

10

Membrane Structure

Cell membranes are crucial to the life of the cell. The **plasma membrane** encloses the cell, defines its boundaries, and maintains the essential differences between the cytosol and the extracellular environment. Inside eucaryotic cells, the membranes of the endoplasmic reticulum, Golgi apparatus, mitochondria, and other membrane-enclosed organelles maintain the characteristic differences between the contents of each organelle and the cytosol. Ion gradients across membranes, established by the activities of specialized membrane proteins, can be used to synthesize ATP, to drive the transmembrane movement of selected solutes, or, as in nerve and muscle cells, to produce and transmit electrical signals. In all cells, the plasma membrane also contains proteins that act as sensors of external signals, allowing the cell to change its behavior in response to environmental cues, including signals from other cells; these protein sensors, or *receptors*, transfer information—rather than molecules—across the membrane.

Despite their differing functions, all biological membranes have a common general structure: each is a very thin film of lipid (fatty) and protein molecules, held together mainly by noncovalent interactions (**Figure 10–1**). Cell membranes are dynamic, fluid structures, and most of their molecules move about in the plane of the membrane. The lipid molecules are arranged as a continuous double layer about 5 nm thick. This *lipid bilayer* provides the basic fluid structure of the membrane and serves as a relatively impermeable barrier to the passage of most water-soluble molecules. Protein molecules that span the lipid bilayer (*transmembrane proteins*; see **Figure 10–1**) mediate nearly all of the other functions of the membrane, transporting specific molecules across it, for example, or catalyzing membrane-associated reactions such as ATP synthesis. In the plasma membrane, some transmembrane proteins serve as structural links that connect the cytoskeleton through the lipid bilayer to either the extracellular matrix or an adjacent cell, while others serve as receptors to detect and transduce chemical signals in the cell's environment. As would be expected, it takes many different membrane proteins to enable a cell to function and interact with its environment, and it is estimated that about 30% of the proteins encoded in an animal cell's genome are membrane proteins.

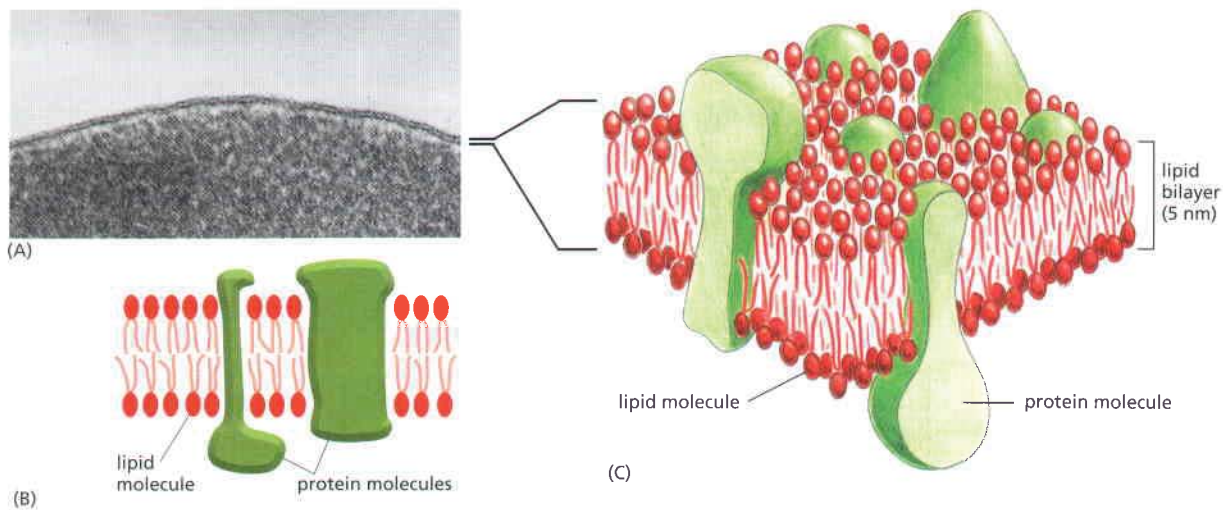
In this chapter, we consider the structure and organization of the two main constituents of biological membranes—the lipids and the proteins. Although we focus mainly on the plasma membrane, most concepts discussed apply to the various internal membranes in cells as well. The functions of cell membranes are considered in later chapters: their role in ATP synthesis, for example, is discussed in Chapter 14; their role in the transmembrane transport of small molecules in Chapter 11; and their roles in cell signaling and cell adhesion in Chapters 15 and 19, respectively. In Chapters 12 and 13, we discuss the internal membranes of the cell and the protein traffic through and between them.

THE LIPID BILAYER

The **lipid bilayer** provides the basic structure for all cell membranes. It is easily seen by electron microscopy, and its structure is attributable exclusively to the

In This Chapter

THE LIPID BILAYER	617
MEMBRANE PROTEINS	629



special properties of the lipid molecules, which assemble spontaneously into bilayers even under simple artificial conditions.

Phosphoglycerides, Sphingolipids, and Sterols Are the Major Lipids in Cell Membranes

Lipid molecules constitute about 50% of the mass of most animal cell membranes, nearly all of the remainder being protein. There are approximately 5×10^6 lipid molecules in a $1 \mu\text{m} \times 1 \mu\text{m}$ area of lipid bilayer, or about 10^9 lipid molecules in the plasma membrane of a small animal cell. All of the lipid molecules in cell membranes are **amphiphilic**—that is, they have a **hydrophilic** (“water-loving”) or *polar* end and a **hydrophobic** (“water-fearing”) or *nonpolar* end.

The most abundant membrane lipids are the **phospholipids**. These have a polar head group and two hydrophobic *hydrocarbon tails*. In animal, plant, and bacterial cells, the tails are usually fatty acids, and they can differ in length (they normally contain between 14 and 24 carbon atoms). One tail typically has one or more *cis*-double bonds (that is, it is *unsaturated*), while the other tail does not (that is, it is *saturated*). As shown in **Figure 10-2**, each *cis*-double bond creates a small kink in the tail. Differences in the length and saturation of the fatty acid tails influence how phospholipid molecules pack against one another, thereby affecting the fluidity of the membrane, as we discuss later.

The main phospholipids in most animal cell membranes are the **phosphoglycerides**, which have a three-carbon *glycerol* backbone (see **Figure 10-2**). Two long-chain fatty acids are linked through ester bonds to adjacent carbon atoms of the glycerol, and the third carbon atom is attached to a phosphate group, which in turn is linked to one of several different types of head group. By combining several different fatty acids and head groups, cells make many different phosphoglycerides. *Phosphatidylethanolamine*, *phosphatidylserine*, and *phosphatidylcholine* are the main ones in mammalian cell membranes (**Figure 10-3A–C**)

Another important phospholipid, called *sphingomyelin*, is built from *sphingosine* rather than glycerol (**Figure 10-3D–E**). Sphingosine is a long acyl chain with an amino group (NH_2) and two hydroxyl groups (OH) at one end of the molecule. In sphingomyelin, a fatty acid tail is attached to the amino group, and a phosphocholine group is attached to the terminal hydroxyl group, leaving one hydroxyl group free. The free hydroxyl group contributes to the polar properties of the adjacent head group, as it can form hydrogen bonds with the head group of a neighboring lipid, with a water molecule, or with a membrane protein. Together, the phospholipids phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin constitute more than half the mass of lipid in most mammalian cell membranes (see **Table 10-1**).

Figure 10-1 Three views of a cell membrane. <TAGC> (A) An electron micrograph of a plasma membrane (of a human red blood cell) seen in cross section. (B and C) These drawings show two-dimensional and three-dimensional views of a cell membrane and the general disposition of its lipid and protein constituents. (A, courtesy of Daniel S. Friend.)

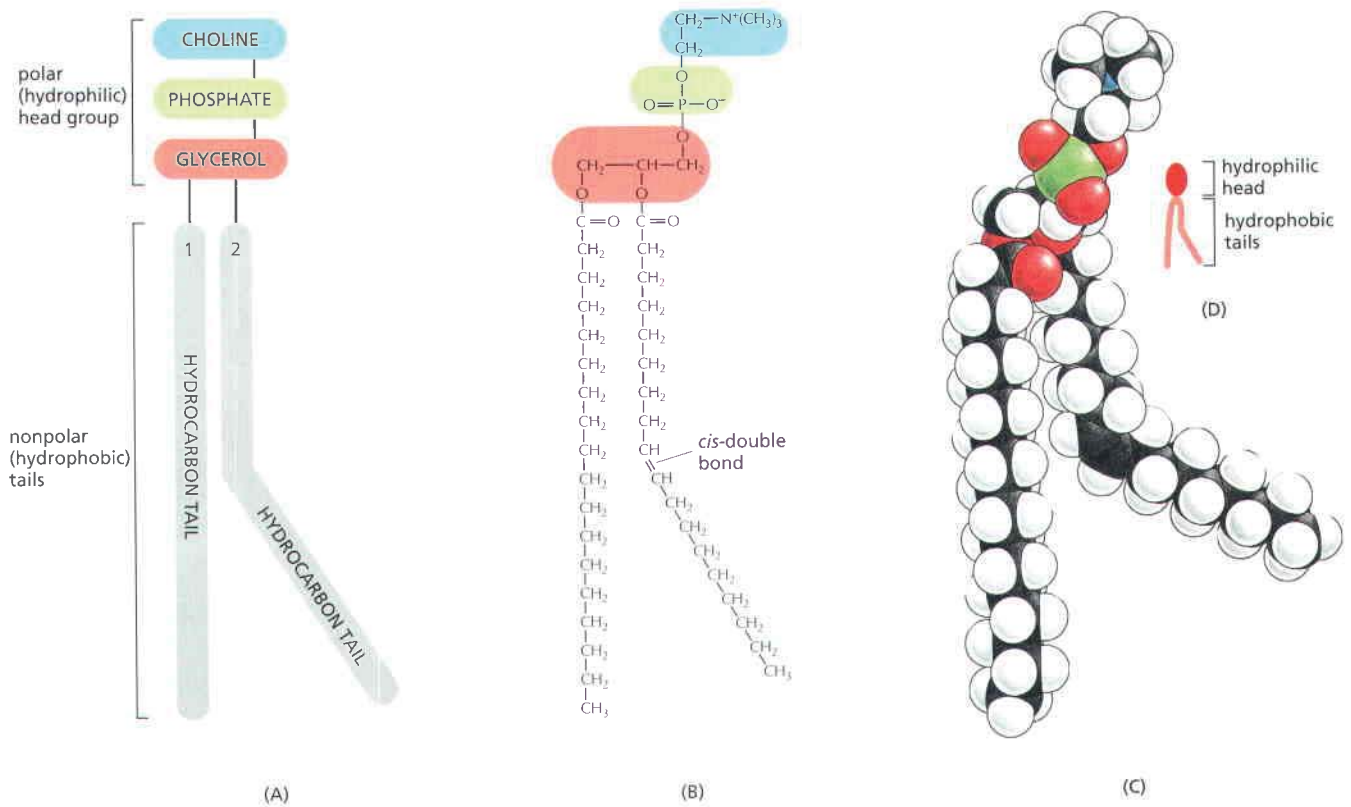


Figure 10-2 The parts of a phosphoglyceride molecule. This example is a phosphatidylcholine, represented (A) schematically, (B) by a formula, (C) as a space-filling model, and (D) as a symbol. The kink resulting from the *cis*-double bond is exaggerated for emphasis.

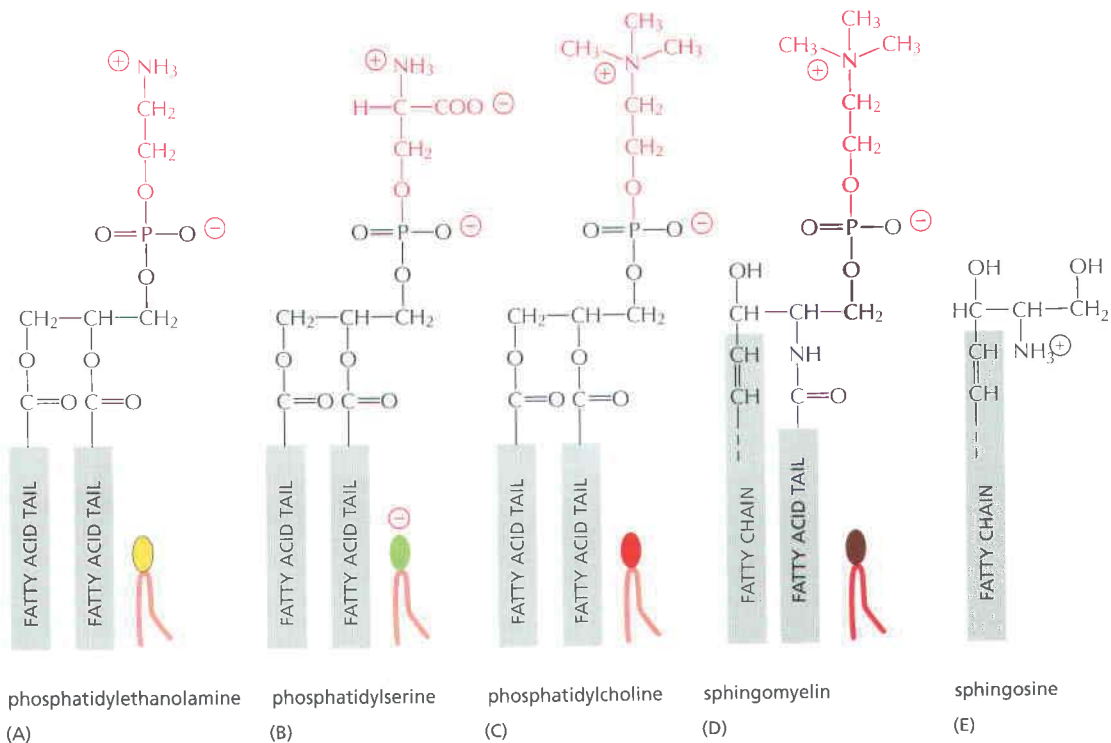


Figure 10-3 Four major phospholipids in mammalian plasma membranes. Different head groups are represented by different colors in the symbols. The lipid molecules shown in (A–C) are phosphoglycerides, which are derived from glycerol. The molecule in (D) is sphingomyelin, which is derived from sphingosine (E) and is therefore a sphingolipid. Note that only phosphatidylserine carries a net negative charge, the importance of which we discuss later; the other three are electrically neutral at physiological pH, carrying one positive and one negative charge.

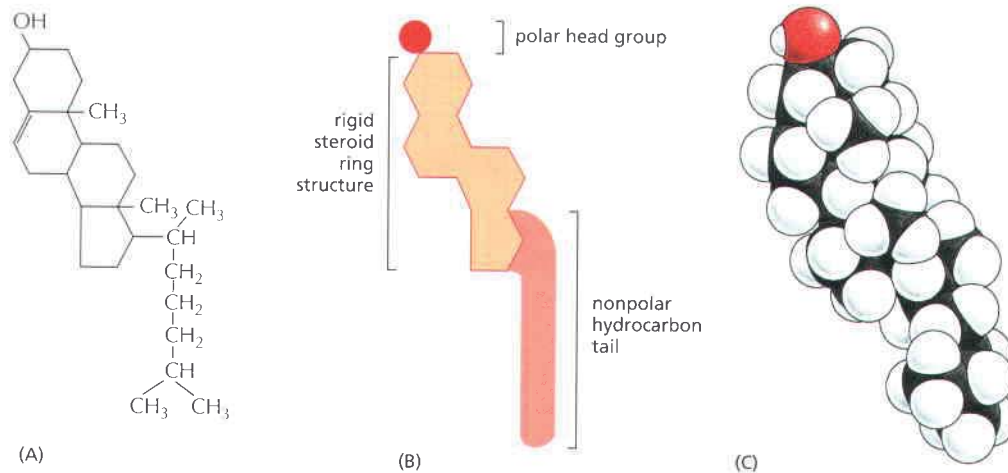


Figure 10-4 The structure of cholesterol. Cholesterol is represented (A) by a formula, (B) by a schematic drawing, and (C) as a space-filling model.

In addition to phospholipids, the lipid bilayers in many cell membranes contain *cholesterol* and *glycolipids*. Eucaryotic plasma membranes contain especially large amounts of **cholesterol** (Figure 10-4)—up to one molecule for every phospholipid molecule. Cholesterol is a sterol. It contains a rigid ring structure, to which is attached a single polar hydroxyl group and a short nonpolar hydrocarbon chain. The cholesterol molecules orient themselves in the bilayer with their hydroxyl group close to the polar head groups of adjacent phospholipid molecules (Figure 10-5).

Phospholipids Spontaneously Form Bilayers

The shape and amphiphilic nature of the phospholipid molecules cause them to form bilayers spontaneously in aqueous environments. As discussed in Chapter 2, hydrophilic molecules dissolve readily in water because they contain charged groups or uncharged polar groups that can form either favorable electrostatic interactions or hydrogen bonds with water molecules. Hydrophobic molecules, by contrast, are insoluble in water because all, or almost all, of their atoms are uncharged and nonpolar and therefore cannot form energetically favorable interactions with water molecules. If dispersed in water, they force the adjacent water molecules to reorganize into ice-like cages that surround the hydrophobic molecule (Figure 10-6). Because these cage structures are more ordered than the surrounding water, their formation increases the free energy. This free energy cost is minimized, however, if the hydrophobic molecules (or the hydrophobic portions of amphiphilic molecules) cluster together so that the smallest number of water molecules is affected.

The hydrophobic and hydrophilic regions of lipid molecules behave in the same way. Thus, lipid molecules spontaneously aggregate to bury their hydrophobic hydrocarbon tails in the interior and expose their hydrophilic heads to water. Depending on their shape, they can do this in either of two ways: they can form spherical *micelles*, with the tails inward, or they can form double-layered sheets, or *bilayers*, with the hydrophobic tails sandwiched between the hydrophilic head groups (Figure 10-7).

Being cylindrical, phospholipid molecules spontaneously form bilayers in aqueous environments. In this energetically most favorable arrangement, the hydrophilic heads face the water at each surface of the bilayer, and the hydrophobic tails are shielded from the water in the interior. The same forces that drive phospholipids to form bilayers also provide a self-healing property. A small tear in the bilayer creates a free edge with water; because this is energetically unfavorable, the lipids tend to rearrange spontaneously to eliminate the free edge. (In eucaryotic plasma membranes, the fusion of intracellular vesicles repairs larger tears.) The prohibition of free edges has a profound consequence: the only way for a bilayer to avoid having edges is by closing in on itself and forming a sealed compartment (Figure 10-8). This remarkable behavior, fundamental

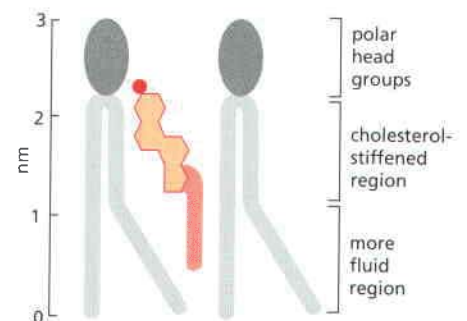


Figure 10-5 Cholesterol in a lipid bilayer. Schematic drawing of a cholesterol molecule interacting with two phospholipid molecules in one monolayer of a lipid bilayer.

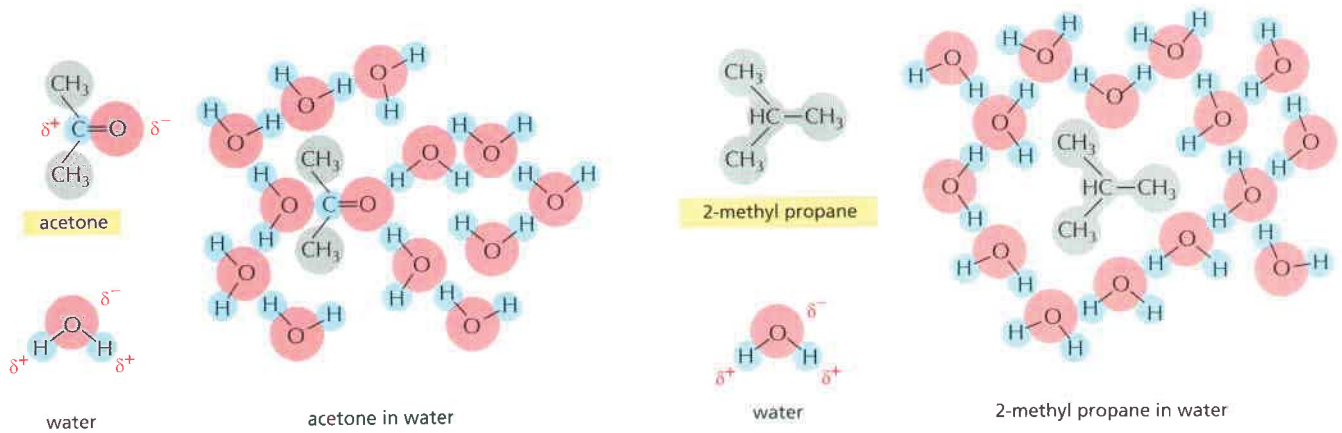


Figure 10-6 How hydrophilic and hydrophobic molecules interact differently with water. (A) Because acetone is polar, it can form favorable electrostatic interactions with water molecules, which are also polar. Thus, acetone readily dissolves in water. (B) By contrast, 2-methyl propane is entirely hydrophobic. Because it cannot form favorable interactions with water, it would force adjacent water molecules to reorganize into icelike cage structures, which increases the free energy. This compound is therefore virtually insoluble in water. The symbol δ^- indicates a partial negative charge, and δ^+ indicates a partial positive charge. Polar atoms are shown in color and nonpolar groups are shown in gray.

to the creation of a living cell, follows directly from the shape and amphiphilic nature of the phospholipid molecule.

