

Type O individuals synthesize a cell-surface tetrasaccharide. Type A individuals augment the tetrasaccharide with an extra Gal-NAc residue, whereas type B individuals add an extra Gal residue. AB type individuals produce both A and B pentasaccharides.

As the immune system develops in an infant, the substances that are present and natural to the body (proteins and glycans, for example) are defined as “self,” and antibodies that recognize them are eliminated from the immune system. If an individual is exposed to non-self antigens, as might happen if mismatched blood is transfused into the body, the cells displaying the foreign antigens are recognized as foreign by the immune system. The ensuing immune reaction can cause lysis of the red blood cells, resulting in renal failure, shock, and sometimes death.

B. LIPIDS AND MEMBRANES

Amphiphiles

Amphiphiles are molecules that have parts that are hydrophilic (loving water) and other parts that are hydrophobic (hating water).

Lipids are a class of biologically important molecules that are grouped by the common property that they are soluble in nonpolar organic solvents, but are insoluble in water as individual molecules. The distinguishing feature in the molecular structures of lipids is that they always have a strongly hydrophobic part (usually hydrocarbon chains or rings, with most of the carbons being saturated) and they also have a hydrophilic part (charged, or very polar, but this part of the molecule may be as small as a single hydroxyl group). This dual character leads to the classification of these molecules as **amphiphiles**.

The very different solvation preferences of these two parts of lipid molecules drives them to form clusters in which the hydrophobic parts are brought together (**Figure 3.27**). This excludes water from the hydrophobic parts of the lipids, while

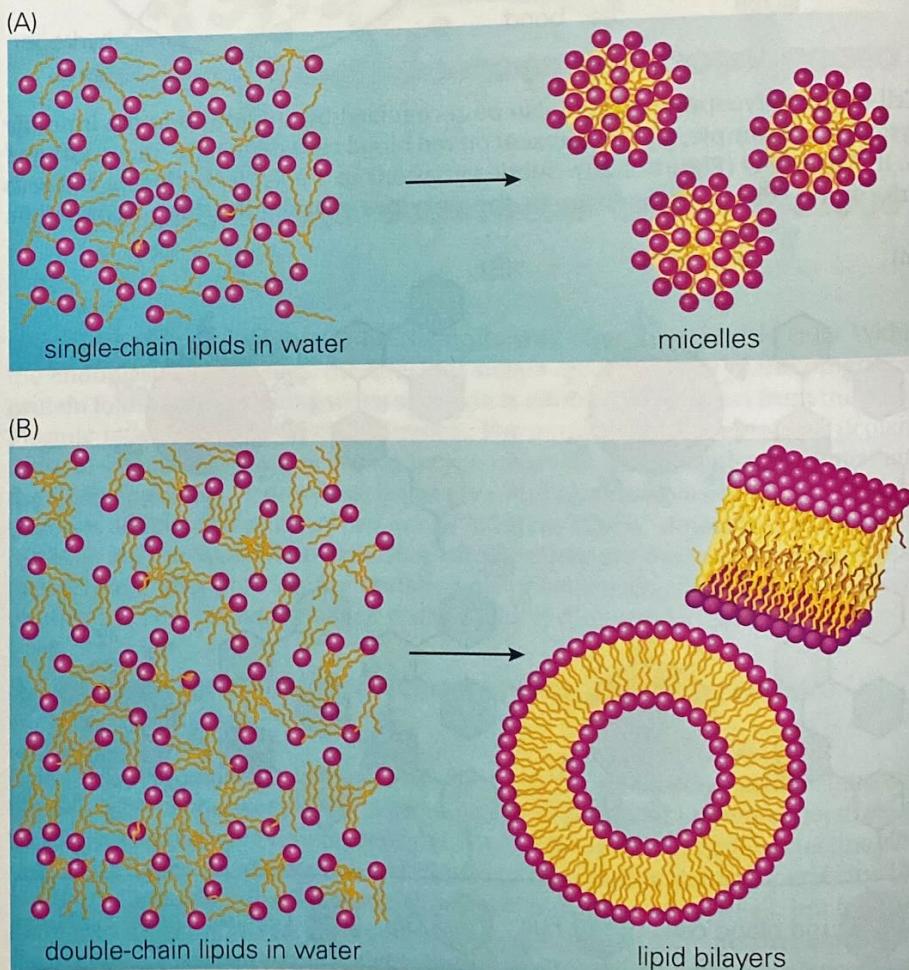


Figure 3.27 The phase separation of lipids in water. Lipids are amphiphilic, so they tend to aggregate (form separate phases from water) when they are dissolved in water. (A) Single-chain lipids aggregate to form micelles. (B) Double-chain lipids aggregate to form planar or spherical bilayer structures.

at the same time allowing the hydrophilic parts to interact well with water. This clustering is a phase separation, just as oil forms a separate phase from water. For many kinds of lipids this phase separation leads to arrays of molecules organized into **lipid bilayers**, called membranes, which are critical to the function of cells. Depending on the specific sizes and shapes of both the hydrophobic and the hydrophilic parts, these molecules can form other structures, including droplets known as **micelles** (see Figure 3.27). Although forming membranes is a critical function of lipids, specific lipids serve many other functions in cells, ranging from energy storage to signaling.

3.13 The most abundant lipids are glycerophospholipids

Glycerophospholipids are the most abundant molecules in biological membranes. As the name suggests, they are built from a glycerol unit, HOCH₂—CHOH—CH₂OH, as shown in Figure 3.28. One of the terminal hydroxyls is linked to a phosphate, which itself carries a polar or charged substituent (an R group). The phosphate group, along with the R group, is hydrophilic and is called the **head group**.

Lipid bilayer

A lipid bilayer is comprised of lipids packed into two parallel layers with the head groups exposed to water and the alkyl chains packed together away from water. Lipid molecules move relatively freely within the plane, but do not easily flip between the two layers.

Head group

A head group is the hydrophilic part of a glycerophospholipid, which is attached to the glycerol. The head group remains in contact with water when the lipids are in bilayers or micelles.

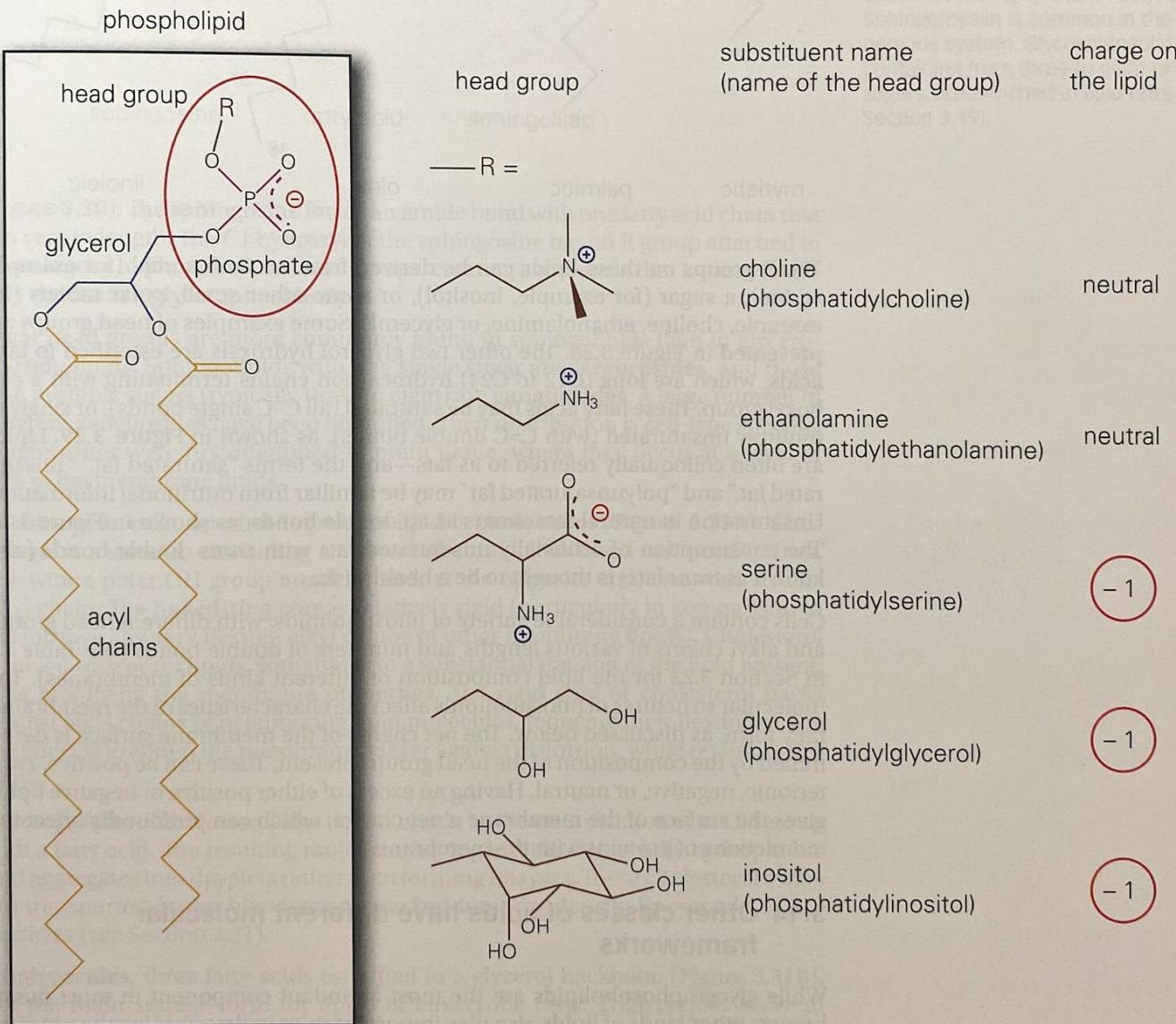
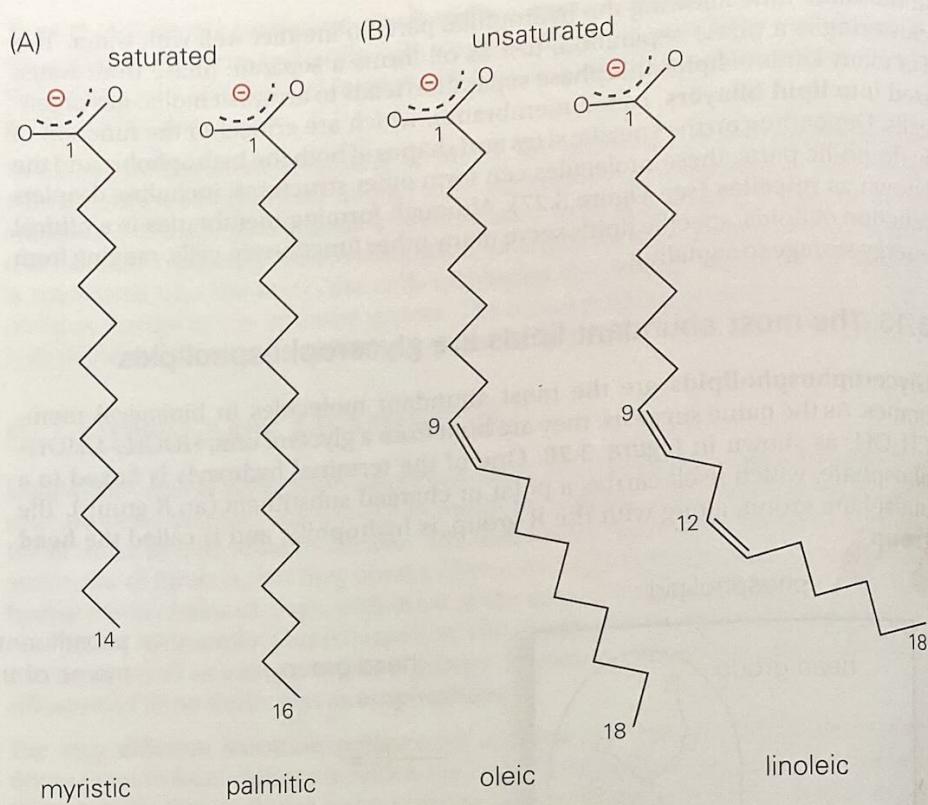


Figure 3.28 Diacylglycerol phospholipid. Phospholipids contain a glycerol backbone linking two acyl chains to head groups, which are phosphoesters. The acyl chains vary in length and in the number of double bonds they contain. The substituents that define some of the different head groups are shown, as are the charges on the lipids with these head groups. Note that because the phosphate group bears a negative charge, lipids with R groups that have a single positive charge are net neutral overall.

