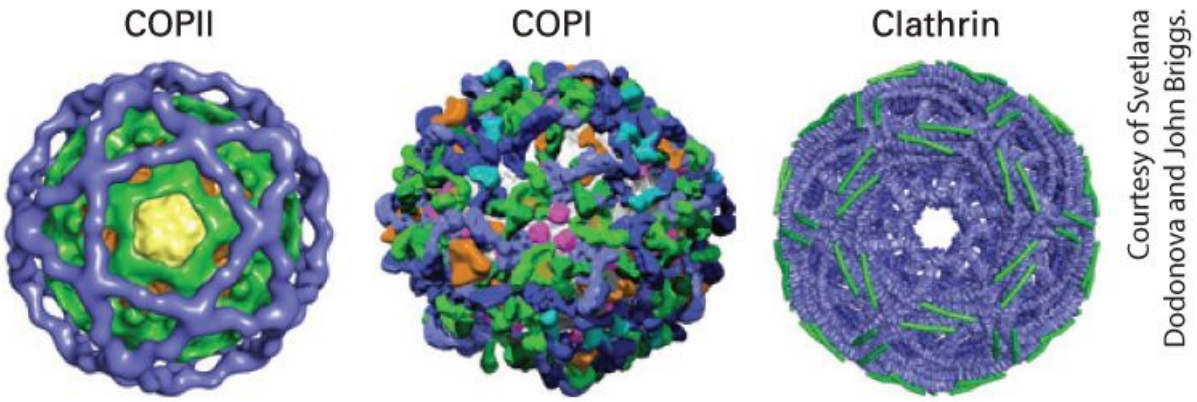
Chapter 14 Vesicular Traffic, Secretion, and Endocytosis



Structural models of the coat protein complexes for three types of transport vesicles. The coat complexes are responsible for sculpting the curvature of the vesicle membrane and for selecting the types of cargo proteins incorporated into the vesicle.

[Data from A. J. Noble and S. M. Stagg, 2015, *Science* **349**(6244):142–143; https://doi.org/10.1126/science.aac6537.]

OUTLINE

In the previous chapter, we explored how proteins are targeted to and translocated across the membranes of different intracellular organelles, including the endoplasmic reticulum (ER), mitochondria, chloroplasts, peroxisomes, and the nucleus. In this chapter, we turn our attention to the **secretory pathway** and the mechanisms of vesicular traffic that allow proteins to be secreted from the cell or delivered to the plasma membrane and the lysosomes. We also discuss the related processes of endocytosis, which delivers proteins and small molecules from outside the cell, or autophagy, which delivers organelles, proteins, and small molecules from the interior of the cell to the lysosome for degradation.

The secretory pathway is so named because it was initially studied in dedicated secretory cells that produce and secrete large quantities of proteins such as insulin or digestive enzymes to the outside of the cell. It was later discovered that the same pathway used for extracellular secretion of proteins delivers almost all newly synthesized membrane lipids and

membrane proteins from the endoplasmic reticulum (ER) to the cell surface. Newly synthesized membrane proteins delivered to the plasma membrane include cell-surface receptors, transporters for nutrient uptake, and ion channels that maintain the proper ionic and electrochemical balance across the plasma membrane. Soluble secreted proteins also follow the secretory pathway to the cell surface, but instead of remaining embedded in the membrane, they are released into the aqueous extracellular environment. Examples of secreted proteins include digestive enzymes, peptide hormones, serum proteins, and collagen.

The secretory pathway is also responsible for the distribution of particular sets of soluble and membrane proteins to the organelles that lie along the secretory pathway including the ER itself, the Golgi, the endosome, and the lysosome. As we saw in Chapter 13, the ER is the site of synthesis and folding of all proteins that enter the secretory pathway. In this chapter, we describe the principal function of the Golgi — remodeling of carbohydrate modifications of secretory proteins. The Golgi along with the endosome are two major hubs for distributing proteins to their final destination at the plasma membrane or the lysosome. The lysosome, as described in Chapter 4, is the organelle with an acidic interior that is used for degradation of proteins and storage of small molecules such as amino acids. Accordingly, the types of proteins delivered to the lysosomal membrane include subunits of the V-class proton pump that pumps H^+ from the cytosol into the acidic lumen of the lysosome, as well as transporters that release small molecules stored in the lysosome into the cytoplasm. Soluble proteins delivered by this pathway include lysosomal digestive enzymes such as proteases, glycosidases, phosphatases, and lipases.