A lipid bilayer also has other characteristics that make it an ideal structure for cell membranes. One of the most important of these is its fluidity, which is crucial to many membrane functions.

The Lipid Bilayer Is a Two-dimensional Fluid

Around 1970, researchers first recognized that individual lipid molecules are able to diffuse freely within lipid bilayers. The initial demonstration came from studies of synthetic lipid bilayers. Two types of preparations have been very useful in such studies: (1) bilayers made in the form of spherical vesicles, called **liposomes**, which can vary in size from about 25 nm to 1 μm in diameter

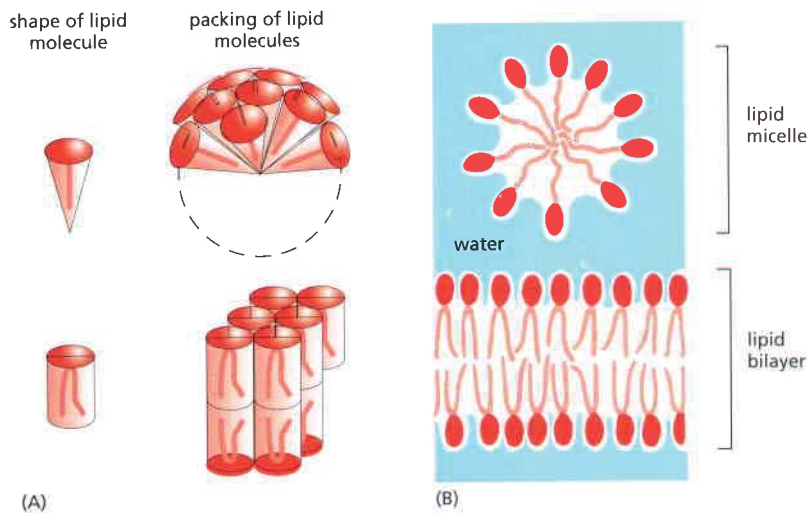


Figure 10-7 Packing arrangements of lipid molecules in an aqueous environment. (A) Cone-shaped lipid molecules (above) form micelles, whereas cylinder-shaped phospholipid molecules (below) form bilayers. (B) A lipid micelle and a lipid bilayer seen in cross section. Lipid molecules spontaneously form one or the other structure in water, depending on their shape.

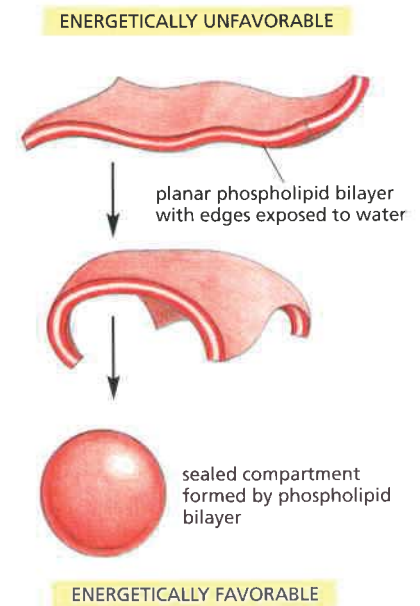


Figure 10-8 The spontaneous closure of a phospholipid bilayer to form a sealed compartment. The closed structure is stable because it avoids the exposure of the hydrophobic hydrocarbon tails to water, which would be energetically unfavorable.

depending on how they are produced (Figure 10–9); and (2) planar bilayers, called **black membranes**, formed across a hole in a partition between two aqueous compartments (Figure 10–10).

Various techniques have been used to measure the motion of individual lipid molecules and their components. One can construct a lipid molecule, for example, with a fluorescent dye or a small gold particle attached to its polar head group and follow the diffusion of even individual molecules in a membrane. Alternatively, one can modify a lipid head group to carry a “spin label,” such as a nitroxyl group (>N-O); this contains an unpaired electron whose spin creates a paramagnetic signal that can be detected by electron spin resonance (ESR) spectroscopy. (The principles of this technique are similar to those of nuclear magnetic resonance, discussed in Chapter 8.) The motion and orientation of a spin-labeled lipid in a bilayer can be deduced from the ESR spectrum. Such studies show that phospholipid molecules in synthetic bilayers very rarely migrate from the monolayer (also called a *leaflet*) on one side to that on the other. This process, known as “flip-flop,” occurs less than once a month for any individual molecule, although cholesterol is an exception to this rule and can flip-flop rapidly. In contrast, lipid molecules readily exchange places with their neighbors *within* a monolayer ($\sim 10^7$ times per second). This gives rise to a rapid lateral diffusion, with a diffusion coefficient (D) of about 10^{-8} cm^2/sec , which means that an average lipid molecule diffuses the length of a large bacterial cell (~ 2 μm) in about 1 second. These studies have also shown that individual lipid molecules rotate very rapidly about their long axis and have flexible hydrocarbon chains. Computer simulations show that lipid molecules in membranes are very disordered, presenting an irregular surface of variously spaced and oriented head groups to the water phase on either side of the bilayer (Figure 10–11).

Similar mobility studies on labeled lipid molecules in isolated biological membranes and in living cells give results similar to those in synthetic bilayers. They demonstrate that the lipid component of a biological membrane is a two-dimensional liquid in which the constituent molecules are free to move laterally. As in synthetic bilayers, individual phospholipid molecules are normally confined to their own monolayer. This confinement creates a problem for their synthesis. Phospholipid molecules are manufactured in only one monolayer of a membrane, mainly in the cytosolic monolayer of the endoplasmic reticulum membrane. If none of these newly made molecules could migrate reasonably promptly to the noncytosolic monolayer, new lipid bilayer could not be made. The problem is solved by a special class of transmembrane enzymes called *phospholipid translocators*, which catalyze the rapid flip-flop of phospholipids from one monolayer to the other, as discussed in Chapter 12.

The Fluidity of a Lipid Bilayer Depends on Its Composition

The fluidity of cell membranes has to be precisely regulated. Certain membrane transport processes and enzyme activities, for example, cease when the bilayer viscosity is experimentally increased beyond a threshold level.

The fluidity of a lipid bilayer depends on both its composition and its temperature, as is readily demonstrated in studies of synthetic bilayers. A synthetic bilayer made from a single type of phospholipid changes from a liquid state to a

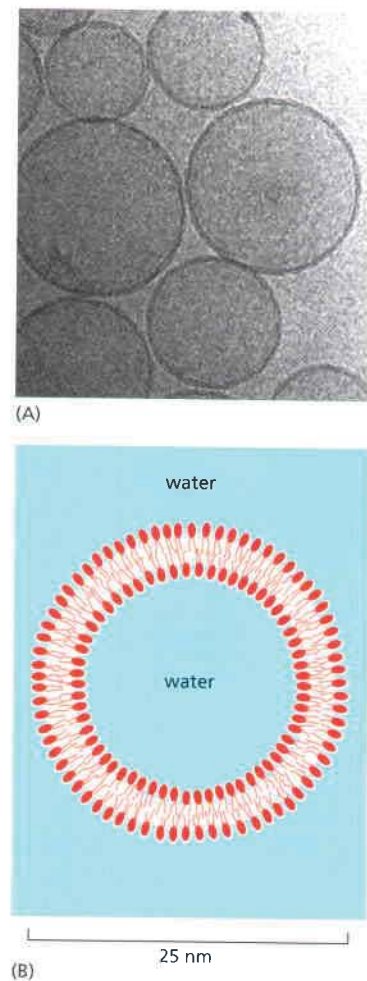


Figure 10–9 Liposomes. (A) An electron micrograph of unfixed, unstained phospholipid vesicles—liposomes—in water rapidly frozen to liquid nitrogen temperature. (B) A drawing of a small spherical liposome seen in cross section. Liposomes are commonly used as model membranes in experimental studies. (A, from P. Frederik and W. Hubert, *Meth. Enzymol.* 391:431, 2005. With permission from Elsevier.)

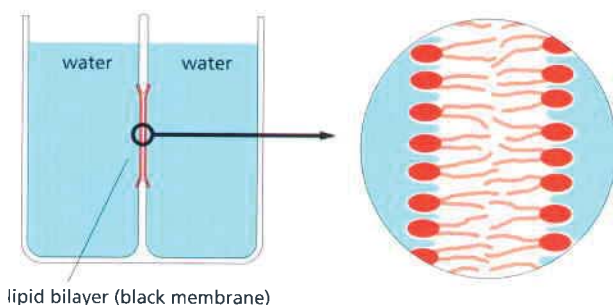


Figure 10–10 A cross-sectional view of a black membrane, a synthetic lipid bilayer. This planar bilayer appears black when it forms across a small hole in a partition separating two aqueous compartments. Black membranes are used to measure the permeability properties of synthetic membranes.

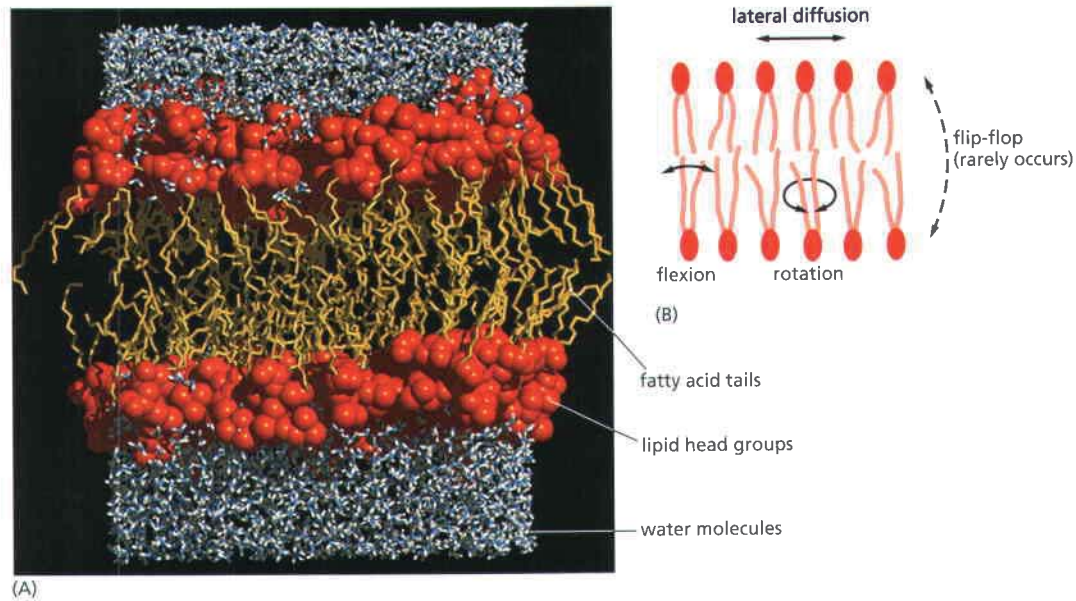


Figure 10–11 The mobility of phospholipid molecules in an artificial lipid bilayer. <CACA> Starting with a model of 100 phosphatidylcholine molecules arranged in a regular bilayer, a computer calculated the position of every atom after 300 picoseconds of simulated time. From these theoretical calculations (taking weeks of processor time in 1995), a model of the lipid bilayer emerges that accounts for almost all of the measurable properties of a synthetic lipid bilayer, such as its thickness, number of lipid molecules per membrane area, depth of water penetration, and unevenness of the two surfaces. Note that the tails in one monolayer can interact with those in the other monolayer, if the tails are long enough. (B) The different motions of a lipid molecule in a bilayer. (A, based on S.W. Chiu et al., *Biophys. J.* 69:1230–1245, 1995. With permission from the Biophysical Society.)

two-dimensional rigid crystalline (or gel) state at a characteristic freezing point. This change of state is called a *phase transition*, and the temperature at which it occurs is lower (that is, the membrane becomes more difficult to freeze) if the hydrocarbon chains are short or have double bonds. A shorter chain length reduces the tendency of the hydrocarbon tails to interact with one another, in both the same and opposite monolayer, and *cis*-double bonds produce kinks in the hydrocarbon chains that make them more difficult to pack together, so that the membrane remains fluid at lower temperatures (**Figure 10–12**). Bacteria, yeasts, and other organisms whose temperature fluctuates with that of their environment adjust the fatty acid composition of their membrane lipids to maintain a relatively constant fluidity. As the temperature falls, for instance, the cells of those organisms synthesize fatty acids with more *cis*-double bonds, and they avoid the decrease in bilayer fluidity that would otherwise result from the temperature drop.

Cholesterol modulates the properties of lipid bilayers. When mixed with phospholipids, it enhances the permeability-barrier properties of the lipid bilayer. It inserts into the bilayer with its hydroxyl group close to the polar head groups of the phospholipids, so that its rigid, platelike steroid rings interact with—and partly immobilize—those regions of the hydrocarbon chains closest to the polar head groups (see Figure 10–5). By decreasing the mobility of the first

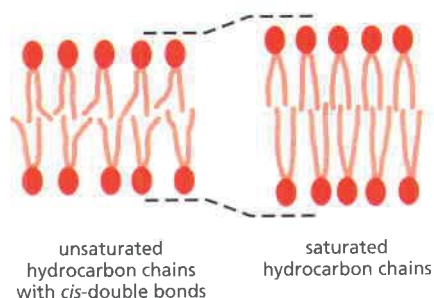


Figure 10–12 The influence of *cis*-double bonds in hydrocarbon chains. The double bonds make it more difficult to pack the chains together, thereby making the lipid bilayer more difficult to freeze. In addition, because the hydrocarbon chains of unsaturated lipids are more spread apart, lipid bilayers containing them are thinner than bilayers formed exclusively from saturated lipids.

Table 10–1 Approximate Lipid Compositions of Different Cell Membranes

LIPID	PERCENTAGE OF TOTAL LIPID BY WEIGHT					
	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM	<i>E. COLI</i> BACTERIUM
Cholesterol	17	23	22	3	6	0
Phosphatidylethanolamine	7	18	15	28	17	70
Phosphatidylserine	4	7	9	2	5	trace
Phosphatidylcholine	24	17	10	44	40	0
Sphingomyelin	19	18	8	0	5	0
Glycolipids	7	3	28	trace	trace	0
Others	22	14	8	23	27	30

few CH₂ groups of the hydrocarbon chains of the phospholipid molecules, cholesterol makes the lipid bilayer less deformable in this region and thereby decreases the permeability of the bilayer to small water-soluble molecules. Although cholesterol tightens the packing of the lipids in a bilayer, it does not make membranes any less fluid. At the high concentrations found in most eucaryotic plasma membranes, cholesterol also prevents the hydrocarbon chains from coming together and crystallizing.

Table 10–1 compares the lipid compositions of several biological membranes. Note that bacterial plasma membranes are often composed of one main type of phospholipid and contain no cholesterol; their mechanical stability is enhanced by an overlying cell wall (see Figure 11–18). In archaea, lipids usually contain 20–25-carbon-long prenyl chains instead of fatty acids, prenyl and fatty acid chains are similarly hydrophobic and flexible (see Figure 10–20F). Thus, lipid bilayers can be built from molecules with similar features but different molecular designs. The plasma membranes of most eucaryotic cells are more varied than those of procaryotes and archaea, not only in containing large amounts of cholesterol but also in containing a mixture of different phospholipids.

Analysis of membrane lipids by mass spectrometry has revealed that the lipid composition of a typical cell membrane is much more complex than originally thought. According to these studies, membranes are composed of a bewildering variety of 500–1000 different lipid species. While some of this complexity reflects the combinatorial variation in head groups, hydrocarbon chain lengths, and desaturation of the major phospholipid classes, membranes also contain many structurally distinct minor lipids, at least some of which have important functions. The *inositol phospholipids*, for example, are present in small quantities but have crucial functions in guiding membrane traffic and in cell signaling (discussed in Chapters 13 and 15, respectively). Their local synthesis and destruction are regulated by a large number of enzymes, which create both small intracellular signaling molecules and lipid docking sites on membranes that recruit specific proteins from the cytosol, as we discuss later.

Despite Their Fluidity, Lipid Bilayers Can Form Domains of Different Compositions

Because a lipid bilayer is a two-dimensional fluid, we might expect most types of lipid molecules in it to be randomly distributed in their own monolayer. The van der Waals attractive forces between neighboring hydrocarbon tails are not selective enough to hold groups of phospholipid molecules together. With certain lipid mixtures, however, different lipids can come together transiently, creating a dynamic patchwork of different domains. In synthetic lipid bilayers composed of phosphatidylcholine, sphingomyelin, and cholesterol, van der Waals forces between the long and saturated hydrocarbon chains of the sphingomyelin molecules can be just strong enough to hold the adjacent molecules together transiently (**Figure 10–13**).

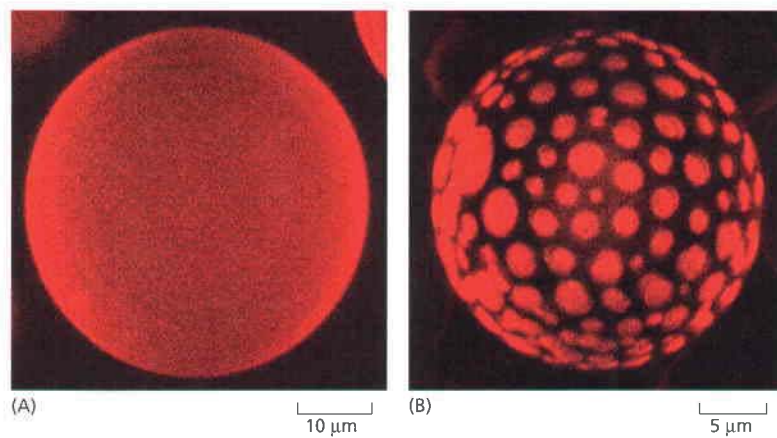


Figure 10-13 Lateral phase separation in artificial lipid bilayers. (A) Giant liposomes produced from a 1:1 mixture of phosphatidylcholine and spingomyelin form uniform bilayers, whereas (B) liposomes produced from a 1:1:1 mixture of phosphatidylcholine, spingomyelin, and cholesterol form bilayers with two immiscible phases. The liposomes are stained with trace concentrations of a fluorescent dye that preferentially partitions into one of the phases. The average size of the domains formed in these giant artificial liposomes is much larger than that expected in biological membranes, where rafts may be as small as a few nanometers in diameter. (A, from N. Kahya et al., *J. Struct. Biol.* 147:77–89, 2004. With permission from Elsevier; B, courtesy of Petra Schwille.)

There has been a long debate among scientists whether the lipid molecules in the plasma membrane of animal cells can transiently assemble into specialized domains, called **lipid rafts**. Certain specialized regions of the plasma membrane, such as the *caveolae* involved in endocytosis (discussed in Chapter 13), are enriched in sphingolipids and cholesterol, and it is thought that the specific proteins that assemble there help stabilize these rafts. Because the hydrocarbon chains of sphingolipids are longer and straighter than those of other membrane lipids, raft domains are thicker than other parts of the bilayer (see Figure 10-12) and better accommodate certain membrane proteins (Figure 10-14). Thus, the lateral segregation of proteins and of lipids into raft domains would, in principle, be a mutually stabilizing process. In this way, lipid rafts could help organize membrane proteins—concentrating them either for transport in membrane vesicles (discussed in Chapter 13) or for working together in protein assemblies, as when they convert extracellular signals into intracellular ones (discussed in Chapter 15).