Figure 3.29 Structures of several fatty acids. These form glycerol esters to become components of lipids. The length and degree of unsaturation are variable. Lipids in cells contain many different fatty acids.



The R groups on these lipids can be derived from an amino acid (for example, serine), a sugar (for example, inositol), or some other small, polar moiety (for example, choline, ethanolamine, or glycerol). Some examples of head groups are presented in Figure 3.28. The other two glycerol hydroxyls are esterified to fatty acids, which are long (C12 to C24) hydrocarbon chains terminating with a carboxyl group. These fatty acids may be saturated (all C–C single bonds), or singly or multiply unsaturated (with C=C double bonds), as shown in Figure 3.29. Lipids are often colloquially referred to as fats—and the terms “saturated fat,” “unsaturated fat,” and “polyunsaturated fat” may be familiar from nutritional information. Unsaturation in natural fats occurs as *cis* double bonds, as shown in Figure 3.29. The consumption of artificially unsaturated fats with *trans* double bonds (also known as *trans* fats) is thought to be a health risk.

Cells contain a considerable variety of phospholipids, with different head groups and alkyl chains of various lengths and numbers of double bonds (see Table 3.2 in Section 3.22 for the lipid composition of different kinds of membranes). The molecular structures of phospholipids affect the characteristics of the membranes they form, as discussed below. The net charge of the membrane surface is determined by the composition of the head groups present. These can be positive, zwitterionic, negative, or neutral. Having an excess of either positive or negative lipids gives the surface of the membrane a net charge, which can profoundly affect the interactions of proteins with the membrane.

3.14 Other classes of lipids have different molecular frameworks

While glycerophospholipids are the most abundant component in most membranes, other kinds of lipids also play important roles in determining the physical properties of the membranes. One set of examples is provided by the **sphingolipids**, which occur in eukaryotes and have structural similarities to phosphoglycerides. Sphingolipids have one “built-in” hydrophobic chain from the molecule sphingosine that in essence replaces the glycerol unit of glycerophospholipids.

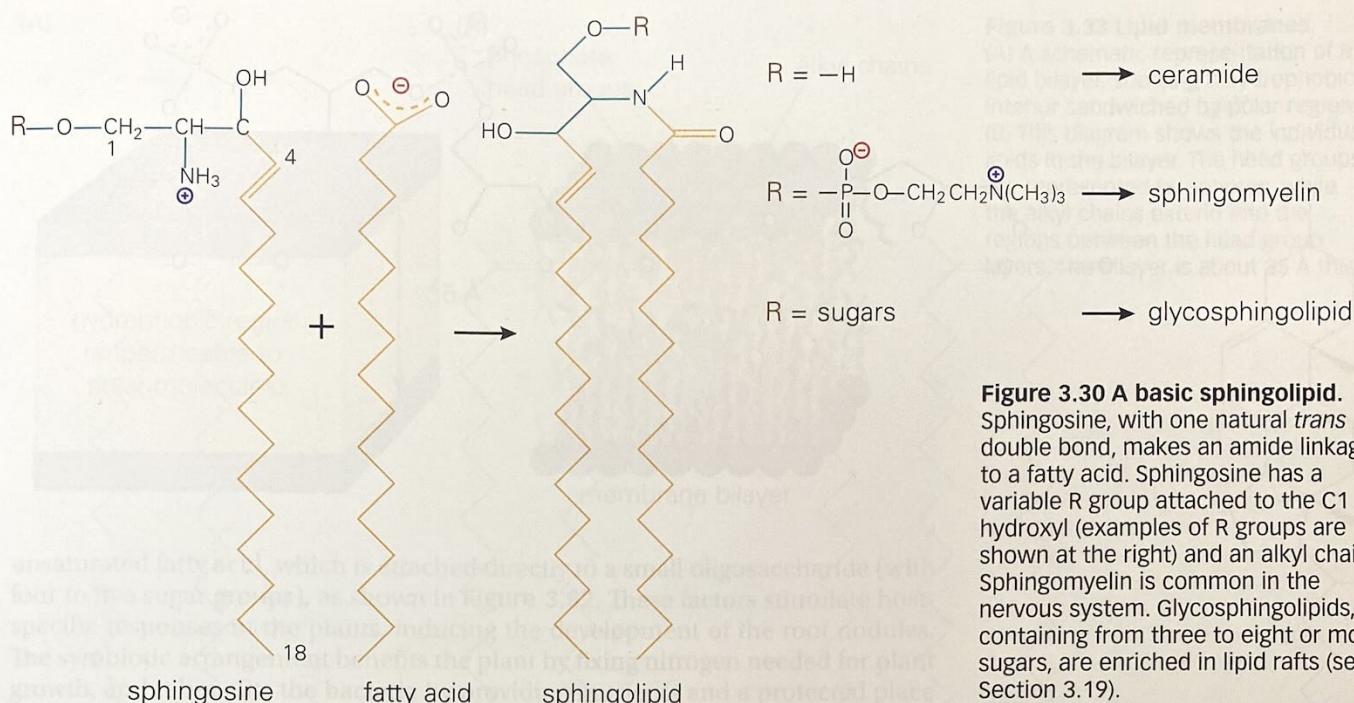


Figure 3.30 A basic sphingolipid. Sphingosine, with one natural *trans* double bond, makes an amide linkage to a fatty acid. Sphingosine has a variable R group attached to the C1 hydroxyl (examples of R groups are shown at the right) and an alkyl chain. Sphingomyelin is common in the nervous system. Glycosphingolipids, containing from three to eight or more sugars, are enriched in lipid rafts (see Section 3.19).

(Figure 3.30). The sphingosine forms an amide bond with one fatty acid chain that can vary in length. The C1 hydroxyl of the sphingosine has an R group attached to it, analogous to phospholipid head groups, creating a molecule that looks remarkably similar to a glycerophospholipid.

Sugar head groups are more commonly found in sphingolipids than in glycerophospholipids. Sphingolipids with one single sugar are **cerebrosides**, and those with multiple sugars (typically three to eight) are **gangliosides**. A large number of different gangliosides have been identified. Their distribution is cell-type specific. Cerebrosides were first identified in brain tissue, where they occur at a level of ~5% of the membrane lipids.

In eukaryotic cells, another important lipid is **cholesterol** (Figure 3.31A). Cholesterol is a **steroid**, containing a conserved core of four fused carbocyclic rings, one with a polar OH group attached and, at the opposite end, one with a short alkyl chain. The linked-ring core is relatively rigid (particularly in comparison to the intrinsically very flexible alkyl chains of other membrane lipids). Cholesterol is mixed with other lipids, sometimes to a substantial fraction of the lipid present, thus modifying the membrane properties. The rigid core of cholesterol packs against alkyl chains of neighboring lipid molecules, reducing their flexibility. The net effect is to stiffen the membrane bilayer against distortion, while retaining fluidity.

A storage form of cholesterol also occurs, made by the esterification of its OH group with a fatty acid. The resulting molecule, having lost the OH, is fully hydrophobic and aggregates into droplets rather than forming bilayers. These cholesterol esters are transported in the bloodstream, packed into low-density lipoprotein (LDL) particles (see Section 3.21).

Triglycerides, three fatty acids esterified to a glycerol backbone (Figure 3.31B), are the main storage form for lipids in eukaryotic cells. Triglycerides serve to transport the dietary fats that are important in metabolism. Triglycerides are a major component of very low density lipoproteins (discussed further in Section 3.21). **Cardiolipin** is a double phospholipid with four alkyl chains, essentially a dimer of a phosphoglycerol lipid (Figure 3.31C). It occurs in mitochondrial membranes.

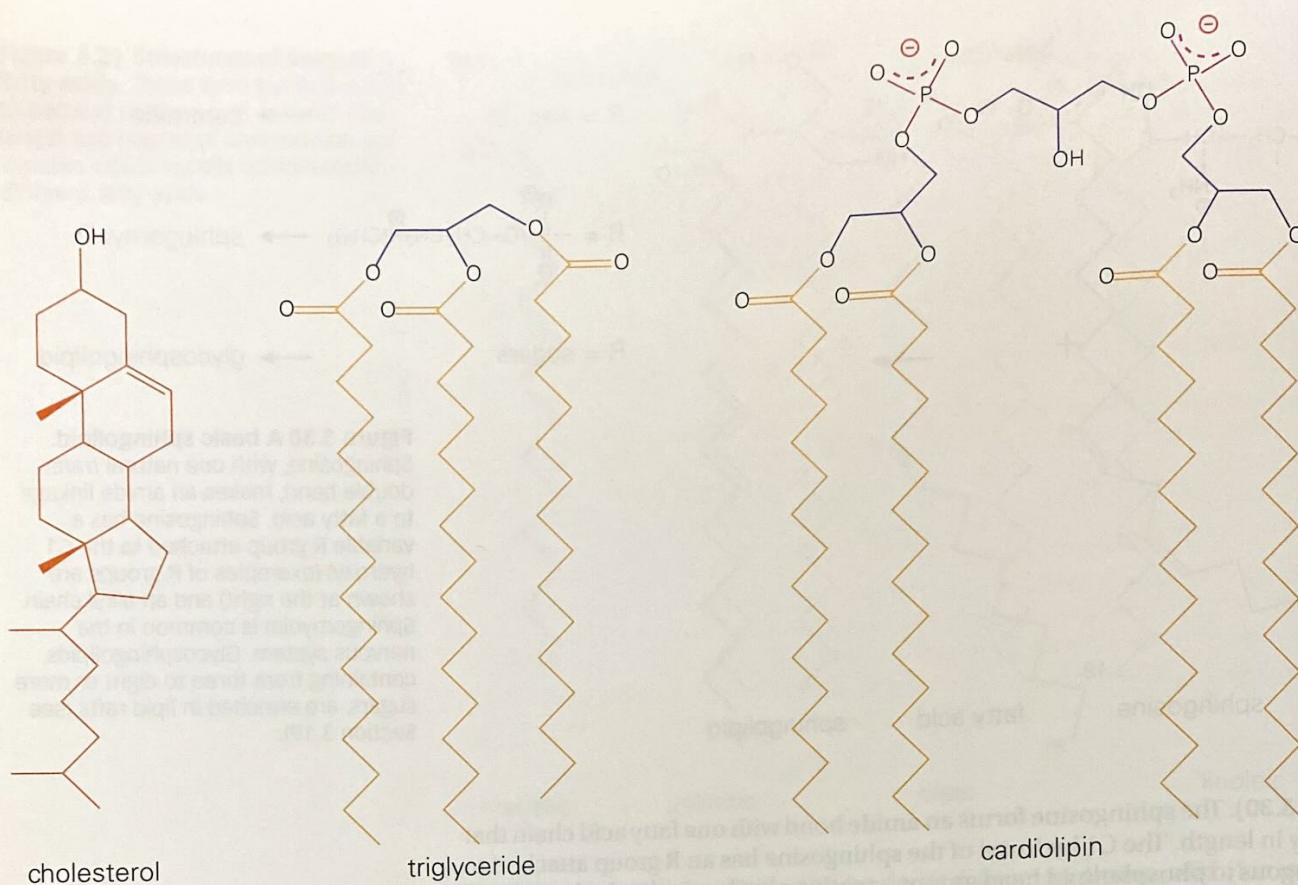


Figure 3.31 Cholesterol, triglyceride, and cardiolipin. Cholesterol is an important component of eukaryotic membranes. Triglycerides are important in fat metabolism. Cardiolipin comprises ~20% of the inner mitochondrial membranes.

