane-spanning region.

The Structure and Functions of Membrane Proteins

- Membrane proteins attach to the bilayer asymmetrically, giving the membrane a distinct "sidedness"
- Membrane proteins can be grouped into three distinct classes:
  2. **Peripheral proteins** are attached to the membrane by weak bonds and are easily solubilized.
     - Located entirely outside of bilayer on either the extracellular or cytoplasmic side; associated with membrane surface by non-covalent bonds.



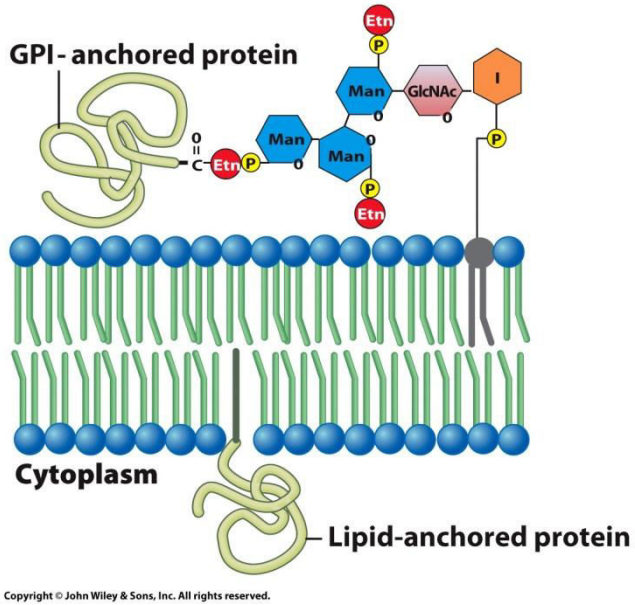
Peripheral proteins

The Structure and Functions of Membrane Proteins

- Membrane proteins attach to the bilayer asymmetrically, giving the membrane a distinct "sidedness"
- Membrane proteins can be grouped into three distinct classes:

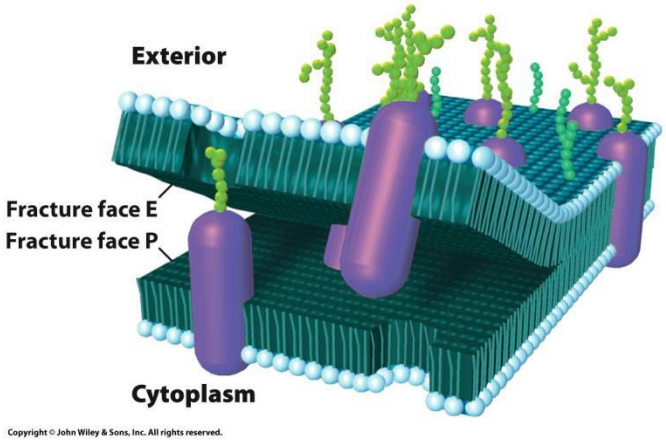
3. Lipid-anchored membrane proteins are distinguished both by the types of lipid anchor and their orientation.

  - Glycophosphatidylinositol **(GPI)-linked proteins** found on the outer leaflet can be released by inositol-specific phospholipases.
  - Some inner-leaflet proteins are anchored to membrane lipids by long hydrocarbon chains.

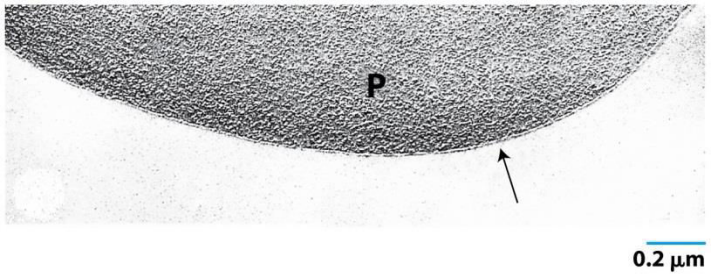


Lipid-anchored proteins

The Structure and Functions of Membrane Proteins

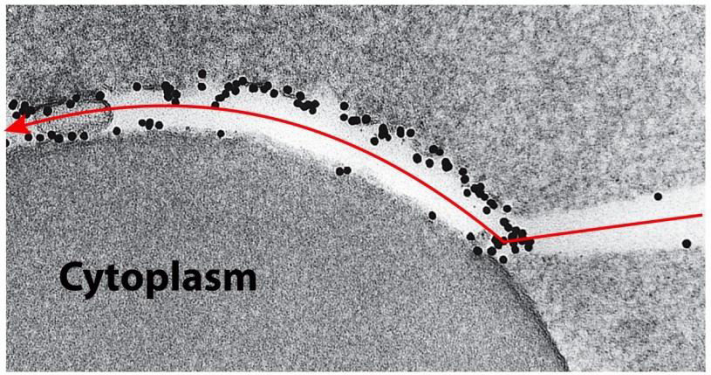


Ectoplasmic vs protoplasmic



From Thomas W. Tillack and Vincent T. Marchesi, J. Cell Biol. 45:651, fig. 1 (top), 1970. Reproduced with permission of Rockefeller University Press

- Distribution of Integral Proteins: Freeze-Fracture Analysis
  - **Freeze-fracture** technique divides the phospholipid leaflets of the membrane.
  - Integral membrane proteins appear as bumps and pits using the electron microscope.
  - The heterogeneity of protein distribution is shown.



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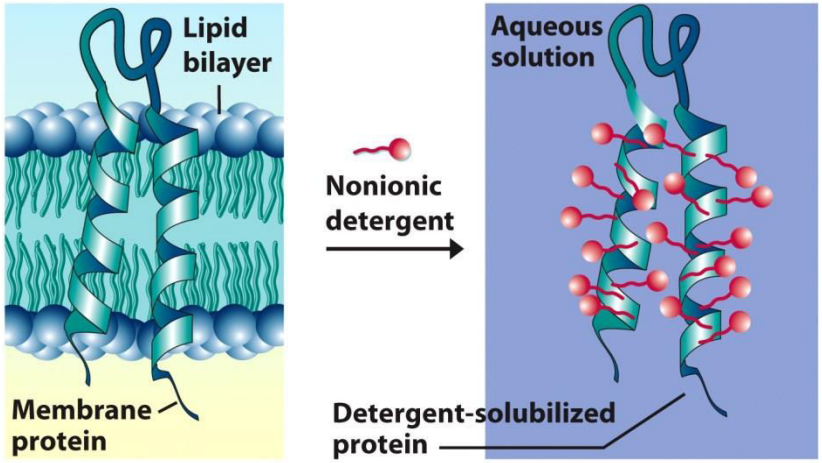
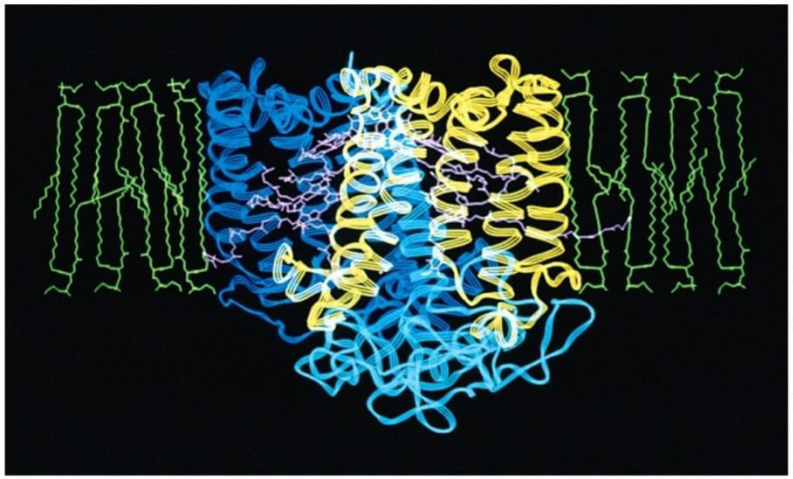
AB: carb group for glycophorin

The Structure and Functions of Membrane Proteins



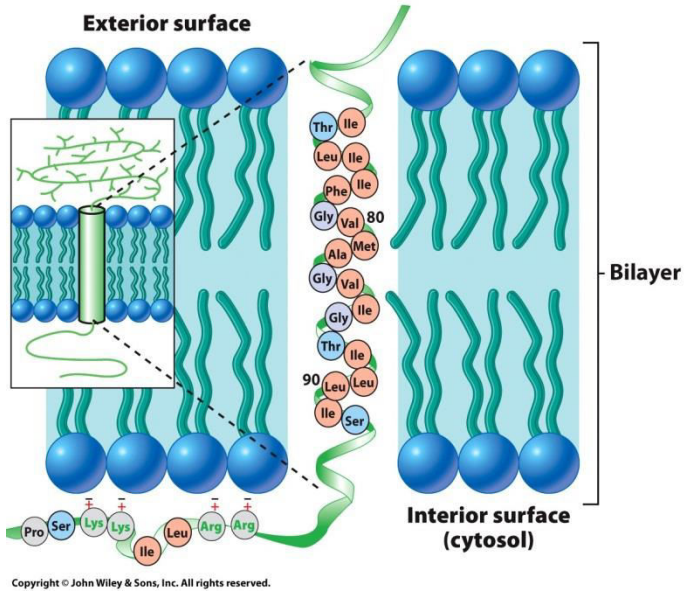
Solubilization of membrane proteins with detergents



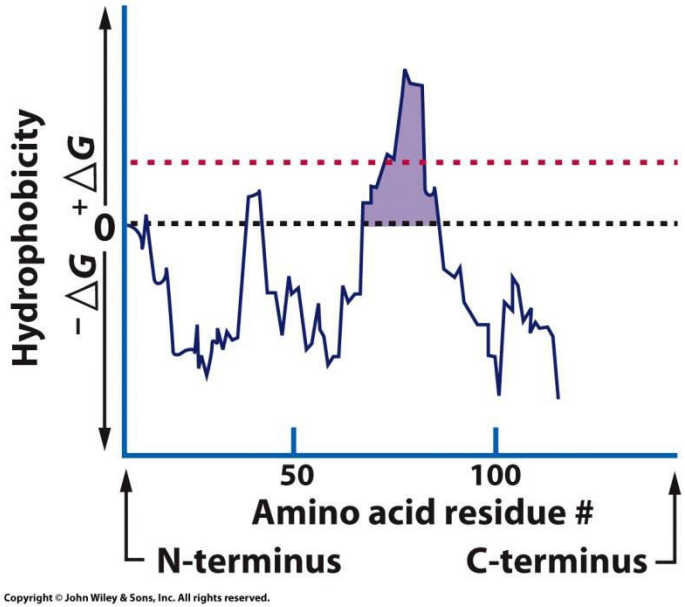
An integral protein as it resides within the plasma membrane

- Studying the Structure and Properties of Integral Membrane Proteins
  - **Determining membrane sidedness**: The orientation of integral proteins can be determined using non-penetrating agents that label the proteins.
  - SDS (ionic)-denatures proteins
  - Triton X-100 (non-ionic)- does not alter protein tertiary structure

The Structure and Functions of Membrane Proteins



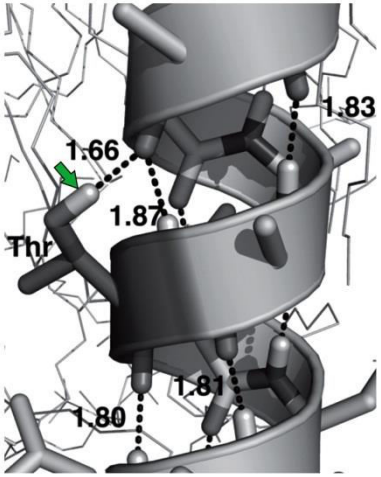
Glycophorin A, an integral protein with a single transmembrane domain with a Gly-X-X-X-Gly sequence



Hydropathy plot for glycophorin A demonstrates a single pass domain

- Studying the Structure and Properties of Integral Membrane Proteins
  - Identifying transmembrane domains: A string of 20-30 hydrophobic amino acids from hydropathy plots identifies a membrane-spanning domain.

The Structure and Functions of Membrane Proteins



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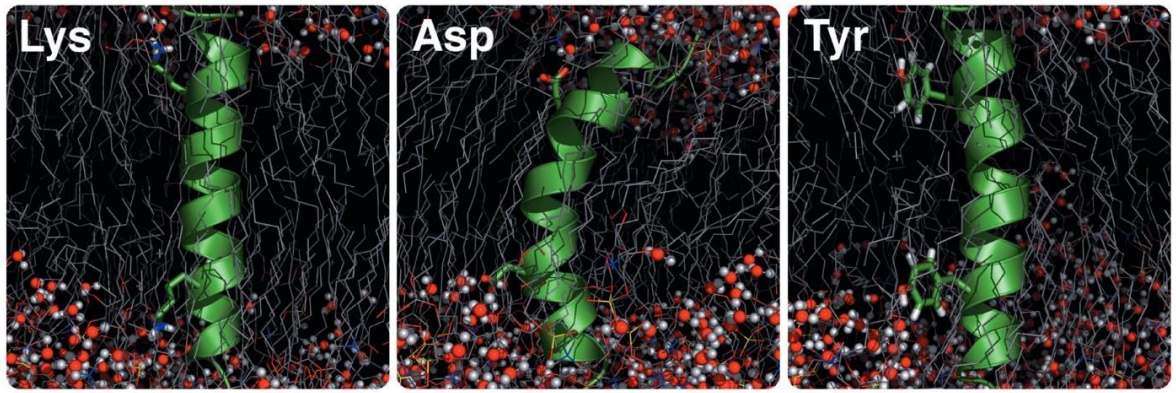

(b) (c) (d)

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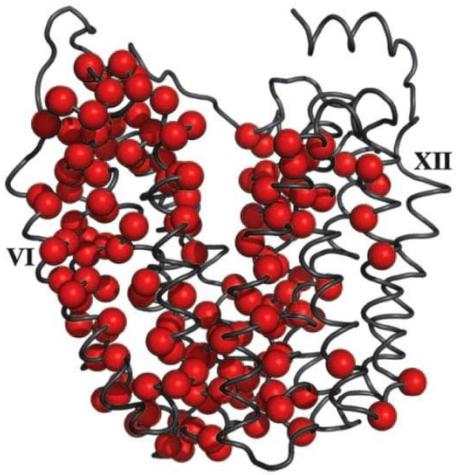
Accommodating nonpolar amino acid residues within transmembrane helices

- Studying the Structure and Properties of Integral Membrane Proteins
  - **Spatial relationships within an integral membrane protein**
    - Site-directed mutagenesis—replacing specific amino acids with others—identifies some spatial relationships.
    - Electron spin resonance identifies some conformational changes that occur when integral proteins function.

The Structure and Functions of Membrane Proteins



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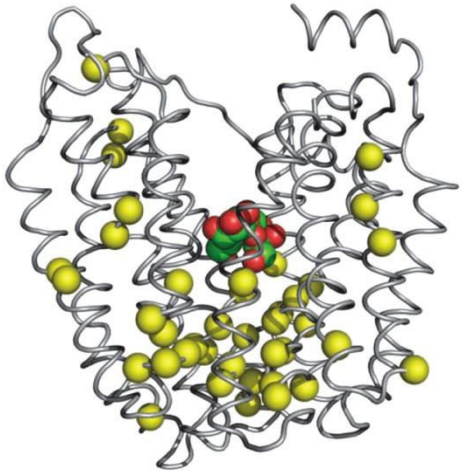

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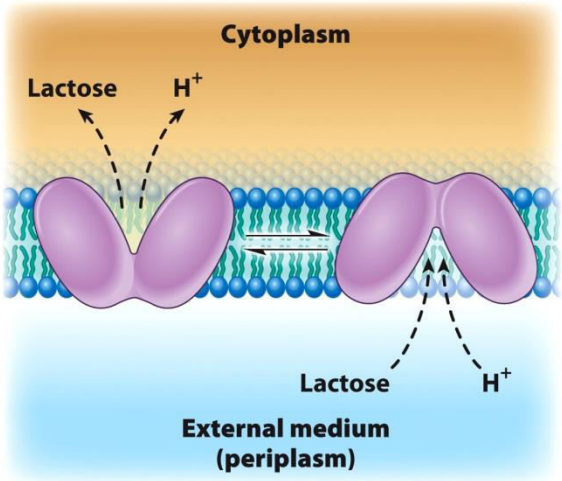
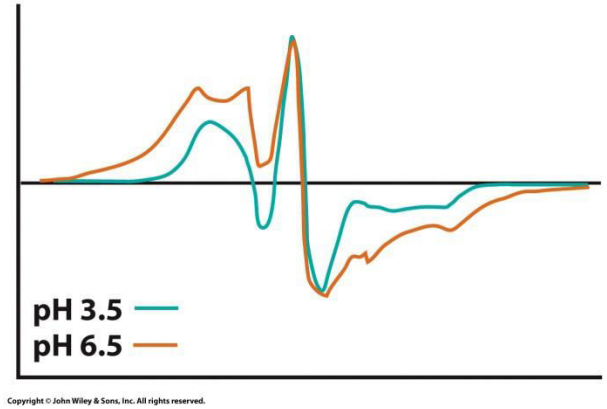
Site-directed mutagenesis to learn about dynamic changes in the conformation of a membrane protein as it carries out its activity

- Determining spatial relationships between amino acids within integral membrane proteins
- Use of **site-directed mutagenesis** to replace amino acids residues
- Replacing residues in neighboring helices with cysteine residues can lead to disulfide bond formation to reveal proximity.

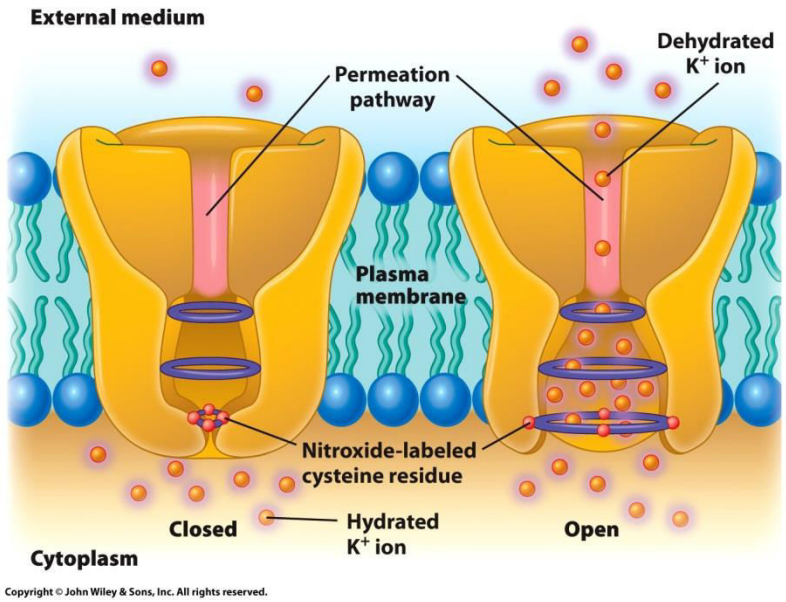


From H. Ronald Kaback, et al., PNAS 104: 492, © 2007. National Academy of Sciences, U.S.A.

