INTERNAL ORGANIZATION OF THE CELL

PART IV

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Cytoplasmic organization. This thin section through the microvilli found on the apical surface of intestinal epithelial cells illustrates the general ordering principles of eucaryotic cells. Cytoskeletal filaments (actin) provide a structural core for the microvilli, the cytosol is filled with membrane-enclosed vesicles and compartments, and adjacent cells are anchored to each other by junctions. (From P.T. Matsudaira and D.R. Burgess, Cold Spring Harbor Symp. Quant. Biol. 46:845–854, 1985.)
Membrane protein. Special proteins inserted in cellular membranes create pores that permit the passage of molecules across them. The bacterial protein shown here uses the energy from light (photons) to activate the pumping of protons across the plasma membrane.
(Adapted from H. Luecke et al., Science 286:255–260, 1999.)
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, 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; these protein sensors, or **receptors**, transfer information—rather than ions or molecules—across the membrane.

Despite their differing functions, all biological membranes have a common general structure: each is a very thin film of lipid and protein molecules, held together mainly by noncovalent interactions. Cell membranes are dynamic, fluid structures, and most of their molecules are able to move about in the plane of the membrane. The lipid molecules are arranged as a continuous double layer about 5 nm thick (Figure 10–1). 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 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 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. In fact, it is estimated that about 30% of the proteins that are 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 membrane proteins. Although we focus mainly on the plasma membrane, most of the concepts discussed are applicable 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 has been firmly established as the universal basis for cell-membrane structure. It is easily seen by electron microscopy, although specialized techniques, such as x-ray diffraction and freeze-fracture electron microscopy, are needed to reveal the details of its organization. The bilayer structure is attributable to the special properties of the lipid molecules, which cause them to assemble spontaneously into bilayers even under simple artificial conditions.

Membrane Lipids Are Amphipathic Molecules, Most of which Spontaneously Form Bilayers

Lipid—that is, fatty—molecules constitute about 50% of the mass of most animal cell membranes, nearly all of the remainder being protein. There are approximately $5 \times 10^6$ lipid molecules in a 1 μm × 1 μm 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 amphipathic (or 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**. The tails are usually fatty acids, and they can differ in length (they normally contain between 14 and 24 carbon atoms). One tail usually has one or more **cis**-double bonds (i.e., it is **unsaturated**), while the other tail does not (i.e., it is **saturated**). As shown in Figure 10–2, each double bond creates a small kink in the tail. Differences in the length and saturation of the fatty acid tails are important because they influence the ability of phospholipid molecules to pack against one another, thereby affecting the fluidity of the membrane (discussed below).

It is the shape and amphipathic nature of the lipid molecules that 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 icelike cages that surround the hydrophobic molecule (Figure 10–3). 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 amphipathic molecules) cluster together so that the smallest number of water molecules is affected.

For the above reason, lipid molecules spontaneously aggregate to bury their hydrophobic 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 bimolecular sheets, or **bilayers**, with the hydrophobic tails sandwiched between the hydrophilic head groups (Figure 10–4).

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

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**Figure 10–2 The parts of a phospholipid molecule.** This example is 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.
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 spontaneously rearrange to eliminate the free edge. (In eucaryotic plasma membranes, larger tears are repaired by the fusion of intracellular vesicles.) The prohibition against 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 10–5). This remarkable behavior, fundamental to the creation of a living cell, follows directly from the shape and amphipathic nature of the phospholipid molecule.

A lipid bilayer has other characteristics beside its self-sealing properties 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.

Figure 10–3 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. It cannot form favorable interactions with water and it would force adjacent water molecules to reorganize into icelike cage structures, which increases the free energy. This compound therefore is virtually insoluble in water. The symbol \( \delta^- \) indicates a partial negative charge, and \( \delta^+ \) indicates a partial positive charge. Polar atoms are shown in color and nonpolar groups are shown in gray.

Figure 10–4 Packing arrangements of lipid molecules in an aqueous environment. (A) Wedge-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 other of these structures in water, depending on their shape.

Figure 10–5 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.
The Lipid Bilayer Is a Two-dimensional Fluid

It was only around 1970 that 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 depending on how they are produced (Figure 10–6); and (2) planar bilayers, called **black membranes**, formed across a hole in a partition between two aqueous compartments (Figure 10–7).

Various techniques have been used to measure the motion of individual lipid molecules and their different parts. One can construct a lipid molecule, for example, whose polar head group carries 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. In contrast, lipid molecules readily exchange places with their neighbors within a monolayer (~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 that their hydrocarbon chains are flexible (Figure 10–8).

Similar studies have been performed with labeled lipid molecules in isolated biological membranes and in living cells. The results are generally the same as for synthetic bilayers, and 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 made in only one monolayer of a membrane, mainly in the cytosolic monolayer of the endoplasmic reticulum (ER) 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 membrane-bound 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 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, 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 10–9). 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, fatty acids with more *cis*-double bonds are synthesized, so the decrease in bilayer fluidity that would otherwise result from the drop in temperature is avoided.

