and in serines and threonines. Proteins destined for the nucleus have internal targeting sequences. A typical nuclear localization signal contains five consecutive positively charged residues such as Lys-Lys-Lys-Arg-Lys. The addition of such a sequence to a protein not found in the nucleus can direct it to the nucleus (Figure 12.38). Other sequences can direct proteins out of the nucleus. The known targeting sequences are given in Table 12.4.

Targeting sequences act by binding to specific proteins associated with each organelle. The determination of the structure of a protein, α-*karyopherin*, that binds to the nuclear localization signal reveals how the protein recognizes such a targeting sequence (Figure 12.39). A peptide containing the appropriate sequence binds to a specific site on the protein. The target peptide is held in an extended conformation through interactions between the target peptide backbone and asparagine side chains of the α-karyopherin while each of the basic residues lies in a deep pocket near the bottom, lined with negatively charged residues. Proteins that bind to the other targeting signal sequences presumably also have structures that allow recognition of the required features. Note that we have considered only how proteins are marked for different compartments. Later, we will consider the mechanisms by which proteins actually cross membranes (Section 11.3.2).

12.7.2. Membrane Budding and Fusion Underlie Several Important Biological Processes

Membranes must be able to separate or join together to take up, transport, and release molecules. Many take up molecules through the process of *receptor- mediated endocytosis* (Figure 12.40). Here, a protein or larger complex initially binds to a receptor on the cell surface. After the protein is bound, specialized proteins act to cause the membrane in the vicinity of the bound protein to invaginate. The invaginated membrane eventually breaks off and fuses to form a *vesicle*.

Receptor-mediated endocytosis plays a key role in cholesterol metabolism (Section 26.3.3). Some cholesterol in the blood is in the form of a lipid-protein complex called *low-density lipoprotein* (LDL). Low density lipoprotein binds to an LDL receptor, an integral membrane protein. The segment of the plasma membrane containing the LDL-LDL receptor complex then invaginates and buds off from the membrane. The LDL separates from the receptor, which is recycled back to the membrane in a separate vesicle. The vesicle containing the LDL fuses with a *lysosome*, an organelle containing an array of digestive enzymes. The cholesterol is released into the cell for storage or use in membrane biosynthesis, and the remaining protein components are degraded. Various hormones, transport proteins, and antibodies employ receptormediated endocytosis to gain entry into a cell. A less advantageous consequence is that this pathway is available to viruses and toxins as a means of entry into cells. The reverse process—the fusion of a vesicle to a membrane—is a key step in the release of neurotransmitters from a neuron into the synaptic cleft (Figure 12.41). Although the processes of budding and fusion appear deceptively simple, the structures of the intermediates in the budding and fusing processes and the detailed mechanisms remain active areas of investigation.

