2.4 Biological Membranes

BIOLOGICAL MEMBRANES SURROUND MOST INTRACELLULAR ORGANELLES

As discussed in Chapter 2.1, cells contain a variety of subcellular organelles and the hallmark of most of them is that they are surrounded by a membrane. These membranes divide the cell into several compartments in which enzymes and substrates are sequestered away from the rest of the cell. This separation into compartments is required for the functioning of these organelles. Maintenance of this compartmentalization requires selective transport of materials across the membranes. Table 2.4.1 lists the various subcellular organelles with their approximate contributions to the cell volume and membrane area. The proportions of cell volume and area represented by the subcellular organelles vary markedly with cell type and activity.

BIOLOGICAL MEMBRANES CONSIST OF A LIPID BILAYER CORE WITH EMBEDDED PROTEINS AND CARBOHYDRATE COATS

The composition of biological membranes varies enormously among the different subcellular organelles, but all biological membranes share a basic structure. The core of the membrane is a lipid bilayer. Embedded in this core are a variety of proteins that carry out many of the activities of the membrane, including selective membrane transport, and some of both the lipids and proteins have carbohydrate coats. This basic structure is shown in Figure 2.4.1. The rest of this chapter expands on this general description.

ORGANIC SOLVENTS CAN EXTRACT LIPIDS FROM MEMBRANES

Gorter and Grendel, in 1925, provided early evidence for the lipid bilayer structure of membranes when they extracted the lipids from erythrocytes with acetone and spread them over the surface of water. They noted that the area occupied by the lipids was about twice the calculated area of the surface of the erythrocytes. Because the only membrane in the erythrocytes is the plasma membrane, they concluded that the membrane was a lipid bilayer. This confirmed earlier observations by Ernst Overton in the 1890s that there is an excellent correlation between the ability of a number of solutes to enter cells and their solubility in olive oil. Overton concluded that the surface of the cell was made up of lipids similar to olive oil.

BIOLOGICAL MEMBRANES CONTAIN MOSTLY *PHOSPHOLIPIDS*

Organelles can be isolated by cellular disruption and differential centrifugation, as described in Appendix 2.1.A1. The lipids in the membranes can be extracted into an organic phase, typically a chloroform/methanol mixture, because these lipids are hydrophobic (see Figure 2.3.7), and then the lipids can be separated into their component classes by chromatography, and the amounts can be measured. Approximate lipid composition of some membranes is given in Table 2.4.2.

TABLE 2.4.1 The Relative Area and Enclosed Volumes of the Major Subcellular Membranes in a Typical Liver Cell

Membrane	Percent of Total Cell Volume Enclosed	Approximate Number per Cell	Percent of Total Cell Membrane
Plasma membrane	100	1	2
Mitochondria	22	1700	39
Rough ER	9	1	35
Smooth ER	2	1	16
Golgi apparatus	4	1	7
Nuclear inner membrane	6	1	0.2
Lysosomes	1	300	0.4
Peroxisomes	1	400	0.4

Values were estimated by quantitative electron microscopy.

FIGURE 2.4.1 Basic structure of biological membranes. Membranes consist of a lipid bilayer core to which proteins are attached in a variety of ways. In addition, some lipids and proteins have carbohydrate groups attached to them.

However, it should be kept in mind that the lipid composition varies not only with the kind of organelle but also with the kind of cell.

PHOSPHOLIPIDS CONTAIN FATTY ACYL CHAINS, GLYCEROL, PHOSPHATE, AND A HYDROPHILIC GROUP

Table 2.4.2 shows that **phospholipids** comprise the most abundant class of lipids in membranes. Each consists of four parts: a **glycerol** backbone, a **phosphate** esterified to one end of the glycerol molecule, a **polar head group** attached to the phosphate, and two **fatty acids** esterified to the other two hydroxyl groups of the glycerol. The glycerol phospholipids form the major subclass of the phospholipids, and they contain phosphate esterified to the C-3 hydroxyl group of glycerol and two fatty acids esterified to the C-2 and C-1 hydroxyl groups. Fatty acids consist of two distinct regions (see Figure 2.4.2): a long hydrocarbon chain and a carboxylic acid group (−COOH). The most frequent length of the carbon chain is 16 or 18 carbon atoms. As discussed in Chapter 1.4, carbon can form four single bonds with bond angles that approximate those between the center of a tetrahedron and its vertices. In a hydrocarbon chain, two of these bonds link a carbon with adjacent carbons and two remain for other bonds. When hydrogen is covalently bound to all of the two remaining bonding orbitals, the hydrocarbon is **saturated**. **Unsaturated** fatty acids contain one or more double bonds between carbon atoms. Those containing more than one double bond are **polyunsaturated.** The variety of phospholipids is produced from the variety of fatty acids and from the different polar head groups. The most common lipid constituent of membranes is phosphatidylcholine, with other phospholipids making major contributions. The components of the simplest phospholipid, phosphatidic acid, are shown in Figure 2.4.2.

A variety of polar groups can be attached to the phosphate of phospholipids. These groups include **serine**, **inositol**, and **ethanolamine**, which can be methylated to form **choline**. The resulting phospholipids are shown in Figure 2.4.3.

TABLE 2.4.2 Approximate Lipid Composition of Different Cell Membranes

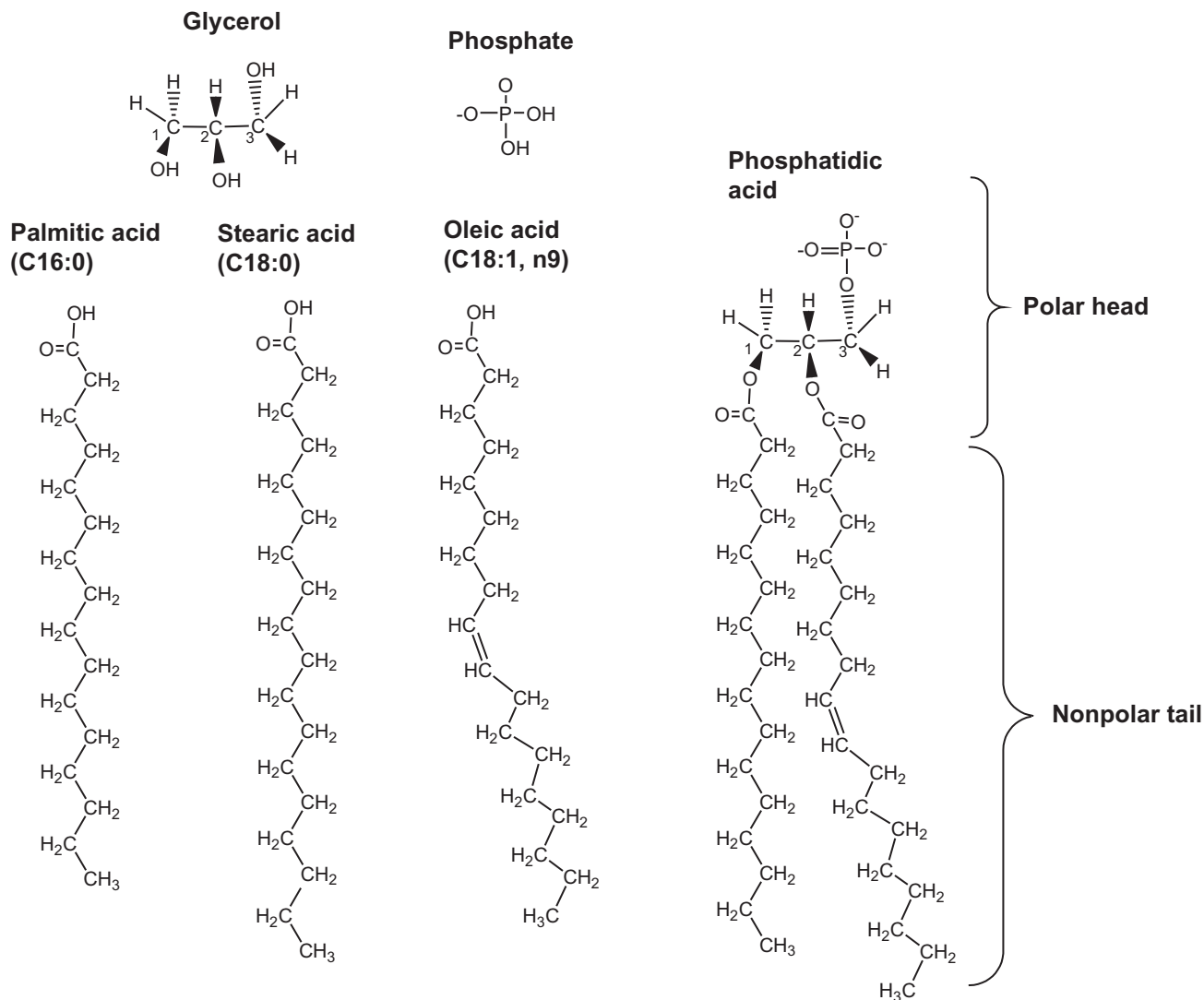| Lipids	Percentage of Total Lipids by Weight		
	Plasma Membrane	Mitochondrial Membrane	Endoplasmic Reticulum Membrane
Phosphatidylcholine	24	39	40
Phosphatidylethanolamine	7	35	17
Phosphatidylserine	4	2	5
Cholesterol	17	3	6
Sphingomyelin	19	0	5
Glycolipids	7	0	0
Other	22	21	27

