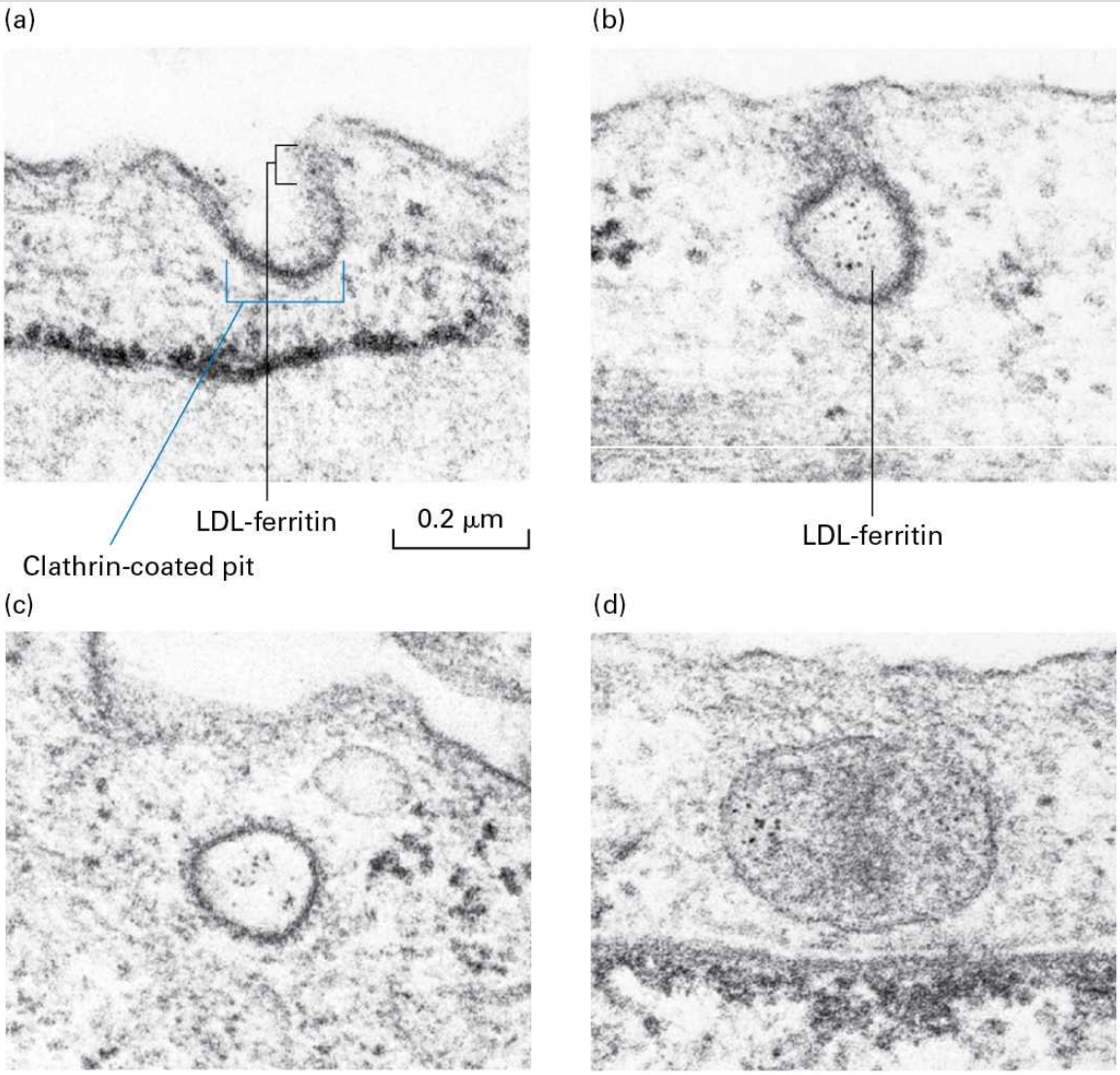
14.5 Receptor-Mediated Endocytosis

In previous sections, we have explored the main pathways of vesicle trafficking responsible for delivering soluble and membrane secretory proteins synthesized on the rough ER to the cell surface. The same basic principle of coated vesicle budding and fusion are responsible for the ability of animal cells to internalize macromolecules from their surroundings and to deliver these macromolecules to particular intracellular destinations. In this process, known as **receptor-mediated endocytosis**, a specific receptor on the cell surface binds tightly to an extracellular macromolecular ligand that it recognizes; then the complex of receptor and ligand are recruited to a plasma-membrane region that buds inward to form a coated transport vesicle. Plant cells and fungal cells take up small molecules by endocytosis, but generally do not take up macromolecules, which cannot readily pass through the cell wall because of their size.