Lipid Droplets Are Surrounded by a Phospholipid Monolayer

Most cells store an excess of lipids in **lipid droplets**, from where they can be retrieved as building blocks for membrane synthesis or as a food source. Fat cells, also called adipocytes, are specialized for lipid storage (see Figure 14-34). They contain vast numbers of large lipid droplets, from which fatty acids can be liberated on demand and exported to other cells through the bloodstream. Lipid droplets store neutral lipids, such as triacylglycerides and cholesterol esters, which are synthesized from fatty acids and cholesterol by enzymes in the

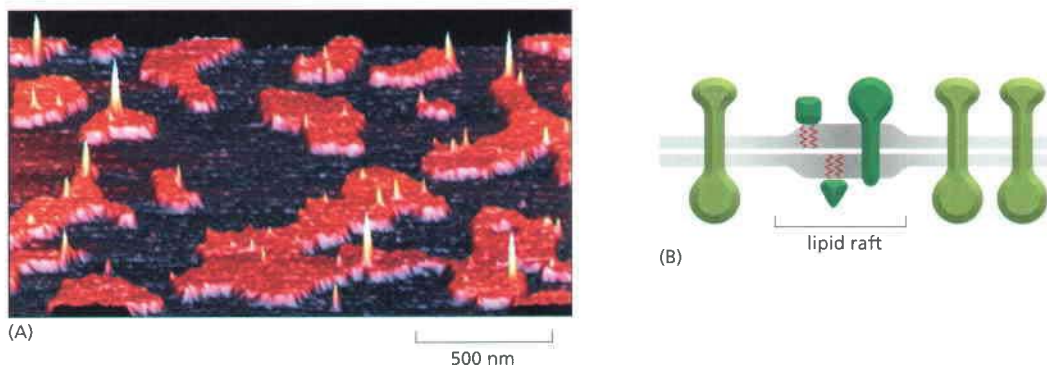


Figure 10-14 The effects of lipid rafts in artificial lipid bilayers. (A) The surface contours of a synthetic bilayer containing lipid rafts, analyzed by atomic force microscopy. Note that the raft areas, shown in orange, are thicker than the rest of the bilayer; as in Figure 10-13, the rafts primarily contain sphingomyelin and cholesterol. The sharp, yellow spikes are incorporated protein molecules, which are attached to the bilayer by a glycosylphosphatidylinositol (GPI) anchor (illustrated in Figure 10-19, example 6), and preferentially partition into the raft domains. (B) Because of both their increased thickness and lipid composition, rafts are thought to concentrate specific membrane proteins (dark green). (A, from D.E. Saslowsky et al., *J. Biol. Chem.* 277:26966–26970, 2002. With permission from the American Society for Biochemistry and Molecular Biology.)

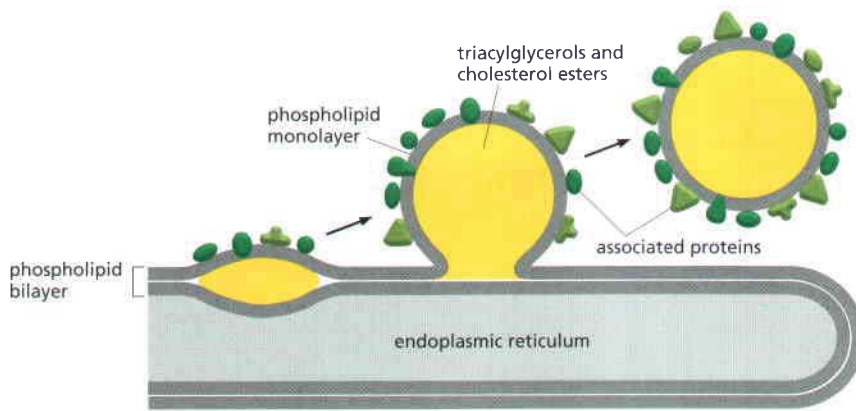


Figure 10–15 A model for the formation of lipid droplets. Neutral lipids are deposited between the two monolayers of the endoplasmic reticulum membrane. There, they aggregate into a three-dimensional droplet, which buds and pinches off from the endoplasmic reticulum membrane as a unique organelle, surrounded by a single monolayer of phospholipids and associated proteins. (Adapted from S. Martin and R.G. Parton, *Nat. Rev. Mol. Cell Biol.* 7:373–378, 2006. With permission from Macmillan Publishers Ltd.)

endoplasmic reticulum membrane. Because these lipids do not contain hydrophilic head groups, they are exclusively hydrophobic molecules, which aggregate into three-dimensional droplets rather than into bilayers.

Lipid droplets are unique organelles because they are surrounded by a single monolayer of phospholipids, which contains a large variety of proteins. Some of the proteins are enzymes involved in lipid metabolism, but the functions of most are unknown. Lipid droplets form rapidly when cells are exposed to high concentrations of fatty acids. They form from discrete regions of the endoplasmic reticulum membrane where many enzymes of lipid metabolism are concentrated. **Figure 10–15** shows one model of how lipid droplets may form and acquire their surrounding monolayer of phospholipids and proteins.

The Asymmetry of the Lipid Bilayer Is Functionally Important

The lipid compositions of the two monolayers of the lipid bilayer in many membranes are strikingly different. In the human red blood cell membrane, for example, almost all of the phospholipid molecules that have choline— $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{OH}$ —in their head group (phosphatidylcholine and sphingomyelin) are in the outer monolayer, whereas almost all that contain a terminal primary amino group (phosphatidylethanolamine and phosphatidylserine) are in the inner monolayer (**Figure 10–16**). Because the negatively charged phosphatidylserine is located in the inner monolayer, there is a significant difference in charge between the two halves of the bilayer. We discuss in Chapter 12 how membrane-bound phospholipid translocators generate and maintain lipid asymmetry.

Lipid asymmetry is functionally important, especially in converting extracellular signals into intracellular ones (discussed in Chapter 15). Many cytosolic proteins bind to specific lipid head groups found in the cytosolic monolayer of the lipid bilayer. The enzyme *protein kinase C* (PKC), for example, is activated in response to various extracellular signals. It binds to the cytosolic face of the plasma membrane, where phosphatidylserine is concentrated, and requires this negatively charged phospholipid for its activity.

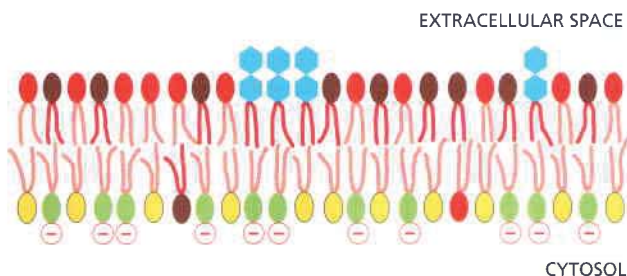


Figure 10–16 The asymmetrical distribution of phospholipids and glycolipids in the lipid bilayer of human red blood cells. The colors used for the phospholipid head groups are those introduced in Figure 10–3. In addition, glycolipids are drawn with hexagonal polar head groups (blue). Cholesterol (not shown) is thought to be distributed roughly equally in both monolayers.

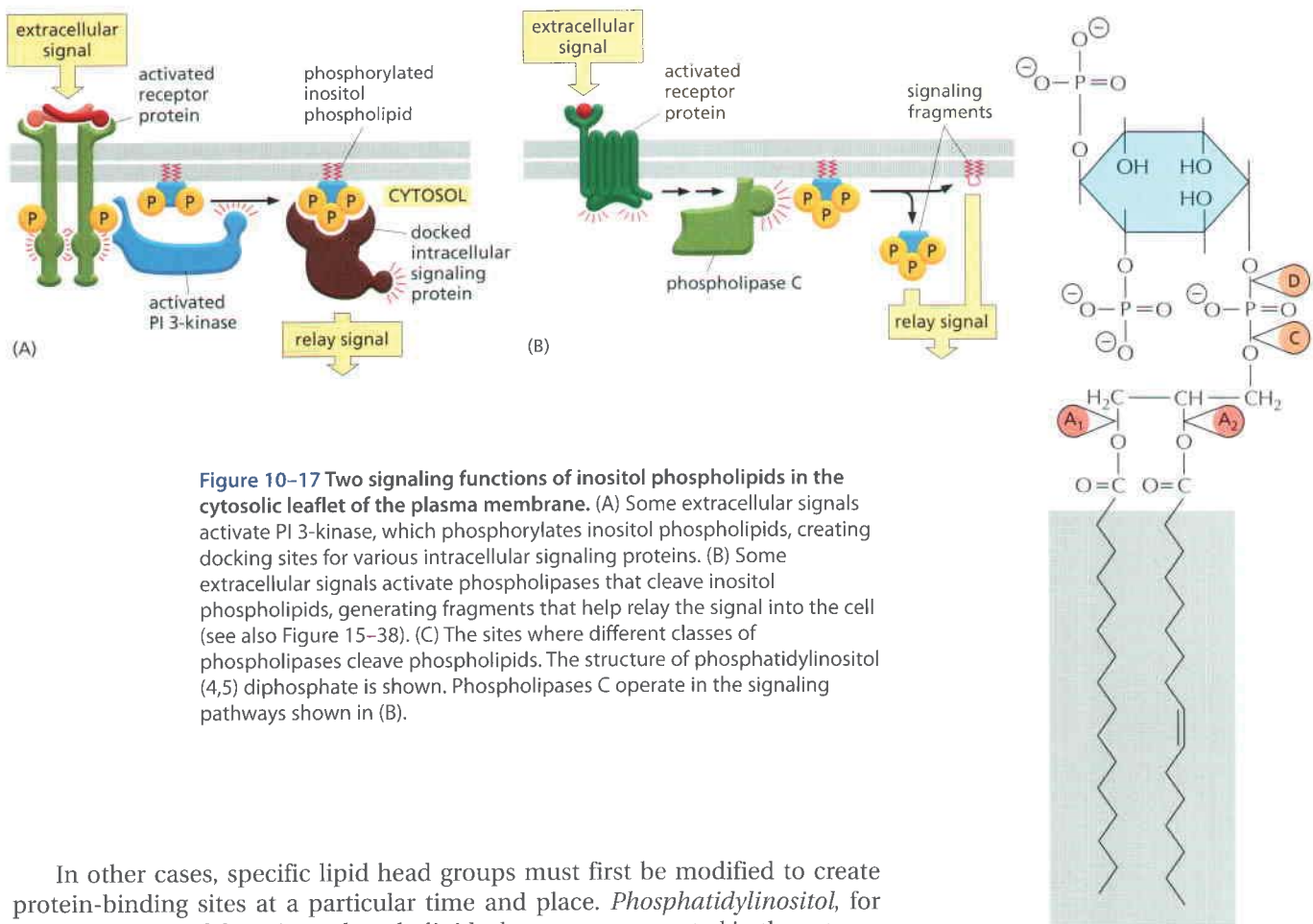


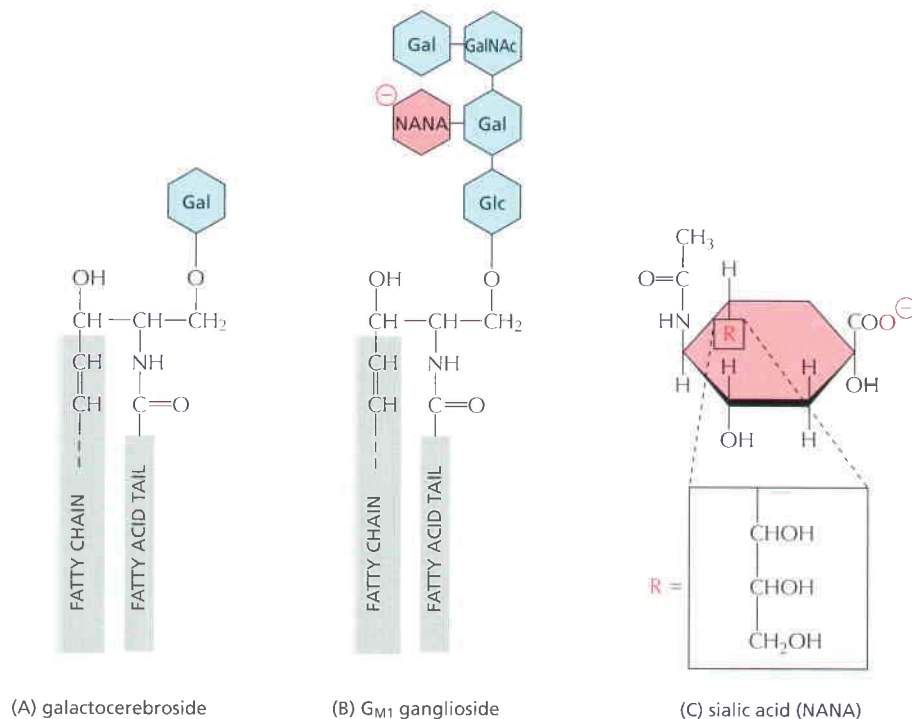
Figure 10–17 Two signaling functions of inositol phospholipids in the cytosolic leaflet of the plasma membrane. (A) Some extracellular signals activate PI 3-kinase, which phosphorylates inositol phospholipids, creating docking sites for various intracellular signaling proteins. (B) Some extracellular signals activate phospholipases that cleave inositol phospholipids, generating fragments that help relay the signal into the cell (see also Figure 15–38). (C) The sites where different classes of phospholipases cleave phospholipids. The structure of phosphatidylinositol (4,5) diphosphate is shown. Phospholipases C operate in the signaling pathways shown in (B).

In other cases, specific lipid head groups must first be modified to create protein-binding sites at a particular time and place. *Phosphatidylinositol*, for instance, is one of the minor phospholipids that are concentrated in the cytosolic monolayer of cell membranes. Various lipid kinases can add phosphate groups at distinct positions in the inositol ring, creating binding sites that recruit specific proteins from the cytosol to the membrane. An important example of such a lipid kinase is *phosphoinositide 3-kinase (PI 3-kinase)*, which is activated in response to extracellular signals and helps to recruit specific intracellular signaling proteins to the cytosolic face of the plasma membrane (Figure 10–17A). Similar lipid kinases phosphorylate inositol phospholipids in intracellular membranes and thereby help to recruit proteins that guide membrane transport.

Phospholipids in the plasma membrane are used in yet another way to convert extracellular signals into intracellular ones. The plasma membrane contains various *phospholipases* that are activated by extracellular signals to cleave specific phospholipid molecules, generating fragments of these molecules that act as short-lived intracellular mediators. *Phospholipase C*, for example, cleaves an inositol phospholipid in the cytosolic monolayer of the plasma membrane to generate two fragments, one of which remains in the membrane and helps activate protein kinase C, while the other is released into the cytosol and stimulates the release of Ca^{2+} from the endoplasmic reticulum (Figure 10–17B–C).

Animals exploit the phospholipid asymmetry of their plasma membranes to distinguish between live and dead cells. When animal cells undergo apoptosis (a form of programmed cell death, discussed in Chapter 18), phosphatidylserine, which is normally confined to the cytosolic monolayer of the plasma membrane lipid bilayer, rapidly translocates to the extracellular monolayer. The phosphatidylserine exposed on the cell surface signals neighboring cells, such as macrophages, to phagocytose the dead cell and digest it. The translocation of the phosphatidylserine in apoptotic cells is thought to occur by two mechanisms:

1. The phospholipid translocator that normally transports this lipid from the noncytosolic monolayer to the cytosolic monolayer is inactivated.
2. A “scramblase” that transfers phospholipids nonspecifically in both directions between the two monolayers is activated.

**Figure 10–18 Glycolipid molecules.**

(A) Galactocerebroside is called a *neutral glycolipid* because the sugar that forms its head group is uncharged. (B) A ganglioside always contains one or more negatively charged sialic acid residues (also called *N*-acetylneuraminic acid, or NANA), whose structure is shown in (C). Whereas in bacteria and plants almost all glycolipids are derived from glycerol, as are most phospholipids, in animal cells almost all glycolipids are based on sphingosine, as is the case for sphingomyelin (see Figure 10–3). Gal = galactose; Glc = glucose, GalNAc = *N*-acetylgalactosamine; these three sugars are uncharged.

Glycolipids Are Found on the Surface of All Plasma Membranes

Sugar-containing lipid molecules called **glycolipids**, found exclusively in the noncytosolic monolayer of the lipid bilayer, have the most extreme asymmetry in their membrane distribution. In animal cells they are made from sphingosine, just like sphingomyelin. These intriguing molecules tend to self-associate, partly through hydrogen bonds between their sugars and partly through van der Waals forces between their long and straight hydrocarbon chains, and they may preferentially partition into lipid rafts. The asymmetric distribution of glycolipids in the bilayer results from the addition of sugar groups to the lipid molecules in the lumen of the Golgi apparatus. Thus, the compartment in which they are manufactured is topologically equivalent to the exterior of the cell (discussed in Chapter 12). As they are delivered to the plasma membrane, the sugar groups are exposed at the cell surface (see Figure 10–16), where they have important roles in interactions of the cell with its surroundings.

Glycolipids probably occur in all animal cell plasma membranes, where they generally constitute about 5% of the lipid molecules in the outer monolayer. They are also found in some intracellular membranes. The most complex of the glycolipids, the **gangliosides**, contain oligosaccharides with one or more sialic acid residues, which give gangliosides a net negative charge (Figure 10–18). The most abundant of the more than 40 different gangliosides that have been identified are in the plasma membrane of nerve cells, where gangliosides constitute 5–10% of the total lipid mass; they are also found in much smaller quantities in other cell types.

Hints as to the functions of glycolipids come from their localization. In the plasma membrane of epithelial cells, for example, glycolipids are confined to the exposed apical surface, where they may help to protect the membrane against the harsh conditions frequently found there (such as low pH and high concentrations of degradative enzymes). Charged glycolipids, such as gangliosides, may be important because of their electrical effects: their presence alters the electrical field across the membrane and the concentrations of ions—especially Ca^{2+} —at the membrane surface. Glycolipids are also thought to function in cell-recognition processes, in which membrane-bound carbohydrate-binding proteins (*lectins*) bind to the sugar groups on both glycolipids and glycoproteins in the process of cell–cell adhesion (discussed in Chapter 19). Surprisingly, however, mutant mice that are deficient in all of their complex gangliosides

show no obvious abnormalities, although the males cannot transport testosterone normally in the testes and are consequently sterile.

Whatever their normal function, some glycolipids provide entry points for certain bacterial toxins. The ganglioside G_{M1} (see Figure 10–18), for example, acts as a cell-surface receptor for the bacterial toxin that causes the debilitating diarrhea of cholera. Cholera toxin binds to and enters only those cells that have G_{M1} on their surface, including intestinal epithelial cells. Its entry into a cell leads to a prolonged increase in the concentration of intracellular cyclic AMP (discussed in Chapter 15), which in turn causes a large efflux of Na^+ and water into the intestine.

Summary

Biological membranes consist of a continuous double layer of lipid molecules in which membrane proteins are embedded. This lipid bilayer is fluid, with individual lipid molecules able to diffuse rapidly within their own monolayer. The membrane lipid molecules are amphiphilic. When placed in water they assemble spontaneously into bilayers, which form sealed compartments.

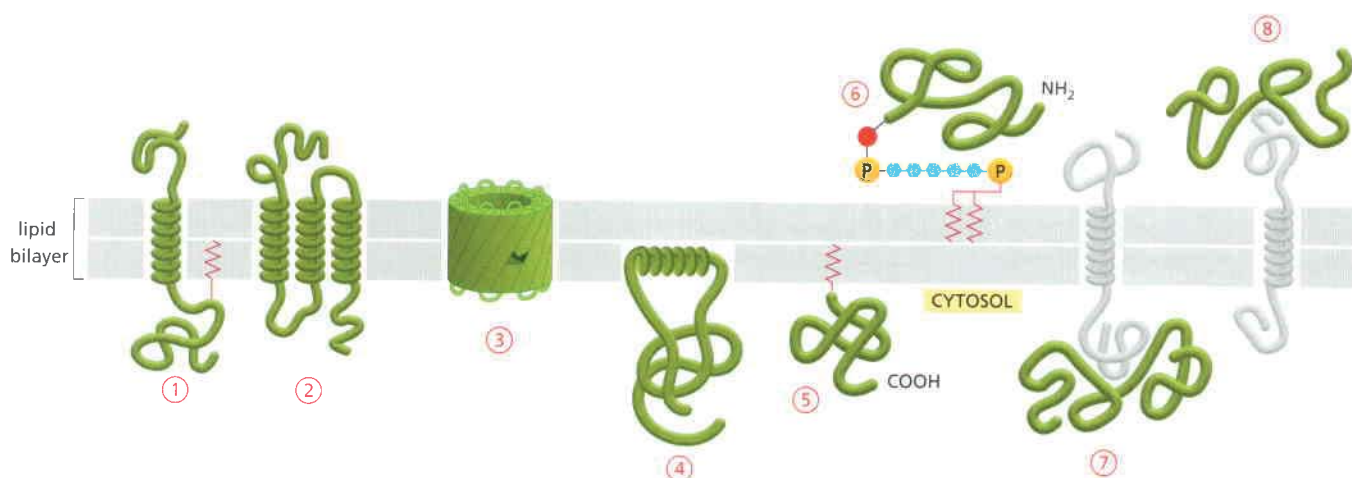
Cells contain 500–1000 different lipid species. There are three major classes of membrane lipids—phospholipids, cholesterol, and glycolipids—and hundreds of minor classes. The lipid compositions of the inner and outer monolayers are different, reflecting the different functions of the two faces of a cell membrane. Different mixtures of lipids are found in the membranes of cells of different types, as well as in the various membranes of a single eucaryotic cell. Inositol phospholipids are a minor class of phospholipids, which in the cytosolic leaflet of the plasma membrane lipid bilayer play an important part in cell signaling: in response to extracellular signals, specific lipid kinases phosphorylate the head groups of these lipids to form docking sites for cytosolic signaling proteins, whereas specific phospholipases cleave certain inositol phospholipids to generate small intracellular signaling molecules.

