14.6 Directing Membrane Proteins and Cytosolic Materials to the Lysosome for Degradation

The major function of lysosomes is to degrade extracellular materials taken up by the cell and to degrade intracellular components under specific conditions. Materials to be degraded must be delivered to the lumen of the lysosome, where the various degradative enzymes reside. As we have just seen, endocytosed ligands (e.g., LDL particles) that dissociate from their receptors in the late endosome subsequently enter the lysosomal lumen when the membrane of the late endosome fuses with the membrane of the lysosome (see Figure 14-29).

It is apparent how the general vesicular trafficking mechanism discussed in this chapter can be used to deliver the luminal contents of an endosomal organelle to the lumen of the lysosome for degradation. In this section, we will discuss two further pathways. Membrane proteins delivered to the lysosome by the typical vesicular trafficking process we have discussed the previous section should ultimately be delivered to the membrane of the lysosome. How, then, are membrane proteins delivered to the interior of the lysosome for degradation? The first pathway discussed in this section, used to degrade endocytosed membrane proteins, utilizes an unusual type of vesicle that buds into the lumen of the endosome to produce a multivesicular endosome. The second pathway that will be discussed is

used by the cell for delivery of cytosolic materials to the lysosomal lumen for degradation. This second pathway, known as *autophagy*, involves the de novo formation of a double-membrane organelle known as an *autophagosome* that envelops cytosolic material, such as soluble cytosolic proteins, or sometimes organelles, such as peroxisomes or mitochondria. Both pathways lead to fusion of either the multivesicular endosome or autophagosome with the lysosome, depositing the contents of these organelles into the lysosomal lumen for degradation.

Multivesicular Endosomes Segregate Membrane Proteins Destined for the Lysosomal Membrane from Proteins Destined for Lysosomal Degradation

Resident lysosomal membrane proteins, such as V-class proton pumps and amino acid transporters, can carry out their functions and remain in the lysosomal membrane, where they are protected from degradation by the soluble hydrolytic enzymes in the lumen. Such proteins are delivered to the lysosomal membrane by transport vesicles that bud from either the *trans*-Golgi network or the endosome by the same basic mechanisms described in earlier sections. In contrast, endocytosed membrane proteins that are to be degraded are transferred in their entirety to the interior of the lysosome by a specialized delivery mechanism. Lysosomal degradation of cell-surface receptors for extracellular signaling molecules is a common mechanism for controlling the sensitivity of cells to extracellular signals

(see Chapter 15). Receptors that become damaged are also targeted for lysosomal degradation.

Early evidence that membranes can be delivered to the lumen of a membrane-bounded compartment came from electron micrographs showing membrane vesicles and fragments of membranes within endosomes and lysosomes. Parallel experiments in yeast revealed that endocytosed receptor proteins targeted to the vacuole (the yeast organelle equivalent to the lysosome) were primarily associated with membrane fragments and small vesicles within the interior of the vacuole rather than with the vacuole surface membrane.

These observations suggest that endocytosed membrane proteins can be incorporated into specialized vesicles that form at the endosomal membrane (Figure 14-31). Although these vesicles are similar in size and appearance to transport vesicles, they differ topologically. Transport vesicles bud *outward* from the surface of a donor organelle into the cytosol, whereas vesicles within the endosome bud *inward* from the surface into the lumen (away from the cytosol). Mature endosomes containing numerous vesicles in their interior are usually called **multivesicular endosomes** (or bodies). The surface membrane of a multivesicular endosome then fuses with the membrane of a lysosome, thereby delivering its internal vesicles and the membrane proteins they contain into the lysosome interior for degradation. Thus the sorting of proteins in the endosomal membrane determines which ones will remain on the lysosome surface (e.g., pumps and transporters) and which ones

will be incorporated into internal vesicles and ultimately degraded in lysosomes.