The lipid bilayer of many cell membranes is not composed exclusively of phospholipids, however; it often also contains *cholesterol* and *glycolipids*. Eucaryotic plasma membranes contain especially large amounts of *cholesterol* (Figure 10–10)—up to one molecule for every phospholipid molecule. The cholesterol molecules enhance the permeability-barrier properties of the lipid bilayer. They orient themselves in the bilayer with their hydroxyl groups close to the polar head groups of the phospholipid molecules. In this position, their rigid, platelike steroid rings interact with—and partly immobilize—those regions of the hydrocarbon chains closest to the polar head groups (Figure 10–11). By decreasing the mobility of the first 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 tends to make lipid bilayers less fluid, at the high concentrations found in most eucaryotic plasma membranes, it also prevents the hydrocarbon chains from coming together and crystallizing. In this way, it inhibits possible phase transitions.
The lipid compositions of several biological membranes are compared in Table 10–1. 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 11–17). The plasma membranes of most eucaryotic cells, by contrast, are more varied, not only in containing large amounts of cholesterol, but also in containing a mixture of different phospholipids.

Four major phospholipids predominate in the plasma membrane of many mammalian cells: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin. The structures of these molecules are shown in Figure 10–12. 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. Together these four phospholipids constitute more than half the mass of lipid in most membranes (see Table 10–1). Other phospholipids, such as the inositol phospholipids, are present in smaller quantities but are functionally very important. The inositol phospholipids, for example, have a crucial role in cell signaling, as discussed in Chapter 15.

One might wonder why eucaryotic membranes contain such a variety of phospholipids, with head groups that differ in size, shape, and charge. One can begin to understand why if one thinks 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 phospholipid head groups, just as many enzymes in aqueous solution require a particular ion for activity. Moreover, some cytosolic enzymes bind to specific lipid head groups exposed on the cytosolic face of a membrane and are thus recruited to and concentrated at specific membrane sites.

The Plasma Membrane Contains Lipid Rafts That Are Enriched in Sphingolipids, Cholesterol, and Some Membrane Proteins

Most types of lipid molecules in cell membranes are randomly mixed in the lipid monolayer in which they reside. The van der Waals attractive forces between neighboring fatty acid tails are not selective enough to hold groups of molecules of this sort together. For some lipid molecules, however, such as the sphingolipids (discussed below), which tend to have long and saturated fatty hydrocarbon chains, the attractive forces can be just strong enough to hold the adjacent molecules together transiently in small microdomains. Such microdomains, or lipid rafts, can be thought of as transient phase separations in the fluid lipid bilayer where sphingolipids become concentrated.

The plasma membrane of animal cells is thought to contain many such tiny lipid rafts (~70 nm in diameter), which are rich in both sphingolipids and

<table>
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<tr>
<th>LIPID</th>
<th>LIVER CELL PLASMA MEMBRANE</th>
<th>RED BLOOD CELL PLASMA MEMBRANE</th>
<th>MYELIN</th>
<th>MITOCHONDRION (INNER AND OUTER MEMBRANES)</th>
<th>ENDOPLASMIC RETICULUM</th>
<th>E. COLE BACTERIUM</th>
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<tr>
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<td>70</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
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<td>7</td>
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<td>13</td>
<td>8</td>
<td>21</td>
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</table>
cholesterol. Because the hydrocarbon chains of the lipids concentrated there are longer and straighter than the fatty acid chains of most membrane lipids, the rafts are thicker than other parts of the bilayer (see Figure 10–9) and can better accommodate certain membrane proteins, which therefore tend to accumulate there (Figure 10–13). In this way, lipid rafts are thought to help organize such proteins—either concentrating them for transport in small vesicles or to enable the proteins to function together, as when they convert extracellular signals into intracellular ones (discussed in Chapter 15).

For the most part, lipid molecules in one monolayer of the bilayer move about independently of those in the other monolayer. In lipid rafts, however, the long hydrocarbon chains of the sphingolipids in one monolayer interact with those in the other monolayer. Thus, the two monolayers in a lipid raft communicate through their lipid tails.

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 lipid molecules that have choline—(CH3)3N+CH2CH2OH—in their head group (phosphatidylcholine and sphingomyelin) are in the outer monolayer, whereas almost all of the phospholipid molecules that contain a terminal primary amino group (phosphatidylethanolamine and phosphatidylserine) are in the inner monolayer (Figure 10–14). 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 lipid asymmetry is generated and maintained by membrane-bound phospholipid translocators.

Lipid asymmetry is functionally important. 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.

In other cases, the lipid head group must first be modified so that protein-binding sites are created at a particular time and place. Phosphatidylinositol, for instance, is a minor phospholipid that is concentrated in the cytosolic monolayer

Figure 10–12 Four major phospholipids in mammalian plasma membranes. Note that different head groups are represented by different colors. All the lipid molecules shown are derived from glycerol except for sphingomyelin, which is derived from serine.

Figure 10–13 A lipid raft. Lipid rafts are small, specialized areas in membranes where some lipids (primarily sphingolipids and cholesterol) and proteins (green) are concentrated. Because the lipid bilayer is somewhat thicker in the rafts, certain membrane proteins accumulate. A more detailed view of a lipid raft is shown in Figure 13–63.
of cell membranes. A variety of lipid kinases can add phosphate groups at distinct positions in the inositol ring. The phosphorylated inositol phospholipids then act as binding sites that recruit specific proteins from the cytosol to the membrane. An important example of a lipid kinase is phosphatidylinositol 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 10–15A). 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 also in another way in the response to extracellular signals. 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 (Figure 10–15B). 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 (see Figure 15–36).