The Structure and Functions of Membrane Proteins



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



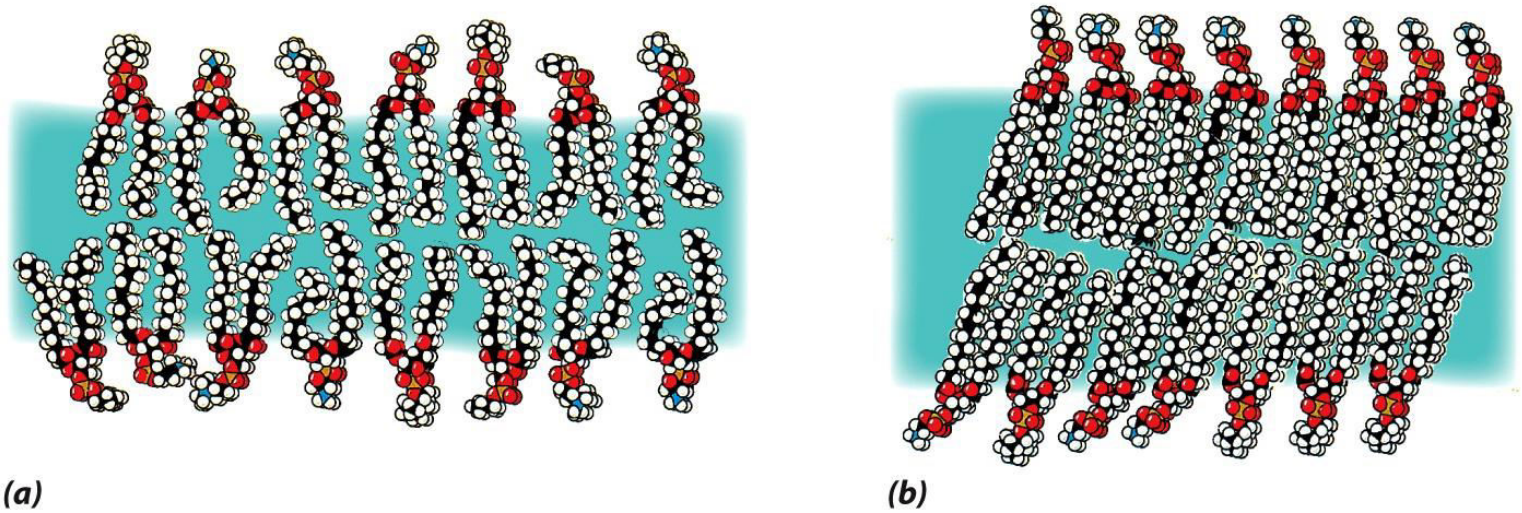
- Dynamic events occur as a protein functions which can be monitored:
  - Introduce chemical groups whose properties are sensitive to distance
  - Introduce nitroxides at any site in protein by first mutating the amino acid residue to cysteine via site-directed mutagenesis, then attach nitroxide to thiol group of cysteine.
  - Monitor by technique called electron paramagnetic resonance [EPR] spectroscopy

(8.5) Membrane Lipids and Membrane Fluidity

- Membrane lipids exist in gel or liquid-crystal phases depending on temperature, lipid composition and saturation in the presence of cholesterol.
  - Liquid-crystal membranes predominate
  - Unsaturated fatty acids lower the temperature at which the liquid-crystal/gel phase transition occurs (**transition temperature**).

Membrane Lipids and Membrane Fluidity

Structure depends on the temperature



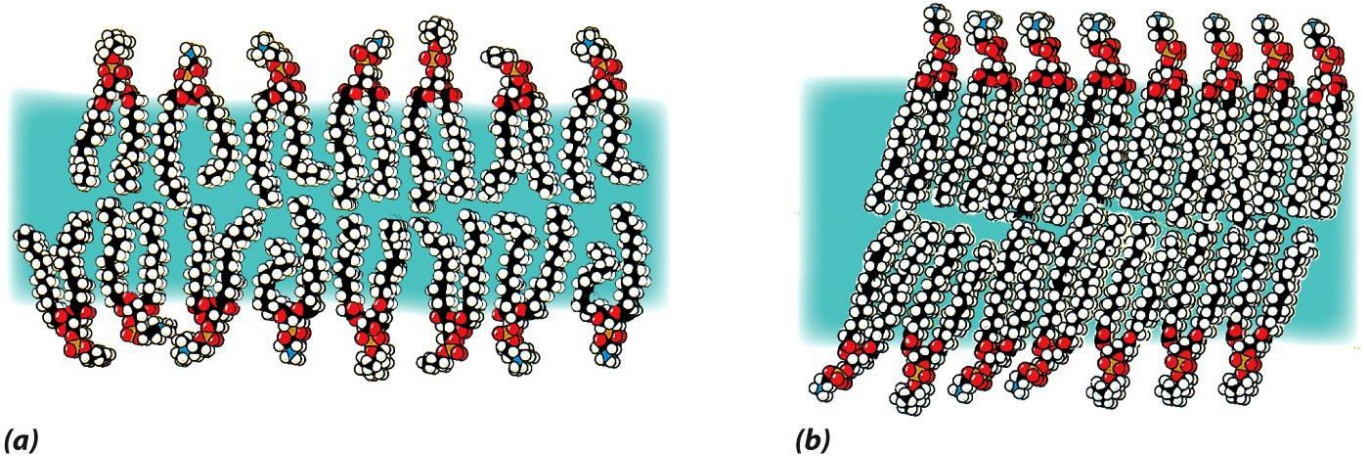
(a) (b)

Structure of the lipid bilayer depends on the temperature: above and below the transition temperature.

- The Importance of Membrane Fluidity
  - The fluidity of membranes is a compromise between structural rigidity and complete fluidity.
  - Membrane fluidity makes it possible for proteins to move in the membrane and for membranes to assemble and grow.

Membrane Lipids and Membrane Fluidity

Structure depends on the temperature



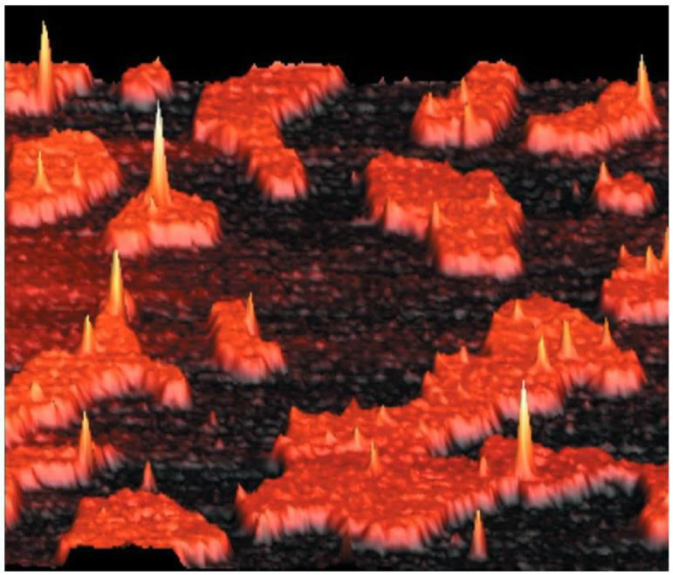
(a) (b)

Structure of the lipid bilayer depends on the temperature: above and below the transition temperature.

- Maintaining Membrane Fluidity
  - Organisms (other than birds and mammals) maintain membrane fluidity as temperature changes by altering the composition of membrane lipids.
  - Remodeling lipid bilayers involves saturation or desaturation of acyl chains and replacement of acyl chains by *phospholipases* or *acyltransferases*.
  - The importance of these mechanisms has been verified using mutants unable to carry out certain desaturation reactions in response to cold.

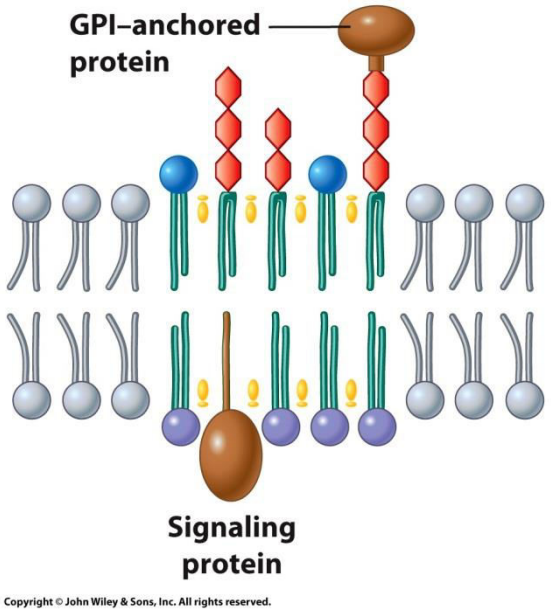
Membrane Lipids and Membrane Fluidity

Lipid rafts



From D.E. Saslowsky, et al, J. Biol. Chem. 277, 26966.26970, July 26, © 2002 The American Society for Biochemistry and Molecular Biology.

Sphingomyelin organizing into orange-colored rafts

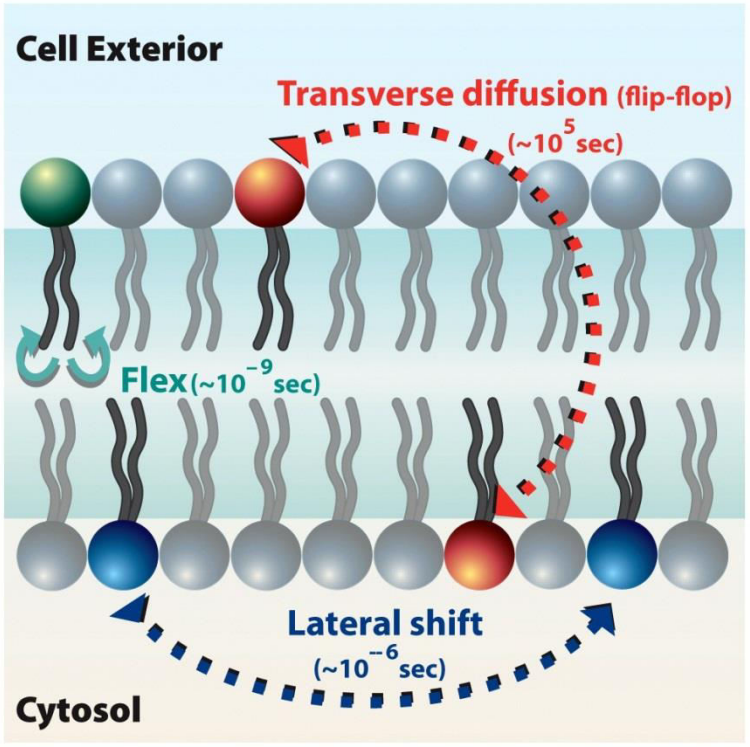


Schematic model of a lipid raft

- Lipid rafts
  - Outer leaflet of plasma membrane contains specialized regions
  - Cholesterol and sphingolipids tend to pack together to form highly ordered microdomains forming **lipids rafts** that float within the more fluid and disordered environment.
  - Provide a favorable environment for cell-surface receptors and GPI-anchored proteins.

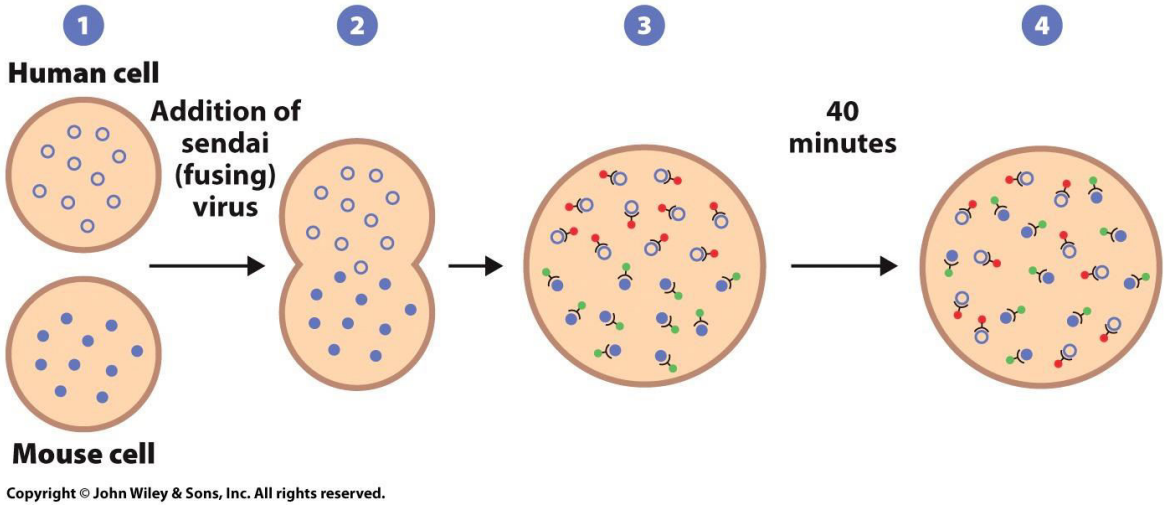
(8.6) The Dynamic Nature of the Plasma Membrane

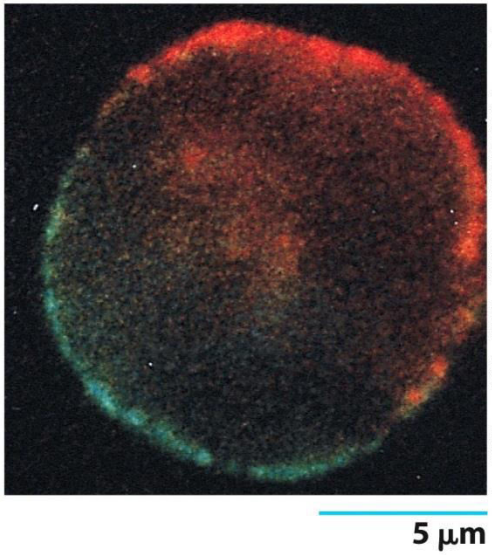
- Lipid bilayer can exist in a relatively fluid state.
- A phospholipid can move laterally within the same leaflet with considerable ease.
- In contrast, it takes a phospholipid molecule a matter of hours to days to move across to the other leaflet (flip-flop).
- The hydrophilic head group of the lipid must pass through the internal hydrophobic sheet of the membrane, which is thermodynamically unfavorable.
- The physical state of the lipid is an important determinant of the mobility of integral proteins.



The possible movements of phospholipids in a membrane

The Dynamic Nature of the Plasma Membrane





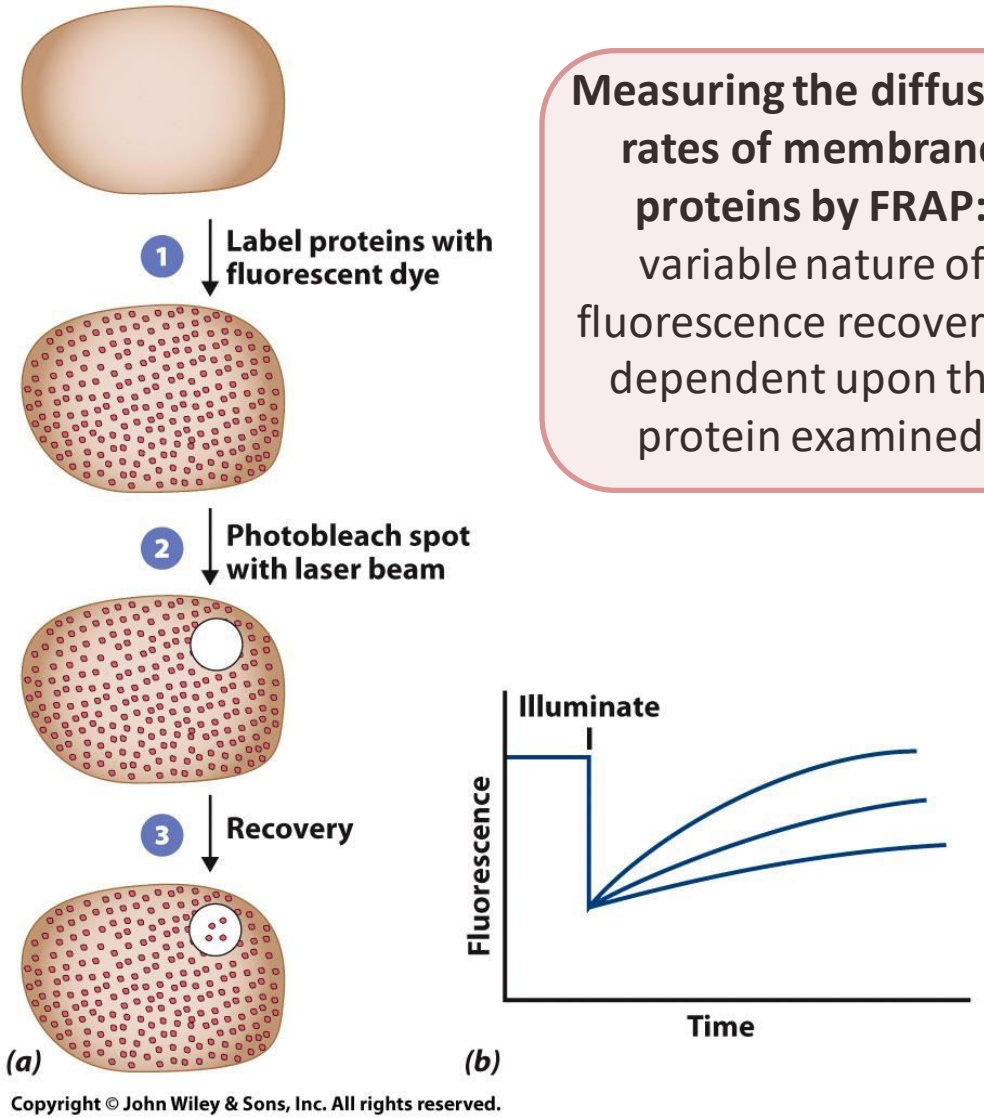
From L.D. Frye and Michael Edidin, J. Cell Science 7:328, 334, 1970; By permission of The Company of Biologists, Ltd. Courtesy of Michael Edidin, Johns Hopkins University. http://jcs.biologists.org/content/7/2/319.full.pdf+html?sid=d93ae648-abca-4f5d-90a6-a6d9726d7d30

Cell fusion to reveal mobility of membrane proteins: fusion of human and mouse cells

- The Diffusion of Membrane Proteins after Cell Fusion
  - **Cell fusion** is a technique whereby two different types of cells, or cells from two different species, can be fused to produce one cell with a common cytoplasm and a single, continuous plasma membrane.
  - **Cell fusion** be induced by certain viruses, or with polyethylene glycol.
  - Labeled proteins have shown that membrane proteins can move between fused cell.