Some lipids have biological activity on their own—that is, activity not associated with forming membranes or micelles. One example of bioactive glycolipids are the Nod factors, which are lipo-chito-oligosaccharide lipids that are produced by bacteria that live symbiotically in the root nodules of plants. These lipids have one

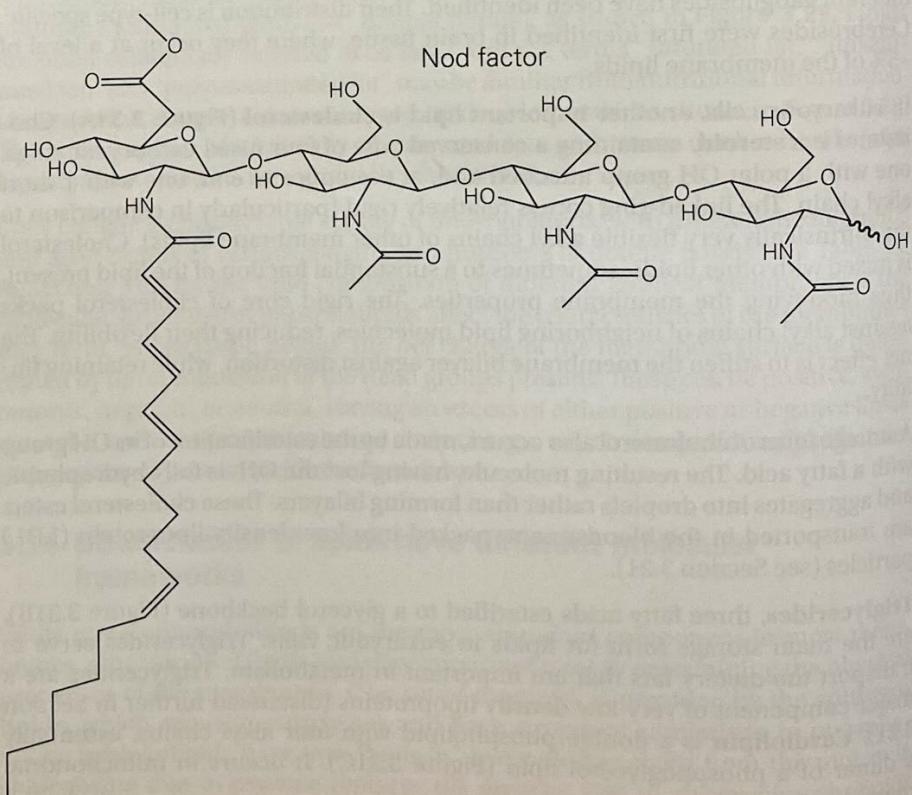
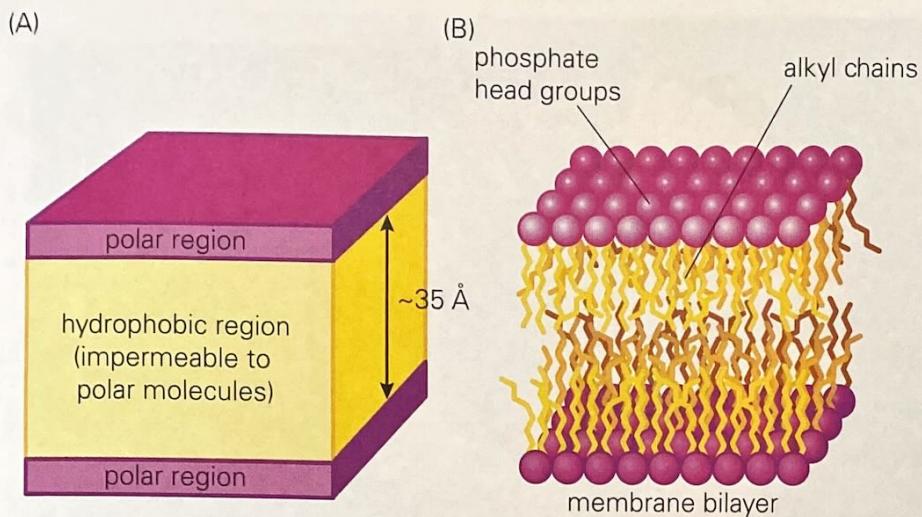


Figure 3.32 A Nod factor. In this case, the fatty acid is 18:4 (18 carbons with four sites of unsaturation), attached to a tetrasaccharide, a derivative of GlcNAc-GalNAc-GalNAc-GalNAc.

**Figure 3.33 Lipid membranes.**

(A) A schematic representation of a lipid bilayer, showing a hydrophobic interior sandwiched by polar regions. (B) This diagram shows the individual lipids in the bilayer. The head groups are represented by spheres, while the alkyl chains extend into the regions between the head group layers. The bilayer is about 35 Å thick.

unsaturated fatty acid, which is attached directly to a small oligosaccharide (with four to five sugar groups), as shown in **Figure 3.32**. These factors stimulate host-specific responses in the plants, inducing the development of the root nodules. The symbiotic arrangement benefits the plant by fixing nitrogen needed for plant growth, and it benefits the bacteria by providing nutrients and a protected place to live.

3.15 Lipids form organized structures spontaneously

The key behavior of lipid molecules is their strong tendency to form separated phases spontaneously when in contact with water. The aggregated lipid molecules are *not* covalently linked (as the amino acids in a protein are), but the same hydrophobic effects that drive protein folding also cause the hydrophobic parts of lipids to cluster together.

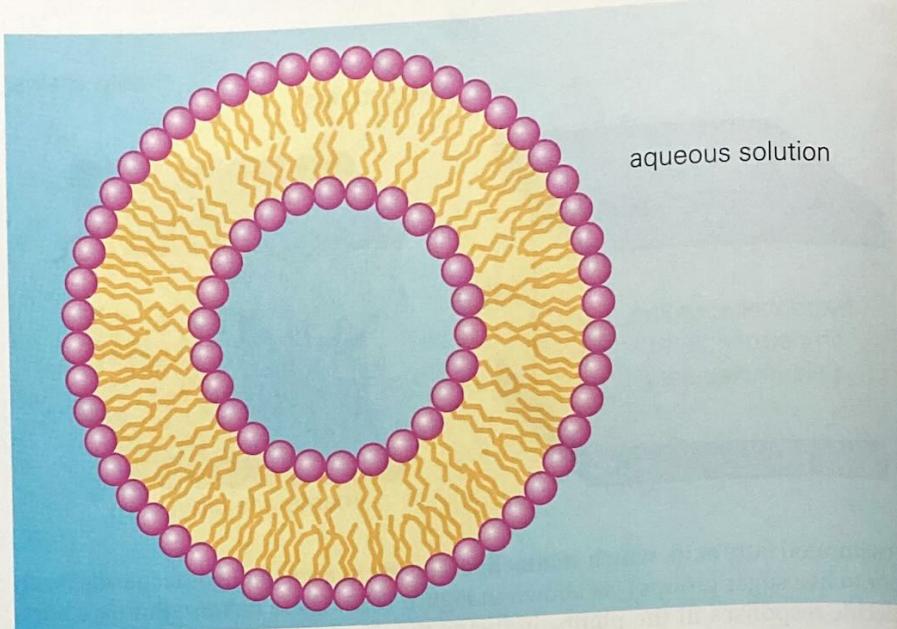
Oil and water separate spontaneously, in a way that minimizes the area of the contact surface. Oil shaken with water forms small droplets, which then gradually merge to give two completely separate layers. For lipids, however, the polar head groups want to remain in water, while the hydrophobic parts want to minimize their interaction with water. Phospholipids, therefore, form organized structures, the most important of which for biology are lipid bilayers, which are illustrated in **Figure 3.33**. In the lipid bilayer, the head groups point out and form a favorable interface with water. The hydrophobic alkyl chains point in and interact with each other laterally, and also pack against the hydrophobic chains of the other layer. Water is excluded completely from the interior region. This structure satisfies, therefore, the interaction preferences for both segments of the lipid molecules.

Bilayers serve as the membranes that partition cells into defined regions. These membranes are effective barriers, and the transport of many molecules (particularly those that are very polar or charged) through the membrane is slow, unless mediated by specific transmembrane proteins. The two layers of the bilayer (often called the **leaflets** of the bilayer) are in contact with different compartments of the cell and may themselves have a rather different composition. For example, glycolipids are not found in the leaflet in contact with the cytoplasm, but are found in the exterior leaflet.

3.16 The shapes of lipid molecules affect the structures they form

Lipids dispersed in water can form different kinds of superstructures that contain membrane bilayers. When placed on a surface that is wetted well by water (indicating that it has hydrophilic groups on its surface), lipids form simple planar

Figure 3.34 A cross section of a vesicle formed by a phospholipid bilayer. Water is both inside and outside the vesicle.



Micelle

A micelle is a spherical array of lipids with head groups on the outside and chains on the inside (see Figures 3.35 and 3.36). Micelles are typically formed by single-chain lipids, or by detergents.

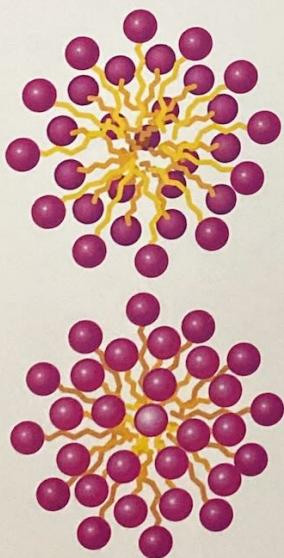


Figure 3.35 A micelle in cross section (top) and from the outside (bottom). Circles represent polar head groups and wavy lines represent alkyl chains.

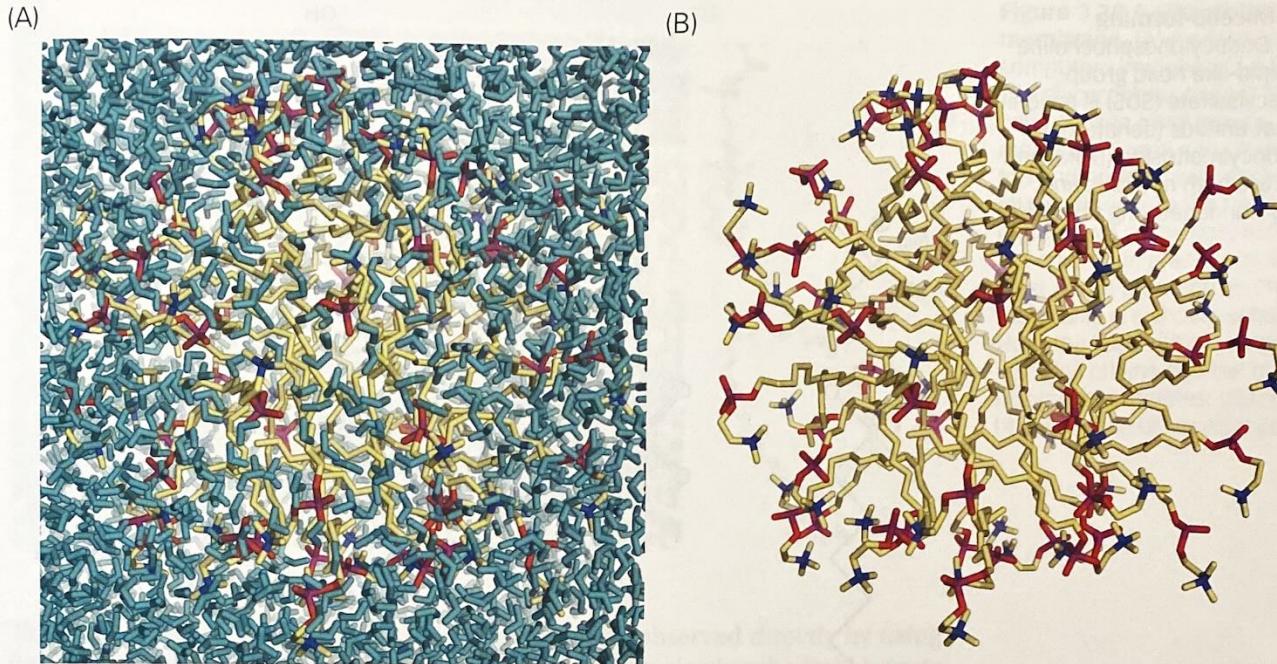
bilayers. If dispersions of lipids in water are sonicated (with an ultrasound source analogous to those used for cleaning jewelry), **lipid vesicles** are formed instead of planar bilayers. These are closed spherical bilayers rather like bubbles, but with water inside and outside rather than air (Figure 3.34). By varying the lipid concentration and conditions, **multilamellar vesicles** can also be formed. These are concentric spheres of bilayers, rather like the layers of an onion.

The curvature of the bilayer (which is highest in small vesicles) makes these structures intrinsically less stable than extended bilayers. However, vesicles are often kinetically trapped, and can therefore be stable for long times. Vesicles can be made with various compositions of lipids and can mimic many aspects of real biological membranes. Lipid vesicles are often used in the laboratory as models for characterizing the physical properties of membranes under well-controlled conditions. Under similar conditions, some lipids will also form long tubes, for which a cross section would also look like that shown in Figure 3.34.

The phase separation of lipids is driven by the hydrophobicity of the alkyl chains and is a common property of strongly amphiphilic molecules. The formation of membrane bilayers is, however, a property of only some lipids. The ability to form a bilayer is determined by the size and shape of both the head groups and the hydrophobic chains. Lipids that have a polar head group but only a single alkyl chain have a higher cross-sectional area in the head group than the hydrophobic section. For these kinds of lipids, packing is optimized by forming a highly curved surface. The radius of curvature is often quite small (the surface is much more curved than for a bilayer vesicle), leading to structures called **micelles**, which are spherical arrays of the molecules with head groups on the outside and chains on the inside, as shown schematically in Figure 3.35.