MEMBRANE PROTEINS

Although the lipid bilayer provides the basic structure of biological membranes, the membrane proteins perform most of the membrane's specific tasks and therefore give each type of cell membrane its characteristic functional properties. Accordingly, the amounts and types of proteins in a membrane are highly variable. In the myelin membrane, which serves mainly as electrical insulation for nerve cell axons, less than 25% of the membrane mass is protein. By contrast, in the membranes involved in ATP production (such as the internal membranes of mitochondria and chloroplasts), approximately 75% is protein. A typical plasma membrane is somewhere in between, with protein accounting for about half of its mass. Because lipid molecules are small compared with protein molecules, there are always many more lipid molecules than protein molecules in cell membranes—about 50 lipid molecules for each protein molecule in cell membranes that are 50% protein by mass. Membrane proteins vary widely in structure and in the way they associate with the lipid bilayer, which reflects their diverse functions.

Membrane Proteins Can Be Associated with the Lipid Bilayer in Various Ways

Figure 10–19 shows the different ways in which membrane proteins can associate with the membrane. Many extend through the lipid bilayer, with part of their mass on either side (Figure 10–19, examples 1, 2, and 3). Like their lipid neighbors, these **transmembrane proteins** are amphiphilic, having hydrophobic and hydrophilic regions. Their hydrophobic regions pass through the membrane and interact with the hydrophobic tails of the lipid molecules in the interior of the bilayer, where they are sequestered away from water. Their hydrophilic regions are exposed to water on either side of the membrane. The covalent attachment of a fatty acid chain that inserts into the cytosolic monolayer of the



lipid bilayer increases the hydrophobicity of some of these transmembrane proteins (see Figure 10–19, example 1).

Other membrane proteins are located entirely in the cytosol and are associated with the cytosolic monolayer of the lipid bilayer, either by an amphiphilic α helix exposed on the surface of the protein (Figure 10–19, example 4) or by one or more covalently attached lipid chains (Figure 10–19, example 5). Yet other membrane proteins are entirely exposed at the external cell surface, being attached to the lipid bilayer only by a covalent linkage (via a specific oligosaccharide) to phosphatidylinositol in the outer lipid monolayer of the plasma membrane (Figure 10–19, example 6).

The lipid-linked proteins in example 5 in Figure 10–19 are made as soluble proteins in the cytosol and are subsequently anchored to the membrane by the covalent attachment of a lipid group. The proteins in example 6, however, are made as single-pass transmembrane proteins in the endoplasmic reticulum (ER). While still in the ER, the transmembrane segment of the protein is cleaved off and a **glycosylphosphatidylinositol (GPI) anchor** is added, leaving the protein bound to the noncytosolic surface of the membrane solely by this anchor (discussed in Chapter 12). Transport vesicles eventually deliver the protein to the plasma membrane (discussed in Chapter 13). Proteins bound to the plasma membrane by a GPI anchor can be readily distinguished by the use of an enzyme called phosphatidylinositol-specific phospholipase C. This enzyme cuts these proteins free from their anchors, thereby releasing them from the membrane.

Some membrane proteins do not extend into the hydrophobic interior of the lipid bilayer at all; they are instead bound to either face of the membrane by noncovalent interactions with other membrane proteins (Figure 10–19, examples 7 and 8). Many of the proteins of this type can be released from the membrane by relatively gentle extraction procedures, such as exposure to solutions of very high or low ionic strength or of extreme pH, which interfere with protein–protein interactions but leave the lipid bilayer intact; these proteins are referred to as **peripheral membrane proteins**. Transmembrane proteins and many proteins held in the bilayer by lipid groups or hydrophobic polypeptide regions that insert into the hydrophobic core of the lipid bilayer cannot be released in these ways. These proteins are called **integral membrane proteins**.

Lipid Anchors Control the Membrane Localization of Some Signaling Proteins

How a membrane protein is associated with the lipid bilayer reflects the function of the protein. Only transmembrane proteins can function on both sides of the bilayer or transport molecules across it. Cell-surface receptors, for example, are transmembrane proteins that bind signal molecules in the extracellular space and generate different intracellular signals on the opposite side of the plasma membrane. To transfer small hydrophilic molecules across a membrane,

Figure 10–19 Various ways in which membrane proteins associate with the lipid bilayer. Most transmembrane proteins are thought to extend across the bilayer as (1) a single α helix, (2) as multiple α helices, or (3) as a rolled-up β sheet (a β barrel). Some of these “single-pass” and “multipass” proteins have a covalently attached fatty acid chain inserted in the cytosolic lipid monolayer (1). Other membrane proteins are exposed at only one side of the membrane. (4) Some of these are anchored to the cytosolic surface by an amphiphilic α helix that partitions into the cytosolic monolayer of the lipid bilayer through the hydrophobic face of the helix. (5) Others are attached to the bilayer solely by a covalently attached lipid chain—either a fatty acid chain or a prenyl group (see Figure 10–20)—in the cytosolic monolayer or, (6) via an oligosaccharide linker, to phosphatidylinositol in the noncytosolic monolayer—called a GPI anchor. (7, 8) Finally, many proteins are attached to the membrane only by noncovalent interactions with other membrane proteins. The way in which the structure in (5) is formed is illustrated in Figure 10–20, while the way in which the GPI anchor shown in (6) is formed is illustrated in Figure 12–56. The details of how membrane proteins become associated with the lipid bilayer are discussed in Chapter 12.

a membrane transport protein must provide a path for the molecules to cross the hydrophobic permeability barrier of the lipid bilayer; the molecular architecture of multipass membrane proteins is ideally suited for this task, as we discuss in Chapter 11.

Proteins that function on only one side of the lipid bilayer, by contrast, are often associated exclusively with either the lipid monolayer or a protein domain on that side. Some intracellular signaling proteins, for example, that are involved in converting extracellular signals into intracellular ones are bound to the cytosolic half of the plasma membrane by one or more covalently attached lipid groups, which can be fatty acid chains or *prenyl groups* (Figure 10–20). In some cases, myristic acid, a saturated 14-carbon fatty acid, is added to the N-terminal amino group of the protein during its synthesis on the ribosome. All members of the *Src family* of cytoplasmic protein tyrosine kinases (discussed in Chapter 15) are myristoylated in this way. Membrane attachment through a single lipid anchor is not very strong, however, and a second lipid group is often added to anchor proteins more firmly to a membrane. For most Src kinases, the second lipid modification is the attachment of palmitic acid, a saturated 16-carbon fatty acid, to a cysteine side chain of the protein. This modification occurs in response to an extracellular signal and helps recruit the kinases to the plasma membrane. When the signaling pathway is turned off, the palmitic acid is removed, allowing the kinase to return to the cytosol. Other intracellular signaling proteins, such as the Ras family small GTPases (discussed in Chapter 15), use a combination of prenyl group and palmitic acid attachment to recruit the proteins to the plasma membrane.

In Most Transmembrane Proteins the Polypeptide Chain Crosses the Lipid Bilayer in an α -Helical Conformation

A transmembrane protein always has a unique orientation in the membrane. This reflects both the asymmetric manner in which it is inserted into the lipid

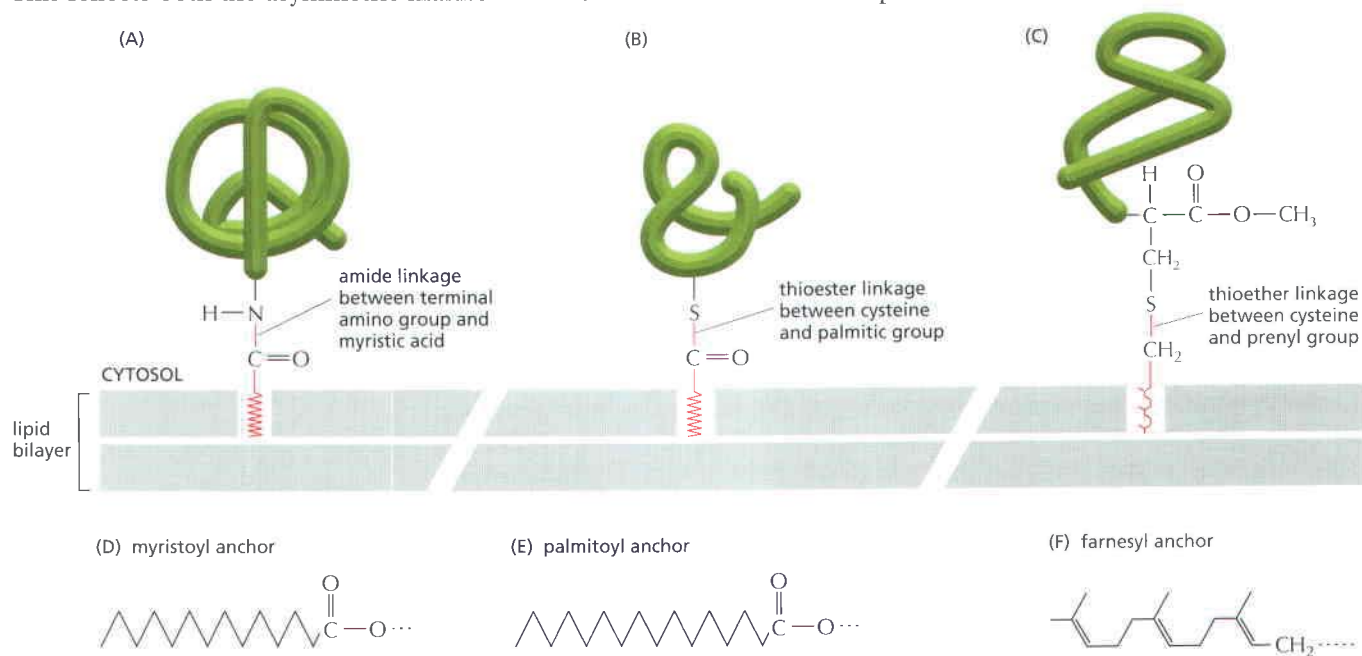


Figure 10–20 Membrane protein attachment by a fatty acid chain or a prenyl group. The covalent attachment of either type of lipid can help localize a water-soluble protein to a membrane after its synthesis in the cytosol. (A) A fatty acid chain (myristic acid) is attached via an amide linkage to an N-terminal glycine. (B) A fatty acid chain (palmitic acid) is attached via a thioester linkage to a cysteine. (C) A prenyl group (either farnesyl or a longer geranylgeranyl group) is attached via a thioether linkage to a cysteine residue that is initially located four residues from the protein's C-terminus. After prenylation, the terminal three amino acids are cleaved off, and the new C-terminus is methylated before insertion of the anchor into the membrane (not shown). The structures of the lipid anchors are shown below: (D) a myristoyl anchor (a 14-carbon saturated fatty acid chain), (E) a palmitoyl anchor (a 16-carbon saturated fatty acid chain), and (F) a farnesyl anchor (a 15-carbon unsaturated hydrocarbon chain).

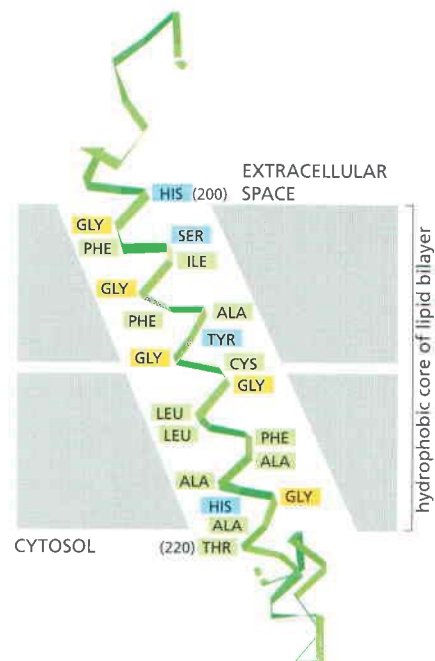
Figure 10–21 A segment of a transmembrane polypeptide chain crossing the lipid bilayer as an α helix. <GTAG> Only the α -carbon backbone of the polypeptide chain is shown, with the hydrophobic amino acids in green and yellow. The polypeptide segment shown is part of the bacterial photosynthetic reaction center illustrated in Figure 10–34, the structure of which was determined by x-ray diffraction. (Based on data from J. Deisenhofer et al., *Nature* 318:618–624, 1985, and H. Michel et al., *EMBO J.* 5:1149–1158, 1986. All with permission from Macmillan Publishers Ltd.)

bilayer in the ER during its biosynthesis (discussed in Chapter 12) and the different functions of its cytosolic and noncytosolic domains. These domains are separated by the membrane-spanning segments of the polypeptide chain, which contact the hydrophobic environment of the lipid bilayer and are composed largely of amino acids with nonpolar side chains. Because the peptide bonds themselves are polar and because water is absent, all peptide bonds in the bilayer are driven to form hydrogen bonds with one another. The hydrogen-bonding between peptide bonds is maximized if the polypeptide chain forms a regular α helix as it crosses the bilayer, and this is how most membrane-spanning segments of polypeptide chains traverse the bilayer (Figure 10–21).

In **single-pass transmembrane proteins**, the polypeptide chain crosses only once (see Figure 10–19, example 1), whereas in **multipass transmembrane proteins**, the polypeptide chain crosses multiple times (see Figure 10–19, example 2). An alternative way for the peptide bonds in the lipid bilayer to satisfy their hydrogen-bonding requirements is for multiple transmembrane strands of a polypeptide chain to be arranged as a β sheet that is rolled up into a closed barrel (a so-called β barrel; see Figure 10–19, example 3). This form of multipass transmembrane structure is seen in the *porin proteins* that we discuss later.

Rapid progress in the x-ray crystallography of membrane proteins has enabled us to determine the three-dimensional structure of many of them. The structures confirm that it is often possible to predict from the protein's amino acid sequence which parts of the polypeptide chain extend across the lipid bilayer. Segments containing about 20–30 amino acids with a high degree of hydrophobicity are long enough to span a lipid bilayer as an α helix, and they can often be identified in *hydropathy plots* (Figure 10–22). From such plots, it is estimated that about 20% of the kind of an organism's proteins are transmembrane proteins, emphasizing their importance. Hydropathy plots cannot identify the membrane-spanning segments of a β barrel, as 10 amino acids or fewer are sufficient to traverse a lipid bilayer as an extended β strand and only every other amino acid side chain is hydrophobic.

The strong drive to maximize hydrogen-bonding in the absence of water means that a polypeptide chain that enters the bilayer is likely to pass entirely through it before changing direction, since chain bending requires a loss of regular hydrogen-bonding interactions. But multipass membrane proteins can also contain regions that fold into the membrane from either side, squeezing into spaces between transmembrane α helices without contacting the hydrophobic core of the lipid bilayer. Because such regions of the polypeptide chain interact only with other polypeptide regions, they do not need to maximize hydrogen-bonding; they can therefore have a variety of secondary structures, including helices that extend only part way across the lipid bilayer (Figure 10–23). Such regions are important for the function of some membrane proteins, including the K^+ and water channels; the regions contribute to the walls of the pores traversing the membrane and confer substrate specificity on the channels, as we discuss in Chapter 11. These regions cannot be identified in hydropathy plots and are only revealed by x-ray crystallography, electron diffraction (a technique similar to x-ray diffraction but performed on two-dimensional arrays of proteins), or NMR studies of the protein's three-dimensional structure.



Transmembrane α Helices Often Interact with One Another

The transmembrane α helices of many single-pass membrane proteins do not contribute to the folding of the protein domains on either side of the membrane.

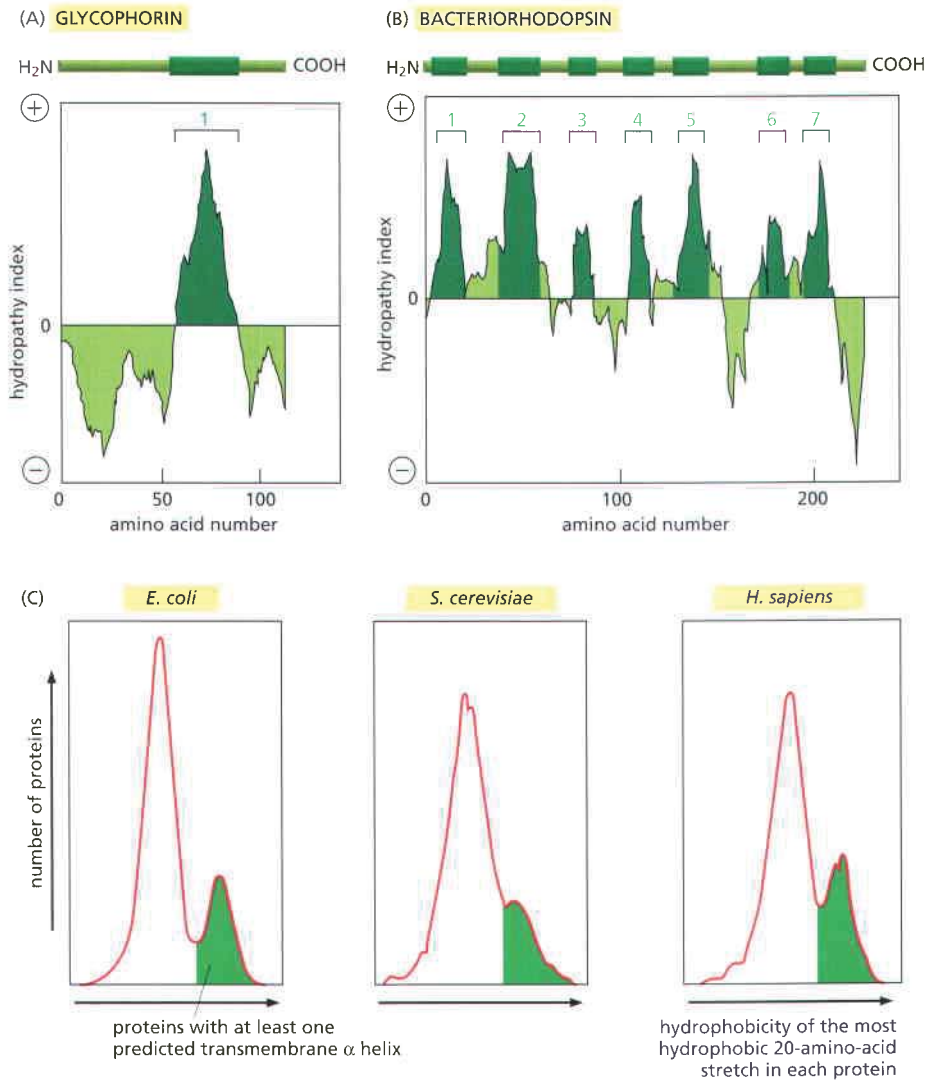
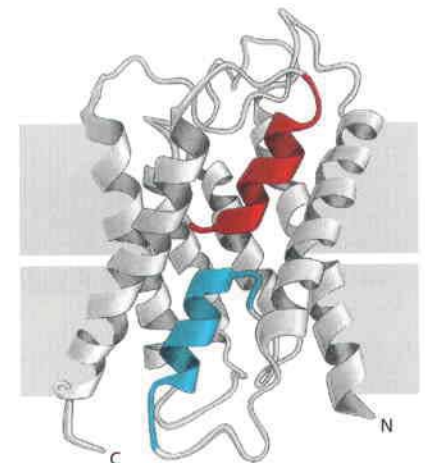


Figure 10-22 Using hydrophathy plots to localize potential α -helical membrane-spanning segments in a polypeptide chain. The free energy needed to transfer successive segments of a polypeptide chain from a nonpolar solvent to water is calculated from the amino acid composition of each segment using data obtained from model compounds. This calculation is made for segments of a fixed size (usually around 10–20 amino acids), beginning with each successive amino acid in the chain. The “hydropathy index” of the segment is plotted on the Y axis as a function of its location in the chain. A positive value indicates that free energy is required for transfer to water (i.e., the segment is hydrophobic), and the value assigned is an index of the amount of energy needed. Peaks in the hydrophathy index appear at the positions of hydrophobic segments in the amino acid sequence. (A and B) Two examples of membrane proteins discussed later in this chapter are shown. Glycophorin (A) has a single membrane-spanning α helix and one corresponding peak in the hydrophathy plot. Bacteriorhodopsin (B) has seven membrane-spanning α helices and seven corresponding peaks in the hydrophathy plot. (C) The proportion of predicted membrane proteins encoded by the genomes of *E. coli*, *S. cerevisiae*, and human. The area shaded in green indicates the fraction of proteins that contain at least one predicted transmembrane helix. The data for *E. coli* and *S. cerevisiae* represent the whole genome; the data for human represent only part of the genome; in each case, the area under the curve is proportional to the number of genes analyzed. (A, adapted from D. Eisenberg, *Annu. Rev. Biochem.* 53:595–624, 1984. With permission from Annual Reviews; C, adapted from D. Boyd et al., *Protein Sci.* 7:201–205, 1998. With permission from The Protein Society.)