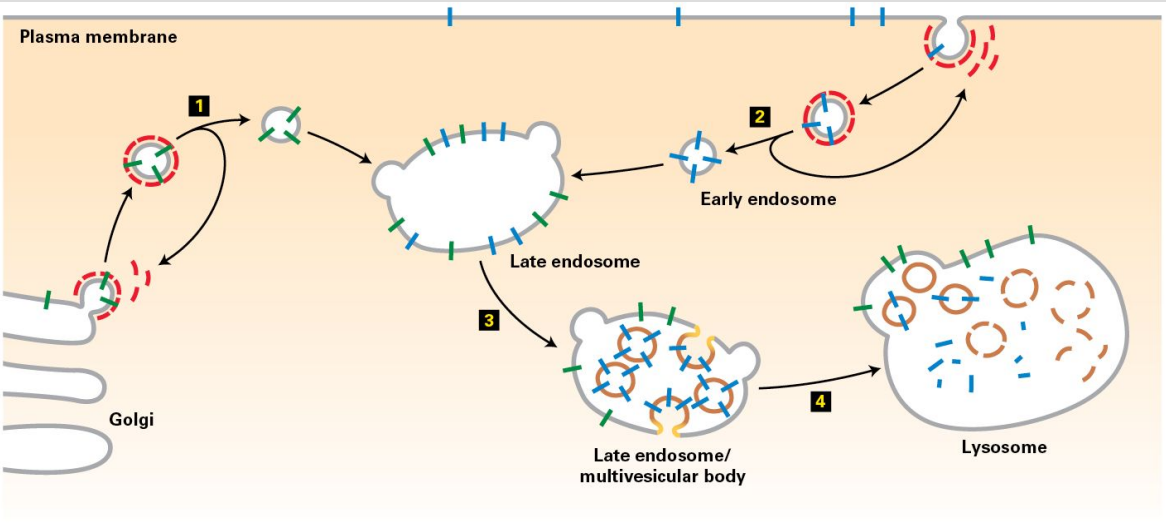

FIGURE 14-31 Delivery of plasma-membrane proteins to the lysosome interior for degradation. Early endosomes carrying endocytosed plasma-membrane proteins (blue) and vesicles carrying lysosomal membrane proteins (green) from the *trans*-Golgi network fuse with the late endosome, transferring their membrane proteins to the endosomal membrane (steps 1 and 2). Proteins to be degraded, such as those from the early endosome, are incorporated into vesicles that bud *into* the interior of the late endosome, eventually forming a multivesicular endosome containing many such internal vesicles (step 3). Fusion of a multivesicular endosome directly with a lysosome releases the internal vesicles into the lumen of the lysosome, where they can be degraded (step 4). Because proton pumps and other lysosomal membrane proteins normally are not incorporated into internal endosomal vesicles, they are delivered to the lysosomal membrane and are protected from degradation. See F. Reggiori and D. J. Klionsky, 2002, *Eukaryot. Cell* **1**:11; and D. J. Katzmann et al., 2002, *Nat. Rev. Mol. Cell Biol.* **3**:893.

Description

Step 1: A vesicle for degradation buds from the Golgi. The coat proteins cover the bud formed in the Golgi. The coated vesicle formed moves towards the late endosome and becomes an uncoated vesicle. The uncoated vesicle fuses with the late endosome membrane.

Step 2: Proteins from the plasma membrane are endocytosed into the cytosol. These vesicles containing proteins for degradation merge with the late endosome, becoming embedded in the membrane of the endosome.

Step 3: The proteins bound to the late endosome membrane bud into the endosome, forming a multivesicular body.

Step 4: Merging with a lysosome, the contents of the multivesicular body, including the membrane proteins, are digested.