FIGURE 2.4.2 Components of a simple phospholipid, phosphatidic acid. Glycerol forms the backbone. Each of the three carbons in glycerol covalently bonds to a hydroxyl group (−OH). Rotation about the C−C single bonds can position the C1 OH or C3 OH in any orientation. The central carbon, C2, is an asymmetric carbon when C1 and C3 are differently substituted, and will show stereoisomerism when different groups attach to the two ends. To distinguish the 1 and 3 positions, the numbering nomenclature shown in the figure is used. The structure of inorganic phosphate is also shown. Fatty acids form the hydrophobic core of phospholipids. The chemical structure of three common fatty acids: palmitic acid, stearic acid, and oleic acid is shown. The single carbon−carbon bonds allow free rotation about the axis connecting the two carbon nuclei. Each carbon atom forms bonds that nearly line up the centers of two tetrahedrons with their respective apices. Palmitic acid is a 16-carbon **saturated fatty acid**, meaning that every carbon's bonds other than the carboxyl carbon are fully occupied with single bonds to carbon or hydrogen. In the nomenclature of fatty acids, it is designated 16:0, indicating a 16-carbon fatty acid with no double C−C bonds. Stearic acid is a 18-carbon saturated fatty acid, designated as 18:0. Oleic acid is a **mono-unsaturated fatty acid**, meaning that one pair of carbon atoms are joined by a double bond. In this case, the double bond is between carbons 9 and 10, numbering down from the carboxyl carbon. Its designation is 18:1 Δ^9, where the 1 indicates one double bond and the Δ^9 indicates the double bond begins at Carbon 9. There is an alternate number system starting from the terminal methyl group of the fatty acid, instead of starting at the carboxyl group. This nomenclature uses the prefix omega (ω); thus oleic acid is 18:1 $\omega - 9$. Here the 18 stands for the length of the hydrocarbon chain, 1 indicates the number of double bonds, and $\omega - 9$ indicates the position of the double bond numbering from the methyl end. Substitution of the omega with the letter n is becoming popularized, with the same meaning. As discussed in Chapter 1.4, double bonds produce kinks in the hydrocarbon chain due to restricted rotation about C＝C double bonds. Oleic acid has a *cis* orientation of the H atoms around the double bond, meaning the two hydrogens are on the same side of the double bond. The structure of a phosphatidic acid is shown on the right. This particular phosphatidic acid has an oleic acid molecule esterified to C-2 of glycerol and a palmitic acid molecule esterified to C-1. The fatty acids differ from molecule to molecule, but this is a typical arrangement in which saturated fatty acids occupy the C-1 position and unsaturated or polyunsaturated fatty acids occupy the C-2 position. Phosphatidic acid illustrates a common property of this class of lipids in that it consists of spatially separated water-soluble polar or hydrophilic groups and water-insoluble nonpolar or hydrophobic groups.
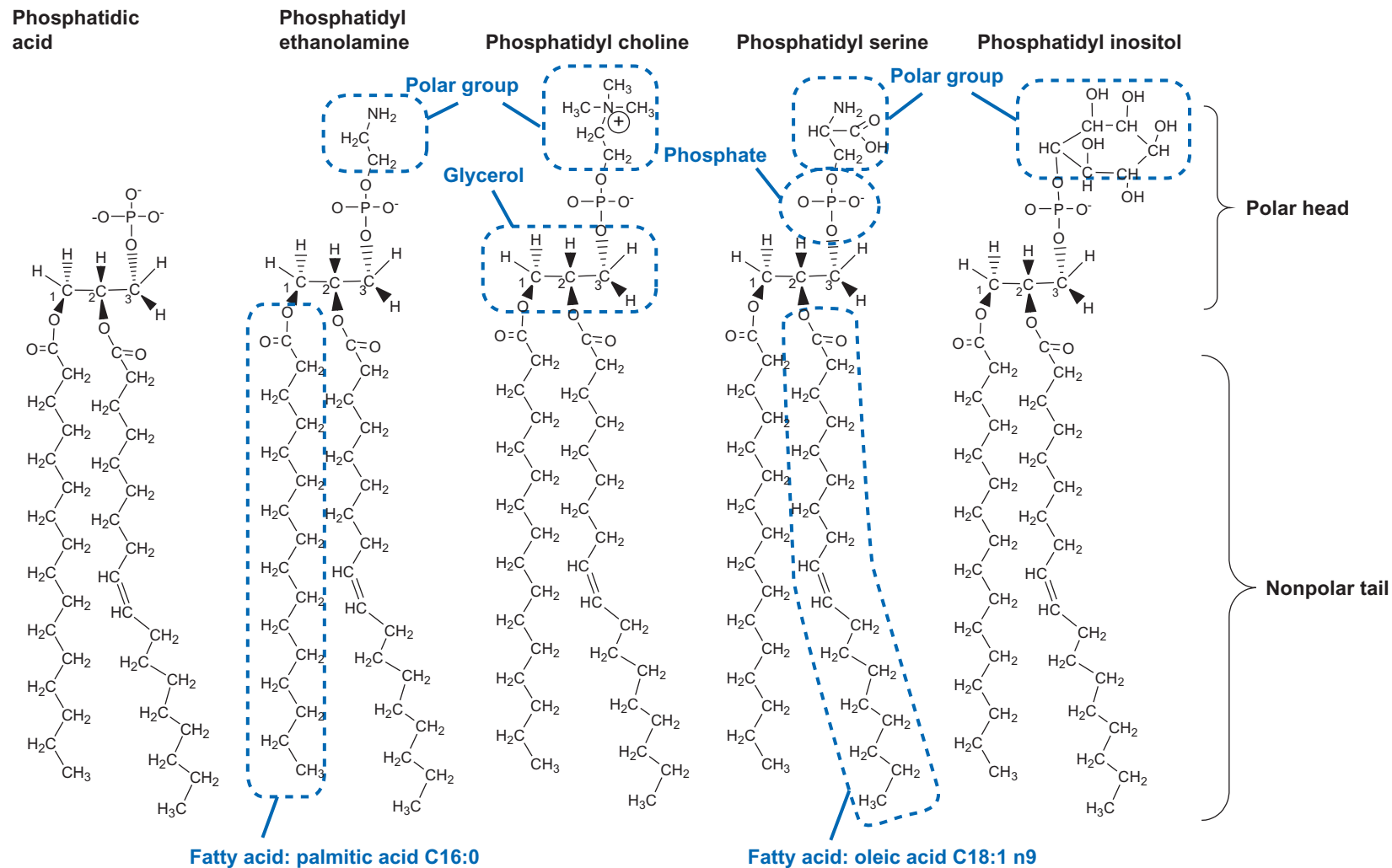
FIGURE 2.4.3 Chemical structures of some common glycerophospholipids. The phosphate group in phosphatidic acid is esterified to the hydroxyl group of several other hydrophilic molecules including ethanolamine, choline, serine, and inositol. These form the lipids shown. Each of these are named for the hydrophilic group and the fatty acids, as in 1-palmitoyl, 2-oleolyl phosphatidylcholine.

PLASMANYL PHOSPHOLIPIDS AND PLASMENYL PHOSPHOLIPIDS USE FATTY ALCOHOLS INSTEAD OF FATTY ACIDS

A major subclass of the phospholipids use fatty alcohols instead of fatty acids, forming an ether linkage with glycerol instead of an ester. These are called plasmanyl glycerol phospholipids. Most of these are modified to contain a vinyl ether linkage, in which the alcohol group is doubly bonded to the rest of the fatty alcohol chain. These are termed plasmenyl glyercol phospholipids or plasmalogens. Their structures are shown in Figure 2.4.4. They make up 15−20% of the total phospholipids of cell membranes.

SPHINGOLIPIDS USE SPHINGOSINE AS A BACKBONE AND ARE PARTICULARLY RICH IN BRAIN AND NERVE TISSUES

Sphingolipids are present in many membranes but they are particularly rich in brain and nerve tissues. There are three classes of sphingolipids: **sphingomyelin**, **cerebrosides**, and **gangliosides**. Sphingomyelin is the only one of these that is a sphingophosphatide. It is analogous to the glycerophosphatides except that it contains sphingosine instead of glycerol as the core structure that links the hydrophilic phosphate and choline to the hydrophobic hydrocarbon chains. Sphingosine is a derivative of the amino acid, serine. The chemical structures of sphingosine and sphingomyelin are shown in Figure 2.4.5.

The fatty acid amide of sphingosine alone is called a **ceramide** (see Figure 2.4.5). Ceramides can be linked through the hydroxyl group to sugar groups to form another class of sphingolipids, the **cerebrosides**. The sugar part contains a number of hydroxyl groups and is hydrophilic. In some cases the sugar part is quite large and branched, forming a **ganglioside**. Both cerebrosides and gangliosides form another class of lipids called glycolipids because they incorporate sugar derivatives.

OTHER LIPID COMPONENTS OF MEMBRANES INCLUDE *CARDIOLIPIN, SPHINGOLIPIDS,* AND *CHOLESTEROL*

CARDIOLIPIN IS TWO GLYCEROLIPIDS LINKED BACK TO BACK

The structure of cardiolipin is shown in Figure 2.4.6. It consists of two phosphatidic acid molecules linked through another glycerol. Mitochondrial membranes are particularly rich in cardiolipin.

CHOLESTEROL CONDENSES MEMBRANES

Cholesterol, shown in Figure 2.4.7, is the most abundant steroid in animal tissues. All steroid hormones are derived from cholesterol. It possesses a rigid ring structure that attracts normally flexible phospholipid chains to itself, causing membranes to become more rigid in the vicinity of this molecule.