As a consequence, it is often possible to engineer cells to produce the cytosolic or extracellular domains of these proteins as water-soluble protein. This approach has been invaluable to study the structure and function of these domains, especially of those in transmembrane receptor proteins (discussed in Chapter 15). A transmembrane α helix, even in a single-pass membrane protein, however, often does more than just anchor the protein to the lipid bilayer. Many single-pass membrane proteins form homodimers, which are held together by strong and highly specific interactions between the two transmembrane α helices; the sequence of the hydrophobic amino acids of these helices contains the information that directs the protein–protein interaction.

Similarly, the transmembrane α helices in multipass membrane proteins occupy specific positions in the folded protein structure that are determined by interactions between the neighboring helices. These interactions are crucial for the structure and function of the many channels and transporters that move molecules across lipid bilayers. In many cases, one can use proteases to cut the loops of the polypeptide chain that link the transmembrane α helices on either side of the bilayer and the helices stay together and function normally. In some

Figure 10-23 Two α helices in the aquaporin water channel, each of which spans only halfway through the lipid bilayer. In the membrane, the protein forms a tetramer of four such two-helix segments, such that the colored surface shown here is buried at an interface formed by protein–protein interactions. The mechanism by which the channel allows the passage of water molecules across the lipid bilayer is discussed in more detail in Chapter 11.



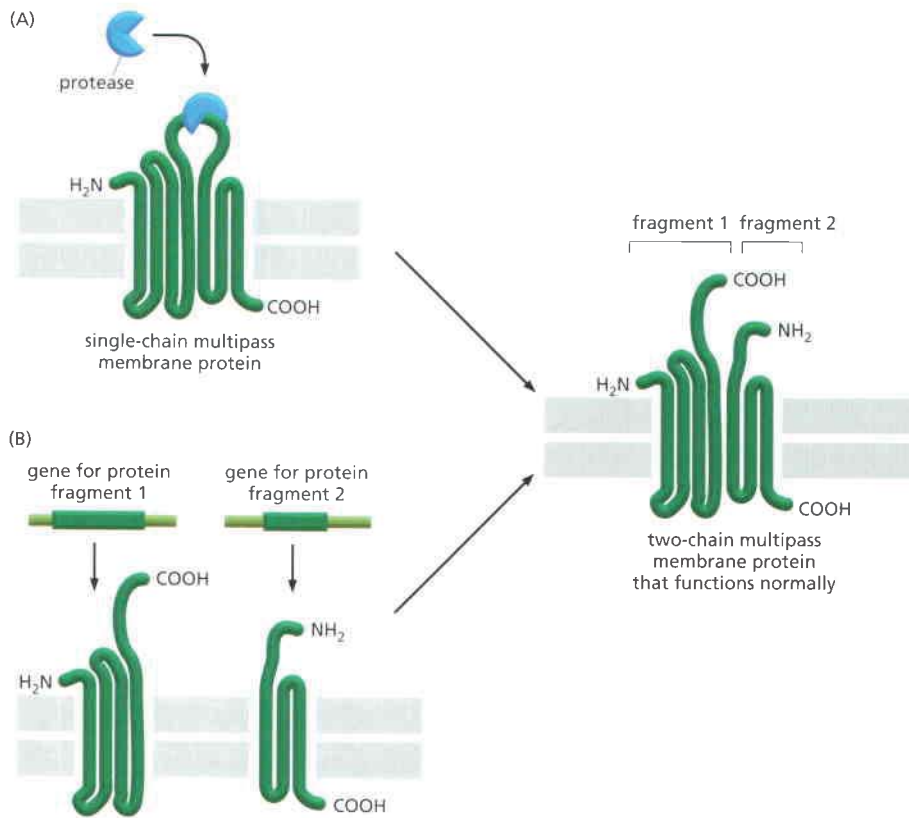


Figure 10–24 Converting a single-chain multipass protein into a two-chain multipass protein. (A) Proteolytic cleavage of one loop to create two fragments that stay together and function normally. (B) Expression of the same two fragments from separate genes gives rise to a similar protein that functions normally.

cases, one can even express engineered genes encoding separate pieces of a multipass protein in living cells, and one finds that the separate pieces assemble properly to form a functional transmembrane protein (**Figure 10–24**), emphasizing the exquisite specificity with which transmembrane α helices can interact.

In multipass membrane proteins, neighboring transmembrane helices in the folded structure of the protein shield many of the transmembrane helices from the membrane lipids. Why, then, are these shielded helices nevertheless composed primarily of hydrophobic amino acids? The answer lies in the way in which multipass proteins are integrated into the membrane during their biosynthesis. As we discuss in Chapter 12, transmembrane α helices are inserted into the lipid bilayer sequentially by a protein translocator. After leaving the translocator, each helix is transiently surrounded by lipids in the bilayer, which requires that the helix be hydrophobic. It is only as the protein folds up into its final structure that contacts are made between adjacent helices and protein–protein contacts replace some of the protein–lipid contacts (**Figure 10–25**).

Some β Barrels Form Large Transmembrane Channels

Multipass transmembrane proteins that have their transmembrane segments arranged as a β barrel rather than as an α helix are comparatively rigid and tend to crystallize readily. Thus, some of them were among the first multipass

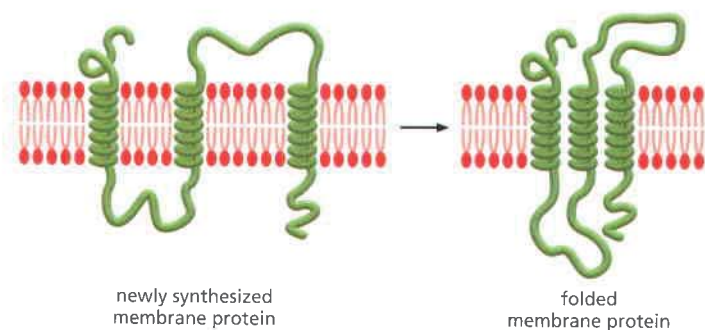


Figure 10–25 Steps in the folding of a multipass transmembrane protein. When the newly synthesized transmembrane α helices are released into the lipid bilayer, they are initially surrounded by lipid molecules. As the protein folds, contacts between the helices displace some of the lipid molecules surrounding the helices.

membrane protein structures to be determined by x-ray crystallography. The number of β strands in a β barrel varies widely, from as few as 8 strands to as many as 22 (Figure 10–26).

β barrel proteins are abundant in the outer membrane of mitochondria, chloroplasts, and many bacteria. Some are pore-forming proteins, which create water-filled channels that allow selected small hydrophilic molecules to cross the lipid bilayer of the bacterial outer membrane. The porins are well-studied examples (example 3 in Figure 10–26). The porin barrel is formed from a 16-strand, antiparallel β sheet, which is sufficiently large to roll up into a cylindrical structure. Polar amino acid side chains line the aqueous channel on the inside, while nonpolar side chains project from the outside of the barrel to interact with the hydrophobic core of the lipid bilayer. Loops of the polypeptide chain often protrude into the lumen of the channel, narrowing it so that only certain solutes can pass. Some porins are therefore highly selective: *maltoporin*, for example, preferentially allows maltose and maltose oligomers to cross the outer membrane of *E. coli*.

The *FepA* protein is a more complex example of a β barrel transport protein (example 4 in Figure 10–26). It transports iron ions across the bacterial outer membrane. It is constructed from 22 β strands, and a large globular domain completely fills the inside of the barrel. Iron ions bind to this domain, which is thought to undergo a large conformational change to transfer the iron across the membrane.

Not all β barrel proteins are transport proteins. Some form smaller barrels that are completely filled by amino acid side chains that project into the center of the barrel. These proteins function as receptors or enzymes (examples 1 and 2 in Figure 10–26), and the barrel serves as a rigid anchor, which holds the protein in the membrane and orients the cytosolic loops that form binding sites for specific intracellular molecules.

Although β barrel proteins have various functions, they are largely restricted to bacterial, mitochondrial, and chloroplast outer membranes. Most multipass transmembrane proteins in eucaryotic cells and in the bacterial plasma membrane are constructed from transmembrane α helices. The helices can slide against each other, allowing conformational changes in the protein that can open and shut ion channels, transport solutes, or transduce extracellular signals into intracellular ones. In β barrel proteins, by contrast, hydrogen bonds bind each β strand rigidly to its neighbors, making conformational changes within the wall of the barrel unlikely.

Figure 10–26 β barrels formed from different numbers of β strands. <TGCT> (1) The *E. coli* OmpA protein serves as a receptor for a bacterial virus. (2) The *E. coli* OMPLA protein is an enzyme (a lipase) that hydrolyzes lipid molecules. The amino acids that catalyze the enzymatic reaction (shown in red) protrude from the outside surface of the barrel. (3) A porin from the bacterium *Rhodobacter capsulatus* forms a water-filled pore across the outer membrane. The diameter of the channel is restricted by loops (shown in blue) that protrude into the channel. (4) The *E. coli* FepA protein transports iron ions. The inside of the barrel is completely filled by a globular protein domain (shown in blue) that contains an iron-binding site (not shown). This domain is thought to change its conformation to transport the bound iron, but the molecular details of the changes are not known.

Many Membrane Proteins Are Glycosylated

Most transmembrane proteins in animal cells are glycosylated. As in glycolipids, the sugar residues are added in the lumen of the ER and the Golgi apparatus

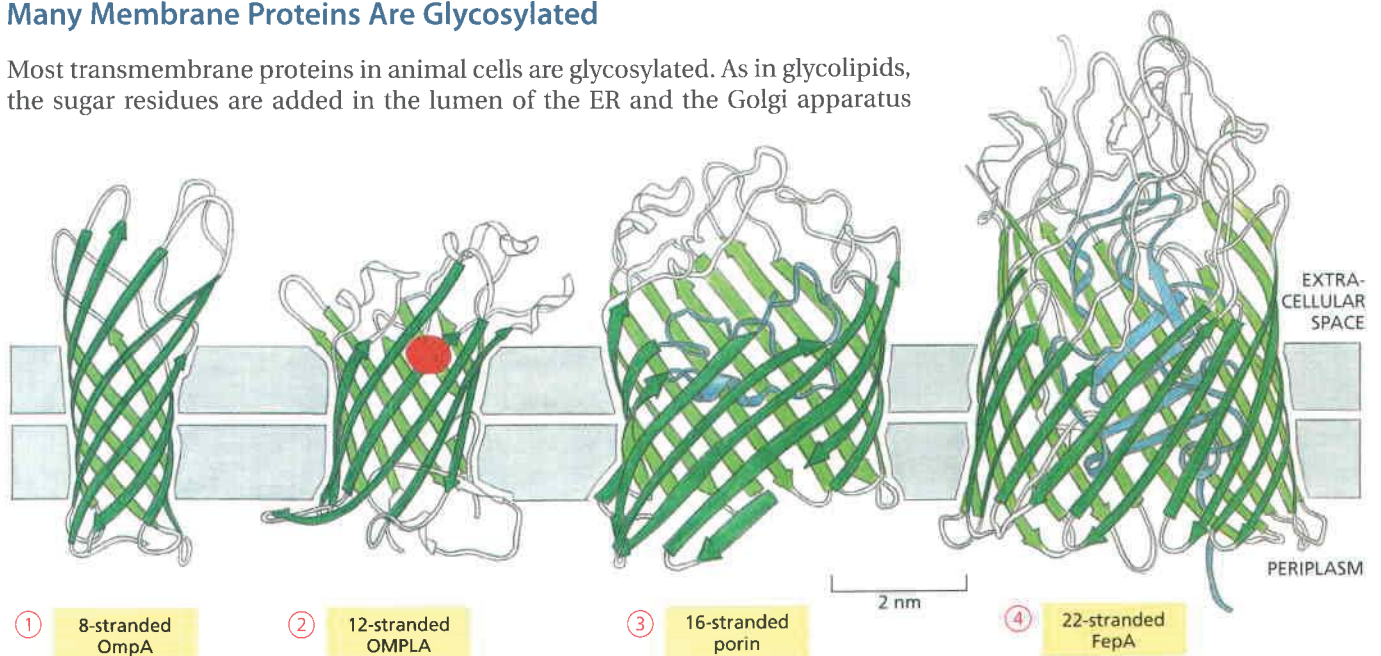


Figure 10–27 A single-pass transmembrane protein. Note that the polypeptide chain traverses the lipid bilayer as a right-handed α helix and that the oligosaccharide chains and disulfide bonds are all on the noncytosolic surface of the membrane. The sulfhydryl groups in the cytosolic domain of the protein do not normally form disulfide bonds because the reducing environment in the cytosol maintains these groups in their reduced ($-SH$) form.

(discussed in Chapters 12 and 13). For this reason, the oligosaccharide chains are always present on the noncytosolic side of the membrane. Another important difference between proteins (or parts of proteins) on the two sides of the membrane results from the reducing environment of the cytosol. This environment decreases the likelihood that intrachain or interchain disulfide ($S-S$) bonds will form between cysteines on the cytosolic side of membranes. These bonds form on the noncytosolic side, where they can help stabilize either the folded structure of the polypeptide chain or its association with other polypeptide chains (**Figure 10–27**).

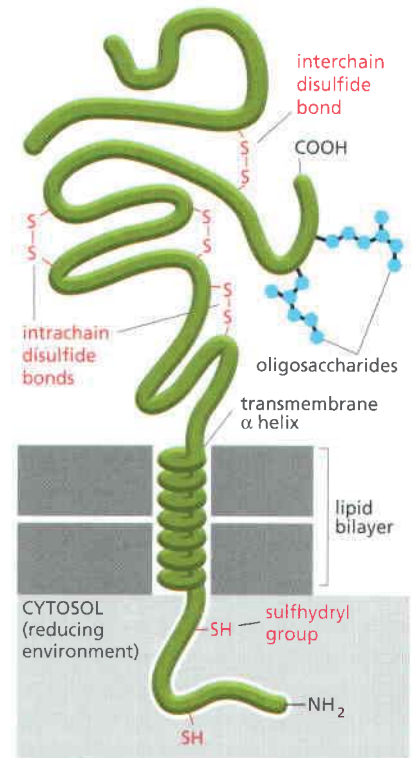
Because most plasma membrane proteins are glycosylated, carbohydrates extensively coat the surface of all eucaryotic cells. These carbohydrates occur as oligosaccharide chains covalently bound to membrane proteins (glycoproteins) and lipids (glycolipids). They also occur as the polysaccharide chains of integral membrane *proteoglycan* molecules. Proteoglycans, which consist of long polysaccharide chains linked covalently to a protein core, are found mainly outside the cell, as part of the extracellular matrix (discussed in Chapter 19). But, for some proteoglycans, the protein core either extends across the lipid bilayer or is attached to the bilayer by a glycosylphosphatidylinositol (GPI) anchor.

The terms cell coat or glycocalyx are sometimes used to describe the carbohydrate-rich zone on the cell surface. This **carbohydrate layer** can be visualized by various stains, such as ruthenium red (**Figure 10–28A**), as well as by its affinity for carbohydrate-binding proteins called **lectins**, which can be labeled with a fluorescent dye or some other visible marker. Although most of the sugar groups are attached to intrinsic plasma membrane molecules, the carbohydrate layer also contains both glycoproteins and proteoglycans that have been secreted into the extracellular space and then adsorbed onto the cell surface (**Figure 10–28B**). Many of these adsorbed macromolecules are components of the extracellular matrix, so that the boundary between the plasma membrane and the extracellular matrix is often not sharply defined. One of the many functions of the carbohydrate layer is to protect cells against mechanical and chemical damage; it also keeps various other cells at a distance, preventing unwanted protein–protein interactions.

The oligosaccharide side chains of glycoproteins and glycolipids are enormously diverse in their arrangement of sugars. Although they usually contain fewer than 15 sugars, they are often branched, and the sugars can be bonded together by various covalent linkages—unlike the amino acids in a polypeptide chain, which are all linked by identical peptide bonds. Even three sugars can be put together to form hundreds of different trisaccharides. Both the diversity and the exposed position of the oligosaccharides on the cell surface make them especially well suited to function in specific cell-recognition processes. As we discuss in Chapter 19, plasma membrane-bound lectins that recognize specific oligosaccharides on cell-surface glycolipids and glycoproteins mediate a variety of transient cell–cell adhesion processes, including those occurring in sperm–egg interactions, blood clotting, lymphocyte recirculation, and inflammatory responses.

Membrane Proteins Can Be Solubilized and Purified in Detergents

In general, only agents that disrupt hydrophobic associations and destroy the lipid bilayer can solubilize transmembrane proteins (and some other tightly bound membrane proteins). The most useful of these for the membrane biochemist are **detergents**, which are small amphiphilic molecules of variable



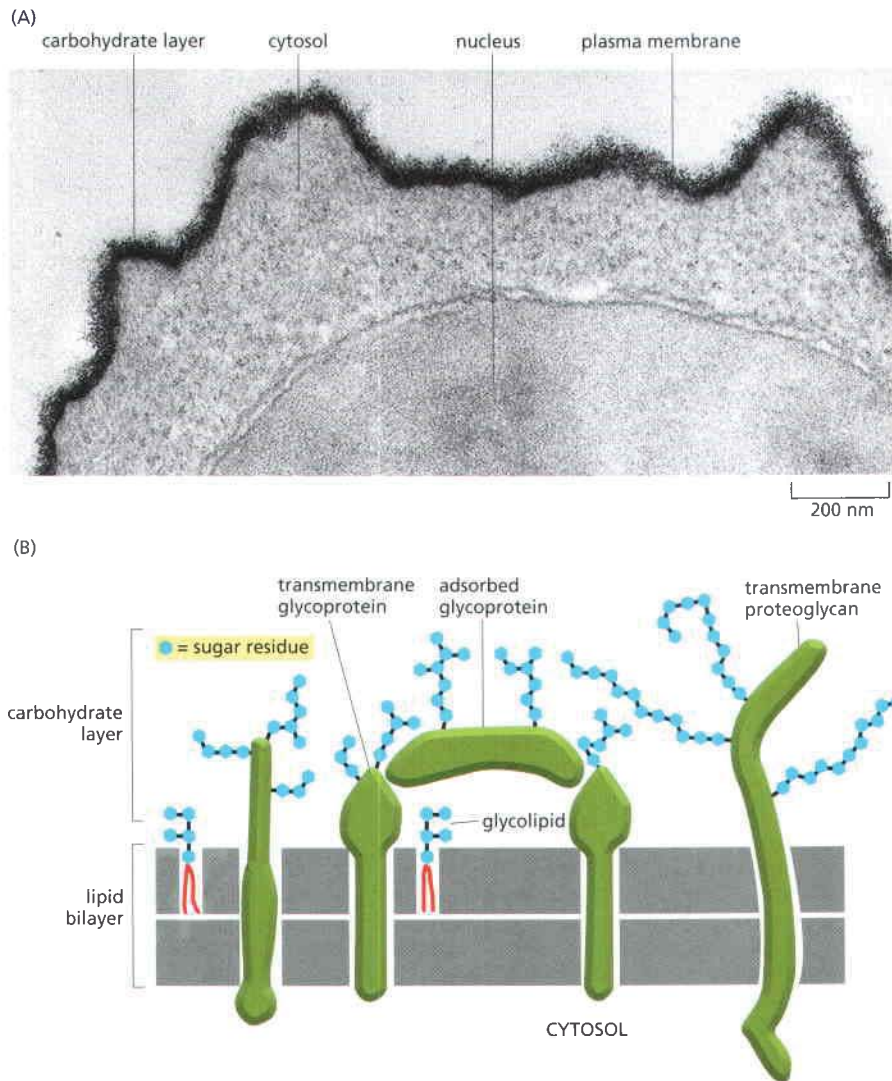


Figure 10–28 The carbohydrate layer on the cell surface. This electron micrograph of the surface of a lymphocyte stained with ruthenium red emphasizes the thick carbohydrate-rich layer surrounding the cell. (B) The carbohydrate layer is made up of the oligosaccharide side chains of glycolipids and integral membrane glycoproteins and the polysaccharide chains on integral membrane proteoglycans. In addition, adsorbed glycoproteins, and adsorbed proteoglycans (not shown) contribute to the carbohydrate layer in many cells. Note that all of the carbohydrate is on the noncytosolic surface of the membrane. (A, courtesy of Audrey M. Glauert and G.M.W. Cook.)

structure. Detergents are much more soluble in water than lipids. Their polar (hydrophilic) ends can be either charged (ionic), as in *sodium dodecyl sulfate* (SDS), or uncharged (nonionic), as in *octylglucoside* and Triton (Figure 10–29A). At low concentration, detergents are monomeric in solution, but when their concentration is increased above a threshold, called the *critical micelle concentration* or CMC, they aggregate to form micelles (Figure 10–29B–C). Detergent molecules rapidly diffuse in and out of micelles, keeping the concentration of monomer in the solution constant, no matter how many micelles are present. Both the CMC and the average number of detergent molecules in a micelle are characteristic properties of each detergent, but they also depend on the temperature, pH, and salt concentration. Detergent solutions are therefore complex systems and are difficult to study.

