21.3 Responding to Changes in the Levels of Cholesterol and Unsaturated Fatty Acids

All cells need to control the amount and composition of membrane lipids. We learned in Chapter 10 that cells must maintain an appropriate fluidity of their membranes and that this is determined by both the amount of cholesterol or other steroids in each cellular membrane and the percentage of unsaturated fatty acids in membrane phospholipids. Indeed, a cell would soon face a crisis if it did not have enough phospholipids to make adequate amounts of membranes or had so much cholesterol that large crystals formed and damaged cellular structures (see Chapter 10). Here we describe how cells maintain cholesterol and fatty acid homeostasis by regulating both the synthesis of enzymes that catalyze key steps in their biosynthesis and the import of exogenous cholesterol from the extracellular fluid. Critical features of this regulation are the sensing of these metabolites by proteins in the endoplasmic reticulum.

Fatty Acid and Cholesterol Biosynthesis as Well as Cholesterol Import Are Regulated at the Level of Gene Transcription

The transcription of genes encoding many enzymes in the cholesterol biosynthetic pathway (see Figure 10-26) is subject to feedback regulation. When cellular cholesterol levels are adequate, cholesterol biosynthesis is suppressed so that excess unesterified cholesterol, which can be toxic, does not accumulate. When cellular cholesterol levels fall too low, threatening the integrity of many cell membranes, biosynthesis is increased. Synthesis of one of the enzymes in the cholesterol biosynthetic pathway, HMG-CoA reductase, is tightly regulated as is the rate-controlling enzyme in cholesterol biosynthesis.

Feedback regulation also controls the import of extracellular cholesterol. As we learned in Chapter 14, lipoproteins are lipid-filled particles that transport hydrophobic molecules through the circulatory system. In humans, the most abundant transporter of cholesterol, in the form of cholesteryl esters, is low-density lipoprotein (LDL; see Figure 14-27). LDL delivers its cholesterol to cells by binding to cell-surface LDL receptors, which mediate the endocytic uptake of LDL particles and their subsequent disassembly in lysosomes, from where the cholesterol enters the cellular pool of cholesterol (see Figure 14-29). The transcription of LDL receptors is transcriptionally regulated to maintain cellular cholesterol homeostasis, and similar systems are used to control unsaturated fatty acid levels.

Sterol Regulatory Elements (SREs)

The levels of sterols such as cholesterol, as well as unsaturated fatty acids, regulate the levels of expression of a number of genes in a cell. The

promoters of most cholesterol-sensitive and unsaturated fatty acid–sensitive genes, including HMG-CoA and the LDL receptor, contain one or more 10-base-pair *sterol regulatory elements (SREs)*, or SRE half-sites, in their promoters. (These SREs differ from the *s*erum *r*esponse *e*lements that control many early response genes, discussed in Section 16.2.) The interaction of lipid-regulated transcription factors called **SRE-binding proteins** (**SREBPs**) with these response elements modulates the expression of the target genes.

Mammals express three isoforms of SREBP: 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 transcription factors control expression of proteins that regulate availability not only of cholesterol, but also of fatty acids and the triglycerides and phospholipids made from fatty acids. 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.

How do cells sense how much cholesterol and unsaturated fatty acids they have, and how are these signals used to control the level of SREBPs in the nucleus and thus gene expression? The SREBP-mediated pathway begins in the membranes of the endoplasmic reticulum (ER) and includes several other proteins besides SREBP. In Chapter 16, we saw how *regulated intramembrane proteolysis* plays a key role in the Notch and EGF signaling pathways; here we see that *regulated intramembrane proteolysis*

also plays an important role in this cellular response to altered cholesterol and fatty acid levels.

The Endoplasmic Reticulum SCAP Protein Senses the Level of Cellular Cholesterol

When cells have adequate levels 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 21-6a). SREBP has three distinct domains: an N-terminal cytosolic domain, containing a basic helix-loop-helix (bHLH) DNA-binding motif (see Figure 8-25d), that functions as a transcription factor when cleaved from the rest of SREBP; a central membrane-anchoring domain containing two transmembrane α helices; and a C-terminal cytosolic-facing regulatory domain.