Animals exploit the phospholipid asymmetry of their plasma membranes to distinguish between live and dead cells. When animal cells undergo programmed cell death, or apoptosis (discussed in Chapter 17), phosphatidylserine,

![Figure 10–14 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 10–12. In addition, glycolipids are drawn with hexagonal polar head groups (blue). Cholesterol (not shown) is thought to be distributed about equally in both monolayers.](image)

![Figure 10–15 Some functions of membrane phospholipids in cell signaling. (A) Extracellular signals can activate PI 3-kinase, which phosphorylates inositol phospholipids in the plasma membrane. Various intracellular signaling molecules then bind to these phosphorylated lipids and are thus recruited to the membrane, where they can interact and help relay the signal into the cell. (B) Other extracellular signals activate phospholipases that cleave phospholipids. The lipid fragments then act as signaling molecules to relay the signal into the cell. (C) Illustration of the sites where different classes of phospholipases cleave phospholipids. As indicated, phospholipases A\(_1\) and A\(_2\) cleave ester bonds, whereas phospholipases C and D cleave at phosphoester bonds.](image)
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 serves as a signal to induce neighboring cells, such as macrophages, to phagocytose the dead cell and digest it. The translocation of the phosphatidylserine in apoptotic cells occurs 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.

Glycolipids Are Found on the Surface of All Plasma Membranes
The lipid molecules with the most extreme asymmetry in their membrane distribution are the sugar-containing lipid molecules called glycolipids. These intriguing molecules are found exclusively in the noncytosolic monolayer of the lipid bilayer, where they are thought to partition preferentially into lipid rafts. The glycolipids tend to self-associate, partly through hydrogen bonds between their sugars and partly through van der Waals forces between their long and mainly saturated hydrocarbon chains. 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, which is topologically equivalent to the exterior of the cell (discussed in Chapter 12). In the plasma membrane, the sugar groups are exposed at the cell surface (see Figure 10–14), 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 10–16). More than 40 different gangliosides have been identified. They are most abundant 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 what the functions of glycolipids might be come from their localization. In the plasma membrane of epithelial cells, for example, glycolipids are

![Figure 10–16 Glycolipid molecules.](image-url)
confined to the exposed apical surface, where they may help protect the membrane against the harsh conditions frequently found there (such as low pH and degradative enzymes). Charged glycolipids, such as gangliosides, may be important for 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 10–16), 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 amphipathic. The most numerous are the phospholipids. When placed in water they assemble spontaneously into bilayers, which form sealed compartments that reseal if torn.*

*There are three major classes of membrane lipid molecules—phospholipids, cholesterol, and glycolipids. 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. Some membrane-bound enzymes require specific lipid head groups in order to function. The head groups of some lipids form docking sites for specific cytosolic proteins. Some extracellular signals that act through membrane receptor proteins activate phospholipases that cleave selected phospholipid molecules in the plasma membrane, thereby generating fragments that act as intracellular signaling molecules.*

**MEMBRANE PROTEINS**

Although the basic structure of biological membranes is provided by the lipid bilayer, membrane proteins perform most of the specific functions of membranes. It is the proteins, therefore, that give each type of membrane in the cell 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 50% of its mass.

Because lipid molecules are small compared with protein molecules, there are always many more lipid molecules than protein molecules in membranes—about 50 lipid molecules for each protein molecule in a membrane that is 50% protein by mass. Like membrane lipids, membrane proteins often have oligosaccharide chains attached to them that face the cell exterior. Thus, the surface that the cell presents to the exterior is rich in carbohydrate, which forms a *cell coat*, as we discuss later.
Membrane Proteins Can Be Associated with the Lipid Bilayer in Various Ways

Different membrane proteins are associated with the membranes in different ways, as illustrated in Figure 10–17. Many extend through the lipid bilayer, with part of their mass on either side (examples 1, 2, and 3 in Figure 10–17). Like their lipid neighbors, these transmembrane proteins are amphipathic, having regions that are hydrophobic and regions that are hydrophilic. 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 hydrophobicity of some of these transmembrane proteins is increased by the covalent attachment of a fatty acid chain that inserts into the cytosolic monolayer of the lipid bilayer (example 1 in Figure 10–17).

Other membrane proteins are located entirely in the cytosol and are associated with the cytosolic monolayer of the lipid bilayer either by an amphipathic α helix exposed on the surface of the protein (example 4 in Figure 10–17) or by one or more covalently attached lipid chains, which can be fatty acid chains or prenyl groups (example 5 in Figure 10–17 and Figure 10–18). 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 (example 6 in Figure 10–17).

The lipid-linked proteins in example 5 in Figure 10–17 are made as soluble proteins in the cytosol and are subsequently directed to the membrane by the covalent attachment of a lipid group (see Figure 10–18). The proteins in example 6, however, are made as single-pass transmembrane proteins in the 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). 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 (examples 7 and 8 in Figure 10–17). 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, many proteins held in the bilayer by lipid groups, and some proteins held on the membrane by unusually tight binding to other proteins cannot be released in these ways. These proteins are called integral membrane proteins.

How a membrane protein associates 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 are transmembrane proteins that bind signal molecules in the extracellular space and generate different intracellular signals on the opposite side of the plasma membrane. 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 of the proteins involved in intracellular signaling, for example, are bound to the cytosolic half of the plasma membrane by one or more covalently attached lipid groups.