PHOSPHOLIPIDS IN WATER SELF-ORGANIZE INTO LAYERED STRUCTURES

All of the glycerophospholipids and sphingolipids we have discussed are characterized by the spatial separation of a polar head group, consisting of ionized groups, hydroxyls and carbonyl oxygens, and a long tail consisting mainly of hydrocarbons. The polar head group is capable of interacting with water and each of the materials there individually is water soluble. This
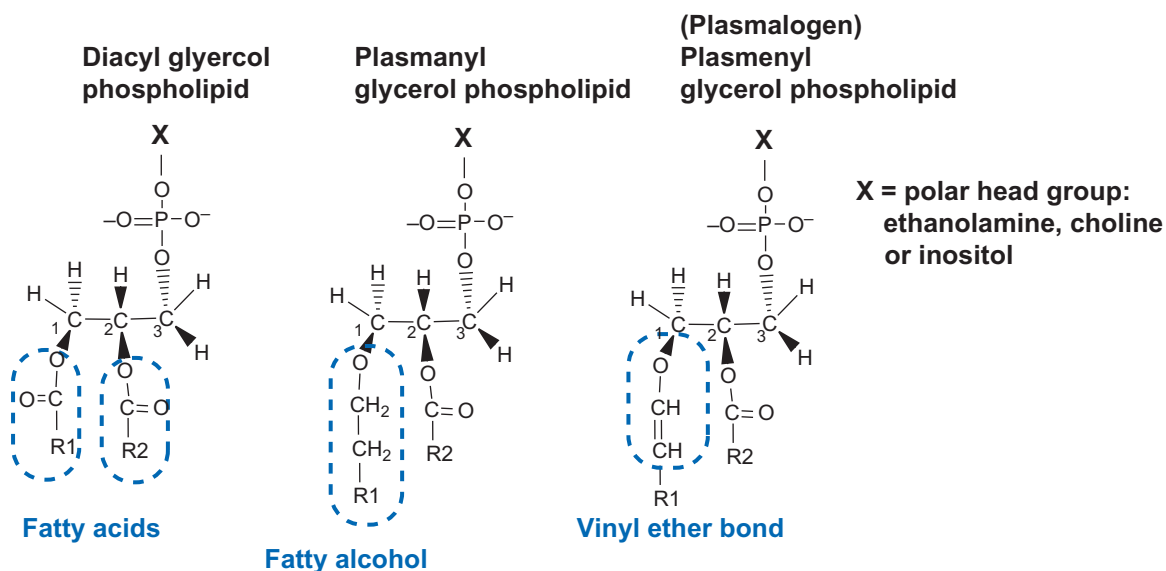

FIGURE 2.4.4 Plasmanyl glycerol phospholipids and plasmenyl glycerol phospholipids. Some long-chain hydrocarbons have an alcohol and not a carboxyl group at their end. These can be joined to glycerol through an ether linkage, forming a plasmanyl glycerol phospholipid which is typically enriched in the sn-2 position with polyunsaturated fatty acids. In most cases, the carbon adjacent to the ether is joined in a double bond, forming a vinyl ether bond that characterizes the plasmenyl glycerol phospholipids, also known as the plasmalogens.
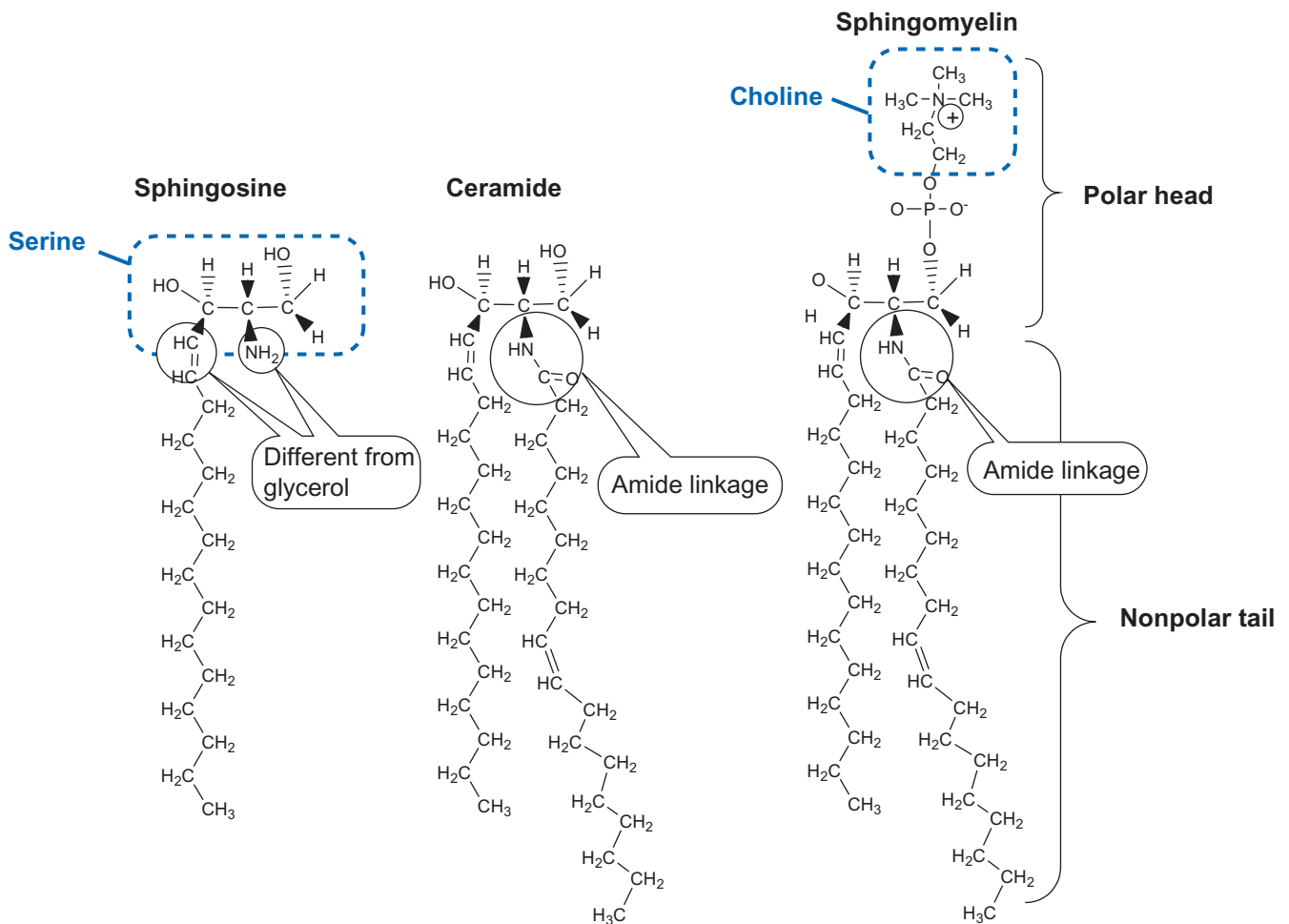
FIGURE 2.4.5 Chemical structures of sphingosine, ceramide, and sphingomyelin. Note that sphingosine does not have glycerol as a core structure and joins a hydrocarbon through an amide linkage instead of an ester to form sphingomyelin.

region of the molecule is **hydrophilic**, meaning *water loving*. The hydrocarbon tail is not soluble in water; it is **hydrophobic** or *water hating*. Hydrophobic parts are also described as **lipophilic** or fat loving. Molecules having both of these separate domains are said to be amphipathic, from the Latin and Greek *amphi* meaning *having two sides*. These two sides are illustrated by the space-filling model in Figure 2.4.8. These two separate domains of phospholipids are crucial to their behavior in cells. When placed in water, the polar heads of these molecules remain associated with water and form hydrogen bonds with it; the long hydrocarbon, nonpolar tails repel the water and associate with each other, forming a self-organized structure, the lipid bilayer. Much of this behavior of the lipids resides in the nature of water. To see this, we need to learn more about the behavior of water at hydrophobic interfaces.

SURFACE TENSION OF WATER RESULTS FROM ASYMMETRIC FORCES AT THE INTERFACE

At the air−water interface, water molecules are subjected to asymmetric forces, as shown in Figure 2.4.9. These molecules have lost some of their bonds connecting

them to the bulk phase and are, therefore, in a higher energy state than water in the bulk phase. These molecules are partially evaporated. Thus it takes energy to promote water molecules to the interface and the energy of the surface increases with its area. At constant temperature and pressure, the change in surface energy is the change in the **Gibbs free energy, G**. The change is given by:

$$[2.4.1] \qquad dG = \gamma\, dA$$

where dA is the increment in area, dG is the increment in Gibbs free energy, and γ is the **surface tension**. Since the energy has units of force × distance, the tension has units of force per unit length. Typical units of γ are dynes cm^{-1}. In SI units, γ is expressed in N m^{-1} or J m^{-2}. The surface tension is a measure of how much more a water molecule at the surface is attracted to the bulk water phase because of the increase in intermolecular forces compared to the surface.