Many of the proteins required for inward budding of the endosomal membrane were first identified by mutations in yeast that blocked the delivery of membrane proteins to the interior of the vacuole. The current model of endosomal budding to form multivesicular endosomes is based primarily on studies in yeast, but mammalian cells appear to have essentially the same mechanism (Figure 14-32). Most cargo proteins that enter a multivesicular endosome are tagged with ubiquitin. Cargo proteins destined to enter a multivesicular endosome usually receive their ubiquitin tags at the plasma membrane, the *trans*-Golgi network, or the endosomal membrane. We have already seen how ubiquitin tagging can serve as a signal for degradation of cytosolic or misfolded ER proteins by proteasomes (see Chapters 3 and 13). When used as a signal for proteasomal degradation, the ubiquitin tag usually consists of a chain of covalently linked ubiquitin molecules (polyubiquitin), whereas ubiquitin used to tag proteins for entry into the multivesicular endosome usually takes the form of a single (monoubiquitin) molecule. In the membrane of the endosome, a ubiquitin-tagged peripheral membrane protein, known as *Hrs*, facilitates recruitment of a set of at least three different protein complexes to the membrane. These **ESCRT** (*e*ndosomal *s*orting

*c*omplexes *r*equired for *t*ransport) **proteins** include the ubiquitin-binding protein *Tsg101*. The membrane-associated ESCRT proteins act to drive vesicle budding directed into the interior of the endosome as well as the loading of specific monoubiquitinylated membrane cargo proteins into the vesicle buds. Finally, the ESCRT proteins pinch off the vesicle by forming a filamentous spiral inside the neck of a vesicle bud, releasing it and the specific membrane cargo proteins it carries into the interior of the endosome. An ATPase, known as *Vps4*, uses the energy from ATP hydrolysis to disassemble the ESCRT proteins, releasing them into the cytosol for another round of budding. In the fusion event that pinches off a completed endosomal vesicle, the ESCRT proteins and Vps4 may function like SNAREs and NSF, respectively, in the typical membrane-fusion process discussed previously (see Figure 14-11).

