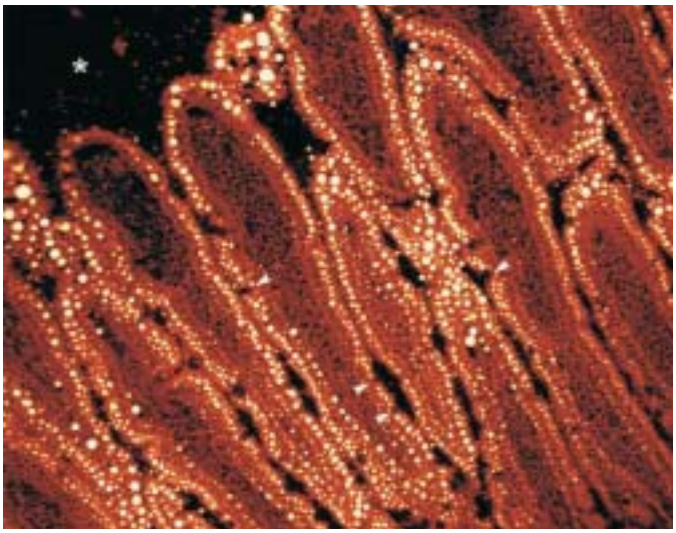
18

METABOLISM AND MOVEMENT OF LIPIDS



Fluorescence micrograph of hamster intestinal epithelium after cellular uptake into lipid droplets of an orally administered fluorescent analog of cholesterol (fluoresterol, dissolved in corn oil) from the intestinal lumen (upper left, unstained). [C. P. Sparrow et al., 1999, *J. Lipid Res.* **40:**1747–1757.]

In this chapter we consider some of the special challenges that a cell faces in metabolizing and transporting **lipids,** which are poorly soluble in the aqueous interior of cells and in extracellular fluids. Cells use lipids for storing energy, building membranes, signaling within and between cells, sensing the environment, covalently modifying proteins, forming specialized permeability barriers (e.g., in skin), and protecting cells from highly reactive chemicals. **Fatty acids,** which are oxidized in mitochondria to release energy for cellular functions (Chapter 8), are stored and transported primarily in the form of **triglycerides.** Fatty acids are also precursors of **phospholipids,** the structural backbone of cellular membranes (Chapter 5). **Cholesterol,** another important membrane component, is a precursor for steroid hormones and other biologically active lipids that function in cell–cell signaling. Also derived from precursors of cholesterol biosynthesis are the fat-soluble vitamins, which have diverse functions including the detection of light by the retinal form of vitamin A in rhodopsin, the control of calcium metabolism by the active hormone form of vitamin D, protection against oxidative damage to cells by vitamin E, and the cofactor activity of vitamin K in the formation of blood clots.

With the exception of a few specialized cells that store large quantities of lipids, the overwhelming majority of lipids within cells are components of cellular membranes. Therefore we focus our discussion of lipid biosynthesis and movement on the major lipids found in cellular membranes and their precursors (Figure 18-1). In lipid biosynthesis, water-soluble precursors are assembled into membrane-associated intermediates that are then converted into membrane lipid products. The movement of lipids, especially membrane components, between different organelles is critical for maintaining the proper composition and properties of membranes and overall cell structure, but our understanding of such intracellular lipid transport is still rudimentary. In contrast, analysis of the transport of lipids into, out of, and between cells is far more advanced, and we describe in some detail these lipid movements mediated by various cell-surface transport proteins and receptors.

We conclude the chapter by examining the connection between cellular cholesterol metabolism and atherosclerosis, which can lead to cardiovascular disease (e.g., heart attack,

OUTLINE

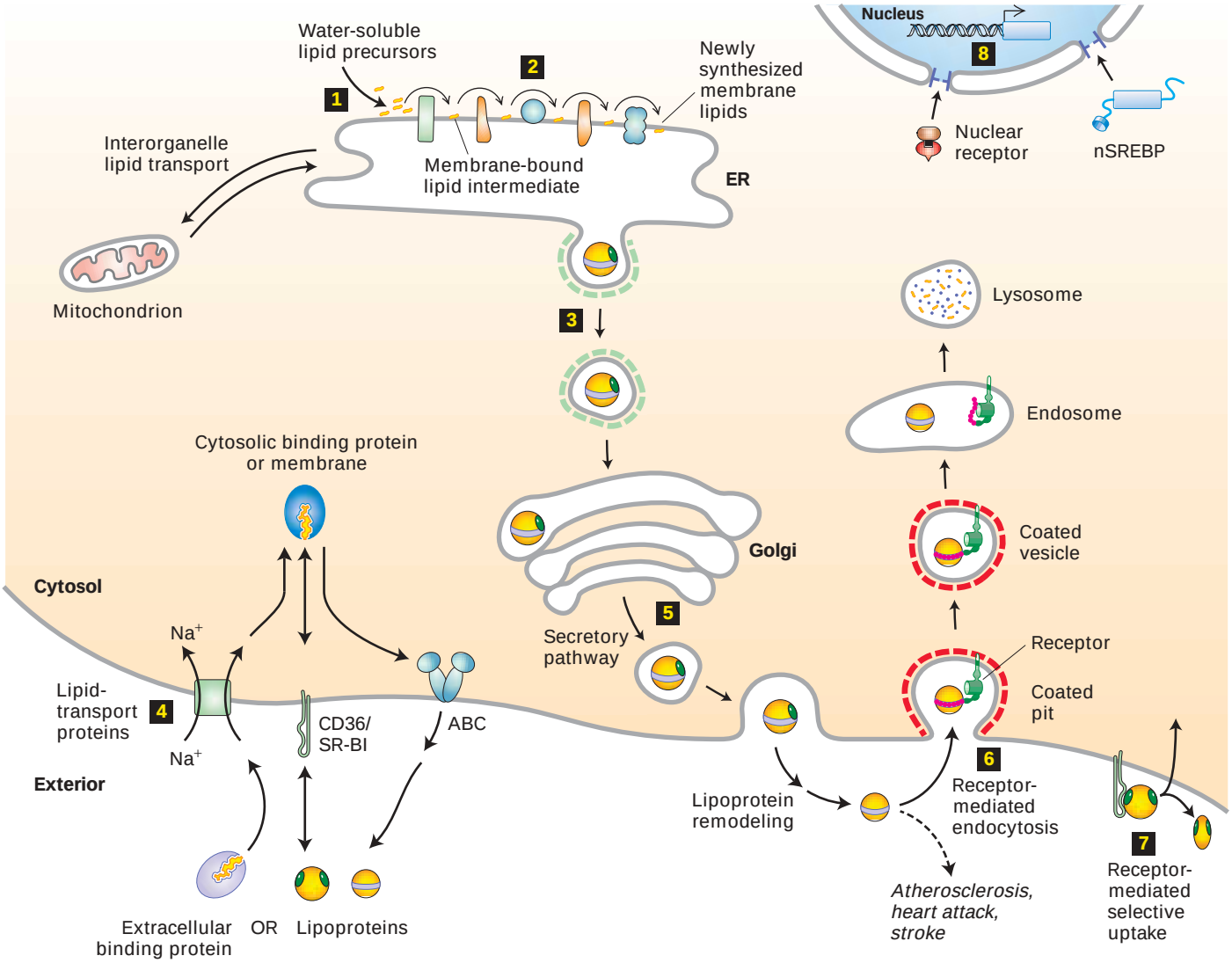
18.1 Phospholipids and Sphingolipids: Synthesis and Intracellular Movement

18.2 Cholesterol: A Multifunctional Membrane Lipid

18.3 Lipid Movement into and out of Cells

18.4 Feedback Regulation of Cellular Lipid Metabolism

18.5 The Cell Biology of Atherosclerosis, Heart Attacks, and Strokes



▲ FIGURE 18-1 **Overview of synthesis of major membrane lipids and their movement into and out of cells.** Membrane lipids (e.g., phospholipids, cholesterol) are synthesized through complex multienzyme pathways that begin with sets of water-soluble enzymes and intermediates in the cytosol (1) that are then converted by membrane-associated enzymes into water-insoluble products embedded in the membrane (2), usually at the interface between the cytosolic leaflet of the endoplasmic reticulum (ER) and the cytosol. Membrane lipids can move from the ER to other organelles (3), such as the Golgi apparatus or the mitochondrion, by either vesicle-mediated or other poorly defined mechanisms. Lipids can move into or out of cells by plasma-membrane transport proteins or by lipoproteins. Transport proteins similar to those described in Chapter 7 that move lipids (4) include sodium-coupled symporters that mediate import; CD36 and SR-BI superfamily proteins that can mediate unidirectional or bidirectional transport; and ABC superfamily proteins that mediate cellular export, the flipping of lipids from the cytosolic to the exoplasmic leaflet of the membrane, or both. Because lipids are insoluble in water, the transport proteins move lipids from and to carrier proteins, lipoproteins, membranes, or other lipid-binding complexes in the extracellular space and the cytosol. Lipoproteins assembled in the ER carry in their hydrophobic cores large amounts of lipid. They are secreted by the classic Golgi-mediated secretory pathway (5), and their lipids are imported either through (6) receptor-mediated endocytosis or through (7) receptor-mediated selective lipid uptake. Cellular lipid metabolism is regulated (8) by nuclear receptor transcription factors that directly bind lipids and by nuclear sterol regulatory element-binding proteins (nSREBPs) that are generated by proteolysis of an integral membrane protein precursor in the Golgi.

stroke), the number one cause of death in Western industrialized societies. We describe current theories about why large arteries can become clogged with cholesterol-containing deposits and how cells recognize the differences between "good" and "bad" cholesterol. As you will see, detailed knowledge of the fundamental cell biology of lipid metabolism has led to the discovery of remarkably effective anti-atherosclerotic drugs.

18.1 Phospholipids and Sphingolipids: Synthesis and Intracellular Movement

A cell cannot divide or enlarge unless it makes sufficient amounts of additional membranes to accommodate the expanded area of its outer surface and internal organelles. Thus the generation of new cell membranes is as fundamentally important to the life of a cell as is protein synthesis or DNA replication. Although the protein components of biomembranes are critical to their biological functions, the basic structural and physical properties of membranes are determined by their lipid components—principally phospholipids, sphingolipids, and sterols such as cholesterol (Table 18-1). Cells must be able to synthesize or import these molecules to form membranes.

A fundamental principle of membrane biosynthesis is that cells synthesize new membranes only by the expansion of existing membranes. Although some early steps in the synthesis of membrane lipids take place in the cytoplasm, the final steps are catalyzed by enzymes bound to preexisting cellular membranes, and the products are incorporated into the membranes as they are generated. Evidence for this phenomenon is seen when cells are briefly exposed to radioactive precursors (e.g., phosphate or fatty acids): all the phospholipids and sphingolipids incorporating these precursor substances are associated with intracellular membranes; none are found free in the cytosol. After they are formed, membrane lipids must be distributed appropriately both in leaflets of a given membrane and among the independent membranes of different organelles in eukaryotic cells. Here, we focus on the synthesis and distribution of phospholipids and sphingolipids; we cover the synthesis of cholesterol in Section 18.2.

Fatty Acids Are Precursors for Phospholipids and Other Membrane Components

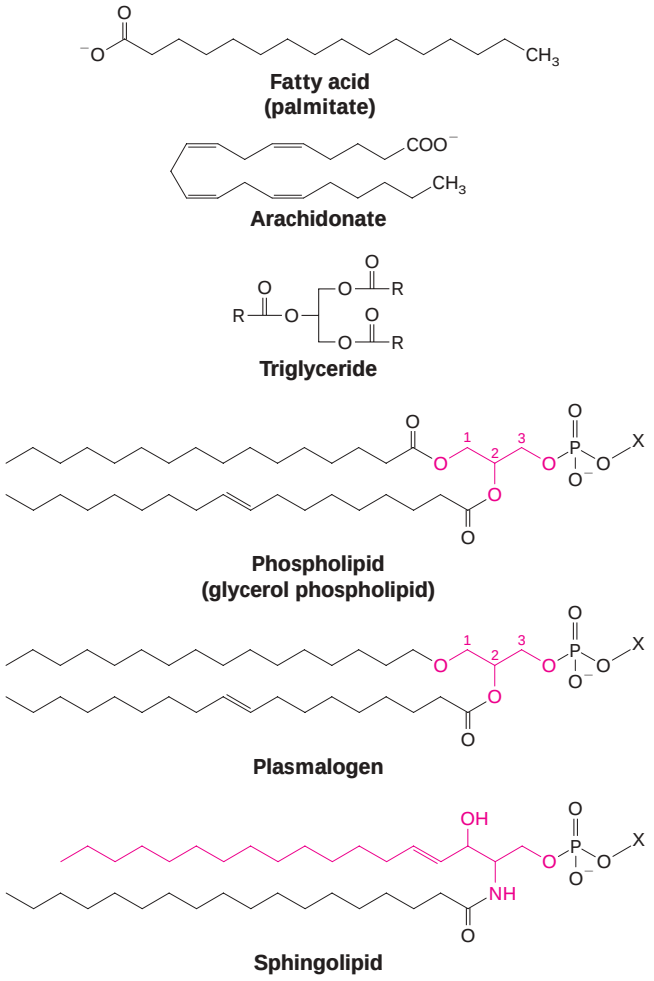
Fatty acids are key components of both phospholipids and sphingolipids; they also anchor some proteins to cellular membranes (see Figure 5-15). Thus the regulation of fatty acid synthesis plays a key role in the regulation of membrane synthesis as a whole. A fatty acid consists of a long hydrocarbon chain with a carboxyl group at one end (Figure 18-2). A *saturated* fatty acid (e.g., palmitate) has only single bonds, and an *unsaturated* fatty acid (e.g., arachidonate) has one or more double bonds in the hydrocarbon chain.

The major fatty acids in phospholipids contain 14, 16, 18, or 20 carbon atoms and include both saturated and unsaturated chains (see Table 18-1). Saturated fatty acids

TABLE 18-1 Synthesis and Transport of Fatty Acids and Major Membrane Lipids in Animal Cells

Lipid Class	Principal Sites of Synthesis	Import or Export Mechanisms	Intercellular Transport
Fatty acids (FAs):* Myristate (C14:0) Palmitate (C16:0) Stearate (C18:0) Oleate (C18:1) Linoleate (C18:2) Arachidonate (C20:4)	Saturated FAs up to 16 carbons long in the cytosol; elongation in the ER and mitochondria; desaturation in the ER	Diffusion and protein-mediated transport of free FAs (FATPs, CD36); secreted in lipoproteins as part of phospholipids, triglycerides and cholesteryl esters	Bound to albumin and other proteins in animal plasma (free FAs); as part of phospholipids, triglycerides, and cholesteryl esters in circulating lipoproteins
Phospholipids (e.g., phosphatidylcholine)†	ER primarily; some in mitochondria	Export by ABC proteins; endocytosis/exocytosis as part of lipoproteins	Packaged into lipoproteins
Plasmalogens	Peroxisomes	—	—
Sphingolipids	ER and Golgi complex	—	Packaged into lipoproteins
Cholesterol	Partly in cytosol and partly in ER	Export by ABC proteins; endocytosis/exocytosis as part of lipoproteins; import by selective lipid uptake from lipoproteins	Packaged into lipoproteins (both unesterified and esterified)

*In Cx:y abbreviation, x is the number of carbons in the chain and y is the number of double bonds.
Other abbreviations are: CD36, a multifunctional cell-surface protein; ER, endoplasmic reticulum; FA, fatty acid; FATP, fatty acid transport protein.
†The common diacyl glycerophospholipids also include phosphatidylethanolamine, phosphatidyl serine, and phosphatidylinositol.



▲ FIGURE 18-2 **Chemical structures of fatty acids and some of their derivatives.** Palmitate, a saturated fatty acid, contains 16 carbon atoms; arachidonate, a polyunsaturated fatty acid, contains 20 carbons atoms. Both saturated and unsaturated fatty acids are stored as triglycerides in which three fatty acyl chains (R = hydrocarbon portion of fatty acid) are esterified to a glycerol molecule. Fatty acids are also components of phospholipids (glycerol phospholipids, plasmalogens, and sphingolipids), which along with cholesterol are the major lipids present in membranes. The common phospholipids (e.g., phosphatidylcholine) have two acyl chains esterified to glycerol; in plasmalogens, one hydrocarbon chain is attached to glycerol by an ether linkage and the other by an ester linkage. Sphingolipids are built from sphingosine, an amino alcohol that contains a long, unsaturated hydrocarbon chain. Several types of polar X groups are found in all three of these classes of membrane lipids (see Figure 5-5).

containing 14 or 16 carbon atoms are made from acetyl CoA by two enzymes, *acetyl-CoA carboxylase* and *fatty acid synthase*. In animal cells, these enzymes are in the cytosol; in plants, they are found in chloroplasts. Palmitoyl CoA (16 carbons) can be elongated to 18–24 carbons by the sequential addition of two-carbon units in the endoplasmic reticulum (ER) or sometimes in the mitochondrion.

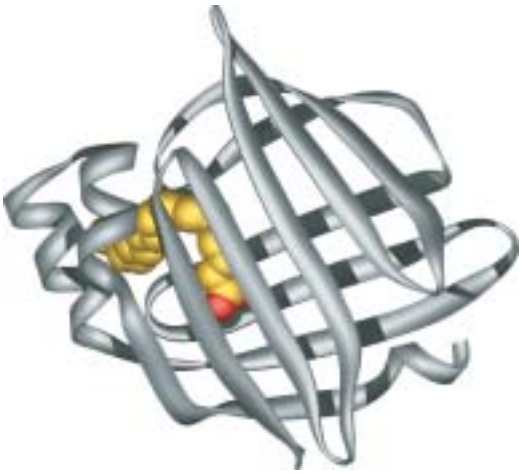
Desaturase enzymes, also located in the ER, introduce double bonds at specific positions in some fatty acids. The presence of a double bond creates a kink in the hydrocarbon chain that interrupts intermolecular packing (see Figure 2-18). As a result, membranes or triglyceride droplets whose components are high in unsaturated fatty acids (e.g., liquid corn and olive oils) tend to be more fluid at room temperature than those with a high proportion of saturated fatty acids (e.g., solid animal fats). Because humans cannot synthesize certain essential polyunsaturated fatty acids, such as linoleic acid and linolenic acid, we must obtain them from our diets.

In addition to de novo synthesis from acetyl CoA, fatty acids can be derived from the enzymatic hydrolysis of triglycerides. The primary form in which fatty acids are stored and transported between cells, triglycerides consist of three fatty acyl chains esterified to glycerol; hence they are also called triacylglycerols (see Figure 18-2). Complete hydrolysis of a triglyceride molecule yields three unesterified fatty acid molecules, or free fatty acids (FFAs), and a glycerol molecule.

Unesterified Fatty Acids Move Within Cells Bound to Small Cytosolic Proteins

Unesterified fatty acids within cells are commonly bound by *fatty acid–binding proteins* (FABPs), which belong to a group of small cytosolic proteins that facilitate the intracellular movement of many lipids. These proteins contain a hydrophobic pocket lined by β sheets (Figure 18-3). A long-chain fatty acid can fit into this pocket and interact noncovalently with the surrounding protein.

The expression of cellular FABPs is regulated coordinately with cellular requirements for the uptake and release of fatty acids. Thus FABP levels are high in active muscles



▲ FIGURE 18-3 **Binding of a fatty acid to the hydrophobic pocket of a fatty acid–binding protein (FABP).** The crystal structure of adipocyte FABP (ribbon diagram) reveals that the hydrophobic binding pocket is generated from two β sheets that are nearly at right angles to each other, forming a clam-shell-like structure. A fatty acid (yellow, oxygens red) interacts noncovalently with hydrophobic amino acid residues within this pocket. [See A. Reese-Wagoner et al., 1999, *Biochim. Biophys. Acta* **23**:1441(2–3):106–116.]

that are using fatty acids for energy and in adipocytes (fat-storing cells) when they are either taking up fatty acids to be stored as triglycerides or releasing fatty acids for use by other cells. The importance of FABPs in fatty acid metabolism is highlighted by the observations that they can compose as much as 5 percent of all cytosolic proteins in the liver and that genetic inactivation of cardiac muscle FABP converts the heart from a muscle that primarily burns fatty acids for energy into one that primarily burns glucose.

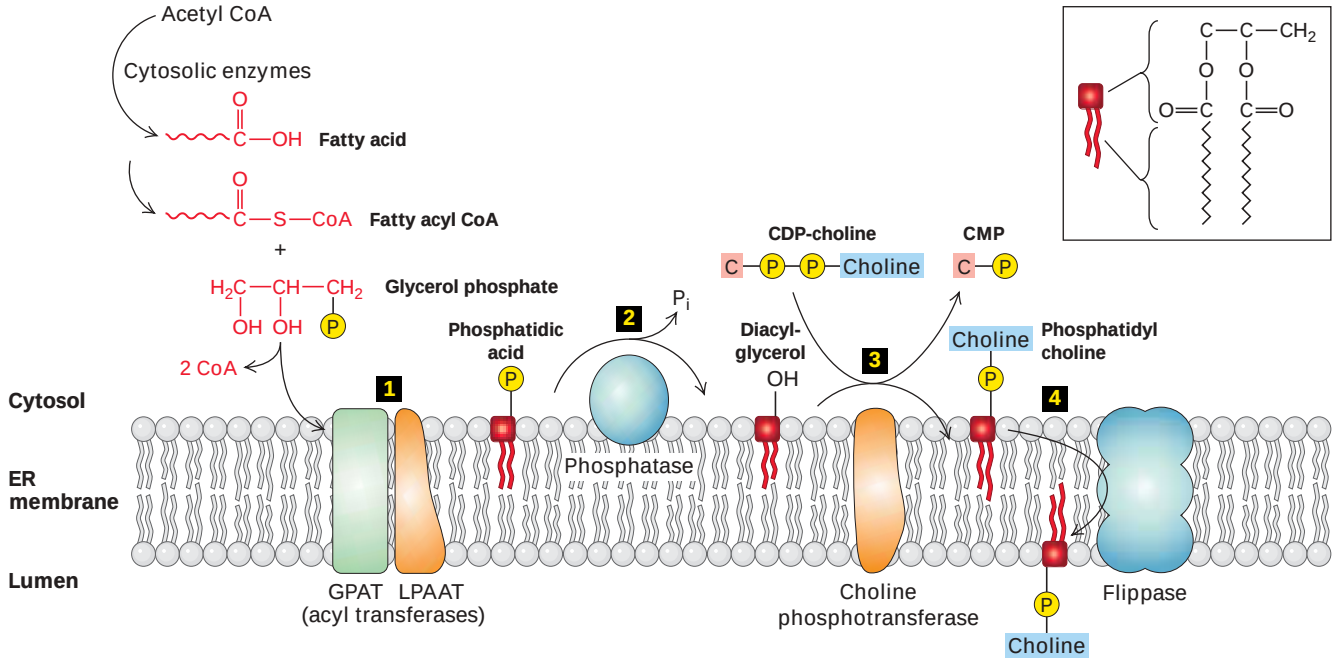
Numerous other small water-soluble proteins with lipid-binding hydrophobic pockets are known. Although some evidence suggests that these proteins participate in intracellular lipid transport, their function in this lipid movement remains to be established with certainty.