Among the common macromolecules that vertebrate cells internalize by receptor-mediated endocytosis are cholesterol-containing low-density lipoprotein (LDL) particles, the iron-carrying protein transferrin, many protein hormones (e.g., insulin), and certain glycoproteins. Receptor-mediated endocytosis of such ligands generally occurs via clathrin/AP2-coated pits and vesicles in a process similar to the packaging of lysosomal

enzymes by the binding of M6P in the *trans*-Golgi network (see Figure 14-23). As noted earlier, some M6P receptors are found on the cell surface, and these receptors participate in the receptor-mediated endocytosis of lysosomal enzymes that are mistakenly secreted. In general, the transmembrane receptor proteins that function in the uptake of extracellular ligands are internalized from the cell surface during endocytosis and are then sorted and recycled back to the cell surface, much as M6P receptors are recycled to the plasma membrane and *trans*-Golgi. The rate at which a ligand is internalized is limited by the amount of its corresponding receptor on the cell surface.

Clathrin/AP2-coated pits make up about 2 percent of the surface of cells such as hepatocytes and fibroblasts. Many internalized ligands have been observed in clathrin/AP2-coated pits and vesicles, which are thought to function as intermediates in the endocytosis of most (though not all) ligands bound to cell-surface receptors (Figure 14-26). Some receptors are clustered over clathrin-coated pits even in the absence of ligand. Other receptors diffuse freely in the plane of the plasma membrane but undergo a conformational change when they bind to ligand, so that when the receptor-ligand complex diffuses into a clathrin-coated pit, it is retained there. Two or more types of receptor-bound ligands, such as LDL and transferrin, can be seen in the same coated pit or vesicle.



Lodish et al., *Molecular Cell Biology*, 9e, © 2021 W. H. Freeman and Company

EXPERIMENTAL FIGURE 14-26 The initial stages of receptor-mediated endocytosis of low-density lipoprotein (LDL) particles are revealed by electron microscopy. Cultured human fibroblasts were incubated in a medium containing LDL particles covalently linked to the electron-dense, iron-containing protein ferritin; each small iron particle in ferritin is visible as a small dot under the electron microscope. Cells were initially incubated at 4 °C; at this temperature LDL can bind to its receptor, but internalization does not occur. After excess LDL not bound to the cells was washed away, the cells were warmed to 37 °C and then prepared for microscopy at periodic intervals. (a) A coated pit, showing the clathrin coat on the inner (cytosolic) surface of the pit, soon after the temperature was raised. (b) A pit containing LDL, apparently closing on itself to form a coated vesicle. (c) A coated vesicle containing ferritin-tagged LDL particles. (d) Ferritin-tagged LDL particles in a

smooth-surfaced early endosome, 6 minutes after internalization began. See M. S. Brown and J. Goldstein, 1986, *Science* **232**:34.

[Republished with permission from *Nature*, from J. Goldstein et al., 1979, "Coated Pits, Coated Vesicles, and Receptor-Mediated Endocytosis," *Nature* **279**:679–685; permission conveyed through the Copyright Clearance Center, Inc.]

Description
The micrograph labeled (a) shows a clathrin-coated U shaped pit containing L D L-ferritin (tiny granules). The vesicle has a diameter of approximately 0.2 micrometers.

The micrograph labeled (b) shows a pit containing L D L ferritin that has almost closed off to form an oval vesicle.

The micrograph labeled (c) shows a fully formed circular vesicle containing L D L ferritin.

The micrograph labeled (d) shows an early endosome full of L D L ferritin. The oval vesicle looks larger and tiny granules are within it.

Cells Take Up Lipids from the Blood in the Form of Large, Well-Defined Lipoprotein Complexes

Lipids absorbed from the diet in the intestines or stored in adipose tissue can be distributed to cells throughout the body. To facilitate the mass transfer of lipids between cells, animals have evolved an efficient way to package hundreds or even thousands of lipid molecules into water-soluble, macromolecular carriers, called *lipoproteins*, which cells can take up from the circulation as an ensemble. A lipoprotein particle has a shell composed

of proteins (*apolipoproteins*) overlying a cholesterol-containing phospholipid monolayer. The shell is amphipathic because its outer surface is hydrophilic, making the particle water soluble, and its inner surface is hydrophobic. Beneath the hydrophobic inner surface of the shell is a core of neutral lipids containing mostly cholesteryl esters, triglycerides, or both. Mammalian lipoproteins fall into different classes, defined by their differing buoyant densities. The class we will consider here is **low-density lipoprotein (LDL)**. A typical LDL particle, depicted in Figure 14-27, is a sphere 20–25 nm in diameter. The amphipathic outer shell is composed of a phospholipid monolayer and a single molecule of a large protein known as *apoB-100*; the core of the particle is packed with cholesterol in the form of cholesteryl esters.