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 synthesized and inserted into the lipid bilayer in the ER 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 acid residues 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

Figure 10–18 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 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 this prenylation, the terminal three amino acids are cleaved off, and the new C-terminus is methylated before insertion into the membrane. Palmitic acid, an 18 carbon saturated fatty acid, can also be attached to some proteins via thioester bonds formed with internal cysteine side chains. This modification is often reversible, allowing proteins to become recruited to membranes only when needed. The structures of two lipid anchors are shown below: (C) a myristyl anchor (a 14-carbon saturated fatty acid chain), and (D) a farnesyl anchor (a 15-carbon unsaturated hydrocarbon chain).
hydrogen bonds with one another. The hydrogen bonding between peptide bonds is maximized if the polypeptide chain forms a regular $\alpha$ helix as it crosses the bilayer, and this is how the great majority of the membrane-spanning segments of polypeptide chains are thought to traverse the bilayer (Figure 10–19).

In **single-pass transmembrane proteins**, the polypeptide crosses only once (see example 1 in Figure 10–17), whereas in **multipass transmembrane proteins**, the polypeptide chain crosses multiple times (see example 2 in Figure 10–17). An alternative way for the peptide bonds in the lipid bilayer to satisfy their hydrogen-bonding requirements is for multiple transmembrane strands of polypeptide chain to be arranged as a $\beta$ sheet in the form of a closed barrel (a so-called $\beta$ barrel; see example 3 in Figure 10–17). This form of multipass transmembrane structure is seen in **porin proteins**, which we discuss later. The strong drive to maximize hydrogen bonding in the absence of water also 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.

Because transmembrane proteins are notoriously difficult to crystallize, relatively few have been studied in their entirety by x-ray crystallography. The folded three-dimensional structures of almost all of the others are uncertain. DNA cloning and sequencing techniques, however, have revealed the amino acid sequences of large numbers of transmembrane proteins, and it is often possible to predict from an analysis of the protein's 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 $\alpha$ helix, and they can often be identified by means of a **hydropathy plot** (Figure 10–20). From such plots it is possible to predict what proportion of the proteins that an organism makes are transmembrane proteins. In budding yeast, for example, where the nucleotide sequence of the entire genome is known, about 20% of the proteins can be identified as transmembrane proteins, emphasizing the importance of membrane protein function. Hydropathy plots cannot identify the membrane-spanning segments of a $\beta$ barrel, as 10 amino acids or fewer are sufficient to traverse a lipid bilayer as an extended $\beta$ strand and only every other amino acid side chain is hydrophobic.

**Some $\beta$ Barrels Form Large Transmembrane Channels**

Multipass transmembrane proteins that have their transmembrane segments arranged as a $\beta$ barrel rather than as an $\alpha$ helix are comparatively rigid and tend to crystallize readily. Thus, the structures of a number of them have been determined by x-ray crystallography. The number of $\beta$ strands varies widely, from as few as 8 strands to as many as 22 (Figure 10–21).

The $\beta$ barrel proteins are abundant in the outer membrane of mitochondria, chloroplasts, and many bacteria. Some are pore-forming proteins, generating water-filled channels that allow selected hydrophilic solutes to cross the lipid bilayer of the bacterial outer membrane. The porins are well-studied examples (example 3 in Figure 10–21). The porin barrel is formed from a 16-stranded antiparallel $\beta$ sheet, which is sufficiently large to roll up into a cylindrical structure. Polar 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 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 transport protein of this kind (example 4 in Figure 10–21). It transports iron ions across the bacterial outer membrane. Its large barrel 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 10–21); here the barrel is used primarily as a rigid anchor that holds the protein in the membrane and orients the cytosolic loop regions that form binding sites for specific intracellular molecules.

Although β barrels can serve many purposes, they are largely restricted to bacterial, mitochondrial, and chloroplast outer membranes. The vast majority of multipass transmembrane proteins in eucaryotic cells and in the bacterial plasma membrane are constructed from transmembrane α helices. The helices within these proteins can slide against each other, allowing the protein to undergo conformational changes that can be exploited to open and shut ion channels, transport solutes, or transduce extracellular signals into intracellular ones. In β barrel proteins, by contrast, each β strand is bound rigidly to its neighbors by hydrogen bonds, making conformational changes of the barrel itself unlikely.
Many Membrane Proteins Are Glycosylated

The great majority of transmembrane proteins in animal cells are glycosylated. As in glycolipids, the sugar residues are added in the lumen of the ER and Golgi apparatus (discussed in Chapters 12 and 13). For this reason, the oligosaccharide chains are always present on the noncytosolic side of the membrane. Another 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 cysteine residues on the cytosolic side of membranes. These intrachain and interchain bonds do form on the noncytosolic side, where they can have an important role in stabilizing either the folded structure of the polypeptide chain or its association with other polypeptide chains, respectively (Figure 10–22).

Membrane Proteins Can Be Solubilized and Purified in Detergents

In general, transmembrane proteins (and some other tightly bound membrane proteins) can be solubilized only by agents that disrupt hydrophobic associations and destroy the lipid bilayer. The most useful of these for the membrane biochemist are detergents, which are small amphipathic molecules that tend to

Figure 10–21 β barrels formed from different numbers of β strands. (1) The E. coli OmpA protein (8 β strands), which serves as a receptor for a bacterial virus. (2) The E. coli OMPLA protein (12 β strands), is a lipase that hydrolyses 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, which forms water-filled pores across the outer membrane (16 β strands). The diameter of the channel is restricted by loops (shown in blue) that protrude into the channel. (4) The E. coli FepA protein (22 β strands), which 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. This domain is thought to change its conformation to transport the bound iron, but the molecular details of the changes are not known.