When mixed with membranes, the hydrophobic ends of detergents bind to the hydrophobic regions of the membrane proteins, where they displace lipid molecules with a collar of detergent molecules. Since the other end of the detergent molecule is polar, this binding tends to bring the membrane proteins into solution as detergent–protein complexes (Figure 10–30). Usually, some lipid molecules also remain attached to the protein.

Strong ionic detergents, such as SDS, can solubilize even the most hydrophobic membrane proteins. This allows the proteins to be analyzed by *SDS polyacrylamide-gel electrophoresis* (discussed in Chapter 8), a procedure that has revolutionized the study of membrane proteins. Such strong detergents unfold (denature) proteins by binding to their internal “hydrophobic cores,”

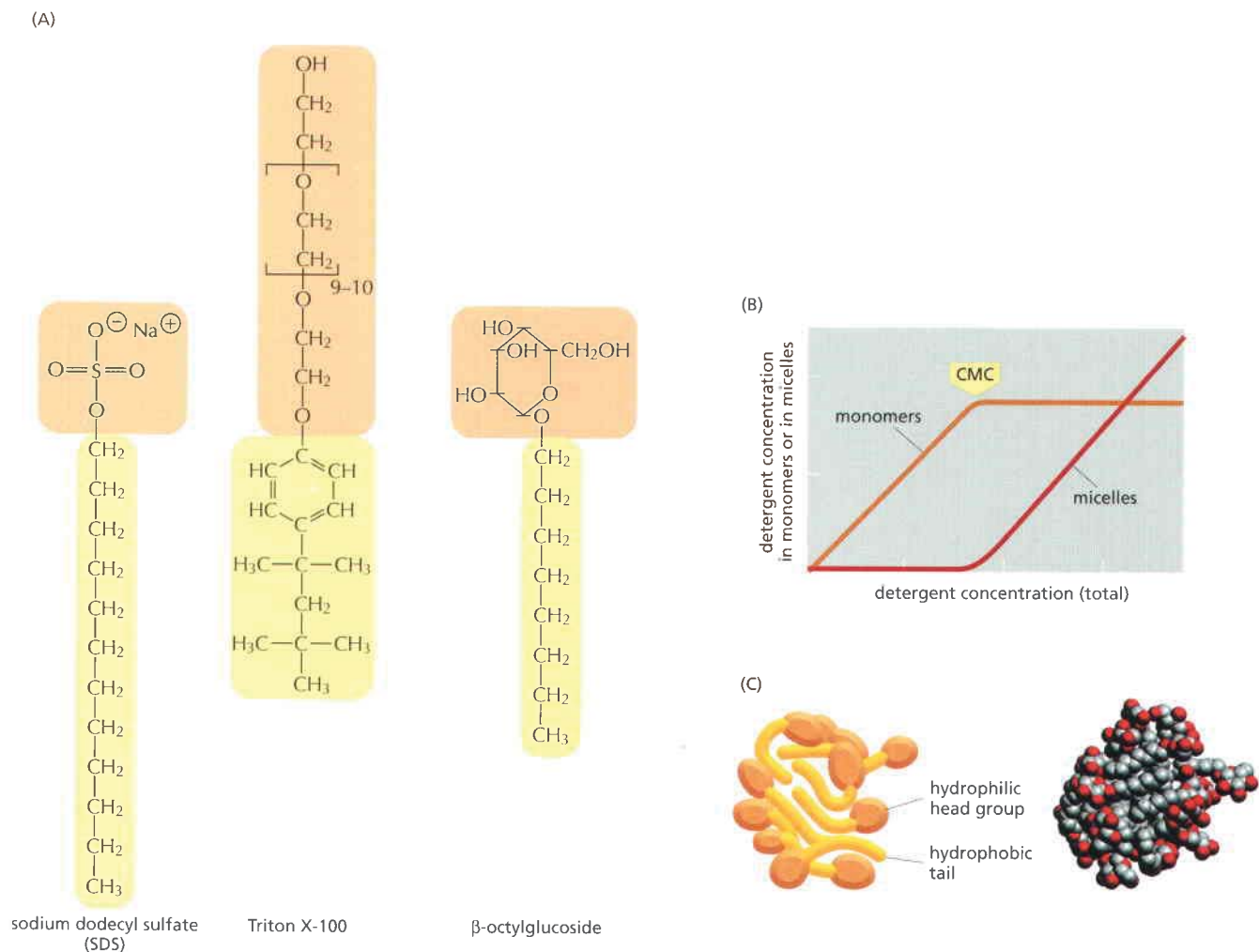


Figure 10-29 The structure and function of detergent micelles. <GCGA> (A) Three commonly used detergents are sodium dodecyl sulfate (SDS), an anionic detergent, and Triton X-100 and β -octylglucoside, two nonionic detergents. Triton X-100 is a mixture of compounds in which the region in brackets is repeated between 9 and 10 times. The hydrophobic portion of each detergent is shown in yellow, and the hydrophilic portion is shown in orange. (B) At low concentration, detergent molecules are monomeric in solution. As their concentration is increased beyond the critical micelle concentration (CMC), some of the detergent molecules form micelles. Note that the concentration of detergent monomer stays constant above the CMC. (C) Because they have both polar and nonpolar ends, detergent molecules are amphiphilic; and because they are cone-shaped, they form micelles rather than bilayers (see Figure 10-7). Detergent micelles have irregular shapes, and, due to packing constraints, the hydrophobic tails are partially exposed to water. The space-filling model shows the structure of a micelle composed of 20 β -octylglucoside molecules, predicted by molecular dynamics calculations (B, adapted from G. Gunnarsson, B. Jönsson and H. Wennerström, *J. Phys. Chem.* 84:3114–3121, 1980; C, from S. Bogusz, R.M. Venable and R.W. Pastor, *J. Phys. Chem. B.* 104:5462–5470, 2000. With permission from the American Chemical Society.)

thereby rendering the proteins inactive and unusable for functional studies. Nonetheless, proteins can be readily separated and purified in their SDS-denatured form. In some cases, removal of the detergent allows the purified protein to renature, with recovery of functional activity.

Many hydrophobic membrane proteins can be solubilized and then purified in an active form by the use of mild detergents. These detergents cover the hydrophobic regions on membrane-spanning segments that become exposed after lipid removal but do not unfold the protein. If the detergent concentration of a solution of solubilized membrane proteins is reduced (by dilution, for example), membrane proteins do not remain soluble. In the presence of an excess of phospholipid molecules in such a solution, membrane proteins incorporate into small liposomes that form spontaneously. In this way, functionally active membrane protein systems can be reconstituted from purified components, providing a powerful means of analyzing the activities of membrane transporters, ion channels, signaling receptors, and so on (Figure 10-31). Such

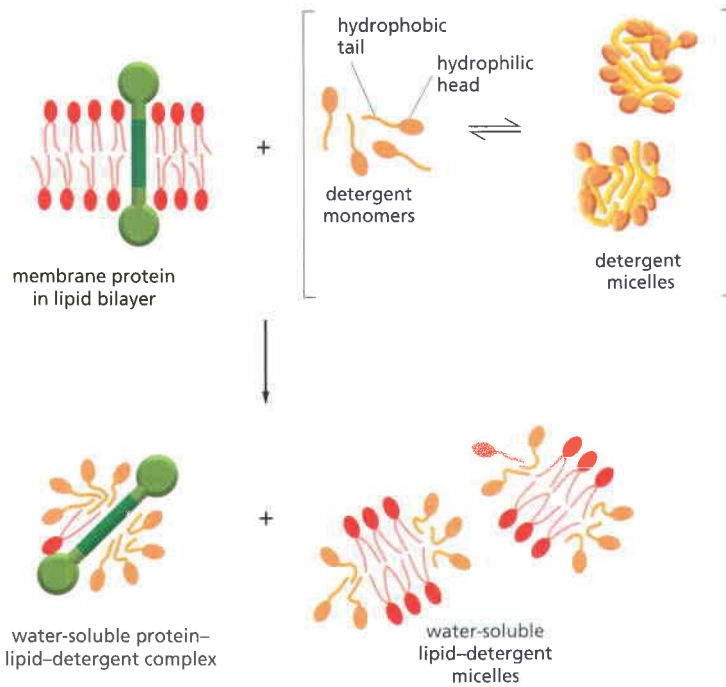


Figure 10–30 Solubilizing membrane proteins with a mild nonionic detergent. The detergent disrupts the lipid bilayer and brings the proteins into solution as protein–lipid–detergent complexes. The phospholipids in the membrane are also solubilized by the detergent.

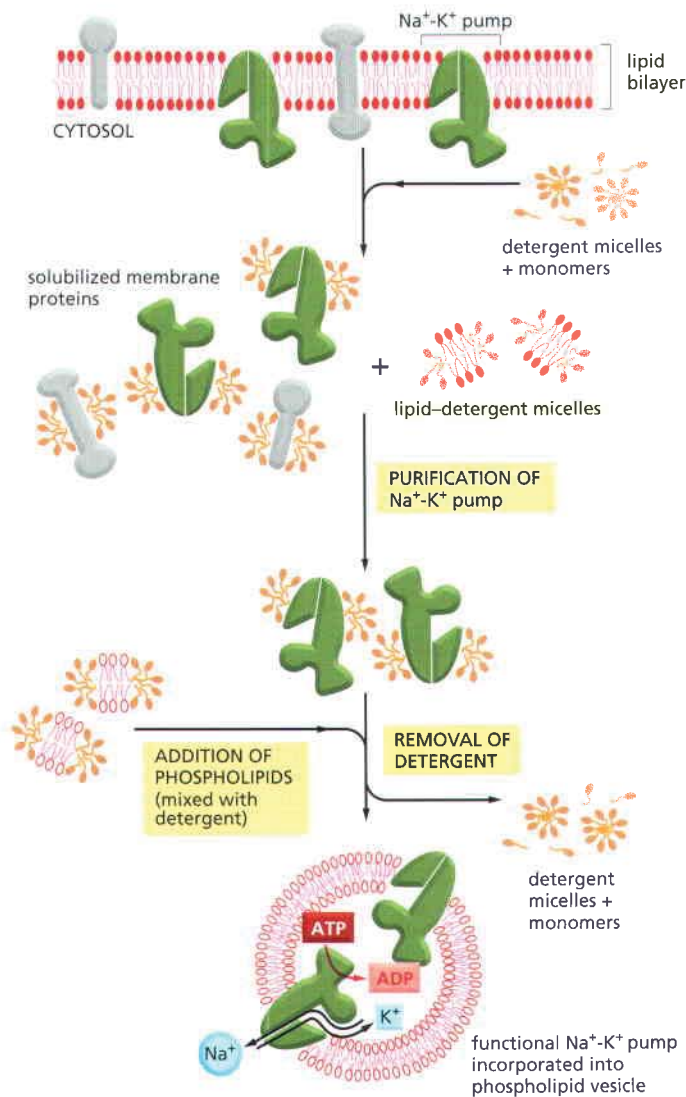


Figure 10–31 The use of mild nonionic detergents for solubilizing, purifying, and reconstituting functional membrane protein systems. In this example, functional Na⁺-K⁺ pump molecules are purified and incorporated into phospholipid vesicles. The Na⁺-K⁺ pump is an ion pump that is present in the plasma membrane of most animal cells; it uses the energy of ATP hydrolysis to pump Na⁺ out of the cell and K⁺ in, as discussed in Chapter 11.

functional reconstitution, for example, provided proof for the hypothesis that the transmembrane ATPases use H^+ gradients in mitochondrial, chloroplast, and bacterial membranes to synthesize ATP.

Detergents have also played a crucial part in the purification and crystallization of membrane proteins. The development of new detergents and new expression systems producing large quantities of membrane proteins from cDNA clones has led to a rapid increase in the number of structures of membrane proteins and protein complexes that are known.

Bacteriorhodopsin Is a Light-Driven Proton Pump That Traverses the Lipid Bilayer as Seven α Helices

In Chapter 11, we consider how multipass transmembrane proteins mediate the selective transport of small hydrophilic molecules across cell membranes. But a detailed understanding of how a membrane transport protein actually works requires precise information about its three-dimensional structure in the bilayer. *Bacteriorhodopsin* was the first membrane transport protein whose structure was determined. It has remained the prototype of many multipass membrane proteins with a similar structure, and it merits a brief digression here.

The “purple membrane” of the archaean *Halobacterium salinarum* is a specialized patch in the plasma membrane that contains a single species of protein molecule, **bacteriorhodopsin** (Figure 10–32). Each bacteriorhodopsin molecule contains a single light-absorbing group, or chromophore (called *retinal*), which gives the protein its purple color. Retinal is vitamin A in its aldehyde form and is identical to the chromophore found in *rhodopsin* of the photoreceptor cells of the vertebrate eye (discussed in Chapter 15). Retinal is covalently linked to a lysine side chain of the bacteriorhodopsin protein. When activated by a single photon of light, the excited chromophore changes its shape and causes a series of small conformational changes in the protein, resulting in the transfer of one H^+ from the inside to the outside of the cell (Figure 10–33). In bright light, each bacteriorhodopsin molecule can pump several hundred protons per second. The light-driven proton transfer establishes an H^+ gradient across the plasma membrane, which in turn drives the production of ATP by a second protein in the cell’s plasma membrane. The energy stored in the H^+ gradient also drives other energy-requiring processes in the cell. Thus, bacteriorhodopsin converts solar energy into a proton gradient, which provides energy to the archaeal cell.

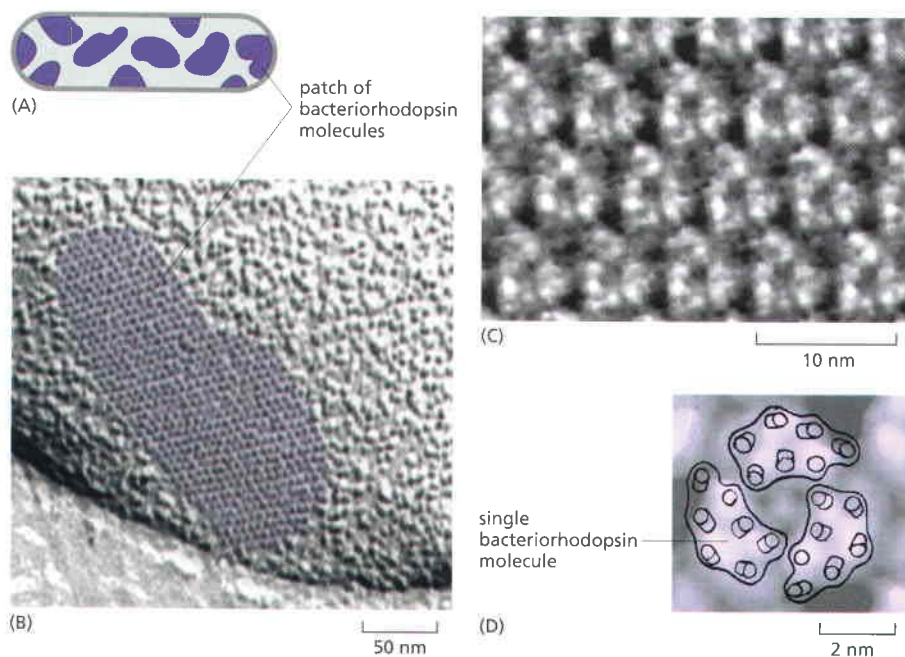


Figure 10–32 Patches of purple membrane, which contain bacteriorhodopsin in the archaean *Halobacterium salinarum*. (A) These archaea live in saltwater pools, where they are exposed to sunlight. They have evolved a variety of light-activated proteins, including bacteriorhodopsin, which is a light-activated proton pump in the plasma membrane. (B) The bacteriorhodopsin molecules in the purple membrane patches are tightly packed into two-dimensional crystalline arrays. (C) Details of the molecular surface visualized by atomic force microscopy. With this technique individual bacteriorhodopsin molecules can be seen. (D) Outline of the approximate locations of three bacteriorhodopsin monomers and their individual α helices in the images shown in (B). (B–D, courtesy of Dieter Oesterhelt.)

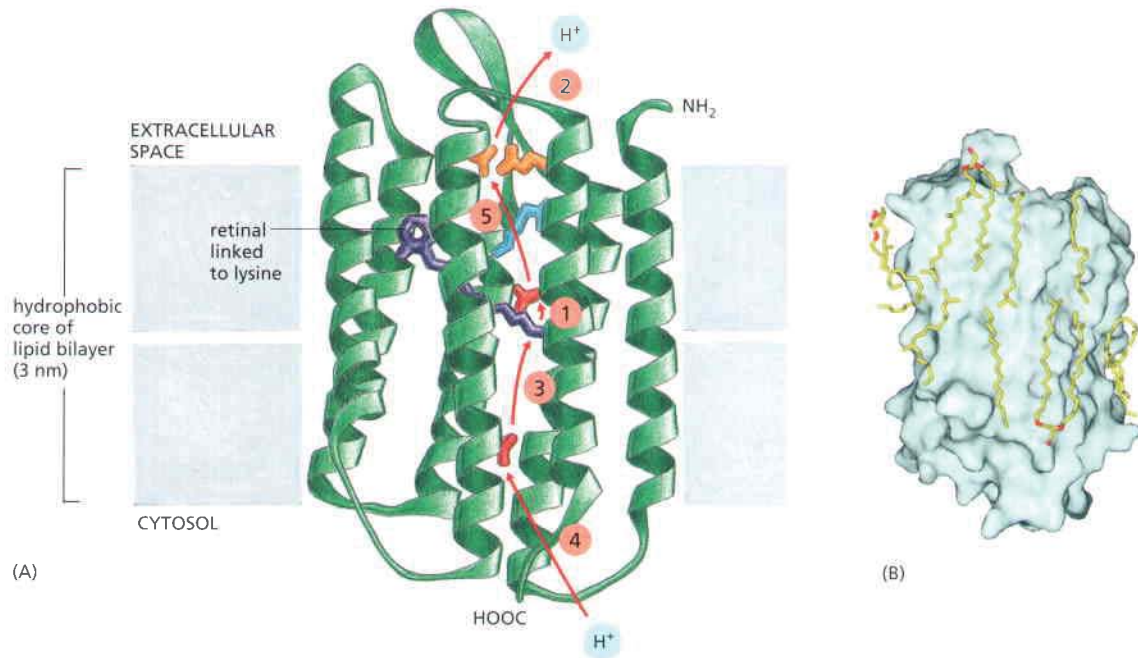


Figure 10-33 The three-dimensional structure of a bacteriorhodopsin molecule. <TTAA> (A) The polypeptide chain crosses the lipid bilayer seven times as α helices. The location of the retinal chromophore (purple) and the probable pathway taken by protons during the light-activated pumping cycle are shown. The first and key step is the passing of a H^+ from the chromophore to the side chain of aspartic acid 85 (red, located next to the chromophore) that occurs upon absorption of a photon by the chromophore. Subsequently, other H^+ transfers—in the numerical order indicated and utilizing the hydrophilic amino acid side chains that line a path through the membrane—complete the pumping cycle and return the enzyme to its starting state. Color code: glutamic acid (orange), aspartic acid (red), arginine (blue). (B) The high-resolution crystal structure of bacteriorhodopsin shows many lipid molecules (yellow with red head groups) that are tightly bound to specific places on the surface of the protein. (A, adapted from H. Luecke et al., *Science* 286:255–260, 1999. With permission from AAAS; B, from H. Luecke et al., *J. Mol. Biol.* 291:899–911, 1999. With permission from Academic Press.)

The numerous bacteriorhodopsin molecules in the purple membrane are arranged as a planar two-dimensional crystal. The regular packing has made it possible to determine the three-dimensional structure and orientation of bacteriorhodopsin in the membrane to moderate resolution (3 Å) by an approach that uses a combination of electron microscopy and electron diffraction analysis. This procedure, known as **electron crystallography**, is analogous to the study of three-dimensional crystals of soluble proteins by x-ray diffraction analysis. It has provided the first structural views of many membrane proteins that were found to be difficult to crystallize from detergent solutions. For bacteriorhodopsin, the structure obtained by electron crystallography was later confirmed and extended to very high resolution by x-ray crystallography. Each bacteriorhodopsin molecule is folded into seven closely packed α helices (each containing about 25 amino acids), which pass through the lipid bilayer at slightly different angles. By obtaining very well ordered protein crystals and freezing them at very low temperatures, it has also been possible to determine the structures of some of the protein's intermediate conformations during its H^+ pumping cycle.