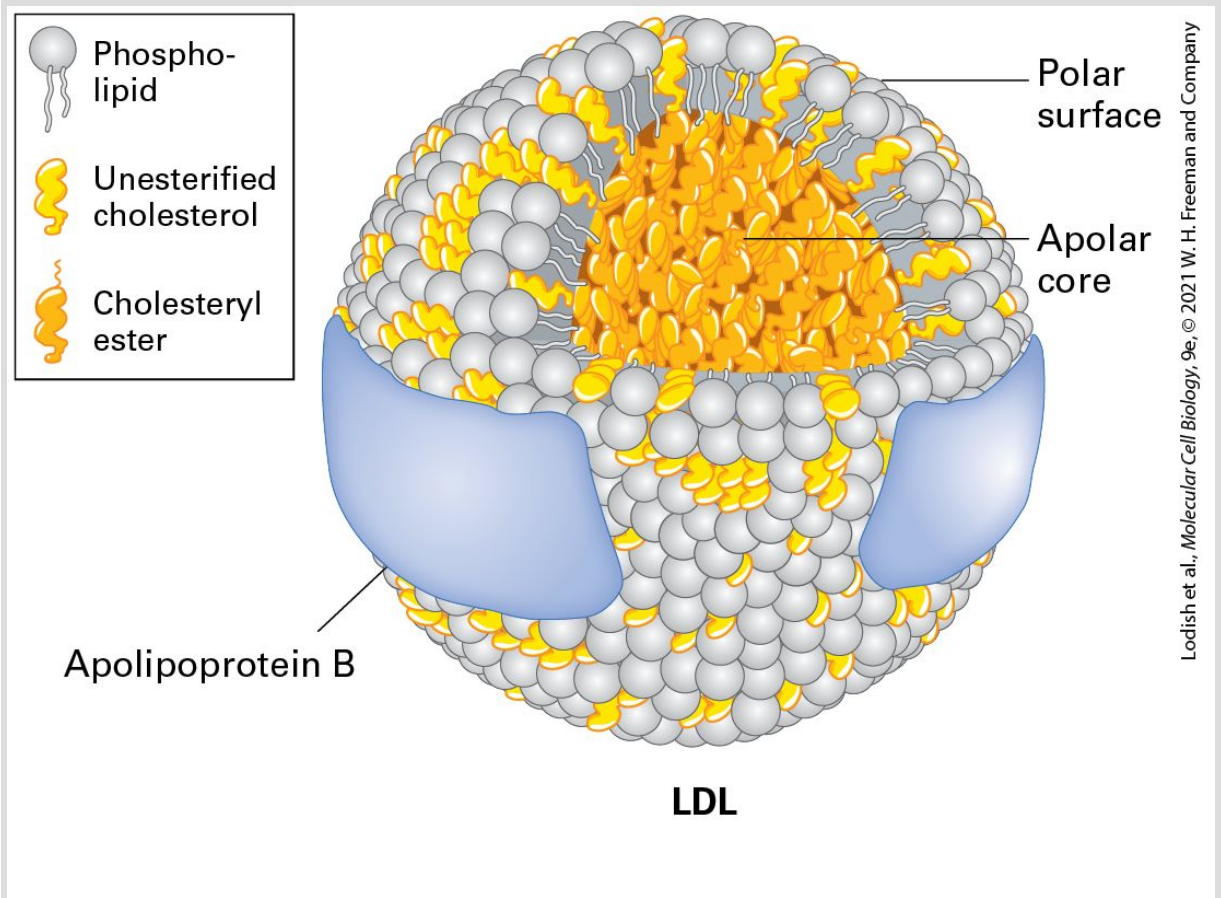
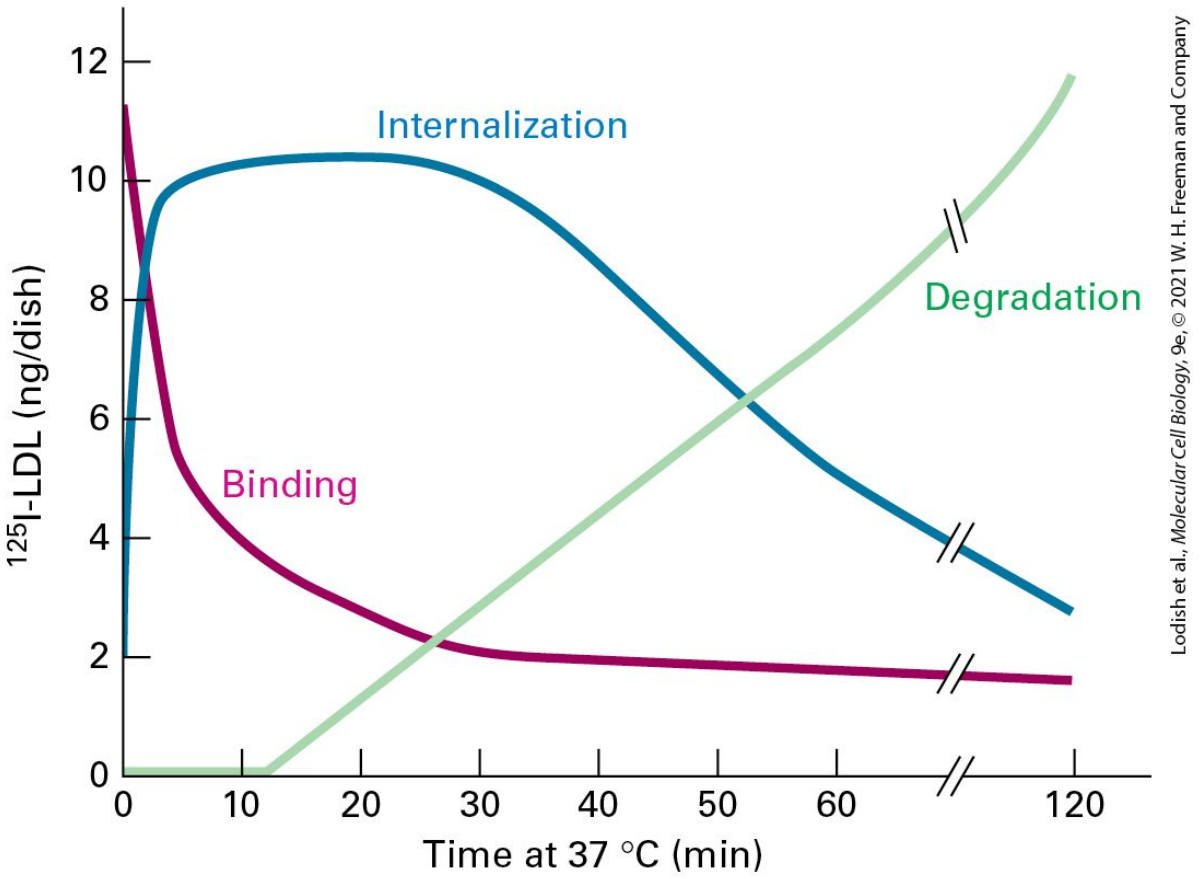