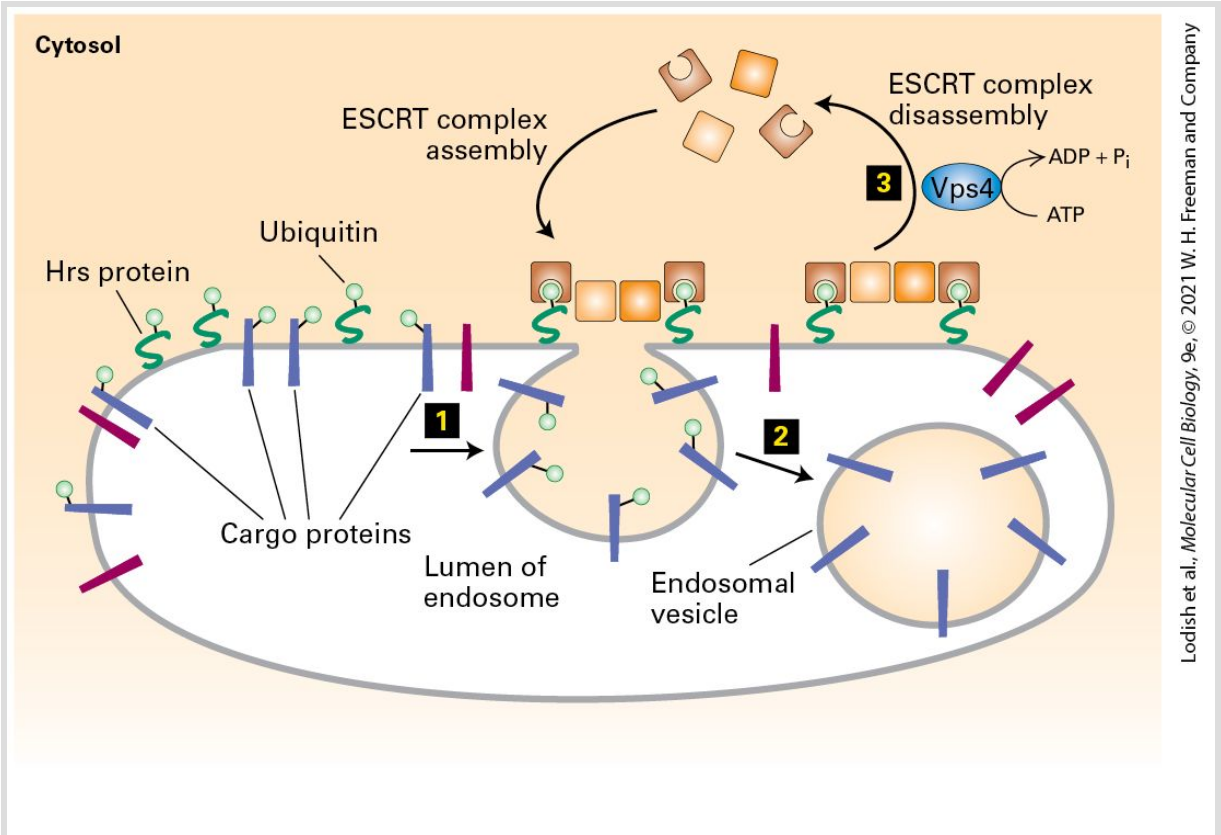

FIGURE 14-32 Model of the mechanism for formation of multivesicular endosomes. In endosomal budding, ubiquitinylated Hrs on the endosomal membrane directs the loading of specific membrane cargo proteins (blue) into vesicle buds and then recruits cytosolic ESCRT protein complexes to the membrane (step 1). Note that both Hrs and the recruited cargo proteins are tagged with ubiquitin. After the set of bound ESCRT complexes mediates the completion and pinching off of the inwardly budding vesicles (step 2), these complexes are disassembled by the ATPase Vps4 and returned to the cytosol (step 3). See text for discussion. See O. Pornillos et al., 2002, *Trends Cell Biol.* **12**:569.

Description
Step 1: An illustration shows an endosome. In the endosomal membrane, many cargo proteins and H r s proteins are embedded. These proteins are labeled with ubiquitin. E S C R T complexes are bound to the ubiquitin-bound h r s proteins. A vesicle starts to bud from the plasma membrane into the lumen of the endosome with the cargo proteins embedded in them.

Step 2: An endosomal vesicle containing cargo proteins is formed from the bud.

Step 3: On the surface of the endosome, the E S C R T complex is disassembled via V P S 4 using an A T P molecule.

Retroviruses Bud from the Plasma Membrane by a Process Similar to Formation of Multivesicular Endosomes

The vesicles that bud into the interior of endosomes have a topology similar to that of enveloped virus particles that bud from the plasma membrane of virus-infected cells. Moreover, recent experiments have

demonstrated that a common set of proteins is required for both types of membrane-budding events. In fact, the two processes so closely parallel each other in mechanistic detail as to suggest that enveloped viruses have evolved mechanisms to recruit the cellular proteins used in inward endosomal budding for their own purposes.

The human immunodeficiency virus (HIV) is an enveloped retrovirus that buds from the plasma membrane of infected cells in a process driven by the viral Gag protein, the major structural component of completed virus particles. Gag protein binds to the plasma membrane of an infected cell, and some 4000 Gag molecules polymerize into a spherical shell, producing a structure that looks like a vesicle bud protruding outward from the plasma membrane. Mutational studies with HIV have revealed that the N-terminal segment of Gag protein is required for association with the plasma membrane, whereas the C-terminal segment is required for pinching off of complete HIV particles. For instance, if the portion of the viral genome encoding the C-terminus of Gag is removed, HIV buds will form in infected cells, but pinching off does not occur, and thus no free virus particles are released.