WATER "SQUEEZES OUT" AMPHIPATHIC MOLECULES

Recall that amphipathic molecules consist of spatially separated water-loving or hydrophilic head group and a
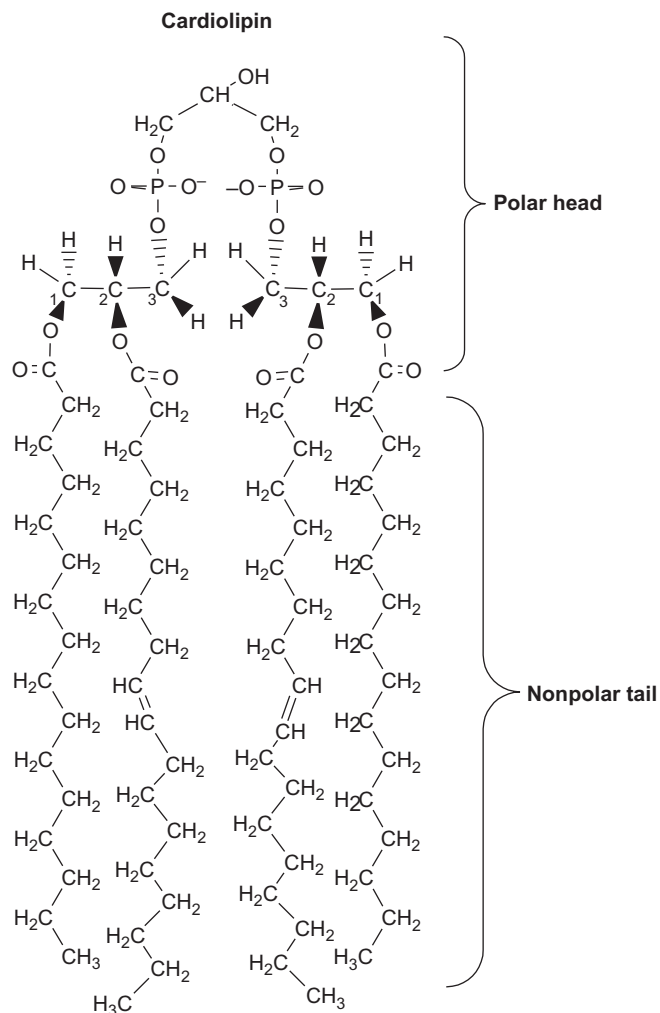
Cardiolipin

Polar head

Nonpolar tail

FIGURE 2.4.6 Chemical structure of cardiolipin.

Phosphatidylcholine

Water

Choline

Phosphate

Glycerol

Fatty acids

Hydrophilic groups

Hydrophobic groups (= Lipophilic groups)

FIGURE 2.4.8 Space-filling model of phosphatidylcholine. Nonpolar surfaces are shown in gray or white. Polar surfaces are dark blue (oxygen), light blue (phosphorus), or intermediate blue (nitrogen). Charged surfaces attract water by dipole—dipole interactions. The hydrophilic or water-loving parts of the molecule are all concentrated at one end. The hydrophobic (water-hating) or lipophilic (fat-loving) groups are located at the opposite end.

Steroid nucleus

Cholesterol

Air

Water

Molecules at the surface have fewer attractive forces than molecules in the bulk phase; they are partially evaporated and have a higher energy
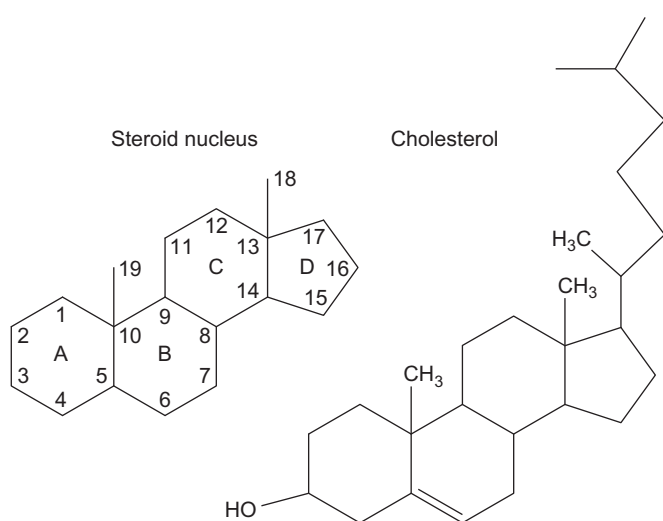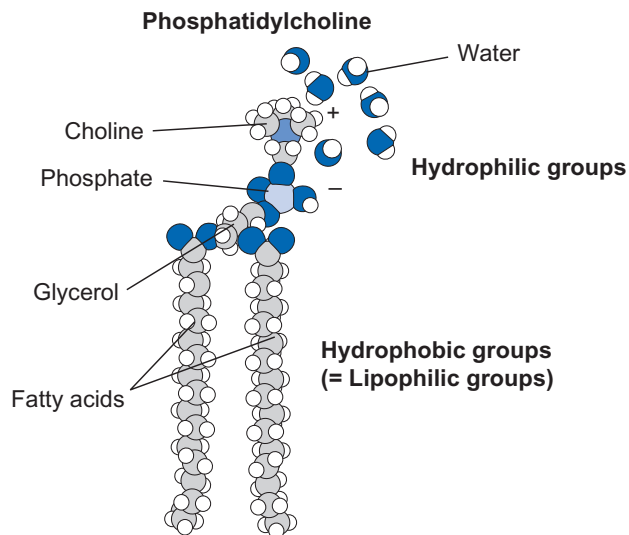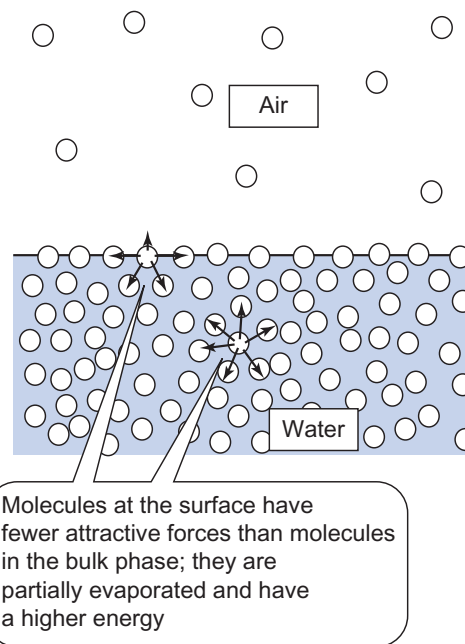
FIGURE 2.4.7 Chemical structure of the steroid nucleus and cholesterol. The steroids are all derived from the steroid nucleus, a perhydrocyclopentanophenanthrene nucleus of four rings: three fused rings in the phenanthrene arrangement and a five-carbon ring attached. Therefore, the nucleus has the name "cyclopentano" to refer to the five-carbon ring; "phenanthrene" refers to the three six-membered rings and "perhydro" indicates that the double bonds of phenanthrene are saturated with hydrogen. The steroid nucleus is numbered as shown.

FIGURE 2.4.9 Asymmetric forces of water molecules at the air—water interface. In the bulk phase of liquid water, the intermolecular forces acting on any water molecule are on average equal in all directions. In the air, intermolecular interactions are markedly reduced. In order to evaporate, energy must be added to the water molecules to break the attractive intermolecular forces. At the air—water interface, water molecules are subject to more intermolecular forces than those water molecules in the air phase, and fewer forces than water molecules in the bulk liquid phase. Therefore, water molecules at the surface have more energy than those in the bulk phase. In a sense, they are partially evaporated, having lost some but not all of their intermolecular bonds. If we were to increase the surface area, we would have to put in energy proportional to the area of increase.
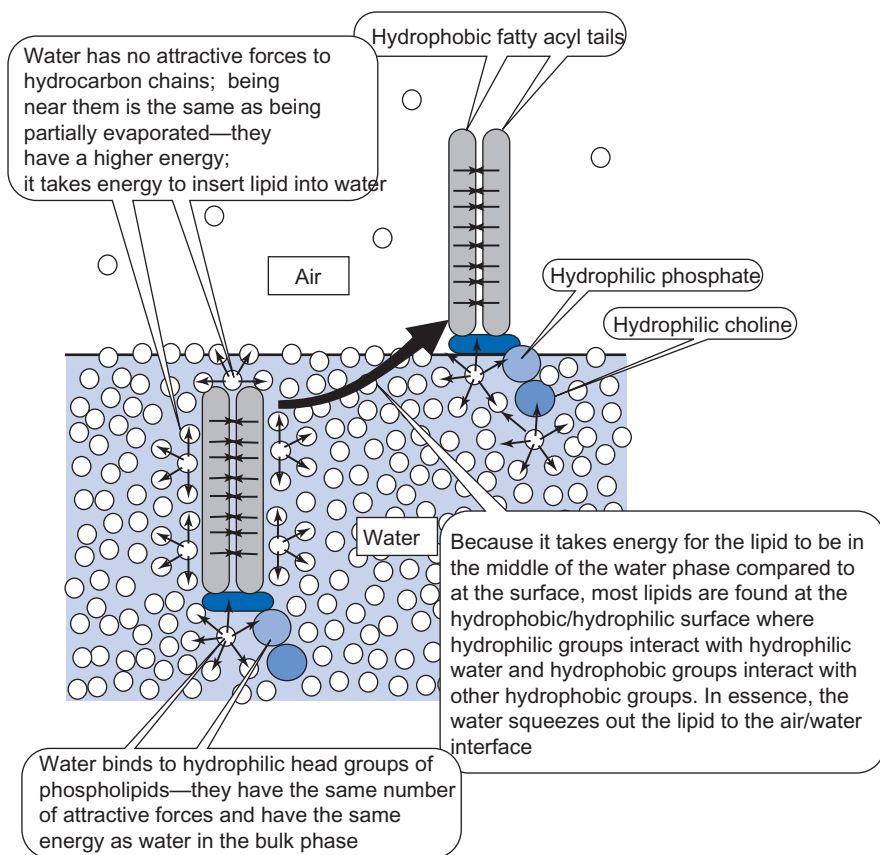
Water has no attractive forces to hydrocarbon chains; being near them is the same as being partially evaporated—they have a higher energy; it takes energy to insert lipid into water