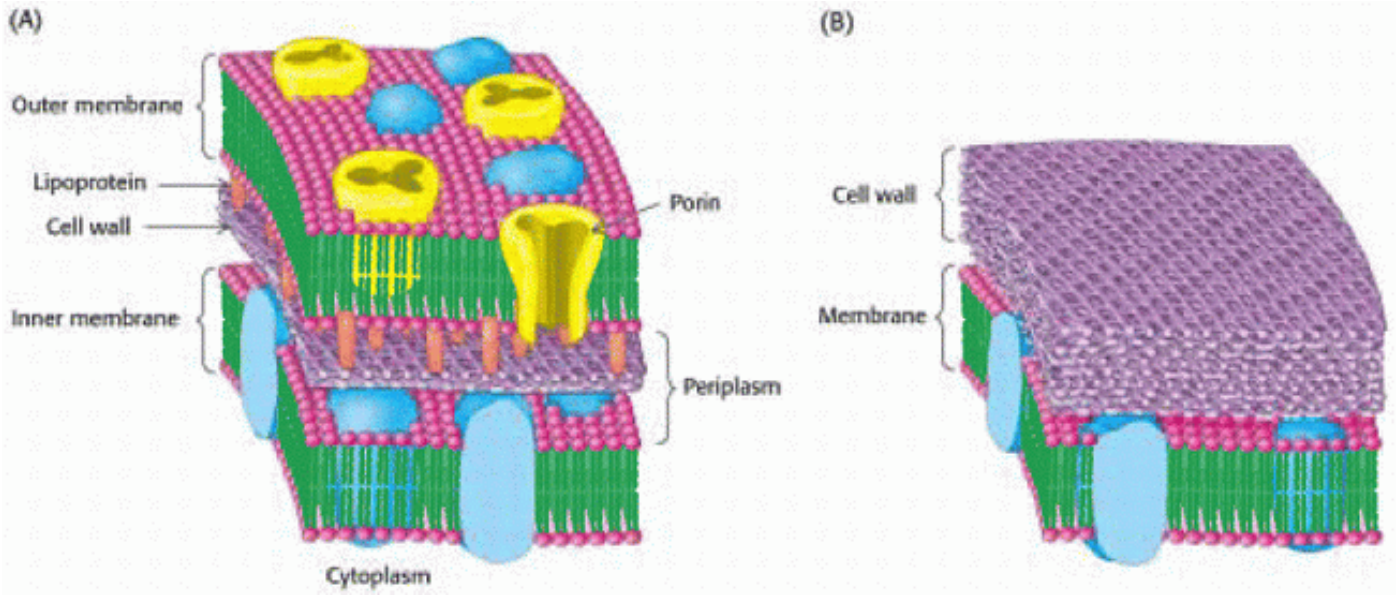


Figure 12.35. Cell Membranes of Prokaryotes. A schematic view of the membrane in bacterial cells surrounded by (A) two membranes or (B) one membrane.

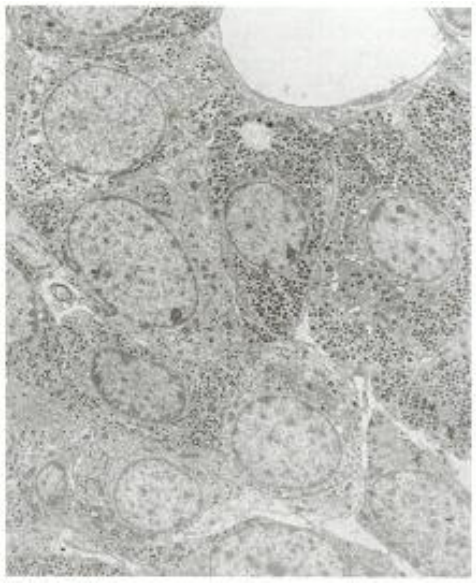
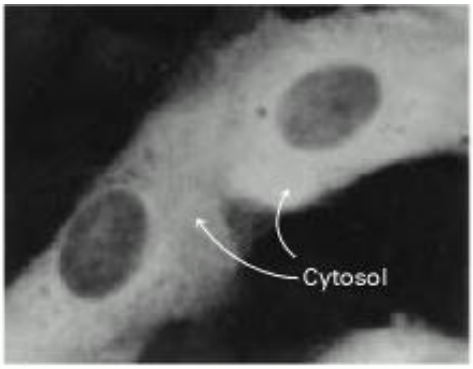
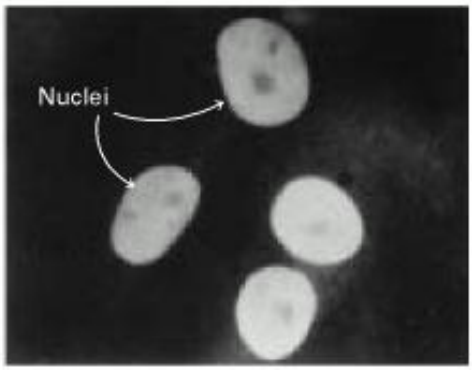


Figure 12.36. Internal Membranes of Eukaryotes. Electron micrograph of a thin section of a hormone-secreting cell for the rat pituitary, showing the presence of internal structures bounded by membranes. [Biophoto Associates/Photo Researchers.]

Figure 12.37. A Mitochondrial Targeting Sequence. This sequence is recognized by receptors on the external face of the outer mitochondrial membrane. A protein bearing the sequence will be imported into the mitochondrion. Hydrophobic residues are shown in yellow, basic ones in blue, and serine and threonine in red.