Incorporation of Fatty Acids into Membrane Lipids Takes Place on Organelle Membranes

Fatty acids are not directly incorporated into phospholipids; rather, they are first converted in eukaryotic cells into CoA esters. The subsequent synthesis of many *diacyl glycerophospholipids* from fatty acyl CoAs, glycerol 3-phosphate, and polar head-group precursors is carried out by enzymes associated with the cytosolic face of the ER membrane, usually the smooth ER, in animal cells (Figure 18-4). Mitochondria synthesize some of their own membrane lipids and import others. In photosynthetic tissues, the chloroplast is the site for the synthesis of all its own lipids. The enzymes that esterify the middle hydroxyl group of glycerol have a preference for adding unsaturated fatty acids.

In addition to diacyl glycerophospholipids, animal cells and some anaerobic microorganisms contain surprisingly large amounts of *plasmalogens*, a different type of glycerol-derived phospholipid. In these molecules, the hydrocarbon chain on carbon 1 of glycerol is attached by an ether linkage, rather than the ester linkage found in diacyl phospholipids (see Figure 18-2). In animal cells, the synthesis of plasmalogens is catalyzed by enzymes bound to the membranes of peroxisomes. Plasmalogens are known to be an important reservoir of arachidonate, a polyunsaturated, long-chain fatty acid that is a precursor for a large group of signaling molecules called eicosanoids (e.g., prostaglandins, thromboxanes, and leukotrienes). The regulated release of arachidonate from membrane glycerophospholipids by the enzyme phospholipase A_2 plays a rate-determining role in many signaling pathways. Also derived from a plasmalogen is platelet-activating factor (PAF), a signaling molecule that plays a key role in the inflammatory response to tissue damage or injury (see Figure 6-32). In addition, plasmalogens may influence the movement of cholesterol within mammalian cells.

Sphingolipids, another major group of membrane lipids, are derivatives of sphingosine, an amino alcohol that contains a long, unsaturated hydrocarbon chain. Sphingosine is



▲ FIGURE 18-4 **Phospholipid synthesis.** Because phospholipids are amphipathic molecules, the last stages of their multistep synthesis take place at the interface between a membrane and the cytosol and are catalyzed by membrane-associated enzymes. Step 1: Fatty acids from fatty acyl CoA are esterified to the phosphorylated glycerol backbone, forming phosphatidic acid, whose two long hydrocarbon chains anchor the molecule to the membrane. Step 2: A phosphatase converts phosphatidic acid into diacylglycerol. Step 3: A polar head group (e.g., phosphorylcholine) is transferred from CDP-choline to the exposed hydroxyl group. Step 4: Flippase proteins catalyze the movement of phospholipids from the cytosolic leaflet in which they are initially formed to the exoplasmic leaflet.

made in the ER, beginning with the coupling of palmitoyl CoA and serine; the addition of a fatty acyl group to form *N*-acyl sphingosine (ceramide) also takes place in the ER. The subsequent addition of a polar head group to ceramide in the Golgi yields *sphingomyelin*, whose head group is phosphorylcholine, and various *glycosphingolipids*, in which the head group may be a monosaccharide or a more complex oligosaccharide (see Figure 18-2). Some sphingolipid synthesis can also take place in mitochondria. In addition to serving as the backbone for sphingolipids, ceramide and its metabolic products are important signaling molecules that can influence cell growth, proliferation, endocytosis, resistance to stress, and apoptosis.

After their synthesis is completed in the Golgi, sphingolipids are transported to other cellular compartments through vesicle-mediated mechanisms similar to those discussed in Chapter 17. In contrast, phospholipids, as well as cholesterol, can move between organelles by different mechanisms, described in Section 18.2.

Flippases Move Phospholipids from One Membrane Leaflet to the Opposite Leaflet

Even though phospholipids are initially incorporated into the cytosolic leaflet of the ER membrane, various phospholipids are asymmetrically distributed in the two leaflets of the ER membrane and of other cellular membranes (see Table 5-1). However, phospholipids spontaneously flip-flop from one leaflet to the other only very slowly, although they can rapidly diffuse laterally in the plane of the membrane. For the ER membrane to expand (growth of both leaflets) and have asymmetrically distributed phospholipids, its phospholipid components must be able to rapidly and selectively flip-flop from one membrane leaflet to the other.

The usual asymmetric distribution of phospholipids in membrane leaflets is broken down as cells (e.g., red blood cells) become senescent or undergo apoptosis. For instance, phosphatidylserine and phosphatidylethanolamine are preferentially located in the cytosolic leaflet of cellular membranes. Increased exposure of these anionic phospholipids on the exoplasmic face of the plasma membrane appears to serve as a signal for scavenger cells to remove and destroy old or dying cells. Annexin V, a protein that specifically binds to anionic phospholipids, can be fluorescently labeled and used to detect apoptotic cells in cultured cells and in tissues.

Although the mechanisms employed to generate and maintain membrane phospholipid asymmetry are not well understood, it is clear that **flippases** play a key role. These integral membrane proteins facilitate the movement of phospholipid molecules from one leaflet to the other (see Figure 18-4, step 4). One of the best-studied flippases is the mammalian ABCB4 protein, a member of the **ABC superfamily** of small-molecule pumps. As discussed in Section 18.3, ABCB4 is expressed in certain liver cells (hepatocytes) and moves phosphatidylcholine from the cytosolic to the exoplasmic leaflet of the plasma membrane for subsequent release into the bile in combination with cholesterol and bile acids. Several other ABC superfamily members participate in the cellular export of various lipids (Table 18-2).

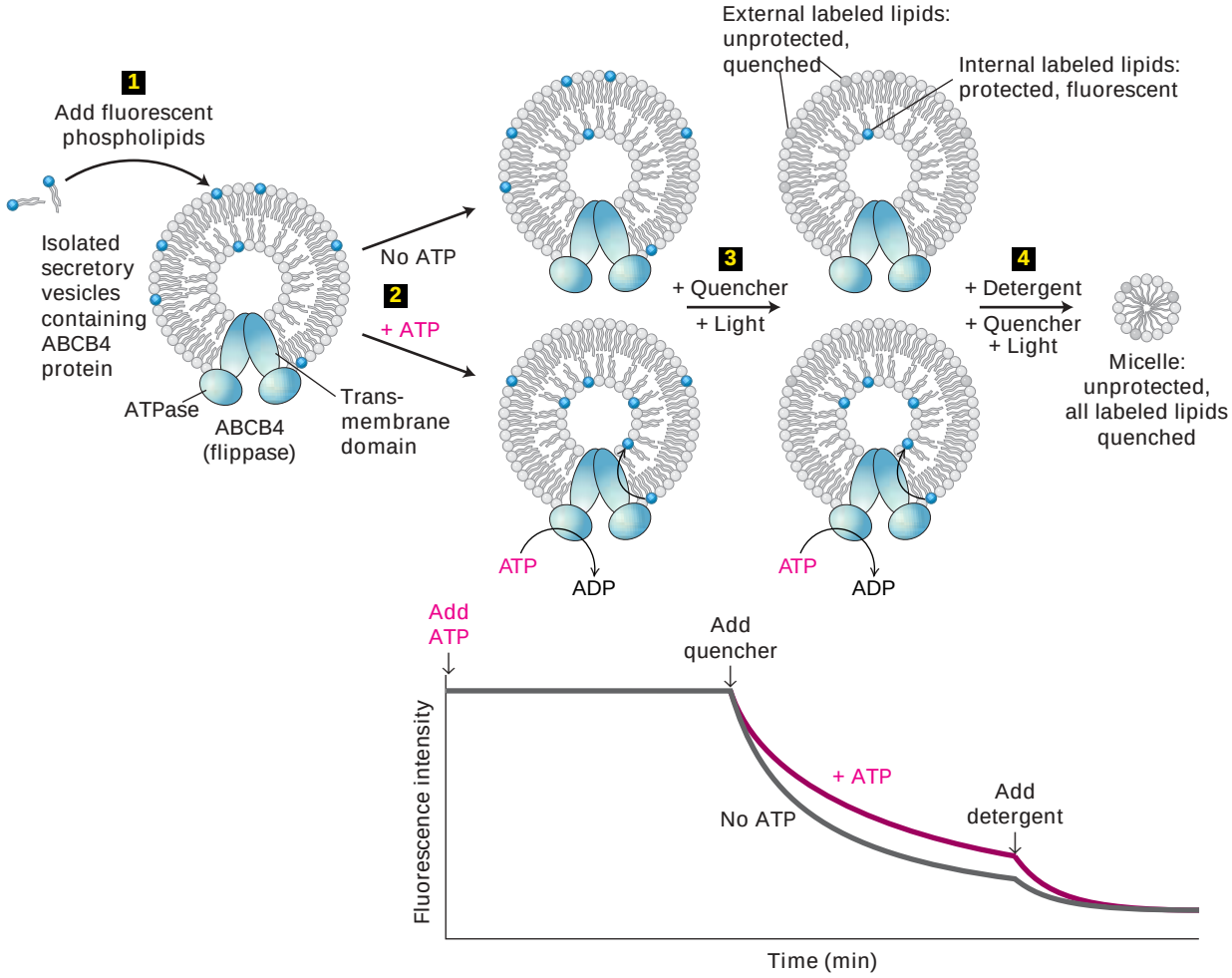
ABCB4 was first suspected of having phospholipid flippase activity because mice with homozygous loss-of-function mutations in the *ABCB4* gene exhibited defects in the secretion of phosphatidylcholine into bile. To determine directly if ABCB4 was in fact a flippase, researchers performed experiments on a homogeneous population of purified vesicles with ABCB4 in the membrane and with the cytosolic face directed outward. These vesicles were obtained by introducing cDNA encoding mammalian ABCB4 into a temperature-sensitive

TABLE 18-2 Selected Human ABC Proteins

Protein	Tissue Expression	Function	Disease Caused by Defective Protein
ABCA1	Ubiquitous	Exports cholesterol and phospholipid for uptake into high-density lipoprotein (HDL)	Tangier's disease
ABCB1 (MDR1)	Adrenal, kidney, brain	Exports lipophilic drugs	
ABCB4 (MDR2)	Liver	Exports phosphatidylcholine into bile	
ABCB11	Liver	Exports bile salts into bile	
CFTR	Exocrine tissue	Transports Cl^- ions	Cystic fibrosis
ABCD1	Ubiquitous in peroxisomal membrane	Influences activity of peroxisomal enzyme that oxidizes very long chain fatty acids	Adrenoleukodystrophy (ADL)
ABCG5/8	Liver, intestine	Exports cholesterol and other sterols	β-Sitosterolemia

yeast *sec* mutant. At the permissive temperature, the ABCB4 protein is expressed by the transfected cells and moves through the secretory pathway to the cell surface (Chapter 17). At the nonpermissive temperature, however, secretory vesicles cannot fuse with the plasma membrane, as they do in wild-type cells; so vesicles containing ABCB4 and other yeast proteins accumulate in the cells. After purifying these secretory vesicles, investigators labeled them in vitro with a fluorescent phosphatidylcholine derivative. The fluorescence-quenching assay outlined in Figure 18-5 was used to demonstrate that the vesicles containing ABCB4 exhibited ATP-dependent some flippase activity, whereas those without ABCB4 did not. The structures and mechanism of action of some flippases are covered in Chapter 7.

Flip-flopping between leaflets, lateral diffusion, and membrane fusion and fission are not the only dynamic processes of phospholipids in membranes. Their fatty acyl chains and, in some cases, their head groups are subject to ongoing covalent remodeling (e.g., hydrolysis of fatty esters by phospholipases and resynthesis by acyl transferases). Another key



▲ EXPERIMENTAL FIGURE 18-5 **In vitro fluorescence quenching assay can detect phospholipid flippase activity of ABCB4.** A homogeneous population of secretory vesicles containing ABCB4 protein was purified from yeast *sec* mutants transfected with the *ABCB4* gene. Step 1: Synthetic phospholipids containing a fluorescently modified head group (blue) were incorporated primarily into the outer, cytosolic leaflets of the purified vesicles. Step 2: If ABCB4 acted as a flippase, then on addition of ATP to the outside of the vesicles a small fraction of the outward-facing labeled phospholipids would be flipped to the inside leaflet. Step 3: Flipping was detected by adding a membrane-impermeable quenching compound called dithionite to the medium surrounding the vesicles. Dithionite reacts with the fluorescent head group, destroying its ability to fluoresce (gray). In the presence of the quencher, only labeled phospholipid in the protected environment on the inner leaflet will fluoresce. Subsequent to the addition of the quenching agent, the total fluorescence decreases with time until it plateaus at the point at which all external fluorescence is quenched and only the internal phospholipid fluorescence can be detected. The observation of greater fluorescence (less quenching) in the presence of ATP than in its absence indicates that ABCB4 has flipped some of the labeled phospholipid to the inside. Step 4: Addition of detergent to the vesicles generates micelles and makes all fluorescent lipids accessible to the quenching agent and lowers the fluorescence to baseline values. [Adapted from S. Ruetz and P. Gros, 1994, *Cell* **77**:1071]

dynamic process is the intracellular movement of phospholipids from one membrane to a different one. Clearly, membranes are dynamic components of the cell that interact with and react to changes in the intracellular and extracellular environments.

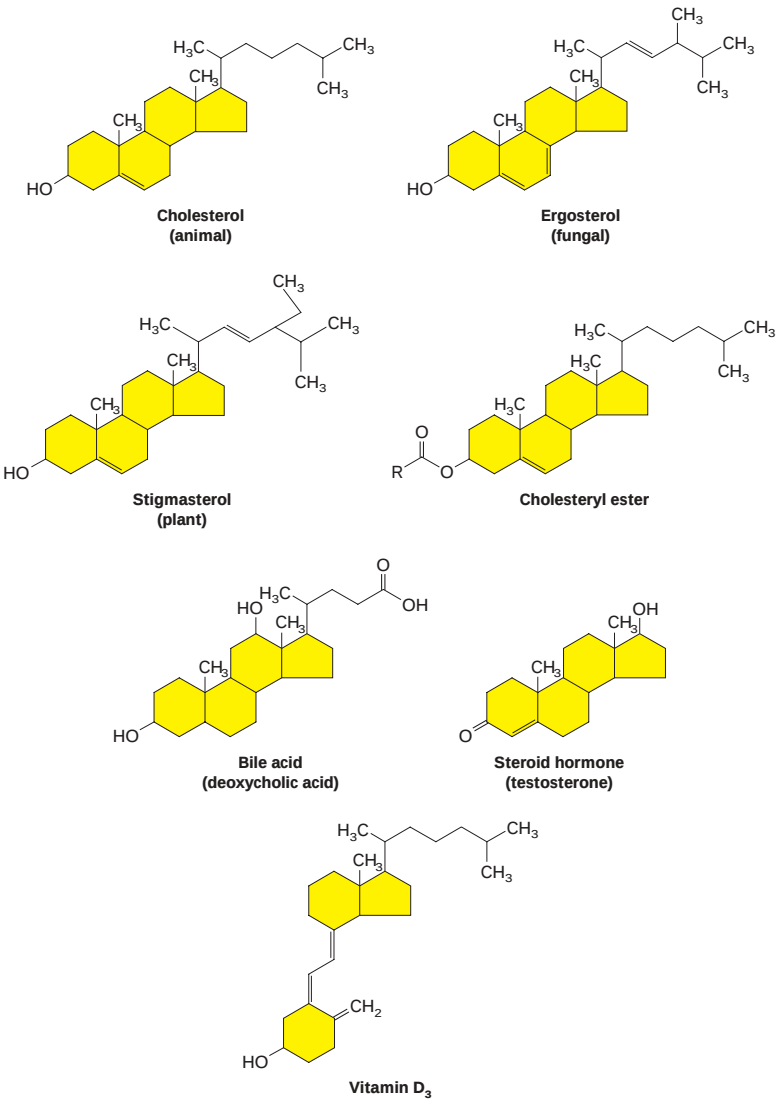
KEY CONCEPTS OF SECTION 18.1

Phospholipids and Sphingolipids: Synthesis and Intracellular Movement

- Saturated and unsaturated fatty acids of various chain lengths are components of phospholipids, sphingolipids, and triglycerides (see Figure 18-2).
- Fatty acids are synthesized by water-soluble enzymes and modified by elongation and desaturation in the endoplasmic reticulum (ER).
- The final steps in the synthesis of glycerophospholipids, plasmalogens, and sphingolipids are catalyzed by membrane-associated enzymes primarily in the ER, but also in the Golgi, mitochondria, and peroxisomes (see Figure 18-4).
- Each type of lipid is initially incorporated into the preexisting membranes on which it is made.
- Most membrane phospholipids are preferentially distributed in either the exoplasmic or the cytosolic leaflet. This asymmetry results in part from the action of flippases such as ABCB4, a phosphatidylcholine flippase contributing to the generation of bile in the liver.

18.2 Cholesterol: A Multifunctional Membrane Lipid

Although phospholipids are critical for the formation of the classic bilayer structure of membranes, eukaryotic cell membranes require other components, including **sterols.** Here, we focus on cholesterol, the principal sterol in animal cells and the most abundant single lipid in the mammalian plasma



▶ FIGURE 18-6 **Chemical structures of major sterols and cholesterol derivatives.** The major sterols in animals (cholesterol), fungi (ergosterol), and plants (stigmasterol) differ slightly in structure, but all serve as key components of cellular membranes. Cholesterol is stored as cholesteryl esters in which a fatty acyl chain (R = hydrocarbon portion of fatty acid) is esterified to the hydroxyl group. Excess cholesterol is converted by liver cells into bile acids (e.g., deoxycholic acid), which are secreted into the bile. Specialized endocrine cells synthesize steroid hormones (e.g., testosterone) from cholesterol, and photochemical and enzymatic reactions in the skin and kidneys produce vitamin D.

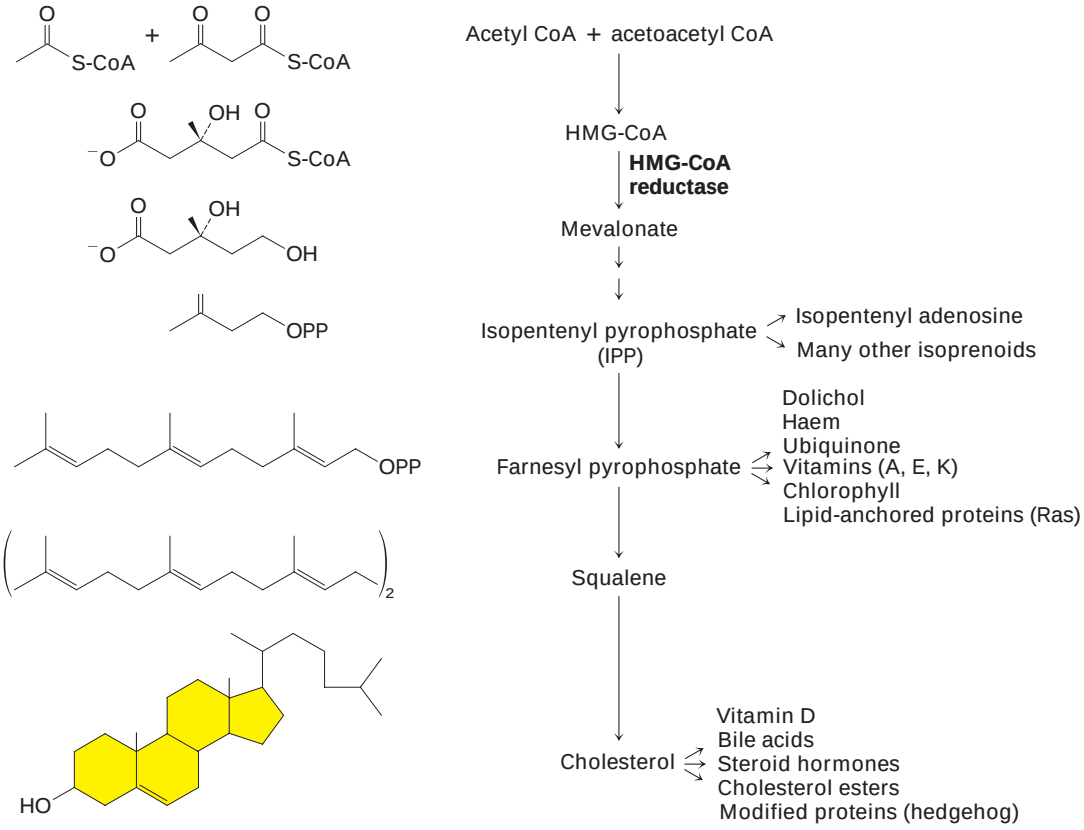
membrane (almost equimolar with all phospholipids). Between 50 and 90 percent of the cholesterol in most mammalian cells is present in the plasma membrane and related endocytic vesicle membranes. Cholesterol is also critical for intercellular signaling and has other functions to be described shortly. The structures of the principal yeast sterol (ergosterol) and plant phytosterols (e.g., stigmasterol) differ slightly from that of cholesterol (Figure 18-6). The small differences in the biosynthetic pathways of fungal and animal sterols and in their structures are the basis of most antifungal drugs currently in use.

Cholesterol Is Synthesized by Enzymes in the Cytosol and ER Membrane

Figure 18-7 summarizes the complex series of reactions that yield cholesterol and several other related biomolecules. The basic features of this pathway are important in the synthesis of other important lipids, and familiarity with these features helps in understanding lipid regulation, discussed later. The first steps of cholesterol synthesis (acetyl CoA → HMG CoA) take place in the cytosol. The conversion of HMG CoA into mevalonate, the key rate-controlling step in cholesterol biosynthesis, is catalyzed by *HMG-CoA reductase,* an ER integral membrane protein, even though both its substrate and its product are water soluble. The water-soluble catalytic domain of HMG-CoA reductase extends into the cytosol, but its eight transmembrane segments firmly embed the enzyme in the ER membrane and act as a regulatory domain. Five of the transmembrane segments compose the so-called *sterol-sensing domain*. As described later, homologous domains are found in other proteins taking part in cholesterol transport and regulation.