Hydrophobic fatty acyl tails

Air

Hydrophilic phosphate

Hydrophilic choline

Water

Because it takes energy for the lipid to be in the middle of the water phase compared to at the surface, most lipids are found at the hydrophobic/hydrophilic surface where hydrophilic groups interact with hydrophilic water and hydrophobic groups interact with other hydrophobic groups. In essence, the water squeezes out the lipid to the air/water interface

Water binds to hydrophilic head groups of phospholipids—they have the same number of attractive forces and have the same energy as water in the bulk phase

FIGURE 2.4.10 Water "squeezes out" phospholipids. The hydrocarbon parts of a phospholipid molecule within the bulk aqueous phase cannot form hydrogen bonds with the adjacent water molecules, so these water molecules cannot form as many hydrogen bonds as the other water molecules in the bulk phase. The set of water molecules surrounding the hydrocarbon are essentially partially evaporated and have a higher energy than the water molecules in the bulk phase. Therefore, to insert the hydrocarbon in the water takes energy. Phospholipids on the surface of the water, on the other hand, are in a lower energy state. The self-association of water thus "squeezes out" the phospholipid to the surface.

hydrophobic or water-fearing hydrocarbon tail. Dissolving an amphipathic molecule in the bulk water phase disrupts the self-association of water, creating an interface within the bulk phase that requires energy. Because of this, the amphipathic molecule is "squeezed out" of the bulk water phase to the surface of the solution adjacent to the air. In this situation, the water molecules at the surface interact with hydrophilic groups in the amphipathic molecule, which lowers the energy of the surface, and the hydrophobic groups in the amphipathic molecule can interact with other hydrophobic groups through London dispersion forces. These ideas are shown diagrammatically in Figures 2.4.10 and 2.4.11.

The free energy change for the transfer of phospholipid from the bulk phase to the surface is given as

[2.4.2] $\Delta G_{\text{bulk} \Rightarrow \text{surface}} = G_{\text{surface}} - G_{\text{bulk}}$

Since G_{surface} is less than G_{bulk}, the free energy change for the transfer is negative, and therefore the transfer occurs spontaneously.

AMPHIPATHIC MOLECULES SPREAD OVER A WATER SURFACE, REDUCE SURFACE TENSION, AND PRODUCE AN APPARENT SURFACE PRESSURE

When amphipathic molecules such as phosphatidylcholine or oleic acid are dissolved in a volatile organic solvent (e.g., hexane, decane) and then layered over water,

the organic solvent evaporates and leaves a thin film of the lipid. As shown in Figures 2.4.10 and 2.4.11, these amphipathic molecules are squeezed out to the surface of the water, forming a layer a single molecule thick. The lipids form a **monolayer**. These lower the surface tension according to Eqn [2.4.1] because they lower the energy required to move water molecules to the surface (see Figure 2.4.11). This lowering of the surface tension can be measured using a **Langmuir trough**, as shown in Figure 2.4.12. This device has two barriers on the surface of the water. One barrier is fixed, the other is movable. Since the monolayer decreases the surface tension, the movable barrier feels a net force in the direction of the clean surfaces. By lowering the surface tension, the lipids appear to exert a **surface pressure** defined as

[2.4.3] $\pi = \gamma_0 - \gamma$

where π is the surface pressure, γ_0 is the surface tension of the clean surface, and γ is the surface tension in the presence of the monolayer. Surface pressure has the same units as the surface tension.

PHOSPHOLIPIDS FORM BILAYER MEMBRANES BETWEEN TWO AQUEOUS COMPARTMENTS

Two monolayers can orient themselves back to back to form a bilayer between two aqueous compartments, as shown in Figure 2.4.13. This is the low energy state for this situation for the reasons we have already described.

If the phospholipids were dispersed throughout the solution, the surface area between the water phase and the hydrophobic, hydrocarbon phase would be large. Since it takes energy to produce this surface, the dispersed hydrocarbon is a high-energy state compared to the condensed one. A secondary cause of this spontaneous organization of the lipids is the attractive interactions among the hydrocarbon chains.
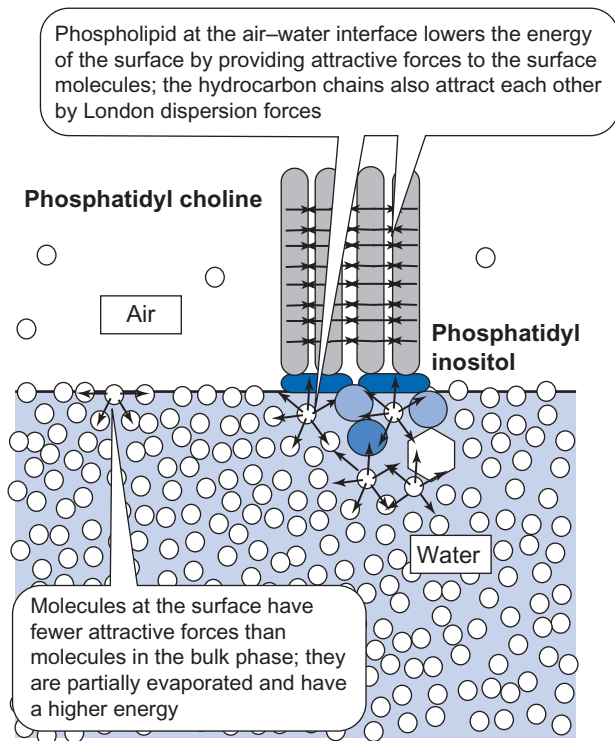

Phospholipid at the air–water interface lowers the energy of the surface by providing attractive forces to the surface molecules; the hydrocarbon chains also attract each other by London dispersion forces

Phosphatidyl choline

Air

Phosphatidyl inositol

Water

Molecules at the surface have fewer attractive forces than molecules in the bulk phase; they are partially evaporated and have a higher energy

FIGURE 2.4.11 Lowering of the surface energy by amphipathic molecules. Water at its ordinary surface is in a higher energy state—it takes energy to break the hydrogen bonds that binds the water molecules in the bulk aqueous phase. When phospholipids are present at the surface, water binds to the hydrophilic groups of the phospholipids, thereby reducing the energy needed to form additional surface. Because the energy of the surface is related to its area by the surface tension, reducing the energy of the surface per unit area is the same as reducing the surface tension.

Macroscopic planar bilayer membranes can be formed across a narrow aperture separating two solutions, as shown in Figure 2.4.14. These membranes are useful because they allow the study of single membrane channels incorporated into the membrane.

LIPID BILAYERS CAN ALSO FORM *LIPOSOMES*

The macroscopic lipid bilayer shown in Figure 2.4.14 is unstable to mechanical forces. When disrupted, the membrane breaks apart to form spherical bilayers separating an internal and external watery compartment. These hollow spheres are called **liposomes** (see Figure 2.4.15). These structures can also be generated directly from phospholipids by soaking them in water and adding sonic energy.

ALTHOUGH LIPIDS FORM THE CORE, MEMBRANE PROTEINS CARRY OUT MANY OF THE FUNCTIONS OF MEMBRANES

So far we have been describing membrane lipids that form the core of biological membranes. These contribute to the barrier function of membranes, but many other functions of biological membranes are performed by protein constituents of the membranes. These functions include:

- **Transport**: Cells must be able to move things into and out of the cell.
- **Signal transduction**: Cell must have mechanisms for responding to signals from other cells or from within the cell. These may be chemical, electrical, or mechanical signals.
- **Recognition**: Cells attach to other cells and to extracellular structures. They must be able to recognize where they should form attachments.
- **Attachment**: Cells must anchor themselves to the extracellular matrix or to each other. Often these attachments also provide a signaling pathway.
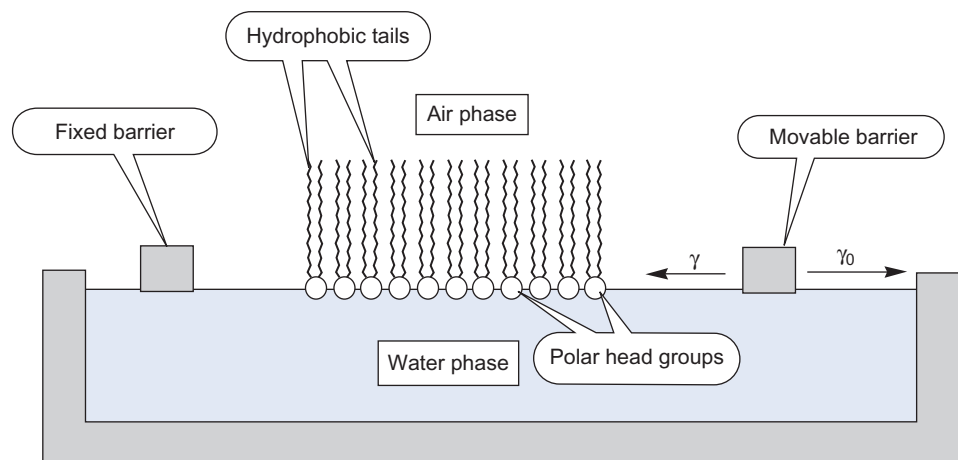