The first indication that HIV budding employs the same molecular machinery as vesicle budding into endosomes came from the observation that Tsg101, an ESCRT protein, binds to the C-terminus of Gag protein. Subsequent findings have clearly established the mechanistic parallels between the two processes. For example, Gag is ubiquitinylated as part of the process of virus budding, and in cells with mutations in Tsg101 or Vps4, HIV virus buds accumulate but cannot pinch off from the membrane

(Figure 14-33). Moreover, when a segment from the cellular Hrs protein is added to a truncated Gag protein by construction of the appropriate hybrid gene, proper budding and release of virus particles is restored. Taken together, these results indicate that Gag protein mimics the function of Hrs, redirecting ESCRT proteins to the plasma membrane, where they can function in the budding of virus particles.

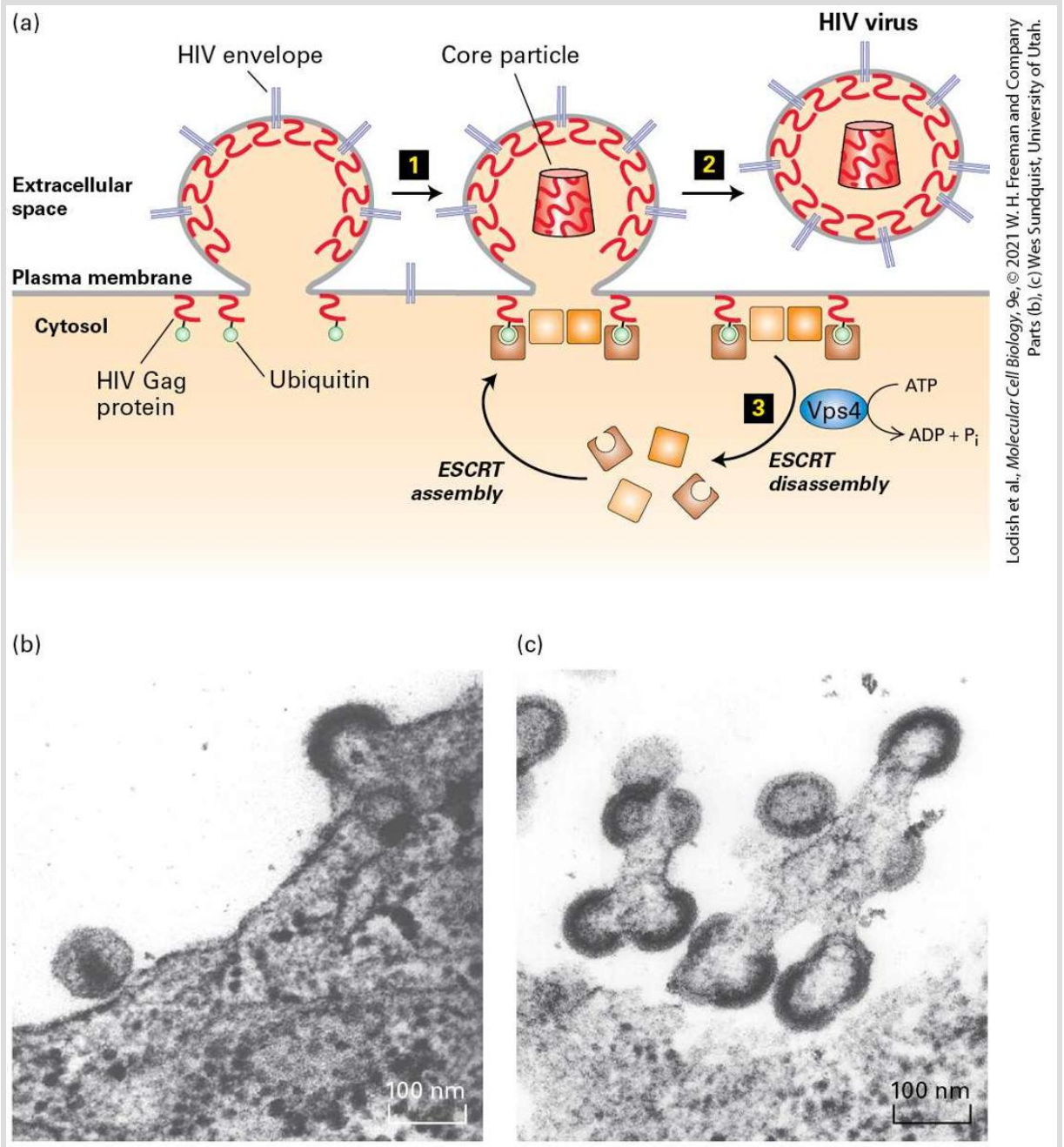

FIGURE 14-33 Mechanism for budding of HIV from the plasma membrane. Proteins required for the formation of multivesicular endosomes are exploited by HIV for virus budding from the plasma membrane. (a) Budding of HIV particles from HIV-infected cells occurs by a mechanism similar to that shown in Figure 14-32, using the virally encoded Gag protein and cellular ESCRT and Vps4 proteins (steps 1–3). Ubiquitinylated Gag located near a budding particle functions like Hrs. (b) In wild-type cells infected with HIV, virus particles bud from the plasma membrane and are rapidly released into the extracellular space. (c) In cells that lack the functional ESCRT protein Tsg101, the viral Gag protein

forms dense viruslike structures, but budding of these structures from the plasma membrane cannot be completed, and chains of incomplete viral buds still attached to the plasma membrane accumulate.

Description