(A)



(B)

Figure 12.38. Movement of a Protein Into the Nucleus. Localization of (A) unmodified pyruvate kinase, and (B) pyruvate kinase containing a nuclear localization signal sequence attached to its amino terminus. The protein was visualized by fluorescence microscopy after staining with a specific antibody. [From W. D. Richardson, B. L. Roberts, and A. E. Smith. *Cell* 44(1986):79.]

Table 12.4. Targeting sequences

Target	Signal
Nucleus	-KKXK or -$(K/R)_2$-X_{10-12}-(K/R)*
Peroxisome	-SKL-COO^-
Mitochondrion	*N*-terminal amphipathic helix
Endoplasmic reticulum	-KDEL-COO^-(ER retention)

* The "/" means that either K or R is required.

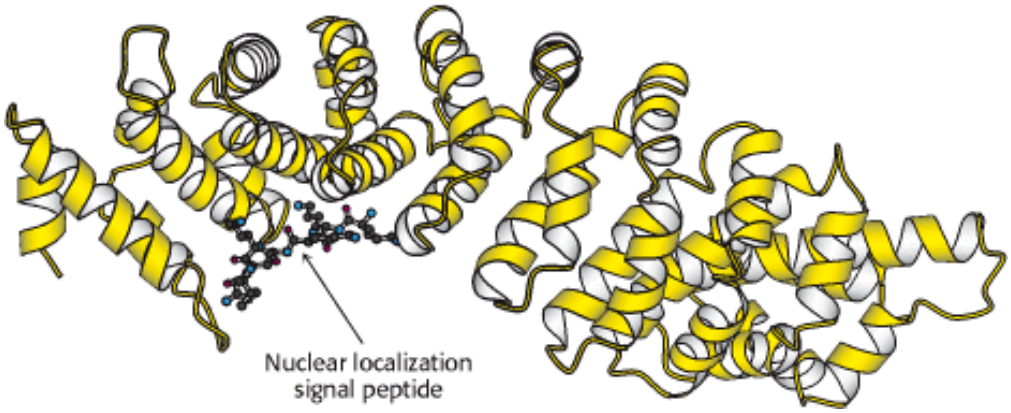


Figure 12.39. Protein Targeting Signal Recognition. The structure of the nuclear localization signal-binding protein α-karyopherin (also known as α-importin) with a nuclear localization signal peptide bound to its major recognition site.

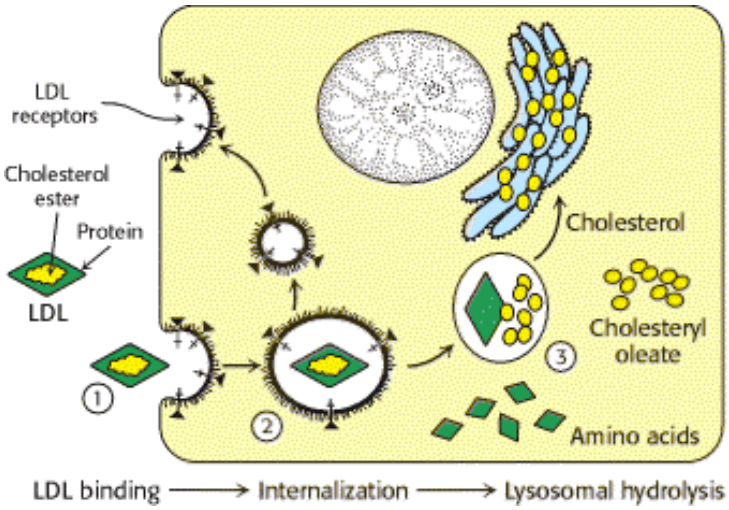


Figure 12.40. Receptor-Mediated Endocytosis. The process of receptor-mediated endocytosis is illustrated for the cholesterol-carrying complex, low-density lipoprotein (LDL): (1) LDL binds to a specific receptor, the LDL receptor; (2) this complex invaginates to form an internal vesicle; (3) after separation from its receptor, the LDL-containing vesicle fuses with a lysosome, leading to degradation of the LDL and release of the cholesterol.