Figure 10–22 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.
form micelles in water (Figure 10–23). When mixed with membranes, the hydrophobic ends of detergents bind to the hydrophobic regions of the membrane proteins, thereby displacing the lipid 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 (although some lipid molecules may remain attached to the protein) (Figure 10–24). The polar (hydrophilic) ends of detergents can be either charged (ionic), as in sodium dodecyl sulfate (SDS), or uncharged (nonionic), as in the Triton detergents. The structures of these two commonly used detergents are illustrated in Figure 10–25.

With strong ionic detergents, such as SDS, even the most hydrophobic membrane proteins can be solubilized. This allows them 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,” thereby rendering the proteins inactive and unusable for functional studies. Nonetheless, proteins can be readily purified in their SDS-denatured form. In some cases the 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, if not entirely normal, form by the use of mild detergents, such as Triton X-100, which covers the membrane-spanning segments of the protein. In this way, functionally active membrane protein systems can be reconstituted from purified components, providing a powerful means of analyzing their activities (Figure 10–26).

The Cytosolic Side of Plasma Membrane Proteins Can Be Studied in Red Blood Cell Ghosts

More is known about the plasma membrane of the human red blood cell (Figure 10–27) than about any other eucaryotic membrane. There are several reasons for this. Red blood cells (also called erythrocytes) are available in large numbers (from blood banks, for example) relatively uncontaminated by other cell types.
Since they have no nucleus or internal organelles, the plasma membrane is their only membrane, and it can be isolated without contamination by internal membranes (thus avoiding a serious problem encountered in plasma membrane preparations from other eukaryotic cell types, in which the plasma membrane typically constitutes less than 5% of the cell’s membrane).

It is easy to prepare empty red blood cell membranes, or “ghosts,” by putting the cells in a medium with a lower salt concentration than the cell interior. Water then flows into the red cells, causing them to swell and burst (lyse) and release

**Figure 10–26** The use of mild 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.

**Figure 10–27** A scanning electron micrograph of human red blood cells. The cells have a biconcave shape and lack a nucleus and other organelles. (Courtesy of Bernadette Chailley.)
their hemoglobin and other soluble cytosolic proteins. Membrane ghosts can be studied while they are still leaky (in which case any reagent can interact with molecules on both faces of the membrane), or they can be allowed to reseal, so that water-soluble reagents cannot reach the internal face. Moreover, since sealed inside-out vesicles can also be prepared from red blood cell ghosts (Figure 10–28), the external and internal (cytosolic) side of the membrane can be studied separately. The use of sealed and unsealed red cell ghosts led to the first demonstration that some membrane proteins extend across the lipid bilayer (discussed below) and that the lipid compositions of the two halves of the bilayer are different. Like most of the basic principles initially demonstrated in red blood cell membranes, these findings were later extended to the various membranes of nucleated cells and bacteria.

The “sidedness” of a membrane protein can be determined in several ways. One method is the use of a covalent labeling reagent (such as a radioactive or fluorescent marker) that is water-soluble and therefore cannot penetrate the lipid bilayer; such a marker attaches covalently only to the portion of the protein on the exposed side of the membrane. The membranes are then solubilized with detergent and the proteins are separated by SDS polyacrylamide-gel electrophoresis. The labeled proteins can be detected either by their radioactivity (by autoradiography of the gel) or by their fluorescence (by exposing the gel to ultraviolet light). By using such vectorial labeling, it is possible to determine how a particular protein, detected as a band on a gel, is oriented in the membrane: for example, if it is labeled from both the external side (when intact cells or sealed ghosts are labeled) and the internal (cytosolic) side (when sealed inside-out vesicles are labeled), then it must be a transmembrane protein. An alternative approach is to expose either the external or internal surface to proteolytic enzymes, which are membrane-impermeant: if a protein is partially digested from both surfaces, it must be a transmembrane protein. In addition, labeled antibodies that bind only to one part of a protein can be used to determine whether that part of a transmembrane protein is exposed on one side of the membrane or the other.

When the plasma membrane proteins of the human red blood cell are studied by SDS polyacrylamide-gel electrophoresis, approximately 15 major protein bands are detected, varying in molecular weight from 15,000 to 250,000 daltons. Three of these proteins—spectrin, glycophorin, and band 3—account for more than 60% (by weight) of the total membrane protein (Figure 10–29). Each of these proteins is arranged in the membrane in a different manner. We shall therefore use them as examples of three major ways in which proteins are associated with membranes, not only in red blood cells but in other cells as well.

**Spectrin Is a Cytoskeletal Protein Noncovalently Associated with the Cytosolic Side of the Red Blood Cell Membrane**

Most of the protein molecules associated with the human red blood cell membrane are peripheral membrane proteins bound to the cytosolic side of the lipid bilayer. The most abundant of these proteins is **spectrin**, a long, thin, flexible rod about 100 nm in length that constitutes around 25% of the membrane-associated proteins.
protein mass (about 2.5 × 10^5 copies per cell). It is the principal component of the protein meshwork (the cytoskeleton) that underlies the red blood cell membrane, maintaining the structural integrity and biconcave shape of this membrane (see Figure 10–26). If the cytoskeleton is dissociated from red blood cell ghosts in low-ionic-strength solutions, the membrane fragments into small vesicles.

