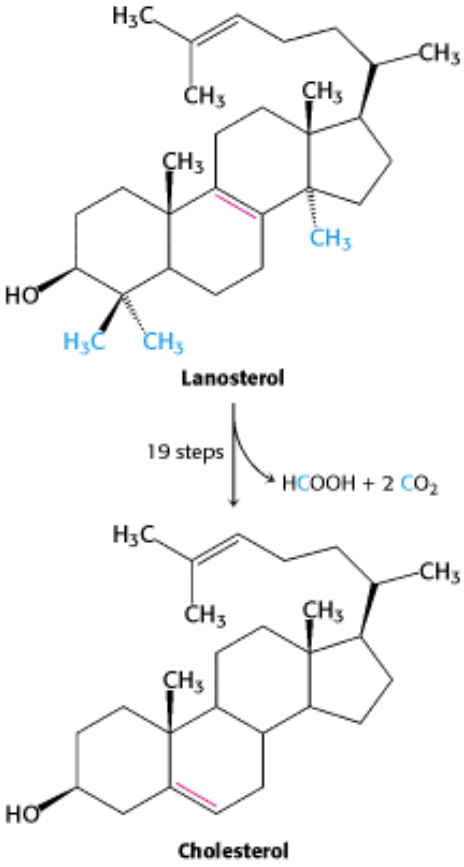

Figure 26.13. Cholesterol Formation. Lanosterol is converted into cholesterol in a complex process.

26.3. The Complex Regulation of Cholesterol Biosynthesis Takes Place at Several Levels

Cholesterol can be obtained from the diet or it can be synthesized de novo. An adult on a low-cholesterol diet typically synthesizes about 800 mg of cholesterol per day. The liver is the major site of cholesterol synthesis in mammals, although the intestine also forms significant amounts. The rate of cholesterol formation by these organs is highly responsive to the cellular level of cholesterol. *This feedback regulation is mediated primarily by changes in the amount and activity of 3-hydroxy-3-methylglutaryl CoA reductase* (Figure 26.14). As discussed in Section 26.2.1, this enzyme catalyzes the formation of mevalonate, the committed step in cholesterol biosynthesis. HMG CoA reductase is controlled in multiple ways:

1. The rate of *synthesis of reductase mRNA* is controlled by the *sterol regulatory element binding protein (SREBP)*. This transcription factor binds to a short DNA sequence called the *sterol regulatory element (SRE)* on the 5′ side of the reductase gene. In its inactive state, the SREBP is anchored to the endoplasmic reticulum or nuclear membrane. When cholesterol levels fall, the amino-terminal domain is released from its association with the membrane by two specific proteolytic cleavages. The released protein migrates to the nucleus and binds the SRE of the HMG-CoA reductase gene, as well as several other genes in the cholesterol biosynthetic pathway, to enhance transcription. When cholesterol levels rise, the proteolytic release of the SREBP is blocked, and the SREBP in the nucleus is rapidly degraded. These two events halt the transcription of the genes of the cholesterol biosynthetic pathways.

2. The rate of *translation of reductase mRNA* is inhibited by nonsterol metabolites derived from mevalonate as well as by dietary cholesterol.

3. The *degradation of the reductase* is stringently controlled. The enzyme is bipartite: its cytosolic domain carries out catalysis and *its membrane domain senses signals that lead to its degradation.* The membrane domain may undergo a change in its oligomerization state *in response to increasing concentrations of sterols such as cholesterol,* making the enzyme more susceptible to proteolysis. Homologous sterol-sensing regions are present in the protease that activates SREBP. The reductase may be further degraded by ubiquitination and targeting to the 26S proteasome under some conditions. A combination of these three regulatory devices can regulate the amount of enzyme over a 200-fold range.

4. *Phosphorylation decreases the activity of the reductase.* This enzyme, like acetyl CoA carboxylase (which catalyzes the committed step in fatty acid synthesis, Section 22.5), is switched off by an AMP-activated protein kinase. Thus, cholesterol synthesis ceases when the ATP level is low.

As we will see shortly, all four regulatory mechanisms are modulated by receptors that sense the presence of cholesterol in the blood.

26.3.1. Lipoproteins Transport Cholesterol and Triacylglycerols Throughout the Organism

Cholesterol and triacylglycerols are transported in body fluids in the form of *lipoprotein particles.* Each particle consists of a core of hydrophobic lipids surrounded by a shell of more polar lipids and apoproteins. The protein components of these macromolecular aggregates have two roles: *they solubilize hydrophobic lipids and contain cell-targeting signals.* Lipoprotein particles are classified according to increasing density (Table 26.1): *chylomicrons, chylomicron remnants, very low density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL),* and *high-density lipoproteins (HDL).* Ten principal apoproteins have been isolated and characterized. They are synthesized and secreted by the liver and the intestine.

Triacylglycerols, cholesterol, and other lipids obtained from the diet are carried away from the intestine in the form of large *chylomicrons* (180– 500 nm in diameter; Section 22.1.2). These particles have a very low density (d<0.94 g cm^{-3}) because triacylglycerols constitute ~99% of their content. Apolipoprotein B-48 (apo B-48), a large protein (240 kd), forms an amphipathic spherical shell around the fat globule; the external face of this shell is hydrophilic. The triacylglycerols in chylomicrons are released through hydrolysis by *lipoprotein lipases.* These enzymes are located on the lining of blood vessels in muscle and other tissues that use fatty acids as fuels and in the synthesis of fat. The liver then takes up the cholesterol-rich residues, known as *chylomicron remnants.*