For all of its apparent complexity, the organization and operation of the secretory pathway can be understood as the consequence of repeated variations of just two basic mechanistic themes. The first theme is that transport of membrane and soluble proteins from one membrane-bounded compartment to another is mediated by coated **transport vesicles** that collect *cargo proteins* in buds arising from the membrane of one compartment and then deliver these cargo proteins to the next compartment by fusing with the membrane of that compartment. As we will see, different types of vesicles are responsible for each step in the secretory pathway, but the underlying mechanisms of budding of a coated vesicle from a donor membrane and the specific fusion with the appropriate target membrane are remarkably conserved, regardless of vesicle type. Importantly, as transport vesicles bud from one membrane and fuse with the next, the same face of the membrane remains oriented toward the cytosol. Therefore, once a protein has been inserted into the membrane or the lumen of the ER, that protein can be carried along the secretory pathway, moving from one organelle to the next without being translocated across another membrane or altering its orientation within the membrane.

The second repeated theme is that each organelle along the secretory pathway both receives new proteins delivered by targeted transport vesicle fusion and donates proteins that are removed from the organelle by transport vesicle budding. Because transport vesicles package and deliver only specific subsets of proteins, the outcome of vesicular trafficking to and from each organelle establishes the composition and thus the identity of each organelle.

The composition of the plasma membrane is determined by two pathways: vesicle transport to the plasma membrane by the secretory pathway, which is counterbalanced by removal of proteins and membrane by the **endocytic pathway** (Figure 14-1). Both selective delivery by secretory vesicles and selective removal of proteins by endocytic vesicles can be regulated, allowing the protein composition of the plasma membrane to be regulated in response to environmental or developmental signals. In addition, the endocytic pathway is used to ingest certain nutrients that are too large to be transported across the plasma membrane by one of the transport mechanisms discussed in Chapter 11. For example, the endocytic pathway is used in the uptake of cholesterol carried in LDL particles. In addition, the endocytic pathway can be used to remove receptor proteins from the cell surface as a way to down-regulate their activity.