The Dynamic Nature of the Plasma Membrane

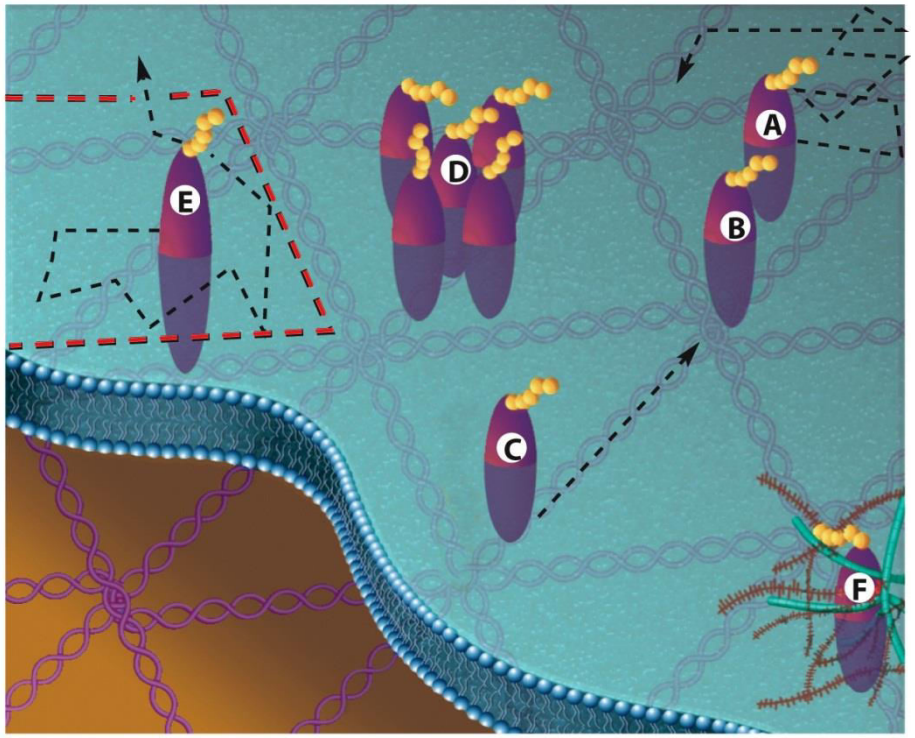
- Restrictions on Protein and Lipid Mobility
  - Proteins can be labeled and tracked by **fluorescence recovery after photobleaching (FRAP)** and **single particle tracking (SPT)**.
  - Proteins can be immobile, mobile in a directed manner, or exhibit random movement.



Measuring the diffusion rates of membrane proteins by FRAP: variable nature of fluorescence recovery is dependent upon the protein examined

The Dynamic Nature of the Plasma Membrane

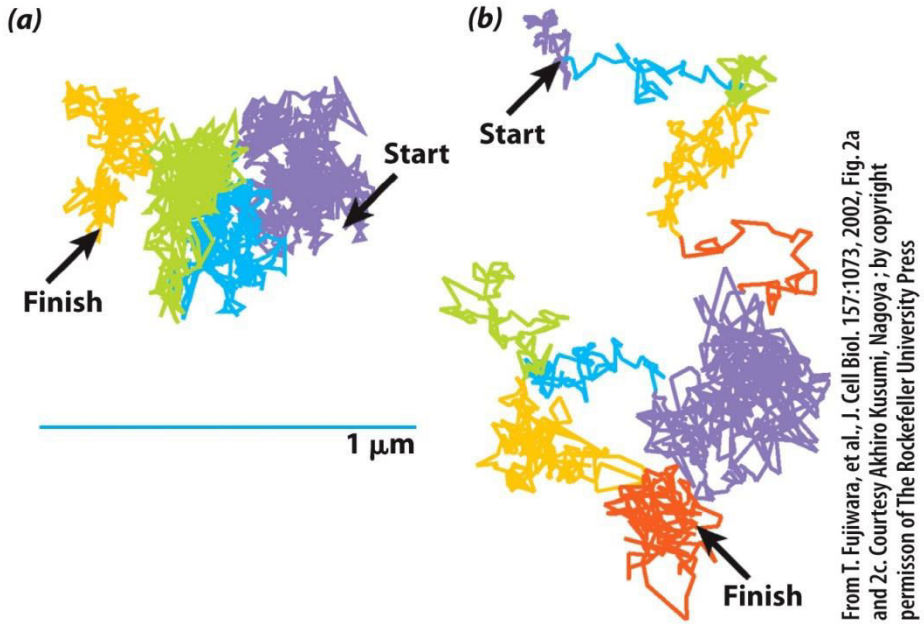
- Control of Membrane Protein Mobility
  - Protein movements are slower than predicted by protein size and membrane viscosity.
  - Protein movements are limited by interactions with the cytoskeleton, other proteins, and extracellular materials.
  - Techniques that can drag tagged proteins within the membrane, indicate that some proteins have barriers to lateral diffusion.
  - Genetically modified proteins missing either intracellular or extracellular domains are less restricted.



Patterns of movement of integral membrane proteins

The Dynamic Nature of the Plasma Membrane

- Membrane Lipid Mobility
  - Phospholipid diffusion is restricted within the bilayer.
  - Phospholipids are confined for very brief periods to certain areas and then hop from one confined area to another.
  - Fences restricting motion are constructed of rows of integral membrane proteins bound to the membrane skeleton by their cytoplasmic domains.

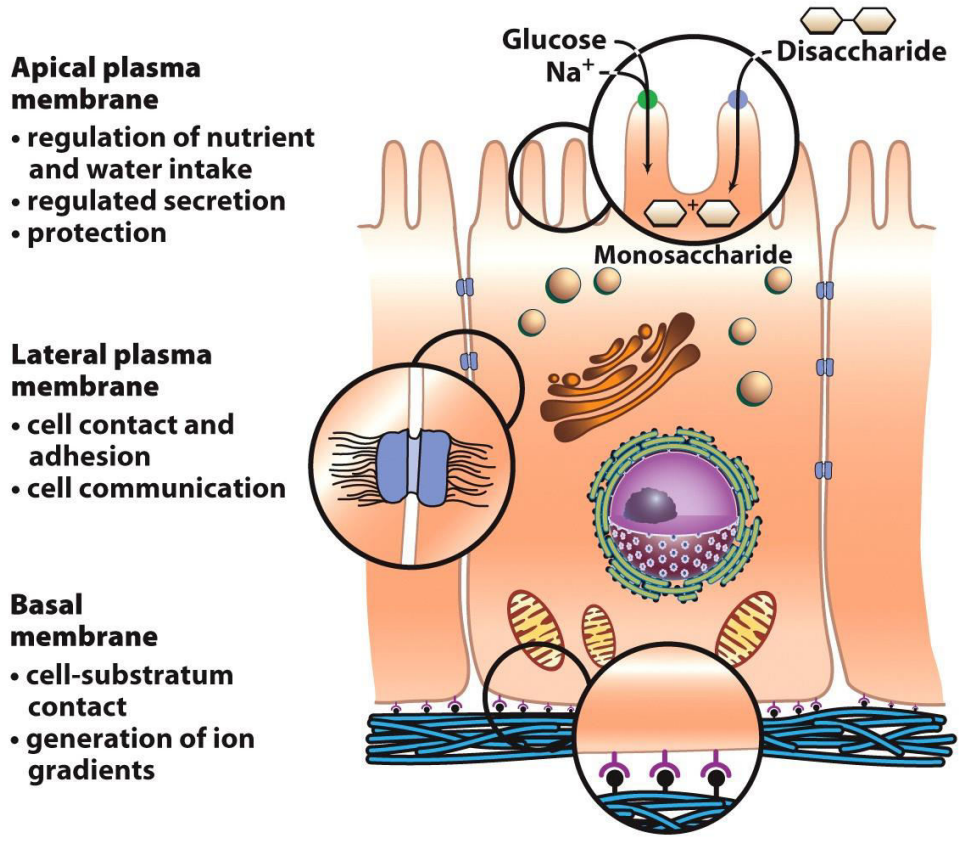


From T. Fujiwara, et al., J. Cell Biol. 157:1073, 2002, Fig. 2a and 2c. Courtesy Akhiro Kusumi, Nagoya ; by copyright permisson of The Rockefeller University Press

Experimental demonstration that diffusion of phospholipids within the plasma membrane is confined

The Dynamic Nature of the Plasma Membrane

- Membrane Domains and Cell Polarity
  - Differences in protein distribution are evident in cells of organized tissues.
  - In epithelia, the proteins of the apical membrane are distinct from those of the lateral and basal membranes
  - Highly differentiated sperm have a head, midpiece, and tail that is covered by a continuous membrane. Can distinguish these regions with antibody staining.



Differentiated functions of the plasma membrane of an epithelial cell.

The Dynamic Nature of the Plasma Membrane

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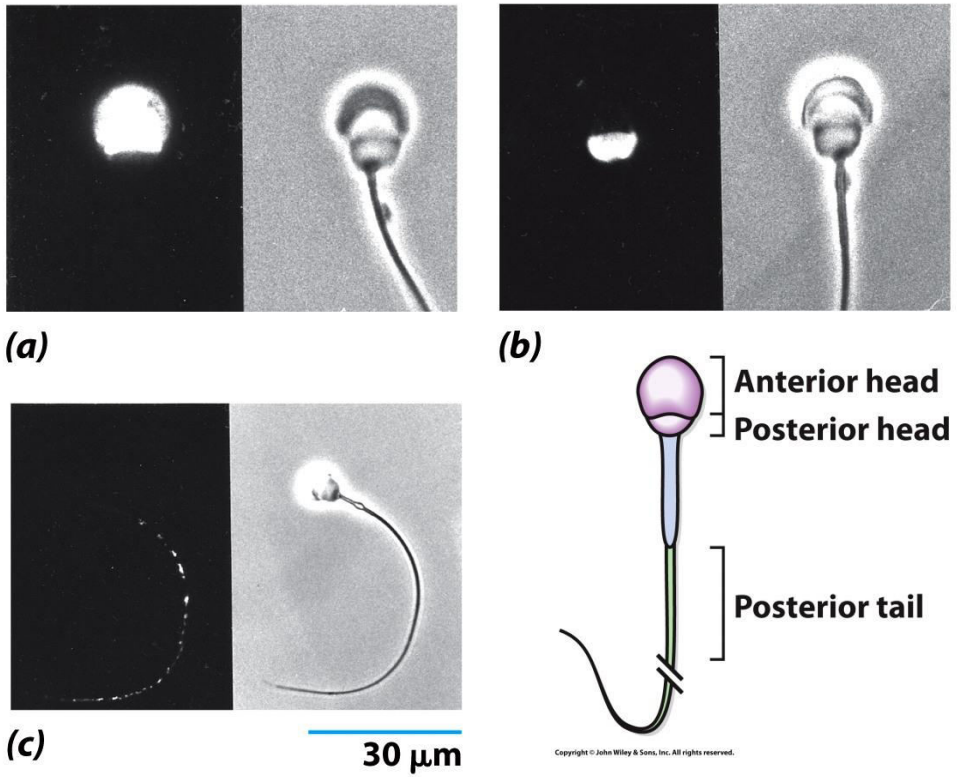
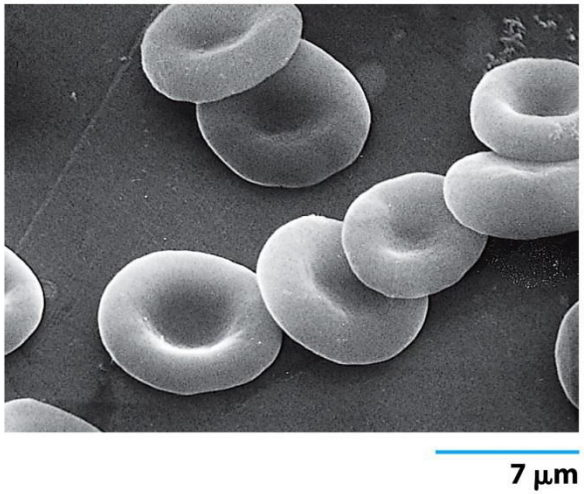


From Diana Gold Myles, Paul Primakoff, and Anthony R. Bellvé©, Cell 23:434, © 1981, with permission from Elsevier.

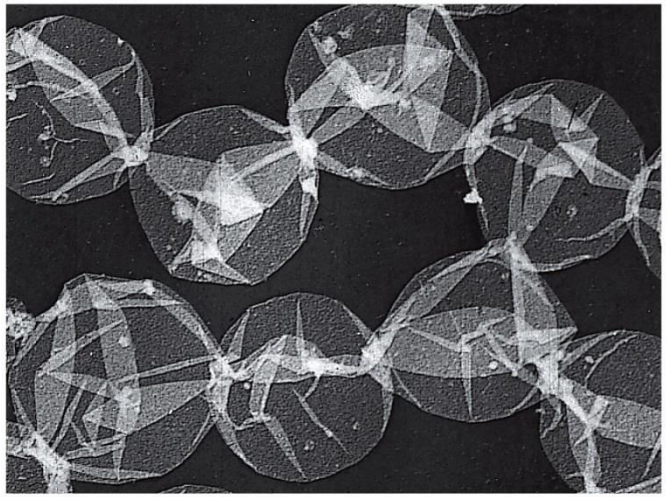
Differentiation of the mammalian sperm plasma membrane as revealed by fluorescent antibodies.

The Dynamic Nature of the Plasma Membrane



Courtesy François M.M. Morel, Richard F. Baker and Harold Wayland.

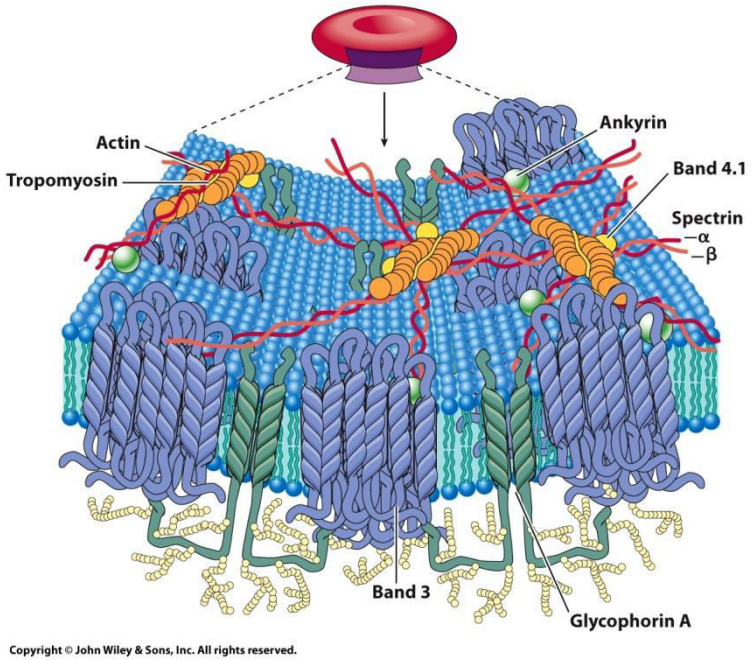
SEM of human erythrocytes and membrane ghosts



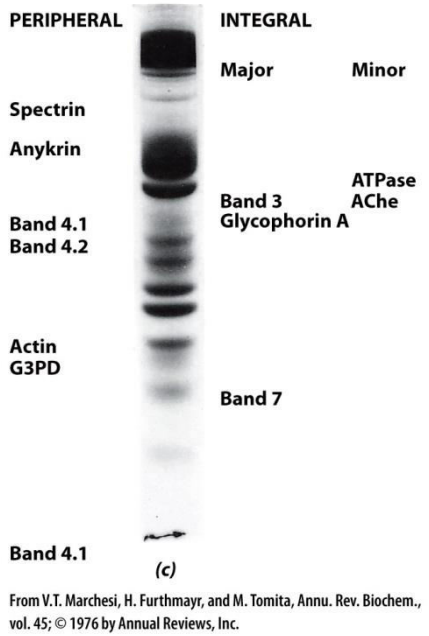
Courtesy Joseph Hoffmann, Yale University

- The Red Blood Cell: An Example of Plasma Membrane Structure
  - Homogeneous preparation of membrane "ghosts" can be prepared by *hemolysis*.
  - Membrane proteins can be purified and characterized by fractionation using SDS-PAGE electrophoresis.