The illustration labeled (a) shows H I V budding from the plasma membrane of a cell. On the cytosolic side of the membrane, many H I V G A G proteins are embedded. The H I V particle also contains H I V envelope proteins. In the cytosol, the G A G proteins are ubiquitinated. The E S C R T assembly leads to pinching off of the vesicle containing the core particle. The H I V buds and the E S C R T complex in the cytosol disassemble by the action of V p s 4 which uses a molecule of A T P.

The electron micrograph labeled (b) shows wild-type H I V budding from a cell membrane into the extracellular space. It is dark and circular.

The electron micrograph labeled (c) shows a clump of buds with dark outlines. These are H I V buds attached to the plasma membrane, unable to leave the plasma membrane.

Other enveloped retroviruses, such as murine leukemia virus and Rous sarcoma virus, have also been shown to require ESCRT complexes for their budding, although each virus appears to have evolved a somewhat different mechanism to recruit ESCRT complexes to the site of virus budding.

The Autophagic Pathway Delivers Cytosolic Proteins or Entire Organelles to Lysosomes

When cells are placed under stressful conditions, such as starvation, they have the capacity to recycle macromolecules for use as nutrients in a process of lysosomal degradation known as **autophagy** ("eating oneself"). The autophagic pathway begins with the formation of a flattened double-membrane cup-shaped structure that envelops a region of the cytosol or an entire organelle (e.g., a mitochondrion), forming an *autophagosome*, or *autophagic vesicle* (Figure 14-34). The outer membrane of an autophagosome can fuse with a lysosome, delivering a large vesicle, bounded by a single membrane bilayer, to the interior of the lysosome. Lipases and proteases within the lysosome degrade the autophagosome and its contents into their molecular components, just as they do when the contents of multivesicular endosomes are delivered to the lysosome. Amino acid permeases in the lysosomal membrane then allow for the transport of free amino acids back into the cytosol for use in synthesis of new proteins.