Bacteriorhodopsin is a member of a large superfamily of membrane proteins with similar structures but different functions. For example, rhodopsin in rod cells of the vertebrate retina and many cell-surface receptor proteins that bind extracellular signal molecules are also built from seven transmembrane α helices. These proteins function as signal transducers rather than as transporters: each responds to an extracellular signal by activating a GTP-binding protein (G protein) inside the cell and are therefore called *G-protein-coupled receptors* (GPCRs), as we discuss in Chapter 15. Although the structures of bacteriorhodopsins and GPCRs are strikingly similar, they show no sequence similarity and thus probably belong to two evolutionarily distant branches of an ancient protein family.

The high-resolution crystal structure of bacteriorhodopsin reveals many lipid molecules that are bound in specific places on the protein surface (Figure 10-33B). Interactions with specific lipids are thought to help stabilize many membrane proteins, which work best and crystallize more readily if some of the lipids remain bound during detergent extraction, or if specific lipids are added back to the proteins in detergent solutions. The specificity of these lipid-protein interactions helps explain why eucaryotic membranes contain such a variety of lipids, with head groups that differ in size, shape, and charge. We can think of the membrane lipids as constituting a two-dimensional solvent for the proteins in the membrane, just as water constitutes a three-dimensional solvent for proteins

in an aqueous solution. Some membrane proteins can function only in the presence of specific lipid head groups, just as many enzymes in aqueous solution require a particular ion for activity.

Membrane Proteins Often Function as Large Complexes

Many membrane proteins function as part of multicomponent complexes, several of which have been studied by x-ray crystallography. One is a bacterial *photosynthetic reaction center*, which was the first transmembrane protein complex to be crystallized and analyzed by x-ray diffraction. The results of this analysis were of general importance to membrane biology because they showed for the first time how multiple polypeptides associate in a membrane to form a complex protein machine (**Figure 10–34**). In Chapter 14, we discuss how such photosynthetic complexes function to capture light energy and use it to pump protons across the membrane. Many of the membrane protein complexes involved in photosynthesis, proton pumping, and electron transport are even larger than the photosynthetic reaction center. The enormous photosystem II complex from cyanobacteria, for example, contains 19 protein subunits and well over 60 transmembrane helices. Membrane proteins are often arranged in large complexes, not only for harvesting various forms of energy, but also for transducing extracellular signals into intracellular ones (discussed in Chapter 15).

Many Membrane Proteins Diffuse in the Plane of the Membrane

Like most membrane lipids, membrane proteins do not tumble (*flip-flop*) across the lipid bilayer, but they do rotate about an axis perpendicular to the plane of the bilayer (*rotational diffusion*). In addition, many membrane proteins are able

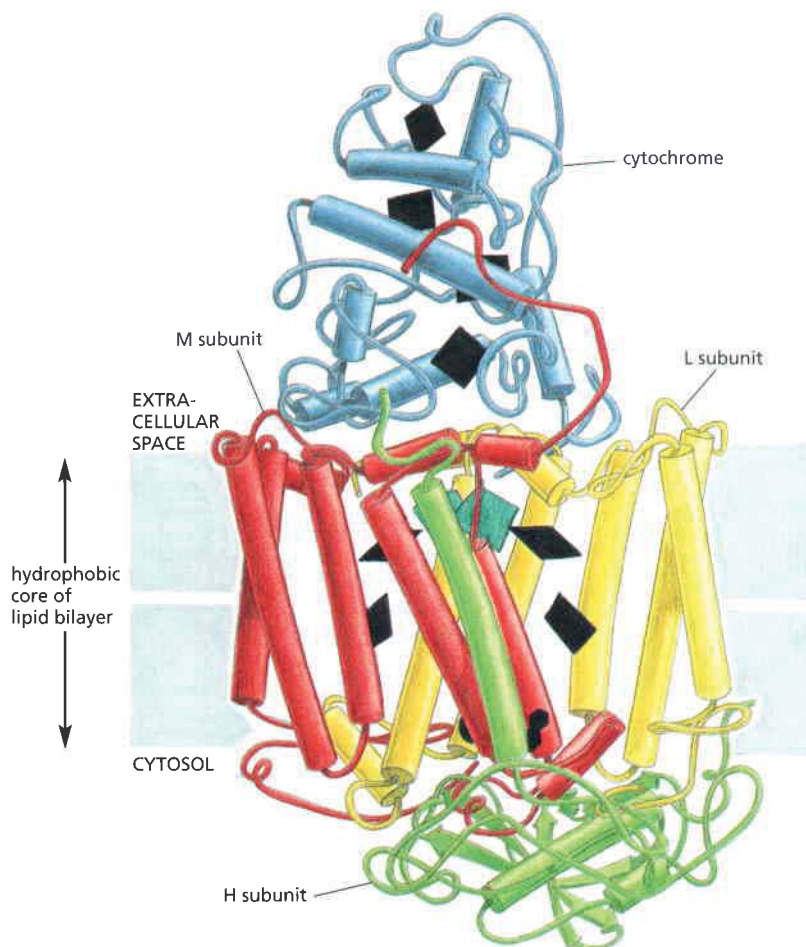


Figure 10–34 The three-dimensional structure of the photosynthetic reaction center of the bacterium

Rhodospseudomonas viridis. <ATCA>

The structure was determined by x-ray diffraction analysis of crystals of this transmembrane protein complex. The complex consists of four subunits L, M, H, and a cytochrome. The L and M subunits form the core of the reaction center, and each contains five α helices that span the lipid bilayer. The locations of the various electron carrier coenzymes are shown in black. Note that the coenzymes are arranged in the spaces between the helices. The *special pair* of chlorophyll molecules (discussed in Chapter 14) is shown in turquoise. (Adapted from a drawing by J. Richardson based on data from J. Deisenhofer et al., *Nature* 318:618–624, 1985. With permission from Macmillan Publishers Ltd.)

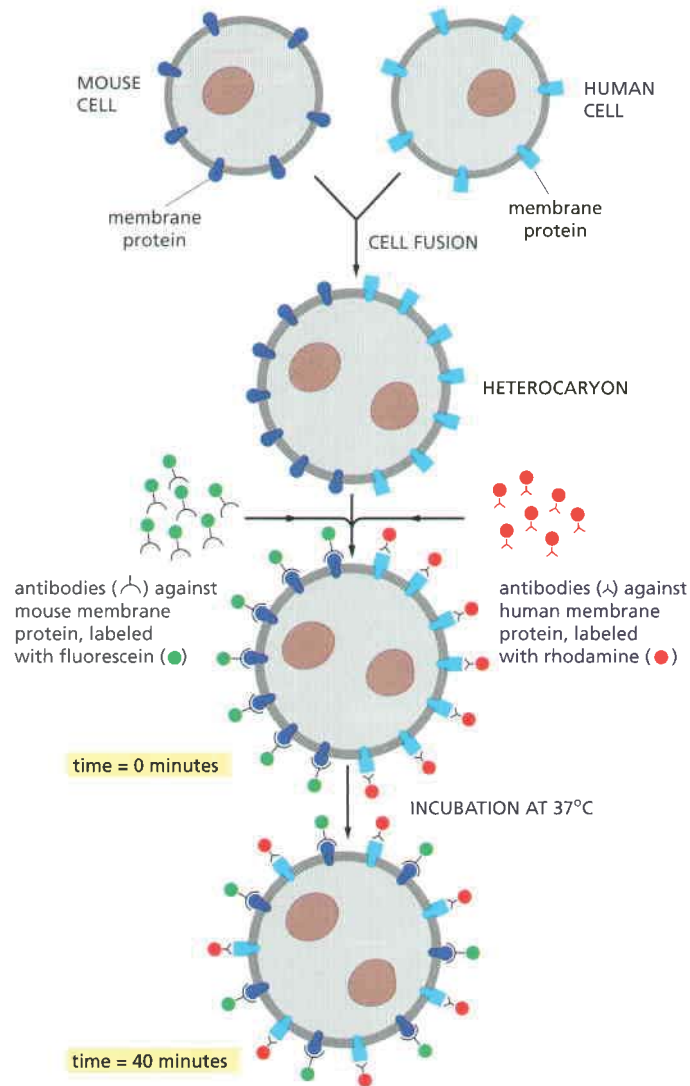


Figure 10–35 An experiment demonstrating the diffusion of proteins in the plasma membrane of mouse–human hybrid cells. The mouse and human proteins are initially confined to their own halves of the newly formed heterocaryon plasma membrane, but they intermix over time. The two antibodies used to visualize the proteins can be distinguished in a fluorescence microscope because fluorescein is green and rhodamine is red. (Based on L.D. Frye and M. Edidin, *J. Cell Sci.* 7:319–335, 1970. With permission from The Company of Biologists.)

to move laterally within the membrane (*lateral diffusion*). An experiment in which mouse cells were artificially fused with human cells to produce hybrid cells (*heterocaryons*) provided the first direct evidence that some plasma membrane proteins are mobile in the plane of the membrane. Two differently labeled antibodies were used to distinguish selected mouse and human plasma membrane proteins. Although at first the mouse and human proteins were confined to their own halves of the newly formed heterocaryon, the two sets of proteins diffused and mixed over the entire cell surface in about half an hour (**Figure 10–35**).

The lateral diffusion rates of membrane proteins can be measured by using the technique of *fluorescence recovery after photobleaching* (FRAP). The method usually involves marking the membrane protein of interest with a specific fluorescent group. This can be done either with a fluorescent ligand such as a fluorophore-labeled antibody that binds to the protein or with recombinant DNA technology to express the protein fused to green fluorescent protein (GFP) (discussed in Chapter 9). The fluorescent group is then bleached in a small area of membrane by a laser beam, and the time taken for adjacent membrane proteins carrying unbleached ligand or GFP to diffuse into the bleached area is measured (**Figure 10–36A**). A complementary technique is *fluorescence loss in photobleaching* (FLIP). Here, a laser beam continuously irradiates a small area of membrane to bleach all the fluorescent molecules that diffuse into it, thereby gradually depleting the surrounding membrane of fluorescently labeled molecules (Figure 10–36B). From such FRAP and FLIP measurements, we can calculate the diffusion coefficient for the marked cell-surface protein. The values

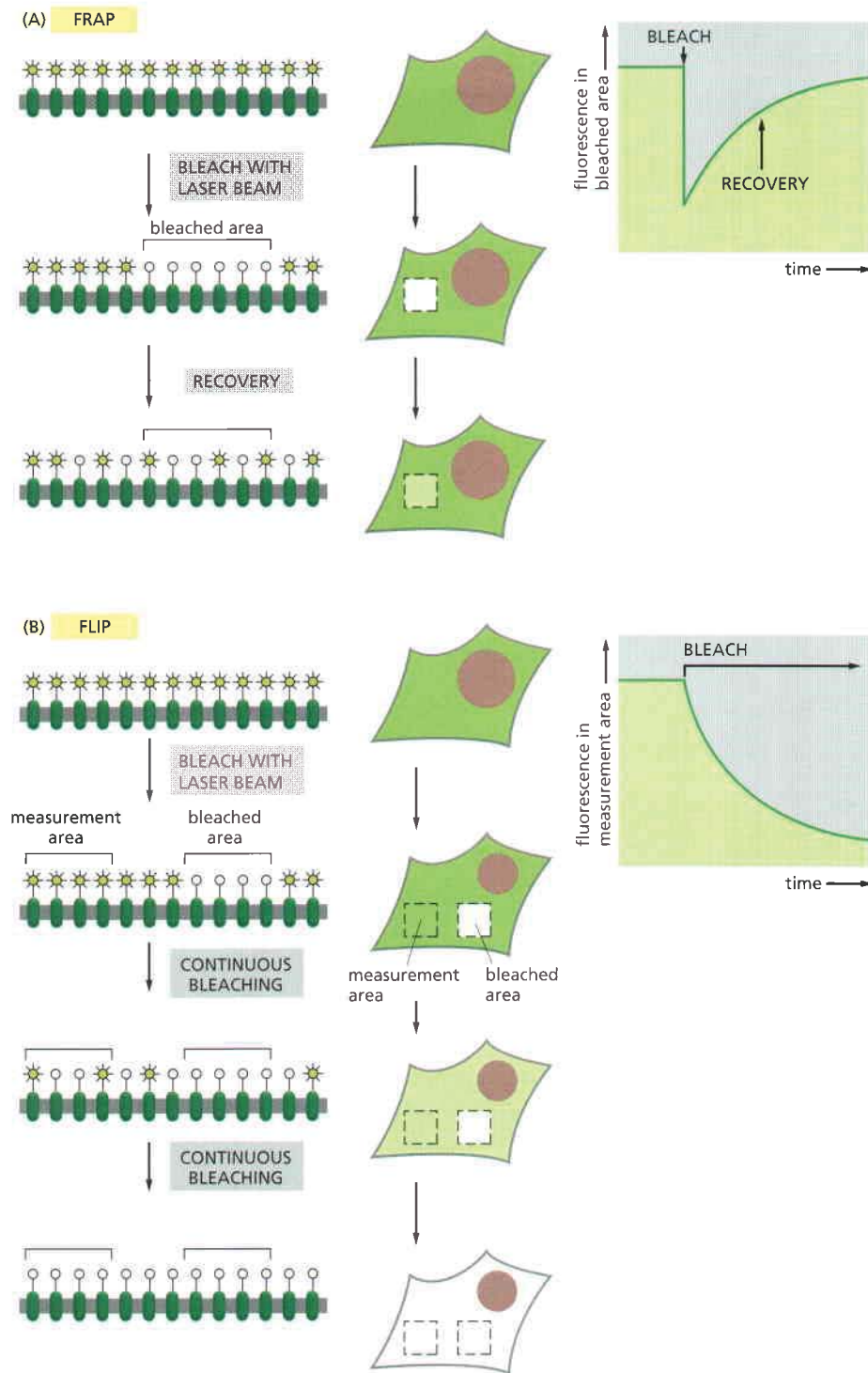


Figure 10-36 Measuring the rate of lateral diffusion of a membrane protein by photo-bleaching techniques. <ATGT> A specific protein of interest can be expressed as a fusion protein with green fluorescent protein (GFP), which is intrinsically fluorescent. (A) In the FRAP technique, fluorescent molecules are bleached in a small area using a laser beam. The fluorescence intensity recovers as the bleached molecules diffuse away and unbleached molecules diffuse into the irradiated area (shown here in side and top views). The diffusion coefficient is calculated from a graph of the rate of recovery: the greater the diffusion coefficient of the membrane protein, the faster the recovery. (B) In the FLIP technique, a small area of membrane is irradiated continuously, and fluorescence is measured in a separate area. Fluorescence in the second area progressively decreases as fluorescent proteins diffuse out and bleached molecules diffuse in; eventually, all of the fluorescent protein molecules are bleached, as long as they are mobile and not permanently anchored to the cytoskeleton or extracellular matrix.

of the diffusion coefficients for different membrane proteins in different cells are highly variable, because interactions with other proteins impede the diffusion of the proteins to varying degrees. Measurements of proteins that are minimally impeded in this way indicate that cell membranes have a viscosity comparable to that of olive oil.

One drawback to the FRAP and FLIP techniques is that they monitor the movement of large populations of molecules in a relatively large area of membrane; one cannot follow individual protein molecules. If a protein fails to migrate into a bleached area, for example, one cannot tell whether the molecule is truly immobile or just restricted in its movement to a very small region of membrane—perhaps by cytoskeletal proteins. *Single-particle tracking* techniques overcome this problem by labeling individual membrane molecules with antibodies coupled to fluorescent dyes or tiny gold particles and tracking their

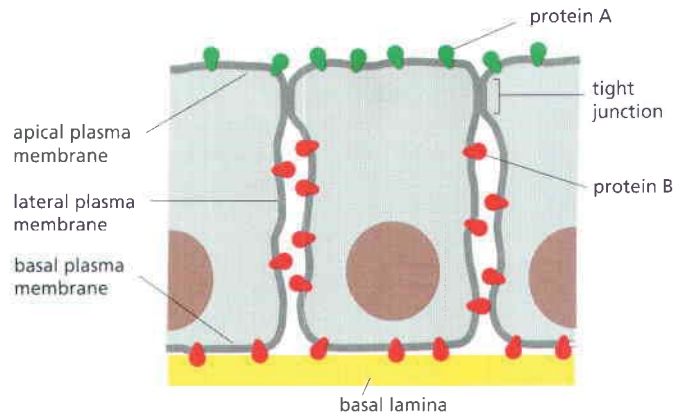


Figure 10–37 How membrane molecules can be restricted to a particular membrane domain. In this drawing of an epithelial cell, protein A (in the apical membrane) and protein B (in the basal and lateral membranes) can diffuse laterally in their own domains but are prevented from entering the other domain, at least partly by the specialized cell junction called a tight junction. Lipid molecules in the outer (noncytosolic) monolayer of the plasma membrane are likewise unable to diffuse between the two domains; lipids in the inner (cytosolic) monolayer, however, are able to do so (not shown). The basal lamina is a thin mat of extracellular matrix that separates epithelial sheets from other tissues (discussed in Chapter 19).

movement by video microscopy. Using single-particle tracking, one can record the diffusion path of a single membrane protein molecule over time. Results from all of these techniques indicate that plasma membrane proteins differ widely in their diffusion characteristics, as we now discuss.

Cells Can Confine Proteins and Lipids to Specific Domains Within a Membrane

The recognition that biological membranes are two-dimensional fluids was a major advance in understanding membrane structure and function. It has become clear, however, that the picture of a membrane as a lipid sea in which all proteins float freely is greatly oversimplified. Many cells confine membrane proteins to specific regions in a continuous lipid bilayer. We have already discussed how bacteriorhodopsin molecules in the purple membrane of *Halobacterium* assemble into large two-dimensional crystals, in which individual protein molecules are relatively fixed in relationship to one another (see Figure 10–32); large aggregates of this kind diffuse very slowly.

In epithelial cells, such as those that line the gut or the tubules of the kidney, certain plasma membrane enzymes and transport proteins are confined to the apical surface of the cells, whereas others are confined to the basal and lateral surfaces (Figure 10–37). This asymmetric distribution of membrane proteins is often essential for the function of the epithelium, as we discuss in Chapters 11 and 19. The lipid compositions of these two membrane domains are also different, demonstrating that epithelial cells can prevent the diffusion of lipid as well as protein molecules between the domains. Experiments with labeled lipids, however, suggest that only lipid molecules in the outer monolayer of the membrane are confined in this way. The barriers set up by a specific type of intercellular junction (called a *tight junction*, discussed in Chapter 19) maintain the separation of both protein and lipid molecules. Clearly, the membrane proteins that form these intercellular junctions cannot be allowed to diffuse laterally in the interacting membranes.

A cell can also create membrane domains without using intercellular junctions. The mammalian spermatozoon, for instance, is a single cell that consists of several structurally and functionally distinct parts covered by a continuous plasma membrane. When a sperm cell is examined by immunofluorescence microscopy with a variety of antibodies, each of which react with a specific cell-surface molecule, the plasma membrane is found to consist of at least three distinct domains (Figure 10–38). Some of the membrane molecules are able to diffuse freely within the confines of their own domain. The molecular nature of the “fence” that prevents the molecules from leaving their domain is not known. Many other cells have similar membrane fences that confine membrane protein diffusion to certain membrane domains. The plasma membrane of nerve cells, for example, contains a domain enclosing the cell body and dendrites, and another enclosing the axon. In this case, it is thought that a belt of actin filaments tightly associated with the plasma membrane at the cell-body–axon junction forms part of the barrier.

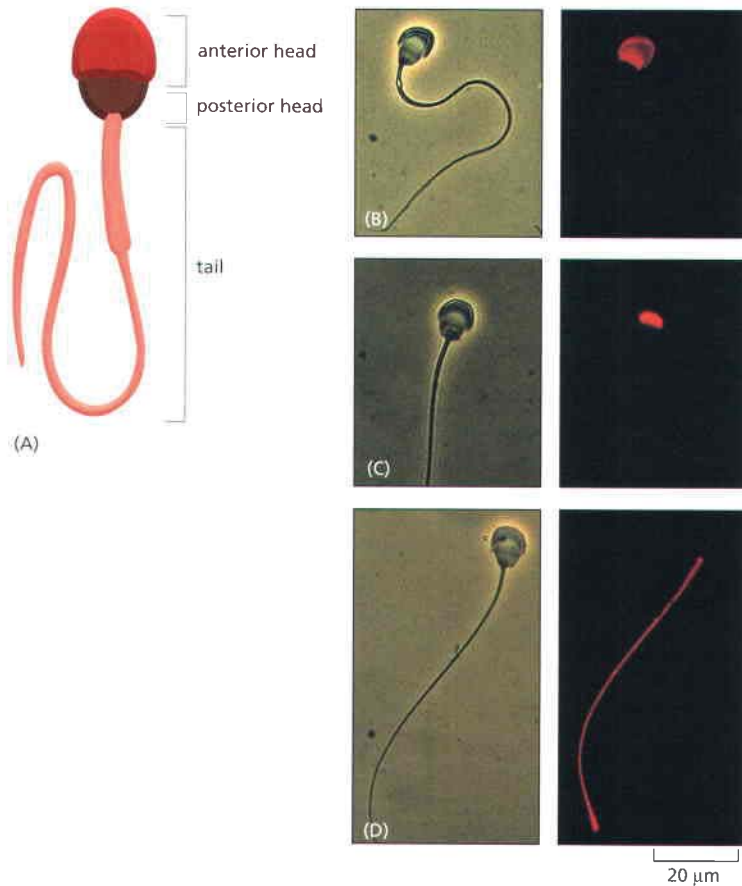


Figure 10–38 Three domains in the plasma membrane of a guinea pig sperm. (A) A drawing of a guinea pig sperm. In the three pairs of micrographs, phase-contrast micrographs are on the left, and the same cell is shown with cell-surface immunofluorescence staining on the right. Different monoclonal antibodies selectively label cell-surface molecules on (B) the anterior head, (C) the posterior head, and (D) the tail. (Micrographs courtesy of Selena Carroll and Diana Myles.)