Hydrophobic tails

Fixed barrier

Air phase

Movable barrier

γ

γ_0

Water phase

Polar head groups

FIGURE 2.4.12 The Langmuir trough. A clean water surface has a fixed barrier and a movable barrier. When only clean water forms the surface, the surface tension is γ_0. Adding a lipid film reduces the surface tension to γ. The movable barrier experiences a net force toward the clean surface without lipid. Thus the lipid appears to exert a surface pressure.
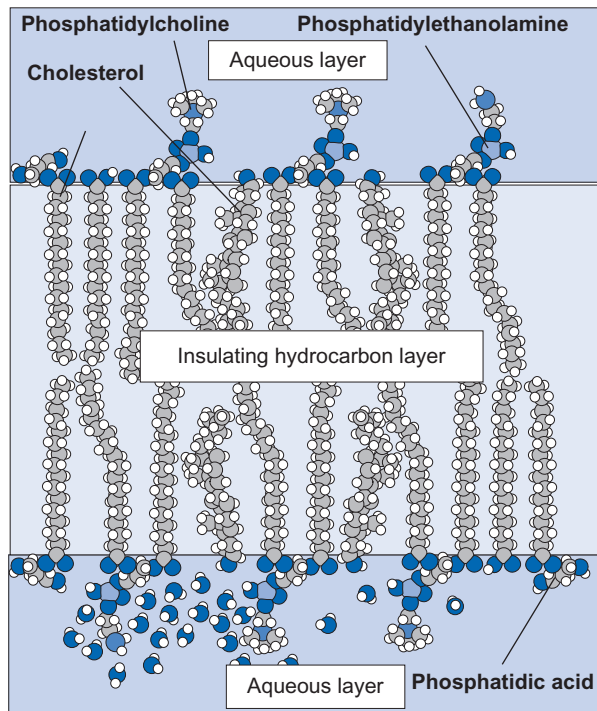
FIGURE 2.4.13 Structure of the lipid bilayer. Only some lipids are shown and in expanded format for clarity. In reality, the lipid bilayer forms a closed surface that approximates a plane. The interior of the lipid bilayer is fluid, consisting of hydrocarbon chains that are saturated (the straight chains in the figure) or unsaturated (bent chains in the figure). The phospholipids form the bulk of the bilayer. Lipids such as cholesterol have a rigid backbone that partially stiffens and solidifies the membrane. Cholesterol may accumulate in heterogeneous patches of membrane called lipid rafts.
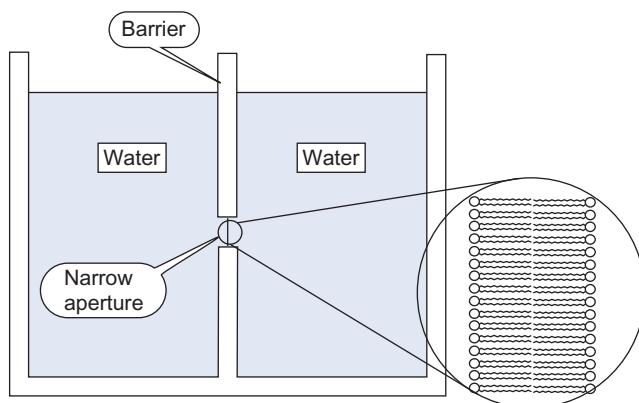

FIGURE 2.4.14 Planar lipid bilayer between two aqueous compartments. Phospholipids were dissolved in hexane and "painted" over a small aperture drilled in a Lexan partition that separated two electrolyte solutions. After thinning, with the passive removal of hexane through the aqueous phase, a lipid bilayer forms between the two compartments.

- **Movement or force production**: Cells often must move or transmit a force from inside the cell to the extracellular matrix. This requires connection of the cell's cytoskeleton through the membrane to the extracellular matrix.
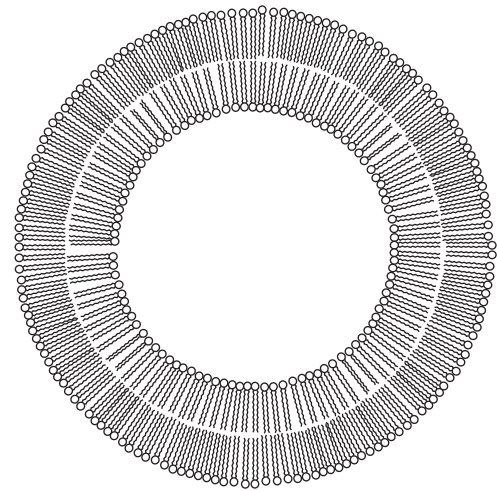

FIGURE 2.4.15 A schematic drawing of a cross-section of a liposome. These are small structures about 50−150 nm across. Because each bilayer is about 7.5−10 nm across, the thickness of the bilayer occupies a considerable portion of the entire liposome volume and the enclosed volume, the lumen, is small. Because of the high curvature and small size, the area on the outside of the liposome is nearly twice the area on the inside, and therefore significantly more lipid faces the outside compared to the inside surface of the liposome. Liposomes may find use someday to deliver drugs to specific locations within the body by incorporating recognition signals into the lipid bilayer.

MEMBRANE PROTEINS BIND TO MEMBRANES WITH VARYING AFFINITY

The proteins that perform the various functions listed above can be loosely classified according to how tightly they are bound to the membrane. Loosely bound proteins are called **peripheral** proteins, also sometimes called **extrinsic** proteins. They can be released from the membrane by relatively gentle procedures such as washing with a salt solution. Other proteins are called **integral** proteins, also sometimes called **intrinsic** proteins. These are tightly bound by the membrane and can be released only by resorting to drastic measures such as dissolving the membrane with a detergent. By coating the hydrophobic parts of the membrane proteins with hydrophilic material, detergents **solubilize** the membrane proteins. Examples of useful detergents in membrane research include the ionic **sodium dodecyl sulfate (SDS)** and the nonionic family of **Triton** detergents. Figure 2.4.16 illustrates integral and peripheral proteins.

Proteins are held in membranes by the same kinds of interactions that hold lipids in the bilayer: hydrophobic and hydrophilic interactions. Many proteins have sequences of amino acids that penetrate all the way across the membrane. These are **transmembrane proteins**. In the parts of the protein exposed to lipid, hydrophobic amino acid side chains appose the lipid core: valine, leucine, isoleucine, phenylalanine, tryptophan, and methionine. Those parts of the protein exposed to water have a preponderance of hydrophilic amino acids: aspartic acid, glutamic acid, lysine, and arginine. The neutral amino acids can be present in
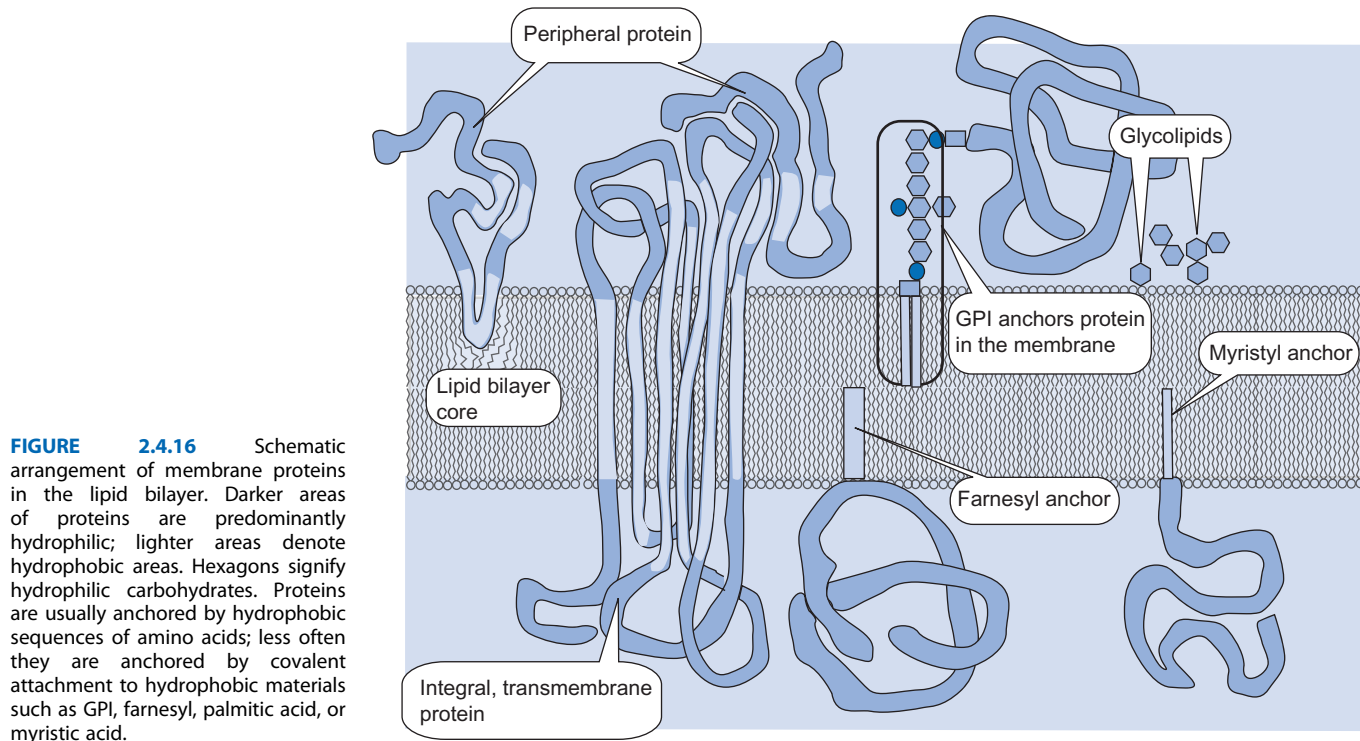
FIGURE 2.4.16 Schematic arrangement of membrane proteins in the lipid bilayer. Darker areas of proteins are predominantly hydrophilic; lighter areas denote hydrophobic areas. Hexagons signify hydrophilic carbohydrates. Proteins are usually anchored by hydrophobic sequences of amino acids; less often they are anchored by covalent attachment to hydrophobic materials such as GPI, farnesyl, palmitic acid, or myristic acid.

either domain. Some proteins have multiple sequences that cross the membrane, with domains facing each of the watery solutions on the two sides of the bilayer. The sequences that cross the membrane are typically arranged as alpha helices, with the hydrophobic amino acids facing the hydrophobic lipid core. Other proteins may bind to the membrane by covalently attached hydrophobic groups. These include **myristic acid** (14:0 fatty acid), **palmitic acid**, (16:0) **farnesyl**, and **glycosyl-phosphatidylinositol** (GPI) (see Figure 2.4.16).