The liver is a major site of triacylglycerol and cholesterol synthesis (Figure 26.15). Triacylglycerols and cholesterol in excess of the liver's own needs are exported into the blood in the form of very low density lipoproteins (d<1.006 g cm^{-3}). These particles are stabilized by two lipoproteins—apo B-100 and apo E (34 kd). Apo B-100, one of the largest proteins known (513 kd), is a longer version of apo B-48. Both apo B proteins are encoded by the same gene and produced from the same initial RNA transcript. In the intestine, RNA editing (Section 28.3.2) modifies the transcript to generate the mRNA for apo B-48, the truncated form. Triacylglycerols in very low density lipoproteins, as in chylomicrons, are hydrolyzed by lipases on capillary surfaces. The resulting remnants, which are rich in cholesteryl esters, are called *intermediate-density lipoproteins*(1.006 < d < 1.019 g cm^{-3}). These particles have two fates. Half of them are taken up by the liver for processing, and half are converted into low-density lipoprotein (1.019 < d < 1.063 g cm^{-3}) by the removal of more triacylglycerol.

Low-density lipoprotein is the major carrier of cholesterol in blood. This lipoprotein particle has a diameter of 22 nm and a mass of about 3 million daltons (Figure 26.16). It contains a core of some 1500 esterified cholesterol molecules; the most common fatty acyl chain in these esters is linoleate, a polyunsaturated fatty acid. A shell of phospholipids and unesterified cholesterols surrounds this highly hydrophobic core. The shell also contains a single copy of apo B-100, which is recognized by target cells. *The role of LDL is to transport cholesterol to peripheral tissues and regulate de novo cholesterol synthesis at these sites,* as described in Section 26.3.3. A different purpose is served by *high-density lipoprotein* (1.063 < d < 1.21 g cm^{-3}), which picks up cholesterol released into the plasma from dying cells and from membranes undergoing turnover. An acyltransferase in HDL esterifies these cholesterols, which are then either rapidly

shuttled to VLDL or LDL by a specific transfer protein or returned by HDL to the liver.

26.3.2. The Blood Levels of Certain Lipoproteins Can Serve Diagnostic Purposes

High serum levels of cholesterol cause disease and death by contributing to the formation of atherosclerotic plaques in arteries throughout the body. This excess cholesterol is present in the form of the low density lipoprotein particle, so-called "bad cholesterol."The ratio of cholesterol in the form of high density lipoprotein, sometimes referred to as "good cholesterol," to that in the form of LDL can be used to evaluate susceptibility to the development of heart disease. For a healthy person, the LDL/HDL ratio is 3.5.

High-density lipoprotein functions as a shuttle that moves cholesterol throughout the body. HDL binds and esterifies cholesterol released from the peripheral tissues and then transfers cholesteryl esters to the liver or to tissues that use cholesterol to synthesize steroid hormones. A specific receptor mediates the docking of the HDL to these tissues. The exact nature of the protective effect of HDL levels is not known; however, a possible mechanism is discussed in Section 26.3.5.

26.3.3. Low-Density Lipoproteins Play a Central Role in Cholesterol Metabolism

Cholesterol metabolism must be precisely regulated to prevent atherosclerosis. The mode of control in the liver, the primary site of cholesterol synthesis, has already been discussed: dietary cholesterol reduces the activity and amount of 3-hydroxy-3-methylglutaryl CoA reductase, the enzyme catalyzing the committed step. The results of studies by Michael Brown and Joseph Goldstein are sources of insight into the control of cholesterol metabolism in nonhepatic cells. In general, cells outside the liver and intestine obtain cholesterol from the plasma rather than synthesizing it de novo. Specifically, *their primary source of cholesterol is the low-density lipoprotein.* The process of LDL uptake, called *receptor-mediated endocytosis,* serves as a paradigm for the uptake of many molecules.

The steps in the receptor-mediated endocytosis of LDL are as follows (see Figure 12.40).

1. Apolipoprotein B-100 on the surface of an LDL particle binds to a specific receptor protein on the plasma membrane of nonhepatic cells. The receptors for LDL are localized in specialized regions called *coated pits,* which contain a specialized protein called *clathrin.*

2. The receptor-LDL complex is internalized by *endocytosis,* that is, the plasma membrane in the vicinity of the complex invaginates and then fuses to form an endocytic vesicle (Figure 26.17).

3. These vesicles, containing LDL, subsequently fuse with *lysosomes,* acidic vesicles that carry a wide array of degradative enzymes. The protein component of the LDL is hydrolyzed to free amino acids. The cholesteryl esters in the LDL are hydrolyzed by a lysosomal acid lipase. The LDL receptor itself usually returns unscathed to the plasma membrane. The round-trip time for a receptor is about 10 minutes; in its lifetime of about a day, it may bring many LDL particles into the cell.

4. *The released unesterified cholesterol can then be used for membrane biosynthesis.* Alternatively, it can be *reesterified for storage inside the cell.* In fact, free cholesterol activates *acyl CoA:cholesterol acyltransferase (ACAT),* the enzyme catalyzing this reaction. Reesterified cholesterol contains mainly oleate and palmitoleate, which are monounsaturated fatty acids, in contrast with the cholesterol esters in LDL, which are rich in linoleate, a polyunsaturated fatty acid (see Table 24.1). It is imperative that the cholesterol be reesterified. High concentrations of unesterified cholesterol disrupt the integrity of cell membranes.

The synthesis of LDL receptor is itself subject to feedback regulation. The results of studies of cultured fibroblasts show that, *when cholesterol is abundant inside the cell, new LDL receptors are not synthesized, and so the uptake of additional cholesterol from plasma LDL is blocked.* The gene for the LDL receptor, like that for the reductase, is regulated by SREBP, which binds to a sterol regulatory element that controls the rate of mRNA synthesis.