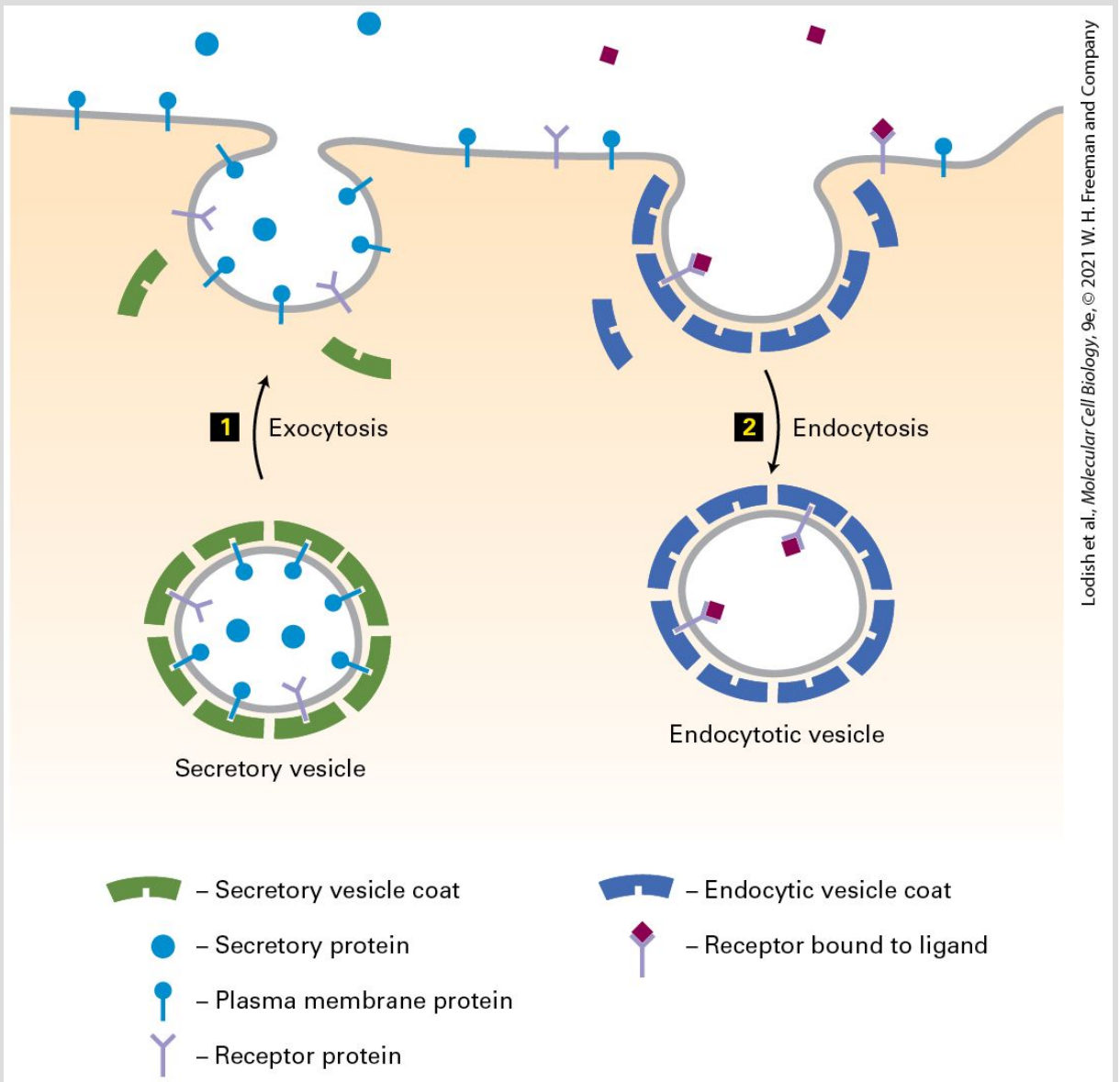


FIGURE 14-1 Basic principles of vesicle trafficking govern the composition of the plasma membrane. 1: Membranes and proteins are delivered to the plasma membrane by exocytosis of coated vesicles that bud from the Golgi complex. The types of proteins incorporated into these vesicles are determined by interactions with vesicle coat proteins. Note that the membrane proteins preserve their orientation in the membrane such that the domain facing the vesicle interior will ultimately face the exoplasmic face of the plasma membrane. Correspondingly, soluble proteins within the vesicle interior will be released into the extracellular space. 2: Proteins and membrane are removed from the plasma membrane by the process of endocytosis by which coated vesicles bud from the plasma membrane into the cytoplasm. In this example, a membrane receptor protein is only

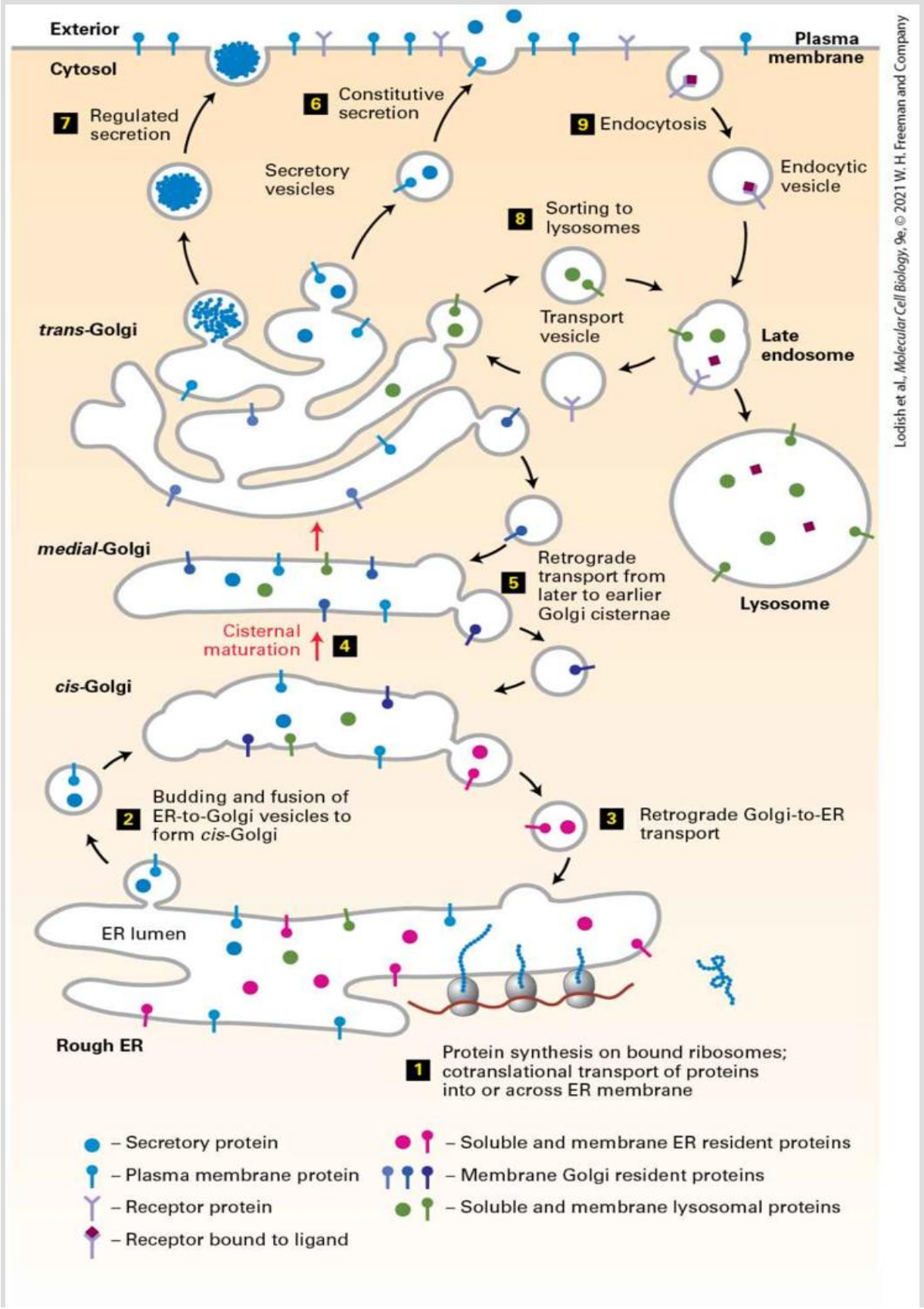
incorporated into endocytic vesicles when bound to ligand. The overall composition of the plasma membrane is controlled by a balance between the proteins that are selectively delivered by exocytic vesicles and the proteins that are removed by endocytic vesicles.