The Dynamic Nature of the Plasma Membrane



Erythrocyte plasma membrane model viewed from the internal surface

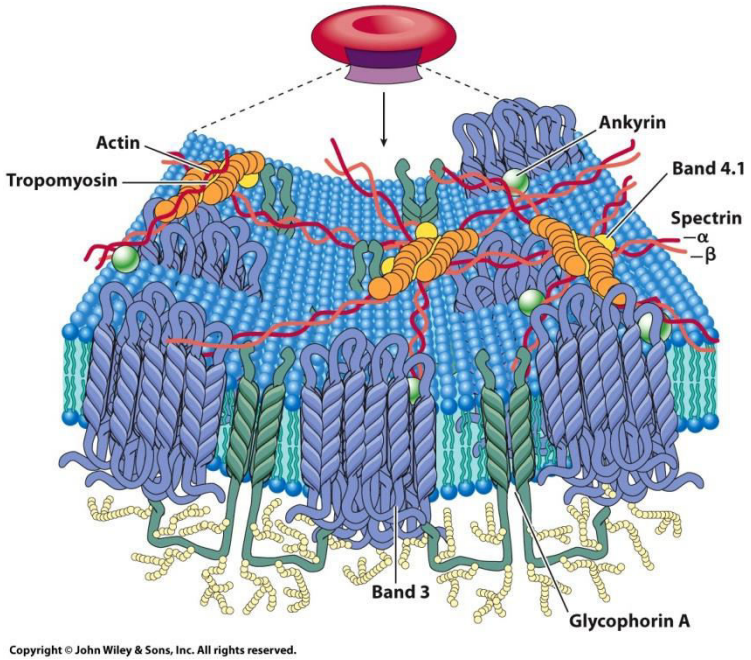


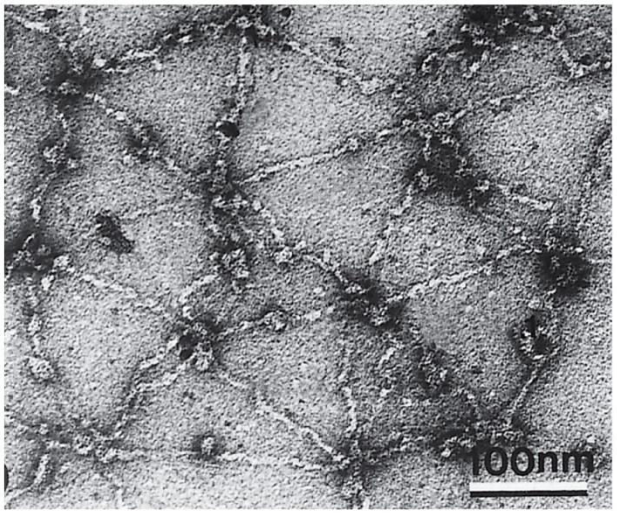
From V.T. Marchesi, H. Furthmayr, and M. Tomita, Annu. Rev. Biochem., vol. 45; © 1976 by Annual Reviews, Inc.

SDS–PAGE of membrane proteins

- Integral Proteins of the Erythrocyte Membrane
  - Band 3 is composed of two *homodimers* of a glycoprotein that exchanges Cl^- and HCO_3^- across the red cell membrane.
  - Glycophorin A is a dimer with 16 oligosaccharide chains bearing negative charges that may prevent red cells from clumping.

The Dynamic Nature of the Plasma Membrane





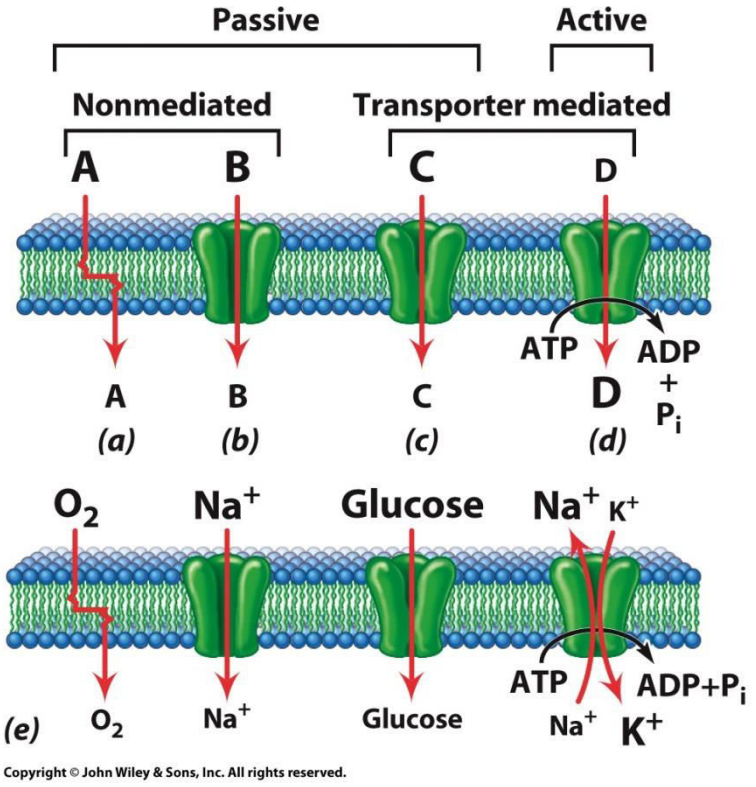
From Shih.Chun Liu, Laura H. Derick and Jiri Palek, J. Cell Biol. 104:529, 1987, Fig 1b. Reproduced with permission of Rockefeller University Press

EM: inner membrane skeleton proteins

- The Erythrocyte Membrane Skeleton
  - The major component of the internal membrane skeleton is *spectrin*.
  - Spectrin molecules are attached to the membrane surface by noncovalent bonds to *ankyrin*, a peripheral membrane protein which is noncovalently bonded to band 3.
  - Spectrin is linked to other cytoplasmic proteins, such as *actin* and *tropomyosin*, which maintains the integrity of the membrane.

(8.7) The Movement of Substances Across Cell Membranes

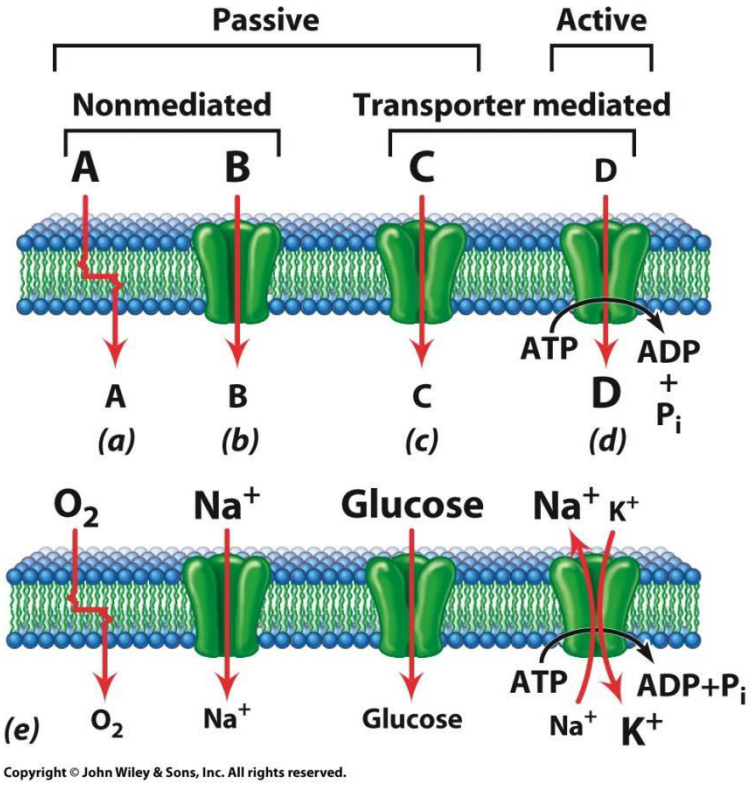
- Selective permeability allows for separation and exchange of materials across the plasma membrane
  - *Net flux* is the difference between *influx* and *efflux* of materials.
  - Flux can occur by passive diffusion and/or active transport.



Four basic mechanisms by which solute molecules move across membranes

The Movement of Substances Across Cell Membranes

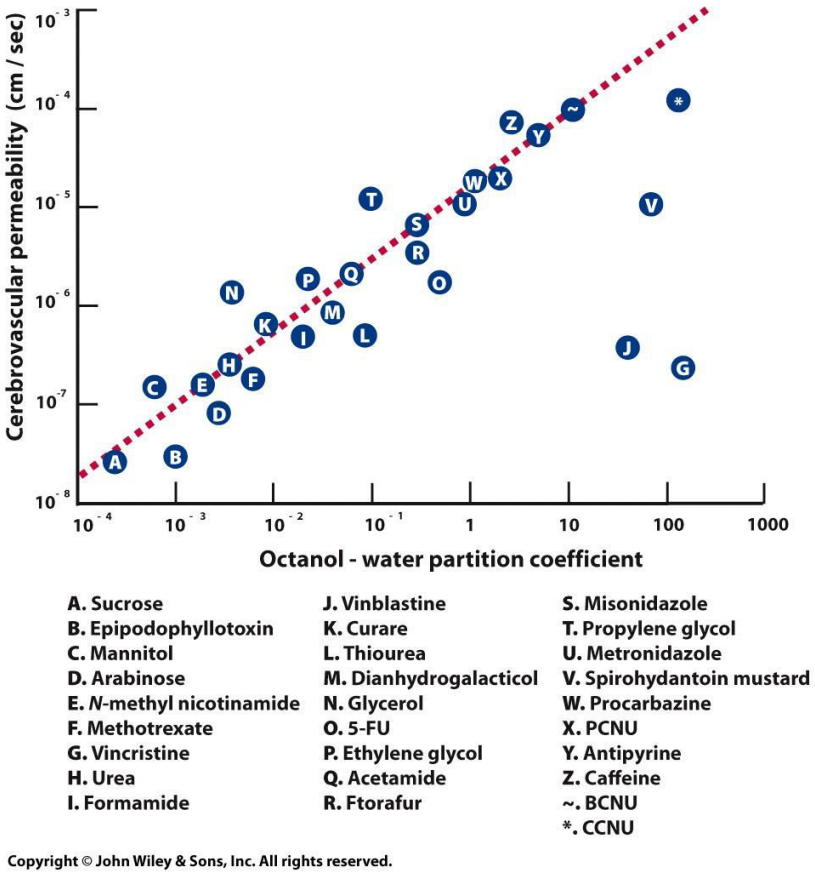
- The Energetics of Solute Movement
  - **Diffusion** is the spontaneous movement of material from a region of high concentration to a region of low concentration.
  - The free-energy change during diffusion of nonelectrolytes depends on the concentration gradient.
  - The free-energy change during diffusion of electrolytes depends on the electrochemical gradient.



Four basic mechanisms by which solute molecules move across membranes

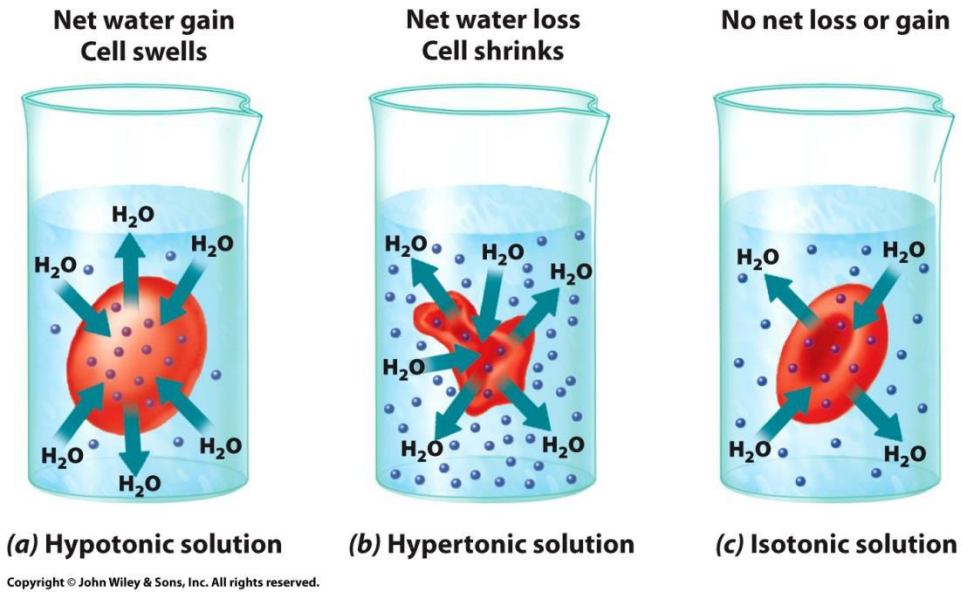
The Movement of Substances Across Cell Membranes

- Diffusion of Substances through Membranes
  - Diffusion requires both a concentration gradient and membrane permeability.
  - Lipid permeability is determined by the **partition coefficient**, molecular size, and polarity.



The relationship between partition coefficient and membrane permeability.

The Movement of Substances Across Cell Membranes

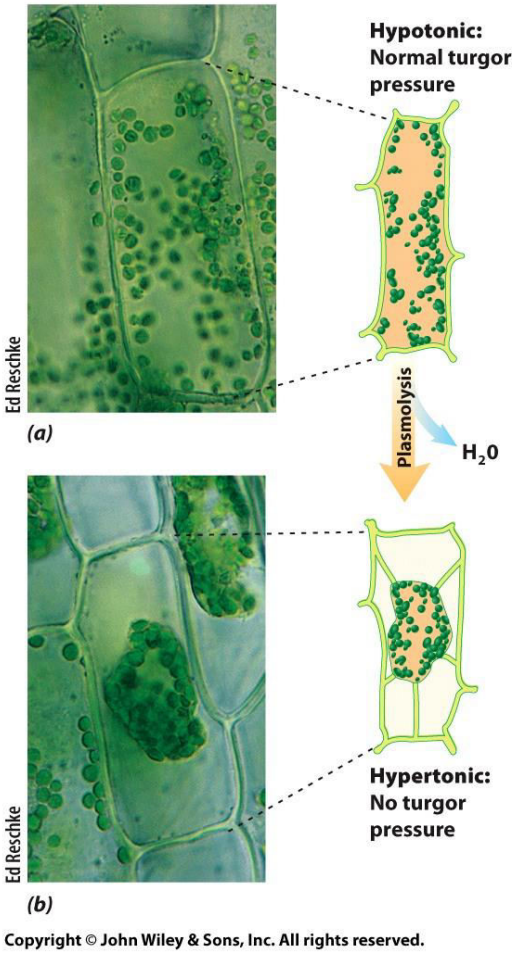


The effects of differences in the concentration of solutes on opposite sides of the plasma membrane

- The Diffusion of Water through Membrane
  - Diffusion of water through a semipermeable membrane is called **osmosis**.
  - Water diffuses from areas of lower solute concentration to areas of higher solute concentration.
  - Cells swell in **hypotonic** solution, shrink in **hypertonic** solutions, and remain unchanged in **isotonic** solutions.

The Movement of Substances Across Cell Membranes

- The Diffusion of Water through Membranes
  - Plant cells develop turgor in hypotonic solutions because cell walls prevent swelling.
  - In hypertonic solutions the plant cell undergoes **plasmolysis**.
  - *Aquaporins* are specialized protein channels that allow passive movement of water.



The effects of osmosis on a plant cell

The Movement of Substances Across Cell Membranes

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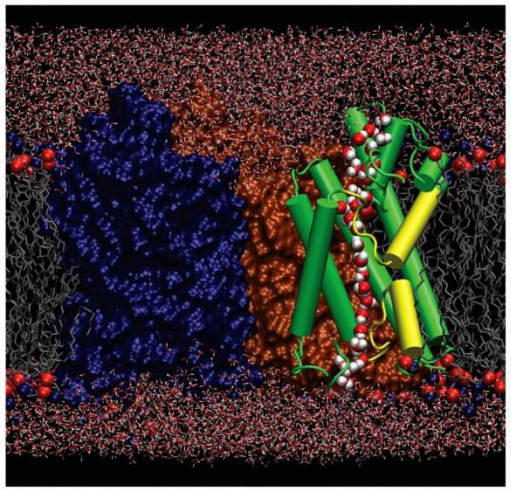
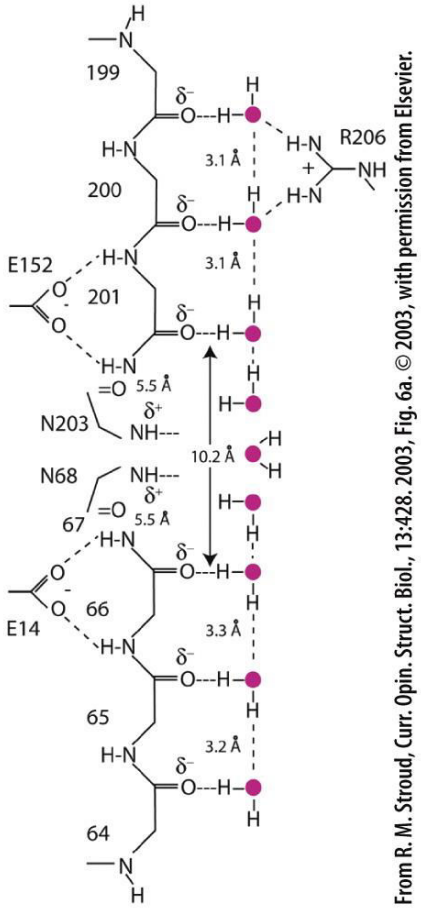


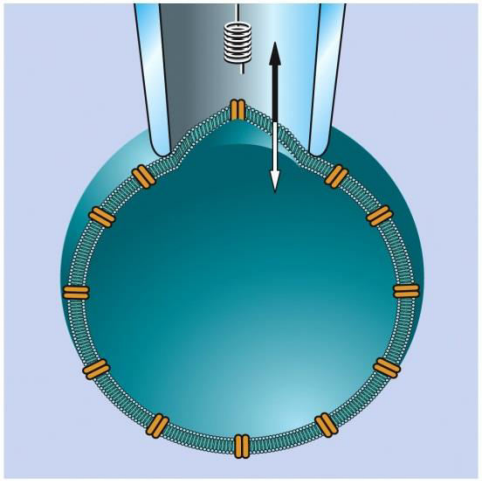
From Benoit Roux and Klaus Schulten, Structure 12:1344, © 2004, by permission of Elsevier.

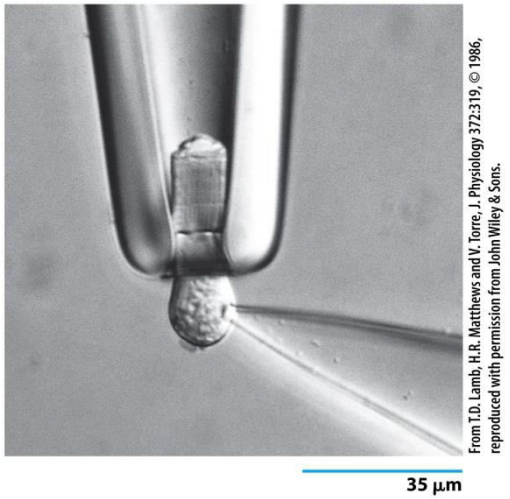
Passage of water molecules through an aquaporin channel



From R. M. Stroud, Curr. Opin. Struct. Biol., 13:428. 2003, Fig. 6a. © 2003, with permission from Elsevier.