Mevalonate, the 6-carbon product formed by HMG-CoA reductase, is converted in several steps into the 5-carbon isoprenoid compound isopentenyl pyrophosphate (IPP) and its stereoisomer dimethylallyl pyrophosphate (DMPP). These reactions are catalyzed by cytosolic enzymes, as are the subsequent reactions in which six IPP units condense to yield squalene, a branched-chain 30-carbon intermediate. Enzymes bound to the ER membrane catalyze the multiple reactions that convert squalene into cholesterol in mammals or into related sterols in other species.

Because an excessive accumulation of cholesterol can lead to the formation of damaging cholesterol crystals, the production and accumulation of cholesterol is tightly



▲ FIGURE 18-7 **Cholesterol biosynthetic pathway.** The regulated rate-controlling step in cholesterol biosynthesis is the conversion of β-hydroxy-β-methylglutaryl CoA (HMG-CoA) into mevalonic acid by HMG-CoA reductase, an ER-membrane protein. Mevalonate is then converted into isopentenyl pyrophosphate (IPP), which has the basic five-carbon isoprenoid structure. IPP can be converted into cholesterol and into many other lipids, often through the polyisoprenoid intermediates shown here. Some of the numerous compounds derived from isoprenoid intermediates and cholesterol itself are indicated. See text for discussion.

controlled. For example, cholesteryl esters (see Figure 18-6) are formed from excess cholesterol and stored as cytosolic lipid droplets. *Acyl:cholesterol acyl transferase (ACAT)*, the enzyme that esterifies fatty acyl CoAs to the hydroxyl group of cholesterol, is located in the ER membrane. Substantial amounts of cholesteryl ester droplets are usually found only in cells that produce steroid hormones and in foam cells, which contribute to atherosclerotic disease in artery walls. Intracellular lipid droplets, whether composed of cholesteryl esters or triglycerides, have an outer protein coat that serves as an interface between the aqueous environment of the cytosol and the lipid. The coat proteins on lipid droplets in mammalian cells are called perilipins or perilipin-related proteins, while the oleosins and their related proteins coat the surfaces of lipid droplets called oil bodies in plants.

Many Bioactive Molecules Are Made from Cholesterol and Its Biosynthetic Precursors

In addition to its structural role in membranes, discussed in Chapter 5, cholesterol is the precursor for several important bioactive molecules. They include **bile acids** (see Figure 18-6), which are made in the liver and help emulsify dietary fats for digestion and absorption in the intestines, steroid hormones produced by endocrine cells (e.g., adrenal gland, ovary, testes), and vitamin D produced in the skin and kidneys. Arthropods need cholesterol or other sterols to produce membranes and ecdysteroid hormones, which control development; however, they cannot make the precursor sterols themselves and must obtain these compounds in their diet. Another critical function of cholesterol is its covalent addition to Hedgehog protein, a key signaling molecule in embryonic development (Chapter 15).

Isopentenyl pyrophosphate and other isoprenoid intermediates in the cholesterol pathway also serve as precursors for more than 23,000 biologically active molecules. Some of these molecules are discussed in other chapters: various hemes, including the oxygen-binding component of hemoglobin and electron-carrying components of cytochromes (see Figure 8-15a); ubiquinone, a component of the mitochondrial electron-transport chain (see Figure 8-16); chlorophylls, the light-absorbing pigments in chloroplasts (see Figure 8-31); and dolichol, a polyisoprenoid in the ER membrane that plays a key role in the glycosylation of proteins (see Figure 16-17).

Isoprenoid derivatives are particularly abundant in plants in which they form fragrances and flavors, rubber and latex, hormones and pheromones, various defensive molecules, the active ingredient in marijuana, the cardioprotective natural drug digitalis, anticancer drugs such as taxol, and many others. Given the importance of isoprenoids as biosynthetic precursors, it is not surprising that a second, mevalonate-independent pathway for IPP synthesis evolved in eubacteria (e.g., *E. coli*), green algae, and higher plants. In plants, this pathway is located in organelles called plastids and operates to synthesize carotenoids, phytol (the isoprenoid side chain of chlorophyll), and other isoprenoids. ▮

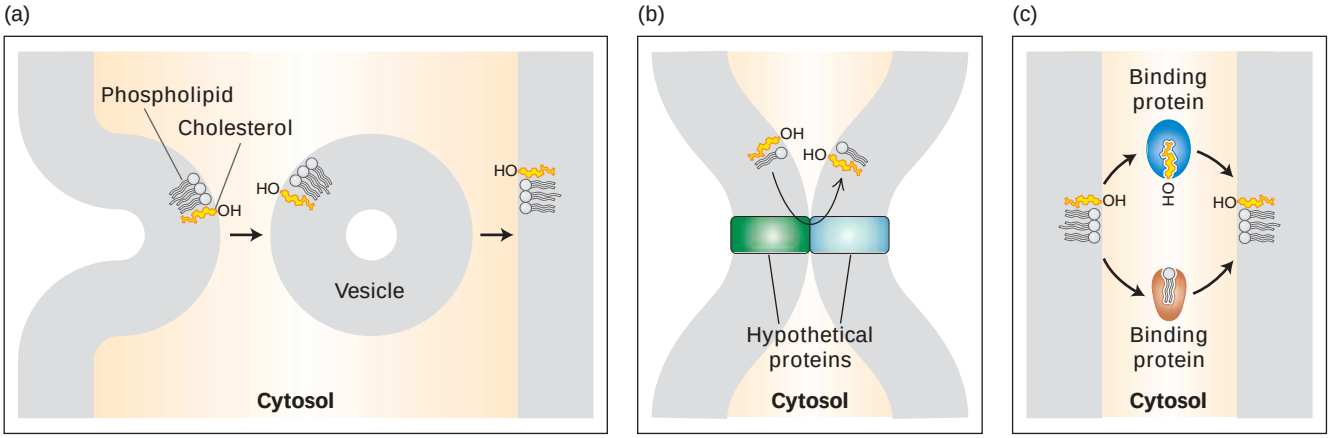
Cholesterol and Phospholipids Are Transported Between Organelles by Golgi-Independent Mechanisms

As already noted, the final steps in the synthesis of cholesterol and phospholipids take place primarily in the ER, although some of these membrane lipids are produced in mitochondria and peroxisomes (plasmalogens). Thus the plasma membrane and the membranes bounding other organelles (e.g., Golgi, lysosomes) must obtain these lipids by means of one or more intracellular transport processes. For example, in one important pathway, phosphatidylserine made in the ER is transported to the inner mitochondrial membrane where it is decarboxylated to phosphatidylethanolamine, some of which either returns to the ER for conversion into phosphatidylcholine or moves to other organelles.

Membrane lipids do accompany both soluble (luminal) and membrane proteins during vesicular trafficking through the Golgi-mediated secretory pathway (see Figure 17-1). However, several lines of evidence suggest that there is substantial interorganelle movement of cholesterol and phospholipids through other, Golgi-independent mechanisms. For example, chemical inhibitors of the classic secretory pathway and mutations that impede vesicular traffic in this pathway do not prevent cholesterol or phospholipid transport between membranes, although they do disrupt the transport of proteins and Golgi-derived sphingolipids. Furthermore, membrane lipids produced in the ER cannot move to mitochondria by means of classic secretory transport vesicles, inasmuch as no vesicles budding from ER membranes have been found to fuse with mitochondria.

Three mechanisms have been proposed for the transport of cholesterol and phospholipids from their sites of synthesis to other membranes independently of the Golgi-mediated secretory pathway (Figure 18-8). First, some Golgi-independent transport is most likely through membrane-limited vesicles or other protein–lipid complexes. The second mechanism entails direct protein-mediated contact of ER or ER-derived membranes with membranes of other organelles. In the third mechanism, small lipid-transfer proteins facilitate the exchange of phospholipids or cholesterol between different membranes. Although such transfer proteins have been identified in assays in vitro, their role in intracellular movements of most phospholipids is not well defined. For instance, mice with a knockout mutation in the gene encoding the phosphatidylcholine-transfer protein appear to be normal in most respects, indicating that this protein is not essential for cellular phospholipid metabolism.

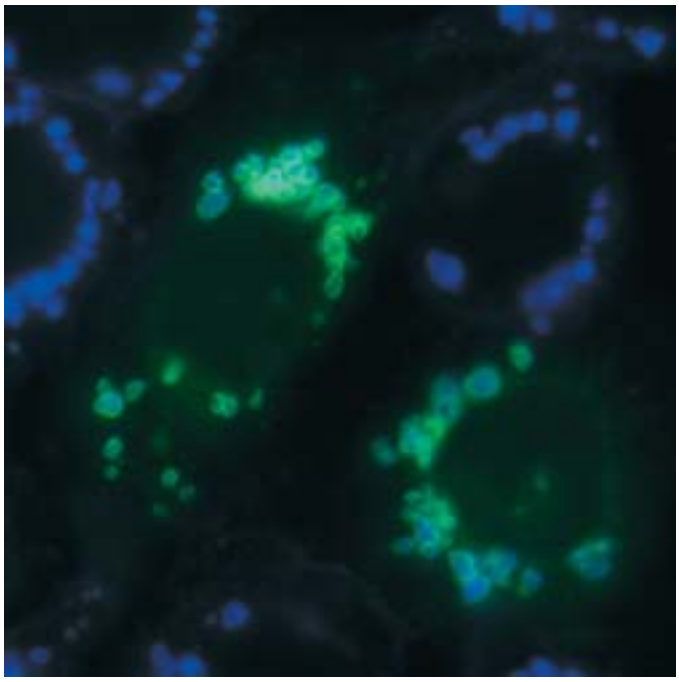
One well-established component of the intracellular cholesterol-transport system is the *steroidogenic acute regulatory (StAR) protein*. This protein, which is encoded in nuclear DNA, controls the transfer of cholesterol from the cholesterol-rich outer mitochondrial membrane to the cholesterol-poor inner membrane, where it undergoes the first steps in its enzymatic conversion into steroid hormones. StAR-mediated cholesterol transport is a key regulated, rate-controlling step in steroid hormone synthesis. StAR contains



▲ FIGURE 18-8 **Proposed mechanisms of Golgi-independent transport of cholesterol and phospholipids between membranes.** In mechanism (a), vesicles transfer lipids between membranes without passing through the Golgi apparatus. In mechanism (b), lipid transfer is a consequence of direct contact between membranes that is mediated by membrane-embedded proteins. In mechanism (c), transfer is mediated by small, soluble lipid-transfer proteins. Some evidence suggests that this mechanism does not account for a significant part of the Golgi-independent flow of phospholipids between membranes. [Adapted from F. R. Maxfield and D. Wustner, 2002, *J. Clin. Invest.* **110**:891.]

an N-terminal targeting sequence that directs the protein to the mitochondrial outer membrane (Chapter 16) and a C-terminal START (StAR-related transfer) domain that has a cholesterol-binding hydrophobic pocket. Similar START domains are found in several proteins implicated in intracellular cholesterol transport, and these domains have been shown to promote cholesterol transfer in cultured cells. Mutations in the StAR gene can cause congenital adrenal hyperplasia, a lethal disease marked by a drastic reduction in the synthesis of steroid hormones. Some other proteins implicated in lipid transport, including the phosphatidylcholine-transfer protein already mentioned, also contain START domains.

A second well-established contributor to intracellular cholesterol movement is the *Niemann-Pick C1 (NPC1) protein*, an integral membrane protein located in the rapidly moving late endosomal/lysosomal compartment. Some of the multiple membrane-spanning segments of NPC1 form a sterol-sensing domain similar to that in HMG-CoA reductase. Mutations in NPC1 cause defects in intracellular cholesterol and glycosphingolipid transport and consequently in the regulation of cellular cholesterol metabolism. Cells without functional NPC1 or cells treated with a drug that mimics loss of NPC1 function accumulate excess cholesterol in the late endosomal/lysosomal compartment (Figure 18-9). Cholesterol transport in NPC1-deficient cells is restored by overexpression of Rab9, a small GTPase implicated in late endosomal vesicular transport (Chapter 17). This finding suggests that vesicular trafficking plays at least some role in NPC1-dependent cholesterol movement.



◀ EXPERIMENTAL FIGURE 18-9 **Cells with nonfunctional Niemann-Pick C1 (NPC1) protein accumulate cholesterol in late endosomal/lysosomal vesicles.** In three cells that express a transgene encoding a hybrid NPC1 protein linked to green fluorescent protein (GFP), the hybrid protein is revealed in the late endosomal/lysosomal compartment by its green fluorescence. Shown in this two-color fluorescence micrograph are cells that had been treated with a drug that inhibits NPC1 function. The cells were also stained with a blue-fluorescing cholesterol-binding drug called fillipin. Note the colocalization of cholesterol (blue) with the NPC1-GFP hybrid protein (green) on the surfaces of vesicles adjacent to the nucleus. Cells not expressing the hybrid NPC1 (blue only) are also seen. The accumulation of cholesterol in these vesicles in the absence of functional NPC1 suggests that late endosomal/lysosomal vesicles play a role in intracellular cholesterol trafficking. [From D. C. Ko et al., 2001, *Mol. Biol. Cell* **12**:601; courtesy of D. Ko and M. Scott.]

MEDICINE In humans, defects in NPC1 function cause abnormal lipid storage in intracellular organelles, resulting in neurologic abnormalities, neurodegeneration, and premature death. Indeed, identification of the gene defective in such patients led to discovery of the NPC1 protein. ∎

The lipid compositions of different organelle membranes vary considerably (see Table 5-1). Some of these differences are due to different sites of synthesis. For example, a phospholipid called cardiolipin, which is localized to the mitochondrial membrane, is made only in mitochondria and little is transferred to other organelles. Differential transport of lipids also plays a role in determining the lipid compositions of different cellular membranes. For instance, even though cholesterol is made in the ER, the cholesterol concentration (cholesterol-to-phospholipid molar ratio) is ~1.5–13-fold higher in the plasma membrane than in other organelles (ER, Golgi, mitochondrion, lysosome). Although the mechanisms responsible for establishing and maintaining these differences are not well understood, the distinctive lipid composition of each membrane has a major influence on its physical properties (Chapter 5).

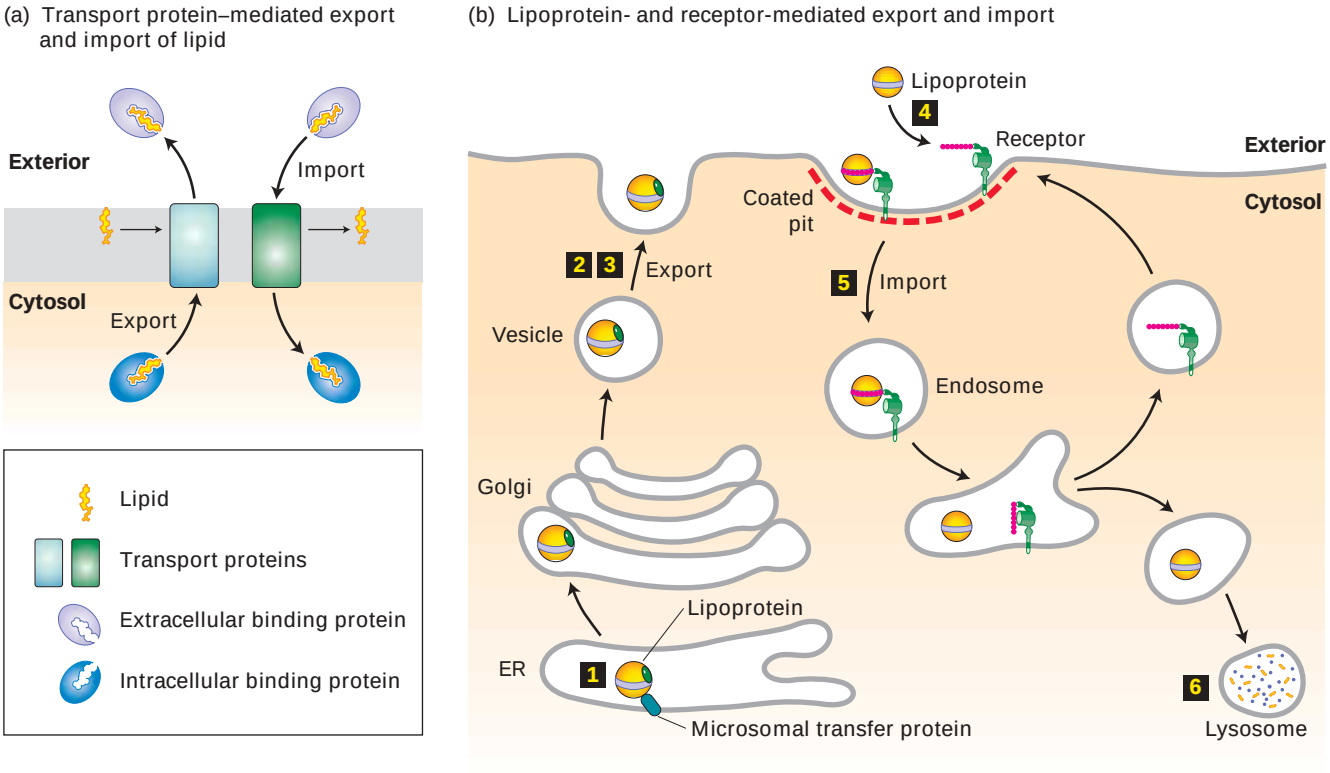
KEY CONCEPTS OF SECTION 18.2

Cholesterol: A Multifunctional Membrane Lipid

- The initial steps in cholesterol biosynthesis take place in the cytosol, whereas the last steps are catalyzed by enzymes associated with the ER membrane.
- The rate-controlling step in cholesterol biosynthesis is catalyzed by HMG-CoA reductase, whose transmembrane segments are embedded in the ER membrane and contain a sterol-sensing domain.
- Cholesterol itself and isoprenoid intermediates in its synthesis are biosynthetic precursors of steroid hormones, bile acids, lipid-soluble vitamins, and numerous other bioactive molecules (see Figure 18-7).
- Considerable evidence indicates that vesicular trafficking through the Golgi complex is not responsible for much cholesterol and phospholipid movement between membranes. Golgi-independent vesicular transport, direct protein-mediated contacts between different membranes, soluble protein carriers, or all three may account for some inter-organelle transport of cholesterol and phospholipids (see Figure 18-8).
- The StAR protein, which has a hydrophobic cholesterol-binding pocket, plays a key role in moving cholesterol into the mitochondrion for steroid hormone synthesis.
- The NPC1 protein, a large, multipass transmembrane protein, contains a sterol-sensing domain similar to that in HMG-CoA reductase. NPC1 is required for the normal movement of cholesterol between certain intracellular compartments.

18.3 Lipid Movement into and out of Cells

In multicellular organisms, particularly mammals, lipids are often imported and exported from cells and transported



among different tissues by the circulation. Such lipid movements help maintain appropriate intracellular and whole-body lipid levels and have other advantages for an organism. For instance, lipids absorbed from the diet in the intestines or stored in adipose tissue can be distributed to cells throughout the body. In this way, cells can obtain essential dietary lipids (e.g., linoleate) and can avoid wasting energy on the synthesis of lipids (e.g., cholesterol) otherwise available from the diet. The ability of some cells to export lipids permits the excretion of excess lipids from the body or their secretion into certain body fluids (e.g., milk in mammary glands). In addition, the coordination of lipid and energy metabolism throughout the organism depends on intercellular lipid transport.

Not surprisingly, intracellular and intercellular lipid transport and metabolism are coordinately regulated, as discussed in Section 18.4. Here, we review the primary ways in which cells import and export lipids. The two main mechanisms of cellular lipid export and import are summarized in Figure 18-10. In the first mechanism, the import or export of individual lipid molecules is mediated by cell-surface transport proteins. The principles underlying this mechanism are similar to those for small water-soluble molecules such as glucose, discussed in Chapter 7. A noteworthy difference is that the hydrophobic lipids, which are poorly soluble in aqueous solution, often associate with lipid-binding proteins in the extracellular space or the cytosol, rather than remaining free in solution. In the second mechanism of lipid import and export, collections of lipids are packaged with proteins into transport particles called lipoproteins. These lipoproteins are exported from cells through the classic secretory pathway (Chapter 17). Lipids carried by extracellular lipoproteins are taken up by cells through surface receptors.

◀ FIGURE 18-10 **The two major pathways for importing and exporting cellular lipids.** (a) Individual lipid molecules cross the plasma membrane with the assistance of transmembrane transport proteins. Intracellular and extracellular lipid-binding proteins (shown here), lipid micelles, or membranes normally participate as the donors and acceptors of the lipids. In some cases, the concentration gradients of the transported substances are sufficient to drive transport (e.g., import of bile acids in the liver and intestines by Na^+-linked symporters). In others, coupled ATP hydrolysis helps drive lipid transport (e.g., secretion of bile lipids from hepatocytes by ABC proteins). (b) Lipids are also transported as components of lipoproteins. These large assemblies of protein and lipids are put together in the ER with the assistance of microsomal transfer protein (1), are exported (2) through the secretory pathway (3) as water-soluble particles, and then circulate in the blood. After circulating lipoproteins bind to certain cell-surface receptors (4), the intact particles can be internalized by endocytosis (5) and the lipids are subsequently hydrolyzed in lysosomes (6), as depicted here. Other receptors mediate the uptake of individual lipid components from lipoproteins, releasing a lipid-depleted particle into the extracellular space.