Figure 10–39 shows four common ways of immobilizing specific membrane proteins through protein–protein interactions.

The Cortical Cytoskeleton Gives Membranes Mechanical Strength and Restricts Membrane Protein Diffusion

As shown in Figure 10–39B and C, a common way in which a cell restricts the lateral mobility of specific membrane proteins is to tether them to macromolecular assemblies on either side of the membrane. The characteristic biconcave shape of a red blood cell (Figure 10–40), for example, results from interactions of its plasma membrane proteins with an underlying *cytoskeleton*, which consists mainly of a meshwork of the filamentous protein **spectrin**. Spectrin is a long, thin, flexible rod about 100 nm in length. Being the principal component of the red cell cytoskeleton, it maintains the structural integrity and shape of the plasma membrane, which is the red cell's only membrane, as the cell has no nucleus or other organelles. The spectrin cytoskeleton is riveted to the membrane through various membrane proteins. The final result is a deformable, net-like meshwork that covers the entire cytosolic surface of the red cell membrane (Figure 10–41). This spectrin-based cytoskeleton enables the red cell to withstand the stress on its membrane as it is forced through narrow capillaries. Mice and humans with genetic abnormalities in spectrin are anemic and have red cells that are spherical (instead of concave) and fragile; the severity of the anemia increases with the degree of spectrin deficiency.

An analogous but much more elaborate and complicated cytoskeletal network exists beneath the plasma membrane of most other cells in our body. This network, which constitutes the cortical region (or **cortex**) of the cytoplasm, is rich in actin filaments, which are attached to the plasma membrane in numerous ways. The cortex of nucleated cells contains proteins that are structurally homologous to spectrin and the other components of the red cell cytoskeleton. We discuss the cortical cytoskeleton in nucleated cells and its interactions with the plasma membrane in Chapter 16.

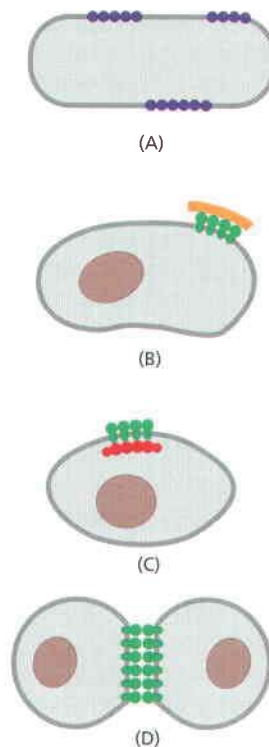


Figure 10–39 Four ways of restricting the lateral mobility of specific plasma membrane proteins. (A) The proteins can self-assemble into large aggregates (as seen for bacteriorhodopsin in the purple membrane of *Halobacterium*); they can be tethered by interactions with assemblies of macromolecules (B) outside or (C) inside the cell; or they can interact with proteins on the surface of another cell (D).

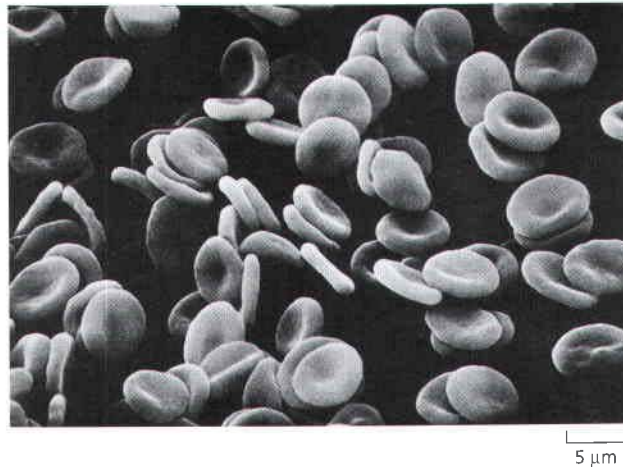


Figure 10–40 A scanning electron micrograph of human red blood cells. **<GTAC>** The cells have a biconcave shape and lack a nucleus and other organelles. (Courtesy of Bernadette Chailley.)

The cortical cytoskeletal network underlying the plasma membrane restricts diffusion of not only the proteins that are directly anchored to it. Because the *cytoskeletal filaments* are often closely apposed to the cytosolic membrane surface, they can form mechanical barriers that obstruct the free diffusion of membrane proteins. These barriers partition the membrane into small domains, or *corrals* (**Figure 10–42**), which can be either permanent, as in the sperm (see **Figure 10–38**), or transient. The barriers can be detected when the diffusion of individual membrane proteins is followed by high-speed, single-particle tracking. The proteins diffuse rapidly but are confined within an individual corral; occasionally,

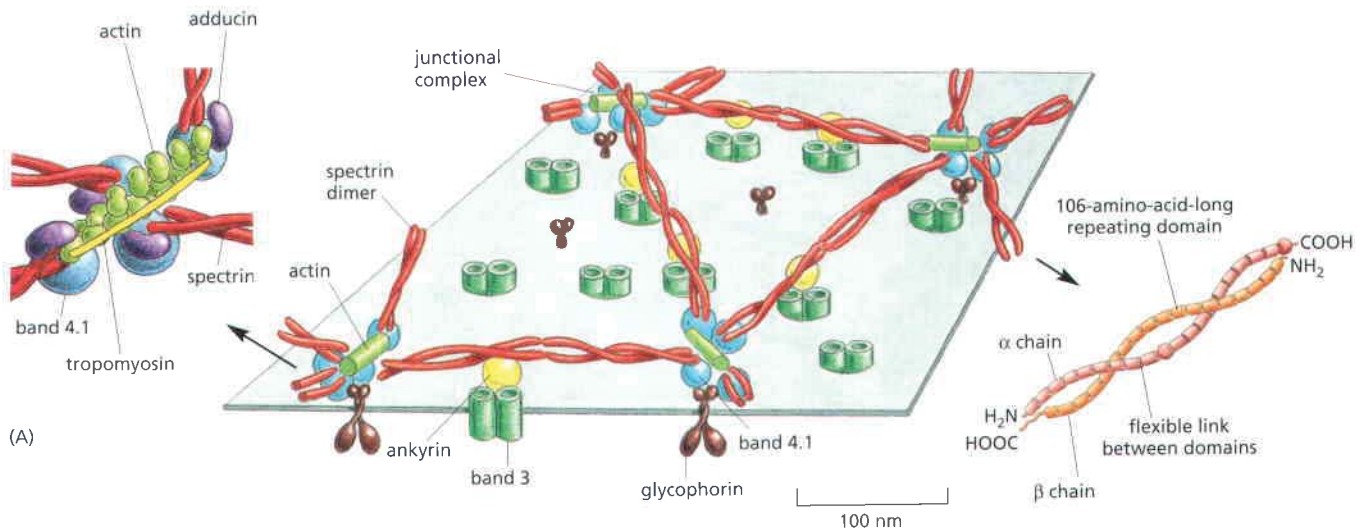
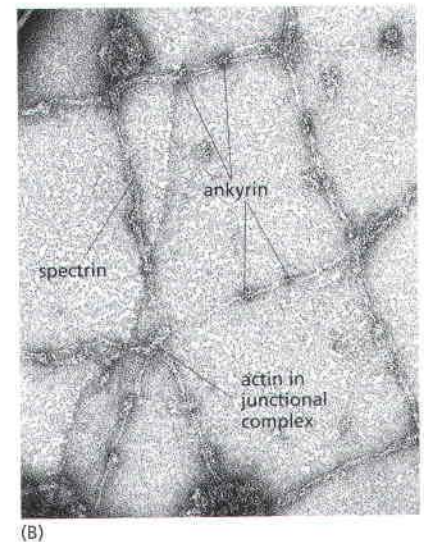


Figure 10–41 The spectrin-based cytoskeleton on the cytosolic side of the human red blood cell plasma membrane. (A) The arrangement shown in the drawing has been deduced mainly from studies on the interactions of purified proteins *in vitro*. Spectrin dimers (enlarged in the box on the right) are linked together into a netlike meshwork by “junctional complexes” (enlarged in the box on the left). Each spectrin heterodimer consists of two antiparallel, loosely intertwined, flexible polypeptide chains called α and β . The two chains are attached noncovalently to each other at multiple points, including at both ends. Both the α and β chains are composed largely of repeating domains. The junctional complexes are composed of short actin filaments (containing 13 actin monomers), *band 4.1*, *adducin*, and a *tropomyosin* molecule that probably determines the length of the actin filaments. The cytoskeleton is linked to the membrane through two transmembrane proteins—a multipass protein called *band 3* and a single-pass protein called *glycophorin*. The spectrin tetramers bind to some *band 3* proteins via *ankyrin* molecules, and to *glycophorin* and *band 3* (not shown) via *band 4.1* proteins. (B) The electron micrograph shows the cytoskeleton on the cytosolic side of a red blood cell membrane after fixation and negative staining. The spectrin meshwork has been purposely stretched out to allow the details of its structure to be seen. In a normal cell, the meshwork shown would be much more crowded and occupy only about one-tenth of this area. (B, courtesy of T. Byers and D. Branton, *Proc. Natl Acad. Sci. U.S.A.* 82:6153–6157, 1985. With permission from National Academy of Sciences.)



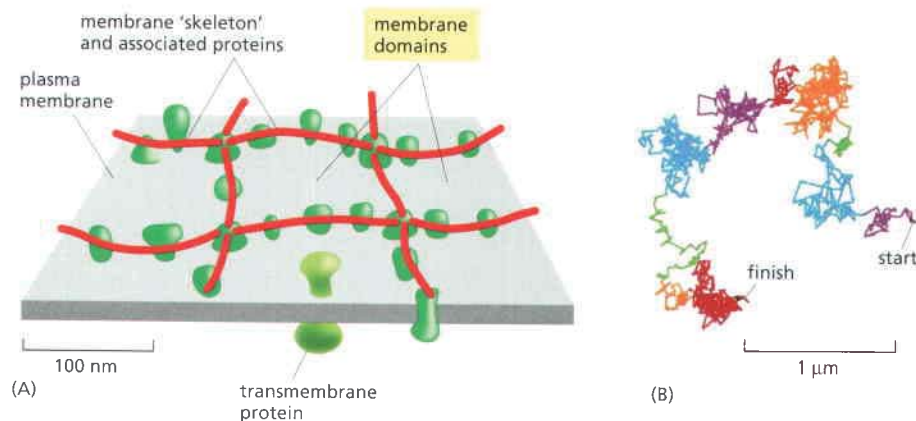


Figure 10-42 Corraling of membrane proteins by cortical cytoskeletal filaments. (A) How cytoskeletal filaments are thought to provide diffusion barriers that divide the membrane into small domains, or corrals. (B) High-speed, single-particle tracking was used to follow the paths of a fluorescently-labeled membrane protein over time. The trace shows that membrane proteins diffuse within a tightly delimited membrane domain (shown by different colors of the trace) and only infrequently escape into a neighboring domain. (Adapted from A. Kusumi et al., *Annu. Rev. Biophys. Biomol. Struct.* 34:351–378, 2005. With permission from Annual Reviews.)

however, thermal motions cause a few cortical filaments to detach transiently from the membrane, allowing the protein to escape into an adjacent corral.

The extent to which a transmembrane protein is confined within a corral depends on its association with other proteins and the size of its cytoplasmic domain; proteins with a large cytosolic domain will have a harder time passing through barriers. When a cell-surface receptor binds its extracellular signal molecules, for example, large protein complexes build up on the cytosolic domain of the receptor, making it more difficult for the receptor to escape from its corral. It is thought that corraling helps concentrate activated signaling complexes, increasing the speed and efficiency of the signaling process (discussed in Chapter 15).

Summary

Whereas the lipid bilayer determines the basic structure of biological membranes, proteins are responsible for most membrane functions, serving as specific receptors, enzymes, transport proteins, and so on. Many membrane proteins extend across the lipid bilayer. Some of these transmembrane proteins are single-pass proteins, in which the polypeptide chain crosses the bilayer as a single α helix. Others are multipass proteins, in which the polypeptide chain crosses the bilayer multiple times—either as a series of α helices or as a β sheet in the form of a closed barrel. All proteins responsible for the transmembrane transport of ions and other small water-soluble molecules are multipass proteins. Some membrane-associated proteins do not span the bilayer but instead are attached to either side of the membrane. Many of these are bound by non-covalent interactions with transmembrane proteins, but others are bound via covalently attached lipid groups. In the plasma membrane of all eucaryotic cells, most of the proteins exposed on the cell surface and some of the lipid molecules in the outer lipid monolayer have oligosaccharide chains covalently attached to them. Like the lipid molecules in the bilayer, many membrane proteins are able to diffuse rapidly in the plane of the membrane. However, cells have ways of immobilizing specific membrane proteins, as well as ways of confining both membrane protein and lipid molecules to particular domains in a continuous lipid bilayer.

PROBLEMS

Which statements are true? Explain why or why not.

10-1 Although lipid molecules are free to diffuse in the plane of the bilayer, they cannot flip-flop across the bilayer unless enzyme catalysts called phospholipid translocators are present in the membrane.

10-2 Whereas all the carbohydrate in the plasma membrane faces outward on the external surface of the cell, all the carbohydrate on internal membranes faces toward the cytosol.

10-3 Although membrane domains with different protein compositions are well known, there are at present no examples of membrane domains that differ in lipid composition.

Discuss the following problems.

10-4 When a lipid bilayer is torn, why does it not seal itself by forming a “hemi-micelle” cap at the edges, as shown in **Figure Q10-1**?

10-5 Margarine is made from vegetable oil by a chemical process. Do you suppose this process converts saturated fatty acids to unsaturated ones, or vice versa? Explain your answer.

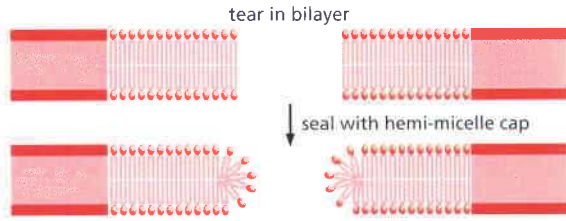


Figure Q10-1 A torn lipid bilayer sealed with a hypothetical “hemi-micelle” cap (Problem 10-4).

10-6 If a lipid raft is typically 70 nm in diameter and each lipid molecule has a diameter of 0.5 nm, about how many lipid molecules would there be in a lipid raft composed entirely of lipid? At a ratio of 50 lipid molecules per protein molecule (50% protein by mass) how many proteins would be in a typical raft? (Neglect the loss of lipid from the raft that would be required to accommodate the protein.)

10-7 A classic paper studied the behavior of lipids in the two monolayers of a membrane by labeling individual molecules with nitroxide groups, which are stable free radicals (Figure Q10-2). These spin-labeled lipids can be detected by electron spin-resonance (ESR) spectroscopy, a technique that does not harm living cells. Spin-labeled lipids are introduced into small lipid vesicles, which are then fused with cells, thereby transferring the labeled lipids into the plasma membrane.

The two spin-labeled phospholipids shown in Figure Q10-2 were incorporated into intact human red cell membranes in this way. To determine whether they were introduced equally into the two monolayers of the bilayer, ascorbic acid (vitamin C), which is a water-soluble reducing agent that does not cross membranes, was added to the medium to destroy any nitroxide radicals exposed on the outside of the cell. The ESR signal was followed as a function of time in the presence and absence of ascorbic acid as indicated in Figure Q10-3A and B.

A. Ignoring for the moment the difference in extent of loss of ESR signal, offer an explanation for why phospholipid 1 (Figure Q10-3A) reacts faster with ascorbate than does phospholipid 2 (Figure Q10-3B). Note that phospholipid 1 reaches a plateau in about 15 minutes, whereas phospholipid 2 takes almost an hour.

B. To investigate the difference in extent of loss of ESR signal with the two phospholipids, the experiments were repeated using red cell ghosts that had been resealed to make them impermeable to ascorbate (Figure Q10-3C and D). Resealed red cell ghosts are missing all of their cytoplasm but have an intact plasma membrane. In these experiments the loss of ESR signal for both phospholipids was negligible in the absence of ascorbate and reached a plateau at 50% in the presence of ascorbate. What

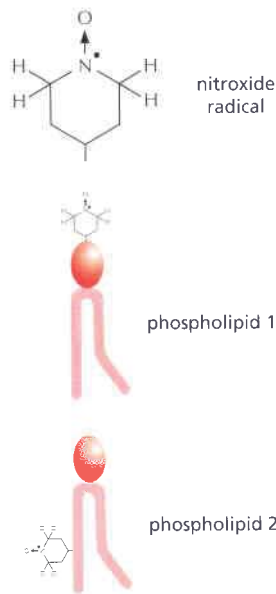


Figure Q10-2 Structures of two nitroxide-labeled lipids (Problem 10-7). The nitroxide radical is shown at the top, and its position of attachment to the phospholipid is shown below.

do you suppose might account for the difference in extent of loss of ESR signal in experiments with red cell ghosts (Figure Q10-3C and D) versus those with normal red cells (Figure Q10-3A and B).

C. Were the spin-labeled phospholipids introduced equally into the two monolayers of the red cell membrane?

10-8 Monomeric single-pass transmembrane proteins span a membrane with a single α helix that has characteristic chemical properties in the region of the bilayer. Which of the three 20-amino acid sequences listed below is the most likely candidate for such a transmembrane segment? Explain the reasons for your choice. (See back of book for one-letter amino acid code; FAMILY VW is a convenient mnemonic for hydrophobic amino acids.)

- A. I T L I Y F G V M A G V I G T I L L I S
- B. I T P I Y F G P M A G V I G T P L L I S
- C. I T E I Y F G R M A G V I G T D L L I S

10-9 You are studying the binding of proteins to the cytoplasmic face of cultured neuroblastoma cells and have found a method that gives a good yield of inside-out vesicles from the plasma membrane. Unfortunately, your preparations are contaminated with variable amounts of right-side-out vesicles. Nothing you have tried avoids this problem. A friend suggests that you pass your vesicles over an affinity column made of lectin coupled to solid beads. What is the point of your friend's suggestion?

10-10 Glycophorin, a protein in the plasma membrane of the red blood cell, normally exists as a homodimer that is held together entirely by interactions between its transmembrane domains. Since transmembrane domains are hydrophobic, how is it that they can associate with one another so specifically?

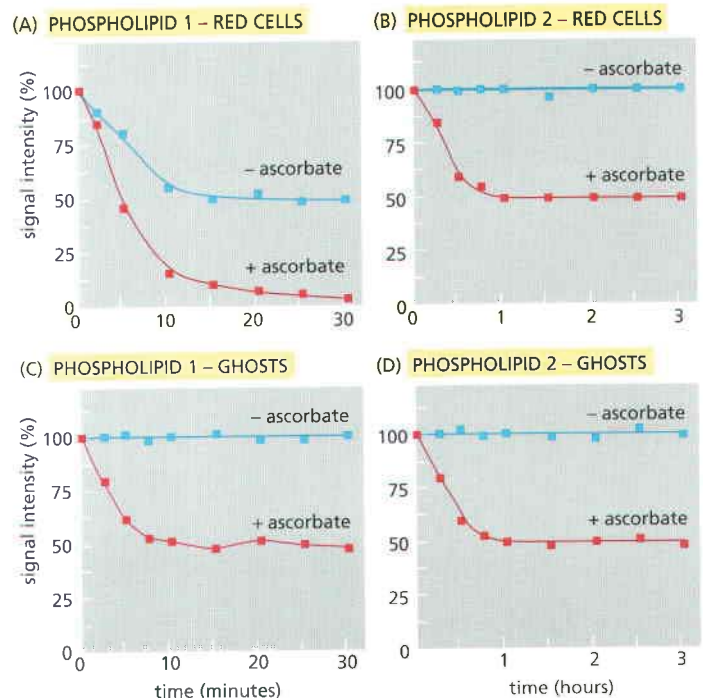


Figure Q10-3 Decrease in ESR signal intensity as a function of time in intact red cells and red cell ghosts in the presence and absence of ascorbate (Problem 10-7). (A and B) Phospholipid 1 and phospholipid 2 in intact red cells. (C and D) Phospholipid 1 and phospholipid 2 in red cell ghosts.

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