Spectrin is a heterodimer formed from two large, structurally similar subunits (Figure 10–30). The heterodimers self-associate head-to-head to form 200-nm-long tetramers. The tail ends of four or five tetramers are linked together by binding to short actin filaments and to other cytoskeletal proteins (including the band 4.1 protein) in a “junctional complex.” The final result is a deformable, netlike meshwork that underlies the entire cytosolic surface of the membrane (Figure 10–31). It is this spectrin-based cytoskeleton that 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 the spectrin deficiency.

The protein mainly responsible for attaching the spectrin cytoskeleton to the red cell plasma membrane was identified by monitoring the binding of radiolabeled spectrin to red cell membranes from which spectrin and various other peripheral proteins had been removed. These experiments showed that the binding of spectrin depends on a large intracellular attachment protein called ankyrin, which attaches both to spectrin and to the cytosolic domain of the transmembrane protein band 3 (see Figure 10–31). By connecting some of the band 3 molecules to spectrin, ankyrin links the spectrin network to the membrane; it also greatly reduces the rate of diffusion of the bound band 3 molecules in the lipid bilayer. The spectrin-based cytoskeleton is also attached to the membrane by a second mechanism, which depends on the band 4.1 protein mentioned above. This protein, which binds to spectrin and actin, also binds to the cytosolic domain of both band 3 and glycophorin, the other major transmembrane protein in red blood cells.

An analogous but much more elaborate and complicated cytoskeletal network exists beneath the plasma membrane of most other cells in our bodies. This network, which constitutes the cortical region (or cortex) of the cytosol, is rich in actin filaments, which are thought to be attached to the plasma membrane in numerous ways. Proteins that are structurally homologous to spectrin, ankyrin, and band 4.1 are present in the cortex of nucleated cells. We discuss the cortical cytoskeleton in nucleated cells and its interactions with the plasma membrane in Chapter 16.

Glycophorin Extends Through the Red Blood Cell Lipid Bilayer as a Single α Helix

Glycophorin is one of the two major proteins exposed on the outer surface of the human red blood cell and was the first membrane protein for which the complete amino acid sequence was determined. Like the model transmembrane protein shown in Figure 10–22, glycophorin is a small, single-pass transmembrane glycoprotein (131 amino acids) with most of its mass on the external surface of the membrane, where its hydrophilic N-terminal end is located. This part of the protein carries all of the carbohydrate (about 100 sugar residues in 16 separate oligosaccharide side chains), which accounts for 60% of the molecule’s mass. In fact, the great majority of the total red blood cell surface carbohydrate (including more than 90% of the sialic acid and therefore most of the negative charge of the surface) is carried by glycophorin molecules. The hydrophilic C-terminal tail of glycophorin is exposed to the cytosol, while a hydrophobic α-helical segment 23 amino acids long spans the lipid bilayer (see Figure 10–20A).

Despite there being nearly a million glycophorin molecules per cell, their function remains unknown. Indeed, individuals whose red blood cells lack a major subset of these molecules seem to be perfectly healthy. Although glycophorin itself is found only in red blood cells, its structure is representative of
Figure 10–30 Spectrin molecules from human red blood cells. The protein is shown (A) schematically and (B) in electron micrographs. Each spectrin heterodimer consists of two antiparallel, loosely intertwined, flexible polypeptide chains called \( \alpha \) and \( \beta \). These are attached noncovalently to each other at multiple points, including both ends. The phosphorylated “head” end, where two dimers associate to form a tetramer, is on the left. Both the \( \alpha \) and the \( \beta \) chains are composed largely of repeating domains 106 amino acids long. In the micrographs, the spectrin molecules have been shadowed with platinum. (A, adapted from D.W. Speicher and V.T. Marchesi, Nature 311:177–180, 1984; B, courtesy of D.M. Shotton, with permission from D.M. Shotton, B.E. Burke, and D. Branton, J. Mol. Biol. 131:303–329, 1979. © Academic Press Inc. [London] Ltd.)

Figure 10–31 The spectrin-based cytoskeleton on the cytosolic side of the human red blood cell membrane. The structure is shown (A) schematically and (B) in an electron micrograph. The arrangement shown in the drawing has been deduced mainly from studies on the interactions of purified proteins in vitro. Spectrin dimers are linked together into a netlike meshwork by junctional complexes (enlarged in the box on the left) 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 by the indirect binding of spectrin tetramers to some band 3 proteins via ankyrin molecules, as well as by the binding of band 4.1 proteins to both band 3 and glycophorin (not shown). 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. USA 82:6153–6157, 1985. © National Academy of Sciences.)
a common class of membrane proteins that traverse the lipid bilayer as a single α helix. Many cell-surface receptors, for example, belong to this class.

Glycophorin normally exists as a homodimer, with its two identical chains linked primarily through noncovalent interactions between the transmembrane α helices. Thus, the transmembrane segment of a membrane protein is often more than just a hydrophobic anchor: the sequence of hydrophobic amino acids can contain information that mediates protein–protein interactions. Similarly, the individual transmembrane segments of a multipass membrane protein occupy defined positions in the folded protein structure that are determined by interactions between neighboring transmembrane α helices. Often, the cytosolic or noncytosolic loops of the polypeptide chain that link the transmembrane segments in multipass transmembrane proteins can be clipped with proteases and the resulting fragments stay together and function normally. In some cases, the separate pieces can be expressed in cells and they assemble properly to form a functional protein (Figure 10–32).