Description
Two numbered pathways are depicted. The information presented is as follows:

1. Exocytosis: A secretory vesicle inside the cell is circular and is made of the secretory vesicle coat. The thumbtack shaped plasma membrane proteins and y-shaped receptor proteins are attached to the insides of the secretory vesicle coat. The secretory vesicle coat also encloses secretory proteins that are represented by tiny spheres. The secretory vesicle coat disintegrates to release the secretory protein outside the cell, while the plasma membrane proteins and the receptor proteins bind to the plasma membrane of the cell.

2. Endocytosis: Endocytic vesicle coat attached to a receptor bound to a ligand in the inner membrane of the plasma membrane. Endocytosis leads to the formation of a circular endocytotic vesicle. The insides of the endocytotic vesicle are attached to two receptors bound to ligands.

The overall organization of the individual vesicle trafficking steps to form the intersecting secretory and endocytic pathways is outlined in Figure 14-2. The first stage of the secretory pathway takes place in the rough endoplasmic reticulum (ER) (Figure 14-2, step **1**). As described in Chapter 13, newly synthesized soluble and membrane proteins are translocated into the ER, where they fold into their proper conformation and receive covalent modifications such as *N*-linked and *O*-linked carbohydrates and disulfide bonds. Once correctly folded and modified in the ER lumen, the newly synthesized proteins progress to the second stage of the secretory pathway: transport to and through the Golgi complex.



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

FIGURE 14-2 Overview of the secretory and endocytic pathways of protein sorting. *Secretory pathway:* Synthesis of proteins bearing an ER signal sequence is completed on the rough ER (step 1), and the newly made polypeptide chains are inserted into the ER membrane or cross it into the ER lumen (see Chapter 13). ER resident proteins such as BiP and PDI remain within the ER. The remainder are packaged into transport vesicles (step 2) that bud from the ER and fuse to form new *cis*-Golgi cisternae. Missorted ER-resident proteins and vesicle membrane proteins that need to be reused are retrieved to the ER by vesicles (step 3) that bud from the *cis*-Golgi and fuse with the ER. Each *cis*-Golgi cisterna, with its protein content, physically moves from the *cis* to the *trans* face of the Golgi complex (step 4) by a nonvesicular process called *cisternal maturation*. Retrograde transport vesicles (step 5) move Golgi-resident proteins to the proper Golgi compartments. In all cells, certain soluble proteins move to the cell surface in transport vesicles (step 6) and are secreted continuously (constitutive secretion). In certain cell types, some soluble proteins are stored in secretory vesicles (step 7) and are released only after the cell receives an appropriate neuronal or hormonal signal (regulated secretion). Lysosome-destined membrane and soluble proteins, which are transported in vesicles that bud from the *trans*-Golgi (step 8), first move to the late endosome and then to the lysosome. *Endocytic pathway:* Plasma membrane proteins such as receptor proteins bound to their ligands can be taken up in vesicles that bud from the plasma membrane (step 9) and move to the endosome from where they can either be recycled to the plasma membrane or be delivered to the lysosome for degradation.