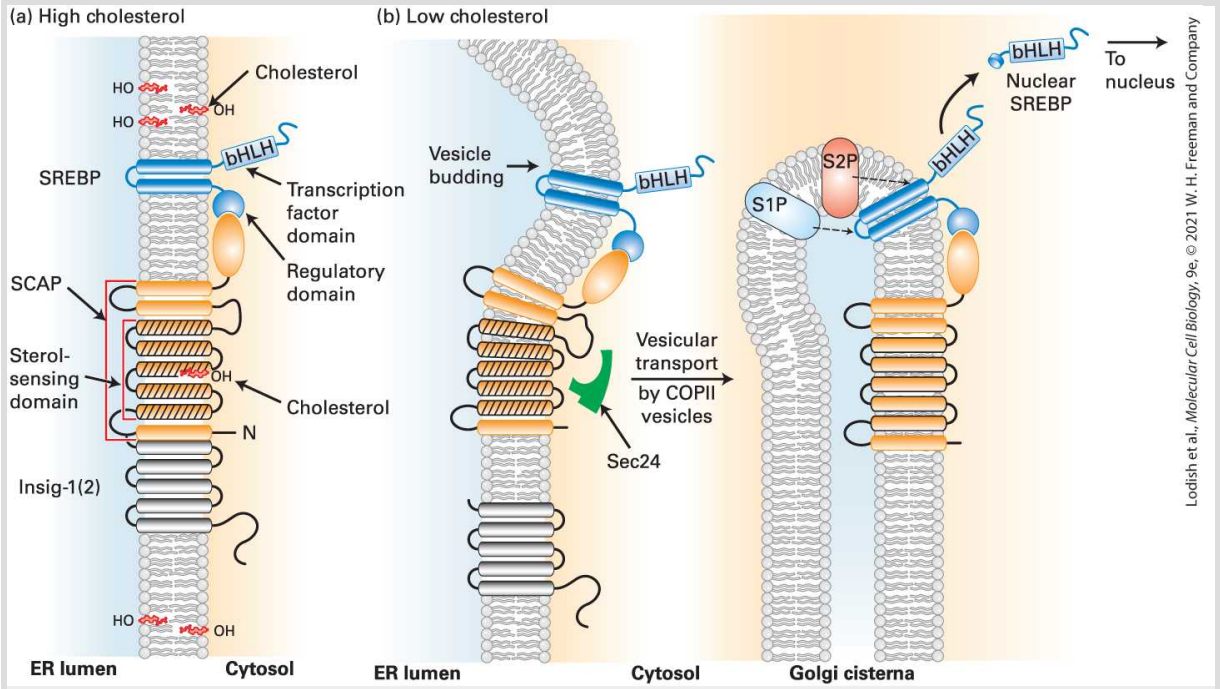

FIGURE 21-6 Cholesterol-sensitive control of SREBP activation. The cellular pool of cholesterol is monitored by the cholesterol-sensor SCAP, located in the ER membrane and stably bound via its C-terminus to the C-terminus of the membrane protein SREBP. Membrane-spanning helices 2–6 of SCAP (orange with black lines) form the sterol-sensing domain. (a) When cholesterol levels are high enough that ER cholesterol exceeds 5 percent of total ER membrane lipid molecules, cholesterol binds to the sterol-sensing domain in SCAP, triggering a conformational change that enables the SREBP/SCAP-cholesterol complex to bind to insig-1(2), anchoring the entire SCAP-SREBP complex in the ER membrane. (b) At low-cholesterol levels, cholesterol dissociates from the SCAP sterol-sensing domain, triggering a reverse conformational change that both dissociates SCAP from insig-1(2) and enables SCAP to bind to Sec24 (green), a subunit of the COPII complex (see Figure 14-9). As a consequence, the SCAP-SREBP complex enters COPII-coated vesicles that transport it to the Golgi. In the Golgi, the sequential cleavage of SREBP by the site 1 and site 2 proteases (S1P, S2P) releases the N-terminal bHLH transcription factor domain of SREBP into the cytosol, which then translocates to the nucleus. There the released bHLH domain of SREBP, called *nuclear SREBP (nSREBP)*, controls the transcription of genes containing sterol regulatory elements (SREs) in their promoters The SCAP protein recycles from the Golgi back to the ER (not shown). See M. S. Brown, A. Radhakrishnan, and J. L. Goldstein, 2018, *Annu. Rev. Biochem.* **87**:783–807; and R. A. DeBose-Boyd and J. Ye, 2018, *Trends Biochem. Sci.* **43**:358–368.

Description
The illustration labeled (a) shows a vertical membrane between the E R and the cytosol. This membrane has little lightning marks labeled cholesterol breaking into the membrane at various points and in the sterol sensing domain. The S R E B P is represented as two blue oblongs above the sterol domain and an orange oblong is labeled S C A P. The illustration labeled (b) shows the same diagram, without cholesterol and the membrane is now flexible and curves backward to form a loop. The b H L H protein is now able to release and translocate to the nucleus.

SCAP has eight transmembrane α helices and a large C-terminal cytosolic domain that binds to the regulatory domain of SREBP. Five of the transmembrane α helices in SCAP form a *sterol-sensing domain* similar to that in HMG-CoA reductase (see Figure 21-6a; also see Section 10.3). When cholesterol binds to the sterol-sensing domain in SCAP, SCAP assumes a conformation such that it binds to insig-1(2). When the SCAP-cholesterol complex is tightly bound to insig-1(2), it is in a conformation that blocks its binding to the Sec24 subunit of the COPII vesicle coat complex (see Figure 14-9). This prevents incorporation of the SCAP-SREBP complex into COPII ER-to-Golgi transport vesicles (see Chapter 14). As a consequence the SCAP-SREBP complex is retained in the ER, and thus the transcription factor in SREBP's N-terminal domain cannot enter the nucleus and regulate gene expression. This occurs when cholesterol levels in the ER membrane exceed 5 percent of total ER membrane lipids.