Band 3 of the Red Blood Cell Is a Multipass Membrane Protein That Catalyzes the Coupled Transport of Anions

Unlike glycophorin, the band 3 protein is known to have an important role in the function of red blood cells. It derives its name from its position relative to the other membrane proteins after electrophoresis in SDS polyacrylamide gels (see Figure 10–29). Like glycophorin, band 3 is a transmembrane protein, but it is a multipass membrane protein, traversing the membrane in a highly folded conformation. The polypeptide chain (about 930 amino acids long) is thought to extend across the bilayer 12 times.

The main function of red blood cells is to carry O₂ from the lungs to the tissues and to help in carrying CO₂ from the tissues to the lungs. The band 3 protein is crucial for the second of these functions. Because CO₂ is only sparingly soluble in water, it is carried in the blood plasma as bicarbonate (HCO₃⁻), which is formed and broken down inside red blood cells by an enzyme that catalyzes the reaction H₂O + CO₂ ⇌ HCO₃⁻ + H⁺. The band 3 protein acts as an anion transporter,

Figure 10–32 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.
which allows $\text{HCO}_3^-$ to cross the membrane in exchange for $\text{Cl}^-$. By making the red cell membrane permeable to $\text{HCO}_3^-$, this transporter increases the amount of $\text{CO}_2$ the blood can deliver to the lungs.

Band 3 proteins can be seen as distinct intramembrane particles by the technique of freeze-fracture electron microscopy. In this procedure, cells are frozen in liquid nitrogen, and the resulting block of ice is fractured with a knife. The fracture plane tends to pass through the hydrophobic middle of membrane lipid bilayers, separating them into their two monolayers (Figure 10–33). The exposed fracture faces are then shadowed with platinum, and the resulting platinum replica is examined with an electron microscope. When examined in this way, human red blood cell membranes appear to be studded with intramembrane particles that are relatively homogeneous in size (7.5 nm in diameter) and randomly distributed (Figure 10–34). The particles are thought to be principally band 3 molecules. When synthetic lipid bilayers are reconstituted with purified band 3 protein molecules, typical 7.5-nm intramembrane particles are observed when the bilayers are fractured. Figure 10–35 illustrates why band 3 molecules are seen in freeze-fracture electron microscopy of red blood cell membranes but glycophorin molecules probably are not.

To transfer small hydrophobic molecules across a membrane, a membrane transport protein must puncture the hydrophobic permeability barrier of the lipid bilayer and provide a path for the hydrophilic molecules to cross. As with the pore-forming $\beta$ barrel proteins discussed earlier, the molecular architecture of multipass transmembrane proteins is ideally suited for this task. In many multipass transmembrane proteins, some of the transmembrane $\alpha$ helices contain both hydrophobic and hydrophilic amino acid side chains. The hydrophobic side chains lie on one side of the helix, exposed to the lipid of the membrane. The hydrophilic side chains are concentrated on the other side, where they form part of the lining of a hydrophilic pore created by packing several such amphipathic $\alpha$ helices side by side in a ring within the hydrophobic interior of the lipid bilayer.

In Chapter 11 we consider how multipass transmembrane proteins are thought to mediate the selective transport of small hydrophilic molecules across membranes. But a detailed understanding of how a membrane transport protein actually works requires precise information about its three-dimensional structure in the bilayer. A plasma membrane transport protein for which such detail is known is bacteriorhodopsin, a protein that serves as a light-activated proton ($\text{H}^+$) pump in the plasma membrane of certain archaea. The structure of bacteriorhodopsin is similar to that of many other membrane proteins, and it merits a brief digression here.
Bacteriorhodopsin Is a Proton Pump That Traverses the Lipid Bilayer as Seven $\alpha$ Helices

The “purple membrane” of the archaeon *Halobacterium salinarum* is a specialized patch in the plasma membrane that contains a single species of protein molecule, bacteriorhodopsin (Figure 10–36). 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 shape and causes a series of small conformational changes in the protein that results in the transfer of one $H^+$ from the inside to the outside of the cell (Figure 10–37). 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, as well as other processes that use the energy stored in the $H^+$ gradient. Thus, bacteriorhodopsin is part of a solar energy transducer that provides energy to the bacterial cell.

To understand the function of a multipass transmembrane protein in molecular detail, it is necessary to locate each of its atoms precisely, which generally requires x-ray diffraction studies of well-ordered three-dimensional crystals of the protein. But because of their amphipathic nature, these proteins are soluble only in detergent solutions and are difficult to crystallize. The numerous bacteriorhodopsin molecules in the purple membrane, however, 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 high resolution (3 Å) by an alternative approach, which 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. The structure obtained by electron crystallography was later confirmed and extended to higher resolution by x-ray crystallography. These studies showed that each bacteriorhodopsin molecule is folded into seven closely packed \( \alpha \) helices (each containing about 25 amino acids), which pass through the lipid bilayer at slightly different angles (see Figure 10–37). By freezing the protein crystals at very low temperatures, it has been possible to solve the structures of some of the intermediate conformations the protein goes through during its 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 \( \alpha \) helices. These proteins function as signal transducers rather than as transporters: each responds to an extracellular signal by activating another protein inside the cell, which generates chemical signals in the cytosol, as we discuss in Chapter 15. Although the structures of bacteriorhodopsins and mammalian signaling receptors are strikingly similar, they show no sequence similarity and thus probably belong to two evolutionarily distant branches of an ancient protein family.