Description

In this illustration, different proteins are represented by various shapes and a legend is given for each one.

1. Protein synthesis on bound ribosomes; and co-translational transport of proteins into or across the E R membrane.

2. Budding and fusion of E R-to-Golgi vesicles to form cis-Golgi

3. Retrograde transport from the Golgi to the E R.

4. Cisternal maturation occurs, transporting proteins from the cis-Golgi to the medial-Golgi and, finally, trans-Golgi.

5. During cisternal maturation, retrograde transport of vesicles from later to earlier Golgi cisternae can occur.

The following setup occurs from the trans-Golgi network:

6. Constitutive secretion.

7. Regulated secretion.

8. Sorting of proteins to lysosomes in transport vesicles.

9. In addition, proteins can be endocytosed from outside of the cell and added to late endosomes destined to become lysosomes or be transported via transport vesicles to the trans-Golgi network.

The transport of cargo proteins from the ER to the Golgi occurs via **anterograde** (forward-moving) transport vesicles (Figure 14-2, step **2**). These vesicles fuse with one another to form a flattened membrane-bounded compartment known as the *cis-Golgi network* or *cis*-Golgi **cisterna** (a "cistern" is a container for holding water or other liquid). Certain proteins, mainly proteins that function in the ER, can be retrieved from the *cis*-Golgi cisterna and returned to the ER via a different set of **retrograde** (backward-moving) transport vesicles (step **3**). In a manner reminiscent of an assembly line, the new *cis*-Golgi cisterna, with its cargo of proteins, physically moves from the *cis* position (nearest the ER) to the *trans* position (farthest from the ER), successively becoming first a *medial*-Golgi cisterna and then a *trans*-Golgi cisterna (step **4**). This process, known as **cisternal maturation**, primarily involves retrograde transport vesicles (step **5**), which retrieve enzymes and other Golgi-resident proteins from later to earlier Golgi cisternae, thereby maturing the *cis*-Golgi cisternae to *medial*-Golgi cisternae, and *medial*-Golgi

cisternae to *trans*-Golgi cisternae. As secretory proteins move through the Golgi, their linked carbohydrates may be further modified by specific glycosyltransferases that are housed in the different Golgi compartments.

Proteins in the Golgi are eventually delivered to a complex network of membranes and vesicles termed the ***trans*-Golgi network**. The *trans*-Golgi network is a major branch point in the secretory pathway. It is at this stage that proteins are loaded into different kinds of vesicles and thereby trafficked to different destinations. Depending on which kind of vesicle the protein is loaded into, it will be transported to the plasma membrane and secreted immediately, stored for later release, or shipped to the lysosome (steps **6**–**8**). The process by which a vesicle moves to and fuses with the plasma membrane and releases its contents is known as **exocytosis**. In all cell types, at least some proteins are secreted continuously (a process commonly called **constitutive secretion**), while others are stored inside the cell in specialized vesicles known as *secretory granules* until a signal for exocytosis causes them to be released (**regulated secretion**). Secretory proteins destined for lysosomes are first transported by vesicles from the *trans*-Golgi network to a compartment usually called the **late endosome**; the proteins are then transferred to the lysosome by direct fusion of the late endosome with the lysosomal membrane.

In this chapter, we first discuss the experimental techniques that have contributed to our knowledge of the secretory pathway and endocytosis. Then we focus on the general mechanisms of membrane budding and fusion. We will see that although different kinds of transport vesicles use

distinct sets of proteins for their formation and fusion, all vesicles use the same general mechanism for budding, selection of particular sets of cargo molecules, and fusion with the appropriate target membrane. In the remaining sections of the chapter, we discuss both the early and late stages of the secretory pathway, including how specificity of targeting to different destinations is achieved. We conclude with a discussion of how proteins are transported to the lysosome by the endocytic pathway.