Cell-Surface Transporters Aid in Moving Fatty Acids Across the Plasma Membrane

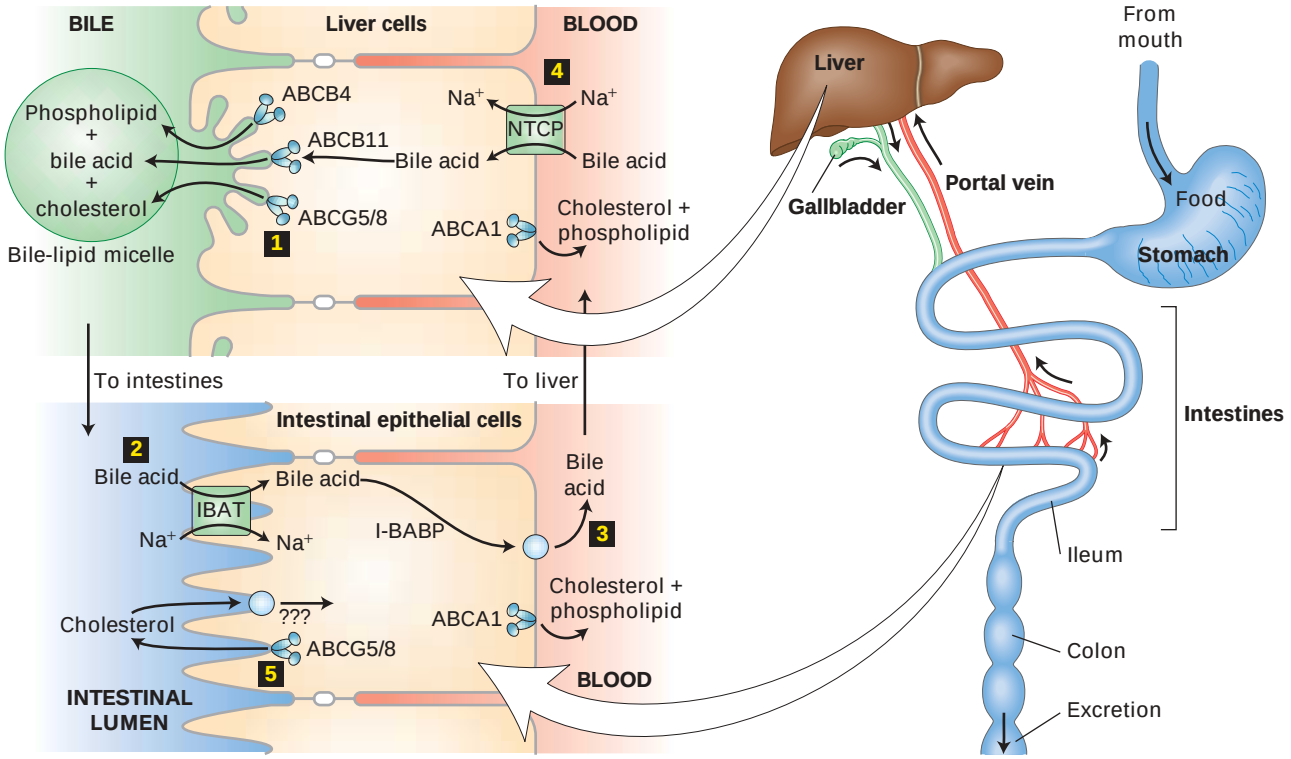
For many years, transmembrane fatty acid transport was thought not to require a protein mediator, because these hydrophobic molecules can diffuse across lipid bilayers. Although some protein-independent fatty acid transport probably does take place, some cells (e.g., intestinal epithelial cells, cardiac muscle cells, and adipocytes) must import or export substantial amounts of fatty acids at rates or with a specificity and regulation or both that are not possible by diffusion alone. Several integral membrane proteins have been shown to participate in fatty acid import in cell culture and whole-animal experiments. These proteins include various *fatty acid transport proteins (FATPs)* and the multifunctional cell-surface protein *CD36*. These transporters mediate the movement of substrates down their concentration gradients. In contrast, transporters that mediate the export of fatty acids have not yet been identified, but at least some transporters may mediate bidirectional transport, depending on the direction of the fatty acid concentration gradient.

Because of their poor water solubility, most fatty acids are bound to a carrier protein in the aqueous cytosol and extracellular space (see Figure 18-10a). The major mammalian extracellular binding protein that donates or accepts fatty acids is serum *albumin*. The most abundant protein in mammalian plasma (fluid part of the blood), albumin has at least one hydrophobic α helix–lined lipid-binding groove or cleft on its surface. In addition to mediating the transport of fatty acids, albumin mediates that of several anionic organic acids (e.g., bilirubin) and other molecules through the bloodstream. The major intracellular carriers of fatty acids are the fatty acid–binding proteins described earlier.

ABC Proteins Mediate Cellular Export of Phospholipids and Cholesterol

The export of phospholipids and cholesterol can be simultaneous owing to the activity of various members of the ABC superfamily (see Table 18-2). The best-understood example of this phenomenon is in the formation of *bile,* an aqueous fluid containing phospholipids, cholesterol, and bile acids, which are derived from cholesterol. After export from liver cells, phospholipids, cholesterol, and bile acids form water-soluble **micelles** in the bile, which is delivered through ducts to the gallbladder, where it is stored and concentrated. In response to a fat-containing meal, bile is released into the small intestine to help emulsify dietary lipids and thus aid in their digestion and absorption into the body. As we shall see later, the alteration of biliary metabolism by drugs can be used to prevent heart attacks.

Figure 18-11 outlines the major transport proteins that mediate the secretion and movement of bile components. Three ABC proteins move phospholipids, cholesterol, and bile acids across the apical surface of liver cells into small ductules (step 1). One of these proteins, the ABCB4 flippase, flips phosphatidylcholine from the cytosolic leaflet to the



▲ FIGURE 18-11 **Major transport proteins in the liver and intestines taking part in the enterohepatic circulation of biliary lipids.** The secretion of bile components and recycling of bile acids are mediated by a diverse array of transport proteins in liver cells (hepatocytes) and intestinal epithelial cells. Both of these polarized cell types import lipids across one surface and export them across the opposite surface. Step 1: Hepatocytes export lipids across their apical membranes into the bile by using three ATP-dependent ABC proteins: ABCB4 (phospholipids), ABCB11 (bile acids), and ABCG5/8 (sterols). Step 2: Intestinal epithelial cells import bile components and dietary lipids from the intestinal lumen by using the ileal bile acid transporter (IBAT), a Na^+-linked symporter, and other less well defined transporters located in the apical membrane. Step 3: Imported bile acids are transported to the basolateral surface bound to intestinal bile acid-binding protein (I-BABP) and are exported into the blood with the aid of unknown transporters. Step 4: Bile acids returned to the liver in the blood are imported by NTCP, another Na^+-linked symporter. Step 5: Absorption of sterols by intestinal cells is reduced by ABCG5/8, which appears to pump plant sterols and cholesterol out of the cells and back into the lumen.

exoplasmic leaflet of the apical membrane in hepatocytes, as described earlier. The precise mechanism by which the excess phospholipid desorbs from the exoplasmic leaflet into the extracellular space is not understood. A related protein, ABCB11, transports bile acids, whereas the ABCG5 and ABCG8 "half" proteins combine into a single ABC protein (ABCG5/8) that exports sterols into the bile.

In the intestine the *ileal bile acid transporter (IBAT)* imports bile acids from the lumen into intestinal epithelial cells (step 2). IBAT is a Na^+-linked symporter (see Figure 7-21) that uses the energy released by the movement of Na^+ down its concentration gradient to power the uptake of about 95 percent of the bile acids. Those bile acids imported on the apical side of intestinal epithelial cells move intracellularly with the aid of *intestinal bile acid–binding protein (I-BABP)* to the basolateral side. There, they are exported into the blood by poorly characterized transport proteins (step 3) and eventually returned to liver cells by another Na^+-linked symporter called NTCP (step 4). This cycling of bile acids from liver to intestine and back, referred to as the **enterohepatic circulation,** is tightly regulated and plays a major role in lipid homeostasis.

Because the amount of dietary cholesterol is normally low, a substantial fraction of the cholesterol in the intestinal lumen comes from the biliary cholesterol secreted by the liver. ABCG5/8 also is expressed in the apical membrane of intestinal epithelial cells, where it helps control the amounts of cholesterol and plant-derived sterols absorbed apparently by pumping excess or unwanted absorbed sterols out of the epithelial cells back into the lumen (see Figure 18-11, step 5). Partly as a result of this activity, only about 1 percent of dietary plant sterols, which are not metabolically useful to mammals, enter the bloodstream. Unabsorbed bile acids (normally <5 percent of the luminal bile acids) and unabsorbed cholesterol and plant sterols are eventually excreted in the feces.

Inactivating mutations in the genes encoding ABCG5 or ABCG8 cause *β-sitosterolemia*. Patients with this rare genetic disease absorb abnormally high amounts of both plant and animal sterols and their livers secrete abnormally low amounts into the bile. Indeed, findings from studies with β-sitosterolemia patients first implicated these ABC proteins in cellular sterol export. ▮

Two other cell-surface transport proteins mediate the export of cellular cholesterol, phospholipid, or both: the ABC superfamily member ABCA1 and a homolog of the fatty acid transporter CD36 called SR-BI. These proteins will be described in detail shortly because of their important roles in lipoprotein metabolism.

Lipids Can Be Exported or Imported in Large Well-Defined Lipoprotein Complexes

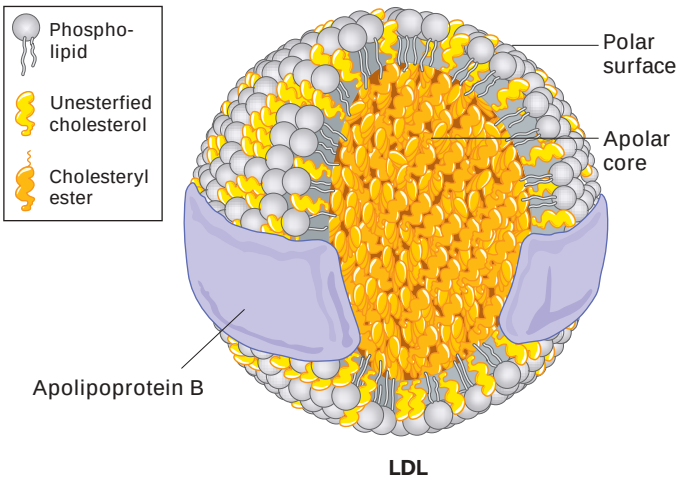
To facilitate the mass transfer of lipids between cells, animals have evolved an efficient alternative to the molecule-by-molecule import and export of lipids mediated by cell-surface transport proteins, such as those depicted in Figure 18-11. This alternative packages from hundreds to thousands of lipid molecules into water-soluble, macromolecular carriers, called **lipoproteins,** that cells can secrete into the circulation or take up from the circulation as an ensemble.

A lipoprotein particle has a shell composed of proteins (*apolipoproteins*) and a cholesterol-containing phospholipid monolayer (Figure 18-12). The shell is **amphipathic** because its outer surface is hydrophilic, making these particles water soluble, and its inner surface is hydrophobic. Adjacent to the hydrophobic inner surface of the shell is a core of neutral lipids containing mostly cholesteryl esters, triglycerides, or both. Small amounts of other hydrophobic compounds (e.g., vitamin E, carotene) also are carried in the lipoprotein core.

Mammalian lipoproteins fall into four major classes. Three of them—**high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low density lipoprotein (VLDL)**—are named on the basis of their differing buoyant densities. The lower the protein-to-lipid ratio, the lower the density. The fourth class, the **chylomicrons,** is the least dense and contains the highest proportion of lipids. Each class of lipoproteins has distinctive apolipoprotein and lipid compositions, sizes, and functions (Table 18-3). VLDLs and chylomicrons carry mainly triglycerides in their cores, whereas the cores of LDLs and HDLs consist mostly of cholesteryl esters. Apolipoproteins help organize the structure of a lipoprotein particle and determine its interactions with enzymes, extracellular lipid-transfer proteins, and cell-surface receptors. Each LDL particle contains a single copy of a large (537-kDa) apolipoprotein called apoB-100 embedded in its outer shell (see Figure 18-12). In contrast, several copies of different apolipoproteins are found in each of the other lipoprotein classes.

TABLE 18-3 Major Classes of Human Plasma Lipoproteins

Property	Chylomicron	VLDL	LDL	HDL
Mass, approx. (kDa)	$50–1000 \times 10^3$	$10–80 \times 10^3$	2.3×10^3	$0.175–0.360 \times 10^3$
Diameter (nm)	75–1200	30–80	18–25	5–12
Triglycerides (% of core lipids)	97	75	12	11
Cholesteryl esters (% of core lipids)	3	25	88	89
Protein:lipid mass ratio	1:100	9:100	25:100	90:100
Major apolipoproteins	A, B-48, C, E	B-100, C, E	B-100	A, C
Major physiological function	Transports dietary triglyceride (Tg) from intestines to extrahepatic tissues (e.g., muscle, adipose tissue); Tg-depleted remnants deliver dietary cholesterol and some Tg to the liver	Transports hepatic Tg to extrahepatic tissues; converted into LDL	Transports plasma cholesterol to liver and to extrahepatic tissues	Takes up cholesterol from extrahepatic tissues and delivers it to liver, steroid-producing tissues, and other lipoproteins



▲ FIGURE 18-12 **Model of low-density lipoprotein (LDL).** This class and the other classes of lipoproteins have the same general structure: an amphipathic shell, composed of a phospholipid monolayer (not bilayer), cholesterol, and protein, and a hydrophobic core, composed mostly of cholesteryl esters or triglycerides or both but with minor amounts of other neutral lipids (e.g., some vitamins). This model of LDL is based on electron microscopy and other low-resolution biophysical methods. LDL is unique in that it contains only a single molecule of one type of apolipoprotein (apoB), which appears to wrap around the outside of the particle as a band of protein. The other lipoproteins contain multiple apolipoprotein molecules, often of different types. [Adapted from M. Krieger, 1995, in E. Haber, ed., *Molecular Cardiovascular Medicine*, Scientific American Medicine, pp. 31–47.]

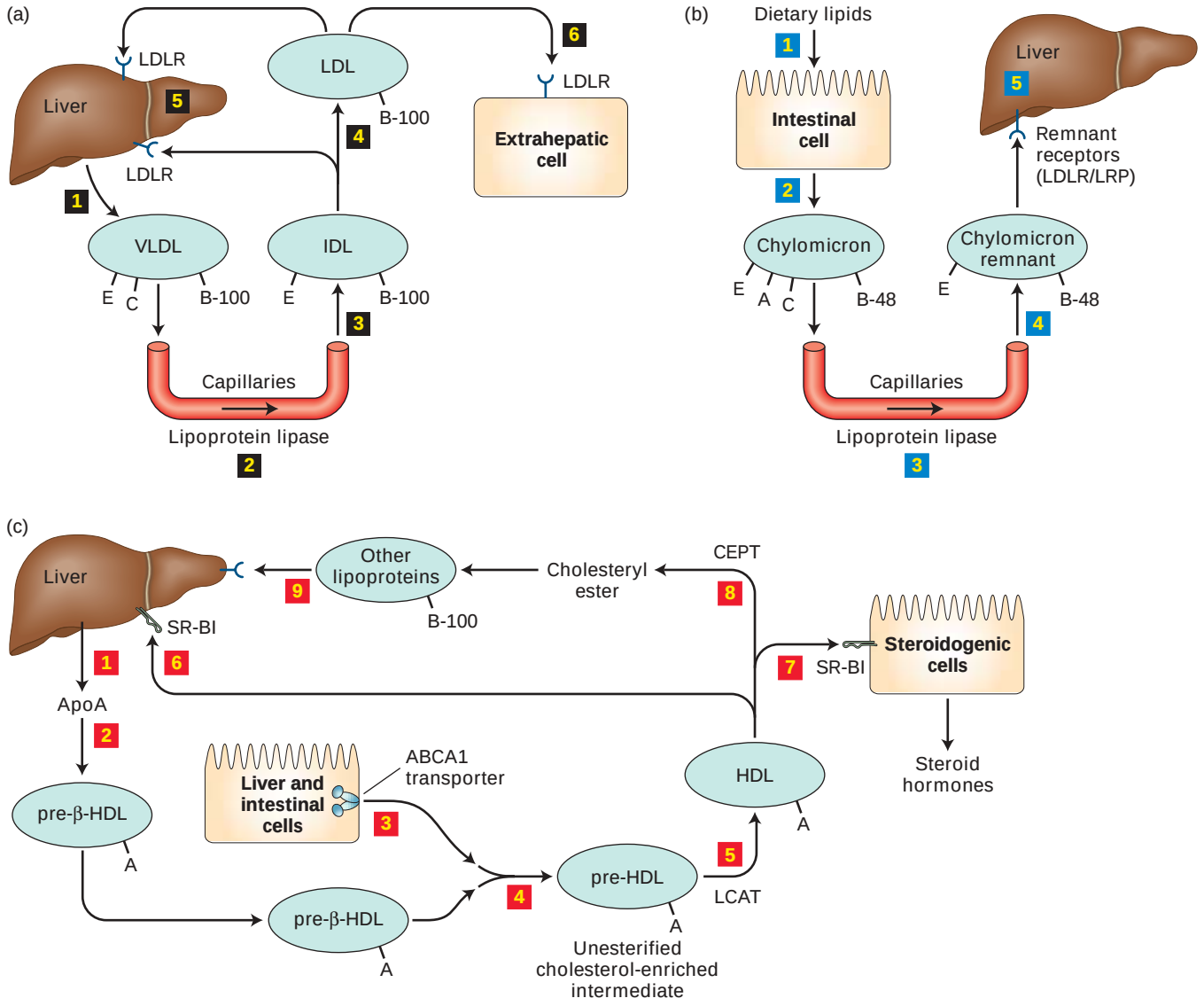
Lipoproteins Are Made in the ER, Exported by the Secretory Pathway, and Remodeled in the Circulation

Only two types of lipoproteins, VLDL and chylomicrons, are fully formed within cells by assembly in the ER, a process that requires the activity of *microsomal transfer protein*. The assembled particles move through the secretory pathway to the cell surface and are released by **exocytosis**—VLDL from liver cells and chylomicrons from intestinal epithelial cells (see Figure 18-10b). LDLs, IDLs (intermediate-density lipoproteins), and some HDLs are generated extracellularly in the bloodstream and on the surfaces of cells by the remodeling of secreted VLDLs and chylomicrons. There are four types of modifications:

- Hydrolysis of triglycerides and phospholipids by lipases and esterification of cholesterol by an acyl transferase
- Transfer of cholesteryl esters, triglycerides, and phospholipids between lipoproteins by specific lipid-transfer proteins
- Uptake by some particles of cholesterol and phospholipids exported from cells
- Association and dissociation of some apolipoproteins from the surfaces of the particles

▶ FIGURE 18-13 **Lipoprotein remodeling and interconversions in the circulatory system.** Apolipoproteins are indicated by capital letters (e.g., A, B-100) projecting from particles. (a) After having been secreted from the liver 1, VLDL's triglycerides are hydrolyzed by lipoprotein lipase, an extracellular enzyme attached to the blood-facing surfaces of vessels 2. The loss of triglycerides and some apolipoproteins from VLDL produces IDL 3, which is converted into LDL 4. Both IDL and LDL can be removed from the circulation by endocytosis by means of LDL receptors (LDLR) on liver (hepatic) cells 5 and nonliver (extrahepatic) cells 6. (b) Dietary lipids are absorbed 1 and packaged into chylomicrons in intestinal epithelial cells, secreted into the lymph, and then enter the bloodstream 2. In the circulation, they are remodeled similarly to the remodeling of VLDL 3, forming smaller, cholesterol-enriched chylomicron remnants 4, which are taken up by hepatocytes through receptor-mediated endocytosis 5. (c) HDL is thought to be formed after secretion of apolipoprotein A from cells 1 by the formation of preβ-HDL particles, which contain apoA but very little lipid 2. Those small particles act as acceptors for phospholipid and cholesterol exported from cells (primarily liver and intestine) by the ABCA1 transporter 3, forming a cholesterol-rich intermediate 4. Lethicin:cholesterol acyl transferase (LCAT), an enzyme in the plasma, esterifies cholesterol after its incorporation into HDL 5. Cholesteryl esters in the core of a large HDL particle can be transferred to cells (especially liver 6 and steroidogenic 7 cells) by the receptor SR-BI or to other lipoproteins by cholesteryl ester–transfer protein (CETP) 8 and subsequently to tissues such as the liver 9. [Adapted from M. S. Brown and J. L. Goldstein, 1984, *Sci. Am.* **251**(5):58, and M. Krieger, 1999, *Ann. Rev. Biochem.* **68**:523.]

For example, VLDL secreted from hepatocytes is converted into IDL and eventually into LDL, which can then deliver its cholesterol to cells through LDL receptors (Figure 18-13a). Similarly, chylomicrons carrying dietary lipids from the intestines are converted by lipase hydrolysis into *chylomicron remnants,* which eventually undergo endocytic uptake by the liver (Figure 18-13b). Small preβ-HDL particles are generated extracellularly from apoA apolipoproteins, secreted mainly by liver and intestinal cells, and from small amounts of cholesterol and phospholipid. They are then further converted into larger, spherical HDL particles, which constitute the bulk of the HDL found in the blood (Figure 18-13c). A major way that preβ-HDL particles grow larger is by accepting phospholipids and cholesterol exported from cells with the aid of yet another ABC protein called *ABCA1* (see Table 18-2). This protein was implicated in the formation of HDL when defects in the *ABCA1* gene were shown to cause Tangier's disease, a very rare genetic disease in which affected persons have almost no HDL in their blood. After being incorporated into HDL, cholesterol is esterified by *lecithin:cholesterol acyl transferase (LCAT),* an enzyme present in the plasma. Large HDL particles can transfer their cholesteryl esters to other lipoproteins through *cholesteryl ester–transfer protein (CETP)* or to cells (especially the liver and steroidogenic cells) through the receptor SR-BI, discussed later.



In Section 18.5, we examine in detail the findings that the plasma LDL cholesterol concentration (including both unesterified and esterified cholesterol) is directly correlated with risk for coronary artery disease, whereas the plasma HDL cholesterol concentration is inversely correlated with risk. For this reason, LDL is popularly called "bad cholesterol" and HDL, "good cholesterol."

Cells Use Several Protein-Mediated Mechanisms to Import Lipoprotein Lipids

For maximum efficiency, the lipids within circulating lipoproteins should be taken up only by those cells that require them for membrane formation (e.g., dividing cells), steroid hormone synthesis (e.g., endocrine cells), energy production (e.g., muscle cells), or storage (adipose cells, endocrine cells). The targeting of lipoprotein lipids to appropriate cells is accomplished in one of two ways: (1) local, partial extracellular hydrolysis of core triglycerides followed by transport protein–mediated uptake of the released fatty acids or (2) the regulated expression of cell-surface lipoprotein receptors that mediate the direct uptake of lipoprotein lipids.

The first targeting mechanism, for example, supplies cells with fatty acids for use as an energy source in muscle and for storage in adipose tissue. The extracellular enzyme lipoprotein lipase is attached by glycosaminoglycan (GAG) chains to the blood-facing surface of endothelial cells in these tissues. Fatty acids released from the hydrolysis of core triglycerides in VLDL and chylomicrons then cross the vessel wall and enter underlying cells through fatty acid transporters such as FATPs and CD36 (see Figure 18-10a). This process results in the delivery of fatty acids to the cells concomitant with remodeling of the lipoprotein particles.

The expression of various lipoprotein receptors by different tissues also ensures that lipids are delivered to cells that need and can use them. In every case of receptor-

facilitated delivery, cholesteryl esters and triglycerides in the lipoprotein core must cross two topological barriers to enter the cytoplasmic space: the phospholipid monolayer shell of the lipoprotein particle and the cell's bilayer plasma membrane. At some point in the lipid-delivery process, the esterified transport forms of the core lipids must be hydrolyzed to unesterified forms (cholesterol, fatty acids) to be usable by the importing cells. Cells have evolved two distinct mechanisms for receptor-facilitated uptake of lipids in lipoprotein cores: **receptor-mediated endocytosis** of entire lipoprotein particles and **selective lipid uptake** of certain lipid components of lipoprotein particles.