FIGURE 14-27 Model of low-density lipoprotein (LDL). All classes of lipoproteins have the same general structure: an amphipathic shell composed of apolipoprotein, a phospholipid monolayer (not bilayer), and cholesterol, and a hydrophobic core composed mostly of cholesteryl esters or triglycerides, or 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. See M. Krieger, 1995, in E. Haber, ed., *Molecular Cardiovascular Medicine, Scientific American Medicine*, pp. 31–47.

Description
The illustration of the L D L model shows a sphere made of tiny spheres having two tail-like projections, called the phospholipids. The phospholipids make up the polar surface. The phospholipids cover the apolar core made from cholesteryl ester. Many unesterified cholesterol molecules are embedded in between the phospholipids. An apolipoprotein B molecule is embedded in the phospholipids.

We have seen that a general feature of coated vesicles is the ability to select specific cargo molecules; this process of cargo selection was first studied in the context of endocytosis of labeled LDL particles added to intact cells. Two general experimental approaches have been used to study how labeled LDL particles enter cells. The first method makes use of LDL that has been 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. After cultured cells are incubated for several hours with the labeled LDL, it is possible to determine how much LDL is bound to the surfaces of cells, how much is internalized, and how much of the apoB-100 component of the LDL is transported to the lysosome and there

degraded by enzymatic hydrolysis to individual amino acids. The degradation of apoB-100 can be detected by the release of ^{125}I-tyrosine into the culture medium. Figure 14-28 shows the time course of these events in receptor-mediated cellular LDL processing, determined by pulse-chase experiments with a fixed concentration of ^{125}I-labeled LDL. These experiments clearly demonstrate the order of events: surface binding of LDL → internalization → degradation. The second approach involves tagging LDL particles with an electron-dense label that can be detected by electron microscopy. Such studies can reveal the details of how LDL particles first bind to the surface of cells at sites of nascent endocytic vesicles known as *clathrin-coated pits*, then remain associated with the coated pits as they invaginate and bud off to form clathrin-coated vesicles, and are finally transported to endosomes.



EXPERIMENTAL FIGURE 14-28 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. 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.

Description

In the graph, the vertical axis represents the amount of 125-iodine labeled L D L in nanograms per dish ranging from 0 to 12 in increments of 2. The vertical axis

represents time at 37 degrees Celsius in minutes ranging from 0 to 60 in increments of 10. After 60 minutes, a break indicates that the experiment is continued for 120 minutes. The binding curve starts at zero minutes, 11 nanograms, and rapidly slopes downward at 5 minutes, 6 nanograms, and plateaus at 30 minutes, 2 nanograms. The internalization curve starts at 0 minutes, 2 nanograms and sharply rises in first few minutes to 10 nanograms, mirroring the binding curve. From 5 to 25 minutes, the internalization curve is flat, then slopes down and ends at 120 minutes, 3 nanograms. The degradation curve starts at 0 minutes, zero nanograms, and does not rise until 11 minutes into the experiment. After 11 minutes, the rise is linear for 70 minutes. Towards the end of the experiment, the gradient of the degradation curve increases.