**Membrane Proteins Often Function as Large Complexes**

Some membrane proteins function as part of multicomponent complexes. A few of these 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 10–38). In Chapter 14 we discuss how such photosynthetic complexes function to capture light energy and use it to pump \( \text{H}^+ \) across the membrane. In that chapter we also discuss the structure and function of membrane protein complexes that are even larger than the photosynthetic reaction center. 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 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 to move laterally within the membrane (lateral diffusion). The first direct evidence that some plasma membrane proteins are mobile in the plane of the membrane was provided by an experiment in which mouse cells were artificially fused with human cells to produce hybrid cells (heterocaryons). 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 within half an hour or so (Figure 10–39).

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 an 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 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 10–40A). A complementary technique is fluorescence loss in photobleaching (FLIP). Here, a laser beam continuously irradiates a small area to bleach all the fluorescent molecules that diffuse into it, thereby gradually depleting the surrounding membrane of fluorescently labeled molecules (Figure 10–40B). From such measurements one can
calculate the diffusion coefficient for the particular cell-surface protein that was marked. The values 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.

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 have ways of confining membrane proteins to specific domains in a continuous lipid bilayer. 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 10–41). This asymmetric distribution of membrane proteins is often essential for the function of the epithelium, as we discuss in Chapter 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. However, experiments with labeled lipids...
suggest that only lipid molecules in the outer monolayer of the membrane are confined in this way. The separation of both protein and lipid molecules is thought to be maintained, at least in part, by the barriers set up by a specific type of intercellular junction (called a **tight junction**, discussed in Chapter 19). 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 reacts with a specific protein of interest can be labeled with a fluorescent antibody (as shown here), or it 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, an area in the 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 will be bleached, as long as they are mobile and not permanently anchored to the cytoskeleton or extracellular matrix.
cell-surface molecule, the plasma membrane is found to consist of at least three distinct domains (Figure 10–42). 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 restrict 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.

In all of these examples, the diffusion of protein and lipid molecules is confined to specialized domains within a continuous plasma membrane. Cells are known to have a variety of ways of immobilizing their membrane proteins. The bacteriorhodopsin molecules in the purple membrane of *Halobacterium* assemble into large two-dimensional crystals in which the individual protein molecules are relatively fixed in relationship to one another; large aggregates of

Figure 10–41 How a plasma membrane protein is 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).

Figure 10–42 Three domains in the plasma membrane of a guinea pig sperm cell. (A) A basic 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 label selectively cell-surface molecules on (B) the anterior head, (C) the posterior head, and (D) the tail. (Micrographs courtesy of Selena Carroll and Diana Myles.)
this kind diffuse very slowly. A more common way of restricting the lateral mobility of specific membrane proteins is to tether them to macromolecular assemblies either inside or outside the cell. We have seen that some red blood cell membrane proteins are anchored to the cytoskeleton inside. In other cell types, plasma membrane proteins can be anchored to the cytoskeleton, or to the extracellular matrix, or to both. The four known ways of immobilizing specific membrane proteins are summarized in Figure 10–43.

**The Cell Surface Is Coated with Sugar Residues**

Plasma membrane proteins, as a rule, do not protrude naked from the exterior of the cell. They are usually decorated by carbohydrates, which 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 a variety of stains, such as ruthenium red (Figure 10–44), 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 on the cell surface (Figure 10–45). Many of these adsorbed macromolecules are components of the extracellular matrix, so that where the plasma membrane ends and the extracellular matrix begins is largely a matter of semantics. One of the likely functions of the carbohydrate layer is to protect cells against mechanical and chemical damage and to keep foreign objects and other cells at a distance, preventing undesirable 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 a variety of 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. In principle, both the diversity and the exposed position of the oligosaccharides on the cell surface make them especially well suited to a function in specific cell-recognition processes. For many years there was little evidence for this suspected function. More recently, however, plasma-membrane-bound lectins have been identified that recognize specific oligosaccharides on cell-surface glycolipids and glycoproteins. As we discuss in Chapter 19, these lectins are now known to mediate a variety of transient cell–cell adhesion processes, including those occurring in sperm–egg interactions, blood clotting, lymphocyte recirculation, and inflammatory responses.

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. In some of these transmembrane proteins, the polypeptide chain crosses the bilayer as a single α helix (single-pass proteins). In others, including those responsible for the transmembrane transport of ions and other small water-soluble molecules, 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 (multipass proteins). Other membrane-associated proteins do not span the bilayer but instead are attached to either side of the membrane. Many of these are bound by noncovalent interactions with transmembrane proteins, but others are bound via covalently attached lipid groups. 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 and of confining both membrane protein and lipid molecules to particular domains in a continuous lipid bilayer.

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. Plasma membranes also contain integral proteoglycan molecules with surface-exposed polysaccharide chains. The resulting sugar coating is thought to protect the cell surface from mechanical and chemical damage. In addition, some of the oligosaccharide chains are recognized by cell-surface carbohydrate-binding proteins (lectins) that help mediate cell–cell adhesion events.