LIPIDS MAINTAIN DYNAMIC MOTION WITHIN THE BILAYER

Researchers have labeled phospholipids with probes that are sensitive indicators of molecular motion and have tracked the mobility of lipids and proteins within the plane of the bilayer. The results of these experiments show a variety of molecular motions, shown in Figure 2.4.17. This has given rise to the fluid mosaic model of biological membranes. The term fluid mosaic model describes a dynamic system in which the lipids form a plane that gradually curves around to form a closed surface—there are no exposed lipid edges. Lipid motion in the plane of the membrane includes rotation, flexion, stretch, and lateral diffusion in the plane. All of these movements are rapid. The proteins in the membrane can also move, unless they are anchored by binding to other components of the membrane. There is a gradient of fluidity of motion from the polar head group to the center of the hydrophobic interior of the membrane, being most fluid in the center and most anchored at the head group. Lipid movement from one side of the membrane to the other—the "flip-flop"
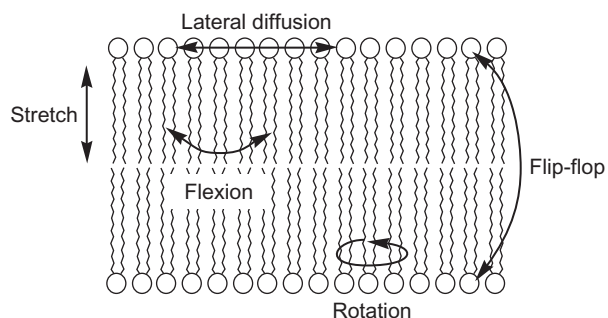

FIGURE 2.4.17 Possible motions of lipids within a bilayer. Lipid molecules can move laterally within the plane of the membrane, rotate about their long axis, flex within the fluid interior of the membrane, or move from one side of the bilayer to the other. Most of these motions are fast, but the "flip-flop" reaction is very slow, occurring less than once in 2 weeks for any individual lipid molecule. Exchange of neighbors occurs very fast, on the order of 10^7 times per second. This rapid exchange gives rise to a rapid lateral movement within each half of the bilayer.

reaction—is slow because it requires lipids to bring their hydrophilic head group through the lipid phase, which is energetically costly. Because of the very slow movement of lipids from one half of the bilayer to another, membranes can maintain an asymmetric composition, but it must actively make and sustain it. In most biological membranes, the two half bilayers differ in their composition. Cells add lipids only to the cytoplasmic side of membranes. An enzyme called a **"scramblase"** flips lipids from the cytoplasmic half to the extracellular half, but this enzyme is not very specific. The different composition of membranes arises from a second enzyme, a **"flippase"**, that flips only some phospholipids from the cytoplasmic half to the extracellular half.

The fluidity of membrane lipids depends on their composition. Saturated fatty acids are known to be very stiff compared to unsaturated fatty acids. Saturated fatty acyl chains can be packed closely together, whereas the kinks produced by *cis* double bonds make it difficult to pack these chains close together. Because of this, unsaturated fatty acids promote fluid movement within the bilayer. Cholesterol is also a very rigid molecule. Cholesterol molecules orient themselves in the membrane with their steroid nucleus adjacent to the hydrophobic tails and the hydroxyl group adjacent to the polar head groups. The steroid nucleus is a flat, plate-like structure that partially immobilizes the nearby fatty acyl chain, thereby reducing the fluidity of the bilayer and increasing the mechanical stability of the bilayer.

The motions of the lipids in the bilayer makes it appear as a two-dimensional fluid. It is fluid within the plane of the membrane, but relatively rigid perpendicular to this plane. The membrane proteins more or less "float" in this lipid see like so many icebergs in the North Atlantic. This combination of fluid lipids and iceberg proteins was the origin of the descriptive term, **fluid mosaic model**.

LIPID RAFTS ARE SPECIAL AREAS OF LIPID AND PROTEIN COMPOSITION

Lipid rafts are microdomains in biological membranes that contain different proportions of lipids and proteins from the rest of the membrane. They were discovered when portions of membranes were found to be less easily solubilized by detergents. Detergents are chemicals that dissolve membranes by providing their constituents with flotation devices: they bind the hydrophobic domains and coat them with water-soluble material. These detergent-resistant areas of membrane accumulate cholesterol and sphingolipids. Sphingolipids generally contain longer and straighter fatty acyl chains. These attract each other more forcefully than do unsaturated fatty acids, because the straight chains can pack more closely without the kinks in their chains. These aggregate into the raft microdomain. Because these chains are straighter, the membranes are also thicker at the rafts. The plasma membrane is thought to have many such rafts about 70 nm in diameter.

CAVEOLAE AND CLATHRIN-COATED PITS ARE STABILIZED BY INTEGRAL PROTEINS

The surface of cells forms a variety of specializations that curve inwardly, forming an indentation of the membrane. Caveolae are one of these. Caveolae are a subset of lipid rafts, but not all lipid rafts are caveolae. Caveolae are 60−80 nm pits in the membrane that contain some 140−150 oligomeric caveolin molecules. There are three mammalian caveolins: CAV1, CAV2, and CAV3, all have parts that bind to membranes. Their oligomeric structure is stabilized by a family of cytoplasmic proteins called cavins. Caveolar membranes are enriched in both cholesterol and phosphatidylserine (another phospholipid in which the head group is serine instead of ethanolamine, choline, or inositol). Depletion of the cholesterol or mechanical flattening of caveolae causes dissociation of cavin from caveolin (see Figure 2.4.18). Flattening of the caveolae occurs upon stretch of skeletal muscle, cardiac myocytes, endothelial cells, and fibroblasts. It may be that caveolae are involved in the sensing or response to mechanical stretch.

Membranes can also form clathrin-coated pits that are involved in receptor-mediated endocytosis, in which parts of the membrane invaginate and pinch off, forming an interior vesicle with enclosed extracellular material. **Clathrin** consists of three heavy chain subunits (CHC17 or CHC22) and three light chains (CLC) that trimerize to form a triskelion, the unit of clathrin. These units then associate on membranes to form a clathrate (lattice) structure. The lattice structure consists of a number of pentagons and hexagons. The clathrin protein itself has a curvature to it, and this imparts a curvature to the clathrate and stabilizes the budding part of the membrane. The membrane is then pinched off by another protein complex called dynamin (see Figure 2.4.19).
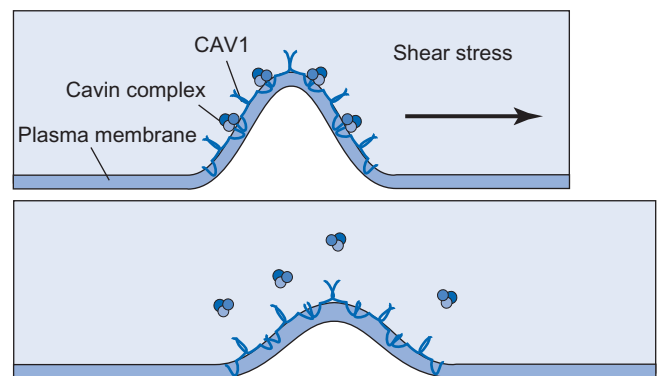

FIGURE 2.4.18 Caveolae response to stretch. Caveolae are indentations or pits in the plasma membrane that are stabilized by a network of integral proteins that include oligomers of caveolin (CAV1). Cytoplasmic proteins called cavins stabilize the caveolin structure. When the membrane is stretched, the caveolae flatten and cavin dissociates from the caveolin. This may be part of how cells sense or respond to stretch.
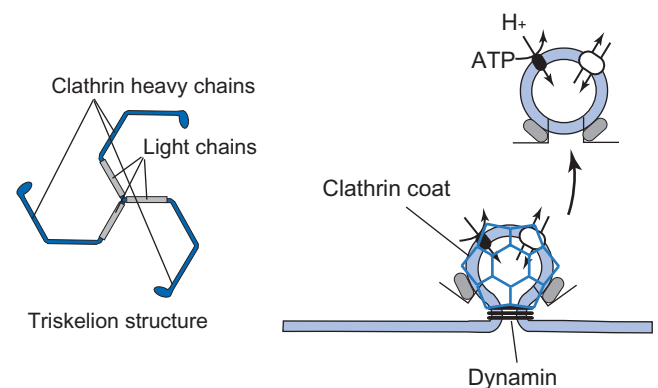