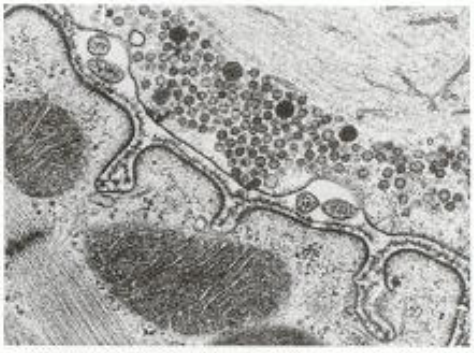


Figure 12.41. Neurotransmitter Release. Neurotransmitter-containing synaptic vesicles are arrayed near the plasma membrane of a nerve cell. Synaptic vesicles fuse with the plasma membrane, releasing the neurotransmitter into the synaptic cleft. [T. Reese/Don Fawcett/ Photo Researchers.]

Summary

Many Common Features Underlie the Diversity of Biological Membranes

Biological membranes are sheetlike structures, typically from 60 to 100 Å thick, that are composed of protein and lipid molecules held together by noncovalent interactions. Membranes are highly selective permeability barriers. They create closed compartments, which may be entire cells or organelles within a cell. Proteins in membranes regulate the molecular and ionic compositions of these compartments. Membranes also control the flow of information between cells.

Fatty Acids Are Key Constituents of Lipids

Fatty acids are hydrocarbon chains of various lengths and degrees of unsaturation that terminate with a carboxylic acid group. The fatty acid chains in membranes usually contain between 14 and 24 carbon atoms; they may be saturated or unsaturated. Short chain length and unsaturation enhance the fluidity of fatty acids and their derivatives by lowering the melting temperature.

There Are Three Common Types of Membrane Lipids

The major classes of membrane lipids are phospholipids, glycolipids, and cholesterol. Phosphoglycerides, a type of phospholipid, consist of a glycerol backbone, two fatty acid chains, and a phosphorylated alcohol. Phosphatidyl choline, phosphatidyl serine, and phosphatidyl ethanolamine are major phosphoglycerides. Sphingomyelin, a different type of phospholipid, contains a sphingosine backbone instead of glycerol. Glycolipids are sugar-containing lipids derived from sphingosine. Cholesterol, which modulates membrane fluidity, is constructed from a steroid nucleus. A common feature of these membrane lipids is that they are amphipathic molecules, having hydrophobic and hydrophilic ends.

Phospholipids and Glycolipids Readily Form Bimolecular Sheets in Aqueous Media

Membrane lipids spontaneously form extensive bimolecular sheets in aqueous solutions. The driving force for membrane formation is the hydrophobic interactions among the fatty acid tails of membrane lipids. The hydrophilic head groups interact with the aqueous medium. Lipid bilayers are cooperative structures, held together by many weak bonds. These lipid bilayers are highly impermeable to ions and most polar molecules, yet they are quite fluid, which enables them to act as a solvent for membrane proteins.

Proteins Carry Out Most Membrane Processes

Specific proteins mediate distinctive membrane functions such as transport, communication, and energy transduction. Many integral membrane proteins span the lipid bilayer, whereas others are partly embedded in the membrane. Peripheral membrane proteins are bound to membrane surfaces by electrostatic and hydrogen-bond interactions. Membrane-spanning proteins have regular structures, including β strands, although the α helix is the most common membrane-spanning domain. Indeed, sequences of 20 consecutive nonpolar amino acids can be diagnostic of a membrane-spanning α-helical region of a protein.

Lipids and Many Membrane Proteins Diffuse Rapidly in the Plane of the Membrane

Membranes are structurally and functionally asymmetric, as exemplified by the restriction of sugar residues to the external surface of mammalian plasma membranes. Membranes are dynamic structures in which proteins and lipids diffuse rapidly in the plane of the membrane (lateral diffusion), unless restricted by special interactions. In contrast, the rotation of lipids from one face of a membrane to the other (transverse diffusion, or flip-flop) is usually very slow. Proteins do not rotate across bilayers; hence, membrane asymmetry can be preserved. The degree of fluidity of a

membrane partly depends on the chain length of its lipids and the extent to which their constituent fatty acids are unsaturated. In animals, cholesterol content also regulates membrane fluidity.