Receptors for Macromolecular Ligands Contain Sorting Signals That Target Them for Endocytosis

The key to understanding how LDL particles bind to the cell surface and are then taken up into endocytic vesicles was the discovery of the *LDL receptor*. The LDL receptor is an 839-residue type I membrane glycoprotein; it has a long N-terminal exoplasmic segment that contains the LDL-binding domain, a single transmembrane segment, and a short C-terminal cytosolic segment. Seven cysteine-rich repeats form the LDL-binding domain, which interacts with the apoB-100 molecule in an LDL particle. Figure 14-29 shows how the LDL receptor facilitates internalization of LDL particles by receptor-mediated endocytosis. 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.

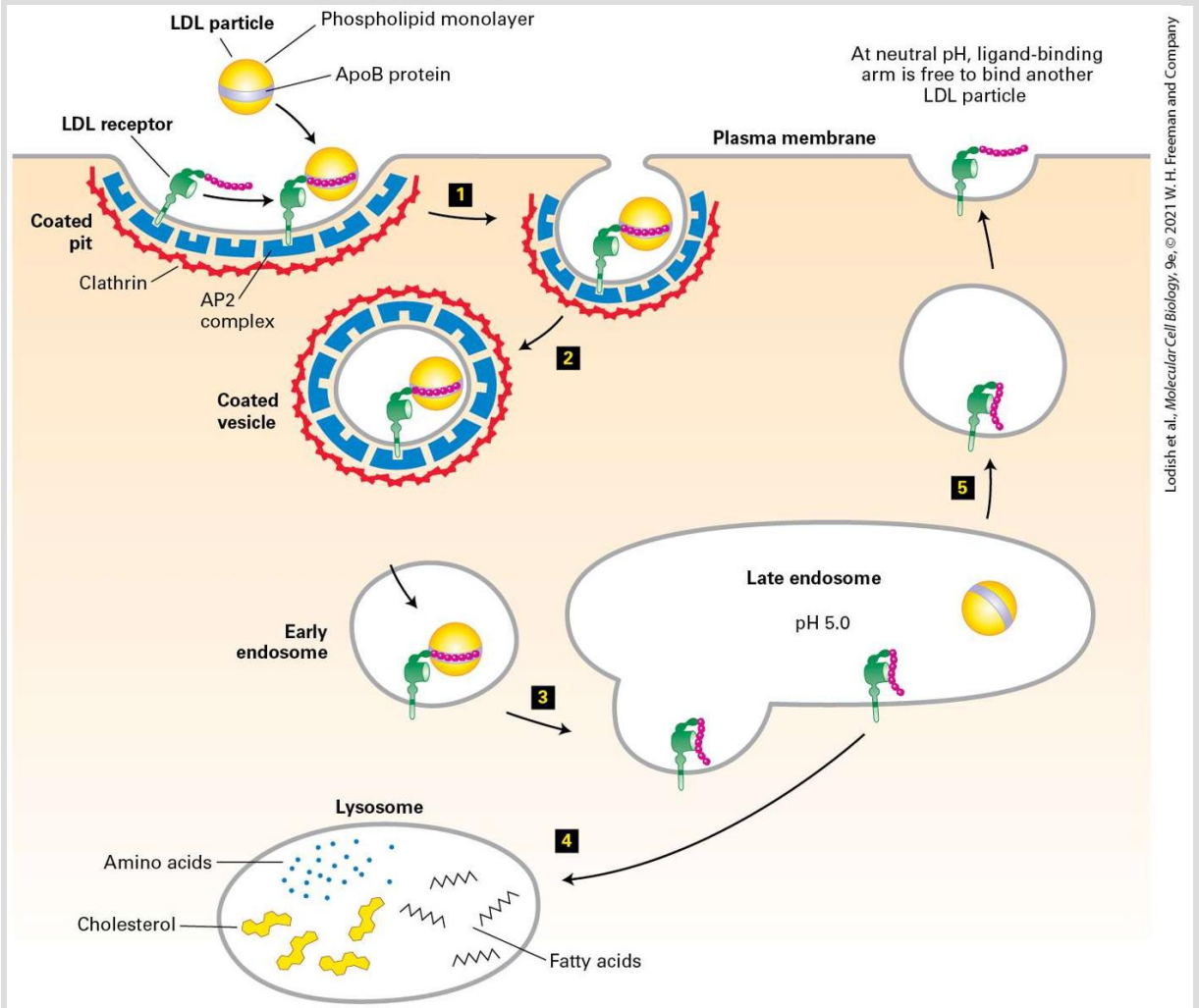


FIGURE 14-29 Endocytic pathway for internalizing low-density lipoprotein (LDL). Step 1: A cell-surface LDL receptor binds to an ApoB protein embedded in the phospholipid outer layer of an LDL particle. Interaction between the NPXY sorting signal in the cytosolic tail of the LDL receptor and the AP2 complex incorporates the receptor-ligand complex into a nascent endocytic vesicle. Step 2: Clathrin-coated pits containing receptor-LDL complexes are pinched off by the same dynamin-mediated mechanism used to form clathrin/AP1-coated vesicles on the *trans*-Golgi network (see Figure 14-19). Step 3: After the vesicle coat is shed, the uncoated endocytic vesicle (early endosome) fuses with a late endosome. The acidic pH in this compartment causes a conformational change in the LDL