The type of structure formed by micelles removes the hydrophobic alkyl groups from water, but unlike membrane bilayers, the micelles are small and move quite freely. The structures and the interface with the solvent water are quite disordered, as shown by a computational model of a dodecylphosphocholine micelle in Figure 3.36.

The tendency of amphiphilic substances to form micelles depends on the size of the hydrophobic portion of the molecule. Amphiphilic compounds are soluble as individual molecules up to a specific concentration, called the **critical micelle concentration**. When that concentration is exceeded, micelles begin to form. The



size of the micelles formed (both in dimensions and number of molecules) is a characteristic of the substance, although it can be affected by solution conditions (that is, by ionic strength and the presence of other compounds).

3.17 Detergents are amphiphilic molecules that tend to form micelles rather than bilayers

The molecules that typically form micelles rather than bilayers are generally called **detergents** rather than lipids, even though they are chemically and structurally similar (Figure 3.37). The idea of a detergent is familiar from everyday life as a substance that helps remove oil and dirt from clothing and household objects. Dirt particles that are hydrophobic are coated effectively by detergents, producing an outer layer around them that allows the particles to be solubilized in water and removed in the process of washing and rinsing (Figure 3.38). Basically, the detergents form a micelle around the dirt particle.

Detergent molecules can also solubilize proteins by surrounding exposed hydrophobic residues, creating hydrophilic surfaces. The ability to bind hydrophobic sidechains can be so effective that some detergents can unfold (denature) water-soluble proteins by clustering around hydrophobic residues in the protein. The tendency to do this varies considerably with the structure of the specific detergent molecule.

Detergents have a wide variety of chemical structures. The simplest of these are the alkyl sulfates. Sodium dodecylsulfate, for example, is a denaturing detergent used commonly in biology labs during electrophoresis, as discussed in Chapter 17. Longer chain alkyl sulfates are also used in products such as shampoo. The head groups of some detergents are the same as those in lipids (for example, DPC; see Figure 3.37). While many detergents are charged, some are not, and these tend to be less strongly denaturing towards proteins than ionic ones. Nondenaturing detergents are particularly useful for extracting membrane proteins from lipid bilayers into micelles for protein purification and structural studies. The hydrophobic surfaces of membrane proteins that would normally be in contact with the alkyl chains of lipids are coated instead with the alkyl chains of the detergent. The hydrophilic parts of the detergent, in turn, make favorable contacts with water, solubilizing the complex.

Figure 3.36 Structure of a micelle.

(A) A solvated micelle made from dodecylphosphocholine. Water molecules are shown in cyan. (B) Water surrounding the micelle in (A) has been removed to show the micelle more clearly. As with lipid bilayers, the positions of both the head groups and the alkyl chains are poorly defined, exchanging rapidly among many conformations. (Atomic coordinates: m54.pdb from <http://moose.bio.ucalgary.ca>)

Figure 3.37 Micelle-forming detergents. Dodecylphosphocholine (DPC) has a lipid-like head group. Sodium dodecylsulfate (SDS) is an ionic detergent that unfolds (denatures) proteins. Dodecylmaltoside (DDM) and Triton X-100 are both nonionic and are relatively nondenaturing towards proteins.

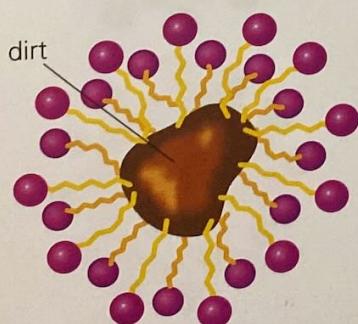
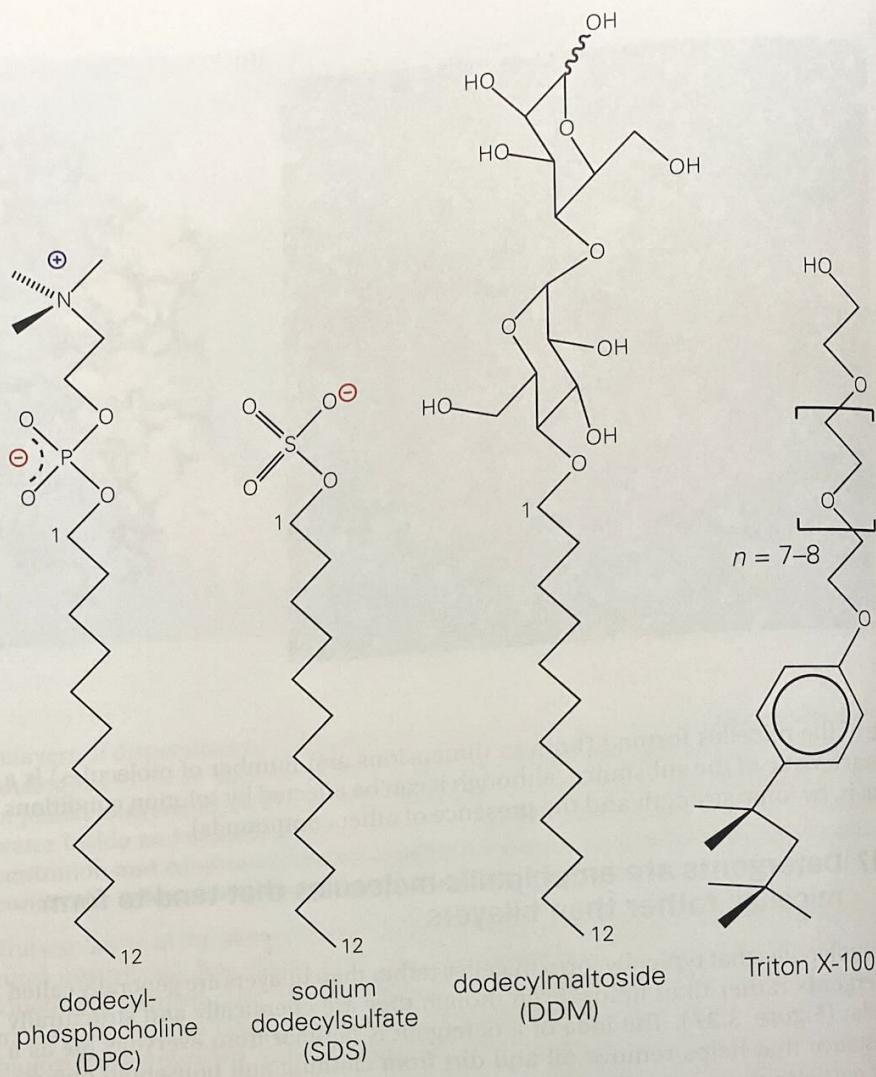


Figure 3.38 A "dirt" particle covered by a detergent micelle. The micelle makes the dirt particle soluble in water.

3.18 Lipids in bilayers move freely in two dimensions

Membrane bilayers are usually drawn as a pair of uniform planes containing the head groups of the lipids (see Figure 3.33). Real membranes are organized into clear layers, but these are not highly ordered at the atomic level. The individual lipid molecules take on many different conformations, and there are significant deviations in the positions of the head groups with respect to the average plane they occupy. The details of water molecules solvating the region around the head groups are also highly variable, with the amount of water present on average decreasing as the region containing the hydrophobic chains is approached.

The hydrophobic center of the membrane is more fluid than the head-group region, with individual alkyl chains undergoing very rapid rotations about the C-C single bonds. The alkyl chains are less densely packed than the head-group region, leaving room for the alkyl chains to exchange rapidly among various conformations. Molecules in a realistic computational membrane model are shown in Figure 3.39, in which many of these features can be seen.

Lipid molecules have no positional order within the planes of the bilayer, although the average spacing between molecules is well defined. The lipid molecules act as a two-dimensional liquid, and individual lipid molecules diffuse relatively rapidly within the bilayer (Figure 3.40).

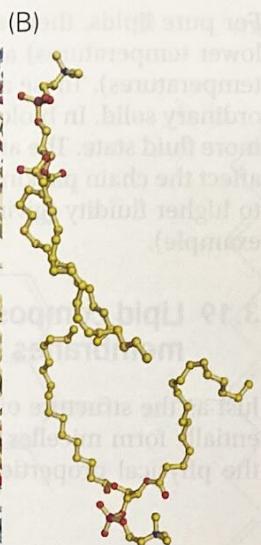
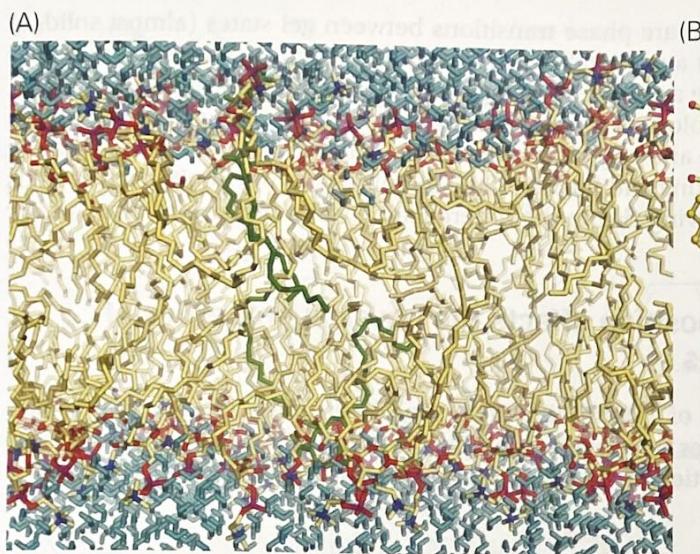


Figure 3.39 A slice through a bilayer membrane. (A) A snapshot from a computer simulation of a lipid bilayer. The glycerophospholipid molecules have choline head groups. Water molecules (cyan) can be seen at the top and bottom, with some waters interspersed into the head group region. Two of the lipid molecules are colored in green (one from each layer of the membrane). These same molecules are shown separately in (B). Note the variability in conformation of the alkyl chains and the head group. (Atomic coordinates: plpc128.pdb from <http://moose.bio.ucalgary.ca>)

The diffusion of molecules in the lipid bilayer can be observed directly by using fluorescent molecules that are attached to the lipids. By viewing the lipid bilayer through a microscope that detects fluorescence, one can observe where the fluorescent molecules are. If the density of fluorescent molecules is high, it can be technically challenging to resolve the positions of individual molecules. Fortunately, there are experimental methods that allow one to deduce how fast the fluorescent molecules are moving.

One such experiment is described in **Figure 3.41**, and it involves a protein that is fluorescent and is attached to a lipid in the membrane. The microscope objective is focused so as to detect fluorescence within a very small region on the membrane (a few microns across). At a certain point in time, a flash of very intense light is transmitted through the microscope and onto the small region under observation. The bright light bleaches the fluorescent molecules, and they lose the ability to absorb and re-emit light. The bleached molecules take an extremely long time to recover their fluorescence, so they become invisible after the flash of light. This leads to a drop in the fluorescence observed through the microscope, as shown in Figure 3.41 (at the 1 second time point). But, because the fluorescent proteins are mobile in the plane of the membrane, within a short time the bleached proteins move out of the field of view of the microscope. At the same time, unbleached proteins move into the field, and the observed fluorescence increases (this takes a few seconds in the experiment shown in Figure 3.41). This kind of experiment, which is called **Fluorescence Recovery After Photobleaching** (FRAP), confirms that lipids in the membrane are diffusing freely.

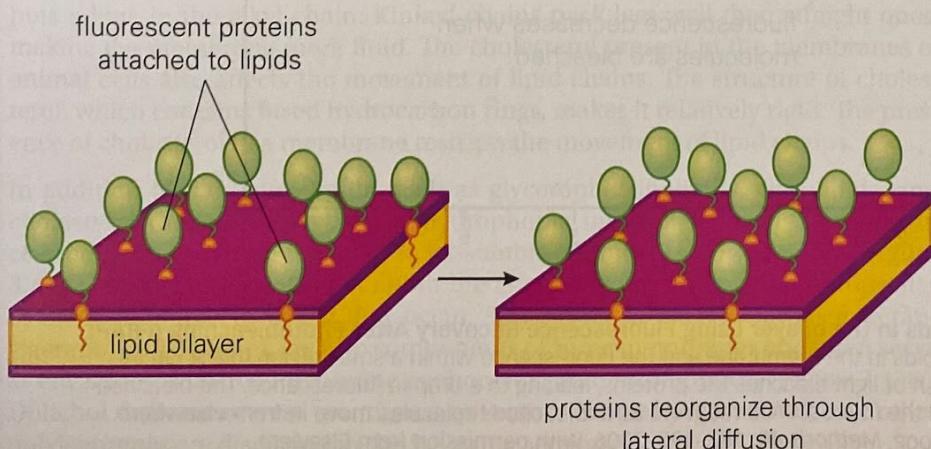


Figure 3.40 Lateral mobility of lipids in a bilayer. The movement of lipids in a bilayer can be monitored by attaching fluorescent molecules or proteins to a few lipids in the membrane. In the example shown here, green fluorescent proteins are attached to lipids. These proteins diffuse rapidly in the plane of the membrane.