The Movement of Substances Across Cell Membranes



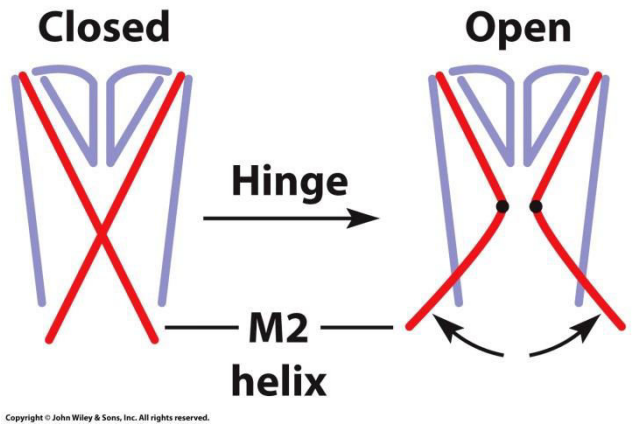


Measuring ion conductance by patch-clamp recording

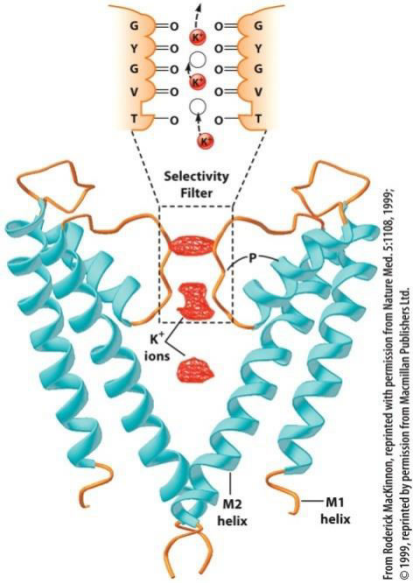
- The Diffusion of Ions through Membranes
  - Ions cross membranes through **ion channels**.
  - Ion channels are selective and bidirectional, allowing diffusion in the direction of the electrochemical gradient.
  - Superfamilies of ion channels have been discovered by cloning analysis of protein sequences, site directed mutagenesis, and patch-clamping experiments.

The Movement of Substances Across Cell Membranes

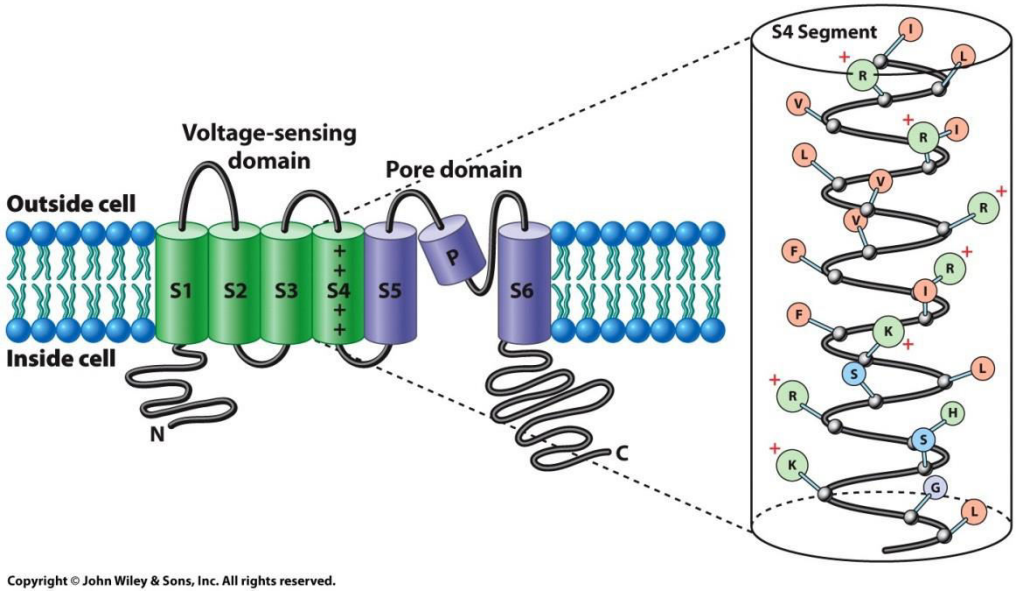
- The **voltage-gated** potassium channel (K_v) contains six membrane-spanning helices.
  - Both Na and C termini are cytoplasmic.
  - A single channel has 4 subunits arranged to create an ion-conducting pore.
  - Channel can be opened, closed, or inactivated.
  - S4 transmembrane helix is voltage sensitive.
  - Crystal structure of bacterial K channel shows that a short amino acid domain selects K and no other ions.



3D structure of the bacterial KcsA channel and K^+ ion selectivity



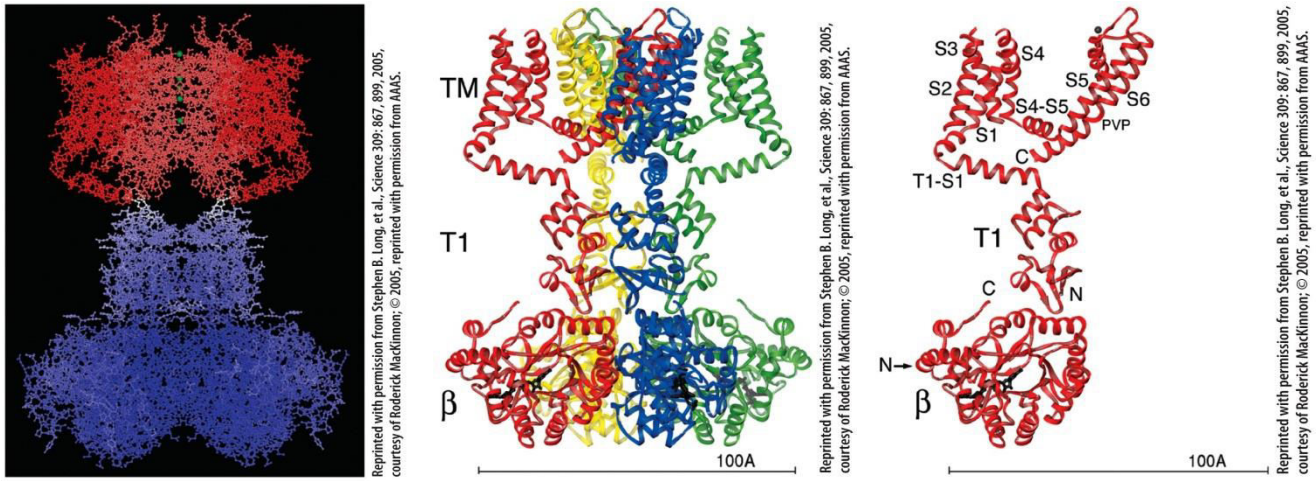
The Movement of Substances Across Cell Membranes



The structure of a eukaryotic, voltage-gated K^+ channel

- Eukaryotic K_v channels
  - Contain six membrane-associated helices (S1-S6).
  - Six helices can be grouped into two domains:
    - **Pore domain** – permits the selective passage of K^+ ions.
    - **Voltage-sensing domain** – consists of helices S1-S4 that senses the voltage across the plasma membrane.

The Movement of Substances Across Cell Membranes



Three-dimensional structure of a voltage-gated mammalian K^+ channel

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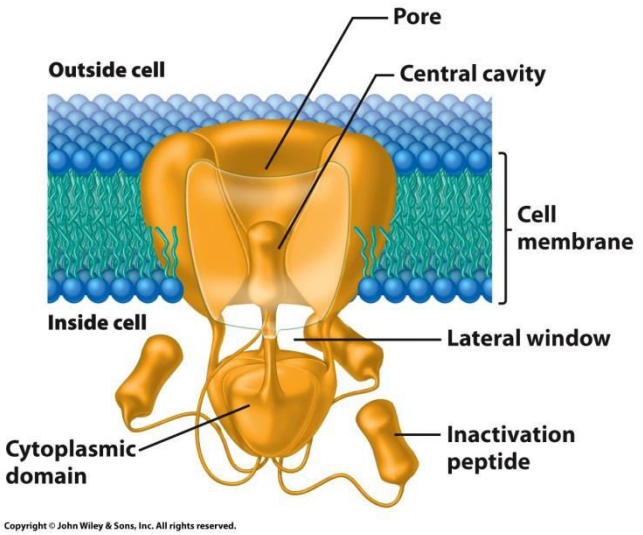
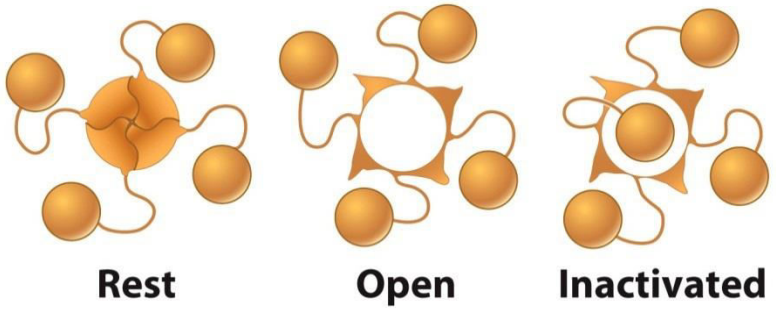
The Movement of Substances Across Cell Membranes

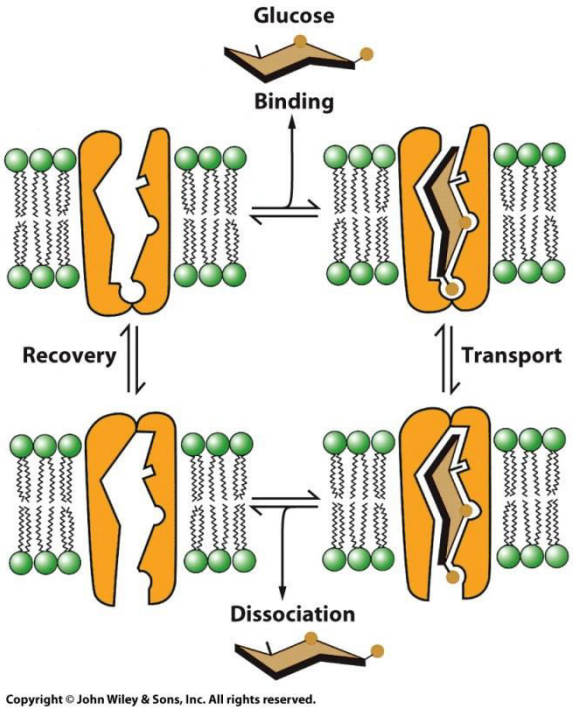
- Eukaryotic K_v channels
  - Once opened, more than 10 million K^+ ions can pass through per second.
  - After the channel is open for a few milliseconds, the movement of K^+ ions is "automatically" stopped by a process known as inactivation.
  - Can exist in three different states: open, inactivated, and closed.



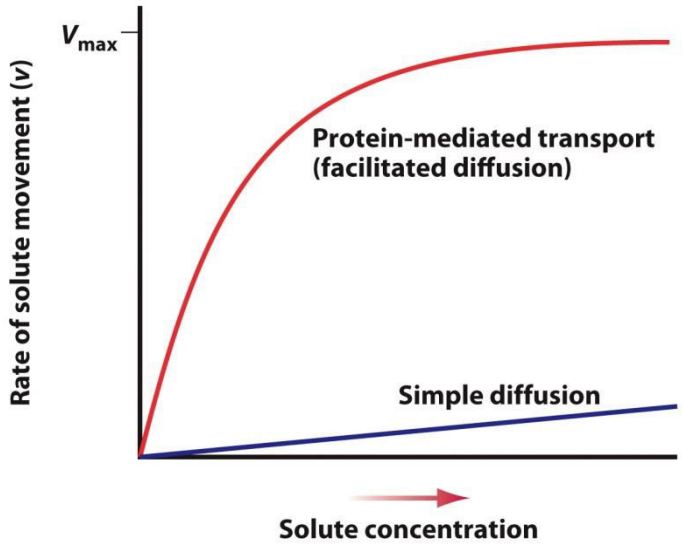
Conformational states of a voltage-gated K^+ ion channel



The Movement of Substances Across Cell Membranes

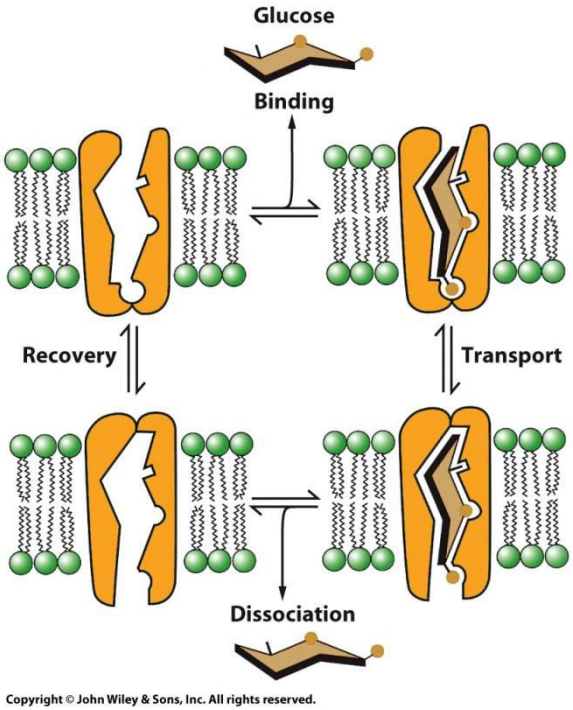


Schematic model and kinetics of facilitated diffusion compared to simple diffusion

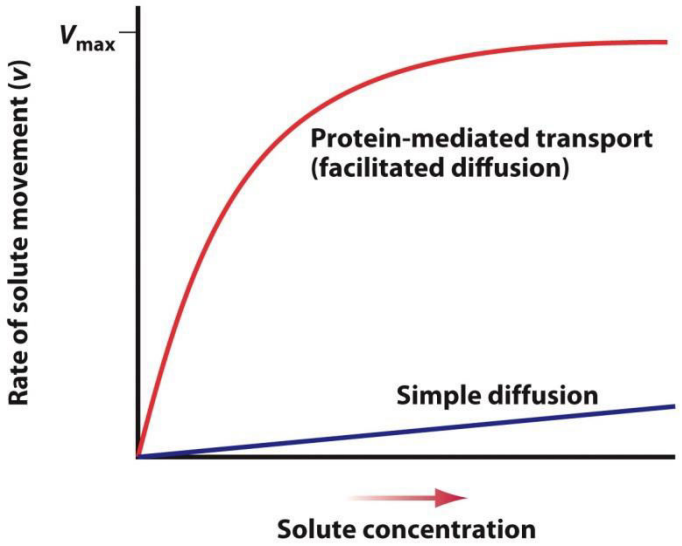


- Facilitated Diffusion
  - Large or hydrophilic substances require a **facilitative transporter** to cross membranes.
  - **Facilitative diffusion** is passive, specific, saturable, and regulated.

The Movement of Substances Across Cell Membranes



Schematic model and kinetics of facilitated diffusion compared to simple diffusion



- The Glucose Transporter: An Example of Facilitated Diffusion
  - The gradient for glucose entry into the cell is maintained by phosphorylation of glucose in the cytoplasm.
  - Insulin stimulates glucose uptake by causing the insertion into the cell membrane of vesicles containing preformed glucose transporters.

The Movement of Substances Across Cell Membranes

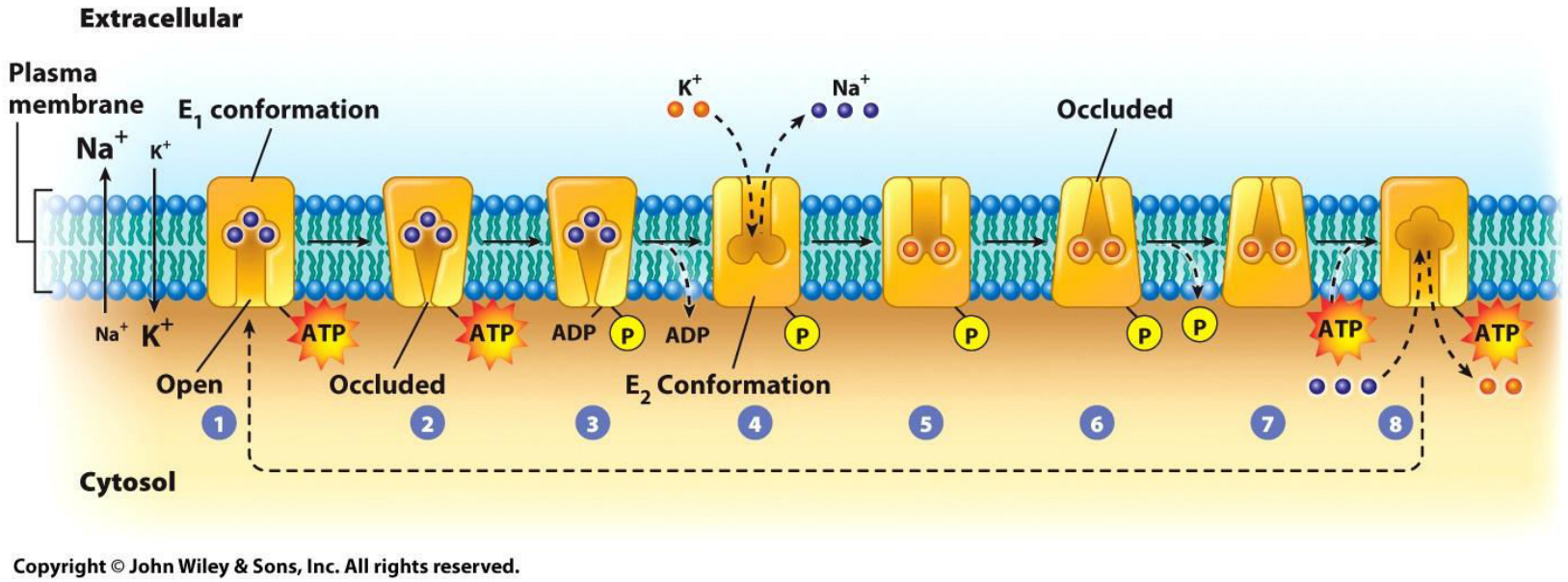
- **Active Transport**
  - Maintains the gradients for potassium, sodium, calcium, and other ions across the cell membrane.
  - Couples the movement of substances against gradients to ATP hydrolysis.

Table 8.3 Ion Concentrations Inside and Outside of a Typical Mammalian Cell

	Extracellular concentration	Intracellular concentration	Ionic gradient
Na^+	150 mM	10 mM	15×
K^+	5 mM	140 mM	28×
Cl^-	120 mM	10 mM	12×
Ca^{2+}	10^{-3} M	10^{-7} M	10,000×
H^+	$10^{-7.4}$ M (pH of 7.4)	$10^{-7.2}$ M (pH of 7.2)	Nearly 2×

The ion concentrations for the squid axon are given on page 363.