FIGURE 2.4.19 Clathrin-coated pits and endocytosis. Clathrin consists of a trimer of heavy chains each of which binds a light chain. These self-assemble to form a clathrin coat that stabilizes budding membranes. The budding membranes are pinched off through the actions of dynamin.
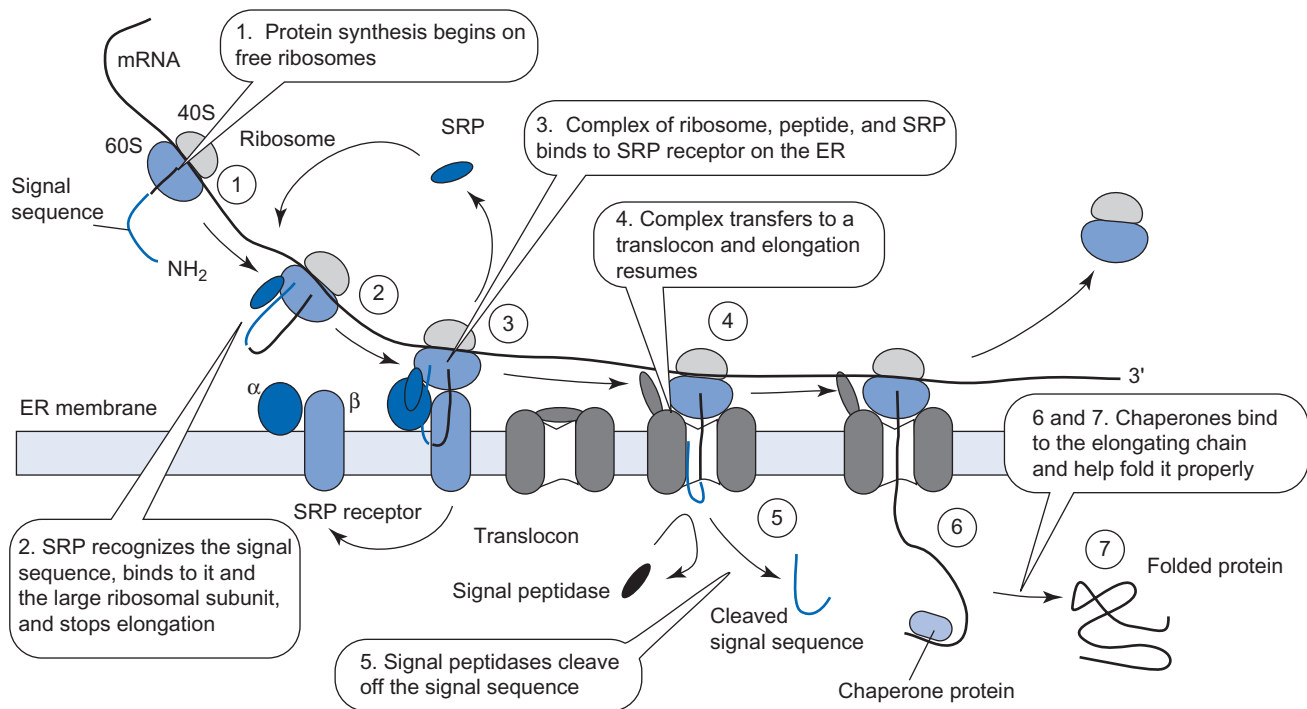
FIGURE 2.4.20 Mechanism of synthesis of secretory proteins into the ER lumen in the pancreas. Synthesis begins on free ribosomes in the cytoplasm (1). The initial N-terminus of the protein contains a signal sequence that is recognized and bound to by cytosolic SRP, signal recognition particle, a nucleoprotein consisting of RNA and a set of six separate proteins (2). Binding of SRP stops elongation. The complex of nascent polypeptide, ribosome, and SRP is bound to the ER by the SRP receptor consisting of a peripheral α-subunit and an integral β-subunit (3). The SRP receptor transfers the ribosome to a translocon, a complex of proteins that spans the ER membrane and provides an aqueous channel for the protein across the membrane (4). The SRP dissociates from the complex in the process. The polypeptide chain grows and translocates across the ER membrane at the same time. The signal sequence is cleaved off (5) by signal peptidases in the ER lumen. The growing polypeptide chain is eventually completed and folds up into its active conformation (7). The folding is assisted by chaperone proteins and enzymes such as Hsp-70, protein disulfide isomerase, and peptidyl prolyl isomerase (6). Source: *Modified from Lodish et al., Molecular Cell Biology, 4th Ed, W.H. Freeman, New York, NY, 2000.*

SECRETED PROTEINS HAVE SPECIAL MECHANISMS FOR GETTING INSIDE THE ENDOPLASMIC RETICULUM

The synthesis of membrane proteins or proteins destined for secretion poses a problem for cells because the ribosomes on which the proteins are made are in the cytosolic compartment but their products are either in the membranes or in the extracellular compartment. This problem is solved by an elaborate mechanism, shown in Figure 2.4.20.

SUMMARY

Lipids readily dissolve in organic solvents such as chloroform while they are sparingly soluble in water. Several classes make up biological lipids including fatty acids, phospholipids, and steroids. They are typically made up of long chains of hydrocarbons or carbon rings. The major constituent of biological membranes are the phospholipids. These consist of a polar head group connected covalently to a nonpolar tail. The polar head group in turn consists of a hydrophilic group like inositol, serine, choline, or ethanolamine linked to a phosphate group, which in turn is esterified to glycerol. All of these are highly water soluble. The other hydroxyls of the glycerol are esterified to two fatty acids, which are highly water insoluble. Thus these phospholipids

spatially separate hydrophilic and hydrophobic parts. When placed in water, the hydrophilic parts associate with the water while the hydrophobic parts associate with other hydrophobic molecules. When placed on top of water, these amphipathic molecules form a lipid monolayer.

The surface tension of the water results from asymmetric forces on the surface from the bulk water phase and the air phase. Because the hydrophilic parts of the lipids attract water molecules on the surface, they reduce the asymmetry in forces. Accordingly, lipids reduce the surface tension. Experimentally, this appears as a surface pressure. Folding such a monolayer back on itself produces a bilayer membrane. This consists of a double layer of molecules in which the hydrophilic domain faces the water phase and the hydrophobic domain faces the interior of the membrane, occupied by other hydrophobic parts of lipid molecules. Other lipid aggregates include the liposome. The liposome is a bilayer that forms a hollow sphere.

As in all molecules, chemical bonds in lipids can stretch, rotate, and flex. These motions along long chains produce motion within the hydrophobic interior of membranes. The hydrophobic chains are relatively well anchored at the polar head group, so there is a gradient of fluidity in the membrane, it being most fluid in the center and less at the periphery. Double bonds in

the hydrocarbon chains make a kink in the chain that disallows close packing of the chains. Thus double bonds promote fluidity within the bilayer. Saturated fats, those that contain no double bonds, make membranes more rigid. Cholesterol is a rigid molecule composed of a plate-like steroid nucleus and a hydrophobic tail. It generally makes membranes more rigid.

Lipids freely move in the plane of the membrane while motion across the membrane, the "flip-flop" reaction, is slow. Cells take advantage of this slow flip-flop to maintain asymmetric distributions of lipids in the two halves of the bilayers. Proteins embed in the membrane and move around, something like icebergs floating in a lipid sea. Thus the membrane is described as a "fluid mosaic." However, thicker microdomains of the membrane contain concentrations of sphingolipids and cholesterol. These microdomains are called lipid rafts.

Proteins can be loosely associated with membranes or tightly bound. The loosely bound proteins are called peripheral or extrinsic proteins and the tightly bound proteins are called integral or intrinsic proteins. Proteins are held in the membrane either by hydrophilic–hydrophobic interactions between their amino acids and the lipid and water phases or by attachment of hydrophobic groups such as myristic acid, palmitic acid, farnesyl, or GPI. Many proteins and lipids have carbohydrate coats.

The synthesis of secreted proteins requires the synthesis of an endoplasmic reticulum (ER) signal sequence that is recognized by a signal recognition particle (SRP), which then binds to a receptor on the ER membrane. This enables transfer of the cytosolic ribosome to the ER membrane and subsequent simultaneous translation and translocation of protein across the ER membrane. Once inside the ER, the synthesized protein undergoes posttranslational modification.

REVIEW QUESTIONS

1. Name the major membranes in the cell. Which membrane accounts for most of the membranes in the cell?
2. What is a saturated fatty acid? What is an unsaturated fatty acid? What effect does unsaturation have on the structure of the fatty acid? What do fatty acids attach to in phospholipids?
3. What is a phospholipid? What are the major types of phospholipids? Which chemical groups on the phospholipid are hydrophilic? Which groups are hydrophobic? What is the significance of spatial separation of hydrophilic and hydrophobic character in lipids?
4. What is the general structure of cardiolipin? What is sphingosine? What is sphingomyelin? What is a ceramide? How do these differ from phosphatidylcholine?
5. What is surface tension? What are its units? What do amphipathic lipids do to the surface tension? Why?
6. Name the various degrees of freedom of lipid motion in a bilayer. Which is the slowest? Which is the fastest?
7. What is meant by the term "fluid mosaic model"?
8. What is an integral protein? What is a peripheral protein? In what ways can proteins be anchored to membranes?
9. What is a liposome? What is a planar lipid bilayer?
10. What are lipid rafts?
11. What is meant by "caveolae"? What is a clathrin-coated pit?
12. How do secreted proteins get inside secretory vesicles? What is meant by "signal sequence"?