For pure lipids, there are phase transitions between gel states (almost solid, at lower temperatures) and liquid-like states (fluid or liquid crystalline, at higher temperatures). These are loosely analogous to the melting phase transition of an ordinary solid. In biological systems, the membranes are probably always in the more fluid state. The amount of unsaturated lipid and the degree of unsaturation affect the chain packing and fluidity of the membrane. More double bonds leads to higher fluidity (giving rise to the difference between butter and olive oil, for example).

3.19 Lipid composition affects the physical properties of membranes

Just as the structure of amphiphilic molecules determines whether they preferentially form micelles or bilayers, the lipids present in a membrane determine the physical properties of that membrane (its thickness, fluidity, and surface

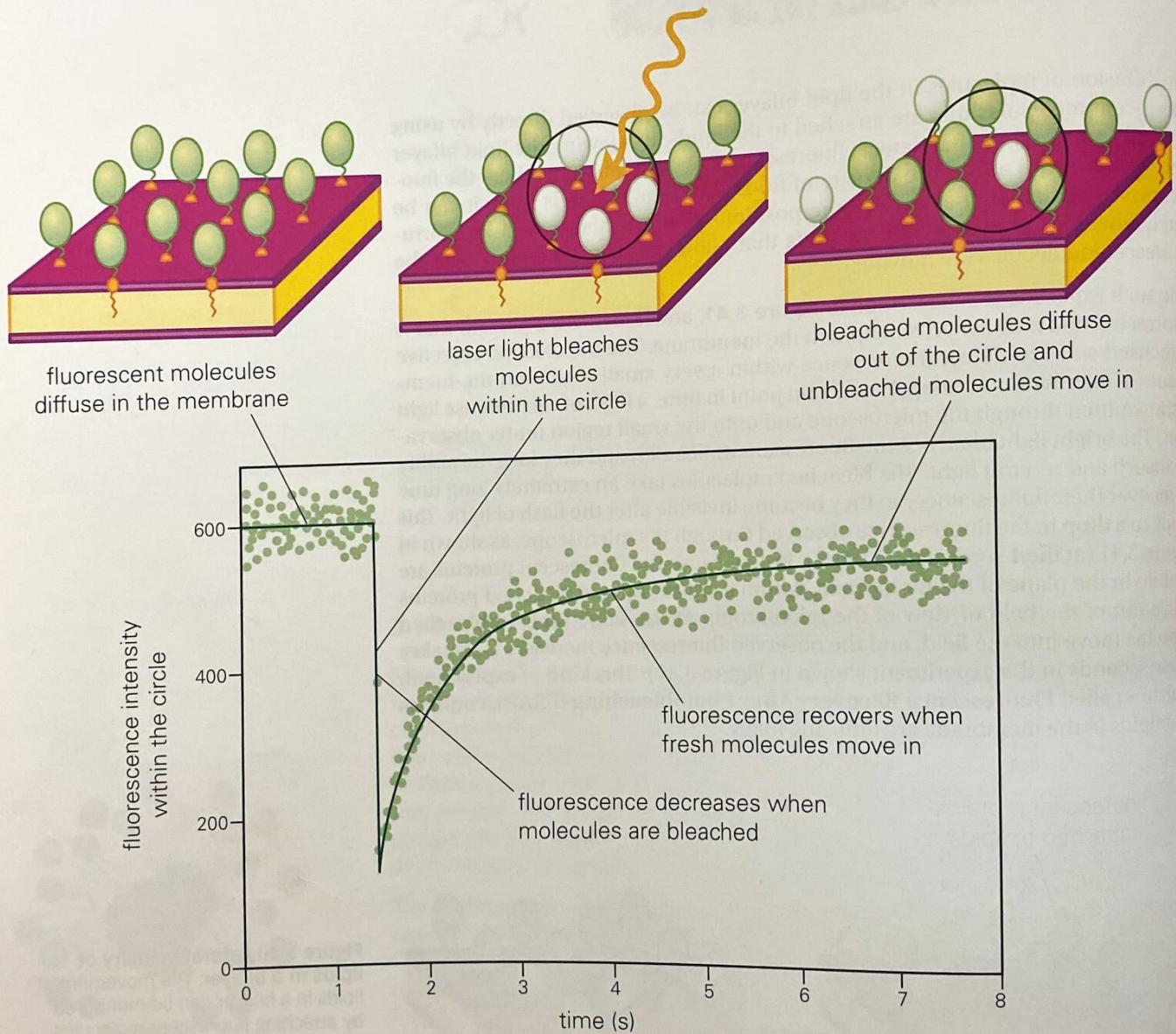
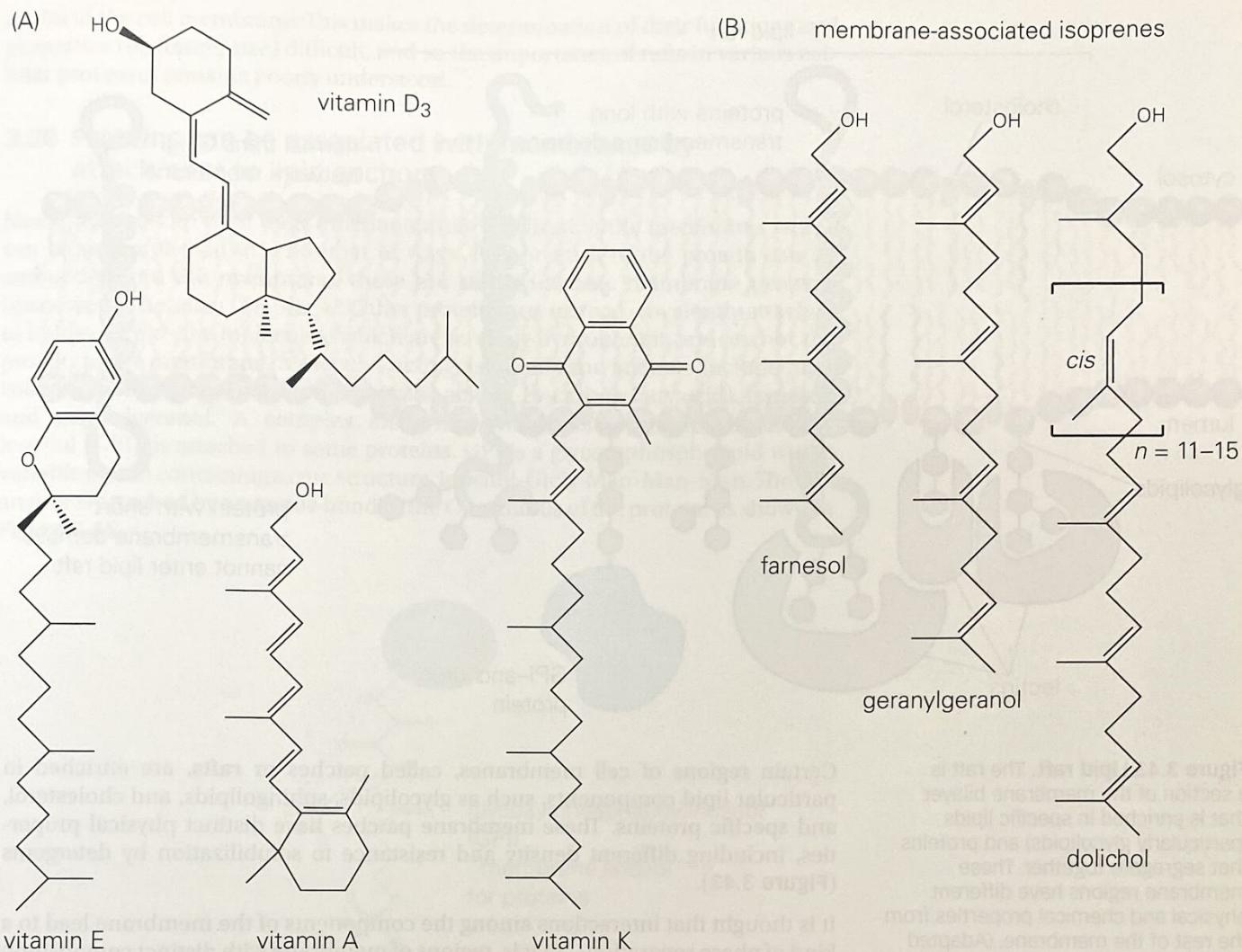


Figure 3.41 Observing the mobility of lipids in the bilayer using Fluorescence Recovery After Photobleaching (FRAP). A green fluorescent protein is attached to lipids in the membrane and the fluorescence within a small region (black circle) is observed using a microscope. A bright flash of light bleaches the proteins, leading to a drop in fluorescence. The bleached molecules move out of the field of view, and the fluorescence recovers as unbleached molecules move in from elsewhere. (Adapted from Y.I. Henis, B. Rotblat, and Y. Kloog, *Methods* 40: 183–190, 2006. With permission from Elsevier.)



charge, for example). Real membranes in cells are complex mixtures of lipids and, by varying the composition of the lipids, the properties of the membrane can be adjusted by the cell.

Many components of membranes have to move (rotate and translate within the membrane) in order to function. As noted in Section 3.13, many of the fatty acid chains within lipids have carbon–carbon double bonds (that is, they are unsaturated). These double bonds are always *cis* in natural lipids, so each double bond puts a kink in the alkyl chain. Kinked chains pack less well than straight ones, making the membrane more fluid. The cholesterol present in the membranes of animal cells also affects the movement of lipid chains. The structure of cholesterol, which contains fused hydrocarbon rings, makes it relatively rigid. The presence of cholesterol in a membrane restricts the movement of lipid chains.

In addition to the major lipids, such as glycerophospholipids, glycolipids, and cholesterol (in eukaryotes), other hydrophobic molecules are also present in cell membranes. These include the fat-soluble vitamins (A, D₃, E, and K; Figure 3.42A). Such compounds affect both the chemistry and the physical properties of the membranes in which they occur. Polyisoprenes, such as farnesol, geranylgeranol and dolichol, are also components of some membranes and often serve as covalently attached membrane anchors for proteins or glycans (Figure 3.42B). Dolichol derivatives were mentioned in Section 3.9 (see Figure 3.19), and other polyisoprenes are discussed in the following section.

Figure 3.42 Fat-soluble vitamins and polyisoprenes. (A) The structures of vitamins D₃, E, A, and K. These are primarily membrane associated, as they have amphiphilic characteristics analogous to lipids (compare vitamin D₃ with cholesterol, Figure 3.31). (B) Isoprene corresponds to a branched five-carbon building block with a double bond. Farnesol (three isoprene units) and geranylgeranol (four isoprene units) occur attached to proteins. The much longer dolichol is used as a carrier during carbohydrate synthesis (see Figure 3.19).