Analysis of Familial Hypercholesterolemia Revealed the Pathway for Receptor-Mediated Endocytosis of LDL Particles

There is no better example of the synergistic relation between basic molecular cell biology and medicine than the story of the discovery of the LDL receptor (LDLR) pathway for delivering cholesterol to cells. The series of elegant and Nobel Prize–winning studies leading to this discovery served as sources of insight into the mechanisms underlying LDL metabolism, the functions and properties of several key organelles, cellular systems for coordinately regulating complex metabolic pathways, and new approaches for treating atherosclerosis.

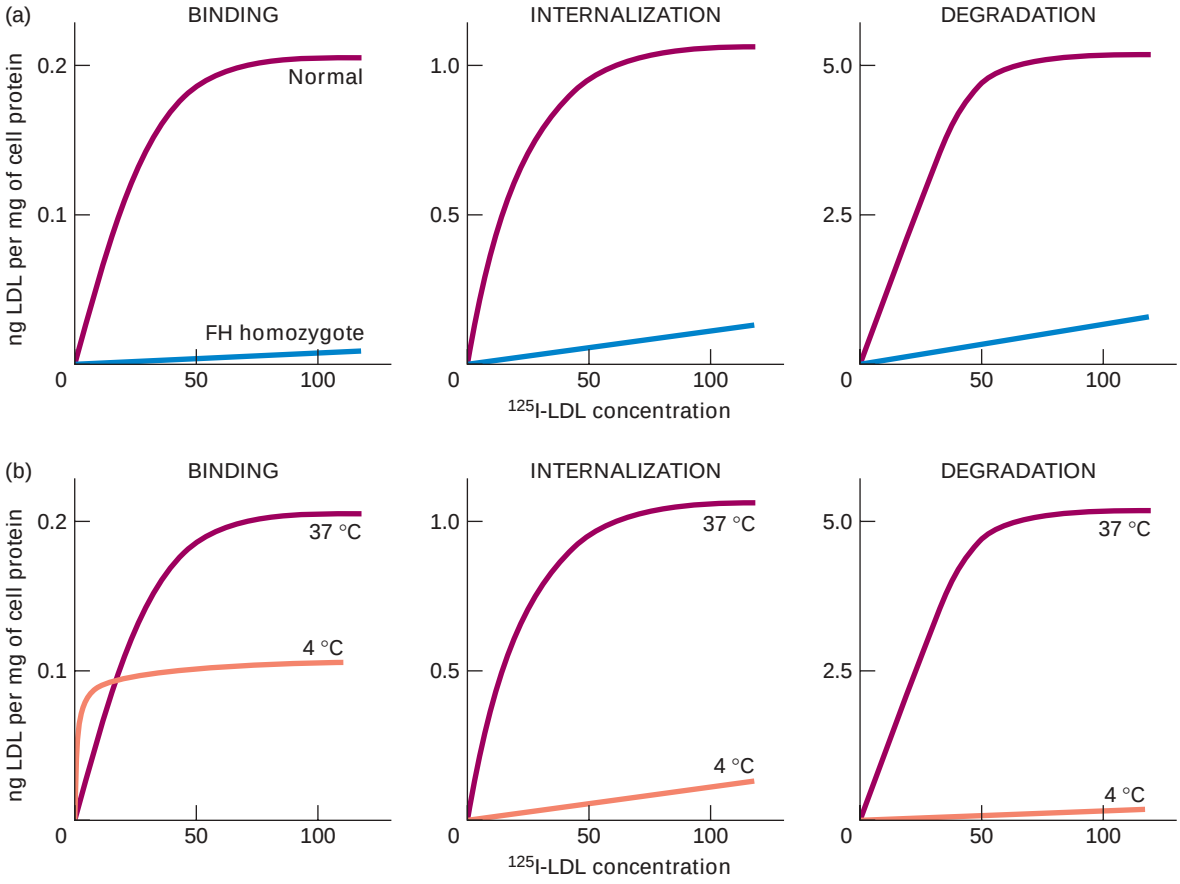
Some of these experiments compared LDL metabolism in normal human cells and in cells from patients with *familial hypercholesterolemia (FH)*, a hereditary disease that is marked by elevated plasma LDL cholesterol and is now known to be caused by mutations in the *LDLR* gene. In patients who have one normal and one defective copy of the *LDLR* gene (heterozygotes), LDL cholesterol is increased about twofold. Those with two defective *LDLR* genes (homozygotes) have LDL cholesterol levels that are from fourfold to sixfold as high as normal. FH heterozygotes commonly develop cardiovascular disease about 10 years earlier than normal people do, and FH homozygotes usually die of heart attacks before reaching their late 20s. ▮

Here, we illustrate how analysis of the cellular defects underlying familial hypercholesterolemia can illuminate normal cellular processes. First let's consider typical cell-culture experiments in which the interactions of LDL with normal and FH homozygous cells were examined as a function of LDL concentration, which defined the high-affinity LDL receptor, and incubation temperature, which established the temperature dependence of LDL uptake. In these experiments, purified LDL was first labeled by the covalent attachment of radioactive ^{125}I to the side chains of tyrosine residues in apoB-100 on the surfaces of the LDL particles. Cultured cells from normal persons and FH patients were incubated for several hours with the labeled LDL. Investigators then determined how much LDL was bound to the surfaces of cells, how much was internalized, and how much of the apoB-100 component of the LDL was degraded by enzymatic hydrolysis to individual amino acids. The degradation of apoB-100 was detected by the release of ^{125}I-tyrosine into the culture medium.

We can see from the results shown in Figure 18-14a that, compared with normal cells, homozygous FH cells are clearly defective in the binding and internalization of added LDL and in the degradation of apoB-100 at the normal physiologic temperature of 37 °C. The homozygous cells exhibit essentially no activity. Heterozygous cells exhibit about half the activity of normal cells. The shape of the binding curve for normal cells is consistent with a receptor that has a high affinity for LDL and is saturable. Note also that the curves for LDL internalization and degradation have the same shape as the binding curve. Moreover, when the experiments were performed with normal cells at 4 °C, LDL binding was observed, but internalization and degradation were inhibited (Figure 18-14b). Low temperature does not normally inhibit the binding of molecules to cell-surface receptors, but it does inhibit processes, such as the internalization and subsequent degradation of molecules, that depend on membrane trafficking (Chapter 17). Thus these results suggest that LDL first binds to cell-surface receptors and is subsequently internalized and degraded. One final feature of these results is worth noting. After cells were incubated for 5 hours at 37 °C, the amounts of internalized LDL and hydrolyzed apoB-100 were substantially greater than those of surface-bound LDL. This result indicates that each receptor molecule bound and mediated the internalization of more than one LDL particle in the incubation period. In other words, the LDL receptor is recycled.

Pulse-chase experiments with normal cells and a fixed concentration of ^{125}I-labeled LDL helped to further define the time course of events in receptor-mediated cellular LDL processing. These experiments clearly demonstrate the order of events: surface binding of LDL → internalization → degradation (Figure 18-15). The results of electron microscopy studies with LDL particles tagged with an electron-dense label revealed that LDL first binds to clathrin-coated endocytic pits that invaginate and bud off to form coated vesicles and then endosomes (see Figure 17-27). Findings from further experiments showed that the LDL receptor recognizes apoB-100 and one or two closely related apolipoproteins; thus binding by this receptor is highly specific for LDL. Binding is also pH dependent: strong binding of LDL occurs at the pH of extracellular fluid (7.4); weak or no binding occurs at the lower pH (≈4.5–6) found in some intracellular organelles (e.g., endosomes and lysosomes). Because of this property, the LDL receptor releases bound LDL within intracellular vesicles and can be recycled to the cell surface.

A variety of mutations in the gene encoding the LDL receptor can cause familial hypercholesterolemia. Some mutations prevent the synthesis of the LDLR protein; others prevent proper folding of the receptor protein in the ER,

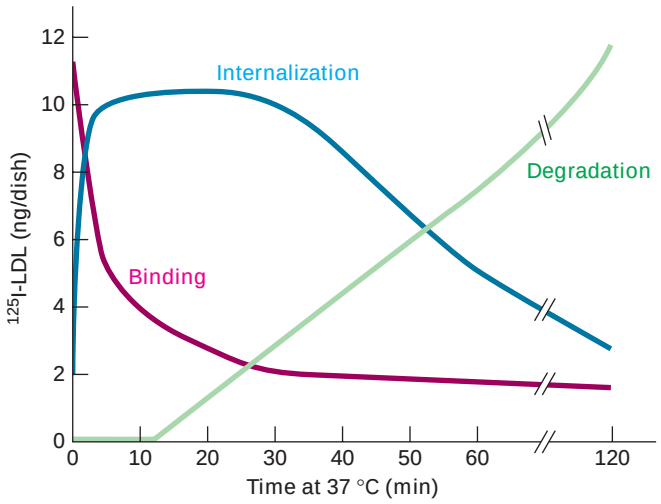


▲ EXPERIMENTAL FIGURE 18-14 **LDL binding, internalization, and degradation are reduced in cells from patients with familial hypercholesterolemia (FH) and are influenced by temperature.** Cultured fibroblasts were incubated for 5 hours in a medium containing LDL whose apolipoprotein B (apoB) component was labeled on tyrosine side chains with ^{125}I. The amounts of LDL bound to the cell surface (binding) and present within cells (internalization), as well as the amount of ^{125}I-tyrosine released into the medium owing to apoB degradation (hydrolysis), were determined as a function of the LDL concentration. (a) The curves shown here represent typical results from experiments at 37 °C with cells from a normal person and from a person homozygous for FH. Note that the y-axis scales are different and that the relative maximal values for binding internalization and hydrolysis are approximately 0.2:1:5. (b) These results for normal cells at 37 °C and 4 °C show that LDL binding is not reduced dramatically at low temperature, whereas subsequent internalization and hydrolysis are blocked. These findings suggest that internalization and hydrolysis entail membrane movements, which are typically inhibited at low temperatures. See text for discussion. [Adapted from M. S. Brown and J. L. Goldstein, 1979, *Proc. Nat'l. Acad. Sci. USA* **76**:3330, and J. L. Goldstein and M. S. Brown, 1989, in C. R. Scriver et al., eds., *The Metabolic Basis of Inherited Disease*, 6th ed, McGraw-Hill, p. 1215.]

leading to its premature degradation (Chapter 16); still other mutations reduce the ability of the LDL receptor to bind LDL tightly. A particularly informative group of mutant receptors are expressed on the cell surface and bind LDL normally but cannot mediate the internalization of bound LDL. Analyses of such defective LDL receptors led to the concept of internalization sequences in cell-surface proteins destined for endocytosis by means of clathrin-coated pits. As discussed in Chapter 17, such sorting signals, located in the cytosolic domains of certain membrane proteins, play a key role in directing these proteins to particular vesicles.

The results of these pioneering studies and other research led to the current model for the receptor-mediated endocytosis of LDL and other receptor–ligand combinations detailed in Chapter 17 (see in particular Figure 17-28) and summarized in Figure 18-10b. After internalized LDL particles reach lysosomes, lysosomal proteases hydrolyze their surface apolipoproteins and lysosomal cholesteryl esterases hydrolyze their core cholesteryl esters. The unesterified cholesterol is then free to leave the lysosome and be used as necessary by the cell in the synthesis of membranes or various cholesterol derivatives. The export of cholesterol from lysosomes depends on the NPC1 protein mentioned previously.

If LDLR-mediated endocytosis were not regulated, cells would continuously take up LDL and accumulate massive amounts of LDL-derived cholesterol because of the recycling

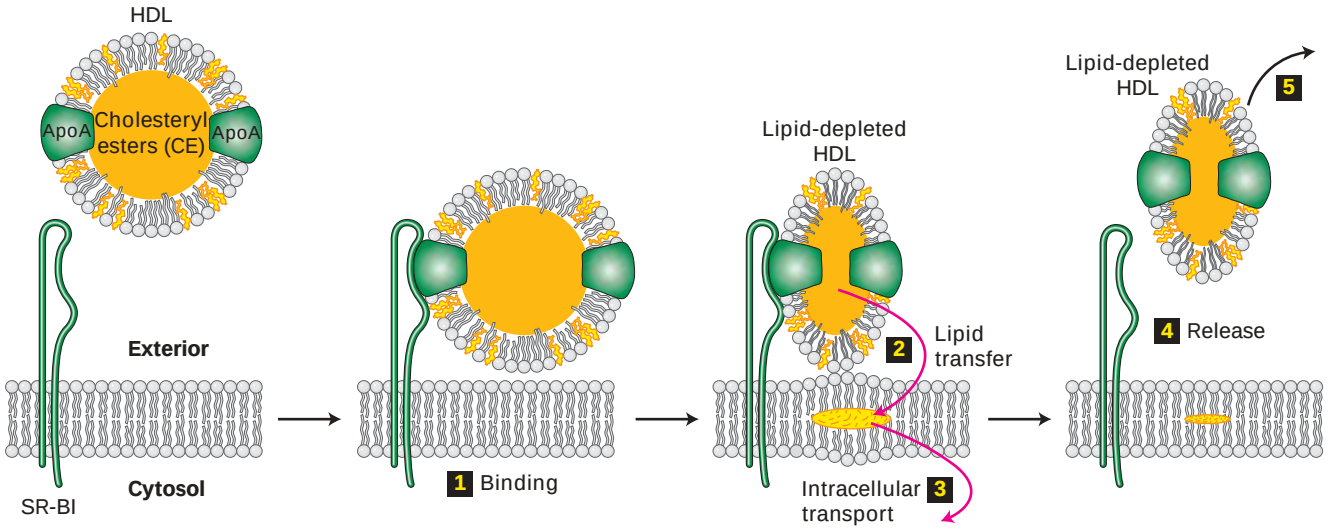


▲ EXPERIMENTAL FIGURE 18-15 **Pulse-chase experiment demonstrates precursor–product relations in cellular uptake of LDL.** Cultured normal human skin fibroblasts were incubated in a medium containing ^{125}I-LDL for 2 hours at 4 °C (the pulse). After excess ^{125}I-LDL not bound to the cells was washed away, the cells were incubated at 37 °C for the indicated amounts of time in the absence of external LDL (the chase). The amounts of surface-bound, internalized, and degraded (hydrolyzed) ^{125}I-LDL were measured as in the experiments presented in Figure 18-14. Binding but not internalization or hydrolysis of LDL apoB-100 occurs during the 4 °C pulse. The data show the very rapid disappearance of bound ^{125}I-LDL from the surface as it is internalized after the cells have been warmed to allow membrane movements. After a lag period of 15–20 minutes, lysosomal degradation of the internalized ^{125}I-LDL commences. [See M. S. Brown and J. L. Goldstein, 1976, *Cell* **9**:663.]

of receptors and the large reservoir of LDL in the bloodstream. However, an elegant regulatory system described in Section 18.4 limits LDL uptake. LDL delivers most of its cholesterol to the liver, which expresses the majority of the body's LDL receptors. The LDL receptor not only interacts with apoB-100 on the surfaces of LDL particles, but also binds to apoE on the surfaces of chylomicron remnants and intermediate-density lipoprotein (IDL) particles. A related receptor called LRP (LDLR-related protein) recognizes apoE but not apoB-100; thus LRP mediates the endocytosis of chylomicron remnants and IDL, but not LDL, by hepatocytes and some other cells (see Figure 18-13b).

Cholesteryl Esters in Lipoproteins Can Be Selectively Taken Up by the Receptor SR-BI

Findings from studies of HDL metabolism led to the discovery of a second, distinct mechanism of receptor-facilitated uptake of lipoprotein lipids. In these studies, experimental animals were injected with purified HDL particles in which the apolipoproteins were labeled with ^{125}I and the core cholesteryl esters were labeled with ^{3}H. The liver and steroid hormone–producing (steroidogenic) tissues in injected animals accumulated substantial amounts of the labeled cholesterol but not the associated labeled apolipoproteins. Conversely, a large amount of the ^{125}I label, but not the ^{3}H label, was ultimately found in the kidneys, where the apolipoproteins are degraded. These findings are inconsistent with receptor-mediated endocytosis of the entire particle. Rather, liver and steroidogenic cells selectively take up cholesteryl esters from the cores of HDL particles without accumulating the components of the outer shells.



▲ FIGURE 18-16 **Model for the selective uptake of cholesteryl esters from HDL mediated by the receptor SR-BI.** After HDL binding to SR-BI 1, cholesteryl esters in the core are selectively transferred to a cell's membrane 2 and then into the cytosol 3 by as-yet-unknown mechanisms. The remaining lipid-depleted HDL particle rapidly dissociates from the receptor 4 and eventually returns to the circulation 5. [See C. Glass et al., 1983, *Proc. Nat'l. Acad. Sci. USA* **80**:5435; Y. Stein et al., 1983, *Biochim. Biophys. Acta* **752**:98; and M. Krieger, 1999, *Ann. Rev. Biochem.* **68**:523.]

Figure 18-16 depicts a model for the selective uptake of cholesteryl esters by a cell-surface receptor called *SR-BI (scavenger receptor, class B, type I)*. SR-BI binds HDL, LDL, and VLDL and can mediate selective uptake from all of these lipoproteins. The detailed mechanism of selective lipid uptake has not yet been elucidated, but it may entail hemifusion of the outer phospholipid monolayer of the lipoprotein and the exoplasmic leaflet of the plasma membrane. The cholesteryl esters initially enter the hydrophobic center of the plasma membrane, are subsequently transferred across the inner leaflet, and are eventually hydrolyzed by cytosolic, not lysosomal, cholesteryl esterases. The lipid-depleted particles remaining after lipid transfer dissociate from SR-BI and return to the circulation; they can then extract more phospholipid and cholesterol from other cells by means of the ABCA1 protein or other cell-surface transport proteins (see Figure 18-13c). Eventually, small lipid-depleted HDL particles circulating in the bloodstream are filtered out by the kidney and bind to a different receptor on renal epithelial cells. After these particles have been internalized by receptor-mediated endocytosis, they are degraded by lysosomes.

The receptor SR-BI differs in two important respects from the LDL receptor. First, SR-BI clusters on microvilli and in cell-surface **lipid rafts** (Chapter 5), not in coated pits as does the LDL receptor. Second, SR-BI mediates the transfer of lipids across the membrane, not endocytosis of entire LDL particles as mediated by the LDL receptor. A multifunctional receptor, SR-BI can mediate the selective uptake from lipoproteins of diverse lipids (e.g., cholesteryl esters, vitamin E); it also functions in the reverse direction to facilitate the export of unesterified cholesterol from cells to bound lipoproteins. SR-BI has a structure similar to that of the fatty acid transporter CD36, and they both belong to the superfamily of *scavenger receptors;* as discussed later, some of these receptors apparently play a role in the onset of atherosclerosis.

KEY CONCEPTS OF SECTION 18.3

Lipid Movement into and out of Cells

- Movement of lipids into and out of cells usually requires either cell-surface transport proteins and water-soluble binding proteins (or micelles), or lipoprotein secretion and lipoprotein receptor–facilitated uptake (see Figure 18-10).
- Fatty acid transporters (FATPs, CD36) in the plasma membrane facilitate the movement of fatty acids between intracellular binding proteins (e.g., FABP) and extracellular carriers (e.g., albumin).
- The ABC superfamily comprises ATP-hydrolyzing small-molecule pumps. Many ABC proteins mediate the export of various lipids from cells (see Table 18-2).
- Several ABC proteins export bile components from hepatocytes: ABCB4 (phospholipids), ABCB11 (bile salts), and ABCG5/8 (cholesterol and plant sterols). Na^+-linked lipid symporters (e.g., IBAT and NTCP), which mediate the cellular uptake of bile acids, also play a key role in the enterohepatic circulation (see Figure 18-11).
- Lipoproteins are large particles with cores of neutral lipids (cholesteryl esters, triglycerides) and amphipathic shells composed of apolipoproteins, a monolayer of phospholipids, and unesterified cholesterol.
- Each class of lipoprotein has a characteristic protein and lipid composition and functions in the cellular export, extracellular transport through the circulatory system, and receptor-mediated cellular import of lipids (see Table 18-3 and Figure 18-13).
- Familial hypercholesterolemia is caused by mutations in the gene encoding the low-density lipoprotein (LDL) receptor. Persons with this disorder have elevated plasma LDL levels and develop cardiovascular disease at abnormally young ages.
- Receptor-mediated endocytosis of lipoproteins, such as LDL, is one mechanism for delivering cholesterol to cells.
- In a second mechanism for delivering cholesterol to cells, the receptor SR-BI mediates the selective uptake of cholesteryl esters from high-density lipoprotein, LDL, and very low density lipoprotein. The resulting lipid-depleted lipoprotein particle is subsequently released from the cell and can be reused (see Figure 18-16).

18.4 Feedback Regulation of Cellular Lipid Metabolism

As is readily apparent, a cell would soon face a crisis if it did not have enough lipids to make adequate amounts of membranes or had so much cholesterol that large crystals formed and damaged cellular structures. To prevent such disastrous events, cells normally maintain appropriate lipid levels by regulating their supply and utilization of lipids. We have seen how cells acquire lipids by biosynthesis or import and how they export lipids. In this section, we consider the regulation of cellular lipid metabolism, focusing on cholesterol. However, the regulatory pathways that control cellular cholesterol levels also function in controlling fatty acid and phospholipid metabolism. Coordinate regulation of the metabolism of these membrane components is necessary to maintain the proper composition of membranes.