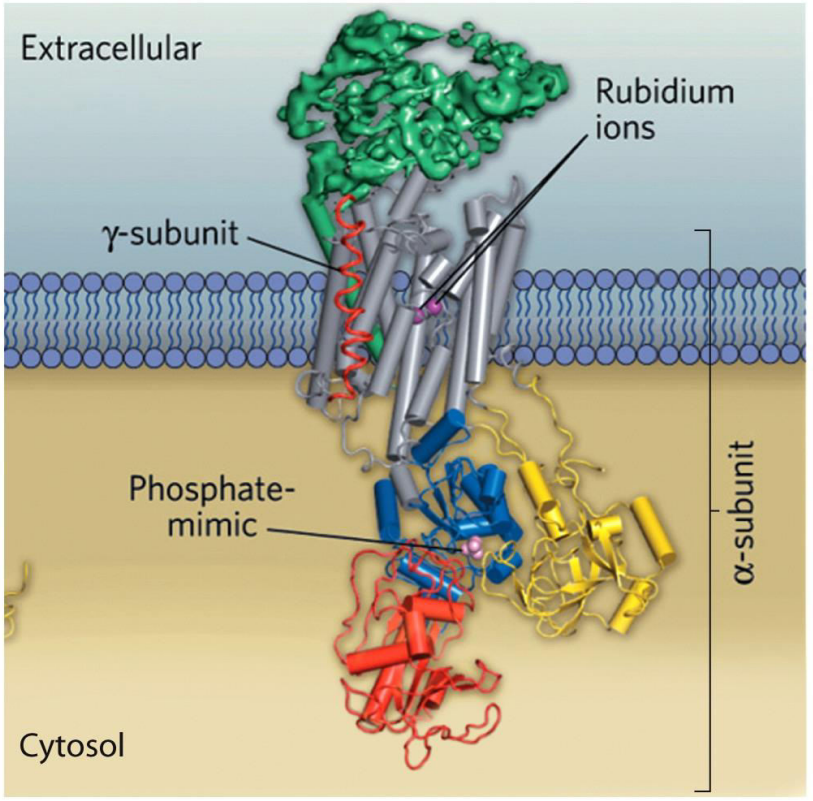
The Movement of Substances Across Cell Membranes



- Coupling Active Transport to ATP Hydrolysis
  - The Na^+/K^+ ATPase (*sodium-potassium pump*)requires K^+ outside, Na^+ inside, and is inhibited by ouabain.
  - The ratio of Na^+:K^+ pumped is 3:2.
  - The ATPase is a P-type pump, in which phosphorylation causes changes in conformation and ion affinity that allow transport against gradients.

The Movement of Substances Across Cell Membranes

- Coupling Active Transport to ATP Hydrolysis
  - The Na^+/K^+ ATPase is found only in animals and evolved early as a means to regulate volume and create large Na^+ and K^+ gradients.



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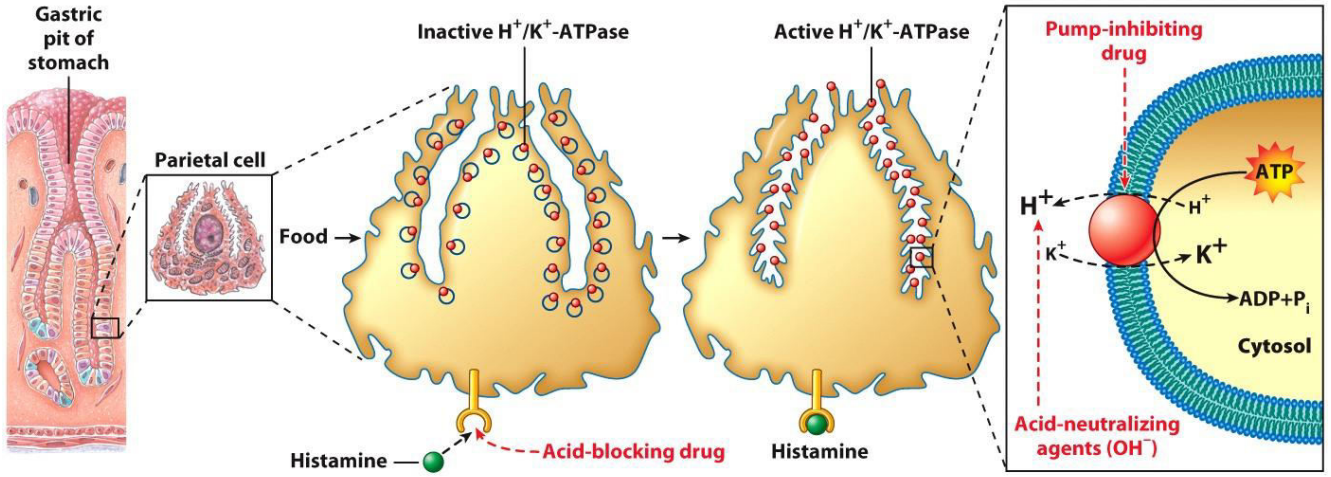
The Na^+/K^+-ATPase pump: A model of the E2 conformation

The Movement of Substances Across Cell Membranes

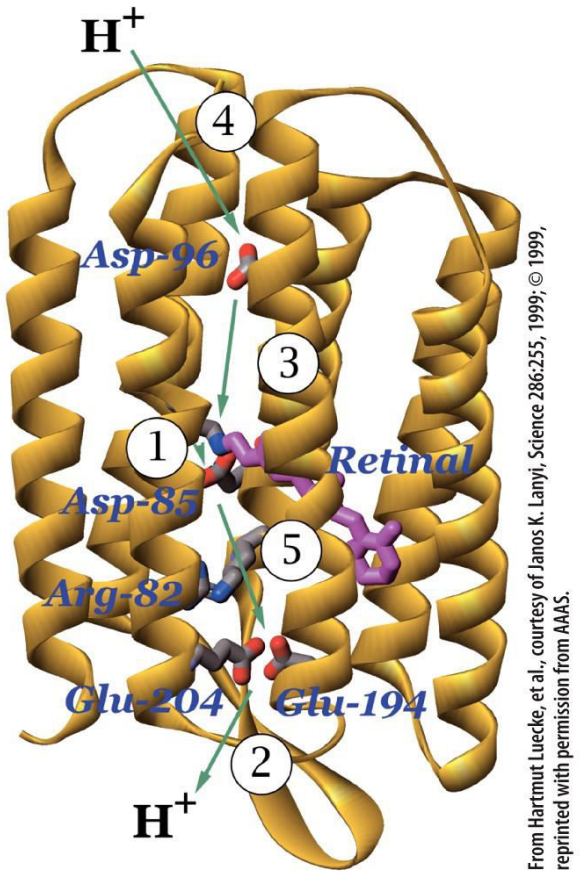


Control of acid secretion in the stomach

- Other Ion Transport Systems
  - Other P-type pumps include H^+ and Ca^{2+} ATPases, and H^+/K^+-ATPases.
  - Vacuolar (V-type) pumps use ATP, but are not phosphorylated during pumping.
  - ATP-binding cassette (ABC) transporters have regulatory ATP-binding sites.

The Movement of Substances Across Cell Membranes

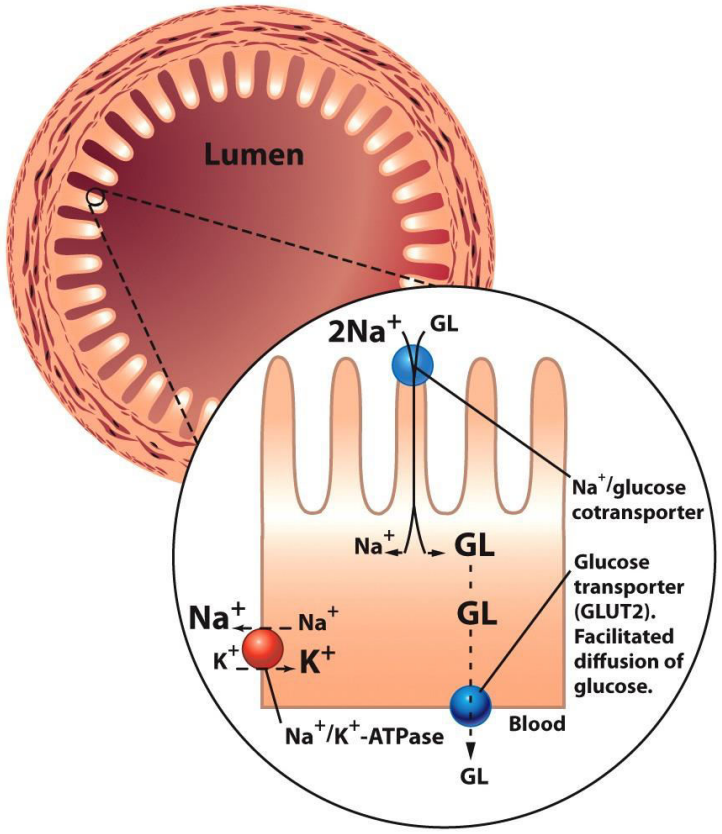
- Using Light Energy to Actively Transport Ions
  - Some archaebacteria use a protein called *bacteriorhodopsin*, which absorbs light energy to transport protons out of the cell.
  - The proton gradient is used to make ATP.



Bacteriorhodopsin: a light-driven proton pump

The Movement of Substances Across Cell Membranes

- **Co-transport:** Coupling Active Transport to Existing Ion Gradients
  - Gradients created by active ion pumping store energy that can be coupled to other transport processes.
- **Secondary transport**: the use of energy stored in an ionic gradient



Secondary transporter: the Na+ gradient helps to transport glucose by a Na+/glucose co-transporter

The Movement of Substances Across Cell Membranes

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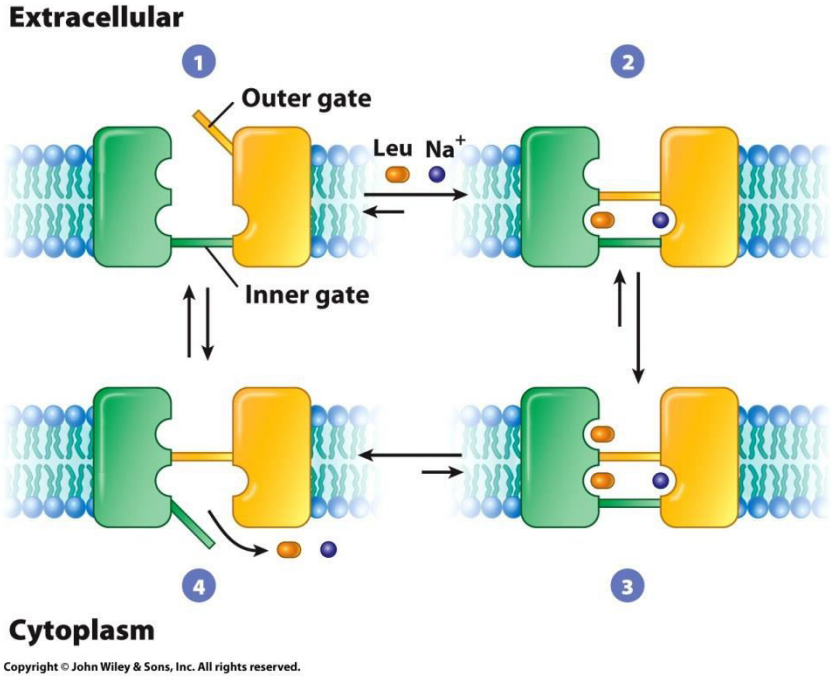
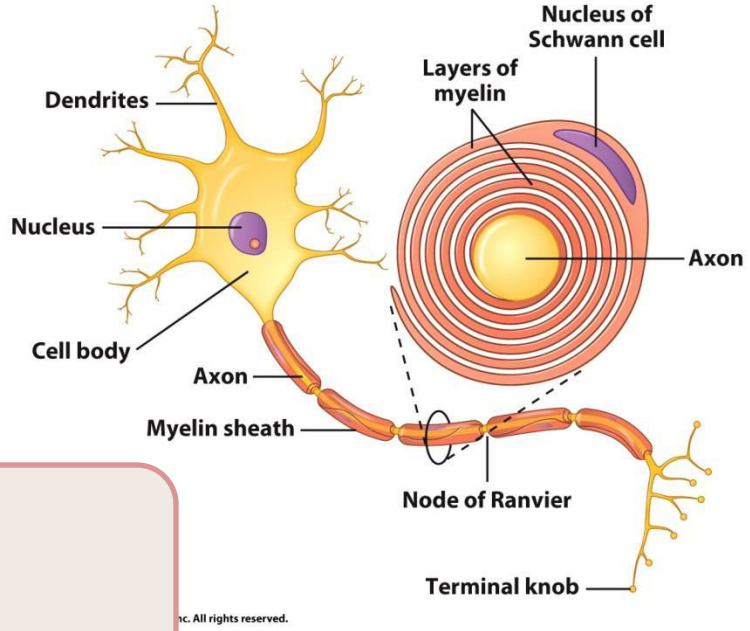


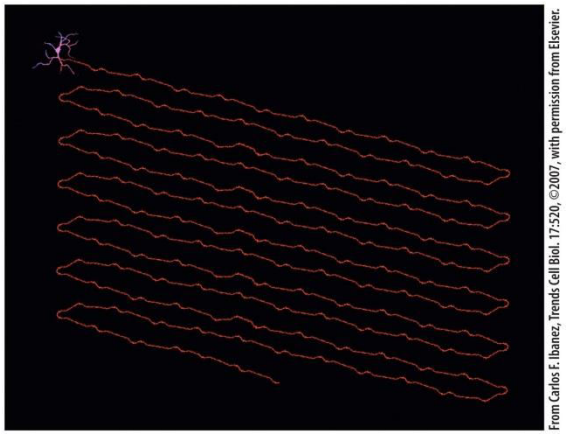
Secondary transporter: the Na+ gradient helps to transport leucine into bacteria

(8.8) Membrane Potentials and Nerve Impulses

- Potential differences exist when charges are separated.
  - Membrane potentials have been measured in all types of cells.
  - *Neurons* are specialized cells for information transmission using changes in membrane potentials.
    - **Dendrites** receive incoming information.
    - Cell body contains the nucleus and metabolic center of the cell.
    - The **axon** is a long extension for conducting outgoing impulses.
    - Most neurons are wrapped by **myelin-sheath.**

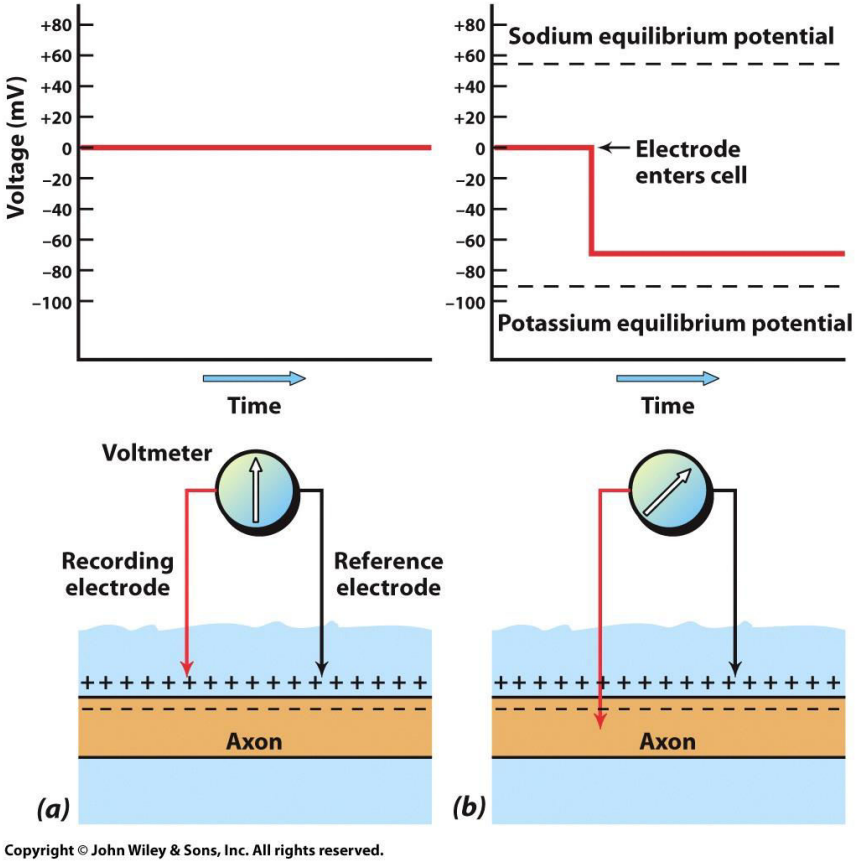


Composite micrograph of one rat hippocampal neuron



Membrane Potentials and Nerve Impulses

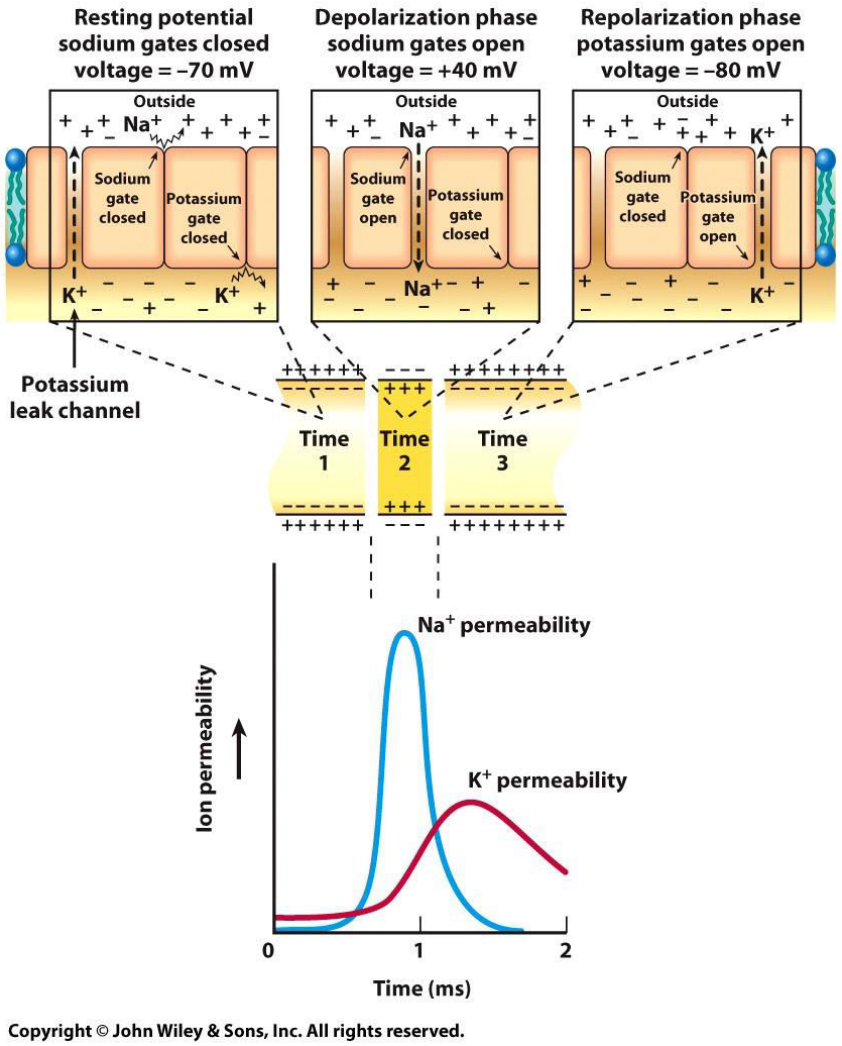
- The **Resting Potential**
  - It is the membrane potential of a nerve or muscle cell, subject to changes when activated.
  - K^+ gradients maintained by the Na^+/K^+-ATPase are responsible for resting potential.
  - Nernst equation used to calculate the voltage equivalent of the concentration gradients for specific ions.
  - Negative resting membrane potential is near the negative Nernst potential for K^+ and far from the positive Nernst potential for Na^+.