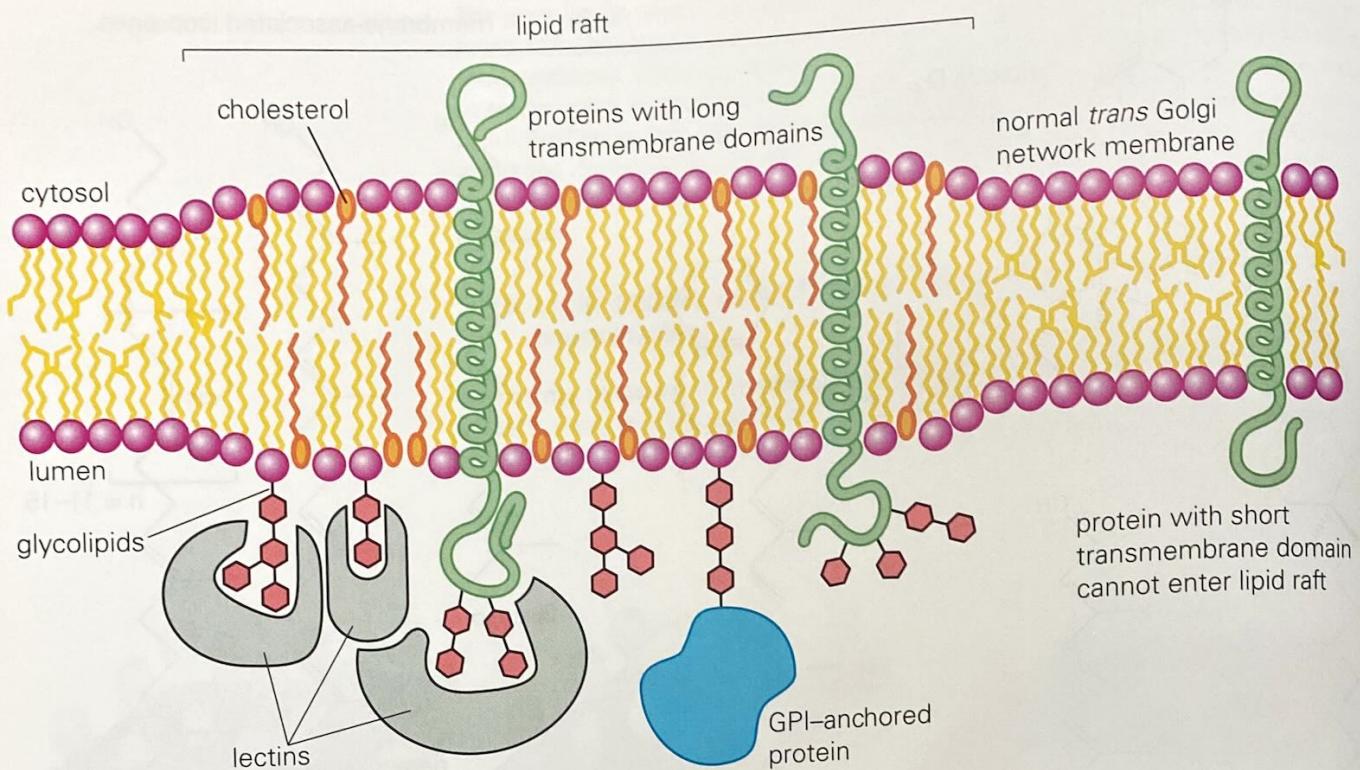
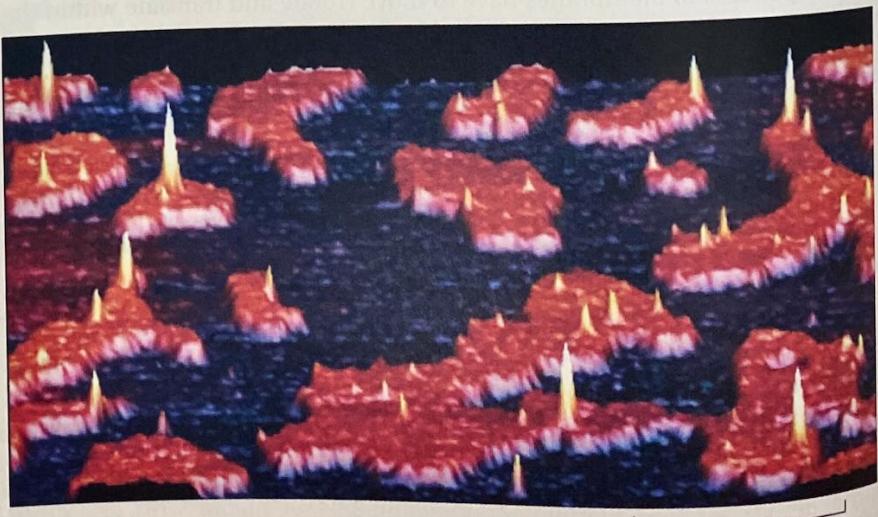


Figure 3.43 Lipid raft. The raft is a section of the membrane bilayer that is enriched in specific lipids (particularly glycolipids) and proteins that segregate together. These membrane regions have different physical and chemical properties from the rest of the membrane. (Adapted from B. Alberts et al., Molecular Biology of the Cell, 4th ed. New York: Garland Science, 2002.)

Certain regions of cell membranes, called patches or **rafts**, are enriched in particular lipid components, such as glycolipids, sphingolipids, and cholesterol, and specific proteins. These membrane patches have distinct physical properties, including different density and resistance to solubilization by detergents (Figure 3.43).

It is thought that interactions among the components of the membrane lead to a kind of phase separation—that is, regions of membrane with distinct composition that separate spontaneously from other regions. This separation allows optimization of the most favorable interactions among particular combinations of molecules. The distinct physical character of these regions is apparent when an image is made using atomic force microscopy, which detects differences in the position of the membrane surface, reflecting its thickness, as shown in Figure 3.44. Nevertheless, these rafts, or patches of distinct composition, are difficult to characterize

Figure 3.44 An atomic force microscope image shows “rafts” of distinct lipid composition. These regions of thicker membrane protrude above the surface of the surrounding regions. (Courtesy of Robert M. Henderson and J. Michael Edwards; data from D.E. Saslowky et al., *J. Biol. Chem.* 277: 26966–26970, 2002.)



in situ in the cell membrane. This makes the determination of their functions and properties (including size) difficult, and so the importance of rafts in various cellular processes remains poorly understood.

3.20 Proteins can be associated with membranes by attachment to lipid anchors

Many proteins carry out their functions while localized to the membrane, which can be accomplished in a number of ways. Part or most of the protein can be embedded into the membrane; these are called intrinsic membrane proteins (discussed in detail in Chapter 4). Other proteins are, instead, covalently attached to lipids or lipid-like molecules, which are strongly hydrophobic and anchor the protein to the membrane. Molecules acting as membrane anchors include myristic acid (a 14-carbon fatty acid), palmitic acid (a 16-carbon fatty acid), farnesol, and geranylgeranol. A complex anchoring molecule, glycosylphosphatidyl-inositol (GPI), is attached to some proteins. GPI is a glycerophospholipid with a variable glycan containing a core structure: inositol-GlcN-Man-Man-Man. The GPI anchor is attached by an amide bond to the C-terminus of the protein, as shown in Figure 3.45.

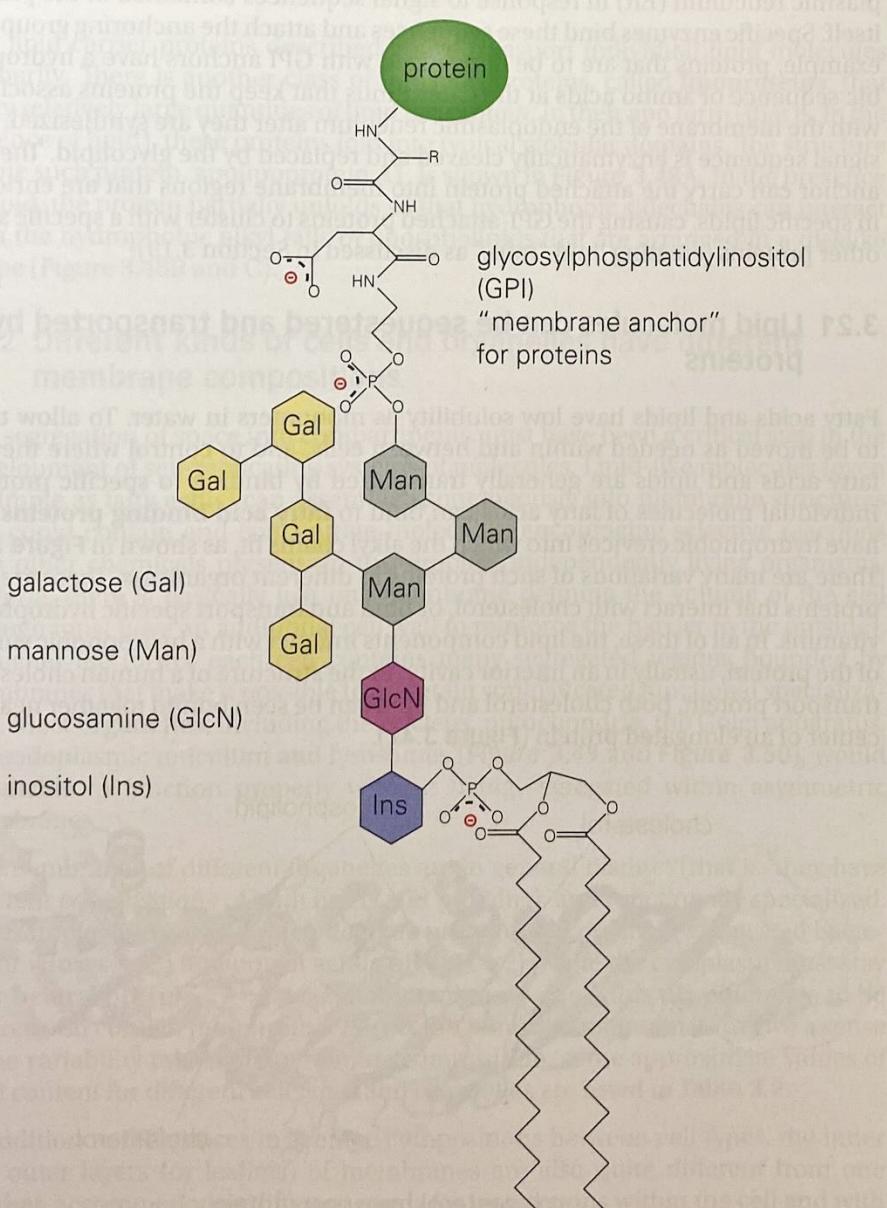
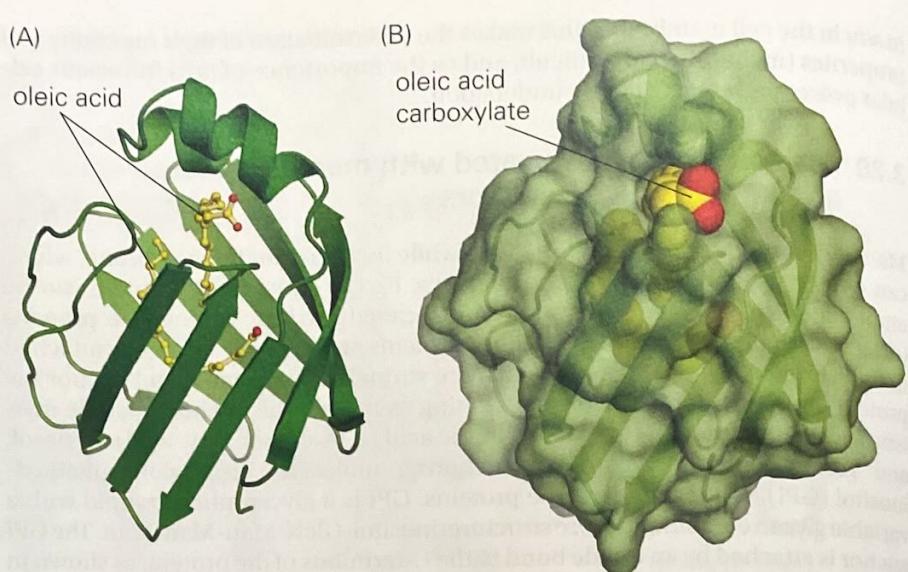


Figure 3.45 A glycosylphosphatidyl-inositol (GPI) membrane anchor.

The C-terminus of the protein is attached via an amide bond to a phosphoethanolamine on the last sugar of the core glycan. The modifications of the mannose nearest the lipid (with four Gal residues in the example shown here) and of the last mannose (no modifications are present in this example) are variable

Figure 3.46 Structure of a fatty acid binding protein. (A) Backbone structure and (B) molecular surface of the protein. The protein forms a hydrophobic cavity that binds two molecules of oleic acid (yellow), sequestering the alkyl chains from water. (PDB code: 1LFO.)



Membrane anchors are attached to proteins post-translationally in the endoplasmic reticulum (ER) in response to signal sequences contained in the protein itself. Specific enzymes bind these sequences and attach the anchoring group. For example, proteins that are to be derivatized with GPI anchors have a hydrophobic sequence of amino acids at the C-terminus that keep the proteins associated with the membrane of the endoplasmic reticulum after they are synthesized. This signal sequence is enzymatically cleaved and replaced by the glycolipid. The GPI anchor can carry the attached protein into membrane regions that are enriched in specific lipids, causing the GPI-attached proteins to cluster with a specific set of other proteins (creating lipid “rafts,” as discussed in Section 3.19).