receptor that leads to release of the bound LDL particle. Step 4: The late endosome fuses with a lysosome, and the proteins and lipids of the free LDL particle are broken down into their constituent parts by enzymes in the lysosome. Step 5: The LDL receptor is recycled to the cell surface, where at the neutral pH of the exterior medium the receptor undergoes a conformational change so that it can bind another LDL particle. See M. S. Brown and J. L. Goldstein, 1986, *Science* **232**:34; and G. Rudenko et al., 2002, *Science* **298**:2353.

Description

Step 1: L D L particle made up of a phospholipid monolayer and a band of Apo B protein binds with the L D L receptor attached to A P 2 complex present along with clathrin forming a coated pit in the plasma membrane. The endings of the coated pit come close together to internalize the L D L particle.

Step 2: A coated vesicle containing L D L is formed inside the cytosol.

Step 3: The coating of the vesicle disintegrates. The uncoated vesicle fuses with the late endosome, which has a pH of 5, causing a conformation change resulting in the release of L D L from its receptor.

Step 4: The late endosome fuses with a lysosome and the L D L particle is broken into amino acids, fatty acids, and cholesterol.

Step 5: A vesicles containing the L D L receptor move from the late endosome to the plasma membrane where the neutral p H of the exterior causes a conformational change and the receptor can bind to another L D L particle.

Mutations in the human gene for the LDL receptor (*LDLR*) can cause a hereditary disease that is marked by elevated plasma LDL known as *familial hypercholesterolemia (FH)*. In patients who have one normal and one defective copy of the *LDLR* gene (heterozygotes), LDL in the blood is increased about twofold. Those with two defective *LDLR* genes (homozygotes) have LDL levels that are from fourfold to sixfold higher

than normal. High levels of blood LDL can lead to excessive deposition of LDL, forming atherosclerotic plaque in coronary arteries. Without medical intervention, FH homozygotes usually die of heart attacks before reaching their late twenties.

A variety of mutations in the *LDLR* gene can cause FH. Some mutations prevent the synthesis of the LDL receptor protein; others prevent proper folding of the receptor protein in the ER, leading to its premature degradation (see Chapter 13); and 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. In individuals with this type of defect, plasma-membrane receptors for other ligands are internalized normally, but the mutant LDL receptor is not recruited into coated pits. Analysis of this mutant receptor and other mutant LDL receptors generated experimentally and expressed in fibroblasts identified a four-residue motif in the cytosolic segment of the receptor that is crucial for its internalization: Asn-Pro-X-Tyr, where X can be any amino acid. This *NPXY sorting signal* binds to the AP2 complex, linking the clathrin/AP2 coat to the cytosolic segment of the LDL receptor in coated pits. A mutation in any of the conserved residues of the NPXY signal abolishes the ability of the LDL receptor to be incorporated into coated pits. ■

The Acidic pH of Late Endosomes Causes Most Receptor-Ligand

Complexes to Dissociate

The overall rate of endocytic internalization of the plasma membrane is quite high: cultured fibroblasts regularly internalize 50 percent of their cell-surface proteins and phospholipids each hour. Most cell-surface receptors that undergo endocytosis will repeatedly deposit their ligands within the cell and then recycle to the plasma membrane to mediate internalization of ligand molecules once again. The LDL receptor, for instance, makes one round trip into and out of the cell interior every 10–20 minutes, for a total of about a hundred trips in its 20-hour life span.

Internalized receptor-ligand complexes commonly follow the pathway depicted for the M6P receptor in Figure 14-22 and the LDL receptor in Figure 14-29. Endocytosed cell-surface receptors typically dissociate from their ligands within late endosomes, which appear as spherical vesicles with tubular branching membranes located a few micrometers from the cell surface.

The dissociation of receptor-ligand complexes in late endosomes occurs not only in the endocytic pathway but also in the delivery of soluble lysosomal enzymes via the secretory pathway (see Figure 14-23). As discussed in Chapter 11, the membranes of late endosomes and lysosomes contain V-class proton pumps that act in concert with Cl^- channels to acidify the vesicle lumen (see Figure 11-13). Most receptors, including the M6P receptor and the LDL receptor, bind their ligands tightly at neutral pH, but release their ligands if the pH is lowered to 6.0 or below. The late

endosome is the first organelle encountered by receptor-ligand complexes whose luminal pH is sufficiently acidic to promote dissociation of most endocytosed receptors from their tightly bound ligands.