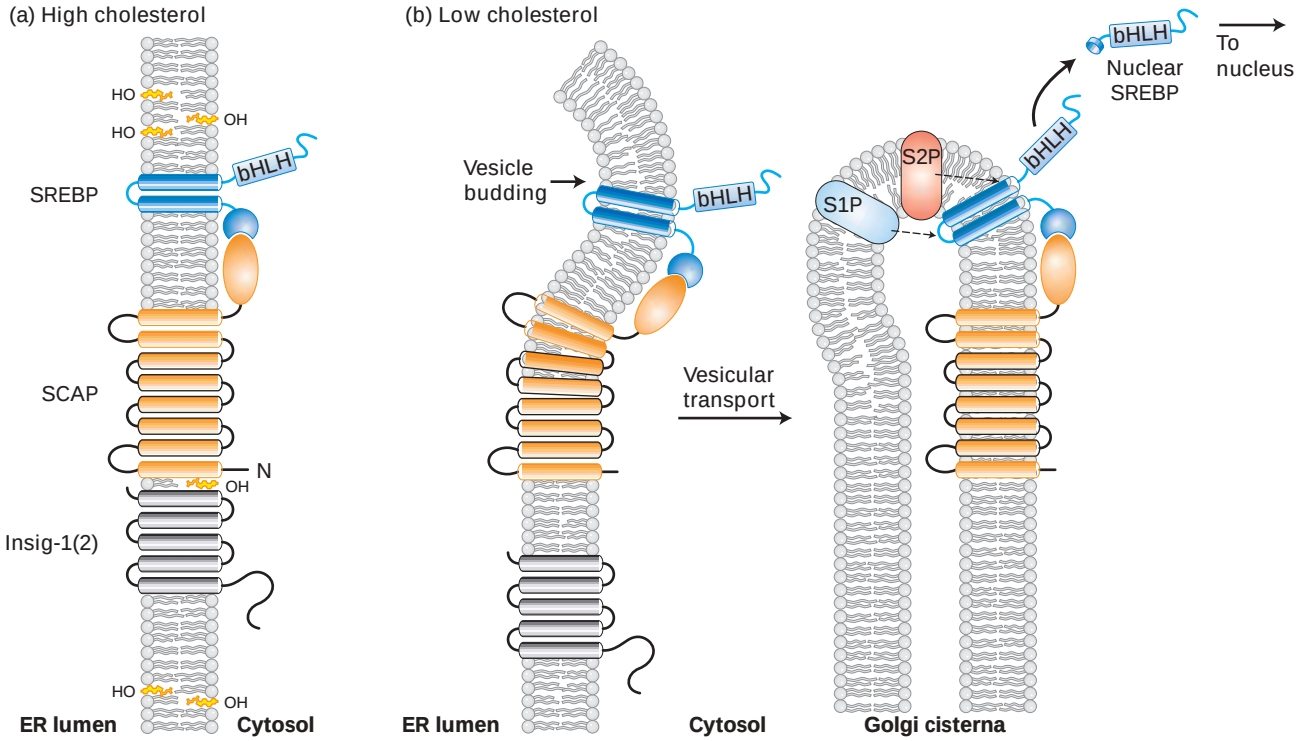
For more than 50 years the cholesterol biosynthetic pathway has been known to be subject to negative feedback regulation by cholesterol. Indeed, it was the first biosynthetic pathway shown to exhibit this type of end-product regulation. As the cellular cholesterol level rises, the need to import cholesterol through the LDL receptor or to synthesize additional cholesterol goes down. As a consequence, transcription of the genes encoding the LDL receptor and cholesterol biosynthetic enzymes decreases. For example, when normal cultured cells are incubated with increasing concentrations of

LDL, the expression and activity of HMG-CoA reductase, the rate-controlling enzyme in cholesterol biosynthesis, is suppressed, whereas the activity of acyl:cholesterol acyl transferase (ACAT), which converts cholesterol into the esterified storage form, is increased. Conversely, when the cellular cholesterol level begins to fall as cells use more cholesterol, expression of the LDL receptor and HMG-CoA reductase increases and the activity of ACAT decreases. Such coordinate regulation is an efficient way for cells to maintain cellular cholesterol homeostasis.

ER-to-Golgi Transport and Proteolytic Activation Control the Activity of SREBP Transcription Factors

Cholesterol-dependent transcriptional regulation often depends on 10-base-pair sterol regulatory elements (SREs), or SRE half-sites, in the promoters of regulated target genes. As you might expect from the discussion of transcriptional control in Chapter 11, the interaction of cholesterol-dependent **SRE-binding proteins (SREBPs)** with these response elements modulates the expression of the target genes. What you might not expect is that the SREBP-mediated pathway, whereby cholesterol controls the expression of proteins engaged in cholesterol metabolism, begins in the ER and includes at least two other proteins besides SREBP.

When cells have adequate concentrations of cholesterol, SREBP is found in the ER membrane complexed with *SCAP* (SREBP cleavage-activating protein), *insig-1* (or its close homolog insig-2), and perhaps other proteins (Figure 18-17, *left*). SREBP has three distinct domains: an N-terminal cytosolic domain that includes a basic helix-loop-helix (bHLH) DNA-binding motif (see Figure 11-22b) and functions as a transcription factor, a central membrane-anchoring domain containing two transmembrane α helices, and a C-terminal cytosolic regulatory domain. SCAP has eight transmembrane α helices and a large C-terminal cytosolic domain that interacts with the regulatory domain of SREBP. Five of the transmembrane helices in SCAP form a sterol-sensing domain, similar to that in HMG-CoA reductase. The sterol-sensing domain in SCAP binds tightly to insig-1(2), but only at high cellular cholesterol levels. When insig-1(2) is tightly bound to SCAP, it blocks the binding of SCAP to COP II vesicle coat proteins and thus prevents incorporation of the SCAP/SREBP complex into ER-to-Golgi transport vesicles (see Chapter 17).



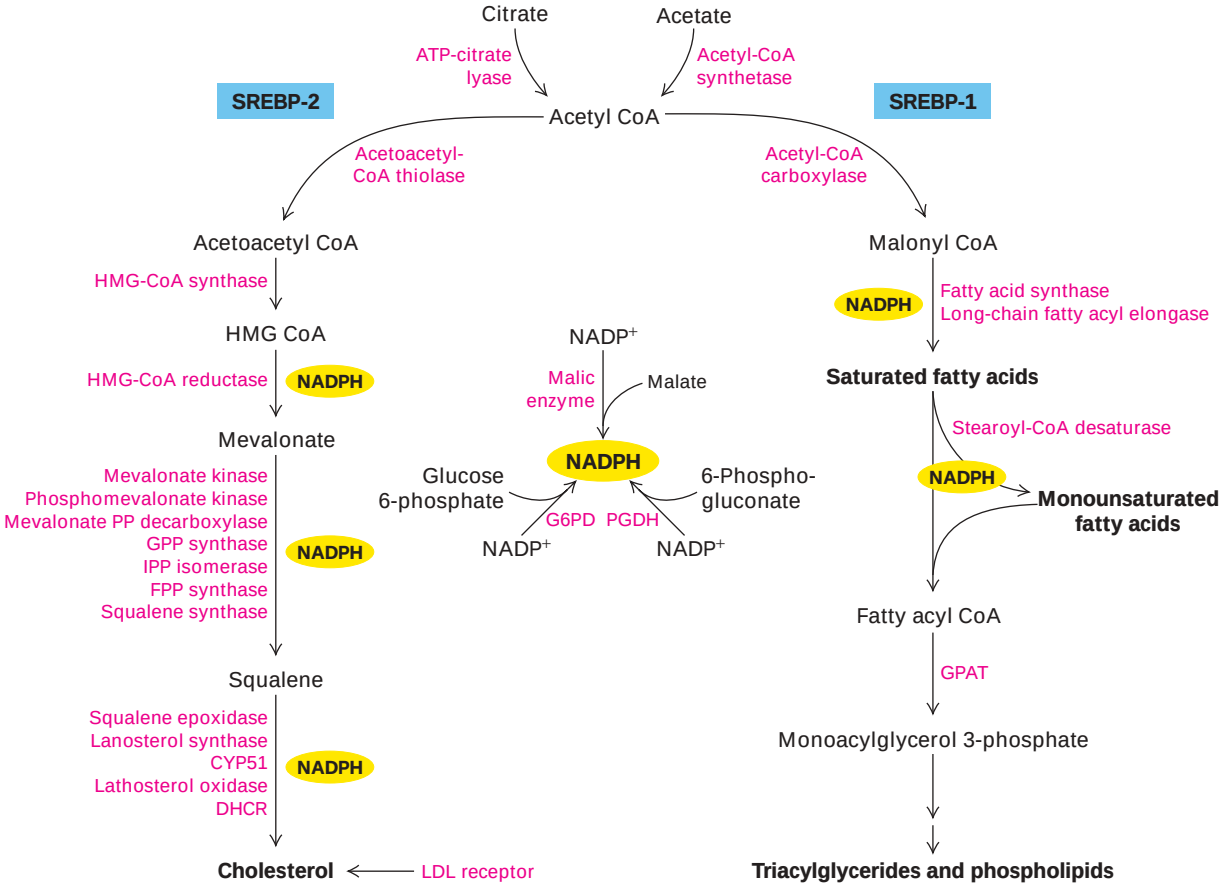
▲ FIGURE 18-17 **Model for cholesterol-sensitive control of SREBP activation mediated by insig-1(2) and SCAP.** The cellular pool of cholesterol is monitored by combined action of insig-1(2) and SCAP, both transmembrane proteins located in the ER membrane. (a) When cholesterol levels are high, insig-1(2) binds to the sterol-sensing domain in SCAP anchoring the SCAP/SREBP complex in the ER membrane. (b) The dissociation of insig-1(2) from SCAP at low cholesterol levels allows the SCAP/SREBP complex to move to the Golgi complex by vesicular transport. (*Right*) The sequential cleavage of SREBP by the site 1 and site 2 proteases (S1P, S2P) associated with the Golgi membrane releases the N-terminal bHLH domain, called nuclear SREBP (nSREBP). After translocating into the nucleus, nSREBP controls the transcription of genes containing sterol regulatory elements (SREs) in their promoters. [Adapted from T. F. Osborne, 2001, *Genes Devel.* **15**:1873; see T. Yang et al., 2002, *Cell* **110**:489.]

When cellular cholesterol levels drop, insig-1(2) no longer binds to SCAP, and the SCAP/SREBP complex can move from the ER to the Golgi apparatus (Figure 18-17, *right*). In the Golgi, SREBP is cleaved sequentially at two sites by two membrane-bound proteases, S1P and S2P. The second intramembrane cleavage at site 2 releases the N-terminal bHLH-containing domain into the cytosol. This fragment, called nSREBP (nuclear SREBP), binds directly to the nuclear import receptor and is rapidly translocated into the nucleus (see Figure 12-21). In the nucleus, nSREBP activates the transcription of genes containing SREs in their promoters, such as those encoding the LDL receptor and HMG-CoA reductase. After cleavage of SREBP in the Golgi, SCAP apparently recycles back to the ER where it can interact with insig-1(2) and another SREBP molecule. High-level transcription of SRE-controlled genes requires the ongoing generation of new nSREBP because it is degraded fairly rapidly by the ubiquitin-mediated proteasomal pathway (Chapter 3). The rapid generation and degradation of nSREBP help cells respond quickly to changes in levels of intracellular cholesterol.

In the *insig-1(2)/SCAP/SREBP pathway* for controlling cellular cholesterol metabolism, the cell exploits intercompartmental movements (ER → Golgi → cytosol → nucleus), regulated by sterol-dependent protein–protein interactions, and post-translational proteolytic cleavage to activate a membrane-bound transcription factor. Cleavage of SREBP in this pathway is one of several known examples of *regulated intramembrane proteolysis (RIP)*. For instance, RIP activates transcription factors in the Notch signaling pathway (Chapter 14) and in the unfolded-protein response (Chapter 16). RIP is also responsible for the generation of the toxic amyloid β peptides that contribute to the onset of Alzheimer's disease (see Figure 14-30).

Multiple SREBPs Regulate Expression of Numerous Lipid-Metabolizing Proteins

Under some circumstances (e.g., during cell growth), cells need an increased supply of all the essential membrane lipids and their fatty acid precursors (coordinate regulation). But



▲ FIGURE 18-18 **Global regulation of cellular lipid metabolism by SREBP.** SREBP controls the transcription of genes indicated here encoding key proteins directly required for the synthesis and import of cholesterol and for the synthesis of fatty acids, phospholipids, and triglycerides. SREBPs also regulate the activity of genes in the production of NADPH, which is an energy source for many of the steps in these biosynthetic pathways. Abbreviations: GPP = geranylgeranyl pyrophosphate; IPP = isopentenyl pyrophosphate; FPP = farnesyl pyrophosphate; CYP51 = lanosterol 14α-demethylase; DHCR = 7-dehydrocholesterol reductase; G6PD = glucose 6-phosphate dehydrogenase; PGDH = 6-phosphogluconate dehydrogenase; GPAT = glycerol 3-phosphate acyl transferase. [Adapted from J. D. Horton, J. L. Goldstein, and M. S. Brown, 2002, *J. Clin. Invest.* **109**:1128.]

cells sometimes need greater amounts of some lipids than others (differential regulation). For example, cells that are producing bile acids or steroid hormones need an increased supply of cholesterol but not of fatty acids or phospholipids. The complex regulation of lipid metabolism characteristic of higher eukaryotes is due largely to a plethora of transcription factors, including multiple SREBPs, that control the expression of proteins taking part in the synthesis, degradation, transport, and storage of lipids.

There are three known isoforms of SREBP in mammals: SREBP-1a and SREBP-1c, which are generated from alternatively spliced RNAs produced from the same gene, and SREBP-2, which is encoded by a different gene. Together these protease-regulated transcription factors control the availability not only of cholesterol but also of fatty acids and their products triglycerides and phospholipids. In mammalian cells, SREBP-1a and SREBP-1c exert a greater influence on fatty acid metabolism than on cholesterol metabolism, whereas the reverse is the case for SREBP-2. As indicated in Figure 18-18, SREBPs can regulate the activities of genes encoding many different proteins. Such proteins include those participating in the cellular uptake of lipids (e.g., the LDL receptor, SR-BI, and lipoprotein lipase) and numerous enzymes in the pathways for synthesizing cholesterol, fatty acids, triglycerides, and phospholipids.

SREBP-1 may play an important role in the development of *fatty liver*, a major pathologic consequence of alcohol abuse. In fatty liver, abnormally high levels of triglycerides and cholesterol accumulate in the cytosol as lipid droplets, which can contribute to alcoholic hepatitis and cirrhosis. The results of experiments using cultured hepatocytes and mice suggest that the metabolism of alcohol to acetaldehyde by hepatic alcohol dehydrogenase leads to the activation of SREBP-1 and the release of nSREBP-1, which in turn induces the synthesis of excess fatty acids and triglycerides. Consistent with this suggestion is the finding that overexpression of a truncated, constitutively active form of SREBP-1 (i.e., nSREBP-1) in the livers of mice significantly increases both fatty acid and cholesterol synthesis, resulting in a fatty liver. ▮

In contrast with the insig-1(2)/SCAP/SREBP pathway in mammalian cells, the homologous pathway in *Drosophila* does not respond to changes in cellular sterol levels. Instead, the SCAP-dependent proteolytic activation of SREBP is suppressed by high levels of phosphatidylethanolamine, the main phospholipid in fruit flies. This finding, the result of an elegant series of experiments using both enzyme inhibitors and RNA interference (RNAi), indicates that the sterol-sensing domain of *Drosophila* SCAP responds to the cellular level of phosphatidylethanolamine, not cholesterol. Thus so-called sterol-sensing domains might more appropriately be called *lipid-sensing* domains. Whether these domains directly bind to their controlling lipids or mediate interaction with other proteins that directly bind the lipids (i.e., sense the levels of the regulatory lipids) is not yet known.

As mentioned previously, HMG-CoA reductase also contains a sterol-sensing domain. This domain senses high levels of cholesterol, some cholesterol derivatives, and certain nonsteroidal precursors of cholesterol, triggering the rapid, ubiquitin-dependent proteasomal degradation of the enzyme. As a consequence, HMG-CoA reductase activity drops, causing reduced cholesterol synthesis. Like SCAP, HMG-CoA reductase is located in the ER membrane and insig-1(2) binds to its sterol-sensing domain. This binding also is cholesterol dependent and is required for the cholesterol-dependent proteasomal degradation of HMG-CoA reductase. Thus mammalian insig-1(2) and the sterol-sensing domain of SCAP or HMG-CoA reductase apparently combine to form a cholesterol sensor. It seems likely that, in the course of evolution, the sterol-sensing domain and its associated proteins proved effective for recognizing various lipid molecules and were incorporated into a variety of regulatory systems for this purpose.

Members of the Nuclear Receptor Superfamily Contribute to Cellular and Whole-Body Lipid Regulation

In addition to SREBPs, several members of the nuclear receptor superfamily regulate lipid metabolism. **Nuclear receptors** are transcription factors that are generally activated when bound to specific small-molecule ligands (Chapter 11). Certain nuclear receptors influence whole-body lipid metabolism by regulating the absorption of dietary lipids, cellular synthesis of lipids, transport protein–mediated import and export of lipids, levels of lipoproteins and their receptors, and catabolism of lipids (e.g., fatty acid oxidation in the peroxisome) and their secretion from the body.

Some ligands for nuclear receptors are extracellular molecules that diffuse across the plasma membrane (e.g., steroid hormones) or enter cells through transporters (e.g., bile acids, fatty acids). Alternatively, ligands generated within a cell, including oxygen-modified cholesterol (*oxysterols*), bile acids, and certain fatty acids and their derivatives, may bind to nuclear receptors within the same cell. Nuclear receptors sense changes in the levels of all key cellular lipids by binding the lipids themselves or their metabolic products. When activated, these receptors stimulate or suppress gene expression to ensure that the proper physiological levels of lipids are maintained (feedback regulation for cellular homeostasis). The binding of multiple types of lipids to an individual nuclear receptor allows the receptor to coordinately control several metabolic pathways.

For example, hepatocytes express *LXR (liver X receptor)*, a nuclear receptor that senses the levels of oxysterols. When cellular cholesterol increases in the liver, oxysterols are generated and activate LXR. Activated LXR stimulates the expression of cholesterol 7α-hydroxylase, the key rate-limiting enzyme in the hepatic conversion of cholesterol into bile acids, a major pathway for disposing of excess cholesterol from the body. LXR also stimulates the expression of the ABC proteins that export cholesterol into the bile (ABCG5/8) or onto lipoproteins in the blood (ABCA1). In

addition, LXR promotes lipoprotein production and modification and the expression of SREBP-1c, which then turns on the transcription of genes required for fatty acid synthesis. The resulting increase in fatty acids can contribute to cholesterol esterification and phospholipid synthesis to maintain the proper ratio of cholesterol to phospholipid. Thus sensing of increased cellular cholesterol by LXR results in diverse responses that prevent the accumulation of excess cholesterol.

Another nuclear receptor, called *FXR*, is activated by the binding of bile acids. Expressed in hepatocytes and intestinal epithelial cells, FXR plays a key role in regulating the enterohepatic circulation of bile acids. Bile acid–activated FXR stimulates the expression of intracellular bile acid–binding protein (I-BABP) and of transport proteins (e.g., ABCB11, NTCP) that mediate cellular export and import of bile acids (see Figure 18-11). In contrast, active FXR represses the expression of cholesterol 7α-hydroxylase, thereby decreasing the synthesis of bile acids from cholesterol in the liver—another example of end-product inhibition of a metabolic pathway. Both FXR and LXR function as heterodimers with the nuclear receptor RXR.

In the next section, we will see how an understanding of the SREBP and nuclear receptor regulatory pathways has contributed to effective strategies for reducing the risk of atherosclerosis and cardiovascular disease.

KEY CONCEPTS OF SECTION 18.4

Feedback Regulation of Cellular Lipid Metabolism

- Two key transcription-control pathways are employed to regulate the expression of enzymes, transporters, receptors, and other proteins taking part in cellular and whole-body lipid metabolism.
- In the insig-1(2)/SCAP/SREBP pathway, the active nSREBP transcription factor is released from the Golgi membrane by intramembrane proteolysis when cellular cholesterol is low (see Figure 18-17). It then stimulates the expression of genes with sterol regulatory elements (SREs) in their promoters (e.g., the genes for LDLR and HMG-CoA reductase). When cholesterol is high, SREBP is retained in the ER membrane complexed with insig-1(2) and SCAP.
- SREBP controls the transcription of numerous genes encoding proteins having roles in cellular lipid metabolism (see Figure 18-18). In mammals, SREBP-1a and SREBP-1c have a greater influence on fatty acid metabolism than on cholesterol metabolism; the reverse is the case for SREBP-2.
- In the nuclear-receptor pathway, transcription factors in the cytosol are activated by intracellular lipids (e.g., high levels of oxysterols or bile acids). The ligand–transcription factor complex then enters the nucleus and regulates the expression of specific target genes that participate in feedback regulation of the synthesis, transport, and catabolism of lipids.
- Homologous transmembrane sterol-sensing domains are present in several integral membrane proteins participating in lipid metabolism (e.g., SCAP, HMG-CoA reductase, NPC1). These domains appear to help detect and respond to changes in the levels of a variety of lipids, not just sterols. Binding to insig-1(2) is essential for sterol-sensing by at least some of these domains.

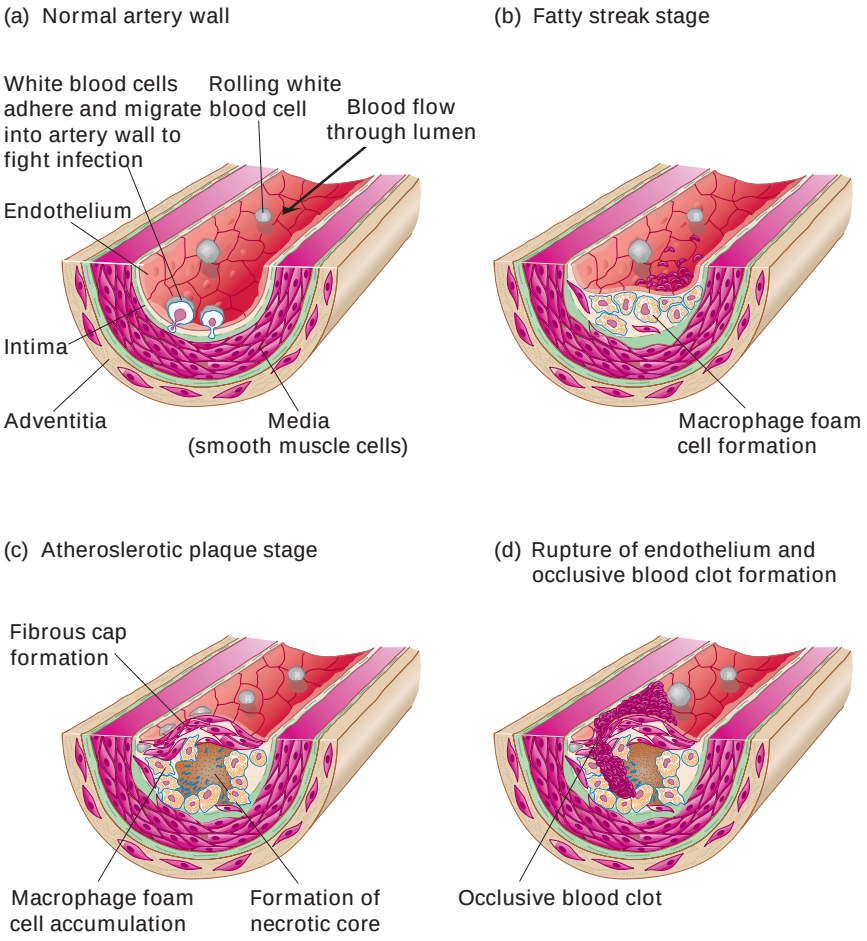
18.5 The Cell Biology of Atherosclerosis, Heart Attacks, and Strokes

In this concluding section we examine the relation between lipid metabolism and *atherosclerosis*, the most common cause of heart attacks and strokes. Atherosclerosis accounts for 75 percent of deaths due to cardiovascular disease in the United States. Advances in our understanding of the molecular mechanisms underlying lipid metabolism and its regulation are having an enormous effect on the treatment and prevention of this major health problem.