3.21 Lipid molecules can be sequestered and transported by proteins

Fatty acids and lipids have low solubility as monomers in water. To allow them to be moved as needed within and between cells, and to control where they go, fatty acids and lipids are generally transported by binding to specific proteins. Individual molecules of fatty acids can bind to **fatty acid binding proteins** that have hydrophobic crevices into which the alkyl chains fit, as shown in Figure 3.46. There are many variations of such proteins in different organisms. There are also proteins that interact with cholesterol, or bind and transport specific hydrophobic vitamins. In all of these, the lipid components interact with a hydrophobic surface of the protein, usually in an interior cavity. In the structure of a human cholesterol transport protein, both cholesterol and lipid can be seen bound together near the center of an elongated protein (Figure 3.47).

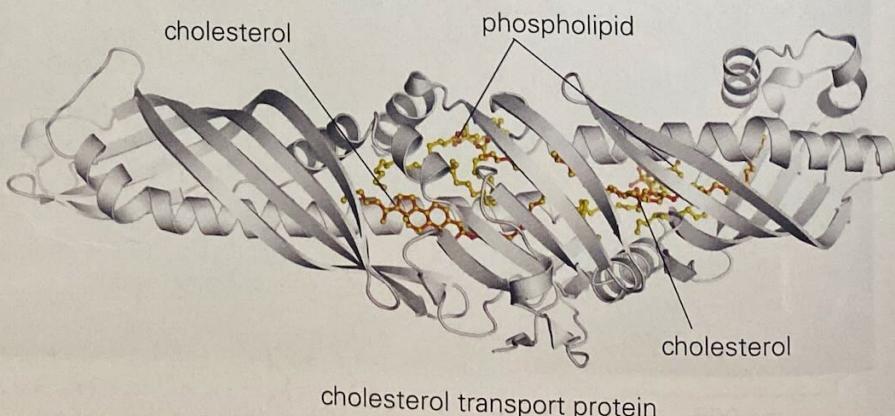
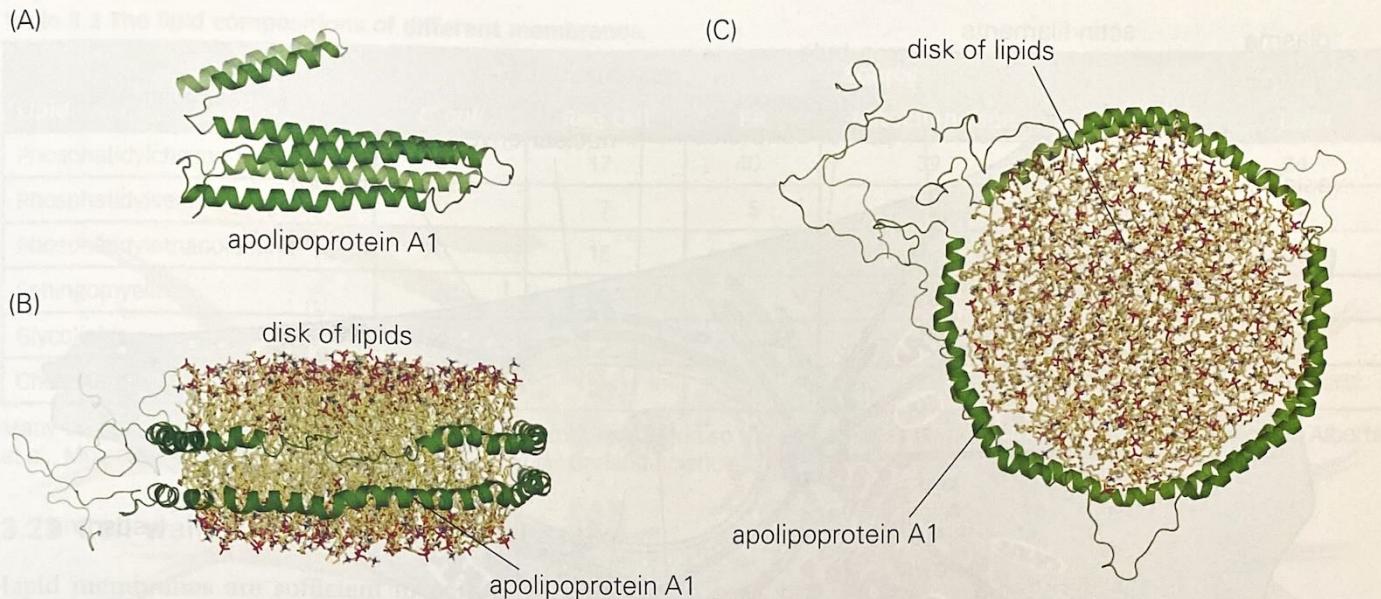


Figure 3.47 A cholesterol transport protein. Two molecules of cholesterol (orange) and two molecules of phospholipid (yellow) are bound to the protein. (PDB code: 2OBD.)



The lipid carrier proteins described above transport individual lipid molecules primarily. There is another class of transport proteins, called **lipoproteins**, that carry relatively large quantities of lipid per protein. In their apo form (that is, in the absence of lipid), these proteins fold into typical globular domains. The structure of one such protein, apolipoprotein A1, is shown in Figure 3.48A. In the presence of lipid, the protein partially unfolds so that hydrophobic sidechains can interact with the hydrophobic alkyl tails of phospholipids that are arranged in a disklike shape (Figure 3.48B and C).

3.22 Different kinds of cells and organelles have different membrane compositions

The segregation of space into compartments must have been a critical step in the development of self-replicating systems of molecules. Lipid-like molecules (even as simple as fatty acids) can assemble spontaneously into membrane structures that could contain and concentrate molecules, encouraging selective reactions with other chemicals present. In the simplest independently living organisms, bacteria, there is basically just one membrane defining the volume of the cell (though most have an additional cell wall to reinforce the barrier to the outside). In higher life forms, each cell contains many segregated regions bounded by membranes that make it possible to maintain sophisticated functional specialization. Most **organelles**, including the nucleus, mitochondria, the Golgi apparatus, the endoplasmic reticulum and lysosomes (Figure 3.49 and Figure 3.50), would be unable to function properly without being segregated within asymmetric membranes.

The membranes of different organelles are in general distinct (that is, they have different compositions of both lipids and proteins), and functionally specialized. For example, lysosomes (which degrade proteins, old organelles, ingested bacteria, or viruses, etc.) function at acidic pH ($\text{pH} < 7$), while the cytoplasm must stay near neutral pH ($\text{pH} = 7$). Lysosomal membranes allow this pH difference to be created and contain proteins that are proton pumps to maintain it. To give a sense of the variability in overall membrane composition, some approximate values of lipid content for different cell types and organelles are listed in Table 3.2.

In addition to differences in average compositions between cell types, the inner and outer layers (or leaflets) of membranes are also quite different from one another, accommodating different needs for interactions within the cell and with

Figure 3.48 Apolipoprotein A1.
 (A) The structure of the protein in the absence of lipid. (B, C) Two views of a model for the structure in the presence of lipid. The helices move apart and expose a surface that interacts with the edge of a small disk of lipid. There are two protein molecules per lipid disk. (See Protein Model Database mi.caspur.it/ PMDB entry PM0074956; Z. Wu and S.L. Hazen, *Nat. Struct. Mol. Biol.* 14: 861–868, 2007.)

Organelle

An organelle is a subcellular structure or chamber, often bounded by a membrane, that is specialized for particular functions. Examples include the nucleus, the endoplasmic reticulum, the mitochondria, the lysosomes, and the Golgi apparatus.

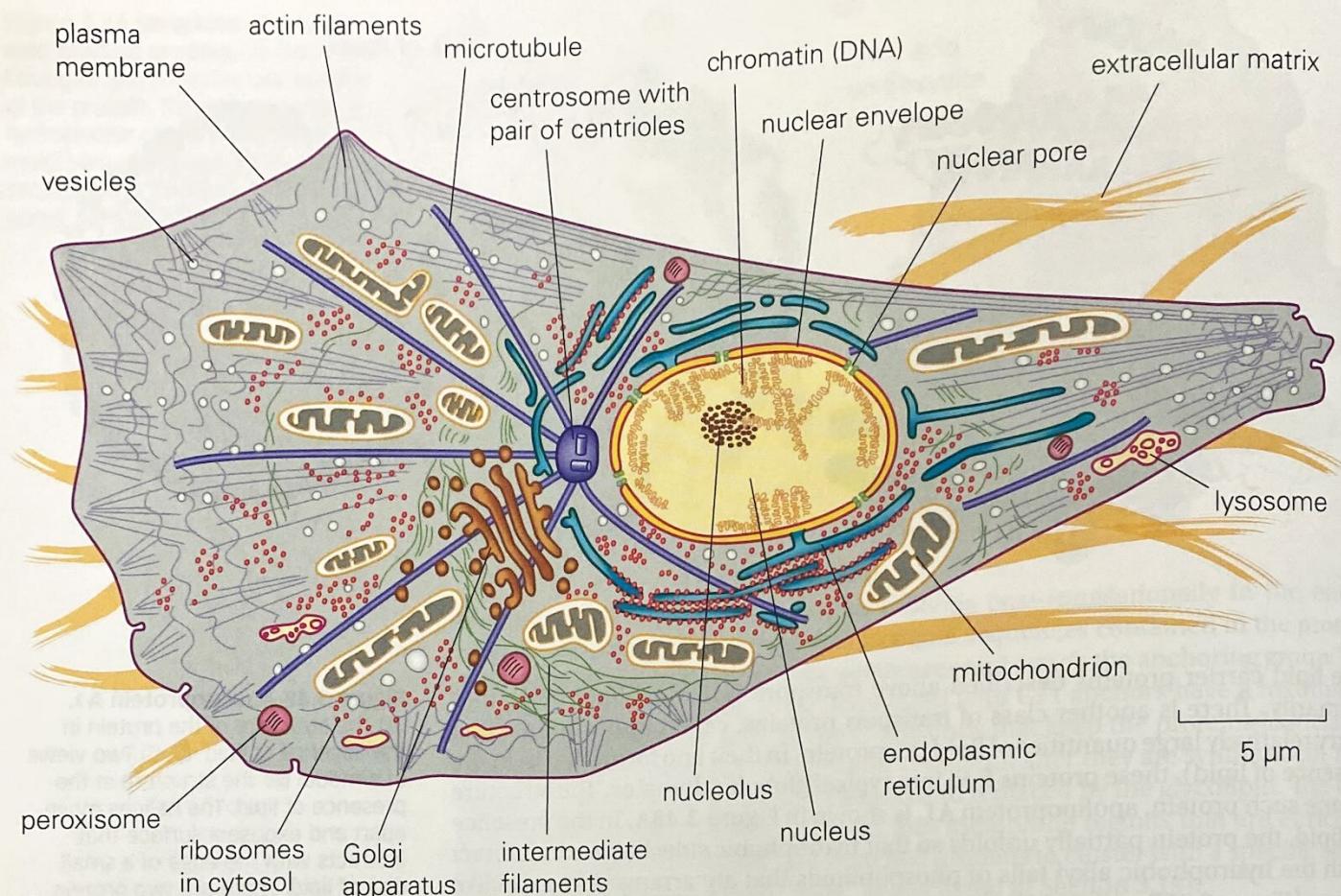


Figure 3.49 A eukaryotic cell. Some of the important structures and organelles are shown. Most organelles are separated from the rest of the cell by membranes, which allows for distinct compositions and, thus, distinct functions in each organelle. (Adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008.)

other cells on the outside. This asymmetry is apparent in Figure 3.43, in which the glycolipids are all on one side of the membrane. As noted above, glycolipids tend to be particularly important for cell-cell interactions, and so glycolipids are found in the outer leaflet of the cell membrane.

We noted in Section 3.18 that membranes are fairly fluid, allowing rapid translation of individual lipid molecules within one leaflet of the membrane. However, the transfer of a lipid molecule from one leaflet to another requires moving the polar (and often charged) head group across the hydrophobic region, a process that is energetically unfavorable, and hence is very slow. Enzymes called **flippases** recognize and “flip” specific lipids between the inner and outer leaflets, a process needed for generating and maintaining membrane asymmetry.