The mechanism by which the LDL receptor releases bound LDL particles is now understood in detail (Figure 14-30). At the endosomal pH of 5.0–5.5, histidine residues in a region known as the *β-propeller domain* of the receptor become protonated, forming a site that can bind with high affinity to the negatively charged cysteine-rich repeats in the LDL-binding domain. This intramolecular interaction sequesters the repeats in a conformation like that of a closed jackknife that cannot simultaneously bind to apoB-100, thus causing release of the bound LDL particle. The LDL receptor in this relatively compact closed state is efficiently recycled back to the plasma membrane for another round of binding and uptake of LDL.

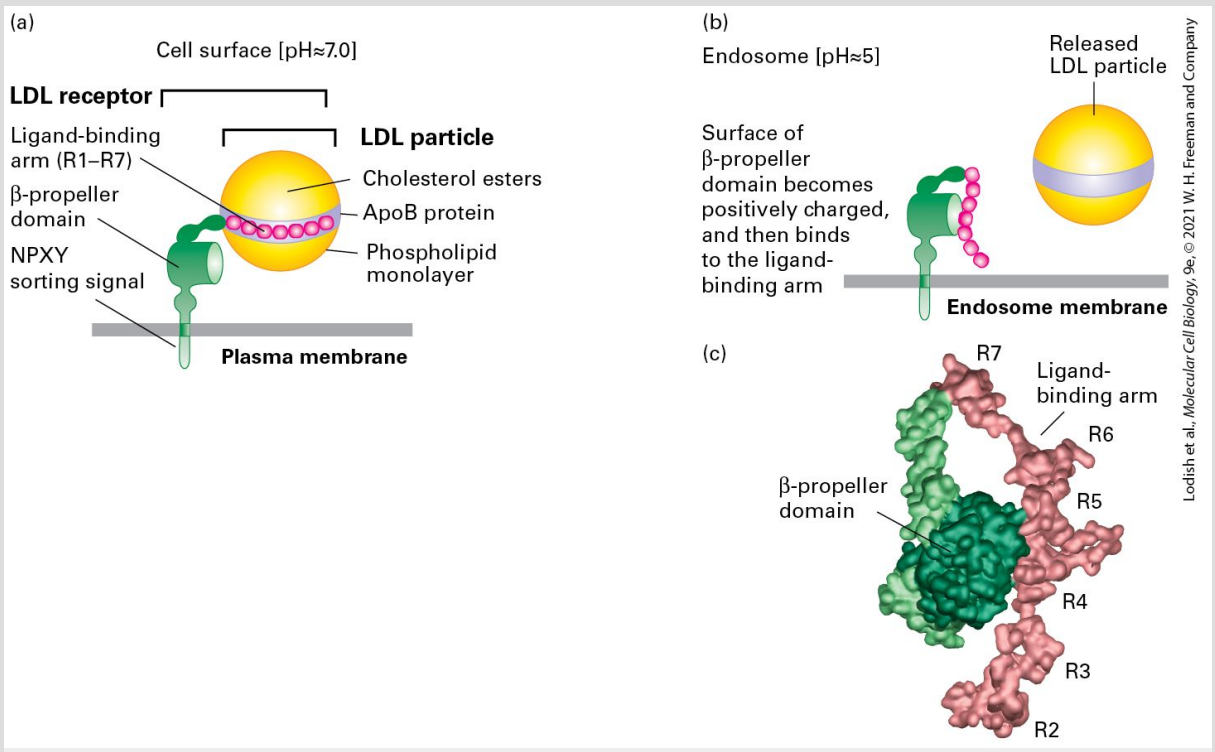


FIGURE 14-30 Model for pH-dependent binding of LDL particles by the LDL receptor. Schematic depiction of an LDL receptor at the neutral pH found at the cell surface and at the acidic pH found in the interior of the late endosome. (a) At the cell surface, apoB-100 on the surface of an LDL particle binds tightly to the receptor. Of the seven cysteine-rich repeats (R1–R7) in the ligand-binding arm, R4 and R5 appear to be most critical for LDL binding. (b) Within the endosome, histidine residues in the β-propeller domain of the LDL receptor become protonated. The positively charged propeller can bind with high affinity to the ligand-binding arm, which contains negatively charged residues, causing release of the LDL particle. (c) Experimental electron density and C_α backbone trace model of the extracellular region of the LDL receptor at pH 5.3 based on x-ray crystallographic analysis. In this conformation, extensive hydrophobic and ionic interactions occur between the β-propeller and the R4 and R5 repeats.

[Part (b) data from G. Rudenko et al., 2002, *Science* **298**:2353; PDB ID 1n7d.]