Regulated Intramembrane Proteolysis of SREBP in the Golgi Releases a

bHLH Transcription Factor That Acts to Maintain Appropriate Phospholipid and Cholesterol Levels

SCAP releases its bound cholesterol when cholesterol levels in the ER membrane drop to less than 5 percent of ER lipids, a value that reflects normal cellular cholesterol levels. Consequently, Insig-1(2) can no longer bind to the cholesterol-free SCAP, and the SCAP-SREBP complex moves from the ER to the Golgi complex via COPII vesicles (Figure 21-6b). SREBP is then cleaved sequentially at two sites by two proteases that are resident in the Golgi membrane. Site-1 protease (S1P) cleaves SREBP in its luminal loop, separating the protein into two membrane-bound halves. S1P-mediated cleavage renders the N-terminal half of SREBP a substrate for the Site-2 protease (S2P), which releases the N-terminal bHLH-containing domain of SREBP into the cytosol by cleaving the intermediate near the cytosol-membrane boundary, an example of *regulated intramembrane proteolysis*. This protein, also called *nSREBP (nuclear SREBP)*, is rapidly translocated into the nucleus, where it activates transcription of genes containing sterol regulatory elements (SREs) in their promoters.

SREBP-2 primarily activates transcription of genes required for cholesterol synthesis and uptake such as those encoding the LDL receptor and HMG-CoA reductase. Thus a reduction in cellular cholesterol, by activating the *Insig-1(2)/SCAP/SREBP pathway*, triggers expression of genes encoding proteins that both import cholesterol into the cell (the LDL

receptor) and synthesize cholesterol from small precursor molecules (HMG-CoA reductase).

SREBP-1a and SREBP-1c preferentially activate transcription of genes required for fatty acid and triglyceride synthesis. The level of unsaturated (but not saturated) fatty acids also regulates cleavage of SREBP-1, but by a slightly different mechanism. In cells deprived of unsaturated fatty acids, Insig-1 becomes a substrate for polyubiquitination, extraction from the ER membrane into the cytosol by the process known as *ERAD (ER-a*ssociated *d*egradation; end of Section 13.3), and subsequent degradation by proteasomes. In the absence of Insig-1, SCAP-1 assumes a conformation enabling it to bind the Sec24 subunit of COPII vesicles, similar to the low-cholesterol situation diagrammed in Figure 21-6b. This initiates transport of SCAP-bound SREBP from the ER to the Golgi, where it undergoes proteolytic cleavage by S1P and S2P that releases its bHLH-containing SREBP-1 transcription factor domain, enabling it to activate expression of genes required for synthesis of fatty acids and triglycerides. In all cases, after cleavage of SREBP in the Golgi, SCAP apparently recycles back to the ER, where it can interact with insig-1(2) and another intact SREBP molecule.

High-level transcription of SRE-controlled genes requires the ongoing generation of new nSREBP because this transcription factor is degraded fairly rapidly by the ubiquitin-mediated proteasomal pathway (see Chapter 3). The rapid generation and degradation of nSREBP also helps cells respond quickly to changes in levels of intracellular cholesterol and fatty acids.

Under some circumstances (e.g., during cell growth), cells need an increased supply of all essential membrane lipids and their fatty acid precursors, processes that require coordinate regulation of many metabolic pathways. In the liver, mTOR, after it becomes activated following stimulation by many growth factor receptors (Section 21.2), directly activates transcription of the *SREB-1c* gene. This in turn results in increased transcription of all of its downstream target genes that encode proteins catalyzing steps in fatty acid synthesis.

KEY CONCEPTS OF SECTION 21.3

Responding to Changes in the Levels of Cholesterol and Unsaturated Fatty Acids

- Vertebrate cells maintain a constant level of cholesterol in their membranes.
- The cellular pool of cholesterol is monitored in the ER membrane by the SCAP protein, which is in a stable complex with full-length SREBP.
- At high-cholesterol levels, cholesterol is bound to the SREBP-SCAP complex, which in turn is bound to the ER protein Insig-1(2), anchoring the entire SREBP-SCAP-cholesterol complex in the ER membrane and preventing its transport to the Golgi and subsequent release of its active nSREBP domain, a transcription factor.
- At low-cholesterol levels, cholesterol dissociates from the SREBP-SCAP complex, triggering conformational changes that lead to the dissociation of SCAP from Insig-1(2), the binding of SREBP and SCAP to the COPII coat, and transport of the SREBP-SCAP complex to the Golgi.
- In the Golgi, cleavage of SREBP by two proteases releases the N-terminal transcription factor, bHLH domain of SREBP (nSREBP), which translocates to the nucleus and activates expression of genes containing sterol regulatory elements (SREs) in their promoters. Among these genes are those that encode proteins that import cholesterol into the cell and enzymes that catalyze key steps in the biosynthesis of cholesterol.
- SREBP-2 primarily regulates cholesterol import and synthesis while SREBP-1a and SREBP-1c primarily regulates unesterified fatty acid synthesis.