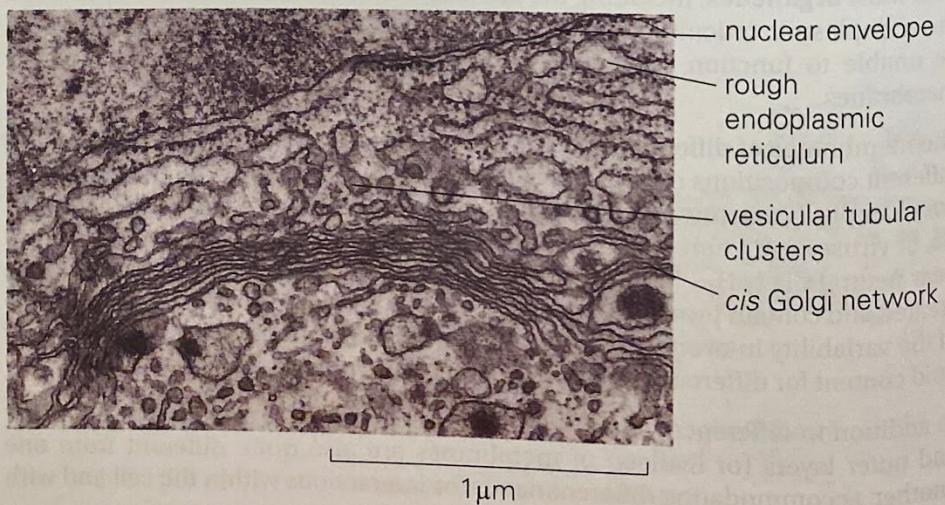


Figure 3.50 Organelle membranes. This electron micrograph of part of a cell includes part of the nucleus (upper left), part of the endoplasmic reticulum with associated ribosomes (dark dots), and part of the Golgi apparatus. Each dark line surrounding a region is a bilayer membrane. (Courtesy of Brij Gupta.)

Table 3.2 The lipid compositions of different membranes.

Lipid	Source					
	E. coli	Red cell	ER	Mitochondrion	Myelin	Liver
Phosphatidylcholine	0	17	40	39	10	24
Phosphatidylserine	0	7	5	2	9	4
Phosphatidylethanolamine	70	18	17	25	15	7
Sphingomyelin	0	18	5	0	8	19
Glycolipids	0	3	~0	~0	28	7
Cholesterol	0	23	6	3	22	17

Many of these membranes have additional minor components, and so the percentages do not add to 100%. (Adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008.)

3.23 Cell walls are reinforced membranes

Lipid membranes are sufficient to partition spaces within cells, but the lipid membranes are relatively fragile because they are only held together by noncovalent interactions. Cells that are exposed to the surroundings often need additional reinforcement to maintain their integrity. This takes the form of a **cell wall** in bacteria and plants.

All bacterial cells are surrounded by a membrane that is the primary barrier to the outside world. A majority also have a cell wall made from a peptidoglycan. This material has a linear polysaccharide [$\beta(1 \rightarrow 4)$ -linked GlcNAc and MurNAc], with peptides containing three to five amino acids attached to the MurNAc residues. The peptides are unusual in that they have D-isomer versions of alanine and glutamate in addition to L-alanine, and glycine in some. The D amino acids reduce susceptibility to protease enzymes, which digest normal proteins but are ineffective against peptides containing D amino acids.

Cell walls in bacteria fall into two classes. Some bacteria have thick peptidoglycan layers and others have thinner peptidoglycan layers associated with a second lipid membrane. In either case, the peptidoglycan matrix is relatively porous, so that small molecules can get to the membrane for transport into the cell, but it is also a dense and strong enough web around the membrane to resist mechanical stress (Figure 3.51).

Cell wall

A cell wall is a polymeric outer layer just outside the cell membrane of some cells (especially in plants, bacteria, fungi, algae, and archaea). The cell wall generally has a high polysaccharide content.

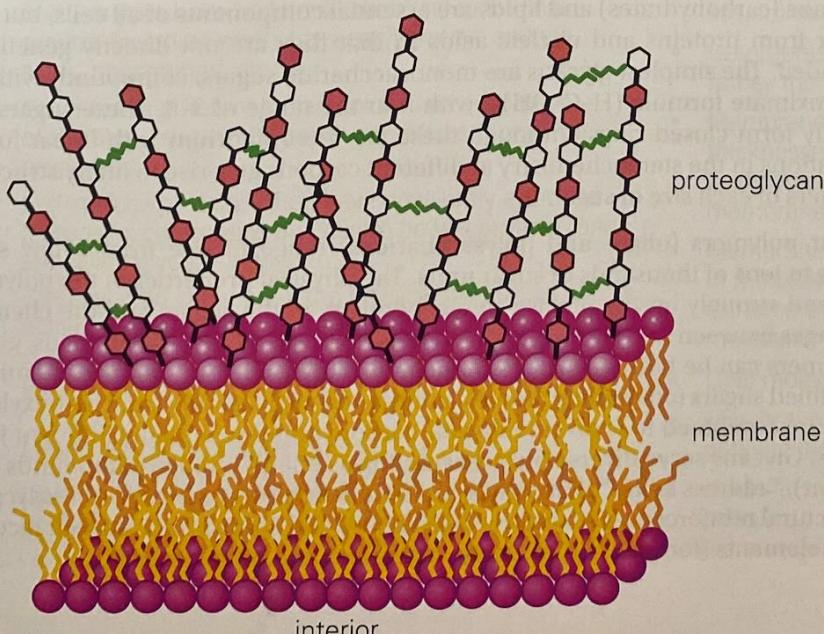
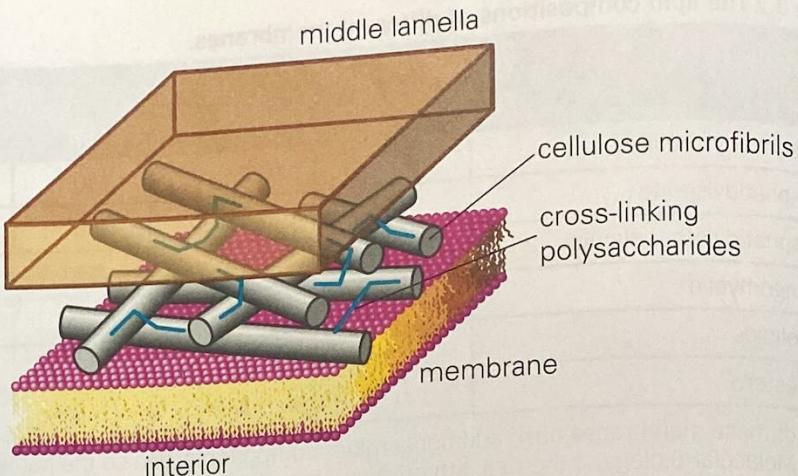


Figure 3.51 The proteoglycan (also called peptidoglycan) cell wall in bacteria. Polysaccharide chains extend from the membrane and are cross-linked by short peptides (green).

Figure 3.52 A plant cell wall.

Outside the cell membrane are cellulose microfibrils with cross-linking polysaccharides. Pectin is interspersed throughout, filling and strengthening the wall. The middle lamella is rich in pectin and helps hold neighboring cells together. (Adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008.)



These two kinds of bacteria were distinguished originally by their color when treated with the “Gram” stain, developed by the bacteriologist Hans Christian Gram. The bacteria with the thinner peptidoglycan layers produce light pink staining when treated with the Gram stain, and were classified as **Gram negative** bacteria. In contrast, the bacteria with the thicker peptidoglycan layers produce dark purple staining and were classified as **Gram positive**.

Plant cell walls are more complex than those of bacteria. They contain microfibrils of cellulose (described in Section 3.7) and hemicellulose (a soft, slightly branched heteropolymer containing many different monomer sugars), with **pectin** [another polysaccharide, $\alpha(1 \rightarrow 4)$ -galacturonic acid with ~4% branching with neutral sugars, galactose, arabinose, and xylose]. Other polysaccharides crosslink the cellulose fibrils to add mechanical strength, as shown in **Figure 3.52**. There is also a filler of lignin, a complex, cross-linked polymer with a high content of aromatic carbons. As noted previously, the cellulose provides high mechanical strength, critical for supporting the complex morphology of the whole plant.

Fungi also have cell walls to protect them. These are made of chitin (described in Section 3.6) and other polysaccharides, but no cellulose. Chitin is also in insect exoskeletons, but there it is mixed with proteins and inorganic mineral deposits.

Summary

Glycans (carbohydrates) and lipids are essential components of all cells, but they differ from proteins and nucleic acids in that they are not directly genetically encoded. The simplest glycans are monosaccharide sugars, compounds with the approximate formula $(H-C-OH)_n$, with n in the range of 3–9. These sugars primarily form closed rings, although these are in equilibrium with linear forms. Variations in the stereochemistry at different carbons give rise to many structural isomers of each size of sugar.

Sugar polymers (oligo- and polysaccharides) range in size from a few sugar units to tens of thousands of sugar units. The physical properties of the polymers depend strongly on the precise stereochemistry of the sugars and the chemical linkages between the sugar rings. In contrast to proteins and nucleic acids, glycan polymers can be both linear and branched. Many cell types produce chemically modified sugars (containing amines, methyl and acetyl groups, and carboxylates) that are combined to form very diverse carbohydrates with many different functions. Glycans serve diverse purposes, acting as energy storage compounds (glycogen), “address labels” that dictate the destinations of proteins (proteoglycans), structural reinforcement in cell walls (cellulose and chitin), and cell-cell recognition elements (for example, lipopolysaccharides and proteoglycans).

Lipids are a critical component of all cell membranes. Although quite variable in their structures, lipids always have a strongly hydrophobic part, as well as some hydrophilic part. The most common membrane lipids, the glycerophospholipids, have a glycerol backbone with ester bonds attaching two fatty acids (commonly with 16–20 carbons) and a head group with a negatively charged phosphate with a variable R group. Unlike proteins, nucleic acids, and glycans, lipids do not form chemically linked polymers; rather, they assemble noncovalently into organized structures.

Natural lipids form bilayer membranes in water, which are planar structures with the head groups exposed to the water and the alkyl chains of the fatty acids clustered together to exclude water completely. Such bilayers are two-dimensional fluids (molecules move quite freely within the plane of the bilayer). Molecules analogous to lipids, but with just one alkyl chain, tend to form spherical droplets (micelles) with the hydrocarbon tails on the inside, rather than bilayers. Such compounds are called detergents, and they can surround other molecules to solubilize them. Because of their largely hydrophobic character, lipid molecules must be associated with proteins to be transported in and between cells.

Membranes play a critical role in partitioning space into distinct regions that can have different chemical properties. Internal regions in eukaryotic cells are partitioned by membranes into organelles, which carry out specific biochemical functions. Many different kinds of proteins are associated with membranes. Some proteins embed into the membrane, while others are covalently attached to lipid molecules and are thereby tethered to the membrane surface.

Key Concepts

A. GLYCANS

- Sugars are primarily “hydrated carbon,” $(H-C-OH)_n$, with $n = 3–9$ (but mostly 5 or 6), and are generally cyclic.
- Individual sugars are covalently linked to form oligomeric or polymeric glycans (also called oligosaccharides or polysaccharides).
- Many sugars are structural isomers with the same chemical formula (for example, $C_6H_{12}O_6$ = glucose, mannose, galactose).
- Some sugars have additional chemical functional groups, including methyl or acetyl groups, amines, acids, phosphates, etc.
- Polymers of different sugars (or different isomers of the same sugar) can have very different physical properties.
- Cell-surface proteins often have covalently attached glycans; such proteins are said to be glycosylated and are called glycoproteins.
- Glycosylation can modify the physical properties and activities of proteins.
- Noncovalent protein–carbohydrate interactions are important for cell–cell recognition and interaction.

B. LIPIDS AND MEMBRANES

- Lipid molecules are amphiphilic (that is, they have both hydrophobic and hydrophilic parts).

- Lipid molecules form the basis of cell membranes, accounting for over 50% by weight of biological membranes.
- Glycerophospholipids, with a variety of attached polar head groups, are abundant in cell membranes.
- Sphingolipids and cholesterol are also common in the cell membranes of eukaryotes.
- Fatty acids in lipids vary in length and the degree of unsaturation.
- Lipids assemble spontaneously into organized bilayer structures.
- Detergents are similar to lipids, but form micelles rather than bilayers.
- Membranes with different lipids have distinct thicknesses and fluidities; thus, regions of different lipid compositions and rafts can exist within cell membranes.
- Membrane-associated proteins can either embed in the bilayer (integral membrane proteins) or be tethered by attachment to a fatty acid or lipid molecule.
- Lipid molecules are generally transported by proteins.
- Membranes define spatial compartments, thus defining individual cells or organelles within cells.
- Lipids and carbohydrates are key components of cell walls.