Frequently called cholesterol-dependent clogging of the arteries, atherosclerosis is characterized by the progressive deposition of lipids, cells, and extracellular matrix material in the inner layer of the wall of an artery. The resulting distortion of the artery's wall can lead, either alone or in combination with a blood clot, to major blockage of blood flow. Thus, to understand the cellular basis of atherosclerosis, we need to first briefly consider the structure of an artery.

Specialized epithelial cells called endothelial cells form a thin layer, the **endothelium,** that lines the blood vessel wall immediately adjacent to the lumen through which the blood flows (Figure 18-19a). Beneath the endothelium are several concentric layers of extracellular matrix and cells that make up the artery wall: the *intima*, composed largely of amorphous collagens, proteoglycans, and elastic fibers; the *media*, a well-organized layer of smooth muscle cells whose contraction controls the diameter of the vessel lumen and thus influences blood pressure; and the *adventitia*, a layer of connective tissue and cells that forms the interface between the vessel and the tissue through which it runs.

Under normal circumstances, the plasma (the fluid part of blood) and many types of blood cells flow smoothly and rapidly through the lumen of an artery, a type of movement termed laminar flow. When an infection or traumatic damage occurs within the walls of an artery or in the underlying tissue, a complex series of events permits white blood cells (leukocytes) to initially adhere loosely to the luminal surface of the artery wall and roll, propelled by the laminar flow of the surrounding plasma (see Figure 6-30). Subsequently, adhesion molecules mediate firm attachment of the white cells to the endothelium and their movement across the endothelium into the wall. Within the artery wall, white blood cells called monocytes differentiate into *macrophages*, which fight infection in a number of ways. For instance, macrophages and other leukocytes release proteins and small molecules



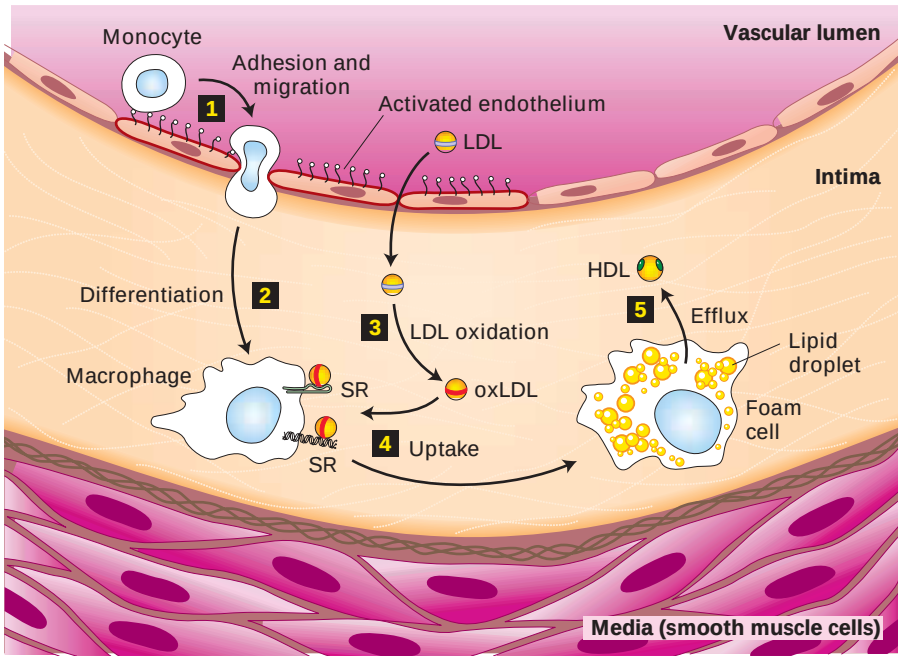
▲ FIGURE 18-19 **Major stages in the onset and progression of atherosclerosis in the artery wall.** (a) The anatomy of a normal artery wall, which is composed of concentric layers of cells and extracellular matrix, is shown. White blood cells adhere to the endothelium, roll along it, and then migrate into an artery wall to fight infection (see Figure 6-30). (b) When plasma LDL is high or plasma HDL is low, or both, macrophages in the intima can accumulate lipoprotein cholesterol, generating foam cells filled with cholesteryl ester droplets (see Figure 18-20). Accumulation of foam cells produces a fatty streak in the vessel wall that is only visible microscopically. (c) Continued generation of foam cells and migration of smooth muscle cells from the media into the intima is followed by cell death, producing an advanced atherosclerotic plaque. This plaque consists of a necrotic core of lipids (including needlelike cholesterol crystals) and extracellular matrix overlain by a fibrous cap of smooth muscle cells and matrix. (d) As an atherosclerotic plaque grows into the lumen of the artery, it disrupts and reduces the flow of blood. In some cases, the plaque alone can fully occlude the artery. In many cases, the fibrous cap ruptures, inducing formation of a blood clot that can fully occlude the artery. [Adapted from R. Russell, 1999, *N. Engl. J. Med.* **340**(2):115.]

that directly attack bacteria and other pathogens. The cells also secrete proteins that help recruit additional monocytes and other immune cells (e.g., T lymphocytes) to join in the fight. Macrophages also engulf and destroy pathogens, damaged macromolecules, and infected or dead body cells. When the infection has been cured, damaged tissue is repaired and the remaining macrophages and other leukocytes move out of the artery wall and reenter the circulation.

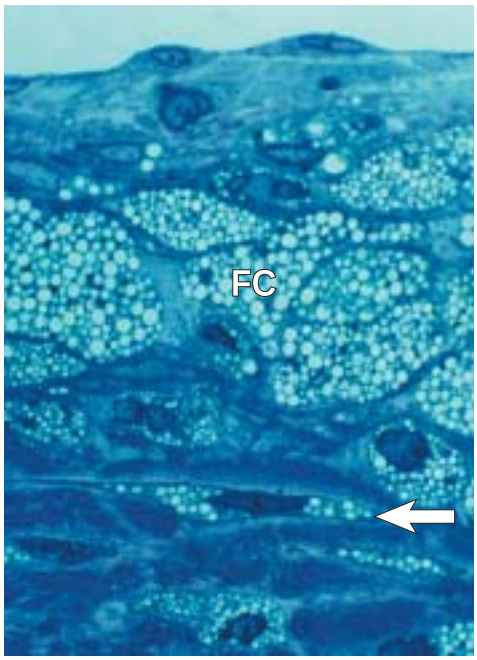
As we will see, atherosclerosis is an "unintended" consequence of this normal physiological *inflammatory response,* which is designed to protect against infection and tissue damage. For this reason and because atherosclerosis most often strikes late in life after the prime reproductive years, there appears to have been little evolutionary selective pressure against the disease. Thus, although atherosclerosis has an enormous negative influence on modern human populations, its high incidence in well-fed, long-lived persons is not surprising.

Arterial Inflammation and Cellular Import of Cholesterol Mark the Early Stages of Atherosclerosis

During an inflammatory response, macrophages in the inflamed artery wall can endocytose substantial amounts of cholesterol from lipoproteins, which accumulate within the artery wall under some circumstances (Figure 18-20a). As macrophages convert the imported cholesterol into the ester



(a)



(b)

▲ FIGURE 18-20 **Generation of macrophage foam cells in an artery wall.** (a) At a site of infection or damage (1), monocytes adhere to and migrate across the activated endothelial cell layer into the intima (2), where they differentiate into macrophages. When plasma LDL levels are high, the concentration of LDL in the intima is high, and some of the LDL is oxidized to oxLDL or otherwise modified (3). Scavenger receptors expressed by macrophages are proposed to bind to and endocytose oxLDL, which is degraded. Its cholesterol accumulates as cholesteryl esters in cytosolic lipid droplets, leading to an accumulation of cholesterol and the formation of foam cells (4). Macrophages also express ABCA1 and SR-BI, which can mediate the efflux of excess cellular cholesterol to HDL in the intima (5). Thus the amount of cholesterol accumulation is determined by the relative uptake of LDL-derived cholesterol and efflux to HDL. (b) Micrograph of a coronary artery with an atherosclerotic plaque containing many intimal macrophage foam cells (FC) filled with spherical cholesteryl ester lipid droplets (light circles). Some smooth muscle cells also are present and also contain lipid droplets (arrow). [Part (a) adapted from C. K. Glass and J. L. Witztum, 2001, *Cell* **104**:503. Part (b) from H. C. Stary, 2003, *Atlas of Atherosclerosis Progression and Regression,* 2d ed., Parthenon Publishing, p. 61.]

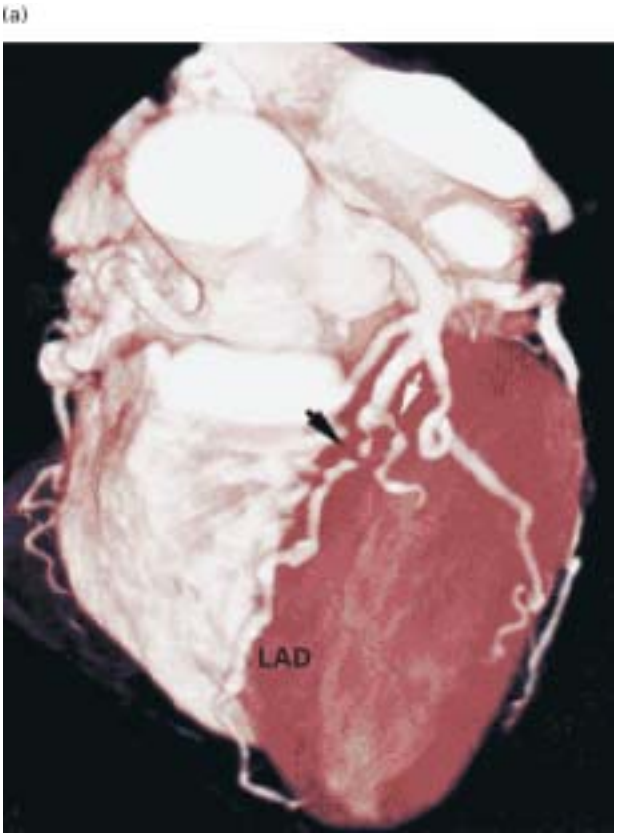
form, they become filled with cholesteryl ester lipid droplets. The resulting lipid-filled macrophages are called *foam cells* because the lipid droplets have a foamy appearance (Figure 18-20b). As macrophage foam cells accumulate in an artery wall, they initially form an early *fatty streak*, the first unique step in atherosclerosis (Figure 18-19b).

The next stage in atherosclerosis is marked by the continued accumulation of macrophage foam cells, proliferation of smooth muscle cells, and migration of these cells from the media into the intima. The smooth muscle cells secrete additional extracellular matrix, and some internalize sufficient amounts of lipoprotein cholesterol to also become foam cells. The initial macroscopically invisible early fatty streak grows bigger as the disease progresses, forming an early **atherosclerotic plaque,** or atheromatous plaque. Cells within the center of the plaque die, producing a necrotic core containing large amounts of cholesteryl esters and unesterified cholesterol (Figure 18-19c). Cholesterol crystals, readily detected microscopically, commonly form within a more advanced plaque, which is eventually covered by a fibrous cap composed of smooth muscle cells and collagen.

Atherosclerotic Plaques Can Impede Blood Flow, Leading to Heart Attacks and Strokes

As an atherosclerotic plaque expands, it projects farther and farther into the lumen of the vessel, narrowing the lumen and distorting the normal shape of the endothelium lining the vessel. Because blood flow through the affected artery is reduced and disturbed, the rate of delivery of nutrient-rich, oxygenated blood to tissues fed by the artery decreases, a condition known as *ischemia.* If sufficiently severe, such partial starvation of the heart can cause pain (angina).

If the endothelial lining covering a plaque ruptures, a large platelet and fibrin blood clot (thrombus) can form very rapidly and block or occlude the artery (Figure 18-19d and Figure 18-21). Tissue downstream of an occlusion soon becomes depleted of oxygen (ischemic hypoxia) and energy sources (e.g., fatty acids in the adult heart, glucose in the brain). The extent of damage, including tissue death, caused by a severe occlusion depends on the length of time that the artery is occluded and the size of the affected area. Severe occlusion of a coronary (heart) artery can cause a



▲ EXPERIMENTAL FIGURE 18-21 **Atherosclerosis narrows and blocks blood flow through coronary arteries.** X-ray multislice computed tomographic image of a human heart reveals a major occlusion (black arrow) of the left anterior descending artery (LAD, arrow) and a narrowing of a nearby vessel (white arrow) as a block in the stream of blood (seen in the arteries as a white tube). [From K. Nieman et al., 2001, *Lancet* **357**:599.]

heart attack; occlusion of an artery feeding the brain can cause a stroke.

Atherosclerosis can begin at or even before puberty but usually takes decades to develop into overt disease. In some cases, the growth of new blood vessels permits sufficient blood flow to tissue downstream of a plaque so that major tissue damage does not occur. Balloon stretching, removal of plaques, insertion of metal scaffolds (stents), and grafting of a bypass vessel are among the surgical treatments for advanced blockage of coronary arteries.

LDLR-Independent Uptake of LDL (Bad Cholesterol) Leads to Formation of Foam Cells

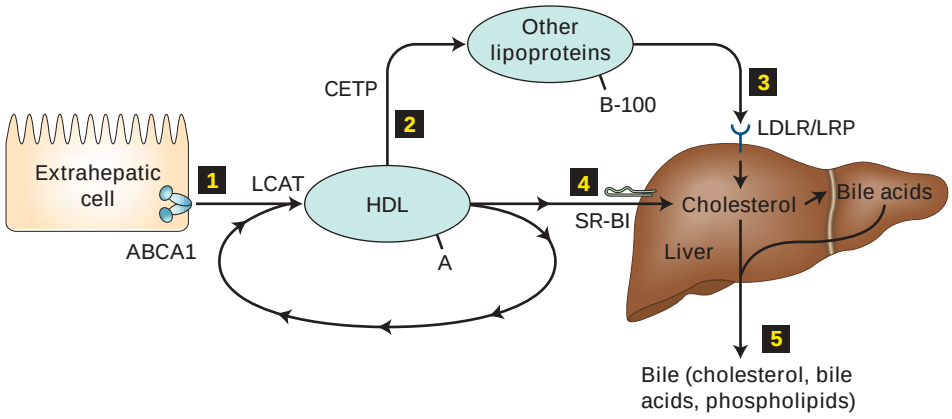
As noted, the first unique step of atherosclerosis is the accumulation in the artery wall of macrophage foam cells filled with lipid droplets containing cholesteryl esters. The greater the plasma LDL concentration and the greater the concentration of LDL in the artery wall, the more rapidly foam cells develop and accumulate to form microscopically visible early fatty streaks. Given these facts, you might initially guess that LDLR-mediated endocytosis is responsible for foam cell formation, but there are two powerful reasons why it cannot be true. First, LDLR activity is under cholesterol-dependent, SREBP-controlled feedback regulation, which maintains cellular cholesterol levels within a narrow range. Intracellular cholesterol levels far below those seen in foam cells prevent transcriptional activation of the LDLR gene by the insig-1(2)/SCAP/SREBP pathway (see Figure 18-17). The consequent low level of LDLR expression prevents massive intracellular cholesterol buildup. Second, in familial hypercholesterolemic patients who lack LDLR activity and consequently have high plasma LDL levels, the onset of atherosclerosis is at much younger ages and its progress is dramatically accelerated compared with normal people. Clearly, the formation of foam cells in these patients does not require LDLR activity.

The obvious conclusion based on this evidence is that an LDLR-independent mechanism is responsible for the cellular uptake of LDL cholesterol that leads to the formation of foam cells. One proposed mechanism is shown in Figure 18-20a. In this model, LDL present in the artery wall at the site of infection or cell damage is subject to oxidation by the products of various oxidative reactions or to other modifications generated by inflammatory cells. For example, the oxidation of unsaturated fatty acyl chains in LDL particles generates reactive aldehyde and other species that can covalently modify the protein and phospholipid components of the outer shells of the lipoprotein particles. Endocytosis of such modified LDL particles by receptors on macrophages not subject to cholesterol-dependent feedback suppression would result in foam cell formation.

Support for this model comes from the finding that macrophages express a diverse array of multiligand receptors belonging to the superfamily of scavenger receptors. Some of these receptors can bind tightly to modified LDLs and, in experiments using transgenic mice, have been implicated in the formation of macrophage foam cells. The normal function of some scavenger receptors (e.g., SR-AI/II) is to recognize and promote the endocytic destruction of damaged macromolecules, pathogens, and injured, aged, or apoptotic cells by macrophages. Because these receptors do not normally take part in cellular cholesterol metabolism, their activities are not subject to regulation by cholesterol. Thus they can mediate the massive accumulation of intracellular cholesterol by macrophages, generally limited only by the amount of extracellular modified LDL.

Reverse Cholesterol Transport by HDL (Good Cholesterol) Protects Against Atherosclerosis

Epidemiological evidence indicates that the concentration of HDL cholesterol in the plasma is inversely correlated with the risk for atherosclerosis and cardiovascular disease. Furthermore, transgenic overexpression of apolipoprotein A-I,



▲ FIGURE 18-22 **HDL-mediated reverse cholesterol transport.** Cholesterol from peripheral tissues is transferred to circulating HDL by ABCA1 (1) and possibly other transporters such as SR-BI and is converted into cholesteryl esters by the plasma enzyme LCAT. Cholesteryl esters in the HDL core can be transferred to other lipoproteins by CETP (2) for subsequent endocytosis by lipoprotein receptors expressed primarily by liver cells (3). Alternatively, the receptor SR-BI, which is present mostly on liver and steroidogenic tissues, can mediate the selective uptake of cholesteryl esters from HDL (4). Cholesterol delivered to the liver and bile acids derived from it are secreted into the bile (5). [Adapted from A. Rigotti and M. Krieger, 1999, *N. Engl. J. Med.* **341**:2011; see also M. Krieger, 1999, *Ann. Rev. Biochem.* **68**:523.]

the major apolipoprotein in HDL, suppresses atherosclerosis in animal models of the disease. Several properties of HDL could contribute to its apparent ability to protect against atherosclerosis.

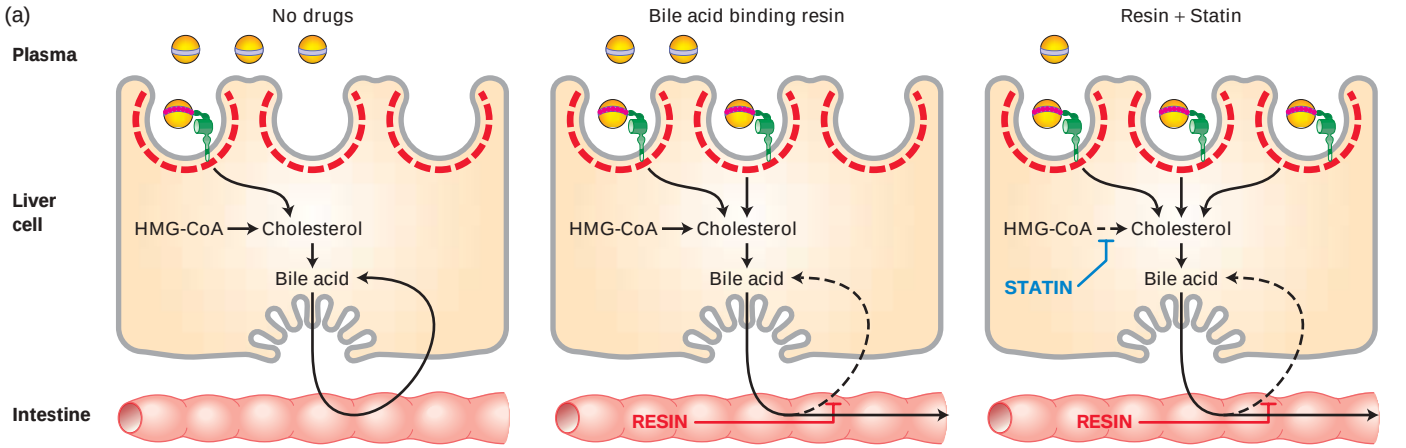
As discussed earlier, HDL can remove cholesterol from cells in extrahepatic tissues, including artery walls, and eventually deliver the cholesterol to the liver either directly by selective lipid uptake mediated by the receptor SR-BI or indirectly by transferring its cholesterol to other lipoproteins that are ligands of hepatic endocytic receptors (see Figure 18-13c). The excess cholesterol can then be secreted into the bile and eventually excreted from the body (see Figure 18-11). Figure 18-22 summarizes this process, called *reverse cholesterol transport,* which lowers both the intracellular cholesterol in macrophages and the total amount of cholesterol carried by the body, thereby directly and indirectly reducing foam cell formation. In a sense, there is a competition between LDL-mediated delivery of cholesterol to cells in the artery wall and HDL-mediated removal of excess cholesterol from those cells. In fact, the ratio of plasma LDL cholesterol to HDL cholesterol is considered a much better indicator of risk for cardiovascular disease than the total plasma cholesterol concentration.