Measuring a membrane's resting potential

Membrane Potentials and Nerve Impulses

- The Action Potential (AP)
  - When cells are stimulated, Na^+ channels open, causing membrane **depolarization**.
  - When cells are stimulated, voltage-gated Na+ channels open, triggering the AP.
  - Na+ channels are inactivated immediately following an AP, producing a short *refractory period* when the membrane cannot be stimulated.
  - Excitable membranes exhibit *all-or-none* behavior.



Formation of an action potential

Membrane Potentials and Nerve Impulses

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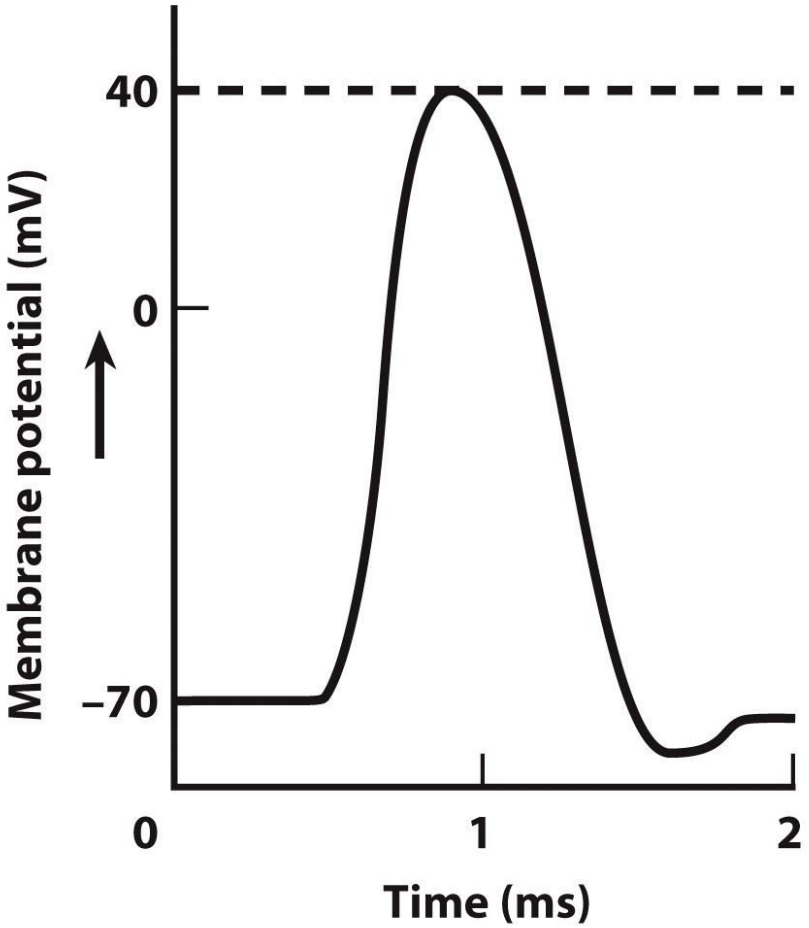
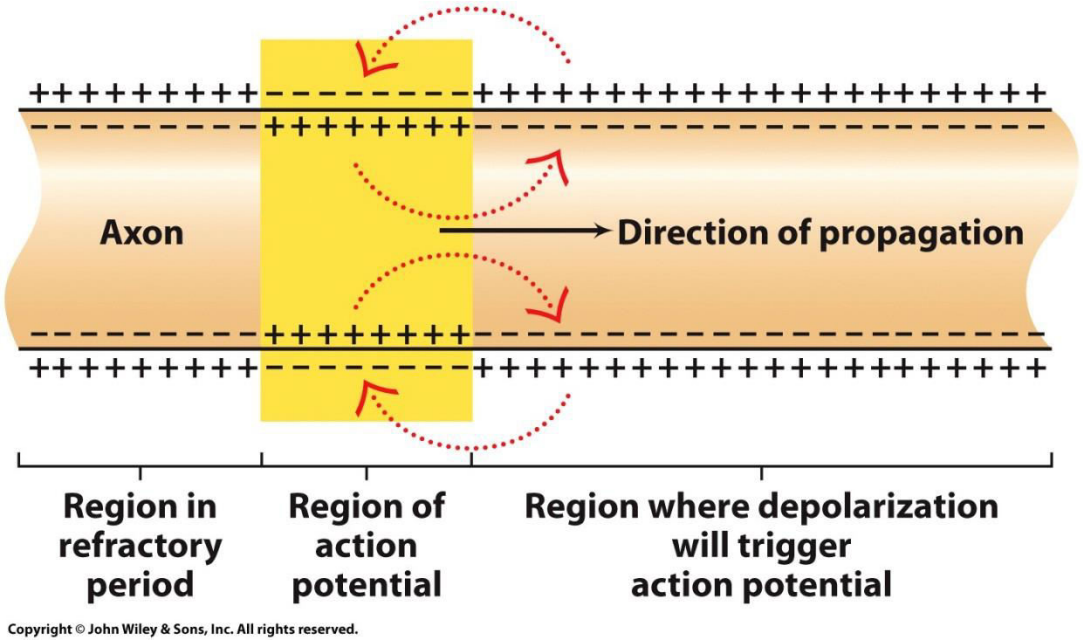


Formation of an action potential

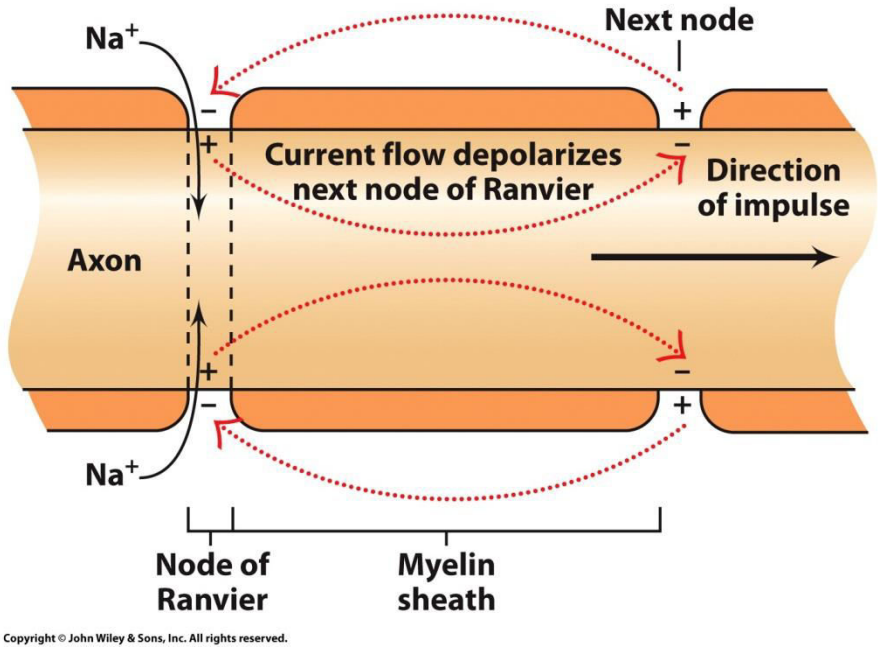
Membrane Potentials and Nerve Impulses



Propagation of an impulse results from the local flow of ions unidirectionally

- Propagation of Action Potentials as an Impulse
  - APs produce local membrane currents depolarizing adjacent membrane regions of the membrane that *propagate* as a **nerve impulse**.
  - *Speed Is of the Essence*: Speed of neural impulse depends on axon diameter and whether axon is myelinated.
    - Resistance to current flow decreases as diameter increases.
    - Myelin sheaths cause **saltatory conduction**.

Membrane Potentials and Nerve Impulses



Saltatory conduction: Propagation of an impulse by forming an action potential only at the nodes of Ranvier

- Propagation of Action Potentials as an Impulse
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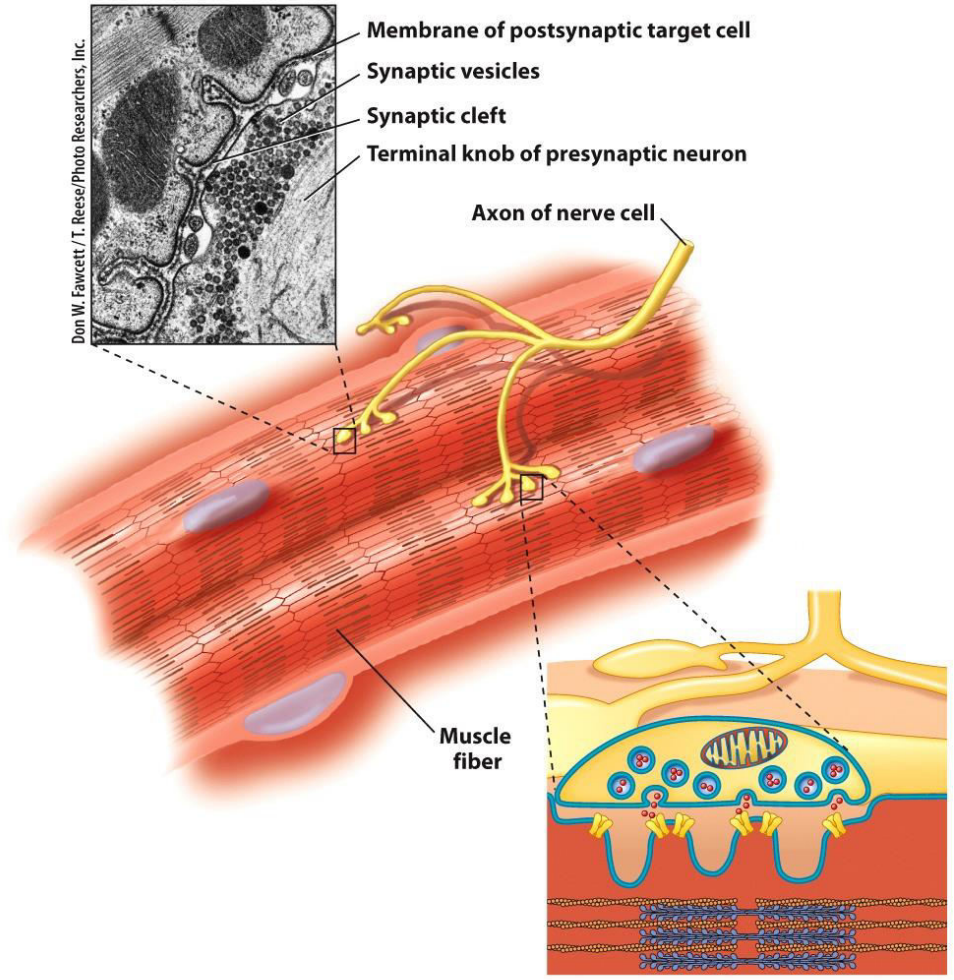
Membrane Potentials and Nerve Impulses

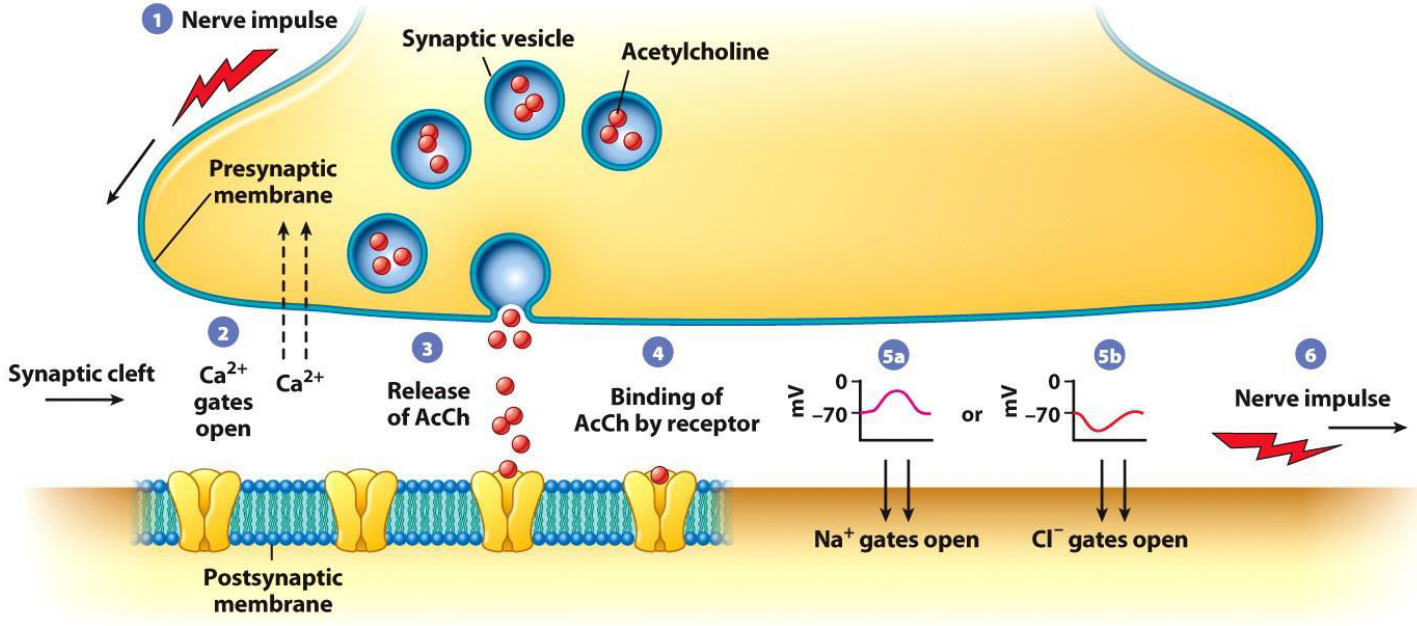
- Neurotransmission: Jumping the Synaptic Cleft
  - **Presynaptic neurons** communicate with **postsynaptic neurons** at a specialized junction, called the **synapse**, across a gap (**synaptic cleft**).
  - Chemicals (**neurotransmitters**) released from the presynaptic cleft diffuse to receptors on the postsynaptic cell.
  - Bound transmitter can depolarize (excite) or hyperpolarize (inhibit) the postsynaptic cell.
  - Transmitter action is terminated by reuptake or enzymatic breakdown.



The neuromuscular junction

Membrane Potentials and Nerve Impulses

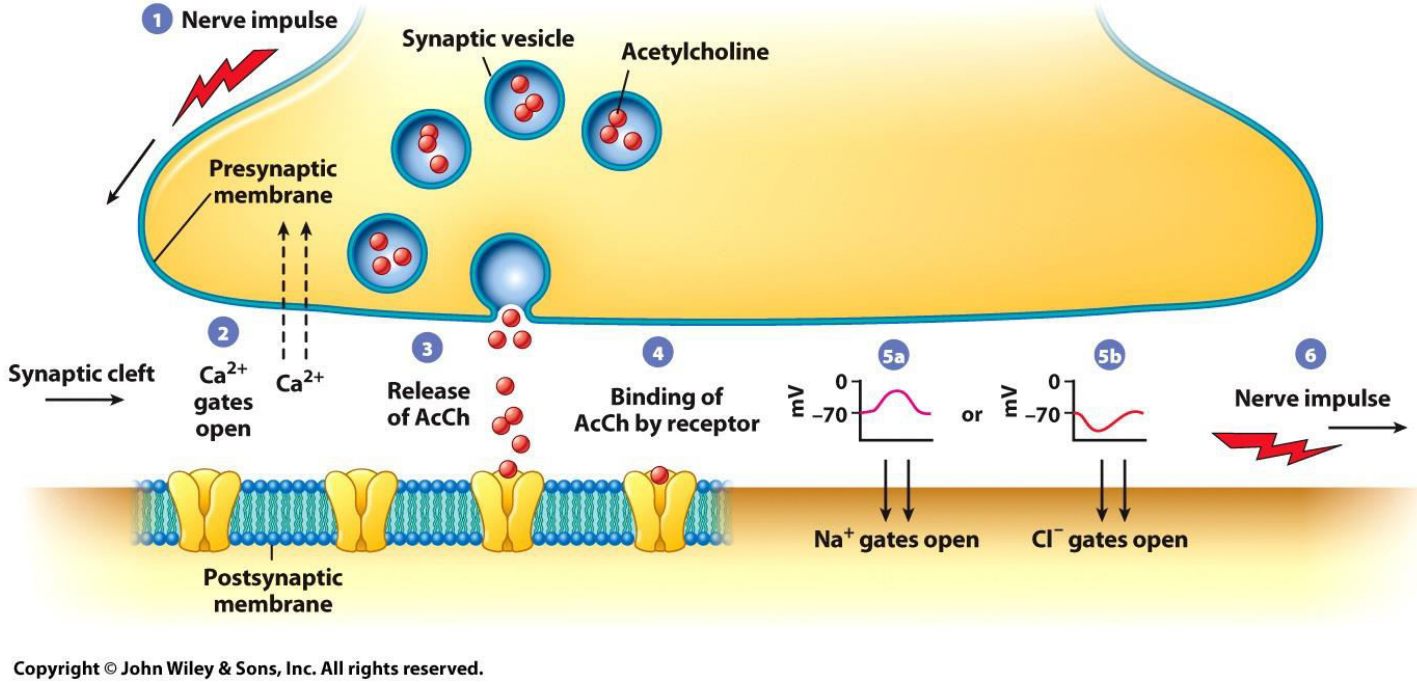
- Depolarization of pre-synaptic cell causes Ca^{2+} channels in membrane to open, Ca^{2+} stimulates fusion of vesicles with membrane
- Neurotransmitter binding to ion channel receptors can either stimulate or inhibit action potential