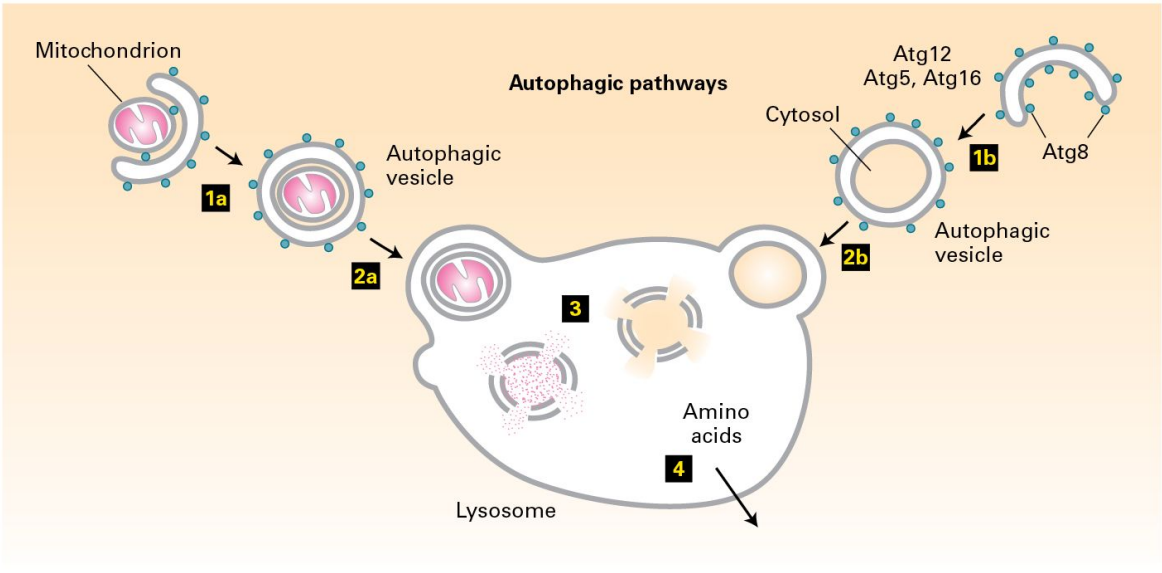

FIGURE 14-34 The autophagic pathway. The autophagic pathway allows cytosolic proteins and organelles to be delivered to the lysosome interior for degradation. In the

autophagic pathway, a cup-shaped structure forms around a portion of the cytosol (*right*) or an organelle such as a mitochondrion (*left*). Continued addition of membrane eventually leads to the formation of an autophagosome that envelops its contents in two complete membranes (steps 1a and 1b). Fusion of the outer membrane with the membrane of a lysosome releases a single-membrane vesicle and its contents into the lysosome interior (steps 2a and 2b). After degradation of the protein and lipid components by hydrolases in the lysosome interior (step 3), the released amino acids are transported across the lysosomal membrane into the cytosol (step 4). Proteins known to participate in the autophagic pathway include Atg8, which forms a coat structure around the autophagosome.

Description
This illustration has a beginning pathway coming in from the top left and labeled with steps 1 a and 2 a, and a second pathway coming down from the top right and labeled 1 b and 2 b.

1 a: In the pathway from top left, the mitochondrion is represented by a pink circle being enclosed by an autophagic vesicle.

2 a: The vesicle fully encloses the mitochondrion to enter into the lysosome.

1 b. A C-shaped autophagic vesicle with tiny A t g 8 spheres attached to it is present in the cytosol.

2 b: The autophagic vesicle encloses cytosol inside it.

3: Inside the lysosome, both vesicles from 2 a and 2 b and the mitochondrion dissolve.

4: Amino acids exit the lysosome.

By studying mutants with defects in the autophagic pathway, scientists have identified processes other than recycling of cellular components during starvation that also depend on autophagy. Experiments carried out principally in *Drosophila* and mice have shown that autophagy participates in a type of quality-control mechanism that removes

organelles that have ceased to function properly. In particular, the autophagic pathway can target dysfunctional mitochondria that have lost their integrity and no longer have an electrochemical gradient across their inner membrane. In certain cell types, pathogenic bacteria and viruses that are multiplying in the cytosol of host cells can be targeted to the autophagic pathway for destruction in the lysosome as part of a host defense mechanism against infection.