Description

The illustration labeled (a) shows an L D L receptor attached to the plasma membrane of a cell. The receptor consists of an N P X Y sorting signal embedded in the plasma membrane, a beta-propeller domain, and a ligand-binding arm with a seven residue-

binding region (R 1 to R 7). The ligand-binding arm is bound to the Apo B band on the L D L particle. The particle has the labels for phospholipid monolayer and cholesterol esters. The cell surface has a p H of 7.

The illustration labeled (b) shows an endosome membrane, which has a p H of 5. The L D L receptor is attached to the endosome membrane, while the L D L particle is released. A label reads, surface of beta-propeller domain becomes positively charged, and then binds to the ligand-binding arm.

A space-filling three-dimensional model labeled (c) shows the L D L receptor with a beta-propeller domain and six residue ligand-binding arms. The six residues are labeled, from top to bottom: R 7, R 6, R 5, R 4, R 3, and R 2.

As described in Chapter 6, human mutations that cause a loss of function of the gene PCSK9 lead to a decrease in blood LDL. We now know that PCSK9 encodes a secreted protein whose normal function is to negatively regulate the LDL receptor. Biochemical and structural studies show that PCSK9 binds to the LDL-binding domain of the LDL receptor, thereby preventing the receptor from closing into a compact state. This more open conformation of the receptor cannot be recycled to the plasma membrane and is transported to the lysosome where it is degraded. Inhibitors of PCSK9 are a promising new class of LDL-lowering drugs.

Receptor-Mediated Endocytosis Can Down-Regulate Signaling Receptors

Signaling receptors are an important class of plasma membrane proteins that can undergo receptor-mediated endocytosis. These receptors bind to extracellular ligands and respond by generating an appropriate

intracellular signal. As described in Chapter 16, the epidermal growth factor receptor (EGFR) activates a signal transduction pathway in response to the extracellular growth factor protein EGF. Treatment of cells with EGF both activates EGFR and stimulates receptor-mediated endocytosis of EGFR. Removal of EGFR from the plasma membrane by endocytosis leads to an attenuation of the signaling response in an adaptive process, known as *down-regulation*, which extends the dynamic range of EGF concentrations over which the system can respond. The mechanism of EGFR down-regulation is one of the best understood examples of regulated endocytosis.

EGFR is a Type I cell surface glycoprotein with a large extracellular domain that binds the ligand EGF, a single transmembrane domain, and a cytoplasmic signaling domain. Normally, EGFR resides in the plasma membrane as a monomer, but when EGF is present, two copies of EGF bind to two copies of EGFR, forming a dimer that brings the cytoplasmic signaling domains of EFGR together and activates signal transduction (see Figure 16-8). Activated EGFR enters the endocytic pathway because the dimeric form of the receptor binds to clathrin/AP2-coated pits. Mutational studies of the cytoplasmic domain of EGFR have identified a di-leucine (Leu-Leu) sorting signal that is necessary for rapid endocytosis. Other proteins employ a cytoplasmic di-leucine sorting signal for entry into the endocytic pathway, but EGFR is unusual in that the cytoplasmic di-leucine signal only interacts with clathrin/AP2 complexes during ligand-induced endocytosis when the cytoplasmic domain is dimeric.

Endocytosis delivers EGFR to the late endosome, and from this location EGFR is available to recycle to the plasma membrane, in a pathway similar to that for the LDL receptor (see Figure 14-29). Complete down-regulation depends on further transport of EGFR to the lysosome where it is degraded. In addition to enabling interaction with clathrin/AP2 complexes, ligand-induced activation of EGFR also triggers covalent modification of the cytoplasmic domain of EGFR by the small protein ubiquitin. As we will see in the next section, monoubiquitinylation marks EGFR for entry into multivesicular bodies that are ultimately degraded in lysosomes (see Figure 14-32).

KEY CONCEPTS OF SECTION 14.5

Receptor-Mediated Endocytosis

- Some extracellular ligands that bind to specific cell-surface receptors are internalized, along with their receptors, in clathrin-coated vesicles whose coats also contain AP2 complexes (see Figure 14-26).
- Sorting signals in the cytosolic domain of cell-surface receptors target them into clathrin/AP2-coated pits for internalization. Known signals include the Asn-Pro-X-Tyr, Tyr-X-X-Φ, and Leu-Leu sequences (see Table 14-2).
- The endocytic pathway delivers some ligands (e.g., LDL particles) to lysosomes, where they are degraded. Transport vesicles from the cell surface first fuse with late endosomes, which subsequently fuse with lysosomes.
- Most receptor-ligand complexes dissociate in the acidic milieu of the late endosome; the receptors are recycled to the plasma membrane, while the ligands are sorted to lysosomes (see Figure 14-29).
- Signaling receptors can be down-regulated by endocytosis. In these cases, the cytoplasmic domain of the receptors only interact with clathrin/AP2-coated pits when the receptor is in a ligand-activated state.