The sequence of events during synaptic transmission with acetylcholine as the neurotransmitter

Membrane Potentials and Nerve Impulses

- Actions of Drugs on Synapses
  - Interference with the destruction or reuptake of neurotransmitters can have dramatic physiological and behavioral effects.
  - Examples include: antidepressants, marijuana

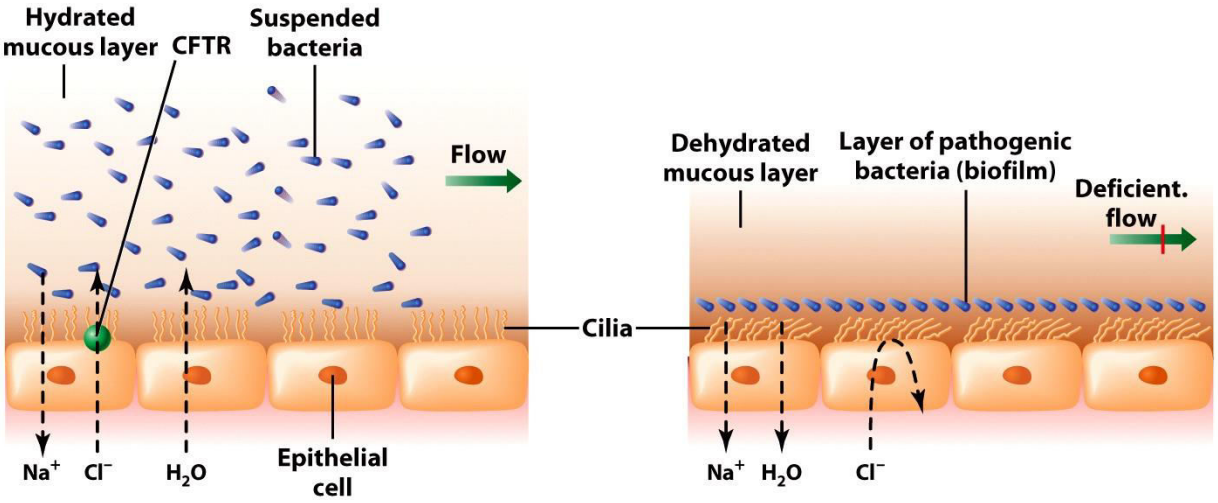


The sequence of events during synaptic transmission with acetylcholine as the neurotransmitter

Membrane Potentials and Nerve Impulses

- Synaptic Plasticity
  - Synapses connecting neurons to their neighbors can become strengthened over time by long term potentiation (LTP).
  - The NMDA receptor binds to the neurotransmitter glutamate and opens an internal cation channel.
  - Subsequent influx of Ca^{2+} ions triggers a cascade of biochemical changes that lead to synaptic strengthening.
  - LTP inhibitors reduce the learning ability of laboratory animals.

The Human Perspective:
Defects in Ion Channels as a Cause of Inherited Disease

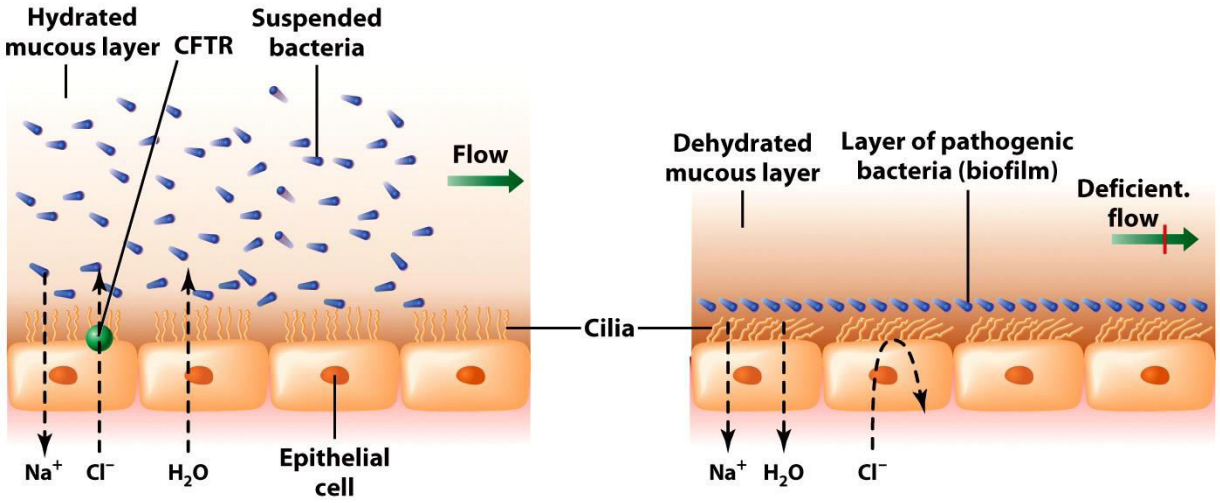


Airway epithelium from normal individual

Airway epithelium from CF patient

- Several inherited disorders have been linked to mutations in genes encoding ion proteins channels.
- *Cystic fibrosis (CF)* is a genetic disease characterized by abnormal fluid secretions from tissues and caused by a defective chloride channel.
- Genetic analysis revealed mutations in an ABC transporter (the *CFTR* polypeptide) with two nucleotide-dependent regulatory sites.

The Human Perspective:
Defects in Ion Channels as a Cause of Inherited Disease



Airway epithelium from normal individual

Airway epithelium from CF patient

- A defect prevents normal insertion of the CFTR polypeptide into the membrane.
- CF has recently been linked to over 1,000 mutations.
- CF is a good candidate for gene therapy and other therapies.
- In total, there have been 25 published clinical trials for CF.

The Human Perspective:
Defects in Ion Channels as a Cause of Inherited Disease

Table 1

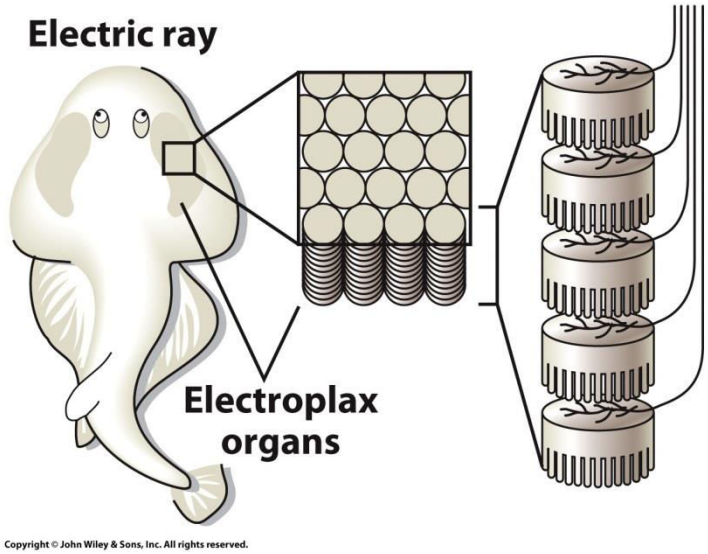
Inherited disorder	Type of channel	Gene	Clinical consequences
Familial hemiplegic migraine (FHM)	Ca^{2+}	*CACNL1A4*	Migraine headaches
Episodic ataxia type-2 (EA-2)	Ca^{2+}	*CACNL1A4*	Ataxia (lack of balance and coordination)
Hypokalemic periodic paralysis	Ca^{2+}	*CACNL1A3*	Periodic myotonia (muscle stiffness) and paralysis
Episodic ataxia type-1	K^+	*KCNA1*	Ataxia
Benign familial neonatal convulsions	K^+	*KCNQ2*	Epileptic convulsions
Nonsyndromic dominant deafness	K^+	*KCNQ4*	Deafness
Long QT syndrome	K^+	*HERG*	Dizziness, sudden death from ventricular fibrillation
	Na^+	*KCNQ1, or SCN5A*	
Hyperkalemic periodic paralysis	Na^+	*SCN4A*	Periodic myotonia and paralysis
Liddle Syndrome	Na^+	*B-ENaC*	Hypertension (high blood pressure)
Myasthenia gravis	Na^+	*nAChR*	Muscle weakness
Dent's disease	Cl^-	*CLCN5*	Kidney stones
Myotonia congenita	Cl^-	*CLC-1*	Periodic myotonia
Bartter's syndrome type IV	Cl^-	*CLC-Kb*	Kidney dysfunction, deafness
Cystic fibrosis	Cl^-	*CFTR*	Lung congestion and infections
Cardiac arrhythmias	Na^+	many different genes	Irregular or rapid heartbeat
	K^+		
	Ca^{2+}		

See *Nature Cell Biol.* 6:1040, 2004, or *Nature* 440:444, 2006, for a more complete list.

Defects in ion Channels

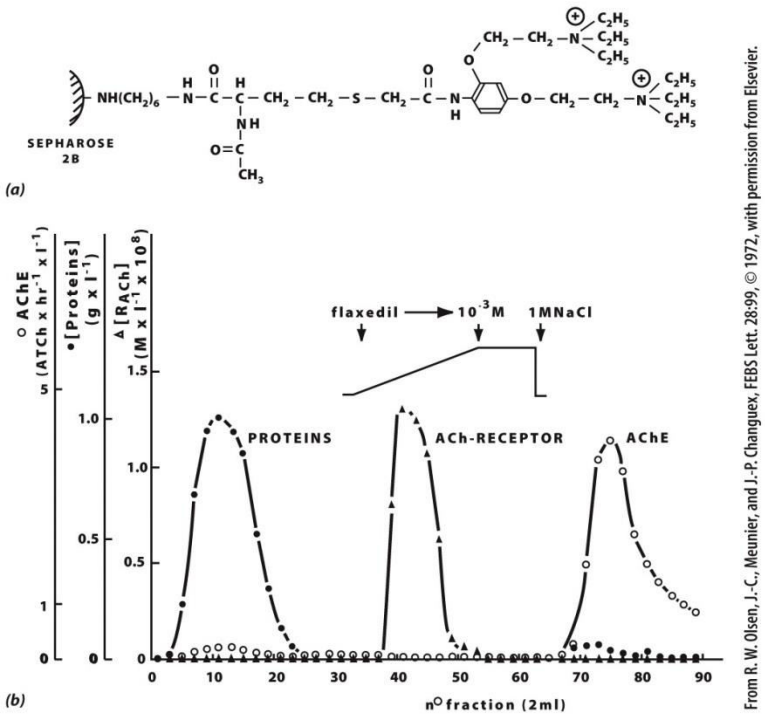
Experimental Pathways: The Acetylcholine Receptor

- Claude Bernard discovered that curare paralyzed muscle function without blocking either nerve or muscle impulses.
- Langley postulated a “chemical transmitter” and “receptive substance” that bound both curare and nicotine.
- Loewi used two hearts to show that “vagusstoff” (acetylcholine) formed in one heart could stop contraction in the second.
- Nachmansohn observed that the electric fish *Torpedo* is an excellent source of nicotinic acetylcholine (nACh) receptors and acetylcholinesterase.



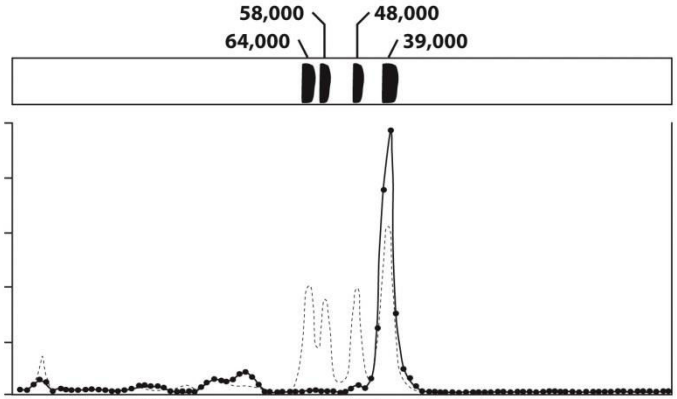
The electric organs of *Torpedo* consist of stacks of modified neuromuscular junctions located on each side of the body.

Experimental Pathways: The Acetylcholine Receptor



From R. W. Olsen, J.-C., Meunier, and J.-P. Changeux, FEBS Lett. 28:99, © 1972, with permission from Elsevier.

Isolation, purification, and electron micrograph characterization of the nAChR



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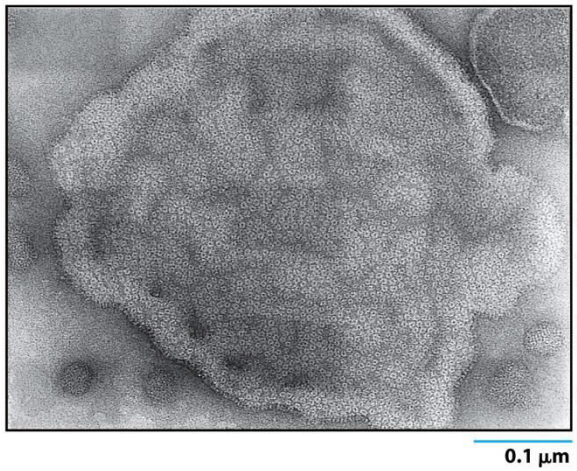
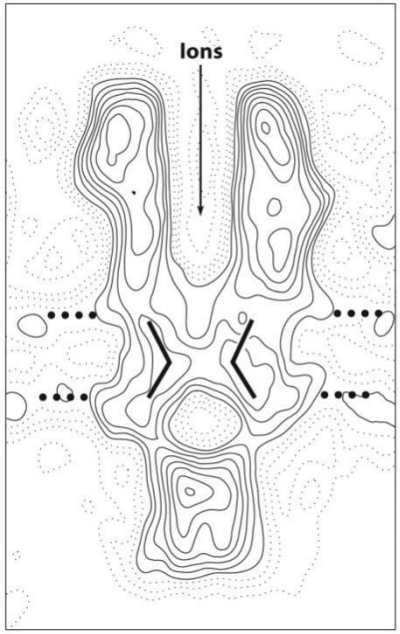


From Werner Schiebler and Ferdinand Hucho, Eur. J. Biochem, 85:58, 1978, reprinted with permission of John Wiley & Sons.

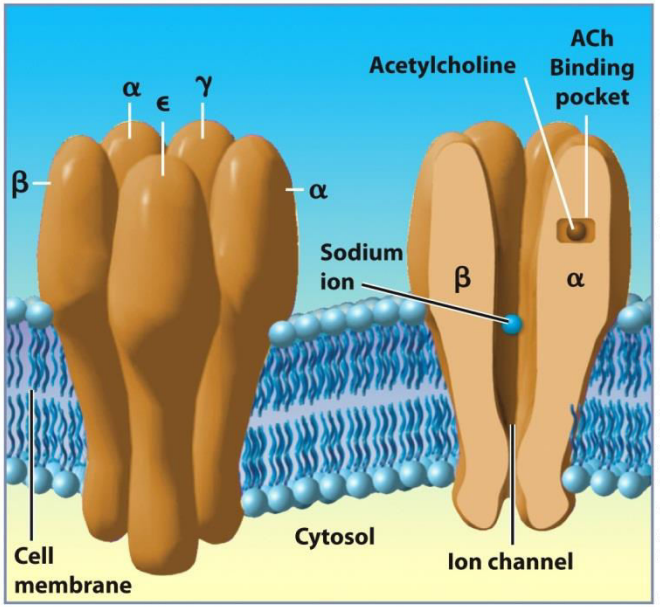
- The snake venom α-bungarotoxin and the detergent Triton X-100 allowed affinity purification of the nACh receptor.

Experimental Pathways: The Acetylcholine Receptor



From N. Unwin, J. Mol. Biol., 229:1118, 1993; copyright 1993; ©1993, by permission of Elsevier

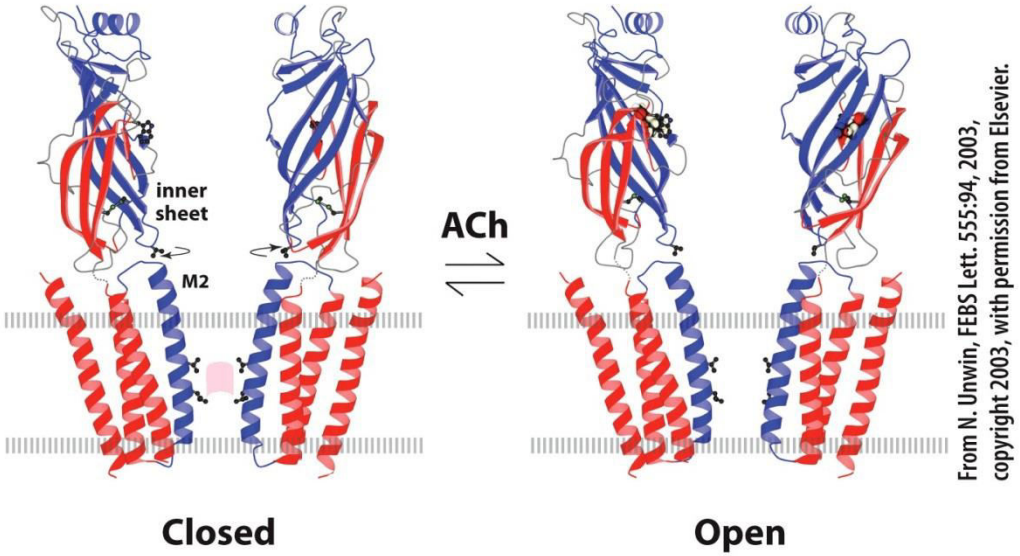
Electron density map of a slice through the nAChR and schematic diagram showing the subunit arrangement



From N. Unwin, J. Mol. Biol., 229:1118, 1993; copyright 1993; © 1993, by permission of Elsevier

- Reconstituting purified receptors into artificial lipids proved that the nACh receptor was a cation channel.
- The structure of the receptor has been studied by both electron microscopy and genetic methods.
- A 43K protein is shown to anchor the receptor to the postsynaptic region.

Experimental Pathways:
The Acetylcholine Receptor



Ribbon drawings illustrating the proposed changes that occur within the nAChR upon binding of acetylcholine

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