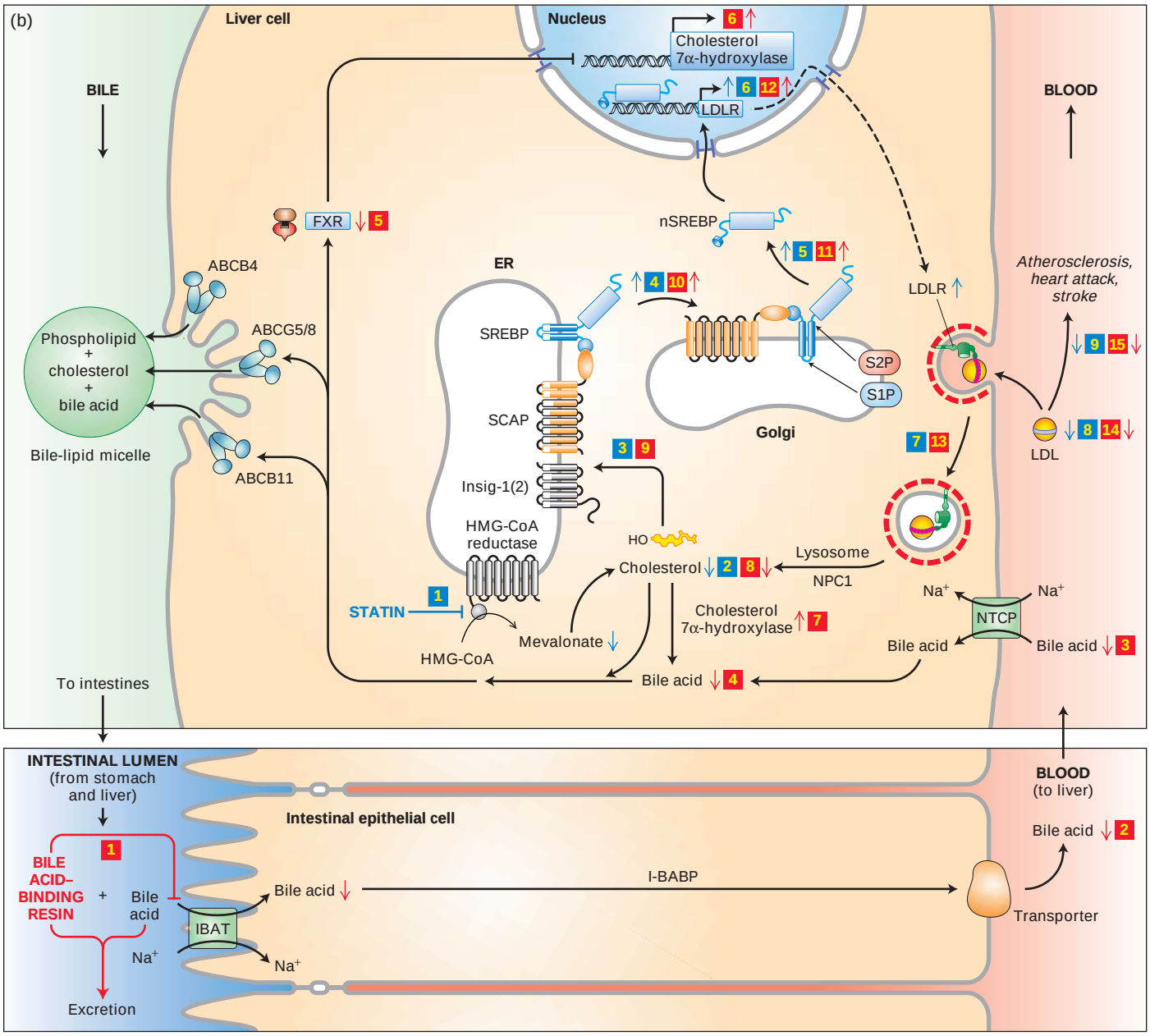
In addition to its role in atheroprotective reverse cholesterol transport, HDL itself and some plasma enzymes associated with HDL can suppress the oxidation of LDL. Decreased LDL oxidation presumably reduces the substrates for scavenger receptors on macrophages, thereby inhibiting their accumulation of LDL cholesterol and thus foam cell formation. HDL also appears to have anti-inflammatory properties, which may contribute to its atheroprotective effect. Finally, the interaction of HDL with the receptor SR-BI can stimulate the activity of endothelial nitric oxide (NO) synthase, leading to increased production of nitric oxide. This potent atheroprotective signaling molecule can diffuse into nearby vascular smooth muscle and induce its relaxation (see Figure 13-30). Relaxation of the smooth muscle around an artery results in dilation (widening) of the artery lumen and consequently increased blood flow, thereby helping to prevent ischemia and tissue damage.

Two Treatments for Atherosclerosis Are Based on SREBP-Regulated Cellular Cholesterol Metabolism

The complex pathogenesis of atherosclerosis, which we have only briefly sketched here, presents a daunting challenge to modern molecular medicine. However, as our understanding of the cell biology underlying this complexity has advanced, many opportunities for intervening in the disease process by modulating cellular pathways have arisen. We conclude this chapter by describing two examples of such interventions affecting cellular lipid metabolism.

Because the risk for atherosclerotic disease is directly proportional to the plasma levels of LDL cholesterol and inversely proportional to those of HDL cholesterol, a major public health goal has been to lower LDL and raise HDL cholesterol levels. The most successful drug interventions to date have been aimed at reducing plasma LDL. The steady-state levels of plasma LDL are determined by the relative rates of LDL formation and LDL removal or clearance. LDL receptors, especially those expressed in the liver, play a major role in clearing LDL from the plasma. The liver is key in cholesterol regulation not only because it is the site of about 70 percent of the body's LDL receptors, but also because it is the site where unesterified cholesterol and its bile acid


(a)
No drugs
Bile acid binding resin
Resin + Statin
Plasma
Liver cell
Intestine
HMG-CoA
Cholesterol
Bile acid
STATIN
RESIN


(b)
Liver cell
Nucleus
BILE
BLOOD
Cholesterol 7α-hydroxylase
LDLR
FXR
nSREBP
ABCB4
ABCG5/8
ABCB11
Phospholipid + cholesterol + bile acid
Bile-lipid micelle
ER
SREBP
SCAP
Insig-1(2)
HMG-CoA reductase
S2P
S1P
Golgi
Atherosclerosis, heart attack, stroke
LDL
Lysosome
NPC1
HO
Cholesterol
Mevalonate
HMG-CoA
STATIN
Cholesterol 7α-hydroxylase
Bile acid
To intestines
Na+
NTCP
INTESTINAL LUMEN (from stomach and liver)
Intestinal epithelial cell
BLOOD (to liver)
BILE ACID–BINDING RESIN
IBAT
I-BABP
Transporter
Excretion

◀ FIGURE 18-23 **Two pharmaceutical approaches for preventing atherosclerosis.** (a) Treatment with statins or bile acid–binding resins lowers hepatic cholesterol levels. This stimulates expression of LDLRs, enhancing LDL removal from the plasma and reducing the risk of atherosclerosis. Combined treatment with both types of drugs often is more effective than using either alone. (b) *Statin therapy:* Step 1: After oral administration, a statin drug inhibits HMG-CoA reductase in liver (*top*) and other cells, lowering production of mevalonate, a precursor of cholesterol. Step 2: Cholesterol levels drop, reducing (3) the interactions of insig-1(2) with the SCAP/SREBP complex, which can then move (4) to the Golgi for processing by the proteases S1P and S2P. As a consequence, there is increased production of the soluble transcription factor nSREBP (5), which moves to the nucleus, binds SREs, and increases expression of genes such as the LDL receptor (LDLR) (6). nSREBP-mediated increases in LDLR activity result in enhanced LDL endocytosis (7), the reduction of plasma LDL concentrations (8), and consequently reduced atherosclerosis, heart attacks, and stroke (9).

Bile acid–binding resin therapy: Oral administration of a bile acid–binding resin, or sequestrant (1), increases the loss of bile acids from the body by preventing their absorption by intestinal epithelial cells through the IBAT transport protein and reduces bile acids delivered to the blood (2) and then to the liver (3) by the transporter NTCP. Step 4: The lower levels of cytoplasmic bile acids reduce the amount of bile acid bound to the nuclear hormone receptor FXR (5) and its suppression (6) of the expression of cholesterol 7α-hydroxylase. The consequent increased levels of expression and activity of cholesterol 7α-hydroxylase (7) reduce the levels of intracellular cholesterol (8). As with the statin treatment, the reduced cellular cholesterol levels (9–15) increase LDLR activity, lower plasma LDL levels, and protect against atherosclerosis. [Part (a) adapted from M. S. Brown and J. L. Goldstein, 1986, *Science* **232**:34.]

derivatives are secreted into bile, some of which is ultimately excreted from the body (see Figure 18-11).

An increase in LDLR activity, especially in the liver, can lower LDL levels and protect against atherosclerosis. We know the insig-1(2)/SCAP/SREBP pathway normally regulates the expression of the LDL receptor in response to intracellular cholesterol (see Figure 18-17). Thus treatments that lower cellular cholesterol levels in the liver will increase the expression of the LDL receptor and consequently lower plasma LDL levels. Two approaches are widely used to lower steady-state hepatic cholesterol levels: (1) reducing cholesterol synthesis and (2) increasing the conversion of cholesterol into bile acids in the liver (Figure 18-23). Both approaches exploit the regulatory mechanisms for controlling cellular cholesterol already described.

Perhaps the most successful anti-atherosclerosis medications are the *statins.* These drugs bind to HMG-CoA reductase and directly inhibit its activity, thereby lowering cholesterol biosynthesis and the pool of hepatic cholesterol. Activation of SREBP in response to this cholesterol depletion promotes increased synthesis of HMG-CoA reductase and the LDL receptor. Of most importance here is the resulting increased numbers of hepatic LDL receptors, which can mediate increased import of LDL cholesterol from the plasma. Statins also appear to inhibit atherosclerosis by suppressing the inflammation that triggers the process. Although the mechanism of this inhibition is not well understood, it apparently contributes to the atheroprotective effect of statins.

The most common drugs used to lower hepatic cholesterol by increasing the formation of bile acids do so by interrupting the enterohepatic circulation. These *bile acid sequestrants* (e.g., cholestyramine) are insoluble resins that bind tightly to bile acids in the lumen of the intestines, forming complexes that prevent IBAT-mediated absorption by intestinal epithelial cells. The complexes are excreted in the feces. The resulting decrease in the return of bile acids to the liver causes a drop in the hepatic bile acid pool. As noted previously, when bile acid levels are high, the nuclear receptor FXR represses the expression of cholesterol 7α-hydroxylase activity, the key enzyme in bile acid synthesis. This repression is lifted as the bile acid pool is being depleted by the drug, leading to an increase in the conversion of cholesterol into bile acids. The resulting drop in the cellular cholesterol pool in hepatocytes engages the SREBP pathway, leading to increased hepatic LDLR synthesis and import of LDL cholesterol (see Figure 18-23).

Thus both the statins and the bile acid sequestrants ultimately reduce plasma LDL cholesterol by increasing the SREBP-mediated expression of the LDL receptor in the liver. When a statin and a bile acid sequestrant are administered simultaneously, they act together to substantially lower hepatic cholesterol, stimulate high levels of hepatic LDLR activity, and thus lower plasma LDL concentrations (see Figure 18-23). Unfortunately, statins and bile acid sequestrants are not particularly effective for LDLR-deficient patients who are homozygous for familial hypercholesterolemia, because both therapies depend on stimulating endogenous LDLR activity. Nevertheless, most people at risk for atherosclerosis are not FH homozygotes and the widespread use of these drugs has been shown in many clinical studies to save lives. ▮

KEY CONCEPTS OF SECTION 18.5

The Cell Biology of Atherosclerosis, Heart Attacks, and Strokes

- Atherosclerosis is the progressive accumulation of cholesterol, inflammatory and other cells, and extracellular matrix in the subendothelial space (intima) of an artery wall, ultimately leading to the formation of a plaque that can occlude the lumen (see Figure 18-19).
- The partial or complete blockage of coronary arteries by atherosclerotic plaques and associated blood clots can starve heart muscle for oxygen and other nutrients and

cause tissue death (heart attack). Similar blockage of arteries supplying the brain causes stroke.

- Normal inflammatory responses in an artery wall, triggered by infection or injury, may lead to the formation and accumulation of cholesterol-filled macrophage foam cells, the first indication of atherosclerosis.
- Plasma LDL (bad cholesterol) promotes foam cell formation and thus atherosclerosis by a LDLR-independent mechanism apparently requiring scavenger receptors (see Figure 18-20).
- Plasma HDL (good cholesterol) reduces the risk for atherosclerosis in part by mediating the reverse transport of cholesterol from peripheral cells to the liver (see Figure 18-22).
- Two types of drugs are widely used for treating or preventing atherosclerosis: statins, which reduce cholesterol biosynthesis by inhibiting HMG-CoA reductase, and bile acid sequestrants, which prevent the enterohepatic recycling of bile acids from the intestine to the liver. Both treatments lower hepatic cholesterol levels, leading to an SREBP-mediated increase in hepatic expression of LDL receptors, which act to lower plasma LDL levels (see Figure 18-23).

PERSPECTIVES FOR THE FUTURE

Despite considerable progress in our understanding of the cellular metabolism and movement of lipids, the mechanisms for transporting cholesterol and phospholipids between organelle membranes remain poorly characterized. Several recent advances may contribute to progress in this area. First, researchers have partly purified two specialized ER membrane compartments, called MAM (mitochondrion-associated membrane) and PAM (plasma membrane–associated membrane), that may play roles in membrane lipid synthesis and Golgi-independent intracellular lipid transport. Both MAM and PAM have a very high capacity for phospholipid or sterol synthesis or both and directly contact mitochondrial membranes and the plasma membrane, respectively. Second, selection for cells with mutations that interfere with intraorganelle lipid transport opens the door to genetic dissection of this system. The results of such genetic studies in yeast have suggested that ER-to-mitochondrion phospholipid transport is regulated by ubiquitination. Third, the demonstration that some Rab proteins can influence NPC1-dependent cholesterol transport will refocus efforts on vesicular mechanisms for lipid traffic. Finally, cholesterol/sphingolipid-rich lipid rafts and related caveolae are under increased experimental scrutiny that is likely to serve as a source of insight into mechanisms of intracellular lipid transport and control of intracellular signaling pathways.

Another fundamental question concerns the generation, maintenance, and function of the asymmetric distribution of lipids within the leaflets of one membrane and the variation in lipid composition among the membranes of different organelles. What are the mechanisms underlying this complexity and why is such complexity needed? We already know that certain lipids can specifically interact with and influence the activity of some proteins. For example, the large multimeric proteins that participate in oxidative phosphorylation in the inner mitochondrial membrane appear to assemble into supercomplexes whose stability may depend on the physical properties and binding of specialized phospholipids such as cardiolipin.

A detailed molecular understanding of how various transport proteins move lipids from one membrane leaflet to another (flippase activity) and into and out of cells is not yet in hand. Such understanding will undoubtedly require a determination of many high-resolution structures of these molecules, their capture in various stages of the transport process, and careful kinetic and other biophysical analyses of their function, similar to the approaches discussed in Chapter 7 for elucidating the operation of ion channels and ATP-powered pumps.

The fruitful transfer of information between cell biology and medicine can work in both directions. For instance, insights into the insig-1(2)/SCAP/SREBP and nuclear receptor pathways provided the basis for treatment of atherosclerosis with statins and bile acid sequestrants. Continuing efforts are underway to develop even more effective drugs based on these pathways. On the other hand, analysis of familial hypercholesterolemia was a source of major insights into receptor-mediated endocytosis, and a new class of anti-hypercholesterolemia drugs (such as Ezetimibe) that inhibit intestinal cholesterol absorption may serve as a useful tool for identifying the proteins that mediate cholesterol absorption and the mechanisms by which they function.

KEY TERMS

ABC superfamily *748*
apolipoprotein *757*
atherosclerotic plaque *769*
"bad cholesterol" *759*
bile acid *752*
chylomicron *757*
enterohepatic circulation *756*
familial hypercholesterolemia (FH) *760*
fatty acid transport protein (FATP) *755*
fatty acid–binding protein (FABP) *746*
flippase *748*
foam cell *769*
"good cholesterol" *759*
high-density lipoprotein *757*
inflammatory response *768*
insig1(2)/SCAP/SREBP pathway *765*
low-density lipoprotein *757*
Niemann-Pick C1 (NPC1) protein *753*
receptor SR-BI *763*
receptor-mediated endocytosis *760*
reverse cholesterol transport *771*
selective lipid uptake *760*
SRE-binding protein (SREBP) *764*
statin *773*
sterol-sensing domain *751*

REVIEW THE CONCEPTS

1. Phospholipid biosynthesis at the interface between the endoplasmic reticulum (ER) and the cytosol presents a number of challenges that must be solved by the cell. Explain how each of the following is handled.

a. The substrates for phospholipid biosynthesis are all water soluble, yet the end products are not.

b. The immediate site of incorporation of all newly synthesized phospholipids is the cytosolic leaflet of the ER membrane, yet phospholipids must be incorporated into both leaflets.

c. Many membrane systems in the cell, for example, the plasma membrane, are unable to synthesize their own phospholipids, yet these membranes must also expand if the cell is to grow and divide.

2. What are the common fatty acid chains in glycerophospholipids, and why do these fatty acid chains differ in their number of carbon atoms by multiples of 2?

3. Plants, fungi, and animals all contain sterols in their membranes. Rather than being absorbed from the intestine, most plant sterols are excreted by humans. Why is this element of the human diet not efficiently absorbed?

4. The biosynthesis of cholesterol is a highly regulated process. What is the key regulated enzyme in cholesterol biosynthesis? This enzyme is subject to feedback inhibition. What is feedback inhibition? How does this enzyme sense cholesterol levels in a cell? Cite one other example of a protein whose activity is sensitive to lipid levels.

5. One source of cholesterol for humans is meat. Vegetarians eliminate this cholesterol contribution. Yet vegetarians can still develop atherosclerosis. How can this be?

6. It is evident that one function of cholesterol is structural because it is the most common single lipid molecule in the plasma membrane of mammals. Yet cholesterol may also have other functions. What aspects of cholesterol and its metabolism lead to the conclusion that cholesterol is a multifunctional membrane lipid?

7. Phospholipids and cholesterol must be transported from their site of synthesis to various membrane systems within cells. One way of doing this is through vesicular transport, as is the case for many proteins in the secretory pathway. However, most phospholipid and cholesterol membrane-to-membrane transport in cells is not by Golgi-mediated vesicular transport. What is the evidence for this statement? What appear to be the major mechanisms for phospholipid and cholesterol transport?

8. The intercellular movement of phospholipids and cholesterol is mediated by various proteins. What are the two major mechanisms by which lipids are exported from cells? What are the three major methods by which lipids are imported into cells?

9. Enterohepatic circulation involves the cycling of bile acids from liver to intestine and back. Explain the role of bile acid cycling in the maintenance of lipid homeostasis.

10. What are the four major classes of lipoproteins in the mammalian circulatory system? Which of these carry mainly cholesterol and which carry mainly triglycerides? Of the cholesterol carriers, a high level of some is considered to be "good" while a high level of some is considered to be "bad." Which are the "good" and "bad" carriers, and why are they considered to be "good" or "bad"?

11. Much health concern is focused on the negative effects of high (or low) levels of lipids in the blood circulatory system. Yet circulating lipids are actually essential to the health and well-being of humans. What are the beneficial effects of circulating lipids, including cholesterol and triglycerides?

12. Atherosclerosis may be thought of as a disease of the organism rather than of the single cell. Do you agree or disagree and why? Explain how hepatic transplantation could, in fact, be one of the strategies for the treatment of familial hypercholesterolemia. How does the fact that atherosclerosis typically manifests itself late in life affect the possibility of whether or not evolution has selected for or against the disease?

13. Increasing concentrations of LDL in the extracellular fluid suppresses HMG-CoA reductase activity and the transcription of genes encoding LDL receptor and HMG-CoA reductase. Conversely, when extracellular LDL levels are low, the transcription of these genes is induced. The responsible transcription factors are SRE-binding proteins (SREBPs). When cells have an adequate level of cholesterol, SREBP is an ER transmembrane protein. To what proteins is SREBP complexed? How does this complex sense the level of sterol? Where within the cell is SREBP released as a soluble protein into the cytosol? What is the mechanism by which SREBP is released? In relation to this process, what is the meaning of RIP?

14. A number of different members of the nuclear-receptor superfamily regulate lipid metabolism. Specific small molecules are typical activators. Such molecules can be either synthesized inside the target cell, diffuse into the cell, or be transported into the cell. One example is FXR, which is activated by binding of bile acids. FXR is expressed in hepatocytes and intestinal epithelial cells. Is the source of bile acid that activates FXR cell synthesized, cell imported, or a combination of the two? Rather than giving specific names of proteins whose synthesis is regulated by FXR, predict classes of proteins whose synthesis should be regulated by a bile acid–activated nuclear receptor.

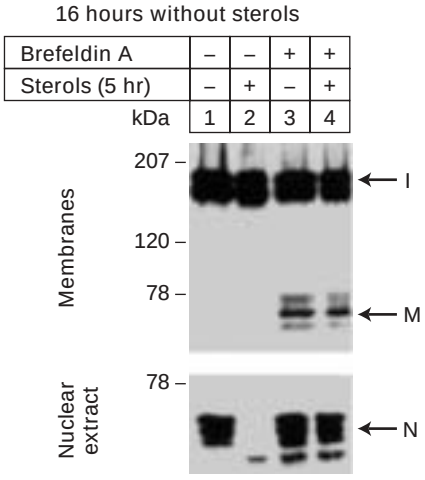
15. Atherosclerosis may be thought of as an unintended consequence of normal physiological responses designed to

protect the body against infection and tissue damage. Explain how inflammation, cholesterol, and foam cells are involved in atherosclerosis.

16. Two different classes of drugs that are in current use to reduce cholesterol levels in the blood have been discussed in detail in this chapter. What are the names of these two drug classes? How does each class act to lower blood cholesterol levels?

ANALYZE THE DATA

You are reviewing the results of a series of recent experiments to determine the regulation of the proteolysis of sterol regulatory element–binding protein (SREBP) by Site-1/2 proteases. In the first experiment, you take an indirect approach to ask if Site-1/2 proteases are components of the Golgi apparatus. You treat CHO cells with the drug brefeldin A (BFA). Brefeldin A induces the redistribution of Golgi proteins to the ER. In the experiment, CHO cells are incubated for 16 hours in the absence of added sterols, then incubated for an additional 5 hours in the absence or presence of added sterols in the absence or presence of BFA (see the first figure). At the end of the 5-hour incubation, the CHO cells are homogenized and separated into a cytoplasm (50 μg protein per gel lane) and nuclear fraction (100 μg protein per gel lane). Proteins are then separated on SDS-polyacrylamide gels and probed for the presence of various molecular forms of SREBP (I = intact, M = intermediate cleavage form, N = nuclear form).



From the observed results, what is the size of M SREBP and N SREBP compared to the size of I SREBP? How can there be an apparent product in this experiment without an apparent loss in intact SREBP? What is the normal effect of added sterols on the production of nuclear SREBP? What is the normal half-life of nuclear SREBP? Why is the instability of nuclear SREBP important to the function of the sterol regulatory pathway? What is the effect of BFA on the level of N SREBP and M SREBP? How does this effect indicate a role of Golgi apparatus in the formation of N SREBP?

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