In each of these processes, and in all eukaryotic organisms, the autophagic pathway takes place in three basic steps. Although the mechanisms underlying each of these steps are relatively poorly understood, they are thought to be related to the basic mechanisms for vesicular trafficking discussed in this chapter.

Autophagosome Nucleation

The autophagosome is thought to originate from a fragment of a membrane-bounded organelle. The origin of this membrane has been difficult to trace because no known unique integral membrane proteins, which might serve to identify the source of this membrane, are known to be required for the formation of the autophagosome. Studies in yeast have shown that some mutants defective in Golgi trafficking are also defective in autophagy, suggesting that the autophagosome is initially derived from a fragment of the Golgi. Autophagy that is induced by starvation appears to be a nonspecific process in which a random portion of the cytoplasm, including organelles, becomes enveloped by an autophagosome. In these cases, the site of nucleation is probably random. In cases in which

organelles are enveloped by the autophagosome in response to nutrient starvation, a signal based on ubiquitin modification on the surface of the organelle targets nucleation of the autophagosome.

Autophagosome Growth and Completion

New membrane must be delivered to the autophagosome membrane in order for this cup-shaped organelle to grow. This growth probably occurs by the fusion of transport vesicles with the membrane of the autophagosome. About 30 proteins that participate in the formation of autophagosomes have been identified in genetic screens for yeast mutants that are defective in autophagy. One of these proteins is Atg8, shown in Figure 14-34, which is covalently linked to the lipid phosphatidylethanolamine and thus becomes attached to the cytoplasmic face of the autophagosome. Association of Atg8 with a membrane vesicle appears to be the key step in enabling a vesicle to fuse with the growing autophagosome.

Fusion of Atg8-containing vesicles with the autophagosome involves the formation of a cytosolic assembly of Atg12, Atg5, and Atg16. Atg12 is similar in structure to ubiquitin, and a set of proteins related to ubiquitin-conjugating enzymes are responsible for covalently joining Atg12 to Atg5 by a process similar to that used for covalently joining ubiquitin to a target protein (see Figure 3-32). The covalently linked Atg12-Atg5 dimer then co-assembles with Atg16 to form a polymeric complex localized to the site of a growing autophagosome. By an unknown mechanism, this

cytosolic complex is thought to bring about the fusion of Atg8-containing vesicles into a cup-shaped autophagosome.

Autophagosome Targeting and Fusion

The outer membrane of the completed autophagosome is thought to contain a set of proteins that target it for fusion with the membrane of a lysosome. Two vesicle-tethering proteins have been found to be required for autophagosome fusion with a lysosome, but the corresponding SNARE proteins have not been identified. Fusion of the autophagosome with the lysosome occurs after Atg8 has been released from the autophagosome membrane by proteolytic cleavage; this proteolysis step occurs only after the autophagosome has completely formed a sealed double-membrane system. Thus Atg8 protein appears to mask fusion proteins and to prevent premature fusion of the autophagosome with the lysosome.

KEY CONCEPTS OF SECTION 14.6

Directing Membrane Proteins and Cytosolic Materials to the Lysosome for Degradation

- Endocytosed membrane proteins destined for degradation in the lysosome are incorporated into vesicles that bud into the interior of the endosome. Multivesicular endosomes, which contain many of these internal vesicles, can fuse with the lysosome to deliver the vesicles to the interior of the lysosome (see Figure 14-31).
- Some of the cellular components (e.g., ESCRT) that mediate inward budding of endosomal membranes are used in the budding and pinching off of enveloped viruses, such as HIV, from the plasma membrane of virus-infected cells (see Figures 14-32 and 14-33).
- In the process of autophagy, a portion of the cytoplasm or an entire organelle (e.g., a mitochondrion) can be enveloped in a flattened membrane and eventually incorporated into a double-membrane autophagosome. Fusion of the outer vesicle

membrane with the lysosome delivers the enveloped contents to the interior of the lysosome for degradation (see Figure 14-34).