Eukaryotic Cells Contain Compartments Bounded by Internal Membranes

An extensive array of internal membranes in eukaryotes creates compartments within a cell for distinct biochemical functions. For instance, a double membrane surrounds the nucleus, the location of most of the cell's genetic material, and the mitochondria, the location of most ATP synthesis. A single membrane defines the other internal compartments, such as the endoplasmic reticulum. Some compartments can exchange material by the process of membrane budding and fusion. As with all membranes, the proteins associated with these membranes determine the specific biochemical function. Specific amino acid sequences in the proteins direct these molecules to the appropriate compartment.

Key Terms

fatty acid

phospholipid

sphingosine

phosphoglyceride

sphingomyelin

glycolipid

cerebroside

ganglioside

cholesterol

amphipathic molecule

lipid bilayer

liposome

integral membrane protein

peripheral membrane protein

hydropathy plot

lateral diffusion

fluid mosaic model

targeting sequence

receptor-mediated endocytosis

Problems

1. *Population density*. How many phospholipid molecules are there in a 1-μm^2 region of a phospholipid bilayer membrane? Assume that a phospholipid molecule occupies 70 $Å^2$ of the surface area.

   See answer

2. *Lipid diffusion*. What is the average distance traversed by a membrane lipid in 1 μs, 1 ms, and 1 s? Assume a diffusion coefficient of 10^{-8} cm^2s^{-1}.

   See answer

3. *Protein diffusion*. The diffusion coefficient, *D*, of a rigid spherical molecule is given by

$$D = kT/6\pi\eta r$$

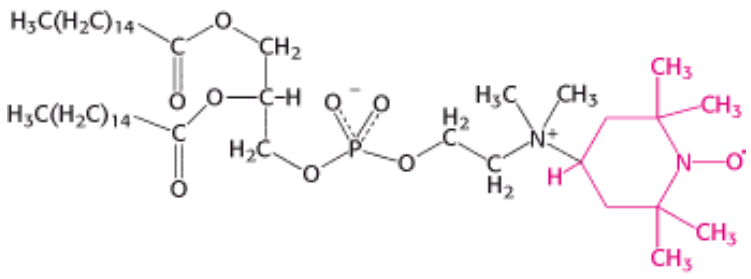
   in which η is the viscosity of the solvent, *r* is the radius of the sphere, *k* is the Boltzman constant (1.38×10^{-16} erg/degree), and *T* is the absolute temperature. What is the diffusion coefficient at 37°C of a 100-kd protein in a membrane that has an effective viscosity of 1 poise (1 poise = 1 erg s^{-1}/cm^{-3})? What is the average distance traversed by this protein in 1 μs, 1 ms, and 1 s? Assume that this protein is an unhydrated, rigid sphere of density 1.35 g cm^{-3}.

   See answer

4. *Cold sensitivity*. Some antibiotics act as carriers that bind an ion on one side of a membrane, diffuse through the membrane, and release the ion on the other side. The conductance of a lipid-bilayer membrane containing a carrier antibiotic decreased abruptly when the temperature was lowered from 40°C to 36°C. In contrast, there was little change in conductance of the same bilayer membrane when it contained a channel-forming antibiotic. Why?

   See answer

5. *Flip-flop*. The transverse diffusion of phospholipids in a bilayer membrane was investigated by using a paramagnetic analog of phosphatidyl choline, called *spin-labeled phosphatidyl choline*.



Spin-labeled phosphatidyl choline

   The nitroxide (NO) group in spin-labeled phosphatidyl choline gives a distinctive paramagnetic resonance spectrum. This spectrum disappears when nitroxides are converted into amines by reducing agents such as ascorbate.

   Lipid vesicles containing phosphatidyl choline (95%) and the spin-labeled analog (5%) were prepared by sonication